# Fear acquisition across the menstrual cycle: The moderating role of vagally mediated heart rate variability

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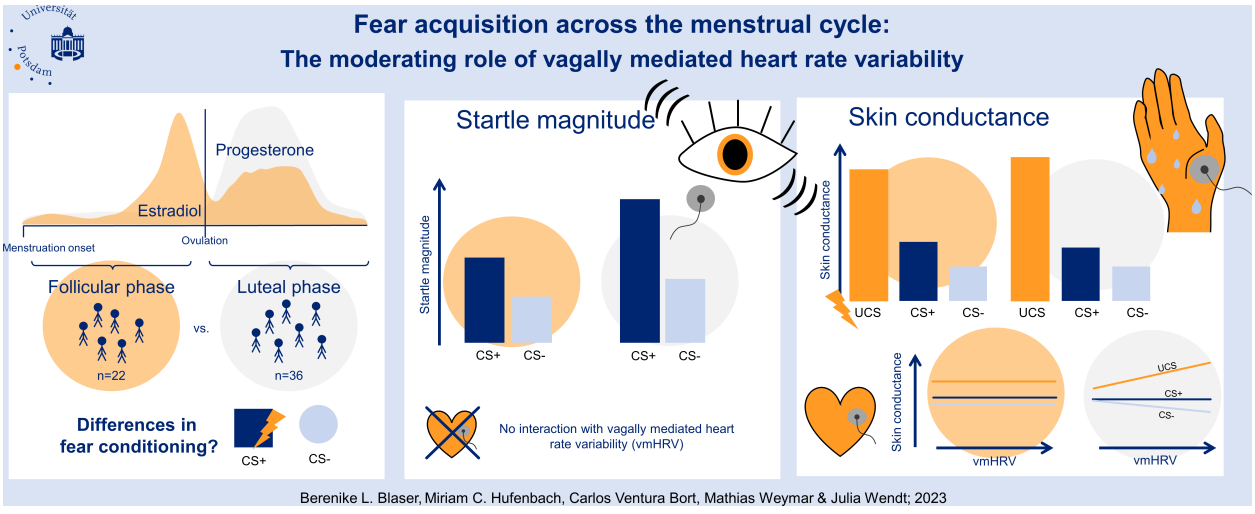
## Abstract

The luteal phase of the menstrual cycle is accompanied by diminished vagally mediated heart rate variability (vmHRV). VmHRV is consistently linked to anxiety, a commonly experienced symptom during the luteal phase. However, fear conditioning, a laboratory model of anxiety, has received limited attention in the context of menstrual cycle fluctuations. This study therefore aims to explore the influence of menstrual cycle phases on instructed fear conditioning and its interactions with vmHRV.

In this study, 58 healthy individuals with regular menstrual cycles, currently in the luteal or follicular phase, participated in a fear conditioning paradigm. During this experiment, two geometric figures were either paired (CS+) or not paired (CS-) with an electric shock. Linear mixed models were used to analyze the modulatory effects of the menstrual cycle phase on the startle magnitude and skin conductance responses (SCRs) to these conditioned stimuli.

Results revealed higher fear differentiation (CS+ vs. CS-) during the luteal phase in the startle magnitude, driven by a startle potentiation to the conditioned stimulus (CS+). In terms of SCR, interacting effects with vmHRV revealed that individuals with high vmHRV exhibited a similar increased fear differentiation during the luteal phase, while low vmHRV individuals showed less fear differentiation.

These findings suggest that during the luteal phase, individuals exhibit stronger fear-related differentiation, a pattern that is partly modulated by vmHRV. These insights shed light on potential origins of varying symptom experiences like increased anxiety during the luteal phase. However, further research is required to investigate associations between these fluctuations and symptomatology.



# Fear acquisition across the menstrual cycle: The moderating role of vagally mediated heart rate variability

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## Introduction

During the luteal phase of their menstrual cycle, most menstruating individuals experience aversive physical and affective symptoms (Tschudin et al., 2010). These symptoms might be linked to cycle phase-dependent neurophysiological and autonomic processing, such as changes in neurotransmitter systems (Nappi et al., 2022) and a decrease in parasympathetic activity (Schmalenberger et al., 2019). Heightened anxiety is a frequently reported symptom during this phase (Allen et al., 1991). Notably, anxiety is consistently associated with reduced vagally mediated heart rate variability (vmHRV) (Chalmers et al., 2014), such that vmHRV fluctuations likely contribute to the mood and affect fluctuations observed during the menstrual cycle (Matsumoto et al., 2007).

VmHRV is an indicator of autonomic processing and is associated with the inhibitory functional connectivity between the medial *prefrontal cortex* (PFC) and the amygdala (Sakaki et al., 2016). In their neurovisceral integration model of fear, Battaglia and Thayer (2022) propose that vmHRV serves as a relevant biomarker for studying inter- and intraindividual differences in fear learning. This is attributed to the fact that vmHRV is considered a peripheral indicator of the interplay among crucial brain structures involved in fear acquisition and processing, with one of the primary regions being the amygdala. Notably, vmHRV is consistently found to be reduced during the luteal phase of the menstrual cycle (Schmalenberger et al., 2019). There is also evidence that suggests that stronger reductions in vmHRV are accompanied by a higher degree of aversive symptoms, including anxiety-related symptomatology, during the late luteal phase, known as premenstrual symptoms (Matsumoto et al., 2007). This may indicate that the heightened aversive symptoms during the luteal phase are linked to reduced amygdala inhibition, as reflected by lower vmHRV.

A research paradigm that serves as a laboratory model for anxiety (Beckers et al., 2023) and in which the amygdala has consistently been found to be involved (Kuhn et al., 2019; Sun et al., 2020) is fear conditioning. Fear conditioning paradigms, therefore, may provide valuable insights into the relationship between anxiety-related symptomatology and cycle-related fluctuations. One of the most commonly used fear conditioning paradigms is the differential conditioning procedure in which neutral stimuli are either paired (CS+) with an aversive unconditioned stimulus (UCS) or not (CS-) (Lonsdorf et al., 2017). The CS+/UCS association (fear acquisition takes place) leads to conditioned responses to the CS+. Additionally, an association between CS- and the absence of UCS is formed, giving the CS- the quality of a safety signal. The difference in reactions to the sole presentation of CS+ and CS- is then termed fear discrimination, representing how effectively the individual distinguishes between the "dangerous" and "safe" conditions. This process of differential fear and safety acquisition can be enhanced by providing verbal instructions about the pairings (Mertens et al., 2018). Two of the most commonly used physiological outcome measures of fear acquisition are skin conductance response (SCR) and the startle blink reflex (i.e., fear-potentiated startle), characterized by larger responses to the CS+ compared to the CS-. SCR is typically interpreted as an indicator of sympathetic activity, while the startle blink reflex is suggested as an indicator of subcortical fear processing in fear-inducing contexts because it is directly mediated by the amygdala (Lonsdorf et al., 2017; Wendt et al., 2023).

In a recent review, Merz et al. (2018) highlighted the potential relevance of menstrual cycle-related fluctuations in fear conditioning paradigms. However, the evidence presented by the authors is inconclusive, and there is little further research on this topic. Armbruster et al. (2018) observed a trend for overall higher startle magnitude in the luteal phase. This effect is especially pronounced in persons experiencing strong affective premenstrual symptoms (Epperson et al., 2007). Critically, Glover et al. (2013) showed higher startle magnitudes for CS+ compared to CS- (i.e., fear discrimination), during the luteal than the follicular phase. In skin conductance measures, however, previous studies showed no overall effects between menstrual phases in SCR (Lonsdorf et al., 2015; Milad et al., 2006), as well as no differential fear discrimination effects (Carpenter et al. (2022); Milad et al., (2006). In a small study of 31 naturally cycling individuals, van der Molen et al. (1988) compared those who were currently in the last week of the luteal phase (premenstrual phase) to those in all other phases (including early and mid-luteal) and found higher fear discrimination in SCR in premenstrual individuals (n = 8).

Overall, despite the potential impact of the menstrual cycle on anxiety-related mechanisms (Merz et al., 2018), the existing research on the relationship between fear acquisition and the menstrual cycle has been rather scarce and characterized by the use of unimodal indicators of fear acquisition, leading to heterogeneous findings. To deepen our understanding of the impact of the menstrual cycle on fear processing, we therefore investigated fear conditioning measures of startle magnitude and SCR in individuals in the follicular phase and individuals in the luteal phase of their menstrual cycle. Furthermore, extending previous findings, we will also investigate the potential interacting role of vmHRV as one of the most relevant modulators of the relationship between anxiety symptomatology and the cycle phase.

## Methods

### Participants

We tested healthy participants who took part in a larger biofeedback intervention study (Wendt et al., 2020, December 16). Participants were recruited through postings at the Universities of Potsdam and Greifswald, online platforms, and through posts on social networks. Exclusion criteria included having a body mass index lower than 18.5 $kg/m^2$ or higher than 30 $kg/m^2$, cardiovascular, neurological, or respiratory diseases, impaired hearing or color vision, claustrophobia, pregnancy, and the use of medications that alter the normal functions of the autonomic nervous system. A total of 128 participants were tested. Of the tested participants, 58 were included in the data analysis who reported a regular active menstrual cycle, no hormonal contraception and were currently either in the luteal or follicular phase of their cycle. All participants gave informed consent and were compensated with course credit or money. The data were collected between June 2020 and October 2022 and the project was approved by the Ethics Committee of the University Medicine Greifswald.

### Procedure and fear conditioning

The testing took place in the context of a large intervention study described elsewhere (Wendt et al., 2020, December 16) which investigates the effects of heart rate variability biofeedback on extinction learning in healthy individuals. The focus of this report is on data from the acquisition phase of the fear conditioning paradigm that was used in this project.

After the introduction to the study, participants were led to a darkened experimental room where sensors were attached to measure the physiological signals. Participants went through a 6-minute electrocardiogram (ECG) measurement with closed eyes, followed by a fear acquisition protocol.

For the fear paradigm, a blue square and an orange circle served as conditioned stimuli. The stimuli were displayed on a black background in the center of the computer screen, each with a duration of 6 seconds. The intertrial intervals (ITI) varied in length between 14, 15, and 16 seconds. The stimulus presentation was realized using Presentation software (Version 20.3, Neurobehavioral Systems, Inc.).

The assignment of the CS+ and CS- stimulus was counterbalanced across participants. The CS+ was partially (50%) reinforced by an electro-tactile stimulus for a 1ms duration (UCS). The intensity was individually adjusted for each participant before training to a level judged to be "very unpleasant but not painful." (M = 6 mA, SD = 3.7, Range [1.3; 21.0]). The CS- was never accompanied by an electro-tactile stimulus. The stimulation electrode was placed on the inside of the right leg, approximately 3 cm above the ankle. Electrical stimulation was controlled using a DS7A Constant Current Stimulator (Digitimer, Hertfordshire, UK) in Potsdam and an S-48K stimulator (Grass Instruments, West Warwick, RI, USA) in Greifswald.

Bursts of white noise (duration of 50 ms duration, 95 dB volume) served as startle acoustic startle probes and were delivered binaurally through headphones (Potsdam: Audio-Technica ATH-PRO700MK2, Greifswald: AKG K 66). The startle stimuli were administered 4.5 or 5.5 seconds after each CS onset and in half of the ITI (i.e., 16 times) 7.5 seconds after CS offset.

The conditioning phase consisted of 16 trials (8 CS+, 8 CS-) of uninstructed acquisition, followed by a slide informing the participants which of the geometric figures was associated with the aversive stimulus. Then 16 trials of instructed acquisition followed. Before and after the acquisition protocol, participants rated the stimuli on valence and arousal using a 9-point visual analog scale ranging from 1 (valence: highly unpleasant, arousal: calm) to 9 (valence: very pleasant, arousal: exciting).

### Startle magnitude, skin conductance and heart rate variability

All physiological recordings were performed using a BIOPAC MP160 amplifier system and AcqKnowledge 5.0.2 software (BIOPAC Systems, Inc., Goleta, CA, USA). All data were sampled at a rate of 2,000 Hz and filtered at various sample rates (see below). A silicon grounding electrode (TerniMed) was attached to the participant's left upper arm.

For the ECG, two Ag/AgCl electrodes (10mm contact surface diameter; Schuler Medizintechnik GmbH) filled with electrode paste (CareFusion) were applied on the right forearm (approximately 2 cm below the elbow) and the left leg (approximately 2 cm proximal to the ankle). The ECG data was digitally sampled at a rate of 400 Hz. The processing of the ECG data was executed using Kubios HRV Software (University of Eastern Finland, Kuopio, Finland) following the recommendations of the Task Force of the European Society of Cardiology (Malik, 1996). The root mean square of successive differences (RMSSD) was used as a vmHRV measure due to its robustness to breathing rate influences, which were not controlled for in the present study (Chapleau & Sabharwal, 2011).

For the Electrodermal Activity (EDA) recording, the non-dominant hand of the participants was used. Two Ag/AgCl sintered biopotential electrodes (8mm contact surface diameter, Easycap GmbH) were filled with isotonic electrode contact gel (0.5% NaCl, GEL101, BIOPAC Systems, Inc.) and then attached palm-side using double-sided adhesive rings over the hypothenar muscles. The EDA was recorded using an EDA100C

module (BIOPAC Systems, Inc.), employing a constant voltage method (0.5V). The EDA data were passed through a 10 Hz low-pass filter.

The startle response was measured using electromyography (EMG) of the Orbicularis Oculi muscle. For this purpose, two electrodes (Ag/AgCl electrodes, 5mm contact surface diameter; Schuler Medizintechnik GmbH) were filled with electrode contact paste (CareFusion) and positioned under the left eye of the participant, with the first electrode located approximately 0.5 to 1 cm below the eye vertically aligned with the pupil, and the second electrode placed laterally adjacent to it (parallel to the eyelid contour) approximately 1 cm from the outer corner of the eye. The EMG signal was recorded using the EMG100C module (BIOPAC Systems, Inc.). The EMG data was digitally sampled at a rate of 1,000 Hz and filtered using 30 Hz high pass, 400 Hz low pass and 50 Hz notch-filter.

The startle magnitude and EDA data were preprocessed using MATLAB.

SCRs were analyzed using the trough-to-peak method (TTP) in Ledalab Version 3.4.9 (Benedek & Kaernbach, 2010). In the TTP method, the SCR amplitude is defined as the difference between the skin conductance at the peak of the response and its preceding trough in a determined time window. Adhering to the guidelines (Boucsein et al., 2012), the response window was set from 1 to 4 seconds after CS onset. The startle magnitude procedure adhered to Blumenthal et al.'s (2005) recommendations. Blink response onset and peak were automatically identified within 20-120 ms after probe onset, with a peak before 150 ms, employing the algorithm of Globisch et al. (1993). In a subsequent visual inspection, trials without blinks were scored as zero, and trials with excessive background activity or artifacts were considered missing. As our emphasis was on interindividual variability in startle magnitude, we decided to use raw data instead of T-transformed data (Bradford et al., 2015).

### Menstrual cycle phase

Cycle phases were assessed through self-report using the forward-count method (Schmalenberger et al., 2021). The follicular phase was assigned when participants reported being in days 1-11 of their cycle. To determine the luteal phase window, 11 days were subtracted from the reported average cycle length. If participants were between these phases or if their cycle day or average cycle length could not be reliably assessed, they were excluded from the analysis.

### Statistical analyses

All analyses were conducted in R version 4.2.2. Linear mixed models were calculated, using SCR and startle magnitude from instructed acquisition trials as dependent variables respectively. Participant intercepts were introduced as random effects to cluster the trials by participant. Predictor variables included trial condition (CS+, CS- or ITI/UCS, contrast-coded), menstrual phase (follicular or luteal, dummy-coded) and their interaction. RMSSD and its interaction with trial condition as well as participants' age (control variable) were included if they improved the model fit as indicated by Likelihood Ratio Tests.

## Results

### Sample description

Out of the 58 individuals included in the analysis, 36 reported being in the follicular phase, while 22 reported being in the luteal phase. The difference in group sizes is attributed to the higher reliability of reporting the follicular phase using the forward-count method, resulting in more individuals currently in the luteal phase being excluded from the analysis. The groups did not differ significantly in terms of age ($m_{luteal}$ = 22.68±2.32, $m_{follicular}$ = 24±2.9).

### Startle response

The results of the model can be viewed in Table 1. There was no significant main effect of the cycle phase, $t(63.4) = 1.54$, $p = .13$., indicating no differential overall startle magnitude between phases. The interaction effect of Condition x Cycle Phase yielded significant terms in the model, $t(1296.1) = -2.58$, $p < .05$ (CS+ vs. CS-), $t(1296.1) = -2.68$, $p < .01$ (CS+ vs. ITI).

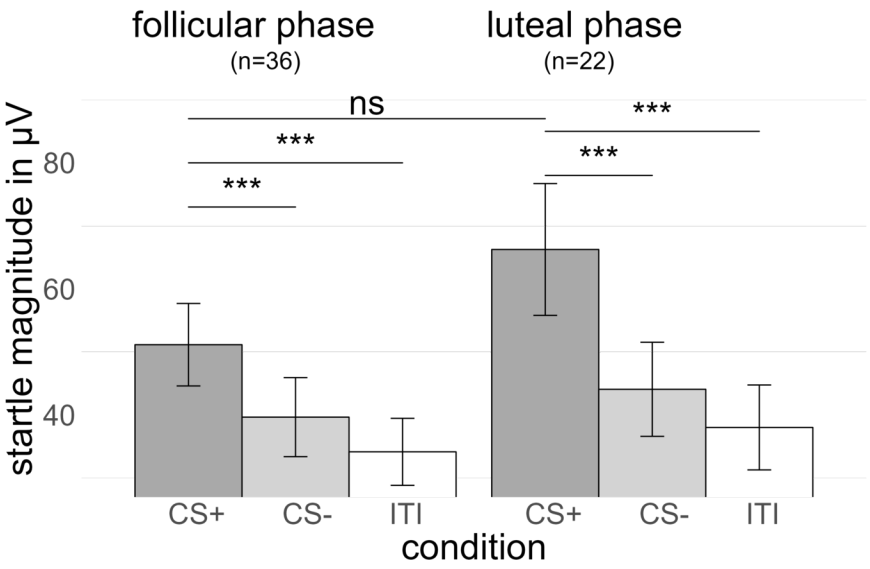
**Table 1** Results of mixed model predicting startle magnitude

| *Predictors* | *Estimates* | *CI* | *p* |
|---|---|---|---|
| (Intercept) | 51.16 | 39.28 – 63.04 | **<0.001*** ** |
| condition [CS-] | -11.38 | -16.43 – -6.33 | **<0.001*** ** |
| condition [ITI] | -17.06 | -22.15 – -11.98 | **<0.001*** ** |
| phase [lut] | 15.19 | -4.11 – 34.48 | 0.123 |
| condition [CS-] × phase [lut] | -10.78 | -18.98 – -2.57 | **0.010*** |
| condition [ITI] × phase [lut] | -11.24 | -19.47 – -3.02 | **0.007**** |
| Observations | 1358 | | |
| Marginal $R^2$ / Conditional $R^2$ | 0.045 / 0.582 | | |

Note. The final model had the following structure: startle magnitude ~ condition * phase + (1|participant). CS+ – stimulus paired with unconditioned stimulus; CS- – stimulus not paired with unconditioned stimulus; ITI – intertrial interval; lut – luteal phase.

A visualization of the interaction effect, including significance levels from post-hoc contrast testing, can be seen in Figure 1. Although CS+ trials evoked higher startle magnitudes compared to CS- and ITI trials in both the follicular and luteal phases, the disparity between CS+ and the other trials was more pronounced during the luteal phase, indicating heightened fear discrimination. Post-hoc contrast testing, however, showed no significant difference between startle responses to the CS+ in the follicular and the luteal phase group, $t(63.4) = -1.54$, $p = 0.13$. Adding the RMSSD as a main effect or interaction to the model did not change the results. Age was not included as it did not improve the model fit.

**Figure 1** Startle magnitude by condition and menstrual cycle phase.




Note. The figure shows a significant interaction effect. Bar plots indicate group means and whiskers standard errors. CS+ – stimulus paired with unconditioned stimulus; CS- – stimulus not paired with unconditioned stimulus; ITI – intertrial interval. ns = not significant; *** = p<.001.

**Skin conductance response**

A Box-Cox analysis indicated the necessity of logarithmic transformation of the SCR data due to left skewness. Therefore, the analyses were conducted using the log-transformed data (log(1+SCR)). In 4 participants, null reactions in UCS trials amounted to more than 50%, which led to their exclusion from the analysis (Lonsdorf et al., 2019).

The results of the model predicting SCR are presented in Table 2. The inclusion of a three-way interaction term (Cycle Phase x Condition x RMSSD) significantly improved the model fit, as evidenced by a Likelihood Ratio Test, $\chi^2$ (2, N = 58) = 8.42, $p > .05$. Adding age or RMSSD as main effects did not improve the model. There was no main effect of the menstrual cycle phase in the final model, $t(75.55) = 0.82$, $p = .42$, indicating no overall differential skin conductance levels between the phases.

**Table 2** Results of mixed model predicting skin conductance

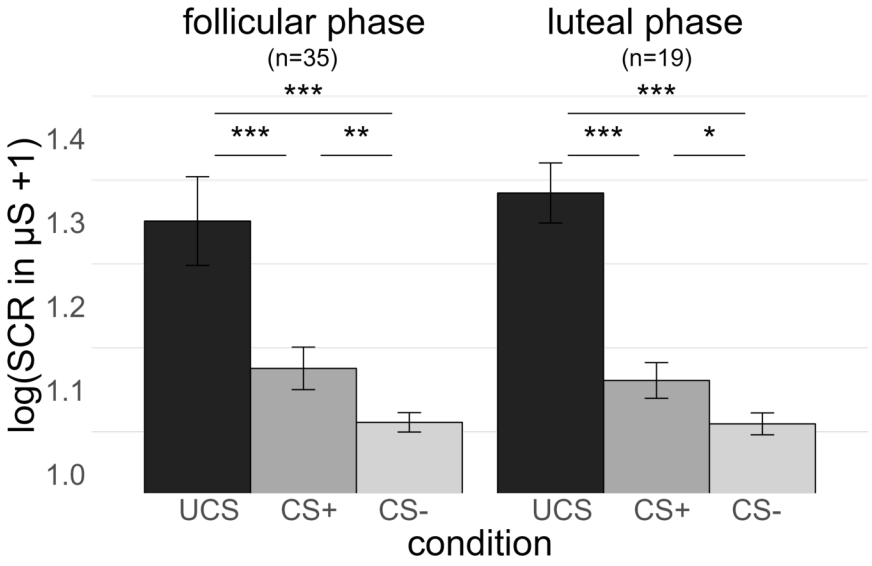
| *Predictors* | *Estimates* | *CI* | *p* |
|---|---|---|---|
| (Intercept) | 1.06 | 1.02 – 1.10 | **<0.001*** ** |
| condition [CS+] | 0.06 | 0.03 – 0.09 | **<0.001*** ** |
| condition [UCS] | 0.24 | 0.20 – 0.28 | **<0.001*** ** |
| phase [lut] | 0.14 | -0.20 – 0.47 | 0.413 |
| condition [CS+] × phase [lut] | -0.16 | -0.42 – 0.10 | 0.219 |
| condition [UCS] × phase [lut] | -0.42 | -0.74 – -0.11 | **0.009** * |
| Condition [CS-] × phase [lut] × log(RMSSD) | -0.04 | -0.13 – 0.05 | 0.398 |
| condition [CS+] × phase [lut] × log(RMSSD) | 0.00 | -0.09 – 0.09 | 0.962 |
| condition [UCS] × phase [lut] × log(RMSSD) | 0.09 | -0.02 – 0.19 | **0.096+** |
| Observations | 1080 | | |

| | |
|---|---|
| Marginal $R^2$ / Conditional $R^2$ | 0.178 / 0.365 |

Note. The final model had the structure: log(skin conductance response) ~ condition * phase + log(RMSSD):condition:phase + (1|participant). CS+ – stimulus paired with unconditioned stimulus; CS- – stimulus not paired with unconditioned stimulus; UCS – unconditioned stimulus; RMSSD – root mean square of successive differences; lut – luteal phase.

However, a two-way interaction of Cycle Phase x Condition (UCS vs. CS-) yielded a significant effect (see Figure 2 for visualization including significance levels from post-hoc contrast testing), $t(1020) = -2.625$, $p < .01$. The effect mirrors the interaction observed in the startle response, albeit less distinctly pronounced. During the luteal phase, a heightened SCR is evident specifically in response to the UCS (unconditioned stimulus) compared to the follicular phase. Although the visual inspection confirms that this difference drives the interaction effect, post-hoc testing did not find a significant difference between the UCS values in the follicular phase group and the luteal phase group, $t(125.7) = -1.084$, $p = 0.28$.

**Figure 2** Skin conductance by condition and menstrual cycle phase

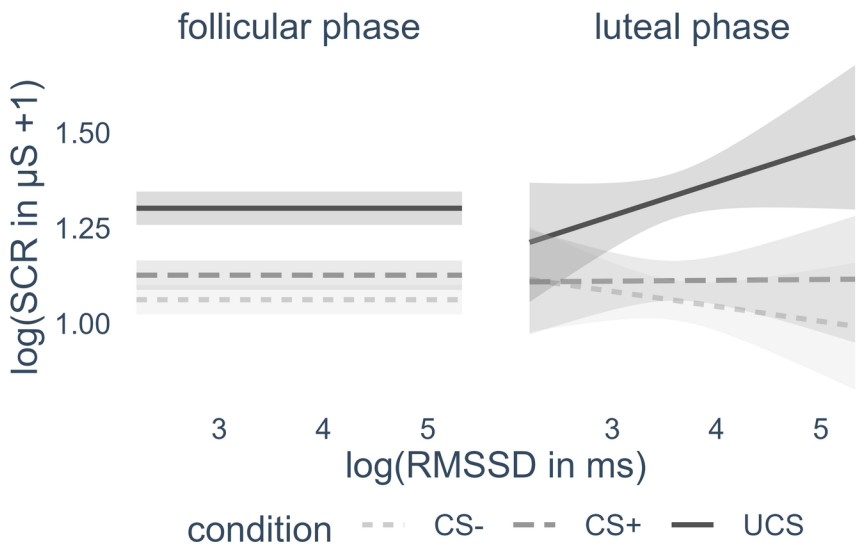



Note. The figure shows a significant interaction effect. Bar plots indicate group means and whiskers standard errors. CS+ – stimulus paired with unconditioned stimulus; CS- – stimulus not paired with unconditioned stimulus; UCS – unconditioned stimulus; SCR – skin conductance response. * = p<.05; ** = p.01 *** = p<.001.

Furthermore, an additional three-way interaction term with RMSSD indicated that this effect was moderated by vagally mediated heart rate variability. While the interaction term was only marginally significant, $t(125.7) = 1.67$, $p = 0.098$, adding the effect significantly improved the overall model fit, indicating a relevant explanation of the data through the three-way interaction. Figure 3 depicts the three-way interaction, illustrating the effect when all other effects are held constant. It revealed that greater fear discrimination

during the luteal phase (resulting in a larger difference between UCS and CS-, as well as CS+ and CS-) is associated with higher vmHRV. Conversely, individuals with very low vmHRV in the luteal phase exhibit less distinction than those in the follicular phase. Post-hoc testing for the beta weights of the interaction showed that this effect is driven by the difference in slopes of the CS- and the UCS in the luteal phase group, $t_{\text{ratio}}(1018) = 2.90$, $p < 0.05$. While the standardized UCS slope in this phase is 0.40, the slope for the CS- is -0.18. This indicates that for each standard deviation (SD) lower vmHRV during the luteal phase, there is a corresponding decrease of 0.4 SDs in the SCR response to the UCS and an increase of 0.2 SDs in the SCR response to the CS-. During the follicular phase, the level of fear discrimination is not linked to resting vmHRV.

**Figure 3** Skin conductance by condition, menstrual cycle phase and vagally mediated heart rate variability




Note. The figure shows a significant interaction effect when all other effects are held constant. RMSSD – root mean square of successive differences; SCR – skin conductance response; CS+ – stimulus paired with unconditioned stimulus; CS- – stimulus not paired with unconditioned stimulus; UCS – unconditioned stimulus.

## Discussion

In the present study, we investigated the effects of the menstrual cycle phase and vagally mediated heart rate variability (vmHRV) on instructed fear conditioning. We found that the menstrual cycle modulated fear discrimination in both startle magnitudes and the SCR. Specifically, an enhanced CS+ vs. CS- differentiation was seen in startle responses in individuals during the luteal phase compared to individuals in the follicular phase. This enhanced discrimination was driven by larger responses particularly to CS+ stimuli. In skin conductance response (SCR) measurements, we observed reduced fear and safety discrimination during the luteal phase in individuals with low resting vmHRV compared to individuals with high vmHRV during the luteal phase and those in the follicular phase.

Firstly, our results indicate higher fear differentiation in startle magnitude during the luteal phase, specifically showing a higher fear-potentiated startle response to the conditioned stimulus (CS+) but not to the unconditioned stimulus (CS-) and intertrial interval (ITI). This finding replicates previous research by Glover

et al. (2013), who similarly observed increased fear discrimination in startle measures during the luteal phase. Importantly, this effect was consistent across all participants, regardless of their resting vmHRV. However, we did not find a significant main effect of the menstrual cycle phase on startle magnitude. This contrasts with the results of Armbruster et al. (2018), who reported higher overall startle magnitudes during the luteal phase compared to the follicular phase.

With regard to skin conductance, we observed that individuals with high vmHRV show the same increased fear differentiation between CS+ and CS- (and UCS) in the luteal phase while low vmHRV individuals showed less fear differentiation. In contrast, during the follicular phase, there were no differences in skin conductance fear responses based on vmHRV. Although this three-way interaction term between condition, cycle phase, and vmHRV was only marginally significant, adding it to the model significantly improved the model fit. This indicates a notable enhancement in the explanation of variance in SCR through this interaction. The two-way interaction between phase and condition was not evident until vmHRV was included in the model, which may be the reason why it was not found in previous studies (Carpenter et al., 2022; Milad et al., 2006). These results emphasize the importance of considering moderating variables that may interact with the menstrual cycle to gain a better understanding of the underlying physiological and psychological changes.

The heightened fear differentiation observed in startle measures across all individuals and in skin conductance in individuals with high vmHRV could potentially be linked to increased estrogen levels during the luteal phase. While some studies have observed increased fear discrimination in individuals with elevated estradiol levels, as seen in skin conductance (Sartin-Tarm et al., 2020) and activation of the amygdala and hypothalamus (Hwang et al., 2015), other studies have not replicated this effect (White & Graham, 2016). In these studies, however, the interpretation of the relation to menstrual cycle phases is limited due to the absence of cycle phase assessment or its exclusion from the analysis, and the reliance on simple median splits of participants' current estradiol levels for group comparisons. It is important to note that high estradiol levels can be observed both during the second half of the follicular phase and throughout the majority of the luteal phase, with a peak during the mid-phase. Consequently, some of the results may be confounded or diluted by including individuals in different phases or predominantly in the luteal phase. To disentangle the effects of the menstrual cycle phase and estradiol and validate the role of estradiol in the amplified fear discrimination during the luteal phase, subsequent studies incorporating evaluations of both phase and hormonal levels would be essential.

We found a positive association of SCR fear discrimination and vmHRV during the luteal phase. Why this association is evident only during the luteal phase of the cycle remains unclear. It is hypothesized that certain individuals possess a lower neuroendocrine reactive threshold to the fluctuating expression of gonadal steroids across the menstrual cycle, contributing to aversive symptomatology during the luteal phase (Nappi et al., 2022). The connection between reduced vmHRV and diminished fear discrimination in skin conductance measures among select participants could potentially reflect this susceptibility.WhileSchmalenberger et al. (2019) reported a general slight decrease in vmHRV during this phase in a meta-analysis, evidence suggests that this reduction in vmHRV is specifically associated with premenstrual symptoms. Persons who reported high premenstrual symptoms showed the characteristic drop in vmHRV from the follicular to the luteal phase, whereas individuals who did not experience premenstrual symptoms also did not exhibit these fluctuations (Matsumoto et al., 2007). Commonly reported premenstrual symptoms encompass anxiety, irritability, and heightened stress sensitivity (Allen et al., 1991). These symptoms align with the less distinct sympathetic reactivity observed in individuals with low vmHRV during the luteal phase in our study, as similar affective states to those experienced during PMS, such as clinical anxiety, have previously been associated with reduced fear discrimination (Cooper et al., 2018). Furthermore, decreased vmHRV is well-known to also be associated with anxiety and anxious states (Chalmers et al., 2014).

Brosschot et al. (2018) present a framework for these findings. In their Generalized Unsafety Theory of Stress, they argue that prolonged stress responses, even in the absence of stressors (such as chronic anxiety), result from a perceived lack of safety even in ostensibly secure environments. Similar to the Neurovisceral Integration Model of Fear, the authors propose the existence of a default stress response, which, in healthy

individuals, is inhibited in safe environments. VmHRV serves as an indicator of this inhibitory capacity. Evidence supporting this theory can be observed in the negative association between vmHRV and SCR in individuals during the luteal phase, as identified in this study ($\beta$ = -.18). The lower an individual's vmHRV was during this phase, the stronger their SCR response to CS- cues, which signal safety. It's noteworthy that all participants were explicitly informed, as part of the instructed fear acquisition paradigm, that there would be no aversive stimuli associated with this symbol. The reduction of vmHRV during the luteal phase accompanied by the altered skin conductance responses may thus be indicative of the origination of premenstrual symptoms in a phasic reduction of this inhibition of the default stress response. This assumption, however, would have to be verified in a paradigm that additionally assesses symptomatology.

In a similar way, our results could also be interpreted within Battaglia and Thayer's (2022) neurovisceral integration theory of fear, which is rooted in the broader neurovisceral integration theory (Thayer & Lane, 2000). This theory highlights the significance of the interplay between the central and autonomic nervous systems in fear processing. This process occurs via a network of interconnected brain structures that facilitate the regulation of a system known as the central autonomic network. A pivotal idea here is that, in healthy individuals, the PFC adeptly gauges the safety or danger of a situation. Through an inhibitory functional connection with limbic structures like the amygdala, fear responses are then dampened in safe scenarios while becoming more pronounced in hazardous assessments. Subsequently, the autonomic nervous system orchestrates the appropriate response, which could manifest as fear or a lack thereof.

The authors emphasize the importance of vmHRV in this context. It may serve as both a real-time indicator of ongoing responses and an index of the strength of the connectivity between the PFC and amygdala on a trait level (Sakaki et al., 2016). This connection, indicated by resting vmHRV, determines an individual's capacity to regulate behaviour effectively and adequately. Battaglia and Thayer (2022) propose that individuals with low resting vmHRV may experience reduced abilities to sufficiently modulate fear responses (Wendt et al., 2019; Wendt et al., 2015).

Our findings partially corroborate this assumption, revealing a connection between vmHRV and effective fear discrimination during the luteal phase of the menstrual cycle. However, this association is only evident in skin conductance and not in startle measures, which partly contradicts the model proposed by Battaglia and Thayer (2022).

## Conclusion

In summary, the menstrual cycle does have an overall effect on instructed fear conditioning, but the effects vary depending on the outcome measure and covariates. While there is a higher differentiation during the luteal phase in startle response, skin conductance shows this differentiation only in individuals with high vmHRV during this phase. Low vmHRV individuals show decreased differentiation in this measure during the luteal phase. These findings may provide valuable insights into the origin of the differential intra- and interindividual experience of premenstrual symptoms. Although in the current study, we did not directly assess whether these fluctuations are directly related to the experience of symptoms, our results clearly suggest that the menstrual cycle phase should be considered in fear conditioning paradigms.

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### Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT 3.5 in order to increase readability of the text. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full

responsibility for the content of the publication.

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