Neurobiology of Learning and Memory 116 (2014) 145–154

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Low levels of estradiol are associated with elevated conditioned responding during fear extinction and with intrusive memories in daily life

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ARTICLE INFO

Article history:
Received 1 April 2014
Revised 8 September 2014
Accepted 9 October 2014
Available online 22 October 2014

Keywords:
Classical conditioning
Trauma film
Intrusions
Anxiety disorders
Estrogen
Menstrual cycle

ABSTRACT

Posttraumatic stress disorder (PTSD) can be conceptualized as a disorder of emotional memory showing strong (conditioned) responses to trauma reminders and intrusive memories among other symptoms. Women are at greater risk of developing PTSD than men. Recent studies have demonstrated an influence of ovarian steroid hormones in both fear conditioning and intrusive memory paradigms. However, although intrusive memories are considered non-extinguished emotional reactions to trauma reminders, none of the previous studies has investigated effects of ovarian hormones on fear conditioning mechanisms and intrusive memories in conjunction. This may have contributed to an overall inconsistent picture of the role of these hormones in emotional learning and memory. To remedy this, we exposed 37 healthy women with a natural menstrual cycle (during early follicular or luteal cycle phase) to a novel conditioned-intrusion paradigm designed to model real-life traumatic experiences. The paradigm included a differential fear conditioning procedure with short violent film clips as unconditioned stimuli. Intrusive memories about the film clips were assessed ambulatorily on subsequent days. Women with lower levels of estradiol displayed elevated differential conditioned skin conductance responding during fear extinction and showed stronger intrusive memories. The inverse relationship between estradiol and intrusive memories was at least partially accounted for by the conditioned responding observed during fear extinction. Progesterone levels were not associated with either fear acquisition/extinction or with intrusive memories. This suggests that lower levels of estradiol might promote stronger symptoms of PTSD through associative processes.

1. Introduction

Posttraumatic stress disorder (PTSD) and other anxiety disorders are highly disabling conditions, with women being at greater risk of PTSD than men (Olff, Langeland, Draijer, & Gersons, 2007; Tolin & Foa, 2006). Contemporary cognitive-behavioral theories assume that PTSD constitutes a disorder of emotional memory with strong responding to trauma reminders and intrusive memories belonging to its most prominent symptoms (Ehlers & Clark, 2000; Mineka & Oehlberg, 2008; VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2014). According to the fear conditioning approach, a traumatic event can be seen as an unconditioned stimulus (UCS) that elicits an unconditioned response (UCR) characterized by strong arousal and fear. Co-occurrence with the traumatic event turns stimuli such as sounds, sights or smells into conditioned stimuli (CSs), signalling impending danger and eliciting a conditioned fear response (CR) even when being presented alone later. Despite sometimes being experienced as 'coming out of the blue', intrusive memories are frequently triggered by such CSs. Based on this account, intrusive memories can be regarded as conditioned emotional reactions to trauma reminders (Foa, Zinbarg, & Rothbaum, 1992; Mineka & Oehlberg, 2008). Ehlers and Clark (2000) suggested that associative learning is particularly strong in patients with PTSD, thus making the triggering of intrusive memories even more likely. Similarly, Orr et al. (2000) proposed that the propensity to acquire larger as well as more persistent autonomic responses to an aversive CS, i.e. stronger fear acquisition and/or weaker extinction (higher fear conditionability), might render individuals more likely to develop symptoms of PTSD. This notion was supported by findings of heightened conditioned

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http://dx.doi.org/10.1016/j.nlm.2014.10.001
1074-7427/

responding during acquisition or extinction in patients with PTSD (Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Lommen, Engelhard, Sijbrandij, van den Hout, & Hermans, 2013; Norrholm et al., 2011; Orr et al., 2000; Peri, Ben-Shakhar, Orr, & Shalev, 2000; Wessa & Flor, 2007).

Owing to the higher risk for PTSD in women, research has recently begun to study fear acquisition and extinction (e.g. Glover et al., 2012; Zeidan et al., 2011) as well as intrusive memories (e.g. Bryant et al., 2011; Ferree, Kamat, & Cahill, 2011) specifically in women and in relation to the ovarian steroid hormones estradiol and progesterone. Localization of estradiol and progesterone receptors is not restricted to the hypothalamus, but receptors have also been identified in a multitude of other brain areas, including the hippocampus, amygdala, and prefrontal cortex (Brinton et al., 2008; Cui, Shen, & Li, 2013; McEwen, Akama, Spencer-Segal, Milner, & Waters, 2012; Montague et al., 2008). Accordingly, estradiol and progesterone are not only involved in the control of reproductive physiology and behavior, but have been found to more broadly modulate cognitive as well as emotional processes (Farage, Osborn, & MacLean, 2008; Lebron-Milad & Milad, 2012; van Wingen, Ossewaarde, Bäckström, Hermans, & Fernández, 2011). The latter is, for example, illustrated by symptoms of anxiety and depression being particularly common during periods with extreme changes in ovarian hormone levels, such as during postpartum and perimenopausal periods (Altshuler, Hendrick, & Cohen, 1998; Moses-Kolko, Berga, Kalro, Sit, & Wisner, 2009; Schmidt & Rubinow, 2009). However, effects of ovarian hormones on cognitive-emotional processing have also become evident on the basis of more subtle fluctuations of these hormones as they occur during the menstrual cycle (Sacher, Okon-Singer, & Villringer, 2013). Estradiol levels are low during the early follicular cycle phase, peak before ovulation in the late follicular phase and decrease to a moderate level during the luteal phase, whereas progesterone levels are low during the follicular phase and peak at the mid-luteal phase (Sacher et al., 2013).

Crucially, with respect to potential relationships between ovarian hormones and cognitive-emotional processing as revealed during *fear conditioning*, an initial study by Milad et al. (2010) reported that naturally cycling women with low as compared to high levels of estradiol exhibited reduced extinction recall (i.e. more fear in an extinction recall session that was conducted one day after initial fear acquisition and extinction training) (but see also Milad et al., 2006). Zeidan et al. (2011) replicated this finding and additionally found higher activation of brain regions such as the ventromedial prefrontal cortex during extinction recall but also during initial extinction learning in women with higher levels of estradiol. Furthermore, it has been shown that the use of hormonal contraceptives – which are known to decrease ovarian production of estradiol and progesterone – impairs extinction recall and that, on the other hand, administration of estradiol has the potential to enhance women's ability to recall extinction memory (Graham & Milad, 2013). Importantly, a role of estradiol in fear extinction is not only supported by studies in healthy participants, but has also been demonstrated in traumatized women (Glover et al., 2012). Furthermore, Glover et al. (2012) found average PTSD symptoms to be higher in traumatized women with low as compared to high estradiol levels. In summary, studies reported above agree on an association between estradiol and fear extinction processes and have led to the conclusion that low levels of estradiol might constitute a vulnerability factor for symptoms of PTSD (respective findings are reviewed by Lebron-Milad, Graham, & Milad, 2012, and Hiroi & Neumaier, 2011).

A separate line of research has investigated associations between estradiol, progesterone, or menstrual cycle phase and *intrusive memories*. Ferree and Cahill (2009) found that women in the luteal phase reported more spontaneous intrusive memories of film clips than women in the follicular phase. Similarly, Bryant et al. (2011) reported that traumatized women were more likely to experience flashback memories if they were in the luteal phase either at the time of trauma or at the time of assessment. However, neither of the two studies assessed ovarian hormone concentrations. This gap was closed by Ferree et al. (2011) observing a positive correlation between progesterone levels and spontaneous intrusive memories that women reported following the viewing of emotional films. More recently, Cheung, Chervonsky, Felmingham, and Bryant (2013) failed to replicate the latter finding. They found that levels of estradiol rather than progesterone were positively related to intrusive memories of negative images. However, it should be noted that Cheung et al. (2013) neither excluded women on hormonal contraceptives nor assessed women's menstrual cycle phase, which could potentially account for the different study outcomes. Finally, a recent study by Soni, Curran, and Kamboj (2013) found a negative relationship between intrusive memories and the estradiol-to-progesterone ratio rather than observing correlations with either estradiol or progesterone alone.

Thus, compared to the emerging picture in fear conditioning research, the intrusive memory literature remains inconsistent. Importantly, despite the theoretical considerations that fear conditioning and intrusive memories are functionally connected (see above), none of the reviewed studies has investigated the two processes in conjunction, thereby possibly contributing to the discrepant findings between these two lines of research. It is likely that methodological differences between these two paradigms precluded their integrated study: Fear conditioning studies mostly rely on UCSs such as electrical stimulation (e.g. Milad et al., 2006, 2010) or air blasts (Glover et al., 2012) that only partially depict the typical features (e.g. dynamic, multimodal events) of situations involved in fear learning in real life. Such UCSs are unlikely to generate the kind of complex memories that could later give rise to intrusive memories and are thus inappropriate to investigate fear conditioning and intrusive memories in conjunction. To overcome this problem, we developed a *conditioned-intrusion paradigm* to model the fear learning process in a more naturalistic manner: Short aversive film clips depicting severe violence serve as UCS and are predicted by neutral sound clips as CSs. Using the paradigm, reliable differential fear conditioning as well as intrusive memories on subsequent days were obtained. Importantly, residual conditioned responding during extinction (indicating both stronger and more persistent conditioned responding, hence higher 'fear conditionability' as defined by Orr et al., 2000) was positively correlated with subsequent intrusive memory strength, thereby encouraging further investigation of intrusive memories within a fear conditioning framework (Wegerer, Blechert, Kerschbaum, & Wilhelm, 2013).

The current study extends this work toward investigating the role of estradiol and progesterone in fear conditioning and intrusive memories, studied together in the conditioned-intrusion paradigm. Lower levels of estradiol were expected to be accompanied by stronger conditioned responding during fear extinction (Glover et al., 2012; Zeidan et al., 2011), whereas acquisition response levels should be unaffected. Furthermore, inasmuch as intrusive memories represent non-extinguished conditioned responding to trauma reminders, low estradiol should also be associated with higher intrusive memory strength and this link should be mediated by the degree of conditioned responding observed during fear extinction. For progesterone predictions were less clear since the reviewed evidence failed to document an effect on fear conditioning. Similarly, predictions for intrusive memories are complicated by inconsistent findings (see above; Cheung et al., 2013; Ferree et al., 2011; Soni et al., 2013) and by the fact that some studies did not assess ovarian hormone levels but analyzed data only with respect to self-reported menstrual cycle phase (Bryant et al., 2011; Ferree & Cahill, 2009). Yet, a related line of research, focusing on deliberate (rather than