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Increased sensitivity to social exclusion during the luteal phase: Progesterone as resilience factor buffering against ostracism?

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ABSTRACT

A woman's social behaviour reportedly varies across the menstrual cycle. In this study, we estimated changes in sensitivity to social exclusion across the menstrual cycle and scrutinized the related role of progesterone. Forty-nine naturally cycling women played a virtual ball-tossing game (Cyberball) to manipulate social inclusion. All participants underwent inclusion and exclusion conditions during the late follicular and the luteal phase. We assessed salivary progesterone concentrations at each cycle phase. After each Cyberball session we measured positive/negative mood using the Multidimensional Mood State Questionnaire (MDMQ). Multilevel analyses indicated that women showed worse mood following exclusion as compared to inclusion conditions ($p = 0.014$). Notably, this exclusion effect was more pronounced during the luteal phase than the late follicular phase ($p = 0.029$). As expected, progesterone concentrations were higher during the luteal phase as compared to the late follicular phase, but interestingly, progesterone concentrations were negatively associated with exclusion effects. When accounting for mediation via progesterone, direct cycle-phase related differences in social exclusion effects even increased as compared to the model without mediator. These findings suggest that progesterone may function as buffer against negative feelings that result from being socially excluded. The relevance of these findings for Premenstrual Dysphoric Disorder (PMDD) are discussed, and we conclude that social exclusion may represent an important research domain criterion (RDoC) of relevance for PMDD, with progesterone pointing to new potential pharmacological targets.

1. Introduction

The need to belong is a fundamental human motive and an essential requirement for security, reproductive success, and mental health (Baumeister and Leary, 1995; Smith et al., 1999). Yet, we all occasionally experience a brief episode of being ignored or excluded and even slight ostracism can be sufficient to cause pain and distress (*e.g.*, Williams, 2007). Using an adaptationist framework, we estimated changes in sensitivity to social exclusion in naturally cycling women once during the late follicular and once during the luteal phase. We then scrutinized the related role of fluctuating estradiol, progesterone, and testosterone concentrations in order to explore physiological resilience factors underlying ostracism.

There is increasing evidence that naturally cycling women undergo a variety of psychological and behavioural changes throughout their menstrual cycle (Derntl et al., 2013; Goldstein et al., 2010; Wolohan et al., 2013). Evolutionary informed scholars have related these changes as serving to increase reproductive fitness: in the high-fertile late follicular phase preceding ovulation, psychological and behavioural changes should support the selection of genetically fit mates (Gangestad et al., 2007; Little et al., 2007) and should increase the chance for reproduction (Davis and Tran, 2001; Krug et al., 2000). Conversely, in the postovulatory (luteal) phase, a woman's body is preparing for potential pregnancy (Müller and Hassel, 2012). During the luteal phase women should hence aim to reduce the risk of harm or disease (Fessler and Navarrete, 2003; Fleischman and Fessler, 2011). At the same time women should show increased affiliation motivation (Jones et al., 2008).

Behavioural changes during the luteal phase have often been linked to progesterone (*e.g.*, Jones et al., 2008; Maner and Miller, 2014). Progesterone helps to secure pregnancy and its concentration increases substantially after ovulation. Increased progesterone levels have been

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shown to be correlated with higher implicit affiliation motivation in men and women (Schultheiss et al., 2003; Wirth and Schultheiss, 2006). Moreover, high progesterone levels during the luteal phase have been associated with increased sensitivity for social information (Maner and Miller, 2014). Another study found that women showed increased progesterone levels after they experienced social exclusion (Seidel et al., 2013; but see Gaffey and Wirth (2014); Radke et al. (2018). During the luteal phase, women often experience a recurrence of negative behavioral (*e.g.* fatigue), psychological (*e.g.* irritability) and physical symptoms (*e.g.* headaches) (Dickerson et al., 2003). Again, these negative symptoms have been associated with elevated progesterone levels (Smith et al., 2006).

The present study aims to investigate whether women are more sensitive to social exclusion during the luteal phase and if so, whether sensitivity to social exclusion can be explained by increased progesterone levels. One way to create situations of social exclusion in a laboratory setting is by using the so-called "Cyberball" game (Williams and Jarvis, 2006). Cyberball is a virtual ball-tossing game in which the participant is excluded from playing at one point. Being excluded during Cyberball results in lower levels of perceived belongingness, control, meaningful existence and self-esteem (Zadro et al., 2004). Furthermore, social exclusion leads to emotional responses such as jealousy (Harmon-Jones et al., 2009) and aggression (Chen et al., 2012).

The present study investigated the reactions to social exclusion across the menstrual cycle and in relation to the cyclic shifts in progesterone, estradiol and testosterone concentrations. While previous studies have reported hormonal reactions after being socially excluded (e.g., Radke et al., 2018; Seidel et al., 2013), the present study examined how levels of progesterone, estradiol and testosterone as measured before social exclusion relate to mood changes experienced after being socially excluded. Specifically, we measured women's progesterone, estradiol and testosterone levels before playing the Cyberball game and assessed mood changes after experiencing social exclusion. Each woman was confronted with social exclusion (Cyberball) twice, once during the late follicular phase and once during the luteal phase. For menstrual cycle studies it is essential to accurately monitor the menstrual cycle, since cycle length can vary substantially between and within women (Jasienska, 2013; Lobmaier and Bachofner, 2018; Munster et al., 1992). We used multiple methods to maximize cycle monitoring accuracy. As a physiologically based fertility predictor we used OvaCUE© to estimate the peri-ovulational phase. Peak fertility was then determined with urine tests measuring the luteinizing hormone and confirmed by the analysis of salivary estradiol, progesterone and testosterone concentrations.

We tested whether the experience of inclusion and exclusion while playing Cyberball varies across the menstrual cycle. We hypothesized that women show a stronger reduction in mood ratings following social exclusion during the luteal phase compared to the late follicular phase. To scrutinize the potential role of sex hormones on negative mood after social exclusion, we assessed the influence of progesterone, estradiol and testosterone concentrations on mood ratings after experiencing social inclusion and exclusion during the two menstrual cycle phases. Because affiliation motivation has been associated with progesterone, we expect progesterone levels to predict mood ratings after social exclusion.

2. Materials and methods

2.1. Participants

Of 86 women who initially showed interest in taking part in this study, datasets of 49 women were eventually included in the analyses (see flow chart Figure S1 in SI for an overview of the participants who dropped out and the reasons for non-participation at each stage). The included participants ranged in age between 18 and 33 years (M = 24.30 years; SD = 3.91 years). Twenty-nine were recuited from the general public via advertisements posted in public amenities and twenty were recruited from a pool of first-year psychology students. They received either course credits (psychology students) or 50 CHF (approximately 50 USD; participants from the general public) for their participation. All participants provided written informed consent to take part in this study and were treated in accordance with the ethical protocol approved by the Faculty of Human Sciences of the University of Bern and with the Code of Ethics of the World Medical Association (Declaration of Helsinki). All women were selected on the basis of the following inclusion criteria: (a) between 18 and 35 years of age, (b) medication-free (including hormonal contraception for at least 3 previous months), (c) regular menstrual cycle (average length of between 25 and 35 days), (d) not pregnant or breastfeeding, and (e) no abortion in the previous six months. Participants indicated neither current nor previous history of psychiatric disorders or alcohol and drug abuse. Using the self-report PMS questionnaire (Ditzen et al., 2011) 14 (29.2%) participants reported premenstrual symptoms that have an impact on daily life. The presence of PMS symptoms was no exclusion criterion, as PMS symptoms are very common in the general population (between 50% and 80% of naturally cycling women; Dickerson et al., 2003; Ditzen et al., 2011; Halbreich et al., 2003).

2.2. Testing order

Participants were randomly assigned to two groups differing only in the order in which they were tested. Group 1 (n = 25) was first tested during the late follicular phase and then during the luteal phase, Group 2 (n = 24) was tested first during the luteal phase and then during the late follicular phase. This was done to control for potential effects of testing order. The groups did not significantly differ with respect to age ($t(47) = 0.059$; $p = 0.953$), PMS-ratings ($t(47) = 0.637$; $p = 0.527$), or trait mood ratings (PANAS positive: $t(47) = -0.854$; $p = 0.397$; PANAS negative: $t(47) = -0.766$; $p = 0.448$, as measured during the screening questionnaire, see Section 2.4, below).

2.3. Menstrual cycle monitoring

The menstrual cycle was monitored using various methods to ensure that women were tested at the right time. After agreeing to take part in this study, women were first interviewed via telephone in which we assessed the dates of the onsets of their last three menses. In cases where these data were not available, we assessed the self-reported cycle length and the approximate date of the onset of the current menstrual cycle. To avoid unnecessary dropouts due to vacation, stressful life-events or a lack of time, we also asked participants to specify months in which study participation would work best for them. Participants were asked to report the onset of menstruation in the cycle in which they were planning to take part in the study.

Four days after menstruation onset participants started using an OvaCUE© fertility monitor (Fairhavenhealth, Bellingham, WA). The OvaCUE© is a hand-held electronic monitor with oral sensors detecting the electrical resistance of salivary secretion (http://www.ovacue.com). The electrical resistance of saliva changes with cyclical variations in oestrogen concentration (Fehring, 1996) and reaches a peak value five to seven days before ovulation; OvaCUE© can therefore be used as an early ovulation predictor (Fehring, 1996). Participants conducted the OvaCUE© measurement daily and immediately after awakening. The assessment takes about three seconds and needs to be carried out for approximately 5 consecutive days, until the device can predict the date of peak fertility.

Two days before the date of predicted peak fertility women started to use urine tests measuring the luteinizing hormone (LH). We used one-step urine LH tests with a reported sensitivity of 10mlU/ml (David One Step Ovulation Tests, Runbio Biotech, China). Women were instructed to perform urine tests twice a day (morning and evening). After

a positive test result participants continued the tests until the results became negative for two subsequent days. After positive testing, the women immediately reported to the laboratory and were then either tested within 48 h of LH surge and then again 7 days later (late follicular-luteal group) or they were scheduled 7 days after the measured peak of the LH surge (luteal-late follicular group). Participants assigned to the luteal-late follicular group again assessed the LH surge in the following cycle and were then tested within 48 h of the next LH peak.

On the days of testing, women additionally provided saliva samples from which we assessed levels of estradiol, testosterone and progesterone. Fifteen minutes after their arrival in the laboratory, participants were asked to collect approximately 7.5 m l of saliva in plastic tubes (Salicaps, IBL International GmbH, Hamburg, Germany). To control for potential factors that are known to influence hormone assessments from saliva, we asked the participants to avoid excessive physical activity and drinking alcohol and to refrain from using drugs on the days of LH testing and 12 h prior to the scheduled session. Participants were further instructed to refrain from eating and to abstain from caffeine and smoking for at least 1 h prior each experimental session. Participants were asked to rinse their mouth with fresh water and to wait approximately 5 min before providing saliva. The plastic tubes were closed and stored at − 20 °C until the salivary samples were analysed for concentrations of estrogen, progesterone and testosterone by an independent laboratory (Dresden Lab Service GmbH, Dresden, Germany) using commercially available radioimmunoassay kits adopted for the analysis of salivary samples (IBL International, Hamburg, Germany). Inter-assay coefficients were below 12% and intra-assay coefficients were below 10%.

2.4. Task and procedure

As soon as participants reported the onset of menstruation, we asked them to complete an online survey (EFS Survey, Questback, Berlin, Germany) with which we collected demographic data such as age, sexual orientation, whether they have any children, whether they currently are in a romantic relationship, and if yes, how long they have been in this relationship. We further assessed subjective ratings of premenstrual symptoms (PMS) using the German PMS questionnaire (Ditzen et al., 2011) and the Positive and Negative Affect Scale (PANAS; Watson et al., 1988). In the PANAS, women were asked to rate how their mood was during the last year (trait).

All test sessions took place in a laboratory at the University of Bern. To control for circadian hormone variability, all participants were tested between 11am and 6 pm, and both test sessions were scheduled to take place at the same time of day. This time window was chosen because hormone levels show less variation in the afternoon than in the early morning (cf., Caufriez et al., 2009)

The lab-testings lasted approximately 30 min each. Upon arrival at the laboratory, participants gave their written informed consent and provided the salivary sample. We applied a modified version of the Cyberball task developed by (Williams and Jarvis, 2006). Participants were informed that they would play a virtual ball tossing game via Internet with two other players. Participants were informed that these players were real players seated in separate rooms. In reality, these players were computer generated. Participants were told that the study examines the effects of mental visualization and that their task was to create a vivid mental image of the game scenery (Williams and Jarvis, 2006; see Figure S2 for a screenshot of the Cyberball scenario). Participants viewed pictures of the players presented on the right (Player 1) and the left side of the screen (Player 3). The picture of Player 1 always showed a man, the picture of Player 3 always was a woman. Different pictures were used in the first and second testing, and all pictures were "medium" attractive as rated in a pre-test. The Cyberball paradigm was presented on a tablet computer (Samsung Galaxy Note, 2.0) and was played online using Google Chrome Explorer (Chrome 35). In each session, participant played three rounds. Each round consisted of thirty ball tosses and took about two minutes to complete. When participants received the ball, they had to choose to whom they wanted to throw the ball by clicking on the respective player's picture using a standard computer mouse. An algorithm controlled the behaviour of the computer-generated players. In the first round, the inclusion round, the participant received 10 out of 30 balls played. In the following exclusion round, the participant received only 4 out of 30 balls played. The rest of the balls were played between the computer-generated players. The last round was an inclusion round and was carried out for ethical reasons. After the first inclusion and the subsequent exclusion round participants completed a questionnaire. The first part of this questionnaire was used to support the cover story and consisted of few questions about the participants' mental images during the game. The second part assessed the experience of the Cyberball manipulation. First participants had to estimate the percentage of balls they received during the round. Then participants answered an abbreviated version of the Need-Threat Questionnaire (NTQ, Williams, 2009) to check whether the exclusion manipulation worked. We included only one question per dimension to ensure that any exclusion induced mood change would still be observable in the mood assessment. Specifically, we included the items "I had the feeling that I belonged to the group during the game" (Belongingness), "I had the feeling that I could influence the direction of the game" (Control), "I was concerned about what the other players thought about me during the game" (Self-Esteem), "I had the feeling that my presence during the game was important" (Meaningful Existence). Mood was assessed using the short form of the German multidimensional-mood-state questionnaire (Mehrdimensionaler Befindlichkeitsfragebogen "MDBF"; Steyer et al., 1994). The MDBF assesses mood state on three dimensions (*good-bad, awake-tired, calm-nervous*) and has been shown to be a time-efficient and reliable instrument for assessing mood in clinical and experimental settings (Heinrichs and Nater, 2002). Questionnaire items were presented on a 15.4-inch laptop monitor (HP Pavilion dv6, Windows 7, 64-bit) using internet explorer (V.11) and unipark software (Questback, Berlin, Germany). Both test sessions followed the exact same procedure except that after the second session participants were fully debriefed.

Before debriefing the participants, we checked whether they knew the paradigm or guessed the aim of the study. One participant knew the paradigm and was hence excluded from the analyses (see Flowchart in supplemental online material). The rest were naïve to the purpose of the study and did not know the paradigm. Participants were told not to talk about this study to their friends and colleagues, so that other participants remained naïve.

3. Statistical analyses

We checked the data for distribution properties and verified normality by inspecting histograms and qq-plots. We log-transformed hormone concentration values to approximate normal distribution. For descriptive analyses, we calculated means and standard deviations for continuous normally distributed variables and absolute and relative frequencies for the categorical variable with categories outlined in Table S1.

To confirm that the two Cyberball conditions 'inclusion' *vs.* 'exclusion' induced the expected contrast, we conducted a manipulation check. To do so, we entered each scale of the Need Threat Questionnaire (NTQ) as outcome in separate linear mixed-effects models (Singer and Willett, 2003), to estimate changes in 'belonging', 'self-esteem', 'meaningful existence', and 'control' between the inclusion and exclusion condition by adding 'ostracism' as predictor. We added the factors 'cycle phase' (luteal phase *vs.* late follicular phase) and 'assessment sequence' (luteal phase assessed first vs. late follicular phase assessed first) as covariates.

Next, to test our main hypotheses that ostracism effects (inclusion vs. exclusion) on mood are more pronounced during the luteal phase as compared to the late follicular phase, we entered each scale of the

MDMQ as outcome variable in separate linear mixed-effects models (Singer and Willett, 2003). We first estimated the effects of the factors 'ostracism' (inclusion *vs.* exclusion) and 'cycle phase' (luteal phase *vs.* late follicular phase) by only including these two categorical predictors. We then repeated the analyses after adding the interaction effect of 'ostracism' and 'cycle phase' as additional predictor. In all these analyses, we added the factor 'assessment sequence' (luteal phase assessed first *vs.* late follicular phase assessed first) as covariate.

To scrutinize changes in salivary concentrations of the hormones progesterone, estradiol, and testosterone between cycle phases we entered each hormone concentration as outcome in separate linear mixed-effects models (Singer and Willett, 2003), and estimated changes in the hormone concentrations between the luteal phase and the late follicular phase by including 'cycle phase' as predictor. We added the factor 'assessment sequence' (luteal phase assessed first vs. late follicular phase assessed first) as covariate.

For all linear mixed-effects models we used an unstructured covariance matrix to account for the time dependence among repeated measures of the within-subjects factors 'ostracism' and 'cycle phase'.

Finally, we estimated whether the associations between cycle phase and ostracism related differences in mood were mediated by progesterone concentrations (see Fig. 1). Therefore, for each scale of the MDMQ, we conducted separate multilevel structural equation models (Preacher et al., 2010), with the predictor 'cycle phase', the outcome 'ostracism-related mood differences' (*i.e.* difference in mood between the inclusion and the exclusion condition), and the mediator 'progesterone concentration', assuming random intercepts and fixed slopes.

Linear mixed-effects models and multilevel structural equation models accommodated missing data. All tests were two-tailed, we set the significance level at 0.05 and calculated 95% confidence intervals (CI) where appropriate. We used the statistical software package Mplus for Mac (version 6.12) for the multilevel structural equation models, and IBM SPSS Statistics for Mac (Version 21) for all other data analyses.

4. Results

Analyses are based on data of 49 women (see Table S1 in SI for characteristics of the study sample).

4.1. Manipulation check

We employed the Need Threat Questionnaire (NTQ, Williams, 2009) to check whether our inclusion/exclusion manipulation worked. In the inclusion condition of the Cyberball paradigm, women reported higher levels of 'belonging', 'meaningful existence', and 'control' than in the exclusion condition, confirming successful induction of subjective ostracism by the Cyberball paradigm. There was no statistically significant difference in 'self-esteem'. Respective results are depicted in Table 1.

4.2. Differences in mood related to ostracism and cycle phase

Linear mixed-effects models revealed an ostracism effect for MDMQ mood dimensions 'good-bad' and 'awake-tired', but not 'calm-nervous', with women reporting worse mood (*Estimate* = 0.224, *standard error, SE* = 0.088, 95%CI [0.047, 0.401], $t = 2.544$, $df = 49$, $p = 0.014$) and being more tired (*Estimate* = 0.276, *SE* = 0.071, 95%CI [0.133, 0.418], $t = 3.908$, $df = 49$, $p < 0.001$) in the exclusion as compared to the inclusion condition of the Cyberball paradigm. There was a significant interaction effect between ostracism and cycle phase for good-bad mood (*Estimate* = 0.332, *SE* = 0.147, 95%CI [0.036, 0.628], $t = 2.255$, $df = 49$, $p = 0.029$), indicating a stronger ostracism effect in the luteal phase as compared to the late follicular phase (see Fig. 2, Table 2).

4.3. Differences in hormone concentrations between cycle phases and mediation of the association between cycle phase and mood via progesterone concentrations

Linear mixed-effect models revealed that salivary progesterone and estradiol concentrations, but not testosterone concentrations were higher during the luteal phase as compared to the late follicular phase (see Table 3).

Results from multilevel structural equation models related to the MDMQ scale 'good-bad mood' are depicted in Table 4, and results related to the MDMQ scales 'awake-tired' and 'calm-nervous' are depicted in supplemental material Table S2, Table S3, Figure S3 and Figure S4, respectively. Please refer to Fig. 1 for the outline of the mediation analyses. As expected and in line with the result from the linear mixed-effect model outlined above (see Table 3), progesterone concentrations were higher during the luteal phase than during the follicular phase (mediation path a). Notably, higher progesterone levels were associated with smaller ostracism effects (mediation path b) with regard to the mood dimensions 'good-bad', 'awake-tired', and 'calm-nervous', when adjusting for cycle phase. This led to 'inconsistent mediation' effects (MacKinnon et al., 2007), in the way that in all three models, the indirect/mediated effects and the direct effects were of opposite sign

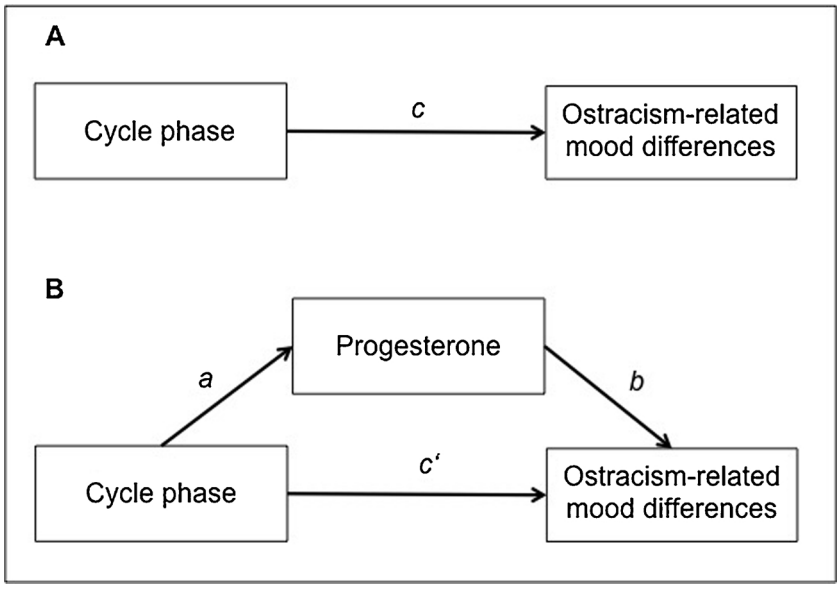

Fig. 1. (A) Illustration of a total effect c of cycle phase on ostracism-related mood differences. (B) Illustration of a mediation design, in which cycle phase is supposed to exert an indirect effect (a*b) on ostracism-related mood differences through progesterone concentrations (a = effect of cycle phase on progesterone concentrations; b = effect of progesterone concentrations on ostracism-related mood differences) and the direct effect of cycle phase on ostracism-related mood differences c' (c' = c – a*b).

Table 1
Results from linear mixed-effect model analyses estimating differences in NTQ scales between inclusion and exclusion condition ($N = 49$).

Comparison: Inclusion vs. exclusion condition						
Outcome: NTQ scale	*Estimate*	*SE*	95%CI [LB, UB]	*t*	*df*	*p*-value
NTQ scale 'belonging'	44.376	3.753	[36.834, 51.919]	11.824	49	< 0.001***
NTQ scale 'self-esteem'	2.538	2.964	[-3.419, 8.496]	0.856	49	0.396
NTQ scale 'meaningful existence'	36.336	3.702	[28.896, 43.775]	9.816	49	< 0.001***
NTQ scale 'control'	34.692	3.581	[27.495, 41.889]	9.687	49	< 0.001***

Notes: Higher estimates indicate stronger ostracism effects; Results adjusted for cycle phase (luteal vs. late follicular) and assessment sequence (luteal phase assessed first *vs.* late follicular phase assessed first) and cycle phase; ***p < 0.001; CI, confidence interval; LB, lower bound; NTQ, need threat questionnaire; SE, standard error; UB, upper bound.

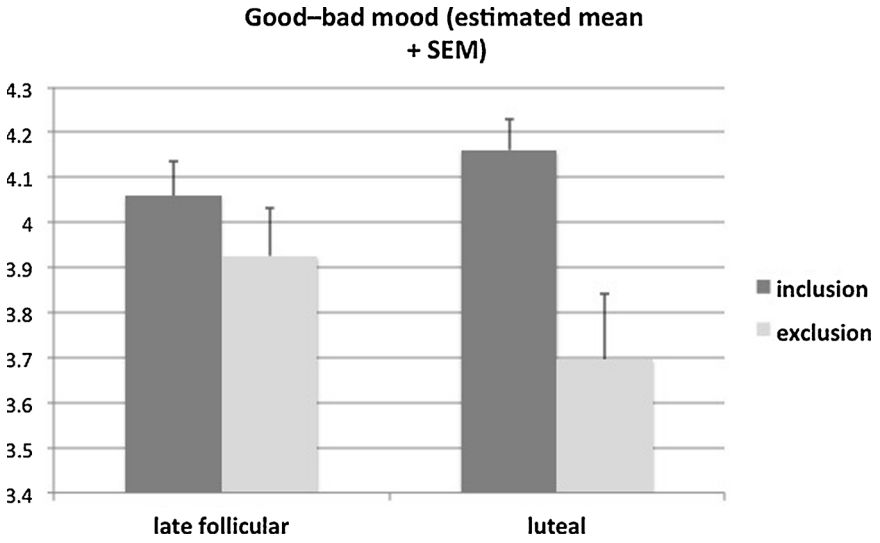

Fig. 2. Good-bad mood assessed using the Multidimensional Mood Questionnaire (MDMQ); SEM, standard error of mean.

Table 2
Results from linear mixed-effect model analyses estimating differences in mood related to ostracism and cycle phase ($N = 49$).

Good–bad mood						
Parameter	*Estimate*	*SE*	95%CI [LB, UB]	*t*	*df*	*p*-value
Ostracism (inclusion vs. exclusion)	0.224[a]	0.088	[0.047, 0.401]	2.544	49	0.014*
Cycle phase (luteal vs. late follicular)	0.065	0.086	[−0.108, 0.237]	0.756	49	0.453
Ostracism (inclusion vs. exclusion) × Cycle phase (luteal vs. late follicular)	0.332	0.147	[0.036, 0.628]	2.255	49	0.029*
Awake–tired mood						
Parameter	*Estimate*	*SE*	95%CI [LB, UB]	*t*	*df*	*p*-value
Ostracism (inclusion vs. exclusion)	0.276[a]	0.071	[0.133, 0.418]	3.908	49	< 0.001***
Cycle phase (luteal vs. late follicular)	−0.189	0.141	[−0.473, 0.095]	−1.340	49	0.186
Ostracism (inclusion vs. exclusion) × Cycle phase (luteal vs. late follicular)	0.020	0.125	[−0.232, 0.273]	0.163	49	0.14
Calm–nervous mood						
Parameter	*Estimate*	*SE*	95%CI [LB, UB]	*t*	*df*	*p*-value
Ostracism (inclusion vs. exclusion)	0.111[a]	0.093	[−0.077, 0.299]	1.192	49	0.239
Cycle phase (luteal vs. late follicular)	−0.098	0.140	[−0.380, 0.185]	0.695	49	0.490
Ostracism (inclusion vs. exclusion) × Cycle phase (luteal vs. late follicular)	0.138	0.144	[−0.153, 0.429]	0.953	49	0.345

Notes: Mood assessed with the Multidimensional Mood Questionnaire (MDMQ); Results adjusted for assessment sequence (luteal phase assessed first vs. late follicular phase assessed first); *p < 0.05; ***p < 0.001; CI, confidence interval; LB, lower bound; SE, standard error; UB, upper bound.

[a] Higher estimates indicate a stronger ostracism effect.

Table 3
Results from linear mixed-effect model analyses, depicting estimated means, and estimated changes in hormone concentrations related to cycle phase ($N = 49$[a]).

	Luteal phase			Late follicular phase			Parameter: Cycle phase (luteal vs. late follicular)					
Parameter	***Mean***	***SE***	**95%CI [LB, UB]**	***Mean***	***SE***	**95%CI [LB, UB]**	***Estimate***	***SE***	**95%CI [LB, UB]**	***t***	***df***	***p-value***
Progesterone	5.369	0.111	[5.146, 5.591]	4.256	0.085	[4.084, 4.428]	1.113	0.138	[0.836, 1.390]	8.084	49.2	< 0.001***
Estradiol	1.567	0.063	[1.441, 1.693]	1.391	0.064	[1.263, 1.520]	0.176	0.085	[0.005, 0.346]	2.070	49	0.044*
Testosterone	2.860	0.127	[2.606, 3.115]	3.029	0.100	[2.827, 3.231]	−0.169	0.094	[−0.358, 0.021]	−1.791	49	0.079

Notes: The outcomes 'hormone concentrations' (progesterone, estradiol, testosterone) have been logarithm transformed, using the formula: outcome = ln (hormone concentration (in pg/ml) + 1); Results adjusted for assessment sequence (luteal phase assessed first vs. late follicular phase assessed first); [a]One subject with missing hormone concentration value during luteal phase; *p < 0.05; ***p < 0.001; CI, confidence interval; LB, lower bound; SE, standard error; UB, upper bound.

Table 4
Results from multilevel structural equation models estimating the mediation of the association between cycle phase and ostracism-related mood changes via progesterone concentrations ($N = 49$).

Outcome: Differences in 'good-bad mood' between the inclusion and the exclusion condition (= ostracism effect[a])					
Parameter	*Estimate*	*SE*	*Standardized estimate*	95%CI [LB, UB]	*p*-value
Association: cycle phase – ostracism effect (total effect, c)	0.354	0.148	2.387	[0.110, 0.598]	0.017*
Association: cycle phase – progesterone (mediation path, a)	1.105	0.139	7.938	[0.876, 1.334]	< 0.001***
Association: progesterone – ostracism effect (mediation path, b)	−0.379	0.119	−3.170	[-0.575, −0.379]	0.002**
Mediation of association: cycle phase – ostracism effect, via progesterone (indirect effect, a*b)	−0.418	0.145	−2.888	[−0.656, −0.180]	0.004**
Association: cycle phase – ostracism effect, accounted for mediation via progesterone (direct effect, c')	0.772	0.225	3.425	[0.401, 1.143]	0.001**

Notes: Mood assessed using the Multidimensional Mood Questionnaire (MDMQ); [a]Higher values indicate a stronger ostracism effect; *p < 0.05, **p < 0.01, ***p < 0.001; CI, confidence interval; LB, lower bound; SE, standard error; UB, upper bound.

(negative vs. positive).

Hence, with regard to 'good-bad mood' there was a statistically significant mediation effect of progesterone concentration (a*b; *Estimate* = − 0.418, *SE* = 0.145, 95%CI [−0.656, − 0.180], $p = 0.004$), resulting in an even stronger direct effect (c'; *Estimate* = 0.772, *SE* = 0.225, 95%CI [0.401, 1.143], $p = 0.001$) than the total effect (c; *Estimate* = 0.354, *SE* = 0.148, 95%CI [0.110, 0.598], $p = 0.017$).

With regard to ostracism-related differences in mood dimensions 'awake-tired' and 'calm-nervous' there were no statistically significant total effects (c) of cycle phase. This is in line with the results from the above-reported linear mixed-effect models (Table 2) indicating no statistically significant interaction between ostracism and cycle phase for the outcomes 'awake-tired' and 'calm-nervous'. However, due to the statistically significant and inconsistent mediation effects of progesterone (a*b), mediation analyses revealed statistically significant direct effects (c') of cycle phase on ostracism-related differences in the dimensions 'awake-tired' (*Estimate* = 0.340, *SE* = 0.170, 95%CI [0.060, 0.619], $p = 0.046$) and 'calm-nervous' (*Estimate* = 0.388, *SE* = 0.187, 95%CI [0.081, 0.695], $p = 0.037$) (see Table S2 and Table S3, respectively).

5. Discussion

The main goals of this study were to estimate the changes in sensitivity to social exclusion (ostracism) in different cycle phases and to scrutinize the related role of fluctuating progesterone concentrations across the menstrual cycle in naturally cycling women. We found that during the more vulnerable luteal phase women were more sensitive to rejection than during the late follicular phase. At the same time sensitivity to rejection was associated with lower progesterone levels. This finding suggests that higher progesterone concentrations buffer against feelings of rejection.

Women in the luteal phase are potentially pregnant. Because pregnancy calls for increased need for social support, an evolutionary informed interpretation of these findings is that social exclusion may represent a higher threat during the luteal phase and may therefore result in more negative mood than during the follicular phase. In this adaptationist framework, progesterone, which coincidentally is raised during the luteal phase, may function as a resilience factor buffering against the negative feelings experienced after being ostracized.

During the luteal phase, naturally cycling women often experience a drop in mood (Dickerson et al., 2003). While most premenopausal women experience some level of negative premenstrual symptoms (Dickerson et al., 2003), up to 8% suffer to such a degree that it interferes with normal functioning (Premenstrual Dysphoric Disorder, PMDD; Bhatia and Bhatia, 2002; Wittchen et al., 2002). Negative mood is one of the most prominent symptoms of PMDD and such dips in mood have been related to progesterone levels which increase during the luteal phase and decrease rapidly at the onset of menses (*e.g.*, Smith et al., 2006). It is striking that the social components of PMDD have received much less attention, despite the fact that the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) explicitly mentions marked affective lability such as increased sensitivity to rejection as well as increased interpersonal conflicts as symptoms of PMDD. From a clinical and research domain criteria (RDoC) perspective, our findings point towards reduced progesterone levels and/or related neurotransmission as a potential target to buffer against suffering related to the symptom dimension 'sensitivity to social rejection' that is of relevance for PMDD and beyond. This converges with recent data suggesting that neurotransmission related to allopregnanolone, a metabolite from progesterone, is a promising target to treat PMDD (Bixo et al., 2017; Martinez et al., 2016). Unfortunately, as our sample includes only 14 women with impairing premenstrual symptoms, it is too small to conduct meaningful statistical analyses in this subgroup. Hence, future studies with a clinical sample should scrutinize, whether our main findings can also be detected in women with PMDD.

The present results substantiate findings of previous work in which social exclusion in Cyberball resulted in more negative mood (*e.g.*, Seidel et al., 2013; Williams and Jarvis, 2006). Furthermore, the results are in line with previous findings suggesting an increased sensitivity for social information during the luteal phase (Maner and Miller, 2014). But while existing literature often argues that this increased sensitivity results from the heightened progesterone concentrations during the luteal phase, we found that progesterone was related to reduced negative mood after experiencing social exclusion. Specifically, in the luteal phase, high progesterone values were associated with less negative mood ratings after experiencing social exclusion. In the late follicular phase we found no relation between progesterone concentrations and mood ratings. Even though we found that during the luteal phase, which is characterized by increased progesterone levels, women reacted more sensitively to social exclusion, our data do not support the view that elevated progesterone levels are responsible for this increased sensitivity to ostracism. Rather, the present findings support the biphasic action model of progesterone metabolites on mood (Andreen et al., 2009). According to this model low concentrations of allopregnanolone increase negative mood changes via GABAa systems, while high concentrations have calming effects. Further studies are needed to verify the role of luteal progesterone in social behaviour.

In the present study, the order of inclusion/exclusion blocks in the Cyberball game was held constant, that is, participants always experienced inclusion before being excluded (see also Masten et al., 2011; Radke et al., 2018). We hence cannot fully rule out that the worse mood and increased tiredness after exclusion vs. inclusion might be confounded with the temporal course of the experiment. We note however, that our most important finding was that worse mood after exclusion was modulated by cycle phase. It is hence unlikely that using this commonly adopted sequence of inclusion followed by exclusion in a constant order detracts from our main finding, namely that worse mood related to the exclusion condition was more pronounced during the luteal phase, compared to the follicular phase. We note that we did not assess mood after the last round (inclusion condition), since our main

outcome variable was mood changes after exclusion. The last round (inclusion condition) was merely included for ethical reasons: Because we could not debrief the participants after the first session, we needed to let them finish the session with the feeling of being included.

The need to belong is a fundamental human motive and feeling left out causes distress. Here we provide evidence that naturally cycling women are more sensitive to social exclusion during the luteal phase as compared to the late follicular phase. Further, high progesterone levels – which characterize the luteal phase – were negatively related to feelings of being left out. During the luteal phase women often experience a dip in mood, which in some cases can lead to Premenstrual Dysphoric Disorder (PMDD). Our findings provide evidence that progesterone acts as resilience factor, buffering against negative feelings that result from being socially excluded, thereby pointing to new potential pharmacological targets to treat PMDD and other premenstrual symptoms.

Author contribution

JSL, FP, VL and GM designed the study, VL, FP and JSL collected the data, AHM and GM analysed the data, JSL, VL, AHM and GM wrote the manuscript. All authors approved the final version of the manuscript.

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Declaration of interest

GM has been acting as consultant for Janssen Research & Development, LLC. JSL, FP, VL and AHM declare that they have no potential conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.05.019.

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