SEX HORMONE FLUCTUATIONS ACROSS THE FEMALE LIFESPAN: MECHANISMS OF ACTION ON BRAIN STRUCTURE, FUNCTION, AND BEHAVIOR

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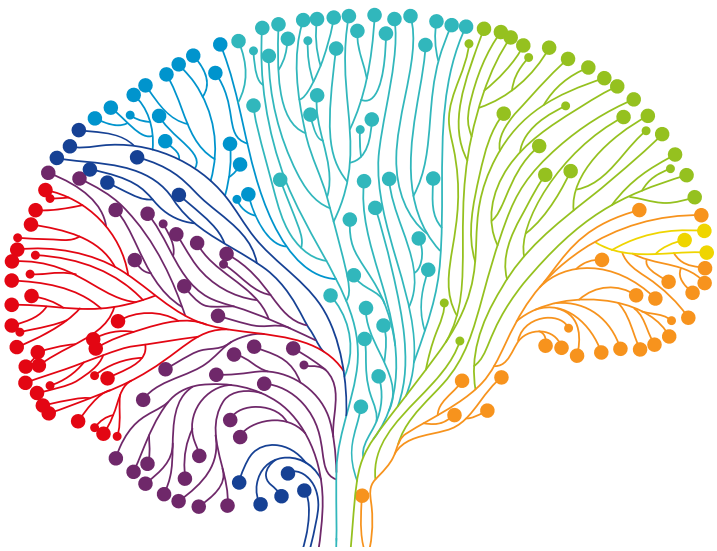
PUBLISHED IN: Frontiers in Behavioral Neuroscience



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ISSN 1664-8714
ISBN 978-2-88976-689-5
DOI 10.3389/978-2-88976-689-5

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SEX HORMONE FLUCTUATIONS ACROSS THE FEMALE LIFESPAN: MECHANISMS OF ACTION ON BRAIN STRUCTURE, FUNCTION, AND BEHAVIOR

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Citation: Koebele, S. V., Taylor, C. M., Herrera, A. Y., Barth, C., Schwarz, J. M., eds. (2022). Sex Hormone Fluctuations Across the Female Lifespan: Mechanisms of Action on Brain Structure, Function, and Behavior. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88976-689-5

Table of Contents

frontiers | Frontiers in Behavioral Neuroscience

EDITORIAL
published: 05 July 2022
doi: 10.3389/fnbeh.2022.964740

Editorial: Sex Hormone Fluctuations Across the Female Lifespan: Mechanisms of Action on Brain Structure, Function, and Behavior

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Keywords: estrogens, progestogens, androgens, menstrual cycle, menopause, women's health, oral contraceptive, sex hormones

Editorial on the Research Topic

Sex Hormone Fluctuations Across the Female Lifespan: Mechanisms of Action on Brain Structure, Function, and Behavior

OPEN ACCESS

Edited and reviewed by:
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Specialty section:
This article was submitted to Behavioral Endocrinology, a section of the journal Frontiers in Behavioral Neuroscience

Received: *08 June 2022*
Accepted: *20 June 2022*
Published: *05 July 2022*

Citation:
Koebele SV, Ycaza Herrera A, Taylor CM, Barth C and Schwarz JM (2022) Editorial: Sex Hormone Fluctuations Across the Female Lifespan: Mechanisms of Action on Brain Structure, Function, and Behavior. Front. Behav. Neurosci. 16:964740. doi: 10.3389/fnbeh.2022.964740

Medical and scientific literature, from preclinical animal models to clinical trials, largely excludes females (Woitowich et al., 2020). The justifications for exclusion are manifold, with the cyclic nature of hormones across the female lifespan targeted as a source of irremediable confound (Shansky, 2019; Rechlin et al., 2022). This dogma has led to an overreliance on data from male participants (Geller et al., 2018). As a result, significant disparities exist in our understanding of the female brain and body across the lifespan (Taylor et al., 2020; Shansky and Murphy, 2021). This perpetuates inequities, such that women frequently experience delays in receiving a diagnosis, which results in inferior healthcare (Vlassoff, 2007; Westergaard et al., 2019; Chinn et al., 2021). Important research over the past several decades has taught the field that sex hormones, including estrogens, androgens, and progesterone, have far-reaching effects beyond their classic reproductive functions. This Research Topic highlights the ways in which sex hormones exert broad systems-levels effects on mammalian biology, including the central nervous system. These effects are not static, but rather fluctuate across time scales ranging from diurnal to decades in both males and females. Major hormone transition periods, including puberty/adolescence, the menstrual cycle, pregnancy, the postpartum period, and the menopause transition impart unique effects on the female brain that can alter the trajectory of brain and cognitive aging, resulting in long lasting structural and functional brain changes (Koebele and Bimonte-Nelson, 2015). The contributions to this collection not only expand our understanding of the impact of sex hormones on the brain and behavior, but will also allow researchers and healthcare professionals to better serve individuals who have an incredible depth and breadth of diverse hormone-related experiences and exposures across the lifespan.

With regard to endogenous hormone fluctuations across the menstrual cycle, Diekhof et al. provide an intriguing report on the influence of variations in 17β-estradiol levels and the COMT-Val158Met genotype on decision-making across the menstrual cycle. Xu et al. also present

novel findings demonstrating that varied workload demands modulate menstrual cycle effects on attentional processes. These studies help reveal the complexity and nuance of sex hormones' influence on behavior.

Preclinical and clinical perspectives have elucidated exogenous hormone effects on the brain and behavior, including hormone-containing contraceptives and menopausal hormone therapies. Despite a majority of women having exposure to exogenous hormones during their lifetime (Centers for Disease Control Prevention, 2019), this is a profoundly understudied research area. In their topic contribution, Beltz et al. explore biopsychosocial influences on spatial skills, including those of oral contraceptive use and gender self-concept on a mental rotation task, expanding our understanding of factors contributing to performance variability often reported from neuropsychological tasks. Kimmig et al. demonstrate that oral contraceptives do not impair emotion recognition, and that progesterone levels were associated with cycle-dependent differences in negativity bias in naturally cycling women during an emotion recognition task. Mentin-Henry et al. also provide novel insights into emotional processing using resting state functional magnetic resonance imaging by reporting both sex and oral contraceptive use alter brain and behavioral outcomes for their emotion recognition task; interestingly, the androgenicity of oral contraceptives significantly impacted results. In a complimentary fashion, Koebele et al. showed that variations in hormone therapy formulation differentially impact working memory, anxiety-like and depressive-like behavior outcomes in a preclinical rat model of transitional menopause. Together, these findings underscore the need to take an individualized approach to exogenous hormone therapies rather than the one-size-fits-all approach that is commonplace today.

Sex hormones modulate all body systems including the brain, which is substantiated by clear sex differences in the incidence of a number of psychiatric, autoimmune, and neurological diseases (Barth et al., 2015; Mauvais-Jarvis et al., 2020). Moreover, mounting evidence suggests that these systemic influences interact. For instance, Engler-Chiurazzi et al. share an in-depth review exploring the convergence of estrogen effects on immune function and affect from a lifespan perspective, advancing the field's knowledge of endocrine–immune interactions in the context of mental health. Furthermore, the critical window hypothesis of hormone loss and cognitive impairment has driven the field's exploration of cognitive changes during aging in recent decades (Maki, 2013). Rodríguez-Landa contributes an insightful commentary on the importance of hormone intervention timing when designing preclinical experiments to investigate affective behavior. This discussion will aid our understanding and interpretation of the time course of hormone and brain changes following surgical menopause intervention.

It is also imperative to illuminate the neurobiological mechanisms underpinning cognitive–behavioral changes across multiple time scales. Beamish and Frick propose a novel mechanism through which estrogen exerts its effects on hippocampal-dependent learning and memory by exploring the role of the ubiquitin proteasome system in synapse remodeling in both sexes. Jiménez-Balado et al. provide new insights into neural mechanisms underlying episodic memory by utilizing magnetic resonance spectroscopy to demonstrate that the relationship between lower hippocampal γ-aminobutyric acid (GABA) concentrations and poorer episodic memory is driven by sex, not apolipoprotein E (ApoE) $\varepsilon 4$ genotype, as the effect was only observed in older community-dwelling women. Gilfarb and Leuner also contribute a thoughtful review summarizing the field's current understanding of cognitive–behavioral effects related to changes in the GABAergic system across major hormonal transition periods from puberty to menopause. Understanding the mechanisms underlying cognitive–behavioral effects of sex hormones is key to ultimately enhancing precision medicine approaches and care at every life stage.

Collectively, investigating the female brain and body across the lifespan with intention not only provides opportunities for advancement in the field of women's health, but also permits us to discover more about the broader human experience through inclusive research practices. Elucidating mechanisms of action of sex hormones on brain structure, function, and behavior allows us to acknowledge the value of these molecules at each life stage. Despite the decades-long axiom that female hormone fluctuations introduce unwanted variability in research, we must recognize that inherent hormone fluctuations across the female lifespan are not pathological or inconsequential, but rather an intrinsic and integral property of the female experience that allows for lifelong neural plasticity and resilience. By emphasizing sex and gender as key biological variables in research inquiries, we will gain knowledge of the human experience as well as improve healthcare and quality of life for women and gender diverse individuals across all life stages. We also hope that this growing awareness of the female experience will provide support for women to have equal and equitable access to essential healthcare across the lifespan.

AUTHOR CONTRIBUTIONS

SK: conceptualization, writing-original draft, and writing—review and editing. AYH, CT, CB, and JS: conceptualization and writing—review and editing. All authors contributed to the article and approved the submitted version.

REFERENCES

Barth, C., Villringer, A., and Sacher, J. (2015). Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. *Front. Neurosci.* 9, 37. doi: 10.3389/fnins.2015.00037

Centers for Disease Control and Prevention (2019). Key Statistics from the National Survey of Family Growth: Contraception. *Natl. Surv. Fam. Growth*. Available online at: https://www.cdc.gov/nchs/nsfg/key_statistics/c.htm#contraception (accessed May 9, 2019).

Chinn, J. J., Martin, I. K., and Redmond, N. (2021). Health equity among black women in the United States. *J. Women's Heal.* 30, 212–219. doi: 10.1089/jwh.2020.8868

Geller, S. E., Koch, A. R., Roesch, P., Filut, A., Hallgren, E., and Carnes, M. (2018). The more things change, the more they stay the same: A study to evaluate compliance with inclusion and assessment of women and minorities in randomized controlled trials. *Acad. Med.* 93, 630–635. doi: 10.1097/ACM.0000000000002027

Koebele, S. V., and Bimonte-Nelson, H. A. (2015). Trajectories and phenotypes with estrogen exposures across the lifespan: What does Goldilocks have to do with it? *Horm. Behav.* 74, 86–104. doi: 10.1016/j.yhbeh.2015.06.009

Maki, P. M. (2013). The critical window hypothesis of hormone therapy and cognition: a scientific update on clinical studies. *Menopause* 20, 695–709. doi: 10.1097/GME.0b013e3182960cf8

Mauvais-Jarvis, F., Bairey Merz, N., Barnes, P. J., Brinton, R. D., Carrero, J. J., DeMeo, D. L., et al. (2020). Sex and gender: modifiers of health, disease, and medicine. *Lancet* 396, 565–582. doi: 10.1016/S0140-6736(20)31561-0

Rechlin, R. K., Splinter, T. F. L., Hodges, T. E., Albert, A. Y., and Galea, L. A. M. (2022). An analysis of neuroscience and psychiatry papers published from 2009 and 2019 outlines opportunities for increasing discovery of sex differences. *Nat. Commun.* 13, 1–14. doi: 10.1038/s41467-022-29903-3

Shansky, R. M. (2019). Are hormones a "female problem" for animal research? *Science*. 364, 825–826. doi: 10.1126/science.aaw7570

Shansky, R. M., and Murphy, A. Z. (2021). Considering sex as a biological variable will require a global shift in science culture. *Nat. Neurosci.* 24, 457–464. doi: 10.1038/s41593-021-00806-8

Taylor, C. M., Pritschet, L., and Jacobs, E. G. (2020). The scientific body of knowledge – whose body does it serve? A spotlight on oral contraceptives and the brain. *Front. Neuroendocrinol.* 60, 874. doi: 10.1016/j.yfrne.2020.100874

Vlassoff, C. (2007). Gender differences in determinants and consequences of health and illness. *J. Heal. Popul. Nutr.* 25, 47–61.

Westergaard, D., Moseley, P., Sørup, F. K. H., Baldi, P., and Brunak, S. (2019). Population-wide analysis of differences in disease progression patterns in men and women. *Nat. Commun.* 10, 1–14. doi: 10.1038/s41467-019-08475-9

Woitowich, N. C., Beery, A. K., and Woodruff, T. K. (2020). A 10-year follow-up study of sex inclusion in the biological sciences. *Elife* 9, 1–8. doi: 10.7554/eLife.56344

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frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 08 July 2021
doi: 10.3389/fnbeh.2021.658769

The Straw That Broke the Camel's Back: Natural Variations in 17β-Estradiol and COMT-Val158Met Genotype Interact in the Modulation of Model-Free and Model-Based Control

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Behavioral Endocrinology, a section of the journal Frontiers in Behavioral Neuroscience

Received: *26 January 2021*
Accepted: *08 June 2021*
Published: *08 July 2021*

Citation:
Diekhof EK, Geana A, Ohm F, Doll BB and Frank MJ (2021) The Straw That Broke the Camel's Back: Natural Variations in 17β-Estradiol and COMT-Val158Met Genotype Interact in the Modulation of Model-Free and Model-Based Control. Front. Behav. Neurosci. 15:658769. doi: 10.3389/fnbeh.2021.658769

The sex hormone estradiol has recently gained attention in human decision-making research. Animal studies have already shown that estradiol promotes dopaminergic transmission and thus supports reward-seeking behavior and aspects of addiction. In humans, natural variations of estradiol across the menstrual cycle modulate the ability to learn from direct performance feedback ("model-free" learning). However, it remains unclear whether estradiol also influences more complex "model-based" contributions to reinforcement learning. Here, 41 women were tested twice – in the low and high estradiol state of the follicular phase of their menstrual cycle – with a Two-Step decision task designed to separate model-free from model-based learning. The results showed that in the high estradiol state women relied more heavily on model-free learning, and accomplished reduced performance gains, particularly during the more volatile periods of the task that demanded increased learning effort. In contrast, model-based control remained unaltered by the influence of hormonal state across the group. Yet, when accounting for individual differences in the genetic proxy of the COMT-Val158Met polymorphism (rs4680), we observed that only the participants homozygote for the methionine allele (n = 12; with putatively higher prefrontal dopamine) experienced a decline in model-based control when facing volatile reward probabilities. This group also showed the increase in suboptimal model-free control, while the carriers of the valine allele remained unaffected by the rise in endogenous estradiol. Taken together, these preliminary findings suggest that endogenous estradiol may affect the balance between model-based and model-free control, and particularly so in women with a high prefrontal baseline dopamine capacity and in situations of increased environmental volatility.

Keywords: reinforcement learning, estrogen, menstrual cycle, dopamine, reward learning, reward volatility, COMT-Val158Met genotype

INTRODUCTION

Neuroactive steroid hormones like 17β-estradiol (estradiol) are important modulators of neural processing (Becker, 2016). As a natural dopamine agonist, estradiol has been implicated in reward processing and basic aspects of reinforcement learning and may modulate activity in the associated frontostriatal circuits (Sakaki and Mather, 2013; Diekhof, 2018). In the striatum, estradiol modulates dopaminergic transmission, which increases the incentive salience of immediate reward and promotes the development of behavioral habits that are inflexible and difficult to overcome. This is why estradiol may also play a central role in the initiation and reinstatement of female addiction (Becker, 2016). However, estradiol is also involved in higher-order prefrontal functions such as working memory (Dumas et al., 2010; Jacobs and D'Esposito, 2011; Hampson and Morley, 2013). This suggests that estradiol could contribute to the more goal-directed aspects of the decision-making process, which enable more structured choices that can override existing habits (Daw et al., 2005). However, this association hasn't been assessed yet and it remains elusive to what extent variations in estradiol influence higher-order cognitive operations during the decision-making process.

Decision-making in complex environments involves different learning strategies. Action selection can be based on previous performance feedback. However, such a "model-free" strategy that requires agents to simply repeat actions that are reinforcing is too inflexible to account for more complex cognitive strategies. These latter strategies become necessary in more structured decision environments that for instance require the prospective anticipation of action consequences using a previously learned map or model (Doll et al., 2015). Recent accounts on reinforcement learning theory thus propose a dual-architecture of two functionally distinct computational processes in decision-making, thereby dissociating "model-free" and "model-based" control (Daw et al., 2005; Dolan and Dayan, 2013; Daw and Dayan, 2014). This dual-architecture is based on the assumption that optimization of reward outcome does not always depend on the most recent choice, but may require taking into consideration the most likely cause of a reward and to do so the learner must represent the task structure. Therefore, a second functionally distinct computational process, "model-based" control, has been proposed to support sequential choice that combines short-term predictions of immediate actions in a sequence of choices that are used to build a prospective model of the world. The process of model-based control is assumed to capture the overall complexity of the environment beyond model-free learning and enables reflective planning (Daw et al., 2005). In humans, the relative dominance of model-based control over the model-free system is correlated with other higher-order cognitive operations like declarative memory (Doll et al., 2015), working-memory span (Potter et al., 2017), and attentional control (Otto, 2013).

Model-free control is believed to rely on the prediction error signal of mesencephalic dopamine neurons (Glimcher, 2011). Transient changes in dopamine thereby signal the difference between received and predicted reward in the striatum, where these signals have opposing effects on the dopamine D1- and D2-receptors, i.e., DRD1 and DRD2, respectively, as well as on the associated processes of approach and avoidance learning (Collins and Frank, 2014). Model-based control depends on central dopamine that modulates activation in both the striatum and the prefrontal cortex. Deserno et al. (2015) found that a higher presynaptic dopamine level in the ventral striatum was associated with a bias toward model-based learning and promoted model-based activation in the lateral prefrontal cortex at the expense of model-free prediction errors in the ventral striatum. Further, the transient enhancement of central dopamine by agonist treatment enhanced model-based learning capacity in healthy young men (Wunderlich et al., 2012; but see Kroemer et al., 2019 for a null finding). In contrast, reductions in the ability to rely on the model-based component have been found in male addicts with a disturbed dopamine system (Sebold et al., 2014), in Parkinson patients in the dopamine-deprived state (Sharp et al., 2016), and following disruptions of the prefrontal cortex by transcranial magnetic stimulation (Smittenaar et al., 2013). These observations fit with the idea that prefrontal and striatal dopaminergic mechanisms interact in higher-order cognitive operations, such as model-based learning, both supporting the stabilization and flexible updating of goal representations (Frank and O'Reilly, 2006; Cools and D'Esposito, 2011).

The COMT-Val158Met polymorphism codes for the activity of the dopamine-degrading enzyme catechol-o-methyltransferase (COMT) (Apud et al., 2007; Käenmäki et al., 2010), which is more active in the prefrontal cortex of carriers of the Val allele than of individuals homozygote for the Met allele. This may lead to higher prefrontal dopamine availability in Met-homozygotes (see also Schacht, 2016). It has been proposed that the Met allele is more beneficial for the stabilization of prefrontal information processing and may protect goal-directed information from interference, supposedly by optimizing signaling through prefrontal DRD1 in relation to DRD2. In contrast, homozygosity for the Val allele may predict less balanced signaling through these receptors, which results in reduced cognitive capacity (Durstewitz and Seamans, 2008; Slifstein et al., 2008; Schacht, 2016). The COMT-Val158Met polymorphism has been associated with model-based control (Doll et al., 2016), as well as with other aspects of higher-order cognition including working memory and executive function (Mier et al., 2010; Schacht, 2016). Homozygosity for the Met allele thereby predicted an overall advantage in prefrontal tasks, especially those with increased cognitive load (Mier et al., 2010).

As an indicator of dopamine baseline capacity in the prefrontal cortex, the COMT-Val158Met polymorphism has further been observed to interact with (pharmacological) agents that transiently enhance dopamine. Notably, the resulting relationship between the combined effect of tonic and phasic dopamine on cognitive performance was not linear, but rather followed an inverted U-shape. This has led to the "Inverted-U-Hypothesis," which presumes that peak cognitive performance is linked to an optimal dopamine level that lies in the intermediate physiological range, while cognitive performance is believed to decline in individuals with either higher or lower than this optimal dopamine range, which has been shown repeatedly

(Cools and D'Esposito, 2011). Therefore, in the present study we decided to account for the COMT-Val158Met polymorphism as a baseline marker of prefrontal dopamine, when assessing the phasic influence of estradiol on model-based control.

Apart from baseline differences in dopamine, our study assessed the role of estradiol as a natural dopamine agonist in model-based reinforcement learning. In rodents, estradiol modulates dopamine within frontostriatal networks. Estradiol increases dopaminergic transmission and amplifies the reward-related dopamine release, for example by augmenting DRD1 action, while concurrently suppressing DRD2 action (Lévesque et al., 1989; Krentzel and Meitzen, 2018; see also Becker, 2016; Yoest et al., 2018 for review). Similarly, estradiol down-regulates the dopamine-degrading enzyme COMT in the female prefrontal cortex, which in turn increases dopamine content in this structure (Xie et al., 1999; Schendzielorz et al., 2011). For these reasons, we expected an interaction between baseline dopamine and the phasic influence of estradiol in women, when comparing distinct high and low estradiol phases of the natural menstrual cycle. We decided to test women twice during the follicular phase. During the follicular phase estradiol level rises from its nadir until it reaches its cyclic peak right before ovulation. Progesterone, another steroid hormone important for female reproductive function, remains at a low concentration throughout the follicular phase. In the second half of the menstrual cycle, estradiol rises again toward the mid luteal phase. But this time, progesterone concentration is also increased (Sakaki and Mather, 2013). This is insofar important, since progesterone inhibits dopaminergic transmission through various physiological mechanisms, and could thus antagonize the dopamine agonistic effect of estradiol during the luteal phase (e.g., Luine and Rhodes, 1983; Dluzen and Ramirez, 1984, 1987; Luine and Hearns, 1990). By restricting our tests to the early (low estradiol) and late (high estradiol) follicular phase, we were able to assess the effect of the dopamine agonist estradiol widely uncontaminated by the dopamine antagonist progesterone.

In line with the dopamine-agonistic properties of estradiol, previous studies with humans showed that estradiol influenced model-free learning, also in interaction with the dopaminergic baseline capacity of the striatum that followed an inverted U-shape relationship (Diekhof, 2015; Jakob et al., 2018; see also Diekhof, 2018). In one study reward sensitivity was compromised when estradiol level reached its peak in the late follicular phase of the menstrual cycle. Conversely, intermediate estradiol levels at the beginning of the follicular phase promoted reward sensitivity. This was especially true for individuals with a lower dopamine baseline capacity in the striatum (Diekhof, 2015), as indicated by lower trait impulsivity (see also Buckholtz et al., 2010). In a similar vein, Jakob et al. (2018) observed that carriers of the 9-repeat-allele of the DAT1 genotype, with a higher dopamine transporter (DAT) density in the striatum, apparently experienced a marked reversal of DAT function as a consequence of rising estradiol, which led to a significant decline in the capacity to avoid negative outcomes in the high estradiol phase.

In the human prefrontal cortex, estradiol has been found to stabilize working memory representations, most likely also through its interaction with dopamine, and particularly so in situations of high cognitive demand (Dumas et al., 2010; Hampson and Morley, 2013). One prominent finding also supported the "Inverted-U-Hypothesis," by demonstrating that the effect of estradiol on working memory performance and prefrontal activity depended on baseline dopamine concentration, and particularly so in high-load conditions (Jacobs and D'Esposito, 2011). A dose-dependency of estradiol could further be observed in ovariectomized rats in that only a moderate dose, but neither a low nor high dosage of estradiol preserved cognitive performance under high-load working memory demands (Bimonte and Denenberg, 1999). This shows that even independent of tonic dopamine, a deficit or abundance of estradiol could destabilize prefrontal working memory representations.

Until now, neurocognitive research has only addressed the role of estradiol in model-free learning (Diekhof, 2018). Considering the modulatory influence of estradiol on frontostriatal networks and dopamine (Becker, 1999; Yoest et al., 2018), and its association with both probabilistic feedback learning (e.g., Diekhof, 2015) and higher-order working memory processes (e.g., Jacobs and D'Esposito, 2011), we hypothesized that estradiol – as a natural dopamine-agonist – should also modulate model-based learning. The major aim of the present study was to examine whether model-based reinforcement learning is affected by the high estradiol state of the late follicular phase compared to the low estradiol state at the beginning of the follicular phase. Further, we also assessed whether the hypothesized effect of estradiol on model-based learning depends on prefrontal dopaminergic baseline capacity, similar to what has been demonstrated for model-free control in relation to striatal dopamine (Diekhof, 2018).

For this purpose, 41 women performed a Two-Step Markov Decision Task (TS-task), once in the low estradiol state of the early follicular phase and once during the high estradiol state of the late follicular phase. The TS-task combined features of a sequential choice task and a probabilistic selection task, which allowed us to assess model-based relative to model-free choice, while participants tried to maximize overall gain (Doll et al., 2016). Each of the 300 experimental trials consisted of two consecutive decision stages. At the initial stage of the TS-task, the participants had to decide between two arbitrary stimuli (a pair of Sanskrit symbols). The initial decision for one of the symbols then stochastically determined a set of second-stage options, i.e., one of the two second-stage stimulus pairs, with fixed transition probabilities (0.7 and 0.3). Depending on the initial choice, one set of options at the second-stage occurred more often, i.e., the "common transition" occurred in 70% of selections of the given first-stage symbol. The other second-stage set is denoted as the "rare transition" that occurred only in 30% of a given first-stage selection. After the selection of a symbol at the second stage, subjects received feedback, either in form of a monetary token or a feedback indicating outcome omission. The outcome was probabilistic. In the first 150 trials (the "drift phase"), outcome probability was slowly and randomly drifting between 0.25 and 0.75, while in the remaining 150 trials (the "stable phase") the reward probabilities for each of the two second-stage sets reached their final values, which was 0.7:0.3

for one and 0.6:0.4 for the other set (see also Doll et al., 2016). This enabled us to dissociate model-free control, i.e., the simple repetition of rewarded choice regardless of the transition, from model-based control, which also takes into account whether the second-stage reward was linked to a rare transition (model-based control would demand a switch to the other option at the first-stage after a rare transition). Additionally, women were genotyped for the COMT-Val158Met polymorphism, a proxy of prefrontal dopamine content, in order to further examine the potentially non-linear relationship between estradiol and model-based control. Since none of the many previous studies on model-based learning controlled for the hormonal state of female subjects nor did they assess the interaction of estradiol with baseline dopamine content, the present study is the first to provide evidence regarding the role of endogenous estradiol in higher-order reinforcement learning.

MATERIALS AND METHODS

Sample

In this study, 41 healthy young women [mean age (±SEM) = 24.6 ± 0.5 years; age range = 20–30 years], were tested with a TS-task in the early follicular phase, when circulating estradiol levels were low, and the late follicular phase, when circulating estradiol levels were high. Women were free of medication and hormonal contraceptives. For the 26 women who had previously taken hormonal contraceptives the mean distance of the first test day to the last intake of hormonal contraception was 15.8 months (SEM = 2.5 months; range = 2–36 months). Four women had stopped the intake 2 months before participation.

Women were included in the study if they had regular menstrual cycles and no gynecological problems, like polycystic ovary syndrome or endometriosis, or any other chronic disorder of the hormone system, e.g., Diabetes, Hashimoto's thyroiditis. Current or previous psychiatric or neurological problems precluded study enrollment as did the present use of hormonal contraceptives. Subjects were of Middle European origin as determined by the place of birth of their parents and grandparents. All subjects gave written informed consent and were paid for participation. The present study was approved by the local Ethics Committee (*Ethikkommission der Ärztekammer Hamburg*).

The women were tested twice within the follicular phase of the menstrual cycle. One test occurred during the first 3 days following the onset of menstruation, i.e., the early follicular phase, which is characterized by low estradiol. The other one took place 2–3 days before expected ovulation in the late follicular phase, when estradiol approached its cyclic maximum. For determination of the actual test day participants stated their average cycle length based on previous menstrual cycles. Upon the onset of menstrual bleeding (cycle day 1) we then used the average cycle length to calculate the last expected cycle day (anticipated cycle end) in the given menstrual cycle. This enabled us to determine the optimal test day with a common counting method: For all subjects with an average cycle length shorter than 28 days, we subtracted 15 days from the anticipated cycle end. For subjects with an average of 28–31 days, 16 days were subtracted, and for cycle lengths longer than 31 days, 17 days were subtracted to schedule the late follicular phase test. Our subjects also tracked the daily concentration of the gonadotrophin Lutropin, which experiences a steep rise approximately 36 h prior to ovulation. For this, a common urine test (One Step® by AIDE Diagnostic Co., Ltd.) was used. The urine test was performed on a daily basis starting 2 days before the scheduled late follicular phase test. In case of a positive result either before or on the day of the scheduled test, the behavioral test was postponed to the subsequent menstrual cycle. Test order was balanced between subjects and half of the subjects started in the early follicular phase. We initially recruited 48 women for the study. Of these, seven women dropped-out after completion of the first test day. Therefore the test order of the repeated tests was slightly biased toward the early follicular phase (24 women started in the early follicular phase). There was no significant interaction between test order and cycle phase when assessing the two learning components as shown in **Table 1**.

Collection and Analysis of Salivary Estradiol

On each test day, subjects collected five samples of morning saliva at home. Starting at their normal wake-up time, each subject collected the samples (2 ml Eppendorf tubes) at regular intervals over 2 h, in order to control for the episodic secretion pattern of steroid hormones. During the sampling period, no consumption of food or beverages other than water was allowed to avoid sample contamination. Also, 12 h before sample collection subjects refrained from eating meat or other animal products. On the same day, the participants brought the samples to the lab, where they were immediately frozen at −20°C until further analyses. The subsequent analysis of free estradiol content was based on the aliquots of the five samples and used a 17beta-Estradiol Luminescence Immunoassay (IBL International, Tecan Group, Hamburg, Germany). The analysis followed the instructions provided by the manufacturer. Altogether, this allowed us to analyze the salivary estradiol level from the repeated tests of 39 subjects. The remaining samples of two women could not be analyzed as the two Immunoassay-plates we used each provided only 39 wells for double sampling.

DNA Collection, Extraction, and Genotypic Analysis

Genotyping was performed by a commercial laboratory (Bioglobe, Hamburg, Germany). DNA was extracted from buccal swabs and purified with a standard commercial extraction kit. The analysis of the single nucleotide polymorphisms (SNP) rs4680 was performed on the MassARRAY® system (Agena Bioscience) applying the iPLEX® method and MALDI-TOF mass spectrometry for analyte detection. In general, all iPLEX reactions were performed according to the standard protocol recommended by the system supplier. The protocol generates allele-specific analytes in a primer extension reaction applying a primer directly adjacent to the SNP site. Assay design was performed with platform-specific software for the SNP sequences, aided by database information accounting

TABLE 1 | Results of the repeated-measures ANOVAs with the factors "cycle phase" and "test-order" separately for drift and stable phase.

Main effect or interaction	*F*-value	df	*p*-value	Partial eta^2
(A) Drift-phase – model-free component				
Cycle phase*	**7.45**	**1, 39**	**0.009**	**0.16**
Test order	0.002	1, 39	0.969	<0.01
Cycle phase × test order	2.60	1, 39	0.115	0.06
(B) Stable-phase – model-free component				
Cycle phase	0.13	1, 39	0.719	<0.01
Test order*,[1]	**4.24**	**1, 39**	**0.046**	**0.10**
Cycle phase × test order	0.84	1, 39	0.365	0.02
(C) Drift-phase – model-based component				
Cycle phase	0.05	1, 39	0.828	<0.01
Test order	0.88	1, 39	0.355	0.02
Cycle phase × test order	0.29	1, 39	0.594	0.01
(D) Stable-phase – model-based component				
Cycle phase	0.21	1, 39	0.648	<0.01
Test order	2.66	1, 39	0.111	0.06
Cycle phase × test order	0.32	1, 39	0.573	<0.01

**Significant effects ($p < 0.05$) are plotted in bold and are marked with an asterisk.*
[1] The direct comparison of subjects who started their first test in the early follicular phase (early-to-late group; n = 24) with those that started in the late follicular phase (late-to-early group; n = 17) yielded a significant difference in the model-free score of the stable phase (mean ± SEM: early-to-late = 20.6 ± 2.7; late-to-early = 11.8 ± 3.4; $t_{(39)} = 2.06$, $p = 0.046$). This suggests that one group of participants used the model-free learning component to a greater extent, yet this effect was independent of cycle phase. This was also supported by the exploratory analysis of the respective test days. It showed that subjects from the early-to-late group, who were in the late follicular phase on the second test day, had a trend-wise higher model-free score than subjects in the early follicular phase (2nd test day: Early = 9.8 ± 4.1; Late = 19.8 ± 3.5; $t_{(39)} = -1.86$, $p = 0.071$). Similarly, on the first test day the subjects from the early-to-late group being in the early follicular phase now had a somewhat higher score on that day, even though again this difference was not significant (1st test day: Early = 21.5 ± 3.0; Late = 13.9 ± 4.6; $t_{(39)} = 1.46$, $p = 0.153$).

for homologous regions and annotated secondary sequence variations in close proximity to the target SNP (proxSNPs). Based on rs-IDs, the multiplex assay design was performed with MassARRAY assay design suite v2.0. The final *in silico* design output was composed of a single multiplex reaction (8plex). The PCR amplification procedure used the following two primers: ACGTTGGATGTTTTCCAGGTCTGACAACGG and ACGTTGGATGACCCAGCGGATGGTGGATTT. The iPLEX primer was tCATGCACACCTTGTCCTTCA. The distribution of genotypes was in Hardy-Weinberg equilibrium ($p = 0.18$; two-tailed) as determined by the HW-Quick Check software by Steven T. Kalinowski[1].

Altogether, four participants were homozygote for the Val allele (Val/Val), while 25 subjects were heterozygote (Met/Val), and 12 subjects were homozygote for the Met allele (Met/Met). Based on the distribution of genotypes, we decided to combine the Val/Val and Met/Val, who constitute the group of "Val-carriers" in all subsequent analyses. The two groups, Met/Met and Val-carriers did not differ in most demographic characteristics like average cycle length, cycle day of early and late follicular test, estradiol level on the respective test day, and trait impulsiveness as determined by Barratt Impulsiveness Scale (BIS-11) (Patton et al., 1995), however, Met allele homozygotes were slightly older than Val allele carriers (see **Table 2**).

Task Description

Participants started the Two-Step task with a computer-based tutorial and a short training of 20 trials, which was supervised by the experimenter. Then the sequential TS-task with 300 trials in total was performed. Participants were tested with the version of the TS-task already employed by Doll et al. (2016). The TS-task incorporates a two-stage choice structure to achieve positive feedback (a virtual 1 Euro-coin) and tests for the individual model-free and model-based learning capacity. It thus captures the distinction between model-free learning behavior, i.e., the ability to adapt behavior based on direct performance feedback, and prospective model-based learning (Daw et al., 2005; Dolan and Dayan, 2013). In the first step of the TS-task, participants choose between two options (two Sanskrit symbols) within a time window of 2 s, which stochastically determines another set of choices with fixed transition probabilities between steps (i.e., 0.7 and 0.3, see Doll et al., 2016).

In the TS-task, model-free control of behavior describes the aspect of learning that increments the value of choices based on the outcome that directly follows, and regardless of the transitions experienced between task stages. In contrast, model-based control takes the history of outcomes as well as the noisy task structure prospectively into account (Daw et al., 2005). Model-based learning is particularly important in the TS-task, since an actual reward can only be reached after the two consecutive choices. The first stage choice thereby determines with a certain probability, which pair of options is available for the second stage choice. For each action at the first stage, one pair of options at stage 2 is more likely to occur (common transition), while the other pair is less likely (rare transition). The model-based component is assumed to take these transition probabilities into account, while the model-free component is not. From this, certain predictions can be

[1] http://www.montana.edu/kalinowski/software/hw-quickcheck.html

TABLE 2 | Demographic data divided by genotype.

	Met allele homozygotes	Val allele carriers	
	Mean ± SEM	**Mean ± SEM**	***t*-value (*p*-value)**
Age (years)*	26.6 ± 0.7	23.8 ± 0.5	**2.95 (0.005)**
Mean length of two consecutive menstrual cycles (days)	29.2 ± 1.1	30.7 ± 0.7	−1.18 (0.245)
Cycle day of early follicular phase	1.8 ± 0.4	2.5 ± 0.3	−1.29 (0.206)
Cycle day of late follicular phase	13.4 ± 0.8	13.2 ± 0.4	0.20 (0.839)
Estradiol level of early follicular phase (pg/ml)	2.98 ± 0.63	3.00 ± 0.27	−0.04 (0.969)
Estradiol level of late follicular phase (pg/ml)	3.81 ± 0.60	4.56 ± 0.35	−1.11 (0.274)
Impulsiveness score (BIS-11)	60.9 ± 2.5	62.7 ± 1.4	−0.64 (0.526)

**Significant differences ($p < 0.05$) are plotted in bold and are marked with an asterisk.*

made: In case of a rare transition, the model-free component would use the feedback at stage 2 to choose the stage 1 stimulus independent of the nature of the transition. It would stay with the previous first-stage choice, even after a rare second-stage reward, which would lower overall reward outcome. In contrast, after receiving a rare reward, model-based control would probably bias the decision toward a switch at stage 1 and the choice of the option more likely to transition to the second-stage state that would have produced reward on the last trial (switch to the common transition) (Doll et al., 2016). Based on these predictions and the stay frequencies from stage 1, we calculated the model-based and model-free learning components according to Sebold et al. (2014), which could then be compared between cycle phases.

The model-free score thereby reflected the main effect of reward on stay frequencies that was calculated by:

$$model - free\ score = \%\ rewarded\ common\ transition + \%\ rewarded\ rare\ transition - \%\ unrewarded\ common\ transition - \%\ unrewarded\ rare\ transition$$

The model-based score mirrored the interaction between transition frequency and reward, which was indicated by:

$$model - based\ score = \%\ rewarded\ common\ transition + \%\ unrewarded\ rare\ transition - \%\ rewarded\ rare\ transition - \%\ unrewarded\ common\ transition$$

In contrast to other versions of the TS-task, the specific version employed by Doll et al. (2016) included two task phases, the drift phase of the first 150 trials and the stable phase of the remaining 150 trials, which were characterized by different degrees of reward uncertainty at the second stage choice. During the drift phase the second stage choice is followed by reward with a slowly and randomly drifting probability set within the boundaries of 0.25 and 0.75. In the present study, one of four sets of drifts was randomly assigned to each person in each cycle phase, whereby the assignment did not differ between cycle phases or COMT genotypes ($p > 0.39$). The design feature of the drift phase emphasized model-free updating, as subjects learned the values of these stimuli incrementally. In the remaining 150 trials (the stable phase) the reward probabilities reached their final values of 0.7 versus 0.3 in state 1, and 0.6 versus 0.4 in state 2.

Statistical Analysis

First, we analyzed the individual stay frequencies at the first stage choice with a repeated-measures analysis of variance (ANOVA). This was done separately for the drift and the stable phase in order to account for the different degrees of reward uncertainty (see task description above). The ANOVA assessed stay frequencies in relation to the reward achieved at stage 2 of the previous trial, i.e., the factor "*previous reward*" (yes, no), and the previous transition that led to this reward, i.e., factor "*previous transition*" (rare or common), as well as their interaction. Additionally, the ANOVA also included the within-subject factor "*cycle phase*" (early or late follicular phase) and the between-subjects factor "*COMT genotype*" (Val-carriers, Met-homozygotes). The effect size is reported as partial eta^2. *Post hoc* tests used paired or independent *t*-tests. For effect sizes we use Cohen's *d* or Hedge's *g* for comparisons including one group with $n < 20$. Statistical significance was assumed at $p < 0.05$, two-tailed, if not indicated otherwise.

In a second step, we looked more specifically at differences in the model-free and model-based learning components. For this, we calculated the model-based and model-free learning components according to Sebold et al. (2014) (see above), which were then compared between cycle phases and genotypes, respectively.

RESULTS

Analysis of Menstrual Cycle Phase Related Changes in Estradiol Level

Estradiol level followed the predicted cycle-typical pattern and significantly increased from the early to the late follicular phase [mean ± SEM: $\text{estradiol}_{\text{early}} = 2.99 \pm 0.26$ pg/ml; $\text{estradiol}_{\text{late}} = 4.35 \pm 0.31$ pg/ml; $t_{(38)} = 4.48$, $p < 0.001$, one-tailed], also within the subgroup of Val-carriers [$t_{(27)} = 4.03$, $p < 0.001$, one-tailed] and in the Met-homozygotes [$t_{(10)} = 2.06$, $p < 0.034$, one-tailed]. In that way, the early follicular and the late follicular phase

can be considered as the low and the high estradiol state, respectively.

Analysis of Stay Frequencies and Learning Scores of the Drift Phase

In the drift phase, the choice at stage 2 was followed by probabilistic reward with a slowly and randomly drifting probability. Thus, the drift phase required a constant updating of the current decision to maximize reward, like in other versions of the TS-task previously employed (e.g., Deserno et al., 2015; Doll et al., 2016; Kroemer et al., 2019).

First, we assessed the influence of hormonal state and genetic variance on the stay frequencies at the first stage choice of the drift phase. The stay frequencies thereby represent the probability that the same stage 1 choice would be made on the next trial. We identified a significant main effect of "previous reward" [$F_{(1,39)}$ = 39.63, $p < 0.001$, partial eta^2 = 0.5] and a significant interaction of "previous reward" by "previous transition" [$F_{(1,39)}$ = 9.80, p = 0.003, partial eta^2 = 0.2], indicating that participants used both model-free and model-based learning while performing the task (Daw et al., 2011). We also found a significant two-way interaction between "cycle-phase" and "previous reward" [$F_{(1,39)}$ = 6.56, p = 0.014, partial eta^2 = 0.14]. This was reflected by enhanced avoidance of non-reward *per se* in the late as opposed to the early follicular phase [non-reward: stay frequency$_{early}$ ± SEM = 73.2 ± 2.3%; stay frequency$_{late}$ ± SEM = 69.1 ± 2.1%; $t_{(40)}$ = 2.23, p = 0.031, d = 0.33]. In addition to that, a four-way interaction between "cycle-phase," "previous reward," "previous transition," and "COMT genotype" was found [$F_{(1,39)}$ = 4.48, p = 0.041, partial eta^2 = 0.1] (see also **Table 3** for the complete ANOVA results). Accordingly, the Val-carriers became better at avoiding the commonly non-rewarded option in the late follicular phase (stay frequency$_{late}$ ± SEM = 65.4 ± 3.0%) compared to the early follicular phase [stay frequency$_{early}$ ± SEM = 71.2 ± 2.6%; $t_{(28)}$ = 2.18, p = 0.038, d = 0.44]. Since the Val-carriers represented the majority of the test group, this change probably drove the above described two-way interaction between "cycle-phase" and "previous reward." Apart from that, we also observed that the stay frequencies of the Met-homozygotes in relation to rare reward showed a trend-wise increase in the late follicular phase [stay frequency$_{early}$ ± SEM = 74.3 ± 5.0%; stay frequency$_{late}$ ± SEM = 83.5 ± 3.8%; $t_{(11)}$ = −2.04, p = 0.065, d = 0.53]. This increase was also significantly different from the delta observed in Val-carriers [Delta stay frequency$_{latevs.early}$ ± SEM: Met/Met = 9.2 ± 4.5%; Val carriers = −3.1 ± 2.8%; $t_{(39)}$ = 2.35, p = 0.025, Hedge's g = −0.81], suggesting that Met-homozygotes became impaired in their ability to adequately integrate the complex task structure in their choices when being in the high estradiol state (see **Figures 1A,B**).

In a second step, we calculated the model-free and the model-based scores based on the stay frequencies (Sebold et al., 2014). We found a significant increase in the model-free score from the early to the late follicular phase in the complete group of subjects [Drift phase: model-free$_{early}$ ± SEM = 15.51 ± 2.18; model-free$_{late}$ ± SEM = 19.81 ± 2.65; $t_{(40)}$ = −2.44, p = 0.019, d = −0.44] (see **Figure 2A**). Notably, the relative increase in model-free learning from the early to the late follicular phase (Delta$_{model-free}$) was related to more suboptimal decision making, reflected by a reduced task success in terms of the total number of acquired coins during the drift phase (r = −0.417, p = 0.007, n = 41) (see **Figure 2C**).

When the sample was dichotomized by genotype we found that the increase in the model-free score from the early to the late follicular phase was only significant in the Met-homozygotes [model-free$_{early}$ ± SEM = 7.2 ± 5.4; model-free$_{late}$ ± SEM = 19.7 ± 5.8; $t_{(11)}$ = −2.71, p = 0.020, d = −0.65], but not in Val-carriers [model-free$_{early}$ ± SEM = 16.1 ± 2.9; model-free$_{late}$ ± SEM = 22.4 ± 3.8; $t_{(28)}$ = −1.47, p = 0.15]. In addition, Met-homozygotes also showed a concurrent decline of the model-based score during the drift phase [model-based$_{early}$ ± SEM = 13.6 ± 6.0; model-based$_{late}$ ± SEM = 0.9 ± 3.2; $t_{(11)}$ = 2.62, p = 0.024, d = 0.67], which was again absent in Val-carriers [model-based$_{early}$ ± SEM = 4.9 ± 3.2; model-based$_{late}$ ± SEM = 8.5 ± 3.2; $t_{(28)}$ = −0.80, p = 0.431]. The magnitude of cycle phase related changes in both the model-free and model-based learning scores, i.e., the Delta value of the score from the late minus the early follicular phase, was also significantly different from zero in the Met-homozygotes (see **Figure 3A**; see also **Table 4**).

Analysis of Stay Frequencies and Learning Scores of the Stable Phase

Following the drift phase, unbeknownst to participants, the reward probabilities at stage two stopped drifting and remained fixed. In principle, this stable phase requires a lower learning rate and the difficulty of learning is reduced, since reward probabilities are reliable now. During the stable part of the TS-task, we also found a significant main effect of "previous reward" [$F_{(1,39)}$ = 45.22, $p < 0.001$, partial eta^2 = 0.54] and a significant interaction of "previous reward" by "previous transition" [$F_{(1,39)}$ = 18.60, $p < 0.001$, partial eta^2 = 0.32]. However, in contrast to the drift phase, we observed a significant three-way interaction between "cycle phase", "previous reward" and "COMT genotype" [$F_{(1,39)}$ = 8.30, p = 0.006, partial eta^2 = 0.18] (see also **Table 5** for a complete list of the ANOVA results). This was reflected by a differential change in avoidance learning capacity between cycle phases and genotypes. We found a reduced avoidance capacity of non-reward (i.e., a higher stay frequency for non-reward) in the late relative to the early follicular phase in Met-homozygotes compared to the Val-carriers [Delta stay frequencies$_{late-early}$ ± SEM: Met/Met = 7.27 ± 3.35%; Val-carriers = −3.8 ± 2.19%; $t_{(39)}$ = 2.74, p = 0.009, Hedge's g = 0.94]. Further conforming to this pattern, the direct comparison of cycle phases within genotype groups revealed two statistical trends, with the Met-homozygotes showing a slight reduction in avoidance learning capacity [stay frequency$_{non-reward}$ ± SEM: Early = 69.2 ± 4.2%; Late = 76.5 ± 3.8%; $t_{(11)}$ = −2.17, p = 0.052, d = 0.60], while the Val-carriers showed a trend-wise increase in this capability

TABLE 3 | Drift phase – Effects of cycle-phase, TS-task manipulation and COMT-genotype on stay frequencies.

Main effect or interaction	*F*-value	df	*p*-value	partial eta^2
Previous reward*	**39.63**	**1, 39**	**<0.001**	**0.50**
Previous transition	0.58	1, 39	0.450	0.02
Cycle phase	0.37	1, 39	0.546	0.01
COMT genotype	0.17	1, 39	0.680	<0.01
Previous reward × previous transition*	**9.80**	**1, 39**	**0.003**	**0.20**
Previous reward × cycle phase*	**6.56**	**1, 39**	**0.014**	**0.14**
Previous reward × COMT genotype	1.25	1, 39	0.270	0.03
Previous transition × cycle phase	0.29	1, 39	0.596	0.01
Previous transition × COMT genotype	0.08	1, 39	0.780	<0.01
Cycle phase × COMT-genotype	2.238	1, 39	0.143	0.05
Previous reward × previous transition × cycle phase	1.39	1, 39	0.245	0.03
Previous reward × previous transition × COMT genotype	0.02	1, 39	0.899	<0.01
Previous reward × cycle phase × COMT genotype	0.70	1, 39	0.408	0.02
Previous transition × cycle phase × COMT genotype	0.53	1, 39	0.469	0.01
Previous reward × previous transition × cycle phase × COMT genotype*	**4.48**	**1, 39**	**0.041**	**0.10**

**Significant effects ($p < 0.05$) are plotted in bold and are marked with an asterisk.*

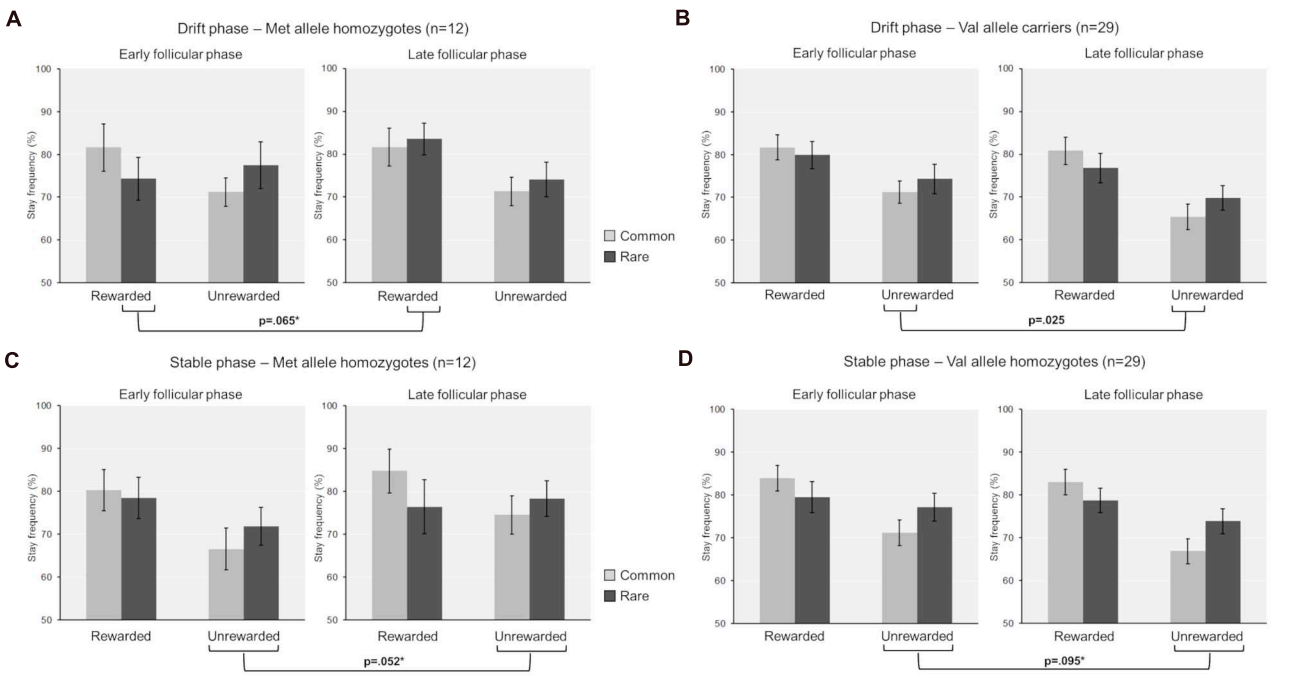

FIGURE 1 | Mean stay frequencies separated by task phase (drift versus stable phase), genotype group (Met-homozygotes versus Val-carriers) and cycle phase (early versus late follicular phase). **(A)** Drift phase, Met-homozygotes. **(B)** Drift phase, Val-carriers. **(C)** Stable phase, Met-homozygotes. **(D)** Stable phase, Val-carriers. The differences between cycle phases are indicated with the respective *p*-value. These also include statistical trends ($p < 0.10$), for which the actual delta-values of stay frequencies (Delta stay frequency$_{\text{latevs.earlyfollicularphase}}$) were significantly different between the genotypes (delta values are not shown here, but are reported in the text). These statistical trends are additionally marked with an asterisk, if the direct comparison between the genotypes yielded a significant difference ($p < 0.05$).

[non-reward stay frequency ± SEM: Early = 74.2 ± 3.0%; Late = 70.4 ± 2.7%; $t_{(28)} = 1.73$, $p = 0.095$, $d = 0.31$] (see **Figures 1C,D**).

With regard to the learning scores, the stable phase yielded partly different results than the drift phase. First, in the analysis of the complete group the model-free score remained unaffected by cycle phase [model-free score ± SEM: Early = 16.7 ± 2.6; Late = 17.3 ± 2.8; $t_{(40)} = -0.21$, $p = 0.835$], like the model-based score [model-based score ± SEM: Early = 9.4 ± 2.7; Late = 11.5 ± 2.9; $t_{(40)} = -0.57$, $p = 0.572$] (see **Figure 2B**). However, similar to the drift phase the increased model-free control in the late follicular phase negatively correlated with the delta of totally acquired coins in the 150 trials of the stable phase ($r = -0.328$, $p = 0.036$, $n = 41$) (see **Figure 2D**).

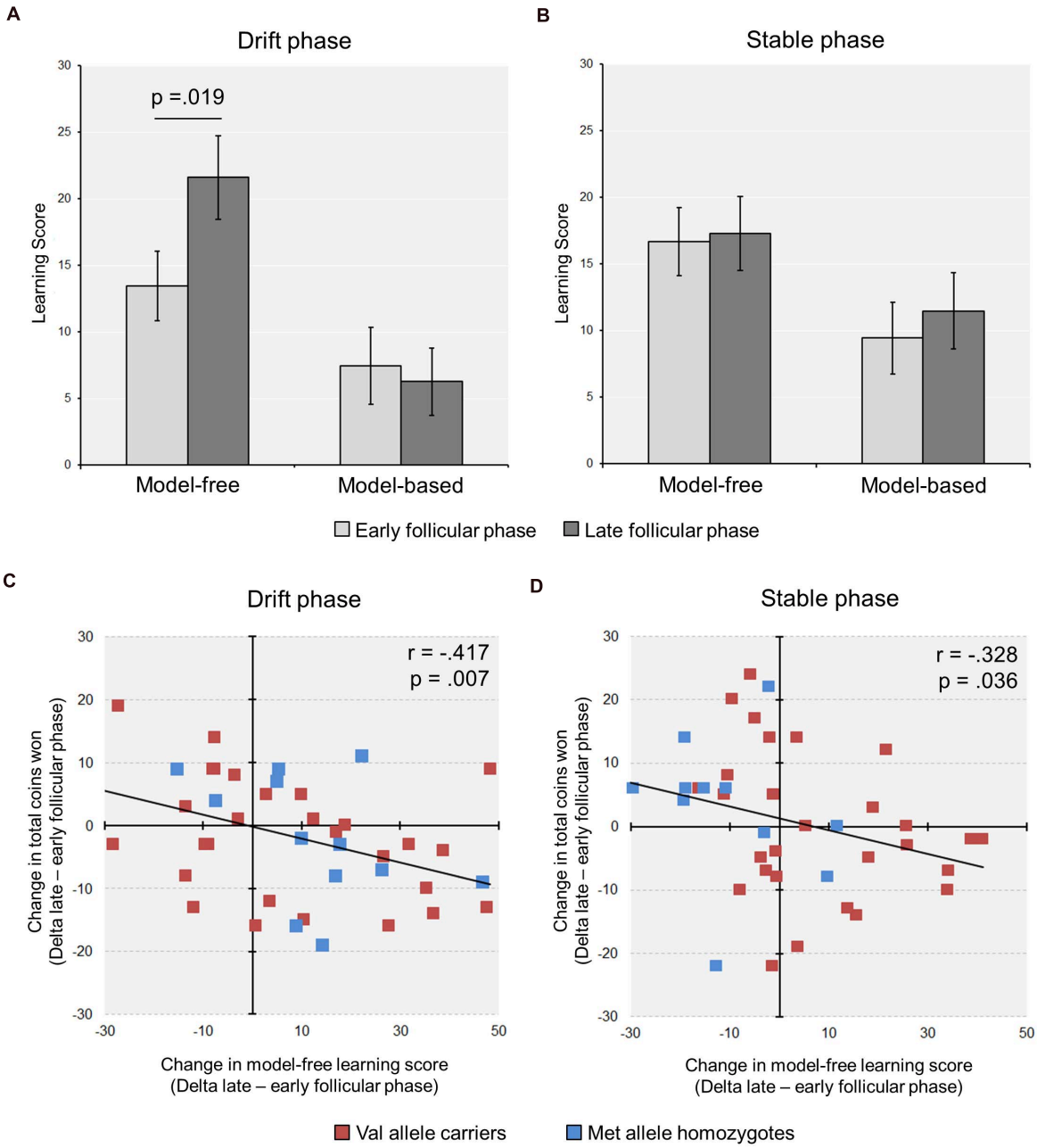

FIGURE 2 | Cycle-phase modulates model-free learning in the Two-Step task (n = 41). **(A)** During the drift phase, a significant increase in model-free learning from the early to the late follicular was observed, while no change in model-based learning occurred. **(B)** During the stable phase, with fixed reward probabilities at stage two, the learning scores remained unchanged between cycle phases. **(C,D)** The relative increase in model-free learning from the early to the late FP was associated with a reduction in the relative amount of coins won, i.e., Δpoints (late minus early follicular phase), in both the drift **(C)** and the stable phase **(D)** (For display purposes, the individual data points of the Met/Met homozygotes and the Val allele carriers are shown in different colors).

Secondly, when separately looking at the two genotypes we found that model-free processing decreased in Met-homozygotes in the high estradiol state [model-free$_{early}$ ± SEM = 20.4 ± 4.6; model-free$_{late}$ ± SEM = 8.4 ± 5.6; $t_{(11)} = 2.93$, $p = 0.014$, $d = 0.67$]. Additionally, this strong decline in model-free processing capacity in the Met-homozygotes (Delta$_{late-early}$ = −12.1 ± 4.1) differed from the delta of the Val-carriers [Delta$_{late-early}$ = 5.9 ± 3.6; $t_{(39)} = -2.88$, $p = 0.006$, Hedge's $g = 0.99$]. In contrast to that, the model-based component remained unchanged in the Met-homozygotes [model-based score ± SEM: Early = 7.1 ± 5.0; Late = 12.2 ± 7.0; $t_{(11)} = -0.76$, $p = 0.462$].

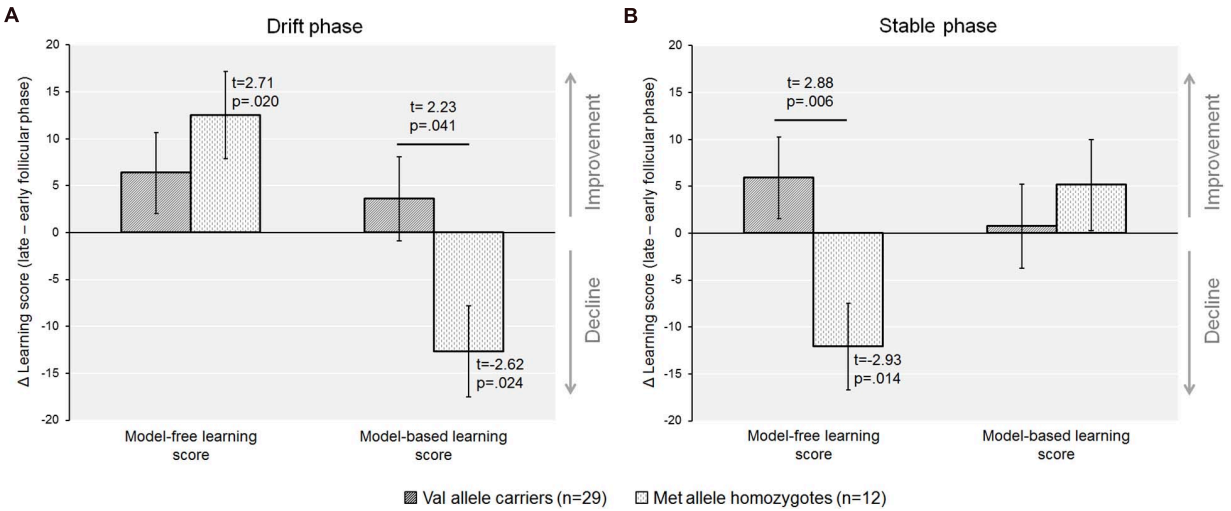

FIGURE 3 | The recruitment of model-free and the model-based control varied between cycle phases when accounting for COMT-Val158Met genotype. **(A)** In the drift phase, Met-homozygotes exhibited a significant decline in model-based control from the early to the late follicular phase, whereas Val-carriers remained unaffected by cycle phase. Further, the $\Delta_{(lateminusearlyfollicularphase)}$ for both the model-based and the model-free score differed from zero in the Met/Met genotype only, indicating that individuals with higher prefrontal dopamine were apparently more negatively affected by the rise in endogenous estradiol. **(B)** In the stable phase, there was a relative decline in model-free control in Met-homozygotes only, that was also significantly different in the comparison of genotypes.

TABLE 4 | Comparison of model-free and model-based scores between genotype and cycle phases.

	Early follicular phase (mean ± SEM)		Late follicular phase (mean ± SEM)		Independent *t*-test	Paired *t*-test
	Met/Met (*n* = 12)	**Val-carriers (*n* = 29)**	**Met/Met (*n* = 12)**	**Val-carriers (*n* = 29)**	**Between genotypes, within cycle phases**	**Between cycle phases, within genotype**
Drift phase						
Model-free score	7.2 ± 5.4	16.1 ± 2.9	19.7 ± 5.8	22.4 ± 3.8	n.s.	**Met/Met**: $t = -2.71$ $p = 0.020$
Model based score	13.6 ± 6.0	4.9 ± 3.2	0.9 ± 3.2	8.5 ± 3.2	n.s.	**Met/Met**: $t = 2.62$ $p = 0.024$
Stable phase						
Model-free score	20.4 ± 4.6	15.1 ± 3.1	8.4 ± 5.6	21.0 ± 3.0	**Late:** $t = -2.16$ $p = 0.037$	**Met/Met**: $t = 2.93$ $p = 0.014$
Model based score	7.1 ± 5.0	10.4 ± 3.3	12.2 ± 6.9	11.2 ± 2.9	n.s.	n.s.

Finally, similar to the drift phase, the Val-carriers did not show significant cycle-related changes in the learning scores during the stable phase [model-free score ± SEM: Early = 15.1 ± 3.1; Late = 21.0 ± 3.0; $t_{(28)} = -1.63$, $p = 0.114$] [model-based score ± SEM: Early = 10.4 ± 3.3; Late = 11.2 ± 2.9; $t_{(28)} = 0.176$, $p = 0.861$] (see **Figure 3B**).

DISCUSSION

Variations in estradiol may influence dopaminergic transmission and basic (model-free) aspects of reinforcement learning as well as higher-order cognition (Jacobs and D'Esposito, 2011; Becker, 2016; Diekhof and Ratnayake, 2016). Here, we examined whether changes in estradiol modulate both model-free and model-based reinforcement learning across the menstrual cycle, also depending on the COMT-Val158Met genotype. The results showed that women relied more heavily on model-free learning in the high compared to the low estradiol state, yet only when reward associations were volatile. This suggests that the increased estradiol level may have led to a disruption of frontostriatal interactions during reinforcement learning. This seems plausible, since estradiol inhibits both striatal DRD2 expression and prefrontal COMT activity, which should interfere with the prospective updating of value representations in the striatum and should reduce the prefrontal signal-to-noise ratio during the maintenance of behavioral goals. At the same time, estradiol enhances dorsolateral striatal dopamine transmission through DRD1, which would also favor habitual model-free control (Lévesque et al., 1989; Xie et al., 1999; Schendzielorz et al., 2011; Krentzel and Meitzen, 2018; see also Becker, 2016; Yoest et al., 2018 for review). When further accounting for individual differences in the prefrontal dopaminergic baseline capacity, we observed that Met-homozygotes with high prefrontal dopamine also experienced a decline in model-based control in the context of volatile reward probabilities. In contrast, the model-based score of Val-carriers remained unaffected by menstrual cycle phase. Altogether, these initial findings lead us to infer that the endogenous change in estradiol does not only affect model-free control, but also modulates

TABLE 5 | Stable phase – Effects of cycle-phase, TS-task manipulation and COMT-genotype on stay frequencies.

Main effect or interaction	*F*-value	df	*p*-value	partial eta^2
Previous reward*	**45.22**	**1, 39**	**<0.001**	**0.54**
Previous transition	0.19	1, 39	0.663	0.01
Cycle phase	0.26	1, 39	0.611	0.01
COMT genotype	0.01	1, 39	0.939	<0.01
Previous reward × previous transition*	**18.60**	**1, 39**	**<0.001**	**0.32**
Previous reward × cycle phase	0.97	1, 39	0.330	0.02
Previous reward × COMT genotype	0.58	1, 39	0.452	0.02
Previous transition × cycle phase	0.52	1, 39	0.474	0.01
Previous transition × COMT genotype	0.56	1, 39	0.460	0.01
Cycle phase × COMT-genotype	3.01	1, 39	0.091	0.07
Previous reward × previous transition × cycle phase	0.55	1, 39	0.461	0.01
Previous reward × previous transition × COMT genotype	0.06	1, 39	0.812	<0.01
Previous reward × cycle phase × COMT genotype*	**8.30**	**1, 39**	**0.006**	**0.18**
Previous transition × cycle phase × COMT genotype	1.04	1, 39	0.315	0.03
Previous reward × previous transition × cycle phase × COMT genotype	0.31	1, 39	0.583	0.01

**Significant effects ($p < 0.05$) are plotted in bold and are marked with an asterisk.*

prospective model-based learning depending on prefrontal baseline capacity.

We observed an increase in the propensity to use model-free control in the high estradiol state in the complete group of our subjects, yet only when reward-outcome was volatile. The increase in model-free control was thereby related to reduced task performance (reduced task success in terms of the total number of acquired coins), suggesting that the predominant use of model-free control was suboptimal for reward maximization in the TS-task. It has been suggested that model-free learning may be primarily mediated by striatal processing, whereas model-based control may recruit both striatal and prefrontal resources (Deserno et al., 2015; Doll et al., 2016). Interestingly, the effect appeared to be specifically driven by the Met-homozygotes, who showed an increase in model-free control as well as a concurrent reduction in the capacity for model-based learning during the drift phase, while the Val-carriers showed no significant change in learning capacity. In that way, the present observations may conform with the notion that higher estradiol could have biased striatal processing toward the model-free, less flexible learning component, and might even have concurrently disrupted frontostriatal interactions necessary for model-based control, at least in the Met-homozygotes. In the striatum of female rodents, estradiol increases stimulated dopamine release, particularly so in the dorsolateral striatum (Becker, 2016). In our study, estradiol may thus have disrupted the balance between model-based and model-free control by favoring model-free processing and the incentive salience of immediate reward during the drift phase. This becomes particularly likely when also considering the environmental volatility of the drift phase. In their theoretical paper on partial reinforcement, Anselme (2015) proposed that incentive motivation may outweigh the effect of actual learning on behavioral choice when a reward outcome is uncertain. In humans, reward uncertainty increases tonic dopamine in the midbrain and promotes reward-related ventral striatal activation (Dreher et al., 2006). One may therefore assume that the combined effect of reward volatility and high estradiol could have biased behavioral choice toward model-free control. In fact, Met-homozygotes also showed an increased stay frequency following rare reward, which could have reflected such a maladaptive increase in the incentive salience of immediate reward.

Our observation of the estradiol-driven increase in model-free control during the drift phase does neither fit with the previously reported result of a disruption of model-free control by the dopamine agonist L-DOPA (Kroemer et al., 2019), nor with another observation of no influence of L-DOPA on model-free learning, yet a positive effect on model-based control (Wunderlich et al., 2012). However, these studies differ in some important aspects from our own: First, any differences to our young female sample (n = 41 women) could have been related to the male predominance in the other two samples [Wunderlich et al. (2012) tested 18 young male undergraduates (mean age = 23 years), and Kroemer et al. (2019) examined a representative adult sample (mean age = 37 years) of 49 men and 16 women], and might therefore reflect biological sex differences in the mechanisms underlying reinforcement learning (see Becker, 2016; Diekhof, 2018). Second, estradiol and L-DOPA modulate different dopaminergic mechanisms. Whereas, L-DOPA increases dopaminergic tone (Harun et al., 2016) and thus reduces local dopamine changes after unexpected reward, estradiol facilitates stimulated dopamine release (Becker, 1990, 1999; Xiao and Becker, 1998; Hu et al., 2006). More specifically, in the prefrontal cortex, estradiol reduces tonic dopamine, yet augments transient dopamine release following stimulation, whereas in the striatum it increases both tonic and phasic dopamine (Almey et al., 2015). Therefore, estradiol would probably increase dopaminergic transmission after unexpected reward, which would in turn increase model-free control, as presently observed.

Only Met-homozygotes exhibited a compromised model-based learning capacity during the drift phase when being in

the late follicular phase. This was expressed by an increase difficulty in the differentiation between common and rare reward, with higher maladaptive stay frequencies after rare rewards. These observations fit with the assumption that, on the one hand, being homozygote for the Met allele is beneficial for the stabilization of prefrontal information processing and may protect goal-directed information from interference, since it may keep the optimal range of dopamine for cognitive processing (Durstewitz and Seamans, 2008; Slifstein et al., 2008; Schacht, 2016). On the other, the estradiol-promoted increase of prefrontal dopamine should then have destabilized information processing, also by disrupting the overall frontostriatal balance (Durstewitz and Seamans, 2008). Even though our sample included only 12 Met-homozygotes, the observed decline in model-based learning during the state of increased reward uncertainty may in fact correspond to this pattern. Jacobs and D'Esposito (2011) found a similar interaction between estradiol and COMT genotype in a working memory task. In their study the 8 Met-homozygotes showed a performance decline and a reduction of prefrontal activation while processing the cognitively demanding lure trials of an N-back task in the late follicular phase. Conversely, in their study the 13 women homozygote for the Val-allele apparently benefited from the higher estradiol and showed enhanced cognitive performance, while prefrontal activation was concurrently increased. In the present study, we did not find a state-related change in the model-based learning component of the 29 Val-carriers. We can only speculate that the predominance of heterozygotes in this group (only 4 Val-homozygotes) may explain this finding. Heterozygosity may place an individual somewhere near or even within the optimal range of prefrontal dopamine (Schacht, 2016) and it could be expected that perturbations of dopamine through an endogenous agonist such as estradiol may not at any case move an individual beyond this range.

Further notably, the decline in model-based learning in the Met-homozygotes was restricted to the state of increased environmental volatility. We assume that this might have been the result of the combined influences of (1) increased task familiarity, and (2) the concurrent reduction of task difficulty. Task familiarity, which can be achieved through extensive training, may automatize model-based learning in the TS-task. Economides et al. (2015) showed that repeated performance of the TS-task on two consecutive days preserved model-based control even in a dual-task condition. We assume that the reduced task difficulty and increased task familiarity rendered model-based learning less vulnerable to the influence of estradiol during the stable phase, even in Met-homozygotes. Further, previous evidence points toward a crucial involvement of striatal DRD2 in the updating of goal-relevant representations, especially in situations of increased task difficulty (Cools and D'Esposito, 2011). High estradiol can suppress DRD2-action and increases stimulated dopamine release (Krentzel and Meitzen, 2018; see also Becker, 1999; Yoest et al., 2018). Therefore, estradiol may particularly interfere with the ability to update changing value representations, which was crucially important for mastering the drift phase. If we further presume that cognitive load was increased by the volatile reward structure, we should also expect an additional load-dependent increase in dopamine (see also Mattay et al., 2003, who reported a similar interaction of increased cognitive load and the dopamine agonist amphetamine on working memory). This would also explain why the Met-homozygotes showed a decline in model-based control during the difficult drift, but not during the relatively easy stable phase.

In the stable part of the TS-task, we found that, in contrast to the drift phase, model-free control decreased from the early to late follicular phase in the Met/Met genotype, i.e., enhanced stay frequencies in relation to non-reward, yet regardless of transition type. Interestingly, this latter finding contrasted that of the Val-carriers, who in the high estradiol state became better at avoiding non-reward. Two previous studies found an interaction between estradiol and avoidance learning capacity. Diekhof and Ratnayake (2016) observed reduced activation of the dorsal anterior cingulate cortex to negative feedback and reduced avoidance learning performance in the late follicular phase. Jakob et al. (2018) reported a similar effect, yet only in subjects with a low striatal dopaminergic baseline. These observations fit with the stable phase result of the Met-homozygotes, but antagonize the observation in Val-carriers. Alternatively, the differences between genotypes may be explained by the interaction between dopamine and the prefrontal signal-to-noise ratio. Firstly, in humans the Val allele has been associated with a reduced prefrontal signal-to-noise ratio (Gallinat et al., 2003; Winterer et al., 2006a,b). Secondly, in rodents dopamine has been observed to increase the signal-to-noise ratio and promote the encoding of aversive stimuli in the medial prefrontal cortex (Weele et al., 2019). Thirdly, according to the inverted U-shape hypothesis the prefrontal deficit of Val-homozygosity can be transiently remedied, while the Met-homozygotes may be thrown out of balance by dopamine agonists (Cools and D'Esposito, 2011; Schacht, 2016). Since estradiol may down-regulate COMT activity (Xie et al., 1999; Schendzielorz et al., 2011), it should in turn increase prefrontal dopaminergic tone. Thus, in the dopamine-deficient Val-carriers higher estradiol might have increased the signal-to-noise ratio leading to a better avoidance of (common) non-reward in both phases (Cools and D'Esposito, 2011).

Nevertheless, this does not explain why Met-homozygotes showed such marked differences in model-free control between phases. We can only speculate that the marked differences in reward volatility might have involved dissimilar cognitive operations and thus taxed different physiological mechanisms to solve the task at hand. On the one hand, the drift phase was characterized by the need to learn stimulus values incrementally, making prospective learning less effective. This emphasized model-free learning from immediate outcome, particularly so in the high estradiol state, and because of the supposedly increased cognitive load, augmenting dopaminergic transmission (Becker, 1999, 2016; Mattay et al., 2003). On the other hand, stable reward contingencies and decreased task difficulty enabled the more effective use of model-based control in the second half of the TS-task. Although the ability to integrate non-reward into behavioral choice declined in the Met-homozygotes it did not impair overall gain in the stable phase. This indicates that the more effective use of model-based control outweighed the need to rely on

model-free control of behavior here. In fact, the model-based system has been shown to cooperate with the model-free system and can "train" the latter by replaying and simulating experience offline. This may in turn allow for choice that appears model-based (see Gershman et al., 2014). Finally, it is possible that the behavioral adaptations to randomly drifting reward probabilities in combination with the increased effort the participants put into responding during the drift phase could have to some extent disguised an estradiol-related deficit in avoidance learning in the state of heightened estradiol.

CONCLUSION

We found that cycle-related differences in reinforcement learning capacity were most pronounced during the state of increased environmental volatility (drift phase) and in Met-homozygotes, whose ability to use model-based learning was significantly reduced in the high estradiol state. Further, model-free learning appeared to be enhanced in the same state and this effect was already evident on the group level, but most pronounced in the Met/Met genotype. In contrast, Val-carriers remained widely unaffected by changes in endogenous estradiol. The present data suggest a disruption of frontostriatal interactions during reinforcement learning in a state of naturally enhanced estradiol. This seems plausible as estradiol may have an inhibitory influence on both striatal DRD2 expression and on prefrontal COMT activity, which should interfere with prospective updating of value representations in the striatum and reduce the prefrontal signal-to-noise ratio during the maintenance of behavioral goals. At the same time, estradiol may enhance dorsolateral striatal dopamine transmission through DRD1, which could decouple behavioral decisions from goal-directed, model-based choice and might favor model-free control. Consequently, the present observations may be important for the better understanding of mechanisms that lead to addiction and substance abuse or promote craving and relapse during abstinence in naturally cycling women.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethikkommission der Hamburger Ärztekammer. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ED contributed to the conceptualization, methodology, investigation, validation, formal analysis, resources, data curation, visualization of results, project administration, project supervision, and wrote the original draft. MF was involved in the project supervision, methodology, and reviewed and edited the first draft. AG was involved in the methodology, formal analysis, and reviewed and edited the first draft. FO contributed to the formal analysis, investigation, data curation, and reviewed and edited the first draft. BD provided the task software and was involved in the methodology. All authors contributed to the article and approved the submitted version.

FUNDING

This research was funded by the regular research budget of ED provided by the Universität Hamburg (Hamburg, Germany).

ACKNOWLEDGMENTS

We would like to thank A. Kroll for her contribution in the analysis of salivary estradiol, and K. Jakob and H. Ehrentreich for help during data acquisition. We would also like to thank all the participants of this research project for their contribution.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnbeh.2021.658769/full#supplementary-material

REFERENCES

Almey, A., Milner, T. A., and Brake, W. G. (2015). Implication for dopamine-dependent cognition in females. *Horm. Behav.* 74, 125–138. doi: 10.1016/j.yhbeh.2015.06.010.Estrogen

Anselme, P. (2015). Incentive salience attribution under reward uncertainty: a pavlovian model. *Behav. Processes* 111, 6–18. doi: 10.1016/j.beproc.2014.10.016

Apud, J. A., Mattay, V., Chen, J., Kolachana, B. S., Callicott, J. H., Rasetti, R., et al. (2007). Tolcapone improves cognition and cortical information processing in normal human subjects. *Neuropsychopharmacology* 32, 1011–1020. doi: 10.1038/sj.npp.1301227

Becker, J. B. (1990). Estrogen rapidly potentiates amphetamine-induced striatal dopamine release and rotational behavior during microdialysis. *Neurosci. Lett.* 118, 169–171. doi: 10.1016/0304-3940(90)90618-J

Becker, J. B. (1999). Gender differences in dopaminergic function in striatum and nucleus accumbens. *Pharmacol. Biochem. Behav.* 64, 803–812. doi: 10.1016/S0091-3057(99)00168-9

Becker, J. B. (2016). Sex differences in addiction. *Dialog. Clin. Neurosci.* 18, 395–402. doi: 10.31887/dcns.2016.18.4/jbecker

Bimonte, H. A., and Denenberg, V. H. (1999). Estradiol facilitates performance as working memory load increases. *Psychoneuroendocrinology* 24, 161–173. doi: 10.1016/S0306-4530(98)00068-7

Buckholtz, J. W., Treadway, M. T., Cowan, R. L., Woodward, N. D., Li, R., Ansari, M. S., et al. (2010). Dopaminergic network differences in human impulsivity. *Science* 329:532. doi: 10.1126/science.1185778

Collins, A. G. E., and Frank, M. J. (2014). Modeling interactive learning and incentive choice effects of striatal dopamine. *Psychol. Rev.* 121, 1–48.

Cools, R., and D'Esposito, M. (2011). Inverted-U shaped dopamine actions on human working memory and cognitive control. *Biol. Psychiatry* 69, e113–e125. doi: 10.1038/jid.2014.371

Daw, N. D., and Dayan, P. (2014). The algorithmic anatomy of model-based evaluation. *Philos. Trans. R. Soc. B Biol. Sci* 369:20130478. doi: 10.1098/rstb.2013.0478

Daw, N. D., Gershman, S. J., Seymour, B., Dayan, P., and Dolan, R. J. (2011). Model-based influences on humans' choices and striatal prediction errors. *Neuron* 69, 1204–1215. doi: 10.1016/j.neuron.2011.02.027

Daw, N. D., Niv, Y., and Dayan, P. (2005). Uncertainty-based competition between prefrontal and dorsolateral striatal systems for behavioral control. *Nat. Neurosci.* 8, 1704–1711. doi: 10.1038/nn1560

Deserno, L., Huys, Q. J. M., Boehme, R., Buchert, R., Heinze, H.-J., Grace, A. A., et al. (2015). Ventral striatal dopamine reflects behavioral and neural signatures of model-based control during sequential decision making. *Proc. Natl. Acad. Sci.U.S.A.* 112, 1595–1600. doi: 10.1073/pnas.1417219112

Diekhof, E. K. (2015). Be quick about it. Endogenous estradiol level, menstrual cycle phase and trait impulsiveness predict impulsive choice in the context of reward acquisition. *Horm. Behav.* 74, 186–193. doi: 10.1016/j.yhbeh.2015.06.001

Diekhof, E. K. (2018). Estradiol and the reward system in humans. *Curr. Opin. Behav. Sci.* 23, 58–64. doi: 10.1016/j.cobeha.2018.03.010

Diekhof, E. K., and Ratnayake, M. (2016). Menstrual cycle phase modulates reward sensitivity and performance monitoring in young women: preliminary fMRI evidence. *Neuropsychologia* 84, 70–80. doi: 10.1016/j.neuropsychologia.2015.10.016

Dluzen, D. E., and Ramirez, V. D. (1984). Bimodal effect of progesterone on in vitro dopamine function of the rat corpus striatum. *Neuroendocrinology* 39, 149–155. doi: 10.1159/000123971

Dluzen, D. E., and Ramirez, V. D. (1987). Intermittent infusion of progesterone potentiates whereas continuous infusion reduces amphetamine-stimulated dopamine release from ovariectomized estrogen-primed rat striatal fragments superfused in vitro. *Brain Res.* 406, 1–9. doi: 10.1016/0006-8993(87)90762-1

Dolan, R. J., and Dayan, P. (2013). Goals and habits in the brain. *Neuron* 80, 312–325. doi: 10.1016/j.neuron.2013.09.007

Doll, B. B., Bath, K. G., Daw, N. D., and Frank, M. J. (2016). Variability in dopamine genes dissociates model-based and model-free reinforcement learning. *J. Neurosci.* 36, 1211–1222. doi: 10.1523/JNEUROSCI.1901-15.2016

Doll, B. B., Duncan, K. D., Simon, D. A., Shohamy, D., and Daw, N. D. (2015). Model-based choices involve prospective neural activity. *Nat. Neurosci.* 18, 767–772. doi: 10.1038/nn.3981

Dreher, J. C., Kohn, P., and Berman, K. F. (2006). Neural coding of distinct statistical properties of reward information in humans. *Cereb. Cortex* 16, 561–573. doi: 10.1093/cercor/bhj004

Dumas, J. A., Kutz, A. M., Naylor, M. R., Johnson, J. V., and Newhouse, P. (2010). Increased memory load-related frontal activation after estradiol treatment in postmenopausal women. *Horm. Behav.* 58, 929–935. doi: 10.1016/j.yhbeh.2010.09.003.Increased

Durstewitz, D., and Seamans, J. K. (2008). The dual-state theory of prefrontal cortex dopamine function with relevance to catechol-o-methyltransferase genotypes and schizophrenia. *Biol. Psychiatry* 64, 739–749. doi: 10.1016/j.biopsych.2008.05.015

Economides, M., Kurth-Nelson, Z., Lübbert, A., Guitart-Masip, M., and Dolan, R. J. (2015). Model-based reasoning in humans becomes automatic with training. *PLoS Comput. Biol.* 11:e1004463. doi: 10.1371/journal.pcbi.1004463

Frank, M. J., and O'Reilly, R. C. (2006). A mechanistic account of striatal dopamine function in human cognition: psychopharmacological studies with cabergoline and haloperidol. *Behav. Neurosci.* 120, 497–517. doi: 10.1037/0735-7044.120.3.497

Gallinat, J., Bajbouj, M., Sander, T., Schlattmann, P., Xu, K., Ferro, E. F., et al. (2003). Association of the G1947A COMT (Val108/158Met) gene polymorphism with prefrontal P300 during information processing. *Biol. Psychiatry* 54, 40–48. doi: 10.1016/S0006-3223(02)01973-X

Gershman, S. J., Markman, A. B., and Otto, A. R. (2014). Retrospective revaluation in sequential decision making: a tale of two systems. *J. Exp. Psychol. Gen.* 143, 182–194. doi: 10.1037/a0030844

Glimcher, P. W. (2011). Understanding dopamine and reinforcement learning: the dopamine reward prediction error hypothesis. *Proc. Natl. Acad. Sci. U.S.A.* 108, 15647–15654. doi: 10.1073/pnas.1014269108

Hampson, E., and Morley, E. E. (2013). Estradiol concentrations and working memory performance in women of reproductive age. *Psychoneuroendocrinology* 38, 2897–2904. doi: 10.1016/j.psyneuen.2013.07.020

Harun, R., Hare, K. M., Brough, E. M., Munoz, M. J., Grassi, C. M., Torres, G. E., et al. (2016). Fast-scan cyclic voltammetry demonstrates that L-DOPA produces dose-dependent, regionally selective bimodal effects on striatal dopamine kinetics in vivo. *J. Neurochem.* 136, 1270–1283. doi: 10.1111/jnc.13444

Hu, M., Watson, C. J., Kennedy, R. T., and Becker, J. B. (2006). Estradiol attenuates the K+-induced increase in extracellular GABA in rat striatum. *Synapse* 59, 122–124. doi: 10.1002/syn.20221

Jacobs, E., and D'Esposito, M. (2011). Estrogen shapes dopamine-dependent cognitive processes: implications for women's health. *J. Neurosci.* 31, 5286–5293. doi: 10.1523/JNEUROSCI.6394-10.2011

Jakob, K., Ehrentreich, H., Holtfrerich, S. K. C., Reimers, L., and Diekhof, E. K. (2018). DAT1-genotype and menstrual cycle, but not hormonal contraception, modulate reinforcement learning: preliminary evidence. *Front. Endocrinol. (Lausanne)* 9:60. doi: 10.3389/fendo.2018.00060

Käenmäki, M., Tammimäki, A., Myöhänen, T., Pakarinen, K., Amberg, C., Karayiorgou, M., et al. (2010). Quantitative role of COMT in dopamine clearance in the prefrontal cortex of freely moving mice. *J. Neurochem.* 114, 1745–1755. doi: 10.1111/j.1471-4159.2010.06889.x

Krentzel, A. A., and Meitzen, J. (2018). Biological sex, estradiol and striatal medium spiny neuron physiology: a mini-review. *Front. Cell. Neurosci.* 12:492. doi: 10.3389/fncel.2018.00492

Kroemer, N. B., Lee, Y., Pooseh, S., Eppinger, B., Goschke, T., and Smolka, M. N. (2019). L-DOPA reduces model-free control of behavior by attenuating the transfer of value to action. *Neuroimage* 186, 113–125. doi: 10.1016/j.neuroimage.2018.10.075

Lévesque, D., Gagnon, S., and Di Paolo, T. (1989). Striatal D1 dopamine receptor density fluctuates during the rat estrous cycle. *Neurosci. Lett.* 98, 345–350. doi: 10.1016/0304-3940(89)90426-6

Luine, V. N., and Rhodes, J. C. (1983). Gonadal hormone regulation of MAO and other enzymes in hypothalamic areas. *Neuroendocrinology* 36, 235–241. doi: 10.1159/000123461

Luine, V., and Hearns, M. (1990). Relationship of gonadal hormone administration, sex, reproductive status and age to monoamine oxidase activity within the hypothalamus. *J. Neuroendocrinol.* 2, 423–428. doi: 10.1111/j.1365-2826.1990.tb00427.x

Mattay, V. S., Goldberg, T. E., Fera, F., Hariri, A. R., Tessitore, A., Egan, M. F., et al. (2003). Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. *Proc. Natl. Acad. Sci. U.S.A.* 100, 6186–6191. doi: 10.1073/pnas.0931309100

Mier, D., Kirsch, P., and Meyer-Lindenberg, A. (2010). Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. *Mol. Psychiatry* 15, 918–927. doi: 10.1038/mp.2009.36

Otto, A. R. (2013). Cognitive control predicts use of model-based reinforcement-learning. *J. Cogn. Neurosci.* 26, 1–10. doi: 10.1162/jocn

Patton, J. H., Stanford, M. S., and Barratt, E. S. (1995). Factor structure of the barratt impulsiveness scale. *J. Clin. Psychol.* 51, 768–774. doi: 10.1111/1467-9310.00265

Potter, T. C. S., Bryce, N. V., and Hartley, C. A. (2017). Cognitive components underpinning the development of model-based learning. *Dev. Cogn. Neurosci.* 25, 272–280. doi: 10.1016/j.dcn.2016.10.005

Sakaki, M., and Mather, M. (2013). How reward and emotional stimuli induce different reactions across the menstrual cycle. *Soc. Pers. Psychol. Compass* 6, 1–17. doi: 10.1111/j.1751-9004.2011.00415.x

Schacht, J. P. (2016). COMT val158met moderation of dopaminergic drug effects on cognitive function: a critical review. *Pharmacogenomics J.* 16, 430–438. doi: 10.1038/tpj.2016.43

Schendzielorz, N., Rysa, A., Reenila, I., Raasmaja, A., and Mannisto, P. T. (2011). Complex estrogenic regulation of catechol-O-methyltransferase (COMT) in rats. *J. Physiol. Pharmacol.* 62, 483–490.

Sebold, M., Deserno, L., Nebe, S., Schad, D. J., Garbusow, M., Hägele, C., et al. (2014). Model-based and model-free decisions in alcohol dependence. *Neuropsychobiology* 70, 122–131. doi: 10.1159/000362840

Sharp, M. E., Foerde, K., Daw, N. D., and Shohamy, D. (2016). Dopamine selectively remediates "model-based" reward learning: a computational approach. *Brain* 139, 355–364. doi: 10.1093/brain/awv347

Slifstein, M., Kolachana, B., Simpson, E. H., Tabares, P., Cheng, B., Duvall, M., et al. (2008). COMT genotype predicts cortical-limbic D1 receptor availability measured with [11C]NNC112 and PET. *Mol. Psychiatry* 13, 821–827. doi: 10.1038/mp.2008.19

Smittenaar, P., FitzGerald, T. H. B., Romei, V., Wright, N. D., and Dolan, R. J. (2013). Disruption of dorsolateral prefrontal cortex decreases model-based in favor of model-free control in humans. *Neuron* 80, 914–919. doi: 10.1016/j.neuron.2013.08.009

Weele, C. M. V., Siciliano, C. A., and Tye, K. M. (2019). Dopamine tunes prefrontal outputs to orchestrate aversive processing. *Brain Res.* 1713, 16–31. doi: 10.1016/j.brainres.2018.11.044

Winterer, G., Egan, M. F., Kolachana, B. S., Goldberg, T. E., Coppola, R., and Weinberger, D. R. (2006a). Prefrontal electrophysiologic "noise" and catechol-o-methyltransferase genotype in schizophrenia. *Biol. Psychiatry* 60, 578–584. doi: 10.1016/j.biopsych.2006.03.023

Winterer, G., Musso, F., Vucurevic, G., Stoeter, P., Konrad, A., Seker, B., et al. (2006b). COMT genotype predicts BOLD signal and noise characteristics in prefrontal circuits. *Neuroimage* 32, 1722–1732. doi: 10.1016/j.neuroimage.2006.05.058

Wunderlich, K., Smittenaar, P., and Dolan, R. J. (2012). Dopamine enhances model-based over model-free choice behavior. *Neuron* 75, 418–424. doi: 10.1016/j.neuron.2012.03.042

Xiao, L., and Becker, J. B. (1998). Effects of estrogen agonists on amphetamine-stimulated striatal dopamine release. *Synapse* 29, 379–391. doi: 10.1002/(sici)1098-2396(199808)29:4<379::aid-syn10>3.0.co;2-m

Xie, T., Ho, S. L., and Ramsden, D. (1999). Characterization and implications of estrogenic down-regulation of human catechol-O-methyltransferase gene transcription. *Mol. Pharmacol.* 56, 31–38. doi: 10.1124/mol.56.1.31

Yoest, K. E., Cummings, J. A., and Becker, J. B. (2018). Oestradiol influences on dopamine release from the nucleus accumbens shell: sex differences and the role of selective estradiol receptor subtypes. *Br. J. Pharmacol.* 176, 4136–4148. doi: 10.1111/bph.14531

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 21 July 2021
doi: 10.3389/fnbeh.2021.696838

Clinically Used Hormone Formulations Differentially Impact Memory, Anxiety-Like, and Depressive-Like Behaviors in a Rat Model of Transitional Menopause

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A variety of U.S. Food and Drug Administration-approved hormone therapy options are currently used to successfully alleviate unwanted symptoms associated with the changing endogenous hormonal milieu that occurs in midlife with menopause. Depending on the primary indication for treatment, different hormone therapy formulations are utilized, including estrogen-only, progestogen-only, or combined estrogen plus progestogen options. There is little known about how these formulations, or their unique pharmacodynamics, impact neurobiological processes. Seemingly disparate pre-clinical and clinical findings regarding the cognitive effects of hormone therapies, such as the negative effects associated with conjugated equine estrogens and medroxyprogesterone acetate vs. naturally circulating 17β-estradiol (E2) and progesterone, signal a critical need to further investigate the neuro-cognitive impact of hormone therapy formulations. Here, utilizing a rat model of transitional menopause, we administered either E2, progesterone, levonorgestrel, or combinations of E2 with progesterone or with levonorgestrel daily to follicle-depleted, middle-aged rats. A battery of assessments, including spatial memory, anxiety-like behaviors, and depressive-like behaviors, as well as endocrine status and ovarian follicle complement, were evaluated. Results indicate divergent outcomes for memory, anxiety, and depression, as well as unique physiological profiles, that were dependent upon the hormone regimen administered. Overall, the combination hormone treatments had the most consistently favorable profile for the domains evaluated in rats that had undergone experimentally induced transitional menopause and remained ovary-intact. The collective results underscore the importance of investigating variations in hormone therapy formulation as well as the menopause background upon which these formulations are delivered.

Keywords: VCD, menopause, estrogen, progesterone, levonorgestrel, memory, anxiety, depression

OPEN ACCESS

Edited by:
Mathias V. Schmidt, Max Planck Institute of Psychiatry (MPI), Germany

Reviewed by:
Victoria Luine, Hunter College (CUNY), United States
Jessica Santollo, University of Kentucky, United States

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Specialty section:
This article was submitted to Behavioral Endocrinology, a section of the journal Frontiers in Behavioral Neuroscience

Received: *17 April 2021*
Accepted: *04 June 2021*
Published: *21 July 2021*

Citation:
Koebele SV, Hiroi R, Plumley ZMT, Melikian R, Prakapenka AV, Patel S, Carson C, Kirby D, Mennenga SE, Mayer LP, Dyer CA and Bimonte-Nelson HA (2021) Clinically Used Hormone Formulations Differentially Impact Memory, Anxiety-Like, and Depressive-Like Behaviors in a Rat Model of Transitional Menopause. Front. Behav. Neurosci. 15:696838. doi: 10.3389/fnbeh.2021.696838

INTRODUCTION

During the midlife transition to menopause, a number of symptoms that negatively impact quality of life and wellbeing may occur. Most commonly, these symptoms originate from natural changes in estrogen production by the ovaries as follicle reserve declines, leading to the onset of vasomotor symptoms (e.g., hot flashes, night sweats), dyspareunia, and urogenital indications (Hoffman et al., 2012; Al-Safi and Santoro, 2014; NAMS, 2014). Benign irregular or heavy bleeding patterns are also common during the transition to menopause (Voorhis et al., 2008; Cornițescu et al., 2011; Pinkerton, 2011). In addition, during the menopause transition many individuals report increased rates of depression and anxiety symptoms, as well as impaired cognition, particularly in the realm of working memory (Kritz-Silverstein et al., 2000; Mitchell and Woods, 2001; Weber and Mapstone, 2009; Maki et al., 2012; Weber et al., 2012, 2014; Worsley et al., 2014; Zilberman et al., 2015; Unkenstein et al., 2016; Rentz et al., 2017; Morgan et al., 2018; Im et al., 2019).

There are a variety of U.S. Food and Drug Administration (FDA)-approved hormone therapy options available that effectively alleviate undesirable symptoms associated with menopause-related changes in the endogenous hormonal milieu (Files et al., 2011; Hoffman et al., 2012; Pinkerton, 2012; Stuenkel et al., 2015; Pinkerton et al., 2017c). If the uterus is intact, a hormone therapy regimen must include a progestogen component (i.e., natural progesterone or one of the many synthetic forms of progesterone; the latter are collectively referred to as progestins) in combination with an estrogen component (e.g., natural 17β-estradiol (E2), synthetic ethinyl estradiol, conjugated equine estrogens). This progestogen component is necessary to mitigate the risk of uterine hyperplasia and cancer (Pinkerton et al., 2017c). If a patient's primary indication for treatment is heavy, irregular, or abnormal uterine bleeding, medical professionals may prescribe a progestogen-only hormone therapy, such as an oral progestogen or an intrauterine device containing the progestin levonorgestrel, a synthetic form of progesterone (Sitruk-Ware, 2002; Marret et al., 2010; Cornițescu et al., 2011; Pinkerton, 2011; Goldstein and Lumsden, 2017). If a patient has undergone hysterectomy with or without ovary removal, they may take estrogen-only hormone therapy, as the removal of uterine tissue eliminates the need for the progestogen component (Haney and Wild, 2007; NAMS, 2014; Pinkerton et al., 2017c). Additionally, low-dose vaginal estrogen-only tablets, creams, and rings are increasing in popularity for the treatment of menopausal genitourinary syndrome even when the uterus is intact (Rahn et al., 2014; Pinkerton et al., 2017b; Biehl et al., 2018; Shifren, 2018). Thus, depending on an individual's circumstance and primary indications for menopausal hormone therapy use, there are a range of possibilities for variations in hormone therapy preparations, including estrogen-only, progestogen-only, or combined estrogen plus progestogen hormone therapy options, which in turn may have variable effects on the brain and periphery.

Sex steroid hormones have been shown to impact learning and memory, although the ideal parameters for individual and combined hormone therapies have proven to be complex (for review, see: Barha and Galea, 2010; Gibbs, 2010; Luine, 2014; Frick, 2015; Koebele and Bimonte-Nelson, 2015, 2017; Korol and Pisani, 2015). Depriving the female system of ovarian-derived hormones leads to cognitive changes in both humans and animal models (e.g., Phillips and Sherwin, 1992; Singh et al., 1994; Bimonte and Denenberg, 1999; Nappi et al., 1999; Heikkinen et al., 2004; Wallace et al., 2006; Rocca et al., 2007; Gibbs and Johnson, 2008; Parker et al., 2009; Ryan et al., 2014). Importantly, ovarian hormone loss also results in an increased susceptibility to anxiety and depression (Parker et al., 2009; Bromberger and Kravitz, 2011; Bromberger et al., 2011; Maki et al., 2012; Weber et al., 2014; Parry, 2020; Soares, 2020; Stute et al., 2020). Under certain parameters or experimental conditions, estrogen supplementation following the surgical removal of the ovaries (ovariectomy; Ovx) reverses or attenuates detriments in cognition and affective behaviors in preclinical models (Bimonte and Denenberg, 1999; Holmes et al., 2002; Foster et al., 2003; Hiroi and Neumaier, 2006; Hiroi et al., 2006, 2016; Fernandez et al., 2008; Harburger et al., 2009; Rodgers et al., 2010; Gleason et al., 2015; Black et al., 2016, 2018; Koebele et al., 2020b). Much emphasis has been placed on exogenous E2 administration following Ovx, and reports show variable effects on cognition depending on the parameters. However, most individuals experience a natural, non-surgical transition to menopause and retain their ovaries. The ovatoxin 4-vinylcyclohexene diepoxide (VCD) induces accelerated follicular atresia, which serves as a rat model of transitional menopause, wherein ovarian tissue is maintained but becomes follicle-deplete (Mayer et al., 2002, 2004; Dyer et al., 2013; Koebele and Bimonte-Nelson, 2016). Using VCD, our laboratory recently demonstrated that compared to follicle-deplete rats that did not receive E2 treatment, tonic E2 had beneficial effects in the learning phase of a complex spatial working memory task. However, some working memory impairments were evident in the E2-treated rats after the rules of the task had been acquired (Koebele et al., 2020a), demonstrating the complex role of estrogens in learning and memory.

Although E2 is a common component in many FDA-approved combined hormone therapy formulations, the progestogen component varies. Progestins are used frequently as an alternative to natural progesterone due to significantly higher oral bioavailability (Sitruk-Ware et al., 1987; Schindler et al., 2003; Kuhl, 2005). All progestins exert progestogenic activity at progesterone receptors, resulting in protective mechanisms for the uterus, which is often their primary clinical application. However, depending on its molecular derivative, a given progestin can also have estrogenic, anti-estrogenic, androgenic, anti-androgenic, and/or glucocorticoid activity to varying extents (Schindler et al., 2003). These unique pharmacological profiles lead to distinct patterns of activity and actions by progestins, including variable cognitive effects (Sitruk-Ware, 2002; Schindler et al., 2003; Braden et al., 2017). Several progestins have been shown by our and other laboratories to negatively affect cognition (Rapp et al., 2003; Shumaker et al., 2003; Rosario et al., 2006; Braden et al., 2010, 2011; Lowry et al., 2010). However, levonorgestrel, a common progestin in hormone therapy

formulations and a hormone-containing intrauterine device, has been reported to have neutral, or even beneficial, effects on cognition in the surgical menopause (i.e., Ovx) rat model when administered independently (Braden et al., 2017; Prakapenka et al., 2018). Levonorgestrel may exhibit these unique effects due to its distinct pharmacodynamic properties; in contrast to natural progesterone or other progestins, levonorgestrel does not elicit glucocorticoid or anti-mineralocorticoid receptor activity, but does have some androgenic activity (Schindler et al., 2003). For example, in middle-aged Ovx rats, we have demonstrated that levonorgestrel alone produced cognitive benefits; however, when levonorgestrel was co-administered with E2, it failed to augment, and in fact attenuated, E2's favorable effects on cognition, producing impairments relative to either hormone alone (Prakapenka et al., 2018). These results highlight the importance of performing translational research in which clinical practices are accurately modeled. Whether a combined E2 + progestogen regimen exerts similar effects in a model of transitional menopause remains to be determined. This is a question of high importance, given that minor alterations in molecular structure can lead to different physiological effects of progestogens (Sitruk-Ware, 2002), and that progestogens are most often given in combination with E2 when an individual undergoing menopause has an intact uterus and ovaries (Pinkerton et al., 2017c). It is critical to methodically compare how daily administration of natural progesterone and the progestin levonorgestrel influence learning and memory independently as well as in combination with E2, and whether progestogen type matters for outcomes with transitional menopause.

To address this question, we administered VCD to permit the retention of follicle-depleted ovarian tissue and to produce a circulating hormone profile more similar to that associated with transitional menopause than would be achievable with Ovx (Koebele and Bimonte-Nelson, 2016). In the current experiment, VCD treatment began at 8 months of age, as we have done in previous publications (Koebele et al., 2020a). Three months later, when rats were middle-aged and considered to be in the early post-menopausal stage after substantial follicular depletion ensued (Lohff et al., 2005; Acosta et al., 2009; Koebele et al., 2020a), daily exogenous hormone treatment began and rats were tested on a behavioral battery assessing spatial memory, anxiety-like, and depressive-like behaviors. Thus, the goals of the current experiment were manifold, as we aimed to systematically evaluate the independent and combined effects of daily E2, progesterone, and levonorgestrel on cognitive, anxiety-like, and depressive-like measures in transitionally menopausal, follicle-deplete, middle-aged rats.

MATERIALS AND METHODS

See **Figure 1** for a detailed experimental timeline.

Subjects

Sixty sexually inexperienced female Fischer-344-CDF rats from the National Institute on Aging colony at Charles River Laboratories (Raleigh, NC) were used in this experiment. Rats were approximately 8 months of age when they arrived at the Arizona State University vivarium facility. Rats were pair-housed upon arrival and had unrestricted access to food and water for the duration of the experiment. Rats were maintained on a 12-h light/dark cycle (lights on at 7 am) and had a 1 week period of acclimation in the vivarium prior to the commencement of experimental procedures. The Institutional Animal Care and Use Committee at Arizona State University approved all procedures, which adhered to National Institutes of Health standards.

VCD Injections

All rats were administered VCD (FYXX Foundation, Flagstaff, AZ) intraperitoneally at a dose of 160 mg/kg/day in 50% dimethyl sulfoxide (DMSO)/50% sterile saline vehicle solution (Sigma-Aldrich, St. Louis, MO, United States) for a total of 15 injection days, based on established protocols (Mayer et al., 2002, 2004; Lohff et al., 2005, 2006; Acosta et al., 2009, 2010; Van Kempen et al., 2011; Frye et al., 2012; Zhang et al., 2016; Koebele et al., 2017, 2020a; Kirshner and Gibbs, 2018; Carolino et al., 2019). Baseline body weight (g) was recorded for all subjects prior to starting injections. VCD injection volume was calculated based on individual daily body weight. If a rat's body weight decreased by 10% or more from its baseline, VCD administration was discontinued until weight was recovered. VCD was administered on Mondays, Tuesdays, Thursdays, and Fridays. Injections were not administered on Wednesdays, Saturdays, or Sundays for weight recovery (Koebele et al., 2017, 2020a). As such, to accommodate injection-related weight loss and recovery, the 15 VCD injections were completed over approximately 9 weeks. Two rats died during VCD injections: one from peritonitis and one from an undetermined cause, likely unrelated to injections.

Hormone Treatment Administration

A 93 day waiting period from the first VCD injection was employed to ensure substantial ovarian follicular depletion (Lohff et al., 2005; Acosta et al., 2009; Koebele et al., 2020a) prior to initiating daily hormone administration, modeling the early post-menopausal time point. Rats were then randomly assigned to one of the following treatment conditions: Vehicle (sesame oil; Sigma Aldrich S3547; n = 10), 17β-estradiol (E2; 3 μg/day; Sigma Aldrich, E8875; n = 10), Progesterone (PROG; 0.7 mg/day; Sigma Aldrich, P0130; n = 9), Levonorgestrel (LEVO; 0.6 μg/day; Sigma Aldrich, N2260; n = 9), E2 + PROG (3 μg E2 + 0.7 mg PROG/day; n = 10), or E2 + LEVO (3 μg E2 + 0.6 μg LEVO/day; n = 10) (summarized in **Figure 1**). All hormone treatments were dissolved in sesame oil, delivered via a 0.10 mL daily subcutaneous injection for 21 days prior to beginning behavioral testing. Hormone or Vehicle injections continued for the duration of the experiment until euthanasia.

Body Weights

Body weights (g) were recorded for all rats at the onset of VCD injections and periodically collected throughout the experiment until euthanasia. Body weight served as a peripheral indicator of general animal health and was used to assess whether hormone

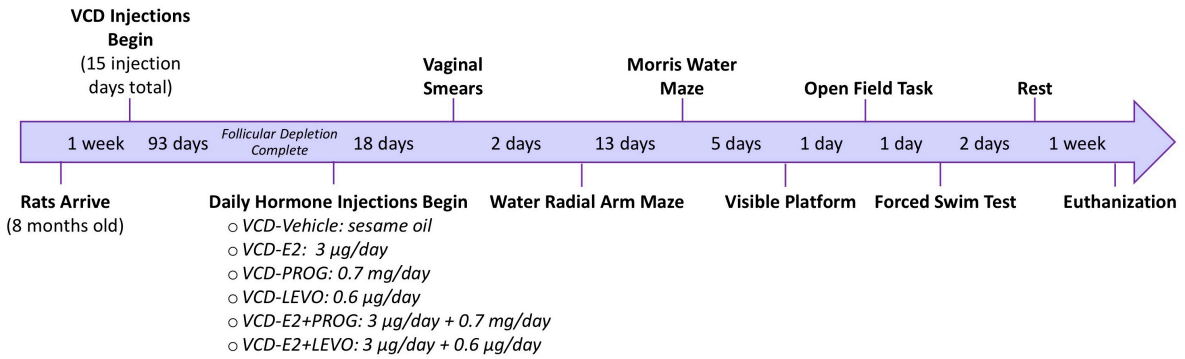


FIGURE 1 | Experimental Timeline. Following accelerated follicular depletion, rats received daily hormone treatments and were evaluated on a series of behavior tasks assessing working memory, reference memory, anxiety-like behavior, and depressive-like behavior.

treatments altered body weight in an ovary-intact, follicle-depleted background.

Vaginal Cytology

Vaginal smears were assessed immediately prior to behavioral testing initiation for two consecutive days, as previously published (Koebele et al., 2020a). The experimenter obtained each swab sample by gently inserting a small cotton-tipped applicator soaked in sterile saline into the vaginal opening. A light microscope (Fisher Scientific Micromaster; CAT #12-561-4B) was used to view the cells at 10× magnification. The experimenter classified samples as proestrus-, estrus-, metestrus-, or diestrus- like as our laboratory and others have previously published (Goldman et al., 2007; Koebele and Bimonte-Nelson, 2016; Koebele et al., 2019).

Behavioral Testing

After 3 weeks of daily hormone administration, 114 days after the first VCD injection, all rats (approximately 11–12 months old) were tested on a series of behavioral tasks assessing spatial working and reference memory, anxiety-like behavior, and depressive-like behavior. These assays included the water radial arm maze (WRAM) to evaluate spatial working and reference memory, the Morris water maze (MM) to assess spatial reference memory, the visible platform (VP) task to confirm motor and visual competency for swim-based tasks, the open field task (OFT) to assess locomotor activity and anxiety-like behavior, and the forced swim task (FST) to evaluate depressive-like behavior. Procedures for each task are described in detail below.

Water Radial Arm Maze

The WRAM evaluated spatial working and reference memory in a water escape paradigm (Bimonte-Nelson et al., 2015). The apparatus had eight arms (38.1 cm × 12.7 cm each) and a circular center, and was filled with water maintained at 18–20°C throughout testing. To assist with spatial navigation, prominent visual cues were placed on the walls around the maze in addition to the tables and heat lamps situated in each room. A pre-selected combination of platform locations was assigned to each rat, wherein hidden escape platforms were submerged 2–3 cm beneath the water's surface in four of the eight maze arms (locations counterbalanced across treatment groups); the other four arms (including the start arm) never contained platforms. Assigned platform locations remained the same across all testing days for a given rat. Black non-toxic powdered paint was added to the water to further obscure submerged platforms. Testing consisted of four trials per day across 13 consecutive days. Day 1 was considered training, days 2–12 were normal testing sessions, and day 13 included a delayed memory retention evaluation. During each daily testing session, the experimenter gently placed the rat in the non-platformed start arm. If the rat did not escape the WRAM via a hidden platform within the allotted 3-min trial time, the experimenter guided the rat to the nearest platform using a lead stick. Upon locating a platform, the rat was allocated 15 s of total platform time before being returned to its heated testing cage to reinforce platform location learning. During a 30 s inter-trial-interval (ITI), the experimenter removed the just-found platform from the maze, swept the water for debris with a net, and stirred the water to diffuse potential olfactory cues. In this way, working memory load progressively became taxed across trials within a daily testing session, as the number of locations to be recalled increased with each trial. On Day 13 of testing, a 6-h delay was implemented between trials two and three to assess delayed working memory retention. During the delay interval, rats were kept in their individual testing cages and given access to water.

Learning and memory performance on the WRAM was quantified by calculating the number of entries into non-platformed arms prior to locating a platform on each trial within a day, which were considered errors. The experimenter logged each arm entry error manually on a testing sheet during the trials. An entry was operationally defined as the tip of the rat's snout crossing a marker 11 cm into the arm (visible on the outside of the maze, but not visible to the rat). Errors were counted and divided into subtypes. Working memory correct (WMC) errors were entries into an arm that previously contained a hidden platform within a daily testing session. Of note, WMC errors can only occur on trials 2–4, as all platforms are present in the maze during the first trial; as such, statistical analyses for WMC errors across trials are inclusive of trials 2, 3, and 4.

Reference memory (RM) errors were the first entries into an arm within a daily testing session that never contained a platform; as such, a total of four RM errors could be made within a daily testing session. Working memory incorrect (WMI) errors were subsequent entries, within a daily testing session, into an arm that never contained a platform (Bimonte-Nelson et al., 2015). RM and WMI errors can be made on any trial; thus, analyses for WMI and RM errors across trials are inclusive of trials 1–4.

Morris Water Maze

Following the WRAM delayed memory retention day, rats were evaluated on the MM, a water-escape task which assesses spatial reference memory (Morris et al., 1982; Morris, 2015). The MM was a circular tub (188 cm in diameter) filled with 18–20°C water made opaque with non-toxic black paint. One platform (11 cm diameter) was placed 2–3 cm below the surface of the water in the northeast quadrant of the tub, where it remained across all days and trials. The rats underwent four trials per day for five consecutive days. During each daily session, rats were dropped off from one of four directions (north, south, east, or west) at the start of each trial. The pattern of the four drop-off locations changed across days but was identical within a day for all rats. Path length (cm) from drop-off to the platform was recorded by a video camera and Ethovision tracking software (Noldus Instruments; Wageningen, Netherlands). Maximum trial time was capped at 1 min. If the rat did not navigate to the platform in the allotted trial time, the experimenter gently guided the rat to the platform using a lead stick. Once the rat located the hidden platform, it was required to stay there for 15 s of platform time before being returned to its heated testing cage for a ∼10-min ITI, during which the other subjects were tested on that trial. On the final testing day of MM, after the fourth trial, rats completed a probe trial wherein the submerged platform was completely removed from the maze. Rats swam freely in the maze for the 1-min probe trial. The proportion of total swim distance covered within the previously platformed quadrant vs. the opposite quadrant was calculated to assess spatial localization to the previous platform location.

Visible Platform

On the day following MM, generalized visual acuity and motor competency necessary for completing swim-based escape tasks were assessed using the VP control task (Morris, 1984; Mennenga et al., 2015a). The VP was a rectangular tub (100 cm × 60 cm) filled with clear water (18–20°C). On the north wall of the tub, a black platform (10 cm diameter) protruded approximately 4 cm above the water's surface and was easily visible to the rats. Opaque curtains surrounded the VP apparatus to obscure spatial and geometric cues within the testing room. Rats underwent six trials in 1 day. Each rat was dropped off from a fixed location in the center of the south wall of the tub. The platform position varied across trials semi-randomly in three possible locations along the north wall: left, center, and right. Each trial was capped at 90 s to reach the visible platform. The experimenter used a stopwatch to obtain latency to the platform and recorded it manually on a testing sheet after each trial. After navigating to the visible platform, the rat was required to stay on the platform for 15 s before the experimenter returned the rat to its heated testing cage outside of the opaque curtains. There was an ITI of approximately 10 min for each rat while the other subjects were tested on that trial.

Open Field Task

The day after VP, rats underwent one evaluation day in the OFT, which measured locomotor activity and anxiety-like behavior. Twenty-four hours before testing, the 100 cm × 100 cm black Plexiglas arena was thoroughly cleaned with Odormute, an enzyme cleaner, to remove potential odors from the apparatus. OFT procedures were carried out in a dark room, a protocol which has previously been found to be sensitive to changes in hormone profiles in female rats (Hiroi and Neumaier, 2006; Hiroi et al., 2016). At the beginning of the testing day, rats were transferred from their home cages to single testing cages and allowed to acclimate in the anteroom of the testing area for at least 30 min. Each subject was brought into the room separately. The experimenter placed the rat into the arena along the center of the north wall and quietly exited the room. Each rat had 10 min to freely explore the arena. Trials were recorded using Samsung infrared night vision cameras connected to an iPad via the SmartCam application. Following each trial, the experimenter reentered the room, removed the rat from the arena, discarded any feces or urine in the arena, and wiped down the entire arena with tap water to distribute odor cues. The box was dried with paper towel prior to the beginning of the next subject's trial. Using an overlay of 25 evenly sized and shaped squares (20 cm × 20 cm), an experimenter blind to treatment conditions manually scored the recorded trials for time spent (s) in the corners, center, and small center of the arena, as well as line crossings into the corners, center, small center, and total line crossings.

Forced Swim Task

The day following the OFT, rats were exposed to 2 days of the FST to evaluate depressive-like behaviors (Huynh et al., 2011; Hiroi et al., 2016). Four clear Plexiglas cylinders (45 cm high and 20 cm in diameter) were filled up to 30 cm in height with fresh water (25°C) and separated by black Plexiglas divider screens. On day one of the FST, rats were acclimated to the testing room for at least 30 min. Each rat was placed in a cylinder for 10 min before being removed, toweled dry, and placed back into a heated testing cage. Twenty-four hours later, rats were given a 5-min trial under the same conditions. Video recordings of the 5-min trial on day two were captured using a GoPro camera connected to an iPad. After the trial was completed, rats were removed from the cylinder and towel dried prior to being placed under an escapable heat lamp. Number of fecal boli were recorded after the trial. The water was drained from the clear cylinder and refilled with fresh water between each subject's trial. Recordings were scored by an independent experimenter blind to treatment conditions for latency to first immobility (s), time immobile (s), time climbing (s), time swimming (s), and number of dives. Immobility was quantified as minor movements necessary to keep the rat's head above water. Climbing was scored as rapid forearm movement to break the surface of the water or upward vertical movement to climb against the cylinder wall. Diving

was defined as a rapid downward movement into the cylinder. Any other motion made by the rats during the 5-min trial was identified as swimming behavior.

Euthanasia

Rats were given 1 week of rest following the FST prior to euthanasia. At approximately 13 months old, all subjects were deeply anesthetized using inhaled isoflurane prior to cardiocentesis and decapitation. Blood was collected from the left ventricle of the heart using a 20 g needle and allowed to clot at 4°C (Vacutainer 367986; Becton Dickinson and Company, Franklin Lakes, NJ, United States) for a minimum of 30 min. Blood vials were maintained on ice and centrifuged at 2000 rpm at 4°C for 20 min at the end of the day. Serum was aliquoted and stored at −20°C until analysis. Ovaries were separated from the uterine horns, trimmed of excess fat, and fixed in 10% buffered formalin for 48 h prior to being transferred to 70% ethanol until analysis. Uteri were dissected from the body cavity, trimmed of excess fat, and wet weight (g) was obtained.

Serum Hormone Measurements

All serum hormone assay processing was completed at the Core Endocrine Laboratory at Pennsylvania State University. E2 levels were detected using a double antibody liquid-phase radioimmunoassay (Beckman Coulter, Brea, CA, United States) as previously described (Acosta et al., 2010; Camp et al., 2012; Mennenga et al., 2015b,c; Koebele et al., 2017, 2019). This RIA used estradiol-specific antibodies with a ^{125}I-labeled estradiol as the tracer. Inter-assay coefficients of variation for the assay averaged 10% at a mean value of 28 pg/ml. E2 assay functional sensitivity was 5 pg/ml. Androstenedione levels were evaluated via ELISA (ALPCO, Salem, NH, United States) based on the typical competitive binding scenario between unlabeled antigen (present in standards, controls, and unknowns) and the enzyme-labeled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. Inter-assay coefficients of variation for the androstenedione assay averaged 9% at a mean value of 0.5 ng/ml. Functional sensitivity of the androstenedione assay was 0.1 ng/ml. Progesterone levels were also evaluated using ELISA (ALPCO, Salem, NH, United States). Progesterone ELISA inter-assay coefficients of variation averaged 13% at a mean value of 2.6 ng/ml. Functional sensitivity of the progesterone assay was 0.3 ng/ml.

Ovarian Follicle Counts

Following post-fixation at euthanasia, one ovary from each rat was randomly selected for processing and quantification. All ovarian follicle histology and quantification was carried out by FYXX Foundation (Flagstaff, AZ, United States). The oviduct was separated from the ovary prior to processing by a Leica TP1020 tissue processor. The ovary was paraffin embedded and serial sectioned at 5 μm on a semi-automatic rotary microtome. Every 10th section was placed on slides, which were stained with Gills 2 hematoxylin and counterstained with eosin Y-phloxine B, then manually cover-slipped. Tissue was scanned for analysis using a 3D HisTech DESK Scanner. Every 20th section was analyzed for viable primordial, primary, secondary and antral follicles. Viable follicles were those with no apparent signs of atresia. Atretic follicles were not counted. Criteria from Haas et al. (2007) was used to classify follicle type. Briefly, a resting-state primordial cell was classified by a single layer of squamous granulosa cells around an oocyte. Primary follicles included a single layer of cuboidal granulosa cells. Secondary follicles were identified by several layers of granulosa cells surrounding the oocyte. Antral follicles had two or more layers of granulosa cells in addition to a fluid-filled antral space within the follicle (Haas et al., 2007). The estimated total number of primordial follicles was obtained using the following formula: $N_t = (N_0 \times S_t \times t_s)/(S_0 \times d_0)$, where N_t = total follicle estimate, N_0 = number of follicles observed in the ovary, S_t = total number of sections in the ovary, t_s = thickness of the section (μm), S_0 = total number of sections observed, and d_0 = mean diameter of the nucleus (Gougeon and Chainy, 1987). Counts for primary, secondary, and antral follicles were summed. Corpora lutea were counted through progression of appearance across the entire sample.

Statistical Analyses

Statview statistical software was used to complete data analyses. All analyses were two-tailed ($\alpha = 0.05$) and presented as means ± S.E.M. A series of two-group planned comparison repeated measures ANOVAs were completed using Treatment as the independent variable. We aimed to answer three key questions with our experimental data. We asked: *(1) What role does daily E2-only treatment have with transitional menopause?* For this question, the VCD-E2 group was compared to the VCD-Vehicle group. *(2) Does daily treatment with an individual progestogen impact cognition with transitional menopause, and is type of progestogen a factor for outcomes?* To address this question, we compared the VCD-Vehicle group to the VCD-PROG group and to the VCD-LEVO group, as well as the VCD-PROG group to the VCD-LEVO group. *(3) What role does combination hormone therapy play for cognition with transitional menopause?* The VCD-E2 group was compared to each combination group (VCD-E2 + PROG and VCD-E2 + LEVO) to assess the impact of adding a progestogen component to E2 therapy in a reproductive tract intact, but follicle-deplete, system. The VCD-PROG and VCD-LEVO groups were compared to their corresponding combination hormone treatment groups (VCD-E2 + PROG or VCD-E2 + LEVO, respectively) to understand how E2 alters progestogen-only effects in a reproductive tract intact, but follicle-deplete, system. Combination groups were also compared to the VCD-Vehicle group, and to each other to evaluate whether different progestogen components of combined hormone therapy matter for cognitive outcomes. Statistically significant two-group comparisons are reported herein, while select non-significant comparisons key to the highlighted questions are provided for context.

Water radial arm maze data were divided into three phase blocks, as previously published (Mennenga et al., 2015c; Braden et al., 2017; Prakapenka et al., 2018; Koebele et al., 2019). Day 1 was considered training and was excluded from the analysis. Days 2–5 were the Early Acquisition Phase, Days 6–9 the Late Acquisition Phase, and Days 10–12 the Asymptotic Phase. Each phase block was analyzed separately, and each error type was

analyzed separately for each phase block, with WMC, WMI, and RM errors as the dependent measures. The three trials for WMC, or four trials for WMI and RM, were nested within days within each phase block (Early Acquisition Phase Block 1: 4 days, Late Acquisition Phase Block 2: 4 days, Asymptotic Phase Block 3: 3 days) as the repeated measures. Thus, these analyses consisted of two-group ANOVAs with Treatment as the independent variable, and two repeated measures variables of trials within days (Trials), and days within block (Days). Separate *a priori* two-group analyses were run for Trial 3 + Trial 4, the high working memory load trials, for WMC and WMI errors on each block based on prior age- and hormone-mediated effects found in our laboratory (Bimonte and Denenberg, 1999; Bimonte et al., 2003; Bimonte-Nelson et al., 2003, 2004; Acosta et al., 2010; Mennenga et al., 2015b,c; Koebele et al., 2017, 2019, 2020b; Prakapenka et al., 2018). Delayed memory retention data were analyzed independently for each treatment group by comparing WMC errors on Trial 3 on the last day of regular testing to Trial 3 on Day 13, the first post-delay trial on the Delay Day.

Morris water maze analyses were completed using the same two-group comparison structure. Swim Distance to the Platform (cm) was the dependent measure, and the four trials per day were nested within the 5 days of the task as the repeated measures. Performance was assessed across all 5 days of the task as well as across the four regular (non-probe trial) trials on Day 5 alone. Probe trial data were analyzed for each treatment group using Proportion Total Swim Distance in the NE (target) vs. SW (opposite) quadrants.

Visible platform analyses were completed for individual treatment groups. Analyses comparing performance on Trial 1 to Trial 6 were compared within each group. Latency to Platform (s) was the dependent measure, and the first and last trials were repeated measures.

Open field task analyses were completed for each two-group comparison. ANOVA was used to analyze total time (s) spent in the corners, center, and small center of the arena, as well as total number of entries made into the corner, center, and small centers of the arena to assess anxiety-like behavior. The total number of line crossings were assessed to evaluate locomotor activity during the task. The number of fecal boli produced during the 10 min trial was quantified.

Forced swim task analyses were completed for each two-group comparison. ANOVA was used to analyze latency to first immobility (s), total immobility duration (s), total swimming duration (s), total climbing duration (s), and number of dives as measures of depressive-like behaviors, as well as the number of fecal boli produced during the trial.

Body weights, uterine weights, serum hormone levels, and ovarian follicle counts were analyzed using ANOVA. For each two-group comparison, Treatment was the independent variable and body weight (g), uterine weight (g), hormone levels (pg/mL or ng/mL), or follicle counts were the dependent measures. An additional set of analyses for ovarian follicle counts were carried out *post hoc* to include a comparison group of ovary-intact, non-VCD treated rats from an independent data set in our laboratory quantified by FYXX Foundation (n = 10). This ovary-intact group received the respective Vehicle injection (50%DMSO/50%Saline) for VCD injections to provide additional context for the VCD-induced follicular depletion in the current study. Unless otherwise noted, the number of subjects per treatment group in the reported analyses was as follows: VCD-Vehicle n = 10, VCD-E2 n = 10, VCD-PROG n = 9, VCD-LEVO n = 9, E2 + PROG n = 10, and E2 + LEVO n = 10.

RESULTS

Water Radial Arm Maze

Figure 2 illustrates the learning curves for WMC (**Figure 2A**), WMI (**Figure 2B**), and RM (**Figure 2C**) errors across Days 2–12 of the WRAM.

Early Acquisition Phase (Days 2–5)

What role does daily E2-only treatment have in spatial learning and memory with transitional menopause?

The VCD E2 vs. VCD-Vehicle groups did not differ for WMC, WMI, or RM errors during the Early Acquisition Phase, suggesting that daily E2 treatment at the given dose did not affect early task learning in a model of transitional menopause compared to follicle-depleted rats that did not receive hormone treatment.

Does daily treatment with an individual progestogen impact cognition with transitional menopause, and does type of progestogen impact outcomes?

There were no differences between the VCD-Vehicle group and the VCD-PROG group or the VCD-LEVO group, nor between the VCD-PROG vs. VCD-LEVO groups for WMC, WMI, or RM during the Early Acquisition Phase. This suggests that with transitional menopause, daily progestogen treatment does not influence early task learning as compared to no hormone treatment, nor does type of progestogen differentially impact outcomes during learning.

What role does daily combination hormone therapy play for spatial learning and memory with transitional menopause?

For RM errors, there was a main effect of Treatment for the VCD-E2 vs. VCD-E2 + LEVO comparison [$F_{(1,18)} = 4.54$, $p < 0.05$], where follicle-deplete rats treated with a combination of E2 and levonorgestrel made fewer RM errors compared to those treated with E2-only (**Figure 3**). For the VCD-E2 + PROG group vs. VCD-E2 + LEVO group, there was also a main effect [$F_{(1,18)} = 9.78$, $p < 0.01$], where follicle-deplete rats treated with a combination of E2 plus levonorgestrel made fewer RM errors than those treated with a combination of E2 plus progesterone during the Early Acquisition Phase. Thus, a daily regimen of E2 plus levonorgestrel combined with transitional menopause may confer benefits to spatial reference memory performance during learning (**Figure 3**).

Late Acquisition Phase (Days 6–9)

There were no significant Treatment differences in WMC, WMI, or RM errors for any two-group comparison during the Late Acquisition Phase.

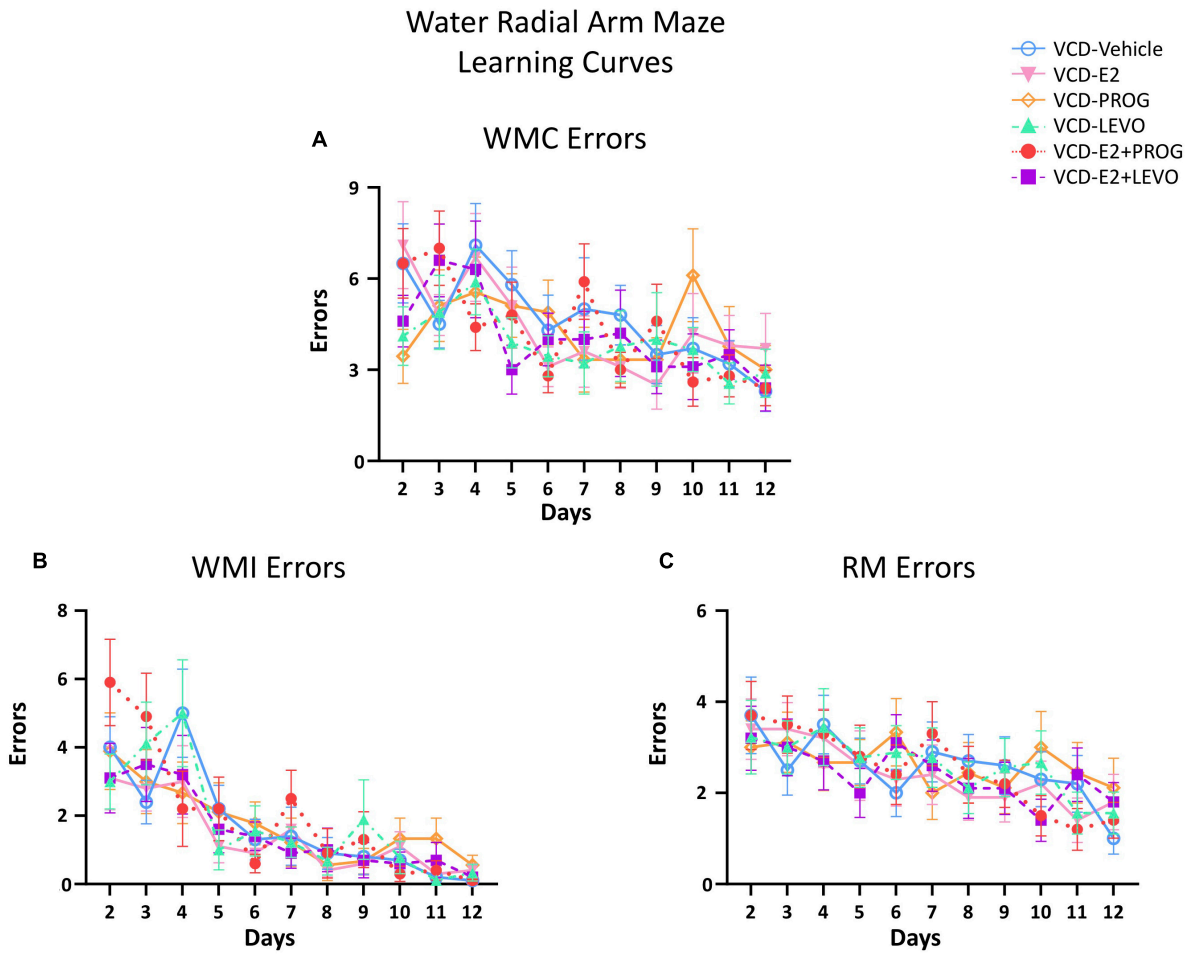


FIGURE 2 | Water Radial Arm Maze Error Subtype Learning Curves. **(A)** Working memory correct errors across days **(B)** Working memory incorrect errors across days **(C)** Reference memory errors across days. For all error types, Day 1 was considered Training and was excluded from data analysis. The Early Acquisition Phase was defined as Days 2–5, the Late Acquisition Phase was defined as Days 6–9, and the Asymptotic Phase was defined as Days 10–12. Performance for each error subtype was analyzed separately. The n/group for all WRAM two-group analyses were: VCD-Vehicle n = 10, VCD-E2 n = 10, VCD-PROG n = 9, VCD-LEVO n = 9, VCD-E2 + PROG n = 10, and VCD-E2 + LEVO n = 10.

Asymptotic Phase (Days 10–12)

What role does daily E2-only treatment have in spatial learning and memory with transitional menopause?

There were no significant differences in WMC, WMI, or RM errors for the VCD-E2 vs. VCD-Vehicle group comparison during the Asymptotic Phase of testing (**Figures 5**, **6**), suggesting that daily E2 treatment at the given dose did not significantly affect memory maintenance with transitional menopause compared to counterparts that did not receive hormone treatment.

Does daily treatment with an individual progestogen impact cognition with transitional menopause, and is type of progestogen a factor for spatial learning and memory?

During the Asymptotic Phase, there were no main effects of Treatment for WMC errors. There was a Trial × Treatment interaction present for WMC errors where follicle-deplete rats treated with progesterone performed worse than those treated with levonorgestrel (VCD-PROG vs. VCD-LEVO: $F_{(2,32)} = 3.76$, $p < 0.05$; **Figure 4A**), indicating that progestogen type has an impact on the ability to handle an increasing working memory load. No significant differences in WMI or RM errors were detected for this comparison in the Asymptotic Phase.

For WMI, there was a main effect of Treatment [$F_{(1,17)} = 5.26$, $p < 0.05$; **Figure 5A**] and a Trial × Treatment interaction [$F_{(3,51)} = 2.87$, $p < 0.05$; **Figure 5B**] whereby follicle-deplete rats treated with progesterone made more WMI errors compared to those without subsequent hormone treatment. When Trial 3 + Trial 4, the highest working memory load trials, were evaluated for WMI errors, there was a main effect of Treatment [$F_{(1,17)} = 5.21$, $p < 0.05$; **Figure 5C**], again indicating that follicle-deplete rats treated with progesterone made more WMI errors when working memory load was burdened compared to transitionally menopausal rats that did not receive subsequent hormone treatment. No differences between WMC or RM errors were present for this comparison.

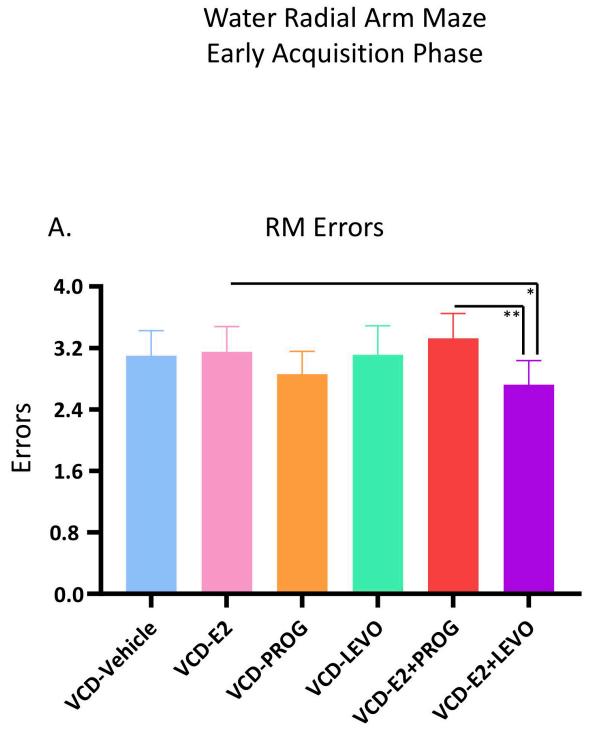


FIGURE 3 | Early Acquisition Phase RM Errors Across All Trials (Two-Group Comparisons). The VCD-E2 + LEVO group showed enhanced reference memory performance compared to the VCD-E2 group ($p < 0.05$) and compared to the VCD-E2 + PROG group ($p < 0.01$) during the Early Acquisition Phase. Significance: * = $p < 0.05$, ** = $p < 0.01$.

What role does daily combination hormone therapy play for spatial learning and memory with transitional menopause?

During the Asymptotic Phase of testing, there was a Trial × Treatment interaction for WMC errors within the VCD-PROG group vs. VCD-E2 + PROG group comparison [$F_{(2,34)} = 3.42$, $p < 0.05$; **Figure 4B**]; when the Trial 3 + Trial 4, the high working memory load trials, were probed for this comparison, there was a main effect of Treatment for WMC errors [$F_{(1,17)} = 4.66$, $p < 0.05$; **Figure 4C**], where rats treated with E2 plus progesterone made fewer errors than progesterone-only counterparts. Similarly, for WMI errors, there was a main effect of Treatment for the VCD-PROG vs. VCD-E2 + PROG comparison [$F_{(1,17)} = 6.64$, $p < 0.05$; **Figure 5A**], indicating that the addition of E2 to progesterone treatment enhanced performance compared to progesterone alone on WMI errors across all trials; a Trial × Treatment interaction [$F_{(3,51)} = 3.17$, $p < 0.05$; **Figure 5D**] was also present for this comparison. When Trial 3 + Trial 4, the high working memory load trials, were probed for WMI errors, a main effect of Treatment persisted [$F_{(1,17)} = 6.67$, $p < 0.05$; **Figure 5E**], where combined E2 plus progesterone treatment enhanced performance compared to progesterone-only treatment, particularly when memory load was highly burdened for WMI errors. A main effect of Treatment was also present for RM errors between VCD-PROG and VCD-E2 + PROG groups [$F_{(1,17)} = 7.56$, $p < 0.05$; **Figure 6A**]. As such, across all error types, a daily combination treatment of E2 plus progesterone treatment enhanced spatial memory performance compared to progesterone-only treatment in transitionally menopausal rats in the Asymptotic Phase. When E2-only treatment was compared to this combination of daily E2 plus progesterone, a Trial × Treatment interaction for RM errors was present [$F_{(3,54)} = 5.72$, $p < 0.01$; **Figure 6B**] with a higher mean error score for the VCD-E2 treated group as compared to the combined VCD-E2+PROG treated group on Trial 4, suggesting a potential benefit for the VCD-E2 + PROG group's spatial reference memory at the highest working memory load compared to E2-only treatment as well, although RM performance across trials should be interpreted with caution given a cap of four possible RM errors. Collectively, when ovaries remained structurally intact but were follicle-deplete, combined E2 plus progesterone treatment improved spatial memory performance compared to treatment with E2 alone or progesterone alone.

Six-Hour Delay

Treatment groups were analyzed separately for delayed memory retention assessment. WMC errors committed on the first post-delay trial (Trial 3) were compared to errors on Trial 3 on the last day of baseline testing. There was a main effect of Delay Day for the VCD-Vehicle group [$F_{(9,1)} = 10.76$, $p < 0.01$; **Figure 7A**], VCD-E2 group [$F_{(9,1)} = 21.00$, $p < 0.01$; **Figure 7B**], VCD-E2 + PROG group [$F_{(9,1)} = 7.36$, $p < 0.05$; **Figure 7E**], and VCD-E2 + LEVO group [$F_{(9,1)} = 19.29$, $p < 0.01$; **Figure 7F**], where most groups made more errors when an extended delay occurred, regardless of hormone therapy regimen. Analyses did not reach statistical significance for the VCD-PROG (**Figure 7C**) or VCD-LEVO group (**Figure 7D**), suggesting that the progestogen-only treatments promoted some level of memory retention across the delay period.

Morris Water Maze

Figure 8A demonstrates MM performance across the 5-day task.

What Role Does Daily E2-Only Treatment Have on a Simple Spatial Reference Memory Task With Transitional Menopause?

There were no Treatment effects across all 5 days of the task or on Day 5 alone between VCD-Vehicle and VCD-E2 groups, indicating that daily E2 treatment at the given dose did not alter spatial reference memory compared to follicle-deplete rats that did not receive subsequent hormone treatment.

Does Daily Treatment With an Individual Progestogen Impact Cognition With Transitional Menopause, and Is Type of Progestogen a Factor for a Simple Spatial Reference Memory Task?

There were no Treatment effects for any planned comparison including the progestogen-only groups across all 5 days of the task or on Day 5 alone.

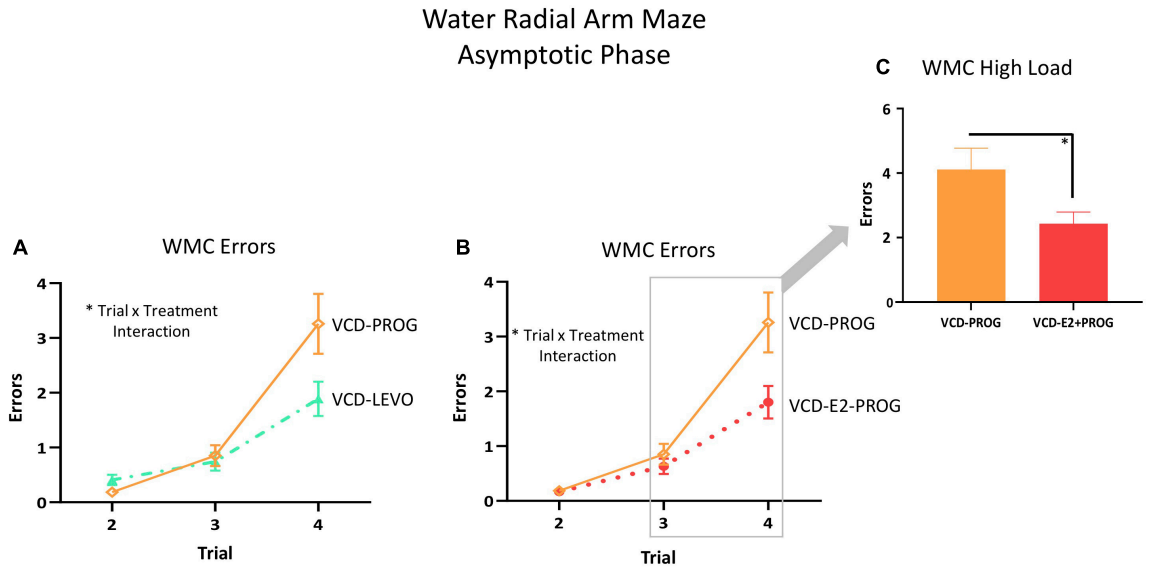


FIGURE 4 | WMC Errors During the Asymptotic Phase. **(A)** While there was no main effect of Treatment collapsed across trials for any two-group comparison, there were Trial × Treatment interactions between the VCD-LEVO and VCD-PROG groups ($p < 0.05$) and **(B)** VCD-PROG and VCD-E2 + PROG groups ($p < 0.05$). **(C)** When the High Load trials (Trials 3 plus 4) were assessed, VCD-PROG rats made more WMC errors than VCD-E2 + PROG rats ($p < 0.05$). Significance: * = $p < 0.05$.

What Role Does Daily Combination Hormone Therapy Play for a Simple Spatial Reference Memory Task With Transitional Menopause?

The VCD-E2 vs. VCD-E2 + PROG comparison yielded a Trial × Treatment interaction across all days of MM testing [$F_{(3,216)} = 2.78$, $p < 0.05$]. On the final testing day, there was a main effect of Treatment for the VCD-E2 vs. VCD-E2 + PROG comparison [$F_{(1,18)} = 7.59$, $p < 0.05$; **Figure 8B**] and the VCD-E2 vs. VCD-E2 + LEVO comparison [$F_{(1,18)} = 5.22$, $p < 0.05$; **Figure 8C**], where follicle-deplete rats treated with only E2 swam less distance to the platform compared to follicle-deplete rats administered a combination hormone therapy treatment. Thus, the addition of an exogenous progestogen, whether it was an endogenous-like progesterone or the synthetic progestin levonorgestrel, in combination with E2 impaired performance compared to E2 administration alone at the end of this simple spatial reference memory task.

Probe Trial

Probe trial analysis demonstrated that each treatment group effectively learned to use a spatial strategy to solve the MM task (**Figures 8D–I**). Indeed, when the platform was removed from the maze, each treatment group spent a greater proportion of total swim distance in the previously platformed target quadrant compared to the opposite quadrant (VCD-Vehicle: [$F_{(9,1)} = 150.44$, $p < 0.0001$]; VCD-E2: [$F_{(9,1)} = 159.271$, $p < 0.0001$]; VCD-PROG: [$F_{(8,1)} = 52.40$, $p < 0.0001$]; VCD-LEVO: [$F_{(8,1)} = 82.03$, $p < 0.0001$]; VCD-E2 + PROG: [$F_{(9,1)} = 66.32$, $p < 0.0001$]; VCD-E2 + LEVO: [$F_{(9,1)} = 159.306$, $p < 0.0001$]).

Visible Platform

When comparing performance on the first trial vs. the last trial for each treatment group, there was a main effect of Trial for the VCD-Vehicle group [$F_{(9,1)} = 9.16$, $p < 0.05$], VCD-E2 group [$F_{(9,1)} = 8.88$, $p < 0.05$], VCD-PROG group [$F_{(8,1)} = 6.59$, $p < 0.05$], VCD-LEVO group [$F_{(8,1)} = 15.67$, $p < 0.01$], and VCD-E2 + LEVO group [$F_{(9,1)} = 15.62$, $p < 0.01$]. The VCD-E2 + PROG group Trial effect was marginal [$F_{(9,1)} = 4.50$, $p = 0.06$], although this was likely due to one subject in that group that took 22 s to reach the platform on Trial 6 (**Figure 9A**). When this subject was excluded from the analysis, the Trial effect became significant [$F_{(8,1)} = 7.08$, $p < 0.05$]. However, all groups, including the VCD-E2 + PROG group, decreased in average trial latency from Trial 1 to Trial 6 of the VP task, with an average latency to platform of 5.3 ± 0.49 s on Trial 6 (**Figure 9B**).

Open Field Task

One subject from the VCD-E2 + PROG group was excluded from OFT analyses due to a technical error. **Figure 10A** provides a schematic of the OFT with boxes overlaid to operationally define the Corners, Center, and Small Center within the arena.

What Role Does Daily E2-Only Treatment Have in Anxiety-Like Behaviors With Transitional Menopause?

Transitionally menopausal rats treated with daily E2-only spent less time in the corners of the OFT when compared to transitionally menopausal rats treated with no hormone [Treatment main effect for the VCD-Vehicle vs. VCD-E2 comparison: $F_{(1,18)} = 5.24$, $p < 0.05$], suggesting decreased

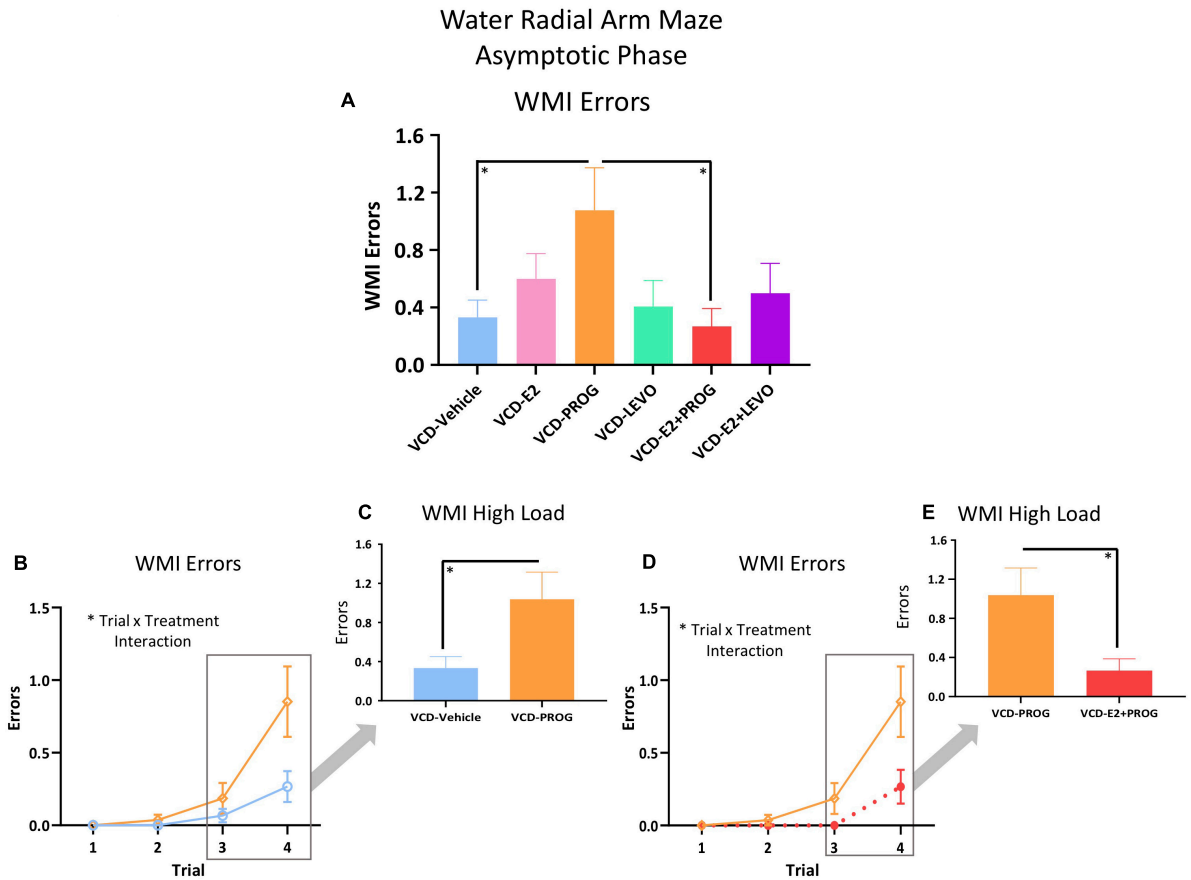


FIGURE 5 | WMI Errors During the Asymptotic Phase. **(A)** Across all trials, a main effect of Treatment was present between the VCD-PROG group and the VCD-Vehicle group ($p < 0.05$) as well as compared to the VCD-E2 + PROG group ($p < 0.05$). **(B)** VCD-PROG vs. VCD-Vehicle comparison: A Trial × Treatment interaction was present for this comparison ($p < 0.05$) **(C)** When High Load trials (Trials 3 + 4) were assessed, VCD-PROG rats made more WMI errors than VCD-Vehicle rats ($p < 0.05$). **(D)** VCD-PROG vs. VCD-E2 + PROG comparison: A Trial × Treatment interaction was present for this comparison ($p < 0.05$). **(E)** When High Load trials (Trials 3 + 4) were assessed, VCD-PROG rats made more WMC errors than VCD-E2 + PROG rats ($p < 0.05$). Significance: * = $p < 0.05$.

anxiety-like behavior when E2-only hormone therapy is given after follicular depletion as compared to no hormone therapy given after follicular depletion (**Figure 10B**). There were no effects present for time in the Center or Small Center for this comparison, nor were there differences in entries into the Corners, Center, or Small Center.

Does Daily Treatment With an Individual Progestogen Impact Anxiety-Like Behavior With Transitional Menopause, and Is Type of Progestogen a Factor for Outcomes?

Regarding Corner Time (s), transitionally menopausal rats treated with daily progesterone alone spent less time in the corners of the OFT when compared to counterparts without hormone treatment [Treatment main effect VCD-Vehicle vs. VCD-PROG comparison: $F_{(1,17)} = 4.80$, $p < 0.05$], suggesting a decrease in anxiety-like behavior for the progesterone-treated group (**Figure 10B**). There were no effects present for time in the Center or Small Center for these comparisons, nor were there differences in entries into the Corners, Center, or Small Center.

What Role Does Daily Combination Hormone Therapy Play for Anxiety-Like Behavior With Transitional Menopause?

Analysis of Center Time (s) revealed a Treatment effect for the VCD-E2 vs. VCD-E2 + LEVO comparison [$F_{(1,18)} = 4.61$, $p < 0.05$], wherein subjects treated with a combination of E2 and levonorgestrel spent significantly more time in the Center of the open field, indicating reduced anxiety-like behavior, compared to rats treated with E2-only (**Figure 10C**). There were no other effects present for Corner time or Small Center time for these comparisons. When assessing entries into the Corners, there were Treatment effects for the VCD-E2 vs. VCD-E2 + LEVO comparison [$F_{(1,18)} = 8.20$, $p < 0.05$] and VCD-E2 + PROG vs. VCD-E2 + LEVO comparison [$F_{(1,17)} = 4.87$, $p < 0.05$]. In both analyses, the VCD-E2 + LEVO group showed increased entries into the corners (**Figure 10F**). A Treatment effect was also indicated within the VCD-E2 vs. VCD-E2 + LEVO comparison for Center entries [$F_{(1,18)} = 7.14$, $p < 0.05$] (**Figure 10G**) and Small Center entries [$F_{(1,18)} = 22.59$, $p < 0.001$] (**Figure 10H**). Increased Small Center entries were also evident

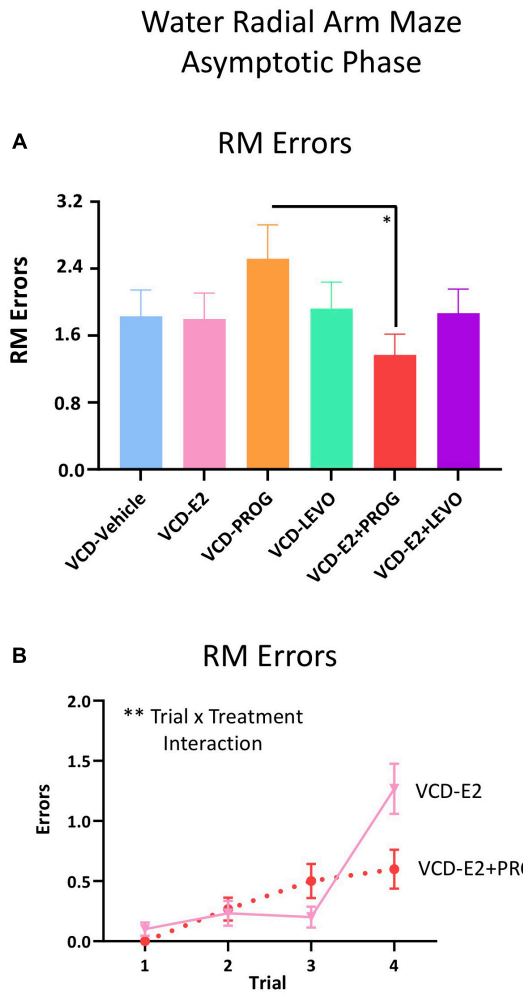


FIGURE 6 | RM Errors During the Asymptotic Phase. **(A)** Across all trials, a main effect of Treatment was present between the VCD-PROG group and the VCD-E2 + PROG group ($p < 0.05$). **(B)** VCD-E2 vs. VCD-E2 + PROG comparison: A Trial × Treatment interaction occurred ($p < 0.01$). Significance: * = $p < 0.05$, ** = $p < 0.01$.

in the VCD-E2 + LEVO group compared to the VCD-Vehicle group [$F_{(1,18)} = 8.10$, $p < 0.05$] and the VCD-E2 + PROG [$F_{(1,17)} = 5.21$, $p < 0.05$] (**Figure 10H**).

Line Crossings Analyses

Total Line Crossings, measuring total locomotion, differed for VCD-Vehicle vs. VCD-E2 + LEVO groups [$F_{(1,18)} = 4.64$, $p < 0.05$], VCD-E2 vs. VCD-E2 + LEVO groups [$F_{(1,18)} = 10.81$, $p < 0.01$], and VCD-E2 + PROG vs. VCD-E2 + LEVO groups [$F_{(1,17)} = 5.11$, $p < 0.05$], with rats treated with a combination of daily E2 plus levonorgestrel exhibiting increased locomotor activity in the OFT overall (**Figure 10I**). Transitionally menopausal rats treated with E2-only produced more fecal boli compared to rats without hormone therapy treatment (VCD-Vehicle vs. VCD-E2: $F_{(1,18)} = 8.27$, $p < 0.05$) and compared to rats treated with a combination of E2 plus progesterone (VCD-E2 vs. VCD-E2 + PROG: [$F_{(1,18)} = 8.87$, $p < 0.01$]) during the 10 min trial (**Figure 10E**).

Forced Swim Task

What Role Does Daily E2-Only Treatment Have in Depressive-Like Behaviors With Transitional Menopause?

Latency to Immobility, Total Immobility Duration, Total Swimming Duration, Total Climbing Duration, Number of Dives, or Number of Fecal Boli did not differ between rats treated with E2 only compared to counterparts not administered subsequent hormone treatment (**Figure 11**).

Does Daily Treatment With an Individual Progestogen Impact Depressive-Like Behavior With Transitional Menopause, and Does Type of Progestogen Have an Impact?

No differences were found in Latency to Immobility, Total Immobility Duration, Total Swimming Duration, Total Climbing Duration, Number of Dives, or Number of Boli for any planned comparison including the VCD-Vehicle group compared to the VCD-PROG or VCD-LEVO group, nor did VCD-PROG and VCD-LEVO groups differ from one another (**Figure 11**).

What Role Does Daily Combination Hormone Therapy Play for Depressive-Like Behavior With Transitional Menopause?

Regarding Latency to Immobility, there was a Treatment effect for the VCD-Vehicle vs. VCD-E2 + PROG comparison [$F_{(1,18)} = 5.51$, $p < 0.05$], the VCD-Vehicle vs. VCD-E2 + LEVO comparison [$F_{(1,18)} = 8.63$, $p < 0.01$], the VCD-E2 vs. VCD-E2 + PROG comparison [$F_{(1,18)} = 5.35$, $p < 0.05$], and the VCD-E2 vs. VCD-E2 + LEVO comparison [$F_{(1,18)} = 8.42$, $p < 0.01$]. In all comparisons, transitionally menopausal rats treated with combined E2 plus progestogen hormone treatment regimens had longer latencies to immobility, indicating that the addition of either natural progesterone or the synthetic progestin levonorgestrel to E2 treatment yields antidepressant-like behavior compared to E2-only treatment or no hormone treatment following transitional menopause (**Figure 11A**). Furthermore, Total Immobility Duration was increased in the VCD-Vehicle group compared to the VCD-E2 + PROG group [$F_{(1,18)} = 4.55$, $p < 0.05$], and compared to the VCD-E2 + LEVO group [$F_{(1,18)} = 6.94$, $p < 0.05$]. In both comparisons, the groups treated with combined E2 plus progestogen hormone regimens spent less total time immobile, indicating that combined hormone therapy regimens induce antidepressant-like behavior compared to no hormone treatment with transitional menopause (**Figure 11B**). Additionally, VCD-LEVO vs. VCD-E2 + LEVO differed for Total Immobility Duration [$F_{(1,17)} = 8.65$, $p < 0.01$], where rats treated with levonorgestrel alone spent more time immobile compared to counterparts treated with a combination of E2 plus levonorgestrel (**Figure 11B**). Although Total Swimming Duration did not differ for any comparison (**Figure 11C**), rats treated with a combination of E2 plus levonorgestrel spent more time presenting with

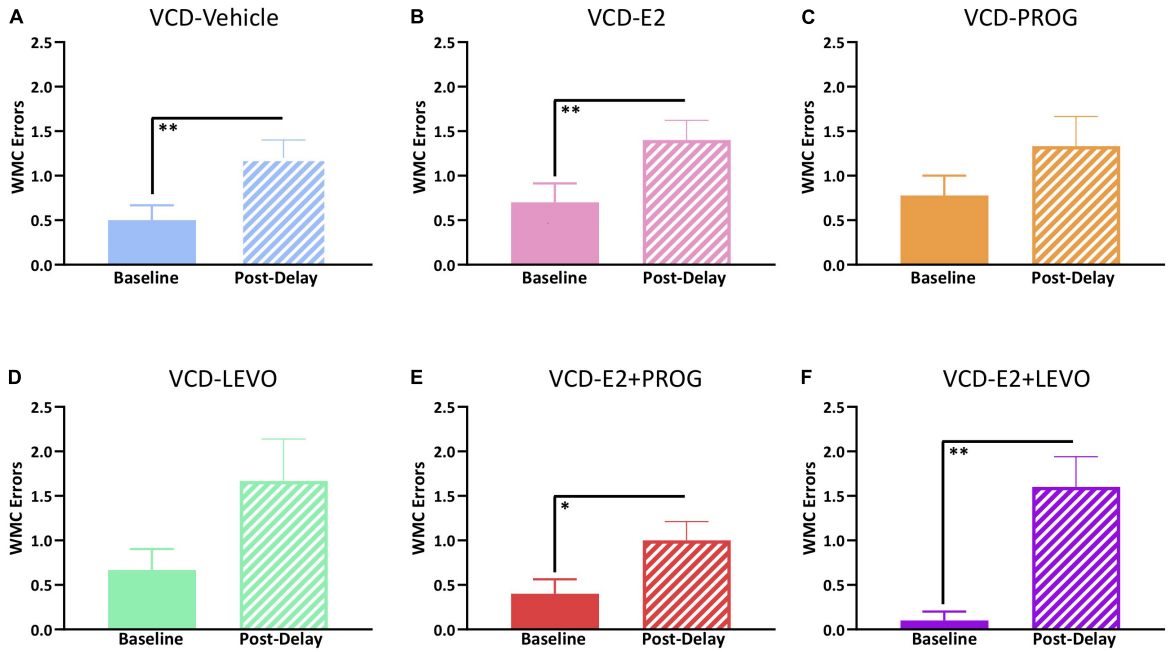


FIGURE 7 | WRAM Six-Hour Delayed Memory Retention Test. **(A)** The VCD-Vehicle group exhibited a delay-induced working memory impairment compared to the previous day's baseline performance ($p < 0.01$). **(B)** The VCD-E2 group exhibited a delay-induced working memory impairment compared to the previous day's baseline performance ($p < 0.01$). **(C)** The VCD-PROG group did not display a delay-induced working memory impairment compared to the previous day's baseline performance. **(D)** The VCD-LEVO group did not display a delay-induced working memory impairment compared to the previous day's baseline performance. **(E)** The VCD-E2 + PROG group exhibited a delay-induced working memory impairment compared to the previous day's baseline performance ($p < 0.05$). **(F)** The VCD-E2 + LEVO group exhibited a delay-induced working memory impairment compared to the previous day's baseline performance ($p < 0.01$). Significance: * = $p < 0.05$, ** = $p < 0.01$.

climbing behavior compared to counterparts that did not receive hormone therapy after follicular depletion (VCD-Vehicle vs. VCD-E2 + LEVO: [$F_{(1,18)} = 6.62, p < 0.05$]) (**Figure 11D**). Taken together, these results suggest that a combined hormone therapy regimen, particularly a combination of E2 and levonorgestrel, results in antidepressant-like effects compared to no hormone treatment, E2-only treatment, or progestogen-only treatment after transitional menopause.

Vaginal Cytology

Across two consecutive days of vaginal cytology monitoring, most VCD-Vehicle-treated rats exhibited mixed cytology resembling metestrus-like smears, suggesting disrupted estrous cyclicity, which is expected following accelerated follicular depletion without subsequent hormone therapy treatment. Rats that received E2 only displayed primarily cornified cells resembling estrus-like smears, which was expected as a result of daily E2 administration. Rats treated with progesterone only or levonorgestrel only had primarily metestrus- or diestrus-like smears, indicative of a relatively higher ratio of circulating progesterone to estrogen levels. The VCD-E2 + PROG group presented with cytology mostly resembling metestrus-like smears, and some diestrus-like smears, while the VCD-E2 + LEVO group showed estrus- and metestrus- like smears. Based on prior data from our and other laboratories, normal estrous cyclicity is disrupted approximately 4 months after VCD injection administration, and vaginal cytology can be modified by a given hormone therapy regimen (Koebele et al., 2020a).

Serum Hormone Levels

One VCD-Vehicle rat, all VCD-E2, and all VCD-E2 + LEVO rats were excluded from the androstenedione analyses because the measured serum hormone level was below the detectable limit of the assay. Additionally, one VCD-Vehicle rat was excluded from the E2 analyses due to insufficient serum volume needed to run the assay. The n per group for each steroid hormone assay is included in the **Figure 12** caption summarizing serum hormone levels.

How Does Daily E2-Only Treatment Affect Serum Hormone Profiles With Transitional Menopause?

Transitionally menopausal rats treated with daily E2 had increased circulating E2 levels compared to the Vehicle-treated

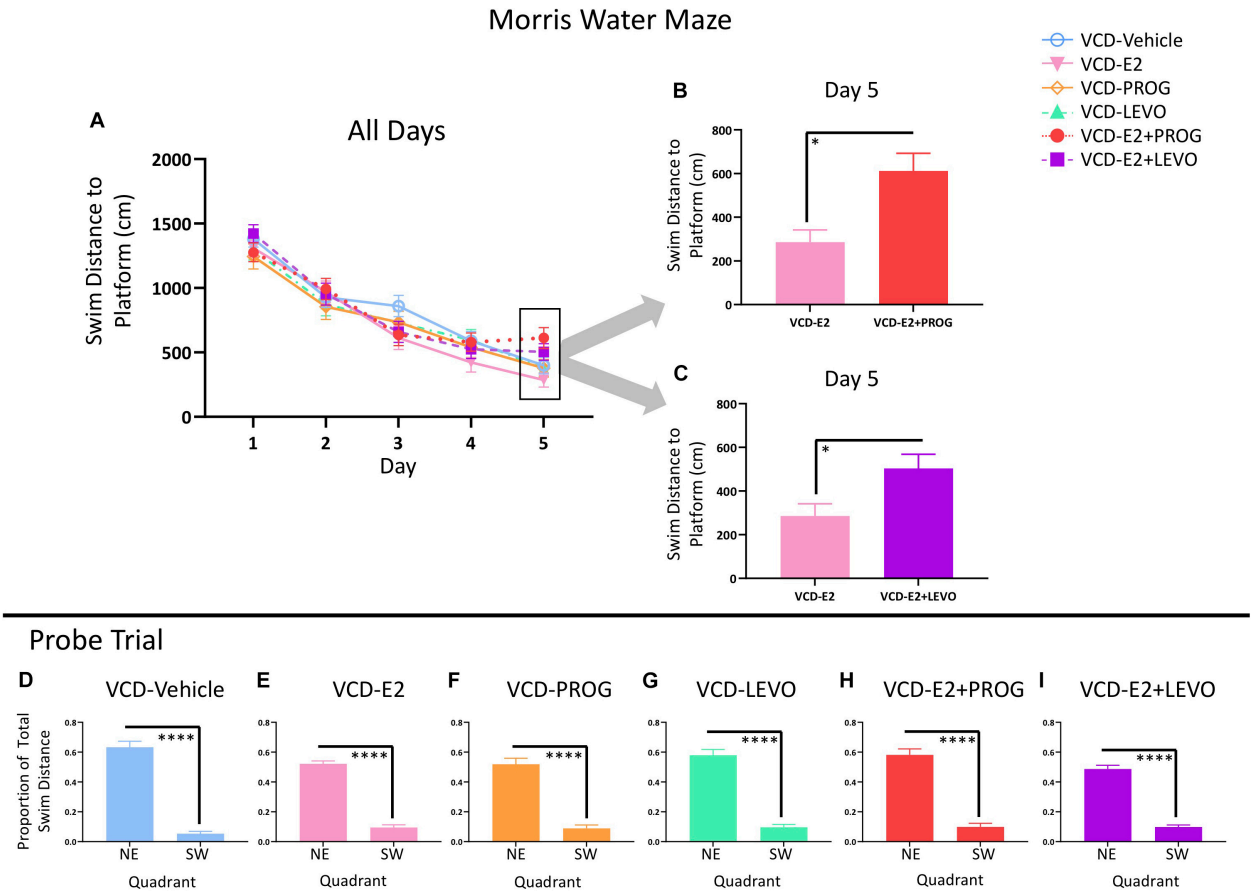


FIGURE 8 | Morris Water Maze. **(A)** Swim Distance to Platform Across All Days. **(B)** VCD-E2 treated rats swam less distance to the platform compared to the VCD-E2 + PROG treated rats ($p < 0.05$). **(C)** VCD-E2 treated rats swam less distance to the platform compared to the VCD-E2 + LEVO treated rats ($p < 0.05$). **(D–I)** Probe trial. All treatment groups swam a greater proportion of total distance in the previously platformed quadrant vs. the opposite quadrant, indicating that all groups spatially localized to the hidden platform location. Significance: * = $p < 0.05$, ****$p < 0.0001$.

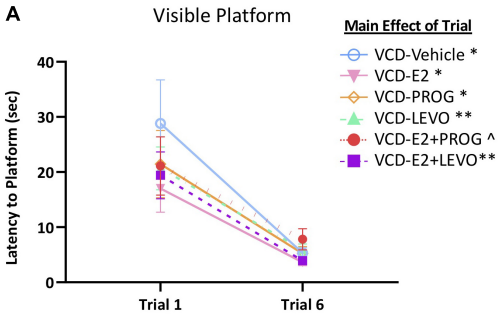
counterparts, as expected [$F_{(1,17)} = 10.82$, $p < 0.01$] (**Figure 12A**). Progesterone levels did not differ between VCD-Vehicle and VCD-E2 groups (**Figure 12B**). Lastly, all subjects within the VCD-E2 group had undetectable levels of androstenedione, and thus the comparison could not be carried out between VCD-Vehicle vs. VCD-E2 groups (**Figure 12C**).

How Does Daily Treatment With Progesterone or Levonorgestrel Affect Serum Hormone Levels With Transitional Menopause, and Does Type of Progestogen Impact Outcomes?

Treatment with progesterone or levonorgestrel did not alter circulating E2 levels compared to transitionally menopausal counterparts that did not receive hormone treatment or compared to each other (**Figure 12A**). The VCD-PROG group had higher circulating progesterone levels than the VCD-Vehicle group [$F_{(1,17)} = 70.95$, $p < 0.0001$] and the VCD-LEVO group [$F_{(1,16)} = 71.26$, $p < 0.0001$] (**Figure 12B**). Rats treated with levonorgestrel had similar circulating progesterone profiles compared to transitionally menopausal rats that did not receive hormone therapy, suggesting that this synthetic progestin did not alter endogenous progesterone levels in follicle-deplete ovary-intact rats. Interestingly, the VCD-PROG group had higher androstenedione levels compared to the VCD-Vehicle group [$F_{(1,16)} = 20.53$, $p < 0.001$], and compared to the VCD-LEVO group [$F_{(1,16)} = 21.49$, $p < 0.001$] (**Figure 12C**), suggesting that follicle-deplete rats with exogenous administration of natural progesterone experience increased circulating androgen levels compared to follicle-deplete rats without hormone treatment, or compared to those treated with the synthetic progestin levonorgestrel. On the other hand, treatment with levonorgestrel alone did not impact circulating androstenedione levels compared to counterparts that did not receive hormone therapy.

How Does Daily Combination Hormone Therapy Affect Serum Hormone Levels With Transitional Menopause?

Compared to rats without hormone treatment, rats in both combined hormone therapy groups demonstrated increased levels of circulating E2 (VCD-Vehicle vs. VCD-E2 + PROG group [$F_{(1,17)} = 14.18$, $p < 0.01$]; VCD-Vehicle vs. VCD-E2 + LEVO [$F_{(1,17)} = 20.21$, $p < 0.0001$]). Circulating E2 did not



B

Treatment	VCD-Vehicle	VCD-E2	VCD-PROG	VCD-LEVO	VCD-E2+PROG	VCD-E2+LEVO
Trial 1 (s)	28.80 ± 7.90	17.00 ± 4.30	21.44 ± 6.10	20.67 ± 3.89	21.10 ± 5.27	19.40 ± 4.25
Trial 6 (s)	5.30 ± 0.96	3.60 ± 0.581	5.22 ± 1.25	5.89 ± 1.07	7.80 ± 1.91	3.90 ± 0.57

FIGURE 9 | Visible Platform. **(A)** All subjects decreased latency to platform from the first to last trial. **(B)** Trial times (means + S.E.M.) for each treatment group are provided. Significance: * = $p < 0.05$, ** = $p < 0.01$, ^ = $p = 0.06$.

differ between VCD-E2 and VCD-E2 + PROG groups or VCD-E2 and VCD-E2 + LEVO groups, indicating that the addition of a progestogen to E2 treatment was insufficient to alter circulating E2 levels, at least at the given doses. Likewise, rats treated with either type of progestogen independently had less circulating E2 compared to their respective combined hormone therapy group (VCD-PROG vs. VCD-E2 + PROG [$F_{(1,17)} = 16.83$, $p < 0.001$]; VCD-LEVO vs. VCD-E2 + LEVO [$F_{(1,17)} = 23.44$, $p < 0.001$]). The VCD-E2 + PROG vs. VCD-E2 + LEVO groups did not differ in circulating E2 levels; thus, the type of progestogen (i.e., natural progesterone or synthetic progestin levonorgestrel) did not impact circulating E2 levels when the hormone therapy was administered in a combined estrogen plus progestogen fashion. Overall, the E2 component is likely the primary driver in determining circulating E2 levels in a given group (**Figure 12A**).

The VCD-E2 + PROG group had increased circulating progesterone levels compared to the VCD-Vehicle group [$F_{(1,18)} = 103.78$, $p < 0.0001$], the VCD-E2 group [$F_{(1,18)} = 62.29$, $p < 0.0001$], the VCD-E2 + LEVO group [$F_{(1,18)} = 74.99$, $p < 0.0001$], and, interestingly, the VCD-PROG alone group [$F_{(1,17)} = 9.36$, $p < 0.01$]; the outcome from this latter comparison indicates that combined E2 plus progesterone therapy may have a synergistic effect on increasing circulating progesterone levels compared to progesterone-only treatment. Circulating progesterone levels did not differ between VCD-Vehicle vs. VCD-E2 + LEVO groups, VCD-E2 vs. VCD-E2 + LEVO groups, or VCD-LEVO vs. VCD-E2 + LEVO groups, suggesting that the synthetic progestin levonorgestrel does not influence endogenous progesterone production itself, at least at the dose given in this experiment (**Figure 12B**).

All subjects in the VCD-E2 + LEVO group had undetectable levels of circulating androstenedione, and thus could not be evaluated relative to respective comparison groups. Because all subjects treated with E2 only likewise had undetectable androstenedione levels, this group also could not be compared to the VCD-E2 + PROG group. The VCD-E2 + PROG group did not differ in androstenedione levels from the VCD-Vehicle group. Androstenedione levels differed between VCD-PROG and VCD-E2 + PROG groups, whereby the combination hormone therapy regimen yielded reduced androstenedione levels compared to progesterone treatment alone [$F_{(1,17)} = 62.90$, $p < 0.0001$] (**Figure 12C**).

Ovarian Follicle Counts

Two subjects from the VCD-Vehicle group, two subjects from the VCD-LEVO group, one subject from the VCD-E2 + PROG group, and one subject from the VCD-E2 + LEVO group were excluded from follicle analyses due to poor tissue quality. Thus, the n/group for all follicle analyses was the following: VCD-Vehicle $n = 8$, VCD-E2 $n = 10$, VCD-PROG $n = 9$, VCD-LEVO $n = 7$, VCD-E2 + PROG $n = 9$, and VCD-E2 + LEVO = 9. The independent ovary-intact Vehicle reference group $n = 10$.

How Does Daily E2-Only Treatment Affect Ovarian Follicle Profiles With Transitional Menopause?

Compared to the VCD-Vehicle group, the VCD-E2 group had significantly fewer primordial follicles [$F_{(1,16)} = 6.10$, $p < 0.05$] and fewer primary follicles [$F_{(1,16)} = 9.89$, $p < 0.01$] (**Figures 13A,B**), an effect we have previously observed in follicle-depleted rats with tonic E2 treatment (Koebele et al., 2020a). Secondary follicles, antral follicles, and corpora lutea counts did not differ between VCD-Vehicle and VCD-E2 groups, although both groups exhibited substantial follicle decline, indicating successful

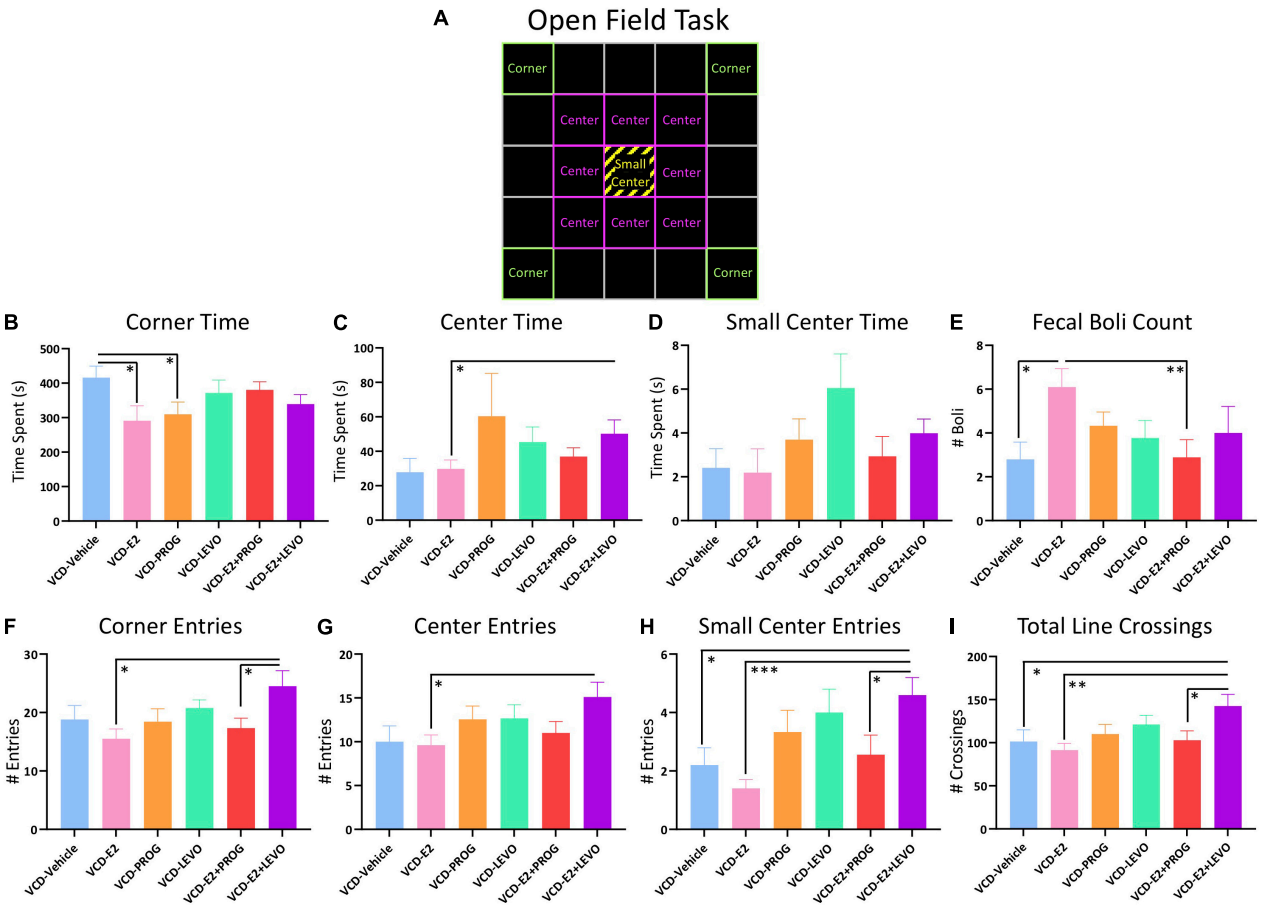


FIGURE 10 | Open Field Task. **(A)** Schematic of the OFT arena. Green squares indicate which boxes were defined as Corners, pink squares indicate which boxes were defined as Center, and yellow stripes indicate the Small Center, which was also included in the "Center" measure. **(B)** The VCD-Vehicle group spent more time in the corners compared to VCD-E2 and VCD-PROG groups. **(C)** VCD-E2 + LEVO treatment increased time spent in the center compared to VCD-E2 treatment. **(D)** No significant differences in Small Center Time were detected. **(E)** The VCD-E2 group had more fecal boli than the VCD-Vehicle and VCD-E2 + PROG group. **(F)** The VCD-E2 + LEVO group made more entries into the corner compared to the VCD-E2 group as well as the VCD-E2 + PROG group. **(G)** The VCD-E2 + LEVO group made more entries into the center compared to VCD-E2 treatment alone. **(H)** The VCD-E2 + LEVO group made more entries into the small center compared to VCD-Vehicle group, VCD-E2 group, and VCD-E2 + PROG group. **(I)** Total Line Crossing analyses indicate that the VCD-E2 + LEVO group moved more in the OFT compared to VCD-Vehicle, VCD-E2, and VCD-E2+PROG groups. Significance: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

VCD-induced follicular depletion. In fact, there were no detectable antral follicles for any subject treated with E2 only (**Figures 13C–E**).

How Does Daily Treatment With Progesterone or Levonorgestrel Affect Ovarian Follicle Profiles With Transitional Menopause, and Does Type of Progestogen Matter?

There were no Treatment group differences in primordial follicles, primary follicles, secondary follicles, antral follicles, or corpora lutea counts in the VCD-Vehicle group vs. the VCD-PROG group or vs. VCD-LEVO group, nor did the VCD-PROG and VCD-LEVO groups differ from each other, indicating that progestogen treatment alone does not impact the composition of the ovarian follicle pool in an accelerated follicular depletion model (**Figures 13A–E**).

How Does Daily Combination Hormone Therapy Affect Ovarian Follicle Profiles in a Model of Transitional Menopause?

Estimated primordial follicle counts did not differ for VCD-Vehicle rats compared to the VCD-E2 + PROG group or compared to the VCD-E2 + LEVO group. Compared to transitionally menopausal rats treated with E2 only, transitionally menopausal rats treated with E2 plus levonorgestrel had more primordial follicles [$F_{(1,17)} = 4.86$, $p < 0.05$] (**Figure 13A**), suggesting that this combined hormone treatment protects remaining healthy follicles in the ovarian reserve during this menopause transition time point compared to treatment with E2 alone. Estimated primordial follicle counts, primary follicles, secondary follicles, and antral follicles did not differ for combined hormone therapy groups compared to their respective progestogen counterparts, nor did they differ from each other. In addition, the VCD-E2 + PROG

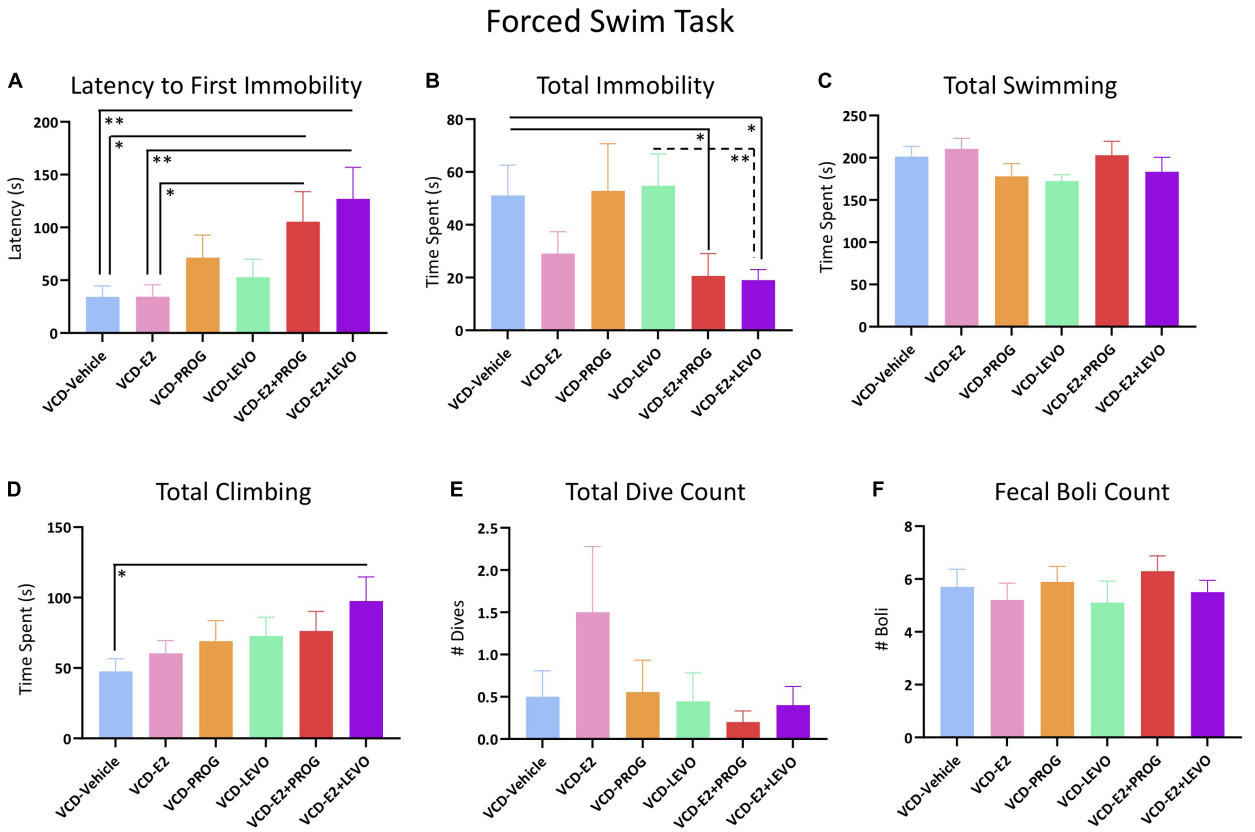


FIGURE 11 | Forced Swim Test. **(A)** Both combination hormone therapy groups had a longer latency to immobility when compared to VCD-Vehicle or VCD-E2 groups, suggesting an antidepressant-like effect of combination hormone therapy compared to no treatment or E2 treatment alone. **(B)** Total immobility was decreased in the combination hormone therapy groups, again suggesting an antidepressant-like effect compared to Vehicle treatment or LEVO-alone treatment. **(C)** No Treatment differences were indicated in time spent swimming. **(D)** The VCD-E2 + LEVO group spent more time climbing compared to the VCD-Vehicle group, indicating antidepressant-like effects. **(E,F)** Total Dive Count and Fecal Boli Counts did not differ among treatment comparisons. Significance: * = $p < 0.05$, ** = $p < 0.01$.

group had more corpora lutea compared to the VCD-E2 group [$F_{(1,17)} = 6.93$, $p < 0.05$], indicating that rats treated with E2 plus progesterone may have occasional ovulatory cycles during the menopause transition, although both groups were all significantly depleted and categorized as infertile (**Figure 13E**).

Confirmation of Follicular Depletion in VCD-Treated Groups: Comparison to an Ovary-Intact Vehicle Reference Group

Overall, groups treated with VCD showed substantial ovarian follicle loss in comparison to normally aging ovary-intact rats that did not receive exposure to VCD. To confirm that VCD treatment depleted the ovarian follicle reserve in all treatment groups in the current study, we utilized an independent data set of ovarian follicle counts collected in our laboratory from rats that received the complementary Vehicle injection for VCD administration, similar to a comparison procedure we have published previously (Koebele et al., 2020a). This ovary-intact Vehicle reference group was compared to each VCD-treated group in the current study (**Figures 13A–E**; specific comparisons below), with analyses showing that each VCD group had fewer primordial follicles, secondary follicles, antral follicles, and corpora lutea than this ovary-intact Vehicle reference group.

For primordial follicles, there was a Treatment main effect for each group comparison with the ovary-intact Vehicle reference group: VCD-Vehicle: [$F_{(1,16)} = 62.55$, $p < 0.0001$]; VCD-E2: [$F_{(1,18)} = 125.72$, $p < 0.0001$]; VCD-PROG: [$F_{(1,17)} = 82.70$, $p < 0.0001$]; VCD-LEVO: [$F_{(1,15)} = 14.79$, $p < 0.01$]; VCD-E2 + PROG: [$F_{(1,17)} = 48.98$, $p < 0.0001$]; VCD-E2 + LEVO: [$F_{(1,17)} = 50.17$, $p < 0.0001$].

For secondary follicles, there was a Treatment main effect for each group comparison with the ovary-intact Vehicle reference group: (VCD-Vehicle: [$F_{(1,16)} = 134.22$, $p < 0.0001$]; VCD-E2: [$F_{(1,18)} = 175.61$, $p < 0.0001$]; VCD-PROG: [$F_{(1,17)} = 130.12$, $p < 0.0001$]; VCD-LEVO: [$F_{(1,15)} = 90.70$, $p < 0.0001$]; VCD-E2 + PROG: [$F_{(1,17)} = 314.74$, $p < 0.0001$]; VCD-E2 + LEVO: [$F_{(1,17)} = 141.85$, $p < 0.0001$]).

For antral follicles, there was a Treatment main effect for each group comparison with the ovary-intact Vehicle reference group (VCD-Vehicle: [$F_{(1,16)} = 40.27$,

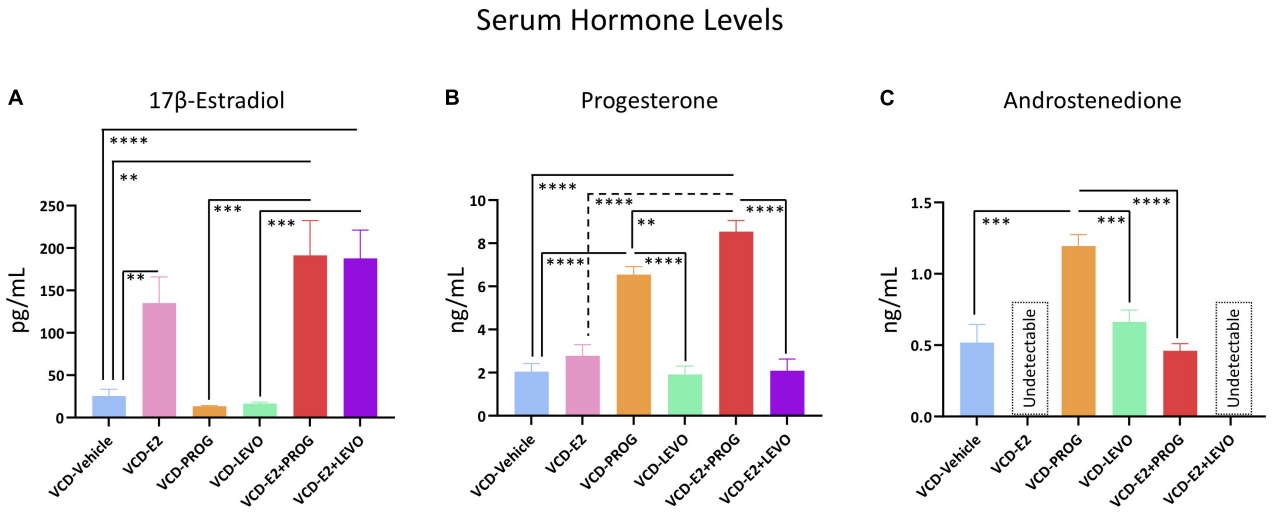


FIGURE 12 | Serum Hormone Levels. **(A)** E2 was elevated in VCD-E2, VCD-E2 + PROG, and VCD-E2 + LEVO groups compared to VCD-Vehicle rats. Additionally, combination hormone therapy groups had elevated E2 compared to their respective progestogen-only groups. E2 analysis n/group: VCD-Vehicle $n = 9$; VCD-E2 $n = 10$; VCD-PROG $n = 9$; VCD-LEVO $n = 9$; VCD-E2 + PROG $n = 10$; VCD-E2 + LEVO $n = 10$. **(B)** Progesterone was elevated in the VCD-PROG group and the VCD-E2 + PROG group compared to the VCD-Vehicle group, VCD-E2 group, and VCD-LEVO group. The combination hormone group had higher progesterone levels compared to the VCD-PROG group alone. Progesterone analysis n/group: VCD-Vehicle $n = 10$; VCD-E2 $n = 10$; VCD-PROG $n = 9$; VCD-LEVO $n = 9$; VCD-E2 + PROG $n = 10$; VCD-E2 + LEVO $n = 10$. **(C)** All subjects in the VCD-E2 group and VCD-E2 + LEVO group had undetectable levels of androstenedione. Androstenedione was elevated in the VCD-PROG group compared to VCD-Vehicle, VCD-LEVO, and VCD-E2 + PROG groups. Androstenedione analysis n/group: VCD-Vehicle $n = 9$; VCD-E2 $n = 0$ [undetectable]; VCD-PROG $n = 9$; VCD-LEVO $n = 9$; VCD-E2 + PROG $n = 10$; VCD-E2 + LEVO $n = 0$ [undetectable]. Significance: ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

$p < 0.0001$]; VCD-E2: [$F_{(1,18)} = 69.44$, $p < 0.0001$]; VCD-PROG: [$F_{(1,17)} = 46.36$, $p < 0.0001$]; VCD-LEVO: [$F_{(1,15)} = 47.66$, $p < 0.0001$]; VCD-E2 + PROG: [$F_{(1,17)} = 62.13$, $p < 0.0001$]; VCD-E2 + LEVO: [$F_{(1,17)} = 62.13$, $p < 0.0001$]).

For corpora lutea, there was a Treatment main effect for each group comparison with the ovary-intact Vehicle reference group: (VCD-Vehicle: [$F_{(1,16)} = 263.46$, $p < 0.0001$]; VCD-E2: [$F_{(1,18)} = 413.27$, $p < 0.0001$]; VCD-PROG: [$F_{(1,17)} = 184.52$, $p < 0.0001$]; VCD-LEVO: [$F_{(1,15)} = 102.73$, $p < 0.0001$]; VCD-E2 + PROG: [$F_{(1,17)} = 278.58$, $p < 0.0001$]; VCD-E2 + LEVO: [$F_{(1,17)} = 314.74$, $p < 0.0001$]).

Interestingly, the ovary-intact vehicle reference group had fewer primary follicles compared to each VCD-treated group: (VCD-Vehicle: [$F_{(1,16)} = 41.99$, $p < 0.0001$]; VCD-E2: [$F_{(1,18)} = 11.85$, $p < 0.01$]; VCD-PROG: [$F_{(1,17)} = 20.16$, $p < 0.001$]; VCD-LEVO: [$F_{(1,15)} = 34.13$, $p < 0.0001$]; VCD-E2 + PROG: [$F_{(1,17)} = 21.74$, $p < 0.001$]; VCD-E2 + LEVO: [$F_{(1,17)} = 16.46$, $p < 0.001$]).

Body Weights

Body Weight measurements across the experiment are illustrated in **Figure 14A**.

How Does Daily E2-Only Treatment Affect Body Weight With Transitional Menopause?

As we have previously observed (Koebele et al., 2020a), there were no body weight differences between the VCD-Vehicle group and the VCD-E2 group at euthanasia, indicating that daily E2 treatment was insufficient to alter body weight compared to a reproductive tract intact, but follicle-deplete, rat not treated with hormone therapy (**Figure 14B**).

How Does Daily Treatment With Progesterone or Levonorgestrel Affect Body Weight With Transitional Menopause, and Does Type of Progestogen Matter?

There were no differences in body weight between the VCD-Vehicle and the VCD-PROG group, or the VCD-LEVO group, at euthanasia. VCD-PROG vs. VCD-LEVO groups did not differ in average body weight either. Overall, this indicates that in reproductive tract intact, follicle-deplete rats, daily progestogen treatment alone did not alter body weight compared to counterparts not treated with hormone therapy. Moreover, body weights from progestogen-only groups did not differ from each other (**Figure 14B**).

How Does Daily Combination Hormone Therapy Affect Body Weight With Transitional Menopause?

The VCD-E2 + PROG group weighed less than the VCD-Vehicle group [$F_{(1,18)} = 6.12$, $p < 0.05$] as well as less than the VCD-PROG group [$F_{(1,17)} = 11.39$, $p < 0.01$] at euthanasia. The VCD-E2 + LEVO group weighed less than LEVO-only treated counterparts as well [$F_{(1,17)} = 7.84$, $p < 0.05$]. However, there were no weight differences indicated between the VCD-Vehicle vs. VCD-E2 + LEVO group at euthanasia. The combination hormone therapy regimens did not have an impact on body weight compared to E2-only treatment, nor did they differ from each other. Overall, these data suggest that a combined

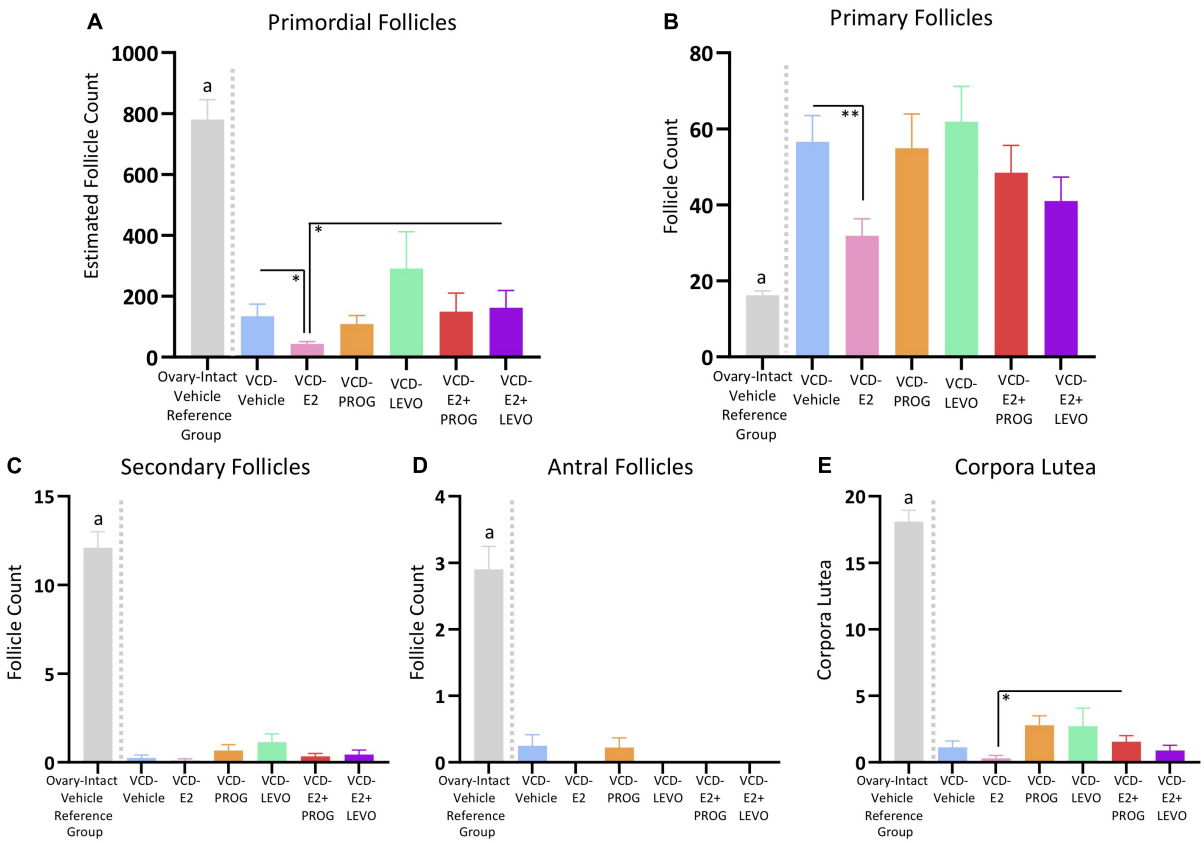


FIGURE 13 | Ovarian Follicle Counts. An independent ovary-intact reference group (n = 10) is included to assess successful follicular depletion following VCD treatment. The letter "a" indicates that this ovary-intact reference group was significantly different from each VCD-treated group. **(A)** Estimated primordial follicle counts were decreased in the VCD-E2 group compared to the VCD-Vehicle group and the VCD-E2 + LEVO group. **(B)** Primary follicles were decreased in the VCD-E2 group compared to the VCD-Vehicle group, replicating prior work. **(C)** Secondary follicle counts were significantly depleted in VCD-treated groups, indicating successful accelerated follicular atresia. **(D)** Antral follicle counts were significantly depleted in VCD-treated groups, indicating successful accelerated follicular atresia. **(E)** The VCD-E2 + PROG group had more corpora lutea compared to the VCD-E2 group, suggesting occasional ovulatory cycles in this group during the transition to reproductive senescence. Significance: * = $p < 0.05$, ** = $p < 0.01$.

hormone therapy regimen, particularly one containing natural progesterone, may lead to weight loss with a follicle-deplete background (**Figure 14B**).

Uterine Weights

How Does Daily E2-Only Treatment Affect Uterine Weight With Transitional Menopause?

The VCD-Vehicle and VCD-E2 groups did not differ in uterine weight (**Figure 14C**). Although we have previously reported an increase in uterine weight with E2-only treatment in a VCD model, that experiment administered E2 tonically using Alzet osmotic pumps (Koebele et al., 2020a). It is possible that transitionally menopausal rats given a low dose of E2 via daily injection is insufficient to induce persistent changes in uterine weight compared to transitionally menopausal rats not receiving hormone therapy treatment.

How Does Daily Treatment With Progesterone or Levonorgestrel Affect Uterine Weight With Transitional Menopause, and Does Type of Progestogen Matter?

While VCD-Vehicle vs. VCD-LEVO groups did not differ in uterine weights, the VCD-PROG group had decreased uterine weights compared to the VCD-Vehicle group [$F_{(1,17)} = 8.14$, $p < 0.05$] and compared to the VCD-LEVO group [$F_{(1,16)} = 6.92$, $p < 0.05$], suggesting that daily natural progesterone treatment attenuates uterine weight in reproductive tract-intact but follicle-deplete rats (**Figure 14C**).

How Does Daily Combination Hormone Therapy Affect Uterine Weight With Transitional Menopause?

Neither combination hormone therapy regimens, E2 plus progesterone nor E2 plus levonorgestrel, had an impact on uterine weight as compared to transitionally menopausal

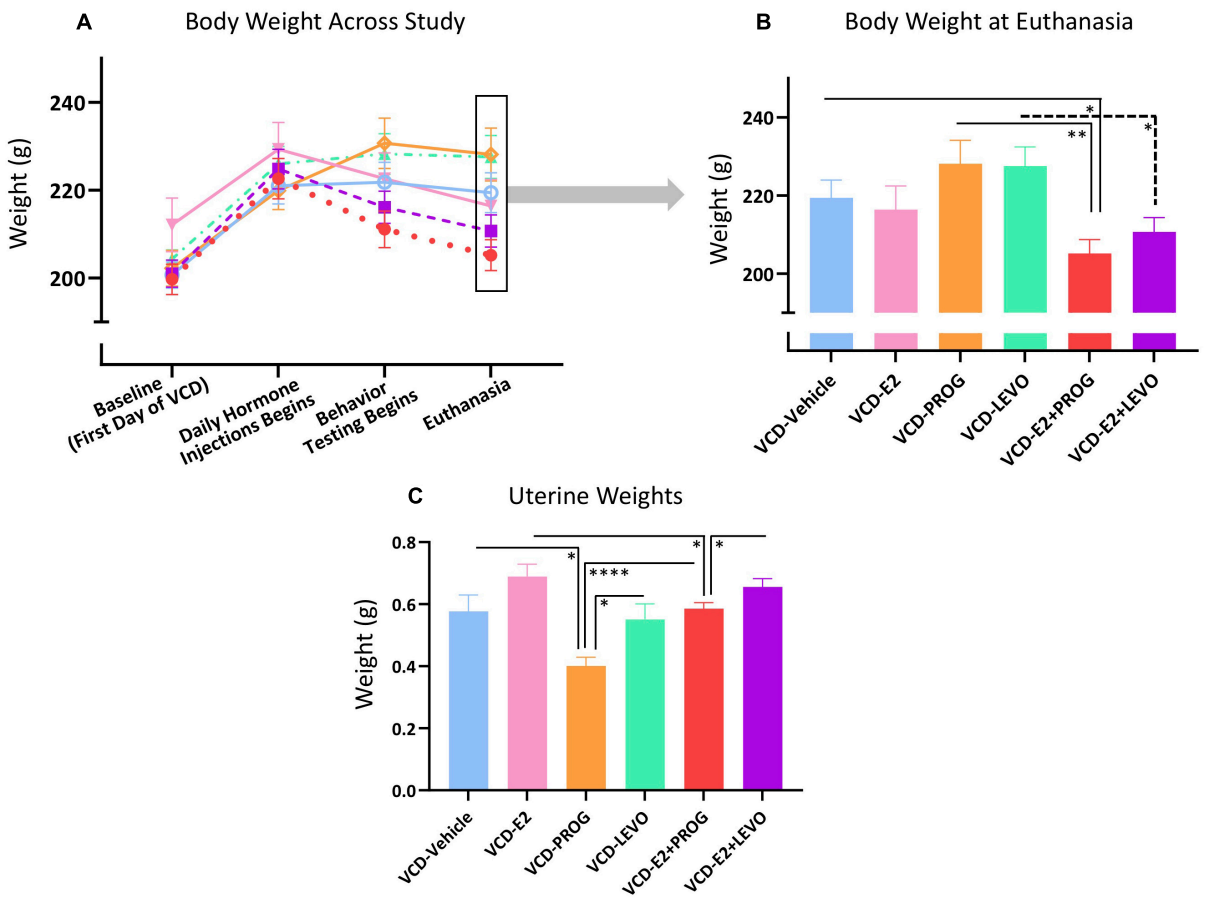


FIGURE 14 | Peripheral markers of overall health and uterine stimulation. **(A)** Body weight changes across the experimental timeline **(B)** At the end of the experiment, the VCD-E2 + PROG group weighed less than the VCD-Vehicle group and the VCD-PROG group, suggesting combination hormone therapy promotes weight maintenance compared to no hormone therapy treatment or progesterone treatment alone. The VCD-E2 + LEVO group also weighed less than its VCD-LEVO alone counterpart, again suggesting combination hormone therapy promotes weight maintenance. **(C)** PROG treatment reduced uterine weight compared to VCD-Vehicle, VCD-LEVO, and VCD-E2 + PROG groups. VCD-E2 + PROG uterine weight was attenuated compared to VCD-E2 treatment along, suggesting progesterone blocked uterine proliferation. The VCD-E2 + LEVO group uteri weighed more than those in the VCD-E2 + PROG group, indicating less progestin-induced attenuation of uterine stimulation compared to natural progesterone when in a combined hormone therapy regimen. Significance: * = $p < 0.05$, ** = $p < 0.01$, **** = $p < 0.0001$.

rats without hormone therapy. The combination of E2 plus progesterone decreased uterine weights compared to E2-only treatment [VCD-E2 group vs. VCD-E2 + PROG group: $F_{(1,18)} = 5.43$, $p < 0.05$], while the combination E2 plus levonorgestrel did not yield this decrease compared to E2-only treatment. Progesterone-only treatment also reduced uterine weights compared to combined E2 plus progesterone treatment [$F_{(1,17)} = 31.58$, $p < 0.0001$]. Uterine weights did not differ between rats treated with levonorgestrel alone and counterparts treated with a combination of E2 plus levonorgestrel. However, when E2 was administered with levonorgestrel, this combination resulted in higher uterine weights than when E2 was combined with natural progesterone [$F_{(1,18)} = 4.627$, $p < 0.05$] (**Figure 14C**).

DISCUSSION

Using the VCD accelerated follicular depletion model of transitional menopause, this experiment evaluated independent and combined effects of daily E2, progesterone, and levonorgestrel treatment on several aspects of cognition, including spatial memory, anxiety-like, and depressive-like behaviors in middle-aged, ovarian follicle-deplete female rats. Endocrine and ovarian follicular profiles were reported in conjunction with general health measures to provide the first comprehensive report of cognitive outcomes associated with independent and combined menopausal hormone therapy regimens in a transitional menopause model. Until now, preclinical investigations into combined hormone therapy regimens have been conducted in Ovx rats (Gibbs, 2000;

Simone et al., 2015; Prakapenka et al., 2018), and evaluations of hormone effects utilizing the VCD model have been limited to estrogen-only (Acosta et al., 2010; Pestana-Oliveira et al., 2018; Long et al., 2019; Koebele et al., 2020a). Divergent cognitive, anxiety-like, and depressive-like profiles were observed dependent upon the type of clinically relevant, daily hormone regimen administered. Overall, under the current experimental parameters, progesterone-only treatment produced detrimental impacts on spatial working memory, while combined E2 plus progestogen treatments resulted in beneficial cognitive effects spanning spatial memory, anxiety-like measures, and depressive-like measures, as well as favorable body and uterine weight profiles in a follicle-deplete, ovary-intact transitional menopause model. Collectively, these findings demonstrate that the presence of follicle-depleted ovarian tissue and the specific formulation of hormone treatment not only yield unique behavioral phenotypes, but are critical considerations when interpreting outcomes in both preclinical and clinical evaluations.

Regarding spatial memory performance, daily E2 treatment in follicle-deplete rats had a neutral effect on working and reference memory compared to counterparts without subsequent hormone treatment. Mode of hormone administration could impact cognitive outcomes in a transitional menopause background. Indeed, we have recently shown that tonic, chronic administration of E2 via a subcutaneous Alzet osmotic pump had beneficial learning effects, and some detrimental memory effects, in follicle-deplete rats of the same age (Koebele et al., 2020a). Thus, although the age of the rats, as well as the VCD treatment, hormone dose, and behavior protocol were constant across studies, varying the drug administration route from a tonic exposure to a daily injection likely altered spatial working memory outcomes.

We also report here that the combined hormone therapy regimen containing E2 plus the synthetic progestin levonorgestrel improved spatial reference memory during task acquisition in the WRAM compared to E2-only treatment. This suggests a unique and broad benefit in the transitionally menopausal model that was not observed in a surgical menopause model, wherein combined E2 plus levonorgestrel treatment attenuated the beneficial effects of E2 alone after Ovx (Prakapenka et al., 2018). Thus, the presence or absence of follicle-deplete ovarian tissue in middle-age plays a role in the cognitive outcomes of E2 plus levonorgestrel combination hormone treatment. Rats that received E2 plus levonorgestrel treatment also had improved reference memory during task acquisition compared to rats treated with E2 plus progesterone concomitantly, suggesting a unique cognitively beneficial role for levonorgestrel when combined with E2 to enhance learning on a complex spatial working memory task. Reference memory benefits observed for the rats treated with daily E2 plus levonorgestrel treatment did not carry over into MM, indicating that the presence of a working memory component in a task alters outcomes on the reference memory measure, an effect we have previously shown in normally aging, ovary-intact rats without hormone treatment (Bernaud et al., 2021). During the latter portion of WRAM testing, transitionally menopausal rats treated with only progesterone showed working memory impairments when working memory was taxed compared to counterparts without hormone treatment, with levonorgestrel treatment, or with E2 plus progesterone treatment. This progesterone-only induced cognitive impairment has been observed in past work from our laboratory and others using the Ovx menopause model (Chesler and Juraska, 2000; Bimonte-Nelson et al., 2006; Harburger et al., 2007; Lowry et al., 2010; Sun et al., 2010; Braden et al., 2015). On the MM, transitionally menopausal rats administered E2-only had significantly better performance on the last day of the task compared to both combination treatment groups, such that in the case of a simple spatial reference memory-only task, the combination of progesterone or levonorgestrel with E2 attenuated performance compared to E2 treatment alone. However, regardless of treatment, all rats spatially localized to the previously platformed area during the probe trial, indicating the effective use of a spatial strategy in the MM. Taken together, the cognitive effects resulting from exogenous hormone treatment may be specific to memory domain, task complexity, and menopause type (Koebele et al., in press). It is also of note that hormone therapy regimens in this study began after follicular depletion was substantial, and cognitive outcomes could have been impacted by the timing of the hormone therapy administration relative to the extent of follicular depletion.

Regarding anxiety-like behavior as measured by the OFT, transitionally menopausal rats treated with a combination of E2 plus levonorgestrel demonstrated less anxiety-like behavior as defined by more time and entries into the open field center compared to E2-only treatment, as well as more entries into the smallest center designation compared to transitionally menopausal rats without hormone therapy, or those given E2-only, or E2 plus progesterone. The E2 plus levonorgestrel group also had increased Total Line Crossings in the OFT, suggesting increased overall locomotor activity with this hormone treatment combination. Increased time in the corners of the open field in the VCD-Vehicle group indicates that the endogenous hormone profile associated with transitional menopause without subsequent hormone therapy increases anxiogenic behavior compared to the profile of transitional menopause with E2-only or progesterone-only administration. This observation corresponds to clinical literature showing increased *de novo* affective disorders during midlife and the transition to menopause, and calls for further evaluations of midlife-aged individuals given these hormone therapies (Bromberger and Kravitz, 2011; Maki et al., 2012; Weber et al., 2014; Soares, 2019; Parry, 2020; Stute et al., 2020). Overall, the combination of E2 and levonorgestrel produced a favorable profile of reduced anxiety-like behaviors compared to other groups. This is particularly noteworthy, as E2-only therapy has been shown to alleviate affective symptoms during the menopause transition, but not in the post-menopausal life stage (Lokuge et al., 2011); perhaps combined hormone regimens could be a novel pathway to alleviate anxiety symptoms in individuals who are reproductive-tract-intact but ovarian follicle-depleted. Regarding depressive-like behavior quantified in the FST, transitionally menopausal rats given combined hormone therapy regimen, irrespective of progestogen type,

exhibited longer latencies to immobility and spent less time immobile overall. This suggests that combined hormone therapy regimens, particularly those containing levonorgestrel, produce advantageous outcomes for depressive-like behaviors with a follicle-deplete, ovary-intact background. It is important to acknowledge that traditional FST measures have more recently been discussed within the context of responsiveness or coping after a severe acute stressor, rather than a pure measure of persistent depressive-like behavior (Commons et al., 2017), and that immobility could be an adaptive response rather than a despair-like behavior (Molendijk and de Kloet, 2015). In the future, it will be important to capture the impact of variations in hormone therapy regimens on additional tasks that encompass varied expressions of anxiety-like and depressive-like behavior in rodents.

In terms of physiological measures, all groups treated with E2 had elevated circulating E2 levels compared to groups that were not treated with E2. Circulating progesterone was increased in groups treated with progesterone. Of particular interest, transitionally menopausal rats treated with a combination of E2 and natural progesterone displayed elevated serum progesterone levels compared to counterparts treated with progesterone alone, which may point to a mechanism by which the combined hormone treatment containing E2 plus progesterone increased natural progesterone production to a greater extent than did the exogenous progesterone treatment alone. Circulating androstenedione levels were undetectable in rats treated with E2 alone or in combination with levonorgestrel, suggesting a potential role of exogenous E2 in mediating endogenous androstenedione production, which is synthesized in the interstitial ovarian tissue. Rats treated with progesterone had elevated circulating androstenedione levels compared to counterparts without hormone treatment, with synthetic levonorgestrel, or with combined E2 plus progesterone regimens, indicating that exogenous progesterone alone promotes the synthesis of endogenous androstenedione.

With regard to ovarian follicle counts, we report that the VCD-E2 treated group had significantly fewer primordial and primary follicles compared to the VCD-Vehicle group, corresponding to recent work from our laboratory showing similar effects with tonically administered E2 (Koebele et al., 2020a). This is a novel phenomenon observed within the middle-aged VCD model, wherein exogenous E2-only treatment may further accelerate follicular depletion by a yet-unknown mechanism. One possibility is that exogenous E2-associated rapid follicular depletion may be moderated, in part, by interactions with estrogen receptor-beta (Chakravarthi et al., 2020). Moreover, a recent report in adult ovary-intact mice revealed that administration of the synthetic estrogen ethinyl estradiol downregulated estrogen receptor expression and oxytocin receptor expression in ovarian tissue, with all receptor downregulation persisting even after treatment was discontinued (Garbett et al., 2020), pointing to a role for exogenous estrogen treatment in accelerated follicular depletion in rodents. Interestingly, the VCD-E2 + PROG group had statistically more corpora lutea present compared to the VCD-E2 alone group, such that the group administered E2 only was largely anovulatory, whereas other groups may have had an occasional ovulatory cycle during depletion, as has been observed in individuals during the human menopause transition (O'Connor et al., 2009; Burger, 2011), resulting in quantifiable corpora lutea at the time of evaluation.

The addition of the ovary-intact vehicle reference group confirmed that primordial, secondary, and antral follicles, as well as corpora lutea, were sufficiently depleted in the VCD-treated groups, regardless of subsequent hormone therapy treatment. In contrast to our previously published findings (Koebele et al., 2020a), the ovary-intact vehicle reference group had significantly *lower* primary follicles counts compared to VCD-treated groups. This may be due to a rat strain difference since the F344-NIH strain utilized in our previously published work has since been retired and replaced with the F344-CDF strain. Six single nucleotide polymorphisms (SNPs) that differ between the strains have been detected, although the effect of these SNPs on the F344-CDF phenotypes is not well defined (National Institute on Aging, 2019). Because primary ovarian follicles are not steroidogenic or responsive to gonadotropins, it is unlikely that there would be a major biologically or behaviorally relevant consequence to the increased primary follicle counts observed in the VCD-treated groups herein. It is notable that the extremely low or undetectable numbers of secondary and antral follicles in all VCD-treated groups demonstrate that the ovatoxin successfully halted any remaining primary follicles from transitioning into later stages of growth, and was thus successful at inducing a transitional menopause model.

Combined hormone therapy regimens containing both an estrogen and progestogen appear to reduce or maintain body weight during the menopause transition. Moreover, natural progesterone-only treatment consistently promoted inhibitory effects of uterine proliferation at the dose given. Follicle-deplete rats administered combined E2 plus progesterone therapy showed decreased uterine weights compared to E2-only therapy, again suggesting that natural progesterone administered exogenously attenuated endometrial growth; of note, we also found that progesterone decreased the uterine weight when combined with E2, while the synthetic progestin levonorgestrel did not. A higher dose of levonorgestrel may prevent uterine weight increases with transitional menopause. Of particular clinical relevance, uterine weights from rats treated with either combination hormone regimen did not differ from transitionally menopausal rats without hormone treatment; thus, the tested combined regimens did not yield substantial E2-induced uterine hyperplasia overall.

Collectively, this experiment demonstrates the remarkable variability that hormone therapy options can have on outcomes associated with memory, anxiety, depression, endocrine, body weight, and reproductive tract profiles during the transition to menopause. In accordance with medical societies providing recommendations for care during the menopause transition, our data support the tenet that hormone therapy is not a one-size-fits-all solution (Neves-E-Castro et al., 2015; Stuenkel et al., 2015; Baber et al., 2016; Pinkerton et al., 2017a). Primary indications for treatment and individual health risk factors must be taken

into account when prescribing hormone therapy; it is clear that formulation and presence of an intact reproductive tract are key to this equation, despite being historically understudied. The neurobiological, pharmacological, and behavioral effects of E2-alone, progestogen-alone, and combined hormone therapy are complex and, in some cases, task-specific. That levonorgestrel has some androgenic receptor activity, but does not have glucocorticoid or anti-mineralocorticoid activity like natural progesterone or other clinically used progestins (Schindler et al., 2003) may play a role in the behavioral phenotypes observed herein. This is particularly important because progesterone-alone had several negative effects on working memory performance in this evaluation, replicating a well-documented effect in the literature in Ovx rats (Chesler and Juraska, 2000; Bimonte-Nelson et al., 2004, 2006; Harburger et al., 2007; Sun et al., 2010; Braden et al., 2015). Moreover, progesterone-only, but not levonorgestrel-only, treatment increased androstenedione levels in the current experiment. Given that androstenedione has been shown to detrimentally impact spatial memory in Ovx rats, likely via its aromatization to estrone (Camp et al., 2012; Mennenga et al., 2015c), interactive effects of levonorgestrel with androgen receptors in conjunction with lower circulating androstenedione levels than seen with progesterone treatment may be a putative mechanism through which levonorgestrel mitigates or prevents negative cognitive effects. Moreover, levonorgestrel has also been shown to have some unique effects on insulin secretion when combined with the synthetic estrogen, ethinyl estradiol (Sitruk-Ware and Nath, 2011), indicating that independent or combined administration may alter biological and behavioral outcomes. Levonorgestrel remains a popular progestin prescribed in intrauterine devices, combined oral contraceptives, emergency contraception, and menopausal hormone therapy formulations; the results described here are promising findings, as a favorable hormone therapy regimen should not compromise cognitive health for the individual (and optimally would provide benefits) while fulfilling its function to alleviate other non-cognitive, unwanted menopause symptoms. Continued exploration into the biological underpinnings of levonorgestrel's unique effects on the brain and periphery will provide critical insight for improving health outcomes across multiple stages in the lifespan. Future investigations should consider additional clinically relevant hormone formulations that take into account a more holistic approach to understanding cognitive-behavioral outcomes, including menopause type (Edwards et al., 2019) and individual life history, with the goal to improve healthy life expectancy outcomes.

DATA AVAILABILITY STATEMENT

The data will be made available by the authors upon reasonable request. Requests to access the datasets should be directed to HB-N, bimonte.nelson@asu.edu.

ETHICS STATEMENT

The animal study was reviewed and approved by Arizona State University Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

SK contributed to conceptualization, data curation, formal analysis, investigation, methodology, project administration, software, supervision, validation, visualization, writing (original draft), and writing (review and editing). RH contributed to conceptualization, data curation, investigation, methodology, project administration, supervision, validation, visualization, and writing (review and editing). ZP, RM, AP, SP, CC, DK, SM, LM, and CD contributed to investigation, validation, and writing (review and editing). HB-N contributed to conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing (original draft), and writing (review and editing). All authors contributed to the article and approved the submitted version.

FUNDING

HB-N, NIA (AG028084), state of Arizona, Arizona Department of Health Services (ADHS14-052688), NIH Alzheimer's Disease Core Center (P30AG019610), and Arizona State University Office of Knowledge Enterprise Development, College of Liberal Arts and Sciences, and Department of Psychology.

ACKNOWLEDGMENTS

Dr. Michael Edwards is thanked for his valuable discussions regarding the statistical analyses in this manuscript. Alicia M. Quihuis is thanked for her assistance with VCD injections.

REFERENCES

Acosta, J. I., Mayer, L., Talboom, J. S., Tsang, C. W. S., Smith, C. J., Enders, C. K., et al. (2009). Transitional versus surgical menopause in a rodent model: etiology of ovarian hormone loss impacts memory and the acetylcholine system. *Endocrinology* 150, 4248–4259. doi: 10.1210/en.2008-1802

Acosta, J. I., Mayer, L. P., Braden, B. B., Nonnenmacher, S., Mennenga, S. E., and Bimonte-Nelson, H. A. (2010). The cognitive effects of conjugated equine estrogens depend on whether menopause etiology is transitional or surgical. *Endocrinology* 151, 3795–3804. doi: 10.1210/en.2010-0055

Al-Safi, Z. A., and Santoro, N. (2014). Menopausal hormone therapy and menopausal symptoms. *Fertil. Steril.* 101, 905–915. doi: 10.1016/j.fertnstert.2014.02.032

Baber, R. J., Panay, N., and Fenton, A. (2016). 2016 IMS Recommendations on women's midlife health and menopause hormone therapy. *Climacteric* 19, 109–150. doi: 10.3109/13697137.2015.1129166

Barha, C. K., and Galea, L. A. M. (2010). Influence of different estrogens on neuroplasticity and cognition in the hippocampus. *Biochim. Biophys. Acta Gen. Subj.* 1800, 1056–1067. doi: 10.1016/j.bbagen.2010.01.006

Bernaud, V. E., Hiroi, R., Poisson, M. L., Castañeda, A. J., Kirshner, Z. Z., Gibbs, R. B., et al. (2021). Age impacts the burden that reference memory imparts on an increasing working memory load and modifies relationships with cholinergic activity. *Front. Behav. Neurosci.* 15:3. doi: 10.3389/fnbeh.2021.610078

Biehl, C., Plotsker, O., and Mirkin, S. (2018). A systematic review of the efficacy and safety of vaginal estrogen products for the treatment of genitourinary syndrome of menopause. *Menopause* 26, 431–453. doi: 10.1097/GME.0000000000001221

Bimonte, H. A., and Denenberg, V. H. (1999). Estradiol facilitates performance as working memory load increases. *Psychoneuroendocrinology* 24, 161–173. doi: 10.1016/S0306-4530(98)00068-7

Bimonte, H. A., Nelson, M. E., and Granholm, A. C. E. (2003). Age-related deficits as working memory load increases: relationships with growth factors. *Neurobiol. Aging* 24, 37–48. doi: 10.1016/S0197-4580(02)00015-5

Bimonte-Nelson, H. A., Daniel, J. M., and Koebele, S. V. (2015). "The mazes," in *The Maze Book: Theories, Practice, and Protocols for Testing Rodent Cognition*, ed. H. A. Bimonte-Nelson (New York, NY: Springer), 37–72.

Bimonte-Nelson, H. A., Francis, K. R., Umphlet, C. D., and Granholm, A. C. (2006). Progesterone reverses the spatial memory enhancements initiated by tonic and cyclic oestrogen therapy in middle-aged ovariectomized female rats. *Eur. J. Neurosci.* 24, 229–242. doi: 10.1111/j.1460-9568.2006.04867.x

Bimonte-Nelson, H. A., Singleton, R. S., Hunter, C. L., Price, K. L., Moore, A. B., and Granholm, A.-C. E. (2003). Ovarian hormones and cognition in the aged female rat: I. Long-term, but not short-term, ovariectomy enhances spatial performance. *Behav. Neurosci.* 117, 1395–1406. doi: 10.1037/h0087876

Bimonte-Nelson, H. A., Singleton, R. S., Williams, B. J., and Granholm, A.-C. E. (2004). Ovarian hormones and cognition in the aged female rat: II. Progesterone supplementation reverses the cognitive enhancing effects of ovariectomy. *Behav. Neurosci.* 118, 707–714. doi: 10.1037/0735-7044.118.4.707

Black, K. L., Baumgartner, N. E., and Daniel, J. M. (2018). Lasting impact on memory of midlife exposure to exogenous and endogenous estrogens. *Behav. Neurosci.* 132, 547–551. doi: 10.1037/bne0000270

Black, K. L., Witty, C. F., and Daniel, J. M. (2016). Previous midlife oestradiol treatment results in long-term maintenance of hippocampal oestrogen receptor α levels in ovariectomised rats: mechanisms and implications for memory. *J. Neuroendocrinol.* 28 doi: 10.1111/jne.12429

Braden, B. B., Andrews, M. G., Acosta, J. I., Mennenga, S. E., Lavery, C., and Bimonte-Nelson, H. A. (2017). A comparison of progestins within three classes: differential effects on learning and memory in the aging surgically menopausal rat. *Behav. Brain Res.* 322, 258–268. doi: 10.1016/j.bbr.2016.06.053

Braden, B. B., Garcia, A. N., Mennenga, S. E., Prokai, L., Villa, S. R., Acosta, J. I., et al. (2011). Cognitive-impairing effects of medroxyprogesterone acetate in the rat: independent and interactive effects across time. *Psychopharmacology* 218, 405–418. doi: 10.1007/s00213-011-2322-4

Braden, B. B., Kingston, M. L., Whitton, E., Lavery, C., Tsang, C. W. S., and Bimonte-Nelson, H. A. (2015). The GABA-A antagonist bicuculline attenuates progesterone-induced memory impairments in middle-aged ovariectomized rats. *Front. Aging Neurosci.* 7:149. doi: 10.3389/fnagi.2015.00149

Braden, B. B., Talboom, J. S., Crain, I. D., Simard, A. R., Lukas, R. J., Prokai, L., et al. (2010). Medroxyprogesterone acetate impairs memory and alters the GABAergic system in aged surgically menopausal rats. *Neurobiol. Learn. Mem.* 93, 444–453. doi: 10.1016/j.nlm.2010.01.002

Bromberger, J. T., and Kravitz, H. M. (2011). Mood and menopause: findings from the Study of Women's Health Across the Nation (SWAN) over ten years. *Obstet. Gynecol. Clin. North Am.* 38, 609–625.

Bromberger, J. T., Kravitz, H. M., Chang, Y.-F., Cyranowski, J. M., Brown, C., and Matthews, K. A. (2011). Major depression during and after the menopausal transition: study of women's health across the nation (SWAN). *Psychol. Med.* 41, 1879–1888. doi: 10.1017/S003329171100016X

Burger, H. G. (2011). Unpredictable endocrinology of the menopause transition: clinical, diagnostic and management implications. *Menopause Int.* 17, 153–154. doi: 10.1258/mi.2011.011026

Camp, B. W., Gerson, J. E., Tsang, C. W. S., Villa, S. R., Acosta, J. I., Blair Braden, B., et al. (2012). High serum androstenedione levels correlate with impaired memory in the surgically menopausal rat: a replication and new findings. *Eur. J. Neurosci.* 36, 3086–3095. doi: 10.1111/j.1460-9568.2012.08194.x

Carolino, R. O. G., Barros, P. T., Kalil, B., and Anselmo-Franci, J. (2019). Endocrine profile of the VCD-induced perimenopausal model rat. *PLoS One* 14:e0226874. doi: 10.1371/journal.pone.0226874

Chakravarthi, V. P., Ghosh, S., Roby, K. F., Wolfe, M. W., and Rumi, M. A. K. (2020). A gatekeeping role of ESR2 to maintain the primordial follicle reserve. *Endocrinology* 161, 1–15. doi: 10.1210/endocr/bqaa037

Chesler, E. J., and Juraska, J. M. (2000). Acute administration of estrogen and progesterone impairs the acquisition of the spatial morris water maze in ovariectomized rats. *Horm. Behav.* 38, 234–242. doi: 10.1006/hbeh.2000.1626

Commons, K. G., Cholanians, A. B., Babb, J. A., and Ehlinger, D. G. (2017). The rodent forced swim test measures stress-coping strategy. not depression-like behavior. *ACS Chem. Neurosci.* 8, 955–960. doi: 10.1021/acschemneuro.7b00042

Cornițescu, F. I., Florentina, T., Simionescu, C., and Iliescu, D. (2011). Clinical, histopathological and therapeutic considerations in non-neoplastic abnormal uterine bleeding in menopause transition. *Rom. J. Morphol. Embryol.* 52, 759–765.

Dyer, C. A., Raymond-Whish, S., Schmuki, S., Fisher, T., Pyzyna, B., Bennett, A., et al. (2013). Accelerated follicle depletion in vitro and in vivo in Sprague-Dawley rats using the combination of 4-vinylcyclohexene diepoxide and triptolide. *J. Zoo. Wildl. Med.* 44, S9–S17. doi: 10.1638/1042-7260-44.4S.S9

Edwards, H., Duchesne, A., Au, A. S., and Einstein, G. (2019). The many menopauses: searching the cognitive research literature for menopause types. *Menopause* 26, 45–65. doi: 10.1097/GME.0000000000001171

Fernandez, S. M., Lewis, M. C., Pechenino, A. S., Harburger, L. L., Orr, P. T., Gresack, J. E., et al. (2008). Estradiol-induced enhancement of object memory consolidation involves hippocampal extracellular signal-regulated kinase activation and membrane-bound estrogen receptors. *J. Neurosci.* 28, 8660–8667. doi: 10.1523/JNEUROSCI.1968-08.2008

Files, J. A., Ko, M. G., and Pruthi, S. (2011). Bioidentical hormone therapy. *Mayo Clin. Proc.* 86, 673–680. doi: 10.4065/mcp.2010.0714

Foster, T. C., Sharrow, K. M., Kumar, A., and Masse, J. (2003). Interaction of age and chronic estradiol replacement on memory and markers of brain aging. *Neurobiol. Aging* 24, 839–852. doi: 10.1016/S0197-4580(03)00014-9

Frick, K. M. (2015). Molecular mechanisms underlying the memory-enhancing effects of estradiol. *Horm. Behav.* 74, 4–18. doi: 10.1016/j.yhbeh.2015.05.001

Frye, J. B., Lukefahr, A. L., Wright, L. E., Marion, S. L., Hoyer, P. B., and Funk, J. L. (2012). Modeling perimenopause in sprague-dawley rats by chemical manipulation of the transition to ovarian failure. *Comp. Med.* 62, 193–202.

Garbett, K. A., Ding, T., Allison, J., Grueter, C. A., Grueter, B. A., Osteen, K. G., et al. (2020). Synthetic female gonadal hormones alter neurodevelopmental programming and behavior in F1 offspring. *Horm. Behav.* 126, 104848. doi: 10.1016/j.yhbeh.2020.104848

Gibbs, R. B. (2000). Long-term treatment with estrogen and progesterone enhances acquisition of a spatial memory task by ovariectomized aged rats. *Neurobiol. Aging* 21, 107–116. doi: 10.1016/S0197-4580(00)00103-2

Gibbs, R. B. (2010). Estrogen therapy and cognition: a review of the cholinergic hypothesis. *Endocr. Rev.* 31, 224–253. doi: 10.1210/er.2009-0036

Gibbs, R. B., and Johnson, D. A. (2008). Sex-specific effects of gonadectomy and hormone treatment on acquisition of a 12-arm radial maze task by Sprague Dawley rats. *Endocrinology* 149, 3176–3183. doi: 10.1210/en.2007-1645

Gleason, C. E., Dowling, N. M., Wharton, W., Manson, J. A. E., Miller, V. M., Atwood, C. S., et al. (2015). Effects of hormone therapy on cognition and mood in recently postmenopausal women: findings from the randomized, controlled KEEPS–cognitive and affective study. *PLoS Med.* 12:e1001833. doi: 10.1371/journal.pmed.1001833

Goldman, J., Murr, A., and Cooper, R. (2007). The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies. *Birth Defects Res. Part B* 80, 84–97.

Goldstein, S., and Lumsden, M. (2017). Abnormal uterine bleeding in perimenopause. *Climacteric* 20, 414–420. doi: 10.1080/13697137.2017.1358921 REVIEW

Gougeon, A., and Chainy, G. B. (1987). Morphometric studies of small follicles in ovaries of women at different ages. *J. Reprod. Fertil.* 81, 433–442.

Haas, J. R., Christian, P. J., and Hoyer, P. B. (2007). Effects of impending ovarian failure induced by 4-vinylcyclohexene diepoxide on fertility in C57BL/6 female mice. *Comp. Med.* 57, 443–449.

Haney, A. F., and Wild, R. A. (2007). Options for hormone therapy in women who have had a hysterectomy. *Menopause* 14, 592–597. doi: 10.1097/gme.0b013e31804154d5

Harburger, L. L., Bennett, J. C., and Frick, K. M. (2007). Effects of estrogen and progesterone on spatial memory consolidation in aged females. *Neurobiol. Aging* 28, 602–610. doi: 10.1016/j.neurobiolaging.2006.02.019

Harburger, L. L., Saadi, A., and Frick, K. M. (2009). Dose-dependent effects of post-training estradiol plus progesterone treatment on object memory consolidation and hippocampal extracellular signal-regulated kinase activation in young ovariectomized mice. *Neuroscience* 160, 6–12. doi: 10.1016/j.neuroscience.2009.02.024

Heikkinen, T., Puoliväli, J., and Tanila, H. (2004). Effects of long-term ovariectomy and estrogen treatment on maze learning in aged mice. *Exp. Gerontol.* 39, 1277–1283. doi: 10.1016/j.exger.2004.05.005

Hiroi, R., McDevitt, R. A., and Neumaier, J. F. (2006). Estrogen selectively increases tryptophan hydroxylase-2 mRNA expression in distinct subregions of rat midbrain raphe nucleus: association between gene expression and anxiety behavior in the open field. *Biol. Psychiatry* 60, 288–295. doi: 10.1016/j.biopsych.2005.10.019

Hiroi, R., and Neumaier, J. F. (2006). Differential effects of ovarian steroids on anxiety versus fear as measured by open field test and fear-potentiated startle. *Behav. Brain Res.* 166, 93–100. doi: 10.1016/j.bbr.2005.07.021

Hiroi, R., Weyrich, G., Koebele, S. V., Mennenga, S. E., Talboom, J. S., Hewitt, L. T., et al. (2016). Benefits of hormone therapy estrogens depend on estrogen type: 17beta-estradiol and conjugated equine estrogens have differential effects on cognitive, anxiety-like, and depressive-like behaviors and increase tryptophan hydroxylase-2 mRNA levels in dorsal. *Front. Neurosci.* 10:517. doi: 10.3389/fnins.2016.00517

Hoffman, B. L., Schorge, J. O., Schaffer, J. I., Halvorson, K. D., Bradshaw, F. G., and Cunningham, L. E. (2012). *Williams Gynecology*, 2nd Edn. New York, NY: McGraw Hill Education.

Holmes, M. M., Wide, J. K., and Galea, L. A. (2002). Low levels of estradiol facilitate, whereas high levels of estradiol impair, working memory performance on the radial arm maze. *Behav. Neurosci.* 116, 928–934. doi: 10.1037/0735-7044.116.5.928

Huynh, T. N., Krigbaum, A. M., Hanna, J. J., and Conrad, C. D. (2011). Sex differences and phase of light cycle modify chronic stress effects on anxiety and depressive-like behavior. *Behav. Brain Res.* 222, 212–222. doi: 10.1016/j.bbr.2011.03.038

Im, E. O., Hu, Y., Cheng, C. Y., Ko, Y., Chee, E., and Chee, W. (2019). Racial/ethnic differences in cognitive symptoms during the menopausal transition. *West. J. Nurs. Res.* 41, 217–237. doi: 10.1177/0193945918767660

Kirshner, Z. Z., and Gibbs, R. B. (2018). Use of the REVERT® total protein stain as a loading control demonstrates significant benefits over the use of housekeeping proteins when analyzing brain homogenates by Western blot: an analysis of samples representing different gonadal hormone states. *Mol. Cell. Endocrinol.* 473, 156–165. doi: 10.1016/j.mce.2018.01.015

Koebele, S. V., and Bimonte-Nelson, H. A. (2015). Trajectories and phenotypes with estrogen exposures across the lifespan: what does Goldilocks have to do with it? *Horm. Behav.* 74, 86–104. doi: 10.1016/j.yhbeh.2015.06.009

Koebele, S. V., and Bimonte-Nelson, H. A. (2016). Modeling menopause: the utility of rodents in translational behavioral endocrinology research. *Maturitas* 87, 5–17. doi: 10.1016/j.maturitas.2016.01.015

Koebele, S. V., and Bimonte-Nelson, H. A. (2017). The endocrine-brain-aging triad where many paths meet: female reproductive hormone changes at midlife and their influence on circuits important for learning and memory. *Exp. Gerontol.* 94, 14–23. doi: 10.1016/j.exger.2016.12.011

Koebele, S. V., Mennenga, S. E., Hiroi, R., Quihuis, A. M., Hewitt, L. T., Poisson, M. L., et al. (2017). Cognitive changes across the menopause transition: a longitudinal evaluation of the impact of age and ovarian status on spatial memory. *Horm. Behav.* 87, 96–114. doi: 10.1016/j.yhbeh.2016.10.010

Koebele, S. V., Mennenga, S. E., Poisson, M. L., Hewitt, L. T., Patel, S., Mayer, L. P., et al. (2020a). Characterizing the effects of tonic 17β-estradiol administration on spatial learning and memory in the follicle-deplete middle-aged female rat. *Horm. Behav.* 126:104854. doi: 10.1016/j.yhbeh.2020.104854

Koebele, S. V., Nishimura, K. J., Bimonte-Nelson, H. A., Kemmou, S., Ortiz, J. B., Judd, J. M., et al. (2020b). A long-term cyclic plus tonic regimen of 17β-estradiol improves the ability to handle a high spatial working memory load in ovariectomized middle-aged female rats. *Horm. Behav.* 118:104656. doi: 10.1016/j.yhbeh.2019.104656

Koebele, S. V., Palmer, J. M., Hadder, B., Melikian, R., Fox, C., Strouse, I. M., et al. (2019). Hysterectomy uniquely impacts spatial memory in a rat model: a role for the non-pregnant uterus in cognitive processes. *Endocrinology* 160, 1–19. doi: 10.1210/en.2018-00709

Koebele, S. V., Quihuis, A. M., Lavery, C. N., Plumley, Z. M. T., Castaneda, A. J., and Bimonte-Nelson, H. A. Estrogen treatment modulates the impact of cognitive experience and task complexity on memory in middle-aged surgically menopausal rats. *J. Neuroendocrinol.* doi: 10.1111/jne.13002

Korol, D. L., and Pisani, S. L. (2015). Estrogens and cognition: Friends or foes?. An evaluation of the opposing effects of estrogens on learning and memory. *Horm. Behav.* 74, 105–115. doi: 10.1016/j.yhbeh.2015.06.017

Kritz-Silverstein, D., Goldani Von, Muhlen, D., and Barrett-Connor, E. (2000). Prevalence and clustering of menopausal symptoms in older women by hysterectomy and oophorectomy status. *J. Women's Heal. Gender Based Med.* 9, 747–755. doi: 10.1089/15246090050147727

Kuhl, H. (2005). Pharmacology of estrogens and progestogens: influence of different routes of administration. *Climacteric* 8, 3–63. doi: 10.1080/13697130500148875

Lohff, J. C., Christian, P. J., Marion, S. L., Arrandale, A., and Hoyer, P. B. (2005). Characterization of cyclicity and hormonal profile with impending ovarian failure in a novel chemical-induced mouse model of perimenopause. *Comp. Med.* 55, 523–527.

Lohff, J. C., Christian, P. J., Marion, S. L., and Hoyer, P. B. (2006). Effect of duration of dosing on onset of ovarian failure in a chemical-induced mouse model of perimenopause. *Menopause* 13, 482–488. doi: 10.1097/01.gme.0000191883.59799.2e

Lokuge, S., Frey, B. N., Foster, J. A., Soares, C. N., and Steiner, M. (2011). Depression in women: windows of vulnerability and new insights into the link between estrogen and serotonin. *J. Clin. Psychiatry* 72, 1563–1569. doi: 10.4088/JCP.11com07089

Long, T., Yao, J. K., Li, J., Kirshner, Z. Z., Nelson, D., Dougherty, G. G., et al. (2019). Estradiol and selective estrogen receptor agonists differentially affect brain monoamines and amino acids levels in transitional and surgical menopausal rat models. *Mol. Cell. Endocrinol.* 496, 110533. doi: 10.1016/j.mce.2019.110533

Lowry, N. C., Pardon, L. P., Yates, M. A., and Juraska, J. M. (2010). Effects of long-term treatment with 17 β-estradiol and medroxyprogesterone acetate on water maze performance in middle aged female rats. *Horm. Behav.* 58, 200–207. doi: 10.1016/j.yhbeh.2010.03.018

Luine, V. N. (2014). Estradiol and cognitive function: past, present and future. *Horm. Behav.* 66, 602–618. doi: 10.1016/j.yhbeh.2014.08.011

Maki, P. M., Rubin, L. H., Cohen, M., Golub, E. T., Greenblatt, R. M., Young, M., et al. (2012). Depressive symptoms are increased in the early perimenopausal stage in ethnically diverse human immunodeficiency virus-infected and human immunodeficiency virus-uninfected women. *Menopause* 19, 1215–1223. doi: 10.1097/gme.0b013e318255434d

Marret, H., Fauconnier, A., Chabbert-Buffet, N., Cravello, L., Golfier, F., Gondry, J., et al. (2010). Clinical practice guidelines on menorrhagia: management of abnormal uterine bleeding before menopause. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 152, 133–137. doi: 10.1016/j.ejogrb.2010.07.016

Mayer, L. P., Devine, P. J., Dyer, C. A., and Hoyer, P. B. (2004). The follicle-deplete mouse ovary produces androgen. *Biol. Reprod.* 71, 130–138. doi: 10.1095/biolreprod.103.016113

Mayer, L. P., Pearsall, N. A., Christian, P. J., Devine, P. J., Payne, C. M., McCuskey, M. K., et al. (2002). Long-term effects of ovarian follicular depletion in rats by 4-vinylcyclohexene diepoxide. *Reprod. Toxicol.* 16, 775–781. doi: 10.1016/S0890-6238(02)00048-5

Mennenga, S. E., Gerson, J. E., Dunckley, T., and Bimonte-Nelson, H. A. (2015a). Harmine treatment enhances short-term memory in old rats: dissociation of cognition and the ability to perform the procedural requirements of maze testing. *Physiol. Behav.* 138, 260–265. doi: 10.1016/j.physbeh.2014.09.001

Mennenga, S. E., Gerson, J. E., Koebele, S. V., Kingston, M. L., Tsang, C. W. S., Engler-Chiurazzi, E. B., et al. (2015b). Understanding the cognitive impact of the contraceptive estrogen ethinyl estradiol: tonic and cyclic administration impairs memory, and performance correlates with basal forebrain cholinergic

system integrity. *Psychoneuroendocrinology* 54, 1–13. doi: 10.1016/j.psyneuen.2015.01.002

Mennenga, S. E., Koebele, S. V., Mousa, A. A., Alderete, T. J., Tsang, C. W. S., Acosta, J. I., et al. (2015c). Pharmacological blockade of the aromatase enzyme, but not the androgen receptor, reverses androstenedione-induced cognitive impairments in young surgically menopausal rats. *Steroids* 99, 16–25. doi: 10.1016/j.steroids.2014.08.010

Mitchell, E. S., and Woods, N. F. (2001). Midlife women's attributions about perceived memory changes: observations from the Seattle Midlife Women's Health Study. *J. Womens. Health Gend. Based. Med.* 10, 351–362. doi: 10.1089/152460901750269670

Molendijk, M. L., and de Kloet, E. R. (2015). Immobility in the forced swim test is adaptive and does not reflect depression. *Psychoneuroendocrinology* 62, 389–391. doi: 10.1016/j.psyneuen.2015.08.028

Morgan, K. N., Derby, C. A., and Gleason, C. E. (2018). Cognitive changes with reproductive aging, perimenopause, and menopause. *Obstet. Gynecol. Clin. North Am.* 45, 751–763.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Methods* 11, 47–60.

Morris, R. (2015). "The watermaze," in *The Maze Book: Theories, Practice, and Protocols for Testing Rodent Cognition*, ed. H. A. Bimonte-Nelson (New York, NY: Springer Publishing Humana Press), 73–92.

Morris, R., Garrud, P., Rawlins, J. N., and O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature* 297, 681–683. doi: 10.1038/297681a0

NAMS (2014). *Menopause Practice: A Clinician's Guide*, 5th Edn. Mayfield Heights, OH.

Nappi, R. E., Sinforiani, E., Mauri, M., Bono, G., Polatti, F., and Nappi, G. (1999). Memory functioning at menopause: impact of age in ovariectomized women. *Gynecol. Obstet. Invest.* 47, 29–36. doi: 10.1159/000010058

National Institute on Aging (2019). *Aged Rodent Colonies Handbook. Available Strains*. Available online at: https://www.nia.nih.gov/research/dab/aged-rodent-colonies-handbook (accessed on 1 June 2019)

Neves-E-Castro, M., Birkhauser, M., Samsioe, G., Lambrinoudaki, I., Palacios, S., Borrego, R. S., et al. (2015). EMAS position statement: the ten point guide to the integral management of menopausal health. *Maturitas* 81, 88–92. doi: 10.1016/j.maturitas.2015.02.003

O'Connor, K. A., Ferrell, R., Brindle, E., Trumble, B., Shofer, J., Holman, D. J., et al. (2009). Progesterone and ovulation accross stages of the transition to menopause. *Menopause* 16, 1178–1187. doi: 10.1097/gme.0b013e3181aa192d. Progesterone

Parker, W. H., Jacoby, V., Shoupe, D., and Rocca, W. (2009). Effect of bilateral oophorectomy on women's long-term health. *Womens. Health* 5, 565–576. doi: 10.2217/whe.09.42

Parry, B. L. (2020). Towards improving recognition and management of perimenopausal depression. *Menopause* 27, 377–379. doi: 10.1097/GME.0000000000001519

Pestana-Oliveira, N., Kalil, B., Leite, C. M., Carolino, R. O. G., Debarba, L. K., Elias, L. L. K., et al. (2018). Effects of estrogen therapy on the serotonergic system in an animal model of perimenopause induced by 4-vinylcyclohexen diepoxide (VCD). *eNeuro* 5:ENEURO.0247-17.2017. doi: 10.1523/ENEURO.0247-17.2017

Phillips, S. M., and Sherwin, B. B. (1992). Effects of estrogen on memory function in surgically meopausal women. *Psychoneuroendocrinology* 17, 485–495.

Pinkerton, J. V. (2011). Pharmacological therapy for abnormal uterine bleeding. *Menopause* 18, 453–461. doi: 10.1097/gme.0b013e318212499c

Pinkerton, J. V. (2012). The truth about bioidentical hormone therapy. *Female Pat.* 37, 16–19.

Pinkerton, J. V., Aguirre, F. S., Blake, J., Cosman, F., Hodis, H., Hoffstetter, S., et al. (2017a). The 2017 hormone therapy position statement of the North American Menopause Society. *Menopause* 24, 728–753. doi: 10.1097/GME.0000000000000921

Pinkerton, J. V., Kaunitz, A. M., and Manson, J. E. (2017b). Vaginal estrogen in the treatment of genitourinary syndrome of menopause and risk of endometrial cancer: an assessment of recent studies provides reassurance. *Menopause* 24, 1329–1332. doi: 10.1097/GME.0000000000000996

Pinkerton, J. V., Sánchez Aguirre, F., Blake, J., Cosman, F., Hodis, H., Hoffstetter, S., et al. (2017c). The 2017 hormone therapy position statement of The North American Menopause Society. *Menopause* 24, 1–26.

Prakapenka, A. V., Hiroi, R., Quihuis, A. M., Carson, C., Patel, S., Berns-Leone, C., et al. (2018). Contrasting effects of individual versus combined estrogen and progestogen regimens as working memory load increases in middle-aged ovariectomized rats: one plus one does not equal two. *Neurobiol. Aging* 64, 1–14. doi: 10.1016/j.neurobiolaging.2017.11.015

Rahn, D. D., Carberry, C., Sanses, T. V., Mamik, M. M., Ward, R. M., Meriwether, K. V., et al. (2014). Vaginal estrogen for genitourinary syndrom of menopause: a systematic review. *Obstet. Gynecol.* 124, 1147–1156. doi: 10.1097/AOG.0000000000000526

Rapp, S. R., Espeland, M. A., Shumaker, S. A., Henderson, V. W., Brunner, R. L., Manson, J. E., et al. (2003). Effect of estrogen plus progestin on global cognitive function in postmenopausal women. *JAMA* 289, 2663–2672.

Rentz, D. M., Weiss, B. K., Jacobs, E. G., Cherkerzian, S., Klibanski, A., Remington, A., et al. (2017). Sex differences in episodic memory in early midlife: impact of reproductive aging. *Menopause* 24, 400–408. doi: 10.1097/GME.0000000000000771

Rocca, W. A., Bower, J. H., Maraganore, D. M., Ahlskog, J. E., Grossardt, B. R., de Andrade, M., et al. (2007). Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. *Neurology* 69, 1074–1083.

Rodgers, S. P., Bohacek, J., and Daniel, J. M. (2010). Transient estradiol exposure during middle age in ovariectomized rats exerts lasting effects on cognitive function and the hippocampus. *Endocrinology* 151, 1194–1203. doi: 10.1210/en.2009-1245

Rosario, E. R., Ramsden, M., and Pike, C. J. (2006). Progestins inhibit the neuroprotective effects of estrogen in rat hippocampus. *Brain Res.* 1099, 206–210. doi: 10.1016/j.brainres.2006.03.127

Ryan, J., Scali, J., Carriere, I., Amieva, H., Rouaud, O., Berr, C., et al. (2014). Impact of a premature menopause on cognitive function in later life. *BJOG An Int. J. Obstet. Gynaecol.* 121, 1729–1739. doi: 10.1111/1471-0528.12828

Schindler, A. E., Campagnoli, C., Druckmann, R., Huber, J., Pasqualini, J. R., Schweppe, K. W., et al. (2003). Classification and pharmacology of progestins. *Maturitas* 46, 7–16. doi: 10.1016/j.maturitas.2003.09.014

Shifren, J. L. (2018). Genitourinary syndrome of menopause. *Clin. Obstet. Gynecol.* 61, 508–516.

Shumaker, S. A., Legault, C., Rapp, S. R., Thal, L., Wallace, R. B., Ockene, J. K., et al. (2003). Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women. *JAMA* 289, 2651–2662. doi: 10.1001/jama.289.20.2651

Simone, J., Bogue, E. A., Bhatti, D. L., Day, L. E., Farr, N. A., Grossman, A. M., et al. (2015). Ethinyl estradiol and levonorgestrel alter cognition and anxiety in rats concurrent with a decrease in tyrosine hydroxylase expression in the locus coeruleus and brain-derived neurotrophic factor expression in the hippocampus. *Psychoneuroendocrinology* 62, 265–278. doi: 10.1016/j.psyneuen.2015.08.015

Singh, M., Meyer, E. M., Millard, W. J., and Simpkins, J. W. (1994). Ovarian steroid deprivation results in a reversible learning impairment and compromised cholinergic function in female Sprague-Dawley rats. *Brain Res.* 644, 305–312. doi: 10.1016/0006-8993(94)91694-2

Sitruk-Ware, R. (2002). Progestogens in hormonal replacement therapy: new molecules, risks, and benefits. *Menopause* 9, 6–15.

Sitruk-Ware, R., Bricaire, C., Lignieres, B. D. E., and Mawais-Jarvis, P. (1987). Oral micronized progesterone. *Contraception* 36, 373–402.

Sitruk-Ware, R., and Nath, A. (2011). Metabolic effects of contraceptive steroids. *Rev. Endocr. Metab. Disord.* 12, 63–75. doi: 10.1007/s11154-011-9182-4

Soares, C. N. (2019). Depression and menopause: an update on current knowledge and clinical management for this critical window. *Med. Clin. North Am.* 103, 651–667.

Soares, C. N. (2020). Taking a fresh look at mood, hormones, and menopause. *Menopause* 27, 371–373. doi: 10.1097/GME.0000000000001506

Stuenkel, C. A., Davis, S. R., Gompel, A., Lumsden, M. A., Murad, M. H., Pinkerton, J. A. V., et al. (2015). Treatment of symptoms of the menopause: an endocrine society clinical practice guideline. *J. Clin. Endocrinol. Metab.* 100, 3975–4011. doi: 10.1210/jc.2015-2236

Stute, P., Spyropoulou, A., Karageorgiou, V., Cano, A., Bitzer, J., Ceausu, I., et al. (2020). Management of depressive symptoms in peri- and postmenopausal women: EMAS position statement. *Maturitas* 131, 91–101. doi: 10.1016/j.maturitas.2019.11.002

Sun, W.-L., Luine, V. N., Zhou, L., Wu, H.-B., Weierstall, K. M., Jenab, S., et al. (2010). Acute progesterone treatment impairs spatial working memory in intact male and female rats. *Ethn. Dis.* 20, S183–S187.

Unkenstein, A. E., Bryant, C. A., Judd, F. K., Ong, B., and Kinsella, G. J. (2016). Understanding women's experience of memory over the menopausal transition: subjective and objective memory in pre-, peri-, and postmenopausal women. *Menopause* 23, 1319–1329. doi: 10.1097/GME.0000000000000705

Van Kempen, T. A., Milner, T. A., and Waters, E. M. (2011). Accelerated ovarian failure: a novel, chemically induced animal model of menopause. *Brain Res.* 1379, 176–187. doi: 10.1016/j.brainres.2010.12.064

Voorhis, B. J., Van, Santoro, N., Harlow, S., Crawford, S. L., and Randolph, J. (2008). The relationship of bleeding patterns to daily reproductive hormones in women approaching menopause. *Obs. Gynecol.* 112, 101–108. doi: 10.1097/AOG.0b013e31817d452b

Wallace, M., Luine, V., Arellanos, A., and Frankfurt, M. (2006). Ovariectomized rats show decreased recognition memory and spine density in the hippocampus and prefrontal cortex. *Brain Res.* 1126, 176–182.

Weber, M. T., Maki, P. M., and McDermott, M. P. (2014). Cognition and mood in perimenopause: a systematic review and meta-analysis. *J. Steroid Biochem. Mol. Biol.* 142, 90–98.

Weber, M. T., and Mapstone, M. (2009). Memory complaints and memory performance in the menopausal transition. *Menopause* 16, 694–700. doi: 10.1097/gme.0b013e318196a0c9

Weber, M. T., Mapstone, M., Staskiewicz, J., and Maki, P. M. (2012). Reconciling subjective memory complaints with objective memory performance in the menopausal transition. *Menopause* 19, 735–741. doi: 10.1097/gme.0b013e318241fd22

Worsley, R., Bell, R., Kulkarni, J., and Davis, S. R. (2014). The association between vasomotor symptoms and depression during perimenopause: a systematic review. *Maturitas* 77, 111–117. doi: 10.1016/j.maturitas.2013.11.007

Zhang, T., Yan, D., Yang, Y., Ma, A., Li, L., Wang, Z., et al. (2016). The comparison of animal models for premature ovarian failure established by several different source of inducers. *Regul. Toxicol. Pharmacol.* 81, 223–232. doi: 10.1016/j.yrtph.2016.09.002

Zilberman, J. M., Cerezo, G. H., Del Sueldo, M., Fernandez-Pérez, C., Martell-Claros, N., and Vicario, A. (2015). Association between hypertension, menopause, and cognition in women. *J. Clin. Hypertens.* 17, 970–976. doi: 10.1111/jch.12643

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

BRIEF RESEARCH REPORT
published: 26 August 2021
doi: 10.3389/fnbeh.2021.695416

Reduced Hippocampal GABA+ Is Associated With Poorer Episodic Memory in Healthy Older Women: A Pilot Study

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OPEN ACCESS

Edited by:
Caroline Gurvich, Monash University, Australia

Reviewed by:
Fei Gao, Shandong University, China
Styliani Vlachou, Dublin City University, Ireland

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Specialty section:
This article was submitted to Behavioral Endocrinology, a section of the journal Frontiers in Behavioral Neuroscience

Received: *15 April 2021*
Accepted: *13 July 2021*
Published: *26 August 2021*

Citation:
Jiménez-Balado J, Ycaza Herrera A, Igwe K, Klem L, Buyukturkoglu K, Irimia A, Liu C, Guo J, Brickman AM, and Eich TS (2021) Reduced Hippocampal GABA+ Is Associated With Poorer Episodic Memory In Healthy Older Women: A Pilot Study. Front. Behav. Neurosci. 15:695416. doi: 10.3389/fnbeh.2021.695416

Background: The current pilot study was designed to examine the association between hippocampal γ-aminobutyric acid (GABA) concentration and episodic memory in older individuals, as well as the impact of two major risk factors for Alzheimer's disease (AD)—female sex and Apolipoprotein ε4 (*ApoE* ε4) genotype—on this relationship.

Methods: Twenty healthy, community-dwelling individuals aged 50–71 (11 women) took part in the study. Episodic memory was evaluated using a Directed Forgetting task, and GABA+ was measured in the right hippocampus using a Mescher-Garwood point-resolved magnetic resonance spectroscopy (MRS) sequence. Multiple linear regression models were used to quantify the relationship between episodic memory, GABA+, *ApoE ε4*, and sex, controlling for age and education.

Results: While GABA+ did not interact with *ApoE* ε4 carrier status to influence episodic memory (p = 0.757), the relationship between GABA+ and episodic memory was moderated by sex: lower GABA+ predicted worse memory in women such that, for each standard deviation decrease in GABA+ concentration, memory scores were reduced by 11% (p = 0.001).

Conclusions: This pilot study suggests that sex, but not *ApoE* ε4 genotype, moderates the relationship between hippocampal GABA+ and episodic memory, such that women with lower GABA+ concentration show worse memory performance. These findings, which must be interpreted with caution given the small sample size, may serve as a starting point for larger studies using multimodal neuroimaging to understand the contributions of GABA metabolism to age-related memory decline.

Keywords: episodic memory, γ-aminobutyric acid, GABA, Alzheimer's disease, sex, apolipoprotein ε4

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder whose hallmark cognitive symptom is episodic memory loss (Tierney et al., 1996). AD is the leading cause of dementia in the elderly, and disproportionately affects women (Miech et al., 2002; Bacigalupo et al., 2018; Dubal, 2020). Despite decades of research investigating β amyloid (Aβ) as the trigger for a cascade of neuropathophysiological events that cause AD dementia (Hardy and Higgins, 1992), the failure of several high-profile late-stage clinical trials targeting Aβ clearance has highlighted the urgent need to explore alternative causal mechanisms for some key aspects of AD pathophysiology (Cummings et al., 2020). While the cholinergic and glutamatergic systems are known to be affected in AD (Hampel et al., 2018; Findley et al., 2019), the gamma-aminobutyric acidergic (GABAergic) system has received less attention (Pike and Cotman, 1993). However, animal models have shown that GABA plays a critical role in long-term memory formation by synchronizing pyramidal neuron activity (Paulsen and Moser, 1998; Lucas and Clem, 2018) and by preventing hyperactivity in the hippocampus (Najm et al., 2019), a brain structure critical for episodic memory formation and retrieval (Nyberg et al., 1996; Schacter et al., 1996). A study by Li et al. (2021) recently showed, using a 5XFAD AD-mouse model, that hyperactivity of pyramidal neurons in the CA1 field of the hippocampus was driven by $GABA_A$ receptor-mediated inhibitory synaptic decline, preceded Aβ-related pathology, was accompanied by cognitive impairments in an episodic-like memory task, and could be reversed *via* administration of a $GABA_A$ receptor agonist (Li et al., 2021). In humans, electrophysiological hyperactivity in the hippocampus—a brain structure that undergoes early and significant morphologic changes in AD (Putcha et al., 2011)—presages episodic memory decline in individuals at-risk for AD (Dickerson et al., 2005; Hämäläinen et al., 2007; Sperling et al., 2010; Yassa et al., 2010). Levetiracetam (Keppra), an anti-epileptic drug thought to enhance the function of GABA indirectly and to target hyperexcitability, reduces hippocampal hyperactivity, as indicated by decreased blood oxygenation level-dependent (BOLD) activation measured *via* functional magnetic resonance imaging (fMRI). Levetiracetam also mitigates memory impairment in patients with amnestic mild cognitive impairment (Bakker et al., 2012, 2015). Together, these findings suggest that GABAergic dysfunction plays a key role in the early hippocampal hyperactivity that is associated with episodic memory impairments in people at risk for, and with, AD.

The prevalence of AD is greater in women than in men (Miech et al., 2002; Bacigalupo et al., 2018; Dubal, 2020). This higher rate may reflect the fact that women typically live longer than men (Mielke, 2018), and/or a sex dimorphism involving either organizational effects that occur during development (Carroll et al., 2010; Luo et al., 2020) or activation effects occurring in mid-to-late life, most notably in the form of age-related estrogen reductions (Pike, 2017; Dubal, 2020). Estradiol (E2), the primary bioactive estrogen in women, increases spontaneous GABA release and increases the expression of $GABA_A$ receptors (Herbison et al., 1990; Herbison and Fénelon, 1995). Along with the decline in E2 levels post-menopause, GABA levels (at least in the anterior cingulate cortex) have been reported as significantly lower than pre-menopausal ones (Wang et al., 2019). Pathology studies in humans have shown lower expression of $GABA_A$ α1, α2, α5, β3 receptor subunits on the membranes of brain neurons in healthy older females in regions like the superior temporal gyrus (Pandya et al., 2019). Furthermore, *in vivo* studies of the frontal cortex suggest that there are stronger negative correlations between GABA levels and age in women than in men (Gao et al., 2013).

The Apolipoprotein ε4 allele (ApoE ε4) is the strongest common genetic risk factor for late-onset AD, being associated with both higher risk and a markedly earlier mean age of AD onset (Corder et al., 1993; Cacabelos, 2003). Several studies comparing cognitively-normal ε4 carriers to non-carriers reported memory-related electrophysiological hyperactivity in the hippocampus and entorhinal cortex (Bondi et al., 2005; Dickerson et al., 2005; Filippini et al., 2009; Sperling et al., 2010). *In vivo* animal studies have shown that learning and memory losses can be rescued through the deletion of ApoE ε4 in GABAergic interneurons (Knoferle et al., 2014) and that GABA-expressing interneurons in the hippocampus are selectively vulnerable to ApoE ε4-mediated neurotoxicity, including decreases in dendritic arborization and spine density (Jain et al., 2013). Indeed, Najm et al. (2019) recently proposed that GABAergic interneurons are selectively vulnerable to *ApoE ε4*, which may translate into a reduction of phasic and tonic inhibition that results in hippocampal excitability (Najm et al., 2019).

Thus, the relation between dysfunction in hippocampal GABA signaling and age-related memory impairment has been widely studied using animal models (Ambrad Giovannetti and Fuhrmann, 2019; Najm et al., 2019), and human studies have revealed interactions between hippocampal hyperactivity and memory which may serve as a biomarker for impending AD. Nevertheless, to our knowledge, no study to date has tested whether hippocampal GABA is associated with episodic memory in cognitively healthy older adults, or considered how such a relationship may be moderated by AD risk factors including sex or ApoE ε4 genotype. The current pilot study explores whether ApoE ε4 and/or sex are associated with decreases in hippocampal GABA concentration and, if so, whether such decreases predict worse episodic memory performance. Briefly, participants completed an episodic memory task, and a Mescher-Garwood point-resolved spectroscopy sequence (MEGA-PRESS) was then used to measure GABA concentration in the right hippocampus, allowing us to interrogate the effects of GABA concentration, ApoE ε4, and sex, as well as their interactions, upon episodic memory.

MATERIALS AND METHODS

Setting and Participants

Healthy older adults were recruited for the study from two participant cohorts maintained by the Cognitive Neuroscience Division at Columbia University, the Cognitive Reserve Study,

and the Reference Ability Neural Network Study. Participants were recruited to these studies by mail-market procedures targeting individuals within 10 miles of the Columbia University Medical Center. Participants were required to be right-handed, native English speakers with at least a fourth-grade reading level. As part of these cohort studies, participants were genotyped for ApoE ε4 and screened for neurological diagnoses and medication use, as detailed elsewhere (Stern et al., 2014), and for dementia using the Dementia Rating Scale (Mattis, 1988). Any participant who scored below 135 was excluded. From this cohort pool, we recruited participants based on their ε4 carrier status (ε4+ and ε4-) and sex (male and female), to obtain a final sample balanced across both variables. Participants performed a Directed Forgetting memory task (MacLeod, 2012) and then underwent MRI scans at the New York State Psychiatric Institute MRI Research Program. Data from 11 women and nine men aged 50–71 years (y) were included. The median age of the sample was 61 years (y; range: 54.5 y to 67.8 y). Ten women self-reported to be postmenopausal. Data on the 11th woman were not available. Twelve participants were ApoE $\varepsilon4^+$ ($\varepsilon2/\varepsilon4 = 1$; $\varepsilon3/\varepsilon4 = 10$; $\varepsilon4/\varepsilon4 = 1$), and eight were ApoE $\varepsilon4^-$ ($\varepsilon3/\varepsilon3 = 7$; $\varepsilon2/\varepsilon3 = 1$). The median education level was 6 (range: 5–7), which corresponds to a bachelor's degree or equivalent, according to the International Standard Classification of Education (ISCE) classification. Written informed consent, as approved by the Institutional Review Board of the Columbia University Medical Center, was obtained prior to study participation.

Directed Forgetting Task

An item-method directed forgetting task was used to assess episodic memory (MacLeod, 2012). In the study phase of the task, participants were presented with unrelated, unambiguous concrete nouns, ranging in length from 3 to 8 letters, one at a time, for 2,500 ms each. Each word was followed by a 500 ms delay, and then a memory cue, presented for 1,500 ms, which indicated whether the preceding word was to be remembered (TBR) or to be forgotten (TBF) for a later memory test. Participants were instructed to remember the TBR words for a later memory test and told that forgetting the TBF words would help them to remember all of the TBR words. The TBR cue consisted of four green R's (for Remember), and the TBF cue consisted of four red F's (for Forget). To minimize primacy and recency effects, six additional buffer trials were presented as the first and last three trials of the experiment and were not scored. Trials were separated by 1,000 ms intervals. Following the study phase, and after a 5-min delay period, memory was tested for all 36 studied words (18 TBR and 18 TBF), as well as 36 words that had not been presented during the study phase. Old and new words were presented in a blocked-randomized design to control for the time between study and test. During this recognition phase, each test word was presented on the screen for 20 s, or until the participant responded. Participants were instructed to press the Y key on the keyboard (for Yes) if they recognized the test word as one of the words that had been presented to them, and to press the N key (for No) if it had not. The current analysis examined only accuracy for TBR items.

Neuroimaging Protocol

Magnetic Resonance Imaging

MRI data were acquired using a 32-channel head coil on a 3 Tesla MR scanner (Discovery, GE Medical Systems). Two anatomical images were acquired for the MRS volume of interest (VOI) placement; the first one was a three-dimensional (3D) brain volume (BRAVO) T_1-weighted sequence (echo time (T_E) = 2,700 ms, repetition time (T_R) = 7,156 ms, inversion time (T_I) = 450 ms, 176 slices, 256 × 256 matrix size, slice thickness = 1 mm, flip angle (FA) = 12°). The second one was a two-dimensional (2D) axially-acquired structural T_1-weighted fluid-attenuated inversion recovery (FLAIR) volume (T_E = 26 ms, T_R = 2,300 ms, T_I = 756 ms, 25 slices, 512 × 512 matrix size, slice thickness = 5 mm, voxel size = 0.4 mm × 0.4 mm × 5 mm, FA = 111°). The VOI with a size of 4 × 2 × 2 cm^3 was centered in the right hippocampus (**Figure 1A**). ^{1}H MRS data were acquired using a MEGA-PRESS sequence (Mullins et al., 2014; T_E = 68 ms, T_R = 1,500 ms, slice thickness = 20 mm, FA = 90°, field of view = 512 × 512) in one acquisition that lasted 768 s. A vendor-provided, semi-automatic shimming procedure was implemented prior to spectroscopic acquisition and was supplemented by interactive manual shimming, resulting in full-width at half-maximum (FWHM) water linewidths ranging from 9 to 22 Hz (mean line width = 13 ± 3.49 Hz).

Anatomical Segmentation

The 3D T1-weighted images were analyzed using FreeSurfer (v5.1.0) an automated segmentation and cortical parcellation software package (Fischl et al., 2002). Boundary lines separating gray matter, white matter, and pial surfaces were visually inspected. When necessary, to ensure accuracy, manual editing of voxel label maps was conducted according to the FreeSurfer manual editing guidelines by a technician blinded to participant demographics. In the second round of quality control, the borders of the parcellated cortical and sub-cortical regions were then overlayed onto the input structural images by a second technician. The Desikan-Killiany Atlas, which includes 34 gyral-based cortical regions, was used for cortical parcellation and for regional identification of clusters (Desikan et al., 2006).

Magnetic Resonance Spectroscopy Quantification

The concentration of resting-state GABA in the right hippocampus was quantified using the Jet algorithm[1] in MATLAB (Mathworks, MA, USA). This algorithm was used to preprocess the spectroscopy data by aligning frequency and phase for ON and OFF spectra, as described previously (Mikkelsen et al., 2018). Then, we edited the GABA peak at 3 parts per million (ppm) and co-edited the glutamine + glutamate (Glx) peak at 3.77 ppm after subtraction of the ON and OFF spectra, as shown in **Figure 1B**. Spectral fitting was performed with a simulated basis-set for metabolites including GABA, Glx, choline (Cho), creatine (Cr), and n-acetylaspartate (NAA). Metabolites were quantified based on the area-under-the-curve (AUC) for each fitted metabolite basis, as illustrated in **Figure 1C**. The signal detected with these parameters will

[1] http://triton.iqfr.csic.es/guide/man/nmrsim/contents.html

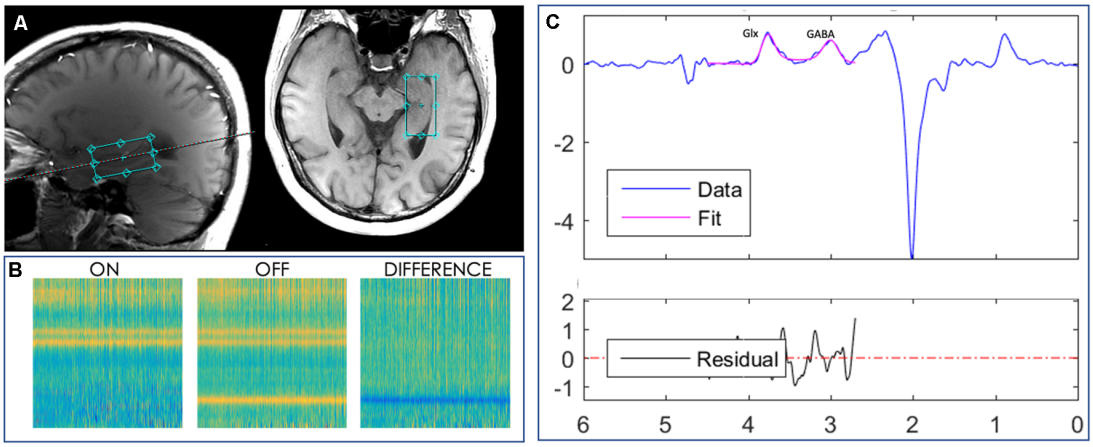

FIGURE 1 | Localized images and representative MR spectra from a 2 × 2 × 4 cm^3 voxel manually placed in the right hippocampus of a subject. **(A)** Axial and sagittal planes showing the hippocampal voxel, outlined in aqua, from one study participant's MPRAGE T1-weighted image. **(B)** Loadings for the GABA edited difference spectrum. **(C)** Representative model fitting showing Glx (a combination of glutamate and glutamine) and GABA spectrum peaks, representing the GABA signal, from the same subject. The blue line represents the actual edited spectrum, whereas the overlaid pink line is the model of best fit. The residual is shown in the black curve below the modeling plot.

contain contributions from both the macromolecules (MM) and homocarnosine in addition to GABA (Rothman et al., 1997), therefore we refer to this signal as GABA+ henceforth. GABA+ concentration was then quantified as a ratio to the reference Cr metabolite concentration.

Statistics

The proportion of correct TBR responses (items that participants were told to remember, which they correctly said were presented), which provides a direct measure of episodic memory, served as the outcome variable. Predictors of interest included MRS-measured GABA+ concentration from the right hippocampus, age, sex, education level (according to the International ISCE classification), and ApoE ε4 genotype, coded as a binary variable (either positive or negative). There were no missing data pertaining to any of these variables.

Univariate analyses were used to assess the association between GABA+, on the one hand, and age, sex, and ApoE ε4 variables, on the other hand, using Spearman's rank correlation coefficients or Student's *t*-tests for continuous or categorical variables, respectively. Multiple linear regression models were used to evaluate the relationship between episodic memory and the predictors of interest. Memory served as the dependent variable, with GABA+, age, sex, education, and ApoE ε4 as independent variables. Independent variables were selected according to *a priori* hypotheses based on the previous literature or on univariate analysis results. To facilitate the interpretation of regression coefficients, GABA concentrations were standardized into *z*-scores. As our hypothesis involved ApoE ε4 and sex effects on GABA+ concentrations, interaction terms for ApoE ε4 × GABA+ and sex × GABA were included. Additionally, to avoid overfitting due to the large number of variables and small sample size, variables were selected *via* backward stepwise elimination according to the Akaike information criterion (AIC). Briefly, the AIC is a metric comparing the goodness of the fit of two models by selecting the one with the highest likelihood after penalizing for the number of parameters in the models. A lower AIC thus corresponds to better goodness of fit. The statistical assumptions (independence and normality of residuals, presence of influential cases, and absence of multicollinearity) of the model obtained through variable selection were verified to confirm that they had been met.

Overall accuracy on the task was high. To investigate as to whether the effect of GABA+ (the predictor variable) on memory was conditioned by the skewed distribution of TBR responses (the dependent variable), we implemented separate quantile regression models in men and women. In quantile regression, instead of fitting a model at the mean of the dependent variable, the effect of the independent variable is tested across the distribution of the dependent variable. Hence, coefficients are calculated at one or more quantiles of the distribution (expressed as τ), which are set *a priori*. In our case, we considered deciles from 10 to 90. This analysis allowed us to observe whether the correlation between GABA+ and memory remained constant across the distribution of TBR responses in men and women, giving robustness to our results. Pairwise comparisons of those models fitted at different τ were compared using Wald tests to assess whether the effect of GABA+ varied across the distribution of TBR responses.

All analyses were conducted using R software (R version 3.6.1, 2019-07-05; © 2019 The R Foundation for Statistical Computing). For all analyses, α was set at 0.05.

RESULTS

Characteristics of the Sample

There was no significant sex-related difference in the sample's age ($U_{(9,11)}$ = 56.5, p = 0.621), educational attainment ($U_{(9,11)}$ = 62.5, p = 0.318), or in its prevalence of the ApoE ε4 allele

TABLE 1 | Linear regression models parameters illustrating the relationship between GABA+ concentration and episodic memory performance.

Variable	β(95% CI)	*t*-value	*p*-value
Baseline Model			
Age [years]	0.00 (−0.01; 0.01)	0.69	0.501
Education, ISCED	−0.01 (−0.07; 0.04)	−0.61	0.551
GABA+ level, SD increase	0.11 (0.04; 0.18)	3.21	0.007
ApoE ε4, Positive	−0.02 (−0.13; 0.10)	−0.32	0.757
Sex, Male	0.12 (0.02; 0.22)	2.72	0.019
GABA+ × *ApoE ε4*	0.00 (−0.11; 0.11)	0.01	0.990
GABA+ × Sex	−0.12 (−0.24; 0.00)	−2.17	0.051
Final model			
GABA+, SD increase	0.11 (0.05; 0.17)	3.92	0.001
Sex, Male	0.12 (0.04; 0.21)	3.10	0.007
GABA+ × Sex	−0.11 (−0.20; −0.03)	−2.74	0.015

Linear regression models were constructed using the proportion of correctly recognized TBR words as the dependent variable, while adjusting for GABA+, age, sex, education, the presence of the ApoE ε4 allele, and for both the GABA+ level × ApoE ε4 and GABA+ level × sex interactions. The final model displayed was obtained after selecting variables via backward stepwise elimination. Female sex is the reference category; thus, the main effect of GABA+ level showed in the final model represents the association between GABA+ concentration and episodic memory performance in females. The adjusted R^2 values were 0.34 and 0.48 for the baseline and final models, respectively. Listed are β coefficients and their 95% confidence intervals, t-statistics, and p-values.

(χ^2_{20} = 0.01, p = 0.927). On the other hand, ApoE ε4 carriers were older [median (interquartile range) = 65.5 y (55.8, 69.0) y] than non-carriers [57.5 y (53.8, 60.3) y], but did not differ in educational attainment ($U_{(8,12)}$ = 64.5, p = 0.194).

Relation Between GABA Concentration and Episodic Memory

The average proportion of correctly recognized TBR words was high, 0.9 ± 0.1. This score did not correlate with age (rs_{18} = 0.32, p = 0.169) or education level (rs_{18} = 0.11, p = 0.632). Further, GABA+ was not associated with either age (rs_{18} = 0.05, p = 0.828), or *ApoE ε4* polymorphism ($t_{(14,5)}$ = 0.26, p = 0.802). However, overall, women had higher GABA+ concentration than did men ($t_{(16,9)}$ = −2.67, p = 0.016).

Multiple linear regression models were used to analyze the relationship between episodic memory and the predictors of interest (age, education, sex, and ApoE ε4 genotype), the results of which are shown in **Table 1**. We did not observe a main effect of ApoE ε4 [β (95% confidence interval) = −0.02 (−0.13, 0.10), p = 0.757], or a ApoE ε4 × GABA+ interaction [β = 0.00 (−0.11, 0.11), p = 0.990; see **Figure 2A**]. We did observe a main effect of sex, such that, on average, women had worse memory performance [0.81 (0.75, 0.86)] than men [0.93 (0.87, 0.99)]. However, this main effect was moderated by a significant interaction between GABA+ concentration and sex, such that lower GABA+ concentrations were associated with worse memory performance in women (**Table 1**), but not in men: β = 0.00 (−0.07, 0.06), p = 0.935. As shown in **Figure 2B**, in women, for each standard deviation decrease in GABA+ concentration, the proportion of correct responses on the memory task decreased by 0.11.

The results of the quantile regression models revealed that GABA+ was not associated with memory performance in men in any portion of the TBR accuracy distribution. By contrast, in women, GABA+ was positively correlated with memory at all deciles except 20 and 50 (τ^{10} = 0.10, t = 2.39, p = 0.040; τ^{20} = 0.20, t = 2.06, p = 0.070; τ^{30} = 0.30, t = 2.32, p = 0.045; τ^{40} = 0.40, t = 2.27, p = 0.049; τ^{50} = 0.50, t = 0.94, p = 0.370; τ^{60} = 0.60, t = 2.43, p = 0.038; τ^{70} = 0.70, t = 2.46, p = 0.036; τ^{80} = 0.80, t = 2.70, p = 0.025; τ^{90} = 0.90, t = 3.47, p = 0.007). As shown in **Figure 3**, GABA+ related regression coefficients in women ranged from 0.08 to 0.15. When significant models were compared by pairs, no significant differences in any comparison were found, suggesting relative stability of the GABA+ concentration effect in women and confirming that these results were not conditioned by a potential ceiling effect observed in TBR accuracy.

DISCUSSION

To our knowledge, this is the first study to test the relation between hippocampal GABA+ and episodic memory in older adults. Contrary to our expectations, *ApoE ε4* status did not moderate the effect of GABA+ concentration on memory. However, sex did: women with lower GABA+ concentrations showed worse episodic memory compared to women with higher GABA concentrations and to men, regardless of the latter's GABA+ concentration. What factors might mediate this effect? The female hippocampus is very responsive to E2. In women, hippocampal volume increases during the high-estradiol late-follicular phase of the menstrual cycle (Protopopescu et al., 2008). In animal models, the dendritic spine density of pyramidal hippocampal neurons increases during the high-estradiol proestrous phase (Woolley et al., 1990), resulting from decreased GABAergic inhibition in the hippocampus (Murphy et al., 1998). The changes in the hippocampal GABA system from pre- to post-menopause—dynamic fluctuations across the menstrual cycle (Protopopescu et al., 2008) to static low levels—may result in static hyperexcitability of hippocampal neurons and to increased risk of pathophysiology.

Other potential overlapping mediating factors are depression and cognitive impairment. While the results are not always consistent, both case-control and cohort studies have reported that a history of depression is a risk factor for cognitive impairment (Kessler, 2003; Ownby et al., 2006), and increases AD risk (Ownby et al., 2006). Women have a higher prevalence of depression (Pehrson and Sanchez, 2015; Flores-Ramos et al., 2017), with symptom risk peaking during major reproductive events (e.g., perimenopausal transition) when fluctuations in sex steroid hormone levels are high (Soares and Zitek, 2008). These transitional phases are associated with dysregulation of the hypothalamic-pituitary-gonadal axis function (Schweizer-Schubert et al., 2021), which is regulated by GABAergic transmission (Flores-Ramos et al., 2017). Interestingly, individuals with major depression have reduced numbers of somatostatin-expressing neurons (a population of GABAergic interneurons playing a key role in memory), and this reduction is exacerbated in women (Fee et al., 2017). Unfortunately, as this was a pilot study, we did not acquire sex hormone levels or screen for depressive symptoms, and

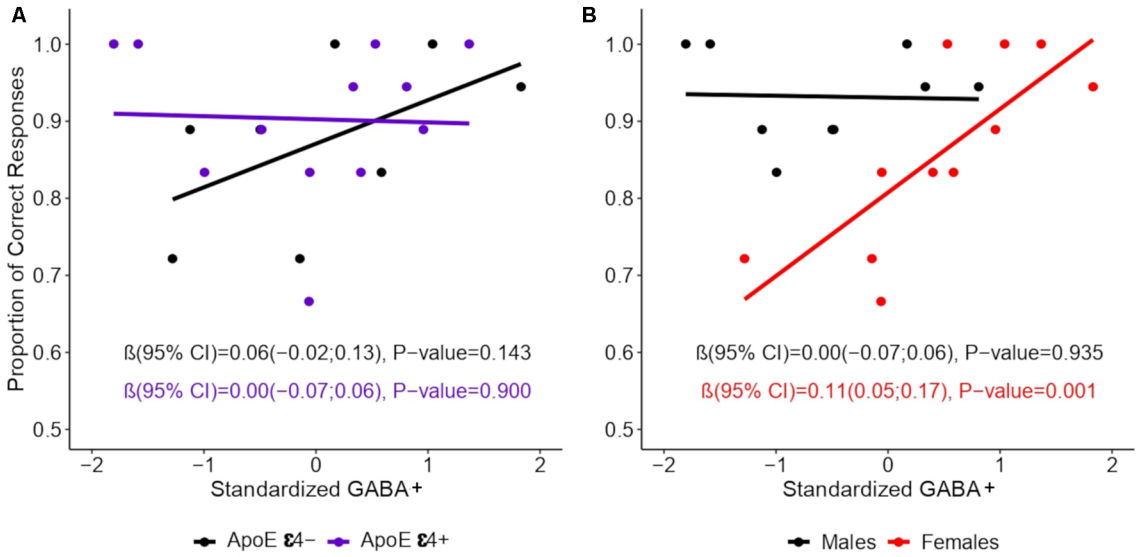

FIGURE 2 | Association between episodic memory and GABA+ concentration by sex. GABA+ concentration was standardized into *z*-scores. Lines show the relation between GABA+ levels and episodic memory performance by **(A)** ApoE ε4 and **(B)** Sex. Linear regression models were constructed using the proportion of the TBR words (those words that participants were instructed to remember) as the dependent variable, and while accounting for the interactions of ApoE ε4 × GABA+ level and of sex × GABA+ level in separate models. Values represent regression coefficients β, their 95% confidence intervals (CIs), and the *p*-values of the standardized GABA main effects.

we were therefore unable directly to test hypotheses on the role of these factors in the relationships between GABA concentrations, episodic memory, and sex that we quantified in this study.

Recently, Schmitz et al. (2017) reported an association between MRS-measured hippocampal GABA+ and the mnemonic control over unwanted thoughts (Schmitz et al., 2017). However, this study included only younger adults (Mean age = 24.7 year). A study by Porges et al. (2017) did investigate the relation between GABA+ and cognitive decline in older individuals (Porges et al., 2017). However, the neuropsychologic measure used in their study (the Montreal Cognitive Assessment, MoCA) was broad and cognitively non-specific, and GABA concentration was assayed in the prefrontal cortex, not in the hippocampus.

Correlations between GABA+ in other brain regions and other cognitive functions have been reported. Riese et al. (2015), for example, reported better performance in a word list task for older individuals with greater GABA+ concentration in the posterior cingulate cortex (Riese et al., 2015). Likewise, several studies reported that GABA+ concentrations in the dorsal anterior cingulate and in the occipital cortex are associated with measures of executive and visuo-perceptual functions, respectively (Marenco et al., 2018; Simmonite et al., 2019). Furthermore, Piras et al. (2019, 2020) found cerebral GABA levels to be associated with performance in phonemic fluency and in the Stroop Color-Word Test, a measure of response inhibition (Piras et al., 2019, 2020). Thus, it is possible that the relation between GABA+ levels and cognition is not specific to memory. However, the data presented here, while drawn from a small sample, support findings from animal models, which have provided strong evidence for the specificity of the age-related, GABA-mediated hippocampal-episodic memory association.

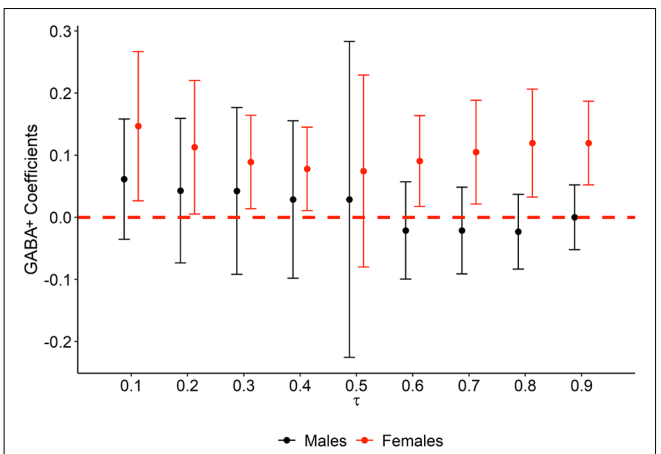

FIGURE 3 | Quantile regression plot of GABA+ statistical effect on memory performance at each decile of TBR accuracy, by sex. The variation of GABA+ regression β coefficients (*y* axis) obtained from quantile regression models is represented at each decile (*x* axis). Colors represent males (black) and females (red). The red dashed line is set at 0, and thus error bars not crossing the red dashed line represent statistically significant associations.

In summary, the data from this pilot study revealed an association between GABA+ levels and episodic memory in women but not men, such that lower levels of GABA+ were associated with worse behavioral performance. Further multimodal neuroimaging studies considering structural, MRS, and fMRI data are needed to determine whether these

GABAergic changes are also associated with hippocampal hyperactivity (Jiménez-Balado and Eich, 2021). Moreover, longitudinal studies with larger samples that consider depression and hormonal balances will help to replicate the findings presented here and test whether GABA-related dysregulation predicts sex-specific incident MCI or dementia risk. Further studies focusing on these questions would be of great interest in confirming the contribution of GABA to age–related cognitive impairment, and clarifying the role of sex in these changes.

LIMITATIONS

This pilot study is preliminary and, as such, has several notable limitations that necessitate the results to be interpreted with caution. First, the sample size was small, which limited statistical power, especially for the critical analyses of group comparisons. Second, we collected neither sex hormone levels (estradiol, progesterone, and testosterone) nor current levels or history of depression. These are important avenues of future inquiry, as they may provide insight into the mechanism driving the sex-specific effects found, and future studies should directly test the role of these factors in the relationship between GABA+ concentration and episodic memory in women. Third, while it is not possible to determine from the ^{1}H MRS estimate where the GABA signal originates, as the measurement reflects a combination of synaptic, intracellular, and extrasynaptic GABA from all types of GABAergic interneurons in our right hippocampal region of interest (Maddock and Buonocore, 2012), the findings reported by Li et al. (2021) suggest that hippocampal CA1 $GABA_A$ postsynaptic pyramidal neuron receptors might be a likely source. Future studies using PET imaging could provide clarity on the precise coupling of the source of the GABA signal and its association with episodic memory deficits. Moreover, fMRI measurements will additionally help to ensure that the effect of GABA reduction or dysfunctional coupling on cognitive impairment is mediated by hippocampal hyperactivity; confirming the main hypothesis of this manuscript. Finally, our sample may not be representative of typical older adults, according to both their self-reported levels of education, and to their objective (high) performance on the memory task. On the other hand, hippocampal volume in our sample ($\mu \pm \sigma = 3.84 \pm 0.5$ cm^3) was on par with recently published normative data acquired from a large sample ($N \simeq 20{,}000$) of clinically healthy older adults (mean age: 62.95 ± 7.48 y; hippocampal volume $\simeq 3.86 \pm 0.4$ cm^3), and these results were similar across sex. Specifically, the women in our sample had average hippocampal volumes of 3.64 ± 0.47 cm^3, vs. 3.77 ± 0.37 cm^3 in the normative sample. Men in our sample had average hippocampal volumes of 4.12 ± 0.35 cm^3, vs. 3.97 ± 0.43 cm^3 in the normative sample, which allays some concern over how representative the participants in our sample are (Nobis et al., 2019). Future studies that include larger samples, use multimodal imaging methods, and also measure depression and hormone levels would help to both remedy these limitations and to facilitate generalization of the results to the general population.

DATA AVAILABILITY STATEMENT

Data supporting the conclusions of this manuscript will be shared under petition of qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Institutional Review Board of the Columbia University Medical Center. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JJ-B performed statistical analysis, created visualizations, and wrote the manuscript. AY wrote the manuscript. LK acquired behavioral and MRS data. KI and CL processed and analyzed the MRS imaging data. KB set up the MRS sequence and helped acquire the MRS data. JG provided technical support for the MRS analysis software. AI and AB edited the manuscript. TE conceived the study, acquired behavioral and MRS data, analyzed and interpreted data, wrote the manuscript, and acquired funding. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported in part by National Institute of Health, National Institute on Aging Grants R00AG055684, R01AG026158, and R01AG038465. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NIH/NIA.

REFERENCES

Ambrad Giovannetti, E., and Fuhrmann, M. (2019). Unsupervised excitation: GABAergic dysfunctions in Alzheimer's disease. *Brain Res.* 15, 216–226. doi: 10.1016/j.brainres.2018.11.042

Bacigalupo, I., Mayer, F., Lacorte, E., Di Pucchio, A., Marzolini, F., Canevelli, M., et al. (2018). A systematic review and meta-analysis on the prevalence of dementia in europe: estimates from the highest-quality studies adopting the DSM IV diagnostic criteria. *J. Alzheimers Dis.* 66, 1471–1481. doi: 10.3233/JAD-180416

Bakker, A., Albert, M. S., Krauss, G., Speck, C. L., and Gallagher, M. (2015). Response of the medial temporal lobe network in amnestic mild cognitive impairment to therapeutic intervention assessed by fMRI and memory task performance. *Neuroimage Clin.* 7, 688–698. doi: 10.1016/j.nicl.2015.02.009

Bakker, A., Krauss, G. L., Albert, M. S., Speck, C. L., Jones, L. R., Stark, C. E., et al. (2012). Reduction of hippocampal hyperactivity improves cognition

in amnestic mild cognitive impairment. *Neuron* 74, 467–474. doi: 10.1016/j.neuron.2012.03.023

Bondi, M. W., Houston, W. S., Eyler, L. T., and Brown, G. G. (2005). fMRI evidence of compensatory mechanisms in older adults at genetic risk for Alzheimer disease. *Neurology* 64, 501–508. doi: 10.1212/01.WNL.0000150885.00929.7E

Cacabelos, R. (2003). The application of functional genomics to Alzheimer's disease. *Pharmacogenomics* 4, 597–621. doi: 10.1517/phgs.4.5.597.23795

Carroll, J. C., Rosario, E. R., Kreimer, S., Villamagna, A., Gentzschein, E., Stanczyk, F. Z., et al. (2010). Sex differences in β-amyloid accumulation in 3xTg-AD mice: role of neonatal sex steroid hormone exposure. *Brain Res.* 17, 233–245. doi: 10.1016/j.brainres.2010.10.009

Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., et al. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261, 921–923. doi: 10.1126/science.8346443

Cummings, J., Lee, G., Ritter, A., Sabbagh, M., and Zhong, K. (2020). Alzheimer's disease drug development pipeline: 2020. *Alzheimers Dement. Transl. Res. Clin. Interv.* 6:e12050. doi: 10.1002/trc2.12050

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., et al. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage* 31, 968–980. doi: 10.1016/j.neuroimage.2006.01.021

Dickerson, B. C., Salat, D. H., Greve, D. N., Chua, E. F., Rand-Giovannetti, E., Rentz, D. M., et al. (2005). Increased hippocampal activation in mild cognitive impairment compared to normal aging and AD. *Neurology* 65, 404–411. doi: 10.1212/01.wnl.0000171450.97464.49

Dubal, D. B. (2020). Sex difference in Alzheimer's disease: an updated, balanced and emerging perspective on differing vulnerabilities. *Handb. Clin. Neurol.* 175, 261–273. doi: 10.1016/B978-0-444-64123-6.00018-7

Fee, C., Banasr, M., and Sibille, E. (2017). Somatostatin-positive gamma-aminobutyric acid interneuron deficits in depression: cortical microcircuit and therapeutic perspectives. *Biol. Psychiatry* 82, 549–559. doi: 10.1016/j.biopsych.2017.05.024

Filippini, N., MacIntosh, B. J., Hough, M. G., Goodwin, G. M., Frisoni, G. B., Smith, S. M., et al. (2009). Distinct patterns of brain activity in young carriers of the APOE-ε4 allele. *Proc. Natl. Acad. Sci. U S A* 106, 7209–7214. doi: 10.1073/pnas.0811879106

Findley, C. A., Bartke, A., Hascup, K. N., and Hascup, E. R. (2019). Amyloid beta-related alterations to glutamate signaling dynamics during Alzheimer's disease progression. *ASN Neuro* 11:1759091419855541. doi: 10.1177/1759091419855541

Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., et al. (2002). Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 33, 341–355. doi: 10.1016/s0896-6273(02)00569-x

Flores-Ramos, M., Salinas, M., Carvajal-Lohr, A., and Rodríguez-Bores, L. (2017). [The role of gamma-aminobutyric acid in female depression]. *Gac. Med. Mex.* 153, 486–495. doi: 10.24875/GMM.17002544

Gao, F., Edden, R. A., Li, M., Puts, N. A., Wang, G., Liu, C., et al. (2013). Edited magnetic resonance spectroscopy detects an age-related decline in brain GABA levels. *NeuroImage* 78, 75–82. doi: 10.1016/j.neuroimage.2013.04.012

Hämäläinen, A., Pihlajamäki, M., Tanila, H., Hänninen, T., Niskanen, E., Tervo, S., et al. (2007). Increased fMRI responses during encoding in mild cognitive impairment. *Neurobiol. Aging* 28, 1889–1903. doi: 10.1055/a-1272-9653

Hampel, H., Mesulam, M.-M., Cuello, A. C., Farlow, M. R., Giacobini, E., Grossberg, G. T., et al. (2018). The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain* 141, 1917–1933. doi: 10.1093/brain/awy132

Hardy, J. A., and Higgins, G. A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256, 184–185. doi: 10.1126/science.1566067

Herbison, A. E., and Fénelon, V. S. (1995). Estrogen regulation of GABAA receptor subunit mRNA expression in preoptic area and bed nucleus of the stria terminalis of female rat brain. *J. Neurosci.* 15, 2328–2337. doi: 10.1523/JNEUROSCI.15-03-02328.1995

Herbison, A. E., Heavens, R. P., and Dyer, R. G. (1990). Oestrogen modulation of excitatory A1 noradrenergic input to rat medial preoptic gamma aminobutyric acid neurones demonstrated by microdialysis. *Neuroendocrinology* 52, 161–168. doi: 10.1159/000125568

Jain, S., Yoon, S. Y., Leung, L., Knoferle, J., and Huang, Y. (2013). Cellular source-specific effects of apolipoprotein (Apo) E4 on dendrite arborization and dendritic spine development. *PLoS One* 8:e59478. doi: 10.1371/journal.pone.0059478

Jiménez-Balado, J., and Eich, T. S. (2021). GABAergic dysfunction, neural network hyperactivity and memory impairments in human aging and Alzheimer's disease. *Semin. Cell Dev. Biol.* doi: 10.1016/j.semcdb.2021.01.005. [Epub ahead of print].

Kessler, R. C. (2003). Epidemiology of women and depression. *J. Affect. Disord.* 74, 5–13. doi: 10.1016/s0165-0327(02)00426-3

Knoferle, J., Yoon, S. Y., Walker, D., Leung, L., Gillespie, A. K., Tong, L. M., et al. (2014). Apolipoprotein E4 produced in GABAergic interneurons causes learning and memory deficits in mice. *J. Neurosci.* 34, 14069–14078. doi: 10.1523/JNEUROSCI.2281-14.2014

Li, Y., Zhu, K., Li, N., Wang, X., Xiao, X., Li, L., et al. (2021). Reversible GABAergic dysfunction involved in hippocampal hyperactivity predicts early-stage Alzheimer disease in a mouse model. *Alzheimers Res. Ther.* 13:114. doi: 10.1186/s13195-021-00859-8

Lucas, E. K., and Clem, R. L. (2018). GABAergic interneurons: the orchestra or the conductor in fear learning and memory? *Brain Res. Bull.* 141, 13–19. doi: 10.1016/j.brainresbull.2017.11.016

Luo, J., Beam, C. R., Karlsson, I. K., Pike, C. J., Reynolds, C. A., and Gatz, M. (2020). Dementia risk in women higher in same-sex than opposite-sex twins. *Alzheimers Dement. Transl. Res. Clin. Interv.* 12:e12049. doi: 10.1002/dad2.12049

MacLeod, C. M. (2012). "Directed forgetting," in *Encyclopedia of the Sciences of Learning*, ed N. M. Seel (Boston, MA: Springer US), 993–995.

Maddock, R. J., and Buonocore, M. H. (2012). MR spectroscopic studies of the brain in psychiatric disorders. *Curr. Top. Behav. Neurosci.* 11, 199–251. doi: 10.1007/7854_2011_197

Marenco, S., Meyer, C., van der Veen, J. W., Zhang, Y., Kelly, R., Shen, J., et al. (2018). Role of gamma-amino-butyric acid in the dorsal anterior cingulate in age-associated changes in cognition. *Neuropsychopharmacology* 43, 2285–2291. doi: 10.1038/s41386-018-0134-5

Mattis, S. (1988). *Dementia Rating Scale Professional Manual.* Odessa, FL: Psychological Assessment Resources.

Miech, R. A., Breitner, J. C., Zandi, P. P., Khachaturian, A. S., Anthony, J. C., and Mayer, L. (2002). Incidence of AD may decline in the early 90s for men, later for women: the Cache County study. *Neurology* 58, 209–218. doi: 10.1212/wnl.58.2.209

Mielke, M. M. (2018). Sex and gender differences in Alzheimer's disease dementia. *Psychiatr. Times* 35, 14–17.

Mikkelsen, M., Loo, R. S. Puts, N. A. J., Edden, R. A. E., and Harris, A. D. (2018). Designing GABA-edited magnetic resonance spectroscopy studies: considerations of scan duration, signal-to-noise ratio and sample size. *J. Neurosci. Methods* 303, 86–94. doi: 10.1016/j.jneumeth.2018.02.012

Mullins, P. G., McGonigle, D. J., O'Gorman, R. L., Puts, N. A., Vidyasagar, R., and Evans, C. J. (2014). Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. *NeuroImage* 86, 43–52. doi: 10.1016/j.neuroimage.2012.12.004

Murphy, D. D., Cole, N. B., Greenberger, V., and Segal, M. (1998). Estradiol increases dendritic spine density by reducing GABA neurotransmission in hippocampal neurons. *J. Neurosci.* 18, 2550–2559. doi: 10.1523/JNEUROSCI.18-07-02550.1998

Najm, R., Jones, E. A., and Huang, Y. (2019). Apolipoprotein E4, inhibitory network dysfunction, and Alzheimer's disease. *Mol. Neurodegener.* 14:24. doi: 10.1186/s13024-019-0324-6

Nobis, L., Manohar, S. G., Smith, S. M., Alfaro-Almagro, F., Jenkinson, M., Mackay, C. E., et al. (2019). Hippocampal volume across age: nomograms derived from over 19,700 people in UK Biobank. *Neuroimage Clin.* 23:101904. doi: 10.1016/j.nicl.2019.101904

Nyberg, L., McIntosh, A. R., Houle, S., Nilsson, L. G., and Tulving, E. (1996). Activation of medial temporal structures during episodic memory retrieval. *Nature* 380, 715–717. doi: 10.1038/380715a0

Ownby, R. L., Crocco, E., Acevedo, A., John, V., and Loewenstein, D. (2006). Depression and risk for Alzheimer disease: systematic review, meta-

analysis, and metaregression analysis. *Arch. Gen. Psychiatry* 63, 530–538. doi: 10.1001/archpsyc.63.5.530

Pandya, M., Palpagama, T. H., Turner, C., Waldvogel, H. J., Faull, R. L., and Kwakowsky, A. (2019). Sex- and age-related changes in GABA signaling components in the human cortex. *Biol. Sex Differ.* 10:5. doi: 10.1186/s13293-018-0214-6

Paulsen, O., and Moser, E. I. (1998). A model of hippocampal memory encoding and retrieval: GABAergic control of synaptic plasticity. *Trends Neurosci.* 21, 273–278. doi: 10.1016/s0166-2236(97)01205-8

Pehrson, A. L., and Sanchez, C. (2015). Altered γ-aminobutyric acid neurotransmission in major depressive disorder: a critical review of the supporting evidence and the influence of serotonergic antidepressants. *Drug Des. Devel. Ther.* 9, 603–624. doi: 10.2147/DDDT.S62912

Pike, C. J. (2017). Sex and the development of Alzheimer's disease. *J. Neurosci. Res.* 95, 671–680. doi: 10.1002/jnr.23827

Pike, C. J., and Cotman, C. W. (1993). Cultured GABA-immunoreactive neurons are resistant to toxicity induced by beta-amyloid. *Neuroscience* 56, 269–274. doi: 10.1016/0306-4522(93)90331-9

Piras, F., Piras, F., Banaj, N., Ciullo, V., Vecchio, D., Edden, R. A. E., et al. (2019). Cerebellar GABAergic correlates of cognition-mediated verbal fluency in physiology and schizophrenia. *Acta Psychiatr. Scand.* 139, 582–594. doi: 10.1111/acps.13027

Piras, F., Vecchio, D., Assogna, F., Pellicano, C., Ciullo, V., Banaj, N., et al. (2020). Cerebellar GABA levels and cognitive interference in Parkinson's disease and healthy comparators. *J. Pers. Med.* 11:16. doi: 10.3390/jpm11010016

Porges, E. C., Woods, A. J., Edden, R. A., Puts, N. A., Harris, A. D., Chen, H., et al. (2017). Frontal gamma-aminobutyric acid concentrations are associated with cognitive performance in older adults. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* 2, 38–44. doi: 10.1016/j.bpsc.2016.06.004

Protopopescu, X., Butler, T., Pan, H., Root, J., Altemus, M., Polanecsky, M., et al. (2008). Hippocampal structural changes across the menstrual cycle. *Hippocampus* 18, 985–988. doi: 10.1002/hipo.20468

Putcha, D., Brickhouse, M., O'Keefe, K., Sullivan, C., Rentz, D., Marshall, G., et al. (2011). Hippocampal hyperactivation associated with cortical thinning in Alzheimer's disease signature regions in non-demented elderly adults. *J. Neurosci.* 31, 17680–17688. doi: 10.1523/JNEUROSCI.4740-11.2011

Riese, F., Gietl, A., Zölch, N., Henning, A., O'Gorman, R., Kälin, A. M., et al. (2015). Posterior cingulate γ-aminobutyric acid and glutamate/glutamine are reduced in amnestic mild cognitive impairment and are unrelated to amyloid deposition and apolipoprotein E genotype. *Neurobiol. Aging* 36, 53–59. doi: 10.1016/j.neurobiolaging.2014.07.030

Rothman, D. L., Behar, K. L., Prichard, J. W., and Petroff, O. A. (1997). Homocarnosine and the measurement of neuronal pH in patients with epilepsy. *Magn. Reson. Med.* 38, 924–929. doi: 10.1002/mrm.1910380611

Schacter, D. L., Alpert, N. M., Savage, C. R., Rauch, S. L., and Albert, M. S. (1996). Conscious recollection and the human hippocampal formation: evidence from positron emission tomography. *Proc. Natl. Acad. Sci. U S A* 93, 321–325. doi: 10.1073/pnas.93.1.321

Schmitz, T. W., Correia, M. M., Ferreira, C. S., Prescot, A. P., and Anderson, M. C. (2017). Hippocampal GABA enables inhibitory control over unwanted thoughts. *Nat. Commun.* 8:1311. doi: 10.1038/s41467-017-00956-z

Schweizer-Schubert, S., Gordon, J. L., Eisenlohr-Moul, T. A., Meltzer-Brody, S., Schmalenberger, K. M., Slopien, R., et al. (2021). Steroid hormone sensitivity in reproductive mood disorders: on the role of the GABAA receptor complex and stress during hormonal transitions. *Front. Med.* 7:479646. doi: 10.3389/fmed.2020.479646

Simmonite, M., Carp, J., Foerster, B. R., Ossher, L., Petrou, M., Weissman, D. H., et al. (2019). Age-related declines in occipital gaba are associated with reduced fluid processing ability. *Acad Radiol.* 26, 1053–1061. doi: 10.1016/j.acra.2018.07.024

Soares, C. N., and Zitek, B. (2008). Reproductive hormone sensitivity and risk for depression across the female life cycle: a continuum of vulnerability? *Psychiatry Neurosci.* 33, 331–343.

Sperling, R. A., Dickerson, B. C., Pihlajamaki, M., Vannini, P., LaViolette, P. S., Vitolo, O. V., et al. (2010). Functional alterations in memory networks in early Alzheimer's disease. *Neuromolecular Med.* 12, 27–43. doi: 10.1007/s12017-009-8109-7

Stern, Y., Habeck, C., Steffener, J., Barulli, D., Gazes, Y., Razlighi, Q., et al. (2014). The reference ability neural network study: motivation, design, and initial feasibility analyses. *NeuroImage* 103, 139–151. doi: 10.1016/j.neuroimage.2014.09.029

Tierney, M. C., Szalai, J. P., Snow, W. G., Fisher, R. H., Nores, A., Nadon, G., et al. (1996). Prediction of probable Alzheimer's disease in memory-impaired patients: a prospective longitudinal study. *Neurology* 46, 661–665. doi: 10.1212/wnl.46.3.661

Wang, D., Wang, X., Luo, M.-T., Wang, H., and Li, Y.-H. (2019). Gamma-aminobutyric acid levels in the anterior cingulate cortex of perimenopausal women with depression: a magnetic resonance spectroscopy study. *Front. Neurosci.* 13:785. doi: 10.3389/fnins.2019.00785

Woolley, C. S., Gould, E., Frankfurt, M., and McEwen, B. S. (1990). Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *J. Neurosci.* 10, 4035–4039. doi: 10.1523/JNEUROSCI.10-12-04035.1990

Yassa, M. A., Stark, S. M., Bakker, A., Albert, M. S., Gallagher, M., and Stark, C. E. (2010). High-resolution structural and functional MRI of hippocampal CA3 and dentate gyrus in patients with amnestic mild cognitive impairment. *NeuroImage* 51, 1242–1252. doi: 10.1016/j.neuroimage.2010.03.040

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frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 21 January 2022
doi: 10.3389/fnbeh.2021.773961

No Evidence for a Role of Oral Contraceptive-Use in Emotion Recognition But Higher Negativity Bias in Early Follicular Women

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Accuracy in facial emotion recognition has shown to vary with ovarian hormones, both in naturally cycling women, as well as in women taking oral contraceptives. It remains uncertain however, if specific – endogenous and exogenous – hormonal levels selectively impact recognition of certain basic emotions (or neutral faces) and if this relationship coincides with certain affective states. Therefore, we investigated 86 women under different hormonal conditions and compared their performance in an emotion recognition task as well as self-reported measures of affective states. Based on self-reported cycle days and ovulation testing, the participants have been split into groups of naturally cycling women during their early follicular phase (fNC, n = 30), naturally cycling women during their peri-ovulatory phase (oNC, n = 26), and women taking oral contraceptives (OC, n = 30). Participants were matched for age and did not differ in education or neuropsychological abilities. Self-reported anxiety and depressive affective state scores were similar across groups, but current affective state turned out to be significantly more negative in fNC women. Independent of negative affective state, fNC women showed a significantly higher negativity bias in recognizing neutral faces, resulting in a lower recognition accuracy of neutral faces compared to oNC and OC women. In the OC group only, negative affective state was associated with lower recognition accuracy and longer response times for neutral faces. Furthermore, there was a significant, positive association between disgust recognition accuracy and negative affective state in the fNC group. Low progesterone levels during the early follicular phase were linked to higher negative affective state, whereas in the peri-ovulatory phase they were linked to elevated positive affective state. Overall, previous findings regarding impaired emotion recognition during OC-use were not confirmed. Synthetic hormones did not show a correlation with emotion recognition performance and affective state. Considering the important role of emotion recognition in social communication, the elevated negativity bias in neutral face recognition found for fNC women may adversely impact social interactions in this hormonal phase.

Keywords: sex hormones, facial emotion recognition, oral contraceptives, menstrual cycle, affective state

OPEN ACCESS

Edited by:
Alexandra Ycaza Herrera, University of Southern California, United States

Reviewed by:
Ursula Stockhorst, University of Osnabrück, Germany
Carolina Villada, University of Guanajuato, Mexico

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Specialty section:
This article was submitted to Behavioral Endocrinology, a section of the journal Frontiers in Behavioral Neuroscience

Received: *10 September 2021*
Accepted: *14 December 2021*
Published: *21 January 2022*

Citation:
Kimmig A-CS, Bischofberger JA, Birrenbach AD, Drotleff B, Lämmerhofer M, Sundström-Poromaa I and Derntl B (2022) No Evidence for a Role of Oral Contraceptive-Use in Emotion Recognition But Higher Negativity Bias in Early Follicular Women. Front. Behav. Neurosci. 15:773961. doi: 10.3389/fnbeh.2021.773961

INTRODUCTION

Women experience significant fluctuations of ovarian hormones over the menstrual cycle. Most notably, 17-β estradiol and progesterone levels change periodically (Becker et al., 2005). During the follicular phase at the beginning of the menstrual cycle estradiol and progesterone levels are low. Estradiol is rising until reaching its peak right before ovulation and abruptly decreasing with ovulation. During the luteal phase, progesterone is rising coinciding with a second yet smaller increase of estradiol, with both hormones declining during the late luteal phase reaching the initial low levels during menstruation. To prevent pregnancy and facilitate safe family planning, millions of women rely on hormonal contraceptives such as oral contraceptives (OCs) during their reproductive years (United Nations [UN], 2020). OCs typically contain ethinyl estradiol (synthetic estrogen) and progestin (synthetic progesterone) that effectively suppress endogenous estradiol and progesterone levels and thus ultimately prevent ovulation (Petitti, 2003). Evidence is accumulating that endogenous as well as synthetic ovarian hormones impact women's socio-affective processing, including facial emotion recognition (Derntl et al., 2008a; Hamstra et al., 2014, 2015, 2017; for reviews see: Montoya and Bos, 2017; Lewis et al., 2019; Pahnke et al., 2019; Gamsakhurdashvili et al., 2021a).

For human communication, the perception and correct interpretation of facial expressions plays a major role. In a fast and direct way, facial expressions project the emotional state of a person to be perceived during social interactions (Horstmann, 2003). Among other functions, facial expressions of emotions can serve as eminent approach- or avoidance signals (Marsh et al., 2005). In naturally cycling (NC) women, several studies revealed superior facial emotion recognition in follicular compared to luteal NC women (Derntl et al., 2008a,b, 2013; Guapo et al., 2009; Rubin et al., 2011; for reviews see: Osório et al., 2018; Gamsakhurdashvili et al., 2021a). However, there are also some studies not finding any menstrual cycle effects on female's emotion recognition (Rubinow et al., 2007; Zhang et al., 2013; Kamboj et al., 2015). These inconsistencies could potentially be explained by different levels of progesterone in the luteal NC women, as all studies that did not report a menstrual cycle effect measured women either during the early or late luteal phase in which progesterone levels are relatively lower than in the mid-luteal phase (Gamsakhurdashvili et al., 2021a). Within the follicular phase, first studies have not found a difference in emotion recognition skills between early follicular and late follicular (i.e., peri-ovulatory) NC women (Guapo et al., 2009; Zhang et al., 2013) except for fear recognition, for which women during the peri-ovulatory phase showed a better performance (Pearson and Lewis, 2005).

In some of these studies endogenous estradiol and progesterone levels were related to emotion recognition performance. Across cycle phases, estradiol was positively associated with facial recognition accuracy of fear (Pearson and Lewis, 2005) and sadness (Hamstra et al., 2017), whereas it was linked with lower performance in anger (Guapo et al., 2009) and disgust recognition (Kamboj et al., 2015). Contradictory findings were reported with respect to neutral face recognition, as it was positively linked to estradiol in one study (Hamstra et al., 2017), but negatively in another study (Shirazi et al., 2020). This incongruency could possibly be due to the inclusion of women in different cycle phases marked by different degrees of estradiol fluctuations as well as levels. In the early follicular phase, estradiol is comparatively low and stable, whereas in the peri-ovulatory phase levels are higher and rapidly fluctuating day by day. For progesterone, lower levels were linked to higher rates of misclassifying emotional faces as neutral (Derntl et al., 2008a; Kamboj et al., 2015). When including multiple cycle phases, progesterone was associated with an increased bias for negative emotions shown by higher recognition rates (Maner and Miller, 2014) as well as longer response times (Kamboj et al., 2015). However, progesterone levels have also been negatively linked with emotion recognition performance across cycle phases and specifically when only considering the luteal phase (Derntl et al., 2008a, 2013). Therefore, the measurement timepoint in the luteal phase may indeed determine whether a higher sensitivity for negative emotions or a general lower face recognition rate can be detected compared to other cycle phases.

Like the midluteal phase, the hormonal milieu in OC-users is marked by a progestogen dominance as high doses of progestogens are needed to inhibit ovulation (Lovett et al., 2017). Therefore, it is not surprising that basic as well as complex facial emotion recognition was repeatedly found to be impaired in OC-users compared to NC women (Hamstra et al., 2014, 2015, 2017; Pahnke et al., 2019). These findings hold especially for negative emotions including anger, sadness, disgust (Hamstra et al., 2014, 2015, 2017). However, there are studies not reporting differences in emotion recognition performance between OC-users and NC women (Radke and Derntl, 2016; Gamsakhurdashvili et al., 2021b), including a large-scale study (n = 395; Shirazi et al., 2020). Interestingly, androgenicity of pill type seems to play no role in the impaired emotion recognition of OC-users (Pahnke et al., 2019). Regarding the modulatory role of endogenous and synthetic ovarian hormone levels not much is known, as previous studies have only measured endogenous but not exogenous ovarian hormone levels in blood or saliva samples. Since exogenous hormones pass the blood brain barrier and bind to hormone-receptors in brain regions involved with socio-emotional processing (Toffoletto et al., 2014; Barth et al., 2015; Louw-du Toit et al., 2017; Rehbein et al., 2021), including them in analyses could aid in shedding light on underlying mechanisms of facial emotion recognition during OC-use.

The aim of this study is to elucidate hormone-based differences in emotion recognition more closely by the incorporation of exogenous in addition to endogenous ovarian hormones. To assess the roles of estrogens and progestogens on facial emotion recognition largely independently, we included three groups of women with different hormonal states: (1) NC-women during the early follicular phase with low concentrations of estradiol and progesterone, (2) NC-women during the peri-ovulatory phase with high estradiol and low progesterone concentration, and (3) women actively taking combined OC-pills, with medium estrogen and high progestogen concentration. Based on previous literature on OC-and menstrual cycle-related differences, we hypothesize that: (1) OC-users show impaired

emotion recognition relative to NC women (see for reviews: Osório et al., 2018; Gamsakhurdashvili et al., 2021a), and for NC women, we hypothesized that: (2) Women in the peri-ovulatory phase show enhanced fear recognition compared to early follicular NC women (Pearson and Lewis, 2005), whilst there is no evidence for an altered fear recognition in OC compared to NC women.

We aim for a systematic investigation of hormone-related effects on female's facial recognition performance. Therefore, we ran explorative analyses with regards to – especially synthetic – ovarian hormones. In addition, affective state supposedly impacts the recognition of valence-congruent emotions but impairs performance for valence-incongruent facial expressions (Schmid and Schmid Mast, 2010). Moreover, current affective state has been associated with fluctuations of ovarian hormones (Reed et al., 2008; Ocampo Rebollar et al., 2017). To account for a possible interplay of affective state and ovarian hormones on emotion recognition performance, we not only exploratively checked for relations of affective state with emotion recognition performance in different hormonal states, but also to ovarian hormone levels.

MATERIALS AND METHODS

To investigate hormone-related differences in facial emotion recognition, we used a quasi-experimental, cross-sectional study design.

Participants

A total of 86 healthy females aged between 18 and 33 years (m_{age} = 23.8, ± 3.1) were recruited via postings at the University of Tübingen, the University Hospital Tübingen, social media, as well as in gynecological practices in Tübingen. Based on self-reported cycle days, the women were divided into three groups: (1) women with long-term (>6 months) OC-use (OC group; n = 30, m_{age} = 23.6 ± 3.0), (2) NC-women (>4 months) during the early follicular phase (fNC group; n = 30, m_{age} = 23.8 ± 3.3), and (3) NC-women (> 4 months) during the peri-ovulatory phase (oNC group; n = 26, m_{age} = 24.0 ± 3.0). The assignment to hormonal status groups was validated by female sex-hormone measurement and described in the Results section ("Sample Description and Hormonal Levels"). The sample size (n = 86) was based on previous, conceptually related studies (Derntl et al., 2013; Radke and Derntl, 2016; Dan et al., 2019; Gurvich et al., 2020; Kimmig et al., 2021). The fNC group was tested 2–5 days after the onset of their menses, the oNC group 3 days before until 2 days after a positive increase of the luteinizing hormone confirmed via LH test (nal van minden GmbH, Germany). The OC group was tested during day 3–21 of active pill intake, expecting to have steady, suppressed estradiol- and progesterone levels. None of the participants were diagnosed with a gynecological illness nor had a lifetime pregnancy. All women gave informed consent, and the study was approved by the ethics committee of the Medical Faculty of the University of Tübingen (331/2016BO2).

TABLE 1 | Sample characteristics (mean and standard deviation if not otherwise specified) and hormone profiles per group (median and interquartile range).

	OC	fNC	oNC	*p*-value
N	30	30	26	
Age (years)	23.6 (3.0)	23.8 (3.3)	24.0 (3.0)	0.906
Education (l/m/h)[1]	1/20/9	0/20/10	1/15/10	0.854
Verbal intelligence (WST, raw scores)	32.4 (2.4)	32.9 (3.1)	32.7 (2.4)	0.563
Cognitive flexibility (TMTB-A, sec)	18.2 (9.9)	16.8 (9.7)	16.4 (7.8)	0.718
Depressive mood (BDI-II, scores)	5.5 (4.3)	7.4 (4.1)	5.2 (3.5)	0.072
Social anxiety (Mini-Spin-R)	7.5 (2.9)	7.9 (1.6)	7.2 (2.2)	0.247
Trait anxiety (STAI)	34.1 (8.6)	34.5 (6.9)	32.8 (6.8)	0.648
State anxiety (STAI)	33.8 (7.1)	35.7 (7.0)	33.8 (8.7)	0.521
Positive affective state (PANAS)	21.3 (8.3)	23.7 (5.9)	24.1 (5.5)	0.308
Negative affective state (PANAS)	2.9 (3.8)	5.3 (4.7)	2.7 (3.0)	0.026 fNC > OC
Hormone profiles				
EndoE2 (pmol/L)	16.9 (7.0)	98.4 (45.2)	444.2 (462.2)	<0.001 oNC > fNC > OC
ExoE2 (pmol/L)	72.7 (36.3)			<0.001[2] oNC > fNC, OC
EndoP (nmol/L)	0.1 (0.6)	0.3 (0.4)	1.0 (4.4)	<0.001 oNC > fNC > OC
ExoP (nmol/L)	33.6 (37.2)			<0.001[3] OC > oNC > fNC
Testosterone (nmol/L)	0.7 (0.4)	0.7 (0.4)	0.9 (0.5)	<0.001 oNC > OC, fNC

[1] *l, no higher education entrance qualification; m, higher education entrance qualification; h, university degree.*

[2,3] *Group differences between endogenous hormone levels for NC women and exogenous hormone levels of OC-users calculated. WST – Wortschatztest; TMT – Trial-making test; BDI – Beck's depression inventory; SPIN-R – social phobia inventory revised; STAI – state-trait anxiety inventory; PANAS – positive and negative affect schedule; EndoE – endogenous estradiol; exoE – exogenous estradiol; endoP – endogenous progesterone; exoP – exogenous progesterone.*

An overview of sociodemographic and neuropsychological characteristics and the plasma hormone profiles for the different groups is provided in **Table 1**.

Procedure

Participants came in for two appointments: (1) a screening (45–60 min) and (2) an experimental session (30–45 min). After a mental health screening, all women performed neuropsychological tests and reported sociodemographic information during the first session. The experimental session took place in the respective hormonal phase (i.e., active OC intake, early follicular or peri-ovulatory phase). At its beginning, participants rated their current affective state. Subsequently, the emotion recognition task was performed. After task completion, plasma samples (2 × 9 ml EDTA) were taken by trained medical staff to obtain the actual hormone status. At the end of the session, participants filled in several questionnaires including state-trait anxiety and depressive mood.

Materials and Measures

Emotion Recognition Task

Stimuli consisted of 36 colored pictures of European-American faces showing five basic emotions (happiness, sadness, anger, fear, and disgust) as well as neutral expressions (i.e., six items per condition, see Gur et al., 2002 for stimulus material). This is a short version of the Vienna Emotion Recognition Task (VERT-K) which has already successfully been carried out to investigate female emotion recognition under varying ovarian hormone concentrations (Derntl et al., 2008a,b, 2013; Radke and Derntl, 2016). In each trial, participants were instructed to choose the correct emotion from six verbal possibilities presented in a random order next to the target face stimulus by button press. A response was necessary to finish the trial. The sequence of stimuli presentation was pseudo-randomized for emotion type and sex of actor. Intertrial intervals lasted 1 s. The variables of interest were emotion recognition accuracy and response time. In total, the task lasted about 2–4 min.

Neuropsychological Tests and Questionnaires

Positive and negative affective state was assessed using the Positive and Negative Affect Scale (PANAS; Watson et al., 1988). Current affective state was included to control for potential confounding effects on emotion recognition. Moreover, we were interested in the interplay of affective state, hormone status and emotion recognition.

The following measures were used for sample characterization and assessing comparability of the hormonal status groups. The absence of current or lifetime mental disorders was checked using a semi-structured interview (SCID screening; Wittchen et al., 1997). The Wortschatztest (WST; Schmidt and Metzler, 1992) was used to assess verbal intelligence and the Trail-Making-Test A and B (TMT; Reitan, 1992) measured cognitive flexibility. Furthermore, several affective measures were taken including state-trait anxiety (STAI-I; Laux et al., 1981), social anxiety with the brief version of the Social Phobia Inventory (Mini-SPIN-R; Aderka et al., 2013) and depressive mood using the Beck's depression inventory (BDI; Hautzinger et al., 2006). These neuropsychological and psychopathological measures were used for sample characterization and assessing comparability of the hormonal status groups.

Hormone Assessment

After blood collection, the sample was centrifuged to obtain plasma, which was aliquoted into microtubes and stored at −70°C. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to determine hormone levels of estradiol (endoE), progesterone (endoP), testosterone, and ethinylestradiol (exoE) as well as progestins (exoP) in pg/mL. Plasma concentrations of the progestins were determined individually for dienogest, levonorgestrel, nomegestrol as well as chlormadinone acetate. The analytical system consisted of a 1290 Infinity II UHPLC (Agilent Technologies, Germany) coupled to a QTRAP 4500 mass spectrometer (Sciex, United States). The hormones were quantified via a surrogate calibrant approach (Li and Cohen, 2003; Drotleff et al., 2018) and the method was validated according to FDA guidelines. The dynamic range of endoE, endoP, testosterone, exoE and the various progestins ranged from 3.45–5179.13, 1.0–47657, 1.9–11438.00, 2.0–3000, and 10–20000 pg/mL, respectively. To evaluate the performance of the method and document the validity of the analytical measurements method, quality control samples (QCs) were analyzed on three consecutive days. Interday precision (i.e., repeatability between different days) and accuracy (as % recovery of QCs' nominal concentration) were 7.0–9.1% and 96.8–100.5% (endoE), 6.4–9.9 and 97.0–104.6% (endoP), 7.4–9.9% and 94.3–106.5% (testosterone), 5.6–12.3% and 97.1–99.9% (exoE), as well as 4.4–11.1% and 93.4–109.2% (progestins), indicating excellent method performance within the acceptance criteria of the FDA bioanalytical method validation guideline. Interday precision measures the repeatability of the concentrations of the quality control samples between different days and interday accuracy the percent recovery (% found/nominal concentration) in the quality control samples on the different days.

Statistical Analyses

All statistical analyses were performed using SPSS 25 (IBM SPSS Statistics) with alpha set to 0.05, if not otherwise specified. All *post hoc* analyses were Bonferroni corrected.

Sample Characteristics and Hormonal Levels

Group differences (OC, fNC, and oNC) in sociodemographic (i.e., age and educational level), neurocognitive (i.e., verbal intelligence and cognitive flexibility) and affective parameters [i.e., affective states (PANAS), state and trait (social) anxiety (STAI and mini-SPIN), as well as depressive mood (BDI)] were either analyzed with an independent ANOVA (age and state anxiety, normality: yes, homogeneity of variances: yes), a Welch's ANOVA (positive affective state, normality: yes, homogeneity of variances: no) or a non-parametric Kruskal-Wallis test [verbal intelligence, cognitive flexibility, negative affective state, trait (social) anxiety and depressive mood, normality: no, homogeneity of variances: yes]. Educational level is a categorical variable (i.e., 1 – no higher education entrance qualification, 2 – higher education entrance qualification, 3 – university degree) and thus analyzed with Fisher's exact test, as not all cells had counts higher than 5.

For OC-users, only exogenous hormone levels were used for analyses as endogenous hormones are suppressed to very low levels. All hormones (endogenous levels for NC groups and exogenous levels of P for OC-users), except for testosterone and exoE, were analyzed using the non-parametric median test, as normality (according to visual inspection and Kolmogorov Smirnov test: $p < 0.05$) as well as homogeneity of variances (Levene's test: $p < 0.05$) were not given. Group differences of testosterone and exoE were assessed with the Kruskal-Wallis test (normality: no, homogeneity of variances: yes).

Emotion Recognition Accuracy

The number of correct responses was calculated for each target emotion, resulting in a mean score of emotion recognition accuracy (percent correct) for each participant per emotion. Kolmogorov-Smirnov tests suggested that the data was not

normally distributed ($p < 0.05$). We therefore used Generalized Estimating Equations (GEE) with emotion as within-subject factor (anger, fear, happiness, sadness, disgust, and neutral) and hormonal group as between-subjects factor (fNC, oNC, and OC) to analyze differences in emotion recognition performance dependent on hormonal status. Significant effects were followed up with Bonferroni-corrected pairwise comparisons. As the groups showed significantly different baseline levels in the scores of the negative affective state scale (PANAS, see section "Sample Description and Hormonal Levels"), we additionally performed an ANCOVA with emotion as within-subject factor, group as between-subjects factor and negative affective states scores as covariate.

Emotion Recognition Response Times

Like the accuracy measure, mean emotion recognition response times were also calculated per emotion for each participant. However, only correct trials and trials with response times larger than 200 ms were considered. A mixed AN(C)OVA with emotion as within-subject factor, group as between-subjects factor and negative affective state as covariate was performed. Due to the violation of the sphericity assumption (Maulchy's test: $p = 0.045$), Huynh-Feldt corrected statistics were reported (Greenhouse $\varepsilon > 0.75$). Bonferroni corrected pairwise comparisons were used as *post hoc* analyses.

Correlational Analyses

Within group associations between overall emotion recognition accuracy and response times with self-reported affective state (i.e., PANAS positive and negative scales) and hormones (endogenous for NC groups, exogenous for OC-users; concentrations of ovarian sex hormones as well as testosterone) were investigated. Besides correlation analyses using the total percent correct for emotion recognition accuracy and total mean response time, exploratory analyses for single emotions were carried out if the GEE or ANOVA analyses revealed significant emotion-specific group differences. Normally distributed data was analyzed with Pearson correlations (OC: overall and neutral emotion recognition response times, exoE, and positive affective state; fNC: overall emotion recognition response time, testosterone, endoE and positive affective state; oNC: overall response time, testosterone, endoE, and positive affective state), whereas Spearman Rank correlations (rho_s) were used to account for non-normality in all other correlational analyses.

RESULTS

Sample Description and Hormonal Levels

Women across the different hormonal status groups did not differ on sociodemographic characteristics such as age [$F(2,83) = 0.10$, $p = 0.906$] and educational level [$p = 0.854$ (Fisher's exact test)]. Furthermore, the groups were similar for neuropsychological parameters including verbal intelligence, cognitive flexibility, depressive mood as well as (social) anxiety (all $|H| \leq 5.26$, all $p \geq 0.072$). Baseline levels of state anxiety and positive affective state at the beginning of the experimental session were comparable amongst women in different hormonal phases (all $|F| \leq 1.20$, all $p \geq 0.308$), whereas fNC women reported significantly higher negative affective state compared to OC-users [main effect: $H(2) = 7.28$, $p = 0.026$; fNC > OC: $p = 0.044$; fNC > oNC: $p = 0.088$; OC > oNC: $p = 1.00$].

Figure 1 depicts the hormonal levels of the different hormonal status groups. Hormonal analyses using median tests confirmed that the women assigned to the respective groups indeed differed in hormonal profiles accounting for endogenous as well as for exogenous sex hormones [EndoE2 vs. ExoE2: $H(2) = 56.73$, $p < 0.001$; EndoP vs. ExoP: $X^2(2) = 49.58$, $p < 0.001$; Testosterone: $H(2) = 7.92$, $p = 0.019$]. As expected, the oNC group had significantly higher levels of estrogens than the OC and fNC group (both $p < 0.001$, OC vs. fNC: $p = 0.097$), whereas the OC group had highest levels of progestogens (OC > oNC, fNC: both $p < 0.001$), followed by the oNC group (oNC > fNC: $p = 0.022$). Testosterone was significantly lower in OC-users compared to the oNC group ($p = 0.020$).

Emotion Recognition Accuracy

The GEE analysis for the emotion recognition accuracy (i.e., percent correct) revealed a main effect of emotion [Wald-$X^2(5) = 468.52$, $p < 0.001$, see **Table 2** for means]. After Bonferroni correction, recognition rates of all emotions differed significantly from each other (all $p \leq 0.026$) except for happiness vs. anger ($p = 1.000$), anger vs. fear ($p = 0.273$), and fear vs. neutral ($p = 1.000$). Happy and angry faces were recognized best, whilst disgusted and sad expressions had the lowest performance scores.

Contrary to our expectation, there was no main effect of group [Wald-$X^2(2) = 1.39$, $p = 0.500$, see **Figure 2A**]. However, the interaction emotion-by-group turned out significant [Wald-$X^2(10) = 25.34$, $p = 0.005$]. To disentangle the significant

TABLE 2 | Emotion recognition performance (in percent) and response times (in ms) across the whole sample and for the individual hormonal groups (presented as mean and standard deviation).

	Whole sample ($n = 86$)	OC ($n = 30$)	fNC ($n = 30$)	oNC ($n = 26$)
Emotion recognition response accuracy (%)				
Happiness	96.1 (9.1)	96.7 (8.1)	96.1 (11.3)	95.5 (7.5)
Anger	95.4 (11.3)	95.6 (8.7)	97.2 (6.3)	93.6 (11.6)
Fear	91.1 (12.2)	91.1 (12.2)	88.3 (13.9)	94.2 (9.4)
Disgust	76.2 (17.6)	78.3 (15.3)	77.2 (18.8)	72.4 (18.8)
Sadness	61.6 (23.2)	61.1 (24.1)	63.3 (18.3)	60.3 (27.5)
Neutral	89.7 (15.4)	93.9 (11.1)	82.2 (19.0)	93.6 (11.6)
Emotion recognition response times (ms)				
Happiness	2204.2 (629.0)	2104.6 (757.4)	2212.7 (428.1)	2309.3 (658.6)
Anger	2837.7 (937.8)	2579.0 (744.9)	2943.6 (966.6)	3013.9 (1066.1)
Fear	3510.4 (1265.2)	3308.5 (977.2)	3628.3 (1459.6)	3607.4 (1337.0)
Disgust	3008.8 (1095.7)*	2868.2 (1185.2)	3200.0 (1118.2)	2953.9 (958.3)*
Sadness	3135.1 (952.1)*	3177.5 (783.6)	3225.5 (1159.3)*	2987.0 (884.3)
Neutral	2570.9 (830.5)*	2327.0 (543.5)	2596.2 (867.1)*	2824.0 (997.0)

One participant missing as no correct answers were recorded.

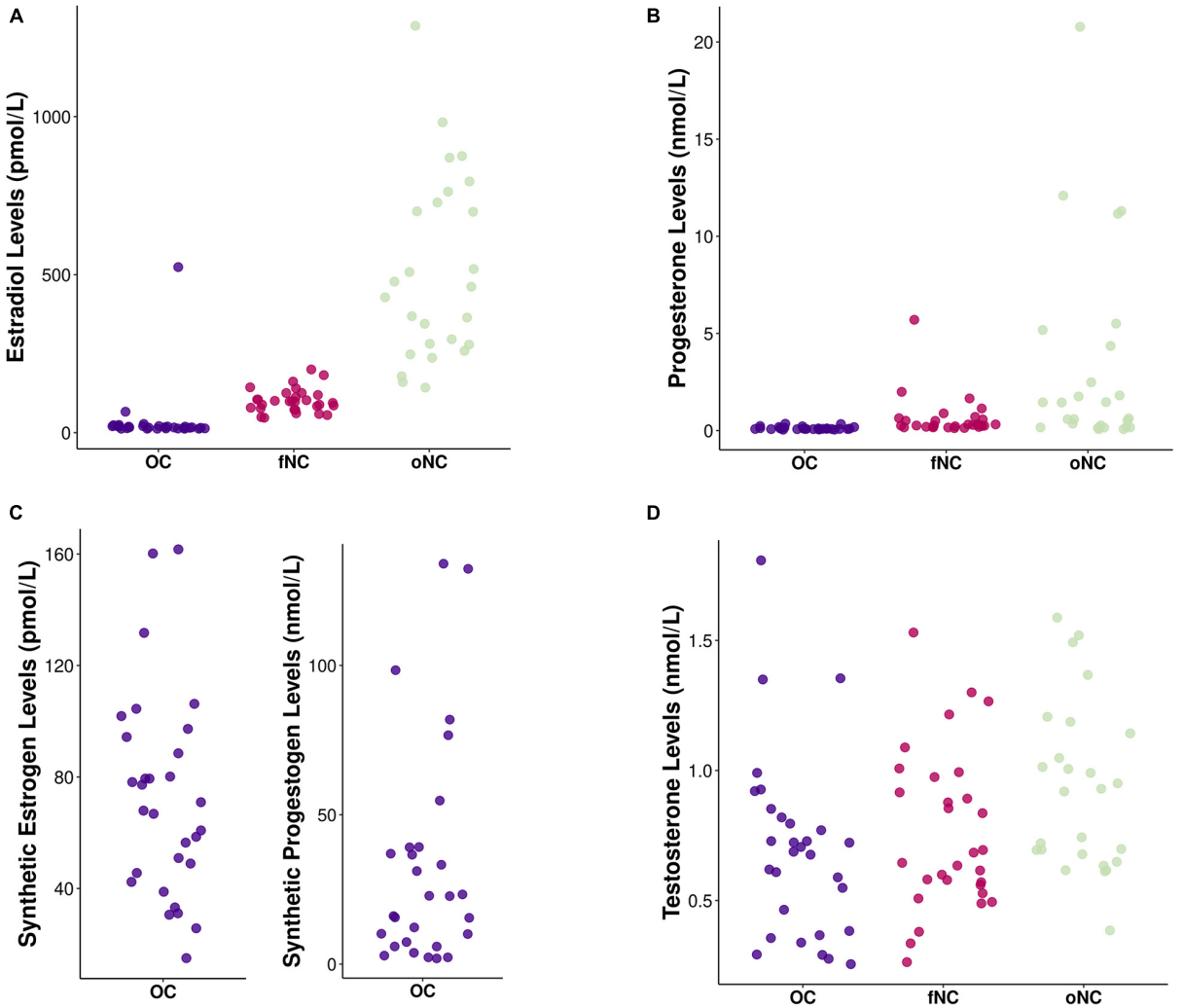

FIGURE 1 | Chart depicting hormone levels of **(A)** endogenous estradiol (in pmol/L), **(B)** endogenous progesterone (in nmol/L), **(C)** exogenous (i.e., synthetic) estrogens (in pmol/L) and progestogens (in nmol/L), and **(D)** endogenous testosterone (in nmol/L) for each hormonal status group [i.e., OC – oral contraceptive users (blue), fNC – naturally cycling women in early follicular phase (magenta), and oNC – naturally cycling women in periovulatory phase (light green)].

interaction, separate GEEs for each group were performed. For the OC and the oNC women, recognition performance of disgust and sadness were significantly worse than for the remaining emotions (all $p \leq 0.001$). Whereas OC women recognized sadness significantly worse than disgust ($p = 0.001$), oNC women's recognition accuracy did not differ between sad and disgust expressions ($p = 0.095$). Happy, angry, neutral, and fearful faces were equally well recognized (all $p \geq 0.116$, except for happy vs. fear in OC: $p = 0.014$). In contrast, fNC women recognized facial expressions of anger and happiness significantly better than fearful, neutral, disgusted, and sad faces (all $p \leq 0.006$). Sad expressions showed the lowest accuracy (all $p \leq 0.006$) in fNC women. Fear was significantly better recognized than disgust ($p = 0.002$), accuracy for neutral faces did not differ significantly from either of the two emotions (all $p \geq 0.116$). Overall, the recognition order per emotion for OC and oNC women was happy, angry, neutral, (>) fearful > disgust, (>) sad. Whereas fNC women's recognition order was angry, happy > fear, neutral (not different from fear or disgust), > disgust > sad. Therefore, the recognition of neutral faces presents the largest difference in the order of emotion recognition between the groups. Congruently, separate GEE analyses looking at between group difference for the specific emotions, revealed no group difference for the five basic emotions (all $|\text{Wald-X}^2| \leq 3.84$, $p \geq 0.147$), while for neutral faces a significant group difference emerged [$\text{Wald-X}^2(2) = 9.57$, $p = 0.008$]. The fNC women had significantly lower accuracy rates for the neutral condition than OC and oNC women (all $p \leq 0.005$, see **Figure 2B**). Neutral faces were mostly misclassified by fNC women as sad or angry instead (66 and 25% of incorrect trials, respectively).

When directly testing for our directed second hypotheses in a GEE only involving fear and the two NC groups, we indeed observed superior fear recognition in oNC compared to fNC women [$\text{Wald-X}^2(1) = 3.66$, $p_{1\,tailed} = 0.028$].

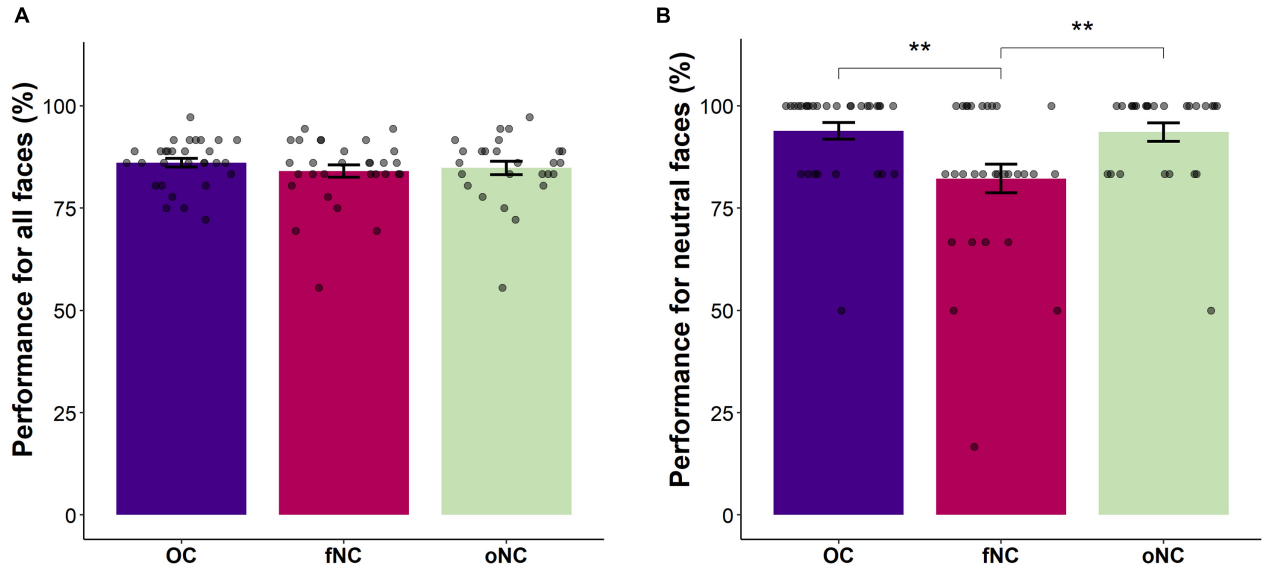

FIGURE 2 | Bar chart depicting the **(A)** overall emotion recognition accuracy (in percent) and **(B)** the emotion recognition accuracy for neutral faces (in percent) per group [i.e., OC – oral contraceptive users (blue), fNC – naturally cycling women in early follicular phase (magenta), and oNC – naturally cycling women in periovulatory phase (light green)]. Error bars with 1 SE. $^{**}p < 0.01$.

Adding negative affective state as a covariate did not change the aforementioned results (except that disgust = sadness for fNC: $p = 0.072$) and had no direct link with emotion recognition accuracy [negative affective state main effect: Wald-$X^2(1) = 0.01$, $p = 0.924$; negative affective state-by-emotion: Wald-$X^2(5) = 3.13$, $p = 0.680$]. However, there was a significant group-by-affective state-by-emotion interaction [Wald-$X^2(10) = 21.34$, $p = 0.019$]. Separate emotion-specific GEE analyses revealed no interaction effect with negative affective state for recognition of all emotional faces as well as for neutral faces (all $|\text{Wald-}X^2| \leq 2.57$, $p \geq 0.277$), except for disgust [group-by-affective state: Wald-$X^2(2) = 6.03$, $p = 0.049$]. Parameter estimates suggest that negative affective state had a significantly larger positive effect on disgust recognition in fNC compared to oNC women [Wald-$X^2(1) = 5.98$, $p = 0.014$], whereas OC-users did not differ from either NC group (all $|\text{Wald-}X^2| \leq 3.05$, $p \geq 0.081$). Negative affective state was positively related with accuracy in the fNC group [$rho_s(30) = 0.38$, $p = 0.036$], whereas there were no significant correlations for the OC [$rho_s(30) = -0.10$, $p = 0.617$] and the oNC group [$rho_s(26) = -0.30$, $p = 0.129$].

Emotion Recognition Response Times

The mixed ANOVA design of emotion recognition response times only from correct trials revealed a significant main effect of emotion [$F(4.9,385.0) = 23.97$, $p < 0.001$]. *Post hoc* analyses revealed that happy faces were recognized the fastest (all $p \leq 0.027$), followed by neutral faces (all $p \leq 0.027$, but neutral vs. angry: $p = 0.074$). Angry and disgusted expressions were significantly faster recognized than fearful ($p = 0.001$ and $p = 0.043$, respectively), but not sad faces (all $p \geq 0.208$). There was no main effect of group [$F(2,79) = 0.93$, $p = 0.401$] nor a group-by-emotion interaction [$F(9.8,385.0) = 0.73$, $p = 0.695$] in the response times including only correct trials.

Adding negative affective state as a covariate did not affect the findings reported above and also had no significant relation with emotion recognition in women with different hormonal states [negative affective state main effect: $F(1,77) = 0.90$, $p = 0.346$; negative affective state-by-emotion interaction: $F(4.9,376.5) = 1.46$, $p = 0.203$].

Within-Group Correlational Analyses: Emotion Recognition, Sex Hormones, and Self-Reported Affective Measures

Correlational analyses were run to assess whether affective states (positive and negative) or hormone levels (endogenous and exogenous ovarian hormones for NC women and OC-users, respectively) are related to emotion recognition performance (i.e., accuracy and response times) within different hormonal states. All correlations between overall accuracy and response times with sex hormones and self-reported positive and negative affective state remained non-significant (all $|r_{(s)}| \leq 0.36$, all $p \geq 0.073$). Since there was a significant difference in emotion recognition accuracy of neutral faces between OC and fNC women, within group correlations between sex hormones, self-reported affective state measures and emotion recognition parameters of neutral faces have been additionally computed. In OC-users, lower negative affective state was associated with higher recognition accuracy [$rho_s(29) = -0.49$, $p = 0.008$] and faster response times [$rho_s(29) = 0.41$, $p = 0.028$], when presented with neutral faces. None of the other self-report measures and sex hormone levels correlated significantly with emotion recognition of neutral faces in the OC group (all $|r_{(s)}| \leq 0.36$,

all $p \geq 0.054$). There were no significant correlations for the fNC group regarding the recognition of neutral faces (all $|rho_s| \leq 0.27$, all $p \geq 0.143$). Fear recognition accuracy was not significantly related to affective states or hormonal levels in the fNC and oNC groups (all $|rho_s| \leq 0.32$, all $p \geq 0.087$). Fear recognition response times showed a positive association with testosterone levels in fNC women [$rho_s(30) = 0.46$, $p = 0.012$], whilst all other correlation remained non-significant in both NC groups (all $|rho_s| \leq 0.30$, all $p \geq 0.143$).

Furthermore, we were interested whether hormone-levels (endogenous and exogenous ovarian hormones in NC women and OC-users, respectively) were related to positive or negative affective state, which in turn could be related to emotion recognition. Spearman rank correlations revealed a negative association of progesterone with negative affective state [$rho_s(30) = -0.47$, $p = 0.009$] in the fNC group, whereas in the oNC group endoP correlated negatively with positive affective state [$rho_s(26) = -0.63$, $p = 0.001$]. Outlier removal did not alter results significantly. All remaining correlations between sex hormones and affective state measures did not reach significance (all $|r_{(s)}| \leq 0.26$, all $p \geq 0.209$).

DISCUSSION

Emotion recognition and other socio-emotional processes have been repeatedly suggested to be associated with fluctuations of endogenous sex hormones as well as with the intake of synthetic ovarian hormones (see reviews: Montoya and Bos, 2017; Osório et al., 2018; Lewis et al., 2019; Gamsakhurdashvili et al., 2021a). However, studies are not entirely conclusive, and the underlying mechanisms remain largely unclear. Therefore, our aim was to systematically investigate the role of hormonal status in facial emotion recognition by linking performance not only to endogenous hormones in NC women, but for the first time also to the more dominant exogenous hormone levels in OC-users. Here the use of the highly recommended LC-MS method for hormone determination is a major strength of this study. Furthermore, we investigated associations to other emotional processes which could impact emotion recognition such as negative and positive affective state.

Overall, women during the early follicular phase, independently of negative affective state differences among groups, showed specific deficits in recognizing neutral faces by misjudging neutral faces as sadness or anger. Furthermore, in a direct comparison peri-ovulatory women, as expected, recognized fearful faces significantly better than early follicular women. There were no significant group-related differences in emotion recognition response times. Endogenous and exogenous sex hormones were, not linked to overall or neutral recognition performance. During the early follicular phase low progesterone levels were linked to higher negative affective state. Notably, during the peri-ovulatory phase progesterone levels were negatively associated with positive affective state.

Contrary to our expectation and previous literature (Hamstra et al., 2014, 2015, 2017; Pahnke et al., 2019), we were not able to replicate inferior emotional face recognition performance in OC-users compared to NC women. Even though studies use the same tasks for basic (i.e., VERT as in the present study) or complex (i.e., Reading the mind in the eye task) emotion recognition, they reveal mixed results. In line with our findings, Radke and Derntl (2016) found no OC-related impairment in basic emotion recognition. Furthermore, the up-to-now largest study on hormonal contraceptives and complex emotion recognition also failed to find any significant differences (Shirazi et al., 2020). This incongruency in findings could be due to the interplay of OC-use with other potential modulatory factors. For instance, Hamstra et al. (2016) found only a significant impairment in emotion recognition relative to NC women in OC-users with a certain genotype of mineralocorticoid receptor (MR-haplotype 1/3). In the present study, we found negative affective state to play a role in neutral face recognition of OC-users. The worse their affective state was, the more likely women misclassified a neutral expression as sadness or anger (i.e., increased negativity bias). The lack of finding any significant associations between endogenous and exogenous hormone levels with emotion recognition performance in OC-users further corroborates the view that OC-related effects may be complex and mediated rather than straightforward.

Regarding menstrual cycle phases, in a direct comparison, we replicated previous findings indicating superior fear recognition in the peri-ovulatory phase compared to the early follicular phase with significantly lower endoE2 levels (Pearson and Lewis, 2005). Interestingly, this superior fear recognition does not translate into increased fear processing in peri-ovulatory women. In fact, high levels of endoE2 have been linked to enhanced fear extinction, whereas low levels (i.e., in the early follicular phase) were associated with enhanced fear conditioning (Montoya and Bos, 2017). Moreover, high levels of estradiol were previously associated with lower disgust (Kamboj et al., 2015) and anger recognition (Guapo et al., 2009), accordingly peri-ovulatory women had lower accuracy in recognizing these facial expressions than early follicular women, however, these differences were only descriptive and did not reach significance. In the early follicular group, disgust recognition accuracy was positively associated with negative affective state. This is congruent with the notion that affective state may enhance emotion recognition of valence-congruent emotions (Schmid and Schmid Mast, 2010). In OC-users and peri-ovulatory women the negative affective state may have not been pronounced and variable enough to reveal such associations.

Independent of negative affective state, early follicular women were significantly worse than OC-users and peri-ovulatory NC women in recognizing neutral faces. The neutral faces were mostly misclassified as being sad or angry instead. However, there were no significant differences in response times. Therefore, suggesting that fNC women were not aware of their difficulty in recognizing these faces correctly, as if they were uncertain, response times should be longer. In previous studies, low endoP levels were associated with a higher number of stimuli falsely classified as neutral (Derntl et al., 2008a), faster response times in correctly identifying neutral faces (Kamboj et al., 2015), and higher amygdala activation during neutral face processing (Derntl et al., 2008b). Therefore, from these studies we could

have expected enhanced neutral face processing of early follicular women, as here endogenous progesterone is low. However, instead we found a greater negativity bias (i.e., misjudging neutral as negative expressions) in this group, which was however not related to ovarian hormone concentrations. This incongruency could be explained by the different menstrual cycle phases included in the studies. The previous studies (Derntl et al., 2008a,b; Kamboj et al., 2015) pooled follicular and luteal women to generate hormone correlations. Therefore, these findings could be rather driven by the inclusion of luteal women with high progesterone levels. Furthermore, the early follicular phase was largely underrepresented in the follicular groups of the previous samples, making a comparison of the previous studies with the present study difficult. Negativity biases in neutral or ambiguous face recognition have been repeatedly implicated in individuals with affective disorders, (social) anxiety and other mental problems (Richards et al., 2002; Leppänen et al., 2004; Yoon and Zinbarg, 2008; Mier et al., 2014; Münkler et al., 2015; Gutiérrez-García and Calvo, 2017; Peschard and Philippot, 2017). These biases or overinterpretations could contribute to the difficulties in social interactions and relations in these individuals. Our analyses, however, revealed no link between affective state and (social) anxiety measures with the negativity bias in the early follicular group. Considering, that the fNC women had no elevated or clinically relevant levels on these scales, these null finding may not be surprising. Nevertheless, fNC women could have felt more menstrual discomfort and pain, which is not evaluated by the PANAS or the STAI, causing a greater precaution in processing of neutral facial expression to account for their increased vulnerability. Indeed, there is evidence of negative interpretation biases associated with pain (Khatibi et al., 2015; Heathcote et al., 2016). Therefore, future studies are needed to examine more closely the possible link between menstrual pain/discomfort and negative interpretation biases.

Independent of emotion recognition, our findings support previous literature reporting a link between menstrual cycle and affective state (Reed et al., 2008; Ocampo Rebollar et al., 2017). As similarly shown by Ocampo Rebollar et al. (2017), the negative link between progesterone and positive affective state in the peri-ovulatory phase implies that pre-ovulatory women have more positive affective state which decreases as ovulation comes closer and progesterone levels start rising. In the early follicular phase, however, lower levels of progesterone were linked with worse affective state. Since progesterone levels in this phase are already low, even lower concentrations could lead to an interruption of the mood stabilizing effects of its metabolite allopregnanolone, by reducing its effect (through lower concentrations) on the GABAeric system (Chen et al., 2021). However, these findings ought to be interpreted with caution, given the generally low levels of progesterone in the follicular phase.

In this study we have only included women using OCs but excluded women using other (hormonal) contraception methods such as intrauterine devices or vaginal ring. To fully capture the impact of (hormonal) contraception on emotion recognition and more general socio-emotional abilities, future studies should systematically investigate their effect. Furthermore, our study investigated women's emotion recognition in a cross-sectional design, comparing different women with different hormonal status once. However, a longitudinal design enabling a within-subject comparison would be beneficial to better characterize the impact of endogenous and exogenous hormones on behavioral outcomes. Additionally, statistical power could be improved this way, without really having to increase the sample size (Gonzales and Ferrer, 2016). Another downside of the sample size per hormonal status group in addition to non-linearity issues of the data was that no mediation analyses using affective state could be carried out to investigate the interplay of sex hormones and affective state on emotion recognition (minimum size per group $n = 72$ for medium effects; Fritz and Mackinnon, 2007). Finally, in this study we only measured emotion recognition of basic emotions. Since in real-life, emotion recognition of complex next to basic emotions plays a major role, the inclusion of complex emotions in the study design could have raised ecological validity.

CONCLUSION

With the current study we shed some light on the role of different hormonal conditions (i.e., OC-use, early follicular and peri-ovulatory phase) in emotion recognition abilities of women. Our results suggest that women in their early follicular phase show both, elevated negative affective state as well as a negativity bias in perceiving neutral faces (i.e., neutral misjudged as sadness or anger), which may impair their success in social interactions. Furthermore, in a direct comparison peri-ovulatory women showed better fear recognition accuracy. Generally, we were not able to replicate OC-related impairments in emotion recognition performance. More importantly, we also found no significant links between endogenous and exogenous hormone levels with emotion recognition, suggesting a more complex mechanism by which emotion recognition is possibly influenced by hormonal contraception. Thus, the study motivates more research to better understand how different hormonal conditions do impact women's social life, and ultimately their mental health. A better understanding of these processes is necessary to provide gynecologists and potential users with details on potential consequences of hormonal contraceptives on female social cognition.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Medical Faculty of the

University Tübingen. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

A-CSK and BiD designed the study and supervised data collection. BiD and IS-P helped with the methodological setup. A-CSK and ADB collected data. BiD, IS-P, and JAB were involved in the planning of data analysis and interpretation of data. BeD carried out all hormone detection analyses under the supervision of ML. A-CSK performed data analyses and wrote the manuscript. All authors contributed to the manuscript.

FUNDING

This work was supported by the German Research Foundation (DFG; DE2319/9-1) and the German Academic Scholarship Foundation (Studienstiftung des deutschen Volkes). We acknowledge support by the Open Access Publishing Fund of the University of Tübingen.

ACKNOWLEDGMENTS

We would like to thank Anna Gärtner, Melina Grahlow, Leonie Matkei, and Theresa Schell for their assistance during participant recruitment and data collection.

REFERENCES

Aderka, I. M., Pollack, M. H., Simon, N. M., Smits, J. A., Van Ameringen, M., Stein, M. B., et al. (2013). Development of a brief version of the Social Phobia Inventory using item response theory: the Mini-SPIN-R. *Behav. Ther.* 44, 651–661. doi: 10.1016/j.beth.2013.04.011

Barth, C., Villringer, A., and Sacher, J. (2015). Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. *Front. Neurosci.* 9:37. doi: 10.3389/fnins.2015.00037

Becker, J. B., Arnold, A. P., Berkley, K. J., Blaustein, J. D., Eckel, L. A., Hampson, E., et al. (2005). Strategies and methods for research on sex differences in brain and behavior. *Endocrinology* 146, 1650–1673. doi: 10.1210/en.2004-1142

Chen, S., Gao, L., Li, X., and Ye, Y. (2021). Allopregnanolone in mood disorders: mechanism and therapeutic development. *Pharmacol. Res.* 169:105682. doi: 10.1016/j.phrs.2021.105682

Dan, R., Canetti, L., Keadan, T., Segman, R., Weinstock, M., Bonne, O., et al. (2019). Sex differences during emotion processing are dependent on the menstrual cycle phase. *Psychoneuroendocrinology* 100, 85–95. doi: 10.1016/j.psyneuen.2018.09.032

Derntl, B., Hack, R. L., Kryspin-Exner, I., and Habel, U. (2013). Association of menstrual cycle phase with the core components of empathy. *Horm. Behav.* 63, 97–104. doi: 10.1016/j.yhbeh.2012.10.009

Derntl, B., Kryspin-Exner, I., Fernbach, E., Moser, E., and Habel, U. (2008a). Emotion recognition accuracy in healthy young females is associated with cycle phase. *Horm. Behav.* 53, 90–95. doi: 10.1016/j.yhbeh.2007.09.006

Derntl, B., Windischberger, C., Robinson, S., Lamplmayr, E., Kryspin-Exner, I., Gur, R. C., et al. (2008b). Facial emotion recognition and amygdala activation are associated with menstrual cycle phase. *Psychoneuroendocrinology* 33, 1031–1040. doi: 10.1016/j.psyneuen.2008.04.014

Drotleff, B., Hallschmid, M., and Lämmerhofer, M. (2018). Quantification of steroid hormones in plasma using a surrogate calibrant approach and UHPLC-ESI-QTOF-MS/MS with SWATH-acquisition combined with untargeted profiling. *Anal. Chim. Acta* 1022, 70–80. doi: 10.1016/j.aca.2018.03.040

Fritz, M. S., and Mackinnon, D. P. (2007). Required sample size to detect the mediated effect. *Psychol. Sci.* 18, 233–239. doi: 10.1111/j.1467-9280.2007.01882.x

Gamsakhurdashvili, D., Antov, M. I., and Stockhorst, U. (2021a). Facial Emotion Recognition and Emotional Memory From the Ovarian-Hormone Perspective: a Systematic Review. *Front. Psychol.* 12:641250. doi: 10.3389/fpsyg.2021.641250

Gamsakhurdashvili, D., Antov, M. I., and Stockhorst, U. (2021b). Sex-hormone status and emotional processing in healthy women. *Psychoneuroendocrinology* 130:105258. doi: 10.1016/j.psyneuen.2021.105258

Gonzales, J. E., and Ferrer, E. (2016). Efficacy of methods for ovulation estimation and their effect on the statistical detection of ovulation-linked behavioral fluctuations. *Behav. Res. Methods* 48, 1125–1144. doi: 10.3758/s13428-015-0638-4

Guapo, V. G., Graeff, F. G., Zani, A. C. T., Labate, C. M., dos Reis, R. M., and Del-Ben, C. M. (2009). Effects of sex hormonal levels and phases of the menstrual cycle in the processing of emotional faces. *Psychoneuroendocrinology* 34, 1087–1094. doi: 10.1016/j.psyneuen.2009.02.007

Gur, R. C., Sara, R., Hagendoorn, M., Marom, O., Hughett, P., Macy, L., et al. (2002). A method for obtaining 3-dimensional facial expressions and its standardization for use in neurocognitive studies. *J. Neurosci. Methods* 115, 137–143. doi: 10.1016/S0165-0270(02)00006-7

Gurvich, C., Warren, A. M., Worsley, R., Hudaib, A. R., Thomas, N., and Kulkarni, J. (2020). Effects of Oral Contraceptive Androgenicity on Visuospatial and Social-Emotional Cognition: a Prospective Observational Trial. *Brain Sci.* 10:194. doi: 10.3390/brainsci10040194

Gutiérrez-García, A., and Calvo, M. G. (2017). Social anxiety and threat-related interpretation of dynamic facial expressions: sensitivity and response bias. *Pers. Individ. Dif.* 107, 10–16. doi: 10.1016/j.paid.2016.11.025

Hamstra, D. A., de Kloet, E. R., Quataert, I., Jansen, M., and Van der Does, W. (2017). Mineralocorticoid receptor haplotype, estradiol, progesterone and emotional information processing. *Psychoneuroendocrinology* 76, 162–173. doi: 10.1016/j.psyneuen.2016.11.037

Hamstra, D. A., de Kloet, E. R., Tollenaar, M., Verkuil, B., Manai, M., Putman, P., et al. (2016). Mineralocorticoid receptor haplotype moderates the effects of oral contraceptives and menstrual cycle on emotional information processing. *J. Psychopharmacol.* 30, 1054–1061. doi: 10.1177/0269881116647504

Hamstra, D. A., de Kloet, E. R., van Hemert, A. M., de Rijk, R. H., and Van der Does, A. J. W. (2015). Mineralocorticoid receptor haplotype, oral contraceptives and emotional information processing. *Neuroscience* 286, 412–422. doi: 10.1016/j.neuroscience.2014.12.004

Hamstra, D. A., De Rover, M., De Rijk, R. H., and Van der Does, W. (2014). Oral contraceptives may alter the detection of emotions in facial expressions. *Eur. Neuropsychopharmacol.* 24, 1855–1859. doi: 10.1016/j.euroneuro.2014.08.015

Hautzinger, M., Keller, F., and Kühner, C. (2006). *BDI-II. Beck depressions inventar revision—Manual.* Frankfurt, Germany: Harcourt Test Services.

Heathcote, L. C., Koopmans, M., Eccleston, C., Fox, E., Jacobs, K., Wilkinson, N., et al. (2016). Negative Interpretation Bias and the Experience of Pain in Adolescents. *J. Pain* 17, 972–981. doi: 10.1016/j.jpain.2016.05.009

Horstmann, G. (2003). What do facial expressions convey: feeling states, behavioral intentions, or actions requests? *Emotion* 3, 150–166. doi: 10.1037/1528-3542.3.2.150

Kamboj, S. K., Krol, K. M., and Curran, H. V. (2015). A specific association between facial disgust recognition and estradiol levels in naturally cycling women. *PLoS One* 10:e0122311. doi: 10.1371/journal.pone.0122311

Khatibi, A., Sharpe, L., Jafari, H., Gholami, S., and Dehghani, M. (2015). Interpretation biases in chronic pain patients: an incidental learning task. *Eur. J. Pain* 19, 1139–1147. doi: 10.1002/ejp.637

Kimmig, A.-C. S., Wildgruber, D., Wendel, S.-M. U., Sundström-Poromaa, I., and Derntl, B. (2021). Friend vs. Foe: cognitive and Affective Empathy in Women With Different Hormonal States. *Front. Neurosci.* 15:608768. doi: 10.3389/fnins.2021.608768

Laux, L., Glanzmann, P., Schaffner, P., and Spielberger, C. D. (1981). *Das State-Trait-Angstinventar (STAI) : theoretische Grundlagen und Handanweisung.* Weinheim: Beltz.

Leppänen, J. M., Milders, M., Bell, J. S., Terriere, E., and Hietanen, J. K. (2004). Depression biases the recognition of emotionally neutral faces. *Psychiatry Res.* 128, 123–133. doi: 10.1016/j.psychres.2004.05.020

Lewis, C. A., Kimmig, A.-C. S., Zsido, R. G., Jank, A., Derntl, B., and Sacher, J. (2019). Effects of Hormonal Contraceptives on Mood: a Focus on Emotion Recognition and Reactivity, Reward Processing, and Stress Response. *Curr. Psychiatry Rep.* 21:115. doi: 10.1007/s11920-019-1095-z

Li, W., and Cohen, L. H. (2003). Quantitation of Endogenous Analytes in Biofluid without a True Blank Matrix. *Anal. Chem.* 75, 5854–5859. doi: 10.1021/ac034505u

Louw-du Toit, R., Perkins, M. S., Hapgood, J. P., and Africander, D. (2017). Comparing the androgenic and estrogenic properties of progestins used in contraception and hormone therapy. *Biochem. Biophys. Res. Commun.* 491, 140–146. doi: 10.1016/j.bbrc.2017.07.063

Lovett, J. L., Chima, M. A., Wexler, J. K., Arslanian, K. J., Friedman, A. B., Yousif, C. B., et al. (2017). Oral contraceptives cause evolutionarily novel increases in hormone exposure: a risk factor for breast cancer. *Evol. Med. Public Health* 2017, 97–108. doi: 10.1093/emph/eox009

Maner, J. K., and Miller, S. L. (2014). Hormones and social monitoring: menstrual cycle shifts in progesterone underlie women's sensitivity to social information. *Evol. Hum. Behav.* 35, 9–16. doi: 10.1016/j.evolhumbehav.2013.09.001

Marsh, A. A., Ambady, N., and Kleck, R. E. (2005). The Effects of Fear and Anger Facial Expressions on Approach- and Avoidance-Related Behaviors. *Emotion* 5, 119–124. doi: 10.1037/1528-3542.5.1.119

Mier, D., Lis, S., Zygrodnik, K., Sauer, C., Ulferts, J., Gallhofer, B., et al. (2014). Evidence for altered amygdala activation in schizophrenia in an adaptive emotion recognition task. *Psychiatry Res.* 221, 195–203. doi: 10.1016/j.pscychresns.2013.12.001

Montoya, E. R., and Bos, P. A. (2017). How Oral Contraceptives Impact Social-Emotional Behavior and Brain Function. *Trends Cogn. Sci.* 21, 125–136. doi: 10.1016/j.tics.2016.11.005

Münkler, P., Rothkirch, M., Dalati, Y., Schmack, K., and Sterzer, P. (2015). Biased Recognition of Facial Affect in Patients with Major Depressive Disorder Reflects Clinical State. *PLoS One* 10:e0129863. doi: 10.1371/journal.pone.0129863

Ocampo Rebollar, A., Menéndez Balaña, F. J., and Conde Pastor, M. (2017). Comparison of affect changes during the ovulatory phase in women with and without hormonal contraceptives. *Heliyon* 3:e00282. doi: 10.1016/j.heliyon.2017.e00282

Osório, F. L., de Paula Cassis, J. M., Machado de Sousa, J. P., Poli-Neto, O., and Martín-Santos, R. (2018). Sex Hormones and Processing of Facial Expressions of Emotion: a Systematic Literature Review. *Front. Psychol.* 9:529. doi: 10.3389/fpsyg.2018.00529

Pahnke, R., Mau-Moeller, A., Junge, M., Wendt, J., Weymar, M., Hamm, A. O., et al. (2019). Oral Contraceptives Impair Complex Emotion Recognition in Healthy Women. *Front. Neurosci.* 12:1041. doi: 10.3389/fnins.2018.01041

Pearson, R., and Lewis, M. B. (2005). Fear recognition across the menstrual cycle. *Horm. Behav.* 47, 267–271. doi: 10.1016/j.yhbeh.2004.11.003

Peschard, V., and Philippot, P. (2017). Overestimation of threat from neutral faces and voices in social anxiety. *J. Behav. Ther. Exp. Psychiatry* 57, 206–211. doi: 10.1016/j.jbtep.2017.06.003

Petitti, D. B. (2003). Combination Estrogen–Progestin Oral Contraceptives. *N. Engl. J. Med.* 349, 1443–1450. doi: 10.1056/NEJMcp030751

Radke, S., and Derntl, B. (2016). Affective responsiveness is influenced by intake of oral contraceptives. *Eur. Neuropsychopharmacol.* 26, 1014–1019. doi: 10.1016/j.euroneuro.2016.03.004

Reed, S. C., Levin, F. R., and Evans, S. M. (2008). Changes in mood, cognitive performance and appetite in the late luteal and follicular phases of the menstrual cycle in women with and without PMDD (premenstrual dysphoric disorder). *Horm. Behav.* 54, 185–193. doi: 10.1016/j.yhbeh.2008.02.018

Rehbein, E., Hornung, J., Sundström Poromaa, I., and Derntl, B. (2021). Shaping of the Female Human Brain by Sex Hormones: a Review. *Neuroendocrinology* 111, 183–206. doi: 10.1159/000507083

Reitan, R. M. (1992). *Trail Making Test*. Tuscon, AZ: Reitan Neuropsychology Laboratory.

Richards, A., French, C. C., Calder, A. J., Webb, B., Fox, R., and Young, A. W. (2002). Anxiety-related bias in the classification of emotionally ambiguous facial expressions. *Emotion* 2, 273–287. doi: 10.1037/1528-3542.2.3.273

Rubin, L. H., Carter, C. S., Drogos, L., Jamadar, R., Pournajafi-Nazarloo, H., Sweeney, J. A., et al. (2011). Sex-specific associations between peripheral oxytocin and emotion perception in schizophrenia. *Schizophr. Res.* 130, 266–270. doi: 10.1016/j.schres.2011.06.002

Rubinow, D. R., Smith, M. J., Schenkel, L. A., Schmidt, P. J., and Dancer, K. (2007). Facial emotion discrimination across the menstrual cycle in women with premenstrual dysphoric disorder (PMDD) and controls. *J. Affect. Disord.* 104, 37–44. doi: 10.1016/j.jad.2007.01.031

Schmid, P. C., and Schmid Mast, M. (2010). Mood effects on emotion recognition. *Motiv. Emot.* 34, 288–292. doi: 10.1007/s11031-010-9170-0

Schmidt, K.-H., and Metzler, P. (1992). *Wortschatztest (WST)*. Weinheim: Beltz.

Shirazi, T. N., Rosenfield, K. A., Cárdenas, R. A., Breedlove, S. M., and Puts, D. A. (2020). No evidence that hormonal contraceptive use or circulating sex steroids predict complex emotion recognition. *Horm. Behav.* 119:104647. doi: 10.1016/j.yhbeh.2019.104647

Toffoletto, S., Lanzenberger, R., Gingnell, M., Sundström-Poromaa, I., and Comasco, E. (2014). Emotional and cognitive functional imaging of estrogen and progesterone effects in the female human brain: a systematic review. *Psychoneuroendocrinology* 50, 28–52. doi: 10.1016/j.psyneuen.2014.07.025

United Nations [UN] (2020). *World Fertility and Family Planning 2020: highlights*. New York: United Nations Department of Economic and Social Affairs.

Watson, D., Clark, L. A., and Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *J. Pers. Soc. Psychol.* 54, 1063–1070.

Wittchen, H.-U., Zaudig, M., and Fydrich, T. (1997). *Strukturiertes klinisches Interview für DSM-IV: achse I und II*. Göttingen: Hogrefe.

Yoon, K. L., and Zinbarg, R. E. (2008). Interpreting neutral faces as threatening is a default mode for socially anxious individuals. *J. Abnorm. Psychol.* 117, 680–685. doi: 10.1037/0021-843X.117.3.680

Zhang, W., Zhou, R., and Ye, M. (2013). Menstrual cycle modulation of the late positive potential evoked by emotional faces. *Percept. Mot. Skills* 116, 707–723. doi: 10.2466/22.27.Pms.116.3.707-723

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

HYPOTHESIS AND THEORY
published: 25 January 2022
doi: 10.3389/fnbeh.2021.807215

A Putative Role for Ubiquitin-Proteasome Signaling in Estrogenic Memory Regulation

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Sex steroid hormones such as 17β-estradiol (E_2) are critical neuromodulators of hippocampal synaptic plasticity and hippocampus-dependent memory in both males and females. However, the mechanisms through which E_2 regulates memory formation in both sexes remain unclear. Research to date suggests that E_2 regulates hippocampus-dependent memory by activating numerous cell-signaling cascades to promote the synthesis of proteins that support structural changes at hippocampal synapses. However, this work has largely overlooked the equally important contributions of protein degradation mediated by the ubiquitin proteasome system (UPS) in remodeling the synapse. Despite being critically implicated in synaptic plasticity and successful formation of long-term memories, it remains unclear whether protein degradation mediated by the UPS is necessary for E_2 to exert its beneficial effects on hippocampal plasticity and memory formation. The present article provides an overview of the receptor and signaling mechanisms so far identified as critical for regulating hippocampal E_2 and UPS function in males and females, with a particular emphasis on the ways in which these mechanisms overlap to support structural integrity and protein composition of hippocampal synapses. We argue that the high degree of correspondence between E_2 and UPS activity warrants additional study to examine the contributions of ubiquitin-mediated protein degradation in regulating the effects of sex steroid hormones on cognition.

Keywords: 17β-estradiol, memory, hippocampus, protein degradation, proteasome, ubiquitin

OPEN ACCESS

Edited by:
Stephanie V. Koebele, Arizona State University, United States

Reviewed by:
Thu Huynh, Midwestern University, United States
Anant Jain, Northwestern University, United States

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Specialty section:
This article was submitted to Behavioral Endocrinology, a section of the journal Frontiers in Behavioral Neuroscience

Received: *01 November 2021*
Accepted: *20 December 2021*
Published: *25 January 2022*

Citation:
Beamish SB and Frick KM (2022) A Putative Role for Ubiquitin-Proteasome Signaling in Estrogenic Memory Regulation. Front. Behav. Neurosci. 15:807215. doi: 10.3389/fnbeh.2021.807215

Abbreviations: E_2, 17β-estradiol; DH, dorsal hippocampus; BLA, basolateral amygdala; LTP, long-term potentiation; UPS, ubiquitin proteasome system; CA1, cornu ammonis 1; CA3, cornu ammonis 3; ER(s), estrogen receptor(s); ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; GPER, G-protein-coupled estrogen receptor; mGluRs, metabotropic glutamate receptors; mGluR1a, metabotropic glutamate receptor 1a; NMDA, N-methyl-D-aspartate; NMDAR(s), NMDA receptor(s); AMPA, α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ERK, extracellular signal-regulated kinase; PI3K, phosphoinositide 3-kinase; mTOR, mechanistic target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; JNK, C-jun N-terminal kinase; PKA, protein kinase A; CaMKII, calcium/calmodulin-dependent protein kinase II; PSD, post-synaptic density; CP, core particle; RP, regulatory particle; K48, lysine 48; Rpt6, regulatory particle triple-ATPase 6 subunit; cAMP, cyclic adenosine monophosphate; LTF, long-term facilitation; CREB, cAMP-response element binding protein; ATF4, activating transcription factor 4; β-lac, clasto-lactacystin β-lactone; Lac, lactacystin; TUBE, tandem ubiquitin binding entity.

INTRODUCTION

The sex steroid hormone 17β-estradiol (E_2) is the most potent and prevalent circulating estrogen and has been studied extensively in the field of hormones and cognition because of its ability to regulate hippocampal synaptic plasticity, spinogenesis, and the storage of long-term memories in males and females. In the early 1990's, seminal work demonstrated that dendritic spine density on pyramidal neurons in the CA1 region of the dorsal hippocampus (DH) fluctuates throughout the rat estrous cycle (Woolley et al., 1990), suggesting for the first time that endogenous sex steroid hormones, such as E_2, alter structural plasticity in brain regions relevant for cognition. Research since then has demonstrated that exogenous E_2 can increase CA1 dendritic spine density in ovariectomized rats and mice as quickly as 30 min following systemic injection (MacLusky et al., 2005; Inagaki et al., 2012) or DH infusion (Tuscher et al., 2016). Likewise, exogenous E_2 also increases intrinsic excitability, excitatory neurotransmission, and long-term potentiation (LTP) in hippocampal neurons (Wong and Moss, 1992; Woolley et al., 1997; Foy et al., 1999; Foy, 2001). These, among other E_2-induced enhancements in hippocampal synaptic function and spinogenesis, are thought to underlie E_2's ability to facilitate the consolidation of multiple hippocampus-dependent memories, including spatial, object recognition, fear, and social memories in both males and females (Tuscher et al., 2015; Taxier et al., 2020).

Despite the ample evidence that E_2 enhances hippocampal function and memory formation in both sexes, the neural mechanisms through which E_2 exerts its effects remain poorly understood. A growing body of research has examined how E_2 activates rapid cell-signaling events to drive increases in protein synthesis to support structural changes at hippocampal synapses (Sarkar et al., 2010; Fortress et al., 2013; Sellers et al., 2015; Tuscher et al., 2016). These studies suggest that local protein synthesis is necessary for E_2 to both increase CA1 spine density and enhance memory consolidation (Fortress et al., 2013; Tuscher et al., 2016). However, this work has largely overlooked the potentially vital and parallel contribution of protein degradation mediated by the ubiquitin proteasome system (UPS). In the UPS, proteins are tagged with ubiquitin and become substrates for degradation by the 26S proteasome. UPS-mediated protein degradation is a necessary counterpart to protein synthesis in driving synaptic plasticity and memory (Jarome and Helmstetter, 2013; Hegde, 2017) because it regulates the destruction of proteins that impose inhibitory constraints on synaptic remodeling, cell signaling, and gene transcription events across subcellular compartments of the neuron (Hegde, 2004).

In this review, we discuss the view that UPS-mediated protein degradation is an overlooked mechanism that may play a key role in regulating E_2's beneficial effects on hippocampal plasticity and memory. The review briefly summarizes E_2's effects on hippocampal function and then describes in some detail how the UPS functions to influence memory. Effects of E_2 on UPS activity are then discussed, as are the numerous ways in which E_2 and UPS signaling overlap to potentially regulate hippocampal function, which include regulation of the structural integrity and protein composition of hippocampal synapses. Finally, we offer some suggestions for future research.

ESTRADIOL AND HIPPOCAMPAL FUNCTION

E_2 has received considerable attention in the past three decades for its role as a powerful modulator of hippocampal synaptic morphology, plasticity, and long-term memory in males and females of various mammalian species (Frick, 2015; Hojo et al., 2015; Hamson et al., 2016; Taxier et al., 2020). However, the mechanisms through which E_2 promotes hippocampal synaptic function in both sexes remain largely unclear. Work to date has demonstrated that E_2 facilitates hippocampal synaptic plasticity and memory consolidation in ovariectomized female rodents by acting at the plasma membrane, where it interacts with membrane-bound estrogen receptors (ERs; Boulware et al., 2005, 2013) to initiate signal transduction events to rapidly modulate synaptic morphology (**Figure 1**). Estrogen receptors alpha and beta (ERα and ERβ) are the canonical intracellular estrogen receptors. Although ERα and ERβ are known for exerting genomic effects in the nucleus, they are also abundantly expressed throughout all segments of hippocampal neurons, including axon terminals, dendrites, and dendritic spines (Milner et al., 2000, 2005), where they are positioned near the plasma membrane to interact with metabotropic glutamate receptors (mGluRs) and other receptors to rapidly regulate synaptic signaling (Mitterling et al., 2010). Data from our lab and others indicate that activation of ERα or ERβ facilitates the consolidation of object recognition and spatial memories in ovariectomized rats and mice (Jacome et al., 2010; Kim and Frick, 2017; Hanson et al., 2018; Fleischer et al., 2021). Additional evidence suggests that glutamate receptors play key roles in mediating these memory-enhancing effects, as ERα and ERβ directly interact with mGluR1a to trigger extracellular signal-regulated kinase (ERK) signaling in the DH to facilitate object placement and object recognition memory consolidation in ovariectomized mice (Boulware et al., 2013). E_2 may also interact with the membrane-bound G-protein-coupled estrogen receptor (GPER) to enhance spatial and object recognition memories, however, GPER agonism appears to facilitate consolidation independently by phosphorylating c-Jun N-terminal kinase (JNK), not ERK (Kim et al., 2016), suggesting that GPER and E_2 use different cell-signaling pathways to regulate memory formation. Additional signaling mechanisms that regulate E_2's memory-enhancing and spinogenic effects depend on the rapid activation of NMDA receptors (NMDARs) and tyrosine receptor kinase B (TrkB) to trigger downstream signaling cascades including calcium/calmodulin-dependent protein kinase II (CaMKII), protein kinase A (PKA), ERK, and phosphoinositide 3-kinase (PI3K; Murakami et al., 2006; Fernandez et al., 2008; Lewis et al., 2008; Fan et al., 2010; Gross et al., 2021).

E_2-induced enhancements in hippocampal synaptic plasticity and memory formation have largely been attributed to its rapid effects on CA1 dendritic spine density (Mukai et al., 2007; Inagaki et al., 2012; Tuscher et al., 2016; Kim et al., 2019). E_2 can promote hippocampal LTP by regulating actin

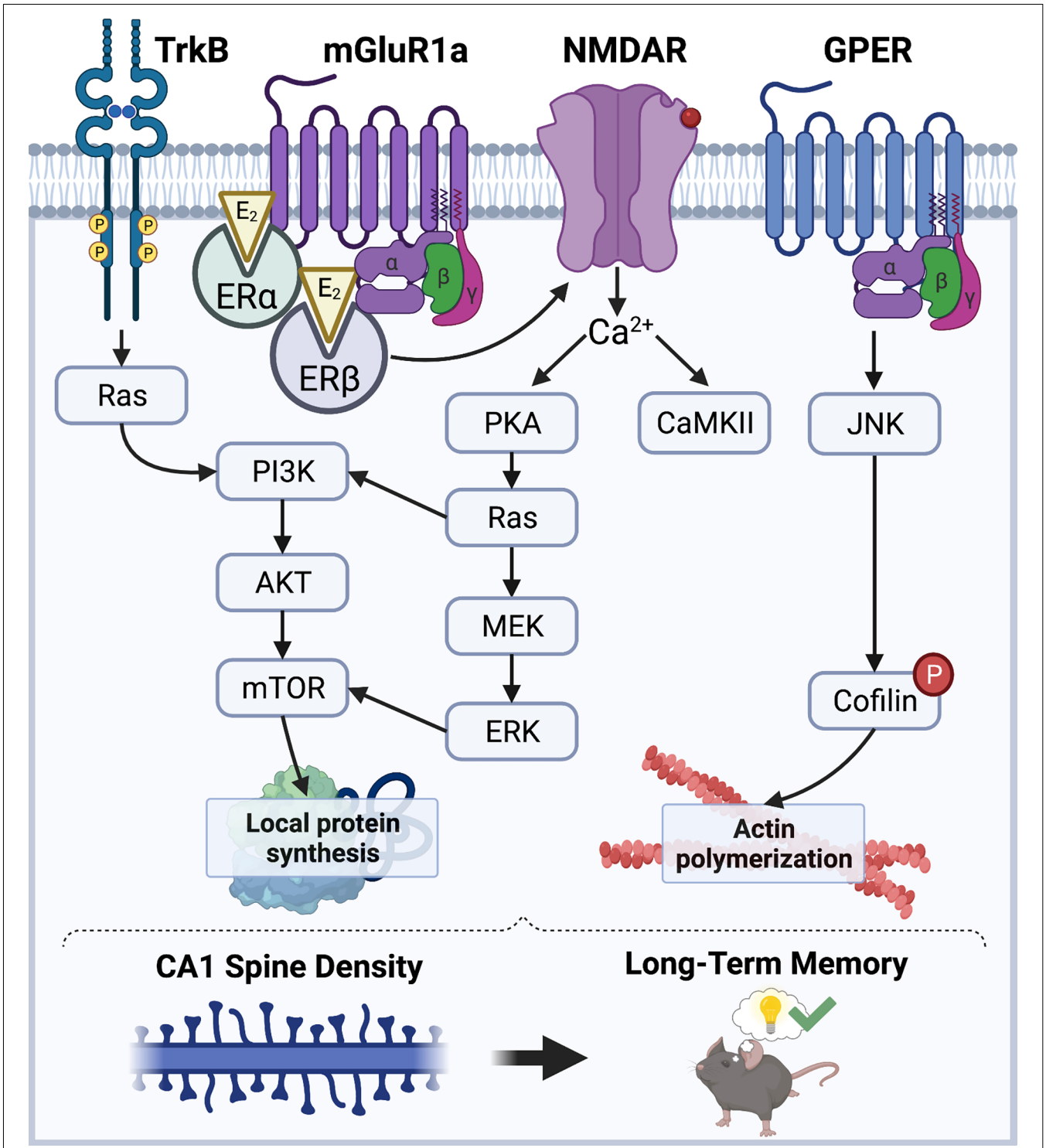

FIGURE 1 | Schematic diagram illustrating a model of the mechanisms through which E_2 regulates CA1 spine density and memory. E_2 acts *via* membrane-associated receptors like mGluRs, GPER, and TrkB, as well as ion channels like NMDAR, to stimulate cell-signaling kinases that promote local protein synthesis and actin polymerization. ERα and ERβ promote protein synthesis *via* ERK and mTOR signaling, whereas GPER promotes actin polymerization through JNK signaling. Illustration created using BioRender.com.

polymerization (Kramár et al., 2009), which is necessary for spine growth and maturation (Penzes and Cahill, 2012). In the DH of ovariectomized mice, E_2 rapidly and transiently increases phosphorylation of cofilin (Kim et al., 2019), which leads to actin stabilization and polymerization (Chen et al., 2007; Babayan and Kramár, 2013). E_2-induced increases in CA1 spine density

also require the rapid synthesis of new proteins within the postsynaptic density (PSD). Several studies have demonstrated that E_2 can activate ERK and Akt signaling to promote local protein synthesis by activating mechanistic target of rapamycin (mTOR) and mTOR complex 1 (mTORC1) signaling (Akama and McEwen, 2003; Sarkar et al., 2010; Fortress et al., 2013; Briz and Baudry, 2014). Interestingly, E_2 increases local protein synthesis of the synaptic scaffolding molecule PSD-95 in cultured neurons in an ERα-, Akt-, and mTOR-dependent manner (Akama and McEwen, 2003), suggesting that these newly synthesized proteins contribute directly to the expanding dendritic architecture. Furthermore, work from our lab suggests that E_2 acts at membrane-localized ERs in the DH to activate ERK and mTORC1 signaling, which is necessary for E_2 to increase CA1 spine density and to enhance object recognition and object placement memory consolidation in ovariectomized female mice (Boulware et al., 2013; Fortress et al., 2013; Tuscher et al., 2016), thereby supporting the hypothesis that local protein synthesis is necessary for E_2-induced spinogenesis and memory.

Thus, evidence to date suggests that E_2 promotes hippocampus-dependent memory by inducing rapid membrane-initiated cell-signaling events that increase CA1 dendritic spine density by reorganizing components of the cytoskeleton, as well as driving increases local protein synthesis. These findings reflect the field's historic focus on identifying the signaling mechanisms that promote protein production to support structural changes at hippocampal synapses. However, this attention on protein synthesis has caused researchers to overlook the potential contributions of protein degradation as an equal, but opposite, regulator of E_2's effects on memory. As will be discussed below, protein degradation mediated by the UPS plays a vital role in hippocampal plasticity and memory by structurally remodeling the synapse and degrading proteins that exert inhibitory constraints to synaptic plasticity. We show that the signaling mechanisms that facilitate proteasomal protein degradation overlap considerably with those that regulate E_2's effects on CA1 spine density and memory, suggesting compelling reasons to explore protein degradation mechanisms as key mediators of E_2's effects on memory.

THE UBIQUITIN-PROTEASOME SYSTEM

The UPS is the primary mechanism for degrading proteins within mammalian cells (Glickman and Ciechanover, 2002). The UPS is comprised of a network of signaling molecules that identify, tag, and degrade substrate proteins within the cell (**Figure 2**). In this system, proteins are first targeted for degradation by the covalent attachment of the small protein modifier ubiquitin *via* the coordinated actions of three separate classes of ubiquitin ligases (E1, E2, and E3). The activating enzyme, E1, binds to and activates free ubiquitin in an ATP-dependent reaction. E1 then transfers activated ubiquitin to an E2 ligase that carries the active ubiquitin to the substrate protein. The substrate proteins to be degraded are recognized by specific E3 ligases which identify degradation signals emitted by the substrate proteins themselves (Nandi et al., 2006). The E2 ligase then binds to the E3-substrate complex, enabling the transfer of activated ubiquitin to the substrate protein (**Figure 2A**). The ubiquitination process is highly complex and involves hundreds of different ligases that interact in a combinatorial manner to achieve substrate specificity. In the human genome, the coding genes for each ligase total 1–2 for E1, 25–30 for E2, and more than 600 for E3. Although substrate specificity is primarily achieved by the vast number of E3 ligases, specificity is also achieved by limited interactions of E2-E3 proteins. For example, E2s bind to numerous different E3s, but not every E3 can interact with every E2. Therefore, the E2s, E3s, and substrate proteins come together to create a unique combinatorial code for the ubiquitin reaction (Hegde, 2017). After the first ubiquitin is bound to the substrate protein, another ubiquitin becomes attached to an internal lysine residue on the first ubiquitin, eventually forming a polyubiquitin chain. Substrate proteins can acquire several different types of ubiquitin "tags," however, those that receive a lysine-48 (K48) polyubiquitin tag become targets for degradation by the 26S proteasome complex (Glickman and Ciechanover, 2002; Musaus et al., 2020).

The 26S proteasome is a multi-subunit structure comprised of a cylindrical 20S core particle (CP) flanked by one or two 19S regulatory particles (RP; Tanaka, 2009). The 19S RP contains an outer lid comprised of a circular ring of non-ATPase subunits where polyubiquitinated protein initially binds (**Figure 2B**). When a polyubiquitinated substrate is bound to the outer segment of the 19S CP, the polyubiquitinated chain becomes hydrolyzed by deubiquitinating enzymes so that the ubiquitin molecules can be reused in the system. The 19S RP also contains an inner cap segment that consists of a circular ring of six ATPase subunits that, when activated, are responsible for initiating unfolding and translocating the protein into the catalytic 20S core of the proteasome. The 20S CP is comprised of two outer rings of α-subunits and two inner rings of β-subunits. The outer α-subunits are connected to the inner ATPase subunits of the 19S cap which gives the proteasome its gate-like mechanism of action. When the 19S ATPase subunits become activated, the α-subunits enable the substrate to pass through its gated channel. However, when a polyubiquitinated substrate is not bound to the proteasome, the α-subunit gate remains closed to prevent the degradation of intact protein, as well as the release of partially degraded substrate protein from the 20S CP. The substrate is then degraded by various catalytic activities (i.e., chymotrypsin-like, trypsin-like, and caspase-like activity) of the 20S CP, which are exerted by the inner β5, β2, and β1 subunits, respectively. The resulting peptide fragments are then expelled through the base of the proteasome.

Regulation of Proteasome Subunits by PKA and CaMKII

As illustrated in **Figure 2**, proteasomes are multi-subunit complexes that must be assembled to exert their chymotrypsin-like, trypsin-like, and caspase-like proteolytic activities. Proteasome subunit phosphorylation is a principal mechanism that regulates proteolysis by altering proteasome assembly, localization, or its catalytic activity (Hegde, 2004; Nandi et al., 2006). Phosphorylation of the 19S *r*egulatory *p*article *t*riple-ATPase *6* subunit at the serine 120 residue (Rpt6 at Ser^{120},

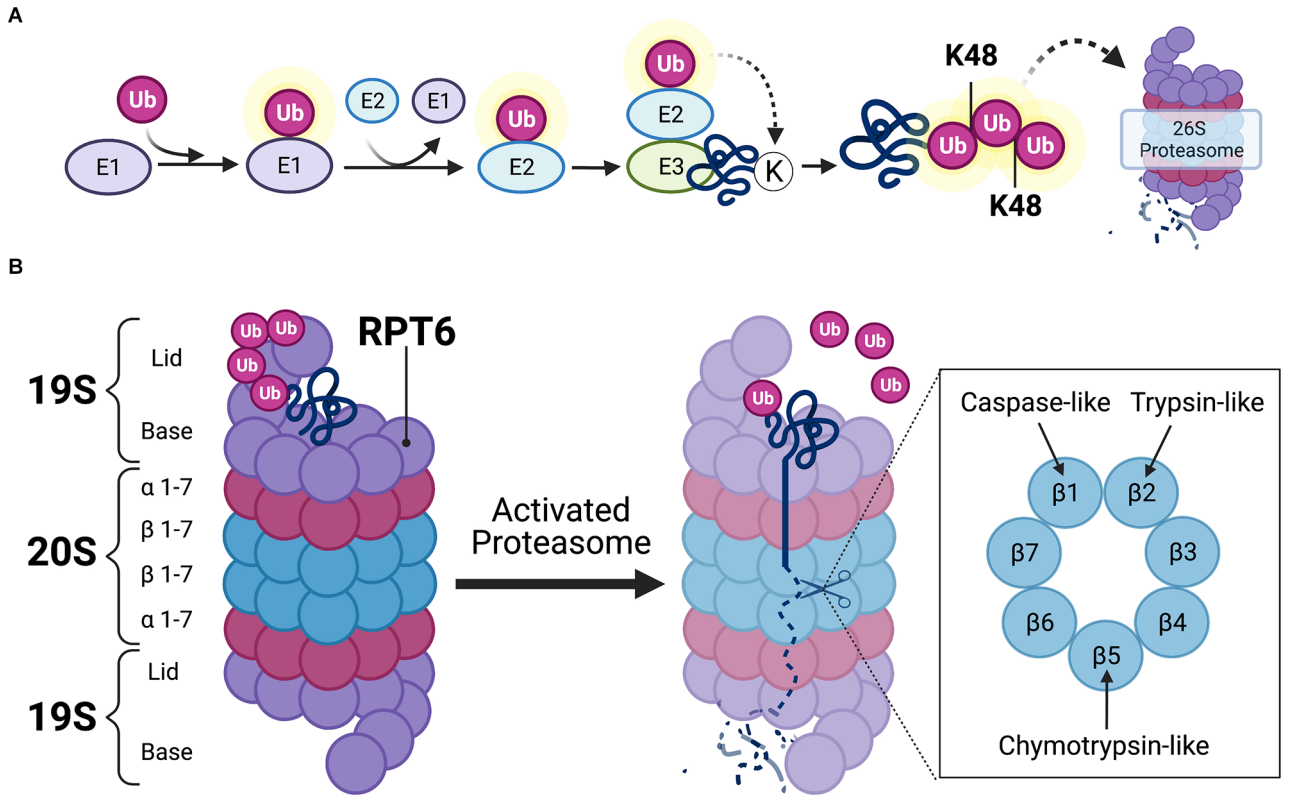

FIGURE 2 | Ubiquitin-mediated proteolysis. **(A)** Schematic illustration of ubiquitin targeting pathway. Free ubiquitin molecules are activated by E1-activating ligases *via* ATP hydrolysis. Activated ubiquitin is then bound to E1, which then transfers the active ubiquitin molecule to E2 carrier enzymes. E3 ligases bind to target substrates destined for degradation. E2 enzymes localize to the E3-substrate complex, enabling the transfer of active ubiquitin to substrate protein. Additional ubiquitin molecules attach at lysine-48 (K48) residues on subsequent ubiquitin, eventually forming a K48-linked polyubiquitin chain that is destined to the 26S proteasome for proteasomal degradation. **(B)** Detailed schematic view of the 26S proteasome. The 26S proteasome contains one or two 19S caps and a 20S core. K48-linked polyubiquitinated protein binds to the 19S regulatory cap at ubiquitin binding sites. Upon activation of the proteasome, which usually occurs at the 19S subunit Rpt6 (Ser^{120} residue), the polyubiquitin chain becomes hydrolyzed and the substrate protein unfolds to allow β subunits in the 20S core to hydrolyze proteins *via* caspase-, trypsin-, and chymotrypsin-like activities. Illustration created using BioRender.com.

hereafter referred to as Rpt6; **Figure 2B**) is the most commonly studied phosphorylation site in the context of synaptic plasticity and memory because this subunit is targeted by PKA and CaMKII. Because these kinases are regulated by E_2, they may mediate its effects on UPS signaling. As such, the regulation of UPS function by PKA and CaMKII will be discussed below.

Thus far, evidence to show that PKA regulates proteasome activity comes from studies of non-neuronal cells or tissues not typically associated with memory. Forskolin, a compound that elevates cyclic AMP (cAMP) levels and activates PKA, stimulates chymotrypsin-like and trypsin-like peptidase activity in nuclear extracts from cultured normal rat kidney cells by phosphorylating Rpt6 (Zhang et al., 2007). Moreover, this forskolin-induced increase in proteasome activity could be blocked pharmacologically, indicating that PKA-dependent phosphorylation is responsible for the increases in peptidase activity (Zhang et al., 2007). Phosphorylation of Rpt6 by the cAMP/PKA pathway is also critical in regulating the neuropathology of Huntington's disease. For example, striatal cells expressing mutant Huntington protein have markedly low PKA activity that prevents phosphorylation of Rpt6 (Lin et al., 2013). Expression of phosphomimetic Rpt6 rescued motor impairments and reduced mutant Huntington protein aggregates in the striatal synaptosome fraction from Huntington's mice (Lin et al., 2013), suggesting an important role for Rpt6 in both pathology and behavior. Additional evidence suggests that PKA regulates proteasome activity by increasing transcriptional levels of proteasome subunits. Artificially increasing cAMP levels in rat spinal cord neurons not only increases chymotrypsin-like activity but also increases mRNA and protein levels of Rpt6 and the 20S proteasome subunit β5 in a PKA-dependent manner (Myeku et al., 2012).

Rpt6 can also be phosphorylated by CaMKIIα in a neuronal activity-dependent manner (Bingol et al., 2010), which then leads to proteasome trafficking into dendritic spines. In cultured hippocampal neurons, synaptic activity causes autophosphorylated CaMKIIα to act as a postsynaptic scaffolding molecule by physically binding to 19S and 20S proteasome complexes and translocating them from dendritic shafts to dendritic spines, where they co-localize to the actin cytoskeleton in an NMDAR-dependent manner (Bingol and Schuman, 2006;

Bingol et al., 2010). These findings are consistent with other work demonstrating that proteasome activity in dendrites of cultured hippocampal neurons is regulated by synaptic activity and that overexpression of a constitutively active form of CaMKII activates proteasomes by phosphorylating Rpt6 (Djakovic et al., 2009). Accordingly, homeostatic scaling of synaptic strength in rodent hippocampal slices was impaired by altered CaMKII-dependent phosphorylation of Rpt6 (Djakovic et al., 2012). CaMKII-induced phosphorylation of Rpt6 also regulates hippocampal spinogenesis, as expression of a phospho-dead mutant of Rpt6 prevented activity-induced hippocampal spine outgrowth on postsynaptic neurons (Hamilton et al., 2012). Furthermore, Rpt6 phosphorylation is also increased in the amygdala of male rats following contextual fear conditioning (Jarome et al., 2013), suggesting that activity-induced Rpt6 phosphorylation may promote the structural plasticity underlying memory formation. Interestingly, the learning-induced increase in Rpt6 phosphorylation depends on CaMKIIα, but not PKA, activity in male rat amygdala tissue (Jarome et al., 2013), indicating greater involvement of CaMKII in mediating the proteasome activity stimulated by learning. It should be noted that one recent study using knock-in mouse models to block or mimic Rpt6 activity indicated no involvement in measures of plasticity, spine growth, or fear conditioning (Scudder et al., 2021), however, compensatory mechanisms may have mitigated the loss of a functional Rpt6 Ser^{120} subunit (Lokireddy et al., 2015; Guo et al., 2016). Overall, these findings suggest that CaMKIIα-dependent phosphorylation of Rpt6 may be critical for regulating the proteasomal protein degradation involved in synaptic remodeling and long-term memory.

PROTEIN DEGRADATION AND SYNAPTIC PLASTICITY

Substantial evidence supports a role for protein degradation as a critical regulator of activity-dependent synaptic plasticity (Fioravante and Byrne, 2010; Jarome and Helmstetter, 2013; Hegde, 2017). Initial studies conducted in *Aplysia* investigated how cAMP-dependent PKA remained persistently active following long-term facilitation (LTF) when levels of cAMP were depleted. This work revealed that the increased pool of PKA regulatory subunits became ubiquitinated and degraded by the proteasome, leaving the catalytic subunits intact and persistently active (Hegde et al., 1993). Additional work in *Aplysia* demonstrated that LTF increased expression of the ubiquitin C-terminal hydrolase (Ap-uch), which is responsible for deubiquitinating proteasome-bound protein, thereby increasing proteasome activity within neurons. Inhibiting the expression of Ap-uch blocked the induction of LTF (Hegde et al., 1997). Similarly, the injection of proteasome inhibitors prevented induction of LTF in *Aplysia* (Chain et al., 1999).

Later studies in hippocampal slices from male rats examined the extent to which the proteasome regulates protein-synthesis-dependent late-phase LTP (L-LTP). L-LTP is comprised of an induction phase that requires translation of pre-existing mRNAs at dendrites, and a maintenance phase that requires *de novo* transcription in the nucleus (Kelleher et al., 2004). In hippocampal slices, L-LTP can be induced by forskolin, thereby supporting a key role for PKA. Interestingly, proteasome inhibition has different effects on forskolin-induced L-LTP based on the location of proteasome inhibition. For example, proteasome inhibition at dendrites enhanced the induction phase of L-LTP, whereas proteasome inhibition at the nucleus blocked the maintenance phase of L-LTP (Dong et al., 2008). These findings led to speculation that proteasome inhibition enhances L-LTP induction by preventing the degradation of proteins synthesized from pre-existing mRNAs, but blocks L-LTP maintenance by preventing the degradation of transcriptional repressors in the nucleus. Subsequent work supported this hypothesis, as inhibition of ERK and mTORC1 signaling in dendrites prevented proteasome inhibition from enhancing the induction phase of L-LTP (Dong et al., 2014). These novel findings showed that the proteasome can paradoxically control local protein translation by regulating the activity of translational activators and repressors throughout the induction and maintenance phases of L-LTP.

Other studies have assessed the effects of proteasome inhibition on L-LTP and found that the maintenance phase, but not the induction phase, of L-LTP was blocked entirely in hippocampal slices from male rats when either protein synthesis or protein degradation was inhibited. These impairments were rescued if protein synthesis and protein degradation inhibitors were applied at the same time (Fonseca et al., 2006; Karpova et al., 2006). These findings conflict with the previous reports discussed above that proteasome inhibition enhanced the induction phase of L-LTP (Dong et al., 2008, 2014), which could result from their use of a lower proteasome inhibitor dose or a less-specific proteasome inhibitor. Nonetheless, these findings collectively demonstrate that a delicate balance between protein synthesis and protein degradation must exist to support L-LTP and maintain long-lasting synaptic plasticity.

This work also began to illustrate that the proteasome regulates hippocampal synaptic plasticity by targeting diverse substrate proteins throughout neuronal synaptic and nuclear compartments (Hegde et al., 2014). For example, the activity-induced translocation of proteasomes to dendritic spines causes other structural changes that influence synaptic plasticity. Synaptic activity in bicuculline-treated cortical neurons significantly increases the number of ubiquitinated proteins in the synapse and PSD (Ehlers, 2003). In particular, the post-synaptic scaffolding proteins Shank, GKAP, and AKAP79 are ubiquitinated and degraded in response to synaptic activity, an effect that is abolished following periods of inactivity (Ehlers, 2003). These cytoskeletal and scaffolding proteins become targets for proteasomal degradation following synaptic activity because they directly support glutamate receptors within the PSD (Sheng and Pak, 2000; Sheng and Kim, 2002). AMPA receptor internalization following NMDAR activation in hippocampal neurons depends on the ubiquitination and subsequent degradation of PSD-95 (Colledge et al., 2003; Patrick et al., 2003; Bingol and Schuman, 2004). Protein degradation also appears to regulate spine shape, as the activity-inducible kinase SNK is targeted to dendritic spines and is responsible

for initiating the degradation of the PSD protein SPAR (Pak and Sheng, 2003). SPAR was also shown to be degraded by the proteasome in an NMDA-dependent manner following LTP induction in CA1 neurons from male rats (Chen et al., 2012). As such, UPS activity provides localized protein degradation within synapses to rapidly remodel the spine morphology that supports enhanced plasticity.

Finally, ubiquitin-mediated proteasomal protein degradation can also influence plasticity by degrading nuclear proteins that inhibit gene transcription. For example, induction of LTF in *Aplysia* depends on the ubiquitination and proteasomal degradation of the cAMP-response element binding protein (CREB) repressor protein activating transcription factor 4 (ATF4; Upadhya et al., 2004). Furthermore, proteasome inhibition following chemically-induced LTP prevents the ubiquitination and degradation of ATF4, effectively preventing the transcription of CREB-inducible genes, such as brain-derived neurotrophic factor (*bdnf*, Dong et al., 2008). More recent work has shown that the E3 ligase β-transducin repeat containing protein (β-TrCP) ubiquitinates ATF4 in a PKA-dependent manner (Smith et al., 2020). Therefore, the proteasome not only serves to degrade synaptic proteins but also to regulate gene expression by degrading machinery that exerts repressive effects on the transcription of genes critical for synaptic plasticity and memory.

PROTEIN DEGRADATION AND LONG-TERM MEMORY

Numerous studies have demonstrated that proteasomal protein degradation is not only critical for activity-dependent synaptic plasticity but also plays an important role in regulating long-term memory. These studies have predominantly used proteasome inhibitors such as lactacystin (lac) and clasto-lactacystin β-lactone (β-lac) to block the catalytic activities of the proteasome (Ōmura and Crump, 2019). Pharmacological blockade of proteasome activity has enabled researchers to examine the extent to which proteasome activity is required for the consolidation and reconsolidation phases of memory storage. The behavioral work to date has primarily examined the involvement of protein degradation in fear learning among male rats (for comprehensive reviews, see Jarome and Helmstetter, 2013, 2014; Hegde, 2017). However, recently published data suggest differences in how males and females regulate and engage in proteasome activity, which will be discussed in more detail below.

Initial work demonstrated that immediate post-training bilateral infusion of lac into the CA1 region of the DH caused full retrograde amnesia for one-trial inhibitory avoidance learning in male rats (Lopez-Salon et al., 2001). Subsequently, bilateral infusion of β-lac into CA1 was shown to impair the extinction, but not consolidation or reconsolidation, of contextual fear memory in male rats (Lee et al., 2008), suggesting a potentially complex role for CA1 protein degradation in fear learning. Protein degradation in other brain regions also contributes to fear learning, as bilateral infusion of lac in the amygdala and insular cortex impaired consolidation of conditioned taste aversion memories (Rodriguez-Ortiz et al., 2011). Moreover, immediate post-training infusion of β-lac into the amygdala impaired the consolidation of both auditory and contextual fear memories (Jarome et al., 2011). Although the same study observed NMDA-dependent increases in polyubiquitination of RISC factor MOV10 and scaffolding protein Shank, β-lac infusion into the amygdala following memory retrieval did not impair auditory or contextual fear memory reconsolidation, but did rescue impairments caused by infusion of protein synthesis inhibitor anisomycin (Jarome et al., 2011), suggesting that protein degradation controls destabilization of retrieved fear memories in the amygdala. Subsequent findings also demonstrated that β-lac in male rats impaired trace fear conditioning when infused into the prefrontal cortex, and impaired contextual memory in a context-preexposure facilitation paradigm when infused into the dorsal and ventral hippocampus (Reis et al., 2013; Cullen et al., 2017). Interestingly, recent work investigating AMPA receptor (AMPAR) exchange at synapses in the amygdala of male rats demonstrated that proteasome activity is critical for the endocytosis of calcium-impermeable AMPARs with calcium-permeable AMPARs during the destabilization phase of reconsolidation (Ferrara et al., 2019). Together, these data suggest an important role for UPS activity in numerous brain regions in mediating fear learning among male rats.

A requirement for proteasomal protein degradation has also become evident for spatial and object recognition memories. Infusion of lac into the hippocampal CA3 subregion of male mice significantly impaired spatial memory consolidation and reconsolidation in Morris water maze when infused immediately, but not 3 h, post-training (Artinian et al., 2008). These findings provided the first demonstration that different phases of non-aversive memory formation require proteasomal protein degradation. Consistent with this conclusion, another study utilizing an object rearrangement task in male mice assessed whether proteasome activity is required for incorporating partially modified information into a pre-existing memory, and found that infusions of β-lac into area CA1 following re-exposure to the context with switched objects disrupted the initial consolidation of spatial information (Choi et al., 2010). Similarly, object recognition memory consolidation in male rats was disrupted by proteasome inhibition, as the infusion of lac into CA1 immediately and 3 h, but not 1.5 or 6 h, post-training significantly reduces the time spent with a novel object during testing (Figueiredo et al., 2015). However, these findings are inconsistent with other work demonstrating that post-training infusion of β-lac into CA1 did not impair consolidation or reconsolidation of object recognition memory in male rats (Furini et al., 2015). Interestingly, however, β-lac infusion reversed reconsolidation impairments caused by anisomycin (Furini et al., 2015). The discrepancies between this and the Figueiredo et al. (2015) study could result from the administration of different proteasome inhibitors at different doses. Nevertheless, the balance of studies conducted so far suggests a potential role for hippocampal proteasomal protein degradation in spatial and object memories among males.

Collectively, these studies suggest that protein degradation mediated by the UPS is essential for different forms of learning across numerous brain regions. Moreover, UPS activity and hippocampal synaptic plasticity are regulated by protein kinases that are also involved in E_2's effects on memory consolidation. This overlap suggests compelling reasons to suspect that E_2's well-documented effects on spatial and object recognition memory consolidation might be regulated in part by proteasomal protein degradation.

EMERGING SEX DIFFERENCES IN UPS ACTIVITY AND MEMORY

Several recent studies have documented notable sex differences in the regulation of, and requirement for, protein degradation following fear memory formation in the basolateral amygdala (BLA) and CA1 region of the DH (Devulapalli et al., 2019, 2021; Martin et al., 2021). This work has also revealed novel sex differences in the number and identity of substrate proteins targeted for proteasomal degradation across BLA and DH tissues (Farrell et al., 2021; Martin et al., 2021).

The first study to examine putative sex differences in UPS activity related to memory showed that CaMKII and PKA differentially regulate proteasome activity in male and female rats across subcellular compartments following contextual fear conditioning (Devulapalli et al., 2019). Tissue in these studies was fractionated to isolate synaptic, cytosolic, nuclear compartments. Chymotrypsin activity, the predominant form of proteasome activity, was decreased in synaptic fractions following CaMKII, but not PKA, inhibition in the male DH, whereas chymotrypsin activity was increased in synapses following CaMKII, but not PKA, inhibition in the female DH. These data suggest that proteasome activity is not only differentially regulated CaMKII and PKA activity across subcellular compartments but is also regulated in a sex-specific manner. Moreover, the regulatory effects of CaMKII and PKA also differed across brain regions. For example, nuclear chymotrypsin activity was decreased following PKA, but not CaMKII, inhibition in the male BLA, whereas nuclear chymotrypsin activity was decreased following CaMKII, but not PKA, inhibition in the female BLA (Devulapalli et al., 2019). These findings are noteworthy because they not only provide support for the idea that proteasome activity can be differentially regulated across subcellular compartments (Upadhya et al., 2006) but also highlight the differences that exist between males and females in the regulation of proteasome function by signaling kinases.

More recently, males and females were found to differ in their engagement and requirement for UPS activity following contextual fear conditioning. For example, trained male, but not female, rats exhibited increased markers of UPS activity, including upregulated proteasome activity and amount of K48 polyubiquitinated proteins, in nuclear BLA extracts relative to behaviorally naïve males (Devulapalli et al., 2021). Interestingly, both naïve and trained females displayed elevated UPS activity relative to naïve males, suggesting higher baseline levels of UPS activity in nuclear BLA extracts among females relative to males (Devulapalli et al., 2021). This finding could have indicated that learning does not engage the UPS in females, however, CRISPR-dCas9-mediated knockdown of UPS activity in BLA was found to impair fear memory in both sexes (Devulapalli et al., 2021), suggesting that males and females differ in their engagement, but not requirement for, UPS activity in the BLA for successful fear memory formation. This conclusion was supported by additional data showing that female rats had elevated levels of free ubiquitin and increased expression of the ubiquitin coding gene *Uba52* in BLA nuclear extracts relative to males (Devulapalli et al., 2021), indicating inherently higher numbers of ubiquitinated targets in females relative to males. Furthermore, naïve female rats exhibited increased 5-hydroxymethylation in the promoter region of the ubiquitin coding gene *Uba52*, suggesting that this gene is more actively transcribed in females than in males. Nevertheless, CRISPR-dCas9-mediated silencing of the ubiquitin coding gene *Uba52* and the proteasome subunit *Psmd14* in the BLA of male and female rats reduced baseline protein degradation levels and impaired contextual fear memory, whereas increasing BLA baseline protein degradation facilitated fear memory in both sexes (Devulapalli et al., 2021). Thus, despite sex differences in baseline ubiquitination in the BLA, fear memory formation in both males and females appears to depend on UPS activity.

Surprisingly, the sex-specific activation of UPS activity by contextual fear conditioning differs strikingly in the DH. In DH nuclear extracts, learning-induced increases in UPS activity were observed in female, but not male, rats (Martin et al., 2021). Moreover, CRISPR-mediated knockdown of UPS activity in DH CA1 blocked fear memory in females, but not males (Martin et al., 2021). These data suggest that females require UPS activity in the DH to form a contextual fear memory, whereas males do not, which contrasts with the BLA in which both sexes require UPS activity for memory formation. Thus, for fear learning, the involvement of protein degradation appears to differ not only by sex by also by brain region across the fear circuit.

Other recent work examined sex differences in UPS activity at 3, 15, and 22 months of age in response to trace fear conditioning to a tone. Age-related memory impairments in trace fear retrieval in male, but not female rats were associated with decreased Rpt6 phosphorylation and increased K48 polyubiquitination in synaptic fractions of BLA tissue (Dulka et al., 2021). Specifically, 22-month-old male rats exhibited impaired memory retrieval 24 h after training, whereas females of all ages displayed relatively poor retrieval at all ages. Among male rats, retrieval-induced Rpt6 phosphorylation was significantly reduced in 22-month-olds relative to 3-month-olds in the BLA, but no changes were observed in the DH or medial prefrontal cortex. Interestingly, 22-month-old females exhibited lower Rpt6 phosphorylation in the cortex relative to 3-month-olds, but no retrieval-induced changes in the other two brain regions. With respect to K48 polyubiquitination, a similar regional pattern was observed, with 22-month-old males having increased levels in the BLA, whereas females had higher levels in the cortex. suggesting

that the memory impairments observed in aged males may arise in part by dysregulated proteasome signaling that results in an accumulation of polyubiquitinated substrate proteins. Together, these findings suggest that the role of UPS activity in memory may differ not only by sex and brain region but by age as well.

To date, most studies examining ubiquitin-proteasome function in the context of learning and memory have examined the factors that regulate proteasome activity itself, leaving unanswered questions about which proteins are targeted for proteasomal degradation following learning. Exciting new work sheds light on the number and identity of protein substrates targeted for proteasomal protein degradation following contextual fear conditioning in both sexes. These studies used an unbiased assay that focuses on K48-specific ubiquitination because this particular lysine tag marks proteins for degradation. This novel K48-specific tandem ubiquitin binding entity (K48-TUBE) liquid chromatography-mass spectrometry analysis captures K48-polyubiquitnated proteins with high affinity, thereby protecting them from proteasomal degradation and deubiquitination, permitting efficient purification, and eliminating non-specific binding. This method has revealed that the number of proteins in the BLA in which K48 polyubiquitination was increased or decreased in response to fear learning overlaps very little between males and females (Farrell et al., 2021). Interestingly, fear learning promoted protein degradation in both sexes, but more so in females, which is in contrast to previous reports that nuclear UPS activity in the BLA was not increased by fear conditioning (Devulapalli et al., 2021); these discrepancies likely result from the increased sensitivity and specificity of the K48-TUBE assay to detect polyubiquitinated proteins relative to immunoblotting (Farrell et al., 2021). In the DH, contextual fear conditioning increased K48 polyubiquitin targeting of only three protein targets in the CA1 of females, whereas learning did not increase K48 polyubiquitination of any proteins in males (Martin et al., 2021). This result is perhaps surprising but is consistent with the finding that contextual fear conditioning did not increase UPS activity in the CA1 of males (Martin et al., 2021). An ingenuity pathway analysis of proteins in the female CA1 showed that fear learning targets the ribosomal binding protein ribosomal RNA processing 12 (RRP12) and chaperone protein heat shock protein 40 (HSP40) for degradation, which has implications for the regulation of intracellular signaling and DNA damage response (Martin et al., 2021). Collectively, these initial findings indicate little overlap between the sexes in how learning influences the targeting of proteins for proteasomal protein degradation in the BLA and CA1 and suggest that a variety of different cellular processes are regulated in a sex-, brain region-, and degradation-specific manner to support the formation of fear memories.

These recent studies not only uncover key sex differences in the regulation of, and requirement for, ubiquitin-proteasome activity in different brain regions for the formation of fear memories, but also reveal sex differences in the number and identity of proteins targeted for proteasomal degradation following learning. It is tempting to speculate, therefore, that sex steroid hormones, such as E_2, play a major role in these effects, although this hypothesis has not yet been tested. In the next sections, we discuss evidence that E_2 can regulate UPS activity and highlight commonalities between E_2- and UPS-signaling that support a potential role for UPS activity in the mnemonic effects of E_2.

ESTROGENIC REGULATION OF UPS ACTIVITY

The data discussed thus far support the conclusion that the UPS is not only involved in regulating synaptic plasticity and long-term memory but also exerts its proteolytic effects to support memory formation in a sex-specific manner. Although not yet examined in the context of learning and memory, evidence also suggests that the UPS can be directly stimulated by E_2.

Data from non-neuronal cells indicate some reciprocal interactions between E_2 and UPS activity. ERα and ERβ are rapidly degraded by the proteasome after they translocate to the nucleus and bind to estrogen response elements on target gene promoters to activate or repress gene transcription (Zhou and Slingerland, 2014; Kondakova et al., 2020). In HeLa cells, estrogen receptors are degraded by the proteasome in an E_2-dependent manner, as the application of proteasome inhibitors MG132 or lactacystin increased ER levels by blocking E_2-induced ER degradation (Nawaz et al., 1999). Additionally, E3 ligases appear to act as transcriptional co-activators for ERs, where they are uniquely positioned to rapidly ubiquitinate E_2-bound ERs for degradation (Shang et al., 2000). Although these data were collected from *in vitro* work assessing breast and endometrial cancers, they lend support to the possibility that E_2 might recruit UPS activity through canonical signaling pathways to promote hippocampal memory formation. This possibility is buoyed by findings showing that E_2 can stimulate the UPS by rapidly activating cell-signaling mechanisms. For example, E_2 causes ERK-dependent phosphorylation of the cyclin-dependent kinase inhibitor p27, which results in the increased ubiquitination and proteasomal degradation of p27, and subsequent unchecked proliferation of endometrial epithelial cells (Lecanda et al., 2007; Huang et al., 2012).

Limited evidence also suggests that E2 signaling in the hippocampus and cortex can directly stimulate UPS activity. For instance, treatment of hippocampal slices with E_2 increased ubiquitination and proteasomal-mediated degradation of GluA1-containing AMPA receptors in the CA3 region of the male rat hippocampus (Briz et al., 2015). Other work has shown that ERα in rat hippocampal CA1 undergoes enhanced proteasomal degradation following long-term E_2 deprivation, an effect that was prevented when E_2 was administered before, but not after, E_2 deprivation (Zhang et al., 2011). A separate study in cultured primary cortical neurons found that Cav1.2, a pore-forming subunit of L-type voltage gated calcium channel, is ubiquitinated by the E3 ligase Mdm2 and degraded by the proteasome in an ERα-dependent manner (Lai et al., 2019). Moreover, this study demonstrated in an ovariectomized Alzheimer's mouse model that systemic administration of an ERα agonist, but not ERβ agonist, reduced Cav1.2 protein in

the hippocampus and cortex by increasing ubiquitination and subsequent degradation of Cav1.2 by Mdm2 (Lai et al., 2019). Thus, although relatively scant, there is some basis on which to speculate that E_2 and the ERs may regulate UPS activity in cognitive brain regions such as the hippocampus and cortex and that the resulting protein degradation may influence memory formation.

OVERLAPPING MECHANISMS IN E_2- AND UPS-SIGNALING

E_2 facilitates hippocampal spine density and memory consolidation in both males and females by interacting with receptors positioned at the plasma membrane to promote a cascade of rapid cell signaling events that regulate protein synthesis to support synaptic plasticity (Frick, 2015; Taxier et al., 2020). Interestingly, the signaling events so far identified as critical for regulating E_2's effects on structural plasticity and memory overlap considerably with those that enable UPS to regulate synaptic plasticity and memory.

For example, E_2 promotes NMDAR signaling by increasing excitatory postsynaptic potential amplitude and receptor binding, and increases hippocampal sensitivity to NMDAR inputs (Woolley et al., 1997; Foy et al., 1999). NMDAR activation is required for many of E_2's effects, including enhanced LTP (Foy et al., 1999), dendritic spine density (Woolley and McEwen, 1994), and hippocampus-dependent memory (Lewis et al., 2008; Vedder et al., 2013). Our lab has shown that DH infusion of an NMDAR antagonist prevents E_2 from enhancing object recognition memory and activating DH cell signaling in ovariectomized mice (Lewis et al., 2008), suggesting that NMDA activity is necessary for E_2 to facilitate memory formation. Similarly, NMDAR activity is required for male rats to increase the amount of polyubiquitinated proteins in the amygdala following auditory fear retrieval (Jarome et al., 2011). Additionally, NMDAR activity is required for proteasomes to be redistributed to hippocampal dendritic spines (Bingol and Schuman, 2006; Ferreira et al., 2021) and for targeting polyubiquitination of synaptic scaffold proteins (Colledge et al., 2003; Guo and Wang, 2007).

In addition to having similar requirements for NMDAR activity, E_2 and the UPS both increase the activity of CaMKII and PKA to exert their beneficial effects on memory. For example, systemic administration of E_2 rapidly increases phosphorylation of CaMKII in ovariectomized mice, a molecular effect that depends on the activation of estrogen receptors (Sawai et al., 2002). Similarly, calcium influx through NMDARs increases CaMKII phosphorylation, which then phosphorylates Rpt6 (Djakovic et al., 2009, 2012), thereby increasing proteasomal activity and proteasome redistribution to synapses (Bingol et al., 2010). CaMKII-mediated phosphorylation of Rpt6 drives hippocampal dendritic spine outgrowth (Hamilton et al., 2012) and fear memory formation in male rats (Jarome et al., 2013). Furthermore, work from our lab demonstrates that E_2 requires PKA activity to enhance object recognition memory consolidation in ovariectomized female mice (Lewis et al., 2008). Other findings show that E_2 requires PKA activity to potentiate synapses in hippocampal slices from female, but not male, rats (Jain et al., 2019). A sex-specific role for PKA activity also appears to be critical for regulating proteasome activity across subcellular compartments of DH and BLA neurons following contextual fear conditioning (Devulapalli et al., 2019). Thus, there are several overlapping mechanisms through which E_2 and the UPS regulate synaptic plasticity and long-term memory which provide support for the possibility that E_2 might require aspects of UPS signaling to exert its neuromodulatory effects.

Although the similarities in the requirements for NMDAR, CaMKII, and PKA activity for both E_2 and the UPS provide compelling reasons to suspect UPS involvement in E_2's ability to facilitate memory consolidation, additional support for this hypothesis comes from the notion that the successful formation long-term memories requires a delicate balance between protein synthesis and protein degradation (Park and Kaang, 2019). Evidence of the involvement of both processes can be seen at the synaptic level, where polyribosomes are transported to dendritic spines to promote local protein synthesis (Bramham and Wells, 2007) at the same time that proteasomes are being translocated to dendrites to promote local protein degradation of synaptic scaffolding molecules (Bingol and Schuman, 2006; Shen et al., 2007; Bingol et al., 2010). Similarly, at the behavioral level, expression levels of mTOR and its downstream effector p70S6 kinase were significantly increased at the same time that levels of K48 polyubiquitination were increased in the amygdala of male rats 1 h following contextual fear conditioning (Jarome et al., 2011). E_2 acts at membrane-localized ERs in the DH to activate mTORC1 signaling, which is necessary for E_2 to increase CA1 dendritic spine density in the DH and enhance the spatial and object recognition memory consolidation in ovariectomized mice (Fortress et al., 2013; Tuscher et al., 2016). Therefore, one might suspect that activity-dependent increases in protein synthesis would precipitate similar increases in the opposite, but equally important process, of protein degradation.

HYPOTHESIZED MECHANISM OF ESTROGENIC REGULATION OF UPS

Based on the literature reviewed above, we hypothesize that E_2 may promote CA1 spine density and hippocampal memory formation in males and females by increasing UPS activity, which would cause the degradation of structural proteins localized in the PSD to allow for synaptic remodeling in response to a learning event. In our model of E_2-induced activation of UPS signaling (**Figure 3**), we propose that E2 stimulates UPS activity by binding to membrane-associated ERα and ERβ which then increase NMDAR activity. E_2-induced activation of NMDARs could trigger an increase in UPS activity by: (1) upregulating the amount of K48-linked polyubiquitinated proteins in the synapse through the actions of E1-E3 ubiquitin ligases; and (2) increasing the assembly and localization of 26S proteasomes to synapses by CaMKII- and PKA-dependent phosphorylation of the Rpt6 26S proteasome subunit. However, it is important to note that the nature of the interaction between E_2 and hippocampal NMDAR activation in males and females remains unclear. Although

we speculate that E_2 activates NMDARs through interaction with membrane-associated ERs, there are alternative putative mechanisms through which NMDARs may be activated by E_2 to initiate UPS signaling. For example, E_2 may indirectly increase NMDAR activity by regulating the activity of AMPAR-mediated currents (Srivastava et al., 2008; Smejkalova and Woolley, 2010; Jain et al., 2019). This E_2-induced activation of AMPARs could theoretically regulate NMDAR activity and, thereby, calcium influx and downstream activation CaMKII and PKA, to facilitate both protein synthesis and protein degradation in ways that increase spine density.

We hypothesize that E_2 promotes local protein degradation in a rapid manner that coincides with the need for local protein synthesis. We have previously documented E_2-induced and mTOR-dependent increases in CA1 spine density in ovariectomized mice 30 min after DH infusion (Tuscher et al., 2016), and other reports show that DH infusion of E_2 or the GPER agonist G-1 increases CA1 spine density in ovariectomized mice within 40 min (Phan et al., 2015; Kim et al., 2019). Because UPS activity relies on many of the same signaling pathways as E_2-induced memory enhancement and spinogenesis, it is plausible that E_2 could increase UPS activity as soon as 30 min following DH infusion. Nevertheless, such rapid action would not exclude the possibility of more long-term activation *via* genomic or epigenomic actions of ERα and ERβ.

We speculate an involvement of ERα and ERβ because work from our lab and others indicates that ERα and ERβ agonism facilitates the consolidation of object recognition and spatial memories in ovariectomized rats and mice (Jacome et al., 2010; Kim and Frick, 2017; Hanson et al., 2018; Fleischer et al., 2021). Moreover, ERα and ERβ in the DH interact directly with mGluR1a to trigger the ERK signaling that is necessary for object placement and object recognition memory consolidation in ovariectomized mice (Boulware et al., 2013), which suggests that these receptors both influence memory at the plasma membrane to promote hippocampal memory formation. Although both ERα and ERβ play discrete roles in regulating synaptic potentiation in male and female rats (Kramár et al., 2009; Smejkalova and Woolley, 2010; Oberlander and Woolley, 2016), the interaction between E_2 and NMDARs may be particularly mediated by ERα, whose agonism has been shown to increase the expression of NMDARs in the DH of ovariectomized rats (Morissette et al., 2008). ERα agonism has also been shown to increase NMDAR-mediated EPSCs and lower the threshold for the induction of NMDA-dependent LTP in the dentate gyrus of male rats (Tanaka and Sokabe, 2013). Thus, ERα may play a larger role in activating UPS signaling than ERβ.

Although we have proposed that UPS-mediated protein degradation is required for the E_2-induced facilitation of CA1 spine density and memory consolidation in both sexes, we suspect that the signaling mechanisms that regulate this activity may differ considerably between males and females. For example, although E_2 can increase synaptic potentiation in both sexes, males and females utilize different ERs at pre- and post-synaptic sites to facilitate synaptic potentiation (Oberlander and Woolley, 2016). In males, presynaptic increase in glutamate release is mediated by ERα and postsynaptic increase in glutamate sensitivity is mediated by ERβ. However, in females, the presynaptic increase in glutamate release is mediated by ERβ and the postsynaptic increase in sensitivity is mediated by GPER (Oberlander and Woolley, 2016). Based on these findings, we speculate that the effects of E_2 on UPS activity in males and females may be regulated in part by different ERs. Furthermore, sex differences may also exist in the signaling mechanisms that occur downstream of our proposed model of E_2-induced NMDAR activation. We speculate that E_2 increases CaMKII and PKA activity in a manner that is dependent on ER-driven activation of NMDARs. There is sufficient evidence to suggest that males and females might differ in their requirements for CaMKII and PKA activity to initiate UPS activity following E_2 exposure. For example, PKA is required for acute E_2-induced initiation of synaptic potentiation in females, but not males (Jain et al., 2019). These findings potentially suggest that PKA activity may be required for E_2 to increase UPS activity in females, but not males, at hippocampal synapses. In males, E_2-mediated UPS activity could be more driven by CaMKII or K48 polyubiquitination.

In sum, our hypothesis posits multiple possible mechanisms through which E_2 might activate the UPS system to facilitate protein degradation, synaptic remodeling, synaptic plasticity, and memory consolidation. Although based largely on circumstantial evidence from the E_2 and UPS literatures, our model provides a framework to empirically test the roles of several UPS mechanisms in the effects of E_2 on memory in both sexes across multiple brain regions and subcellular sites.

DISCUSSION

This review has summarized evidence suggesting that protein degradation is an important regulator of synaptic plasticity and memory (Kaang and Choi, 2012; Hegde, 2017), yet the role that UPS-mediated protein degradation plays in regulating E_2's modulatory effects on memory formation in either sex remains unexplored. E_2 may facilitate hippocampal structural plasticity and memory consolidation in part by regulating protein degradation mediated by the UPS. This hypothesis is supported by evidence that E_2 can directly stimulate UPS activity in hippocampal slices (Briz et al., 2015), cultured neurons (Lai et al., 2019), and in a rat model of long-term E_2 deprivation (Zhang et al., 2011). This notion is further strengthened by data suggesting that the UPS is regulated in part by sex steroid hormones, such as E_2, as males and females appear to have different baseline regulation of, and requirement for, proteasome activity following fear learning, and target different proteins for proteasomal degradation after learning (Devulapalli et al., 2019, 2021; Farrell et al., 2021; Martin et al., 2021). As such, there is sufficiently plausible evidence to support future studies exploring a role for the UPS in estrogenic memory modulation.

When speculating why E_2 might stimulate UPS-mediated protein degradation to regulate hippocampal memory formation, we have proposed that E_2 triggers degradation of proteins in the synapse to promote structural remodeling of CA1 dendritic spines. However, E_2 may also stimulate UPS activity to

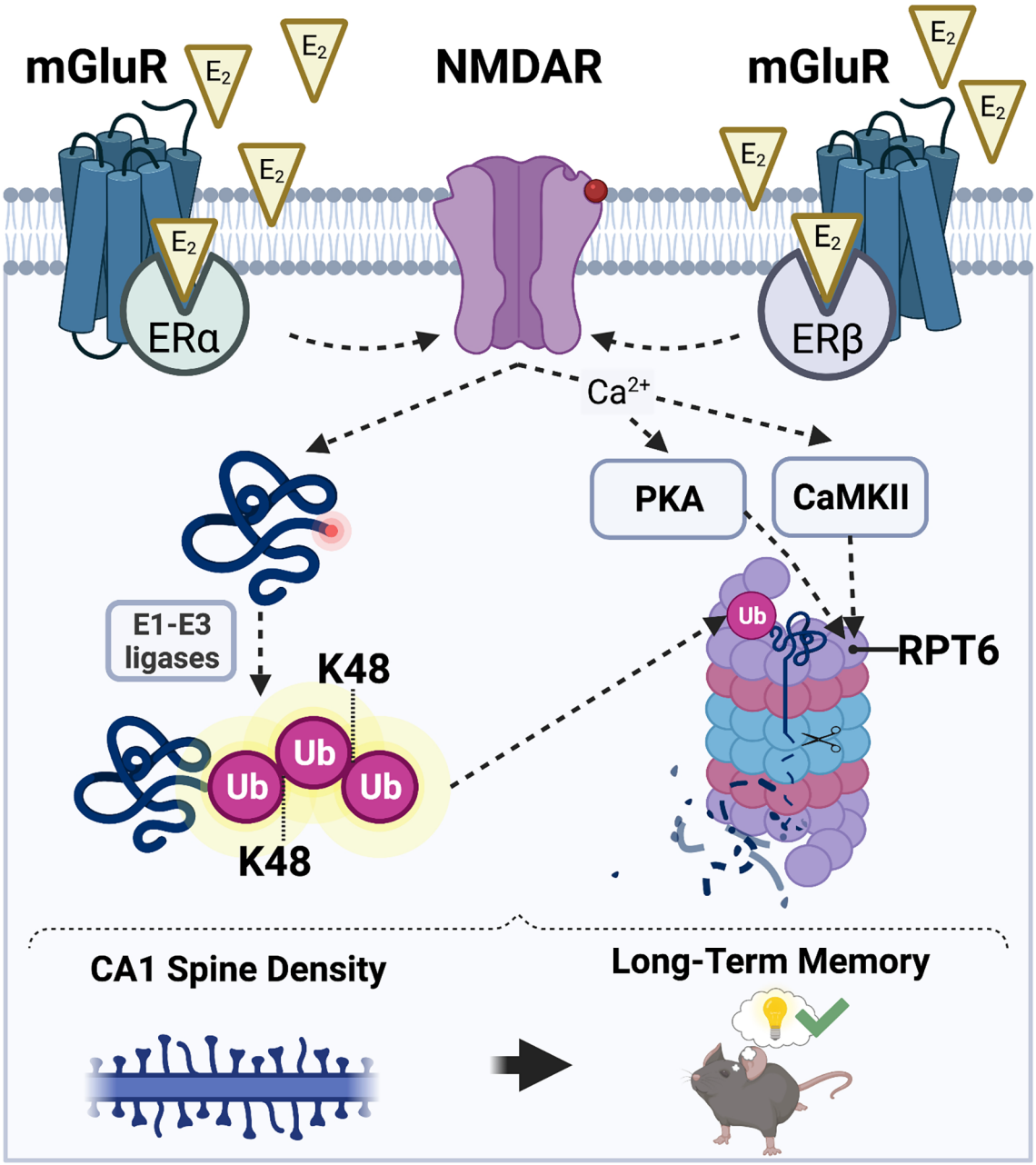

FIGURE 3 | Hypothesized mechanisms through which E_2 may activate the UPS to regulate CA1 dendritic spine density and hippocampal memory. E_2 acts *via* membrane-associated estrogen receptors including ERα and ERβ to promote postsynaptic sensitivity to glutamate (red circle on NMDAR) and opening of NMDARs. E_2-induced NMDAR activation promotes an increase in the amount of substrate proteins that acquire a K48-polyubiquitin tag through the actions of E1-E3 ubiquitin ligases (left). NMDAR activation also simultaneously permits an influx of intracellular Ca^{2+} which results in an E_2-induced increase in CaMKII and PKA activity (right). CaMKII and PKA then phosphorylate the Rpt6 subunit of the 26S proteasome complex, which mobilizes proteasomes to dendritic spine shafts to initiate the breakdown of K48-tagged substrate proteins. Illustration created using BioRender.com.

compensate for the enhanced synaptic potentiation caused by E_2 administration. Interestingly, some evidence suggests that E_2 activates the UPS to regulate the expression of proteins involved in synaptic transmission. For example, GluA1-containing AMPA receptors are ubiquitinated and degraded in the CA3 region of the male rat hippocampus following E_2 administration (Briz et al., 2015). Similarly, the pore-forming subunit of L-type voltage gated calcium channel, Cav1.2, is ubiquitinated and degraded in cultured primary cortical neurons in an ERα-dependent manner (Lai et al., 2019). These findings might suggest that E_2 can also regulate UPS activity in a manner that promotes homeostasis following E_2-induced excitatory synaptic potentiation. It is of course possible that E_2 can promote UPS activity in a manner to support both structural remodeling of synapses and to permit

cellular homeostasis. However, the time course of E_2-UPS interactions for the latter would likely occur at a time point later than 30 min, as we have proposed for structural remodeling.

Future work should examine the extent to which E_2 requires UPS activity in males and females to support changes in hippocampal plasticity and memory. To our knowledge, no studies to date have examined whether sex differences exist in the requirement for proteasome activity in males and females following non-aversive forms of learning. As such, it remains unclear whether non-aversive tasks, such as the object placement and object recognition paradigms, activate the same cell signaling mechanisms and proteasome subunits to upregulate protein degradation in males and females that have been documented in aversive tasks. Work in this direction could potentially reveal critical baseline and learning-induced sex differences that may provide an impetus to examine the contributions of sex steroid hormones.

Moreover, we speculate that the cellular mechanisms that signal a need for protein degradation might differ between males and females, as previous work from our lab and others demonstrates that the molecular mechanisms through which E_2 mediates DH plasticity and memory consolidation differ between the sexes (Oberlander and Woolley, 2016; Koss et al., 2018; Jain et al., 2019; Koss and Frick, 2019). Much less is known about the potential time course through which E_2 requires UPS activity, although data suggest that the need for protein degradation during consolidation overlaps with that for protein synthesis (Park and Kaang, 2019), potentially indicating that E2 stimulates UPS activity rapidly following DH infusion, as we previously documented for local protein synthesis (Fortress et al., 2013; Tuscher et al., 2016). Finally, it remains unclear which proteins could be targeted within the DH following E_2 treatment, and whether these protein targets differ between the sexes. Future studies in this realm would provide more direct insights into how E_2 modifies the existing molecular framework to support hippocampal plasticity and memory.

In conclusion, this review has provided an overview of the signaling mechanisms so far identified as critical for E_2 and UPS function, with particular emphasis on the ways in which these mechanisms overlap to support structural integrity and protein composition of hippocampal synapses. If UPS activity is integral to E_2's effects on memory, then this could lead to exciting new avenues of basic research into hormonal regulation of cognition that could have important clinical implications for treating psychiatric and neurodegenerative diseases in which sex or E_2 play a role.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by University of Wisconsin–Milwaukee Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

KF and SB conceived the hypothesis that is the focus of the manuscript. KF and SB were both responsible for reviewing relevant literature, preparing the manuscript, and approving the submitted version. Both authors contributed to the article and approved the submitted version.

FUNDING

We gratefully acknowledge National Institutes of Health, R01MH107886 and the UW-Milwaukee College of Letters and Sciences for support of this review.

ACKNOWLEDGMENTS

We wish to thank Dr. Kellie S. Gross for helpful discussions about UPS and estradiol function in preparing this manuscript.

REFERENCES

Akama, K., and McEwen, B. (2003). Estrogen stimulates Postsynaptic Density-95 rapid protein synthesis via the Akt/Protein Kinase B pathway. *J. Neurosci.* 23, 2333–2339. doi: 10.1523/JNEUROSCI.23-06-02333.2003

Artinian, J., McGauran, A., De Jaeger, X., Mouledous, L., Frances, B., Roullet, P., et al. (2008). Protein degradation, as with protein synthesis, is required during not only long-term spatial memory consolidation but also reconsolidation. *Eur. J. Neurosci.* 27, 3009–3019. doi: 10.1111/j.1460-9568.2008.06262.x

Babayan, A., and Kramár, E. (2013). Rapid effects of oestrogen on synaptic plasticity: interactions with actin and its signaling proteins. *J. Neuroendocrinol.* 25, 1163–1172. doi: 10.1111/jne.12108

Bingol, B., and Schuman, E. (2004). A proteasome-sensitive connection between PSD-95 and GluR1 endocytosis. *Neuropharmacology* 47, 755–763. doi: 10.1016/j.neuropharm.2004.07.028

Bingol, B., and Schuman, E. (2006). Activity-dependent dynamics and sequestration of proteasomes in dendritic spines. *Nature* 441, 1144–1148. doi: 10.1038/nature04769

Bingol, B., Wang, C., Arnott, D., Cheng, D., Peng, J., and Sheng, M. (2010). Autophosphorylated CaMKIIα acts as a scaffold to recruit proteasomes to dendritic spines. *Cell* 140, 567–578. doi: 10.1016/j.cell.2010.01.024

Boulware, M. I., Heisler, J. D., and Frick, K. M. (2013). The memory-enhancing effects of hippocampal estrogen receptor activation involve metabotropic glutamate receptor signaling. *J. Neurosci.* 33, 15184–15194. doi: 10.1523/JNEUROSCI.1716-13.2013

Boulware, M., Weick, J., Becklund, B., Kuo, S., Groth, R., and Mermelstein, P. (2005). Estradiol activates group I and II metabotropic glutamate receptor signaling, leading to opposing influences on cAMP response element-binding protein. *J. Neurosci.* 25, 5066–5078. doi: 10.1523/JNEUROSCI.1427-05.2005

Bramham, C., and Wells, D. (2007). Dendritic mRNA: transport, translation and function. *Nat. Rev. Neurosci.* 8, 776–789. doi: 10.1038/nrn2150

Briz, V., and Baudry, M. (2014). Estrogen regulates protein synthesis and actin polymerization in hippocampal neurons through different molecular mechanisms. *Front. Endocrinol. (Lausanne)* 5:22. doi: 10.3389/fendo.2014.00022

Briz, V., Liu, Y., Zhu, G., Bi, X., and Baudry, M. (2015). A novel form of synaptic plasticity in field CA3 of hippocampus requires GPER1 activation and BDNF release. *J. Cell Biol.* 210, 1225–1237. doi: 10.1083/jcb.201504092

Chain, D., Casadio, A., Schacher, S., Hegde, A., Valbrun, M., Yamamoto, N., et al. (1999). Mechanisms for generating the autonomous cAMP-dependent protein kinase required for long-term facilitation in Aplysia. *Neuron* 22, 147–156. doi: 10.1016/s0896-6273(00)80686-8

Chen, L., Rex, C., Casale, M., Gall, C., and Lynch, G. (2007). Changes in synaptic morphology accompany actin signaling during LTP. *J. Neurosci.* 27, 5363–5372. doi: 10.1523/JNEUROSCI.0164-07.2007

Chen, Y., Yuanxiang, P., Knöpfel, T., Thomas, U., and Behnisch, T. (2012). Hippocampal LTP triggers proteasome-mediated SPAR degradation in CA1 neurons. *Synapse* 66, 142–150. doi: 10.1002/syn.20994

Choi, J., Kim, J., and Kaang, B. (2010). Protein synthesis and degradation are required for the incorporation of modified information into the pre-existing object-location memory. *Mol. Brain* 3:1. doi: 10.1186/1756-6606-3-1

Colledge, M., Snyder, E., Crozier, R., Soderling, J., Jin, Y., Langeberg, L., et al. (2003). Ubiquitination regulates PSD-95 degradation and AMPA receptor surface expression. *Neuron* 40, 595–607. doi: 10.1016/s0896-6273(03)00687-1

Cullen, P., Ferrara, N., Pullins, S., and Helmstetter, F. (2017). Context memory formation requires activity-dependent protein degradation in the hippocampus. *Learn. Mem.* 24, 589–596. doi: 10.1101/lm.045443.117

Devulapalli, R., Jones, N., Farrell, K., Musaus, M., Kugler, H., McFadden, T., et al. (2021). Males and females differ in the regulation and engagement of, but not requirement for, protein degradation in the amygdala during fear memory formation. *Neurobiol. Learn. Mem.* 180:107404. doi: 10.1016/j.nlm.2021.107404

Devulapalli, R. K., Nelsen, J., Orsi, S., McFadden, T., Navabpour, S., Jones, N., et al. (2019). Males and females differ in the subcellular and brain region dependent regulation of proteasome activity by CaMKII and protein kinase A. *Neuroscience* 418, 1–14. doi: 10.1016/j.neuroscience.2019.08.031

Djakovic, S., Marquez-Lona, E., Jakawich, S., Wright, R., Chu, C., Sutton, M., et al. (2012). Phosphorylation of Rpt6 regulates synaptic strength in hippocampal neurons. *J. Neurosci.* 32, 5126–5131. doi: 10.1523/JNEUROSCI.4427-11.2012

Djakovic, S., Schwarz, L., Barylko, B., DeMartino, G., and Patrick, G. (2009). Regulation of the proteasome by neuronal activity and calcium/calmodulin-dependent protein kinase II. *J. Biol. Chem.* 284, 26655–26665. doi: 10.1074/jbc.M109.021956

Dong, C., Bach, S., Haynes, K., and Hegde, A. (2014). Proteasome modulates positive and negative translational regulators in long-term synaptic plasticity. *J. Neurosci.* 34, 3171–3182. doi: 10.1523/JNEUROSCI.3291-13.2014

Dong, C., Upadhya, S., Ding, L., Smith, T., and Hegde, A. (2008). Proteasome inhibition enhances the induction and impairs the maintenance of late-phase long-term potentiation. *Learn. Mem.* 15, 335–347. doi: 10.1101/lm.984508

Dulka, B., Trask, S., and Helmstetter, F. J. (2021). Age-related memory impairment and sex-specific alterations in phosphorylation of the Rpt6 proteasome subunit and polyubiquitination in the basolateral amygdala and medial prefrontal cortex. *Front. Aging Neurosci.* 13:656944. doi: 10.3389/fnagi.2021.656944

Ehlers, M. (2003). Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nat. Neurosci.* 6, 231–242. doi: 10.1038/nn1013

Fan, L., Zhao, Z., Orr, P. T., Chambers, C. H., Lewis, M. C., and Frick, K. M. (2010). Estradiol-induced object memory consolidation in middle-aged female mice requires dorsal hippocampal extracellular signal-regulated kinase and phosphatidylinositol 3-kinase activation. *J. Neurosci.* 30, 4390–4400. doi: 10.1523/JNEUROSCI.4333-09.2010

Farrell, K., Musaus, M., Navabpour, S., Martin, K., Ray, W., Helm, R., et al. (2021). Proteomic analysis reveals sex-specific protein degradation targets in the amygdala during fear memory formation. *Front. Mol. Neurosci.* 14:716284. doi: 10.3389/fnmol.2021.716284

Fernandez, S. M., Lewis, M. C., Pechenino, A. S., Harburger, L. L., Orr, P. T., Gresack, J. E., et al. (2008). Estradiol-induced enhancement of object memory consolidation involves hippocampal extracellular signal-regulated kinase activation and membrane-bound estrogen receptors. *J. Neurosci.* 28, 8660–8667. doi: 10.1523/JNEUROSCI.1968-08.2008

Ferrara, N., Jarome, T., Cullen, P., Orsi, S., Kwapis, J., Trask, S., et al. (2019). GluR2 endocytosis-dependent protein degradation in the amygdala mediates memory updating. *Sci. Rep.* 9:5180. doi: 10.1038/s41598-019-41526-1

Ferreira, J., Kellermayer, B., Carvalho, A., and Groc, L. (2021). Interplay between NMDA receptor dynamics and the synaptic proteasome. *Eur. J. Neurosci.* 54, 6000–6011. doi: 10.1111/ejn.15427

Figueiredo, L., Dornelles, A., Petry, F., Falavigna, L., Dargél, V., Köbe, L., et al. (2015). Two waves of proteasome-dependent protein degradation in the hippocampus are required for recognition memory consolidation. *Neurobiol. Learn. Mem.* 120, 1–6. doi: 10.1016/j.nlm.2015.02.005

Fioravante, D., and Byrne, J. (2010). Protein degradation and memory formation. *Brain Res. Bull.* 85, 14–20. doi: 10.1016/j.brainresbull.2010.11.002

Fleischer, A. W., Schalk, J. C., Wetzel, E. A., Hanson, A. M., Sem, D. S., Donaldson, W. A., et al. (2021). Long-term oral administration of a novel estrogen receptor beta agonist enhances memory and alleviates drug-induced vasodilation in young ovariectomized mice. *Horm. Behav.* 130:104948. doi: 10.1016/j.yhbeh.2021.104948

Fonseca, R., Vabulas, R., Hartl, F., Bonhoeffer, T., and Nägerl, U. (2006). A balance of protein synthesis and proteasome-dependent degradation determines the maintenance of LTP. *Neuron* 52, 239–245. doi: 10.1016/j.neuron.2006.08.015

Fortress, A. M., Fan, L., Orr, P. T., Zhao, Z., and Frick, K. M. (2013). Estradiol-induced object recognition memory consolidation is dependent on activation of mTOR signaling in the dorsal hippocampus. *Learn. Mem.* 20, 147–155. doi: 10.1101/lm.026732.112

Foy, M. (2001). 17beta-estradiol: effect on CA1 hippocampal synaptic plasticity. *Neurobiol. Learn. Mem.* 76, 239–252. doi: 10.1006/nlme.2001.4018

Foy, M., Xu, J., Xie, X., Brinton, R., Thompson, R., and Berger, T. (1999). 17beta-estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. *J. Neurophysiol.* 81, 925–929. doi: 10.1152/jn.1999.81.2.925

Frick, K. M. (2015). Molecular mechanisms underlying the memory-enhancing effects of estradiol. *Horm. Behav.* 74, 4–18. doi: 10.1016/j.yhbeh.2015.05.001

Furini, C., Myskiw, J., Schmidt, B., Zinn, C., Peixoto, P., Pereira, L., et al. (2015). The relationship between protein synthesis and protein degradation in object recognition memory. *Behav. Brain Res.* 294, 17–24. doi: 10.1016/j.bbr.2015.07.038

Glickman, M., and Ciechanover, A. (2002). The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol. Rev.* 82, 373–428. doi: 10.1152/physrev.00027.2001

Gross, K. S., Alf, R. L., Polzin, T. R., and Frick, K. M. (2021). 17β-estradiol activation of dorsal hippocampal TrkB is independent of increased mature BDNF expression and is required for enhanced memory consolidation in female mice. *Psychoneuroendocrinology* 125:105110. doi: 10.1016/j.psyneuen.2020.105110

Guo, L., and Wang, Y. (2007). Glutamate stimulates glutamate receptor interacting protein 1 degradation by ubiquitin-proteasome system to regulate surface expression of GluR2. *Neuroscience* 145, 100–109. doi: 10.1016/j.neuroscience.2006.11.042

Guo, X., Wang, X., Wang, Z., Banerjee, S., Yang, J., Huang, L., et al. (2016). Site-specific proteasome phosphorylation controls cell proliferation and tumorigenesis. *Nat. Cell Biol.* 18:212. doi: 10.1038/ncb3289

Hamilton, A., Oh, W., Vega-Ramirez, H., Stein, I., Hell, J., Patrick, G., et al. (2012). Activity-dependent growth of new dendritic spines is regulated by the proteasome. *Neuron* 74, 1023–1030. doi: 10.1016/j.neuron.2012.04.031

Hamson, D., Roes, M., and Galea, L. (2016). Sex hormones and cognition: neuroendocrine influences on memory and learning. *Compr. Physiol.* 6, 1295–1337. doi: 10.1002/cphy.c150031

Hanson, A., Perera, K. L., Iresha, S., Kim, J., Pandey, R. K., Sweeney, N., et al. (2018). A-C estrogens as potent and selective estrogen receptor-beta agonists (SERBAs) to enhance memory consolidation under low-estrogen conditions. *J. Med. Chem.* 61, 4720–4738. doi: 10.1021/acs.jmedchem.7b01601

Hegde, A. (2004). Ubiquitin-proteasome-mediated local protein degradation and synaptic plasticity. *Prog. Neurobiol.* 73, 311–357. doi: 10.1016/j.pneurobio.2004.05.005

Hegde, A. (2017). Proteolysis, synaptic plasticity and memory. *Neurobiol. Learn. Mem.* 138, 98–110. doi: 10.1016/j.nlm.2016.09.003

Hegde, A., Goldberg, A., and Schwartz, J. (1993). Regulatory subunits of cAMP-dependent protein kinases are degraded after conjugation to ubiquitin: a molecular mechanism underlying long-term synaptic plasticity. *Proc. Nat. Acad. Sci. U S A* 90, 7436–7440. doi: 10.1073/pnas.90.16.7436

Hegde, A., Haynes, K., Bach, S., and Beckelman, B. (2014). Local ubiquitin-proteasome-mediated proteolysis and long-term synaptic plasticity. *Front. Mol. Neurosci.* 7:96. doi: 10.3389/fnmol.2014.00096

Hegde, A., Inokuchi, K., Pei, W., Casadio, A., Ghirardi, M., Chain, D., et al. (1997). Ubiquitin C-terminal hydrolase is an immediate-early gene essential for long-term facilitation in aplysia. *Cell* 89, 115–126. doi: 10.1016/s0092-8674(00)80188-9

Hojo, Y., Munetomo, A., Mukai, H., Ikeda, M., Sato, R., Hatanaka, Y., et al. (2015). Estradiol rapidly modulates spinogenesis in hippocampal dentate gyrus: involvement of kinase networks. *Horm. Behav.* 74, 149–156. doi: 10.1016/j.yhbeh.2015.06.008

Huang, K., Pavlides, S., Lecanda, J., Blank, S., Mittal, K., and Gold, L. (2012). Estrogen and progesterone regulate p27kip1 levels via the ubiquitin-proteasome system: pathogenic and therapeutic implications for endometrial cancer. *PLoS One* 7:e46072. doi: 10.1371/journal.pone.0046072

Inagaki, T., Frankfurt, M., and Luine, V. (2012). Estrogen-induced memory enhancements are blocked by acute bisphenol A in adult female rats: role of dendritic spines. *Endocrinology* 153, 3357–3367. doi: 10.1210/en.2012-1121

Jacome, L., Gautreaux, G., Inagaki, T., Mohan, G., Alves, S., Lubbers, L., et al. (2010). Estradiol and ERβ agonists enhance recognition memory and DPN, an ERβ agonist, alters brain monoamines. *Neurobiol. Learn. Mem.* 94, 488–498. doi: 10.1016/j.nlm.2010.08.016

Jain, A., Huang, G., and Woolley, C. (2019). Latent sex differences in molecular signaling that underlies excitatory synaptic potentiation in the hippocampus. *J. Neurosci.* 39, 1552–1565. doi: 10.1523/JNEUROSCI.1897-18.2018

Jarome, T., and Helmstetter, F. (2013). The ubiquitin-proteasome system as a critical regulator of synaptic plasticity and long-term memory formation. *Neurobiol. Learn. Mem.* 105, 107–116. doi: 10.1016/j.nlm.2013.03.009

Jarome, T., and Helmstetter, F. (2014). Protein degradation and protein synthesis in long-term memory formation. *Front. Mol. Neurosci.* 7:61. doi: 10.3389/fnmol.2014.00061

Jarome, T., Kwapis, J., Ruenzel, W., and Helmstetter, F. (2013). CaMKII, but not protein kinase A, regulates Rpt6 phosphorylation and proteasome activity during the formation of long-term memories. *Front. Behav. Neurosci.* 7:115. doi: 10.3389/fnbeh.2013.00115

Jarome, T., Werner, C., Kwapis, J., and Helmstetter, F. (2011). Activity dependent protein degradation is critical for the formation and stability of fear memory in the amygdala. *PLoS One* 6:e24349. doi: 10.1371/journal.pone.0024349

Kaang, B., and Choi, J. (2012). Synaptic protein degradation in memory reorganization. *Adv. Exp. Med. Biol.* 970, 221–240. doi: 10.1007/978-3-7091-0932-8_10

Karpova, A., Mikhaylova, M., Thomas, U., Knöpfel, T., and Behnisch, T. (2006). Involvement of protein synthesis and degradation in long-term potentiation of schaffer collateral CA1 synapses. *J. Neurosci.* 26, 4949–4955. doi: 10.1523/JNEUROSCI.4573-05.2006

Kelleher, R., Govindarajan, A., and Tonegawa, S. (2004). Translational regulatory mechanisms in persistent forms of synaptic plasticity. *Neuron* 44, 59–73. doi: 10.1016/j.neuron.2004.09.013

Kim, J., and Frick, K. M. (2017). Distinct effects of estrogen receptor antagonism on object recognition and spatial memory consolidation in ovariectomized mice. *Psychoneuroendocrinology* 85, 110–114. doi: 10.1016/j.psyneuen.2017.08.013

Kim, J., Schalk, J. C., Koss, W. A., Gremminger, R. L., Taxier, L. R., Gross, K. S., et al. (2019). Dorsal hippocampal actin polymerization is necessary for activation of G-protein-coupled estrogen receptor (GPER) to increase CA1 dendritic spine density and enhance memory consolidation. *J. Neurosci.* 39, 9598–9610. doi: 10.1523/JNEUROSCI.2687-18.2019

Kim, J., Szinte, J., Boulware, M., and Frick, K. (2016). 17β-estradiol and agonism of G-protein-coupled estrogen receptor enhance hippocampal memory via different cell-signaling mechanisms. *J. Neurosci.* 36, 3309–3321. doi: 10.1523/JNEUROSCI.0257-15.2016

Kondakova, I., Shashova, E., Sidenko, E., Astakhova, T., Zakharova, L., and Sharova, N. (2020). Estrogen receptors and ubiquitin proteasome system: mutual regulation. *Biomolecules* 10:500. doi: 10.3390/biom10040500

Koss, W. A., and Frick, K. M. (2019). Activation of androgen receptors protects intact male mice from memory impairments caused by aromatase inhibition. *Horm. Behav.* 111, 96–104. doi: 10.1016/j.yhbeh.2019.01.002

Koss, W. A., Haertel, J. M., Philippi, S. M., and Frick, K. M. (2018). Sex differences in the rapid cell signaling mechanisms underlying the memory-enhancing effects of 17β-estradiol. *eNeuro* 5:ENEURO.0267-18.2018. doi: 10.1523/ENEURO.0267-18.2018

Kramár, E., Chen, L., Brandon, N., Rex, C., Liu, F., Gall, C., et al. (2009). Cytoskeletal changes underlie estrogen's acute effects on synaptic transmission and plasticity. *J. Neurosci.* 29, 12982–12993. doi: 10.1523/JNEUROSCI.3059-09.2009

Lai, Y., Zhu, B., Sun, F., Luo, D., Ma, Y., Luo, B., et al. (2019). Estrogen receptor α promotes Cav1.2 ubiquitination and degradation in neuronal cells and in APP/PS1 mice. *Aging Cell* 18:e12961. doi: 10.1111/acel.12961

Lecanda, J., Parekh, T., Gama, P., Lin, K., Liarski, V., Uretsky, S., et al. (2007). Transforming growth factor-beta, estrogen and progesterone converge on the regulation of p27 Kip1 in the normal and malignant endometrium. *Cancer Res.* 67, 1007–1018. doi: 10.1158/0008-5472.CAN-06-0235

Lee, S., Choi, J., Lee, N., Lee, H., Kim, J., Yu, N., et al. (2008). Synaptic protein degradation underlies destabilization of retrieved fear memory. *Science* 319, 1253–1256. doi: 10.1126/science.1150541

Lewis, M. C., Kerr, K. M., Orr, P. T., and Frick, K. M. (2008). Estradiol-induced enhancement of object memory consolidation involves NMDA receptors and protein kinase a in the dorsal hippocampus of female C57BL/6 mice. *Behav. Neurosci.* 122, 716–721. doi: 10.1037/0735-7044.122.3.716

Lin, J., Chang, W., Chen, H., Lai, H., Chen, C., Tao, M., et al. (2013). Regulation of feedback between protein kinase A and the proteasome system worsens Huntington's disease. *Mol. Cell. Biol.* 33:1084. doi: 10.1128/MCB.01434-12

Lokireddy, S., Kukushkin, N., and Goldberg, A. (2015). cAMP-induced phosphorylation of 26S proteasomes on Rpn6/PSMD11 enhances their activity and the degradation of misfolded proteins. *Proc. Nat. Acad. Sci. U S A* 112, E7176–E7185. doi: 10.1073/pnas.1522332112

Lopez-Salon, M., Alonso, M., Vianna, M., Viola, H., Souza, T., Izquierdo, I., et al. (2001). The ubiquitin-proteasome cascade is required for mammalian long-term memory formation. *Eur. J. Neurosci.* 14, 1820–1826. doi: 10.1046/j.0953-816x.2001.01806.x

MacLusky, N., Luine, V., Hajszan, T., and Leranth, C. (2005). The 17α and 17β isomers of estradiol both induce rapid spine synapse formation in the CA1 hippocampal subfield of ovariectomized female rats. *Endocrinology* 146, 287–293. doi: 10.1210/en.2004-0730

Martin, K., Musaus, M., Navabpour, S., Gustin, A., Ray, K., Helm, R., et al. (2021). Females, but not males, require protein degradation in the hippocampus for contextual fear memory formation. *Learn. Mem.* 28, 248–253. doi: 10.1101/lm.053429.121

Milner, T., Ayoola, K., Drake, C., Herrick, S., Tabori, N., McEwen, B., et al. (2005). Ultrastructural localization of estrogen receptor beta immunoreactivity in the rat hippocampal formation. *J. Compar. Neurol.* 491, 81–95. doi: 10.1002/cne.20724

Milner, T., McEwen, B., Hayashi, S., Chen, L., Lawrence, R., and Alves, S. (2000). Ultrastructural evidence that hippocampal alpha estrogen receptors are located at extranuclear sites. *J. Comp. Neurol.* 429, 355–371.

Mitterling, K., Spencer, J., Dziedzic, N., Shenoy, S., McCarthy, K., Waters, E., et al. (2010). Cellular and subcellular localization of estrogen and progestin receptor immunoreactivities in the mouse hippocampus. *J. Comp. Neurol.* 518, 2729–2743. doi: 10.1002/cne.22361

Morissette, M., Le Saux, M., and Di Paolo, T. (2008). Effect of oestrogen receptor alpha and beta agonists on brain N-methyl-D-aspartate receptors. *J. Neuroendocrinol.* 20, 1006–1014. doi: 10.1111/j.1365-2826.2008.01754.x

Mukai, H., Tsurugizawa, T., Murakami, G., Kominami, S., Ishii, H., Ogiue-Ikeda, M., et al. (2007). Rapid modulation of long-term depression and spinogenesis via synaptic estrogen receptors in hippocampal principal neurons. *J. Neurochem.* 100, 950–967. doi: 10.1111/j.1471-4159.2006.04264.x

Murakami, G., Tsurugizawa, T., Hatanaka, Y., Komatsuzaki, Y., Tanabe, N., Mukai, H., et al. (2006). Comparison between basal and apical dendritic spines in estrogen-induced rapid spinogenesis of CA1 principal neurons in the adult hippocampus. *Biochem. Biophys. Res. Commun.* 351, 553–558. doi: 10.1016/j.bbrc.2006.10.066

Musaus, M., Navabpour, S., and Jarome, T. (2020). The diversity of linkage-specific polyubiquitin chains and their role in synaptic plasticity and memory formation. *Neurobiol. Learn. Mem.* 174:107286. doi: 10.1016/j.nlm.2020.107286

Myeku, N., Wang, H., and Figueiredo-Pereira, M. (2012). cAMP stimulates the ubiquitin/proteasome pathway in rat spinal cord neurons. *Neurosci. Lett.* 527, 126–131. doi: 10.1016/j.neulet.2012.08.051

Nandi, D., Tahiliani, P., Kumar, A., and Chandu, D. (2006). The ubiquitin-proteasome system. *J. Biosci.* 31, 137–155. doi: 10.1007/BF02705243

Nawaz, Z., Lonard, D., Dennis, A., Smith, C., and O'Malley, B. (1999). Proteasome-dependent degradation of the human estrogen receptor. *Proc. Natl. Acad. Sci. U S A* 96, 1858–1862. doi: 10.1073/pnas.96.5.1858

Oberlander, J. G., and Woolley, C. S. (2016). 17β-estradiol acutely potentiates glutamatergic synaptic transmission in the hippocampus through distinct mechanisms in males and females. *J. Neurosci.* 36, 2677–2690. doi: 10.1523/JNEUROSCI.4437-15.2016

Ōmura, S., and Crump, A. (2019). Lactacystin: first-in-class proteasome inhibitor still excelling and an exemplar for future antibiotic research. *J. Antibiot. (Tokyo)* 72, 189–201. doi: 10.1038/s41429-019-0141-8

Pak, D., and Sheng, M. (2003). Targeted protein degradation and synapse remodeling by an inducible protein kinase. *Science* 302, 1368–1373. doi: 10.1126/science.1082475

Park, H., and Kaang, B. (2019). Balanced actions of protein synthesis and degradation in memory formation. *Learn. Mem.* 26, 299–306. doi: 10.1101/lm.048785.118

Patrick, G., Bingol, B., Weld, H., and Schuman, E. (2003). Ubiquitin-mediated proteasome activity is required for agonist-induced endocytosis of GluRs. *Curr. Biol.* 13, 2073–2081. doi: 10.1016/j.cub.2003.10.028

Penzes, P., and Cahill, M. (2012). Deconstructing signal transduction pathways that regulate the actin cytoskeleton in dendritic spines. *Cytoskeleton (Hoboken)* 69, 426–441. doi: 10.1002/cm.21015

Phan, A., Suschkov, S., Molinaro, L., Reynolds, K., Lymer, J., Bailey, C., et al. (2015). Rapid increases in immature synapses parallel estrogen-induced hippocampal learning enhancements. *Proc. Natl. Acad. Sci. U S A* 112, 16018–16023. doi: 10.1073/pnas.1522150112

Reis, D. S., Jarome, T. J., and Helmstetter, F. J. (2013). Memory formation for trace fear conditioning requires ubiquitin-proteasome mediated protein degradation in the prefrontal cortex. *Front. Behav. Neurosci.* 7:150. doi: 10.3389/fnbeh.2013.00150

Rodriguez-Ortiz, C., Balderas, I., Saucedo-Alquicira, F., Cruz-Castañeda, C., and Bermudez-Rattoni, F. (2011). Long-term aversive taste memory requires insular and amygdala protein degradation. *Neurobiol. Learn. Mem.* 95, 311–315. doi: 10.1016/j.nlm.2010.12.010

Sarkar, S., Smith, L., Logan, S., and Simpkins, J. (2010). Estrogen-induced activation of extracellular signal-regulated kinase signaling triggers dendritic resident mRNA translation. *Neuroscience* 170, 1080–1085. doi: 10.1016/j.neuroscience.2010.07.035

Sawai, T., Bernier, F., Fukushima, T., Hashimoto, T., Ogura, H., and Nishizawa, Y. (2002). Estrogen induces a rapid increase of calcium-calmodulin-dependent protein kinase II activity in the hippocampus. *Brain Res.* 950, 308–311. doi: 10.1016/s0006-8993(02)03186-4

Scudder, S., Gonzales, F., Howell, K., Stein, I., Dozier, L., Anagnostaras, S., et al. (2021). Altered phosphorylation of the proteasome subunit Rpt6 has minimal impact on synaptic plasticity and learning. *eNeuro* 8:ENEURO.0073-20.2021. doi: 10.1523/ENEURO.0073-20.2021

Sellers, K., Erli, F., Raval, P., Watson, I., Chen, D., and Srivastava, D. (2015). Rapid modulation of synaptogenesis and spinogenesis by 17β-estradiol in primary cortical neurons. *Front. Cell Neurosci.* 9:137. doi: 10.3389/fncel.2015.00137

Shang, Y., Hu, X., DiRenzo, J., Lazar, M., and Brown, M. (2000). Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell* 103, 843–852. doi: 10.1016/s0092-8674(00)00188-4

Shen, H., Korutla, L., Champtiaux, N., Toda, S., LaLumiere, R., Vallone, J., et al. (2007). NAC1 regulates the recruitment of the proteasome complex into dendritic spines. *J. Neurosci.* 27, 8903–8913. doi: 10.1523/JNEUROSCI.1571-07.2007

Sheng, M., and Kim, M. (2002). Postsynaptic signaling and plasticity mechanisms. *Science* 298, 776–780. doi: 10.1126/science.1075333

Sheng, M., and Pak, D. (2000). Ligand-gated ion channel interactions with cytoskeletal and signaling proteins. *Annu. Rev. Physiol.* 62, 755–778. doi: 10.1146/annurev.physiol.62.1.755

Smejkalova, T., and Woolley, C. (2010). Estradiol acutely potentiates hippocampal excitatory synaptic transmission through a presynaptic mechanism. *J. Neurosci.* 30, 16137–16148. doi: 10.1523/JNEUROSCI.4161-10.2010

Smith, S., Haynes, K., and Hegde, A. (2020). Degradation of transcriptional repressor ATF4 during long-term synaptic plasticity. *Int. J. Mol. Sci.* 21:8543. doi: 10.3390/ijms21228543

Srivastava, D. P., Woolfrey, K., Jones, K. A., Shum, C. Y., Lash, L. L., Swanson, G. T., et al. (2008). Rapid enhancement of two-step wiring plasticity by estrogen and NMDA receptor activity. *Proc. Natl. Acad. Sci.* 105, 14650–14655. doi: 10.1073/pnas.0801581105

Tanaka, K. (2009). The proteasome: overview of structure and functions. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* 85, 12–36. doi: 10.2183/pjab.85.12

Tanaka, M., and Sokabe, M. (2013). Bidirectional modulatory effect of 17β-estradiol on NMDA receptors via ERα and ERβ in the dentate gyrus of juvenile male rats. *Neuropharmacology* 75, 262–273. doi: 10.1016/j.neuropharm.2013.07.029

Taxier, L. R., Gross, K. S., and Frick, K. M. (2020). Oestradiol as a neuromodulator of learning and memory. *Nat. Rev. Neurosci.* 21, 535–550. doi: 10.1038/s41583-020-0362-7

Tuscher, J. J., Fortress, A. M., Kim, J., and Frick, K. M. (2015). Regulation of object recognition and object placement by ovarian sex steroid hormones. *Behav. Brain Res.* 285, 140–157. doi: 10.1016/j.bbr.2014.08.001

Tuscher, J. J., Luine, V., Frankfurt, M., and Frick, K. M. (2016). Estradiol-mediated spine changes in the dorsal hippocampus and medial prefrontal cortex of ovariectomized female mice depend on ERK and mTOR activation in the dorsal hippocampus. *J. Neurosci.* 36, 1483–1489. doi: 10.1523/JNEUROSCI.3135-15.2016

Upadhya, S., Ding, L., Smith, T., and Hegde, A. (2006). Differential regulation of proteasome activity in the nucleus and the synaptic terminals. *Neurochem. Int.* 48, 296–305. doi: 10.1016/j.neuint.2005.11.003

Upadhya, S., Smith, T., and Hegde, A. (2004). Ubiquitin-proteasome-mediated CREB repressor degradation during induction of long-term facilitation. *J. Neurochem.* 91, 210–219. doi: 10.1111/j.1471-4159.2004.02707.x

Vedder, L., Smith, C., Flannigan, A., and McMahon, L. (2013). Estradiol-induced increase in novel object recognition requires hippocampal NR2B-containing NMDA receptors. *Hippocampus* 23, 108–115. doi: 10.1002/hipo.22068

Wong, M., and Moss, R. (1992). Long-term and short-term electrophysiological effects of estrogen on the synaptic properties of hippocampal CA1 neurons. *J. Neurosci.* 12, 3217–3225. doi: 10.1523/JNEUROSCI.12-08-03217.1992

Woolley, C., Gould, E., Frankfurt, M., and McEwen, B. (1990). Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *J. Neurosci.* 10, 4035–4039. doi: 10.1523/JNEUROSCI.10-12-04035.1990

Woolley, C., and McEwen, B. (1994). Estradiol regulates hippocampal dendritic spine density via an N-methyl- D-aspartate receptor-dependent mechanism. *J. Neurosci.* 14, 7680–7687. doi: 10.1523/JNEUROSCI.14-12-07680.1994

Woolley, C., Weiland, N., McEwen, B., and Schwartzkroin, P. (1997). Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: correlation with dendritic spine density. *J. Neurosci.* 17, 1848–1859. doi: 10.1523/JNEUROSCI.17-05-01848.1997

Zhang, F., Hu, Y., Huang, P., Toleman, C., Paterson, A., and Kudlow, J. (2007). Proteasome function is regulated by cyclic AMP-dependent protein kinase through phosphorylation of Rpt6. *J. Biol. Chem.* 282, 22460–22471. doi: 10.1074/jbc.M702439200

Zhang, Q., Han, D., Wang, R., Dong, Y., Yang, F., Vadlamudi, R. K., et al. (2011). C terminus of Hsc70-interacting protein (CHIP)-mediated degradation of hippocampal estrogen receptor α and the critical period hypothesis of estrogen neuroprotection. *Proc. Natl. Acad. Sci. U S A* 108, E617–E624. doi: 10.1073/pnas.1104391108

Zhou, W., and Slingerland, J. (2014). Links between between oestrogen receptor activation and proteolysis: relevance to hormone-regulated cancer therapy. *Nat. Rev. Cancer* 14, 26–38. doi: 10.1038/nrc3622

Conflict of Interest: KF is a co-founder of, and shareholder in, Estrigenix Therapeutics, Inc., a company which aims to improve women's health by developing safe, clinically proven treatment for the mental and physical effects of menopause. She also serves as the company's Chief Scientific Officer.

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frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 27 January 2022
doi: 10.3389/fnbeh.2021.777911

The Link Between Masculinity and Spatial Skills Is Moderated by the Estrogenic and Progestational Activity of Oral Contraceptives

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OPEN ACCESS

Edited by:
Caitlin M. Taylor, University of California, Santa Barbara, United States

Reviewed by:
Elaine M. Hull, Florida State University, United States
Jesse Lacasse, Concordia University, Canada
Elizabeth Chrastil, University of California, Irvine, United States

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Specialty section:
This article was submitted to Behavioral Endocrinology, a section of the journal Frontiers in Behavioral Neuroscience

Received: *16 September 2021*
Accepted: *14 December 2021*
Published: *27 January 2022*

Citation:
Beltz AM, Loviska AM, Kelly DP and Nielson MG (2022) The Link Between Masculinity and Spatial Skills Is Moderated by the Estrogenic and Progestational Activity of Oral Contraceptives. Front. Behav. Neurosci. 15:777911. doi: 10.3389/fnbeh.2021.777911

Conversations about gender and spatial skills frequently dissolve into a hackneyed debate over nature and nurture. This is particularly true for conversations concerning three-dimensional (3D) mental rotations skill, which shows the largest gender difference of all aspects of cognition, with men—on average—outperforming women. To advance this empirical area of inquiry, biopsychosocial influences on spatial skills should be considered, and a unique opportunity do to that is provided by combined oral contraceptives (OCs). OCs with relatively low estradiol doses and with highly androgenic progestins have been positively related to spatial skills. Gender self-concepts, including masculine and feminine self-perceptions, have also been positively related to spatial skills. It is wholly unknown, however, whether the exogenous sex hormones contained in OCs moderate the link between self-perceived masculinity and 3D mental rotations. This study filled that knowledge gap by utilizing a sample of 141 naturally cycling (NC) women and 229 OC users who completed a computerized survey and cognitive tests. A series of moderation analyses examined whether the link between masculinity and 3D mental rotations depended on pill use or on the estrogenic, progestational, or androgenic activity in OCs, which were operationalized using a novel coding scheme. Results showed that the positive masculinity-3D mental rotations link was only present for NC women, presumably because it was altered by the exogenous hormones in OCs. Indeed, the link was accentuated in users of OCs with relatively low estrogenic and high progestational activity. Future research on menstrual cycle and pill phase is needed, but these findings importantly delineate ways in which biological and psychosocial factors combine to explain variation in spatial skills among women. They also suggest that focus should be placed on the under-investigated progestational activity of OCs, which is facilitated by the novel quantification of OC action used in this study. Thus, this research increases understanding of the neurocognitive and behavioral correlates of ovarian hormones and has implications for the betterment of women's health.

Keywords: androgenic activity, estrogenic activity, progestational activity, mental rotation (MR), masculinity, femininity, hormonal contraceptive, ovarian hormones

INTRODUCTION

Interest in the biopsychosocial correlates of gender differences in spatial skills has been persistent across time and pervasive across scientists, parents, educators, and policy makers (see Newcombe, 2020). There is particular interest in three-dimensional (3D) mental rotations skill in which men outperform women on average, despite substantial variability within each gender (Voyer, 2011; Halpern, 2013; Beltz et al., 2020). Sex hormones, such as androgens and estradiol, have been consistently shown to be biological contributors to this gender difference. For instance, prenatal androgens facilitate spatial skills throughout life, and the gender difference in mental rotations skill is reduced during low estradiol phases of the menstrual cycle (Berenbaum et al., 2012; Peragine et al., 2020). There are also several psychosocial contributors to gender differences in spatial skills, including parent socialization (e.g., use of spatial language; Pruden and Levine, 2017) and a person's own gender self-concept, or how they think about and use gender labels like *masculine* and *feminine* (Nash, 1979; Reilly and Neumann, 2013). Despite emerging evidence and hypotheses concerning the gendered and interactive nature of biological and psychosocial influences on cognition (e.g., Hausmann et al., 2009; Berenbaum et al., 2018), research that concurrently examines these influences with respect to spatial skills is limited. Combined oral contraceptive (OC) users provide a unique opportunity to fill this knowledge gap because their pills contain exogenous estradiol and progestins that vary in androgenicity, yet they do not have different gender self-concepts (e.g., self-perceived femininity and masculinity) from naturally cycling (NC) women (Nielson and Beltz, 2021). Thus, the goal of this study was to determine whether ovarian hormonal milieu (marked by NC vs. OC status and the hormone activity of OCs) moderates links between women's self-perceived masculinity and mental rotations skill.

There is strong empirical support for a positive association between masculinity and spatial skills. Indeed, the *sex-role mediation hypothesis* attempts to explain some mean-level gender differences in cognition through this purported mechanism (Nash, 1979), such that masculinity is positively related to spatial skills, whereas femininity is positively related to verbal skills. Empirical evidence from meta-analyses and well-powered studies ($N > 300$) has borne out this general pattern of results for masculinity and spatial skills, but findings are less consistent for femininity and verbal skills (Signorella and Jamison, 1986; Reilly and Neumann, 2013; Reilly et al., 2016; Kelly and Beltz, under review). The most frequently assessed spatial skill in the extant literature is 3D mental rotations skill, which shows the largest effects, and masculinity is often assessed by stereotypical gendered personality traits (e.g., "assertive" or "independent"; Bem, 1974), but also by self-perceptions of masculinity (e.g., "How masculine is your personality?"; Storms, 1979).

Although there is empirical support for an association between masculinity and spatial skills, the extant literature is unclear concerning potential gender differences in this link. This is somewhat surprising because the link between masculinity and spatial skills is an explanatory mechanism for cognitive gender differences, according to the sex-role mediation hypothesis. For instance, some studies report stronger relations between masculinity and spatial skills for men than for women (Reilly and Neumann, 2013), and other studies report that the relation is stronger for women than for men (Signorella and Jamison, 1986; Kelly and Beltz, under review).

This mixed evidence for a gender difference is not entirely surprising, though, because masculinity and spatial skills are complex, multi-determined constructs (Ruble et al., 2006; Verdine et al., 2017). This is apparent in the conceptualization of the sex-role mediation hypothesis and in work examining it. For instance, Nash (1979) suggested that cultural norms surrounding gender impact masculinity, and this predisposes participation in gender stereotypical activities that hone spatial skillsets. Consistent with that notion, recent work shows that gendered experiences and interests (operationalized as the extent to which college majors have a science, technology, engineering, and mathematics, or STEM, focus) partially explain the relation between masculinity and spatial skills, but only for women and only for particular skills (e.g., 3D mental rotations; Kelly and Beltz, under review). Thus, there is a pressing need for research that explicates the relation between masculinity and spatial skills—not just by identifying which social factors underlie it, but also by identifying which biological factors might qualify it. Indeed, the multidimensionality of gender (Ruble et al., 2006) and a compelling literature on sex hormone contributions to spatial skills suggest biology could play a significant role (reviewed in Beltz et al., 2020).

A unique opportunity to study biological, particularly neuroendocrinological, associations with masculinity and spatial skills is afforded by women who use OCs, which are a widely used natural experiment for ovarian hormone manipulations (see Beltz and Moser, 2020; Hampson, 2020). All OCs contain a progestin (synthetic progesterone), and combined OCs also contain a synthetic estrogen (typically ethinyl estradiol; Beltz and Moser, 2020; Hampson, 2020). Progestins in OCs vary (e.g., there are at least 12 types), and they have hormonal activity other than progestational activity, with degrees of androgenic or anti-androgenic activity most frequently studied (Beltz and Moser, 2020; Hampson, 2020). Most OC regimens attempt to mimic the average menstrual cycle, with about 21 active pills (containing a progestin and perhaps ethinyl estradiol) followed by about seven placebo pills, instigating menses. Monophasic OCs have consistent progestin and ethinyl estradiol doses in all active pills, whereas biphasic and triphasic OCs typically have consistent ethinyl estradiol doses but progestin doses that increase once or twice, respectively, across active pill days. Through neuroendocrine feedback mechanisms, OCs effectively halt ovulation, both preventing pregnancies and easing menstrual cycle-induced symptoms (e.g., dysmenorrhea). Thus, OCs also reduce *endogenous* levels of estradiol and progesterone in the body, while increasing levels of the *exogenous* hormones contained in the pills (Hampson, 2020). Though enlightening, comparisons between endogenous and exogenous hormone activity in OC users vs. NC women are not straight-forward because assays for the exogenous hormones contained in OCs are not widely available.

Nonetheless, comparisons between OC users and NC women are effective at marking whether general ovarian hormonal milieus are related to behavior and neurocognition (consistent with the approach of others; e.g., Peragine et al., 2020). These comparisons also indicate whether there may be differences between women who do and do not use OCs, as self-selection into OC use has been posited as a potential confound of research using NC-OC comparisons to make inferences about ovarian hormone influences (Oinonen et al., 2008). The limited research comparing NC women and OC users (that included women from this sample) indicates that groups do not differ in Big Five personality traits (i.e., neuroticism, extraversion, openness, agreeableness, and conscientiousness; Beltz et al., 2019), nor in gendered personality qualities most closely tied to the sex-role mediation hypothesis, such as instrumentality and expressivity as well as self-perceptions of masculinity and femininity (Nielson and Beltz, 2021). Moreover, these studies showed that the androgenicity of progestins, which vary across different types of OCs, did not impact results (Beltz et al., 2019; Nielson and Beltz, 2021). Progestin androgenicity is the extent to which progestins have androgenic properties and actions that are primarily due to their structural derivatives. Thus, studies of ovarian hormone links to gender self-concept suggest OC users of various pill formations do not differ from NC women.

Similarly for spatial skills, many studies do not report differences between OC users and NC women when OC users are considered as a single, heterogeneous group (Rosenberg and Park, 2002; Islam et al., 2008; Mordecai et al., 2008; Wharton et al., 2008; Puts et al., 2010; Griksiene and Ruksenas, 2011; Beltz et al., 2015). One explanation for the lack of differences is that research including NC women is challenged by menstrual cycle phase. Although endogenous hormone levels fluctuate throughout the cycle (i.e., with low estradiol and progesterone during menses, heightened estradiol during the follicular phase, followed by ovulation and slightly decreasing estradiol levels and concomitant peak in progesterone during the luteal phase), extant research examining how those phases are related to spatial skills is inconsistent, likely owing to poor research methodology. Some studies report improved spatial skills, especially mental rotations performance, during the follicular phase (e.g., Hausmann et al., 2000; McCormick and Teillon, 2001; Maki et al., 2002), but others do not (e.g., Mordecai et al., 2008; Griksiene and Ruksenas, 2011). These studies have small samples (most $N < 40$) and largely use unreliable count methods (e.g., estimating days since menses) to determine menstrual cycle phase (see Hampson, 2020; Gloe et al., under review). Another explanation for the lack of differences when OC users are considered as a single, heterogeneous group is that the variable hormone activities of OCs "cancel out" (e.g., the effects of androgenic and anti-androgenic progestins; Pletzer and Kerschbaum, 2014).

There is, however, indication that OC users and NC women differ in spatial skills, particularly 3D mental rotations skill, when OC users are categorized into smaller, homogenous groups informed by their pharmacokinetics (described in Beltz et al., 2015). These groups have primarily been formed based upon progestin androgenicity, as androgens have been shown to facilitate spatial skills in other natural experiments (reviewed in Beltz et al., 2020). Increasingly precise progestin-linked groups have led to increasingly precise inferences about OC influences on spatial skills. For instance, groups based solely on progestin generation, or the timing of a specific progestin's introduction to the United States or European markets (Petitti, 2003), have shown mixed results: Studies reported no differences between NC women and androgenic generation or antiandrogenic generation OC users (Griksiene and Ruksenas, 2011) as well as increased spatial skills for androgenic early generation OC users compared to antiandrogenic newer generation users (Gurvich et al., 2020). Including information about progestin dose along with generation led to consistent inferences, though, with studies reporting that NC women outperformed monophasic antiandrogenic new generation OC users in mental rotations (Wharton et al., 2008; Griksiene et al., 2018), potentially suggesting that endogenous androgens in NC women contribute to performance. Finally, groups based on even more complete information about OC pharmacokinetics, including the exact type of progestin and estrogen as well as phase [according to Food and Drug Administration (FDA) criteria; USDHHS, 2018], revealed even more specific neuroendocrine effects: Women using monophasic pills containing ethinyl estradiol and the moderately androgenic progestin norethindrone acetate outperformed NC women and women using triphasic pills containing ethinyl estradiol and the mildly androgenic progestin norgestimate on a 3D mental rotations test (Beltz et al., 2015). This study using FDA criteria was also unique among OC studies in exploring exogenous hormone doses in OCs, finding that ethinyl estradiol dose was inversely related to mental rotations performance, but that progestin dose was not related to cognition (Beltz et al., 2015).

Thus, findings across OC studies converge in suggesting that there is a significant link between progestin androgenicity and spatial skills, especially mental rotations skill; ethinyl estradiol dose may also be important. These findings, however, overwhelmingly come from studies focused solely on the androgenicity of progestins, leaving unanswered questions about the roles of hormones that largely prevent ovulation, that is, the degree of estrogenic and progestational activity in different OCs. This may be an unfortunate byproduct of the methods researchers have used to account for OC heterogeneity, which rely almost exclusively on homogenous groups based on progestin generation or type. This approach certainly has benefits, but even these groups are not consistently defined across studies. Thus, future research that addresses heterogeneity in OCs without dropping information about degree of hormone activity, especially estrogenic and progestational activity, is sorely needed.

Despite a biological literature linking ovarian hormones (e.g., progestin androgenicity) to spatial skills and a psychosocial literature linking masculinity to spatial skills, the two perspectives have yet to be combined. Thus, the goal of this study was to take a biopsychosocial approach to the study of spatial skills, particularly 3D mental rotations skill, in NC women and OC users. Specifically, the link between self-perceived masculinity

and mental rotations skill was examined in all women, and then potential moderation of the link by hormonal milieu was investigated to determine if its direction or magnitude differed for NC women and OC users. Menstrual cycle phase was not considered due to its unreliable determination in cross-sectional data (see Hampson, 2020; Gloe et al., under review), but the pharmacokinetics of OCs (i.e., estrogenic, progestational, and androgenic activity, according to levels reported in Dickey, 2020) were examined as moderators of the masculinity-mental rotations link within OC users, leveraging a novel four-point coding scheme. These activities "are dependent on the biological activities and the doses of individual estrogen and progestin components and by potentiating and antagonistic effects of one steroid component upon the other" (Dickey, 2020, p. 25). Based on past research, it was expected that the link would differ for NC women and OC users due to the progestin androgenicity of pills.

MATERIALS AND METHODS

Previous reports use some data from this sample (e.g., to show that NC women and OC users do not differ in personality or gender self-concept; Beltz et al., 2019; Nielson and Beltz, 2021), but study variables have not been investigated in concert, and the coding scheme for examining unique effects of estrogenic, progestational, and androgenic activity in OCs is novel. All participants provided informed consent before contributing to this research, which was conducted under the auspices of the University of Michigan Institutional Review Board.

Participants

Participants were 370 women aged 18–28 years (M_{age} = 20.54 years; SD_{age} = 2.28) recruited from a United States university community via an established subject pool, online announcements, and posted flyers. Most identified as White (74%) and non-Hispanic (93%), with some identifying as Asian (16%), Black/African American (8%), or multiracial (2%). They were selected for inclusion in the current analyses from the full sample of 473 women and 221 men. Men were excluded from analyses because the primary research question on the role of ovarian hormone milieu in the link between behavior and cognition only concerned women. Women were excluded from analyses for the following reasons: having a reproductive health or medical condition that could impact ovarian hormone milieu, including past pregnancy, irregular menstrual cycles, the use of hormone-containing medications other than OCs (n = 64), being inattentive during data collection, including sleeping (n = 14) or failing to follow directions on the mental rotations test (n = 19), or being statistical outliers (i.e., three standard deviations from the sample mean) on age (n = 6); outliers bias error terms and age is related to masculinity and especially to femininity (Barrett and White, 2002; Jones et al., 2011).

Women in the analytic sample were grouped according to whether they were naturally cycling (n = 141) or using OCs (n = 229). OC users had been using the same pill for at least 3 months, and NC women had not used any hormonal contraceptive for at least 3 months and had regular menstrual cycles. OC users and NC women did not differ significantly in age, $t(368) = -1.36$, $p = 0.174$, or ethnicity, $\chi^2(1) = 0.25$, $p = 0.615$, but there were disproportionately more Asian identified women in the NC (26%) vs. OC group (11%), $\chi^2(4) = 21.02$, $p < 0.001$.

The OCs used by women in the sample represented a wide range of formulations. All included ethinyl estradiol, but progestins ranged from the highly androgenic levonorgestrel (n = 15) and norethindrone acetate (n = 71) to the mildly androgenic norgestimate (n = 71) to the antiandrogenic drospirenone (n = 45), among a variety of others (n = 18). Importantly, however, OCs were not merely grouped according to progestin types, but rather, they were coded according to their estrogenic, progestational, and androgenic activity, which are based on hormone types and doses, particularly their biological (e.g., receptor) actions (Dickey, 2020). Each hormone was considered to have *low*, *low-intermediate*, *intermediate-high*, or *high* activity in each OC formulation based on assays conducted in rodent and human tissues; estrogenic activity was determined by mouse uterine assay, progestational activity was determined by human endometrial response, and androgenic activity was determined by rat prostate assay (detailed in Dickey, 2020). These levels were converted into a four-point Likert scale to facilitate their inclusion in quantitative analyses (1 = *Low*, 2 = *Low-intermediate*; 3 = *Intermediate-high*, and 4 = *High*). For example, the commonly used OC Loestrin FE 1/20 (ethinyl estradiol, norethindrone acetate) had activity codes of estrogenic = 2, progestational = 3, and androgenic = 4, the triphasic Ortho Tri-Cyclen (ethinyl estradiol, norgestimate) had activity codes of estrogenic = 4, progestational = 1, and androgenic = 2, and Yaz (ethinyl estradiol, drospirenone) had activity codes of estrogenic = 2, progestational = 4, and androgenic = 1.

Nine OC users had pill types that could not be coded because their specific formulations were not contained in the coding system (Dickey, 2020). For the remaining 220 OC users (which makes this study among the largest—if not the largest—in terms of OC users with defined pharmacokinetic pill properties; see Warren et al., 2014), average estrogenic activity was 2.67 (SD = 1.03), progestational activity was 2.66 (SD = 1.29), and androgenic activity was 2.50 (SD = 1.18), and all activity levels ranged from 1 to 4, suggesting notable variability. Moreover, the activity levels showed both overlap and distinction, with estrogenic and progestational activity correlated at $r(218) = -0.64$, $p < 0.001$, estrogenic and androgenic activity at $r(218) = -0.36$, $p < 0.001$, and progestational and androgenic activity at $r(218) = 0.11$, $p = 0.114$. Thus, estrogenic and progestational activity were most highly related in this sample, but their relation only resulted in 41% overlap between the codes.

Procedures

Participants came to a university research laboratory for an hour-long test session. During the session, they provided informed consent, described their reproductive history in a brief interview, recorded information about their OC formulation from the pill packet they brought with them (if applicable), and completed

a monitored online survey. They were compensated with either course credit or $15.

Measures

Participants responded to the questionnaire and completed the two cognitive tests described below as part of this study's survey. All responses were provided on laboratory computers.

Masculinity

Self-perceived masculinity was assessed with the six-item Sex Role Identity Scale (Storms, 1979). This measure assesses gender self-concept, specifically gender self-labels, as items concern the extent to which individuals feel masculine or feminine in general, in their behavior, and in their dress. Specifically, three items correspond with masculinity (e.g., "How masculine is your personality?"), and three items correspond with femininity (e.g., "How feminine do you act, appear, and come across to others?"). Participants were asked to respond to each item on a five-point scale (1 = *Not at all* to 5 = *Extremely*). Following recent studies and current conceptualizations of gender as a continuum (Beltz, 2018; Gülgöz et al., 2019; Beltz et al., 2021), the three feminine items were reverse coded and averaged with the three masculine items, so that high scores reflect masculinity and low scores reflect femininity. Cronbach's α for the scale was excellent at 0.89.

3D Mental Rotations Skill

3D mental rotations skill was assessed with the Vandenberg and Kuse test (Vandenberg and Kuse, 1978). Each of 20 items consists of a 3D object composed of small blocks portrayed in 2D space and four response options that were similarly composed. Two of the response options were the same shape as the target but rotated in 3D space, which participants were instructed to identify. Participants had 10 min to complete the test. Following the "single scoring" procedure, participants received a single point for each correct response; thus, scores could range from 0 to 40.

General Cognition

General cognition was assessed with the advanced vocabulary test (Ekstrom et al., 1976), which is significantly correlated with overall intelligence (Sattler and Ryan, 2009). General cognition is important to consider in studies of spatial skills to isolate those skills from other aspects of cognition; this has been done in some previous neuroendocrine research (e.g., Beltz et al., 2015) and in research on the sex-role mediation hypothesis (Kelly and Beltz, under review). Each of 36 items consists of a target word or phrase and five response options. Participants were instructed to select which option is the best synonym of the target word, and they had 4 min to complete each half of the test. They received one point for each correct response, and were deducted a quarter point for each incorrect response; thus, scores could range from −9 to 36.

Analysis Plan

Analyses were conducted in three parts. First, a regression was used to examine the relation between self-perceived masculinity and 3D mental rotations skill for all women. Second, a moderation analysis was used to examine whether the masculinity-mental rotations relation differed between NC women (coded 0) and OC users (coded 1). Third, three separate moderation analyses in only OC users were used to examine whether the masculinity-mental rotations relation varied by pill estrogenic, progestational, or androgenic activity. Age and general cognition were covariates in all analyses, and all predictor variables were centered prior to analyses. Moderations were conducted using the PROCESS macro in SPSS (see Hayes, 2013), with follow-up simple slopes analyses conducted separately for each group (i.e., NC women vs. OC users) or for hormone activity and masculinity scores at the mean plus or minus one standard deviation. Type I error was set at 0.05 for all analyses.

RESULTS

Prior to testing study hypotheses on the link between masculinity and spatial skills and its potential moderation by hormonal milieu, NC and OC group descriptives, differences, and correlations among study variables were examined. Descriptives are shown on the left of **Table 1**. Independent *t*-tests revealed no significant differences for general cognition, $t(368) = 0.81$, $p = 0.421$, masculinity, $t(368) = 0.21$, $p = 0.837$, or 3D mental rotations, $t(368) = -0.08$, $p = 0.934$. Correlations for each group are shown on the right of **Table 1**, revealing consistent expected relations between age and general cognition, and between general cognition and mental rotations. The patterns of relations between masculinity and mental rotations differed across NC women and OC users; these differences are directly tested in moderations below.

Link Between Masculinity and Spatial Skills in All Women

The first inferential analysis examined the link between masculinity and spatial skills in all women. Results of the regression revealed a significant overall model, $F(3, 366) = 9.44$, $p < 0.001$, $R^2 = 0.07$, due to the significant covariate of general cognition, $b = 0.34$, $p < 0.001$; age was not a significant covariate, $b = 0.14$, $p = 0.431$. Importantly, masculinity was not a significant predictor of 3D mental rotations, $b = 0.59$, $p = 0.331$.

Oral Contraceptive Moderation of the Link Between Masculinity and Spatial Skills

The second inferential analysis was a moderation examining whether the link between masculinity and spatial skills differed for NC women and OC users; in other words, it examined whether the lack of an association in the full sample was due in part to women's hormonal milieu. Results of the regression revealed a significant overall model, $F(5, 364) = 6.76$, $p < 0.001$, $R^2 = 0.09$. General cognition continued to be a significant covariate, $b = 0.33$, $p < 0.001$, and age was not, $b = 0.17$, $p = 0.338$. Although there was a significant main effect of masculinity, $b = 2.35$, $p = 0.017$ and not NC-OC group, $b = 0.17$, $p = 0.819$,

TABLE 1 | Descriptive statistics and correlations among study variables by group.

	Descriptives				Correlations			
	NC women *N* = 141		OC users *N* = 229					
Variables	***M***	***SD***	***M***	***SD***	**Age**	**General cognition**	**Masculinity**	**Mental rotations**
Age	20.33	2.28	20.66	2.27		0.43***	0.12	0.25**
General cognition	13.42	5.70	12.97	4.88	0.31***		0.08	0.36***
Masculinity	2.07	0.61	2.06	0.63	0.28***	0.05		0.21*
Mental rotations	26.41	7.90	26.48	6.88	0.06	0.17**	−0.02	

Correlations for NC women are above the gray diagonal, and correlations for OC users are below the gray diagonal.
NC, Naturally cycling; OC, Oral contraceptive; M, Mean; SD, Standard deviation.
**p < 0.05, **p < 0.01, ***p < 0.001.*

these main effects must be considered in the context of the significant interaction between masculinity and group on 3D mental rotations, $b = -2.80$, $p = 0.023$. Follow-up simple slopes analyses revealed the expected significant positive association for NC women, $b = 2.35$, $p = 0.017$, but not for OC users, $b = -0.44$, $p = 0.558$. The nature of the interaction is also shown in the scatterplot in **Figure 1**. Individual women are shown by the gray circles (NC women) and black squares (OC users), with the zero-order linear relation for each group indicated by the gray (NC women) and black (OC users) lines.

Oral Contraceptive Pharmacokinetic Moderation of the Link Between Masculinity and Spatial Skills

The third inferential analysis was a moderation among only OC users examining whether the link between masculinity and spatial skills depended upon the pharmacokinetic properties of the pills. Similar to the previous moderation, it examined whether the lack of an association among OC users was due in part to the heterogeneity of OCs, specifically their estrogenic, progestational, and androgenic activity. Complete results are listed in **Table 2** and plotted in **Figure 2**, and significant findings are discussed here.

For estrogenic activity (top third of **Table 2**), the overall model of the relation between masculinity and mental rotations skill was not significant, but the interaction of masculinity and estrogenic activity was significant. Estrogenic activity at one standard deviation below the mean (dotted line in **Figure 2A**) was positively related to masculinity and 3D mental rotations, $b = 1.45$, $p = 0.201$, whereas estrogenic activity one standard deviation above the mean (solid line in **Figure 2A**) was inversely related to masculinity, $b = -1.69$, $p = 0.094$. Thus, the masculinity-mental rotations relation became increasingly positive as levels of OC estrogenic activity *decreased*.

For progestational activity (middle third of **Table 2**), the overall model was significant. General cognition was a significant, positive covariate, and the interaction of masculinity and progestational activity was significant, with effects in the opposite direction of estrogenic activity. Progestational activity at one standard deviation below the mean (dotted line in **Figure 2B**) was inversely related to masculinity and 3D mental rotations, $b = -1.66$, $p = 0.093$, whereas progestational activity one standard deviation above the mean (solid line in **Figure 2B**) was positively related to masculinity, $b = 1.52$, $p = 0.169$. Thus, the masculinity-mental rotations relation became increasingly positive as levels of OC progestational activity *increased*.

For androgenic activity (bottom third of **Table 2**), the overall model was not significant. The interaction of masculinity and androgenic activity was also not significant, but general cognition was a significant covariate (see **Figure 2C**).

Sensitivity analyses were conducted to determine whether length of OC use altered these relations; thus, moderations were repeated with length of OC use (in months) as a covariate. The pattern of results, including inferences about the significance of the interactions, did not change (estrogenic activity: $b = -1.51$, $p = 0.039$, progestational activity: $b = 1.17$, $p = 0.035$, androgenic activity: $b = 0.64$, $p = 0.315$).

DISCUSSION

The goal of this study was to leverage a natural experiment—varying hormonal milieus through OC use—to reveal biopsychosocial links to spatial skills in women. Based on research showing that masculinity and the progestin androgenicity of OCs are independently and positively associated with those skills, this study aimed to—for the first time—examine their combined influence. In a large sample of NC women and OC users, whose pill pharmacokinetic properties were innovatively coded on a four-point scale for estrogenic, progestational, and androgenic activity (see Dickey, 2020), hormonal milieu was found to moderate the relation between self-perceived masculinity and 3D mental rotations skill. Specifically, there was a positive relation for NC women and virtually no relation for OC users. The lack of an association among the heterogenous group of OC users was partly due to the varying pharmacokinetic properties of OCs, as the masculinity-mental rotations relation increased with both decreasing pill estrogenic activity and with increasing pill progestational activity. In contrast, androgenic activity of OCs was not significantly related to the relation between masculinity and mental rotations skill.

Interpretation of Findings

Across the full sample (i.e., NC women and OC users combined), there was not a significant relation between masculinity and

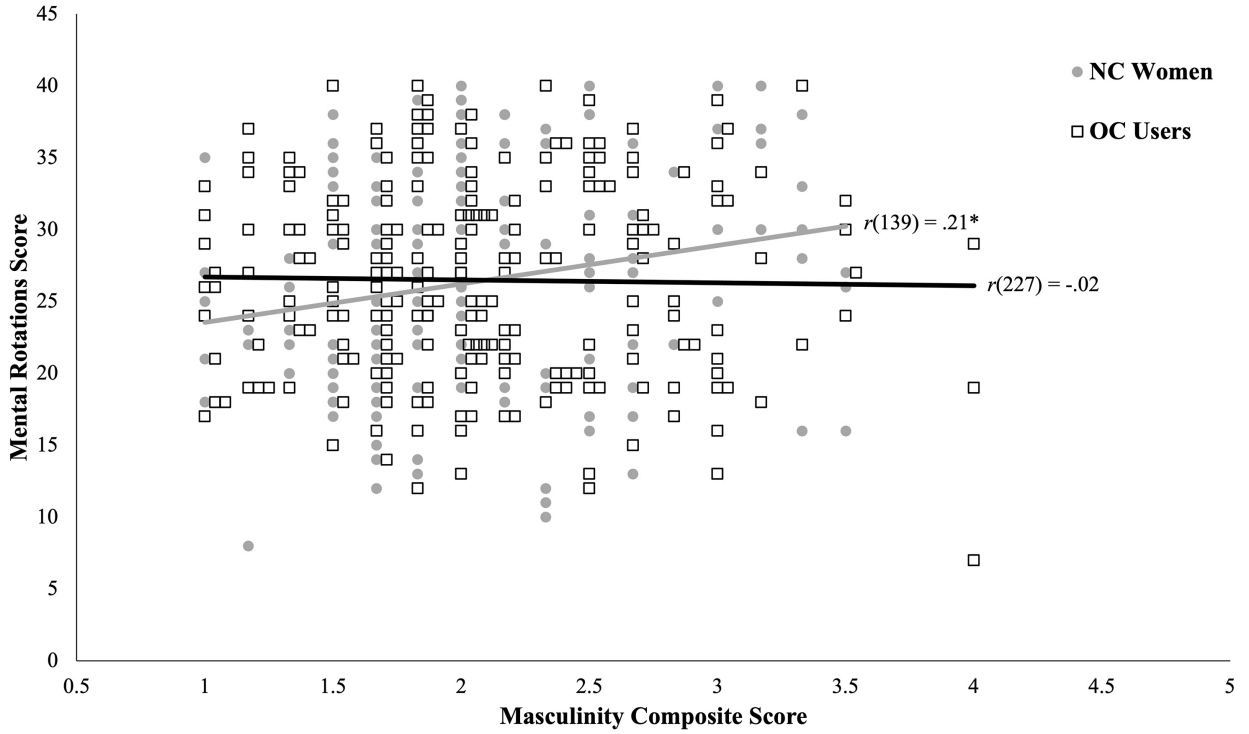

FIGURE 1 | Relation between masculinity and spatial skills (operationalized by three-dimensional mental rotations skill) plotted by hormonal milieu (operationalized by NC vs. OC status). Gray circles and black squares show individual data points of NC women and OC users, respectively, with like-colored lines reflecting the linear trends for each group (i.e., zero-order correlations). NC, naturally cycling; OC, oral contraceptive. *$p < 0.05$.

spatial skills. Even though a significant relation was expected according to the sex-role mediation hypothesis (Nash, 1979), the non-significance is not necessarily at odds with the extant literature. Past empirical studies have led to somewhat inconsistent results by statistically controlling for gender and even occasionally finding a stronger relation in men than women (Reilly and Neumann, 2013; Reilly et al., 2016). Importantly, results of moderation analyses qualify that non-significant masculinity-spatial skills relation in revealing that it depends upon ovarian hormonal milieu (marked by NC vs. OC status): Masculinity was significantly and positively related to mental rotations skill for NC women, but not for OC users. The robustness of this interaction effect is buttressed by the fact that NC women and OC users did not differ in mean levels in any study variables, including gender self-concept or mental rotations skill, consistent with past research in heterogenous samples (e.g., Beltz et al., 2015; Nielson and Beltz, 2021). Thus, the sex-role mediation hypothesis may only describe women with some hormonal milieus (e.g., those experiencing the typical fluctuations in endogenous ovarian hormones that characterize the menstrual cycle). As past studies on the sex-role mediation hypothesis did not report the hormonal milieu of study participants (e.g., whether all women were NC), it is possible that varying hormonal milieus contributed to their inconsistent findings. Additionally, varying hormonal milieus may even contribute to the inconsistent findings among men for whom the masculinity-spatial skills relation is not consistently found (e.g., Signorella and Jamison, 1986; Kelly and Beltz, under review), and who experience fluctuations in endogenous sex hormones levels depending upon season of the year, life experiences like

TABLE 2 | Results of moderation analyses examining whether sex hormone activity in OCs moderates the link between masculinity and mental rotations skill.

Moderation model	*b*	*F*	df	R^2
Estrogenic activity		2.12$^+$	5, 214	0.05
Age	0.04			
General cognition	0.19$^+$			
Masculinity	−0.12			
Estrogenic activity	0.14			
Interaction	−1.52*			
Progestational activity		2.45*	5, 214	0.05
Age	−0.07			
General cognition	0.20*			
Masculinity	−0.07			
Progestational activity	0.38			
Interaction	1.23*			
Androgenic activity		1.61	5, 214	0.04
Age	0.03			
General cognition	0.22*			
Masculinity	−0.27			
Androgenic activity	−0.41			
Interaction	0.65			

N = 220 OC users, and unstandardized b's are shown in table.
*$^+p < 0.10$; *$p < 0.05$.*

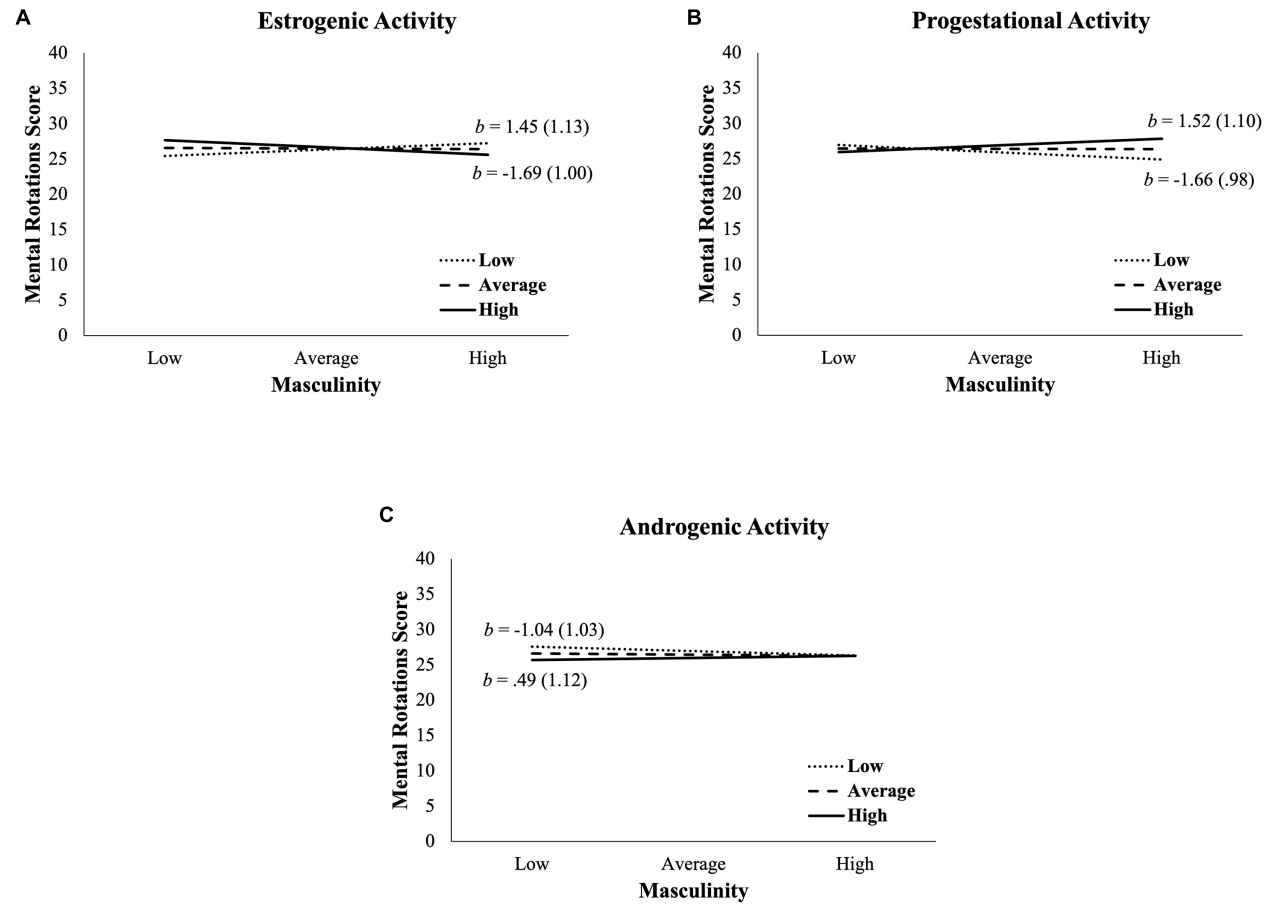

FIGURE 2 | Relation between masculinity and spatial skills (operationalized by three-dimensional mental rotations skill) plotted by oral contraceptive hormone activity (operationalized by a novel 4-point coding scheme). **(A)** The masculinity and spatial skills relation was significantly moderated by OC estrogenic activity. **(B)** The masculinity and spatial skills relation was significantly moderated by OC progestational activity. **(C)** The masculinity and spatial skills relation was not moderated by OC androgenic activity. Dotted, dashed, and solid black lines reflect low, average, and high hormone activity, respectively. Low vs. high hormone activity and masculinity reflect the conditional effect for the model estimated for scores ± one standard deviation from the mean, respectively. Unstandardized coefficients (*b*) reflect the masculinity-mental rotations relation for the low and high hormone activity levels with standard errors in parentheses. See **Table 2** for results of statistical interactions.

fatherhood, and even daily experiences like winning a game (e.g., Zilioli and Watson, 2012; Smith et al., 2013; Grebe et al., 2019).

To directly investigate the extent to which ovarian hormonal milieus may have contributed to the null masculinity-spatial skills relation in OC users, the OCs of all users in the study were coded on a four-point Likert scale for their estrogenic, progestational, and androgenic activity; the codes were based on assays conducted in rodent models and human tissue response, largely reflecting the action of OCs at receptors in the context of other hormones (see Dickey, 2020). Moderation analyses then revealed that the relation between masculinity and 3D mental rotations depended upon both the estrogenic and progestational—but not significantly upon the androgenic—activity in OCs, although effects were clearly small (as seen in **Figure 2**) and require replication. Specifically, as OC estrogenic activity decreased but as progestational activity increased, there was an increasingly positive association between masculinity and spatial skills. Findings are consistent with the inverse association between OC estrogenic and progestational activity in this sample. Also, findings regarding estrogenic activity are generally consistent with past work among OC users in showing an inverse association between pill estradiol dose and spatial skills (Beltz et al., 2015), and are also consistent with past menstrual cycle studies showing an inverse relation between endogenous estradiol and spatial skills (Hausmann et al., 2000; Courvoisier et al., 2013; Hampson et al., 2014; Griksiene et al., 2018, 2019), as well as with studies that show improvements in spatial skills when estradiol levels are low (Maki et al., 2002; Courvoisier et al., 2013).

Findings regarding progestational activity are novel, as there is hardly any consideration of the effects of the progestins in OCs—beyond their androgenicity—in the extant literature (for an exception and null findings confounded by androgenicity, see Beltz et al., 2015). Furthermore, findings from past research on endogenous progesterone (e.g., from menstrual cycle studies that often have notable methodological limitations, such as small

sample size) are mixed (reporting null, positive, and inverse associations; Hausmann et al., 2000; Maki et al., 2002; Griksiene and Ruksenas, 2011; Courvoisier et al., 2013; Hampson et al., 2014; Noreika et al., 2014; Pletzer et al., 2019; Shirazi et al., 2021). There does seem to be a potential indirect role for progesterone in spatial skills through attention, though. Progesterone has been linked to the local vs. global processing of visual stimuli (Pletzer et al., 2014), and similar global-local processing notions have been used to explain gender differences in spatial skills, especially mental rotations (Peña et al., 2008; Pletzer et al., 2014).

Therefore, this research is important not only for the empirical findings it uncovered, but also for introducing and illustrating the utility of a new coding scheme for OCs that disentangles the estrogenic, progestational, and androgenic properties of the pills. Past work has highlighted the importance of considering heterogeneity in ovarian hormonal milieus afforded by different OCs (Beltz and Moser, 2020), but most work has accomplished this by classifying users based on the types of hormones in their pills, such as androgenic or anti-androgenic progestins (e.g., Wharton et al., 2008; Griksiene and Ruksenas, 2011; Gurvich et al., 2020) or according to active ingredients specified by the FDA (e.g., Puts et al., 2010; Beltz et al., 2015, 2019). Some studies have also accomplished this by considering the exogenous dose of one hormone in the pills (e.g., ethinyl estradiol) without simultaneously considering other hormones (e.g., Beltz et al., 2015; Griksiene et al., 2018). The novel four-point coding scheme used in this study may be an improvement upon all of these approaches. It is based on the biological activity of the exogenous hormones in animal tissue, and it does not require the creation of inconsistent group classifications that could result in some participants being excluded from analyses (e.g., if they are using an OC that is not widely represented in the sample). Although this study did not have power to detect higher order interactions, this coding scheme permits the examination of interaction effects between different hormone activities in OCs in future studies with even larger samples (e.g., whether progestational and androgenic activity combine masculinity to influence outcomes).

Because OC androgenic activity did not moderate the masculinity-spatial skills relation, findings from this study may seem inconsistent with past work showing that OCs with highly androgenic progestins facilitate spatial skills, whereas those with anti-androgenic progestins reduce spatial skills (Beltz et al., 2015; Griksiene et al., 2018; Gurvich et al., 2020), and with work showing a positive association between endogenous testosterone and spatial skills (Hausmann et al., 2000; Pletzer et al., 2019). Not all studies find links between progestin androgenicity and spatial skills, though (e.g., Griksiene and Ruksenas, 2011), and no studies have investigated potential neuroendocrine modulation of the relation between masculinity and spatial skills. Moreover, and as noted above, the OC groupings used in past research may conflate androgenic and progestational activity by focusing on groups of OC users determined by progestin androgenicity. It could very well be that past work focused on androgenic properties of pills was actually reflecting (at least to some meaningful degree) progestational activity. For instance, OCs containing the anti-androgenic progestin drospirenone have among the highest progestational activity (Dickey, 2020). Such conflation is less likely in this study because of the small correlation between androgenic and progestational activity.

Study Considerations

Study findings and presumed methodological advances should be considered in light of other characteristics of the sample and approach. Regarding the sample, it is likely not representative of all OC users, as most participants were White, from a Midwestern university community, and six women who were deemed statistical age outliers were excluded from analyses. Women who identified as Asian were also disproportionately underrepresented among OC users (compared to NC women). It is vital that future research on ovarian hormone links to the brain and behavior include increasingly diverse and longitudinal samples to best inform women's health across the lifespan. Hopefully, such research will be facilitated by the advances in OC-related research methods proposed in this and other recent work (reviewed in Beltz and Moser, 2020; Hampson, 2020).

Regarding the study approach, a common, validated, and reliable measure of spatial skills, specifically the Vandenberg and Kuse (1978) 3D mental rotations test, was used. This test shows an established gender difference (Voyer, 2011; Halpern, 2013; Beltz et al., 2020), making it ideal for this study's goal of detecting gendered links (via masculinity) to mental rotations performance. From these findings, however, it is not clear what aspect of 3D mental rotations skill is linked to masculinity. Although general cognitive ability can be largely discounted (as it was a covariate in all analyses), other possibilities are not easily parsed, including actual mental visualization, strategy use including global vs. local processing, or working memory interference caused by time constraints (see Peters, 2005; Pletzer, 2014; Boone and Hegarty, 2017). It is also not clear if the pattern of findings would be the same if a different mental rotations test was used, particularly a test that shows smaller average gender differences than does the Vandenberg and Kuse (1978) measure; this includes 2D tests and the Shepard and Metzler (1971) 3D test. Thus, future work aimed at replicating and potentially decomposing mental rotations skill and its links to masculinity in the context of varying hormonal milieus is needed.

Also, the focus of this study was on the overarching hormonal milieu afforded by having a natural menstrual cycle or using OCs with particular pharmacokinetic formulations, both of which holistically reflect neuroendocrine function. This leads to some unique considerations for NC women and OC users. Regarding NC women, we did not include menstrual cycle phases in analyses, as methods for determining them are error-ridden without many repeated assessments (Hampson, 2020; Gloe et al., under review). Nonetheless, study findings are broadly consistent with menstrual cycle research on spatial skills, suggesting small roles for estradiol and progesterone. Regarding OC users, we similarly did not assess active vs. placebo pill phase, as placebo phases and adherence to them vary greatly across users. Moreover, we did not assess time of pill ingestion; it is linked to spikes in bloodstream hormone concentrations, but the spikes have unclear implications for concentrations

across days, and the temporal association between bloodstream concentrations and central nervous system function is largely unknown (Jusko, 2017; Hampson, 2020). Thus, future research using valid and reliable measures of cycle phase and investigating the pharmacokinetic properties of OCs on neurally mediated processes is sorely needed.

Moreover, this study—like all complex biopsychosocial research on OCs—has some unique considerations. For instance, this study was novel in leveraging a coding scheme for estrogenic, androgenic, and progestational activity of OCs, there are undoubtedly individual differences in the biopsychosocial impacts and correlates of these exogenous hormones, as humans are likely more complex than the animal models upon which the coding scheme is based. Thus, there is a pressing need for future work that considers individual women and their daily OC use across time (e.g., intensive longitudinal studies, such as Weigard et al., 2021; Gloe et al., under review).

Finally, the vast majority of the extant research on the sex-role mediation hypothesis (and on gender, more broadly) has operationalized *masculinity* in terms of explicitly defined gendered personality characteristics (e.g., independent vs. patient), which are to some extent culturally determined and time-varying (for a meta-analysis, see Donnelly and Twenge, 2017). In the present study and consistent with established research (e.g., Egan and Perry, 2001; Beltz et al., 2021; Nielson and Beltz, 2021; Kelly and Beltz, under review), masculinity was operationalized by gender self-expression, with its meaning (and the meaning of femininity) being implicitly defined by the participant. Gendered personality qualities and self-expressions are certainly related, but they are distinct constructs with distinct outcomes (Eagly and Wood, 2017; Hyde et al., 2019). Thus, future biopsychosocial research will likely benefit from having multidisciplinary teams who grapple with psychosocial measurement alongside biological indices of hormone activity.

CONCLUSION

This biopsychosocial study examined the sex-role mediation hypothesis in the context of women's ovarian hormonal milieus, revealing that exogenous hormones in OCs moderate the relation between self-perceived masculinity and 3D mental rotations skill: There was a positive relation for NC women and women using OCs with low estrogenic and high progestational activity, but not for women using OCs with intermediate exogenous hormone activity. Moreover, the androgenic activity of OCs was not a significant moderator of the masculinity-spatial skills relation, which was unexpected but plausible, as androgenic and progestational activity were likely confounded in past research. Findings are important not only in delineating the ways in which biological (i.e., ovarian hormone milieu) and psychosocial (i.e., self-perceived masculinity) factors combine to explain variation in spatial skills among women, but also in demonstrating the utility of a new approach for indexing the hormone activity of OCs. In these innovative ways, this study may propel forward research on women's health, particularly the neurocognitive and behavioral correlates of ovarian hormones.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because data use agreements should be established. Requests to access the datasets should be directed to AB, abeltz@umich.edu.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of Michigan IRB (Health Sciences and Behavioral Sciences). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AB conceptualized and directed the study. AL collected the data. AB analyzed the data with critical input from DK and MN. All authors drafted the manuscript, provided critical revisions, and approved the final version.

FUNDING

AB was supported by the Jacobs Foundation. MN was supported by the National Institutes of Health grant T32HD007109 (PIs: V. C. McLoyd and C. S. Monk).

ACKNOWLEDGMENTS

We thank the following people who contributed to the success of this study: Members of the Methods, Sex differences, and Development—M(SD)—Lab at the University of Michigan helped with data collection and management; Jovan Jande and Julianna King assisted with the conceptualization of a preliminary investigation; and the participants took time and effort to share their thoughts, experiences, and skills.

REFERENCES

Barrett, A. E., and White, H. R. (2002). Trajectories of gender role orientations in adolescence and early adulthood: a prospective study of the mental health effects of masculinity and femininity. *J. Health Soc. Behav.* 43, 451–468. doi: 10.2307/3090237

Beltz, A. M. (2018). Gendered mechanisms underlie the relation between pubertal timing and adult depressive symptoms. *J. Adolesc. Health* 62, 722–728. doi: 10.1016/j.jadohealth.2017.12.019

Beltz, A. M., Loviska, A. M., and Kelly, D. P. (2019). No personality differences between oral contraceptive users and naturally cycling women: implications for research on sex hormones.

Psychoneuroendocrinology 100, 127–130. doi: 10.1016/j.psyneuen.2018.09.034

Beltz, A. M., Hampson, E., and Berenbaum, S. A. (2015). Oral contraceptives and cognition: a role for ethinyl estradiol. *Horm. Behav.* 74, 209–217. doi: 10.1016/j.yhbeh.2015.06.012

Beltz, A. M., Kelly, D. P., and Berenbaum, S. A. (2020). "Sex differences in brain and behavioral development," in *Neural Circuit Development And Function In The Healthy And Diseased Brain*, 2nd Edn, ed. H. Tager-Flusberg (Amsterdam: Elsevier), 585–638. doi: 10.1016/B978-0-12-814411-4.00027-5

Beltz, A. M., Loviska, A. M., and Weigard, A. (2021). Daily gender expression is associated with psychological adjustment for some people, but mainly men. *Sci. Rep.* 11:9114. doi: 10.1038/s41598-021-88279-4

Beltz, A. M., and Moser, J. S. (2020). Ovarian hormones: a long overlooked but critical contributor to cognitive brain structures and function. *Ann. N. Y. Acad. Sci.* 146, 156–180. doi: 10.1111/nyas.14255

Bem, S. L. (1974). The measurement of psychological androgyny. *J. Consult. Clin. Psychol.* 42, 155–162. doi: 10.1037/h0036215

Berenbaum, S. A., Beltz, A. M., Bryk, K., and McHale, S. (2018). Gendered peer involvement in girls with congenital adrenal hyperplasia: effects of prenatal androgens, gendered activities, and gender cognitions. *Arch. Sex. Behav.* 47, 915–929. doi: 10.1007/s10508-017-1112-4

Berenbaum, S. A., Korman Bryk, K. L., and Beltz, A. M. (2012). Early andrgen effects on spatial and mechanical abilities: evidence from congenital adrenal hyperplasia. *Behav. Neurosci.* 126, 86–96. doi: 10.1037/a0026652

Boone, A. P., and Hegarty, M. (2017). Sex differences in mental rotation tasks: not just in the mental rotation process! *J. Exp. Psychol. Learn. Mem. Cogn.* 43, 1005–1019. doi: 10.1037/xlm0000370

Courvoisier, D. S., Renaud, O., Geiser, C., Paschke, K., Gaudy, K., and Jordan, K. (2013). Sex hormones and mental rotation: an intensive longitudinal investigation. *Horm. Behav.* 63, 345–351. doi: 10.1016/j.yhbeh.2012.12.007

Dickey, R. P. (2020). *Managing Contraceptive Pill Patients And Managing Hormone Replacement*, 16th Edn. New Orleans: Emis Medical Publishing.

Donnelly, K., and Twenge, J. M. (2017). Masculine and feminine traits on the Bem Sex-Role Inventory, 1993-2012: a cross-temporal meta-analysis. *Sex Roles* 76, 556–565. doi: 10.1007/s11199-016-0625-y

Eagly, A. H., and Wood, W. (2017). Janet Taylor Spence: innovator in the study of gender. *Sex Roles* 77, 725–733. doi: 10.1007/s11199-017-0835-y

Egan, S. K., and Perry, D. G. (2001). Gender identity: a multidimensional analysis with implications for psychosocial adjustment. *Dev. Psychol.* 37, 451–463. doi: 10.1037//0012-I649.37.4.45I

Ekstrom, R. B., French, J. W., and Harman, H. H. (1976). *Kit of Factor-Referenced Cognitive Tests*. Princeton NJ: Educational Testing Service.

Grebe, N. M., Sarafin, R. E., Strenth, C. R., and Zilioli, S. (2019). Pair-bonding, fatherhood, and the role of testosterone: a meta-analytic review. *Neurosci. Biobehav. Rev.* 98, 221–233. doi: 10.1016/j.neubiorev.2019.01.010

Griksiene, R., Arnatkeviciute, A., Monciunskaite, R., Koenig, T., and Ruksenas, O. (2019). Mental rotation of sequentially presented 3D figures: sex and sex hormones related differences in behavioural and ERP measures. *Sci. Rep.* 9:18843. doi: 10.1038/s41598-019-55433-y

Griksiene, R., Monciunskaite, R., Arnatkeviciute, A., and Ruksenas, O. (2018). Does the use of hormonal contraceptives affect the mental rotation performance? *Horm. Behav.* 100, 29–38. doi: 10.1016/j.yhbeh.2018.03.004

Griksiene, R., and Ruksenas, O. (2011). Effects of hormonal contraceptives on mental rotation and verbal fluency. *Psychoneuroendocrinology* 36, 1239–1248. doi: 10.1016/j.psyneuen.2011.03.001

Gurvich, C., Warren, A. M., Worsley, R., Hudaib, A. R., Thomas, N., and Kulkarni, J. (2020). Effects of oral contraceptive androgenicity on visuospatial and social-emotional cognition: a prospective observational trial. *Brain Sci.* 10:194. doi: 10.3390/brainsci10040194

Gülgöz, S., Glazier, J. J., Enright, E. A., Alonso, D. J., Durwood, L. J., Fast, A. A., et al. (2019). Similarity in transgender and cisgender children's gender development. *Proc. Natl. Acad. Sci. U. S. A.* 116, 24480–24485. doi: 10.1073/pnas.1909367116

Halpern, D. F. (2013). *Sex Differences In Cognitive Abilities*. Hove, East Sussex: Psychology Press, doi: 10.4324/978141060

Hampson, E. (2020). A brief guide to the menstrual cycle and oral contraceptive use for researchers in behavioral endocrinology. *Horm. Behav.* 119:104655. doi: 10.1016/j.yhbeh.2019.104655

Hampson, E., Levy-Cooperman, N., and Korman, J. M. (2014). Estradiol and mental rotation: relation to dimensionality, difficulty, or angular disparity? *Horm. Behav.* 65, 238–248. doi: 10.1016/j.yhbeh.2013.12.016

Hausmann, M., Schoofs, D., Rosenthal, H. E. S., and Jordan, K. (2009). Interactive effects of sex hormones and gender stereotypes on cognitive sex differences: a psychobiosocial approach. *Psychoneuroendocrinology* 34, 289–401. doi: 10.1016/j.psyneuen.2008.09.019

Hausmann, M., Slabbekoorn, D., Van Goozen, S. H. M., Cohen-Kettenis, P. T., and Gunturkun, O. (2000). Sex hormones affect spatial abilities during the menstrual cycle. *Behav. Neurosci.* 114, 1245–1250. doi: 10.1037/0735-7044.114.6.1245

Hayes, A. F. (2013). *Introduction to Mediation, Moderation, and Conditional Process Analysis*. New York, NY: The Guilford Press.

Hyde, J. S., Bigler, R. S., Joel, D., Tate, C. C., and van Anders, S. M. (2019). The future of sex and gender in psychology: five challenges to the gender binary. *Am. Psychol.* 74, 171–193. doi: 10.1037/amp0000307

Islam, F., Sparkes, C., Roodenrys, S., and Astheimer, L. (2008). Short-term changes in endogenous estrogen levels and consumption of soy isoflavones affect working and verbal memory in young adult females. *Nutr. Neurosci.* 11, 251–262. doi: 10.1179/147683008X301612

Jones, C. J., Peskin, H., and Livson, N. (2011). Men's and women's change and individual differences in change in femininity from age 33 to 85: results from the intergenerational studies. *J. Adult Dev.* 18, 155–163. doi: 10.1007/s10804-010-9108-5

Jusko, W. J. (2017). Perspectives on variability in pharmacokineatics of an oral contraceptive product. *Contraception* 95, 5–9. doi: 10.1016/j.contraception.2016.07.019

Maki, P. M., Rich, J. B., and Rosenbaum, R. S. (2002). Implicit memory varies across the menstrual cycle: estrogen effects in young women. *Neuropsychologia* 40, 518–529. doi: 10.1016/s0028-3932(01)00126-9

McCormick, C. M., and Teillon, S. M. (2001). Menstrual cycle variation in spatial ability: relation to salivary cortisol levels. *Horm. Behav.* 39, 29–38. doi: 10.1006/hbeh.2000.1636

Mordecai, K. L., Rubin, L. H., and Maki, P. M. (2008). Effects of menstrual cycle phase and oral contraceptive use on verbal memory. *Horm. Behav.* 54, 286–293. doi: 10.1016/j.yhbeh.2008.03.006

Nash, S. C. (1979). "Sex role as a mediator of intellectual functioning," in *Sex-Related Differences In Cognitive Functioning*, eds M. A. Wittig and A. C. Peterson (Cambridge, Massachusetts: Academic Press).

Newcombe, N. S. (2020). The puzzle of spatial sex differences: current status and prerequisites to solutions. *Child Dev. Perspect.* 14, 251–257. doi: 10.1111/cdep.12389

Nielson, M. G., and Beltz, A. M. (2021). Oral contraceptive use is not related to gender self-concept. *Psychoneuroendocrinology* 129:105271. doi: 10.1016/j.psyneuen.2021.105271

Noreika, D., Griskova-Bulanova, I., Alaburda, A., Baranauskas, M., and Griksiene, R. (2014). Progesterone and mental rotation task: is there any effect? *Biomed Res. Int.* 2014:741758. doi: 10.1155/2014/741758

Oinonen, K. A., Jarva, J. A., and Mazmanian, D. (2008). "Pre-existing hormonal differences between oral contraceptive users and nonusers? Evidence from digit ratio, age of menarche, and sociosexual orientation," in *Progress In Biological Psychology Research*, ed. G. A. Conti (Hauppauge, NY: Nova Science Publisher).

Peña, D., Contreras, M. J., Shih, P. C., and Santacreu, J. (2008). Solution strategies as possible explanations of individual and sex differences in a dynamic spatial task. *Acta Psychol.* 128, 1–14. doi: 10.1016/j.actpsy.2007.09.005

Peragine, D., Simeon-Spezzaferro, C., Brown, A., Gervais, N. J., Hampson, E., and Einstein, G. (2020). Sex difference or hormonal difference in mental rotation? The influence of ovarian milieu. *Psychoneuroendocrinology* 115:104488. doi: 10.1016/j.psyneuen.2019.104488

Peters, M. (2005). Sex differences and the factor of time in solving Vandenberg and Kuse mental rotation problems. *Brain Cogn.* 57, 176–184. doi: 10.1016/j.bandc.2004.08.052

Petitti, D. B. (2003). Combination estrogen-progestin oral contraceptives. *N. Engl. J. Med.* 349, 1443–1450. doi: 10.1056/NEJMcp030751

Pletzer, B. (2014). Sex-specific strategy use and global-local processing: a perspective toward integrating sex differences in cognition. *Front. Neurosci.* 8:425. doi: 10.3389/fnins.2014.00425

Pletzer, B., Petasis, O., and Cahill, L. (2014). Switching between forest and trees: opposite relationship of progesterone and testosterone to global-local processing. *Horm. Behav.* 66, 257–266. doi: 10.1016/j.yhbeh.2014.05.004

Pletzer, B., Steinbeisser, J., van Laak, L., and Harris, T. (2019). Beyond biological sex: interactive effects of gender role and sex hormones on spatial abilities. *Front. Neurosci.* 13:675. doi: 10.3389/fnins.2019.00675

Pletzer, B. A., and Kerschbaum, H. H. (2014). 50 years of hormonal contraception—time to find out, what it does to our brain. *Front. Neurosci.* 8:256. doi: 10.3389/fnins.2014.00256

Pruden, S. M., and Levine, S. C. (2017). Parents' spatial language mediates a sex difference in preschoolers' spatial-language use. *Psychol. Sci.* 28, 1583–1596. doi: 10.1177/0956797617711968

Puts, D. A., Cárdenas, R. A., Bailey, D. H., Burriss, R. P., Jordan, C. L., and Breedlove, S. M. (2010). Salivary testosterone does not predict mental rotation performance in men or women. *Horm. Behav.* 58, 282–289. doi: 10.1016/j.yhbeh.2010.03.005

Reilly, D., and Neumann, D. L. (2013). Gender-role differences in spatial ability: a meta-analytic review. *Sex Roles* 68, 521–535. doi: 10.1007/s11199-013-0269-0

Reilly, D., Neumann, D. L., and Andrews, G. (2016). Sex and sex-role differences in specific cognitive abilities. *Intelligence* 54, 147–158. doi: 10.1016/j.intell.2015.004

Rosenberg, L., and Park, S. (2002). Verbal and spatial functions across the menstrual cycle in healthy young women. *Psychoneuroendocrinology* 27, 835–841. doi: 10.1016/s0306-4530(01)00083-x

Ruble, D. N., Martin, C. L., and Berenbaum, S. A. (2006). "Gender development," in *Handbook of Child Psychology*, 6th Edn, eds N. Eisenberg, W. Damon, and R. M. Lerner (Hoboken: John Wiley & Sons, Inc), 858–932. doi: 10.1002/9780470147658.chpsy0314

Sattler, J. M., and Ryan, J. J. (2009). *Assessment with the WAIS-IV*. San Diego: Jerome M. Sattler.

Shepard, R. N., and Metzler, J. (1971). Mental rotation of three-dimensional objects. *Science* 171, 701–703. doi: 10.1126/science.171.3972.701

Shirazi, T. N., Levenberg, K., Cunningham, H., Self, H., Dawood, K., Cardenas, R., et al. (2021). Relationships between ovarian hormone concentrations and mental rotations performance in naturally-cycling women. *Horm. Behav.* 127:104886. doi: 10.1016/j.yhbeh.2020.104886

Signorella, M. L., and Jamison, W. (1986). Masculinity, femininity, androgyny, and cognitive performance: a meta-analysis. *Psychol. Bull.* 100, 207–228. doi: 10.1037/0033-2909.100.2.207

Smith, R. P., Coward, R. M., Kovac, J. R., and Lipshultz, L. I. (2013). The evidence for seasonal variations of testosterone in men. *Maturitas* 74, 208–212. doi: 10.1016/j.maturitas.2012.12.003

Storms, M. D. (1979). Sex-role identity and its relationship to sex role attributes and sex role stereotypes. *J. Pers. Soc. Psychol.* 37, 1779–1789. doi: 10.1037/0022-3514.37.10.1779

USDHHS (2018). *Approved Drug Products With Therapeutic Equivalence Evaluations*, 38th Edn. Silver Spring, Maryland: Food and Drug Administration.

Vandenberg, S. G., and Kuse, A. R. (1978). Mental rotations, a group test of three-dimensional spatial visualization. *Percept. Mot. Skills* 47, 599–604. doi: 10.2466/pms.1978.47.2.599

Verdine, B. N., Golinkoff, R. M., Hirsh-Pasek, K., and Newcombe, N. S. (2017). Spatial skills, their development, and their links to mathematics. *Monogr. Soc. Res. Child Dev.* 82, 7–30. doi: 10.1111/mono.12280

Voyer, D. (2011). Time limits and gender differences on paper-and-pencil tests of mental rotation: a meta-analysis. *Psychon. Bull. Rev.* 18, 267–277. doi: 10.3758/s13423-010-0042-0

Warren, A. M., Gurvich, C., Worsley, R., and Kulkarni, J. (2014). A systematic review of the impact of oral contraceptives on cognition. *Contraception* 90, 111–116. doi: 10.1016/j.contraception.2014.03.015

Weigard, A., Loviska, A. M., and Beltz, A. M. (2021). Little evidence for sex or ovarian hormone influences on affective variability. *Sci. Rep.* 11:20925. doi: 10.1038/s41598-021-00143-7

Wharton, W., Hishman, E., Merritt, P., Doyle, L., Paris, S., and Gleason, C. (2008). Oral contraceptives and androgenicity: influences on visuospatial task performance in younger individuals. *Exp. Clin. Psychopharmacol.* 16, 156–164. doi: 10.1037/1064-1297.16.2.156

Zilioli, S., and Watson, N. V. (2012). The hidden dimensions of the competition effect: basal cortisol and basal testosterone jointly predict changes in salivary testosterone after social victory in men. *Psychoneuroendocrinology* 37, 1855–1865. doi: 10.1016/j.psyneuen.2012.03.022

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

OPINION
published: 04 March 2022
doi: 10.3389/fnbeh.2022.829274

Considerations of Timing Post-ovariectomy in Mice and Rats in Studying Anxiety- and Depression-Like Behaviors Associated With Surgical Menopause in Women

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Keywords: anxiety, depression, oophorectomy, psychopharmacology, surgical menopause model

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Specialty section:
This article was submitted to Behavioral Endocrinology, a section of the journal Frontiers in Behavioral Neuroscience

Received: *05 December 2021*
Accepted: *09 February 2022*
Published: *04 March 2022*

Citation:
Rodríguez-Landa JF (2022) Considerations of Timing Post-ovariectomy in Mice and Rats in Studying Anxiety- and Depression-Like Behaviors Associated With Surgical Menopause in Women. Front. Behav. Neurosci. 16:829274. doi: 10.3389/fnbeh.2022.829274

INTRODUCTION

Menopause is a clinical term that indicates the end of the reproductive period of women that occurs naturally; however, it can be induced by bilateral oophorectomy, which is commonly referred to as surgical menopause. Both types of menopause in women are characterized by low plasma levels and brain concentrations of estradiol and progesterone, and a marked increase in follicle-stimulating hormone (FSH) levels. This likely affects several brain neurotransmitter systems and some peripheral physiological processes, impacting the quality of life of women (Monteleone et al., 2018; Giannini et al., 2021). In addition, the long-term absence of steroid hormones is associated with physiological changes that predispose women to genitourinary, cardiovascular, osseous, and mood disorders (Giannini et al., 2021). While such changes occur gradually and require a long time to stabilize in natural menopause, they are established in a shorter time in surgical menopause, thereby influencing the severity of symptoms in comparison to natural menopause (Rodríguez-Landa et al., 2015; Kingsberg et al., 2020; Giannini et al., 2021).

In preclinical research, ovariectomy performed on mice and rats is used as a surgical menopausal model to study the effects of permanent reduction in the levels of steroid hormones. This model has been used for studying the effects of surgical menopause on the central nervous system, which is responsible for cognitive deterioration, irritability, anxiety- and depression-like behaviors (Zakaria et al., 2019; Georgieva et al., 2021). In this way, the present article discusses the influence of time post-ovariectomy on hormonal, neurochemical, and neuroanatomical changes in rodents and its relation with anxiety- and depression-like behaviors that have been reported among women subjected to bilateral oophorectomy. This would help in enhancing the understanding of behavioral changes associated with surgical menopause and help evaluate potential pharmacological strategies to ameliorate the negative effects produced by long-term ovariectomy.

MENOPAUSE, ANXIETY, AND DEPRESSION SYMPTOMS

Throughout the reproductive life of the human female, significant changes in the peripheral and central concentrations of estradiol and progesterone have been reported. These hormonal changes interfere with their physiological, endocrine, and neurochemical processes, and often have negative implications on their quality of life (Kingsberg et al., 2020). Reduced concentrations of estradiol and

progesterone during the premenstrual, postpartum, and menopausal periods predispose women to anxiety and depressive symptoms (Albert et al., 2015). Generally, natural menopause occurs at 45–55 years of age and indicates the end of the reproductive period in women. However, surgical menopause, which is artificially induced by bilateral oophorectomy (Rodríguez-Landa et al., 2015), also exists as an option. Oophorectomy is recommended to women for averting the potential development of estrogen-dependent ovarian/breast cancer or to reduce pain from endometriosis (Kingsberg et al., 2020). Both types of menopause are known to predispose women to neuropsychiatric disorders (Georgieva et al., 2021), which is attributed to low concentrations of estradiol, progesterone, and their reduced metabolites such as allopregnanolone, among others (Zsakai et al., 2016).

Clinical studies have shown that women who undergo bilateral oophorectomy develop physiological and psychological changes similar to those that occur with natural menopause. One report suggest that women can immediately develop symptoms of severe anxiety that impair their quality of life (Chung-Park, 2006). The emotional and affective symptoms associated with surgical menopause vary among women; however, it has been reported that performing bilateral oophorectomy before natural menopause is associated with the development of anxiety and depression disorders and an increased risk in cognitive impairment, compared with women who experience natural menopause (Rocca et al., 2018; Olmos-Vázquez et al., 2020). Rocca et al. (2018) reported an increased long-term risk of depression and anxiety symptoms in women who underwent bilateral oophorectomy, which was principally associated with estrogen deficiency. Interestingly, the incidence of anxiety symptoms in bilateral oophorectomized women was higher in the long-term as compared to that of non-oophorectomized women, which exponentially increased as time post-oophorectomy passed, even, 10, 20, and 30 years after the removal of both ovaries. As such, preclinical and clinical studies are required to understand the neurobiological bases of the disorders associated with surgical menopause in the long-term, thus enabling the evaluation of new therapeutic strategies to ameliorate symptoms and improve the quality of life of this particular group of women. In this case, neuroendocrine changes produced by long-term ovariectomy should be considered when this surgical procedure is used for evaluating the disorders associated with surgical menopause and their potential treatments.

EFFECTS OF LONG-TERM OVARIECTOMY ON ANXIETY- AND DEPRESSION-LIKE BEHAVIOR

It is important to emphasize that multiple changes in the levels of diverse hormones and other substances (e.g., estradiol, progesterone, testosterone, cortisol, prolactin, insulin, glucose, and lipids) occur during natural or surgical menopause in the human female (Huerta et al., 1995; Kingsberg et al., 2020), which contribute to multiple symptoms associated with menopause. However, the most significant changes in natural or surgical menopause among women include a decrease in early cycle inhibin B and anti-Müllerian hormone levels. The decline in inhibin B produces an increase in FSH levels, which regulate the production of estradiol. Therefore, in menopausal women, FSH levels are markedly raised, and estradiol levels are significantly lowered (Burger et al., 2007; Kingsberg et al., 2020); these are associated with characteristic symptoms during menopause. Nonetheless, these hormonal changes are different between natural and surgical menopause. These changes occur gradually in natural menopause, while in surgical menopause, they occur in a shorter time.

In preclinical research, ovariectomy is a surgical procedure that involves the removal of one or both ovaries in females, which in the medium- and long-term significantly reduces plasma and brain concentrations of steroid hormones and other molecules that negatively impact brain function (Alagwu and Nneli, 2005). Interestingly, in ovariectomized mice and rats, similar hormonal changes to those of the human female occur. The FSH levels are increased as more time passes following ovariectomy, which is higher at 4 weeks than at 1–2 weeks (Moiety et al., 2015). Contrarily, progesterone and estradiol levels are undetectable at 3 months and 15 months post-ovariectomy, respectively, and they are lower at 4 weeks, than they are at 1–2 weeks post-ovariectomy (de Chaves et al., 2009; Moiety et al., 2015).

It has been reported that apparition of anxiety- and depression-like behavior associated with ovariectomy depends on the time elapsed after the procedure. For instance, the incidence of anxiety-like behavior was higher in rats at 12 weeks than at 3 weeks post-ovariectomy (Picazo et al., 2006); however, a limitation of this study was that these results were not compared with those of non-ovariectomized rats. Interestingly, 1 week post-ovariectomy did not produce significant changes on anxiety-like behavior with respect to non-ovariectomized rats; however, 3 weeks post-ovariectomy, a high incidence of anxiety-like behavior was detected in comparison with that of rats in the proestrus-estrus phase. This effect was similarly detected in rats at 6, 9, 12, and 15 weeks post-ovariectomy (Puga-Olguín et al., 2019), which coincides with estradiol and progesterone levels reduction. It was reported that rats at 12 weeks post-ovariectomy were more responsive to diazepam, an anxiolytic drug, than rats at 3 weeks post-ovariectomy (Picazo et al., 2006). This emphasizes the importance of considering the time post-ovariectomy in the evaluation of anxiety-like behavior and the effect of anxiolytic drugs.

Moreover, in the forced swim test an increase in the time of immobility was detected 2 weeks post-ovariectomy (Fedotova et al., 2016). Contrarily, Puga-Olguín et al. (2019) did not detect depression-like behaviors (increased time of immobility) in rats at 1 and 3 weeks post-ovariectomy, with respect to non-ovariectomized rats. Nonetheless, rats from 6 weeks post-ovariectomy significantly increased the incidence of depression-like behavior, with respect to rats in proestrus-estrus and metestrus-diestrus phases, and this effect was similar in rats at 9, 12, and 15 weeks post-ovariectomy.

As mentioned above, the time post-ovariectomy in mice and rats plays a significant role in the expression of anxiety- and depression-like behavior, which can be partially explained

by the hormonal, neurochemical, and neuroanatomical changes associated with ovariectomy in the long term. It is noteworthy that all these changes are simply an overview of how hormones affect the brain in the context of anxiety and depression symptoms, but we cannot disregard the other physiological changes that may influence symptoms related to menopause.

EFFECTS OF LONG-TERM OVARIECTOMY ON FOLLICLE-STIMULATING HORMONE AND STEROID HORMONES IN RATS AND MICE

Early studies have explored endocrine changes associated with ovariectomy in rats and mice. Particularly in rats, 2 weeks post-ovariectomy, the plasma concentrations of estradiol and progesterone did not differ significantly from the basal conditions (Ratka and Simpkins, 1990). However, 3 weeks post-ovariectomy, there was a significant reduction in estradiol and progesterone levels and an increase in luteinizing hormone and FSH levels in plasma (Wise and Ratner, 1980). To note, the reduced concentration of estradiol after 2 weeks post-ovariectomy remained until 7 weeks when the study ended (Li et al., 2014). Six weeks post-ovariectomy, a significant reduction in the plasma levels of progesterone, estradiol, and testosterone was detected in rats (Alagwu and Nneli, 2005).

On the other hand, Moiety et al. (2015) evaluated the effects at 1 and 4 weeks after hysterectomy and unilateral and bilateral ovariectomy on FSH and estradiol levels in rats. With respect to the baseline, FSH levels increased approximately to 26.19 and 73.81%, after 1 and 4 weeks post-hysterectomy, respectively. One week post unilateral ovariectomy, FSH levels increased approximately to 44.68%, while after 4 weeks FSH increased to ∼80.85% with respect to the baseline. Interestingly, a more significant effect on FSH levels was observed when bilateral ovariectomy was performed. In this case, 1 week post bilateral ovariectomy FSH levels significantly increased to approximately 91.11% vs. the baseline, but more significant effects were detected after 4 weeks, increasing FSH approximately to 222.22% with respect to the baseline. As for the concentration of estradiol at 1 week post hysterectomy, a slight reduction of ∼5.21% was noted, while 4 weeks after, estradiol levels were reduced to ∼16.94%, compared with the baseline. One week after the unilateral ovariectomy, estradiol was reduced to 7.26%, while 4 weeks after it was reduced to ∼29.19%, with respect to the baseline. Interestingly, when bilateral ovariectomy was performed, the concentration of estradiol was reduced to ∼21.45% of the baseline. However, estradiol was further reduced to 60.81% 4 weeks post bilateral ovariectomy with respect to the baseline. Importantly, the greater effects on FSH and estradiol were produced after 4 weeks of bilateral ovariectomy, followed by unilateral ovariectomy and hysterectomy. These results are consistent with those of previous studies that found a significant reduction in plasma concentrations of estradiol and testosterone at 4 weeks post-ovariectomy and was undetectable at 24 weeks post-ovariectomy (Zhao et al., 2005). The same effect was detected in rats at 3 months and 15 months post-ovariectomy (de Chaves et al., 2009).

Despite few studies on endocrine changes occurring in ovariectomized mice, it has been reported that after 4 days and 1 week post-ovariectomy, the concentrations of FSH are significantly increased with respect to intact cycling mice (Bronson, 1976; Chandrashekar and Bartke, 1996; Xu et al., 2000), but greater effects are detected after 2–4 weeks post-ovariectomy (Rodin et al., 1990), as it occurs in ovariectomized rats. Accordingly, after 2–14 weeks post-ovariectomy in mice, serum estradiol levels were significantly decreased by approximately 31% with respect to the sham-operated mice, which was accompanied by a significantly increased concentration of FSH (Park et al., 2014; Lee et al., 2020; Canuas-Landero et al., 2021). These data clearly show the importance of considering and standardizing the post-ovariectomy time frame to identify behavioral and hormonal changes associated with this surgical manipulation, thus avoiding erroneous interpretation of results and compare results from different laboratories.

NEUROANATOMICAL AND NEUROCHEMICAL CHANGES ASSOCIATED WITH LONG-TERM OVARIECTOMY: EFFECTS ON ANXIETY AND DEPRESSION-LIKE BEHAVIOR

The lower concentrations of progesterone and estrogen that occur during surgical or natural menopause produce an imbalance in neurochemical brain communication that subsequently affects neurotransmitter pathways (Smith et al., 1998; Rodríguez-Landa et al., 2015; García-Ríos et al., 2017; Kingsberg et al., 2020), wherein reduced concentrations of dopamine, serotonin, androstenedione, testosterone estradiol, GABA, β-endorphin, and allopregnanolone, and increased concentrations of noradrenaline and cortisol, among others, have been reported (Monteleone et al., 2018). All these changes produce neuroanatomical modifications in brain structures involved in anxiety and depression such as the raphe nucleus, hippocampus, and cerebral cortex (Monteleone et al., 2018).

In experimental research, bilateral ovariectomy in rats has also been used to evaluate the effects of the post-ovariectomy time frame on anxiety- and depression-like behavior, as well as the effect of anxiolytic and antidepressant compounds under these experimental conditions (**Table 1**). However, different post-ovariectomy time frames in mice and rats have been used, which can hinder the comparison of the results of different studies. Therefore, it is important to establish the different changes associated with the time of post-ovariectomy in rodents.

Ovarian hormones, estradiol, and progesterone, regulate several neurochemical processes in brain structures involved in the physiopathology of anxiety and depression, such as the prefrontal cortex, hypothalamus, amygdala, septal nucleus, and hippocampus (Giannini et al., 2021). Studies among laboratory animals and humans have reported that progesterone and its reduced metabolite allopregnanolone target the $GABA_A$ receptor

TABLE 1 | Effects of timing post-ovariectomy and some pharmacological treatments on anxiety- and depression-like behaviors in mice and rats.

Effects of ovariectomy and anxiolytic drugs on anxiety-like behavior

Subjects/Weeks post-OVX	OVX effect/Test	Treatment	Treatment effect*	References
NMRI mice/1	ne/EPM	–	–	Galeeva and Tuohimaa, 2001
Wistar rats/1	ne/EPM	–	–	Puga-Olguín et al., 2019
Sprague-Dawley rats/2	ne/OFT	–	–	Hiroi and Neumaier, 2006
C57BL/6 mice/2	ne/CUS+EPM	–	–	Lagunas et al., 2010
Wistar rats/3	ne/EPM	–	–	Marcondes et al., 2001
Wistar Rats/8	↑/EPM	–	–	Dornellas et al., 2018
Wistar rats/3,6,9,12,15	↑/EPM	–	–	Puga-Olguín et al., 2019
Sprague-Dawley rats/4	↑/EPM	–	–	Zoladz et al., 2019
C57BL/6 mice/16	↑/CUS+EPM	–	–	Lagunas et al., 2010
Wistar rats/12	ne/EPM	–	–	de Chaves et al., 2009
Wistar rats/60	↑/EPM	–	–	de Chaves et al., 2009
Long-Evans rats/1	↑/DBT	4 mg/kg P 10 μg/rat E2	P reduces anxiety-like behavior, E2 without effect	Llaneza and Frye, 2009
Sprague-Dawley rats/2	ne/EPM	6 μM/rat Allo	ne	Laconi et al., 2001
Sprague-Dawley rats/2	↑/EPM	25 mg/kg P 10 μg/kg E2B	ne	Díaz-Véliz et al., 1997
Wistar rats/3	↑/LDB	1 mg/kg DZ	Reduces anxiety-like behavior	Zuluaga et al., 2005
Wistar rats/3, 12	↑/DBT	0.5, 1, and 2 mg/kg DZ 0.5 and 0.50 mg/kg 8-OH-DPAT	Both treatments reduce anxiety-like behavior	Picazo et al., 2006
Wistar rats/4	↑/EPM	10 μg/kg E2 10 mg/kg Flx	E2 reduces anxiety-like behavior, Flx without effect	Charoenphandhu et al., 2011
Wistar rats/4	↑/OFT	150 μg/rat/week E2	Reduces anxiety-like behavior	Diz-Chaves et al., 2012
Wistar rats/8	↑/EPM	0.09 mg/kg E2	Reduces anxiety-like behavior	Puga-Olguín et al., 2019
Wistar rats/12	↑/EPM	0.5, 1, 2, and 4 mg/kg Chry	Only 1, 2, and 4 mg/kg Chry reduce anxiety-like behavior	Rodríguez-Landa et al., 2019
Wistar rats/12	↑/LDB	0.25, 0.5, and 1 mg/kg Gen	Reduces anxiety-like behavior	Rodríguez-Landa et al., 2009
Wistar rats/12	↑/LDB	2 mg/kg Dz	Reduces anxiety-like behavior	Rodríguez-Landa et al., 2019
Wistar rats/12	↑/EPM	0.45, 0.09, and 0.18 mg/kg E2 and Gen	Only 0.09 and 0.18 mg/kg reduce anxiety-like behavior	Rodríguez-Landa et al., 2017

Effects of ovariectomy and antidepressant substances on depression-like behavior

Animal/Weeks post-OVX	OVX effect/Test	Treatment	Treatment effect*	References
C57BL/6 mice/2	ne/CUS+FST	–	–	Lagunas et al., 2010
C57BL/6 mice/2	ne/FST ↑/TST	–	–	Carrier et al., 2015
Wistar rats/1, 3	ne/FST	–	–	Puga-Olguín et al., 2019
Wistar rats/8	ne/FST	–	–	Dornellas et al., 2018
Wistar rats/12, 60	ne/FST	–	–	de Chaves et al., 2009
Wistar rats/6, 9, 12, 15	↑/FST	–	–	Puga-Olguín et al., 2019
C57BL/6 mice/16	↑/CUMS+FST	–	–	Lagunas et al., 2010
C57BL/6 mice /1-2	ne/TST	10 mg/kg P	Reduces depression-like behavior	Frye, 2011
Wistar rats/2	ne/FST	0.8, 1.6, and 3.0 mg/kg P	Reduces depression-like behavior	Martínez-Mota et al., 1999

(Continued)

TABLE 1 | Continued

Effects of ovariectomy and antidepressant substances on depression-like behavior

Animal/Weeks post-OVX	OVX effect/Test	Treatment	Treatment effect*	References
Wistar rats/2	↑/FST	0.3, 1, and 3 μg/rat E2 and 0.6 mg/rat Map	Reduces depression-like behavior	Okada et al., 1997
Wistar rats/2	↑/FST	1 mg/kg P, Allo, Chry	Reduces depression-like behavior	Cueto-Escobedo et al., 2020
Wistar rats/7	↑/FST	150 μg/rat/week E2	Reduces depression-like behavior	Diz-Chaves et al., 2012
Wistar rats/8	↑/FST	0.09 mg/kg E2	Reduces depression-like behavior	Puga-Olguín et al., 2019
Wistar rats/8	↑/FST	0.5, 1, and 2 mg/kg P	Only 1 and 2 mg/kg P reduce depression-like behavior	Rodríguez-Landa et al., 2020
ICR mice/8	↑/FST /TST	1 μg/kg E2 and 100 mg/kg Pue	Both treatments reduce depression-like behavior	Tantipongpiradet et al., 2019
Wistar rats/8	↑/FST	5 mg/kg P	Reduces depression-like behavior	Rodríguez-Landa et al., 2020
Wistar rats/9	↑/FST	25 μg/day/6 weeks E2	Reduces depression-like behavior	Khayum et al., 2020
Wistar rats/12	ne/FST	0.25 mg/rat E2	ne	Boldarine et al., 2019

*Behavioral tests: CUS, chronic unpredictable stress; OFT, open field test; DBT, defensive burying test; LDB, light/dark box; FST, forced swim test; TST, tail suspension test. Evaluated substances: E2, estradiol; E2B, estradiol benzoate; P, progesterone; Allo, neurosteroid allopregnanolone; Flx, fluoxetine; Map, maprotiline; Dz, diazepam; Chry, flavonoid chrysin; Gen, isoflavone genistein; Pue, isoflavone puerarin. Effects of timing post-ovariectomy: ne, no effects on anxiety- or depression-like behaviors; ↑, OVX increase anxiety-like behavior by decreasing time in open arms and increasing anxiety index; ↑, OVX increase anxiety-like behavior by decreasing time in open arms; ↑, OVX increase anxiety-like behavior by increasing time spent burying; ↑, OVX increase anxiety-like behavior by decreasing time spent in light compartment; ↑, OVX increase anxiety-like behavior in aged rats by decreasing time in central areas; ↑, OVX increase depression-like behavior by increasing total time of immobility; *Anxiety- and depression-like behaviors are reduced when treatment produce the contrary effect on the variable described in the second column of the table (indicated by the different arrow colors). OVX, ovariectomy.*

and facilitate the activation of the GABAergic system, which can modulate dopaminergic and serotonergic pathways (Chen et al., 2021), producing anxiolytic- and antidepressant-like effects (Fernández-Guasti and Picazo, 1995; Estrada-Camarena et al., 2002; Frye and Walf, 2002; Rodríguez-Landa et al., 2007). Estrogen appears to mediate anxiety- and depression-like behavior by stimulating tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of serotonin (Bethea et al., 2000; Giannini et al., 2021). In rats, estrogen also mediates the regulation of the 5-hydroxytryptamine-1A (5-HT_{1A}) receptor expression in the dorsal raphe nucleus. Furthermore, it increases the density of postsynaptic 5-HT_2 receptors in the forebrain (Sumner et al., 1999), thereby affecting the receptors involved in the neurobiology of depressive disorders and the mechanisms of action of antidepressant drugs (García-Ríos et al., 2017).

Interestingly, at 1 week post-ovariectomy in rats, there was a reduction in the activation of glutamatergic receptors in the basolateral amygdala (De Jesús-Burgos et al., 2012), a structure involved in the regulation of anxiety. At 1–2 weeks post-ovariectomy, a critical reduction of dendritic spines and synaptophysin density occurred in the pyramidal neurons of the CA1 layer of the hippocampus in rats (Velázquez-Zamora et al., 2012), negatively impacting neuronal communication and the regulation of emotional and cognitive processes. This reduction in dendritic spine density in CA1 also occurs in rats at 3 weeks post-ovariectomy (McLaughlin et al., 2008). It is important to note that neurochemical changes in the brain are dependent on the post-ovariectomy time frame. In this way, after 4 weeks post-ovariectomy, genomic changes occur in the brain structures involved in the neurobiology of anxiety and depression. As studied in rats, the mRNA expression of the α-2 and α-3 subunits of the amygdala $GABA_A$ receptor (De Jesús-Burgos et al., 2012) was reduced, along with tryptophan hydroxylase mRNA, and serotonergic transporter mRNA of the dorsal raphe nucleus (Charoenphandhu et al., 2011). In addition, the concentration of serotonin in the hippocampus and nucleus accumbens (Pandaranandaka et al., 2009) is reduced. Additionally, other neurochemical and molecular changes appear post-ovariectomy. It is noteworthy that all these changes are reported from 4 weeks post-ovariectomy, but it is unknown whether these changes occur earlier than 4 weeks post-ovariectomy, which remains to be explored.

Five weeks after ovariectomy in mice, there is a reduced thickness of the CA1 layer in the hippocampus and the cerebral prefrontal cortex (Xu and Zhang, 2006). While 6 weeks after ovariectomy in rats, there is a reduction in the dopamine concentration in the central nucleus of the amygdala (Izumo et al., 2012) and a significant reduction in the expression of Fos protein immunoreactivity in the dorsal, intermediate, and ventral areas of the lateral septum. However, this was negatively correlated with anxiety- and depression-like behavior (Puga-Olguín et al., 2019). Interestingly, at 8 and 9 weeks

post-ovariectomy, a reduced expression of estrogen receptors (ER), αER and βER mRNA was detected in the hippocampus and cerebral cortex along with a reduced density of dendritic spines in the cerebral cortex of rats (Jin et al., 2005).

All these data clearly show that ovariectomy in mice and rats produces significant neuroanatomical and neurochemical changes in brain structures involved in the physiopathology of anxiety and depression, in which anxiolytic and antidepressant drugs play a contributory role, similar to women experiencing early surgical menopause (Kingsberg et al., 2020; Georgieva et al., 2021). Although few studies have correlated neurochemical and neuroanatomical changes that occur in ovariectomy with anxiety- and depression-like behaviors, these results support that long-term ovariectomy can be a useful tool to evaluate the neurobiological substrates that underlie anxiety- and depression-like behaviors associated with a reduced concentration of ovarian hormones induced by surgical menopause. Further, consideration of these factors will aid in studying the effect of new compounds with potential anxiolytic and antidepressant activity in women whose ovaries are removed.

EVALUATION OF ANXIETY- AND DEPRESSION-LIKE BEHAVIOR ASSOCIATED WITH LONG TERM OVARIECTOMY: EFFECTS OF ANXIOLYTIC AND ANTIDEPRESSANT DRUGS

Long-term ovariectomy in mice and rats increases the incidence of anxiety-like behavior in behavioral tests of anxiety, such as the elevated plus maze (EPM), open field test, burying defensive test, and light/dark box, as presented in **Table 1**. In the EPM, ovariectomy decreases the time spent into the open arms, which is considered as higher anxiety and depends on the time post-ovariectomy (Puga-Olguín et al., 2019). Contrarily, anxiolytic drugs like diazepam, hormones like progesterone and estradiol, or natural products like flavonoids, significantly increase the time in the open arms, thereby producing anxiolytic effects (Charoenphandhu et al., 2011; Rodríguez-Landa et al., 2017, 2019). Similar effects are observed in the other behavioral tests for anxiety. Ovariectomy reduces the time spent in the area where anxiogenic stimuli are present, and this time increases when anxiolytic drugs are injected to mice or rats, such as diazepam, estradiol, progesterone, allopregnanolone, or phytoestrogen genistein (Picazo et al., 2006; Llaneza and Frye, 2009; Rodríguez-Landa et al., 2009; Diz-Chaves et al., 2012).

Ovariectomy also increases the incidence of depression-like behavior in some animal models to evaluate despair behavior, including the tail suspension test (TST), forced swim test (FST), and chronic unpredictable stress (CUS). In the TST and FST, ovariectomy increases the time of immobility depending on the time-post ovariectomy (Lagunas et al., 2010; Puga-Olguín et al., 2019), which is considered as an indicator of depression-like behavior. Interestingly, ovariectomized mice and rats treated with antidepressant drugs like maprotiline, or some hormones like progesterone, allopregnanolone, and estradiol, had reduced time of immobility among other behavioral variables (Okada et al., 1997; Tantipongpiradet et al., 2019; Cueto-Escobedo et al., 2020; Khayum et al., 2020; Rodríguez-Landa et al., 2020), which is considered as an antidepressant-like effect. In the particular case of CUS, rats are subjected to several stressors for 4 weeks, which subsequently produces depression-like behavior identified by an increase in the time of immobility in the FST (Lagunas et al., 2010). This effect is dependent on the time post-ovariectomy, which was higher at 20 weeks than at 6 weeks post-ovariectomy. This shows that time post-ovariectomy is an important variable that should be considered in studying the effects of ovariectomy on anxiety- and depression-like behavior, and their potential treatments.

CONCLUDING REMARKS

Studying the effect of the long-term absence of ovarian hormone produced by ovariectomy in mice and rats on anxiety- and depression-like behaviors and the underlying neurochemical and anatomical changes is necessary in understanding neuropsychiatric disorders among women undergoing oophorectomy. Considering that in experimental animals, it is possible to discard the socio-cultural influence that menopause could have on women and its contribution to their emotional and affective disorders (Afridi, 2017; Zhang et al., 2019), the surgical menopause model in mice and rats may provide new evidence for the neurobiological mechanism resulting from ovariectomy (oophorectomy in women) in the long-term. Therefore, this model can be used to explore potential therapeutic strategies to ameliorate emotional and affective symptoms, in addition to physiological, histological, structural, and neuropsychiatric disorders occurring in women subjected to surgical menopause.

As mentioned above, the time elapsed after ovariectomy in rats plays a significant role in the expression of anxiety- and depression-like behavior, and possibly in the effects produced by anxiolytic and antidepressant drugs. Therefore, it is important to consider the time post-ovariectomy when we use such procedure to explore anxiety- and depression-like behavior or potential anxiolytic or antidepressant drugs. Additionally, including control groups of non-ovariectomized rats or referring to the control groups in previously published studies would ensure suitable comparations to progress our research. This would help in enhancing the understanding of behavioral changes associated with surgical menopause and help develop potential pharmacological strategies to ameliorate the negative effects produced by long-term ovariectomy. The model also allows consideration of the post-ovariectomy time frame to achieve better control of the evaluated variables and ensure the reproducibility and comparison of results.

AUTHOR CONTRIBUTIONS

JFR-L conceived and wrote this article.

FUNDING

This article is part of the SIREI project (DGI: 266502020108) registered by JFR-L, with partial financial support from the Sistema Nacional de Investigadores (SNI, Exp. 32753), CONACyT, Mexico.

ACKNOWLEDGMENTS

The author would like to thank the reviewers and the editor for their suggestions and comments that greatly contributed to improving this article. Additionally, the author would like to thank Editage (www.editage.com) for English language editing.

REFERENCES

Afridi, I. (2017). "Psychological and social aspects of menopause," in *A Multidisciplinary Look at Menopause,* eds J. F. Rodríguez-Landa and J. Cueto-Escobedo (Rijeka: Intech), 49–61. doi: 10.5772/intechopen.69078

Alagwu, E. A., and Nneli, R. O. (2005). Effect of ovariectomy on the level of plasma sex hormones in albino rats. *Niger J. Physiol. Sci.* 20, 90–94.

Albert, K., Pruessner, J., and Newhouse, P. (2015). Estradiol levels modulate brain activity and negative responses to psychosocial stress across the menstrual cycle. *Psychoneuroendocrinology* 59, 14–24. doi: 10.1016/j.psyneuen.2015.04.022

Bethea, C. L., Gundlah, C., and Mirkes, S. J. (2000). Ovarian steroid action in the serotonin neural system of macaques. *Novartis Found. Symp.* 230, 112–130. doi: 10.1002/0470870818.ch9

Boldarine, V. T., Pedroso, A. P., Neto, N. I. P., Dornellas, A. P. S., Nascimento, C. M. O., Oyama, L. M., et al. (2019). High-fat diet intake induces depressive-like behavior in ovariectomized rats. *Sci. Rep.* 9:10551. doi: 10.1038/s41598-019-47152-1

Bronson, F. H. (1976). Serum, FSH, LH, and prolactin in adult ovariectomized mice bearing silastic implants of estradiol: responses to social cues. *Biol. Reprod.* 15, 147–152. doi: 10.1095/biolreprod15.2.147

Burger, H. G., Hale, G. E., Robertson, D. M., and Dennerstein, L. (2007). A review of hormonal changes during the menopausal transition: focus on findings from the Melbourne Women's Midlife Health Project. *Hum. Reprod. Update* 13, 559–565. doi: 10.1093/humupd/dmm020

Canuas-Landero, V. G., George, C. N., Lefley, D. V., Corness, H., Muthana, M., Wilson, C., et al. (2021). Oestradiol contributes to differential antitumour effects of adjuvant zoledronic acid observed between pre- and post-menopausal women. *Front. Endocrinol.* 12:749428. doi: 10.3389/fendo.2021.749428

Carrier, N., Wang, X., Sun, L., and Lu, X. Y. (2015). Sex-specific and estrous cycle-dependent antidepressant-like effects and hippocampal Akt signaling of leptin. *Endocrinology* 156, 3695–3705. doi: 10.1210/EN.2015-1029

Chandrashekar, V., and Bartke, A. (1996). Influence of hypothalamus and ovary on pituitary function in transgenic mice expressing the bovine growth hormone gene and in growth hormone-deficient Ames dwarf mice. *Biol. Reprod.* 54, 1002–1008. doi: 10.1095/biolreprod54.5.1002

Charoenphandhu, J., Teerapornpuntakit, J., Nuntapornsak, A., Krishnamra, N., and Charoenphandhu, N. (2011). Anxiety-like behaviors and expression of SERT and TPH in the dorsal raphe of estrogen- and fluoxetine-treated ovariectomized rats. *Pharmacol. Biochem. Behav.* 98, 503–510. doi: 10.1016/j.pbb.2011.02.023

Chen, S., Gao, L., Li, X., and Ye, Y. (2021). Allopregnanolone in mood disorders: mechanism and therapeutic development. *Pharmacol. Res.* 169:105682. doi: 10.1016/j.phrs.2021.105682

Chung-Park, M. (2006). Anxiety attacks following surgical menopause. *Nurse Pract.* 31, 44–49. doi: 10.1097/00006205-200605000-00008

Cueto-Escobedo, J., Andrade-Soto, J., Lima-Maximino, M., Maximino, C., Hernández-López, F., and Rodríguez-Landa, J. F. (2020). Involvement of GABAergic system in the antidepressant-like effects of chrysin (5,7-dihydroxyflavone) in ovariectomized rats in the forced swim test: comparison with neurosteroids. *Behav. Brain Res.* 386:112590. doi: 10.1016/j.bbr.2020.112590

de Chaves, G., Moretti, M., Castro, A. A., Dagostin, W., da Silva, G. G., Boeck, C. R., et al. (2009). Effects of long-term ovariectomy on anxiety and behavioral despair in rats. *Physiol. Behav.* 97, 420–425. doi: 10.1016/j.physbeh.2009.03.016

De Jesús-Burgos, M., Torres-Llenza, V., and Pérez-Acevedo, N. L. (2012). Activation of amygdalar metabotropic glutamate receptors modulates anxiety, and risk assessment behaviors in ovariectomized estradiol-treated female rats. *Pharmacol. Biochem. Behav.* 101, 369–378. doi: 10.1016/j.pbb.2012.01.016

Díaz-Véliz, G., Alarcón, T., Espinoza, C., Dussaubat, N., and Mora, S. (1997). Ketanserin and anxiety levels: influence of gender, estrous cycle, ovariectomy and ovarian hormones in female rats. *Pharmacol. Biochem. Behav.* 58, 637–642. doi: 10.1016/S0091-3057(97)90004-6

Diz-Chaves, Y., Kwiatkowska-Naqvi, A., Von Hülst, H., Pernía, O., Carrero, P., and Garcia-Segura, L. M. (2012). Behavioral effects of estradiol therapy in ovariectomized rats depend on the age when the treatment is initiated. *Exp. Gerontol.* 47, 93–99. doi: 10.1016/j.exger.2011.10.008

Dornellas, A. P. S., Boldarine, V. T., Pedroso, A. P., Carvalho, L. O. T., de Andrade, I. S., Vulcani-Freitas, T. M., et al. (2018). High-fat feeding improves anxiety-type behavior induced by ovariectomy in rats. *Front. Neurosci.* 12:557. doi: 10.3389/fnins.2018.00557

Estrada-Camarena, E., Contreras, C. M., Saavedra, M., Luna-Baltazar, I., and López-Rubalcava, C. (2002). Participation of the lateral septal nuclei (LSN) in the antidepressant-like actions of progesterone in the forced swimming test (FST). *Behav. Brain Res.* 134, 175–183. doi: 10.1016/S0166-4328(02)00023-2

Fedotova, J., Dudnichenko, T., Kruzliak, P., and Puchavskaya, Z. (2016). Different effects of vitamin D hormone treatment on depression-like behavior in the adult ovariectomized female rats. *Biomed. Pharmacother.* 84, 1865–1872. doi: 10.1016/j.biopha.2016.10.107

Fernández-Guasti, A., and Picazo, O. (1995). Flumazenil blocks the anxiolytic action of allopregnanolone. *Eur. J. Pharmacol.* 281, 113–115. doi: 10.1016/0014-2999(95)00311-8

Frye, C. A. (2011). Progesterone reduces depressive behavior of young ovariectomized, aged progestin receptor knockout, and aged wild type mice in the tail suspension test. *J. Psychopharmacol.* 25, 421–428. doi: 10.1177/0269881109349836

Frye, C. A., and Walf, A. A. (2002). Changes in progesterone metabolites in the hippocampus can modulate open field and forced swim test behavior of proestrous rats. *Horm Behav.* 41, 306–315. doi: 10.1006/hbeh.2002.1763

Galeeva, A., and Tuohimaa, P. (2001). Analysis of mouse plus-maze behavior modulated by ovarian steroids. *Behav. Brain Res.* 119, 41–47. doi: 10.1016/S0166-4328(00)00341-7

García-Ríos, R. I., Mora-Pérez, A., and Soria-Fregozo, C. (2017). "Depression and serotonergic changes during the climacteric and postmenopause stags: hormonal influences," in *A Multidisciplinary Look at Menopause,* eds J. F. Rodríguez-Landa and J. Cueto-Escobedo (Rijeka: Intech), 63–80. doi: 10.5772/intechopen.69786

Georgieva, A., Eftimov, M., Todorova, M., Kuzmanova, V., Kuzmanov, A., Kuzmanov, K., et al. (2021). Effects of ovariectomy-induced estrogen deficit on rat behaviour, lipid metabolism, inflammation, bone mineral density, and turnover. *Folia Med.* 63, 385–391. doi: 10.3897/folmed.63.e55267

Giannini, A., Caretto, M., Genazzani, A. R., and Simoncini, T. (2021). Neuroendocrine changes during menopausal transition. *Endocrines* 2, 405–416. doi: 10.3390/endocrines2040036

Hiroi, R., and Neumaier, J. F. (2006). Differential effects of ovarian steroids on anxiety versus fear as measured by open field test and fear-potentiated startle. *Behav. Brain Res.* 166, 93–100. doi: 10.1016/j.bbr.2005.07.021

Huerta, R., Mena, A., Malacara, J. M., and de León, J. D. (1995). Symptoms at the menopausal and premenopausal years: their relationship with insulin, glucose, cortisol, FSH, prolactin, obesity and attitudes towards sexuality. *Psychoneuroendocrinology* 20, 851–864. doi: 10.1016/0306-4530(95)00030-5

Izumo, N., Ishibashi, Y., Ohba, M., Morikawa, T., and Manabe, T. (2012). Decreased voluntary activity and amygdala levels of serotonin and dopamine in ovariectomized rats. *Behav. Brain Res.* 227, 1–6. doi: 10.1016/j.bbr.2011.10.031

Jin, M., Jin, F., Zhang, L., Chen, Z., and Huang, H. (2005). Two estrogen replacement therapies differentially regulate expression of estrogen receptors alpha and beta in the hippocampus and cortex of ovariectomized rat.

Brain Res. Mol. Brain Res. 142, 107–114. doi: 10.1016/j.molbrainres.2005.09.013

Khayum, M. A., Moraga-Amaro, R., Buwalda, B., Koole, M., den Boer, J. A., Dierckx, R. A. J. O., et al. (2020). Ovariectomy-induced depressive-like behavior and brain glucose metabolism changes in female rats are not affected by chronic mild stress. *Psychoneuroendocrinology* 115:104610. doi: 10.1016/j.psyneuen.2020.104610

Kingsberg, S. A., Larkin, L. C., and Liu, J. H. (2020). Clinical effects of early or surgical menopause. *Obstet. Gynecol.* 135, 853–868. doi: 10.1097/AOG.0000000000003729

Laconi, M. R., Casteller, G., Gargiulo, P. A., Bregonzio, C., and Cabrera, R. J. (2001). The anxiolytic effect of allopregnanolone is associated with gonadal hormonal status in female rats. *Eur. J. Pharmacol.* 417, 111–116. doi: 10.1016/S0014-2999(01)00865-2

Lagunas, N., Calmarza-Font, I., Diz-Chaves, Y., and Garcia-Segura, L. M. (2010). Long-term ovariectomy enhances anxiety and depressive-like behaviors in mice submitted to chronic unpredictable stress. *Horm. Behav.* 58, 786–791. doi: 10.1016/j.yhbeh.2010.07.014

Lee, E., Jang, M., Lim, T. G., Kim, T., Ha, H., Lee, J. H., et al. (2020). Selective activation of the estrogen receptor-β by the polysaccharide from Cynanchum wilfordii alleviates menopausal syndrome in ovariectomized mice. *Int. J. Biol. Macromol.* 165, 1029–1037. doi: 10.1016/j.ijbiomac.2020.09.165

Li, L. H., Wang, Z. C., Yu, J., and Zhang, Y. Q. (2014). Ovariectomy results in variable changes in nociception, mood and depression in adult female rats. *PLoS ONE* 9:e94312. doi: 10.1371/journal.pone.0094312

Llaneza, D. C., and Frye, C. A. (2009). Progestogens and estrogen influence impulsive burying and avoidant freezing behavior of naturally cycling and ovariectomized rats. *Pharmacol. Biochem. Behav.* 93, 337–342. doi: 10.1016/j.pbb.2009.05.003

Marcondes, F. K., Miguel, K. J., Melo, L. L., and Spadari-Bratfisch, R. C. (2001). Estrous cycle influences the response of female rats in the elevated plus-maze test. *Physiol. Behav.* 74, 435–440. doi: 10.1016/S0031-9384(01)00593-5

Martínez-Mota, L., Contreras, C. M., and Saavedra, M. (1999). Progesterone reduces immobility in rats forced to swim. *Arch. Med. Res.* 30, 286–289. doi: 10.1016/S0188-0128(99)00024-X

McLaughlin, K. J., Bimonte-Nelson, H., Neisewander, J. L., and Conrad, C. D. (2008). Assessment of estradiol influence on spatial tasks and hippocampal CA1 spines: evidence that the duration of hormone deprivation after ovariectomy compromises 17β-estradiol effectiveness in altering CA1 spines. *Horm. Behav.* 54, 386–395. doi: 10.1016/j.yhbeh.2008.04.010

Moiety, F. M., Salem, H. A., Mehanna, R. A., and Abdel-Ghany, B. S. (2015). Comparative study on induction and effects of surgical menopause in a female rat model: a prospective case control study. *Int. J. Clin. Exp. Med.* 8, 9403–9411.

Monteleone, P., Mascagni, G., Giannini, A., Genazzani, A. R., and Simoncini, T. (2018). Symptoms of menopause - global prevalence, physiology and implications. *Nat. Rev. Endocrinol.* 14, 199–215. doi: 10.1038/nrendo.2017.180

Okada, M., Hayashi, N., Kometani, M., Nakao, K., and Inukai, T. (1997). Influences of ovariectomy and continuous replacement of 17β-estradiol on the tail skin temperature and behavior in the forced swimming test in rats. *Jpn. J. Pharmacol.* 73, 93–96. doi: 10.1254/jjp.60.93

Olmos-Vázquez, O. J., Rovirosa-Hernández, M. J., Pulido-Criollo, F., Soria-Fregozo, C., Thangavel, P., and Rodríguez-Landa, J. F. (2020). "Antidepressant effects of fluoxetine in natural and surgical menopause: preclinical and clinical research," in *Advance in Medicine and Biology,* ed L. V. Berhardt (New York, NY: Nova Science Publisher), 139–170.

Pandaranandaka, J., Poonyachoti, S., and Kalandakanond-Thongsong, S. (2009). Differential effects of exogenous and endogenous estrogen on anxiety as measured by elevated T-maze in relation to the serotonergic system. *Behav. Brain. Res.* 198, 142–148. doi: 10.1016/j.bbr.2008.10.043

Park, B. W., Pan, B., Toms, D., Huynh, E., Byun, J. H., Lee, Y. M., et al. (2014). Ovarian-cell-like cells from skin stem cells restored estradiol production and estrus cycling in ovariectomized mice. *Stem Cells Dev.* 23, 1647–1658. doi: 10.1089/scd.2014.0029

Picazo, O., Estrada-Camarena, E., and Hernández-Aragon, A. (2006). Influence of the post-ovariectomy time frame on the experimental anxiety and the behavioural actions of some anxiolytic agents. *Eur. J. Pharmacol.* 530, 88–94. doi: 10.1016/j.ejphar.2005.11.024

Puga-Olguín, A., Rodríguez-Landa, J. F., Rovirosa-Hernández, M. J., Germán-Ponciano, L. J., Caba, M., Meza, E., et al. (2019). Long-term ovariectomy increases anxiety- and despair-like behaviors associated with lower Fos immunoreactivity in the lateral septal nucleus in rats. *Behav. Brain Res.* 360, 185–195. doi: 10.1016/j.bbr.2018.12.017

Ratka, A., and Simpkins, J. W. (1990). Dose-dependent effects of chronic treatment with estradiol or progesterone on LH secretion in ovariectomized rats. *Endocr. Res.* 16, 165–184. doi: 10.1080/07435809009032997

Rocca, W. A., Grossardt, B. R., Geda, Y. E., Gostout, B. S., Bower, J. H., Maraganore, D. M., et al. (2018). Long-term risk of depressive and anxiety symptoms after early bilateral oophorectomy. *Menopause* 25, 1275–1285. doi: 10.1097/GME.0000000000001229

Rodin, D. A., Abbot, S. D., Saade, G., and Clayton, R. N. (1990). Comparison of the pretranslational regulation of FSH synthesis by gonadal steroids in rats and mice. *J. Mol. Endocrinol.* 4, 159–167. doi: 10.1677/jme.0.0040159

Rodríguez-Landa, J. F., Contreras, C. M., Bernal-Morales, B., Gutiérrez-García, A. G., and Saavedra, M. (2007). Allopregnanolone reduces immobility in the forced swimming test and increases the firing rate of lateral septal neurons through actions on the GABAA receptor in the rat. *J. Psychopharmacol.* 21, 76–84. doi: 10.1177/0269881106064203

Rodríguez-Landa, J. F., Cueto-Escobedo, J., Puga-Olguín, A., Rivadeneyra-Domínguez, E., Bernal-Morales, B., Herrera-Huerta, E. V., et al. (2017). The phytoestrogen genistein produces similar effects as 17β-estradiol on anxiety-like behavior in rats at 12 weeks after ovariectomy. *Biomed. Res. Int.* 2017:9073816. doi: 10.1155/2017/9073816

Rodríguez-Landa, J. F., Hernández-Figueroa, J. D., Hernández-Calderón, B. C., and Saavedra, M. (2009). Anxiolytic-like effect of phytoestrogen genistein in rats with long-term absence of ovarian hormones in the black and white model. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33, 367–372. doi: 10.1016/j.pnpbp.2008.12.024

Rodríguez-Landa, J. F., Hernández-López, F., Cueto-Escobedo, J., Herrera-Huerta, E. V., Rivadeneyra-Domínguez, E., Bernal-Morales, B., et al. (2019). Chrysin (5,7-dihydroxyflavone) exerts anxiolytic-like effects through GABAA receptors in a surgical menopause model in rats. *Biomed. Pharmacother.* 109, 2387–2395. doi: 10.1016/j.biopha.2018.11.111

Rodríguez-Landa, J. F., Olmos-Vázquez, O. J., Dutra da Costa, B. P., Lima-Maximino, M., Maximino, C., and Guillén-Ruiz, G. (2020). Actions of progesterone on depression-like behavior in a model of surgical menopause are mediated by GABAA receptors. *Salud Mental* 43, 43–53. doi: 10.17711/SM.0185-3325.2020.007

Rodríguez-Landa, J. F., Puga-Olguín, A., Germán-Ponciano, L. J., García-Ríos, R. I., and Soria-Fregozo, C. (2015). "Anxiety in natural and surgical menopause: physiologic and therapeutic bases," in *A Fresh Look at Anxiety Disorders,* ed F. Durbano (Rijeka: Intech), 173–198. doi: 10.5772/60621

Smith, S. S., Gong, Q. H., Li, X., Moran, M. H., Bitran, D., Frye, C. A., et al. (1998). Withdrawal from 3α-OH-5α-pregnan-20-one using a pseudopregnancy model alters the kinetics of hippocampal GABAA-gated current and increases the GABAA receptor α4 subunit in association with increased anxiety. *J. Neurosci.* 18, 5275–5284. doi: 10.1523/JNEUROSCI.18-14-05275.1998

Sumner, B. E., Grant, K. E., Rosie, R., Hegele-Hartung, C., Fritzemeier, K. H., and Fink, G. (1999). Effects of tamoxifen on serotonin transporter and 5-hydroxytryptamine(2A) receptor binding sites and mRNA levels in the brain of ovariectomized rats with or without acute estradiol replacement. *Brain Res. Mol. Brain Res.* 73, 119–128. doi: 10.1016/S0169-328X(99)00243-0

Tantipongpiradet, A., Monthakantirat, O., Vipatpakpaiboon, O., Khampukdee, C., Umehara, K., Noguchi, H., et al. (2019). Effects of puerarin on the ovariectomy-induced depressive-like behavior in ICR mice and its possible mechanism of action. *Molecules* 24:4569. doi: 10.3390/molecules24244569

Velázquez-Zamora, D. A., González-Tapia, D., González-Ramírez, M. M., Flores-Soto, M. E., Vázquez-Valls, E., Cervantes, M., et al. (2012). Plastic changes in dendritic spines of hippocampal CA1 pyramidal neurons from ovariectomized rats after estradiol treatment. *Brain Res.* 1470, 1–10. doi: 10.1016/j.brainres.2012.06.012

Wise, P. M., and Ratner, A. (1980). Effect of ovariectomy on plasma LH, FSH, estradiol, and progesterone and medial basal hypothalamic

LHRH concentrations old and young rats. *Neuroendocrinology* 30, 15–19. doi: 10.1159/000122968

Xu, M., Hill, J. W., and Levine, J. E. (2000). Attenuation of luteinizing hormone surges in neuropeptide Y knockout mice. *Neuroendocrinology* 72, 263–271. doi: 10.1159/000054595

Xu, X., and Zhang, Z. (2006). Effects of estradiol benzoate on learning-memory behavior and synaptic structure in ovariectomized mice. *Life Sci*. 79, 1553–1560. doi: 10.1016/j.lfs.2006.04.020

Zakaria, R., Rahbi, B. A., Ahmad, A. H., Said, R. M., Othman, Z., Azman, K. F., et al. (2019). Menopause rodent models: suitability for cognitive aging research. *Int. Med. J*. 26, 450–452.

Zhang, Y., Zhao, X., Leonhart, R., Nadig, M., Wang, J., Zhao, Y., et al. (2019). A cross-cultural comparison of climacteric symptoms, health-seeking behavior, and attitudes towards menopause among Mosuo women and Han Chinese women in Yunnan, China. *Transcult. Psychiatry* 56, 287–301. doi: 10.1177/1363461518804094

Zhao, H., Tian, Z., Hao, J., and Chen, B. (2005). Extragonadal aromatization increases with time after ovariectomy in rats. *Reprod. Biol. Endocrinol*. 3:6. doi: 10.1186/1477-7827-3-6

Zoladz, P. R., D'Alessio, P. A., Seeley, S. L., Kasler, C. D., Goodman, C. S., Mucher, K. E., et al. (2019). A predator-based psychosocial stress animal model of PTSD in females: influence of estrous phase and ovarian hormones. *Horm. Behav*. 115:104564. doi: 10.1016/j.yhbeh.2019.104564

Zsakai, A., Karkus, Z., Utczas, K., Biri, B., Sievert, L. L., and Bodzsar, E. B. (2016). Body fatness and endogenous sex hormones in the menopausal transition. *Maturitas* 87, 18–26. doi: 10.1016/j.maturitas.2016.02.006

Zuluaga, M. J., Agrati, D., Pereira, M., Uriarte, N., Fernández-Guasti, A., and Ferreira, A. (2005). Experimental anxiety in the black and white model in cycling, pregnant and lactating rats. *Physiol. Behav*. 84, 279–286. doi: 10.1016/j.physbeh.2004.12.004

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 14 March 2022
doi: 10.3389/fnbeh.2022.775796

Oral Contraceptives Modulate the Relationship Between Resting Brain Activity, Amygdala Connectivity and Emotion Recognition – A Resting State fMRI Study

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Recent research into the effects of hormonal contraceptives on emotion processing and brain function suggests that hormonal contraceptive users show (a) reduced accuracy in recognizing emotions compared to naturally cycling women, and (b) alterations in amygdala volume and connectivity at rest. To date, these observations have not been linked, although the amygdala has certainly been identified as core region activated during emotion recognition. To assess, whether volume, oscillatory activity and connectivity of emotion-related brain areas at rest are predictive of participant's ability to recognize facial emotional expressions, 72 participants (20 men, 20 naturally cycling women, 16 users of androgenic contraceptives, 16 users of anti-androgenic contraceptives) completed a brain structural and resting state fMRI scan, as well as an emotion recognition task. Our results showed that resting brain characteristics did not mediate oral contraceptive effects on emotion recognition performance. However, sex and oral contraceptive use emerged as a moderator of brain-behavior associations. Sex differences did emerge in the prediction of emotion recognition performance by the left amygdala amplitude of low frequency oscillations (ALFF) for anger, as well as left and right amygdala connectivity for fear. Anti-androgenic oral contraceptive users (OC) users stood out in that they showed strong brain-behavior associations, usually in the opposite direction as naturally cycling women, while androgenic OC-users showed a pattern similar to, but weaker, than naturally cycling women. This result suggests that amygdala ALFF and connectivity have predictive values for facial emotion recognition. The importance of the different connections depends heavily on sex hormones and oral contraceptive use.

Keywords: emotion recognition, amygdala, limbic system, resting state fMRI, brain connectivity, ALFF, hormonal contraceptives, progestins

OPEN ACCESS

Edited by:
Jaclyn M. Schwarz, University of Delaware, United States

Reviewed by:
Virginie Rappeneau, Osnabrück University, Germany
Jennifer Strafford Stevens, Emory University, United States
Alyssa Roeckner, Emory University, United States, in collaboration with reviewer JS

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Specialty section:
This article was submitted to Behavioral Endocrinology, a section of the journal Frontiers in Behavioral Neuroscience

Received: *14 September 2021*
Accepted: *24 January 2022*
Published: *14 March 2022*

Citation:
Menting-Henry S, Hidalgo-Lopez E, Aichhorn M, Kronbichler M, Kerschbaum H and Pletzer B (2022) Oral Contraceptives Modulate the Relationship Between Resting Brain Activity, Amygdala Connectivity and Emotion Recognition – A Resting State fMRI Study. Front. Behav. Neurosci. 16:775796. doi: 10.3389/fnbeh.2022.775796

INTRODUCTION

Emotion recognition, specifically the recognition of facial expressions, is central to social interaction (Mannava, 2012) and important for both the ontogenetic and phylogenetic development of our species (Schmidt and Cohn, 2001; Shenk et al., 2013). It is also selectively impaired in a variety of disorders associated with poor social functioning, e.g., autism or schizophrenia (Kohler et al., 2003; Kuusikko et al., 2009; Comparelli et al., 2013; Uljarevic and Hamilton, 2013). We are now looking back at centuries of extensive research on emotion recognition dating back to Darwin's seminal work Darwin (1989). The recognition of at least five basic emotional expressions identified by Ekman (1992)—happiness, sadness, anger, fear, disgust—is considered universal across cultures (Elfenbein and Ambady, 2002; Scherer et al., 2011; but see Russell, 1994; and Barrett et al., 2019).

Neuroimaging studies demonstrate that emotion recognition is associated with the activation of limbic regions including amygdala, basal ganglia, hippocampus, parahippocampus, anterior cingulate cortex, and the orbitofrontal cortex (Lane et al., 1998; Bush et al., 2000; Adolphs, 2002; Gur et al., 2002; Streit et al., 2003; Lancaster et al., 2019). The response to facial emotions by these limbic regions is modulated by the relevance of the emotional content (Gur et al., 2002). The amygdala in particular seems to be a key structure in the recognition of emotions and consistently activates during the processing of facial emotion expressions, especially during fearful expressions (Thomas et al., 2001; Adolphs, 2002; Gur et al., 2002; Hariri et al., 2002; Derntl et al., 2008b). This group of nuclei, functionally connected to extensive subcortical and cortical regions (Roy et al., 2009), has been the focus of a multitude of neuroimaging studies describing how the brain responds to recognizing different emotions in various contexts. Subcortical connections of the amygdala allow the processing of subliminal or unconscious facial emotion expressions, while cortical connections are involved in the conscious recognition of facial emotion expressions (Adolphs, 2002). Bilateral amygdala activity is stronger when there is explicit emotion recognition, and thus conscious emotion processing (Gur et al., 2002; Habel et al., 2007). The activation of the amygdala in response to fear and anger is lateralized with stronger reactivity in the right hemisphere, and decreases after habituation (Thomas et al., 2001; Hariri et al., 2002).

Previous research indicates that emotion recognition and amygdala reactivity and connectivity are modulated by sex and participant's hormonal status (Engman et al., 2016). In most studies, women display a higher accuracy in recognizing facial emotions, particularly negative emotions, than men (Thayer and Johnsen, 2000; Montagne et al., 2005; Hoffmann et al., 2010; Rukavina et al., 2018; Connolly et al., 2019; Olderbak et al., 2019). Limbic areas respond with stronger activation to emotional expressions in men compared to women (Weisenbach et al., 2014) and amygdala reactivity to emotional expressions is more lateralized in men (Thomas et al., 2001).

However, these sex differences are further modulated by women's hormonal status, i.e., their menstrual cycle phase or hormonal contraceptive use. An average menstrual cycle lasts 29 days, divided into follicular and luteal phase (Fehring et al., 2006). Ovarian hormone levels are lowest at the beginning of each cycle, i.e., during menses (Abraham et al., 1972). During the follicular phase estrogen levels rise and peak right before ovulation, while progesterone levels remain low. The consecutive luteal phase is characterized by high progesterone levels and medium estradiol levels. Androgen levels also vary over the menstrual cycle, with lower levels of testosterone at the beginning and end of the menstrual cycle (Judd and Yen, 1973). These fluctuations in hormonal levels are not seen in women using combined oral contraceptives (COCs). COCs contain a synthetic estrogen, mostly ethinylestradiol, and a synthetic progestin (Pletzer and Kerschbaum, 2014). These synthetic steroids downregulate the hypothalamic–pituitary–gonadal axis and decrease the production of endogenous sex hormones, including testosterone (Wiegratz et al., 2003). Therefore, COC users show reduced and stable levels of endogenous ovarian hormones over time (Fleischman et al., 2010), most comparable with levels seen during menses in naturally cycling women. However, the synthetic steroids show strong estrogenic and progestogenic activity due to their high binding affinities to the estrogen and progesterone receptors, respectively (Sitruk-Ware, 2008; Stanczyk et al., 2013). Accordingly, it is hard to discern, whether the effects of COCs on emotion recognition are attributable to the reduction of endogenous hormones or the estrogenic and progestogenic actions of the synthetic hormones.

Ovarian hormonal fluctuations along the menstrual cycle have been related to emotion recognition and associated brain activation. During the follicular phase there is a higher emotion recognition accuracy compared to the luteal phase (Derntl et al., 2008a). On the contrary, it appears that women are more sensitive to facial cues signaling nearby threats during the luteal phase, when progesterone levels are high (Conway et al., 2007). A neuroimaging review of Toffoletto et al. (2014) showed that emotional processing leads to different activation across the distinct cycle phases in the amygdala, medial prefrontal cortex, orbitofrontal cortex, dorsolateral prefrontal cortex and inferior frontal gyrus. The most consistent finding in fMRI studies is that the amygdala has a stronger response to negative emotional stimuli during the luteal phase (Sundström Poromaa and Gingnell, 2014). The influence of ovarian hormones on emotion recognition and concurrent amygdala activation in naturally cycling women is not always found (Sundström Poromaa and Gingnell, 2014; Shirazi et al., 2020).

Hormonal contraceptive use also influences emotion recognition in women (Hamstra et al., 2014; Pahnke et al., 2019). Some studies suggest that women using COCs are less accurate in recognizing emotions (Hamstra et al., 2014; Pahnke et al., 2019), while other studies report no significant differences (Radke and Derntl, 2016; Shirazi et al., 2020). These inconsistencies between studies may arise from a lack of control for the type of COCs used. Apart from their progestogenic activity, progestins can be classified by their interaction with androgen receptors (Pletzer and Kerschbaum, 2014). Androgenic progestins are derived from 19-nortestosterone and act as agonists of the androgen receptor, whereas anti-androgenic

progestins bind selectively to the progesterone receptor or act as antagonists of the androgen receptor (Sitruk-Ware, 2008). Accordingly, androgenic progestins have a more androgenic side effect profile than anti-androgenic progestins (Gurvich et al., 2020). Differential effects of androgenic and anti-androgenic progestins on brain structure have already been reported (Pletzer et al., 2015). To the best of our knowledge, Gurvich et al. (2020) are the only group that also examined the effect of oral contraceptive type on emotion recognition performance. They found an effect of androgenic vs. anti-androgenic oral contraceptive use on facial emotion recognition, in advantage of androgenic oral contraceptive users. Since men have higher androgen levels compared to women, their performance on facial emotion recognition is of interest to compare with women using androgenic and anti-androgenic COCs.

Pahnke et al. (2019) suggest that COCs impair the recognition of emotions *via* changes in the activity and connectivity in the prefrontal and temporal brain regions, caused by the reduction in endogenous hormone levels. It has indeed been observed that in hormonal contraceptive users, the amygdala shows not only reduced reactivity to emotional stimuli (Petersen and Cahill, 2015), but reduced gray matter volumes and altered connectivity to pre-frontal and central areas during the resting state (Lisofsky et al., 2016; Engman et al., 2018). However, to the best of our knowledge, these alterations in resting state connectivity patterns in hormonal contraceptive users has not been related to their ability to recognize facial emotional expressions. Despite the extensive research into the brain reactivity to emotional expressions, no study has so far assessed whether certain characteristics of the resting brain, like the size and functional connectivity of limbic areas such as the amygdala, are predictive of emotion recognition performance.

Understanding these brain-behavior associations and their modulation by androgenic vs. anti-androgenic oral contraceptives use is meaningful in the larger context of women's mental health. Hormonal contraceptives are used by 150 million women worldwide (Petitti, 2003) and though their effects on the brain are not yet fully understood (Brønnick et al., 2020), they have been implicated in cognitive, emotional and social functioning (Montoya and Bos, 2017). Of particular interest with regards to mental health are their effects on emotion processing. Although long-term users of COC appear to experience stabilizing effects on mood (Oinonen and Mazmanian, 2002), about 4–10% of women report severe adverse mood effects (Sundström Poromaa and Segebladh, 2012). Accordingly, some studies report an increased risk of COC users to develop depression (e.g., Skovlund et al., 2016), while other studies suggest a protective effect of COCs regarding mood disorders (Cheslack-Postava et al., 2015). It is yet unclear, why these effects of COCs on mood appear to be bidirectional, but a differential responsiveness of the brain to synthetic steroids seems to be a plausible explanation. Accordingly, it is important to identify brain areas, which are (i) associated with emotional processing already at rest and (ii) modulated by COC use. Since the risk for adverse mood effects appears to be increased in adolescent compared to adult participants (de Wit et al., 2020) and with androgenic compared to anti-androgenic COCs (Sundström Poromaa and Segebladh, 2012), age and androgenicity of progestins appear to be important modulators in that respect.

The present manuscript focuses on identifying the neural bases of emotion recognition performance in the resting brain in relation to individual hormonal status. In order to do so, we assess the gray matter volumes and the resting state oscillatory activity in relation to emotion recognition performance. We also considered whether this association was modulated by sex and hormonal status. Furthermore, bilateral amygdalae were defined as regions of interest (ROIs) and its volume, oscillatory activity and functional connectivity at rest assessed in relation to the participant's ability to recognize facial emotional expressions. We hypothesize that larger amygdalae, along with higher resting activity or connectivity of these areas are predictive of better emotion recognition performance. Taking into account the hormonal status of participants, we hypothesize that any behavioral differences in emotion recognition performance between different groups of hormonal contraceptive users (androgenic vs. anti-androgenic) and non-users can be explained by differences in the resting brain. In order to clearly characterize the differences between androgenic and anti-androgenic contraceptives, men are used as a comparison group.

MATERIALS AND METHODS

Participants

Seventy-two healthy young participants (mean age: 25.34 ± 6.35 years). 20 men (mean age: 28.35 ± 8.83 years), 20 women with natural menstrual cycle (mean age: 25.95 ± 6.10 years) and 32 hormonal contraceptive users (mean age: 23.09 ± 3.25 years) took part in this study. The hormonal contraceptive group can be divided into 16 androgenic users (mean age: 24.56 ± 3.03 years) and 16 anti-androgenic users (mean age: 21.63 ± 2.83 years). All participants were white Caucasian and college students or employees at university. Naturally cycling women had a regular menstrual cycle with a mean duration of 29.08 days (SD = 1.56 days). Within the natural cycling (NC) group, only participants who had not been using any hormonal contraceptives or intrauterine device for the past 6 months were included. Of the 32 hormonal contraceptive users, 16 were taking older generation hormonal contraceptives containing androgenic progestins (Desogestrel, Levonorgestrel or Gestoden) and 16 were taking newer generation hormonal contraceptives containing anti-androgenic progestins (Drospirenone, Chlormadinone Acetate, Dienogest). Within the androgenic oral contraceptive (OC) and anti-androgenic OC group, participants needed be on their current OC for at least 6 months before start of the study. All participants gave their signed written consent to participate in the study. The study was approved by the University of Salzburg's ethic committee and conforms to the Code of Ethics of the World Medical Association (Declaration of Helsinki). Participants had no psychological, endocrinological or neurological disorders and did not display any brain structural abnormalities on structural MRI.

Behavioral Data Acquisition

In order to assess a trait value of emotion recognition performance, participants completed three different versions of an emotion recognition task with approximately 10 days between the respective sessions. Performance measures were then averaged across the three test sessions. The order of versions across test sessions was counterbalanced. During each session, participants viewed 60 faces from the FACES database[1] on a computer screen, 10 each displaying either a neutral expression or happiness, sadness, anger, fear or disgust (see **Figure 1** for example faces). The order of emotions was randomized during each session. Participants had 4 s to rate the emotional expression of each face as neutral, happy, sad, angry, fearful or disgusted by pressing the, respectively, marked keys on a computer keyboard. Mean reaction times (RT) and accuracy were recorded for each emotion. The inter-stimulus-interval was 500 ms.

In order to control for potential hormonal influences on emotion recognition performance, hormonal status of the participating women was counterbalanced across test sessions. Naturally cycling women completed the three sessions during the following phases of their menstrual cycle: early follicular/menstrual phase (cycle days 1–5; low estradiol and progesterone), pre-ovulatory phase (1–3 days before ovulation; high estradiol), mid-luteal phase (4–10 days past ovulation; high estradiol and progesterone). Ovulation was assumed 14 days before the onset of the next period as expected by participants' self-reports of cycle length and onset of the last period and was confirmed by commercial ovulation tests. The menses session on average took place on day 5 (SD = 2.92), the ovulation session on average took place on day 15 (SD = 2.34), the luteal session on average took place on day 23 (SD = 4.70). Cycle phases were confirmed by the assessment of the sex hormones from saliva samples using DeMediTec ELISA kits for estradiol, progesterone and testosterone, as reported in Pletzer et al. (2016). Two naturally cycling women were excluded due to a mismatch between the expected and the actual cycle phase. Hormonal contraceptive users completed one test session during the active pill phase (hormone containing pills) and one test session during the placebo phase (placebo pills or no pills). The third test session was scheduled randomly either in the active pill or placebo phase.

Behavioral Analyses

In order to explore whether the hormonal status was predictive of emotion recognition performance we investigated menstrual cycle phase and oral contraceptive pill effects through linear mixed models in R version 3.6.1.[2] (R Core Team, 2019) with *nlme* (Pinheiro et al., 2014) and *multcomp* (Hothorn et al., 2008) packages. Using *performance* as dependent variable and *participant number* (PNr) as random effect, for naturally cycling women *cycle phase* and *session number* were used as fixed effects (independent variables): e.g., RT $\sim$ 1|PNr + cycle phase + session. For pill users, *pill phase, pill type* and its *interactive effect* alongside the *session* number were used as the independent variables: e.g., RT $\sim$ 1|PNr + pill phase*pill type + session. We then address whether the groups (men, naturally cycling women, A-OC women and AA-OC women) differed from each other in emotion recognition performance using *group* and *session number* as fixed effects, and *participant number* as random effect: e.g., RT $\sim$ 1|PNr + group + session. In all the aforementioned cases, we accounted for multiple testing by first, FDR-correcting for the 5 emotions (anger, sadness, disgust, fear, happiness), and second, conducting Tukey-corrected all-pairwise comparisons between the different levels of the factors *cycle phase*, *session* and *group*.

MRI Data Acquisition

Depending on the group participants were assigned to, they were scanned during one, two or all three test sessions, although only one scan is relevant to the current study. Of the 72 participants, two participants did not complete all planned

[1]http://faces.mpib-berlin.mpg.de/

[2]https://www.R-project.org/

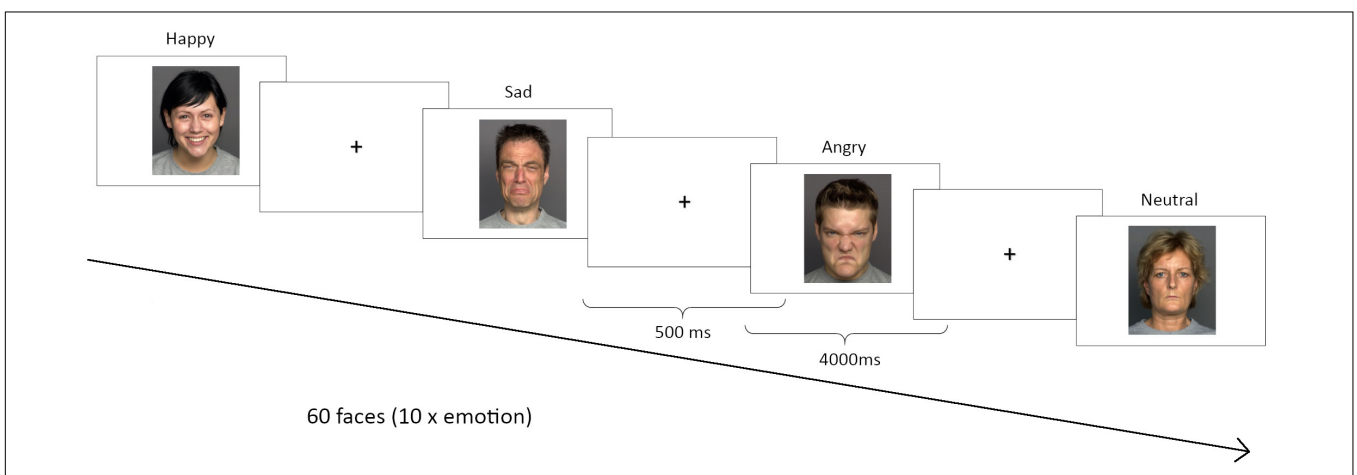

FIGURE 1 | Emotion recognition task. Three different versions were performed in each correspondent session. During each session, participants viewed 60 faces from the FACES database (http://faces.mpib-berlin.mpg.de/), 10 for each emotion: anger, sadness, happiness, disgust, fear or neutral.

scans and were therefore excluded from further analysis. Brain images from one male participant were not included due to bad quality, resulting in a final sample of 69 participants (19 men, 18 women with a natural menstrual cycle and 32 hormonal contraceptive users). Men were scanned during one visit only. Naturally cycling women were scanned during their menses, pre-ovulatory and luteal cycle phase. Women using oral contraceptives were scanned during pill intake and during pill pause.

However, changes in brain structure and resting brain activity related to cycle phase or pill phase were described elsewhere (Pletzer et al., 2015, 2016) and are not within the scope of the current manuscript. Therefore, only the menses scan from naturally cycling women, and the active pill scan from oral contraceptive users were used for the analyses. Given that oral contraceptives use downregulates the hypothalamic–pituitary–gonadal axis, decreasing the endogenous ovarian hormones production to levels comparable to those observed in naturally cycling women during menses, the menses session of naturally cycling women was chosen as the most comparable in terms of endogenous hormone levels.

Functional and high resolution structural images were acquired on a Siemens Magnetom TIM Trio 3 Tesla scanner (Siemens Healthcare) at the Christian Doppler Klinik (Salzburg, Austria). For resting state a T2-weighted gradient echo planar (EPI) sequence with 36 transversal slices orientated parallel to the AC-PC line (whole-brain coverage, TE = 30 ms, TR = 2,250 ms, flip angle 70°, slice thickness 3.0 mm, matrix 192 × 192, FOV 192 mm, in-plane resolution 2.6 mm × 2.6 mm). Participants were instructed to close their eyes, relax and let their mind flow. For structural images we acquired a T1-weigthed 3D MPRAGE sequence of 5 min 58 s (160 sagital slices, slice thickness = 1 mm, TE 291 ms, TR 2,300 ms, TI delay 900 ms, FA 9°, FOV 256 mm × 256 mm).

MRI Data Analysis

Preprocessing of Structural Images

In order to analyze the structural data, we performed voxel-based morphometry (VBM) using the CAT12 toolbox[3] of the SPM12 software[4] (Ashburner and Friston, 2000). During this procedure, scans are corrected for bias-field inhomogeneities, spatially registered to an anatomical template, and each voxel is classified as gray matter, white matter or cerebrospinal fluid, accounting for partial volume effects. CAT12 segmentation was applied through default options, including affine registration to SPM12 tissue probability maps and European brain templates, light affine preprocessing and moderate (0.5) strength of local adaptive segmentation, skull stripping and final clean-up for segmentation. Spatial normalization to the MNI template was used to correct intra-subject bias, and non-linear normalization parameters to control for inter-subject variability (Luders et al., 2004). For smoothing, we used an 8 mm full width at half maximum Gaussian kernel.

Preprocessing of Functional Images

In order to pre-process the functional images, we first applied a 3d-despiking as implemented in AFNI[5], and then the standard procedures and templates from SPM12 including realignment and unwarping of the functional images using the fieldmap, co-registration of the functional images to the segmented structural images, normalization of functional images using the parameters as estimated by CAT12, and spatial smoothing using a 6 mm kernel. Finally, we perform ICA-AROMA non-aggressive removal of artifactual components on the resulting images (Pruim et al., 2015).

Calculation of ALFF Maps

In order to assess how strongly the BOLD- signal fluctuates, and as a measure of spontaneous neuronal activity, the amplitude of low-frequency fluctuations (ALFF) was calculated using the DPABI toolbox (Yan et al., 2016). In order to do so, the signal was first filtered (bandpass, 0.01–0.08 Hz) to remove effects of very-low-frequency drift and high frequency noise as caused e.g., by respiratory and heart rhythms. Then, ALFF maps were calculated as the average square root of the power spectrum within this range of frequencies.

ROI Analyses

In a first step, we focused on the amygdala as a region of interest (ROI). Masks were constructed for the left and right amygdala *via* the wfu-pickatlas toolbox, using the Neuromorphometrics atlas. Gray matter (GM) volumes from the left and right amygdala were extracted using the get_totals script by G. Ridgeway[6]. ALFF in the left and right amygdala was extracted from a one-sample *t*-test over all subjects using eigenvalues. In order to address whether left and right amygdala volumes, as well as left and right amygdala ALFF were predictive of emotion recognition performance and whether this association was modulated by hormonal status, we ran linear models in R 3.6.1. Each emotion was explored using RT/Accuracy as dependent variable and GM volume/ALFF as well as its interaction with group as independent variable (e.g., RT ∼ GM*Group, Acc ∼ ALFF*Group). We accounted for multiple testing by FDR-correcting for the 5 emotions (anger, sadness, disgust, fear, happiness). If a significant interaction between performance and group was observed, correlation (Pearson's *r*) of performance with GM/ALFF was calculated for each group.

Calculation of Seed-Based Connectivity Maps

In order to investigate the connectivity of each of the left and right amygdala to the rest of the brain, we assessed seed-to-voxel connectivity using the CONN-toolbox[7] (Whitfield-Gabrieli and Nieto-Castanon, 2012). For each subject we calculated connectivity maps through standard procedures and templates, using 6 movement parameters as well as 5 white matter and cerebrospinal fluid components as regressors in a first-level analysis and a band-pass filter of 0.008 to 0.09 Hz.

[3]http://dbm.neuro.uni-jena.de/vbm/

[4]http://www.fil.ion.ucl.ac.uk/spm/

[5]https://afni.nimh.nih.gov/

[6]http://www0.cs.ucl.ac.uk/staff/gridgway/vbm/get_totals.m

[7]http://www.nitrc.org/projects/conn

Whole Brain Analyses

In a second step, we used SPM second level analyses at the whole brain level to assess whether emotion recognition performance related to gray matter volumes or ALFF outside the amygdala on the one hand, and whether emotion recognition performance related to amygdala connectivity on the other hand. To that end, and separately for each emotion, we performed full factorial models on modulated GM maps, ALFF maps and connectivity maps, using either emotion recognition RT or emotion recognition accuracy as regressors and modeling their interaction with group. If a significant interaction between performance and group was observed, brain parameters were extracted from significant clusters and their correlation (Pearson's *r*) with performance explored for each group. We also explored whether accounting for age *via* partial correlations affected any correlation coefficients. However, due to the age-homogeneity of the sample, this was not the case. Accordingly, age was not considered further in the analyses. In order to account for multiple testing, the uncorrected *p*-value threshold was divided by the number of emotions (5) and therefore set to $p = 0.0002$. Results are reported when Family-Wise Error (FWE) corrected $p < 0.05$.

RESULTS

Behavior

Within the naturally cycling group of women (NC), there was no cycle phase effect on either emotion recognition reaction time (RT) or emotion recognition accuracy for any of the emotions (all $F_{2,31} < 2.15$, $p_{FDR} > 0.05$. For women on oral contraceptives (OC) there were no significant effects of the pill phase (all $F_{1,46} < 5.10$, $p_{FDR} > 0.05$), the pill type (A or AA) (all $F_{1,27} < 6.10$, $p_{FDR} > 0.05$) or their interaction (all $F_{1,46} < 4.40$, $p_{FDR} > 0.05$) on either emotion recognition RT or emotion recognition accuracy. When considering the whole sample, there were no differences in performance (RT or accuracy) between the groups for any of the emotions (all $F_{3,65} < 3.35$, $p_{FDR} > 0.05$). Emotion recognition RT and accuracy on the different emotions for the four groups can be found in **Tables 1**, **2**.

The number of sessions had an effect on RT for anger ($F_{2,133} = 7.78$, $p_{FDR} = 0.003$), fear ($F_{2,133} = 3.78$, $p_{FDR} = 0.04$), and disgust ($F_{2,133} = 4.70$, $p_{FDR} = 0.03$). For these emotions participants were faster during the second and third session than the first session, irrespective of the group (SE < 0.15, $|z| > 2.3$, $p_{tukey} < 0.05$). The number of sessions also had an effect on the accuracy for sadness ($F_{2,133} = 7.04$, $p_{FDR} = 0.006$). Participants were more accurate during the second and third session than the first session, irrespective of the group (SE < 0.14, $|z| > 2.5$, $p_{tukey} < 0.03$).

ROI-Based Analysis

In order to address, whether left and right amygdala volumes, as well as left and right amygdala ALFF were predictive of emotion recognition performance and whether this association was modulated by hormonal status, we ran linear models with *performance* as dependent variable and *GM volume/ALFF* as well as its interaction with *group* as fixed effects: e.g., RT ~ GM*Group. Here, we also accounted for multiple testing by first, FDR-correcting for the 5 emotions (anger, sadness, disgust, fear or happiness), and second, conducting Tukey-corrected all-pairwise comparisons between the different levels of *group*.

Gray Matter Volumes

Neither left nor right amygdala volumes were predictive of either emotion recognition reaction time or emotion recognition accuracy (all $F_{3,61} < 3.00$, $p_{FDR} > 0.05$) and no interactions with group were observed (all $F_{3,61} < 2.18$, $p_{FDR} > 0.05$).

ALFF

The ALFF of the left amygdala showed a trend interactive effect with group on the RT for anger ($F_{3,60} = 3.58$, $p = 0.018$), however, it did not survive the multiple comparison correction ($p_{FDR} = 0.09$). ALFF and RT were found to be moderately positively correlated for women on A-OC ($r_{14} = 0.50$; **Figure 2**). The higher the ALFF in the left amygdala, the slower anger recognition in women using A-OC.

No further main effects or interaction with group was observed for left nor right amygdala ALFF on emotion recognition RT (all $F_{3,61} < 3.58$, $p_{FDR} > 0.05$).

Neither left nor right amygdala ALFF were predictive of emotion recognition accuracy and no interactions with group were observed (all $F_{3,60} < 2.90$, $p_{FDR} > 0.05$).

TABLE 1 | Reaction time for emotion recognition.

Group	Session	Angry	Sad	Fear	Happy	Disgust
Men	1	1,960.68 (317.08)	1,993.27 (445.51)	2,328.84 (487.99)	1,303.05 (170.46)	2,073.35 (522.52)
	2	1,686.76 (370.36)*	1,875.31 (386.45)	2,078.09 (567.13)*	1,278.01 (268.55)	1,920.69 (480.46)*
	3	1,713.39 (282.43)*	1,952.4 (401.38)	2,021.63 (500.02)*	1,272.04 (167.5)	1,832.67 (434.06)*
NC women	1	1,677.46 (306.76)	1,846.7 (414.7)	1,991.86 (395.68)	1,229.62 (238.71)	1,820.05 (471.46)
	2	1,615.58 (221.99)*	1,890.15 (382.19)	1,993.44 (484.65)*	1,147.72 (211.22)	1,713.28 (391.88)*
	3	1,566.16 (348.84)*	1,873.44 (362.66)	1,955.08 (441.67)*	1,221.95 (205.67)	1,787.83 (308.96)*
A-OC women	1	1,709.23 (304.54)	1,900.21 (498.44)	2,010.89 (519.72)	1,203.37 (241.76)	1,671.62 (257.6)
	2	1,618.56 (300.64)*	1,810.91 (453.7)	1,858.49 (356.62)*	1,108.9 (219.32)	1,585.55 (298.76)*
	3	1,637.84 (384.47)*	1,773.26 348.54)	1,834.54 (447)*	1,152.74 (276.16)	1,622.3 (287.85)*
AA-OC women	1	1,834.08 (400.94)	1,982.83 (439.01)	2,255.06 (611.17)	1,244 (192.94)	1,887.47 (323.63)
	2	1,669.09 (237.88)*	1,989.74 449.24)	2,014.23 (390.7)*	1,207.06 (171.7)	1,817.52 (303.47)*
	3	1,733.11 (231.83)*	1,889.88 (349.86)	2,120.76 (446.16)*	1,175.14 (141.91)	1,761.33 (200.02)*

*Mean in milliseconds (SD) *p < 0.05 for the second and third session compared to the first one.*

TABLE 2 | Accuracy for emotion recognition.

Group	Session	Angry	Sad	Fear	Happy	Disgust
Men	1	80.00 (17)	50.00 (24.49)	56.32 (27.53)	98.95 (3.15)	72.11 (17.18)
	2	87.89 (12.73)	64.21 (19.53)*	70.00 (30.55)	98.42 (5.01)	76.84 (21.1)
	3	88.95 (15.6)	52.63 (25.79)*	67.37 (25.79)	97.89 (4.19)	75.26 (20.1)
NC women	1	88.89 (12.78)	55.56 (19.77)	78.33 (14.65)	100 (0)	82.78 (14.47)
	2	89.44 (9.38)	66.67 (27.65)*	81.67 (22.03)	99.44 (2.36)	73.33 (18.15)
	3	87.06 (12.13)	61.76 (28.34)*	78.24 (28.34)	97.65 (5.62)	78.82 (18.67)
A-OC women	1	88.13 (14.71)	51.25 (21.25)	76.25 (22.17)	98.75 (3.42)	72.50 (22.06)
	2	90.67 (12.23)	63.33 (23.5)*	83.26 (14.44)	98.67 (3.52)	76.00 (21.31)
	3	92.67 (10.33)	67.67 (19.17)*	88.00 (15.68)	100 (0)	77.67 (16.57)
AA-OC women	1	89.38 (10.63)	63.75 (17.08)	78.75 (21.56)	98.13 (4.03)	78.75 (17.84)
	2	87.50 (14.38)	71.25 (16.28)*	72.50 (22.66)	98.13 (4.03)	86.25 (14.55)
	3	93.75 (9.75)	71.25 (20.62)*	80.00 (16.73)	98.75 (3.42)	87.12 (16.01)

*Mean percentage hits (SD) *p < 0.05 for the second and third session compared to the first one.*

Whole Brain Analyses

No main effects of performance were observed for the gray matter volume or ALFF. No interaction of group by performance was found for the gray matter volume. Regarding the ALFF, we observed a significant group*accuracy interaction in the left posterior cingulate gyrus (PCC) [0 −28 34], k = 48 voxels, F = 13.59, $p_{FWE} < 0.001$, for the emotion of disgust. ALFF and accuracy were found to be moderately negatively correlated for women on AA-OC ($r_{14} = -0.53$; **Figure 3**). The lower the ALFF in the left PCC, the higher disgust recognition accuracy in women using AA-OC.

We also observed a significant group*accuracy interaction for the ALFF in the right superior parietal lobe (SPL) [33 −43 64], k = 20 voxels, F = 4.09, p_{FWE} = 0.02, for the emotion of sadness. ALFF and accuracy were found to be positively correlated for naturally cycling women (r_{16} = 0.69) whereas it was negatively correlated for women on AA-OC ($r_{14} = -0.82$) (**Figure 4**).

The lower the ALFF in the right SPL, the lower sadness recognition accuracy in naturally cycling women, while the highest sadness recognition accuracy in women using AA-OC.

No interaction of group*RT of any emotion was observed for the ALFF.

Amygdala Connectivity

For the emotion of fear, we observed a significant group*RT interaction for the connectivity between the left amygdala and the left anterior cingulate cortex (ACC) [−3 29 −8], k = 18 voxels, F = 12.35, p_{FWE} = 0.02. Connectivity strength and RT were found to be positively correlated for men (r_{17} = 0.82), whereas it was negatively correlated for naturally cycling women ($r_{16} = -0.61$) (**Figure 5**). The lower the left amygdala-ACC connectivity strength, the faster fear recognition in men, while the slower fear recognition in naturally cycling women.

Also for the emotion of fear, we observed a significant group*accuracy interaction for the connectivity between the right amygdala and the left middle frontal gyrus (MFG) [−33 41 19], k = 15 voxels, F = 10.61, p_{FWE} = 0.04. Connectivity strength and accuracy were found to be negatively correlated for naturally cycling women ($r_{16} = -0.75$), whereas it was positively correlated for women on AA-OC (r_{14} = 0.82) (**Figure 6**). The lower the right amygdala-left MFG connectivity strength, the higher fear recognition accuracy in naturally cycling women, while the lower fear recognition accuracy in women using AA-OC.

In summary, we found the ALFF in the left amygdala, the left PCC and the right SPL related to anger, disgust and sadness

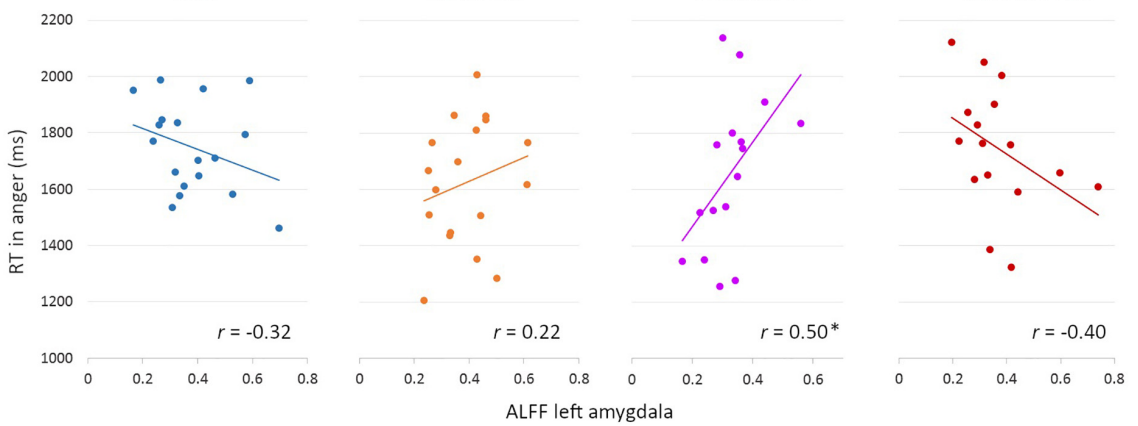

FIGURE 2 | Relationship between the amplitude of low-frequency fluctuations (ALFF) in the left amygdala and reaction time (RT) for angry faces by group. ALFF and RT were found to be moderately positively correlated for women on A-OC. *$p < 0.05$.

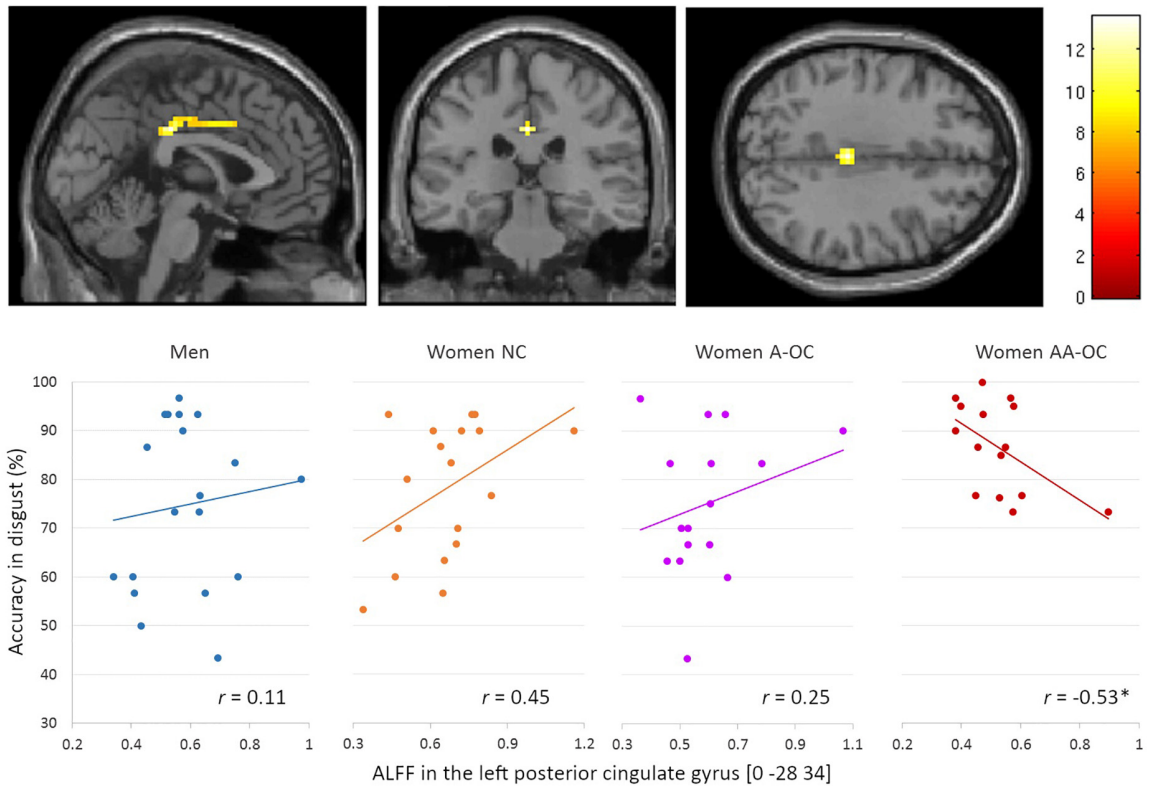

FIGURE 3 | Relationship between the amplitude of low-frequency fluctuations (ALFF) in the left posterior cingulate gyrus and accuracy for disgust faces by group. ALFF and accuracy were found to be moderately negatively correlated for women on AA-OC. *$p < 0.05$.

recognition performance (respectively), depending on hormonal status in women (**Figure 7**). Regarding the connectivity from the amygdalae, the connectivity strength to ACC and left MFG was related to fear recognition performance, depending on sex and hormonal status (**Figure 7**).

DISCUSSION

The current study set out to investigate whether hormonal contraceptive effects on emotion recognition performance were related to gray matter volume, oscillatory activity and connectivity of emotion-related brain areas at rest. Notably, no behavioral differences according to sex, menstrual cycle phase or hormonal contraceptive use emerged. These results are in accordance with previous studies who did not find a link between emotion recognition performance and hormonal status or pill use (Sundström Poromaa and Gingnell, 2014; Radke and Derntl, 2016; Shirazi et al., 2020), but differ from other studies who did find an effect (Conway et al., 2007; Derntl et al., 2008a; Hamstra et al., 2014; Pahnke et al., 2019; Gurvich et al., 2020). Habituation over sessions could be a possible explanation for the lack of differences between different genders and pill type. Participants recognized anger, fear and disgust faster during the second and third session and were more accurate in their identification of sadness during the second and third session compared to the first session, which could explain the similar emotion recognition performance in the combined data.

Regarding the question, whether certain characteristics of the resting brain were predictive of participants ability to recognize five basic emotions (anger, fear, sadness, happiness, disgust), while controlling for participant's sex and hormonal status, no general pattern of emotion recognition predictability emerged. Instead, results were strongly dependent on (a) the respective emotion and (b) the hormonal status of participants. Thus, contrary to our predictions, resting brain characteristics did not mediate oral contraceptive effects on performance, but oral contraceptive use emerged as a moderator of brain-behavioral associations. This suggests that depending on participants' hormonal status, certain brain areas tune to the recognition of specific emotions. In the following, we will first discuss the differences that emerged between men and naturally cycling women, and then discuss the differences between naturally cycling women and OC users.

Sex differences did emerge in the prediction of emotion recognition performance by the left amygdala amplitude of low frequency oscillations (ALFF) for anger, as well as left and right amygdala connectivity for fear. The recognition of anger was faster in men with higher ALFF, but slower in naturally cycling women with higher ALFF. This suggests that a stronger oscillatory activity of the amygdala at rest facilitates the recognition of anger in men, but impairs the recognition of anger

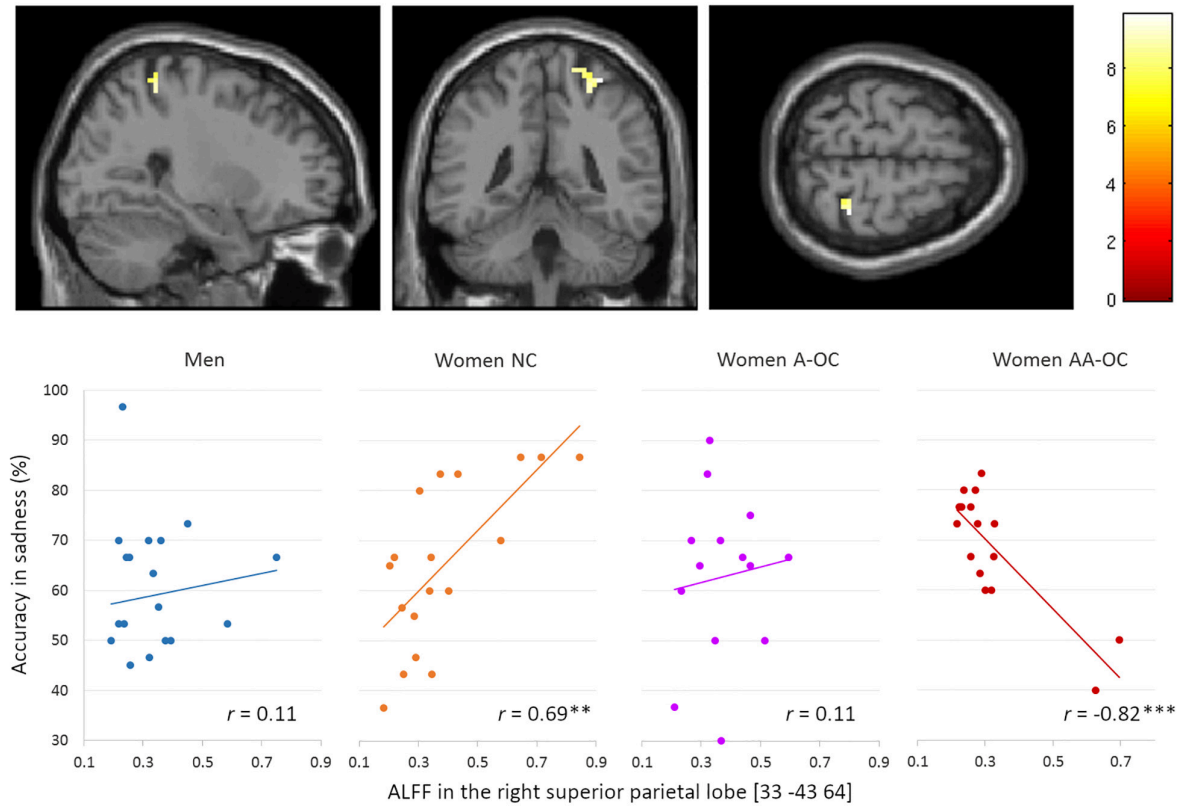

FIGURE 4 | Relationship between the amplitude of low-frequency fluctuations (ALFF) in the in the right superior parietal lobe and accuracy of sadness by group. ALFF and accuracy were found to be positively correlated for naturally cycling women, whereas they were negatively correlated for women on AA-OC. $^{**}p < 0.01$, $^{***}p < 0.001$.

in naturally cycling women. Repple et al. (2018) show that men have a stronger response in the amygdala after provocation, and that this response correlated with trait anger. They also showed a positive association between the anterior cingulate cortex activity when provoked and a more aggressive response.

The connectivity between the amygdala and anterior cingulate cortex (ACC) is involved in aversive learning and important for threatening stimuli processing (Klavir et al., 2013). Inhibitory functional coupling during threatening stimuli processing has been shown (Toyoda et al., 2011) and in rodents inactivation of the ACC inputs to the amygdala lead to an enhanced fear response (Jhang et al., 2018). Projections from the ACC might control anxiety in threatening situations (Jhang et al., 2018), explaining the differential connectivity between the amygdala and ACC found in this manuscript. The more strongly the left amygdala recruits the ACC, the slower men are in recognizing fear, but the faster are naturally cycling women. These results suggest that a stronger connectivity of the amygdala at rest impairs the recognition of fear in men, but facilitates the recognition of fear in women. An interesting finding of Kogler et al. (2016) shows that cortisol levels are negatively associated with resting state functional connectivity of the amygdala with the ACC in women, and positively associated in men. Higher levels of cortisol can lower connectivity in women, hence making their response slower, while the opposite occurs for men with high cortisol levels. This is also of interest in the light of the findings of Bouma et al. (2009) who showed reduced cortisol reaction following hormonal contraceptive use.

Apart from that, the coupling of the amygdala and ACC during face processing shifts from positive to negative over age (Kujawa et al., 2016). Young people display greater ACC activation to emotional faces due to inhibitory effects on amygdala activation (Kujawa et al., 2016). Although in the present sample, brain behavior association were not modulated by age and this does not explain the faster recognition by women, where the opposite effect was found, it could be that such an effect appears more strongly in adult men than women. Another possible explanation is that women use the connection between the ACC and the amygdala to recognize fear faster, because a fearful face is indicative of a threat and elicits a fear response. Rahman et al. (2004) reported a faster response to facial emotional stimuli for women compared to men. They suggest that the faster response in women is a by-product of face recognition, which has a faster response to non-congruent gender faces than men do.

In addition, the more strongly the right amygdala recruits the left middle frontal gyrus (MFG), the more accurate men are in recognizing fear, but the worse naturally cycling women

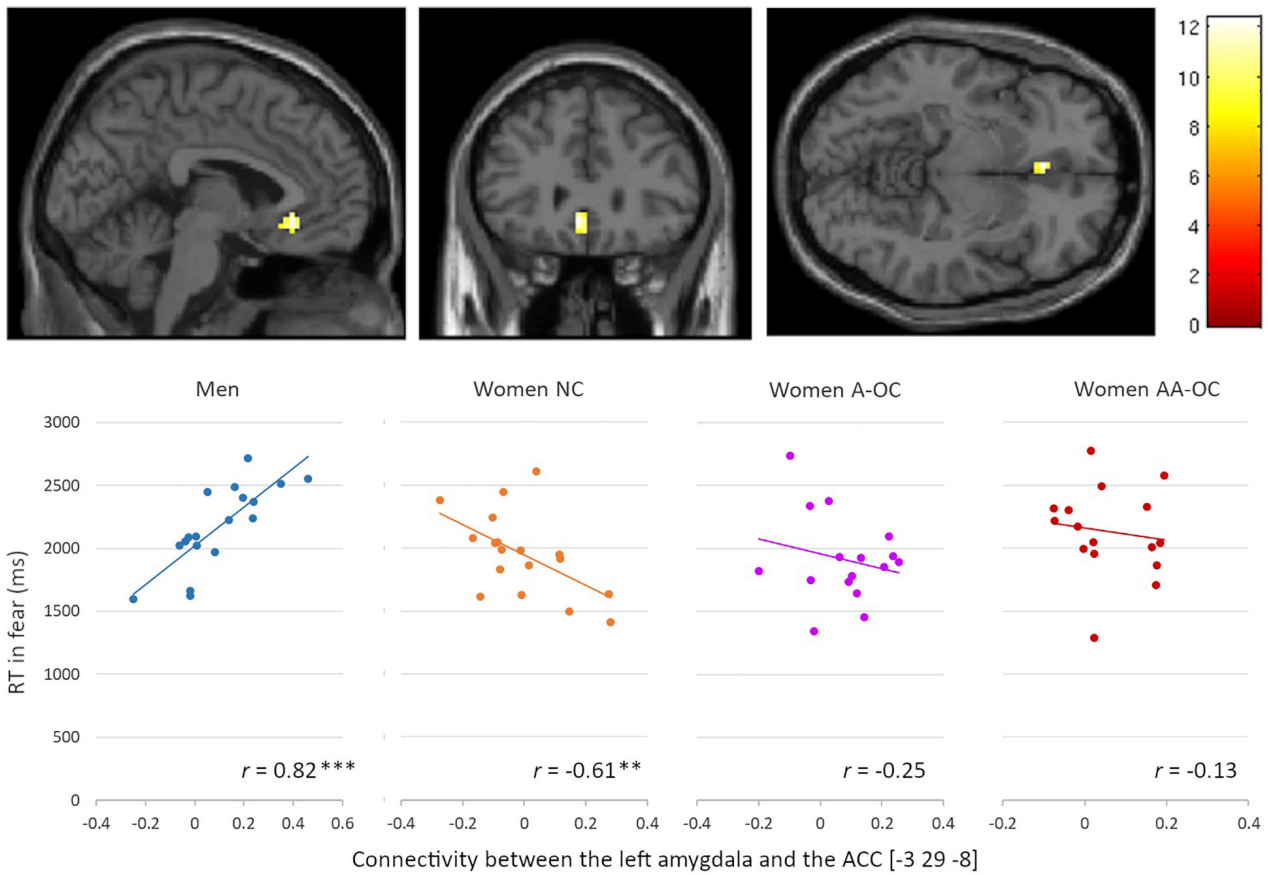

FIGURE 5 | Relationship between the left amygdala-anterior cingulate cortex connectivity and reaction time (RT) for fearful faces by group. Connectivity strength and RT were found to be positively correlated for men, whereas they were negatively correlated for naturally cycling women. $^{**}p < 0.01$, $^{***}p < 0.001$.

recognize fear. Studies have shown that the MFG is involved in fearful engagement (Thielscher and Pessoa, 2007), and that signaling from the middle frontal gyrus to the amygdala is suppressed in response to emotional distractors (Yamasaki et al., 2002). Since the MFG inhibits the right amygdala, it is possible that the less inhibition there is of the amygdala, the better men recognize fear.

Regarding OC effects the pattern of results was opposite as expected. While we hypothesized a more masculinized pattern of brain-behavior associations in androgenic OC users, but a feminized pattern in anti-androgenic OC users, the opposite pattern emerged. Anti-androgenic OC-users stood out in that they showed strong brain-behavior associations, usually in the opposite direction as naturally cycling women, while androgenic OC-users showed a pattern similar to, but weaker, than naturally cycling women. This was observed in (i) the ALFF of the left amygdala, (ii) the ALFF of the posterior cingulate gyrus (PCC), (iii) the ALFF of the superior parietal lobe (SPL), and (iv) the connectivity between right amygdala and left MFG.

For most of the sample, higher ALFF in the PCC and SPL was associated with higher accuracy in recognizing disgust and sadness respectively. On the contrary, in anti-androgenic OC-users, emotion recognition accuracy dropped with higher ALFF in these areas. Regarding the left amygdala, anti-androgenic OC users showed a similar increase in anger recognition speed as men with higher oscillatory activity. This is of interest in relation to possible effects of hormonal contraceptive use on adverse mood symptoms and related disorders (Cheslack-Postava et al., 2015; Skovlund et al., 2016; de Wit et al., 2020). The cingulate cortex is known to play a role in emotional processing (Vogt, 2005), and activity in its posterior section is higher when observing disgusting stimuli compared to neutral stimuli (Benuzzi et al., 2008). Activation in PCC in a face-related has been related to both estradiol and testosterone levels in NC women (Rupp et al., 2009), which may explain its modulation by OCs with estrogenic, but anti-androgenic activity. As for the SPL, previous research has shown that the display of sadness leads to increased activity in the left SPL (McLellan et al., 2012). Functional resting-state connectivity between the right basolateral amygdala and the SPL are associated with the personality trait sadness (Deris et al., 2017). Finally, regarding the connectivity of the right amygdala and left MFG, the MFG seems to suppress emotional stimuli (Yamasaki et al., 2002) and inhibits the right amygdala, explaining the better fear recognition in anti-androgenic oral contraceptives.

While these results are somewhat surprising given that masculinizing effects were expected in androgenic OC users

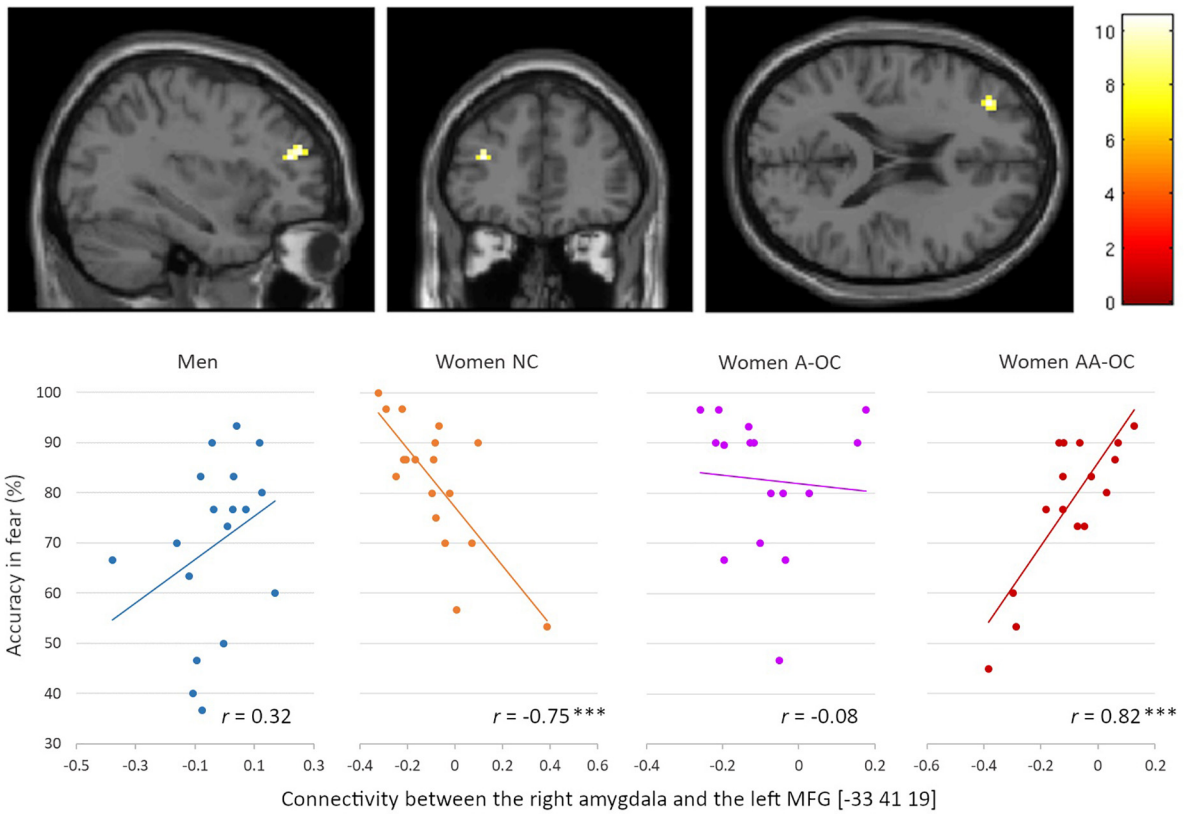

FIGURE 6 | Relationship between the right amygdala-left middle frontal gyrus connectivity and accuracy for fearful faces by group. Connectivity strength and accuracy were found to be negatively correlated for naturally cycling women, whereas they were positively correlated for women on anti-androgenic oral contraceptives. ***$p < 0.001$.

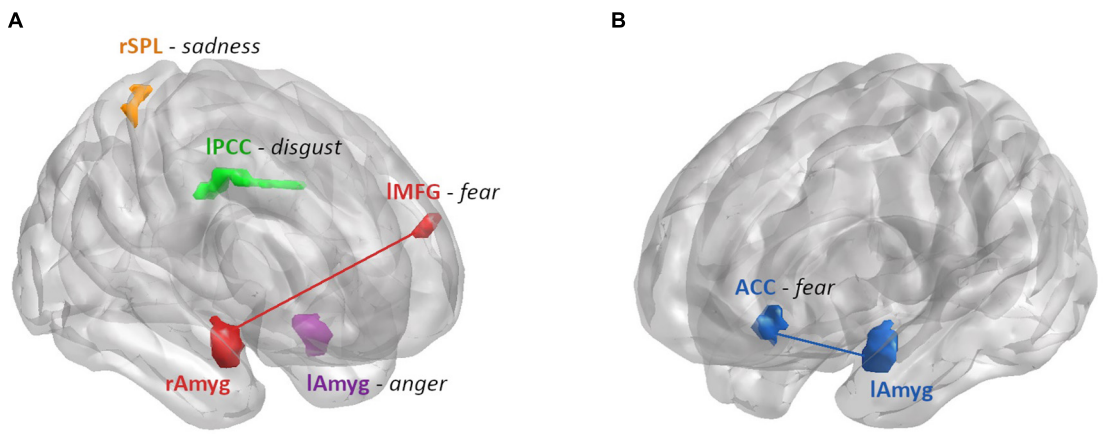

FIGURE 7 | Summary of the brain areas that showed a differential relationship between ALFF or connectivity strength and emotion recognition performance by hormonal status in women **(A)** and sex **(B)**. **(A)** Areas modulated by hormonal status: ALFF and RT for anger were found to be moderately positively correlated for women on A-OC in the left amygdala (purple). ALFF and accuracy for disgust were found to be moderately negatively correlated for women on AA-OC in the left PCC (green). ALFF and accuracy for sadness were found to be positively correlated for naturally cycling women, whereas they were negatively correlated for women on AA-OC in the right SPL (orange). Connectivity strength between right amygdala-left MFG (red) and accuracy for fear were found to be negatively correlated for naturally cycling women, whereas they were positively correlated for women on AA-OC. **(B)** Areas modulated by sex: Connectivity strength between the left amygdala-ACC (blue) and RT for fear were found to be positively correlated for men, whereas they were negatively correlated for naturally cycling women.

due to the binding affinity of androgenic progestins to the androgen receptor, there are two possible explanations for this pattern. First, multiple mechanisms might facilitate androgenic actions in OC users, some of which are also present in anti-androgenic OC users. For instance, OC-use reduces the levels of progesterone, which has a higher affinity for the enzyme 5α-dehydrogenase compared to testosterone (Wright et al., 1983). Thus, in OC-users more testosterone can be converted into the physiologically more active dihydrotestosterone, which has a higher binding affinity for the androgen receptor. Second, it is possible that these differences between groups are not due to activational effects of sex hormones in adulthood, but the result of organizational effects of sex hormones. Anti-androgenic progestins are often prescribed in women who present with at least slight androgenic symptoms, e.g., acne or hirsutism (Fuchs et al., 2019). It is thus possible that our groups were subject to a selection bias and women in the group of anti-androgenic OC-users either had higher androgen levels until they started taking their contraceptive or have a higher sensitivity to androgens.

Despite the extended use of OCs, only few studies have investigated their effects on brain activity and connectivity of emotion-related brain regions. Relatedly, the impact of OCs use on psychological well-being and emotional regulation, and a mechanistic approach on how this effects may be exerted is lacking. Understanding the hormonal contraceptive effects on emotion recognition performance related to brain activity and connectivity at rest provide some groundwork for future studies. In the present study, we showed that the oscillatory activity in emotion-related brain areas, such as PCC, SPL and amygdala were indeed predictive of participant's ability to recognize facial emotional expressions, particularly the emotions anger, disgust, fear and sadness (**Figure 7**). Furthermore, these results were dependent on the use and type of COCs. Amygdalae connectivity were also predictive of fear recognition performance.

DATA AVAILABILITY STATEMENT

Data and scripts are openly available online at http://webapps.ccns.sbg.ac.at/OpenData/. MR images are available upon request from the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the University of Salzburg. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

BP designed the study together with HK, acquired the data and supervised the data analysis and manuscript preparation. SM-H and EH-L performed the data analysis and wrote the first draft of the manuscript. MK and MA provided input on the data analysis. All authors read and approved the final manuscript.

FUNDING

This research was funded by the Austrian Science Fund (FWF) Project P32276 supporting BP, the European Research Council (ERC) Starting Grant 850953 supporting BP and EH-L, and FWF-Ph.D. Program "Imaging the Mind: Connectivity and Higher Cognitive Function" (W 1233-G17) supporting SM-H.

REFERENCES

Abraham, G. E., Odell, W. D., Swerdloff, R. S., and Hopper, K. (1972). Simultaneous radioimmunoassay of plasma FSH, LH, progesterone, 17-hydroxyprogesterone, and estradiol-17β during the menstrual cycle. *J. Clin. Endocrinol. Metab.* 34, 312–318. doi: 10.1210/jcem-34-2-312

Adolphs, R. (2002). Neural systems for recognizing emotion. *Curr. Opin. Neurobiol.* 12, 169–177. doi: 10.1016/s0959-4388(02)00301-x

Ashburner, J., and Friston, K. J. (2000). Voxel-based morphometry - the methods. *NeuroImage* 11(6 Pt. 1), 805–821. doi: 10.1006/nimg.2000.0582

Barrett, L. F., Adolphs, R., Marsella, S., Martinez, A. M., and Pollak, S. D. (2019). Emotional expressions reconsidered: challenges to inferring emotion from human facial movements. *Psychol. Sci. Public Interest* 20, 1–68.

Benuzzi, F., Lui, F., Duzzi, D., Nichelli, P. F., and Porro, C. A. (2008). Does it look painful or disgusting? Ask your parietal and cingulate cortex. *J. Neurosci.* 28, 923–931.

Bouma, E. M., Riese, H., Ormel, J., Verhulst, F. C., and Oldehinkel, A. J. (2009). Adolescents' cortisol responses to awakening and social stress; effects of gender, menstrual phase and oral contraceptives. The TRAILS study. *Psychoneuroendocrinology* 34, 884–893. doi: 10.1016/j.psyneuen.2009.01.003

Brønnick, M. K., Økland, I., Graugaard, C., and Brønnick, K. K. (2020). The effects of hormonal contraceptives on the brain: a systematic review of neuroimaging studies. *Front. Psychol.* 11:556577. doi: 10.3389/fpsyg.2020.556577

Bush, G., Luu, P., and Posner, M. I. (2000). Cognitive and emotional influences in anterior cingulate cortex. *Trends Cogn. Sci.* 4, 215–222. doi: 10.1016/s1364-6613(00)01483-2

Cheslack-Postava, K., Keyes, K. M., Lowe, S. R., and Koenen, K. C. (2015). Oral contraceptive use and psychiatric disorders in a nationally representative sample of women. *Arch. Womens Ment. Health* 18, 103–111. doi: 10.1007/s00737-014-0453-4

Comparelli, A., Corigliano, V., De Carolis, A., Mancinelli, I., Trovini, G., Ottavi, G., et al. (2013). Emotion recognition impairment is present early and is stable throughout the course of schizophrenia. *Schizophr. Res.* 143, 65–69. doi: 10.1016/j.schres.2012.11.005

Connolly, H. L., Lefevre, C. E., Young, A. W., and Lewis, G. J. (2019). Sex differences in emotion recognition: evidence for a small overall female superiority on facial disgust. *Emotion* 19:455. doi: 10.1037/emo0000446

Conway, C. A., Jones, B. C., Debruine, L. M., Welling, L. L. M., Smith, M. L., Perrett, D. I., et al. (2007). Salience of emotional displays of danger and contagion in faces is enhanced when progesterone levels are raised. *Horm. Behav.* 51, 202–206. doi: 10.1016/j.yhbeh.2006.10.002

Darwin, C. R. (1989). *The Expression of the Emotions in Man and Animals, With an Introduction, Afterword and Commentaries by Paul Ekman. Essay on the History of the Illustrations by Phillip Prodger*, 3rd Edn. London: Harper Collins.

de Wit, A. E., Booij, S. H., Giltay, E. J., Joffe, H., Schoevers, R. A., and Oldehinkel, A. J. (2020). Association of use of oral contraceptives with depressive symptoms

among adolescents and young women. *JAMA Psychiatry* 77, 52–59. doi: 10.1001/jamapsychiatry.2019.2838

Deris, N., Montag, C., Reuter, M., Weber, B., and Markett, S. (2017). Functional connectivity in the resting brain as biological correlate of the affective neuroscience personality scales. *NeuroImage* 147, 423–431. doi: 10.1016/j.neuroimage.2016.11.063

Derntl, B., Kryspin-Exner, I., Fernbach, E., Moser, E., and Habel, U. (2008a). Emotion recognition accuracy in healthy young females is associated with cycle phase. *Horm. Behav.* 53, 90–95. doi: 10.1016/j.yhbeh.2007.09.006

Derntl, B., Windischberger, C., Robinson, S., Lamplmayr, E., Kryspin-Exner, I., Gur, R. C., et al. (2008b). Facial emotion recognition and amygdala activation are associated with menstrual cycle phase. *Psychoneuroendocrinology* 33, 1031–1040. doi: 10.1016/j.psyneuen.2008.04.014

Ekman, P. (1992). An argument for basic emotions. *Cogn. Emot.* 6, 169–200. doi: 10.1080/02699939208411068

Elfenbein, H. A., and Ambady, N. (2002). On the universality and cultural specificity of emotion recognition: a meta-analysis. *Psychol. Bull.* 128:203. doi: 10.1037/0033-2909.128.2.203

Engman, J., Linnman, C., Van Dijk, K. R., and Milad, M. R. (2016). Amygdala subnuclei resting-state functional connectivity sex and estrogen differences. *Psychoneuroendocrinology* 63, 34–42. doi: 10.1016/j.psyneuen.2015.09.012

Engman, J., Poromaa, I. S., Moby, L., Wikström, J., Fredrikson, M., and Gingnell, M. (2018). Hormonal cycle and contraceptive effects on amygdala and salience resting-state networks in women with previous affective side effects on the pill. *Neuropsychopharmacology* 43, 555–563. doi: 10.1038/npp.2017.157

Fehring, R. J., Schneider, M., and Raviele, K. (2006). Variability in the phases of the menstrual cycle. *J. Obstet. Gynecol. Neonatal Nurs.* 35, 376–384.

Fleischman, D. S., Navarrete, C. D., and Fessler, D. M. (2010). Oral contraceptives suppress ovarian hormone production. *Psychol. Sci.* 21, 750–752. doi: 10.1177/0956797610368062

Fuchs, A., Matonóg, A., Sieradzka, P., Pilarska, J., Hauzer, A., Czech, I., et al. (2019). Anti-androgenic therapy in young patients and its impact on intensity of hirsutism, acne, menstrual pain intensity and sexuality - a preliminary study. *Ginekol. Pol.* 90, 520–526. doi: 10.5603/GP.2019.0091

Gur, R. C., Schroeder, L., Turner, T., McGrath, C., Chan, R. M., Turetsky, B. I., et al. (2002). Brain activation during facial emotion processing. *Neuroimage* 16, 651–662. doi: 10.1006/nimg.2002.1097

Gurvich, C., Warren, A. M., Worsley, R., Hudaib, A. R., Thomas, N., and Kulkarni, J. (2020). Effects of oral contraceptive androgenicity on visuospatial and social-emotional cognition: a prospective observational trial. *Brain Sci.* 10:194. doi: 10.3390/brainsci10040194

Habel, U., Windischberger, C., Derntl, B., Robinson, S., Kryspin-Exner, I., Gur, R. C., et al. (2007). Amygdala activation and facial expressions: explicit emotion discrimination versus implicit emotion processing. *Neuropsychologia* 45, 2369–2377. doi: 10.1016/j.neuropsychologia.2007.01.023

Hamstra, D. A., De Rover, M., De Rijk, R. H., and Van der Does, W. (2014). Oral contraceptives may alter the detection of emotions in facial expressions. *Eur. Neuropsychopharmacol.* 24, 1855–1859. doi: 10.1016/j.euroneuro.2014.08.015

Hariri, A. R., Tessitore, A., Mattay, V. S., Fera, F., and Weinberger, D. R. (2002). The amygdala response to emotional stimuli: a comparison of faces and scenes. *Neuroimage* 17, 317–323. doi: 10.1006/nimg.2002.1179

Hoffmann, H., Kessler, H., Eppel, T., Rukavina, S., and Traue, H. C. (2010). Expression intensity, gender and facial emotion recognition: women recognize only subtle facial emotions better than men. *Acta Psychol.* 135, 278–283. doi: 10.1016/j.actpsy.2010.07.012

Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biom. J.* 50, 346–363. doi: 10.1002/bimj.200810425

Jhang, J., Lee, H., Kang, M. S., Lee, H. S., Park, H., and Han, J. H. (2018). Anterior cingulate cortex and its input to the basolateral amygdala control innate fear response. *Nat. Commun.* 9:2744. doi: 10.1038/s41467-018-05090-y

Judd, H. L., and Yen, S. S. (1973). Serum androstenedione and testosterone levels during the menstrual cycle. *J. Clin. Endocrinol. Metab.* 36, 475–481.

Klavir, O., Genud-Gabai, R., and Paz, R. (2013). Functional connectivity between amygdala and cingulate cortex for adaptive aversive learning. *Neuron* 80, 1290–1300. doi: 10.1016/j.neuron.2013.09.035

Kogler, L., Müller, V. I, Seidel, E. M., Boubela, R., Kalcher, K., Moser, E., et al. (2016). Sex differences in the functional connectivity of the amygdalae in association with cortisol. *Neuroimage* 134, 410–423. doi: 10.1016/j.neuroimage.2016.03.064

Kohler, C. G., Turner, T. H., Bilker, W. B., Brensinger, C. M., Siegel, S. J., Kanes, S. J., et al. (2003). Facial emotion recognition in schizophrenia: intensity effects and error pattern. *Am. J. Psychiatry* 160, 1768–1774. doi: 10.1176/appi.ajp.160.10.1768

Kujawa, A., Wu, M., Klumpp, H., Pine, D. S., Swain, J. E., Fitzgerald, K. D., et al. (2016). Altered development of amygdala-anterior cingulate cortex connectivity in anxious youth and young adults. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* 1, 345–352. doi: 10.1016/j.bpsc.2016.01.006

Kuusikko, S., Haapsamo, H., Jansson-Verkasalo, E., Hurtig, T., Mattila, M. L., Ebeling, H., et al. (2009). Emotion recognition in children and adolescents with autism spectrum disorders. *J. Autism dev. Disord.* 39, 938–945. doi: 10.1007/s10803-009-0700-0

Lancaster, K., Venkatesan, U. M., Lengenfelder, J., and Genova, H. M. (2019). Default mode network connectivity predicts emotion recognition and social integration after traumatic brain injury. *Front. Neurol.* 10:825. doi: 10.3389/fneur.2019.00825

Lane, R. D., Reiman, E. M., Axelrod, B., Yun, L. S., Holmes, A., and Schwartz, G. E. (1998). Neural correlates of levels of emotional awareness: evidence of an interaction between emotion and attention in the anterior cingulate cortex. *J. Cogn. Neurosci.* 10, 525–535. doi: 10.1162/089892998562924

Lisofsky, N., Riediger, M., Gallinat, J., Lindenberger, U., and Kühn, S. (2016). Hormonal contraceptive use is associated with neural and affective changes in healthy young women. *Neuroimage* 134, 597–606. doi: 10.1016/j.neuroimage.2016.04.042

Luders, E., Gaser, C., Jancke, L., and Schlaug, G. (2004). A voxel-based approach to gray matter asymmetries. *NeuroImage.* 22, 656–664. doi: 10.1016/j.neuroimage.2004.01.032

Mannava, S. (2012). Age-related differences in emotion recognition ability: visual and auditory modalities. *Vanderbilt Undergraduate Res. J.* 8, 1–5. doi: 10.15695/vurj.v8i0.3570

McLellan, T. L., Wilcke, J. C., Johnston, L., Watts, R., and Miles, L. K. (2012). Sensitivity to posed and genuine displays of happiness and sadness: a fMRI study. *Neurosci. Lett.* 531, 149–154. doi: 10.1016/j.neulet.2012.10.039

Montagne, B., Kessels, R. P., Frigerio, E., de Haan, E. H., and Perrett, D. I. (2005). Sex differences in the perception of affective facial expressions: do men really lack emotional sensitivity? *Cogn. Process.* 6, 136–141. doi: 10.1007/s10339-005-0050-6

Montoya, E. R., and Bos, P. A. (2017). How oral contraceptives impact social-emotional behavior and brain function. *Trends Cogn. Sci.* 21, 125–136. doi: 10.1016/j.tics.2016.11.005

Oinonen, K., and Mazmanian, D. (2002). To what extent do oral contraceptives influence mood and affect? *J. Affect. Dis.* 70, 229–240. doi: 10.1016/s0165-0327(01)00356-1

Olderbak, S., Wilhelm, O., Hildebrandt, A., and Quoidbach, J. (2019). Sex differences in facial emotion perception ability across the lifespan. *Cogn. Emot.* 33, 579–588. doi: 10.1080/02699931.2018.1454403

Pahnke, R., Mau-Moeller, A., Junge, M., Wendt, J., Weymar, M., Hamm, A. O., et al. (2019). Oral contraceptives impair complex emotion recognition in healthy women. *Front. Neurosci.* 12:1041. doi: 10.3389/fnins.2018.01041

Petersen, N., and Cahill, L. (2015). Amygdala reactivity to negative stimuli is influenced by oral contraceptive use. *Soc. Cogn. Affect. Neurosci.* 10, 1266–1272. doi: 10.1093/scan/nsv010

Petitti, D. (2003). Combination estrogen–progestin oral contraceptives. *N. Engl. J. Med.* 349, 1443–1450.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team (2014). *Nlme: Linear and Nonlinear Mixed EFFECTS Models. R Package Version 3.1–117.*

Pletzer, B. A., and Kerschbaum, H. H. (2014). 50 years of hormonal contraception—time to find out, what it does to our brain. *Front. Neurosci.* 8:256. doi: 10.3389/fnins.2014.00256

Pletzer, B., Crone, J. S., Kronbichler, M., and Kerschbaum, H. (2016). Menstrual cycle and hormonal contraceptive-dependent changes in intrinsic connectivity of resting-state brain networks correspond to behavioral changes due to hormonal status. *Brain Connect.* 6, 572–585. doi: 10.1089/brain.2015.0407

Pletzer, B., Kronbichler, M., and Kerschbaum, H. (2015). Differential effects of androgenic and anti-androgenic progestins on fusiform and frontal gray matter

volume and face recognition performance. *Brain Res.* 1596, 108–115. doi: 10.1016/j.brainres.2014.11.025

Pruim, R. H. R., Mennes, M., van Rooij, D., Llera, A., Buitelaar, J. K., and Beckmann, C. F. (2015). ICA-AROMA: a robust ICA-based strategy for removing motion artifacts from fMRI data. *NeuroImage* 112, 267–277. doi: 10.1016/J.NEUROIMAGE.2015.02.064

R Core Team (2019). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Radke, S., and Derntl, B. (2016). Affective responsiveness is influenced by intake of oral contraceptives. *Eur. Neuropsychopharmacol.* 26, 1014–1019. doi: 10.1016/j.euroneuro.2016.03.004

Rahman, Q., Wilson, G. D., and Abrahams, S. (2004). Sex, sexual orientation, and identification of positive and negative facial affect. *Brain Cogn.* 54, 179–185. doi: 10.1016/j.bandc.2004.01.002

Repple, J., Habel, U., Wagels, L., Pawliczek, C. M., Schneider, F., and Kohn, N. (2018). Sex differences in the neural correlates of aggression. *Brain Struct. funct.* 223, 4115–4124. doi: 10.1007/s00429-018-1739-5

Roy, A. K., Shehzad, Z., Margulies, D. S., Kelly, A. M., Uddin, L. Q., Gotimer, K., et al. (2009). Functional connectivity of the human amygdala using resting state fMRI. *Neuroimage* 45, 614–626. doi: 10.1016/j.neuroimage.2008.11.030

Rukavina, S., Sachsenweger, F., Jerg-Bretzke, L., Daucher, A. E., Traue, H. C., Walter, S., et al. (2018). Testosterone and its influence on emotion recognition in young, healthy males. *Psychology* 9:1814.

Rupp, H. A., James, T. W., Ketterson, E. D., Sengelaub, D. R., Janssen, E., and Heiman, J. R. (2009). Neural activation in women in response to masculinized male faces: mediation by hormones and psychosexual factors. *Evol. Hum. Behav.* 30, 1–10.

Russell, J. A. (1994). Is there universal recognition of emotion from facial expression? A review of the cross-cultural studies. *Psychol. Bull.* 115, 102–141. doi: 10.1037/0033-2909.115.1.102

Scherer, K. R., Clark-Polner, E., and Mortillaro, M. (2011). In the eye of the beholder? Universality and cultural specificity in the expression and perception of emotion. *Int. J. Psychol.* 46, 401–435. doi: 10.1080/00207594.2011.626049

Schmidt, K. L., and Cohn, J. F. (2001). Human facial expressions as adaptations: evolutionary questions in facial expression research. *Am. J. Phys. Anthropol.* Suppl 33, 3–24. doi: 10.1002/ajpa.2001

Shenk, C. E., Putnam, F. W., and Noll, J. G. (2013). Predicting the accuracy of facial affect recognition: the interaction of child maltreatment and intellectual functioning. *J. Exp. Child. Psychol.* 114, 229–242. doi: 10.1016/j.jecp.2012.08.007

Shirazi, T. N., Rosenfield, K. A., Cárdenas, R. A., Breedlove, S. M., and Puts, D. A. (2020). No evidence that hormonal contraceptive use or circulating sex steroids predict complex emotion recognition. *Horm. Behav.* 119:104647. doi: 10.1016/j.yhbeh.2019.104647

Sitruk-Ware, R. (2008). Reprint of pharmacological profile of progestins. *Maturitas* 61, 151–157.

Skovlund, C. W., Mørch, L. S., Kessing, L. V., and Lidegaard, Ø (2016). Association of hormonal contraception with depression. *JAMA Psychiatry* 73, 1154–1162.

Stanczyk, F. Z., Archer, D. F., and Bhavnani, B. R. (2013). Ethinyl estradiol and 17β-estradiol in combined oral contraceptives: pharmacokinetics, pharmacodynamics and risk assessment. *Contraception* 87, 706–727. doi: 10.1016/j.contraception.2012.12.011

Streit, M., Dammers, J., Simsek-Kraues, S., Brinkmeyer, J., Wölwer, W., and Ioannides, A. (2003). Time course of regional brain activations during facial emotion recognition in humans. *Neurosci. Lett.* 342, 101–104. doi: 10.1016/s0304-3940(03)00274-x

Sundström Poromaa, I., and Gingnell, M. (2014). Menstrual cycle influence on cognitive function and emotion processing—from a reproductive perspective. *Front. Neurosci.* 8:380. doi: 10.3389/fnins.2014.00380

Sundström Poromaa, I., and Segebladh, B. (2012). Adverse mood symptoms with oral contraceptives. *Acta Obstet. Gynecol. Scand.* 91, 420–427.

Thayer, J., and Johnsen, B. H. (2000). Sex differences in judgement of facial affect: a multivariate analysis of recognition errors. *Scand. J. Psychol.* 41, 243–246. doi: 10.1111/1467-9450.00193

Thielscher, A., and Pessoa, L. (2007). Neural correlates of perceptual choice and decision making during fear–disgust discrimination. *J. Neurosci.* 27, 2908–2917. doi: 10.1523/JNEUROSCI.3024-06.2007

Thomas, K. M., Drevets, W. C., Whalen, P. J., Eccard, C. H., Dahl, R. E., Ryan, N. D., et al. (2001). Amygdala response to facial expressions in children and adults. *Biol. Psychiatry* 49, 309–316. doi: 10.1016/s0006-3223(00)01066-0

Toffoletto, S., Lanzenberger, R., Gingnell, M., Sundström-Poromaa, I., and Comasco, E. (2014). Emotional and cognitive functional imaging of estrogen and progesterone effects in the female human brain: a systematic review. *Psychoneuroendocrinology* 50, 28–52. doi: 10.1016/j.psyneuen.2014.07.025

Toyoda, H., Li, X. Y., Wu, L. J., Zhao, M. G., Descalzi, G., Chen, T., et al. (2011). Interplay of amygdala and cingulate plasticity in emotional fear. *Neural Plasticity* 2011:813749. doi: 10.1155/2011/813749

Uljarevic, M., and Hamilton, A. (2013). Recognition of emotions in autism: a formal meta-analysis. *J. Autism dev. Disord.* 43, 1517–1526. doi: 10.1007/s10803-012-1695-5

Vogt, B. A. (2005). Pain and emotion interactions in subregions of the cingulate gyrus. *Nat. Rev. Neurosci.* 6, 533–544. doi: 10.1038/nrn1704

Weisenbach, S. L., Rapport, L. J., Briceno, E. M., Haase, B. D., Vederman, A. C., Bieliauskas, L. A., et al. (2014). Reduced emotion processing efficiency in healthy males relative to females. *Soc. Cogn. Affect. Neurosci.* 9, 316–325. doi: 10.1093/scan/nss137

Whitfield-Gabrieli, S., and Nieto-Castanon, A. (2012). Conn?: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect.* 2, 125–141. doi: 10.1089/brain.2012.0073

Wiegratz, I., Kutschera, E., Lee, J. H., Moore, C., Mellinger, U., Winkler, U. H., et al. (2003). Effect of four different oral contraceptives on various sex hormones and serum-binding globulins. *Contraception* 67, 25–32. doi: 10.1016/s0010-7824(02)00436-5

Wright, F., Giacomini, M., Riahi, M., and Mowszowicz, I. (1983). "Antihormone activity of progesterone and progestins," in *Progesterone and Progestins*, eds C. W. Bardin, E. Milgröm, and P. Mauvais-Jarvis (New York, NY: Raven Press), 121–134.

Yamasaki, H., LaBar, K. S., and McCarthy, G. (2002). Dissociable prefrontal brain systems for attention and emotion. *Proc. Natl. Acad. Sci. U.S.A.* 99, 11447–11451.

Yan, C. G., Wang, X. D., Zuo, X. N., and Zang, Y. F. (2016). DPABI: data processing & analysis for (resting-state) brain imaging. *Neuroinformatics* 14, 339–351. doi: 10.1007/s12021-016-9299-4

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Behavioral Neuroscience

REVIEW
published: 15 April 2022
doi: 10.3389/fnbeh.2022.850623

Estrogen, the Peripheral Immune System and Major Depression – A Reproductive Lifespan Perspective

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Major depression is a significant medical issue impacting millions of individuals worldwide. Identifying factors contributing to its manifestation has been a subject of intense investigation for decades and several targets have emerged including sex hormones and the immune system. Indeed, an extensive body of literature has demonstrated that sex hormones play a critical role in modulating brain function and impacting mental health, especially among female organisms. Emerging findings also indicate an inflammatory etiology of major depression, revealing new opportunities to supplement, or even supersede, currently available pharmacological interventions in some patient populations. Given the established sex differences in immunity and the profound impact of fluctuations of sex hormone levels on the immune system within the female, interrogating how the endocrine, nervous, and immune systems converge to impact women's mental health is warranted. Here, we review the impacts of endogenous estrogens as well as exogenously administered estrogen-containing therapies on affect and immunity and discuss these observations in the context of distinct reproductive milestones across the female lifespan. A theoretical framework and important considerations for additional study in regards to mental health and major depression are provided.

Keywords: estrogen, sex differences, major depressive disorder, peripheral immune system, mood

OPEN ACCESS

Edited by:
Stephanie V. Koebele, Arizona State University, United States

Reviewed by:
Benedetta Leuner, The Ohio State University, United States
Angela Garcia, Arizona State University, United States

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Specialty section:
This article was submitted to Behavioral Endocrinology, a section of the journal Frontiers in Behavioral Neuroscience

Received: *07 January 2022*
Accepted: *17 March 2022*
Published: *15 April 2022*

Citation:
Engler-Chiurazzi EB, Chastain WH, Citron KK, Lambert LE, Kikkeri DN and Shrestha SS (2022) Estrogen, the Peripheral Immune System and Major Depression – A Reproductive Lifespan Perspective. Front. Behav. Neurosci. 16:850623. doi: 10.3389/fnbeh.2022.850623

INTRODUCTION

Mood disorders, including major depressive disorder (MDD), are a significant global health issue (Krishnan and Nestler, 2008). Worldwide lifetime prevalence of mood disorders has been reported to be nearly 10% (Steel et al., 2014), and in 2010, the nearly 300 million global cases of MDD accounted for 8.2% of all disease-induced years lived with disability (Ferrari et al., 2013a). In the US alone, one in six adults will receive an MDD diagnosis in their lifetime and more than 13 million

Abbreviations: 5-HT, serotonin; E2, estradiol; ABC, age-associated B cell; CNS, central nervous system; ER, estrogen receptor; HRT, hormone replacement therapy; IL, interleukin; Ig, Immunoglobulin; LPS, lipopolysaccharide; MDD, major depressive disorder; NK, natural killer (cell); rTMS, repetitive transcranial magnetic stimulation; SSRI, selective serotonin reuptake inhibitor; TNF, tumor necrosis factor.

Americans experience a major depressive episode with severe impairment each year (Kessler et al., 2005; Ferrari et al., 2013b; Brody et al., 2018). Annual costs associated with this condition are estimated at ∼$210 billion (Greenberg et al., 2015). MDD is generally considered a brain-targeted disease associated with persistent sadness, guilt, anhedonia (reduced interest in rewarding stimuli), despair, and in some cases, suicide (Krishnan and Nestler, 2008). Due to a happenstance discovery of psychiatric patients showing improved mood when treated with monoamine oxidase inhibitors (Schildkraut, 1965), MDD has historically been associated with deficiencies in serotonergic (5-HT), dopaminergic, and noradrenergic signaling within limbic, reward, and brainstem structures (Krishnan and Nestler, 2008). Problematically, available pharmacologic treatments targeting these presumed dysregulated monoamine systems are associated with delayed and inadequate symptom alleviation in a large proportion of patients (Trivedi et al., 2006; Al-Harbi, 2012; Akil et al., 2018). This led the field to conclude that the pathology of MDD is more complex than previously appreciated, that the neurotransmitters thought to underlie MDD-associated brain pathology may not be the sole contributors to its presentation, *and* that the therapeutic interventions targeting these systems will likely remain insufficient at imparting symptomatic relief.

Emerging data strongly implicate additional mechanisms in the manifestation of mood disorders. As a result, many researchers have begun considering biological factors that could significantly contribute to the development and persistence of MDD. One of these factors is that of genetic sex and the accompanying differences in sex hormone secretion across the lifespan. A substantial amount of research attention has been paid to the role of sex hormones, especially the steroid hormone estrogen, in driving development of MDD in women (Wharton et al., 2012; Eid et al., 2019). In addition to sex hormones, converging data amassed over the past few decades also support significant immune contributions to brain function and mood (Leonard, 2010; Dantzer, 2018). Indeed, it is now accepted that inflammatory cascades mediated by innate and adaptive arms of the immune system significantly contribute to MDD, at least in some patient subsets (Maes, 2011; Wohleb et al., 2016; Herkenham and Kigar, 2017). Sex differences in the susceptibility to certain infections, the presence of sex hormone receptors on immune cells, and shifts in the function of the immune system during distinct periods of the reproductive lifespan all point to a critical role of sex hormones in modulating immunity (Pennell et al., 2012; Klein and Flanagan, 2016).

Given the known sex differences in the prevalence of mood disorders, emerging support for the immune system's role in mediating susceptibility or resilience to psychosocial stress, and the potentially profound impacts of sex hormones (especially estrogens) on impacting immunity, the consideration of neuro-immuno-endocrine interactions in the context of mood and MDD across the female lifespan, is warranted. Here, we will review evidence regarding the mood impacts of these factors individually, describe shifts in immune responses during key reproductive milestones, highlight a few examples of potential autoimmune consequences of estrogenic stimulation in females, and summarize the small but growing collection of findings exploring the convergence of sex, sex hormones and immune function in the context of mood and MDD. Finally, we present important experimental considerations when the convergence of these factors is investigated.

MANIFESTATION OF DISORDERED MOOD ACROSS THE FEMALE LIFESPAN: ROLE FOR ESTROGENS

Women shoulder a disproportionate burden of mood disorders and the role of estrogen in modulating mood has been well studied. Estrogens are generally thought to improve mood in many, but not all, circumstances. Below, we highlight major observations driving this conclusion. Though a thorough discussion of this extensive literature is beyond the scope of the current review, the reader is directed to several excellent reviews specifically addressing this topic (Wharton et al., 2012; Altemus et al., 2014; Eid et al., 2019; LeGates et al., 2019).

Sex Differences in Depression

Differences in the prevalence of MDD, phenotypic manifestations of depression, and the efficacy of antidepressant therapy between the sexes are well established (Altemus et al., 2014; LeGates et al., 2019). Rates of MDD are substantially higher among females compared to males (Weissman and Klerman, 1977), though this sex difference appears to be critically dependent on age. Prior to puberty, boys are more likely to have a mood disorder than girls (Faravelli et al., 2013). This incidence shifts during the pubertal transition as girls display depression at a rate double that of boys between the ages of 15 to 19 (Faravelli et al., 2013). MDD is nearly twice as prevalent in adult women than men, at rates of 10.4 and 5.5%, respectively (Brody et al., 2018). However, following reproductive senescence during the fifth decade of life, aging men and women tend to have similar prevalence rates of mood disorders (Faravelli et al., 2013).

Throughout life, men and women may also differ in their MDD endophenotypes. Results of several studies, including the large-scale Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial, indicate that women display higher rates of atypical and anxious depressive phenotypes. These are characterized by increased appetite, weight gain, comorbid eating disorder, rumination, hypersomnia, gastrointestinal complaints, and a higher rate of past suicide attempts relative to male patients (Marcus et al., 2008; Shors et al., 2017). Men are more likely to display comorbid substance use coping strategies and have higher rates of successful suicide, likely due to their use of more lethal means (e.g., firearms). Reports of irritability and the melancholic depressive subtype are similar in both men and women (Marcus et al., 2008).

Finally, though MDD treatments are available, barriers to treatment access as well as intervention type playing a role in the realization of symptom relief (LeGates et al., 2019) leaves the affective symptoms of many patients poorly controlled. Indeed, a recent study assessing nearly 250,000 depressed adults noted that only about 30% of MDD patients obtained pharmacological antidepressant treatment within three

months of diagnosis (Waitzfelder et al., 2018), and of those, antidepressant efficacy is often delayed and highly variable (Trivedi et al., 2006; Al-Harbi, 2012; Akil et al., 2018). Sex may account for some of this variability as women appear to experience better symptom remission from selective serotonin (SSRI) or norepinephrine reuptake inhibitors, while men respond better when treated with tricyclic antidepressants (LeGates et al., 2019). Sex differences were not readily observed among adult patients with refractory bipolar/MDD undergoing repetitive transcranial magnetic stimulation (Huang et al., 2008). This observation appears to be age- and hormone status-dependent. Older women may display a poor response to rTMS (Huang et al., 2008) or the SSRI, venlafaxine (Thase et al., 2005), and this effect was reversed by estrogen supplementation. Sex differences in response to newly developed, fast-acting, glutamatergic-modulating, antidepressant interventions such as ketamine, are only beginning to be assessed. Emerging findings suggests conflicting results. Some groups have reported needing lower ketamine doses in female rats to impact affective behaviors under basal conditions while others report that male mice may be more responsive than females following exposure to stress (Saland et al., 2017; Okine et al., 2020). This is noteworthy given the recent Federal Drug Administration approval of nasally-administered esketamine for MDD patients treatment-resistant to traditional antidepressant interventions FDA (2019). It is also important to note that these sex-specific antidepressant treatment responses could also be explained, at least in part, by differences in the observed MDD endophenotypes in men versus women described above; further interrogation of this possibility is needed.

Hormone Effects on Depression and Mood During and After the Reproductive Years in Women

These observations along with the dynamic shifts in reproductive capacity that take place across the female lifespan implicate ovarian hormones in modulating mood and mood disorders in women. Indeed, between 10 and 80% of women experience mood disruptions that are related to their menstrual cycle, (Baker and Driver, 2007), and 3-8% of women can experience premenstrual dysphoric disorder, characterized by extreme premenstrual anxiety, decreased mood, and irritability (Robakis et al., 2019). These observations have been reported for the past several decades in both human and preclinical populations, though not all studies have consistently found an association between cycle stage and affect (Moos et al., 1969; Laessle et al., 1990; Jenkins et al., 2001; D'Souza and Sadananda, 2017; Sundström-Poromaa, 2018; Zhao et al., 2021). As well, the peripartum period is associated with dynamic shifts in sex hormone levels, and one of the most common complications of pregnancy, observed to impact one in seven mothers, are postpartum mood and anxiety disorders (Wenzel, 2016; Luca et al., 2019). Among menopausal women, (Maartens et al., 2002; Bekku et al., 2006; Gordon et al., 2016; Soares, 2017; Gracia and Freeman, 2018) and ovariectomized rodents (de Chaves et al., 2009; Li et al., 2014; Schoenrock et al., 2016), in whom levels of key sex hormones are substantially lower, increased anxiety and depressive behaviors have been noted.

Effects of Exogenous Estrogen Therapies on Mood

Estrogen-containing treatments have been shown to improve mood or attenuate depressive symptoms in humans (Schmidt et al., 2000; Soares et al., 2001; Poromaa and Segebladh, 2012; Maki et al., 2019) and to reverse at least some ovariectomy-induced pro-depressive changes in rodents (Bernardi et al., 1989; Galea et al., 2001; Walf and Frye, 2009; Schiller et al., 2013; Li et al., 2014; Hiroi et al., 2016), suggesting pro-resilience benefits. Estrogens, especially the most potent naturally circulating estrogen 17β-estradiol (E2), are known to induce dendritic spine plasticity and neuronal complexity, facilitate neurogenesis, regulate brain region volume and activity levels, and impact key neurotransmitter and growth factor systems implicated in depression, to name just a few examples (Galea et al., 2001; Maki and Resnick, 2001; Brinton, 2009; Walf and Frye, 2009; Wharton et al., 2012; Marrocco and McEwen, 2016; Engler-Chiurazzi et al., 2017). Yet, not all studies report beneficial impacts of exogenously administered estrogens on mood. Several studies have noted increased depression among women taking hormonal contraceptives (Duke et al., 2007; Skovlund et al., 2016; de Wit et al., 2020) though collective findings generally suggest that contraception exerts minimal effects on mood (Robakis et al., 2019). The realization of neurobiological and behavioral effects of estrogen-containing treatments depends on a number of factors including, but not limited to, age of the organism, etiology and duration of hormone depletion, type of estrogen, treatment route of administration, treatment regimen, and functional domain targeted (Engler-Chiurazzi et al., 2017). Consideration of these factors is of key importance when assessing mood-impacting effects of this hormone.

EVIDENCE OF IMMUNE IMPACTS ON THE DEVELOPMENT AND PERSISTENCE OF DEPRESSION

The immune system supports the body's response against infection, injury, and disease. This complex network of intercommunicating, interactive cells and their secretory factors coordinates across multiple organs to mount a rapid and appropriate response to a threat to homeostasis through complex signaling cascades and activation/regulation sequences; the reader is directed to several excellent reviews that thoroughly describe the complexities of this system in detail (Chaplin, 2010; Marshall et al., 2018). Understanding of the complexity of neuroimmune mechanisms within the central nervous system (CNS) has grown rapidly in recent years. Although once considered "immune privileged", a compelling body of literature indicates that the CNS and the peripheral immune systems engage in bidirectional communication, profoundly influencing one another during homeostasis and in pathological/diseased states (Lucas et al., 2006; Pavlov et al., 2018), including those

associated with chronic stress and MDD (Dantzer, 2018). Microglial cells, the resident immune cells of the CNS, represent a particularly well-studied neuroimmune cascade mediator. Their actions as well as the contributions of other key CNS components (i.e., astrocytes, oligodendrocytes, perivascular macrophages, neurons, and endothelial cells) to the local neuroinflammatory cascade in response to CNS perturbation have been extensively described elsewhere (Ousman and Kubes, 2012; Ransohoff et al., 2015; Morimoto and Nakajima, 2019). Therefore, we will focus our discussion on the contributions of peripheral immune components to mood and MDD.

The peripheral innate immune response is characterized by rapid and non-specific activation of pattern/danger recognition receptors on innate immune cells to initiate phagocytosis of non-self antigens, secrete a variety of signaling factors including cytokines and chemokines, and/or function as antigen presenting cells to trigger adaptive immune activation (Chaplin, 2010; Marshall et al., 2018). Inflammation driven by innate immune system components, particularly macrophages, in modulating mood is now well established (Adzic et al., 2018). Chronic inflammation is implicated in a variety of mood disorders, leading to the emergence of the "macrophage/monokine theory of depression" (Dey and Hankey Giblin, 2018). For instance, depressive phenotypes have been consistently reported both among patients receiving proinflammatory cytokine treatment regimens and in preclinical models (Pryce and Fontana, 2017). As well, elevated levels of circulating cytokines, principally tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6, have been repeatedly reported among some subsets of depressed clinical populations (Dowlati et al., 2010; Köhler et al., 2017). Elevated levels of these inflammatory biomarkers are often associated with poor responsiveness to 5-HT targeting interventions (Arteaga-Henríquez et al., 2019), and anti-depressant treatment has been shown to reduce proinflammatory cytokine levels among treatment-responders or in preclinical models of immune challenge (Roumestan et al., 2007; Arteaga-Henríquez et al., 2019). Finally, compared to placebo, antidepressant treatment with co-administration of agents of anti-inflammatory action, such as non-steroidal anti-inflammatory drugs, statins, or cytokine inhibitors, improved depressive symptoms and MDD remission rates (Köhler-Forsberg et al., 2019). Mood benefits among depressed patients were even realized when these anti-inflammatory agents were administered as monotherapies (Köhler-Forsberg et al., 2019).

The complement system, an innate Immune arm that amplifies the recruitment signals initiated by other innate immune players, labels non-self antigens to facilitate immune-induced attack on these cells and mitigates the spread of the infection via membrane dysfunction-induced cell death (Rus et al., 2005), is also impacted by stress and depression. Indeed, levels of C3c and C4 complement as well as several other positive acute phase proteins including α1-antitrypsin and haptoglobin are elevated in depressed populations, while negative acute phase proteins like albumin are reduced (Kronfol and House, 1989; Maes et al., 1992b; Song et al., 1994).

The adaptive immune arm represents a delayed, antigen-specific response that targets intracellular infection/damage, amplifies and also resolves inflammatory cascade responses, and facilitates antigen memory (Chaplin, 2010; Marshall et al., 2018). Though evidence supporting a role for the peripheral adaptive immune system in modulating mood was slower to evolve due in part to the historical perception that lymphocytes are largely absent from brain parenchyma, T and B cells have also been implicated in response to CNS injury and disease, in the control of some normal brain functions and more recently, in MDD (Maes, 2011; Herkenham and Kigar, 2017; Dantzer, 2018). Indeed, many adaptive immune cells express the cellular machinery to respond to stimulation by the stress hormone, cortisol, and elevated cortisol levels, like those associated with a host of mood disorders, tend to be immunosuppressive (Gruver-Yates et al., 2014; Kovacs, 2014). Importantly, chronic stress is known to affect lymphocyte numbers/function in both humans suffering from mood disorders and in preclinical populations exposed to stressful conditions (Yin et al., 2000; Domínguez-Gerpe and Rey-Méndez, 2001; Frick et al., 2009; Scheinert et al., 2016). Lymphocytes are also profoundly impacted by 5-HT, at least in the periphery (Herr et al., 2017).

That peripherally derived T cells are now appreciated to be present in healthy brain parenchyma and can also infiltrate CNS tissue in response to injury or autoimmune disease has fostered major interest in the role of antigen-specific adaptive immunity in normal and abnormal brain function, including within the context of chronic stress and depression (Fletcher, 2010; Maes, 2011; Filiano et al., 2017; Herkenham and Kigar, 2017; Rayasam et al., 2018). Several seminal observations among depressed patient populations reported increased numbers of T helper/inducer cells and shifted ratios of $CD4^+/CD8^+$ T cells (Darko et al., 1988; Schleifer et al., 1989; Maes et al., 1990). Further, studies in lymphocyte-deficient mice (nude, scid or $Rag^{-/-}$ mice) have noted deficits in adaptability to stress and reconstitution with lymphocyte populations generally implicated the absence of T cells in mediating these deficits in a subset-specific way (Cohen et al., 2006; Beurel et al., 2013; Rattazzi et al., 2013; Brachman et al., 2015; Clark et al., 2016). For example, (primarily) T lymphocytes from stress-exposed mice can modify the behavioral response to stress when adoptively transferred into lymphocyte deficient subjects (Brachman et al., 2015). T cells also robustly respond to glutamatergic signaling (Ganor and Levite, 2012), a neurotransmitter system that is emerging as a key contributor to MDD and a principle target for novel, fast acting antidepressants (Wang Y. T. et al., 2021).

The B cell component of the adaptive immune system may also play an important role in modulating both normal CNS function as well as the response to stress. Historically there were inconsistencies with regards to whether B cells were changed in depressed populations. However, methodological advances in measurement of these populations has revealed blood B cell number alterations in the context of mood disorders, including chronic academic stress, MDD, bipolar disorder, and panic disorder (Darko et al., 1988; Maes et al., 1992b; Schleifer et al., 2002; Robertson et al., 2005; Pavón et al., 2006; McGregor et al., 2016; Ahmetspahic et al., 2018). Further, some studies have reported B cell responsiveness among MDD patients given monoamine-modulating antidepressant

interventions (Hernandez et al., 2010; Ahmetspahic et al., 2018). These observations have been successfully recapitulated in a recently published preclinical study leveraging the chronic social defeat stress model (Lynall et al., 2021). Indeed, pioneering work from the Clathworhy group (Lynall et al., 2021) reported that chronic stress increased splenic B cell activation and increased meningeal monocytes, while meningeal B cell counts were reduced. From a mechanistic perspective, like T cells, B cells have been shown to express 5-HT receptors and the 5-HT transporter, indicating that these cells may even take up this key MDD-associated neurotransmitter and transport it to distant sites (Meredith et al., 2005; Herr et al., 2017). Whether the brain is one of these is yet to be determined. As well, growth factors, such as brain derived neurotrophic factor, have been implicated in the manifestation of MDD (Yang et al., 2020), and their stimulation is critical for B cell development (Schuhmann et al., 2005; Fauchais et al., 2008). Given the crucial role of B cells in antigen presentation to T cells, their ability to facilitate T cell activation, and emerging understanding of their immunoregulatory impacts, additional exploration of their role in the response to stress is warranted.

Key functional activities of B cells, such as antibody secretion, may also be altered by stress in an antibody subclass-specific way (Kronfol and House, 1989; Joyce et al., 1992; Song et al., 1994; Gold et al., 2012). For example, relative to mentally healthy control subjects, Gold and colleagues (Gold et al., 2012) noted that depressed populations displayed reductions in serum IgA, but not IgM or IgG levels, while Joyce et al. (Joyce et al., 1992) reported increased IgA. Methodological differences between sample populations and measurement approaches may account for some of the discrepancy between these studies. The critical role of hypothalamic-pituitary-adrenal axis dysregulation and altered cortisol secretion in the manifestation of MDD is well established (Krishnan and Nestler, 2008). Physiological states associated with high levels of circulating cortisol, such as hypercortisolism (Sarcevic et al., 2020) or treatment of patients with corticosteroid-based interventions, shifts serum antibody profiles relative to healthy controls (Griggs et al., 1972; Settipane et al., 1978). As well, neuronal surface autoantibody expression has been implicated in a number of neuropsychiatric conditions, MDD included (Zong et al., 2017).

ESTROGENIC IMPACTS ON IMMUNE FUNCTION DURING DISTINCT REPRODUCTIVE MILESTONES ACROSS THE FEMALE LIFESPAN

Sex differences in immunity are well documented, and hormone influences, including those of estrogens, have been shown to impact immune function throughout adulthood (Pennell et al., 2012; Klein and Flanagan, 2016). Immunological impacts of genetic sex and of estrogenic stimulation across key reproductive milestones are described in the following sections and have been summarized in **Figure 1**.

Mechanisms of Estrogen Regulation of Immunity

Estrogenic signaling is regulated by two nuclear estrogen receptors (ER), ERα and ERβ, both of which are expressed on a variety of immune cell types and tissues. For instance, ERα is widely expressed in bone marrow thymocytes and hematopoietic cells, while ERβ expression appears to be limited to the thymus, lymphocytes in lymph nodes, and the spleen in mid-gestational fetuses (Khan and Ansar Ahmed, 2015; Moulton, 2018; Rubinow, 2018; Zhang et al., 2020). Estrogens regulate immune cell number and function likely via an ER-dependent mechanism. When human lymphocytes were administered 17β-E2, CD45 and CD45RO isoform RNA expression were increased, an effect that was blocked with co-treatment of ER antagonists (Zhang et al., 2020). Less potent naturally circulating estrogens also appear to exert similar regulatory effects on immune cells. For example, estriol, at levels similar to the first trimester of pregnancy (2 ng/mL), increased levels of venous blood $CD4^+FoxP3^+$ T regulatory cells and decreased levels of $CD4^+RORC^+$ Th17 lymphocytes were seen in women of reproductive age (Shirshev et al., 2019).

Sex Differences in Immunity During Early Life and Puberty

Some subtle sex differences in childhood immunity have been reported. For example, splenocyte response to cell surface-receptor-independent mitogenic combination of phorbol ester and ionomycin was greater in female mice at 3 weeks old, but was greater for 4-6 week old male mice (Rosen et al., 1999). Furthermore, a study on healthy Asian children noted that male babies showed 8% more natural killer (NK) cells at birth than females, while female newborns showed higher levels of $CD3^+$ T cells (Lee et al., 1996). Between 1 and 6 years of age, girls had somewhat higher numbers of lymphocytes, B cells, and $CD3^+$, $CD4^+$, and $CD8^+$ T cells, while boys had higher NK, activated T cells, and $CD4^+$ T cell counts (Lee et al., 1996). In contrast, Lisse et al. found that West African boys show higher levels of $CD8^+$ cells and lower $CD4^+/CD8^+$ ratios than girls (Lisse et al., 1997). Despite the discrepancy between these studies, prior to the onset of puberty, it is generally thought that the immune systems of male and female organisms exhibit few robust sex differences in immune cell counts or function (Robinson et al., 2014; Sharma et al., 2019). Indeed, splenic expression of some innate immune response genes was greater in pre-pubescent male mice, though the differences were not statistically significant and expression of adaptive immune response genes was generally similar between the sexes (Lamason et al., 2006). There also appear to be no sex differences in the vaccine response during childhood (Vom Steeg et al., 2019). This variability in the literature warrants future study to clarify the extent to which these observations replicate across study populations and translate to impact immunity overall during childhood.

The pubertal transition to reproductive capacity and the associated dramatic increases in sex hormone levels marks a period of substantial change in the immune system, changes that may exert functionally significant effects with regard to

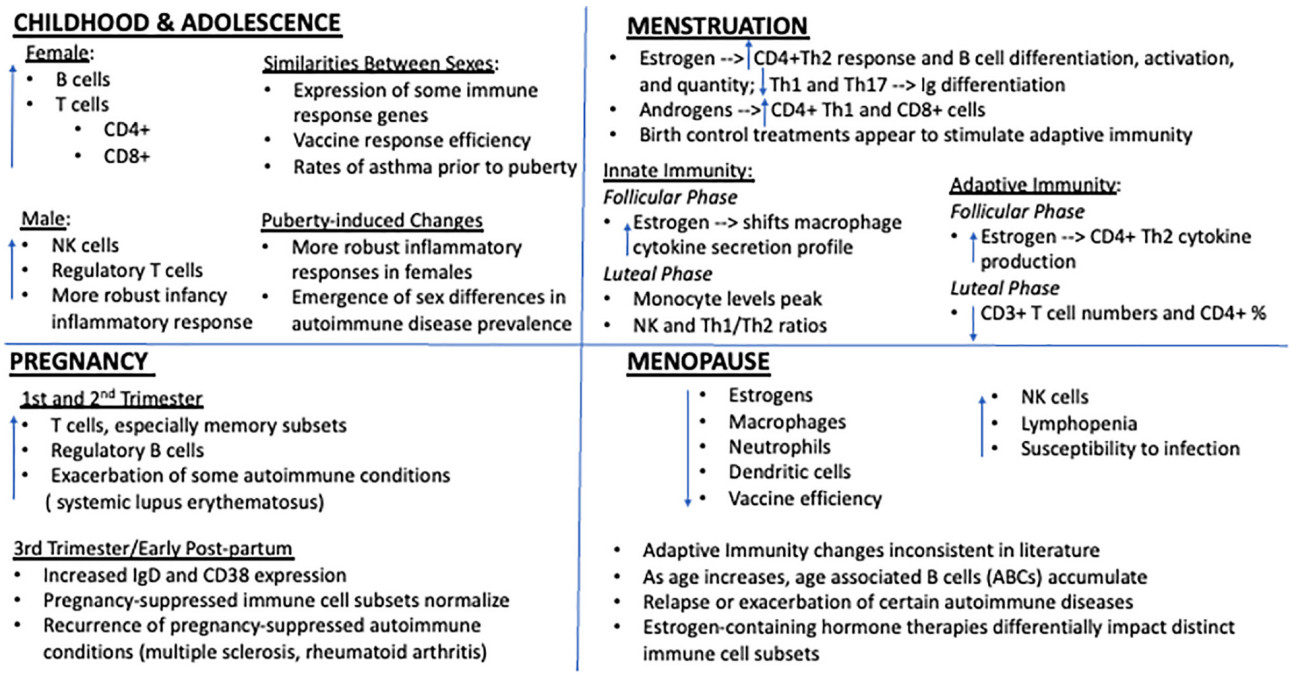

FIGURE 1 | Key immune system impacts of estrogen at distinct female reproductive milestones. Immune function is profoundly impacted by genetic sex and variations in estrogen. Though a few subtle differences have been reported during childhood, prior to puberty onset immune cell counts are generally similar between males and females and any differences appear to have little functional impact on overall immunity. However, beginning with adolescence and the onset of menstruation, marked sex differences in immune cell ratios and response profiles emerge. Generally, females display a more robust inflammatory response to immune challenge, rendering them potentially more resilient to the negative consequences of infection but also more susceptible to certain autoimmune conditions. High concentrations of estrogen, whether they be due to natural shifts in circulating levels across the cycle or via administration of estrogen containing exogenous treatments, appear to exert cell-type specific effects with regards to key immune players, generally potentiating adaptive immunity. Falling estrogen levels with the transition to reproductive and immunosenescence also imparts profound consequences for immunity and is associated with dramatic shifts in peripheral immune cell profiles, autoimmune disease manifestation, and susceptibility to immune challenge.

immune function in developing children. For instance, studies have noted increased numbers of circulating NK cells, $CD4^+$ T cells, and B cells among girls, but higher $CD8^+$ T cell numbers among adolescent boys as well as distinct response profiles of cultured peripheral blood mononuclear cells derived from male vs. female donors to phytohaemagglutinin stimulation (Lee et al., 1996; Uppal et al., 2003; Abdullah et al., 2012). There are numerous functional consequences of these puberty-induced sex differences in response to antigen challenge. Inflammatory responses to infection or toll-like receptor stimulation appear to be stronger in females than in males (Seillet et al., 2012; Robinson et al., 2014), with females showing increased gene expression of interferon-gamma, lymphotoxin beta granzyme A, IL-12 receptor beta2, and granulysin (Hewagama et al., 2009). Similar findings have been found preclinically where, in post-pubertal mice, following stimulation with ovalbumin and anti-CD3/CD28 antibodies, IL-4, IL-5, IL-13 were all significantly higher in female bronchial lymph node cells than in male cells (Okuyama et al., 2013). Additionally, IL-5 production from stimulated $CD4^+$ T cells was significantly increased in females compared to males. Viral challenge with the mimetic polyinosinic:polycytidylic acid induced greater sickness behavior in post-pubertal males than females (Sharma et al., 2019). However, changes in body temperature and central c-fos expression were more prevalent in female mice, and gonadectomy both worsened sickness behavior and altered temperature in both sexes. Efficiency of vaccination has also been tested in murine models with adult female mice having greater antibody response to the vaccination and an increased number of antigen-specific hepatic $CD8^+$ T cells compared to young mice (Vom Steeg et al., 2019). Another functional consequence relates to the prevalence of immune-associated diseases, especially asthma. Indeed, despite having similar numbers during childhood, adult females exhibit a 6.2% prevalence of asthma while males exhibit a 4.3% prevalence (Vink et al., 2010). Evidence supports that asthma responses and estrogen are largely correlated (Melgert et al., 2007) and that estrogen contributes to the innate macrophage polarization, thus leading to greater allergy response (Keselman et al., 2017).

Immune Variation Across the Ovulatory Cycle of Reproductively Capable Organisms

Innate immune cell number and function display a complex pattern throughout the menstrual cycle. For example, peripheral levels of NK cells along with their cytotoxic potential became heightened during the luteal phase, when estrogen levels begin to decline but progesterone levels tend to be high (Lee et al., 2010).

Monocyte numbers also appear to peak during the luteal phase while circulating neutrophil levels decline during menstruation (Pennell et al., 2012). Overall, estrogen seems to enhance, while progesterone and androgens tend to suppress proinflammatory innate immune responses (Roberts et al., 2001; Musabak et al., 2003; Arruvito et al., 2008; Shepherd et al., 2020).

Adaptive immunity also displays dynamic changes across the menstrual cycle. Estrogens generally have stimulatory effects on lymphocyte presence, concentration, and function (Lee et al., 2010; Oertelt-Prigione, 2012; Pennell et al., 2012; Rodriguez-Garcia et al., 2013; Moulton, 2018) though cell type and tissue-specific effects of estrogen stimulation have also been suggested (Pung et al., 1985; Chen et al., 2015). For instance, increased levels of estrogen are thought to stimulate overall $CD4^+$ Th2 cytokine production in females (Ackerman, 2006; Pennell et al., 2012; Shah, 2012). Peripheral regulatory T cell counts were shown to be higher during the follicular phase when estrogen levels are typically highest (Moulton, 2018). Wegienka et al. (2011) noted that blood levels of the less potent naturally circulating estrogen, estrone, were positively correlated with regulatory T cell counts in asthmatic women. Peripheral blood $CD3^+$ and $CD4^+$ T cell percentages decrease in the luteal phase, when estrogen levels are low relative to those of progesterone (Lee et al., 2010). Inhibitory effects of estrogens have also been noted within certain immune cell subtypes. Indeed, estrogen exposure inhibits Th1 cytokine proliferation and Th17 differentiation (Chen et al., 2015).

Though information regarding B cell changes across the menstrual cycle is more limited, converging evidence suggests that estrogen stimulates B cell differentiation and activation, increases B cell numbers, and enhances their function (Verthelyi, 2001; Oertelt-Prigione, 2012; Moulton, 2018). For example, B cell activation by 17β-E2 generally induces higher levels of Ig synthesis (Franklin and Kutteh, 1999; Pennell et al., 2012) specifically in B cells found in bone marrow and the spleen (Moulton, 2018). In mice treated with sustained slow-release 17β-E2-containing silastic implants (4-6 mg) resulting in levels comparable to those achieved during murine pregnancy, numbers of antibody-secreting plasma cell numbers increased dramatically, and secretion of various immunoglobulins and autoantibodies increased (Verthelyi and Ahmed, 1998). This estrogen-driven B cell hyperactivity may contribute to the development of autoimmune diseases (Pennell et al., 2012).

Sex hormone type also appears to influence female immunity in a cell subtype-specific way. Indeed, while it is widely accepted that estrogens usually correspond with an increased $CD4^+$ Th2 cell response, androgens promote $CD4^+$ Th1 and $CD8^+$ cell responses (Ackerman, 2006; Pennell et al., 2012; Guven Yorgun and Ozakbas, 2019). As for progesterone, increased levels during the luteal phase sometimes correspond to increased NK cell levels, unchanged Th1/Th2 ratios, decreased $CD3^+$ and $CD4^+$ T cell percentages, and increased serum levels of the anti-inflammatory IL-1 receptor antagonist (Lee et al., 2010; Vetrano et al., 2020). Despite this, in one study, serum $CD4^+/IL10^+$ regulatory T cells displayed heightened responses when progesterone levels were elevated in the late follicular and luteal phases (Weinberg et al., 2011). These findings reveal the significance of distinguishing between the different immune cell subtypes in how they react to steroid hormone stimulation in distinct target tissues and in response to various immunological challenges.

Pregnancy-Associated Impacts to the Immune System

Tight regulation of the maternal immune response are key contributors to pregnancy success; historically, immune responses during pregnancy were thought to be suppressed to allow for a semi-allogeneic fetus (Racicot et al., 2014). However, this previously held notion has been reevaluated as additional findings implicating sex hormone regulation of immune responses have emerged in recent years (Mor and Cardenas, 2010). Indeed, immune contributions to the development of the decidua and placenta and the maintenance of the maternal-fetal interface is required for a successful pregnancy (Hsu and Nanan, 2014). Nair et al. (2017), and there is growing appreciation that dynamic shifts in maternal sex hormone levels may, at least in part, contribute to observed shifts in gestational immunity (Robinson and Klein, 2012). Uterine immune cells, including NK cells, macrophages, T cells, dendritic cells, mast cells, and B cells, are necessary for the normal formation of placenta beds and appear to play a key role in converting high-resistance, low-flow vessels to low-resistance, high-flowing vessels in spiral arteries in the placental bed (Faas and De Vos, 2018). Maternal monocytes and macrophages obtain a unique phenotype throughout pregnancy that allows them to retain immunological tolerance and permit hormone–immune cell interactions, both of which are required for progression of the fetus inside the uterus (Mendoza-Cabrera et al., 2020).

It is thought that the increase in steroid hormone levels throughout pregnancy modulates inflammatory responses at the maternal fetal interface, and E2, estriol, and progesterone influence the transcriptional signaling of those responses (Robinson and Klein, 2012). During the first trimester, levels of placental-derived estrogen increase sharply and contribute significantly to the development of organs and other bodily systems in the fetus. T cell subsets are profoundly affected by these changes. Early in pregnancy, the increase of regulatory T cells supports the development of a semi-allogeneic fetus protected from maternal immune rejection by restraining inflammation during the shift from proinflammatory to anti-inflammatory immunity (Krop et al., 2020). $CD25^+/CD4^+$ T regulatory cell numbers reach a peak during the second trimester, and it is thought that these cells allow the maternal immune system to respond to the developing fetal organs within the uterus (Somerset et al., 2004; Lima et al., 2017). Further, appropriately titrated T cell populations early in pregnancy may contribute to fetal viability. Indeed, when Lissauer and colleagues (Lissauer et al., 2014) evaluated circulating T cell subsets across distinct pregnancy stages, they observed that about 60% of Th17 cells in the body during pregnancy were found during the first trimester of pregnancy, though no changes in Th1 or Th2 T cell subsets were noted across the gestational and postpartum period. Th1 and Th17 cells numbers were elevated among women with recurrent miscarriage, suggesting that these cell types may serve

as important targets to improve gestational success (Lissauer et al., 2014). Memory T cells are also increased during the first trimester and promote fetal-maternal tolerance (Kieffer et al., 2019). It is thought that insufficient numbers of memory $CD4^+$ T cells contribute to pregnancy complications such as preeclampsia, gestational diabetes, and premature labor (Lim et al., 2019). Following pregnancy, it can take three to four months for cells to return to normal function after delivery, and inhibition of helper T cells and NK cells appears to last for the first few months.

Like T cells, B cells also support the semi-allogeneic fetus while protecting the mother and fetus against infection (Muzzio et al., 2013). Regulatory B cell numbers similarly increase during the first trimester, limiting proinflammatory responses (Esteve-Solé et al., 2018). B cell activation factors, which facilitate the inflammatory response, are also increased, likely in support immune tolerance of the semi-allogeneic fetus (Wang L. et al., 2021). B cells also appear to impact immunity during later stages of pregnancy and following parturition. Lima and colleagues examined healthy pregnancies and determined the degree of activation of different B cell subsets, reporting increases in $CD38^+$ and IgD markers of B cell activation during the third trimester of pregnancy and postpartum period (Lima et al., 2016).

Immune Shifts During Female Reproductive Senescence and Aging

Aging plays a significant role in modulating the function of the immune system and is associated with deterioration of immunity seen in the elderly (Fulop et al., 2017). Immunosenescence cascades have been reviewed elsewhere (Xu et al., 2020) but in brief, key immunosenescence characteristics include inflammaging, lymphopenia, higher susceptibility to infection and poor vaccine response (Ghosh et al., 2014). Within the adaptive immune system, aging is associated with an increase in differentiated memory T cells, effector T cells, senescent $CD8^+CD28^-$ T cells, and age-associated innate-like B cells, but a decrease in most B cell subsets and the ratio of CD4:CD8 T cells, to name just a few examples (Weyand and Goronzy, 2016). Collectively, these and other senescence-related changes diminish the ability of the immune system to protect against certain infections and cancers and may accelerate the development of certain diseases, rendering older populations at-risk for a host of immunological challenges. It was historically presumed that this senescence-associated shift in immunity occurred at a similar rate and manner, regardless of sex (Aiello et al., 2019). However, rising life expectancies have revealed that men and women experience these consequences along different trajectories; emerging evidence suggests sex-specific and potentially profound consequences of immunosenescence (Gubbels Bupp et al., 2018; Márquez et al., 2020). Indeed, it is now appreciated that female innate immune systems appear to age at a faster rate, whereas the adaptive immune systems of men age at a faster rate (Ghosh et al., 2014).

Age-related shifts in reproductive function likely influence the function of the immune system during aging, and the sex-specific nature of this transition period may account for differences in male and female immunosenescence. Indeed, while men experience andropause, a gradual reduction in circulating testosterone over the course of several decades (Kevorkian, 2007), women experience a more accelerated transition. Menopause marks a period of reproductive senescence in a woman's life when the ovarian oocytes have become depleted, and sex hormones are no longer produced by the ovaries (Keppel and Wickens, 2004). As a result, the menstrual cycle becomes irregular and eventually terminates while levels of estrogens and progestins drastically decline. Natural menopause is a normal part of aging that typically occurs in the fourth and fifth decade of life and can take place over the course of only a few years. Still others undergo surgical menopause to remove the ovaries when there is an increased likelihood of cancer, infection or endometriosis ("Medical Causes of Menopause"), resulting in an accelerated reproductive senescence.

The distinct trajectory of female reproductive senescence has important impacts with regard to immune function during aging. In comparison to men of a similar age or to reproductively capable women, post-menopausal women are disproportionately affected by certain autoimmune disorders and have an increased susceptibility to infection with aging (Fairweather et al., 2008; Gubbels Bupp et al., 2018; Maglione et al., 2019). Estrogen deficiency has been implicated in many senescence-associated changes seen in the immune cells, such as the increase in proinflammatory markers IL-1, IL-6 and TNF-α (Gameiro et al., 2010), and low levels of estrogen are linked to higher levels of IL-17 produced by Th17 cells (Molnár et al., 2014). Following menopause, women undergo various changes in the levels of innate immune cells. Whereas the number of NK cells increases, their cytotoxic capacity is diminished (Albrecht et al., 1996; Ghosh et al., 2014; Toniolo et al., 2015). Further, the number of macrophages, neutrophils and dendritic cells decreases (Ghosh et al., 2014; Toniolo et al., 2015). Macrophages are vital, as they aid in the conversion of proinflammatory phenotypes to anti-inflammatory phenotypes, and estrogens help to prevent the effects of proinflammatory agents on the functions of macrophages by accelerating the resolution phase of inflammation in these cells (Toniolo et al., 2015; Villa et al., 2015). E2 also seems to decrease the rate of apoptosis in neutrophils as following menopause, neutrophils numbers have been shown to decrease as the rate of apoptosis increases (Chen et al., 2016).

Menopause is also associated with significant shifts in adaptive immunity. As reviewed in Gubbels Bupp et al. (2018), some studies report a decrease in total lymphocyte counts in postmenopausal women (Giglio et al., 1994; Kamada et al., 2000), while other studies have shown that numbers of some lymphocyte subsets are significantly higher in postmenopausal women (Chen et al., 2016; Abildgaard et al., 2020). Further, levels of functioning $CD4^+$ T and B cells decrease, while numbers of exhausted and senescent cells rise, whether the etiology of menopause is surgical or transitional (Giglio et al., 1994; Gameiro et al., 2010; Gubbels Bupp et al., 2018; Maglione et al., 2019; Abildgaard et al., 2020; Vrachnis et al., 2021). However, it has also been shown that thirty days after surgical menopause via total abdominal hysterectomy and bilateral salpingo-oopherectomy, patients displayed increased levels of $CD8^+$, but decreased levels

of B cells and a reduced $CD4^+/CD8^+$ T cell ratio (Kumru et al., 2004). Other conflicting literature has noted a decrease in naïve $CD8^+$ T cells, but an increase in memory or activated T cells in postmenopausal women compared to pre-menopausal women or women taking hormone replacement therapy (HRT) (Kamada et al., 2000; Engelmann et al., 2016; Vrachnis et al., 2021). In regards to the function of B cells, E2 enhances certain aspects of humoral immunity (Gameiro et al., 2010). Aged women tend to accumulate more innate-like age-associated B cells (ABCs) than young women and men of any age, and there is a relationship between ABCs, viral infections, autoimmunity and a proinflammatory state (Rubtsova et al., 2015; Gubbels Bupp et al., 2018). ABCs are known to originate from follicular B cells and show a bias in females, due to hormones and X chromosome-encoded genes, but the mechanisms that cause the production and accumulation of ABCs are still unknown and need to be further investigated (Rubtsova et al., 2015).

Effects of Exogenous Estrogen-Containing Treatments on Immune Function

Immune cells not only respond to endogenously secreted estrogens; estrogen-containing contraceptives, commonly used for pregnancy prevention, hormonal imbalances, and menstrual cycle regulation, also impact immunity. For example, compared to untreated women, women taking the oral contraceptive pill, Ortho Novum 777 (containing ethinyl estradiol and norethindrone), had higher Ig levels, implicating these hormones in promoting B cell activity (Franklin and Kutteh, 1999). In another small study evaluating respiratory performances of thirteen asthmatic women, blood regulatory T cell counts were higher among contraceptive treated women, and this was associated with less intense asthmatic symptoms (Wegienka et al., 2011; Vélez-Ortega et al., 2013). Estrogen-containing contraceptives administered vaginally also impact the local immune environment. Indeed, Hughes et al. noted that the NuvaRing® (0.12 mg etonogestrel/0.015 mg E2 per day) was associated with increased T cell- related proteins, granulysin and granzyme B in cervicovaginal fluid, indicating that, similar to during phases of heightened estrogen in the menstrual cycle, estrogen has a stimulatory effect on vaginal T cell response when locally administered. Yet in mice, when the synthetic estrogen, diethylstilbestrol, was administered subcutaneously for five consecutive days, T cell proliferation and IL-2 production in the spleen both declined (Pung et al., 1985), implicating species or estrogen subtype differences with regards to exogenous estrogen impacts to immunity.

Though menopausal HRT is commonly prescribed to attenuate the negative vasomotor and vaginal symptoms of menopause, it may also be a potential therapeutic option to modify menopause-related shifts in immune system function (Ghosh et al., 2014); the complexities associated with HRT impacts to the brain and immunity have been extensively reviewed elsewhere (Abdi et al., 2016). As an example, postmenopausal women taking estrogen and progestin-containing HRT have been reported to have higher numbers of lymphocytes and B cells specifically, but maintain low levels of $CD4^+$ T cells, and exhibit a decrease in $CD8^+$ cells resulting in an increase in the ratio of $CD4^+/CD8^+$ T cells; naïve and memory/activated T cell numbers generally remained consistent (Kamada et al., 2000; Yang et al., 2000; Porter et al., 2001; Kumru et al., 2004). These HRT-induced immune cell impacts may be effective in alleviating the symptoms associated with menopause or autoimmune disease, as well as the risk for developing certain disorders, especially when used within 10 years of experiencing symptoms if the woman is under 60 years old (Stopińska-Głuszak et al., 2006; Cagnacci and Venier, 2019). Taken together, these data indicate that exogenous estrogen treatment has significant immunological consequences, which may in turn impact women's susceptibility to systemic infection or autoimmune disease (see below); additional investigation in this research domain is clearly warranted.

SEX, ESTROGEN AND AUTOIMMUNITY – CONSEQUENCES OF ESTROGENIC IMPACTS TO THE FEMALE IMMUNE SYSTEM

Though the evidence described above reveals robust sex-specific differences in immune responses, may at times, provide some advantages to infection for female organisms, maladaptive consequences have also been indicated. Indeed, women shoulder a disproportionate burden of some autoimmune diseases and several reviews extensively explore the topic of sex differences in the prevalence of autoimmunity (Lateef and Petri, 2012; Pennell et al., 2012; Ortona et al., 2016; Moulton, 2018; Keestra et al., 2021). For instance, with a typical age of disease onset occurring during puberty, the prevalence of systemic lupus erythematosus (SLE), an autoimmune condition associated with widespread inflammation, in prepubertal girls is only double that of boys; by adulthood, the ratio of female to male patients has increased to 9:1 (Ngo et al., 2014; Moulton, 2018). Further, SLE-associated flare-ups during pregnancy are common (Petri, 2020). That estrogen stimulation promotes a Th2 immune phenotype may further contribute to the increased prevalence of Th2-mediated autoimmune diseases such as SLE (Ackerman, 2006). For instance, regulatory $CD4^+$ T cells from female SLE patients showed reduced FoxP3 expression when incubated with physiological levels of E2, suggesting that high E2 levels may place women at an increased risk due to the presence of fewer immune regulatory cells (Singh and Bischoff, 2021). Among SLE patients, HRT has been found to increase the amount of mild, but not severe, flares (Lateef and Petri, 2012).

A sex-specific burden of multiple sclerosis (MS), a chronic, progressive, demyelinating inflammatory autoimmune disease associated with a myriad of degenerative sensori/locomotor and cognitive deficits, has also been documented (Goldenberg, 2012). Indeed, a woman's risk for developing MS increases after the pubertal transition, an effect linked to increasing levels of estrogens given that MS symptomology appears to decrease in intensity during the luteal phase of the menstrual cycle, when

estrogen levels are low (Moulton, 2018; Keestra et al., 2021). Additional clarity regarding the contributions of sex hormones alone and in combination is warranted as perplexingly, some studies note greater MS symptomology and worsened cognitive function in the premenstrual phase when sex hormones are generally at their lowest levels (Guven Yorgun and Ozakbas, 2019; Keestra et al., 2021). As well, though relapse rates increase significantly by three months post-partum, pregnancy is typically associated with symptom remission (Confavreux et al., 1998). Short-term corticosteroid treatment to manage MS symptoms during late pregnancy is considered safe with regards to fetal outcomes such as risk of pre-term birth and low birth weight (Ramo-Tello et al., 2021). Whether this treatment impacts affective outcomes in the pregnant or post-partum mother is not clear and represents an important area of investigation, given that corticosteroid treatments are known to induce psychiatric symptoms such as mania, depression, psychosis, and cognitive changes (Brown and Chandler, 2001).

Rheumatoid arthritis (RA) is an autoimmune disease characterized by joint pain, painful swelling, fatigue and fever due to the immune system attacking its own healthy tissue (Bullock et al., 2018). RA is both more common and may be more severe in women than men (Walker, 2011; Pennell et al., 2012). Like MS patients, women with RA experience symptom remission during pregnancy but these effects are short-lived as women often experience disease aggravation following parturition (Ostensen et al., 1983). The typical age of RA onset in women is during the menopausal transition, and an early age at menopause is associated with an increased likelihood of RA (Goemaere et al., 1990; Desai and Brinton, 2019). This observation may be attributed to the loss of endogenous estrogen that women experience during menopause. Menopausal RA patients taking HRT do not appear to display increased flare-ups and may even experience improved disease symptomology (Holroyd and Edwards, 2009). Similar effects have been demonstrated among pre-menopausal women, where oral contraceptive use did not prevent emergence of new disease but did reduce transformation of cases from mild to severe, suggesting beneficial effects of exogenously-administered estrogen-containing therapies against disease progression.

CONVERGENCE OF SEX, ESTROGEN AND IMMUNITY IN STRESS AND DEPRESSION

The complexities of how biological sex or sex hormones and peripheral immunity converge to impact mood are beginning to be revealed. Sex-specific affective responses to peripheral inflammatory challenge generally suggest that female organisms may respond more robustly to immune activation (Bekhbat and Neigh, 2018). Indeed, inflammatory challenge with lipopolysaccharide (LPS; bacterial infection mimic) was associated with mood disruptions in women but not men (Moieni et al., 2015) and intranasal LPS administration induced depressive-like behavior and elevated hippocampal proinflammatory cytokine expression only in female rodents (Tonelli et al., 2008). However, this effect has not consistently been observed. For instance, following LPS challenge, while women displayed greater increases in proinflammatory IL-6 and TNF-α levels than men and men displayed higher levels of the typically anti-inflammatory cytokine IL-10, surprisingly affective consequences were similar among both sex groups (Engler et al., 2016). Similar observations were noted in preclinical studies where male and female rodents displayed similar depressive-like behavioral phenotypes despite robust sex-distinct effects on inflammatory and growth factor cascades in response to peripheral immune stimulation (Adzic et al., 2015; Brkic et al., 2017). Still, other reports suggest that males may be more susceptible to affective impacts of peripheral immune activation. Indeed, in male mice exposed to a LPS challenge, depressive-like behavioral changes along with altered brain proinflammatory cytokine mRNA levels were observed at 24 h, and hippocampal apoptosis was shown at 28 days later, effects not observed in female mice (Millett et al., 2019; Rossetti et al., 2019).

Sex differences in peripheral circulating cytokine levels among clinically depressed populations or in preclinical models have also been reported. For instance, higher levels of C-reactive protein were associated with an increased risk of depressive transformation, and increased psychopathology among depressed women was associated with elevated levels of C-reactive protein where no such association was noted in depressed men (Köhler-Forsberg et al., 2017; Kim et al., 2021; Zainal and Newman, 2021). Genetic predispositions related to the immune system also appear to induce sex-specific risk factors for development of a depressive phenotype as IL-18 haplotype in women, but not men, is associated with increased threat-induced central amygdala reactivity (Swartz et al., 2017). Other cytokines that are associated with depressive phenotypes in females, or the responsiveness of depressed patients to antidepressant treatment, include IL-1β, and IL-6 (Carboni et al., 2019; Kim et al., 2021; Zainal and Newman, 2021). However, some inconsistencies regarding sex-specific differences in peripheral inflammation among depressed populations have been reported. For example, while Piantella and colleagues (Piantella et al., 2021) agreed with other literature that IL-6 was associated with higher depressive symptoms in women exposed to workplace stress, they observed that higher C-reactive protein levels were associated with depression only in men. Further, in a study of more than 1,800 patient samples, C-reactive protein was associated with MDD state only in men (Ramsey et al., 2016). The experimental heterogeneity associated with the study population and sample size, the stressor nature and severity being evaluated, the approach to measure cytokine levels, the post-stress measurement timeframe, etc., among evaluations reported in the literature indicate that additional work is needed to discern the utility of sex-specific cytokine biomarkers for depression.

Taken together, it appears that immune activation cascades in response to psychosocial stress differ between males and females, though whether the consequences of these distinct trajectories reliably manifest in differential mood-related disruptions between the sexes is not altogether clear. Further clarification of the parameters in which sex-specific mood impacts may be

realized in the context of antigen-driven or sterile immune challenges is needed.

DISCUSSION: CHALLENGES IN EXPLORING NEURO-IMMUNO-ENDOCRINE INTERACTIONS IN THE CONTEXT OF MOOD

As summarized above, genetic sex, estrogen, and the immune system significantly contribute to mood and mood disorders both individually and as converging, interactive factors (**Figure 2**). As this exciting field further develops, consideration of a number of limitations and challenges to probing these complex interactions in the context of mental health is warranted.

Consideration of Relevant Biological Variables

First, though historical representation of both sexes in biomedical research has been lacking, there is increasing awareness among researchers regarding the need to consider sex as a biological variable and moreover consider how biological phenomena change as reproductive capacity shifts across the lifespan (Arnegard et al., 2020). Indeed, in 2015, the NIH (2015). announced requirements for the appropriate consideration of sex as a biological variable, incorporating this as a review criteria for all proposals submitted shortly thereafter. This policy change included requirements for the use of both sexes within study populations unless strong justification is provided as to why research questions being assessed could only be evaluated in one sex (e.g., exploration of ovarian function would preclude the use of only female organisms) as well as disaggregation of data analyses to observe sex-related trends and accurate reporting of data based on sex As part of NIH's larger initiative to improve experimental rigor and reproducibility (Price and Duman, 2020), due consideration of other relevant biological variables is now also strongly advised (Lauer, 2016).

At present, the majority of research addressing the convergence of immune cells and sex or sex hormones on mood outcomes does not regularly factor in cyclicity stage, parturition experience, nor circulating levels of steroid hormones. As well, even when females are included in experimental designs, the majority of work in this area is conducted in young adult subjects prior to initiation of age-related immunosenescence cascades, potentially limiting translatability of the findings to older cohorts. While due consideration of key biological variables is not without its methodological challenges, there exists numerous aging or sex-based research centers of excellence around the United States (e.g., Nathan Shock Centers of Excellence in the Basic Biology of Aging, Tulane Center for Excellence In Sex-Based Biology and Medicine, and several workshops (e.g., International Symposium on the Neurobiology and Neuroendocrinology of Aging) providing training in the conduct of aging and/or sex-based research have been developed in recent years. In addition to informal laboratory based training, several publications laying out strategies are readily available (Bale and Epperson, 2017; Joel and McCarthy, 2017; Clayton, 2018). Especially given the profound age-related shifts in immune function, there exist numerous opportunities for productive research collaborations between immunologists, neuroendocrinologists, and biostatisticians to thoroughly address the convergence of sex, sex hormones, age, immune function, and stress responses. Continued progress is still needed (Woitowich and Woodruff, 2019; Arnegard et al., 2020), and additional incentivization of research specifically aimed at systematically addressing sex differences and the influence of sex hormones within the scope of mental health research will likely benefit the field.

Complexities of Evaluating Mood and Modeling Human Mental Health Disorders Preclinically

Another significant challenge facing this area of study is that effectively modeling complex mood disorders such as MDD in a rodent is difficult (Krishnan and Nestler, 2008; Nestler and Hyman, 2010; Wang et al., 2017). Whereas the etiology of MDD can be varied in humans, 'depressive-like' states in rodents are typically experimentally induced via environmental, experiential, genetic, pharmacological, physical, social, or surgical manipulations. Many of the classic induction approaches, developed at the height of the monoamine hypothesis of depression, were aimed at revealing antidepressant efficacy novel drugs (Nestler and Hyman, 2010; Wang et al., 2017). Unfortunately, no single stress-induction approach fully recapitulates the heterogeneity of disease susceptibility and no one readout fully captures the behavioral and neurobiological pathology seen in human populations, though some newer paradigms have been developed that display better translational validity. For instance, the long-leveraged forced swim stressor results in near ubiquitous floating behavior, thought to be an indicator of behavioral despair/learned helplessness, while the chronic social defeat paradigm can effectively discriminate susceptible from resilient populations (Golden et al., 2011; Bogdanova et al., 2013).

As well, MDD is a psychiatric disorder associated with a variety of phenotypes, and many symptoms experienced by human patients (i.e., sadness, guilt, suicide ideation) cannot be directly evaluated in rodents. When MDD symptomology can be more effectively recapitulated preclinically (anhedonia, behavioral despair), the available murine tests of depressive-like behavior generally only probe one dimension of this heterogeneity. Evaluating depression phenotypes through the assessment of other impacted functions, such as cognitive domains, may provide additional insights (Hales et al., 2014; Price and Duman, 2020). Further, in contrast with the delayed response of antidepressants prescribed to patients in the clinic, acute treatment of mice with antidepressants is sufficient to alleviate depressive-like behavior in some of the commonly employed preclinical tests, though again some paradigms show response timing profiles similar to those observed in clinical populations (Golden et al., 2011; Willner, 2017).

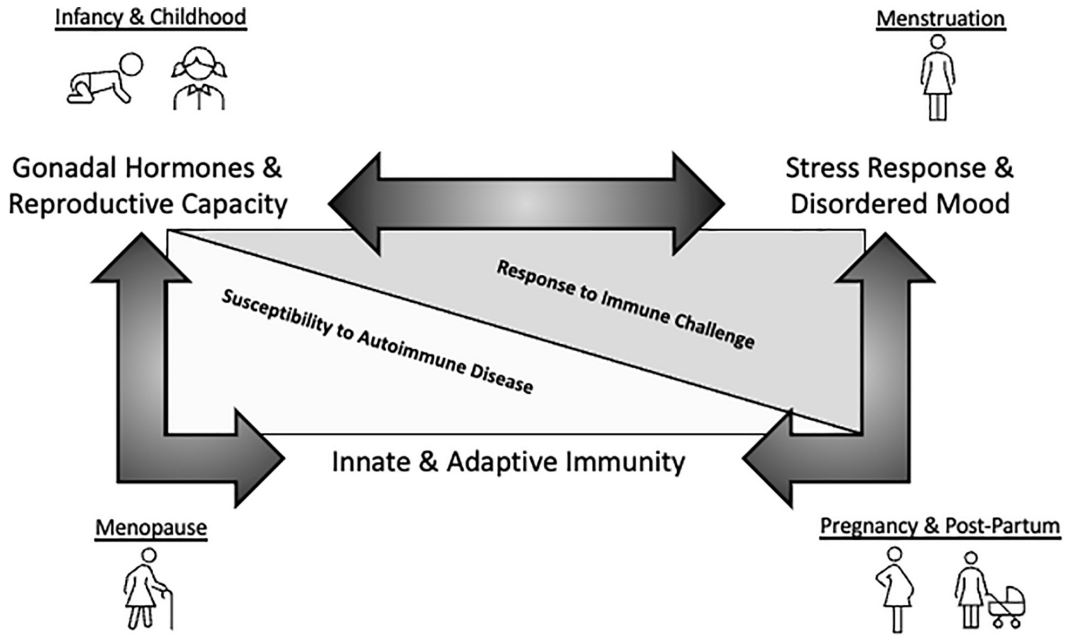

FIGURE 2 | Schematic representation of mood-immune convergence across the female reproductive lifespan. A substantial amount of research has been dedicated to exploring how endocrine and immune factors impact mood separately. For instance, neuroprotective effects of estrogen in regards to MDD are well-established. Further, inflammatory insult and immune dysfunction are emerging as key contributors to disordered mood. Finally, genetic sex and estrogen clearly modulate immune system components, having important functional consequences for immunity across the reproductive lifespan. However, insight regarding how these two systems converge to impact mental health, especially during aging, is currently limited. This knowledge gap may be driven by experimental challenges associated with exploring these complicated interactions including, but not limited to, heterogeneity associated with the study population and sample size, the species used, the stressor nature and severity being evaluated, the approach to measure cytokine levels, the post-stress measurement timeframe, to name a few examples. Whether estrogenic influences on inflammatory activation cascades in the context of 'sterile' psychosocial stress-induced immune challenges result in sex-specific susceptibility to MDD during key reproductive milestones remains to be further interrogated and represents an exciting area of study.

Finally, methods used to induce stress phenotypes in rodents may confound readouts. For instance, a common behavioral readout of the chronic variable stress paradigm is sucrose preference, a measure of anhedonia, the results of which may be profoundly impacted by metabolic changes associated with brief food restriction, a commonly leveraged component of that stress-induction approach (Willner, 2017). As such, no single preclinical stressor paradigm or test for 'depression' fully recapitulates the complexity of the MDD phenotype nor the response profile to typically prescribed treatments given to alleviate symptomology.

Selection of the method to induce stress as well as the approach to determine behavioral, physiological, and neurobiological responses require careful consideration of the research question being posed. In alignment with recent guidance from the NIMH (2019) and in pursuit of current Research Domain Criteria (RDoC) recommendations (Maes, 2011), it is advisable to leverage tests where the underlying neurobiological circuitry is well understood rather than on the basis of "presumed congruence to human symptoms of mental illness". Homological validity, that is capturing behavioral readouts that are species-relevant, should also be prioritized. The use of a behavioral battery of readouts within affective domains rather than a single assessment is also highly recommended to accurately capture the breadth and depth of a phenomenon, though test order should be an important consideration in their deployment (Powell et al., 2012). Composite behavioral battery z-scores should also be leveraged to capture overall impacts of stress on an organism as there is often substantial individual performance variability on unique readouts, especially among controls (Johnson et al., 2021). Behavioral readouts are best coupled with physiological readouts of stress, such as circulating corticosterone levels, metabolic alterations (such as attenuated weight gain), reduced self-care and health metrics, and shifts in circadian activity. There are also logistical complexities in preclinically modeling depression specifically in female organisms, including whether to consider stage of estrous cycle and reproductive capacity. Rigorous research portends the quantification of vaginal lavage to determine cyclicity and inclusion of Cycle Stage as an additional factor in statistical assessment of stress responses. Importantly, Johnson et al. (2021) did not identify a predicitive relationship between cycle stage and the stress response of their experimental and control animals. Further, though 'non-brain' measures showed more female-associated variability, a recent metanalysis of 311 articles did not report large-scale sex differences in neuroscience outcome measures and failed to identify increased variability in female rodents due to estrous cycle (Becker et al., 2016), suggesting that the impact of cycle stage on stress/neuroscience readouts may be relatively small.

It is also important to consider that some stress paradigms are extremely difficult to apply to females and may need significant modification to be applied to appropriately. For example, chromogenic activation of the ventromedial hypothalamus was required to induce male aggressors to attack female test mice (Takahashi et al., 2017). When addressing age interactions in response to stress within females, careful consideration of species differences in the trajectory of reproductive senescence as well as the biological consequences of surgical hormone depletion is also warrented (Engler-Chiurazzi et al., 2017). Of note, there are potentially independent cognitive contributions of ovaries versus uterus (Koebele et al., 2019) and consideration of the entire reproducitve system is necessary to comprehensively discern immune-sex hormone interactions with mood. Finally, investigators should not limit themselves to studying only populations that display maladaptive stress response profiles but should also consider exploration of subjects that display stress resiliency; important understanding in this domain is actively being advanced (Russo et al., 2012; Faye et al., 2018).

Sick as a Mouse: Can Rodent Models Effectively Recapitulate Human Immunity?

From an immunological perspective, consideration of the limitations of the experimental model leveraged to study the convergence of endocrine-immune factors within mental health is of paramount importance. First, species differences among humans vs. rodents in the development, total numbers, and functional ability of a variety of immune cell subsets have been long established and comprehensively discussed (Mestas and Hughes, 2004). For example, notable differences in innate immune responses including neutrophil defensin expression, toll-like receptor distribution, and macrophage function as it relates to nitric oxide, and natural killer cell inhibitor receptors for major histocompatibility complex I molecules between humans and rodents have been observed (Mestas and Hughes, 2004). Peripheral leukocyte profiles also vary by species such that up to 70% of immune cells in human blood are granulocytes (such as neutrophils) while lymphocytes make up approximately 30% of cells; monocytes are up to 10%, and other cell populations are more rare (Mestas and Hughes, 2004; Olin et al., 2018). In contrast, rodents display some sex differences in total blood leukocyte counts though importantly in both sexes lymphocytes, at between ∼75-90% for males and females, respectively, were the dominant immune cell type in circulation while neutrophil counts ranged between 24 and 8% (Doeing et al., 2003). Species differences in adaptive immune responses have also been reported, including variations in Fc receptor and Ig isotype expression, the regulation of T and B cell development, and the functional response of lymphocytes to antigen challenge (Mestas and Hughes, 2004). The consequences of these variations may be significant when attempting to address consequences of immunogens that exhibit host-specific patterns of infection, such as cytomegalovirus (Mestas and Hughes, 2004; Masopust et al., 2017), leading some researchers to suggest that rodents poorly recapitulate human injury or disease-associated inflammatory cascades and to advocate caution in the utilization of rodent models for immune-focused research questions (Seok et al., 2013).

As well, research mice are raised in specific pathogen free vivarium facilities that abide guidelines for cleanliness from regulatory organizations such as the United States Department of Agriculture and Association for the Assessment and Accreditation of Laboratory Animal Care accrediting bodies. While such practices support the health and welfare of laboratory rodents and promote reproducibility of data generated in a variety of fields, it is now recognized that pathogen free mice have immature immune systems that are functionally distinct from laboratory mice deliberately exposed to pathogens, from wild caught or pet-store reared mice, and from the human populations they are meant to model (Abolins et al., 2017; Masopust et al., 2017; Tao and Reese, 2017). For instance, adult humans have differentiated memory $CD8^+$ T cell subsets that are not observed in laboratory mice raised in typical pathogen-free conditions; co-housing mice with more antigen experienced pet-store mice can "humanize" their immune profiles, potentially improving their translational validity (Beura et al., 2016). Importantly, antigen exposure history shapes the function of the immune system (Beura et al., 2016; Tao and Reese, 2017), an important consideration given that emerging evidence implicates a higher infection burden with several negative neurological and cognitive consequences across the aging trajectory. Whether these factors manifest in functionally significant impacts for immunity as it relates to mental health is not yet clear and will be an important area of future study as the field evolves. Increased interest among both scientists and funding organizations in the rethinking of the research pipeline, the utilization of "dirty" mice, and the deploying of novel sequencing methods that capture the complexity of immune responses to explore key research questions may reveal more translational insights in the coming years (Shultz, 2016; Tao and Reese, 2017; Wagar et al., 2018).

Challenges Investigating Mood and Immunity in Human Populations

Many factors contribute to challenges in successful translation of preclinical findings to human populations. Here we will highlight variability in human immune profiles (Brodin and Davis, 2017) as well as mood disorder manifestations (Altemus et al., 2014). Immune profiles in middle aged adults evaluated longitudinally over the course of one year display some intra-individual variability that varies in magnitude from subject to subject and may be predictive of overall health (Lakshmikanth et al., 2020). Immune variability is also prevalent across individuals as immune profiles of the very young (Olin et al., 2018) and the very old (Kaczorowski et al., 2017) exhibit more heterogenous composition than do those of adults. As immune composition of monozygotic twins become increasingly distinct with time, the shaping of individual immune profiles is likely due to a combination of heritable and environmental influences (Brodin et al., 2015). Further, the numerous and sometimes vague or opposing diagnostic criteria used to identify clinically depressed

patients leads to a potentially highly variable subject pool that likely reflects distinct MDD sub-phenotypes (Zimmerman et al., 2015; LeGates et al., 2019). To account for this variability when evaluating variables of interest, a straightforward statistical solution is to increase sample size (Keppel and Wickens, 2004). However, many of the seminal papers exploring immune profile variations among depressed and mentally healthy populations had stressed/depressed participant numbers of less than 50 (Darko et al., 1988; Maes et al., 1990, 1992a; Petitto et al., 1993; McGregor et al., 2016; Ahmetspahic et al., 2018). This potential under-sampling not only presents challenges to replicability of the significant differences of immune readouts revealed in each study, but could also indicate a lack of statistical power to detect more subtle differences (Keppel and Wickens, 2004). However, many studies do not report observed power nor effect sizes, limiting the ability to make such determinations. These collective factors may contribute to potentially large intra-individual differences that make evaluation of the convergence of mood and immune function a significant logistical challenge. Robust assessment of immune-mood-sex interactions with statistically powerful meta-analysis approaches will become more feasible as additional investigations are conducted.

CONCLUSION

In summary, collective evidence addressing the unique affective contributions of genetic sex, sex hormones, reproductive capacity, and immunity has already expanded the prevailing 'monoamine theory of depression' and yielded improved understanding of the mechanisms driving disordered mood. Given the complex interactions that take place across the female lifespan between these systems, due consideration of how these factors acting in concert may converge to modulate mood is necessary. This will be made possible by adherence to new policies in the consideration of key biological variables, the inclusion of diverse subject populations and the reporting of findings based on population factors such as sex, reproductive experience, and age. The goal of this expanded appreciation for neuro-endo-immune factors in modulating mood is an increased appreciation for the mechanisms driving the manifestation of MDD and other mood disorders and revelation of novel, potentially sex or age-specific therapeutic interventions; we look forward to this outcome.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was supported by funds awarded to EE-C including NIH K01 MH117343, NIH P20 GM109036, the Tulane Brain Institute Research Fund Award and start-up funds provided by Tulane University.

REFERENCES

Abdi, F., Mobedi, H., Mosaffa, N., Dolatian, M., and Ramezani Tehrani, F. (2016). Effects of hormone replacement therapy on immunological factors in the postmenopausal period. *Climacteric* 19, 234–239. doi: 10.3109/13697137.2016.1164136

Abdullah, M., Chai, P. S., Chong, M. Y., Tohit, E. R., Ramasamy, R., Pei, C. P., et al. (2012). Gender effect on in vitro lymphocyte subset levels of healthy individuals. *Cell Immunol.* 272, 214–219. doi: 10.1016/j.cellimm.2011.10.009

Abildgaard, J., Tingstedt, J., Zhao, Y., Hartling, H. J., Pedersen, A. T., Lindegaard, B., et al. (2020). Increased systemic inflammation and altered distribution of T-cell subsets in postmenopausal women. *PLoS One* 15:e0235174. doi: 10.1371/journal.pone.0235174

Abolins, S., King, E. C., Lazarou, L., Weldon, L., Hughes, L., Drescher, P., et al. (2017). The comparative immunology of wild and laboratory mice, Mus musculus domesticus. *Nat. Commun.* 8:14811. doi: 10.1038/ncomms14811

Ackerman, L. S. (2006). Sex hormones and the genesis of autoimmunity. *Arch. Dermatol.* 142, 371–376. doi: 10.1001/archderm.142.3.371

Adzic, M., Brkic, Z., Mitic, M., Francija, E., Jovicic, M. J., Radulovic, J., et al. (2018). Therapeutic strategies for treatment of inflammation-related depression. *Curr. Neuropharmacol.* 16, 176–209. doi: 10.2174/1570159x15666170828163048

Adzic, M., Djordjevic, J., Mitic, M., Brkic, Z., Lukic, I., and Radojcic, M. (2015). The contribution of hypothalamic neuroendocrine, neuroplastic and neuroinflammatory processes to lipopolysaccharide-induced depressive-like behaviour in female and male rats: involvement of glucocorticoid receptor and C/EBP-β. *Behav. Brain Res.* 291, 130–139. doi: 10.1016/j.bbr.2015.05.029

Ahmetspahic, D., Schwarte, K., Ambrée, O., Bürger, C., Falcone, V., Seiler, K., et al. (2018). Altered B cell homeostasis in patients with major depressive disorder and normalization of CD5 surface expression on regulatory B cells in treatment responders. *J. Neuroimmune Pharmacol.* 13, 90–99. doi: 10.1007/s11481-017-9763-4

Aiello, A., Farzaneh, F., Candore, G., Caruso, C., Davinelli, S., Gambino, C. M., et al. (2019). Immunosenescence and its hallmarks: how to oppose aging strategically? A review of potential options for therapeutic intervention. *Front. Immunol.* 10:2247. doi: 10.3389/fimmu.2019.02247

Akil, H., Gordon, J., Hen, R., Javitch, J., Mayberg, H., McEwen, B., et al. (2018). Treatment resistant depression: a multi-scale, systems biology approach. *Neurosci. Biobehav. Rev.* 84, 272–288. doi: 10.1016/j.neubiorev.2017.08.019

Albrecht, A. E., Hartmann, B. W., Scholten, C., Huber, J. C., Kalinowska, W., and Zielinski, C. C. (1996). Effect of estrogen replacement therapy on natural killer cell activity in postmenopausal women. *Maturitas* 25, 217–222. doi: 10.1016/s0378-5122(96)01063-8

Al-Harbi, K. S. (2012). Treatment-resistant depression: therapeutic trends, challenges, and future directions. *Patient Prefer Adherence* 6, 369–388. doi: 10.2147/ppa.S29716

Altemus, M., Sarvaiya, N., and Neill Epperson, C. (2014). Sex differences in anxiety and depression clinical perspectives. *Front. Neuroendocrinol.* 35:320–330. doi: 10.1016/j.yfrne.2014.05.004

Arnegard, M. E., Whitten, L. A., Hunter, C., and Clayton, J. A. (2020). Sex as a biological variable: a 5-year progress report and call to action. *J. Womens Health (Larchmt)* 29, 858–864. doi: 10.1089/jwh.2019.8247

Arruvito, L., Giulianelli, S., Flores, A. C., Paladino, N., Barboza, M., Lanari, C., et al. (2008). NK cells expressing a progesterone receptor are susceptible to progesterone-induced apoptosis. *J. Immunol.* 180, 5746–5753. doi: 10.4049/jimmunol.180.8.5746

Arteaga-Henríquez, G., Simon, M. S., Burger, B., Weidinger, E., Wijkhuijs, A., Arolt, V., et al. (2019). Low-grade inflammation as a predictor of antidepressant and anti-inflammatory therapy response in mdd patients: a systematic review of the literature in combination with an analysis of experimental data collected in the EU-MOODINFLAME Consortium. *Front. Psychiatry* 10:458. doi: 10.3389/fpsyt.2019.00458

Baker, F. C., and Driver, H. S. (2007). Circadian rhythms, sleep, and the menstrual cycle. *Sleep Med.* 8, 613–622. doi: 10.1016/j.sleep.2006.09.011

Bale, T. L., and Epperson, C. N. (2017). Sex as a biological variable: who, what, when, why, and how. *Neuropsychopharmacology* 42, 386–396. doi: 10.1038/npp.2016.215

Becker, J. B., Prendergast, B. J., and Liang, J. W. (2016). Female rats are not more variable than male rats: a meta-analysis of neuroscience studies. *Biol. Sex Differ.* 7, 34. doi: 10.1186/s13293-016-0087-5

Bekhbat, M., and Neigh, G. N. (2018). Sex differences in the neuro-immune consequences of stress: focus on depression and anxiety. *Brain Behav. Immun.* 67, 1–12. doi: 10.1016/j.bbi.2017.02.006

Bekku, N., Yoshimura, H., and Araki, H. (2006). Factors producing a menopausal depressive-like state in mice following ovariectomy. *Psychopharmacology (Berl)* 187, 170–180. doi: 10.1007/s00213-006-0395-2

Bernardi, M., Vergoni, A. V., Sandrini, M., Tagliavini, S., and Bertolini, A. (1989). Influence of ovariectomy, estradiol and progesterone on the behavior of mice in an experimental model of depression. *Physiol. Behav.* 45, 1067–1068. doi: 10.1016/0031-9384(89)90238-2

Beura, L. K., Hamilton, S. E., Bi, K., Schenkel, J. M., Odumade, O. A., Casey, K. A., et al. (2016). Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature* 532, 512–516. doi: 10.1038/nature17655

Beurel, E., Harrington, L. E., and Jope, R. S. (2013). Inflammatory T helper 17 cells promote depression-like behavior in mice. *Biol .Psychiatry* 73, 622–630. doi: 10.1016/j.biopsych.2012.09.021

Bogdanova, O. V., Kanekar, S., D'Anci, K. E., and Renshaw, P. F. (2013). Factors influencing behavior in the forced swim test. *Physiol. Behav.* 118, 227–239. doi: 10.1016/j.physbeh.2013.05.012

Brachman, R. A., Lehmann, M. L., Maric, D., and Herkenham, M. (2015). Lymphocytes from chronically stressed mice confer antidepressant-like effects to naive mice. *J. Neurosci.* 35, 1530–1538. doi: 10.1523/jneurosci.2278-14.2015

Brinton, R. D. (2009). Estrogen-induced plasticity from cells to circuits: predictions for cognitive function. *Trends Pharmacol. Sci.* 30, 212–222. doi: 10.1016/j.tips.2008.12.006

Brkic, Z., Francija, E., Petrovic, Z., Franic, D., Lukic, I., Mitic, M., et al. (2017). Distinct modifications of hippocampal glucocorticoid receptor phosphorylation and FKBPs by lipopolysaccharide in depressive female and male rats. *J. Psychopharmacol.* 31, 1234–1249. doi: 10.1177/0269881117725914

Brodin, P., and Davis, M. M. (2017). Human immune system variation. *Nat. Rev. Immunol* 17, 21–29. doi: 10.1038/nri.2016.125

Brodin, P., Jojic, V., Gao, T., Bhattacharya, S., Angel, C. J., Furman, D., et al. (2015). Variation in the human immune system is largely driven by non-heritable influences. *Cell* 160, 37–47. doi: 10.1016/j.cell.2014.12.020

Brody, D. J., Pratt, L. A., and Hughes, J. P. (2018). Prevalence of depression among adults aged 20 and Over: United States, 2013-2016. *NCHS Data Brief.* 303, 1–8.

Brown, E. S., and Chandler, P. A. (2001). Mood and cognitive changes during systemic corticosteroid therapy. *Prim. Care Companion J. Clin. Psychiatry* 3, 17–21. doi: 10.4088/pcc.v03n0104

Bullock, J., Rizvi, S. A. A., Saleh, A. M., Ahmed, S. S., Do, D. P., Ansari, R. A., et al. (2018). Rheumatoid arthritis: a brief overview of the treatment. *Med. Princ. Pract.* 27, 501–507. doi: 10.1159/000493390

Cagnacci, A., and Venier, M. (2019). The controversial history of hormone replacement therapy. *Medicina (Kaunas)* 55, 602. doi: 10.3390/medicina55090602

Carboni, L., McCarthy, D. J., Delafont, B., Filosi, M., Ivanchenko, E., Ratti, E., et al. (2019). Biomarkers for response in major depression: comparing paroxetine and venlafaxine from two randomised placebo-controlled clinical studies. *Transl. Psychiatry* 9, 182. doi: 10.1038/s41398-019-0521-7

Chaplin, D. D. (2010). Overview of the immune response. *J. Allergy Clin. Immunol.* 125(2 Suppl. 2), S3–S23. doi: 10.1016/j.jaci.2009.12.980

Chen, R. Y., Fan, Y. M., Zhang, Q., Liu, S., Li, Q., Ke, G. L., et al. (2015). Estradiol inhibits Th17 cell differentiation through inhibition of RORγT transcription by recruiting the ERα/REA complex to estrogen response elements of the RORγT promoter. *J. Immunol.* 194, 4019–4028. doi: 10.4049/jimmunol.1400806

Chen, Y., Zhang, Y., Zhao, G., Chen, C., Yang, P., Ye, S., et al. (2016). Difference in leukocyte composition between women before and after menopausal age, and distinct sexual dimorphism. *PLoS One* 11:e0162953. doi: 10.1371/journal.pone.0162953

Clark, S. M., Soroka, J. A., Song, C., Li, X., and Tonelli, L. H. (2016). CD4(+) T cells confer anxiolytic and antidepressant-like effects, but enhance fear memory processes in Rag2(-/-) mice. *Stress* 19, 303–311. doi: 10.1080/10253890.2016.1191466

Clayton, J. A. (2018). Applying the new SABV (sex as a biological variable) policy to research and clinical care. *Physiol. Behav.* 187, 2–5. doi: 10.1016/j.physbeh.2017.08.012

Cohen, H., Ziv, Y., Cardon, M., Kaplan, Z., Matar, M. A., Gidron, Y., et al. (2006). Maladaptation to mental stress mitigated by the adaptive immune system via depletion of naturally occurring regulatory CD4+CD25+ cells. *J. Neurobiol.* 66, 552–563. doi: 10.1002/neu.20249

Confavreux, C., Hutchinson, M., Hours, M. M., Cortinovis-Tourniaire, P., and Moreau, T. (1998). Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in Multiple Sclerosis Group. *N. Engl. J. Med.* 339, 285–291. doi: 10.1056/NEJM199807303390501

Dantzer, R. (2018). Neuroimmune Interactions: from the Brain to the Immune System and Vice Versa. *Physiol. Rev.* 98, 477–504. doi: 10.1152/physrev.00039.2016

Darko, D. F., Lucas, A. H., Gillin, J. C., Risch, S. C., Golshan, S., Hamburger, R. N., et al. (1988). Cellular immunity and the hypothalamic-pituitary axis in major affective disorder: a preliminary study. *Psychiatry Res.* 25, 1–9. doi: 10.1016/0165-1781(88)90152-7

de Chaves, G., Moretti, M., Castro, A. A., Dagostin, W., da Silva, G. G., Boeck, C. R., et al. (2009). Effects of long-term ovariectomy on anxiety and behavioral despair in rats. *Physiol. Behav.* 97, 420–425. doi: 10.1016/j.physbeh.2009.03.016

de Wit, A. E., Booij, S. H., Giltay, E. J., Joffe, H., Schoevers, R. A., and Oldehinkel, A. J. (2020). Association of use of oral contraceptives with depressive symptoms among adolescents and young women. *JAMA Psychiatry* 77, 52–59. doi: 10.1001/jamapsychiatry.2019.2838

Desai, M. K., and Brinton, R. D. (2019). Autoimmune disease in women: endocrine transition and risk across the lifespan. *Front. Endocrinol. (Lausanne)* 10:265. doi: 10.3389/fendo.2019.00265

Dey, A., and Hankey Giblin, P. A. (2018). Insights into macrophage heterogeneity and cytokine-induced neuroinflammation in major depressive disorder. *Pharmaceuticals (Basel)* 11:64. doi: 10.3390/ph11030064

Doeing, D. C., Borowicz, J. L., and Crockett, E. T. (2003). Gender dimorphism in differential peripheral blood leukocyte counts in mice using cardiac, tail, foot, and saphenous vein puncture methods. *BMC Clin Pathol* 3:3. doi: 10.1186/1472-6890-3-3

Domínguez-Gerpe, L., and Rey-Méndez, M. (2001). Alterations induced by chronic stress in lymphocyte subsets of blood and primary and secondary immune organs of mice. *BMC Immunol* 2:7. doi: 10.1186/1471-2172-2-7

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K., et al. (2010). A meta-analysis of cytokines in major depression. *Biol. Psychiatry* 67, 446–457. doi: 10.1016/j.biopsych.2009.09.033

D'Souza, D., and Sadananda, M. (2017). Estrous cycle phase-dependent changes in anxiety- and depression-like profiles in the late adolescent wistar-kyoto rat. *Ann. Neurosci.* 24, 136–145. doi: 10.1159/000477151

Duke, J. M., Sibbritt, D. W., and Young, A. F. (2007). Is there an association between the use of oral contraception and depressive symptoms in young Australian women? *Contraception* 75, 27–31. doi: 10.1016/j.contraception.2006.08.002

Eid, R. S., Gobinath, A. R., and Galea, L. A. M. (2019). Sex differences in depression: insights from clinical and preclinical studies. *Prog. Neurobiol.* 176, 86–102. doi: 10.1016/j.pneurobio.2019.01.006

Engelmann, F., Rivera, A., Park, B., Messerle-Forbes, M., Jensen, J. T., and Messaoudi, I. (2016). Impact of estrogen therapy on lymphocyte homeostasis and the response to seasonal influenza vaccine in post-menopausal women. *PLoS One* 11:e0149045. doi: 10.1371/journal.pone.0149045

Engler, H., Benson, S., Wegner, A., Spreitzer, I., Schedlowski, M., and Elsenbruch, S. (2016). Men and women differ in inflammatory and neuroendocrine responses to endotoxin but not in the severity of sickness symptoms. *Brain Behav. Immun.* 52, 18–26. doi: 10.1016/j.bbi.2015.08.013

Engler-Chiurazzi, E. B., Brown, C. M., Povroznik, J. M., and Simpkins, J. W. (2017). Estrogens as neuroprotectants: estrogenic actions in the context of cognitive aging and brain injury. *Prog. Neurobiol.* 157, 188–211. doi: 10.1016/j.pneurobio.2015.12.008

Esteve-Solé, A., Luo, Y., Vlagea, A., Deyà-Martínez, Á, Yagüe, J., Plaza-Martín, A. M., et al. (2018). B regulatory cells: players in pregnancy and early life. *Int. J. Mol. Sci.* 19:2099. doi: 10.3390/ijms19072099

Faas, M. M., and De Vos, P. (2018). Innate immune cells in the placental bed in healthy pregnancy and preeclampsia. *Placenta* 69, 125–133. doi: 10.1016/j.placenta.2018.04.012

Fairweather, D., Frisancho-Kiss, S., and Rose, N. R. (2008). Sex differences in autoimmune disease from a pathological perspective. *Am. J. Pathol.* 173, 600–609. doi: 10.2353/ajpath.2008.071008

Faravelli, C., Alessandra Scarpato, M., Castellini, G., and Lo Sauro, C. (2013). Gender differences in depression and anxiety: the role of age. *Psychiatry Res.* 210, 1301–1303. doi: 10.1016/j.psychres.2013.09.027

Fauchais, A. L., Lalloué, F., Lise, M. C., Boumediene, A., Preud'homme, J. L., Vidal, E., et al. (2008). Role of endogenous brain-derived neurotrophic factor and sortilin in B cell survival. *J. Immunol.* 181, 3027–3038. doi: 10.4049/jimmunol.181.5.3027

Faye, C., McGowan, J. C., Denny, C. A., and David, D. J. (2018). Neurobiological mechanisms of stress resilience and implications for the aged population. *Curr. Neuropharmacol.* 16, 234–270. doi: 10.2174/1570159x15666170818095105

FDA (2019). *FDA Approves New Nasal Spray Medication for Treatment-Resistant Depression*. Silver Spring, MD: FDA.

Ferrari, A. J., Charlson, F. J., Norman, R. E., Flaxman, A. D., Patten, S. B., Vos, T., et al. (2013a). The epidemiological modelling of major depressive disorder: application for the Global Burden of Disease Study 2010. *PLoS One* 8:e69637. doi: 10.1371/journal.pone.0069637

Ferrari, A. J., Charlson, F. J., Norman, R. E., Patten, S. B., Freedman, G., Murray, C. J., et al. (2013b). Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. *PLoS Med.* 10:e1001547. doi: 10.1371/journal.pmed.1001547

Filiano, A. J., Gadani, S. P., and Kipnis, J. (2017). How and why do T cells and their derived cytokines affect the injured and healthy brain? *Nat. Rev. Neurosci.* 18, 375–384. doi: 10.1038/nrn.2017.39

Fletcher, H. (2010). Han JY, Choi JS, Chun JM, Park HD, Lee SY, Kim CH, et al. 2009. Pregnancy outcome of women transfused during pregnancy with blood products inadvertently obtained from donors treated with acitretin. Journal of Obstetric and Gynaecology. 29:694-697. *J. Obstet. Gynaecol.* 30, 333; author reply 334. doi: 10.3109/01443610903506198

Franklin, R. D., and Kutteh, W. H. (1999). Characterization of immunoglobulins and cytokines in human cervical mucus: influence of exogenous and endogenous hormones. *J. Reprod. Immunol.* 42, 93–106. doi: 10.1016/s0165-0378(98)00086-2

Frick, L. R., Arcos, M. L., Rapanelli, M., Zappia, M. P., Brocco, M., Mongini, C., et al. (2009). Chronic restraint stress impairs T-cell immunity and promotes tumor progression in mice. *Stress* 12, 134–143. doi: 10.1080/10253890802137437

Fulop, T., Larbi, A., Dupuis, G., Le Page, A., Frost, E. H., Cohen, A. A., et al. (2017). Immunosenescence and inflamm-aging as two sides of the same coin: friends or foes? *Front. Immunol.* 8:1960. doi: 10.3389/fimmu.2017.01960

Galea, L. A., Wide, J. K., and Barr, A. M. (2001). Estradiol alleviates depressive-like symptoms in a novel animal model of post-partum depression. *Behav. Brain Res.* 122, 1–9. doi: 10.1016/s0166-4328(01)00170-x

Gameiro, C. M., Romão, F., and Castelo-Branco, C. (2010). Menopause and aging: changes in the immune system–a review. *Maturitas* 67, 316–320. doi: 10.1016/j.maturitas.2010.08.003

Ganor, Y., and Levite, M. (2012). "Glutamate in the immune system: glutamate receptors in immune cells, potent effects, endogenous production and involvement in disease," in *Nerve-Driven Immunity: Neurotransmitters and Neuropeptides in the Immune System*, ed. M. Levite (Vienna: Springer), 121–161. doi: 10.1007/978-3-7091-0888-8_4

Ghosh, M., Rodriguez-Garcia, M., and Wira, C. R. (2014). The immune system in menopause: pros and cons of hormone therapy. *J. Steroid Biochem. Mol. Biol.* 142, 171–175. doi: 10.1016/j.jsbmb.2013.09.003

Giglio, T., Imro, M. A., Filaci, G., Scudeletti, M., Puppo, F., De Cecco, L., et al. (1994). Immune cell circulating subsets are affected by gonadal function. *Life Sci.* 54, 1305–1312. doi: 10.1016/0024-3205(94)00508-7

Goemaere, S., Ackerman, C., Goethals, K., De Keyser, F., Van der Straeten, C., Verbruggen, G., et al. (1990). Onset of symptoms of rheumatoid arthritis in relation to age, sex and menopausal transition. *J. Rheumatol.* 17, 1620–1622.

Gold, P. W., Pavlatou, M. G., Carlson, P. J., Luckenbaugh, D. A., Costello, R., Bonne, O., et al. (2012). Unmedicated, remitted patients with major depression have decreased serum immunoglobulin A. *Neurosci. Lett.* 520, 1–5. doi: 10.1016/j.neulet.2012.04.072

Golden, S. A., Covington, H. E. III., Berton, O., and Russo, S. J. (2011). A standardized protocol for repeated social defeat stress in mice. *Nat. Protoc.* 6, 1183–1191. doi: 10.1038/nprot.2011.361

Goldenberg, M. M. (2012). Multiple sclerosis review. *P T* 37, 175–184.

Gordon, J. L., Rubinow, D. R., Eisenlohr-Moul, T. A., Leserman, J., and Girdler, S. S. (2016). Estradiol variability, stressful life events, and the emergence of depressive symptomatology during the menopausal transition. *Menopause* 23, 257–266. doi: 10.1097/gme.0000000000000528

Gracia, C. R., and Freeman, E. W. (2018). Onset of the menopause transition: the earliest signs and symptoms. *Obstet. Gynecol. Clin. North Am.* 45, 585–597. doi: 10.1016/j.ogc.2018.07.002

Greenberg, P. E., Fournier, A. A., Sisitsky, T., Pike, C. T., and Kessler, R. C. (2015). The economic burden of adults with major depressive disorder in the United States (2005 and 2010). *J. Clin. Psychiatry* 76, 155–162. doi: 10.4088/JCP.14m09298

Griggs, R. C., Condemi, J. J., and Vaughan, J. H. (1972). Effect of therapeutic dosages of prednisone on human immunoglobulin G metabolism. *J. Allergy Clin. Immunol.* 49, 267–273. doi: 10.1016/0091-6749(72)90094-2

Gruver-Yates, A. L., Quinn, M. A., and Cidlowski, J. A. (2014). Analysis of glucocorticoid receptors and their apoptotic response to dexamethasone in male murine B cells during development. *Endocrinology* 155, 463–474. doi: 10.1210/en.2013-1473

Gubbels Bupp, M. R., Potluri, T., Fink, A. L., and Klein, S. L. (2018). The confluence of sex hormones and aging on immunity. *Front. Immunol.* 9:1269. doi: 10.3389/fimmu.2018.01269

Guven Yorgun, Y., and Ozakbas, S. (2019). Effect of hormonal changes on the neurological status in the menstrual cycle of patient with multiple sclerosis. *Clin. Neurol. Neurosurg.* 186:105499. doi: 10.1016/j.clineuro.2019.105499

Hales, C. A., Stuart, S. A., Anderson, M. H., and Robinson, E. S. (2014). Modelling cognitive affective biases in major depressive disorder using rodents. *Br. J. Pharmacol.* 171, 4524–4538. doi: 10.1111/bph.12603

Herkenham, M., and Kigar, S. L. (2017). Contributions of the adaptive immune system to mood regulation: mechanisms and pathways of neuroimmune interactions. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 79(Pt A), 49–57. doi: 10.1016/j.pnpbp.2016.09.003

Hernandez, M. E., Martinez-Fong, D., Perez-Tapia, M., Estrada-Garcia, I., Estrada-Parra, S., and Pavón, L. (2010). Evaluation of the effect of selective serotonin-reuptake inhibitors on lymphocyte subsets in patients with a major depressive disorder. *Eur. Neuropsychopharmacol.* 20, 88–95. doi: 10.1016/j.euroneuro.2009.11.005

Herr, N., Bode, C., and Duerschmied, D. (2017). The effects of serotonin in immune cells. *Front. Cardiovasc. Med.* 4:48. doi: 10.3389/fcvm.2017.00048

Hewagama, A., Patel, D., Yarlagadda, S., Strickland, F. M., and Richardson, B. C. (2009). Stronger inflammatory/cytotoxic T-cell response in women identified by microarray analysis. *Genes Immun.* 10, 509–516. doi: 10.1038/gene.2009.12

Hiroi, R., Weyrich, G., Koebele, S. V., Mennenga, S. E., Talboom, J. S., Hewitt, L. T., et al. (2016). Benefits of hormone therapy estrogens depend on estrogen type: 17β-estradiol and conjugated equine estrogens have differential effects on cognitive, anxiety-like, and depressive-like behaviors and increase tryptophan hydroxylase-2 MRNA levels in dorsal raphe nucleus subregions. *Front. Neurosci.* 10:517. doi: 10.3389/fnins.2016.00517

Holroyd, C. R., and Edwards, C. J. (2009). The effects of hormone replacement therapy on autoimmune disease: rheumatoid arthritis and systemic lupus erythematosus. *Climacteric* 12, 378–386. doi: 10.1080/13697130903025449

Hsu, P., and Nanan, R. K. (2014). Innate and adaptive immune interactions at the fetal-maternal interface in healthy human pregnancy and pre-eclampsia. *Front. Immunol.* 5:125. doi: 10.3389/fimmu.2014.00125

Huang, C. C., Wei, I. H., Chou, Y. H., and Su, T. P. (2008). Effect of age, gender, menopausal status, and ovarian hormonal level on rTMS in treatment-resistant depression. *Psychoneuroendocrinology* 33, 821–831. doi: 10.1016/j.psyneuen.2008.03.006

Jenkins, J. A., Williams, P., Kramer, G. L., Davis, L. L., and Petty, F. (2001). The influence of gender and the estrous cycle on learned helplessness in the rat. *Biol. Psychol.* 58, 147–158. doi: 10.1016/s0301-0511(01)00111-9

Joel, D., and McCarthy, M. M. (2017). Incorporating sex as a biological variable in neuropsychiatric research: where are we now and where should we be? *Neuropsychopharmacology* 42, 379–385. doi: 10.1038/npp.2016.79

Johnson, A., Rainville, J. R., Rivero-Ballon, G. N., Dhimitri, K., and Hodes, G. E. (2021). Testing the limits of sex differences using variable stress. *Neuroscience* 454, 72–84. doi: 10.1016/j.neuroscience.2019.12.034

Joyce, P. R., Hawes, C. R., Mulder, R. T., Sellman, J. D., Wilson, D. A., and Boswell, D. R. (1992). Elevated levels of acute phase plasma proteins in major depression. *Biol. Psychiatry* 32, 1035–1041. doi: 10.1016/0006-3223(92)90065-8

Kaczorowski, K. J., Shekhar, K., Nkulikiyimfura, D., Dekker, C. L., Maecker, H., Davis, M. M., et al. (2017). Continuous immunotypes describe human immune variation and predict diverse responses. *Proc. Natl. Acad. Sci. U.S.A.* 114, E6097–E6106. doi: 10.1073/pnas.1705065114

Kamada, M., Irahara, M., Maegawa, M., Yasui, T., Takeji, T., Yamada, M., et al. (2000). Effect of hormone replacement therapy on post-menopausal changes of lymphocytes and T cell subsets. *J. Endocrinol. Invest.* 23, 376–382. doi: 10.1007/bf03343741

Keestra, S. M., Male, V., and Salali, G. D. (2021). Out of balance: the role of evolutionary mismatches in the sex disparity in autoimmune disease. *Med. Hypotheses* 151, 110558. doi: 10.1016/j.mehy.2021.110558

Keppel, G. W., and Wickens, T. D. (2004). *Design and Analysis*. Saddle River, NJ: Pearson/Prentice Hall.

Keselman, A., Fang, X., White, P. B., and Heller, N. M. (2017). Estrogen signaling contributes to sex differences in macrophage polarization during asthma. *J. Immunol.* 199, 1573–1583. doi: 10.4049/jimmunol.1601975

Kessler, R. C., Chiu, W. T., Demler, O., Merikangas, K. R., and Walters, E. E. (2005). Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch. Gen. Psychiatry* 62, 617–627. doi: 10.1001/archpsyc.62.6.617

Kevorkian, R. (2007). Andropause. *Mol. Med.* 104, 68–71; quiz71.

Khan, D., and Ansar Ahmed, S. (2015). The Immune system is a natural target for estrogen action: opposing effects of estrogen in two prototypical autoimmune diseases. *Front. Immunol.* 6:635. doi: 10.3389/fimmu.2015.00635

Kieffer, T. E. C., Laskewitz, A., Scherjon, S. A., Faas, M. M., and Prins, J. R. (2019). Memory T cells in pregnancy. *Front. Immunol.* 10:625. doi: 10.3389/fimmu.2019.00625

Kim, J., Kim, J. H., and Chang, K. A. (2021). Sex difference in peripheral inflammatory biomarkers in drug-naïve patients with major depression in young adulthood. *Biomedicines* 9:708. doi: 10.3390/biomedicines9070708

Klein, S. L., and Flanagan, K. L. (2016). Sex differences in immune responses. *Nat. Rev. Immunol.* 16, 626–638. doi: 10.1038/nri.2016.90

Koebele, S. V., Palmer, J. M., Hadder, B., Melikian, R., Fox, C., Strouse, I. M., et al. (2019). Hysterectomy uniquely impacts spatial memory in a rat model: a role for the nonpregnant uterus in cognitive processes. *Endocrinology* 160, 1–19. doi: 10.1210/en.2018-00709

Köhler, C. A., Freitas, T. H., Maes, M., de Andrade, N. Q., Liu, C. S., Fernandes, B. S., et al. (2017). Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. *Acta Psychiatr. Scand.* 135, 373–387. doi: 10.1111/acps.12698

Köhler-Forsberg, O., Buttenschøn, H. N., Tansey, K. E., Maier, W., Hauser, J., Dernovsek, M. Z., et al. (2017). Association between C-reactive protein (CRP) with depression symptom severity and specific depressive symptoms in major depression. *Brain Behav. Immun.* 62, 344–350. doi: 10.1016/j.bbi.2017.02.020

Köhler-Forsberg, O., Lydholm, C. N., Hjorthøj, C., Nordentoft, M., Mors, O., and Benros, M. E. (2019). Efficacy of anti-inflammatory treatment on major depressive disorder or depressive symptoms: meta-analysis of clinical trials. *Acta Psychiatr. Scand.* 139, 404–419. doi: 10.1111/acps.13016

Kovacs, W. J. (2014). To B or not to B? Glucocorticoid impact on B lymphocyte fate and function. *Endocrinology* 155, 339–342. doi: 10.1210/en.2013-2085

Krishnan, V., and Nestler, E. J. (2008). The molecular neurobiology of depression. *Nature* 455, 894–902. doi: 10.1038/nature07455

Kronfol, Z., and House, J. D. (1989). Lymphocyte mitogenesis, immunoglobulin and complement levels in depressed patients and normal controls. *Acta Psychiatr. Scand.* 80, 142–147. doi: 10.1111/j.1600-0447.1989.tb01316.x

Krop, J., Heidt, S., Claas, F. H. J., and Eikmans, M. (2020). Regulatory T cells in pregnancy: it is not all about foxP3. *Front. Immunol.* 11:1182. doi: 10.3389/fimmu.2020.01182

Kumru, S., Godekmerdan, A., and Yilmaz, B. (2004). Immune effects of surgical menopause and estrogen replacement therapy in peri-menopausal women. *J. Reprod. Immunol.* 63, 31–38. doi: 10.1016/j.jri.2004.02.001

Laessle, R. G., Tuschl, R. J., Schweiger, U., and Pirke, K. M. (1990). Mood changes and physical complaints during the normal menstrual cycle in healthy young women. *Psychoneuroendocrinology* 15, 131–138. doi: 10.1016/0306-4530(90)90021-z

Lakshmikanth, T., Muhammad, S. A., Olin, A., Chen, Y., Mikes, J., Fagerberg, L., et al. (2020). Human Immune System Variation during 1 Year. *Cell Rep.* 32:107923. doi: 10.1016/j.celrep.2020.107923

Lamason, R., Zhao, P., Rawat, R., Davis, A., Hall, J. C., Chae, J. J., et al. (2006). Sexual dimorphism in immune response genes as a function of puberty. *BMC Immunol.* 7:2. doi: 10.1186/1471-2172-7-2

Lateef, A., and Petri, M. (2012). Hormone replacement and contraceptive therapy in autoimmune diseases. *J. Autoimmun.* 38, J170–J176. doi: 10.1016/j.jaut.2011.11.002

Lauer, M. (2016). *Consideration of Relevant Biological Variables in NIH Grant Applications [Online]*. Bethesda, MD: NIH.

Lee, B. W., Yap, H. K., Chew, F. T., Quah, T. C., Prabhakaran, K., Chan, G. S., et al. (1996). Age- and sex-related changes in lymphocyte subpopulations of healthy Asian subjects: from birth to adulthood. *Cytometry* 26, 8–15. doi: 10.1002/(SICI)1097-0320(19960315)26:1<8::AID-CYTO2>3.0.CO;2-E

Lee, S., Kim, J., Jang, B., Hur, S., Jung, U., Kil, K., et al. (2010). Fluctuation of peripheral blood T, B, and NK cells during a menstrual cycle of normal healthy women. *J. Immunol.* 185, 756–762. doi: 10.4049/jimmunol.0904192

LeGates, T. A., Kvarta, M. D., and Thompson, S. M. (2019). Sex differences in antidepressant efficacy. *Neuropsychopharmacology* 44, 140–154. doi: 10.1038/s41386-018-0156-z

Leonard, B. E. (2010). The concept of depression as a dysfunction of the immune system. *Curr. Immunol. Rev.* 6, 205–212. doi: 10.2174/157339510791823835

Li, L. H., Wang, Z. C., Yu, J., and Zhang, Y. Q. (2014). Ovariectomy results in variable changes in nociception, mood and depression in adult female rats. *PLoS One* 9:e94312. doi: 10.1371/journal.pone.0094312

Lim, A. I., Harrison, O. J., and Belkaid, Y. (2019). Pre-birth memory. *Nat. Immunol.* 20, 254–256. doi: 10.1038/s41590-019-0326-0

Lima, J., Martins, C., Leandro, M. J., Nunes, G., Sousa, M. J., Branco, J. C., et al. (2016). Characterization of B cells in healthy pregnant women from late pregnancy to post-partum: a prospective observational study. *BMC Pregnancy Childbirth* 16:139. doi: 10.1186/s12884-016-0927-7

Lima, J., Martins, C., Nunes, G., Sousa, M. J., Branco, J. C., and Borrego, L. M. (2017). Regulatory T cells show dynamic behavior during late pregnancy, delivery, and the postpartum period. *Reprod. Sci.* 24, 1025–1032. doi: 10.1177/1933719116676395

Lissauer, D., Goodyear, O., Khanum, R., Moss, P. A., and Kilby, M. D. (2014). Profile of maternal CD4 T-cell effector function during normal pregnancy and in women with a history of recurrent miscarriage. *Clin. Sci. (Lond)* 126, 347–354. doi: 10.1042/cs20130247

Lisse, I. M., Aaby, P., Whittle, H., Jensen, H., Engelmann, M., and Christensen, L. B. (1997). T-lymphocyte subsets in West African children: impact of age, sex, and season. *J Pediatr* 130, 77–85. doi: 10.1016/s0022-3476(97)70313-5

Luca, D. L., Garlow, N., Staatz, C., Margiotta, C., and Zivin, K. (2019). *Societal Costs of Untreated Perinatal Mood and Anxiety Disorders in the United States*. Princeton, NJ: Mathematica Policy Research.

Lucas, S. M., Rothwell, N. J., and Gibson, R. M. (2006). The role of inflammation in CNS injury and disease. *Br. J. Pharmacol.* 147 Suppl 1(Suppl 1), S232–S240. doi: 10.1038/sj.bjp.0706400

Lynall, M. E., Kigar, S. L., Lehmann, M. L., DePuyt, A. E., Tuong, Z. K., Listwak, S. J., et al. (2021). B-cells are abnormal in psychosocial stress and regulate meningeal myeloid cell activation. *Brain Behav. Immun.* 97, 226–238. doi: 10.1016/j.bbi.2021.08.002

Maartens, L. W., Knottnerus, J. A., and Pop, V. J. (2002). Menopausal transition and increased depressive symptomatology: a community based prospective study. *Maturitas* 42, 195–200. doi: 10.1016/s0378-5122(02)00038-5

Maes, M. (2011). Depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 35, 664–675. doi: 10.1016/j.pnpbp.2010.06.014

Maes, M., Bosmans, E., Suy, E., Vandervorst, C., De Jonckheere, C., and Raus, J. (1990). Immune disturbances during major depression: upregulated expression of interleukin-2 receptors. *Neuropsychobiology* 24, 115–120. doi: 10.1159/000119472

Maes, M., Scharpé, S., Van Grootel, L., Uyttenbroeck, W., Cooreman, W., Cosyns, P., et al. (1992b). Higher alpha 1-antitrypsin, haptoglobin, ceruloplasmin and lower retinol binding protein plasma levels during depression: further evidence for the existence of an inflammatory response during that illness. *J. Affect. Disord.* 24, 183–192. doi: 10.1016/0165-0327(92)90066-f

Maes, M., Lambrechts, J., Bosmans, E., Jacobs, J., Suy, E., Vandervorst, C., et al. (1992a). Evidence for a systemic immune activation during depression: results of leukocyte enumeration by flow cytometry in conjunction with monoclonal antibody staining. *Psychol. Med.* 22, 45–53. doi: 10.1017/s0033291700032712

Maglione, A., Rolla, S., Mercanti, S. F., Cutrupi, S., and Clerico, M. (2019). The adaptive immune system in multiple sclerosis: an estrogen-mediated point of view. *Cells* 8:280. doi: 10.3390/cells8101280

Maki, P. M., Kornstein, S. G., Joffe, H., Bromberger, J. T., Freeman, E. W., Athappilly, G., et al. (2019). Guidelines for the evaluation and treatment of perimenopausal depression: summary and recommendations. *J. Womens Health (Larchmt)* 28, 117–134. doi: 10.1089/jwh.2018.27099.mensocrec

Maki, P. M., and Resnick, S. M. (2001). Effects of estrogen on patterns of brain activity at rest and during cognitive activity: a review of neuroimaging studies. *Neuroimage* 14, 789–801. doi: 10.1006/nimg.2001.0887

Marcus, S. M., Kerber, K. B., Rush, A. J., Wisniewski, S. R., Nierenberg, A., Balasubramani, G. K., et al. (2008). Sex differences in depression symptoms in treatment-seeking adults: confirmatory analyses from the Sequenced Treatment Alternatives to Relieve Depression study. *Compr. Psychiatry* 49, 238–246. doi: 10.1016/j.comppsych.2007.06.012

Márquez, E. J., Chung, C. H., Marches, R., Rossi, R. J., Nehar-Belaid, D., Eroglu, A., et al. (2020). Sexual-dimorphism in human immune system aging. *Nat. Commun.* 11:751. doi: 10.1038/s41467-020-14396-9

Marrocco, J., and McEwen, B. S. (2016). Sex in the brain: hormones and sex differences. *Dialogues Clin. Neurosci.* 18, 373–383. doi: 10.31887/DCNS.2016.18.4/jmarrocco

Marshall, J. S., Warrington, R., Watson, W., and Kim, H. L. (2018). An introduction to immunology and immunopathology. *Allergy Asthma. Clin. Immunol.* 14(Suppl. 2), 49. doi: 10.1186/s13223-018-0278-1

Masopust, D., Sivula, C. P., and Jameson, S. C. (2017). Of mice, dirty mice, and men: using mice to understand human immunology. *J. Immunol.* 199, 383–388. doi: 10.4049/jimmunol.1700453

McGregor, B. A., Murphy, K. M., Albano, D. L., and Ceballos, R. M. (2016). Stress, cortisol, and B lymphocytes: a novel approach to understanding academic stress and immune function. *Stress* 19, 185–191. doi: 10.3109/10253890.2015.1127913

Melgert, B. N., Ray, A., Hylkema, M. N., Timens, W., and Postma, D. S. (2007). Are there reasons why adult asthma is more common in females? *Curr. Allergy Asthma Rep.* 7, 143–150. doi: 10.1007/s11882-007-0012-4

Mendoza-Cabrera, M. I., Navarro-Hernández, R. E., Santerre, A., Ortiz-Lazareno, P. C., Pereira-Suárez, A. L., and Estrada-Chávez, C. (2020). Effect of pregnancy hormone mixtures on cytokine production and surface marker expression in naïve and LPS-activated THP-1 differentiated monocytes/macrophages. *Innate Immun.* 26, 84–96. doi: 10.1177/1753425919864658

Meredith, E. J., Holder, M. J., Chamba, A., Challa, A., Drake-Lee, A., Bunce, C. M., et al. (2005). The serotonin transporter (SLC6A4) is present in B-cell clones of diverse malignant origin: probing a potential anti-tumor target for psychotropics. *FASEB J.* 19, 1187–1189. doi: 10.1096/fj.04-3477fje

Mestas, J., and Hughes, C. C. (2004). Of mice and not men: differences between mouse and human immunology. *J. Immunol.* 172, 2731–2738. doi: 10.4049/jimmunol.172.5.2731

Millett, C. E., Phillips, B. E., and Saunders, E. F. H. (2019). The sex-specific effects of lps on depressive-like behavior and oxidative stress in the hippocampus of the mouse. *Neuroscience* 399, 77–88. doi: 10.1016/j.neuroscience.2018.12.008

Moieni, M., Irwin, M. R., Jevtic, I., Olmstead, R., Breen, E. C., and Eisenberger, N. I. (2015). Sex differences in depressive and socioemotional responses to an inflammatory challenge: implications for sex differences in depression. *Neuropsychopharmacology* 40, 1709–1716. doi: 10.1038/npp.2015.17

Molnár, I., Bohaty, I., and Somogyiné-Vári, É (2014). High prevalence of increased interleukin-17A serum levels in postmenopausal estrogen deficiency. *Menopause* 21, 749–752. doi: 10.1097/gme.0000000000000125

Moos, R. H., Kopell, B. S., Melges, F. T., Yalom, I. D., Lunde, D. T., Clayton, R. B., et al. (1969). Fluctuations in symptoms and moods during the menstrual cycle. *J Psychosom. Res.* 13, 37–44. doi: 10.1016/0022-3999(69)90017-8

Mor, G., and Cardenas, I. (2010). The immune system in pregnancy: a unique complexity. *Am. J. Reprod. Immunol.* 63, 425–433. doi: 10.1111/j.1600-0897.2010.00836.x

Morimoto, K., and Nakajima, K. (2019). Role of the immune system in the development of the central nervous system. *Front. Neurosci.* 13:916. doi: 10.3389/fnins.2019.00916

Moulton, V. R. (2018). Sex hormones in acquired immunity and autoimmune disease. *Front. Immunol.* 9:2279. doi: 10.3389/fimmu.2018.02279

Musabak, U., Bolu, E., Ozata, M., Oktenli, C., Sengul, A., Inal, A., et al. (2003). Gonadotropin treatment restores in vitro interleukin-1beta and tumour necrosis factor-alpha production by stimulated peripheral blood mononuclear cells from patients with idiopathic hypogonadotropic hypogonadism. *Clin. Exp. Immunol.* 132, 265–270. doi: 10.1046/j.1365-2249.2003.02141.x

Muzzio, D., Zenclussen, A. C., and Jensen, F. (2013). The role of B cells in pregnancy: the good and the bad. *Am. J. Reprod. Immunol.* 69, 408–412. doi: 10.1111/aji.12079

Nair, R. R., Verma, P., and Singh, K. (2017). Immune-endocrine crosstalk during pregnancy. *Gen. Comp. Endocrinol.* 242, 18–23. doi: 10.1016/j.ygcen.2016.03.003

Nestler, E. J., and Hyman, S. E. (2010). Animal models of neuropsychiatric disorders. *Nat. Neurosci.* 13, 1161–1169. doi: 10.1038/nn.2647

Ngo, S. T., Steyn, F. J., and McCombe, P. A. (2014). Gender differences in autoimmune disease. *Front. Neuroendocrinol.* 35:347–369. doi: 10.1016/j.yfrne.2014.04.004

NIH (2015). *Consideration of Sex as a Biological Variable in NIH-funded Research [Online]*. Bethesda, MD: NIH.

NIMH (2019). *Notice of NIMH's Considerations Regarding the Use of Animal Neurobehavioral Approaches in Basic and Pre-Clinical Studies [Online]*. Bethesda, MD: NIMH.

Oertelt-Prigione, S. (2012). Immunology and the menstrual cycle. *Autoimmun. Rev.* 11, A486–A492. doi: 10.1016/j.autrev.2011.11.023

Okine, T., Shepard, R., Lemanski, E., and Coutellier, L. (2020). Sex differences in the sustained effects of ketamine on resilience to chronic stress. *Front. Behav. Neurosci.* 14:581360. doi: 10.3389/fnbeh.2020.581360

Okuyama, K., Suenaga, M., Furuki, S., Kawano, T., Ohkawara, Y., Takayanagi, M., et al. (2013). Contribution of CD4+ T cells and dendritic cells to female-dominant antigen-induced T helper type 2 cytokine production by bronchial lymph node cells. *Int. Arch. Allergy Immunol.* 161(Suppl. 2), 58–65. doi: 10.1159/000350426

Olin, A., Henckel, E., Chen, Y., Lakshmikanth, T., Pou, C., Mikes, J., et al. (2018). Stereotypic Immune System Development in Newborn Children. *Cell 174(5)* 127, e1214. doi: 10.1016/j.cell.2018.06.045

Ortona, E., Pierdominici, M., Maselli, A., Veroni, C., Aloisi, F., and Shoenfeld, Y. (2016). Sex-based differences in autoimmune diseases. *Ann. Ist Super Sanita* 52, 205–212. doi: 10.4415/ann_16_02_12

Ostensen, M., Aune, B., and Husby, G. (1983). Effect of pregnancy and hormonal changes on the activity of rheumatoid arthritis. *Scand. J. Rheumatol.* 12, 69–72. doi: 10.3109/03009748309102886

Ousman, S. S., and Kubes, P. (2012). Immune surveillance in the central nervous system. *Nat. Neurosci.* 15, 1096–1101. doi: 10.1038/nn.3161

Pavlov, V. A., Chavan, S. S., and Tracey, K. J. (2018). Molecular and functional neuroscience in immunity. *Annu. Rev. Immunol.* 36, 783–812. doi: 10.1146/annurev-immunol-042617-053158

Pavón, L., Sandoval-López, G., Eugenia Hernández, M., Loría, F., Estrada, I., Pérez, M., et al. (2006). Th2 cytokine response in Major Depressive Disorder patients before treatment. *J. Neuroimmunol.* 172, 156–165. doi: 10.1016/j.jneuroim.2005.08.014

Pennell, L. M., Galligan, C. L., and Fish, E. N. (2012). Sex affects immunity. *J. Autoimmun.* 38, J282–J291. doi: 10.1016/j.jaut.2011.11.013

Petitto, J. M., Folds, J. D., and Evans, D. L. (1993). Abnormal diurnal variation of B lymphocyte circulation patterns in major depression. *Biol. Psychiatry* 34, 268–270. doi: 10.1016/0006-3223(93)90082-o

Petri, M. (2020). Pregnancy and systemic lupus erythematosus. *Best Pract. Res. Clin. Obstet Gynaecol* 64, 24–30. doi: 10.1016/j.bpobgyn.2019.09.002

Piantella, S., Dragano, N., McDonald, S. J., and Wright, B. J. (2021). Depression symptoms mediate the association between workplace stress and interleukin 6 in women, but not men: the Whitehall II study. *Brain Behav. Immun. Health* 12:100215. doi: 10.1016/j.bbih.2021.100215

Poromaa, I. S., and Segebladh, B. (2012). Adverse mood symptoms with oral contraceptives. *Acta Obstet. Gynecol. Scand.* 91, 420–427. doi: 10.1111/j.1600-0412.2011.01333.x

Porter, V. R., Greendale, G. A., Schocken, M., Zhu, X., and Effros, R. B. (2001). Immune effects of hormone replacement therapy in post-menopausal women. *Exp. Gerontol.* 36, 311–326. doi: 10.1016/s0531-5565(00)00195-9

Powell, T. R., Fernandes, C., and Schalkwyk, L. C. (2012). Depression-related behavioral tests. *Curr. Protoc. Mouse Biol.* 2, 119–127. doi: 10.1002/9780470942390.mo110176

Price, R. B., and Duman, R. (2020). Neuroplasticity in cognitive and psychological mechanisms of depression: an integrative model. *Mol. Psychiatry* 25, 530–543. doi: 10.1038/s41380-019-0615-x

Pryce, C. R., and Fontana, A. (2017). Depression in autoimmune diseases. *Curr. Top. Behav. Neurosci.* 31, 139–154. doi: 10.1007/7854_2016_7

Pung, O. J., Tucker, A. N., Vore, S. J., and Luster, M. I. (1985). Influence of estrogen on host resistance: increased susceptibility of mice to Listeria monocytogenes correlates with depressed production of interleukin 2. *Infect. Immun.* 50, 91–96. doi: 10.1128/iai.50.1.91-96.1985

Racicot, K., Kwon, J. Y., Aldo, P., Silasi, M., and Mor, G. (2014). Understanding the complexity of the immune system during pregnancy. *Am. J. Reprod. Immunol.* 72, 107–116. doi: 10.1111/aji.12289

Ramo-Tello, C., Blanco, Y., Brieva, L., Casanova, B., Martinez-Caceres, E., Ontaneda, D., et al. (2021). Recommendations for the diagnosis and treatment of multiple sclerosis relapses. *J. Pers. Med.* 12:6. doi: 10.3390/jpm12010006

Ramsey, J. M., Cooper, J. D., Bot, M., Guest, P. C., Lamers, F., Weickert, C. S., et al. (2016). Sex differences in serum markers of major depressive disorder in the Netherlands Study of Depression and Anxiety (NESDA). *PLoS One* 11:e0156624. doi: 10.1371/journal.pone.0156624

Ransohoff, R. M., Schafer, D., Vincent, A., Blachère, N. E., and Bar-Or, A. (2015). Neuroinflammation: ways in which the immune system affects the brain. *Neurotherapeutics* 12, 896–909. doi: 10.1007/s13311-015-0385-3

Rattazzi, L., Piras, G., Ono, M., Deacon, R., Pariante, C. M., and D'Acquisto, F. (2013). $CD4^+$ but not $CD8^+$ T cells revert the impaired emotional behavior of immunocompromised RAG-1-deficient mice. *Transl. Psychiatry* 3:e280. doi: 10.1038/tp.2013.54

Rayasam, A., Kijak, J. A., Dallmann, M., Hsu, M., Zindl, N., Lindstedt, A., et al. (2018). Regional distribution of cns antigens differentially determines T-Cell mediated neuroinflammation in a CX3CR1-dependent manner. *J. Neurosci.* 38, 7058–7071. doi: 10.1523/jneurosci.0366-18.2018

Robakis, T., Williams, K. E., Nutkiewicz, L., and Rasgon, N. L. (2019). Hormonal contraceptives and mood: review of the literature and implications for future research. *Curr. Psychiatry Rep.* 21:57. doi: 10.1007/s11920-019-1034-z

Roberts, C. W., Walker, W., and Alexander, J. (2001). Sex-associated hormones and immunity to protozoan parasites. *Clin. Microbiol. Rev.* 14, 476–488. doi: 10.1128/cmr.14.3.476-488.2001

Robertson, M. J., Schacterle, R. S., Mackin, G. A., Wilson, S. N., Bloomingdale, K. L., Ritz, J., et al. (2005). Lymphocyte subset differences in patients with chronic fatigue syndrome, multiple sclerosis and major depression. *Clin. Exp. Immunol.* 141, 326–332. doi: 10.1111/j.1365-2249.2005.02833.x

Robinson, D. P., Hall, O. J., Nilles, T. L., Bream, J. H., and Klein, S. L. (2014). 17β-estradiol protects females against influenza by recruiting neutrophils and increasing virus-specific CD8 T cell responses in the lungs. *J. Virol.* 88, 4711–4720. doi: 10.1128/jvi.02081-13

Robinson, D. P., and Klein, S. L. (2012). Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis. *Horm. Behav.* 62, 263–271. doi: 10.1016/j.yhbeh.2012.02.023

Rodriguez-Garcia, M., Biswas, N., Patel, M. V., Barr, F. D., Crist, S. G., Ochsenbauer, C., et al. (2013). Estradiol reduces susceptibility of CD4+ T cells and macrophages to HIV-infection. *PLoS One* 8:e62069. doi: 10.1371/journal.pone.0062069

Rosen, J. L., Tran, H. T., Lackey, A., and Viselli, S. M. (1999). Sex-related immune changes in young mice. *Immunol. Invest.* 28, 247–256. doi: 10.3109/08820139909060859

Rossetti, A. C., Paladini, M. S., Trepci, A., Mallien, A., Riva, M. A., Gass, P., et al. (2019). Differential neuroinflammatory response in male and female mice: a role for BDNF. *Front. Mol. Neurosci.* 12:166. doi: 10.3389/fnmol.2019.00166

Roumestan, C., Michel, A., Bichon, F., Portet, K., Detoc, M., Henriquet, C., et al. (2007). Anti-inflammatory properties of desipramine and fluoxetine. *Respir. Res.* 8:35. doi: 10.1186/1465-9921-8-35

Rubinow, K. B. (2018). An intracrine view of sex steroids, immunity, and metabolic regulation. *Mol. Metab.* 15, 92–103. doi: 10.1016/j.molmet.2018.03.001

Rubtsova, K., Rubtsov, A. V., Cancro, M. P., and Marrack, P. (2015). Age-associated b cells: a T-bet-dependent effector with roles in protective and pathogenic immunity. *J. Immunol.* 195, 1933–1937. doi: 10.4049/jimmunol.1501209

Rus, H., Cudrici, C., and Niculescu, F. (2005). The role of the complement system in innate immunity. *Immunol. Res.* 33, 103–112. doi: 10.1385/ir:33:2:103

Russo, S. J., Murrough, J. W., Han, M. H., Charney, D. S., and Nestler, E. J. (2012). Neurobiology of resilience. *Nat. Neurosci.* 15, 1475–1484. doi: 10.1038/nn.3234

Saland, S. K., Duclot, F., and Kabbaj, M. (2017). Integrative analysis of sex differences in the rapid antidepressant effects of ketamine in preclinical models for individualized clinical outcomes. *Curr. Opin. Behav. Sci.* 14, 19–26. doi: 10.1016/j.cobeha.2016.11.002

Sarcevic, J., Cavelti-Weder, C., Berger, C. T., and Trendelenburg, M. (2020). Case report-secondary antibody deficiency due to endogenous hypercortisolism. *Front. Immunol.* 11:1435. doi: 10.3389/fimmu.2020.01435

Scheinert, R. B., Haeri, M. H., Lehmann, M. L., and Herkenham, M. (2016). Therapeutic effects of stress-programmed lymphocytes transferred to chronically stressed mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 70, 1–7. doi: 10.1016/j.pnpbp.2016.04.010

Schildkraut, J. J. (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. *Am. J. Psychiatry* 122, 509–522. doi: 10.1176/ajp.122.5.509

Schiller, C. E., O'Hara, M. W., Rubinow, D. R., and Johnson, A. K. (2013). Estradiol modulates anhedonia and behavioral despair in rats and negative affect in a subgroup of women at high risk for postpartum depression. *Physiol. Behav.* 119, 137–144. doi: 10.1016/j.physbeh.2013.06.009

Schleifer, S. J., Bartlett, J. A., Keller, S. E., Eckholdt, H. M., Shiflett, S. C., and Delaney, B. R. (2002). Immunity in adolescents with major depression. *J. Am. Acad. Child. Adolesc. Psychiatry* 41, 1054–1060. doi: 10.1097/00004583-200209000-00005

Schleifer, S. J., Keller, S. E., Bond, R. N., Cohen, J., and Stein, M. (1989). Major depressive disorder and immunity. Role of age, sex, severity, and hospitalization. *Arch. Gen. Psychiatry* 46, 81–87. doi: 10.1001/archpsyc.1989.01810010083011

Schmidt, P. J., Nieman, L., Danaceau, M. A., Tobin, M. B., Roca, C. A., Murphy, J. H., et al. (2000). Estrogen replacement in perimenopause-related depression: a preliminary report. *Am. J. Obstet. Gynecol.* 183, 414–420. doi: 10.1067/mob.2000.106004

Schoenrock, S. A., Oreper, D., Young, N., Ervin, R. B., Bogue, M. A., Valdar, W., et al. (2016). Ovariectomy results in inbred strain-specific increases in anxiety-like behavior in mice. *Physiol. Behav.* 167, 404–412. doi: 10.1016/j.physbeh.2016.09.026

Schuhmann, B., Dietrich, A., Sel, S., Hahn, C., Klingenspor, M., Lommatzsch, M., et al. (2005). A role for brain-derived neurotrophic factor in B cell development. *J. Neuroimmunol.* 163, 15–23. doi: 10.1016/j.jneuroim.2005.01.023

Seillet, C., Laffont, S., Trémollières, F., Rouquié, N., Ribot, C., Arnal, J. F., et al. (2012). The TLR-mediated response of plasmacytoid dendritic cells is positively regulated by estradiol in vivo through cell-intrinsic estrogen receptor α signaling. *Blood* 119, 454–464. doi: 10.1182/blood-2011-08-371831

Seok, J., Warren, H. S., Cuenca, A. G., Mindrinos, M. N., Baker, H. V., Xu, W., et al. (2013). Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc. Natl. Acad. Sci. U.S.A.* 110, 3507–3512. doi: 10.1073/pnas.1222878110

Settipane, G. A., Pudupakkam, R. K., and McGowan, J. H. (1978). Corticosteroid effect on immunoglobulins. *J. Allergy Clin. Immunol.* 62, 162–166. doi: 10.1016/0091-6749(78)90101-x

Shah, S. (2012). Hormonal link to autoimmune allergies. *ISRN Allergy* 2012:910437. doi: 10.5402/2012/910437

Sharma, R., Kearns, M. M., Sarr, F., and Ismail, N. (2019). The adaptive immune and stress responses of adult female CD1 mice following exposure to a viral mimetic. *Immunol. Lett.* 208, 30–38. doi: 10.1016/j.imlet.2019.03.005

Shepherd, R., Cheung, A. S., Pang, K., Saffery, R., and Novakovic, B. (2020). Sexual dimorphism in innate immunity: the role of sex hormones and epigenetics. *Front. Immunol.* 11:604000. doi: 10.3389/fimmu.2020.604000

Shirshev, S. V., Nekrasova, I. V., Gorbunova, O. L., and Orlova, E. G. (2019). Effect of estriol, chorionic gonadotropin, and oncostatin M on the expression of recombinase RAG-1 in regulatory T lymphocyte subpopulations. *Bull. Exp. Biol. Med.* 167, 57–61. doi: 10.1007/s10517-019-04460-w

Shors, T. J., Millon, E. M., Chang, H. Y., Olson, R. L., and Alderman, B. L. (2017). Do sex differences in rumination explain sex differences in depression? *J. Neurosci. Res.* 95, 711–718. doi: 10.1002/jnr.23976

Shultz, D. (2016). *Can 'Dirty Mice' Save Animal Research?*. Available online at: https://www.science.org/content/article/can-dirty-mice-save-animal-research (accessed February 25, 2022).

Singh, R. P., and Bischoff, D. S. (2021). Sex Hormones and Gender Influence the Expression of Markers of Regulatory T Cells in SLE Patients. *Front Immunol* 12:619268. doi: 10.3389/fimmu.2021.619268

Skovlund, C. W., Morch, L. S., Kessing, L. V., and Lidegaard, O. (2016). Association of hormonal contraception with depression. *JAMA Psychiatry* 73, 1154–1162. doi: 10.1001/jamapsychiatry.2016.2387

Soares, C. N. (2017). Depression and menopause: current knowledge and clinical recommendations for a critical window. *Psychiatr. Clin. North Am.* 40, 239–254. doi: 10.1016/j.psc.2017.01.007

Soares, C. N., Almeida, O. P., Joffe, H., and Cohen, L. S. (2001). Efficacy of estradiol for the treatment of depressive disorders in perimenopausal women: a double-blind, randomized, placebo-controlled trial. *Arch. Gen. Psychiatry* 58, 529–534. doi: 10.1001/archpsyc.58.6.529

Somerset, D. A., Zheng, Y., Kilby, M. D., Sansom, D. M., and Drayson, M. T. (2004). Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. *Immunology* 112, 38–43. doi: 10.1111/j.1365-2567.2004.01869.x

Song, C., Dinan, T., and Leonard, B. E. (1994). Changes in immunoglobulin, complement and acute phase protein levels in the depressed patients and normal controls. *J. Affect. Disord.* 30, 283–288. doi: 10.1016/0165-0327(94)90135-x

Steel, Z., Marnane, C., Iranpour, C., Chey, T., Jackson, J. W., Patel, V., et al. (2014). The global prevalence of common mental disorders: a systematic review and meta-analysis 1980-2013. *Int. J. Epidemiol.* 43, 476–493. doi: 10.1093/ije/dyu038

Stopińska-Głuszak, U., Waligóra, J., Grzela, T., Głuszak, M., Jóźwiak, J., Radomski, D., et al. (2006). Effect of estrogen/progesterone hormone replacement therapy on natural killer cell cytotoxicity and immunoregulatory cytokine release by peripheral blood mononuclear cells of postmenopausal women. *J. Reprod. Immunol.* 69, 65–75. doi: 10.1016/j.jri.2005.07.006

Sundström-Poromaa, I. (2018). The menstrual cycle influences emotion but has limited effect on cognitive function. *Vitam Horm* 107, 349–376. doi: 10.1016/bs.vh.2018.01.016

Swartz, J. R., Prather, A. A., Di Iorio, C. R., Bogdan, R., and Hariri, A. R. (2017). A Functional interleukin-18 haplotype predicts depression and anxiety through increased threat-related amygdala reactivity in women but not men. *Neuropsychopharmacology* 42, 419–426. doi: 10.1038/npp.2016.129

Takahashi, A., Chung, J. R., Zhang, S., Zhang, H., Grossman, Y., Aleyasin, H., et al. (2017). Establishment of a repeated social defeat stress model in female mice. *Sci. Rep.* 7:12838. doi: 10.1038/s41598-017-12811-8

Tao, L., and Reese, T. A. (2017). making mouse models that reflect human immune responses. *Trends Immunol.* 38, 181–193. doi: 10.1016/j.it.2016.12.007

Thase, M. E., Entsuah, R., Cantillon, M., and Kornstein, S. G. (2005). Relative antidepressant efficacy of venlafaxine and SSRIs: sex-age interactions. *J. Womens Health (Larchmt)* 14, 609–616. doi: 10.1089/jwh.2005.14.609

Tonelli, L. H., Holmes, A., and Postolache, T. T. (2008). Intranasal immune challenge induces sex-dependent depressive-like behavior and cytokine expression in the brain. *Neuropsychopharmacology* 33, 1038–1048. doi: 10.1038/sj.npp.1301488

Toniolo, A., Fadini, G. P., Tedesco, S., Cappellari, R., Vegeto, E., Maggi, A., et al. (2015). Alternative activation of human macrophages is rescued by estrogen treatment in vitro and impaired by menopausal status. *J. Clin. Endocrinol. Metab.* 100, E50–E58. doi: 10.1210/jc.2014-2751

Trivedi, M. H., Rush, A. J., Wisniewski, S. R., Nierenberg, A. A., Warden, D., Ritz, L., et al. (2006). Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am. J. Psychiatry* 163, 28–40. doi: 10.1176/appi.ajp.163.1.28

Uppal, S. S., Verma, S., and Dhot, P. S. (2003). Normal values of CD4 and CD8 lymphocyte subsets in healthy indian adults and the effects of sex, age, ethnicity, and smoking. *Cytometry B Clin. Cytom.* 52, 32–36. doi: 10.1002/cyto.b.10011

Vélez-Ortega, A. C., Temprano, J., Reneer, M. C., Ellis, G. I., McCool, A., Gardner, T., et al. (2013). Enhanced generation of suppressor T cells in patients with asthma taking oral contraceptives. *J. Asthma* 50, 223–230. doi: 10.3109/02770903.2012.761231

Verthelyi, D. (2001). Sex hormones as immunomodulators in health and disease. *Int. Immunopharmacol.* 1, 983–993. doi: 10.1016/s1567-5769(01)00044-3

Verthelyi, D. I., and Ahmed, S. A. (1998). Estrogen increases the number of plasma cells and enhances their autoantibody production in nonautoimmune C57BL/6 mice. *Cell Immunol.* 189, 125–134. doi: 10.1006/cimm.1998.1372

Vetrano, M., Wegman, A., Koes, B., Mehta, S., and King, C. A. (2020). Serum IL-1RA levels increase from follicular to luteal phase of the ovarian cycle: a pilot study on human female immune responses. *PLoS One* 15:e0238520. doi: 10.1371/journal.pone.0238520

Villa, A., Rizzi, N., Vegeto, E., Ciana, P., and Maggi, A. (2015). Estrogen accelerates the resolution of inflammation in macrophagic cells. *Sci. Rep.* 5:15224. doi: 10.1038/srep15224

Vink, N. M., Postma, D. S., Schouten, J. P., Rosmalen, J. G., and Boezen, H. M. (2010). Gender differences in asthma development and remission during transition through puberty: the TRacking Adolescents' Individual Lives Survey (TRAILS) study. *J. Allergy Clin. Immunol.* 126, .498–504.e1–6. doi: 10.1016/j.jaci.2010.06.018

Vom Steeg, L. G., Flores-Garcia, Y., Zavala, F., and Klein, S. L. (2019). Irradiated sporozoite vaccination induces sex-specific immune responses and protection against malaria in mice. *Vaccine* 37, 4468–4476. doi: 10.1016/j.vaccine.2019.06.075

Vrachnis, N., Zygouris, D., Vrachnis, D., Antonakopoulos, N., Fotiou, A., Panagopoulos, P., et al. (2021). Effects of hormone therapy and flavonoids capable on reversal of menopausal immune senescence. *Nutrients* 13:2363. doi: 10.3390/nu13072363

Wagar, L. E., DiFazio, R. M., and Davis, M. M. (2018). Advanced model systems and tools for basic and translational human immunology. *Genome Med.* 10:73. doi: 10.1186/s13073-018-0584-8

Waitzfelder, B., Stewart, C., Coleman, K. J., Rossom, R., Ahmedani, B. K., Beck, A., et al. (2018). Treatment initiation for new episodes of depression in primary care settings. *J. Gen. Intern. Med.* 33, 1283–1291. doi: 10.1007/s11606-017-4297-2

Walf, A. A., and Frye, C. A. (2009). Effects of two estradiol regimens on anxiety and depressive behaviors and trophic effects in peripheral tissues in a rodent model. *Gend. Med.* 6, 300–311. doi: 10.1016/j.genm.2009.04.004

Walker, S. E. (2011). Estrogen and autoimmune disease. *Clin. Rev Allergy Immunol.* 40, 60–65. doi: 10.1007/s12016-010-8199-x

Wang, L., Jiang, P., Zhao, S., Liu, H., Liu, L., Mor, G., et al. (2021). The dynamic profile and potential function of B-cell subsets during pregnancy. *Cell Mol. Immunol.* 18, 1082–1084. doi: 10.1038/s41423-020-00535-1

Wang, Q., Timberlake, M. A. II, Prall, K., and Dwivedi, Y. (2017). The recent progress in animal models of depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 77, 99–109. doi: 10.1016/j.pnpbp.2017.04.008

Wang, Y. T., Wang, X. L., Feng, S. T., Chen, N. H., Wang, Z. Z., and Zhang, Y. (2021). Novel rapid-acting glutamatergic modulators: targeting the synaptic plasticity in depression. *Pharmacol. Res.* 171:105761. doi: 10.1016/j.phrs.2021.105761

Wegienka, G., Bobbitt, K. R., Woodcroft, K. J., and Havstad, S. (2011). Regulatory T cells vary over bleeding segments in asthmatic and non-asthmatic women. *J. Reprod. Immunol.* 89, 192–198. doi: 10.1016/j.jri.2011.03.002

Weinberg, A., Enomoto, L., Marcus, R., and Canniff, J. (2011). Effect of menstrual cycle variation in female sex hormones on cellular immunity and regulation. *J. Reprod. Immunol.* 89, 70–77. doi: 10.1016/j.jri.2010.11.009

Weissman, M. M., and Klerman, G. L. (1977). Sex differences and the epidemiology of depression. *Arch. Gen. Psychiatry* 34, 98–111. doi: 10.1001/archpsyc.1977.01770130100011

Wenzel, A. (2016). *The Oxford Handbook of Perinatal Psychology*. Oxford: Oxford University Press.

Weyand, C. M., and Goronzy, J. J. (2016). Aging of the immune system. mechanisms and therapeutic targets. *Ann. Am. Thorac. Soc.* 13 Suppl 5(Suppl 5), S422–S428. doi: 10.1513/AnnalsATS.201602-095AW

Wharton, W., Gleason, C. E., Olson, S. R., Carlsson, C. M., and Asthana, S. (2012). Neurobiological underpinnings of the estrogen – mood relationship. *Curr. Psychiatry Rev.* 8, 247–256. doi: 10.2174/157340012800792957

Willner, P. (2017). The chronic mild stress (CMS) model of depression: history, evaluation and usage. *Neurobiol. Stress* 6, 78–93. doi: 10.1016/j.ynstr.2016.08.002

Wohleb, E. S., Franklin, T., Iwata, M., and Duman, R. S. (2016). Integrating neuroimmune systems in the neurobiology of depression. *Nat. Rev. Neurosci.* 17, 497–511. doi: 10.1038/nrn.2016.69

Woitowich, N. C., and Woodruff, T. K. (2019). Opinion: research community needs to better appreciate the value of sex-based research. *Proc. Natl. Acad. Sci. U.S.A.* 116, 7154–7156. doi: 10.1073/pnas.1903586116

Xu, W., Wong, G., Hwang, Y. Y., and Larbi, A. (2020). The untwining of immunosenescence and aging. *Semin. Immunopathol.* 42, 559–572. doi: 10.1007/s00281-020-00824-x

Yang, J. H., Chen, C. D., Wu, M. Y., Chao, K. H., Yang, Y. S., and Ho, H. N. (2000). Hormone replacement therapy reverses the decrease in natural killer cytotoxicity but does not reverse the decreases in the T-cell subpopulation or interferon-gamma production in postmenopausal women. *Fertil Steril* 74, 261–267. doi: 10.1016/s0015-0282(00)00622-1

Yang, T., Nie, Z., Shu, H., Kuang, Y., Chen, X., Cheng, J., et al. (2020). The role of BDNF on neural plasticity in depression. *Front. Cell Neurosci.* 14:82. doi: 10.3389/fncel.2020.00082

Yin, D., Tuthill, D., Mufson, R. A., and Shi, Y. (2000). Chronic restraint stress promotes lymphocyte apoptosis by modulating CD95 expression. *J. Exp. Med.* 191, 1423–1428. doi: 10.1084/jem.191.8.1423

Zainal, N. H., and Newman, M. G. (2021). Increased inflammation predicts nine-year change in major depressive disorder diagnostic status. *J. Abnorm Psychol.* 130, 829–840. doi: 10.1037/abn0000716

Zhang, T., Dong, Z., Cai, H., Rong, J., and Su, Z. (2020). Estradiol regulates the expression of CD45 splicing isoforms in lymphocytes. *Mol. Biol. Rep.* 47, 3025–3030. doi: 10.1007/s11033-020-05373-y

Zhao, W., Li, Q., Ma, Y., Wang, Z., Fan, B., Zhai, X., et al. (2021). Behaviors related to psychiatric disorders and pain perception in C57BL/6J mice during different phases of estrous cycle. *Front. Neurosci.*15:650793. doi: 10.3389/fnins.2021.650793

Zimmerman, M., Ellison, W., Young, D., Chelminski, I., and Dalrymple, K. (2015). How many different ways do patients meet the diagnostic criteria for major depressive disorder? *Compr Psychiatry* 56, 29–34. doi: 10.1016/j.comppsych.2014.09.007

Zong, S., Hoffmann, C., Mané-Damas, M., Molenaar, P., Losen, M., and Martinez-Martinez, P. (2017). Neuronal surface autoantibodies in neuropsychiatric disorders: are there implications for depression? *Front. Immunol.* 8:752. doi: 10.3389/fimmu.2017.00752

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frontiers | Frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 02 June 2022
doi: 10.3389/fnbeh.2022.856276

The Cycling Brain in the Workplace: Does Workload Modulate the Menstrual Cycle Effect on Cognition?

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Recent decades have witnessed increased research efforts to clarify how the menstrual cycle influence females' cognitive and emotional functions. Despite noticeable progress, the research field faces the challenges of inconsistency and low generalizability of research findings. Females of reproductive ages are a heterogeneous population. Generalizing the results of female undergraduates to women in the workplace might be problematic. Furthermore, the critical cognitive processes for daily life and work deserve additional research efforts for improved ecological validity. Thus, this study investigates cognitive performance across the menstrual cycle using a sample of young nurses with similar duties. We developed a mini-computerized cognitive battery to assess four mental skills critical for nursing work: cognitive flexibility, divided attention, response inhibition, and working memory. Participants completed the cognitive battery at menses, late-follicular, and mid-luteal phases. In addition, they were classified into low- and high workload groups according to their subjective workload ratings. Our results demonstrate a general mid-luteal cognitive advantage. Besides, this study reveals preliminary evidence that workload modulates the menstrual cycle effect on cognition. Only females of low workload manifest the mid-luteal cognitive advantage on divided attention and response inhibition, implying that a suitable workload threshold might be necessary for regular neuro-steroid interactions. Thus, this study advocates the significance of research focusing on the cycling brain under workloads.

Keywords: menstrual cycle, workload, cognitive flexibility, inhibitory control, divided attention

OPEN ACCESS

Edited by:
Jaclyn M. Schwarz, University of Delaware, United States

Reviewed by:
Caroline Gurvich, Monash University, Australia
Belinda Pletzer, University of Salzburg, Austria

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Specialty section:
This article was submitted to Behavioral Endocrinology, a section of the journal Frontiers in Behavioral Neuroscience

Received: *17 January 2022*
Accepted: *06 May 2022*
Published: *02 June 2022*

Citation:
Xu M, Chen D, Li H, Wang H and Yang L-Z (2022) The Cycling Brain in the Workplace: Does Workload Modulate the Menstrual Cycle Effect on Cognition? Front. Behav. Neurosci. 16:856276. doi: 10.3389/fnbeh.2022.856276

INTRODUCTION

Ovarian hormones, such as estradiol and progesterone, fluctuate during the menstrual cycle in healthy females of reproductive age. The estradiol levels gradually increase after the menses phase, peaking in the late follicular phase and then dropping after ovulation and rising again in the mid-luteal phase to moderate levels. The progesterone levels increase after ovulation and peak in the middle of the luteal phase. Then, the two ovarian hormones drop to the lowest levels before the onset of the next menses. Thus, the menstrual cycle is a convenient and ecological model of ovarian hormones. Recent years have witnessed an explosion of research on how sex hormones and the menstrual cycle shape female brains (Barth et al., 2015; Ycaza Herrera et al., 2019; Beltz and Moser, 2020; Le et al., 2020; Dubol et al., 2021; Hidalgo-Lopez and Pletzer, 2021).

Estradiol and progesterone have neuroactive effects. The hypothalamic-pituitary-gonadal (HPG) axis regulates the reproductive processes and modulates cognitive and emotional functions through direct or indirect projection to the prefrontal cortex, hippocampus, thalamus, and brainstem (Morrison et al., 2006; Le et al., 2020). It has long been hypothesized that cognitive performance across the menstrual cycle might vary due to the fluctuation of ovarian hormones. The effect of the menstrual cycle has been found on social preference (Durante et al., 2014; Zhuang and Wang, 2014; Wang and Chen, 2020; Wang et al., 2021), cognitive ability (Hussain et al., 2016; Hidalgo-Lopez and Pletzer, 2017; Leeners et al., 2017; Pletzer et al., 2017; Scheuringer and Pletzer, 2017), motor learning (Ikarashi et al., 2020), cortical structures (Lisofsky et al., 2015; Catenaccio et al., 2016; Pletzer et al., 2018), and brain functions (Barth et al., 2016; Diekhof and Ratnayake, 2016; Hidalgo-Lopez and Pletzer, 2019, 2021; Pletzer et al., 2019; Wang et al., 2020a). Moreover, the late follicular or luteal phase advantage on cognition has always been advocated because of high neuroprotective steroids (Sundstrom-Poromaa and Gingnell, 2014; Zhuang et al., 2020; Wang et al., 2021). For example, previous studies suggest that females show superior social cognitive performance during their luteal than menses or follicular phase (Wang and Chen, 2020; Wang et al., 2020a,b, 2021). Although the neuroprotective role of estradiol has been advocated, the role of progesterone remains ambiguous (Baudry et al., 2013). Some studies suggest that progesterone may antagonize rather than synergize estradiol effects (Rosario et al., 2006; Carroll et al., 2008). It is hard to separate the effects of progesterone and estradiol across the menstrual cycle because both hormone levels are high in the mid-luteal phase. Thus, studying the three different phases across the menstrual cycle is necessary to clarify the relationship between hormones.

Despite the appealing association between the menstrual cycle and cognition, the empirical evidence is far from consistent (Sundstrom-Poromaa and Gingnell, 2014; Sundstrom-Poromaa, 2018; Beltz and Moser, 2020; Le et al., 2020). The evolutionary hypothesis implicated that women might show visuospatial ability advantage during their early follicular phase (low ovarian steroids) and verbal ability advantage in the luteal phase (high ovarian steroids). However, Sundstrom-Poromaa and Gingnell (2014) summarized that the supporting evidence is insufficient in the literature. In addition, their following review further suggests that the menstrual cycle might influence emotion, but has a limited effect on cognitive function (Sundstrom-Poromaa, 2018). However, recent neuroimaging studies have revealed consistent evidence that the menstrual cycle modulates the structure, functional activation, and connectivity of brain regions that are responsible for cognitive control (e.g., prefrontal cortex) and memory (e.g., hippocampus) (Beltz and Moser, 2020; Dubol et al., 2021). Thus, it might be too soon to reject the menstrual cycle's potential effect on "cold" cognition.

The inconsistency might be because the effect of the menstrual cycle is too transient to be captured by behavioral assessment or noise due to methodological flaws (Le et al., 2020). However, it has been long overlooked in the field that healthy females of reproductive age are a heterogeneous population with huge variability in their social-economic status, years of education, social support, occupation, and work pressure. Many studies recruited undergraduate or graduated female students from the campus or females in nearby communities of different professions. These findings might not generalize seamlessly to some specific populations. Only a few studies have employed homogenous samples within a particular workplace, such as nurses (Hatta and Nagaya, 2009). Investigating the cycling brain in specific workplaces is, thus, a valuable research direction.

Furthermore, the inconsistency might be due to the menstrual cycle's interaction with other factors (Bernal and Paolieri, 2022). It has been proposed that estrogen and progesterone interact with cognition-related neurotransmitter systems, including serotoninergic, dopaminergic, gamma-aminobutyric-acid (GABA)-ergic, and glutamatergic pathways, with profound effects on brain structure and function (Barth et al., 2015). For example, recent evidence shows that the effect of estradiol status on working memory function depends on the baseline dopamine levels (Jacobs and D'Esposito, 2011). Using the eye blink rate (EBR), an indicator of striatal dopamine levels, one following study reveals that females with lower EBR showed superior Stroop performance during their luteal phase and vice versa (Hidalgo-Lopez and Pletzer, 2017). Hidalgo-Lopez and Pletzer (2019) recent work also suggests that baseline performance modulates the menstrual cycle effect on the inhibitory control ability. Besides the factors mentioned above, there might be many contextual and individual factors deserving increasing research attention.

Female nurses account for 90% of the global nursing workforce and play irreplaceable roles in public health (World Health Organization, 2020). Meanwhile, female nurses undertake noticeable workloads. For example, in China, on average, a nurse in a general hospital takes care of 8 patients in the daytime and 23 patients at night (Shen et al., 2020). Compared with other careers, the nursing job characterizes by mental pressures induced by multitasking and attentional interferences. Potter et al. (2005) use a cognitive task analysis methodology to reveal that a nurse must hold 11 activities in mind in the acute care work setting. The nursing job is also full of interruptions associated with procedure failures and clinical errors (Westbrook et al., 2010). Thus, an efficient nurse needs to switch flexibly among tasks (cognitive flexibility), attend to patients and clinical signals simultaneously (divided attention), inhibit automatic, habitual but inappropriate actions (response inhibition), and store necessary information in mind (working memory). Research focusing on the female nurse population is, thus, valuable for promoting their occupational health.

Previous studies have suggested that hormones from the hypothalamic-pituitary-adrenal (HPA) axis regulate the HPG axis (Oyola and Handa, 2017). The HPA axis is the coordinator of the brain's fight-or-flight response, which increases cortisol production to deal with stressful events. Previous studies have also demonstrated an inverted U-shaped relationship between workload and task performance (Ma et al., 2020). However, it is still ambiguous whether workload would interact with the menstrual cycle to affect cognitive performance. This study investigates whether workload modulates the cycling brain using a homogenous nurse sample. The workload here refers to the

cognitive, emotional, and physiological resources expended to complete the task requirement (Alghamdi, 2016). We chose four representative cognitive paradigms (task-switching, divided attention, spatial Stroop, and multiple change detection) to target core mental skills necessary for nursing work. In addition, a self-report measure, namely the National Aeronautics and Space Administration Task Load Index (NASA-TLX), quantifies the nursing workload, which can tease apart six sources of work pressures (Hart, 2016). Although the workload can be evaluated physiologically (Borghini et al., 2014), self-report measures are helpful to provide a convenient, inexpensive, reliable, and valid sampling (Wickens, 2008). We hypothesized that female nurses perform better during their mid-luteal phase. In addition, workload might be a potential modulatory factor of the menstrual cycle effect.

MATERIALS AND METHODS

Participants

We recruited 96 healthy right-handed female registered nurses in a local hospital. All of them had a regular menstrual cycle of 24–35 days (Le et al., 2020) and variability between cycles of less than 7 days in the past 3 months, with normal or corrected-to-normal vision, had not taken oral contraceptive or other hormonal medications within the previous 3 months, no history of nicotine or alcohol abuse, no sleep disorders, and no neurological, psychiatric, or endocrine disorders, including premenstrual dysphoric disorder (PMDD) and premenstrual syndrome (PMS). Ten participants were excluded due to their actual cycle phase falling out of the normal range during the experiment session according to their follow-up report on the onset of the next cycle. Seven participants dropped out for personal reasons, leaving a final sample of 79 nurses (M = 25.52 years, SD = 4.33 years) with a mean cycle length of 29.42 days (SD = 1.69). The study was approved by the local ethics committee and was conducted following the Declaration of Helsinki. All participants gave written informed consent and received monetary compensation.

Research Procedure

Participants completed an online screening questionnaire to determine whether they were eligible to participate in the study. Eligible participants enrolled in the test session were required to record their menses' start date and duration for at least 3 months to double-check whether their menstrual cycle is regular. The first author (MX) interviewed them privately to survey their subjective nursing workload and check their menstrual cycle information and calculate their cycle phase for those participants. The menstrual cycle mapping was determined using the backward counting procedure widely used in the literature (Zhuang and Wang, 2014; Hidalgo-Lopez and Pletzer, 2017; Schaumberg et al., 2017; Wang et al., 2021). Specifically, we defined the menses phase (low estradiol levels, low progesterone levels) as the 1–4 days after the onset of menstruation; the late-follicular phase (estradiol levels peak and low progesterone levels) as the 3 days before the predicted ovulation; and the mid-luteal phase (moderate estradiol levels and high progesterone levels) as 3 days after the expected ovulation to 3 days before the next onset of the menstruation. We calculated the predicted ovulation by subtracting 14 days from the expected next menstruation onset, determined using each participant's average cycle length in the last 3 months.

The study was a within-subject, longitudinal design. Thus, participants attended three behavior test sessions during the menses, late-follicular, and mid-luteal phases. These cycle phases were set apart by at least 6 days. The starting session was counterbalanced among participants. About one-third of participants started their first session in the menses, one-third in the late-follicular, and one-third in the mid-luteal phase. For each test session, participants first rated their negative emotions of the past week. Then, they completed a ∼50-min mini-computerized cognitive battery, including inhibitory control, cognitive flexibility, divided attention, and working memory. The experiment environment was a quiet room. A well-trained graduate student (MX) instructed and monitored tests of all participants. After the third test session, participants were tracked for their subsequent menses to validate their predicted cycle phase falling into the normal range. Those who violated were excluded from analysis even after completing the study.

Self-Assessment Scales

National Aeronautics and Space Administration Task Load Index

The NASA-TLX measured the subjective workload using six subscales: mental demand, physical demand, temporal demand, performance, effort, and frustration levels (Tubbs-Cooley et al., 2018). Each subscale is rated on a 20-point scale (0 = low, 20 = high), but for the performance scale (0 = good, 20 = poor). Higher scores indicate increased workloads.

Depression Anxiety Stress Scale-21

The Depression Anxiety Stress Scale-21 (DASS-21) measured negative emotions in the past week, including depression, anxiety, and stress subscale. Each subscale contains seven items. Each item was scored on a 4-point scale ranging from 0 to 3. The final score was the summed score multiplied by two (Henry and Crawford, 2005). Higher scores indicate higher depression, anxiety, or stress levels, respectively.

The Mini-Computerized Cognitive Battery

The mini-computerized cognitive battery was developed using GNU Octave and Psychtoolbox 3.16 (Brainard, 1997; Pelli, 1997) under the UBUNTU 18.04 system on a Thinkpad T61 laptop (12-inch, 1024 × 768 pixels, 50 Hz refresh rate). Participants completed the battery sitting about 50 cm in front of the laptop screen in a quiet room. The battery included four tasks measuring inhibitory control, cognitive flexibility, divided attention, and working memory capacity.

Measure of Inhibitory Control

The spatial Stroop task is a paradigm measuring inhibitory control (Aidman et al., 2019). A typical trial starts with a fixation

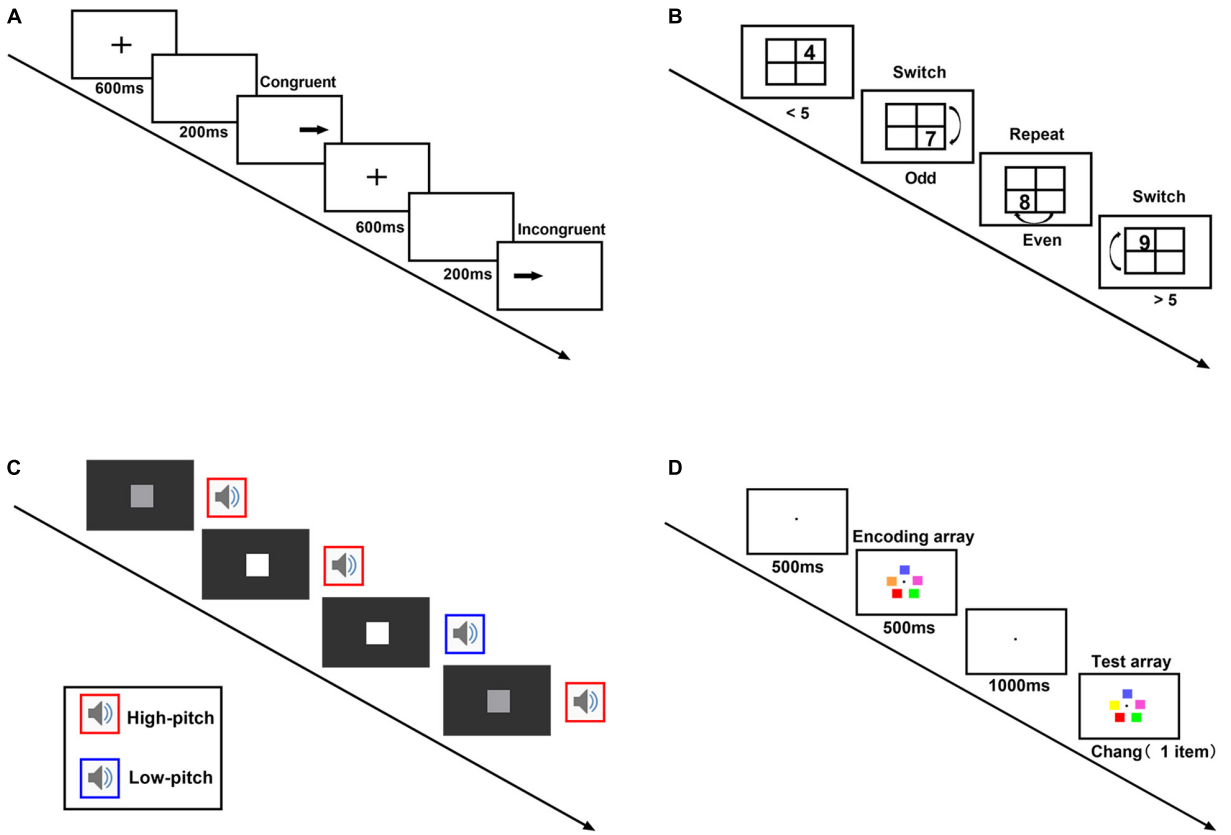

FIGURE 1 | The mini-computerized cognitive battery. **(A)** Spatial Stroop task. A typical trial starts with a fixation (600 ms). After a blank screen (200 ms), an arrow appears on the left or the right side of the screen. Participants needed to report the arrow direction by pressing corresponding keys as soon as possible with the accuracy ensured. **(B)** Task-switch task. On each trial, a number (randomly selected from 1, 2, 3, 4, 6, 7, 8, 9) appeared in a cell of a 2 × 2 grid. If the number appeared in the upper row, participants needed to respond whether it was greater or less than 5. If the number appeared in the lower row, they answered whether it was even or odd. The first number appeared in the right-top cell and changed location clock-wisely in the subsequent trials. **(C)** Divided attention task. A square appears at regular intervals on the screen, and at the same time, participants listen to a sound. Every trial appeared every 1 s and lasted 1 s each. Participants needed to detect changes in either the visual sequence or the audio sequence. Whenever the square gets noticeably lighter, or the sound gets noticeably higher pitch twice in a row, they need to press the space bar as soon as possible. **(D)** Working memory task. A typical trial starts with a fixation (500 ms), followed by an encoding array (5 colored squares). The colored squares appeared on an imaginary circle (500 ms). A test array appeared after a 1,000 ms delay. The test array could have 0, 1, 2, or 5 changed items with equal probability. Participants had to indicate whether the test array and the encoding array differed.

in the screen center for 600 ms. After a blank screen of 200 ms, an arrow pointing leftward or rightward appeared on the left or the right side of the screen. Participants needed to report the arrow direction by pressing corresponding keys as soon as possible with accuracy ensured. The primary interest variable was the congruency between the arrow direction (leftward, rightward) and their position (left side, right side) (**Figure 1A**). There were 10 practice trials and 60 test trials, including 24 congruent and 36 incongruent trials. The error rate for each condition was summarized. We also calculated the mean reaction time after removal of error trials and trials too fast (< 150 ms) or too slow (> 1,500 ms).

Measure of Cognitive Flexibility

The task-switching paradigm measures cognitive flexibility (Monsell, 2003; Aidman et al., 2019). A number randomly chosen from 1 to 9 appeared in one cell of a 2 × 2 grid for each trial. If the number appeared in the upper row, participants needed to respond whether it was greater or less than 5. If the number appeared in the lower row, they answered whether it was even or odd. The first number appeared in the right-top cell and changed location clockwise in the subsequent trials. The trial was in repeat condition (same task rule) if the number appeared on the top-right and bottom-left cell; other trials were in switch condition (change of task rule) (**Figure 1B**). There were 12 practice trials and 60 test trials, including 29 repeat trials (with the first trial discarded) and 30 switching trials. We calculated error rates and mean reaction time for each condition. Error trials and trials too fast (< 150 ms) or low (> 1,500 ms) were excluded from reaction time analysis.

Measure of Divided Attention

The audiovisual cross-modal monitoring task measures divided attention ability (Himi et al., 2019). A square appears at regular intervals on the screen center, and, at the same time, participants listen to a sound. Participants needed to detect changes in either

the visual sequence or the audio sequence. Sometimes the square gets noticeably lighter, and sometimes the sound gets a noticeably higher pitch. Whenever the square gets noticeably lighter or the sound gets noticeably higher pitch twice in a row, they need to press the space bar as soon as possible (**Figure 1C**). Thirty-two practice trials were followed by the test trials, which consisted of 200 trials. Every trial appeared every 1 s and lasted 1 s each. The index of the task was the sensitivity calculated according to signal detection theory using the non-parametric sensitivity measure (A') (Stanislaw and Todorov, 1999) and the mean reaction time of correct responses.

Measure of Working Memory Capacity

The multiple change detection paradigm estimates working memory capacity (Gold et al., 2019). A typical trial starts with a fixation in the screen center for 500 ms, followed by an encoding array (5 colored squares). The colored squares appeared on an imaginary circle with a radius of 120 pixels centered on the center of the screen for 500 ms. A test array appeared after a 1,000-ms delay. The test array could have 0, 1, 2, or 5 changed items with equal probability. Participants had to indicate whether the test array and the encoding array differed (**Figure 1D**). There were 240 trials with 60 trials for each change type. The working memory capacity (K) was estimated according to a computational model (Feuerstahler et al., 2019) and used R (R Core Team, 2021) and the est_KAG function.[1]

Statistical Analysis

Participants were classified into high and low workload groups using the mean value of NASA TLX total scores of all participants as the cut-off criterion. The group difference was examined using the independent-samples t-test and chi-square test. We conducted an omnibus mixed factorial analysis of variance (ANOVA) first for each emotion and task measure and performed *post hoc* comparisons using the LSD method if necessary. The p-value was adjusted using the Greenhouse–Geisser procedure in case of violation of the sphericity hypothesis. We translate p-values in the language of evidence to avoid the black-or-white null-hypothesis testing with an arbitrary p-value cut-off (Muff et al., 2022). The statistical analysis software was IBM SPSS Statistics for Windows (Version 22.0. Armonk, NY, United States: IBM). To exclude the practice effect and potential confounding effect of age, we also conducted linear mixed model analyses by controlling the effect of the session and participants' age. The supplementary analysis, in the form of an *Rnotebook*, is available online (see Data Availability Statement). The linear mixed-effect model was conducted using *R (version 4.1.1)* (R Core Team, 2021), *afex* (Singmann et al., 2021), and *lme4* (Bates et al., 2015) package.

RESULTS

Subjective Workload

We divided participants into low (n = 41) and high (n = 38) workload groups according to the mean NASA-TLX score (M = 69.65, SD = 14.81). **Table 1** compares the low and high workloads groups on demographic information and mental health measures.

Effect of Workload and Menstrual Cycle on Negative Emotion

A mixed factorial ANOVA of 2 (group: low, high workload) × 3 (cycle phase: menses, late follicular, mid-luteal) was conducted for depression, anxiety, and stress scores, respectively. The results revealed no evidence that the high and the low workload group differed on each emotion subscale (all $ps > 0.29$, see **Supplementary Table 1** for detailed information). There was no evidence of the effect of the cycle phase, no matter in terms of main effect or interaction effect, on the depression and stress scores (all $ps > 0.1$). However, there was weak evidence of the main effect of the cycle phase on the anxiety score ($p = 0.071$).

Effect of Workload and Menstrual Cycle on Inhibitory Control

A mixed factorial ANOVA of 2 (group: low, high workload) × 3 (cycle phase: menses, late follicular, mid-luteal) × 2 (congruency: congruent, incongruent) was conducted on error rate and reaction time, respectively. See **Supplementary Table 2** for a summary of the ANOVA analysis.

On the measure of error rate, there was moderate evidence for a main effect of congruency [$F(1, 77) = 4.873$, $p = 0.03$, $\eta^2 = 0.06$], indicating generally more errors in the incongruent ($M = 0.020$, SE = 0.003) than the congruent condition ($M = 0.014$, SE = 0.002). The statistical evidence supporting the main effect of group and cycle phase was little or no (all $ps > 0.2$). Besides, we found little or no evidence for the two-way interactions (all $ps > 0.05$). However, there was moderate evidence for the interaction effect among the three factors [$F(2, 154) = 3.885$, $p = 0.023$, $\eta^2 = 0.048$]. A repeated-measures ANOVA of 3 (cycle phase: menses, late follicular, and mid-luteal) × 2 (congruency: congruent, incongruent) was then performed for the low workload group and the high workload group, respectively.

There was only weak evidence for the low workload group for the main effect of the cycle phase [$F(1.56, 62.3) = 2.87$, $p = 0.076$, $\eta^2 = 0.067$]. However, there was strong evidence for the main effect of congruency [$F(1, 40) = 7.76$, $p = 0.008$, $\eta^2 = 0.163$], and moderate evidence for the interaction effect between the cycle phase and the congruency [$F(2, 80) = 4.56$, $p = 0.013$, $\eta^2 = 0.102$]. Follow-up analyses found no evidence that performance on the congruent condition varied among the cycle phase [$F(2, 39) = 0.39$, $p = 0.68$, $\eta^2 = 0.02$]. However, on the error rate of the incongruent condition, there was strong evidence for the main effect of the cycle phase [$F(2, 39) = 5.76$, $p = 0.006$, $\eta^2 = 0.23$]. Error rates of the mid-luteal phase were lower than the menses ($p = 0.003$) and the late follicular phase ($p = 0.037$). There was little or no evidence for the difference between the menses and the late follicular phase on the incongruent condition ($p = 0.183$). We found little or no evidence for the main effect and interaction term involving the cycle phase in the high

[1] https://github.com/leahfeuerstahler/vwm

TABLE 1 | Demographics and work characteristics of the sample.

Variables	Low (*n* = 41)	High (*n* = 38)	*p*-value
Cycle length, mean (SD)	29.6 (1.81)	29.2 (1.64)	0.224
Age, mean (SD)	24.1 (3.81)	27.1 (4.40)	0.002
BMI, mean (SD)	20.6 (2.65)	21.2 (2.07)	0.286
Marital status, *n* (%)			0.093
Single	32 (78.0%)	22 (57.9%)	
Married	9 (22.0%)	16 (42.1%)	
Education level, *n* (%)			0.098
Specialist qualification	29 (70.7%)	19 (50.0%)	
Bachelor degree	12 (29.3%)	19 (50.0%)	
Monthly income, *n* (%)			0.126
< 4000 RMB	30 (73.2%)	19 (50.0%)	
< 6000 RMB	9 (22.0%)	11 (28.9%)	
< 8000 RMB	1 (2.44%)	4 (10.5%)	
> 8000 RMB	1 (2.44%)	4 (10.5%)	
BDI, mean (SD)	3.05 (2.77)	2.66 (2.81)	0.536
GAD-7, mean (SD)	3.34 (2.52)	3.76 (2.89)	0.493

BMI, Body mass index; BDI, Beck depression inventory; GAD-7, Generalized anxiety disorder 7-item scale; SD, Standard deviation.

workload group [cycle: $F(2, 74) = 0.17$, $p = 0.846$, $\eta^2 = 0.005$, cycle × congruency: $F(2, 74) = 1.18$, $p = 0.312$, $\eta^2 = 0.031$]. See **Figure 2** for an illustration.

On the measure of reaction time, there was strong evidence for the main effect of congruency [$F(1, 77) = 12.759$, $p = 0.001$, $\eta^2 = 0.142$], indicating faster responses in the congruent condition ($M = 487$ ms, SE = 8 ms) than the incongruent condition ($M = 499$ ms, SE = 8 ms). We also found moderate evidence for the main effect of group [$F(1, 77) = 5.353$, $p = 0.023$, $\eta^2 = 0.065$], suggesting that the high workload group ($M = 476$ ms, SD = 11 ms) responded faster than the low workload group ($M = 511$ ms, SD = 11 ms) in general. However, we found little or no evidence for the main effect or interaction effect involving cycle phase (all $ps > 0.05$).

Effect of Workload and Menstrual Cycle on Cognitive Flexibility

A mixed 2 (group: low, high workload) × 3 (cycle phase: menses, late follicular, mid-luteal) × 2 (condition: repeat, switch) factorial ANOVA was conducted on error rate and reaction time, respectively. See **Supplementary Table 3** for a summary of the ANOVA analysis.

On the measure of error rate, there was very strong evidence for the main effect of condition [$F(1, 77) = 18.058$, $p < 0.001$, $\eta^2 = 0.19$], suggesting more errors in the switch condition ($M = 0.028$, SE = 0.003) than the repeat condition ($M = 0.016$, SE = 0.002). We also found strong evidence for the main effect of cycle phase [$F(1.703, 131.121) = 5.271$, $p = 0.009$, $\eta^2 = 0.064$], indicating fewer errors in the mid-luteal phase than the menses phase ($p = 0.006$) and late follicular phase ($p = 0.007$). However, there was little or no evidence that the menses and the late follicular phase differ ($p = 0.317$). See **Figure 3** for an illustration. The results revealed little or no evidence for the main effect of group [$F(1, 77) = 0.628$, $p = 0.431$, $\eta^2 = 0.008$]. In addition, we found little or no evidence for the two-way and three-way interactions (all $ps > 0.2$, see **Supplementary Table 3**).

On the measure of reaction time, there was a strong evidence for the main effect of condition [$F(1, 77) = 445.941$, $p < 0.001$, $\eta^2 = 0.853$], indicating slower responses in the switch ($M = 987$ ms, SE = 15 ms) than the repeat condition ($M = 825$ ms, SE = 15 ms). However, we found little or no evidence for the main effect of the group and cycle phase and the two-way and three-way interactions (all $ps > 0.45$, see **Supplementary Table 3**).

Effect of Workload and Menstrual Cycle on Divided Attention

A mixed 2 (group: low, high workload) × 3 (cycle phase: menses, late follicular, mid-luteal) factorial ANOVA was conducted on the sensitivity measure (A') and reaction time, respectively. See **Supplementary Table 4** for a summary of the ANOVA analysis.

The ANOVA on the sensitivity measure (A') revealed moderate evidence for the main effect of cycle phase [$F(2,$

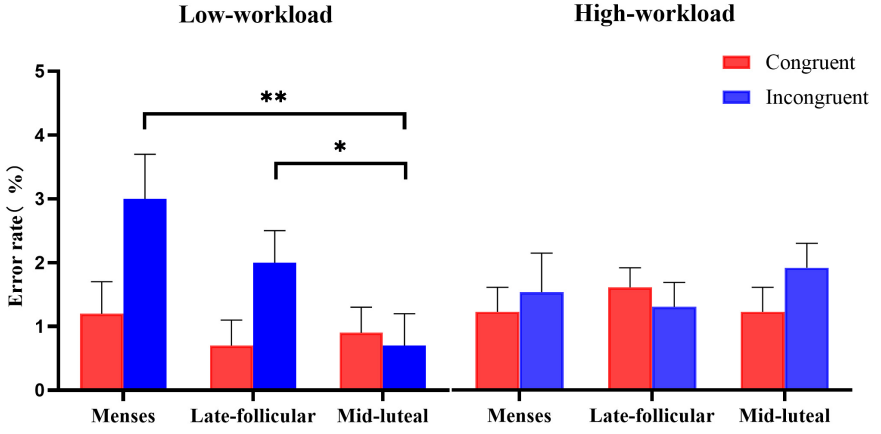

FIGURE 2 | The interactive effect of workload and the menstrual cycle on the error rate measure of the spatial Stroop task. Error bars represent the standard error of the mean. * and ** indicates $p < 0.05$ and $p < 0.01$, correspondingly.

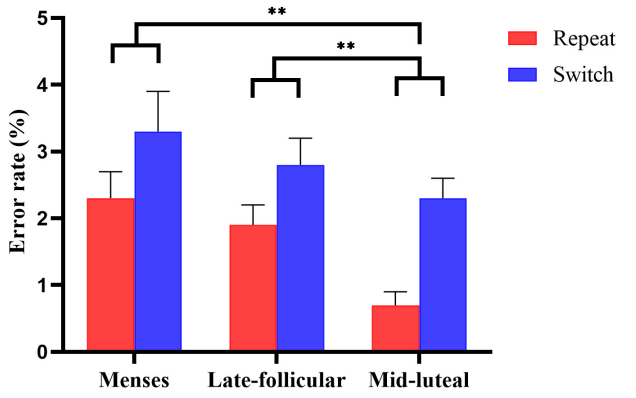

FIGURE 3 | The main effect of the menstrual cycle on the error rate measure of the task-switching task. Error bars represent the standard error of the mean. ** indicates $p < 0.01$.

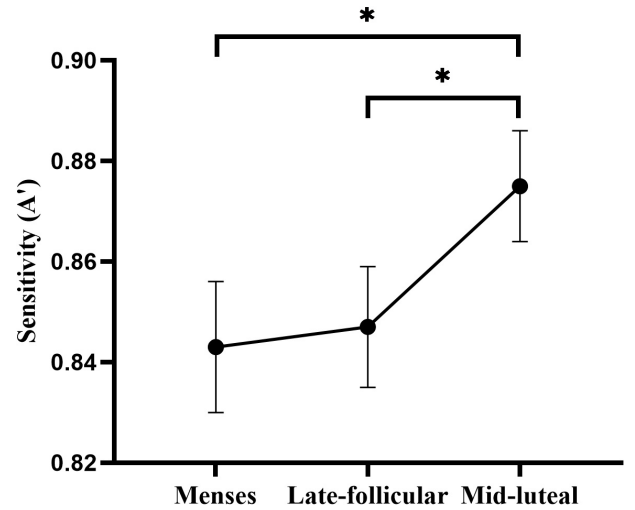

FIGURE 4 | The main effect of the menstrual cycle on the sensitivity measure of the divided attention task. * indicates $p < 0.05$.

154) = 3.29, $p = 0.035$, $\eta^2 = 0.042$]. *Post hoc* analysis indicated higher *A'* in the mid-luteal phase than in the menses phase ($p = 0.021$) and late follicular phase ($p = 0.030$). However, there was no evidence of the difference between the menses and the late follicular phase ($p = 0.780$) (**Figure 4**). We also found little or no evidence for the main effect and interaction terms involving the group (all *ps* > 0.17, see **Supplementary Table 4**).

On the measure of reaction time, there was little or no evidence for the main effect of cycle phase [$F(2, 154) = 0.74$, $p = 0.479$, $\eta^2 = 0.01$] or group [$F(1, 77) = 0.473$, $p = 0.494$, $\eta^2 = 0.006$]. However, the results revealed moderate evidence for the interaction between the group and cycle phase [$F(2, 154) = 3.21$, $p = 0.043$, $\eta^2 = 0.04$]. Follow-up analyses revealed that moderate evidence that participants of low workload responded faster during the mid-luteal phase than the menses ($p = 0.037$) and little or no evidence for other comparisons (mid-L vs. late-F: $p = 0.12$; late-F vs. menses: $p = 0.635$). However, there was no evidence for the cycle effect in the high workload group (all *ps* > 0.11) (**Figure 5**).

Effect of Workload and Menstrual Cycle on Working Memory

A mixed 2 (group: low, high workload) × 3 (cycle phase: menses, late follicular, mid-luteal) factorial ANOVA on the working memory capacity estimation (*K*) was conducted. See **Supplementary Table 5** for a summary of ANOVA results. However, there was no evidence for the main effects [group: $F(1, 77) = 0.563$, $p = 0.455$, $\eta^2 = 0.007$; cycle phase: $F(2, 154) = 0.705$, $p = 0.496$, $\eta^2 = 0.009$] and the interaction effect [$F(2, 154) = 0.009$, $p = 0.991$, $\eta^2 < 0.001$]. See **Supplementary Table 5** for detailed information.

DISCUSSION

The present study investigates cognitive performance across the menstrual cycle using a sample of nurses with similar duties. As summarized in **Table 2**, our results demonstrate evidence for the general cognitive advantage of the mid-luteal phase, as manifested by the main effect of the menstrual cycle on the error rate measure of the task-switch task and the sensitivity measure of the divided attention task. Moreover, the present study demonstrates that workload might be a modulatory factor of the menstrual cycle effect, with preliminary evidence that the cycle phase effect on the reaction time measure of divided attention task and the error rate measure of response inhibition task is only manifested in the low workload group.

The Main Effect of the Menstrual Cycle

Our results demonstrate a mid-luteal phase advantage on the error rate measure of task-switching. The task-switching paradigm adopted in the current research is a classical measure of cognitive flexibility (Monsell, 2003). Our results replicate a typical switch-cost phenomenon: people make slower responses and more errors when the task rule is switched compared with repeat. Using the Wisconsin Card Sorting Task, Solis-Ortiz et al. (2004) reported a similar finding that females show superior cognitive flexibility during their luteal phase. A recent study using a similar task-switching task in functional MRI (fMRI) demonstrated that enhanced prefrontal activations after hormone therapy (sequential estradiol-plus-progesterone) were associated with improved task-switching performance in a sample of early menopausal women (Girard et al., 2017). However, Girard et al. (2017) did not observe beneficial effects on behavioral measures. Their failure to detect behavioral effects might be due to the task design tailored for fMRI or the small sample size.

It should be noteworthy that we found no evidence for the interaction between the menstrual cycle and the switch condition. In other words, the menstrual cycle modulates the general task performance, but not the switching cost in our study. Thus, the performance improvement in the mid-luteal phase might not specifically suggest the ovarian hormone's effect

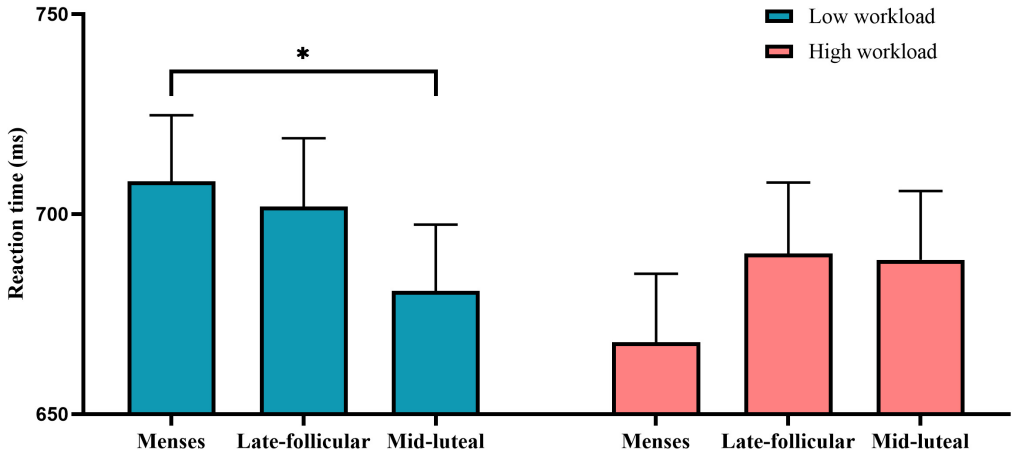

FIGURE 5 | The interactive effect of workload and the menstrual cycle on the reaction time measure of the divided attention task. Error bars represent the standard error of the mean. * indicates $p < 0.05$.

TABLE 2 | Summary of main findings involving menstrual cycle.

Task	Measure	Cycle	Cycle × Workload	Cycle × Workload × Condition
Inhibitory control	Error rate	0.274	0.096	**0.023**
	RT	0.224	0.660	0.066
Cognitive flexibility	Error rate	**0.009**	0.701	0.876
	RT	0.531	0.599	0.498
Divided attention	Sensitivity	**0.035**	0.224	–
	RT	0.479	**0.043**	–
Working memory	Capacity	0.496	0.991	–

RT, Reaction time; The Cycle × Workload × Condition is not available as no experimental contrast was defined; The values in the table refer to p-values and are shown in bold when p < 0.05.

on the task-switching process. The general task performance improvement in the task-switch task is likely due to an attentional augment mechanism. Female participants in our study might show relatively good skills in neglecting task-irrelevant and focusing on task-relevant information during their mid-luteal phase. This hypothesis is consistent with our finding on the divided attention task.

On the divided attention task, female nurses in our research show superior sensitivity in detecting the visual and auditory changes during their mid-luteal phase. We adopted a cross-modal monitoring task to assess participants' ability to simultaneously attend to visual and auditory modalities (Himi et al., 2019). The effect of hormones or the menstrual cycle on different facets of attention has been explored, such as sustained attention (Solis-Ortiz and Corsi-Cabrera, 2008), selective attention (Thimm et al., 2014; Brotzner et al., 2015; Wang and Chen, 2020), and divided attention (Leeners et al., 2017; Pletzer et al., 2017). A seminal work from Pletzer et al. (2017) systematically examined the sex and menstrual cycle effect on three aspects of attention, which reports a follicular phase advantage on the accuracy measure of divided and sustained attention. Unlike us, Pletzer et al. (2017) use paper-pencil tests to assess selective and divided attention. Moreover, Pletzer et al. (2017) only compared the luteal and follicular phases, making a direct comparison with us impossible. Leeners et al. (2017) adopted a similar bimodal attention task as us, but they failed to detect any association between hormone levels and divided attention (Leeners et al., 2017). However, Leeners et al. (2017) used a very heterogeneous sample, including both endocrine disorders and healthy females, making a direct comparison with us impossible.

The general cognitive advantage of the mid-luteal phase, manifested in the task switch and divided attention task, is consistent with recent evidence of the progesterone effect on prefrontal function (Dubol et al., 2021). The prefrontal cortex plays essential roles in cognitive control, influencing attention, impulse inhibition, prospective memory, and cognitive flexibility. A recent systematic review of multimodal neuroimaging studies suggests that enhanced prefrontal activations in the middle luteal phase are a convergent finding in the literature (Dubol et al., 2021). For example, Pletzer et al. (2019) investigated brain activations and functional connectivity changes when women perform a spatial navigation task and a verbal fluency task during the menstrual cycle. Intriguingly their study reveals that progesterone increases the BOLD responses of the dorsal prefrontal cortex and caudate during the luteal cycle phase irrespective of the task (Pletzer et al., 2019). Whether the main effect of the menstrual cycle on task switching and divided attention performance was driven by progesterone's impact on the prefrontal cortex requires additional research efforts. Future studies might use fMRI to clarify this issue.

Workload as a Modulatory Factor

This study reveals intriguing interactions between the error rate measure of the spatial Stroop task and the reaction time measure of the divided attention task. Analysis of the two tasks reveals a similar finding that only low workload groups performed better during their mid-luteal phases. However, the mid-luteal cognitive advantage disappeared in high workload groups.

In the spatial Stroop task, participants make a speeded response to the arrow direction and inhibit the dominant tendency to respond with the ipsilateral hand matching the arrow position when direction and position information conflict. Cognitive control is necessary to focus on the task-relevant information (selective attention) and inhibit the dominant response tendency (response inhibition) (Pires et al., 2018). Unlike us, previous studies mainly used the color Stroop task, and the results were inconsistent (Hatta and Nagaya, 2009; Hidalgo-Lopez and Pletzer, 2017). For example, a study reported that females performed worse during their luteal phase than during the menses phase (Hatta and Nagaya, 2009). In contrast, we did not find evidence for the main effect but evidence for the interactive effect of the menstrual cycle. There was also moderate evidence for the interaction effect on the reaction time measure of the divided attention task.

Our findings parallel recent studies on modulatory factors (Jacobs and D'Esposito, 2011; Hidalgo-Lopez and Pletzer, 2017, 2019; Bernal and Paolieri, 2022). A recent study demonstrates that the menstrual cycle effect on color Stroop task performance is modulated by the baseline dopamine levels (Hidalgo-Lopez and Pletzer, 2017). Their following research used the stop-signal fMRI task to measure inhibitory control and associated brain activity, indicating the baseline inhibitory control might also be a potential modulating variable (Hidalgo-Lopez and Pletzer, 2019). In addition, the recent review proposes that it is crucial to consider modulating factors to avoid confounding findings (Bernal and Paolieri, 2022). Those pieces of evidence, along with us, advocate research attention to potential modulating factors that might change the direction or strength of the menstrual cycle effect.

It is noteworthy that the mid-luteal phase advantage was eliminated in the high workload group on both the error rate measure of the spatial Stroop task and the reaction time measure of the divided attention task. These preliminary findings imply that work-related stress might offset the protective effect of ovarian steroids. Previous studies have suggested that hormones from the HPA axis are involved in regulating the HPG axis at different levels (Oyola and Handa, 2017). The HPA axis is the coordinator of the brain's fight-or-flight response, which increases cortisol production to deal with stressful events. A recent study indicates that the hair cortisol concertation predicts work-related stress only in the high workload condition but not in the normal workload condition (van der Meij et al., 2018). The increased perceived workload in the high workload group did not affect emotion yet as we did not find the workload effect on the DASS scores. We failed to detect the workload effect on emotion, but "cold" prefrontal-mediated tasks might be due to the rating scale's insensitivity. Another explanation is a complex interaction among the HPA axis, HPG axis, and the prefrontal network underneath women's cycling cognitive and affective performance. Our preliminary findings advocate future research efforts to tease apart the potential dynamics among the "cold"/"hot" brain systems and the neuroendocrine system.

Contrary to our expectations, we did not find an effect of workload and menstrual cycle on working memory. Visual working memory is essential for cognitive performance (Luck and Vogel, 2013). The present study estimates participants' visual working memory capacity (K) for each test session using a multiple-change detection paradigm (Gold et al., 2019) and a computational model (Feuerstahler et al., 2019). However, this study did not reveal the workload or the menstrual cycle's effect on the K index, consistent with a recent study using a single probe change detection paradigm (Wassell et al., 2015). Although Wassell et al. (2015) revealed that progesterone levels in the mid-luteal phase modulate mental imagery ability, but they failed to find any association between cycle phase, hormone concentration, and working memory performance.

Previous studies on the menstrual cycle effect primarily used verbal working memory tasks (Joseph et al., 2012; Hidalgo-Lopez and Pletzer, 2021). A recent study found enhanced frontal activity and disinhibition of the salience brain network and striatum in a verbal working memory task (letter N-back) during the luteal phase (Hidalgo-Lopez and Pletzer, 2021). Hampson and Morley (2013) suggest that estradiol, but not progesterone levels, is associated with spatial working memory performance using a sample of women of reproductive age. Their study implies that females might perform best during their follicular phase when the estradiol levels are high. However, using a working memory task for emotional expressions, Gasbarri et al. (2008) indicated that working memory is impaired in the follicular phase.

The inconsistent findings in the literature might be due to methodological differences. Another potential explanation might be complex interactions among the HPA axis, HPG axis, and neurotransmitter systems, such as the dopaminergic system. Previous studies have suggested an inverted U-shape relationship between dopamine concentration and prefrontal cortex mediated cognitive function, such as working memory and cognitive control (Cools and D'Esposito, 2011). Recent studies have found that the mid-luteal phase and progesterone levels drive the effects of dopamine and cycle interactions on cognitive control (Hidalgo-Lopez and Pletzer, 2017). The picture gets increasingly complex by considering another inverted U-shape association between workload and task performance (Ma et al., 2020). It is possible that complex interactions among progesterone, dopamine, and workload obscure the findings of this study. Alternatively, it may be due to other mechanisms, such as functional compensation in the brain. Although this study provides insights on potential intriguing modulating mechanisms, clarifying the exact mechanism is far from our reach. Increasing research efforts are necessary for the future.

Limitations and Future Directions

This study used a validated backward-counting procedure to determine the late-follicular and mid-luteal phases. To further minimize the impact of menstrual cycle mapping error, we increase the sample sizes. As far as we know, few studies have a sample size bigger than us (n = 79) if they used the longitudinal design with a homogenous sample like us. In addition, we double-checked and excluded participants if their actual menses onset deviated from the normal range during the experiment. Despite this, we admit that it might comprise a

potential limitation without saliva, urine, or blood test to verify the hormone levels.

Although our results indicate workload as a modulatory factor on the menstrual cycle's effect on cognition, caution should be made that the evidence is preliminary. Future research is still necessary to replicate the role of workload with samples of females in other workplace settings. In addition, the workload is a too complex construct that might confound many other concepts. Moreover, participants in the present study rated their generally experienced workload in the past 3 months, not their workload at the moment. Thus, the current findings might not answer how acute work stress impacts the menstrual cycling effect. Future studies might use new research methodology, such as experience sampling (Bos et al., 2015) and wearable neurophysiological recordings (Yokota et al., 2017), to provide an objective and immediate measure of workload.

This study contributes a mini-computerized cognitive battery specifically designed to evaluate four cognitive skills critical for nursing performance. We make it publicly available to make replicative and collaborative research works possible. However, we need to emphasize that the tasks in the battery are only a tiny subset of cognitive assessment and may not capture the cognitive performance at work. We suggest that it is valuable to assess cognitive performance by tracking operational errors when nurses perform routine tasks in their workplace.

CONCLUSION

How the menstrual cycle impacts the cognitive performance of females in the workplace is less understood. The present study employed a sample of nurses with similar duties and tracked their cognitive performance during their menses, late-follicular, and mid-luteal phases. Our results demonstrate a general mid-luteal advantage in error rate measure of task-switching and sensitivity measure of divided attention. Moreover, the present study reveals preliminary evidence that workload modulates the menstrual cycle effect on cognition. Only females with low workload manifest the mid-luteal cognitive advantage on the reaction time measure of divided attention and the error rate measure of response inhibition, implying that a suitable workload threshold might be necessary for regular neuro-steroid interactions. Thus, this study advocates the significance of research focused on the brain cycle under workloads.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.doi.org/10.11922/sciencedb.o00068.00001.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Hefei Cancer Hospital, Chinese Academy of Sciences. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

L-ZY, MX, HW, and HL designed and made the concept of the study and performed the analysis and interpretation of the data. L-ZY, HW, and HL contributed research tools. MX and DC were responsible for data acquisition. MX and L-ZY drafted the manuscript, which was critically revised and approved by all authors. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

FUNDING

This work was supported by the Natural Science Fund of Anhui Province (2008085MC69), the Natural Science Fund of Hefei City (2021033), the General scientific research project of Anhui Provincial Health Commission (AHWJ2021b150), Collaborative Innovation Program of Hefei Science Center, CAS (2020HSC-CIP001 and 2021HSC-CIP013), and Anhui Province Key Laboratory of Medical Physics and Technology (LMPT201904).

ACKNOWLEDGMENTS

We appreciate our participants very much for their patience and kindness during the research. We appreciate Liu-Yang Kuang and Fei-Yan Chen for their generous help in data acquisition. We also acknowledge Jin-Wei Lang, Zheng-Lin Zhang, Jin Liu, and Zheng-Kang Zhang for their invaluable technique support in developing the mini-computerized cognitive battery.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnbeh.2022.856276/full#supplementary-material

REFERENCES

Aidman, E., Jackson, S. A., and Kleitman, S. (2019). Effects of sleep deprivation on executive functioning, cognitive abilities, metacognitive confidence, and decision making. *Appl. Cogn. Psych.* 33, 188–200. doi: 10.1002/acp.3463

Alghamdi, M. G. (2016). Nursing workload: a concept analysis. *J. Nurs. Manag.* 24, 449–457. doi: 10.1111/jonm.12354

Barth, C., Steele, C. J., Mueller, K., Rekkas, V. P., Arelin, K., Pampel, A., et al. (2016). In-vivo dynamics of the human hippocampus across the menstrual cycle. *Sci. Rep.* 6:32833. doi: 10.1038/srep32833

Barth, C., Villringer, A., and Sacher, J. (2015). Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. *Front. Neurosci.* 9:37. doi: 10.3389/fnins.2015.00037

Bates, D., Machler, M., Bolker, B. M., and Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Baudry, M., Bi, X., and Aguirre, C. (2013). Progesterone-estrogen interactions in synaptic plasticity and neuroprotection. *Neuroscience* 239, 280–294. doi: 10.1016/j.neuroscience.2012.10.051

Beltz, A. M., and Moser, J. S. (2020). Ovarian hormones: a long overlooked but critical contributor to cognitive brain structures and function. *Ann. N. Y. Acad. Sci.* 1464, 156–180. doi: 10.1111/nyas.14255

Bernal, A., and Paolieri, D. (2022). The influence of estradiol and progesterone on neurocognition during three phases of the menstrual cycle: modulating factors. *Behav. Brain Res.* 417:113593. doi: 10.1016/j.bbr.2021.113593

Borghini, G., Astolfi, L., Vecchiato, G., Mattia, D., and Babiloni, F. (2014). Measuring neurophysiological signals in aircraft pilots and car drivers for the assessment of mental workload, fatigue and drowsiness. *Neurosci. Biobehav. Rev.* 44, 58–75. doi: 10.1016/j.neubiorev.2012.10.003

Bos, F. M., Schoevers, R. A., and Aan het Rot, M. (2015). Experience sampling and ecological momentary assessment studies in psychopharmacology: a systematic review. *Eur. Neuropsychopharmacol.* 25, 1853–1864. doi: 10.1016/j.euroneuro.2015.08.008

Brainard, D. H. (1997). The psychophysics toolbox. *Spat. Vis.* 10, 433–436.

Brotzner, C. P., Klimesch, W., and Kerschbaum, H. H. (2015). Progesterone-associated increase in ERP amplitude correlates with an improvement in performance in a spatial attention paradigm. *Brain Res.* 1595, 74–83. doi: 10.1016/j.brainres.2014.11.004

Carroll, J. C., Rosario, E. R., and Pike, C. J. (2008). Progesterone blocks estrogen neuroprotection from kainate in middle-aged female rats. *Neurosci. Lett.* 445, 229–232. doi: 10.1016/j.neulet.2008.09.010

Catenaccio, E., Mu, W., and Lipton, M. L. (2016). Estrogen- and progesterone-mediated structural neuroplasticity in women: evidence from neuroimaging. *Brain Struct. Funct.* 221, 3845–3867. doi: 10.1007/s00429-016-1197-x

Cools, R., and D'Esposito, M. (2011). Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biol. Psychiatry* 69, e113–e125. doi: 10.1016/j.biopsych.2011.03.028

Diekhof, E. K., and Ratnayake, M. (2016). Menstrual cycle phase modulates reward sensitivity and performance monitoring in young women: preliminary fMRI evidence. *Neuropsychologia* 84, 70–80. doi: 10.1016/j.neuropsychologia.2015.10.016

Dubol, M., Epperson, C. N., Sacher, J., Pletzer, B., Derntl, B., Lanzenberger, R., et al. (2021). Neuroimaging the menstrual cycle: a multimodal systematic review. *Front. Neuroendocrinol.* 60:100878. doi: 10.1016/j.yfrne.2020.100878

Durante, K. M., Griskevicius, V., Cantú, S. M., and Simpson, J. A. (2014). Money, status, and the ovulatory cycle. *J. Market. Res.* 51, 27–39. doi: 10.1509/jmr.11.0327

Feuerstahler, L. M., Luck, S. J., MacDonald, A. III, and Waller, N. G. (2019). A note on the identification of change detection task models to measure storage capacity and attention in visual working memory. *Behav. Res. Methods* 51, 1360–1370. doi: 10.3758/s13428-018-1082-z

Gasbarri, A., Pompili, A., d'Onofrio, A., Cifariello, A., Tavares, M. C., and Tomaz, C. (2008). Working memory for emotional facial expressions: role of the estrogen in young women. *Psychoneuroendocrinology* 33, 964–972. doi: 10.1016/j.psyneuen.2008.04.007

Girard, R., Metereau, E., Thomas, J., Pugeat, M., Qu, C., and Dreher, J. C. (2017). Hormone therapy at early post-menopause increases cognitive control-related prefrontal activity. *Sci. Rep.* 7:44917. doi: 10.1038/srep44917

Gold, J. M., Barch, D. M., Feuerstahler, L. M., Carter, C. S., MacDonald, A. W., Ragland, J. D., et al. (2019). Working memory impairment across psychotic disorders. *Schizophr. Bull.* 45, 804–812. doi: 10.1093/schbul/sby134

Hampson, E., and Morley, E. E. (2013). Estradiol concentrations and working memory performance in women of reproductive age. *Psychoneuroendocrinology* 38, 2897–2904. doi: 10.1016/j.psyneuen.2013.07.020

Hart, S. G. (2016). Nasa-Task load index (NASA-TLX); 20 years later. *Proceed. Hum. Fact. Ergon. Soc. Annu. Meeting* 50, 904–908. doi: 10.1177/154193120605000909

Hatta, T., and Nagaya, K. (2009). Menstrual cycle phase effects on memory and Stroop task performance. *Arch. Sex Behav.* 38, 821–827. doi: 10.1007/s10508-008-9445-7

Henry, J. D., and Crawford, J. R. (2005). The short-form version of the Depression Anxiety Stress Scales (DASS-21): construct validity and normative data in a large non-clinical sample. *Br. J. Clin. Psychol.* 44(Pt 2), 227–239. doi: 10.1348/014466505X29657

Hidalgo-Lopez, E., and Pletzer, B. (2017). Interactive effects of dopamine baseline levels and cycle phase on executive functions: the role of progesterone. *Front. Neurosci.* 11:403. doi: 10.3389/fnins.2017.00403

Hidalgo-Lopez, E., and Pletzer, B. (2019). Individual differences in the effect of menstrual cycle on basal ganglia inhibitory control. *Sci. Rep.* 9:11063. doi: 10.1038/s41598-019-47426-8

Hidalgo-Lopez, E., and Pletzer, B. (2021). Fronto-striatal changes along the menstrual cycle during working memory: effect of sex hormones on activation and connectivity patterns. *Psychoneuroendocrinology* 125:105108. doi: 10.1016/j.psyneuen.2020.105108

Himi, S. A., Buhner, M., Schwaighofer, M., Klapetek, A., and Hilbert, S. (2019). Multitasking behavior and its related constructs: executive functions, working memory capacity, relational integration, and divided attention. *Cognition* 189, 275–298. doi: 10.1016/j.cognition.2019.04.010

Hussain, D., Hanafi, S., Konishi, K., Brake, W. G., and Bohbot, V. D. (2016). Modulation of spatial and response strategies by phase of the menstrual cycle in women tested in a virtual navigation task. *Psychoneuroendocrinology* 70, 108–117. doi: 10.1016/j.psyneuen.2016.05.008

Ikarashi, K., Sato, D., Iguchi, K., Baba, Y., and Yamashiro, K. (2020). Menstrual cycle modulates motor learning and memory consolidation in humans. *Brain Sci.* 10:696. doi: 10.3390/brainsci10100696

Jacobs, E., and D'Esposito, M. (2011). Estrogen shapes dopamine-dependent cognitive processes: implications for women's health. *J. Neurosci.* 31, 5286–5293. doi: 10.1523/JNEUROSCI.6394-10.2011

Joseph, J. E., Swearingen, J. E., Corbly, C. R., Curry, T. E. Jr., and Kelly, T. H. (2012). Influence of estradiol on functional brain organization for working memory. *Neuroimage* 59, 2923–2931. doi: 10.1016/j.neuroimage.2011.09.067

Le, J., Thomas, N., and Gurvich, C. (2020). Cognition, the menstrual cycle, and premenstrual disorders: a review. *Brain Sci.* 10:198. doi: 10.3390/brainsci10040198

Leeners, B., Kruger, T. H. C., Geraedts, K., Tronci, E., Mancini, T., Ille, F., et al. (2017). Lack of associations between female hormone levels and visuospatial working memory. divided attention and cognitive bias across two consecutive menstrual cycles. *Front. Behav. Neurosci.* 11:120. doi: 10.3389/fnbeh.2017.00120

Lisofsky, N., Martensson, J., Eckert, A., Lindenberger, U., Gallinat, J., and Kuhn, S. (2015). Hippocampal volume and functional connectivity changes during the female menstrual cycle. *Neuroimage* 118, 154–162. doi: 10.1016/j.neuroimage.2015.06.012

Luck, S. J., and Vogel, E. K. (2013). Visual working memory capacity: from psychophysics and neurobiology to individual differences. *Trends Cogn. Sci.* 17, 391–400. doi: 10.1016/j.tics.2013.06.006

Ma, S., Zhang, W., Yang, Z., Kang, C., Wu, C., Chai, C., et al. (2020). Promote or inhibit: an inverted U-shaped effect of workload on driver takeover performance. *Traffic Inj. Prev.* 21, 482–487. doi: 10.1080/15389588.2020.1804060

Monsell, S. (2003). Task switching. *Trends Cogn. Sci.* 7, 134–140. doi: 10.1016/s1364-6613(03)00028-7

Morrison, J. H., Brinton, R. D., Schmidt, P. J., and Gore, A. C. (2006). Estrogen, menopause, and the aging brain: how basic neuroscience can inform hormone therapy in women. *J. Neurosci.* 26, 10332–10348. doi: 10.1523/JNEUROSCI.3369-06.2006

Muff, S., Nilsen, E. B., O'Hara, R. B., and Nater, C. R. (2022). Rewriting results sections in the language of evidence. *Trends Ecol. Evol.* 37, 203–210. doi: 10.1016/j.tree.2021.10.009

Oyola, M. G., and Handa, R. J. (2017). Hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes: sex differences in regulation of stress responsivity. *Stress* 20, 476–494. doi: 10.1080/10253890.2017.1369523

Pelli, D. G. (1997). The VideoToolbox software for visual psychophysics: transforming numbers into movies. *Spat .Vis.* 10, 437–442.

Pires, L., Leitao, J., Guerrini, C., and Simoes, M. R. (2018). Cognitive control during a spatial Stroop task: comparing conflict monitoring and prediction

of response-outcome theories. *Acta Psychol.* 189, 63–75. doi: 10.1016/j.actpsy.2017.06.009

Pletzer, B., Harris, T. A., and Ortner, T. (2017). Sex and menstrual cycle influences on three aspects of attention. *Physiol. Behav.* 179, 384–390. doi: 10.1016/j.physbeh.2017.07.012

Pletzer, B., Harris, T. A., Scheuringer, A., and Hidalgo-Lopez, E. (2019). The cycling brain: menstrual cycle related fluctuations in hippocampal and fronto-striatal activation and connectivity during cognitive tasks. *Neuropsychopharmacology* 44, 1867–1875. doi: 10.1038/s41386-019-0435-3

Pletzer, B., Harris, T., and Hidalgo-Lopez, E. (2018). Subcortical structural changes along the menstrual cycle: beyond the hippocampus. *Sci. Rep.* 8:16042. doi: 10.1038/s41598-018-34247-4

Potter, P., Wolf, L., Boxerman, S., Grayson, D., Sledge, J., Dunagan, C., et al. (2005). Understanding the cognitive work of nursing in the acute care environment. *J. Nurs. Adm.* 35, 327–335.

R Core Team (2021). *R: A Language And Environment For Statisticalcomputing*. Vienna: R Foundation for Statistical Computing.

Rosario, E. R., Ramsden, M., and Pike, C. J. (2006). Progestins inhibit the neuroprotective effects of estrogen in rat hippocampus. *Brain Res.* 1099, 206–210. doi: 10.1016/j.brainres.2006.03.127

Schaumberg, M. A., Jenkins, D. G., Janse de Jonge, X. A. K., Emmerton, L. M., and Skinner, T. L. (2017). Three-step method for menstrual and oral contraceptive cycle verification. *J. Sci. Med. Sport* 20, 965–969. doi: 10.1016/j.jsams.2016.08.013

Scheuringer, A., and Pletzer, B. (2017). Sex differences and menstrual cycle dependent changes in cognitive strategies during spatial navigation and verbal fluency. *Front. Psychol.* 8:381. doi: 10.3389/fpsyg.2017.00381

Shen, Y., Jian, W., Zhu, Q., Li, W., Shang, W., and Yao, L. (2020). Nurse staffing in large general hospitals in China: an observational study. *Hum. Resour. Health* 18:3. doi: 10.1186/s12960-020-0446-5

Singmann, H., Bolker, B., Westfall, J., and Aust, F. (2021). *Afex: Analysis of Factorial Experiments". R Package Version 1.0*.

Solis-Ortiz, S., and Corsi-Cabrera, M. (2008). Sustained attention is favored by progesterone during early luteal phase and visuo-spatial memory by estrogens during ovulatory phase in young women. *Psychoneuroendocrinology* 33, 989–998. doi: 10.1016/j.psyneuen.2008.04.003

Solis-Ortiz, S., Guevara, M. A., and Corsi-Cabrera, M. (2004). Performance in a test demanding prefrontal functions is favored by early luteal phase progesterone: an electroencephalographic study. *Psychoneuroendocrinology* 29, 1047–1057. doi: 10.1016/j.psyneuen.2003.10.007

Stanislaw, H., and Todorov, N. (1999). Calculation of signal detection theory measures. *Behav. Res. Methods Instrum. Comput.* 31, 137–149. doi: 10.3758/bf03207704

Sundstrom-Poromaa, I. (2018). The menstrual cycle influences emotion but has limited effect on cognitive function. *Vitam Horm.* 107, 349–376. doi: 10.1016/bs.vh.2018.01.016

Sundstrom-Poromaa, I., and Gingnell, M. (2014). Menstrual cycle influence on cognitive function and emotion processing-from a reproductive perspective. *Front. Neurosci.* 8:380. doi: 10.3389/fnins.2014.00380

Thimm, M., Weis, S., Hausmann, M., and Sturm, W. (2014). Menstrual cycle effects on selective attention and its underlying cortical networks. *Neuroscience* 258, 307–317. doi: 10.1016/j.neuroscience.2013.11.010

Tubbs-Cooley, H. L., Mara, C. A., Carle, A. C., and Gurses, A. P. (2018). The NASA Task Load Index as a measure of overall workload among neonatal, paediatric and adult intensive care nurses. *Intensive Crit. Care Nurs.* 46, 64–69. doi: 10.1016/j.iccn.2018.01.004

van der Meij, L., Gubbels, N., Schaveling, J., Almela, M., and van Vugt, M. (2018). Hair cortisol and work stress: importance of workload and stress model (JDCS or ERI). *Psychoneuroendocrinology* 89, 78–85. doi: 10.1016/j.psyneuen.2017.12.020

Wang, J. X., Zhuang, J. Y., Fu, L., and Lei, Q. (2021). Social orientation in the luteal phase: increased social feedback sensitivity. Inhibitory response, interpersonal anxiety and cooperation preference. *Evol. Psychol.* 19:1474704920986866. doi: 10.1177/1474704920986866

Wang, J. X., Zhuang, J. Y., Fu, L., Lei, Q., Fan, M., and Zhang, W. (2020a). How ovarian hormones influence the behavioral activation and inhibition system through the dopamine pathway. *PLoS One* 15:e0237032. doi: 10.1371/journal.pone.0237032

Wang, J. X., Zhuang, J. Y., Fu, L., Lei, Q., and Zhang, W. (2020b). Association of ovarian hormones with mapping concept of self and others in the brain's default mode network. *Neuroreport* 31, 717–723. doi: 10.1097/WNR.0000000000001477

Wang, J., and Chen, A. (2020). High progesterone levels facilitate women's social information processing by optimizing attention allocation. *Psychoneuroendocrinology* 122:104882. doi: 10.1016/j.psyneuen.2020.104882

Wassell, J., Rogers, S. L., Felmingam, K. L., Bryant, R. A., and Pearson, J. (2015). Sex hormones predict the sensory strength and vividness of mental imagery. *Biol. Psychol.* 107, 61–68. doi: 10.1016/j.biopsycho.2015.02.003

Westbrook, J. I., Woods, A., Rob, M. I., Dunsmuir, W. T., and Day, R. O. (2010). Association of interruptions with an increased risk and severity of medication administration errors. *Arch. Intern. Med.* 170, 683–690. doi: 10.1001/archinternmed.2010.65

Wickens, C. D. (2008). Multiple resources and mental workload. *Hum. Fact.* 50, 449–455. doi: 10.1518/001872008X288394

World Health Organization (2020). *The State of The World's Nursing 2020: Investing In Education, Jobs And Leadership*. Available online at: https://www.who.int/publications/i/item/9789240003279 (accessed April 6, 2020).

Ycaza Herrera, A., Wang, J., and Mather, M. (2019). The gist and details of sex differences in cognition and the brain: how parallels in sex differences across domains are shaped by the locus coeruleus and catecholamine systems. *Prog. Neurobiol.* 176, 120–133. doi: 10.1016/j.pneurobio.2018.05.005

Yokota, Y., Tanaka, S., Miyamoto, A., and Naruse, Y. (2017). Estimation of human workload from the auditory steady-state response recorded via a wearable electroencephalography system during walking. *Front. Hum. Neurosci.* 11:314. doi: 10.3389/fnhum.2017.00314

Zhuang, J. Y., and Wang, J. X. (2014). Women ornament themselves for intrasexual competition near ovulation, but for intersexual attraction in luteal phase. *PLoS One* 9:e106407. doi: 10.1371/journal.pone.0106407

Zhuang, J. Y., Wang, J. X., Lei, Q., Zhang, W., and Fan, M. (2020). Neural basis of increased cognitive control of impulsivity during the mid-luteal phase relative to the late follicular phase of the menstrual cycle. *Front. Hum. Neurosci.* 14:568399. doi: 10.3389/fnhum.2020.568399

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Behavioral Neuroscience

REVIEW
published: 16 June 2022
doi: 10.3389/fnbeh.2022.802530

GABA System Modifications During Periods of Hormonal Flux Across the Female Lifespan

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The female lifespan is marked by periods of dramatic hormonal fluctuation. Changes in the ovarian hormones estradiol and progesterone, in addition to the progesterone metabolite allopregnanolone, are among the most significant and have been shown to have widespread effects on the brain. This review summarizes current understanding of alterations that occur within the GABA system during the major hormonal transition periods of puberty, the ovarian cycle, pregnancy and the postpartum period, as well as reproductive aging. The functional impacts of altered inhibitory activity during these times are also discussed. Lastly, avenues for future research are identified, which, if pursued, can broaden understanding of the GABA system in the female brain and potentially lead to better treatments for women experiencing changes in brain function at each of these hormonal transition periods.

Keywords: GABA, estradiol, progesterone, puberty, ovarian cycle, pregnancy, postpartum, menopause

OPEN ACCESS

Edited by:
Jaclyn M. Schwarz, University of Delaware, United States

Reviewed by:
Debra A. Bangasser, Temple University, United States
Mary Holder, Georgia Institute of Technology, United States

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Specialty section:
This article was submitted to Behavioral Endocrinology, a section of the journal Frontiers in Behavioral Neuroscience

Received: *26 October 2021*
Accepted: *21 February 2022*
Published: *16 June 2022*

Citation:
Gilfarb RA and Leuner B (2022) GABA System Modifications During Periods of Hormonal Flux Across the Female Lifespan. Front. Behav. Neurosci. 16:802530. doi: 10.3389/fnbeh.2022.802530

INTRODUCTION

The female lifespan is marked by periods of dramatic hormonal flux. The first of these periods is puberty, which is when the ovaries begin to secrete increasing amounts of estrogens and progestins (Jost et al., 1973), and when the ovarian cycle emerges in spontaneously ovulating species, such as humans, mice, and rats (Herbison, 2016, 2020). Fluctuations in 17β-estradiol (e.g., estradiol or E2), the predominant circulating estrogen in females, and progesterone, the main progestin, continue with each ovarian cycle until either: 1) pregnancy, when high levels of estradiol and progesterone are sustained for an extended period of time, followed by a precipitous drop at birth (Stewart et al., 1993; Tal and Taylor, 2000; Nair et al., 2017), or 2) the menopausal transition, when steep declines in ovarian hormones mark reproductive senescence (Wise, 1999; Genazzani et al., 2005; Nappi and Cucinella, 2020). These times are also accompanied by shifts in the neuroactive progesterone metabolite allopregnanolone (also known as 3α-Hydroxy-5α-pregnan-20-one, 3α,5α-Tetrahydroprogesterone, 3α,5α-THP, or ALLO).

The brain is highly responsive to changes in estradiol, progesterone, and ALLO, resulting in heightened plasticity during periods of hormonal flux (Sisk and Foster, 2004; Shanmugan and Epperson, 2014; Juraska and Willing, 2017; Piekarski et al., 2017b; Barrientos et al., 2019;

Abbreviations: ALLO, allopregnanolone, 3α-Hydroxy-5α-pregnan-20-one, 3α,5α-Tetrahydroprogesterone, or 3α,5α-THP; CSF, cerebrospinal fluid; Estradiol, 17β-estradiol; E/I, excitatory/inhibitory; GABA, gamma-Aminobutyric acid; $GABA_AR$, $GABA_A$ receptor; GAD, glutamate decarboxylase; HPO, hypothalamic-pituitary-ovarian; HRT, hormone replacement therapy; mPFC, medial prefrontal cortex; mPOA, medial preoptic area; OVX, ovariectomy; PAM, positive allosteric modulator; PFC, prefrontal cortex; PNNs, perineuronal nets; PPD, postpartum depression; PV, parvalbumin; SST, somatostatin; VCD, vinylcyclohexene diepoxide; VGAT, vesicular GABA transporter; VIP, vasointestinal peptide.

Duarte-Guterman et al., 2019). Among the systems which exhibit plasticity across such periods is the GABA (γ-aminobutyric acid) system, which traffics the principal inhibitory neurotransmitter GABA (Shen et al., 2007; Smith et al., 2009; Kilb, 2012; Smith, 2013). Activity of the GABAergic system changes during hormonal transition periods due to the actions of ovarian hormones and their metabolites such as ALLO (Maguire and Mody, 2008; Smith, 2013; MacKenzie and Maguire, 2014; Wang et al., 2016, 2019). During times of hormonal change, adaptations of the GABA system are necessary to maintain excitatory and inhibitory balance (E/I balance). Failure to regulate E/I balance has been linked to changes in cognitive functioning, mood alterations, and susceptibility to the development of psychiatric disorders (Scharfman and MacLusky, 2006; Smith, 2013; MacKenzie and Maguire, 2014; Page and Coutellier, 2018; Sohal and Rubenstein, 2019).

The GABA system consists of multiple components that together modulate inhibitory tone and aid in maintaining E/I balance (**Figure 1**). The GABA system includes GABA neurons, which are largely inhibitory interneurons expressing the proteins parvalbumin (PV), somatostatin (SST), or vasointestinal peptide (VIP) (Rudy et al., 2011; Tremblay et al., 2016). GABA neurons synapse on pyramidal neurons or each other to form a network in which GABA neurons control neural output (Meyer et al., 2011; Buzsáki and Wang, 2012). Resulting neural outputs synchronize and contribute to the production of gamma oscillations, which further influence the activity of surrounding neurons (Buzsáki and Wang, 2012). The GABAergic system is also comprised of perineuronal nets (PNNs), which primarily (though not exclusively) surround PV neurons and regulate the formation of synaptic connections to affect inhibitory gain (Karetko and Skangiel-Kramska, 2009; Bosiacki et al., 2019). GABA neurons and PNNs are hormonally sensitive (Hart et al., 2001; Blurton-Jones and Tuszynski, 2002; Maguire and Mody, 2008; Smith et al., 2009; MacKenzie and Maguire, 2014; Wu et al., 2014; Równiak, 2017; Drzewiecki et al., 2020; Uriarte et al., 2020), making them prime targets during periods of hormonal flux throughout the female lifespan. $GABA_A$ receptors ($GABA_A$Rs) are another facet of the GABA system that can modulate inhibition within the neurons that they populate (MacKenzie and Maguire, 2014; Braden et al., 2015). Synaptic and extrasynaptic $GABA_A$Rs are ionotropic, pentameric receptors containing different subunits [α(1–6), β(1–4), γ(1–3), δ, ε, θ, π and ρ(1–3)] that create pores through which Cl- ion gradients form (Maguire and Mody, 2009). α and β subunits are present in all different receptor compositions; the γ subunit is mainly associated with $GABA_A$Rs expressed in the synaptic compartment and mediates "phasic" inhibition, while the δ subunit is associated with extrasynaptic receptors and mediates "tonic" inhibition (Licheri et al., 2015). $GABA_A$Rs are hormonally responsive, thus they can further influence inhibition within the female brain during times of hormonal change (Maguire and Mody, 2008; Smith et al., 2009; MacKenzie and Maguire, 2014). For example, ALLO can act as a positive allosteric modulator (PAM) at $GABA_A$Rs to potentiate inhibitory activity and alter inhibitory tone (MacKenzie and Maguire, 2014).

This review explores how fluctuations in estradiol, progesterone, and ALLO promote alterations within the GABA system of the female brain during puberty, the ovarian cycle, pregnancy and the postpartum period, as well as reproductive senescence considering data from both humans and rodents. Additionally, this review discusses the functional consequences of altered inhibitory activity and suggests avenues for future research. Broadening our understanding of the GABA system across the female lifespan has important implications for mood regulation and cognition, which are also known to be modified during periods of hormonal flux (Maguire and Mody, 2009; MacKenzie and Maguire, 2014; Wu et al., 2014). Ultimately, expanding what is known about GABA system alterations during hormonal transition periods may further aid in the development of targeted strategies to maintain E/I balance and could lead to better treatments for women experiencing changes in brain function during these times.

PUBERTY

Puberty, the transition to reproductive maturity, is a developmental stage when the ovaries begin to secrete increasing amounts of estrogens and progestins due to a rise in activity of the hypothalamic-pituitary-ovarian (HPO) axis (Jost et al., 1973; Keating et al., 2019; Delevich et al., 2021). In addition, ALLO gradually increases before and rapidly drops off with pubertal onset in rodents and humans (Fadalti et al., 1999; McCartney et al., 2007; Shen et al., 2007; Smith, 2013). Some evidence suggests that changes in estradiol, progesterone, and ALLO during puberty may play a key role in brain and behavioral maturation by altering inhibitory tone (Schulz et al., 2009; Holder and Blaustein, 2014; Jones and Lopez, 2014; Juraska and Willing, 2017; Delevich et al., 2021).

Changes to the Gamma-Aminobutyric Acid System Following Pubertal Onset in Young Women

Adolescence, the developmental period beginning with pubertal onset, is characterized by protracted development of inhibition within late-developing brain regions like the prefrontal cortex (PFC) (Chugani et al., 2001; Fung et al., 2010; Silveri et al., 2013; Laube et al., 2020). Various changes within the human GABA system likely contribute to increases in inhibition over the course of adolescence. For example, cortical expression of the enzyme responsible for GABA synthesis (glutamate acid decarboxylase, GAD) peaks around puberty (Pinto et al., 2010). In addition, shifts in $GABA_A$R subunit expression occur during adolescence (Pinto et al., 2010; Silveri et al., 2013). Interneurons in the PFC also undergo protracted maturation throughout adolescence and into adulthood exhibiting increased expression of PV, calcium binding proteins which expand fast-spiking capabilities of GABA neurons to promote neural synchrony and cortical maturation (Fung et al., 2010). Moreover, PNN density increases within the human PFC after pubertal onset (Mauney et al., 2013) and as PNNs primarily enwrap PV neurons, likely contribute to the regulation of both cortical plasticity and PV expression (Carceller et al., 2020). Together, these changes to the GABA system during adolescence increase cortical inhibition to facilitate maturation of PFC functions such as working memory, executive abilities,

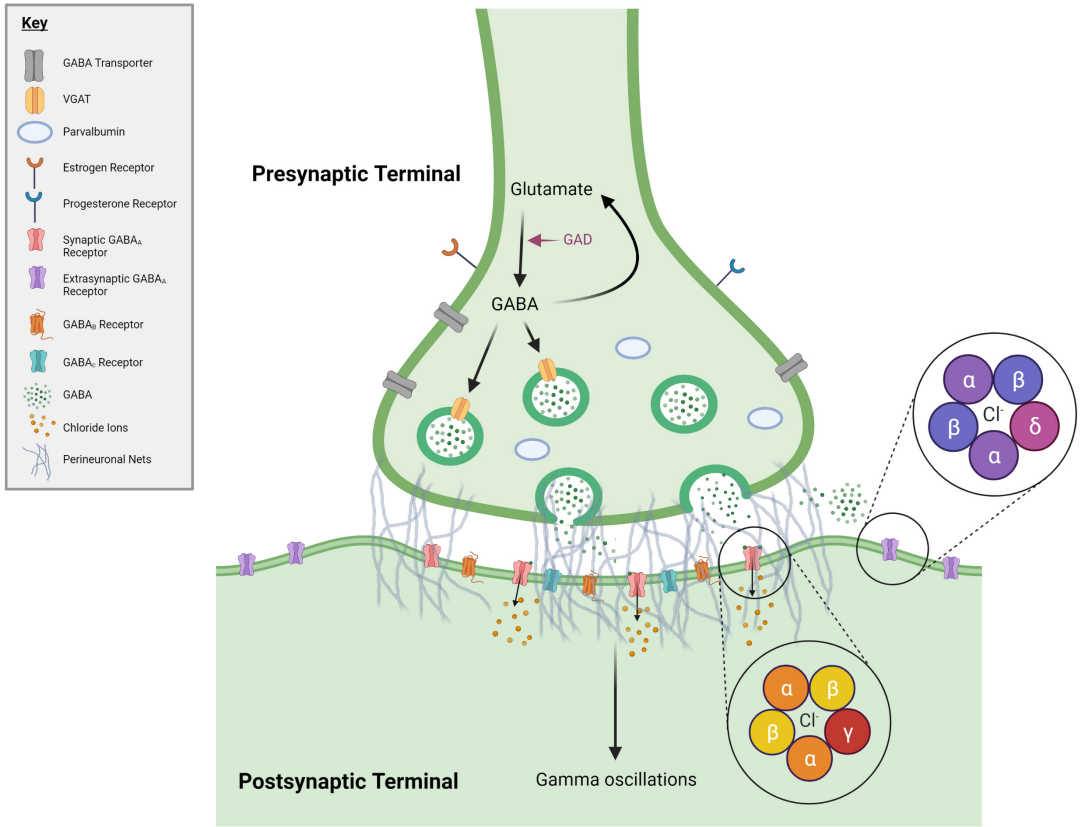

FIGURE 1 | *GABAergic synapse.* During periods of hormonal flux across the female lifespan, numerous changes to the GABA system occur. Many of these changes occur within GABA synapses, represented here. Included in this figure are the elements of the GABA system discussed in this manuscript. Pink text denotes an enzyme. GABA = gamma-Aminobutyric acid, GAD = glutamate decarboxylase, VGAT = vesicular GABA transporter. Created with BioRender.com.

impulse inhibition, and emotional control (Luna et al., 2004; Silveri et al., 2013; Laube et al., 2020).

The extent to which modifications in the GABA system described above are driven by pubertal changes in ovarian hormones and/or ALLO has yet to be determined. Indeed, the focus of many studies is on how the GABA system and brain function develop over adolescence, which is a more protracted developmental period compared to the shorter timeframe following pubertal onset. Further, many studies do not examine sex as a biological variable thereby limiting what conclusions can be drawn concerning the implications of estradiol, progesterone or ALLO on GABA system activity in young women following pubertal onset.

Changes to the Gamma-Aminobutyric Acid System Associated With Puberty in Rodents

Pubertal increases in inhibitory tone are also seen in the rodent brain, providing an avenue to study the direct effects of ovarian hormones and ALLO on GABA system activity following pubertal onset in a mammalian system. Rodent studies have shown that the underlying mechanisms that contribute to the development of greater inhibitory tone in the brain following pubertal onset are multifaceted and due in part to increases in GAD expression, as well as increases in the production of inhibitory synapses and GABA transporters (Kilb, 2012; Shen et al., 2020). As described below, growing inhibitory tone following pubertal onset also results from alterations in PV neurons within the medial prefrontal cortex (mPFC, analog to human PFC) and hippocampus (Sisk and Zehr, 2005; Smith et al., 2009; Buzsáki and Wang, 2012; Kilb, 2012; Juraska et al., 2013; Caballero and Tseng, 2016; Zimmermann et al., 2019; Delevich et al., 2021). Inhibitory tone in these regions is further influenced by changes in subunit expression of $GABA_AR$s, which affect $GABA_AR$ activity (Smith et al., 2009). Evidence suggests that changes to the GABA system within the mPFC and hippocampus of the adolescent female brain may depend on the presence of estradiol, progesterone, and/or ALLO and impact behavior (Sisk and Foster, 2004; Smith et al., 2007; Shen et al., 2010; Juraska et al., 2013; Wu et al., 2014; Caballero and Tseng, 2016; Piekarski et al., 2017a,b).

Changes to PV Neurons Associated With Puberty

Expression of PV increases within the rodent mPFC and hippocampus following pubertal onset (Cruz et al., 2003; Kilb, 2012; Caballero et al., 2014; Glausier et al., 2014; Wu et al., 2014; Delevich et al., 2021). In addition, evidence in rodents indicates that both mPFC and hippocampal PV neuronal

complexity are enhanced following pubertal onset resulting in more synaptic connections with neighboring neurons over adolescence despite no concomitant increase in PV neuron number (Caballero et al., 2014; Honeycutt et al., 2016; Baker et al., 2017; Shen et al., 2020). During adolescence, PV neurons also begin promoting neural synchrony and the production of gamma oscillations within the mPFC and hippocampus, both of which are key to maturation of cognitive capacity and flexibility as well as emotional regulation (Buzsáki and Wang, 2012; Kilb, 2012). These shifts in PV expression, morphology, and activity enhance inhibitory tone following pubertal onset, positioning PV neurons as pacers of brain maturation during adolescence.

Though PV expression increases within the hippocampus and mPFC of both male and female rats during adolescence (Caballero et al., 2014; Wu et al., 2014; Caballero et al., 2020; Gildawie et al., 2020), rodent studies have shown that gonadal hormones play especially critical roles in promoting PV expression and activity in the female pubertal brain. A study by Wu et al. (2014) showed that ovariectomy (OVX), but not castration, prior to pubertal onset reduces hippocampal PV expression in adult mice (Wu et al., 2014), illustrating the importance of gonadal hormones in organizing patterns of PV expression specifically in the female hippocampus. Activity of PV neurons is also dependent on ovarian hormones. Piekarski et al. (2017a) found that pre-pubertal, but not post-pubertal, OVX in female mice impairs inhibitory signaling in the mPFC (Piekarski et al., 2017a). Additionally, this same study demonstrated that pre-pubertal hormone treatment with estradiol and progesterone accelerates maturation of mPFC inhibitory tone and cognitive development. Together this work suggests that ovarian steroids play a role in pubertal maturation of inhibitory activity within the female mouse mPFC through specific modulation of GABA neurons to affect behavior (Caballero et al., 2014; Piekarski et al., 2017a). Though the type of GABA neuron affected in the Piekarski et al. (2017a) study was not identified, evidence strongly suggests that they were PV neurons due to their patterns of activity, increased activity following puberty, and vulnerability to manipulation of ovarian hormones (Caballero et al., 2014, 2016; Caballero and Tseng, 2016). The effects of ovarian hormones on PV neurons enumerated here are perhaps not surprising, as co-localization of PV neurons and estrogen receptors in the rodent brain (including the hippocampus and mPFC) suggests that estradiol acts directly on PV neurons to influence their activity (Hart et al., 2001; Blurton-Jones and Tuszynski, 2002; Wu et al., 2014; Równiak, 2017). However, increases in both estradiol and progesterone accompany pubertal onset (Piekarski et al., 2017b; Filice et al., 2018; Delevich et al., 2021). Thus, the role of progesterone in PV neuron maturation within the pubertal/adolescent female brain requires further consideration.

In addition to their role in facilitating cognitive maturation during adolescence, mPFC PV neurons also contribute to emotional behaviors specifically in female mice. Following chronic stress during adolescence, PV neuron expression in the mPFC increases, as do some anxiety-like behaviors (Page and Coutellier, 2018). Further, chemogenetic activation of PV neurons within the mPFC was shown to enhance anxiety-like behaviors in adult female, but not male, mice (Page et al., 2019). As adolescence is associated with the emergence of sex differences in mood disorders (Smith et al., 2009), interactions between ovarian hormones and the GABA system during this time may render the female brain exceptionally sensitive to stress and predispose adolescent females to developing anxiety and depression.

Along with increases in both expression and activity of PV neurons, PNN expression also increases following pubertal onset within the mPFC (Drzewiecki et al., 2020). Though this increase is observed in both male and female rats, PNN expression in the mPFC transiently drops at pubertal onset exclusively in females (Drzewiecki et al., 2020), an effect which may serve to facilitate neurite growth and formation of new synapses in the female mPFC. As PNN presence regulates the activity of the PV neurons they surround (Carceller et al., 2020), sex differences in patterns of PNN expression may also reflect modulation of PV neuron activity by ovarian hormones through control of PNN formation. PNN density also increases over adolescence in the human PFC (Mauney et al., 2013) and thus, additional animal research can aid in determining the function of pubertal changes in PNN expression and the underlying hormonal mechanisms.

Together, available evidence suggests a critical role for ovarian hormones in both the expression and function of PV neurons following pubertal onset in females. However, additional investigation of PV neuron development within the female pubertal brain is needed, since many of the studies examining inhibitory maturation during adolescence either do not include female subjects or do not analyze the data by sex. Further, most studies focus on the mPFC or hippocampus and thus it remains to be determined if the pubertal pattern of PNN expression is recapitulated in other brain regions (Caballero et al., 2014, 2020; Caballero and Tseng, 2016). Understanding the role of hormones in maturation of GABA neurons in the developing brain is critical, as activity of these neurons contribute to E/I balance and the neural signatures necessary for the changes in cognitive function and mood associated with adolescent brain development (Piekarski et al., 2017a; Page and Coutellier, 2018; Page et al., 2019).

Changes in $GABA_AR$ Expression Associated With Puberty

In addition to modifications in the expression and activity of PV neurons, pubertal onset induces shifts in $GABA_AR$ subunit expression, as illustrated in mouse models of puberty (Smith et al., 2009). Changes in the expression of $GABA_AR$ subunits can affect ion gradients and thus, excitability of the neurons expressing $GABA_ARs$ (Smith et al., 2007, 2009; Smith, 2013; Keating et al., 2019).

In the hippocampus of female mice, withdrawal of ALLO at puberty leads to a transient increase in α4βδ $GABA_AR$ expression on pyramidal neurons to affect behavior (Shen et al., 2007; Smith et al., 2007, 2009; Smith, 2013; Keating et al., 2019). Specifically, pubertal enhancements in hippocampal α4βδ $GABA_ARs$ are associated with impairments in hippocampal-dependent learning tasks (Shen et al., 2010). Such impairments in learning appear to be mediated by the δ subunit, the site of neurosteroid action during periods of hormonal flux, and can be reversed by administration of ALLO which reduces tonic inhibition at puberty (Shen et al., 2010). Additional research in mice suggests

that pubertal ALLO facilitates spontaneous spiking of pyramidal neurons through reductions of inhibitory tone mediated by hippocampal α4βδ $GABA_A$Rs to promote anxiety-like behavior (Shen et al., 2007; Smith, 2013). Together, these data suggest that ALLO *via* its actions at α4βδ $GABA_A$Rs affect inhibition during puberty, as well as cognitive and emotional behavior (Shen et al., 2007; Smith et al., 2009; Afroz et al., 2016; Parato et al., 2019).

Pubertal changes in $GABA_A$R subunit expression also occur on hippocampal PV neurons (Shen et al., 2020). While expression of δ subunits increases on hippocampal pyramidal neurons, δ subunit expression decreases on hippocampal PV neurons following pubertal onset (Shen et al., 2010, 2020; Keating et al., 2019). Since there are fewer δ subunits on PV neurons following pubertal onset to diminish their activity, these neurons are disinhibited and subsequently promote brain maturation by enhancing inhibitory tone (Smith et al., 2007; Torres-Reveron et al., 2009; Wu et al., 2014; Piekarski et al., 2017b; Shen et al., 2020). Though the behavioral implications of these changes are not yet known, reductions in δ subunit expression on PV neurons may be another mechanism contributing to maturation of cognitive function and emotional regulation during adolescence (Shen et al., 2007, 2010).

In conclusion, existing data point to a role for ovarian hormones and the progesterone metabolite ALLO in the development of inhibition within the female pubertal brain. Continued investigation into the hormonal mechanisms of adolescent brain maturation is important, as the hormonal environment during adolescence influences the trajectory of GABA system development to potentially have long-lasting effects on brain function and behavior (Shen et al., 2007, 2010; Piekarski et al., 2017a,b).

THE OVARIAN CYCLE

Following pubertal onset, an infradian rhythm known as the ovarian cycle begins. Fluctuations in estradiol, progesterone, and ALLO affect the GABA system to determine inhibitory tone over the ovarian cycle with implications for cycle-related variations in behavior (Maguire and Mody, 2009; MacKenzie and Maguire, 2014).

Changes to the Gamma-Aminobutyric Acid System Over the Menstrual Cycle

In humans, the menstrual cycle takes place over the course of approximately one month and is comprised of the follicular and luteal phases. Estradiol levels rise during the follicular phase due to the complex interactions of both positive and negative feedback loops within the HPO axis (Oyola and Handa, 2017). The follicular phase ends at ovulation when a mature follicle within the ovaries ruptures and subsequently becomes the corpus luteum, which produces high amounts of progesterone and ALLO during the luteal phase. Estradiol also increases during the luteal phase, though less robustly than during the follicular phase. At menses, estradiol, progesterone, and ALLO levels plummet and remain low for the a few days to mark the beginning of the follicular phase of the following menstrual cycle (Reed and Carr, 2018).

Inhibitory tone changes over the menstrual cycle in association with fluctuations in estradiol, progesterone, and ALLO (Harada et al., 2011; Vigod et al., 2014). Increases in cortical inhibition, gamma oscillation frequency, and neural synchrony are observed during the luteal phase relative to the follicular phase despite a reduction in GABA concentrations (Smith et al., 1999; Epperson et al., 2002; Sumner et al., 2018), while at ovulation GABA concentrations peak within the PFC (De Bondt et al., 2015). Alterations in GABAergic activity and inhibitory tone likely contribute to changes in mood reported across the menstrual cycle associated with specific hormonal profiles, such as increased calmness during the luteal phase and greater sensitivity to psychosocial stress during the follicular phase (Albert et al., 2015; Welz et al., 2016). In contrast, negative changes in mood are observed during the luteal phase in women with premenstrual dysphoric disorder (PMDD). It has been hypothesized that impaired mood regulation in women with PMDD may result from enhanced cortical GABA levels and reduced sensitivity of $GABA_A$Rs to fluctuations in ALLO, thereby preventing establishment of typical E/I balance (Epperson et al., 2002; Hantsoo and Epperson, 2020).

Changes to the Gamma-Aminobutyric Acid System Over the Estrous Cycle

Though similar hormonal shifts occur during the estrous cycle of lab rodents compared to the menstrual cycle of women, there are notable differences in the timing of these shifts which may further depend on species of lab rodent. Rats and mice, the most common models in studies examining neurobiological effects of the ovarian cycle, have 4-6 day estrous cycles although the duration of the cycle may be less consistent in mice (Lovick and Zangrossi, 2021). The estrous cycle consists of four phases: metestrus, diestrus, proestrus, and estrus. Though the names of these phases are the same between rats and mice, the hormonal profiles during these phases differ. In mice, estradiol levels peak during proestrus and progesterone levels peak during diestrus (Wood et al., 2007; McLean et al., 2012; Wu et al., 2013). In rats, peaks in estradiol and progesterone coincide during proestrus (Levine, 2015). ALLO levels also fluctuate across the estrous cycle in relation to progesterone in both rats and mice.

Changes in PV and GABAergic Activity Across the Estrous Cycle

Across the estrous cycle, ovarian hormones modify inhibitory tone through PV neuron expression and activity to affect behavior. For example, within the amygdala, PV expression in rats decreases during proestrus, compared to diestrus, contributing to an overall reduction in inhibition (Blume et al., 2017). As the amygdala has been implicated in arousal and stress reactivity, these inhibitory changes across the cycle may explain, at least in part, estrous cycle-related variations in arousal and stress-related behaviors (Lovick and Zangrossi, 2021). Hormonal status can also influence sensory encoding through activity of PV neurons in the barrel cortex of rats, as estradiol enhances both fast spiking interneuron activity (thought to be PV neurons due to their patterns of activity and sensitivity to ovarian hormones) and frequency of inhibitory post-synaptic potentials following social touch during estrus (Clemens et al., 2019). Other work

examining PNNs in the medial preoptic area (mPOA), a region of the hypothalamus important for the expression of sexual and parental behaviors, found no changes in PNN number or intensity across the rat estrous cycle (Uriarte et al., 2020). As PNNs preferentially surround PV neurons, these data suggest that though PV neuron expression and activity are responsive to changes in ovarian hormones, plasticity of the neurons may not change with shifting hormones during the estrous cycle. However, these data were derived from separate brain regions and thus it remains unclear the extent to which findings in one region generalize to others (Uriarte et al., 2020).

Changes to $GABA_A$Rs Over the Estrous Cycle

Ovarian hormones and their metabolites can further modulate inhibitory tone across the estrous cycle through $GABA_A$Rs (Löscher et al., 1992; Maguire et al., 2005; Maguire and Mody, 2007, 2009; Lovick, 2012; Wu et al., 2013; Sabaliauskas et al., 2015). Notably, ALLO can act as a PAM at $GABA_A$Rs over the estrous cycle to modulate inhibition, an effect that is thought to be mediated by expression of the ALLO-sensitive δ subunit of the $GABA_A$R (Maguire et al., 2005; Wu et al., 2013; Sabaliauskas et al., 2015). Expression of this subunit is critically dependent on the cyclic nature of the estrous cycle and ALLO levels, as changes in δ subunit expression do not occur in acyclic mice and are prevented in mice treated with finasteride, a drug which inhibits the metabolism of progesterone to reduce ALLO production (Barth et al., 2014; Wu et al., 2013). These cyclic changes in the expression of neurosteroid-sensitive δ subunit of the $GABA_A$R affect neuronal activity and inhibitory tone to influence behavior. For example, enhancements in hippocampal δ subunit expression during late diestrus results in increased tonic inhibition within dentate gyrus granule cells from the female mouse hippocampus, along with reductions in anxiety-like behavior (Maguire et al., 2005; Maguire and Mody, 2007). Changes in δ subunit expression are also seen on inhibitory neurons in the female mouse hippocampus across the estrous cycle (Barth et al., 2014). Specifically, increases in δ subunit expression on PV neurons during diestrus compared to estrus diminish production of gamma oscillations and impair neuronal synchrony within the hippocampus which may have consequence for cognitive function (Barth et al., 2014; Shen et al., 2020). Together, these data show that changes in $GABA_A$Rs subunit composition depend on hormonal status and can subsequently affect activity of hippocampal interneurons to modulate inhibitory tone over the estrous cycle. In addition to the hippocampus, other work in rats has examined $GABA_A$ receptor subunit composition within the midbrain periaqueductal gray (PAG), a region important for integrating anxiety responses. Within the PAG, expression of α4β1δ $GABA_A$ receptors fluctuates over the estrous cycle in relation to changing levels of ALLO to modify GABAergic tone and anxiety-like behavior (Griffiths and Lovick, 2005; Lovick, 2012).

Much of the research examining the effects of shifting ovarian hormones on the GABA system across the estrous cycle has focused on different brain regions and has used different animal model species with variations in hormonal profiles across the ovarian cycle, making direct comparisons across studies difficult. Additionally, most of the existing research addresses the influence of ALLO on the GABA system. Therefore, determining the mechanisms by which estradiol and progesterone affect the GABA system is warranted, as levels of these hormones change over the ovarian cycle and have been shown to affect GABAergic activity and brain functions dependent on proper E/I balance during other hormonal transition periods (Löscher et al., 1992; Maffucci and Gore, 2009; Barth et al., 2014; Blume et al., 2017; Clemens et al., 2019). It is also worth mentioning that there are a limited number of studies which have looked at the effects of hormonal contraceptives on the GABA system and these have found altered cortical GABA concentrations in human hormonal contraceptive users as well as changes in $GABA_A$ receptor subunit expression in rodents after prolonged treatment with hormones found in hormonal contraceptives (Follesa et al., 2002; De Bondt et al., 2015). Hormonal contraceptives may give rise to these effects because they contain synthetic estradiol and progesterone analogs that inhibit the HPO axis through negative feedback leading to lower endogenous levels of estradiol and progesterone and a suppression in the fluctuation of ovarian hormones across the cycle as well as a decrease in ALLO (Montoya and Bos, 2017; Porcu et al., 2019). Although hormonal contraceptives are used by millions of women worldwide, their impact on the brain and behavior have not been well studied and warrant greater consideration (Porcu et al., 2019; Taylor et al., 2021).

PREGNANCY AND THE POSTPARTUM PERIOD

As during puberty and the ovarian cycle, major hormonal shifts occur over the course of pregnancy and the postpartum period (Bloch et al., 2003; De Bonis et al., 2012; Duarte-Guterman et al., 2019). In humans, pregnancy induces profound increases in both plasma estradiol and progesterone, which can reach levels 50- and 10-fold higher, respectively, than at peak concentration during the menstrual cycle due to placental hormone production (Bloch et al., 2003). In rodents, levels of estradiol and progesterone similarly surge during late pregnancy (Concas et al., 1998, 1999; Brunton and Russell, 2010). Likewise, ALLO concentrations rise throughout gestation in rodents and humans reaching peak concentrations during late pregnancy (Concas et al., 1999; Luisi et al., 2000). This accumulation in hormones over the course of pregnancy rapidly declines just prior to (rats) or following (humans) delivery, resulting in a period of hormonal change that affects different aspects of the GABA system and, in turn, inhibitory tone (Concas et al., 1998, 1999; Epperson et al., 2006; Maguire et al., 2009; MacKenzie and Maguire, 2014; Vigod et al., 2014; Maguire, 2019; Deems and Leuner, 2021). As described below, dysregulation in GABAergic signaling has been linked to deficits in maternal care, as well as heightened anxiety- and depression-like behaviors, during the postpartum period.

Changes in Gamma-Aminobutyric Acid Concentration During Pregnancy and the Postpartum Period in Humans

Central GABA concentrations have been reported to be altered in pregnant and postpartum women. For example, GABA levels

in cerebrospinal fluid (CSF) decrease during the last few weeks of pregnancy (Altemus et al., 2004; Vigod et al., 2014) and then significantly increase during labor (Sethuraman et al., 2006). In addition, cortical GABA levels decrease after birth and begin to normalize over the course of the postpartum period (Epperson et al., 2006; Vigod et al., 2014). Though these data do not directly support a causal relationship, they nonetheless suggest that changes in GABA concentrations over late pregnancy and into the postpartum period are related to changes in ovarian hormones and ALLO given their coincident timing.

It has been proposed that aberrant GABAergic activity and a failure to properly maintain E/I balance during the transition into the postpartum period may be a precipitating factor leading to postpartum depression (PPD), a disorder in new mothers characterized by symptoms such as sadness, cognitive impairment, and strained mother-infant interactions (Deligiannidis et al., 2013, 2016; Stewart and Vigod, 2019; Deems and Leuner, 2021). In particular, PPD is thought to arise, at least in part, when there is a failure of $GABA_AR$s to adapt to the abrupt decline in ALLO after birth (Faden and Citrome, 2020; Meltzer-Brody and Kanes, 2020).

Changes in Inhibition During Pregnancy and the Postpartum Period in Rodents

Changes in GABA During Pregnancy and the Postpartum Period in Rodents

GABA concentrations are also altered in the rodent brain during pregnancy and the postpartum period. For example, GABA concentrations significantly decline during late pregnancy in the mouse hippocampus and subsequently normalize following parturition (Smolen et al., 1993). In postpartum rats, CSF concentrations of GABA increase following offspring interaction (Qureshi et al., 1987; Lonstein et al., 2014). GABA modifications during pregnancy and the postpartum period are not limited to GABA concentrations with increases in GABA synthesis (as indicated by higher expression of GAD) reported in the lateral septum of postpartum mice (Zhao et al., 2012) and the mPFC of postpartum rats (Ahmed and Lonstein, 2012; Lonstein et al., 2014). Other work has shown both lower GABA release in the basolateral amygdala of pregnant rats (Young and Cook, 2006) and reduced turnover in the cerebral cortex of late pregnant/early postpartum mice (Smolen et al., 1993), while postpartum rats have both higher basal GABA release and turnover in the mPFC in comparison to virgins (Kornblatt and Grattan, 2000; Arriaga-Avila et al., 2014; Ragan et al., 2022). Overall, these data show complex effects on GABA that are highly dependent on species and brain region. The regions analyzed in these studies have been implicated in behavioral functions that are altered postpartum such as anxiety, fear, maternal care, and maternal aggression, with some work pointing to GABAergic involvement (Lee and Gammie, 2009; Sabihi et al., 2021).

Changes in Expression of Perineuronal Nets During Pregnancy and the Postpartum Period in Rodents

Over the course of pregnancy and into the postpartum period, PNN number and intensity change in the rat mPOA, a region that is both critical to establishment of maternal care behaviors and highly plastic during this time (Keyser-Marcus et al., 2001; Duarte-Guterman et al., 2019). Specifically, expression of PNNs in the mPOA steadily increases from mid-gestation to late pregnancy and peaks immediately prior to parturition (Uriarte et al., 2020). PNN expression subsequently drops following parturition, increases again to peak levels one week into the postpartum period, and remains elevated in comparison to cycling rats until weaning (Uriarte et al., 2020). These changes in PNN expression during pregnancy and the postpartum period may be due to extended exposure to ovarian hormones, as induction of hormonal pseudopregnancy recapitulates a similar pattern, though to a lesser extent (Uriarte et al., 2020). Since PNN presence typically limits neuroplasticity (Karetko and Skangiel-Kramska, 2009), changes in PNN expression in the mPOA suggests that PNNs may play a time-dependent role in contributing to both maintenance of established circuitry in this region during pregnancy and transient permission of plasticity to establish the circuitry critical to postpartum maternal behaviors.

Recent data also show that PNN expression in postpartum rats changes in the primary somatosensory cortex (S1) following viral-mediated knockdown of receptors for oxytocin, a neuropeptide important for social bonding and maternal behavior (Grieb et al., 2021). In oxytocin receptor knockdown rats exhibiting disrupted postpartum social and affective behaviors, more plasticity-restricting PNNs were found in the S1 rostral region, and fewer in the S1 caudal region. It is possible that such alterations in PNN expression may disrupt cortical neuroplasticity to affect maternal sensitivity to tactile cues from the young and impact behavior (Grieb et al., 2021). Other work in never-pregnant mice housed in proximity to pups demonstrates that pup interaction, and not hormonal milieu, drive sub-regional differences in PNN density within the somatosensory cortex (Lau et al., 2020; Grieb et al., 2021). Additional studies are needed to better understand PNNs in the maternal brain and the extent to which they contribute to behavior during this time.

Changes to $GABA_AR$s During Pregnancy and the Postpartum Period in Rodents

$GABA_AR$s and their capacity to mediate GABAergic inhibition are highly plastic across pregnancy and the postpartum period. Within rat forebrain, $GABA_AR$ binding affinity is enhanced during late pregnancy and is further enhanced in the postpartum period (Majewska et al., 1989). The brain region(s) driving these effects are unknown but likely do not include the cortex as this region shows a decrease in binding affinity during late pregnancy (Concas et al., 1999). A postpartum reduction in total forebrain $GABA_AR$ density has also been reported although again, specific brain sites remain to be elucidated (Miller and Lonstein, 2011; Lonstein et al., 2014).

Much of the work examining $GABA_AR$s during pregnancy and the postpartum period has focused on ALLO's effects on the expression of specific $GABA_AR$ subunits. Overall this body of work shows that during late pregnancy and the postpartum period, shifts in ALLO lead to changes in subunit composition of $GABA_AR$s to modulate $GABA_AR$ activity and influence

inhibitory tone (Concas et al., 1999; Maguire and Mody, 2008; Licheri et al., 2015). For example, in the hippocampal dentate gyrus of late pregnant rats, high levels of ALLO enhance granule cell expression of the δ subunit to increase tonic inhibition, effects which can be blocked by finasteride treatment (Sanna et al., 2009). Postpartum expression of the δ subunit in the rat hippocampus, as well as tonic inhibition, diminishes following a postpartum decline in ALLO (Smith et al., 1998; Sanna et al., 2009). Though the functional effects of these changes in the rat maternal hippocampus remain to be studied, they likely contribute to the development of behaviors that have been shown to be altered during pregnancy and the postpartum period and that are hippocampal-dependent, such as working memory and anxiety (Pawluski et al., 2006).

Like in rats, female mice show fluctuations in ALLO levels over the course of pregnancy which affects hippocampal δ subunit expression and modulates activity at $GABA_A$Rs to influence E/I balance. However, in contrast to rats, expression in mice is instead reduced in the hippocampus as well as in the striatum and thalamus, while remaining stable in the cortex (Maguire et al., 2009). Within 48 hours of delivery, hippocampal δ subunit expression subsequently recovers to virgin levels (Maguire et al., 2009). This pattern of δ subunit expression is critical, as data from mice deficient in the $GABA_A$R δ subunit suggest that the inability to regulate δ subunit-containing $GABA_A$Rs during pregnancy and the postpartum period results in increased anxiety- and depression-like behaviors, as well as abnormal mothering (Maguire and Mody, 2008; Melón et al., 2018). Importantly, treatment of $GABA_A$R δ subunit deficient mice with a synthetic neuroactive steroid $GABA_A$R PAM (SGE-516) was shown to decrease PPD-like behaviors and improve maternal care (Melón et al., 2018).

Localized changes in δ subunit expression also occur on hippocampal interneurons during late pregnancy in mice (Maguire and Mody, 2008; Melón et al., 2018). As δ subunit-containing $GABA_A$Rs often populate PV neurons in this region and are vulnerable to changes in ALLO, reductions in δ subunit expression during pregnancy in mice lead to a modification of hippocampal PV neuron activity (Ferando and Mody, 2013). Indeed, *in vitro* electrophysiological analyses indicate that the downregulation in δ subunit expression during pregnancy enhances gamma oscillation frequency in hippocampal slices from pregnant mice because of the acute withdrawal from ALLO (Ferando and Mody, 2013). However, application of ALLO at concentrations physiologically equivalent to those seen during pregnancy reduces the frequency of gamma oscillations in the hippocampi of pregnant mice to that observed in virgin mice, suggesting that ALLO acts as a PAM to normalize PV neuron activity during pregnancy through regulation of the δ subunit of $GABA_A$Rs (Ferando and Mody, 2013). Following parturition and the subsequent decline in ALLO, δ subunit expression on hippocampal interneurons returns to baseline, as does the frequency of induced gamma oscillations (Ferando and Mody, 2013). This evidence points to modulation of interneuron activity by ALLO at δ subunits, and not changes in interneuron density or connectivity, as main determinants of inhibitory tone in the hippocampus during pregnancy (Ferando and Mody, 2013). Overall, despite differences in mice and rats, these data indicate that high levels of ALLO during pregnancy modulate expression of neurosteroid-sensitive $GABA_A$Rs δ subunits to determine E/I balance in the rodent hippocampus to affect behavior.

Other evidence points to modifications in γ subunits of $GABA_A$Rs, which are responsive to changing levels of neurosteroids (Maguire and Mody, 2008; Licheri et al., 2015). For example, expression of the γ2L subunit is reduced in the rat hippocampus and cortex, as well as in the mouse hippocampus, with rising ALLO levels during pregnancy which, along with observed changes in expression of the δ subunit, may serve to potentiate inhibitory tone (Concas et al., 1998, 1999; Maguire and Mody, 2008; Maguire et al., 2009; Sanna et al., 2009). Following a postpartum drop in ALLO levels, expression of γ2L in the rat hippocampus and cortex increases to likely facilitate a return of inhibitory tone to a pre-pregnancy state (Concas et al., 1998, 1999; Sanna et al., 2009). In addition to the postpartum changes seen in the hippocampus, γ2 subunit expression increases in the rat paraventricular nucleus of the hypothalamus during lactation (Fénelon and Herbison, 1996), an effect that could be necessary to ensure sufficient stimulation of oxytocin neurons in this region to establish maternal care behaviors (Giovenardi et al., 1997).

Along with δ and γ, α subunit expression also changes over pregnancy and the postpartum period. In rats, hippocampal α4 expression increases during the postpartum period and likely contributes to changes in hippocampal inhibitory tone and anxiety-like behavior (Smith et al., 1998; Sanna et al., 2009). However, some studies report no change in α subunit expression in the rat hippocampus or cortex during pregnancy and the postpartum period (Concas et al., 1998, 1999; Follesa et al., 1998). Thus, despite some heterogeneity, the data overall show cell-, region-, and species-specific changes in δ, γ, and α subunit composition which are important for the establishment of inhibitory tone during pregnancy and the postpartum period.

Together, evidence points to major changes in the GABA system over the course of pregnancy and the postpartum period. While some findings implicate ovarian hormones in these changes, much of the research during pregnancy and the postpartum period to date has focused on ALLO and changes in $GABA_A$R subunit composition. There is also work which suggests that another important mediator of inhibition in the pregnant and/or postpartum brain to consider is oxytocin, which aligns with known oxytocin-GABA interactions within the hypothalamus (Giovenardi et al., 1997; Fénelon and Herbison, 2000; Kornblatt and Grattan, 2000; Herbison, 2001; Lonstein et al., 2014) and the mPFC (Sabihi et al., 2014, 2021) to affect maternal, cognitive, and emotional behavior.

MENOPAUSAL TRANSITION AND REPRODUCTIVE SENESCENCE

Aging gives way to reproductive senescence caused by HPO axis disruption (Wise, 1999). In women, the decline in HPO axis activity that characterizes the menopausal transition occurs following ovarian follicle depletion, which in turn promotes production of gonadotropins in an effort to rescue falling

concentrations of estradiol, progesterone, and progesterone metabolites (Wise, 1999; Morrison et al., 2006; Andréen et al., 2009; Kimball et al., 2020). Failure of such rescue causes menstrual irregularity and eventually amenorrhea which over time will render an individual reproductively senescent and menopausal, typically around 52 years of age (Jacobs and Goldstein, 2018). Reproductive senescence represents another period of the female lifespan during which hormonal changes impact the GABA system with consequences for declining cognition and mood often experienced during this time (Harden et al., 1999; Andréen et al., 2009; Erel and Guralp, 2011; Jacobs and Goldstein, 2018).

Changes Within the GABA System During the Menopausal Transition in Women

Like during other periods of hormonal fluctuation, the brain experiences shifts in GABAergic tone during the menopausal transition. Recent work examining pre- and post-menopausal women shows that GABA concentrations in the cortex decline over the menopausal transition and decline further in postmenopausal women with depression (Wang et al., 2016, 2019). Postmenopausal reductions in cortical GABA concentrations may also be relevant to cognitive function, as a recent study shows that hippocampal GABA positively correlates with memory performance in older women (Jiménez-Balado et al., 2021). Other than these few reports, we are unaware of any other work assessing GABAergic activity of postmenopausal women, though decreases in ALLO associated with reproductive senescence suggest that additional changes are likely. As ALLO is both reduced in postmenopausal women (Kimball et al., 2020) and is a PAM of $GABA_A$Rs, changes in functional inhibition most likely occur over the course of the menopausal transition (Gordon et al., 2015).

Models of Reproductive Senescence in Rodents

Comparisons between humans and rodents are difficult in the context of reproductive senescence due to various factors. First, rodents do not undergo menopause but instead as they enter middle age, will experience irregular cycles and eventually will cease ovulation and become acyclic at which time they are either in persistent diestrus or persistent estrus (Kermath and Gore, 2012). Following this transition, which typically begins at approximately 10–12 months of age, estradiol levels depend on if a rodent transitions into persistent diestrus (lower estrogen) or persistent estrus (higher estrogen). This stands in contrast to the to the eventual decline in estradiol observed universally in women (Wise, 1999; Koebele and Bimonte-Nelson, 2016). Second, rodents do not undergo follicular depletion to the same extent as in humans nor do they depend expressly on follicular content within the ovary to determine reproductive senescence (Peng and Huang, 1972; Wise, 1999; Diaz Brinton, 2012). Lastly, many research groups choose to model menopause through OVX, which does not capture the steady decline in hormones that characterize the transition to menopause and thus limits translatability as most women undergo natural, not surgical, menopause (Diaz Brinton, 2012). In fact, many studies modeling menopause through OVX frequently use young rodents, which is inconsistent with the middle-age timing of menopausal transition in women. OVX as a model of menopause is also problematic when studying the GABAergic system, as OVX in rats can contribute to reductions in hippocampal GABA concentrations (Tominaga et al., 2001) and enhances GABA binding across various brain regions dependent on time between OVX and assessment (Bosse and Paolo, 1995; Bossé and Paolo, 1996). Thus, simply reducing ovarian hormones has substantial effects itself on inhibitory tone within the brain. Other work investigating the menopausal transition and reproductive senescence centers on the use of hormone replacement therapy (HRT) to address menopausal symptoms, such as onset of hot flashes and vaginal dryness (Shanmugan and Epperson, 2014; Toffol et al., 2015). This is reiterated in rodent neuroscience research, as the combination of OVX and ovarian hormone supplementation is a commonly used model to study the effects of HRT during menopause on the brain and behavior, including cognition and mood (Dumitriu et al., 2010; Rao et al., 2013; Koebele et al., 2017, 2021; Jacobs and Goldstein, 2018; Prakapenka et al., 2018).

Changes Within the GABA System During the Transition to Reproductive Senescence in Rodents and in Response to Hormone Replacement Therapy

The effects of HRT on the GABA system have only been examined in a few studies. These studies have shown that supplementation of both estradiol and progesterone following OVX reduces hippocampal GAD expression in rats (Weiland, 1992) and attenuates GABAergic gene expression within the hippocampus and amygdala of rhesus macaques (Noriega et al., 2010). It has also been shown that exogenous hormone administration following OVX in aged female rats ameliorates depressive-like behaviors through a GABAergic mechanism (Rodríguez-Landa et al., 2020), suggesting that HRT may modulate the GABA system to positively affect behavior in reproductively senescent rodents. However, not all studies find beneficial effects of HRT on brain function. One research group used OVX female rats over one year of age to study how supplementation of either progesterone or a synthetic progestin (medroxyprogesterone acetate) commonly used in HRT immediately following OVX affects GAD expression and brain function during reproductive senescence. Using this model, GAD was found to be reduced in the hippocampus and increased in the entorhinal cortex following progesterone or progestin supplementation compared to rats not supplemented with any hormones (Braden et al., 2010). Further, progesterone supplementation following OVX in aged female rats was associated with a decline in working memory, which later studies demonstrated was caused by excessive activity at $GABA_A$Rs (Braden et al., 2015). Although other work has shown that HRT can improve working memory, these typically use HRT with estrogenic components suggesting that is important to consider both estrogens and progestins when studying the GABA system during reproductive senescence (Koebele et al., 2021).

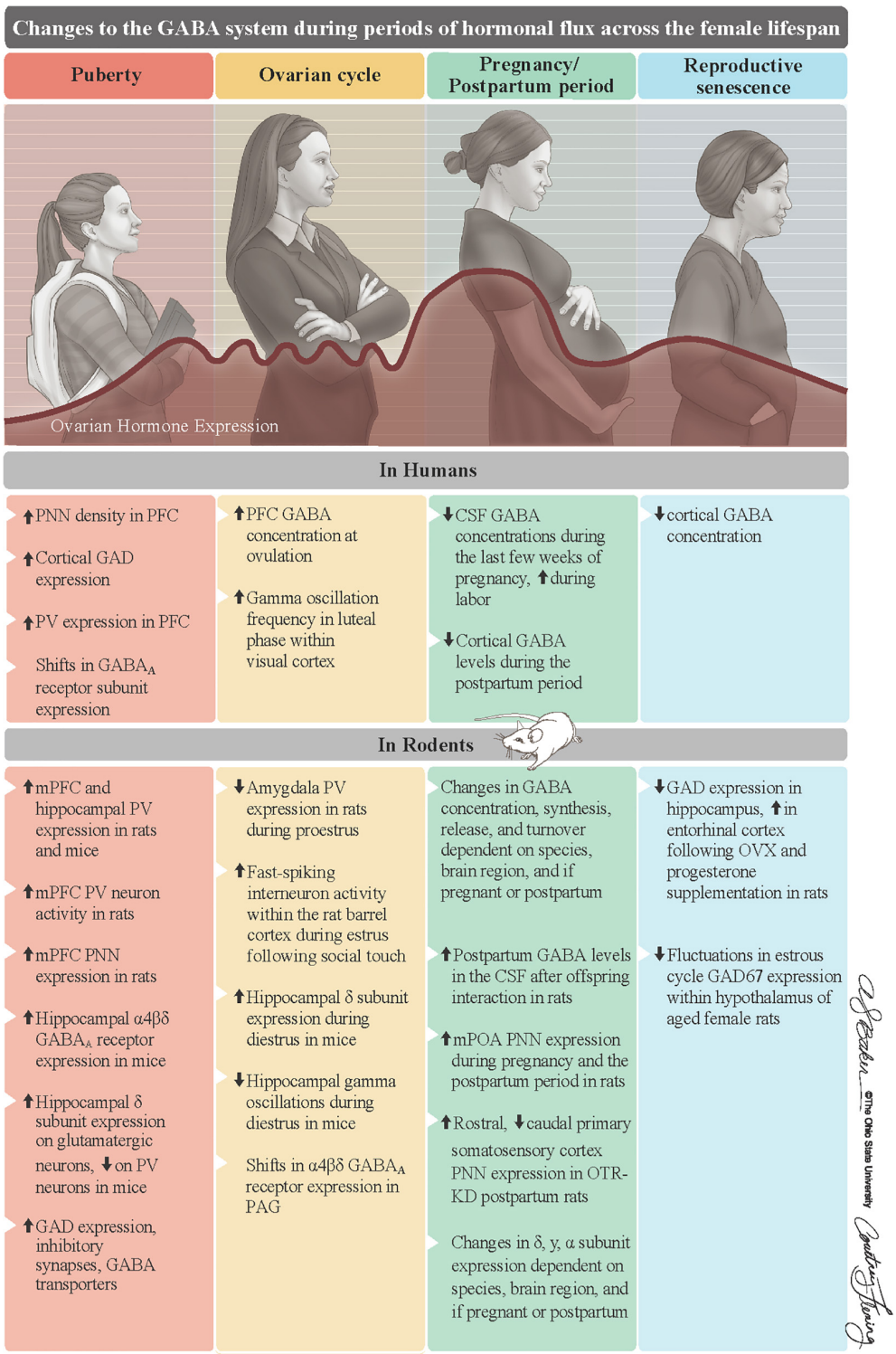

FIGURE 2 | *Summary of changes to the GABA system of females during periods of hormonal flux.* Ovarian hormones substantially change across the female lifespan. They first increase at puberty, fluctuate on an infradian cycle during reproductive years, surge to their lifelong peak during pregnancy, and steadily decline into reproductive senescence. Along with ALLO, these fluctuations in ovarian hormones, which are mostly recapitulated in rodents, are associated with changes to GABA system in both humans (top row) and rodents (bottom row). Reproduced with the permission of The Ohio State University, patterned after Figure 1 in Barrientos et al. (2019). ALLO = allopregnanolone, CSF = cerebrospinal fluid, GAD = glutamate decarboxylase, OTR-KD = oxytocin receptor knockdown, mPFC = medial prefrontal cortex, mPOA = medial preoptic area, PAG = periaqueductal gray, PNNs = perineuronal nets, PV = parvalbumin.

It is also worth noting studies that have provided additional insight into how activity of the GABA system within the hypothalamus changes with age (Cashion et al., 2004; Grove-Strawser et al., 2007; Neal-Perry et al., 2008). For example, in middle-aged rats, fluctuations in hypothalamic GAD are attenuated over the course of the estrous cycle resulting in reduced hypothalamic GAD expression compared to adult rats on the day of proestrus (Cashion et al., 2004; Grove-Strawser et al., 2007; Neal-Perry et al., 2008). Estrogen and progesterone receptors may be involved since their expression changes across different regions of the hypothalamus during aging in rats (Wilson et al., 2002; Chakraborty et al., 2003; Kermath and Gore, 2012). Overall, this work suggests possible mechanisms contributing to HPO axis disruption during the transition to reproductive senescence.

Together, these data point to changes occurring in the GABA system during the transition to reproductive senescence in both humans and animals. However, the existing evidence is limited. For example, no studies have examined if and how PV expression or PNNs change over the course of reproductive aging in females. Also lacking are studies looking at $GABA_A$ receptor plasticity specifically during the menopausal transition. Furthermore, additional research using animal models that better reflect the steady withdrawal from ovarian hormones are needed to advance understanding of GABAergic changes across the menopausal period as well as in the preceding perimenopausal period. In this regard, the ovarian toxin vinylcyclohexene diepoxide (VCD), which causes a steady decline in ovarian follicles as seen in human menopausal transitions, may be useful (Diaz Brinton, 2012; Koebele and Bimonte-Nelson, 2016; Koebele et al., 2021). Some preliminary work has shown effects of VCD on the brain, though no work to date has examined the GABA system (Koebele et al., 2021). Studies using more translational models of reproductive senescence could provide critical insights into the mechanisms by which changes to the GABA system can impair mood and cognitive function during this critical stage of the female lifespan.

TARGETING GABA SIGNALING FOR THE TREATMENT OF MOOD DYSREGULATION ACROSS THE FEMALE LIFEPSAN

Recent advances have been made in the treatment of PPD through FDA approval for use of the GABA-modulating drug Brexanolone, a synthetic version of ALLO which stabilizes fluctuations in ALLO levels allowing $GABA_A$Rs to steadily adjust. Brexanolone, a PAM of $GABA_A$Rs, tempers the dramatic changes in E/I balance that arise postpartum due to ALLO withdrawal and, as a result, has been shown to improve mood, cognition, and mother-infant bonding in women with PPD (Kanes et al., 2017; Dacarett-Galeano and Diao, 2019; Faden and Citrome, 2020; Meltzer-Brody and Kanes, 2020). The success of Brexanolone in treating PPD adds further credence to GABAergic dysfunction and withdrawal from ALLO as contributing factors involved in the pathophysiology of PPD. While Brexanolone must be intravenously administered in a hospital setting, recent data suggest another neuroactive steroid $GABA_A$R PAM drug, Zuranolone, is also effective in reducing PPD symptoms and unlike Brexanolone, can be orally administered (Deligiannidis et al., 2021). Additional GABA-modulating drugs are also under investigation for the treatment of mood dysregulation associated with other times of hormonal change including PMDD (Bixo et al., 2018; Bäckström et al., 2021; Schweizer-Schubert et al., 2021) and menopausal depression (Saripalli et al., 2021; Schweizer-Schubert et al., 2021) but more data are needed.

CONCLUSION

The ovarian hormones estradiol and progesterone, as well as the progesterone metabolite ALLO, exert substantial influence over the GABA system during periods of hormonal flux that characterize the female lifespan (**Figure 2**). However, there remain gaps in our understanding of the GABA system during these hormonally dynamic periods. This is in part because our current knowledge is based on results that often span different brain regions or focus on only one aspect of the GABA system. There are also components of the GABA system that remain almost completely unexplored. For example, PV neurons have been the focus but are not the sole GABA neurons in the brain. Thus, whether other GABA neurons, like SST and VIP neurons, are affected in relation to hormonal fluctuations across the female lifespan should be examined. In addition, the role of $GABA_B$ and $GABA_C$ receptors in modulating inhibitory tone during periods of hormonal transition periods warrants exploration, as some evidence suggests that GABA binding capacity at $GABA_B$ receptors differ between stages of the estrous cycle (Al-Dahan et al., 1994). It is also important to consider that different types of estrogen predominate during different phases of the female lifespan, yet the focus of ongoing research centers on estradiol, the most common type in women of child-bearing age (Stillwell, 2016). For example, minimal research has examined the effects of estriol, the main estrogen during pregnancy (Falah et al., 2015), and estrone, the predominant form of estrogen during menopause (Cui et al., 2013), on the GABA system. Further insight into how hormones affect the GABA system will broaden our understanding of the female brain and could lead to better treatments for women experiencing changes in brain function at each of these hormonal transition periods.

AUTHOR CONTRIBUTIONS

Both authors contributed to the article and approved the submitted version.

FUNDING

BL was supported by a grant from the National Institute of Health (MH117482).

REFERENCES

Afroz, S., Parato, J., Shen, H., and Smith, S. S. (2016). Synaptic pruning in the female hippocampus is triggered at puberty by extrasynaptic $GABA_A$ receptors on dendritic spines. *ELife* 5:e15106. doi: 10.7554/eLife.15106

Ahmed, E., and Lonstein, J. (2012). Vesicular GABA transporter and glutamic acid decarboxylase expression in the neural anxiety network of postpartum and virgin female rats. *Soc. Neurosci. Abstr.* 483:10.

Albert, K., Pruessner, J., and Newhouse, P. (2015). Estradiol levels modulate brain activity and negative responses to psychosocial stress across the menstrual cycle. *Psychoneuroendocrinology* 59, 14–24. doi: 10.1016/j.psyneuen.2015.04.022

Al-Dahan, M. I., Jalilian Tehrani, M. H., and Thalmann, R. H. (1994). Regulation of y-aminobutyric acidB ($GABA_B$) receptors in cerebral cortex during the estrous cycle. *Brain Res.* 640, 33–39. doi: 10.1016/0006-8993(94)91854-6

Altemus, M., Fong, J., Yang, R., Damast, S., Luine, V., and Ferguson, D. (2004). Changes in cerebrospinal fluid neurochemistry during pregnancy. *Biol. Psychiatry* 56, 386–392. doi: 10.1016/j.biopsych.2004.06.002

Andréen, L., Nyberg, S., Turkmen, S., van Wingen, G., Fernández, G., and Bäckström, T. (2009). Sex steroid induced negative mood may be explained by the paradoxical effect mediated by $GABA_A$ modulators. *Psychoneuroendocrinology* 34, 1121–1132. doi: 10.1016/j.psyneuen.2009.02.003

Arriaga-Avila, V., Martínez-Abundis, E., Cárdenas-Morales, B., Mercado-Gómez, O., Aburto-Arciniega, E., Miranda-Martínez, A., et al. (2014). Lactation Reduces Stress-Caused Dopaminergic Activity and Enhances GABAergic Activity in the Rat Medial Prefrontal Cortex. *J. Mol. Neurosci.* 52, 515–524. doi: 10.1007/s12031-013-0104-7

Bäckström, T., Ekberg, K., Hirschberg, A. L., Bixo, M., Epperson, C. N., Briggs, P., et al. (2021). A randomized, double-blind study on efficacy and safety of sepranolone in premenstrual dysphoric disorder. *Psychoneuroendocrinology* 133:105426. doi: 10.1016/j.psyneuen.2021.105426

Baker, K. D., Gray, A. R., and Richardson, R. (2017). The development of perineuronal nets around parvalbumin gabaergic neurons in the medial prefrontal cortex and basolateral amygdala of rats. *Behav. Neurosci.* 131, 289–303. doi: 10.1037/bne0000203

Barrientos, R. M., Brunton, P. J., Lenz, K. M., Pyter, L., and Spencer, S. J. (2019). Neuroimmunology of the female brain across the lifespan: Plasticity to psychopathology. *Brain Behav. Immun.* 79, 39–55 doi: 10.1016/j.bbi.2019.03.010

Barth, A. M. I., Ferando, I., and Mody, I. (2014). Ovarian cycle-linked plasticity of δ-$GABA_A$ receptor subunits in hippocampal interneurons affects gamma oscillations in vivo. *Front. Cell. Neurosci.* 8:222. doi: 10.3389/fncel.2014.00222

Bixo, M., Johansson, M., Timby, E., Michalski, L., and Bäckström, T. (2018). Effects of GABA active steroids in the female brain with a focus on the premenstrual dysphoric disorder. *J. Neuroendocrinol.* 30:e12553. doi: 10.1111/jne.12553

Bloch, M., Daly, R. C., and Rubinow, D. R. (2003). Endocrine factors in the etiology of postpartum depression. *Comp. Psychiatry* 44, 234–246. doi: 10.1016/S0010-440X(03)00034-8

Blume, S. R., Freedberg, M., Vantrease, J. E., Chan, R., Padival, M., Record, M. J., et al. (2017). Sex- and Estrus-Dependent Differences in Rat Basolateral Amygdala. *J. Neurosci.* 37, 10567–10586. doi: 10.1523/JNEUROSCI.0758-17.2017

Blurton-Jones, M., and Tuszynski, M. H. (2002). Estrogen receptor-beta colocalizes extensively with parvalbumin-labeled inhibitory neurons in the cortex, amygdala, basal forebrain, and hippocampal formation of intact and ovariectomized adult rats. *J. Comp. Neurol.* 452, 276–287. doi: 10.1002/cne.10393

Bosiacki, M., Gąssowska-Dobrowolska, M., Kojder, K., Fabiańska, M., Jeżewski, D., Gutowska, I., et al. (2019). Perineuronal Nets and Their Role in Synaptic Homeostasis. *Int. J. Mol. Sci.* 20:4108. doi: 10.3390/ijms20174108

Bosse, R., and Paolo, T. D. (1995). Dopamine and $GABA_A$ Receptor Imbalance after Ovariectomy in Rats: Model of Menopause. *J. Psychiatry* 20, 364–372.

Bossé, R., and Paolo, T. D. (1996). The modulation of brain dopamine and $GABA_A$ receptors by estradiol: A clue for CNS changes occurring at menopause. *Cell. Mol. Neurobiol.* 16, 199–212. doi: 10.1007/BF02088176

Braden, B. B., Kingston, M. L., Koenig, E. N., Lavery, C. N., Tsang, C. W. S., and Bimonte-Nelson, H. A. (2015). The $GABA_A$ antagonist bicuculline attenuates progesterone-induced memory impairments in middle-aged ovariectomized rats. *Front. Aging Neurosci.* 7:149. doi: 10.3389/fnagi.2015.00149

Braden, B. B., Talboom, J. S., Crain, I. D., Simard, A. R., Lukas, R. J., Prokai, L., et al. (2010). Medroxyprogesterone acetate impairs memory and alters the GABAergic system in aged surgically menopausal rats. *Neurobiol. Learn. Memory* 93, 444–453. doi: 10.1016/j.nlm.2010.01.002

Brunton, P. J., and Russell, J. A. (2010). Endocrine induced changes in brain function during pregnancy. *Brain Res.* 1364, 198–215. doi: 10.1016/j.brainres.2010.09.062

Buzsáki, G., and Wang, X.-J. (2012). Mechanisms of Gamma Oscillations. *Annu. Rev. Neurosci.* 35, 203–225. doi: 10.1146/annurev-neuro-062111-150444

Caballero, A., Flores-Barrera, E., Cass, D. K., and Tseng, K. Y. (2014). Differential regulation of parvalbumin and calretinin interneurons in the prefrontal cortex during adolescence. *Brain Struct. Funct.* 219, 395–406. doi: 10.1007/s00429-013-0508-8

Caballero, A., Flores-Barrera, E., Thomases, D. R., and Tseng, K. Y. (2020). Downregulation of parvalbumin expression in the prefrontal cortex during adolescence causes enduring prefrontal disinhibition in adulthood. *Neuropsychopharmacology* 45, 1527–1535. doi: 10.1038/s41386-020-0709-9

Caballero, A., Granberg, R., and Tseng, K. Y. (2016). Mechanisms contributing to prefrontal cortex maturation during adolescence. *Neurosci. Biobehav. Rev.* 70, 4–12. doi: 10.1016/j.neubiorev.2016.05.013

Caballero, A., and Tseng, K. Y. (2016). GABAergic Function as a Limiting Factor for Prefrontal Maturation during Adolescence. *Trends Neurosci.* 39, 441–448. doi: 10.1016/j.tins.2016.04.010

Carceller, H., Guirado, R., Ripolles-Campos, E., Teruel-Marti, V., and Nacher, J. (2020). Perineuronal Nets Regulate the Inhibitory Perisomatic Input onto Parvalbumin Interneurons and γ Activity in the Prefrontal Cortex. *J. Neurosci.* 40, 5008–5018. doi: 10.1523/JNEUROSCI.0291-20.2020

Cashion, A. B., Smith, M. J., and Wise, P. M. (2004). Glutamic Acid Decarboxylase 67 (GAD67) Gene Expression in Discrete Regions of the Rostral Preoptic Area Change During the Oestrous Cycle and with Age. *J. Neuroendocrinol.* 16, 711–716. doi: 10.1111/j.1365-2826.2004.01225.x

Chakraborty, T. R., Ng, L., and Gore, A. C. (2003). Age-Related Changes in Estrogen Receptor β in Rat Hypothalamus: A Quantitative Analysis. *Endocrinology* 144, 4164–4171. doi: 10.1210/en.2003-0052

Chugani, D. C., Muzik, O., Juhász, C., Janisse, J. J., Ager, J., and Chugani, H. T. (2001). Postnatal maturation of human $GABA_A$ receptors measured with positron emission tomography. *Ann. Neurol.* 49, 618–626. doi: 10.1002/ana.1003

Clemens, A. M., Lenschow, C., Beed, P., Li, L., Sammons, R., Naumann, R. K., et al. (2019). Estrus-Cycle Regulation of Cortical Inhibition. *Curr. Biol.* 29, 605–615. doi: 10.1016/j.cub.2019.01.045

Concas, A., Follesa, P., Barbaccia, M. L., Purdy, R. H., and Biggio, G. (1999). Physiological modulation of $GABA_A$ receptor plasticity by progesterone metabolites. *Eur. J. Pharmacol.* 375, 225–235. doi: 10.1016/S0014-2999(99)00232-0

Concas, A., Mostallino, M. C., Porcu, P., Follesa, P., Barbaccia, M. L., Trabucchi, M., et al. (1998). Role of brain allopregnanolone in the plasticity of -aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. *Proc. Natl. Acad. Sci.* 95, 13284–13289. doi: 10.1073/pnas.95.22.13284

Cruz, D. A., Eggan, S. M., and Lewis, D. A. (2003). Postnatal development of pre- and postsynaptic GABA markers at chandelier cell connections with pyramidal neurons in monkey prefrontal cortex. *J. Comp. Neurol.* 465, 385–400. doi: 10.1002/cne.10833

Cui, J., Shen, Y., and Li, R. (2013). Estrogen synthesis and signaling pathways during aging: From periphery to brain. *Trends Mol. Med.* 19, 197–209. doi: 10.1016/j.molmed.2012.12.007

Dacarett-Galeano, D. J., and Diao, X. Y. (2019). Brexanolone: A Novel Therapeutic in the Treatment of Postpartum Depression. *Am. J. Psychiatry Residents* 15, 2–4. doi: 10.1176/appi.ajp-rj.2019.150201

De Bondt, T., De Belder, F., Vanhevel, F., Jacquemyn, Y., and Parizel, P. M. (2015). Prefrontal GABA concentration changes in women—Influence of menstrual

cycle phase, hormonal contraceptive use, and correlation with premenstrual symptoms. *Brain Res.* 1597, 129–138. doi: 10.1016/j.brainres.2014.11.051

De Bonis, M., Torricelli, M., Severi, F. M., Luisi, S., De Leo, V., and Petraglia, F. (2012). Neuroendocrine aspects of placenta and pregnancy. *Gynecol. Endocrinol.* 28, 22–26. doi: 10.3109/09513590.2012.651933

Deems, N., and Leuner, B. (2021). Pregnancy, Postpartum and Parity: Resilience and Vulnerability in Brain Health and Disease. *Front. Neuroendocrinol.* 57, 1–43. doi: 10.1016/j.yfrne.2020.100820

Delevich, K., Klinger, M., Okada, N. J., and Wilbrecht, L. (2021). Coming of age in the frontal cortex: The role of puberty in cortical maturation. *Semin. Cell Devel. Biol.* 118, 64–72. doi: 10.1016/j.semcdb.2021.04.021

Deligiannidis, K. M., Kroll-Desrosiers, A. R., Mo, S., Nguyen, H. P., Svenson, A., Jaitly, N., et al. (2016). Peripartum neuroactive steroid and γ-aminobutyric acid profiles in women at-risk for postpartum depression. *Psychoneuroendocrinology* 70, 98–107. doi: 10.1016/j.psyneuen.2016.05.010

Deligiannidis, K. M., Meltzer-Brody, S., Gunduz-Bruce, H., Doherty, J., Jonas, J., Li, S., et al. (2021). Effect of Zuranolone vs Placebo in Postpartum Depression: A Randomized Clinical Trial. *JAMA Psychiatry* 78, 951–959. doi: 10.1001/jamapsychiatry.2021.1559

Deligiannidis, K. M., Sikoglu, E. M., Shaffer, S. A., Frederick, B., Svenson, A. E., Kopoyan, A., et al. (2013). GABAergic neuroactive steroids and resting-state functional connectivity in postpartum depression: A preliminary study. *J. Psychiatric Res.* 47, 816–828. doi: 10.1016/j.jpsychires.2013.02.010

Diaz Brinton, R. (2012). Minireview: Translational Animal Models of Human Menopause: Challenges and Emerging Opportunities. *Endocrinology* 153, 3571–3578. doi: 10.1210/en.2012-1340

Drzewiecki, C. M., Willing, J., and Juraska, J. M. (2020). Influences of age and pubertal status on number and intensity of perineuronal nets in the rat medial prefrontal cortex. *Brain Struct. Funct.* 225, 2495–2507. doi: 10.1007/s00429-020-02137-z

Duarte-Guterman, P., Leuner, B., and Galea, L. A. M. (2019). The long and short term effects of motherhood on the brain. *Front. Neuroendocrinol.* 53:100740. doi: 10.1016/j.yfrne.2019.02.004

Dumitriu, D., Hao, J., Hara, Y., Kaufmann, J., Janssen, W. G. M., Lou, W., et al. (2010). Selective Changes in Thin Spine Density and Morphology in Monkey Prefrontal Cortex Correlate with Aging-Related Cognitive Impairment. *J. Neurosci.* 30, 7507–7515. doi: 10.1523/JNEUROSCI.6410-09.2010

Epperson, C. N., Gueorguieva, R., Czarkowski, K. A., Stiklus, S., Sellers, E., Krystal, J. H., et al. (2006). Preliminary evidence of reduced occipital GABA concentrations in puerperal women: A 1H-MRS study. *Psychopharmacology* 186, 425–433. doi: 10.1007/s00213-006-0313-7

Epperson, C. N., Haga, K., Mason, G. F., Sellers, E., Gueorguieva, R., Zhang, W., et al. (2002). Cortical gamma-Aminobutyric Acid Levels Across the Menstrual Cycle in Healthy Women and Those With Premenstrual Dysphoric Disorder. *Arch. Gen. Psychiatry* 59, 851–859. doi: 10.1001/archpsyc.59.9.851

Erel, T., and Guralp, O. (2011). Epilepsy and menopause. *Arch.Gynecol. Obst.* 284, 749–755. doi: 10.1007/s00404-011-1936-4

Fadalti, M., Petraglia, F., Luisi, S., Bernardi, F., Casarosa, E., Ferrari, E., et al. (1999). Changes of Serum Allopregnanolone Levels in the First 2 Years of Life and during Pubertal Development. *Pediatric Res.* 46, 323–327.

Faden, J., and Citrome, L. (2020). Intravenous brexanolone for postpartum depression: What it is, how well does it work, and will it be used? *Ther. Adv. Psychopharmacol.* 10:12045125320968658. doi: 10.1177/2045125320968658

Falah, N., Torday, J., Quinney, S. K., and Haas, D. M. (2015). Estriol review: Clinical applications and potential biomedical importance. *Clin. Res. Trials* 1, 29–33. doi: 10.15761/CRT.1000109

Fénelon, V. S., and Herbison, A. E. (1996). Plasticity in $GABA_A$ Receptor Subunit mRNA Expression by Hypothalamic Magnocellular Neurons in the Adult Rat. *J. Neurosci.* 16, 4872–4880. doi: 10.1523/JNEUROSCI.16-16-04872.1996

Fénelon, V. S., and Herbison, A. E. (2000). Progesterone regulation of $GABA_A$ receptor plasticity in adult rat supraoptic nucleus. *Eur. J. Neurosci.* 12, 1617–1623. doi: 10.1046/j.1460-9568.2000.00053.x

Ferando, I., and Mody, I. (2013). Altered gamma oscillations during pregnancy through loss of δ subunit-containing $GABA_A$ receptors on parvalbumin interneurons. *Front. Neural Circuits* 7:114. doi: 10.3389/fncir.2013.00144

Filice, F., Lauber, E., Vörckel, K. J., Wöhr, M., and Schwaller, B. (2018). 17-β estradiol increases parvalbumin levels in Pvalb heterozygous mice and attenuates behavioral phenotypes with relevance to autism core symptoms. *Mol. Autism* 9:15. doi: 10.1186/s13229-018-0199-3

Follesa, P., Porcu, P., Sogliano, C., Cinus, M., Biggio, F., Mancuso, L., et al. (2002). Changes in $GABA_A$ receptor γ2 subunit gene expression induced by long-term administration of oral contraceptives in rats. *Neuropharmacol.* 42, 325–336. doi: 10.1016/s0028-3908(01)00187-3

Follesa, P., Floris, S., Tuligi, G., Mostallino, M. C., Concas, A., and Biggio, G. (1998). Molecular and functional adaptation of the $GABA_A$ receptor complex during pregnancy and after delivery in the rat brain. *Eur. J. Neurosci.* 10, 2905–2912. doi: 10.1111/j.1460-9568.1998.00300.x

Fung, S. J., Webster, M. J., Sivagnanasundaram, S., Duncan, C., Elashoff, M., and Weickert, C. S. (2010). Expression of Interneuron Markers in the Dorsolateral Prefrontal Cortex of the Developing Human and in Schizophrenia. *Am. J. Psychiatry* 167, 1479–1488. doi: 10.1176/appi.ajp.2010.09060784

Genazzani, A. R., Bernardi, F., Pluchino, N., Begliuomini, S., Lenzi, E., Casarosa, E., et al. (2005). Endocrinology of Menopausal Transition and Its Brain Implications. *CNS Spectrums* 10, 449–457. doi: 10.1017/S1092852900023142

Gildawie, K. R., Honeycutt, J. A., and Brenhouse, H. C. (2020). Region-specific Effects of Maternal Separation on Perineuronal Net and Parvalbumin-expressing Interneuron Formation in Male and Female Rats. *Neuroscience* 428, 23–37. doi: 10.1016/j.neuroscience.2019.12.010

Giovenardi, M., Padoin, M. J., Cadore, L. P., and Lucion, A. B. (1997). Hypothalamic Paraventricular Nucleus, Oxytocin, and Maternal Aggression in Rats. *Ann. N.Y. Acad. Sci.* 807, 606–609. doi: 10.1111/j.1749-6632.1997.tb51981.x

Glausier, J. R., Fish, K. N., and Lewis, D. A. (2014). Altered parvalbumin basket cell inputs in the dorsolateral prefrontal cortex of schizophrenia subjects. *Mol. Psychiatry* 19, 30–36. doi: 10.1038/mp.2013.152

Gordon, J. L., Girdler, S. S., Meltzer-Brody, S. E., Stika, C. S., Thurston, R. C., Clark, C. T., et al. (2015). Ovarian Hormone Fluctuation, Neurosteroids, and HPA Axis Dysregulation in Perimenopausal Depression: A Novel Heuristic Model. *Am. J. Psychiatry* 172, 227–236. doi: 10.1176/appi.ajp.2014.14070918

Grieb, Z. A., Ford, E. G., Yagan, M., Lau, B. Y. B., Manfredsson, F. P., Krishnan, K., et al. (2021). Oxytocin receptors in the midbrain dorsal raphe are essential for postpartum maternal social and affective behaviors. *Psychoneuroendocrinology* 131:105332. doi: 10.1016/j.psyneuen.2021.105332

Griffiths, J. L., and Lovick, T. A. (2005). GABAergic neurones in the rat periaqueductal grey matter express α4, β1 and δ $GABA_A$ receptor subunits: Plasticity of expression during the estrous cycle. *Neuroscience* 136, 457–466. doi: 10.1016/j.neuroscience.2005.08.013

Grove-Strawser, D., Jimenez-Linan, M., and Rubin, B. S. (2007). Middle-Aged Female Rats Lack the Dynamic Changes in GAD $_{67}$ mRNA Levels Observed in Young Females on the Day of a Luteinising Hormone Surge. *J. Neuroendocrinol.* 19, 708–716. doi: 10.1111/j.1365-2826.2007.01579.x

Hantsoo, L., and Epperson, C. N. (2020). Allopregnanolone in premenstrual dysphoric disorder (PMDD): Evidence for dysregulated sensitivity to $GABA_A$ receptor modulating neuroactive steroids across the menstrual cycle. *Neurobiol. Stress* 12:100213. doi: 10.1016/j.ynstr.2020.100213

Harada, M., Kubo, H., Nose, A., Nishitani, H., and Matsuda, T. (2011). Measurement of variation in the human cerebral GABA level by in vivo MEGA-editing proton MR spectroscopy using a clinical 3 T instrument and its dependence on brain region and the female menstrual cycle. *Hum. Brain Mapp.* 32, 828–833. doi: 10.1002/hbm.21086

Harden, C. L., Pulver, M. C., Ravdin, L., and Jacobs, A. R. (1999). The Effect of Menopause and Perimenopause on the Course of Epilepsy. *Epilepsia* 40, 1402–1407. doi: 10.1111/j.1528-1157.1999.tb02012.x

Hart, S. A., Patton, J. D., and Woolley, C. S. (2001). Quantitative analysis of ER alpha and GAD colocalization in the hippocampus of the adult female rat. *J. Comp. Neurol.* 440, 144–155. doi: 10.1002/cne.1376

Herbison, A. E. (2001). Physiological roles for the neurosteroid allopregnanolone in the modulation of brain function during pregnancy and parturition. *Progress in Brain Research* 133, 39–47. doi: 10.1016/S0079-6123(01)33003-0

Herbison, A. E. (2016). Control of puberty onset and fertility by gonadotropin-releasing hormone neurons. *Nat. Rev. Endocrinol.* 12, 452–466. doi: 10.1038/nrendo.2016.70

Herbison, A. E. (2020). A simple model of estrous cycle negative and positive feedback regulation of GnRH secretion. *Front. Neuroendocrinol.* 57:100837. doi: 10.1016/j.yfrne.2020.100837

Holder, M. K., and Blaustein, J. D. (2014). Puberty and adolescence as a time of vulnerability to stressors that alter neurobehavioral processes. *Front. Neuroendocrinol.* 35:89–110. doi: 10.1016/j.yfrne.2013.10.004

Honeycutt, J. A., Keary, III, K. M., Kania, V. M., and Chrobak, J. J. (2016). Developmental Age Differentially Mediates the Calcium-Binding Protein Parvalbumin in the Rat: Evidence for a Selective Decrease in Hippocampal Parvalbumin Cell Counts. *Devel. Neurosci.* 38, 105–114. doi: 10.1159/000444447

Jacobs, E. G., and Goldstein, J. M. (2018). The middle-aged brain: Biological sex and sex hormones shape memory circuitry. *Curr. Opin. Behav. Sci.* 23, 84–91. doi: 10.1016/j.cobeha.2018.03.009

Jiménez-Balado, J., Ycaza Herrera, A., Igwe, K., Klem, L., Buyukturkoglu, K., Irimia, A., et al. (2021). Reduced Hippocampal GABA+ Is Associated With Poorer Episodic Memory in Healthy Older Women: A Pilot Study. *Front. Behav. Neurosci.* 15:695416. doi: 10.3389/fnbeh.2021.695416

Jones, R. E., and Lopez, K. H. (2014). "Puberty," in *Human Reproductive Biology*, eds R.E. Jones and K.H. Lopez (Amsterdam: Elsevier), 103–118. doi: 10.1016/B978-0-12-382184-3.00006-4

Jost, A., Vigier, B., Prépin, J., and Perchellet, J. P. (1973). "Studies on Sex Differentiation in Mammals," in *In Proceedings of the 1972 Laurentian Hormone Conference* (Amsterdam: Elsevier), 1–41. doi: 10.1016/B978-0-12-571129-6.50004-X

Juraska, J. M., Sisk, C. L., and DonCarlos, L. L. (2013). Sexual differentiation of the adolescent rodent brain: Hormonal influences and developmental mechanisms. *Horm. Behav.* 64, 203–210. doi: 10.1016/j.yhbeh.2013.05.010

Juraska, J. M., and Willing, J. (2017). Pubertal onset as a critical transition for neural development and cognition. *Brain Res.* 1654, 87–94. doi: 10.1016/j.brainres.2016.04.012

Kanes, S., Colquhoun, H., Gunduz-Bruce, H., Raines, S., Arnold, R., Schacterle, A., et al. (2017). Brexanolone (SAGE-547 injection) in post-partum depression: A randomised controlled trial. *Lancet* 390, 480–489. doi: 10.1016/S0140-6736(17)31264-3

Karetko, M., and Skangiel-Kramska, J. (2009). Diverse functions of perineuronal nets. *Neurobiol. Exper.* 69, 564–577. https://doi.org/PMID:20048772

Keating, N., Zeak, N., and Smith, S. S. (2019). Pubertal hormones increase hippocampal expression of α4βδ $GABA_A$ receptors. *Neurosci. Lett.* 701, 65–70. doi: 10.1016/j.neulet.2019.02.005

Kermath, B. A., and Gore, A. C. (2012). Neuroendocrine Control of the Transition to Reproductive Senescence: Lessons Learned from the Female Rodent Model. *Neuroendocrinol.* 96, 1–12. doi: 10.1159/000335994

Keyser-Marcus, L., Stafisso-Sandoz, G., Gerecke, K., Jasnow, A., Nightingale, L., Lambert, K. G., et al. (2001). Alterations of medial preoptic area neurons following pregnancy and pregnancy-like steroidal treatment in the rat. *Brain Res. Bull.* 55, 737–745. doi: 10.1016/S0361-9230(01)00554-8

Kilb, W. (2012). Development of the GABAergic System from Birth to Adolescence. *Neuroscientist* 18, 613–630. doi: 10.1177/1073858411422114

Kimball, A., Dichtel, L. E., Nyer, M. B., Mischoulon, D., Fisher, L. B., Cusin, C., et al. (2020). The allopregnanolone to progesterone ratio across the menstrual cycle and in menopause. *Psychoneuroendocrinology* 112:104512. doi: 10.1016/j.psyneuen.2019.104512

Koebele, S. V., and Bimonte-Nelson, H. A. (2016). Modeling menopause: The utility of rodents in translational behavioral endocrinology research. *Maturitas* 87, 5–17. doi: 10.1016/j.maturitas.2016.01.015

Koebele, S. V., Hiroi, R., Plumley, Z. M. T., Melikian, R., Prakapenka, A. V., Patel, S., et al. (2021). Clinically Used Hormone Formulations Differentially Impact Memory, Anxiety-Like, and Depressive-Like Behaviors in a Rat Model of Transitional Menopause. *Front. Behav. Neurosci.* 15:696838. doi: 10.3389/fnbeh.2021.696838

Koebele, S. V., Mennenga, S. E., Hiroi, R., Quihuis, A. M., Hewitt, L. T., Poisson, M. L., et al. (2017). Cognitive changes across the menopause transition: A longitudinal evaluation of the impact of age and ovarian status on spatial memory. *Horm. Behav.* 87, 96–114. doi: 10.1016/j.yhbeh.2016.10.010

Kornblatt, J. J., and Grattan, D. R. (2000). Lactation Alters Gamma-Aminobutyric Acid Neuronal Activity in the Hypothalamus and Cerebral Cortex in the Rat. *Neuroendocrinology* 73, 175–184. doi: 10.1159/000054634

Lau, B. Y. B., Krishnan, K., Huang, Z. J., and Shea, S. D. (2020). Maternal Experience-Dependent Cortical Plasticity in Mice Is Circuit- and Stimulus-Specific and Requires MECP2. *J. Neurosci.* 40, 1514–1526. doi: 10.1523/JNEUROSCI.1964-19.2019

Laube, C., van den Bos, W., and Fandakova, Y. (2020). The relationship between pubertal hormones and brain plasticity: Implications for cognitive training in adolescence. *Devel. Cogn. Neurosci.* 42:100753.. doi: 10.1016/j.dcn.2020.100753

Lee, G., and Gammie, S. C. (2009). $GABA_A$ receptor signaling in the lateral septum regulates maternal aggression in mice. *Behav. Neurosci.* 123, 1169–1177. doi: 10.1037/a0017535

Levine, J. E. (2015). "Neuroendocrine control of the ovarian cycle of the rat," in *Knobil and Neill's Physiology of Reproduction*, eds T. Plant and A. Zeleznik (San Diego, CA: Elsevier), 1199–1257.

Licheri, V., Talani, G., Gorule, A. A., Mostallino, M. C., Biggio, G., and Sanna, E. (2015). Plasticity of $GABA_A$ receptors during pregnancy and postpartum period: from gene to function. *Neural Plast.* 2015:170435. doi: 10.1155/2015/170435

Lonstein, J. S., Maguire, J., Meinlschmidt, G., and Neumann, I. D. (2014). Emotion and Mood Adaptations in the Peripartum Female: Complementary Contributions of GABA and Oxytocin. *J. Neuroendocrinol.* 26, 649–664. doi: 10.1111/jne.12188

Löscher, W., Wahnschaffe, U., Rundfeldt, C., Hönack, D., and Hoppen, H.-O. (1992). Regional alterations in brain amino acids during the estrous cycle of the rat. *Neurochem. Res.* 17, 973–977. doi: 10.1007/BF00966823

Lovick, T. A. (2012). Estrous cycle and stress: influence of progesterone on the female brain. *Braz. J. Med. Biol. Res.* 45, 314–320. doi: 10.1590/s0100-879x2012007500044

Lovick, T. A., and Zangrossi, H. (2021). Effect of Estrous Cycle on Behavior of Females in Rodent Tests of Anxiety. *Front. Psychiatry* 12:711065. doi: 10.3389/fpsyt.2021.711065

Luisi, S., Petraglia, F., Benedetto, C., Nappi, R. E., Bernardi, F., Fadalti, M., et al. (2000). Serum Allopregnanolone Levels in Pregnant Women: Changes during Pregnancy, at Delivery, and in Hypertensive Patients. *J. Clin. Endocrinol. Metab.* 85, 2429–2433.

Luna, B., Garver, K. E., Urban, T. A., Lazar, N. A., and Sweeney, J. A. (2004). Maturation of Cognitive Processes From Late Childhood to Adulthood. *Child Devel.* 75, 1357–1372. doi: 10.1111/j.1467-8624.2004.00745.x

MacKenzie, G., and Maguire, J. (2014). The role of ovarian hormone-derived neurosteroids on the regulation of $GABA_A$ receptors in affective disorders. *Psychopharmacology* 231, 3333–3342. doi: 10.1007/s00213-013-3423-z

Maffucci, J. A., and Gore, A. C. (2009). "Hypothalamic neural systems controlling the female reproductive life cycle: gonadotropin-releasing hormone, glutamate, and GABA," in *International Review of Cell and Molecular Biology*, ed. K. W. Jeon (Burlington, MA: Elsevier), 69–127. doi: 10.1016/S1937-6448(08)02002-9

Maguire, J. (2019). "Hormonal and immunological factors in postpartum psychosis," in *Biomarkers of Postpartum Psychiatric Disorders*, eds J L. Payne and L M. Osborne (Cambridge: Academic Press), 159–179.

Maguire, J., Ferando, I., Simonsen, C., and Mody, I. (2009). Excitability Changes Related to $GABA_A$ Receptor Plasticity during Pregnancy. *J. Neurosci.* 29, 9592–9601. doi: 10.1523/JNEUROSCI.2162-09.2009

Maguire, J., and Mody, I. (2007). Neurosteroid Synthesis-Mediated Regulation of $GABA_A$ Receptors: Relevance to the Ovarian Cycle and Stress. *J. Neurosci.* 27, 2155–2162. doi: 10.1523/JNEUROSCI.4945-06.2007

Maguire, J., and Mody, I. (2008). $GABA_A$R Plasticity during Pregnancy: Relevance to Postpartum Depression. *Neuron* 59, 207–213. doi: 10.1016/j.neuron.2008.06.019

Maguire, J., and Mody, I. (2009). Steroid hormone fluctuations and $GABA_A$R plasticity. *Psychoneuroendocrinology* 34, S84–S90. doi: 10.1016/j.psyneuen.2009.06.019

Maguire, J., Stell, B. M., Rafizadeh, M., and Mody, I. (2005). Ovarian cycle–linked changes in $GABA_A$ receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat. Neurosci.* 8, 797–804. doi: 10.1038/nn1469

Majewska, M. D., Ford-Rice, F., and Falkay, G. (1989). Pregnancy-induced alterations of $GABA_A$ receptor sensitivity in maternal brain: An antecedent of post-partum 'blues'? *Brain Res.* 482, 397–401. doi: 10.1016/0006-8993(89)91208-0

Mauney, S. A., Athanas, K. M., Pantazopoulos, H., Shaskan, N., Passeri, E., Berretta, S., et al. (2013). Developmental Pattern of Perineuronal Nets in the Human Prefrontal Cortex and Their Deficit in Schizophrenia. *Biol. Psychiatry* 74, 427–435. doi: 10.1016/j.biopsych.2013.05.007

McCartney, C. R., Blank, S. K., Prendergast, K. A., Chhabra, S., Eagleson, C. A., Helm, K. D., et al. (2007). Obesity and Sex Steroid Changes across Puberty: Evidence for Marked Hyperandrogenemia in Pre- and Early Pubertal Obese Girls. *J. Clin. Endocrinol. Meta.* 92, 430–436. doi: 10.1210/jc.2006-2002

McLean, A. C., Valenzuela, N., Fai, S., and Bennett, S. A. L. (2012). Performing Vaginal Lavage, Crystal Violet Staining, and Vaginal Cytological Evaluation for Mouse Estrous Cycle Staging Identification. *J. Visualized Experiments* 67:e4389. doi: 10.3791/4389

Melón, L., Hammond, R., Lewis, M., and Maguire, J. (2018). A Novel, Synthetic, Neuroactive Steroid Is Effective at Decreasing Depression-Like Behaviors and Improving Maternal Care in Preclinical Models of Postpartum Depression. *Front. Endocrinol.* 9:703. doi: 10.3389/fendo.2018.00703

Meltzer-Brody, S., and Kanes, S. J. (2020). Allopregnanolone in postpartum depression: Role in pathophysiology and treatment. *Neurobiol. Stress* 12:100212. doi: 10.1016/j.ynstr.2020.100212

Meyer, H. S., Schwarz, D., Wimmer, V. C., Schmitt, A. C., Kerr, J. N. D., Sakmann, B., et al. (2011). Inhibitory interneurons in a cortical column form hot zones of inhibition in layers 2 and 5A. *Proc. Natl. Acad. Sci.* 108, 16807–16812. doi: 10.1073/pnas.1113648108

Miller, S. M., and Lonstein, J. S. (2011). Autoradiographic analysis of $GABA_A$ receptor binding in the neural anxiety network of postpartum and non-postpartum laboratory rats. *Brain Res. Bull.* 86, 60–64. doi: 10.1016/j.brainresbull.2011.05.013

Montoya, E. R., and Bos, P. A. (2017). How oral contraceptives impact social-emotional behavior and brain function. *Trends Cogn. Sci.* 21, 125–136. doi: 10.1016/j.tics.2016.11.005

Morrison, J. H., Brinton, R. D., Schmidt, P. J., and Gore, A. C. (2006). Estrogen, Menopause, and the Aging Brain: How Basic Neuroscience Can Inform Hormone Therapy in Women. *J. Neurosci.* 26, 10332–10348. doi: 10.1523/JNEUROSCI.3369-06.2006

Nair, R. R., Verma, P., and Singh, K. (2017). Immune-endocrine crosstalk during pregnancy. *Gen. Comp. Endocrinol.* 242, 18–23. doi: 10.1016/j.ygcen.2016.03.003

Nappi, R. E., and Cucinella, L. (2020). "Long-Term Consequences of Menopause," in *Female Reproductive Dysfunction*, eds F. Petraglia and B. C. Fauser (Midtown Manhattan: Springer International Publishing), 1–13. doi: 10.1007/978-3-030-03594-5_17-1

Neal-Perry, G. S., Zeevalk, G. D., Shu, J., and Etgen, A. M. (2008). Restoration of the Luteinizing Hormone Surge in Middle-Aged Female Rats by Altering the Balance of $GABA_A$ and Glutamate Transmission in the Medial Preoptic Area. *Biol. Reprod.* 79, 878–888. doi: 10.1095/biolreprod.108.069831

Noriega, N. C., Eghlidi, D. H., Garyfallou, V. T., Kohama, S. G., Kryger, S. G., and Urbanski, H. F. (2010). Influence of 17β-estradiol and progesterone on GABAergic gene expression in the arcuate nucleus, amygdala and hippocampus of the rhesus macaque. *Brain Res.* 1307, 28–42. doi: 10.1016/j.brainres.2009.10.011

Oyola, M. G., and Handa, R. J. (2017). Hypothalamic–pituitary–adrenal and hypothalamic–pituitary–gonadal axes: Sex differences in regulation of stress responsivity. *Stress* 20, 476–494. doi: 10.1080/10253890.2017.1369523

Page, C. E., and Coutellier, L. (2018). Adolescent Stress Disrupts the Maturation of Anxiety-related Behaviors and Alters the Developmental Trajectory of the Prefrontal Cortex in a Sex- and Age-specific Manner. *Neuroscience* 390, 265–277. doi: 10.1016/j.neuroscience.2018.08.030

Page, C. E., Shepard, R., Heslin, K., and Coutellier, L. (2019). Prefrontal parvalbumin cells are sensitive to stress and mediate anxiety-related behaviors in female mice. *Sci. Rep.* 9:19772 doi: 10.1038/s41598-019-56424-9

Parato, J., Shen, H., and Smith, S. S. (2019). A4βδ $GABA_A$ Receptors Trigger Synaptic Pruning and Reduce Dendritic Length of Female Mouse CA3 Hippocampal Pyramidal Cells at Puberty. *Neuroscience* 398, 23–36. doi: 10.1016/j.neuroscience.2018.11.032

Pawluski, J. L., Walker, S. K., and Galea, L. A. M. (2006). Reproductive experience differentially affects spatial reference and working memory performance in the mother. *Horm. Behav.* 49, 143–149. doi: 10.1016/j.yhbeh.2005.05.016

Peng, M.-T., and Huang, H.-H. (1972). Aging of Hypothalamic-Pituitary-Ovarian Function in the Rat. *Fertil. Steril.* 23, 535–542. doi: 10.1016/S0015-0282(16)39131-2

Piekarski, D. J., Boivin, J. R., and Wilbrecht, L. (2017a). Ovarian Hormones Organize the Maturation of Inhibitory Neurotransmission in the Frontal Cortex at Puberty Onset in Female Mice. *Curr. Biol.* 27, 1735.e–1745.e. doi: 10.1016/j.cub.2017.05.027

Piekarski, D. J., Johnson, C. M., Boivin, J. R., Thomas, A. W., Lin, W. C., Delevich, K., et al. (2017b). Does puberty mark a transition in sensitive periods for plasticity in the associative neocortex? *Brain Res.* 1654, 123–144. doi: 10.1016/j.brainres.2016.08.042

Pinto, J. G. A., Hornby, K. R., Jones, D. G., and Murphy, K. M. (2010). Developmental changes in GABAergic mechanisms in human visual cortex across the lifespan. *Front. Cell. Neurosci.* 4:16. doi: 10.3389/fncel.2010.00016

Porcu, P., Serra, M., and Concas, A. (2019). The brain as a target of hormonal contraceptives: Evidence from animal studies. *Front. Neuroendocrinol.* 55:100799. doi: 10.1016/j.yfrne.2019.100799

Prakapenka, A. V., Hiroi, R., Quihuis, A. M., Carson, C., Patel, S., Berns-Leone, C., et al. (2018). Contrasting effects of individual versus combined estrogen and progestogen regimens as working memory load increases in middle-aged ovariectomized rats: One plus one does not equal two. *Neurobiol. Aging* 64, 1–14. doi: 10.1016/j.neurobiolaging.2017.11.015

Qureshi, G. A., Hansen, S., and Södersten, P. (1987). Offspring control of cerebrospinal fluid GABA concentrations in lactating rats. *Neurosci. Lett.* 75, 85–88. doi: 10.1016/0304-3940(87)90080-2

Ragan, C. M., Ahmed, E. I., Vitale, E. M., Linning-Duffy, K., Miller-Smith, S. M., Maguire, J., et al. (2022). Postpartum State, but Not Maternal Caregiving or Level of Anxiety, Increases Medial Prefrontal Cortex GAD65 and vGAT in Female Rats. *Front. Glob. Women's Health* 2, 1–10. doi: 10.3389/fgwh.2021.746518

Rao, Y. S., Mott, N. N., Wang, Y., Chung, W. C. J., and Pak, T. R. (2013). MicroRNAs in the Aging Female Brain: A Putative Mechanism for Age-Specific Estrogen Effects. *Endocrinology* 154, 2795–2806. doi: 10.1210/en.2013-1230

Reed, B. G., and Carr, B. R. (2018). "The normal menstrual cycle and the control of ovulation," in *Endotext*, eds K. R. Feingold, B. Anawalt, and A. Boyce (South Dartmouth: Endotext). Available online at: https://www.ncbi.nlm.nih.gov/books/NBK279054/ (accessed August 18, 2021).

Rodríguez-Landa, J. F., Olmos-Vázquez, O. J., Dutra da Costa, B. P., Lima-Maximino, M., Maximino, C., et al. (2020). Actions of progesterone on depression-like behavior in a model of surgical menopause are mediated by $GABA_A$ receptors. *Salud Mental* 43, 43–53. doi: 10.17711/SM.0185-3325.2020.007

Równiak, M. (2017). The neurons expressing calcium-binding proteins in the amygdala of the guinea pig: Precisely designed interface for sex hormones. *Brain Struct. Funct.* 222, 3775–3793. doi: 10.1007/s00429-017-1432-0

Rudy, B., Fishell, G., Lee, S., and Hjerling-Leffler, J. (2011). Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. *Devel. Neurobiol.* 71, 45–61. doi: 10.1002/dneu.20853

Sabaliauskas, N., Shen, H., Molla, J., Gong, Q. H., Kuver, A., Aoki, C., et al. (2015). Neurosteroid effects at α4βδ $GABA_A$ receptors alter spatial learning and synaptic plasticity in CA1 hippocampus across the estrous cycle of the mouse. *Brain Res.* 1621, 170–186. doi: 10.1016/j.brainres.2014.12.026

Sabihi, S., Dong, S. M., Durosko, N. E., and Leuner, B. (2014). Oxytocin in the medial prefrontal cortex regulates maternal care, maternal aggression and anxiety during the postpartum period. *Front. Behav. Neurosci.* 8:258. doi: 10.3389/fnbeh.2014.00258

Sabihi, S., Goodpaster, C., Maurer, S., and Leuner, B. (2021). GABA in the medial prefrontal cortex regulates anxiety-like behavior during the postpartum period. *Behav. Brain Res.* 398:112967. doi: 10.1016/j.bbr.2020.112967

Sanna, E., Mostallino, M. C., Murru, L., Carta, M., Talani, G., Zucca, S., et al. (2009). Changes in Expression and Function of Extrasynaptic $GABA_A$ Receptors in the Rat Hippocampus during Pregnancy and after Delivery. *J. Neurosci.* 29, 1755–1765. doi: 10.1523/JNEUROSCI.3684-08.2009

Saripalli, A., Amar, P., Duffee, L. E., and Clayton, A. H. (2021). $GABA_A$ Receptor Positive Allosteric Modulators as a Novel Approach to Treating Depression: A Review of Available Data. *Touchrev. Neurol.* 17, 77–84.

Scharfman, H. E., and MacLusky, N. J. (2006). The Influence of Gonadal Hormones on Neuronal Excitability. Seizures, and Epilepsy in the Female. *Epilepsia* 47, 1423–1440. doi: 10.1111/j.1528-1167.2006.00672.x

Schulz, K. M., Molenda-Figueira, H. A., and Sisk, C. L. (2009). Back to the future: The organizational–activational hypothesis adapted to puberty and adolescence. *Horm. Behav.* 55, 597–604. doi: 10.1016/j.yhbeh.2009.03.010

Schweizer-Schubert, S., Gordon, J. L., Eisenlohr-Moul, T. A., Meltzer-Brody, S., Schmalenberger, K. M., Slopien, R., et al. (2021). Steroid Hormone Sensitivity in Reproductive Mood Disorders: On the Role of the $GABA_A$ Receptor Complex and Stress During Hormonal Transitions. *Front. Med.* 7:479646. doi: 10.3389/fmed.2020.479646

Sethuraman, R., Lee, T.-L., Chui, J.-W., and Tachibana, S. (2006). Changes in Amino Acids and Nitric Oxide Concentration in Cerebrospinal Fluid During Labor Pain. *Neurochem. Res.* 31, 1127–1133. doi: 10.1007/s11064-006-9133-8

Shanmugan, S., and Epperson, C. N. (2014). Estrogen and the prefrontal cortex: Towards a new understanding of estrogen's effects on executive functions in the menopause transition: Estrogen and the Prefrontal Cortex. *Hum. Brain Mapp.* 35, 847–865. doi: 10.1002/hbm.22218

Shen, H., Gong, Q. H., Aoki, C., Yuan, M., Ruderman, Y., Dattilo, M., et al. (2007). Reversal of neurosteroid effects at α4β2δ $GABA_A$ receptors triggers anxiety at puberty. *Nat. Neurosci.* 10, 469–477. doi: 10.1038/nn1868

Shen, H., Kenney, L., and Smith, S. S. (2020). Increased Dendritic Branching of and Reduced δ-$GABA_A$ Receptor Expression on Parvalbumin-Positive Interneurons Increase Inhibitory Currents and Reduce Synaptic Plasticity at Puberty in Female Mouse CA1 Hippocampus. *Front. Cell. Neurosci.* 14:203. doi: 10.3389/fncel.2020.00203

Shen, H., Sabaliauskas, N., Sherpa, A., Fenton, A. A., Stelzer, A., Aoki, C., et al. (2010). A Critical Role for $GABA_A$ Receptors in Shaping Learning Deficits at Puberty in Mice. *Science* 327, 1515–1518. doi: 10.1126/science.1184245

Silveri, M. M., Sneider, J. T., Crowley, D. J., Covell, M. J., Acharya, D., Rosso, I. M., et al. (2013). Frontal Lobe γ-Aminobutyric Acid Levels During Adolescence: Associations with Impulsivity and Response Inhibition. *Biol. Psychiatry* 74, 296–304. doi: 10.1016/j.biopsych.2013.01.033

Sisk, C. L., and Foster, D. L. (2004). The neural basis of puberty and adolescence. *Nat. Neurosci.* 7, 1040–1047. doi: 10.1038/nn1326

Sisk, C. L., and Zehr, J. L. (2005). Pubertal hormones organize the adolescent brain and behavior. *Front. Neuroendocrinol.* 26:163–174. doi: 10.1016/j.yfrne.2005.10.003

Smith, M., Keel, J., Greenberg, B., Adams, L., Schmidt, P., Rubinow, D., et al. (1999). Menstrual cycle effects on cortical excitability. *Neurology* 53, 2069-72. doi: 10.1212/wnl.53.9.2069

Smith, S. (2013). The influence of stress at puberty on mood and learning: Role of the α4βδ $GABA_A$ receptor. *Neuroscience* 249, 192–213. doi: 10.1016/j.neuroscience.2012.09.065

Smith, S., Aoki, C., and Shen, H. (2009). Puberty, steroids and $GABA_A$ receptor plasticity. *Psychoneuroendocrinology* 34, S91–S103. doi: 10.1016/j.psyneuen.2009.05.011

Smith, S. S., Gong, Q. H., Li, X., Moran, M. H., Bitran, D., Frye, C. A., et al. (1998). Withdrawal from 3alpha-OH-5alpha-Pregnan-20-One Using a Pseudopregnancy Model Alters the Kinetics of Hippocampal $GABA_A$-Gated Current and Increases the $GABA_A$ Receptor alpha4 Subunit in Association with Increased Anxiety. *J. Neurosci.* 18, 5275–5284.

Smith, S. S., Shen, H., Gong, Q. H., and Zhou, X. (2007). Neurosteroid regulation of $GABA_A$ receptors: Focus on the α4 and δ subunits. *Pharmacol. Ther.* 116, 58–76. doi: 10.1016/j.pharmthera.2007.03.008

Smolen, A., Smolen, T. N., and Han, P. C. (1993). Alterations in regional brain GABA concentration and turnover during pregnancy. *Pharmacol. Biochem. Behav.* 44, 63–69. doi: 10.1016/0091-3057(93)90281-W

Sohal, V. S., and Rubenstein, J. L. R. (2019). Excitation-inhibition balance as a framework for investigating mechanisms in neuropsychiatric disorders. *Mol. Psychiatry* 24, 1248–1257. doi: 10.1038/s41380-019-0426-0

Stewart, D. E., and Vigod, S. N. (2019). Postpartum Depression: Pathophysiology. Treatment, and Emerging Therapeutics. *Ann. Rev. Med.* 70, 183–196. doi: 10.1146/annurev-med-041217-011106

Stewart, D. R., Overstreet, J. W., Nakajima, S. T., and Lasley, B. L. (1993). Enhanced ovarian steroid secretion before implantation in early human pregnancy. *J. Clin. Endocrinol. Metab.* 76, 1470–1477. doi: 10.1210/jcem.76.6.8501152

Stillwell, W. (2016). *Bioactive Lipids. In An Introduction to Biological Membranes*. Amsterdam: Elsevier, 453–478. doi: 10.1016/B978-0-444-63772-7.00020-8

Sumner, R. L., McMillan, R. L., Shaw, A. D., Singh, K. D., Sundram, F., and Muthukumaraswamy, S. D. (2018). Peak visual gamma frequency is modified across the healthy menstrual cycle. *Hum. Brain Mapp.* 39, 3187–3202. doi: 10.1002/hbm.24069

Tal, M. D and Taylor, M. D. (2000). "Endocrinology of pregnancy" in *Endotext*, eds K. R. Feingold, B. Anawalt, and A. Boyce (South Dartmouth: Endotext). Available online at: https://www.ncbi.nlm.nih.gov/books/NBK278962/?report=reader (accessed July 20, 2021).

Taylor, C. M., Pritschet, L., and Jacobs, E. G. (2021). The scientific body of knowledge – Whose body does it serve? A spotlight on oral contraceptives and women's health factors in neuroimaging. *Front. Neuroendocrinol.* 60:100874. doi: 10.1016/j.yfrne.2020.100874

Toffol, E., Heikinheimo, O., and Partonen, T. (2015). Hormone therapy and mood in perimenopausal and postmenopausal women: a narrative review. *Menopause* 22, 564–578. doi: 10.1097/GME.0000000000000323

Tominaga, K., Yamauchi, A., Shuto, H., Niizeki, M., Makino, K., Oishi, R., et al. (2001). Ovariectomy aggravates convulsions and hippocampal γ-aminobutyric acid inhibition induced by cyclosporin A in rats. *Eur. J. Pharmacol.* 430, 243–249. doi: 10.1016/s0014-2999(01)01377-2

Torres-Reveron, A., Williams, T. J., Chapleau, J. D., Waters, E. M., McEwen, B. S., Drake, C. T., et al. (2009). Ovarian steroids alter mu opioid receptor trafficking in hippocampal parvalbumin GABAergic interneurons. *Exper. Neurol.* 219, 319–327. doi: 10.1016/j.expneurol.2009.06.001

Tremblay, R., Lee, S., and Rudy, B. (2016). GABAergic Interneurons in the Neocortex: From Cellular Properties to Circuits. *Neuron* 91, 260–292. doi: 10.1016/j.neuron.2016.06.033

Uriarte, N., Ferreño, M., Méndez, D., and Nogueira, J. (2020). Reorganization of perineuronal nets in the medial Preoptic Area during the reproductive cycle in female rats. *Scie. Rep.* 10:5479. doi: 10.1038/s41598-020-62163-z

Vigod, S. N., Strasburg, K., Daskalakis, Z. J., and Blumberger, D. M. (2014). Systematic review of gamma-aminobutyric-acid inhibitory deficits across the reproductive life cycle. *Arch. Women's Ment. Health* 17, 87–95. doi: 10.1007/s00737-013-0403-6

Wang, D., Wang, X., Luo, M.-T., Wang, H., and Li, Y.-H. (2019). Gamma-Aminobutyric Acid Levels in the Anterior Cingulate Cortex of Perimenopausal Women With Depression: A Magnetic Resonance Spectroscopy Study. *Front. Neurosci.* 13:785. doi: 10.3389/fnins.2019.00785

Wang, Z., Zhang, A., Zhao, B., Gan, J., Wang, G., Gao, F., et al. (2016). GABA+ levels in postmenopausal women with mild-to-moderate depression: A preliminary study. *Medicine* 95:e4918. doi: 10.1097/MD.0000000000004918

Weiland, N. G. (1992). Glutamic acid decarboxylase messenger ribonucleic acid is regulated by estradiol and progesterone in the hippocampus. *Endocrinology* 131, 2697–2702. doi: 10.1210/en.131.6.2697

Welz, A., Huffziger, S., Reinhard, I., Alpers, G. W., Ebner-Priemer, U., and Kuehner, C. (2016). Anxiety and rumination moderate menstrual cycle effects on mood in daily life. *Women Health* 56, 540–560. doi: 10.1080/03630242.2015.1101739

Wilson, M. E., Rosewell, K. L., Kashon, M. L., Shughrue, P. J., Merchenthaler, I., and Wise, P. M. (2002). Age differentially influences estrogen receptor-α (ERα) and estrogen receptor-β (ERβ) gene expression in specific regions of the rat brain. *Mech. Ageing Dev.* 123, 593–601.

Wise, P. M. (1999). Neuroendocrine modulation of the "menopause": Insights into the aging brain. *Am. J. Physiol.Endocrinol. Metab.* 277, E965–E970. doi: 10.1152/ajpendo.1999.277.6.E965

Wood, G. A., Fata, J. E., Watson, K. L. M., and Khokha, R. (2007). Circulating hormones and estrous stage predict cellular and stromal remodeling in murine uterus. *Reproduction* 133, 1035–1044. doi: 10.1530/REP-06-0302

Wu, X., Gangisetty, O., Carver, C. M., and Reddy, D. S. (2013). Estrous Cycle Regulation of Extrasynaptic δ -Containing $GABA_A$ Receptor-Mediated Tonic Inhibition and Limbic Epileptogenesis. *J. Pharmacol. Exper. Ther.* 346, 146–160. doi: 10.1124/jpet.113.203653

Wu, Y., Du, X., van den Buuse, M., and Hill, R. (2014). Sex differences in the adolescent developmental trajectory of parvalbumin interneurons in the hippocampus: A role for estradiol. *Psychoneuroendocrinology* 45, 167–178. doi: 10.1016/j.psyneuen.2014.03.016 0306-4530/#

Young, B. J., and Cook, C. J. (2006). Reduced basal GABA concentrations in the rat amygdala during pregnancy. *Physiol. Behav.* 87, 817–820. doi: 10.1016/j.physbeh.2006.01.011

Zhao, C., Driessen, T., and Gammie, S. C. (2012). Glutamic acid decarboxylase 65 and 67 expression in the lateral septum is up-regulated in association with the postpartum period in mice. *Brain Res.* 1470, 35–44. doi: 10.1016/j.brainres.2012.06.002

Zimmermann, K., Richardson, R., and Baker, K. (2019). Maturational Changes in Prefrontal and Amygdala Circuits in Adolescence: Implications for Understanding Fear Inhibition during a Vulnerable Period of Development. *Brain Sci.* 9:65. doi: 10.3390/brainsci9030065

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