Lasting effects of oral contraceptives on fear responses to a safe context.

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Abstract

Estradiol (E2), whether endogenous or administered via oral contraceptives (OCs), modulates fear regulation. However, its role in contextual fear signaling, critical for distinguishing threat from safety, remains poorly understood in humans. While prior human studies focused on cue-related extinction recall, animal research suggests that a single high-dose of exogenous E2 generalizes fear to safe contexts. This study tested whether hormonal status and OC use influence contextual fear responses in humans, and whether OC-related effects are long-lasting and dose-dependent. In a two-day fear conditioning and extinction protocol, 147 healthy participants (men, cycling women, OC users) underwent fear conditioning in a threat-associated context (CX+) and extinction in a safety-associated context (CX-). On day 2, cues were presented in both contexts. Fear return to each context was assessed via skin conductance responses and brain activations. Groups were formed sequentially using the same participants: (A) by endogenous/exogenous E2 status, (B) by OC use history, and (C) by current ethinyl estradiol (EE) dose. CX- fear returns were elevated in current (p=.015, d=0.64) and past OC users (p=.014, d=0.83) compared to never users. High-EE, but not low-EE users, showed greater fear returns than never users (p=.021, d=0.81). Biological sex and endogenous E2 were unrelated to contextual fear. Across participants, CX- fear returns were negatively associated with hippocampal and ventromedial prefrontal cortex activation. OC use, particularly at higher EE doses, may impair retrieval of safety signals from context, with effects persisting beyond discontinuation. These findings highlight exogenous hormones as a modulator of contextual fear regulation in women.

Introduction

Effective fear regulation depends on the ability to contextualize threat-related cues, such as recognizing the difference between seeing a snake (cue) in the forest (threat context) or at the zoo (safety context) [1]. While cue-related and context-related fear processes share neural substrates including the ventromedial prefrontal cortex (vmPFC), amygdala, and anterior cingulate cortex (ACC), contextual fear expression depends more critically on the hippocampus [1–10]. Although most research has focused on cue-related fear, disruptions in contextualization can produce maladaptive fear in safe environments and are implicated in psychiatric conditions such as post-traumatic stress disorder [PTSD; 1,2,11–15]. Notably, PTSD and fear-related disorders are nearly twice as prevalent in women than in men, with this sex difference emerging in adolescence [16–18]. While sociocultural factors undoubtedly contribute, accumulating evidence implicates the ovarian hormone estradiol (E2) as a biological mediator of this female-biased vulnerability [19–21].

Rodent studies demonstrate a robust sex difference in contextual fear conditioning protocols [7]. For instance, females typically show reduced discrimination between neutral and threat-associated contexts compared to males, with E2 playing a central role [3,22,23]. While ovariectomized females can differentiate between contexts, exogenous E2 replacement promotes fear generalization to neutral contexts, through effects in the dorsal hippocampus (posterior hippocampus in humans) and ACC [3,23–25]. These effects are specific to higher-dose beta-estrogen receptor (ERb) agonists [23,26] and absent across natural hormonal fluctuations during the estrous cycle [3], suggesting that high-dose exogenous E2 exposure may uniquely disrupt contextual fear processing.

Hormonal contraceptives allow to investigate the neurobehavioral effects of exogenous hormones in humans. The most prevalent form in North America, combined oral contraceptives (OCs), delivers synthetic E2 (mainly ethinyl estradiol or EE; 10-35 μg) and a progestin daily [27–29]. This induces both low endogenous and high exogenous hormonal states via suppression of hypothalamic-pituitary-gonadal function by exogenous hormones [27,30,31]. By contrast, cycling women experience dynamic endogenous E2 fluctuations, with levels peaking before ovulation and reaching a nadir during early follicular menstrual phase [32].

Human research typically assesses contextual modulation of fear through cue-in-context extinction protocols. In these protocols, extinction recall is assessed by presenting previously

extinguished cues in a safety-associated context (CX-) and fear renewal is assessed in a threat-associated context (CX+) [33]. Successful fear regulation relies on accurate retrieval of contextual information to suppress (extinction recall) or re-engage (fear renewal) conditioned responses to cues [4,5,33–37]. Women with low endogenous E2 levels (naturally or through hormonal contraceptives) consistently show reduced extinction recall compared to women with high endogenous E2 levels or men [38–44,but see 45]. Similar effects are reported in male and female rodents, where causal roles of E2 have been demonstrated using pharmacological and surgical manipulations [40,42,44,46–52,see 20,21 for reviews]. Fewer studies have addressed fear renewal, and E2-related differences are less consistent in that phase [39,45,53]. Thus, E2 may selectively enhance fear regulation in safe contexts by facilitating context-dependent extinction memory retrieval.

Together, findings highlight a complex role for E2: while it reliably enhances cued extinction recall across species [20,21,40,54], it paradoxically impairs contextual discrimination in rodents [3,23–26]. This contradiction may reflect differences in the neural circuits and temporal dynamics underlying cued versus contextual fear [1,55,56], or differences in the functional impact of endogenous versus exogenous E2 [57–59]. Notably, no human study has directly examined context-specific fear responses as a function of hormonal status, a critical gap given robust sex differences in fear-related disorders and fear extinction recall. In addition, emerging evidence suggests that hormonal contraceptives may have long-lasting effects among former users, notably on extinction recall, anxiety symptoms, depression, brain morphology, and cognition [39,60–64], raising the possibility of enduring alterations in context-sensitive fear processes.

This study included an initial exploratory analysis of sex differences in context-related fear, followed by three main objectives. First, we examined context-related fear responses in relation to endogenous and exogenous E2 levels in women, including men as a comparison group. Second, we investigated the potential long-term effects of OCs on context-related fear by accounting for OC history. Third, given that E2 generalization effects emerge primarily at higher doses [26], we explored possible dose-dependent effects of OC use. A validated 2-day extinction protocol was used, with skin conductance responses (SCRs) and brain activity indexing fear. Based on animal research, we hypothesized that natural E2 fluctuations would not impact context-related fear, but that female sex and OC use would be associated with elevated SCRs in safe contexts, with possible

lasting effects and dose-dependence related to OC use. Finally, we anticipated that SCR-level differences would parallel changes in hippocampal and ACC activity.

Materials and Methods

Participants

This study was part of a larger project involving three sessions, with some data previously published [65–68]. From the initial 218 participants, 39 discontinued their involvement in this study (see Supplementary Materials and Methods). Participants were aged 23-35, French-speaking, and free of current or past psychiatric, neurological, or endocrine disorders, pregnancy, endocrine therapy, and medications affecting the endocrine system. Women had exclusively used combined OCs.

Participants included men, cycling women, and current OC users (**Figure 1**). In E2-based analyses, cycling women were randomly assigned to the early follicular (E2-; days 1–5 of menstruation, except one on day 7) or pre-ovulatory phase (E2+; 1-4 days before ovulation, calculated as cycle length minus 14). In OC-history-based groups, cycling women were classified as never or past users (≥1 year since discontinuing OCs). For EE-based groups, current users were split by median dose into low (EE-; 10-25 µg) and high (EE+; 30-35 µg) dose users [as in 65]. Only current dosages were analyzed, as fewer than half participants maintained a consistent dose across time. Descriptive data on past and current OC users are available in our previous publications [65,66] and Table S1.

Fear extinction protocol

Participants completed a validated 2-day fear extinction protocol [**Figure S1A**; 4,5,69–71]. On day 1, fear conditioning occurred in the threat-associated context (office; CX+), where two cues (CS+) were paired with an electric shock, and one was not (CS-). Extinction learning followed in a different, safety-associated context (library; CX-), where one CS+ and the CS- were unreinforced. On day 2, extinction recall and fear renewal were tested by re-presenting cues in the CX- and the CX+ without reinforcement. For more details, see Supplementary Materials and Methods.

Data collection

SCR recordings

Context-related SCRs were recorded with AcqKnowledge (Biopac; MP160). Phasic responses were extracted with Ledalab [72], using a 0.01 µS minimum amplitude and a 1-3 s

latency to avoid overlap with cue presentations (**Figure S1B**). SCRs were square-root transformed [37,73] and visually inspected for irregularities (e.g., flat signal, noise). Participants with no response in >75% of trials in any phase were excluded. Their non-response patterns did not align with typical extinction profiles (i.e., measurable responses followed by decline). Since contexts might elicit weaker conditioned responses than cues [1,2,74], these criteria were applied to cue-related responses, resulting in the same sample as used in a separate publication [39]. Nonetheless, sensitivity analyses including all participants were performed [37,75].

Sex hormones

Salivary sex hormone concentrations were measured using liquid chromatography-tandem mass spectrometry. Collection and assay details are in Supplementary Materials and Methods.

Descriptive data

To assess pre-existing psychological differences between-groups, questionnaires assessed state/trait anxiety [76], depressive symptoms [77], religious beliefs [78,79], emotion regulation [80,81], and personality [82]. Details are in Supplementary Materials and Methods.

Procedure

The study received approval from institutional ethics boards (CIUSSS-EMTL and UQAM). Participants provided informed consent and were compensated CAD $140. This study is part of a larger project involving three sessions and online questionnaires; data presented here are from the final two sessions. On day 1, saliva samples were collected. Before MRI each day, participants completed the State Anxiety Inventory. SCRs were recorded from the left palm (Ag/AgCl electrodes, 11 mm, EL509, Biopac Systems) and electrical stimulations were delivered to the right index and middle fingers. Participants chose a non-painful but highly aversive stimulation level (0.8-6.0 mA). Day 1 included structural imaging [65,66], habituation, fear conditioning and extinction learning. Day 2 comprised extinction recall, fear renewal, and completion of the Beck Depression Inventory-II.

Analyses

Statistical analyses were conducted in R [v4.3.1; 83]. Significance was defined as $p<.05$ and trends ($p<.08$) were reported. For outcome variables, outlier values ($z>\pm3.29$ within group) were winsorized using the next highest value of each group [84].

Preliminary analyses

Between-group differences in demographic and psychological measures were tested. Variables with $p<.10$ were included as covariates in follow-up sensitivity analyses on statistically significant models [61,65,66,68]. Associations between potential covariates and study outcomes were also examined.

SCR analyses

Trial-level SCRs were analyzed using multilevel modeling with maximum likelihood estimation [85,86]. Separate models were fit for each experimental phase and each set of groups (E2, OC history, EE dose). For each model, the random effect structure and the best-fitting polynomial term for Trial (linear, quadratic, or cubic) were selected with the Bayesian information criterion (BIC). Group and Group X Trial fixed effects were added. Marginal and conditional R^2 were computed for significant models and post-hoc between-group comparisons were conducted. Residual diagnostics and multicollinearity checks were performed. Notably, 1.7% of the data were winsorized.

Unlike differential designs where responses to CS+ and CS- can be compared [37], responses to contexts reflect an absolute level of physiological reactivity, challenging the interpretation of between-group contrasts [37,73,87]. Therefore, fear return metrics were used to index relative SCR changes.

Fear return metrics

Fear return metrics are widely used to quantify relative fear levels [5,37,40,43,44,88]. We computed these indices as follows for the CX-/CX+: (SCR on the first extinction recall or fear renewal trial / mean SCR on the last 4 extinction learning or fear conditioning trials) x 100. These metrics are debated due to inconsistent operationalization across studies [88], prompting sensitivity analyses to test the robustness of results. For numerators, although the first recall/renewal trial is often viewed as minimally contaminated by re-extinction or habituation [37], we tested alternatives using the mean of the first 2, 3, or 4 trials. For denominators, although the final four trials of extinction learning are frequently used to index extinction learning [51,89–91], restricting analyses to a small trial window has been reported to inflate effects [37]. We therefore varied trial windows (last 8, 16, or 32 extinction learning/fear conditioning trials) [37]. Three outliers were winsorized for the CX- (1 E2- past user, 1 E2+ past user, 1 EE+ current user) and the CX+ (1 men, 1 E2- past user, 1 EE+ current user).

To evaluate the neural correlates of fear returns, bilateral BOLD signal was extracted from each region of interest (ROI; see following section), defined as the average beta value across all voxels of each mask. Across all participants, two multiple regression models were conducted (CX-, CX+), with all ROI activations as predictors and fear return percentages as outcome variables. Fear returns were compared between groups using univariate ANOVAs, with Games-Howell corrections for unequal variances. *P*-values, 95% confidence intervals (CI), eta-squared, and Cohen's *d*s were reported [92].

Modeling of fMRI data

Information on image acquisition and preprocessing is provided in Supplementary Materials and Methods. First-level general linear models were specified for each participant and experimental phase. Models specified event-related onsets of contexts, conditioned stimuli and shocks. Second-level general linear models focused on two contrasts of interest mirroring fear return calculations: CX- (first 4 CX- presentations of extinction recall vs. last 4 CX- presentations of extinction learning) and CX+ (first 4 CX+ presentations of fear renewal vs. last 4 CX+ presentations of fear conditioning). First, CX- and CX+ contrasts were assessed across all participants. Then, between-group differences were assessed with three ANOVAs (E2, OC history, EE dose) per contrast in the following ROIs: hippocampus, ACC, vmPFC and amygdala (for ROI definitions, see Supplementary Materials and Methods). An initial height threshold of p=.001 and a minimum cluster size of 10 voxels were applied [93]. Clusters surviving family-wise error (FWE) correction at p<.05 were retained. For these, Tukey-corrected tests assessed group differences in beta values.

Results

Preliminary analyses

Exclusions. Thirty-nine participants were excluded (e.g., technical problems; Supplementary Results). The final sample included 147 participants for SCR analyses and 143 participants for fMRI (as in 37).

Preliminary analyses. Per-group descriptive information is presented in **Tables 1 and S1**. Groups differed as expected on hormone concentrations. In E2-based groups, E2+ women had higher E2 levels than E2- women (p_M=.008), OC users (p_M<.001), and men (p_M=.019), with the latter three groups not differing (p_Ms≥.282). In OC-history-based groups, never and past users had similar E2

levels (p_M=.553). Additional post-hoc analyses and correlations between psychological or demographic variables and outcome measures are presented in the Supplementary Results.

Neural correlates of fear returns. Neural correlates of fear returns were investigated across all participants. For the CX-, the regression model was significant (F(4,138)=2.74, p=.031, R^2=.07; **Figure S2**), with hippocampus (β=-0.19, SE=0.09, p=.034) and vmPFC activity (β=-0.19, SE=0.09, p=.044) negatively predicting fear returns. For the CX+, the model was marginally significant (F(4,138)=2.42, p=.051, R^2=.07), with the vmPFC negatively predicting fear returns (in trend; β=-0.17, SE=0.10, p=.074). In addition, BOLD contrasts of the CX- performed across all participants notably revealed activation in bilateral posterior hippocampus and right dorsal ACC (**Table S2**).

Main analyses

Models with random intercepts and cubic Trial effects best fit SCR data (**Table S3**). Intra-individual variability explained 22.4%-32.2% of SCR variance. Sex differences in SCRs and BOLD activation were examined first. No group differences emerged (Supplementary Results).

Objective A: E2-based groups. Group X Trial interactions were significant for extinction learning (F(9,4557)=2.80, p=.003, marginal R^2=.03) and recall (F(9,4556)=4.18, p<.001, marginal R^2=.08), but not for fear conditioning (F(9,4557)=1.69, p=.085) and renewal (F(9,2205)=1.73, p=.078; **Figure 2A**). Extinction learning revealed higher SCRs in OC users compared to E2-women on trials 1-3 (ps≤.035) and 22-30 (ps≤.034). Men also showed higher SCRs than E2-women on trial 1 (p=.013). Extinction recall indicated elevated SCRs in OC users compared to men (trials 1-2; ps≤.017), E2- women (trials 1-6; ps≤.017), and E2+ women (trials 1-3; ps≤.033). For fear renewal, a main effect of Group was observed (F(3,147)=3.53, p=.017), with no significant post-hoc comparison (ps≥.124).

Groups were then compared on their fear returns. Main effects of Group were non-significant for fear returns to the CX- (F(3,143)=1.46, p=.227, η^2_p=0.03; **Figure 2B**) and the CX+ (F(3,143)=0.35, p=.792, η^2_p=0.01; **Figure 2C**). In addition, no significant between-group difference emerged on BOLD contrasts (CX- and CX+; Supplementary Results).

Objective B: OC-history-based groups. Group X Trial interactions were significant for fear conditioning (F(9,4557)=2.12, p=.025, marginal R^2=.14), extinction learning (F(9,4557)=2.80, p=.003, marginal R^2=.03), and extinction recall (F(9,4557)=3.74, p<.001, marginal R^2=.08), but not for fear renewal (F(9,2205)=0.87, p=.556; **Figure 3A**). Fear conditioning revealed higher

SCRs in current users compared to past users on trials 2-4 ($ps \leq .040$). Extinction learning showed higher SCRs in current users compared to past users on trials 1-3 ($ps \leq .040$) and 21-30 ($ps \leq .037$). Men also showed higher SCRs than past users on trials 1-3 ($ps \leq .040$). Extinction recall revealed higher SCRs in current users than men (trials 1-2; see above), never users (trials 1-6; $ps \leq .031$), and past users (trials 1-5; $ps \leq .045$). For fear renewal, a main effect of Group emerged ($F(3,147)=3.53$, $p=.017$), with no significant post-hoc comparison ($ps \geq .157$).

Groups differed on their fear return to the CX- ($F(3,143)=3.60$, $p=.015$, $\eta^2_p=0.07$; **Figure 3B**), with current ($p=.015$, 95%CI=[27.42, 350.11], $d=0.64$) and past OC users ($p=.014$, 95%CI=[31.40, 367.33], $d=0.83$) showing higher fear returns than never users. For the CX+, no between-group differences emerged ($F(3,143)=0.60$, $p=.614$, $\eta^2_p=0.01$; **Figure 3D**). Temporal aspects of OC exposure (duration of use, time since discontinuation, time since last pill ingestion) were not associated with fear returns, whether current and past users were analyzed jointly or separately ($|rs| \leq .24$, $ps \geq .092$).

In BOLD analyses for the CX+, left dorsal ACC activation was reduced in never users relative to current users, past users, and men. This was driven by heightened ACC activity in never users during fear conditioning (Supplementary Results). For the CX-, no significant contrast emerged. Given the significant correlations between SCRs and activation in the hippocampus and vmPFC across participants, we conducted exploratory between-group analyses on beta values extracted from the full ROI surface of these regions. Never users exhibited greater hippocampal activation compared to men (Supplementary Results).

Objective C: EE-based groups. These analyses compared low-EE and high-EE users. Never users were included as a reference condition given previous findings (Objective B). Group X Trial interactions were significant for extinction learning ($F(6,2542)=3.69$, $p=.001$, marginal $R^2=.03$) and extinction recall ($F(6,2542)=6.14$, $p<.001$, marginal $R^2=.09$), but not for fear conditioning ($F(6,2542)=1.84$, $p=.087$) and fear renewal ($F(6,1230)=0.96$, $p=.453$). Extinction learning showed higher SCRs in EE+ users relative to never users on trial 1 ($p=.045$). Extinction recall revealed higher SCRs in EE+ users than never users (trials 1-7, 20-29; $ps \leq .037$) and EE- users (trials 1-3; $ps \leq .028$). EE- users also had higher SCRs than never users on trials 1-3 ($ps \leq .028$). For fear renewal, a main effect of Group emerged ($F(2,82)=3.57$, $p=.033$), with elevated SCRs in EE+ women relative to never users ($p=.047$).

Groups differed on their fear return to the CX- ($F(2,79)=5.71$, $p=.005$, $\eta^2_p=0.13$), with elevated fear returns in EE+ users relative to never users ($p=.021$, 95%CI=[38.25, 542.08], $d=0.81$; **Figure 3C**). EE- users did not differ from EE+ ($p=.209$) or never users ($p=.199$). To explore the contribution of other hormonal components, OC users were re-classified according to progestin dose and androgenicity and compared on their fear return to the CX- (Supplementary Results). Moreover, for the CX+, no significant Group effect was found ($F(2,79)=1.94$, $p=.150$, $\eta^2_p=0.05$; **Figure 3E**).

In BOLD analyses for the CX+, the left dorsal ACC cluster (see Objective B) showed increased activation in both EE groups relative to never users (Supplementary Results). For the CX-, no significant contrast emerged. Exploratory analyses specifically targeting the hippocampus and vmPFC revealed marginally greater hippocampal activation in never users compared to EE+ women (Supplementary Results).

Sensitivity analyses. We tested the robustness of results across varying fear return operationalizations (see Materials and Methods). Between-group results remained stable. We also added variables that differed across groups (**Table 1**) as covariates in significant models, removed OC users in their placebo/inactive phase, and reintroduced excluded participants with poor conditioned responding. Results remained unchanged (see Supplementary Results).

Discussion

Although endogenous E2 and OC use have been associated with extinction recall performance, this study was the first to directly examine their association with context-related fear in humans. An initial analysis of sex differences found no significant prediction of context-related fear, diverging from rodent models [3,22,23] and underscoring the need for hormone-based profiling in human emotion research. Instead, our findings revealed elevated fear responses in safe contexts among OC users, with effects persisting after discontinuation and most pronounced in users of high-EE or high-progestin formulations.

Both current and past OC users exhibited greater fear returns in the safe context relative to never users, whose mean levels of fear return were nominally lower than those of men. This extends previous findings in the same sample [39], where current users showed increased fear to extinguished cues during extinction recall relative to never users, with past users not differing from either group. Based on prior research, these results speculatively suggest that current OC use may disrupt extinction recall by impairing both 1) context-related safety signaling via high exogenous

E2 levels and 2) cue-related extinction memory via suppressed endogenous E2 levels. By contrast, past OC use appears to exert more persistent effects on context processes, raising the possibility that cue-related disruptions resolve more readily following discontinuation. Because cue- and context-related processes rely on partially distinct neural circuits (1), OC effects may reverse on different neurobiological timescales post-discontinuation [e.g.,64]. Context-dependent processes rely heavily on the hippocampus, which is highly plastic, sensitive to both endogenous E2 and OC exposure, and causally implicated in contextual fear generalization in female rodents [1,5,25,64,94–99]. In this study, hippocampal activation negatively correlated with fear returns and was elevated in never users as compared to EE+ women (marginally) and men, supporting its role in the current findings. Therefore, although hippocampal structural changes can reverse [64,65,94,100], the current results raise the possibility of longer-lasting effects on hippocampus-dependent functions following OC discontinuation. Notably, OC use duration and time since discontinuation were not associated with fear returns, indicating possible non-linear relationships [e.g.,66,101], threshold effects emerging after prolonged periods [e.g.,66], heterogeneity in OC histories, or individual differences in hormonal sensitivity.

Building on preclinical evidence, we stratified OC users by EE dose and compared them to never users. High-EE users showed greater fear returns than never users in the safe context, echoing rodent data showing contextual generalization only at higher E2 doses [26] and broadly supporting the well-established role of E2 in fear regulation [25,26,40,44,46]. Interestingly, animal work suggests that these effects are mediated through ERb activation [23,26]. Although EE likely preferentially binds alpha receptors over ERb [57,102,103], EE is up to 100 times more potent than E2 and bioaccumulates with chronic use [103,104], plausibly enhancing ERb activity as well at higher doses. Moreover, prior work in this sample showed reduced vmPFC thickness in low-EE users relative to men [65]. While vmPFC thinning has been linked to impaired fear extinction and increased generalization [105–107], our current psychophysiological results in low-EE users contrast with this pattern. Yet, recent replication failures challenge the reliability of such structure-function associations in healthy samples [108,109]. In contrast, we observed a negative correlation between vmPFC activation and fear returns, consistent with its role in fear inhibition [110–112] and suggesting that functional activation may better index vmPFC involvement in context-related fear regulation than morphology.

To tentatively isolate EE-specific effects, we reclassified OCs by 1) progestin dose and 2) progestin androgenicity. These two hormonal components have been broadly linked to fear-related outcomes but remain understudied due to progestin heterogeneity across formulations [104,113,114]. Relative to never users, greater fear returns were observed in 1) users of high-progestin dose and 2) users of anti-androgenic progestins. Although EE dose explained unique variance beyond progestins, model fits were comparable, suggesting no single hormonal component was decisively superior. These findings underscore the need to consider both estrogenic and progestogenic influences as well as their combined effects on fear regulation, beyond the typical EE focus. Progestin-only contraceptives could help disentangle these contributions. If replicated in clinical samples, our results may guide individualized OC prescribing, with lower-dose or androgenic options being potential alternatives for women vulnerable to fear-related disorders.

Naturally fluctuating E2 did not predict context-related fear, aligning with animal data showing minimal effects of estrous phase on contextual generalization [3]. Moreover, time since pill ingestion (reflecting exogenous hormone bioavailability) was not related to context-related fear in current OC users, further suggesting that transient or acute hormonal states have limited influence on these processes. Contrasting with these findings, previous human research has reliably reported impairments in cued extinction recall among women with low endogenous E2 levels, often attributed to difficulty suppressing fear in safe contexts [38–44]. While contexts can elicit conditioned responses (as shown in OC-history and EE-based findings), they may also act as 'occasion setters', modulating the expression of learned cue-shock associations without generating conditioned responses themselves [1,35,74]. Thus, cue-evoked responses may be more effectively captured than context-evoked responses in E2-based groups. Supporting this, the between-group response pattern remained stable across all experimental phases (**Figure 2A**; OC>men>E2+>E2-), indicating a lack of learning-driven differentiation, which would be reflected on day 2 by reduced fear in the safe context and enhanced fear in the threat context. The absence of such modulation suggests that the recorded context-evoked responses may represent general arousal rather than conditioned responses. Protocols directly pairing shocks with contexts (without intermediate cues) may allow to better elicit context-specific responses.

Overall, psychophysiological between-group differences were not mirrored in BOLD contrasts using standard analytic approaches [e.g.,95,97,115]. The lack of effects may reflect the

smaller magnitude of context-related versus cue-related conditioned responses, the conservative thresholds and corrections applied, or the health homogeneity of the sample. However, targeting brain regions showing correlations with SCRs revealed hippocampal between-group differences that paralleled SCR findings. The only between-group activation difference emerged during early fear renewal (vs. late fear conditioning), where men and current/past OC users exhibited greater dorsal ACC activity than never users. Post-hoc tests indicated that this was driven by heightened dorsal ACC responses in never users during late fear conditioning. The dorsal ACC is implicated in fear expression and salience detection [71,112]. Prior studies have shown greater dorsal ACC volume and activation during fear conditioning in women compared to men [65,90,116–119], a pattern also observed between never users and men in this sample (see 65 for volumetric analyses). At first glance, elevated dorsal ACC activation and volume in never users may seem paradoxical given their association with symptom severity and treatment resistance in PTSD [2,11,15,120–122]. However, PTSD is characterized by ACC hyperactivation not only in threat contexts, but also in safe contexts [2]. In our sample, heightened dorsal ACC activation in never users was specific to fear conditioning, consistent with accurate threat-safety discrimination. Conversely, current and past OC users may exhibit dampened neural specificity, potentially affecting the processing of contextual threat signals in these groups.

This study has limitations. The brief 3-second context exposure may have limited full engagement of contextual processes; future work should consider longer or immersive paradigms [123]. Nonetheless, correlations between fear return and vmPFC/hippocampal activity support the validity of the protocol in capturing context-evoked conditioned responses [1]. Second, pre-existing differences among never, past, and current users are likely [101,124,125], and while some confounds (e.g., relationship status, personality) were measured, others were not [e.g., sexual orientation; 126]. However, the sample was homogeneously healthy, and groups were similar on key mental health variables. On the other hand, the homogeneous sample limits generalizability, warranting replication in clinical populations with PTSD or anxiety, and across hormonally distinct groups (e.g., women with premenstrual dysphoric disorder). Future work should also explore OC history and dose effects on exposure therapy, longitudinal prediction of psychopathology, and connectivity analyses (e.g., vmPFC-hippocampus).

Nonetheless, this is the first human study to investigate context-related fear while accounting for sex hormones and OC use. Our findings highlight contextualization as an

underappreciated dimension of fear regulation, modulated by exogenous hormones. In addition, this study used psychophysiological-neural correlations and sensitivity analyses to support the validity of fear return metrics, acknowledging the need for justified and empirically supported statistical decisions.

Conclusion

The inability to suppress fear in safe environments is a hallmark of fear-related disorders. Our findings suggest that hormonal contraceptives may impair this capacity, with effects persisting after discontinuation. These results underscore the importance of examining exogenous hormonal influences on fear contextualization and sex-specific vulnerability in fear-related conditions.

Author contributions

AB and MFM contributed to the conception and design of the study. LMD and AB managed data collection. LMD organized the database, performed the statistical analyses, and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon request.

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Competing interests

The authors have nothing to disclose.

Figure legends

Figure 1. Schematic of the three sets of between-group analyses. Analyses based on endogenous estradiol (E2) levels (A) compare men, early follicular women (E2-), pre-ovulatory women (E2+), and current oral contraceptive (OC) users. E2- and E2+ menstrual phases are represented on the menstrual cycle diagram. Analyses based on OC history of use (B) compare men, never users of hormonal contraception, past OC users, and current OC users. Analyses based on ethinyl estradiol (EE) doses (C) compare current OC users using low-dose formulations and current OC users using high-dose formulations. Never users were included as a reference group in (C) based on findings from (B) indicating intact contextual fear regulation in this group. Color circles represent the legend.

Figure 2. Skin conductance responses (SCRs) to contexts in estradiol (E2)-based groups. **(A)** Trial-by-trial SCRs across the protocol. **(B)** Percentages of fear return to the safe context (CX-). No between-group differences were observed. **(C)** Percentages of fear return to the threatening context (CX+). No between-group differences were observed. E2- = women in the early follicular phase, E2+ = women in the pre-ovulatory phase, OC = oral contraceptive users. *Indicates significant pairwise contrasts (p<.05); colors denote the group with the highest SCR in each contrast.

Figure 3. Skin conductance responses (SCRs) across oral contraceptive (OC) history-based (panels A, B, D) and ethinyl estradiol (EE) dose-based (panels C, E) groupings. **(A)** Trial-by-trial SCRs across the protocol in groups based on OC history. **(B)** Percentages of fear return in the safety-associated context (CX-) in groups based on OC history. Both current (n = 52; p = .014, d = 0.64) and past OC users (n = 29; p = .015, d = 0.83) showed elevated fear returns relative to never users (n = 30). **(C)** Percentages of fear return in the CX- in groups based on EE dose. High-EE users (n = 24) showed elevated fear returns relative to never users (p = .021, d = 0.81). **(D)** Percentages of fear return in the threat-associated context (CX+) in groups based on OC history. No between-group differences were observed. **(E)** Percentages of fear return in the CX+ in groups based on EE dose. No between-group differences were observed. The same never-user group was used in both OC-history and EE-based analyses. In the OC-history grouping, current users include both low-EE (EE–) and high-EE (EE+) users (see Figure 1 for group classification details). Never = never users of hormonal contraception, Past = past OC users, Current = current OC users. *Indicates significant pairwise contrasts (p<.05); #indicates a statistical trend (p<.08); colors denote the group with the highest SCR in each contrast.

Table 1. Sample descriptives for the different types of groupings based on (A) endogenous and exogenous estradiol levels, (B) oral contraceptive history, and (C) ethinyl estradiol doses.

			Naturally cycling women						Current OC users		
			(A) E2 levels			(B) OC history			(C) EE dosage		
	Men	Current OC users	E2-	E2+	[1]*p*-value	Never users	Past users	[2]*p*-value	EE-	EE+	[3]*p*-value
n	36	52	36	23		30	29		28	24	
E2 (pg/ml)	0.2 (0.1)	0.1 (0.2)	0.2 (0.2)	0.9 (0.6)	< .001	0.4 (0.5)	0.5 (0.6)	.008	0.1 (0.3)	0.1 (0.1)	.601
P4 (pg/ml)	4.2 (6.8)	3.9 (8.5)	4.0 (6.4)	1.8 (2.4)	.548	3.4 (5.3)	3.0 (5.4)	.491	4.7 (11.3)	3.1 (3.3)	.506
T (pg/ml)	53.3 (21.6)	2.0 (1.5)	3.5 (1.6)	7.6 (11.9)	< .001	6.0 (10.6)	4.2 (2.0)	< .001	2.3 (1.6)	1.6 (1.3)	.312
EE (pg/ml)		3.3 (17.5)							0.4 (0.8)	6.4 (25.1)	.003
Shock level (mA)	2.2 (1.5)	1.9 (1.1)	1.9 (1.2)	1.8 (1.1)	.747	1.6 (0.8)	2.2 (1.3)	.261	1.9 (1.3)	1.9 (1.0)	.929
Unconditioned response to shocks (sqrt µS)	1.5 (0.5)	1.6 (0.7)	1.4 (0.7)	1.7 (0.8)	.381	1.4 (0.7)	1.6 (0.7)	.492	1.8 (0.8)	1.5 (0.6)	.173
Age	26.4 (3.7)	26.3 (2.8)	26.6 (3.4)	27.1 (3.2)	.759	26.1 (3.2)	27.4 (3.3)	.367	25.3 (2.1)	27.5 (3.1)	.004
Age of menarche		12.5 (1.6)	12.5 (1.7)	12.7 (1.6)	.820	12.8 (1.9)	12.4 (1.4)	.614	12.4 (1.7)	12.7 (1.3)	.479
Years of education	17.1 (2.4)	18.0 (2.0)	17.3 (1.8)	17.5 (2.4)	.207	17.3 (2.2)	17.4 (1.9)	.208	17.8 (1.5)	18.3 (2.4)	.330
Ethnicity (White)	23 (64%)	43 (83%)	26 (72%)	11 (48%)	.018	15 (50%)	22 (76%)	.013	24 (86%)	19 (79%)	.799
Religiosity (SCSRFQ)	16.7 (8.7)	13.5 (6.9)	15.1 (8.3)	15.0 (6.4)	.276	16.0 (8.2)	14.1 (6.9)	.184	12.9 (6.3)	14.1 (7.6)	.553
Relationship status (single)	27 (75%)	17 (33%)	21 (58%)	14 (61%)	.001	20 (67%)	15 (52%)	< .001	6 (21%)	11 (46%)	.137
Anxiety trait (STAI-T)	39.3 (10.2)	41.1 (10.4)	38.7 (6.6)	42.7 (10.4)	.350	41.2 (7.8)	39.2 (9.1)	.712	42.1 (8.5)	40.0 (12.3)	.462
Anxiety state – Day 1 (STAI-S)	30.6 (5.9)	32.6 (7.4)	33.0 (8.2)	30.2 (6.4)	.281	33.4 (8.4)	30.3 (6.4)	.207	32.4 (5.3)	32.8 (9.4)	.862
Anxiety state – Day 2 (STAI-S)	28.6 (6.5)	30.6 (8.8)	31.0 (8.6)	30.0 (8.3)	.594	32.4 (9.0)	28.8 (7.4)	.212	29.8 (5.8)	31.6 (11.3)	.455
Depressive symptoms (BDI-II)	8.0 (6.5)	8.2 (5.2)	7.8 (6.3)	9.3 (5.6)	.815	8.6 (6.7)	8.1 (5.4)	.981	8.1 (4.4)	8.3 (6.0)	.905
Cognitive reappraisal (ERQ)	28.9 (6.0)	29.4 (5.1)	28.7 (6.8)	29.8 (7.4)	.902	27.6 (7.6)	30.7 (6.1)	.287	30.1 (4.5)	28.5 (5.8)	.263
Expressive suppression (ERQ)	14.7 (5.4)	12.4 (5.0)	13.2 (5.4)	12.5 (5.9)	.214	13.9 (5.4)	11.9 (5.7)	.095	11.5 (4.5)	13.3 (5.5)	.199
Neuroticism (NEO-PI-3)	32.9 (9.5)	35.9 (8.7)	35.2 (7.6)	38.3 (9.8)	.144	36.6 (8.3)	36.2 (8.9)	.293	38.0 (7.7)	33.5 (9.4)	.066
Extraversion (NEO-PI-3)	44.6 (6.9)	43.7 (6.3)	44.7 (5.8)	42.1 (8.8)	.480	41.0 (6.5)	46.5 (6.9)	.015	42.6 (6.5)	45.0 (6.0)	.170
Conscientiousness (NEO-PI-3)	42.3 (7.5)	45.8 (6.3)	45.1 (5.5)	45.3 (8.7)	.134	44.1 (7.4)	46.2 (6.2)	.073	46.1 (5.9)	45.4 (6.8)	.693
Openness (NEO-PI-3)	45.4 (5.3)	43.8 (5.8)	46.0 (7.4)	45.8 (6.8)	.380	46.7 (6.6)	45.1 (7.7)	.260	43.1 (5.8)	44.7 (5.9)	.330
Agreeableness (NEO-PI-3)	42.4 (4.9)	45.8 (4.3)	44.8 (4.6)	43.2 (6.4)	.010	44.1 (5.5)	44.2 (5.4)	.019	44.1 (4.3)	47.8 (3.6)	.002

Notes. (A) Data represent *M* (*SD*), except for categorical variables, where data represent *n* (%) of participants in the most prevalent category (indicated in brackets). (B) *P*-values refer to main effects of Groups. The first *p*-value ([1]*p*-value) refers to estradiol (E2)-based comparisons between men, women in the early follicular menstrual phase (E2-), women in the pre-ovulatory menstrual phase (E2+),

and current oral contraceptive (OC) users. The second *p*-value (^2p-value) refers to OC-history-based comparisons between men, never users of hormonal contraception, past OC users, and current OC users. The third *p*-value (^3p-value) refers to ethinyl estradiol (EE)-dose-based comparisons between current users of low-EE OCs (EE- users) and current users of high-EE OCs (EE+ users). Figure 1 illustrates the different groupings. (C) For hormone levels, raw *M*s and *SD*s are reported to ease data interpretation, but *p*-values were computed on log-transformed data. E2 = estradiol, P4 = progesterone, T = testosterone, EE = ethinyl estradiol, SCSRFQ = Santa Clara Strength of Religious Faith Questionnaire, STAI-T = State and Trait Anxiety Inventory (Trait), STAI-S = State and Trait Anxiety Inventory (State), BDI-II = Beck Depression Inventory II, ERQ = Emotion Regulation Questionnaire, NEO-PI-3 = NEO Personality Inventory-3.