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Identification of a narrow post-ovulatory window of vulnerability to distressing involuntary memories in healthy women

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ABSTRACT

Psychological disorders characterised by intrusive memories are more prevalent in women than men. The biological, social and cognitive processes underlying this gender-difference have yet to be fully elucidated. Some evidence suggests that (fluctuations in) ovarian hormone levels are responsible for altered sensitivity to emotional stimuli during certain phases in the menstrual-cycle and this may form the basis of a specific vulnerability to psychological disorders in women. The post-ovulatory (luteal) phase has been identified as a period of particular vulnerability to the development of Post-Traumatic Stress Disorder (PTSD).

Using an experimental model of PTSD, we examine whether differences are detectable between discrete phases in the menstrual-cycle in the experience of intrusive memories. Women (18–35 years-old) in one of three tightly-defined periods within the menstrual cycle – mid-follicular ($n = 15$), early-luteal ($n = 15$) and late-luteal ($n = 11$) – provided saliva samples for ovarian-hormone assay and watched a distressing film. Subsequent intrusive memories, assessed using a daily online-diary, occurred significantly more frequently in the early-luteal group compared to mid-follicular and late-luteal groups. Intrusion frequency was negatively correlated with the estradiol-to-progesterone ratio, but not estradiol or progesterone alone, suggesting that the interactive effect of low estradiol and high progesterone at encoding contributes to the observed effect. Our results support the need for further research in a clinical context with naturally-cycling women who experience a traumatic event, since assessment of days-since-last-menses and ovarian hormone levels may help to identify those at greatest risk of developing re-experiencing symptoms akin to those seen in psychological disorder such as depression and PTSD.

1. Introduction

Recurrent, involuntary negative thoughts and images are a common feature of many psychiatric disorders (Brewin, Gregory, Lipton, & Burgess, 2010; Reynolds & Brewin, 1998). Anxiety and mood disorders characterised by such intrusive memories are significantly more prevalent among women than men (e.g. Breslau, Davis, Peterson, & Schultz, 1997; Kendler, Thornton, & Prescott, 2001; McLean & Anderson, 2009). A number of psychosocial and biological factors have been implicated in this gender imbalance (McLean & Anderson, 2009).

The observation that prevalence of psychological disorders fluctuates across the life span in women (e.g. Ditlevsen & Elklit, 2010) may hold some clues about the nature of biological risk factors. In particular, periods of vulnerability coincide with significant changes in ovarian hormone levels, for example during and after pregnancy as well as menopause. More commonly, vulnerability to psychological symptoms follows a cyclical pattern which is temporally tied to fluctuations in progesterone and estradiol (e.g. Nillni, Toufexis, & Rohan, 2011).

Recent studies suggest that menstrual phase may influence the encoding and/or retrieval of negative emotional events via data-driven, sensory representations which are encoded at the expense of contextualised episodic memories (Bryant et al., 2011; Ferree & Cahill, 2009 Ferree, Kamat, & Cahill, 2011). The typical menstrual cycle in humans ranges from between 25 and 35 days (see Nillni et al., 2011). In the modal example of a 28 day cycle, day 14 marks ovulation, an event preceded by the follicular-, and followed by the luteal-phase. Many healthy women experience predictable fluctuations in physical sensation and mood across their menstrual cycle (Clayton, 2008). In particular, the late luteal phase (i.e. the week prior to menstruation) is commonly linked to increased likelihood of mood swings, sleep disturbances, anxiety and depressive symptoms (Steiner, Peer, Macdougall, & Haskett, 2011) with approximately 80% of healthy women regularly experiencing at least one premenstrual symptom (Wittchen, Becker, Lieb, & Krause, 2002). Taken as a whole, the luteal phase is also associated with increased physiological responsivity to psychological stressors in healthy women (Kirshbaum, Kudielka, Gaab, Schommer, & Hellhammer,

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1999) as well as exacerbation of symptoms in women with depression (Kornstein et al., 2005) and social anxiety disorder (van Veen, Jonker, van Vliet, & Zitman, 2009).

The cycling nature of psychological and physical symptoms is closely linked to variations in ovarian hormones, particularly progesterone (along with its neuroactive metabolite, allopregnanolone) and estradiol. These hormones have been implicated in the amelioration (e.g. Wirth, 2011), as well as exacerbation (e.g. Bäckström et al., 2011) of stress responses. The nature of the dependence of psychological symptoms on ovarian hormone levels therefore remains unclear. An elegant way to examine this dependence is by examining the effects of a stressful life event at different phases of the menstrual cycle, while ovarian hormone levels vary naturalistically.

Recent research on the effect of cycle phase on involuntary memories suggests that women experiencing a stressful or traumatic event during the luteal phase have reported a greater number of negative intrusive thoughts than those who have such experiences in the follicular phase (Bryant et al., 2011; Ferree & Cahill, 2009; Ferree et al., 2011). These findings have significant clinical implications because they suggest the presence of a temporal 'window of vulnerability' that may be targeted in efforts to prevent the onset of psychological disorders (e.g. PTSD) in women. The idea of a window of vulnerability representing a 'window of opportunity' for prevention or treatment is a familiar one to PTSD researchers. For example, Holmes and colleagues (Holmes, James, Kilford, & Deeprose, 2010) showed that participants viewing distressing film footage who engaged in a visuospatial task within a critical timeframe (30 min–4 h) during which memory consolidation occurs, were inoculated against the formation (or recall) of unpleasant involuntary memories of the film. Clearly the entire luteal phase represents a substantial period of vulnerability (14 days). It is therefore of interest to determine whether there are specific periods of especially elevated risk for intrusive memories within the luteal phase. Furthermore, it is important to use an experimental protocol that allows the identification of intrusive memories that have characteristics relevant to psychopathology. For example, intrusive memories in PTSD depression and social anxiety are often characterised by *sensory* phenomena (i.e. as predominantly mental *images*) rather than verbal processing. These phenomena should be assessed – as far as possible – in the absence of cuing by other (explicit) memory tasks (Brewin et al., 2010).

Here we used the stressful film paradigm (Holmes & Bourne, 2008) to compare involuntary recollection of distressing film footage over a 72 h period in three separate groups, each consisting of participants in a discrete phase of their menstrual cycle: mid follicular, early luteal and late luteal. No previous clinical or experimental study has examined the effect of a stressful life event (or simulation of such an event) on the expression of psychological symptoms in two distinct *and short* epochs within the luteal phase (compared to a short period in the follicular phase).

Whilst previous studies have compared memory effects in follicular and luteal phases as a whole, predictable fluctuations in ovarian hormone levels during the luteal phase suggest that it can be characterised as having an early and late phase separated by the 'progesterone peak.' (e.g. Gandara, Leresche, & Mancl, 2007). For example, while progesterone levels are low in the mid follicular phase, they rise and fall (at different rates) in the early and late luteal phases respectively. On the other hand, estradiol levels are expected on average, to be similar across these three cycle phases over the specific periods of assessment chosen for this study (see below). Since these distinct patterns of hormone activity may give rise to differential memory effects, we also examined the relationship between intrusion frequency and salivary estradiol and progesterone levels separately, as well as their interaction in the form of the estradiol–progesterone (E:P) ratio. We distinguished between sensory and verbal intrusions and assessed intrusion frequency in the absence of any additional (prior) memory test related to the distressing film in order to avoid biasing of intrusion estimates. These methodological considerations are refinements of the methods used by Ferree et al. (2011) who showed a greater number of 'spontaneous intrusive recollections' over the course of the entire luteal period compared to the follicular. A more temporally fine-grained understanding of the luteal phase in onset or maintenance of key psychological symptoms may help to efficiently target preventative interventions towards individuals most vulnerable to psychological disorder.

2. Method

2.1. Participants and design

The study was advertised on a university internet site as an investigation of emotional information processing. Participants who responded to the advert underwent screening via an internet survey, to ensure they met inclusion criteria. These were to have fluency in English, predictable menstrual cycle length of between 26 and 34 days (Nillni et al., 2011), and daily access to a mobile phone and internet. Exclusion criteria were use of hormonal contraception within the past three months, history of mental health difficulty requiring treatment (psychologically/pharmacologically), and phobia of blood, injury or injection. Before participating, all participants were informed of the distressing nature of the film and were told that graphical scenes may be remembered involuntarily after the experiment. The study was approved by the University College London/University College London Hospital Ethics Committee. At the end of the study, all participants received £20 in compensation for their time. Forty-five participants aged between18 and 35 ($M = 23.34$; $SD = 3.86$) completed the study. An independent group design was used where participants were tested during one of three discrete periods in the menstrual cycle (mid follicular, early luteal or late luteal). Cycle length was taken into account when determining menstrual phase, therefore all participants were asked to measure one full cycle (i.e. number of days between menses in two consecutive cycles) prior to taking part in the study. For a 28-day cycle, the three periods were as follows: mid follicular: days 7–11 (25–39% of way through cycle), early luteal: days 16–20 (57–71% of cycle) and late luteal: days 24–28 (86–100% of cycle). For cycle lengths longer or shorter than 28 days, cycle phase was adjusted proportionately (e.g. in a 25-day cycle the early luteal phase (57–71% of cycle) would be days 14–17). Overall, the average mid follicular test day (±SEM) was: day 9 ± 0.45; early luteal: day 17 ± 0.34; late luteal: day 24 ± 0.47.

Three participants were excluded (all in the late luteal group): two due to diary non-compliance and one with outlying intrusion values (>2 SDs). Final analysis involved data from 42 women, with $n = 11$ in the late luteal phase, and $n = 15$ in both mid follicular (36.5%) and early luteal groups. Participants attended the laboratory for two sessions separated by one week.

Upon arrival at session 1, participants read the relevant information sheet and provided written informed consent. After providing a saliva sample, an unrelated task involving facial affect recognition was completed. Standardised questionnaires were then administered to determine level of equivalence between groups on psychological variables, which are either associated with risk of development of PTSD symptoms (trait anxiety) or are relevant to physical and psychological premenstrual symptoms (ASI; PMTS-VAS; see Section 2.3 below). A number of additional visual analogue scale (VAS) measures of current emotional states (see Section 2.3) were taken immediately before and after participants watched the stressful film.

After the film each participant was given a full explanation of the nature of intrusive thoughts and images (see Section 2.3) as well as verbal and written instructions detailing how they should complete the diary each evening. They were told that over the next few days they may experience intrusive memories about the film and that they should record these on the online diary or state if they experienced no intrusions. Participants were sent an SMS message at 6 pm each day to remind them to complete the diary that evening. When they returned for the second session, they completed a diary compliance rating, were debriefed and received payment.

2.2. Stressful film

A fourteen minute film containing real life film footage of five separate scenes of similar length involving death, mutilation and severe injury, was displayed on a 15 in. computer monitor. Participants were asked to attend to the scenes as if observing the events as an actual witness. They were also asked to attend to their own emotional responses. Each scene was preceded by a brief commentary, which provided context to the scene and people involved. Before watching the film participants were reminded that they could terminate the study at any point and were encouraged to contact the experimenter if they felt distressed after the study.

2.3. Measures

Intrusive memories. In an effort to simultaneously ensure compliance and accuracy of recording of intrusions participants were asked to use an online diary (Bisby, King, Brewin, Burgess, & Curran, 2010) to record the number and content of any spontaneous intrusions after watching the stressful film. For each recorded intrusion, participants were also instructed to rate vividness, distress and extent to which the scene was 're-experienced' (all rated on a 0–100 scale). Furthermore, they differentiated whether the intrusion was a verbal thought, visual image, or a combination of the two. Participants were asked to set a regular time each evening to complete the diary.

Intrusions experienced in the 72 h after viewing the film were examined. This time window was chosen specifically to increase the likelihood that retrieval was occurring in a similar hormonal milieu to that prevailing during encoding (memory encoding and recording occurred within same period as defined in Section 2.1, for the mid follicular, early luteal and late luteal phases).

Diary Compliance. Participants indicated how accurately they completed the diary from 1 (*not at all accurately*) to 10 (*extremely accurately*).

Trait anxiety. The trait version of the State-Trait Anxiety Inventory (STAI-T; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) was used to assess trait anxiety.

Anxiety Sensitivity. The 16-item Anxiety Sensitivity Index (ASI; Reiss, Peterson, Gursky, & McNally, 1986) indexed the degree to which participants feared the potential negative consequences of anxiety-related psychological (e.g. *'it is important for me not to appear nervous'*) and physiological (e.g. *'it scares me when my heart beats rapidly'*) situations.

State mood ratings. Immediately before and after viewing the stressful film, participants completed visual analogue scales (VASs) indicating levels of discrete emotions: disgusted, frightened, angry, sad and happy (ranging from 1 = *not at all* to 10 = *extremely*). These scores reflected how participants were feeling *currently* and aimed to measure state changes in mood pre- to post-film.

Premenstrual symptoms. A visual analogue premenstrual tension rating scale (PMTS–VAS; Steiner et al., 2011) measured premenstrual symptom severity. This scale has been shown to be a sensitive measure of symptoms, including negative mood, restlessness, anxiety, insomnia, hypersomnia, irritability, eating habits and other physical symptoms.

Salivary levels of estradiol and progesterone. Samples were collected in polypropylene cryovials (Salicaps) approved for use with steroid hormone analysis as they reduced the likelihood of contaminants (IBL International, Germany). Participants were told not to eat or drink anything for at least thirty minutes prior to taking part in the study; however they were able to drink water up to 10 min before saliva was collected. Participants were instructed to supply by 'passive drool' at least 1 ml of saliva (excluding foam/bubbles). All saliva samples were immediately frozen at −80° after collection and later shipped on dry ice for analysis (University of Dresden, Germany). Hormone levels were assessed using Salimetrics luminescence immunoassay kits. Salivary analysis of estradiol and progesterone has previously been shown to provide a non-invasive, reliable assessment of menstrual cycle profile (Gandara et al., 2007; Gröschl, 2008).

2.4. Data analysis

Using cycle phase as the between-subjects factor, one-way ANOVAs were used to compare group means of demographics, intrusion data, trait mood variables, premenstrual symptom severity and salivary hormone levels. Tukey's tests were used for post hoc comparisons. Where equal variances could not be assumed, a Welch's F statistic is reported (with adjusted degrees of freedom) and Games–Howell tests are used for post hoc comparisons. ANOVA degrees of freedom (total group numbers) varied slightly across tests where participants missed out questionnaire items or only partially completed the online diary.

Repeated measures ANOVAs were used to assess main effects of state VAS mood scores (before and after watching a stressful film) and also interactions between these mood scores and cycle phase. Relationships between salivary estradiol and progesterone levels and intrusion frequency were examined using multiple regression (predictors were mean centred to avoid collinearity).

All analyses were performed with IBM Statistic Package for Social Sciences (version 19).

3. Results

3.1. Demographics

There were no significant differences in the age [$F(2,38) = 0.80$, $p = 0.46$] or years in education [χ^2 (6, $N = 41$) = 3.81, $p = 0.70$] of participants across cycle phases.

3.2. Salivary hormone levels

All participants had salivary hormonal levels within expected values for self-reported cycle phase (Gandara et al., 2007). Salivary progesterone significantly differed across groups (see Table 1) [$F(2,37) = 3.25$, $p = 0.05$]. Planned comparisons revealed that progesterone levels were higher in the luteal groups compared to the mid follicular phase [$t(37) = -3.21$, $p = 0.004$], but there were no significant differences between the early *versus* late luteal phases [$t(37) = 1.15$, $p = 0.15$]. Estradiol levels were higher in the early luteal group compared to other cycle phases, but this did not reach statistical significance [$F(2,37) = 0.15$, $p = 0.86$].

3.3. Mood and premenstrual symptoms

Analysis of VAS scores (pre- and post-film) showed main effects of time across all state variables, reflecting mood deterioration after watching the stressful film, however there were no

Table 1
Participants' mean (± standard deviation) salivary progesterone and estradiol levels across the three phases of menstrual cycle on session 1 (i.e. at encoding).[a]

	Mid-follicular (n = 15)	Early luteal (n = 15)	Late luteal (n = 11)
Progesterone (pg/ml)	47.25 ± 48.93	125.95 ± 124.24	200.18 ± 183.88
Estradiol (pg/ml)	5.63 ± 2.02	6.11 ± 2.57	5.86 ± 2.52

[a] The expected range of progesterone concentrations for the follicular phase (as a whole) is 28–82 pg/mL and for the luteal phase (as a whole), 127–446 pg/mL. For estrogen, expected concentrations for the follicular phase (as a whole) are 0.8–7.7 pg/mL and for the luteal phase (as a whole) 3.4–14.3 pg/mL.

Table 2
Subjective mood state VAS scores (1–10 scale; higher values indicate more intense emotion) for disgust, fear, anger, sadness, happiness by cycle phase. Means(± standard deviation) are indicated. The right-sided columns indicate outcomes of ANOVAs.

	Mid follicular (n = 15)		Early luteal (n = 15)		Late luteal (n = 11)		ANOVA (Main effect of state mood score)	ANOVA (State mood scores × cycle phase)
	Pre	Post	Pre	Post	Pre	Post		
Disgust	0.79 ± 1.63	4.79 ± 3.12	1.43 ± 1.99	6.07 ± 1.98	1.27 ± 1.95	6.27 ± 2.00	[$F(1,36) = 83.03$, $p < 0.001$]	[$F(2,36) = 0.34$, $p > 0.05$]
Frightened	0.50 ± 0.76	4.29 ± 2.81	0.93 ± 1.14	4.57 ± 2.68	1.27 ± 2.10	4.55 ± 2.46	[$F(1,36) = 42.22$, $p < 0.001$]	[$F(2,36) = 0.07$, $p > 0.05$]
Anger	0.36 ± 0.63	3.71 ± 2.73	0.71 ± 0.99	2.71 ± 2.30	0.45 ± 0.93	3.45 ± 3.21	[$F(1,36) = 33.91$, $p < 0.001$]	[$F(2,36) = 0.78$, $p > 0.05$]
Sadness	0.50 ± 0.86	6.64 ± 2.59	1.43 ± 1.79	5.43 ± 2.38	1.09 ± 1.87	6.45 ± 2.66	[$F(1,36) = 112.81$, $p < 0.001$]	[$F(2,36) = 1.80$, $p > 0.05$]
Happiness	5.64 ± 2.95	2.43 ± 2.07	4.86 ± 1.79	2.64 ± 2.02	6.09 ± 2.88	3.00 ± 2.15	[$F(1,36) = 49.54$, $p < 0.001$]	[$F(2,36) = 0.65$, $p > 0.05$]

interactions between mood scores and cycle phase (see Table 2). Premenstrual symptoms as assessed using the PMTS–VAS scores were not related to cycle phase [$F(2,38) = 0.22$, $p = 0.80$]. STAI scores were not related to cycle phase [$F(2,38) = 2.38$, $p = 0.12$] and neither were ASI scores [$F(2,37) = 0.56$, $p = 0.58$].

3.4. Intrusions and cycle phase

There was a significant effect of cycle phase on the total number of intrusions, across intrusion types (i.e. the combination of visual images, verbal thoughts and mixed image/verbal thought) [Welch's $F(2,24) = 6.04$, $p = 0.01$]. Post-hoc tests revealed that women in the early luteal group (n = 15) reported significantly more intrusions than those in the mid follicular phase (n = 15; $p = 0.01$) and late luteal phase (n = 11; $p = 0.008$) (Fig. 1). However, there was no statistical difference between intrusions reported in mid follicular and late luteal groups ($p > 0.1$).

Given this, we examined the different intrusion types (i.e. thought, visual image, or mixed thought/image) separately in exploratory analysis and found that while participants reported more intrusions in the early luteal phase compared to the other two phases across intrusion types, only images varied significantly across cycle phase [$F(2,37) = 6.86$, $p = 0.003$]. Those in the early luteal (n = 14) group reported significantly more images than those in mid follicular (n = 15, $p = 0.01$) or late luteal (n = 11, $p = 0.01$) groups. There were no significant differences in the number of verbal thoughts [Welch's $F(2,23) = 2.38$, $p = 0.12$] or mixed thoughts/images [$F(2,37) = 1.43$, $p = 0.25$] across cycle phase.

Although subjective ratings of vividness, distress and feelings of re-experiencing intrusions were higher in the early luteal phase compared to other stages of the cycle, these differences did not reach significance (see Table 3).

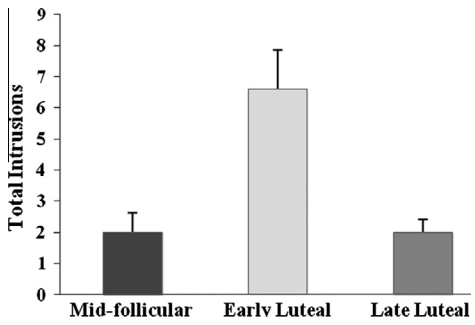

Fig. 1. Relationship between cycle phase and total intrusions over the course of three days post-stressful film. Mean values (± SEM) are indicated for total intrusions (all intrusion types: intrusive images, thoughts and mixed intrusive phenomena).

Table 3
Means of intrusion subjective ratings (± SEM) by cycle phase.

	Mid follicular (n = 15)	Early luteal (n = 14)	Late luteal (n = 11)
Distress	28.41 (±6.50)	46.41 (±5.72)	37.04 (±9.47)
Vividness	31.00 (±6.91)	50.17 (±6.80)	35.23 (±7.37)
Re-experience	26.64 (±7.56)	43.74 (±6.72)	30.91 (±9.01)

3.5. Intrusion frequency and ovarian hormones

Intrusion frequency and estradiol–progesterone ratio were significantly associated (see Fig. 2). Correlations between intrusions and both estradiol and progesterone were non-significant ($p > 0.1$). In a multiple regression model including estradiol, progesterone and the E:P interaction term, the coefficient was significant only for the E:P term ($\beta = -28.26$, $p = 0.015$). The overall model did not reach statistical significance [$R^2 = 0.16$, $F(3,36) = 2.26$, $p = 0.098$].

4. Discussion

This is the first study to demonstrate a discrete period of psychological vulnerability that is temporally linked to a well defined cyclical biological event (ovulation), which may contribute to the development of intrusive memories following a stressful experience. The finding of enhanced intrusion frequency in the early luteal phase was particularly striking for sensory, image-based intrusions, although verbal and mixed phenomena showed a similar (though non-significant) pattern. Additionally, a significant negative correlation between intrusion frequency and E:P ratio was demonstrated. While intrusive images are considered to be prototypical of PTSD, they are in fact a transdiagnostic symptom of psychological disorders (Brewin et al., 2010); our results may therefore inform further research into the general prevention and treatment of psychological disorders in women who experience a stressful life event in the early luteal phase of the menstrual cycle. Although we used a between-groups design, these findings suggest pronounced cyclical *intra-individual* variation in vulnerability to intrusive thoughts over an unusually short time scale.

These effects on intrusions were found in the absence of differences between groups in subjective mood, physical symptoms (assessed using the PMTS–VAS) or changes in emotion ratings as

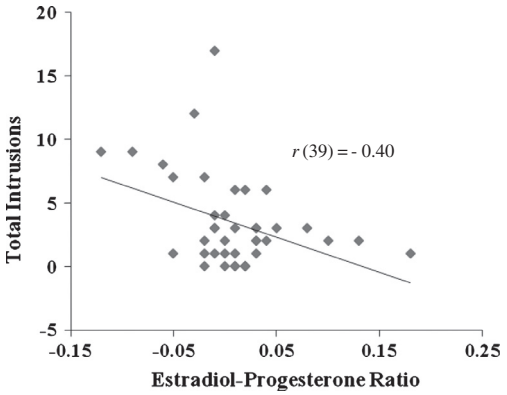

Fig. 2. The relationship between total intrusions (all intrusion types) showing a significant negative correlation between intrusion frequency and centred estradiol–progesterone ratio ($p = 0.006$).

a result of viewing the film. The film produced marked subjective effects of decreasing happiness ratings and increasing ratings of disgust, fear, anger and sadness however these changes were seen across all three groups.

Our results add to existing literature demonstrating a relationship between ovarian hormones and emotional memory. They highlight the importance of the interactive effects of estradiol and progesterone, which is particularly relevant considering their seemingly opposite effects. For instance, low levels of estrogen are associated with reduced extinction of fear responding (Glover et al., 2012; Graham & Milad, 2013) whereas the higher levels of progesterone appear to be associated with enhanced emotional memory (Adreano, Ariomandi, & Cahill, 2008; Ferree et al., 2011). Furthermore, both of these ovarian hormones interact with the hypothalamic–pituitary–adrenal (HPA) axis to modulate the stress response (Kudielka & Kirschbaum, 2005) and brain areas involved in emotional processing, such as the amygdala and hippocampus (Brinton et al., 2008; Lebron-Milad & Milad, 2012; van Wingen et al., 2008; Österlund & Herd, 2001).

Only one previous experimental study has examined the role of ovarian hormones and menstrual phase on putative intrusive memories (Ferree et al., 2011). Our findings are consistent with those of Ferree et al. (2011): these authors also found higher levels of 'spontaneous intrusive recollections' in the luteal phase, although they investigated the effects of the entire luteal phase, comparing it to the entire follicular phase. This may account for the small effect reported in that study (mean number of intrusion over 48 h: 1.29 (follicular phase) *versus* 1.87 (luteal phase)). Thus our results complement and extend those of Ferree et al. (2011) by delineating a specific vulnerable period within the luteal phase during which the risk of formation or expression of intrusive memories is especially elevated. In contrast to Ferree et al. (2011), we used an established experimental procedure for recording intrusions[1] and importantly, recorded these independently of other memory assessments[2]. As such we can be more confident that we were indeed measuring intrusive memories, that is: involuntary (non-effortful), spontaneous memory events. Furthermore, we assessed a range of potentially spontaneous memory phenomena (images, verbal thoughts and mixture of both). The fact that the cycle phase dependent effect was most obvious for images (rather than verbal intrusions) again strengthens our claim that we were assessing sensory representations (which most often characterise intrusive memories) relevant for maintenance of psychopathology.

[1] This procedure has been validated against the prevailing models of memory dysfunction in PTSD (e.g. see Bisby et al., 2010; Holmes, Brewin, & Hennersey, 2004).

[2] Ferree et al. (2011) recorded intrusions after a declarative memory task.

Our findings are also consistent with those reported in a cross sectional study examining symptoms of PTSD in women admitted to hospital after traumatic injury (Bryant et al., 2011). However the sample in that previous study included women who were taking hormone-based contraceptives. Given that estradiol levels may play a significant role in the formation of fear memories (Glover et al., 2012; Graham & Milad, 2013) artificial modulation of estrogen through use of hormone-based contraceptives may have affected the occurrence of intrusive memories in Bryant et al.'s (2011) study. Nevertheless, those findings are important as they suggest our experimental findings on cycle phase and intrusive memories may extend to clinical populations.

Influential models of psychopathology tend to evoke 'dual representation' of emotional information in memory (e.g. Dalgleish, 2004). Such information is proposed to be stored as sensory representations – which when activated by internal or external cues – result in intrusive memories, and also as contextualized information, which forms the basis of deliberately recalled memories (declarative memories). These two memory representations are likely to have different neuroanatomical substrates (Brewin et al., 2010; Dalgleish, 2004) and may be subject to differing influences of ovarian hormones and neurosteroids. For example, recall of declarative, contextualised memory may depend on progesterone levels at encoding (Adreano et al., 2008) while our study suggests that this relationship does not hold for spontaneous recollections of sensory representations. Alternatively, other research suggests that estradiol levels (and not progesterone) correlate with performance on sensory/perceptually-based memories, at least when tested in the mid luteal phase (Maki, Rich, & Rosenbaum, 2002).

Most research examining the effects of menstrual phase or hormone modulation on emotional memory has focused on contextualised, declarative representations (e.g. Adreano et al., 2008; Nielsen, Ertman, Lakhani, & Cahill, 2011). However, in contrast to *intrusive* emotional memories (which are involuntary, repetitive, lacking contextual detail, rich in sensory detail and distressing); contextualised, deliberately recalled memories of distressing events may be less a symptom of psychopathology after a stressful life event, than a potential antidote to it (Brewin et al., 2010). Future research examining the effects of menstrual phase (and associated hormonal changes) on intrusive memories should therefore more clearly describe the phenomenological characteristics of these intrusions by distinguishing between intrusive *verbal thoughts versus intrusive images* (*c.f.* Ferree et al., 2011).

Turning briefly to clinical relevance: the number of intrusions over the memory recording period and the associated distress may seem relatively low. However, it should be noted that the diagnostic criteria for PTSD and other psychological disorders do not specify number of intrusive thoughts as relevant to diagnosis, presumably because a single intrusion (especially in the form of a flashback) can be associated with high levels of distress and impairment. Furthermore, intrusive thoughts (meaning negatively valenced, repetitive and unwanted thoughts) do not have to be highly vivid or distressing to be associated with psychopathology (Watkins, 2008).

Limitations of the current study are acknowledged. Most obviously our results relate only to pre-menopausal women who do not use ovarian-hormone-based contraception. Our participants were in a relatively narrow young age bracket limiting the generalisability of our findings. Ageing (in men and women) is associated with significant endocrine changes (e.g. Harman, Metter, Tobin, Pearson, & Blackman, 2001) and hormonal regulation of cognition (Craig & Murphy, 2007). The effects of ageing (especially when marked by hormonal changes following menopause) on vulnerability to intrusive thoughts has yet to be examined. This is an important research area, especially considering that the greatest prevalence of PTSD in women is in the early 1950s (Ditlevsen & Elklit, 2010).

Our assessment of mood variables was limited to the laboratory session where participants viewed the distressing film. While the groups did not differ on any of these baseline measures on this session (i.e. the groups were well matched at encoding) it is possible that groups diverged in mood symptoms over the 72 h during which intrusions were recorded. In addition, assays of salivary estradiol and progesterone were only taken once (before the film). A second assay (after 72 h) would have allowed us to examine the effect of rising/falling levels of these hormones across the testing period.

While the stressful video paradigm is widely used in experimental psychopathology research (Holmes & Bourne, 2008), it relies on retrospective recall of the occurrence of intrusions, which is subject to error. In relation to the current findings, we cannot rule out the possibility that the metacognitive abilities underlying the retrospective recall of memory events do not also vary as a function of menstrual phase, and that such changes may underlie the observed effect on intrusions. Development of technologies to assist in the (near-) real-time recording of intrusive memories would significantly enhance accuracy of recording of intrusive memories, although such technologies would need to be minimally intrusive to avoid cueing of memories. Another potential criticism of stressful video paradigm as used here, was the detailed instructions participants received to help them distinguish between intrusive memories (involuntary; cued by signals below the threshold for awareness) from other (voluntary) memory events. This may have artificially increased the salience of these events or resulted in an over-reporting bias across groups.

Finally, due to relatively small group samples, our study may have been underpowered to detect some effects. For example, the difference between cycle-phase groups on verbal intrusions was not significant, although the pattern of results was the same as for intrusive images. The absence of an effect must therefore be treated with caution and further work on potential differences of menstrual cycle phase on verbal *versus* image-based/sensory processes is merited (see Hagenaars, Brewin, van Minnen, Holmes, & Hoogduin, 2010).

Overall, the present findings suggest that there is a specific, short period in the menstrual cycle, where susceptibility to developing intrusive memories appears to be enhanced after experience of a distressing event. Interactive effects of ovarian hormones may explain this finding. From a clinical perspective, this could translate to a specific temporal vulnerability to developing symptoms that have the hallmark of PTSD: namely, re-experiencing symptoms. Accordingly, extension of these findings to a clinical population is an important next step.

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