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Stress exposure prior to fear acquisition interacts with estradiol status to alter recall of fear extinction in humans

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Summary Classical fear acquisition and extinction are important models for the etiology and treatment of anxiety disorders such as posttraumatic stress disorder (PTSD). Women are at a higher risk for PTSD than men. Levels of circulating 17-β estradiol (E2) in women have been linked to deficits in fear extinction and extinction recall. In PTSD, fear learning coincides with acute traumatic stress. However, little is known about the possible interaction between stress exposure and hormone status on fear acquisition and extinction learning. In a 2-day, 2×3 between-subjects design with healthy participants, we examined the effects of stress (psychosocial stressor vs. control, placed 45 min prior to conditioning) and natural E2-status on differential fear conditioning, covering fear acquisition, immediate extinction (Day 1), and 24 h-delayed extinction recall (Day 2). To operationalize E2-status, we compared women in the early follicular phase (EF) of their menstrual cycle (low E2, low progesterone plasma levels), women in the midcycle phase (MC, high E2, low progesterone), and men. Conditioning was indicated by differential skin conductance responses. We found an interaction between stress exposure and natural E2-status in women only: In MC-women, extinction recall on Day 2 (24 h after initial extinction training) was better when fear acquisition had been preceded by stress. In EF-women, the inverse was true. We show that extinction recall of conditioned fear acquired after stress depends on estrogen status in women. Therefore, extinction-based exposure therapy in free-cycling female anxiety patients should take cycle status into account.

1. Introduction

In classical fear conditioning, an initially neutral stimulus (conditioned stimulus, CS) is paired with a biologically relevant stimulus (unconditioned stimulus, US) capable of eliciting a fear response. After pairing, the CS alone comes to elicit a fear response. In fear extinction, the CS is repeatedly presented without the US and conditioned

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responses decline. Extinction is assumed to involve the formation of a new inhibitory memory trace, rather than ''forgetting'' (Bouton, 2004).

Fear conditioning and extinction are of clinical importance: Severe traumatic stress can lead to posttraumatic stress disorder (PTSD). Fear conditioning is proposed to play a central role in the etiology of PTSD (Mineka and Zinbarg, 2006; Mineka and Oehlberg, 2008) and to account for re-experiencing symptoms (Ehlers and Clark, 2000). Accordingly, PTSD is associated with enhanced conditionability (Orr et al., 2000). Fear extinction is the main target of exposure therapy for anxiety disorders. Failure to extinguish conditioned fear is assumed to contribute to the maintenance of anxiety disorders (Milad et al., 2010). Correspondingly, PTSD was shown to be associated with deficits in fear extinction (Lissek et al., 2005; Blechert et al., 2007), and impaired recall of fear extinction (Milad et al., 2008, 2009b).

Women are at a much higher risk to develop PTSD than men (Tolin and Foa, 2006; Zoladz and Diamond, 2013). Female PTSD-patients show enhanced fear acquisition as compared to males (Inslicht et al., 2013). One explanation is the different level of circulating sex hormones. There is increasing evidence that estrogens influence learning, memory, and emotion, via their actions in hippocampus and amygdala (Ter Horst, 2010). Functional magnetic resonance imaging (fMRI) suggests a role for estrogen in the reactivity of the brain's fear and arousal circuitry including the amygdala, the medial prefrontal cortex (PFC), the orbitofrontal cortex, and the anterior cingulate cortex (ACC). These structures show attenuated responses to emotional stimuli during late follicular (midcycle) phase of the menstrual cycle, where estrogen (but not progesterone) levels are high (Goldstein et al., 2005, 2010). There is also evidence for an important role of 17β-estradiol (E2) in fear extinction: low E2 levels are associated with extinction deficits in female PTSD-patients (Glover et al., 2012), and impaired fear inhibition in healthy and traumatized women (Glover et al., 2013). In rats, E2 facilitated contextual fear extinction via actions on estrogen receptor β (Chang et al., 2009). Human neuroimaging shows altered fear extinction in women using oral contraceptives: they exhibit higher reactivity toward the CS+ in the amygdala, ACC, and ventromedial PFC during extinction compared to men and women in the luteal phase (Merz et al., 2012a). Furthermore, while E2 level had no effect on fear acquisition or extinction learning itself (Milad et al., 2010), both, animal and human studies show that female sex hormones are involved in extinction recall: Low circulating E2 levels impaired whereas high E2 levels enhanced the recall of fear extinction (Milad et al., 2006, 2009a, 2010; Zeidan et al., 2011). Correspondingly, both, female rats and women under hormonal contraceptives show impaired extinction recall (Graham and Milad, 2013). Brain regions involved in the acquisition and consolidation of fear extinction, i.e., the amygdala, the ventromedial PFC (vmPFC), and the hippocampus are sexually dimorphic and contain a high density of estrogen receptors (Milad et al., 2010). Accordingly, E2 levels in free-cycling women modulated the fMRI reactivity during extinction and extinction recall in the vmPFC and in the amygdala (Zeidan et al., 2011). Thus, there is strong evidence that estrogens influence emotional reactivity, fear extinction and extinction recall.

From an associative-learning point of view, traumatic situations comprise classical fear conditioning under severe stress. There is evidence that effects of stress on fear conditioning are also sex-specific in animals (Dalla and Shors, 2009 for a review). In humans, sex-specific conditioning effects after noradrenergic stimulation were not found so far (Soeter and Kindt, 2011, 2012). Psychosocial stress (inducing cortisol increases) prior to acquisition enhanced conditioned skin conductance responses (SCRs) in men, but not in women (Jackson et al., 2006). However, psychosocial stress reduced conditioned BOLD responses and SCRs during acquisition in the nucleus accumbens, amygdala and ACC in men, but enhanced BOLD responses in women (Merz et al., 2013b). Similar results were found after cortisol administration, where conditioned BOLD responses in the orbitofrontal and mPFC (Stark et al., 2006) and insula, hippocampus and thalamus (Merz et al., 2010) were impaired in men, but enhanced in women. When sex-hormone status of women was taken into account, cortisol enhanced conditioned BOLD responses in women using oral contraceptives, but decreased them in free-cycling women and men (Merz et al., 2012b). Furthermore, basal cortisol level was positively correlated with differential amygdala activity during fear acquisition in OC-women and men, but not in women in the luteal phase (Merz et al., 2013a). Thus, there is preliminary evidence that the effects of psychosocial stress and/or cortisol administration on fear conditioning are modified by the level of circulating female sex hormones.

By now, there is no human study that examined the combined effects of psychosocial stress, estrogen level, and their interaction on fear extinction and extinction recall. Specifically, a fear-conditioning study that compares women in the early follicular phase (EF, low estrogen and progesterone levels) vs. midcycle phase (MC, high estrogen but low progesterone) while simultaneously assessing hormone levels is missing. Consequently, in a 2×3 between-subjects design (factors ''Treatment'' and ''Hormonal Status'') we tested EF-women, MC-women, and men. Half of the participants per group underwent a psychosocial stressor (Deinzer et al., 2004) while the other half underwent a control procedure 45 min before fear conditioning. Conditioning covered acquisition and immediate extinction on Day 1, and extinction recall on Day 2. Since stress-induced cortisol negatively correlated with acquisition performance (Antov et al., 2013) and stress and cortisol reduced conditioned BOLD-responses (Stark et al., 2006; Merz et al., 2010, 2012b, 2013b) we expected stress to reduce fear acquisition in men. In EF-women (low E2, low progesterone), psychosocial stress should decrease fear acquisition, as inferred from data on cortisol administration in women using oral contraceptives (low E2, low progesterone) (Merz et al., 2012b). With regard to Hormonal Status (Milad et al., 2006, 2010; Zeidan et al., 2011; Graham and Milad, 2013) EF-women should show poorer extinction recall than MC-women and men.

2. Methods

2.1. Participants

Volunteers eligible for the study were students, recruited on the campus of the University of Osnabrück. Participants were screened for posttraumatic stress disorder (PTSD) using