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THE INFLUENCE OF GONADAL HORMONES ON CONDITIONED FEAR EXTINCTION IN HEALTHY HUMANS

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Abstract—Recent rodent studies suggest that gonadal hormones influence extinction of conditioned fear. Here we investigated sex differences in, and the influence of estradiol and progesterone on, fear extinction in healthy humans. Men and women underwent a two-day paradigm in which fear conditioning and extinction learning took place on day 1 and extinction recall was tested on day 2. Visual cues were used as the conditioned stimuli and a mild electric shock was used as the unconditioned stimulus. Skin conductance was recorded throughout the experiment and used to measure conditioned responses (CRs). Blood samples were obtained from all women to measure estradiol and progesterone levels. We found that higher estradiol during extinction learning enhanced subsequent extinction recall but had no effects on fear acquisition or extinction learning itself. Sex differences were only observed during acquisition, with men exhibiting significantly higher CRs. After dividing women into low- and high-estradiol groups, men showed comparable extinction recall to high-estradiol women, and both of these groups showed higher extinction recall than low-estradiol women. Therefore, sex differences in extinction memory emerged only after taking into account women's estradiol levels. Lower estradiol may impair extinction consolidation in women. These findings could have practical applications in the treatment of anxiety disorders through cognitive and behavioral therapies.

Key words: estrogen, progesterone, sex difference, fear, menstrual cycle, learning and memory.

Failure to extinguish conditioned fear may play an important role in the pathogenesis of anxiety disorders (Davis et al., 2006; Milad et al., 2006b, 2008; Milad and Rauch, 2007; Orr et al., 2000; Rauch et al., 2006). Extinction forms the theoretical basis of treatment by exposure (Rothbaum and Davis, 2003). A substantial number of studies conducted in rodents and more recently in humans indicate that brain regions associated with the acquisition and consolidation of conditioned fear extinction are implicated in anxiety disorders. These areas include the amygdala, ventromedial prefrontal cortex (vmPFC), and hippocampus (for review, see Quirk and Mueller, 2008). These brain regions are sexually dimorphic and contain a high density of gonadal hormone receptors (Giedd et al., 1996; Goldstein et al., 2001; Ostlund et al., 2003). This may explain why measures of fear and arousal are associated with changes in hormonal levels over the menstrual cycle (Cahill, 2003; Goldstein et al., 2005; Gupta et al., 2001; Jasnow et al., 2005), and sex differences in arousal (Goldstein et al., 2010). Such findings also hold promise for clarifying important sex differences in anxiety disorders (Kinrys and Wygant, 2005; Pigott, 2003).

We recently showed that female rats undergoing extinction learning during the proestrous phase of the estrus cycle (when estradiol and progesterone levels are elevated) exhibited better extinction memory during subsequent extinction recall (i.e., retention) testing (Milad et al., 2009a). Moreover, exogenously administered estradiol and progesterone facilitated extinction recall, whereas estradiol and progesterone receptor antagonists impaired it (Milad et al., 2009a). A recent study found that estradiol administration into the hippocampus in female rats facilitated fear extinction (Chang et al., 2009). Collectively, these data suggest that gonadal hormones influence the consolidation of extinction memory.

In a study of healthy humans, we showed that phase of the menstrual cycle influences consolidation of fear extinction (Milad et al., 2006a), but gonadal hormones were not measured. The present goal was to explore the associations of estradiol and progesterone with physiological aspects of acquisition and extinction of conditioned fear in healthy women at different phases of their menstrual cycles, and to compare their responses to those of healthy men. Estradiol and progesterone levels were measured from blood samples obtained prior to the initiation of a previously described two-day fear conditioning and extinction experiment (Milad et al., 2005, 2007; Rauch et al., 2005). On Day 1, participants underwent fear conditioning in virtual context A and extinction learning in virtual context B. On Day 2, extinction recall (context B) was tested using

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Abbreviations: ASI, anxiety sensitivity index; CR, conditioned response; CS, conditioned stimulus; HE, high estrogen; HP, high progesterone; LE, low estrogen; LP, low progesterone; PTSD, post-traumatic stress disorder; SCL, skin conductance level; SCR, skin conductance response; UCR, unconditioned response; US, unconditioned stimulus; vmPFC, ventromedial prefrontal cortex.

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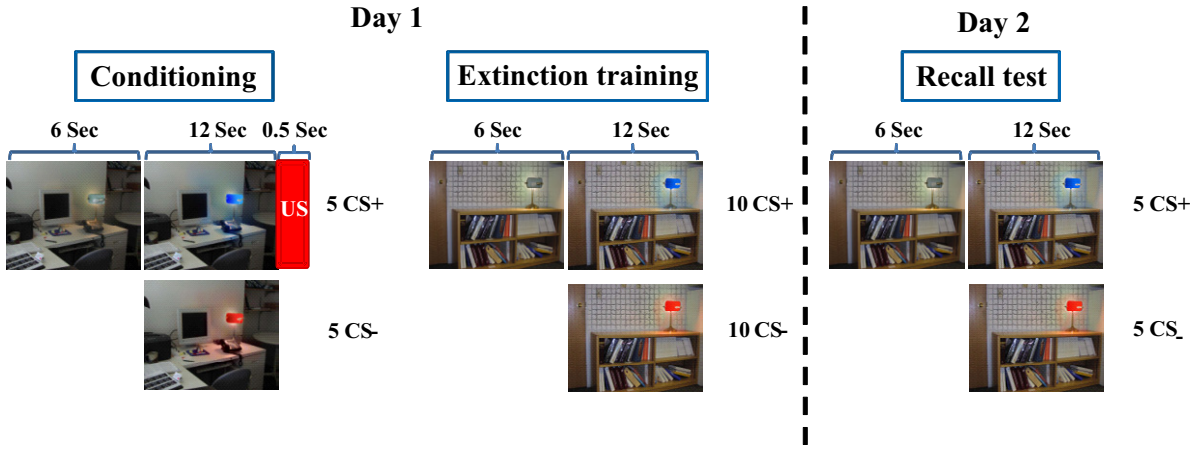

Fig. 1. Fear conditioning and extinction protocol. CS+, conditioned stimulus; CS−, conditioned stimulus never paired with shock (US, unconditioned stimulus). Adapted from Milad et al., 2005.

skin conductance response (SCR) as a measure of fear (Milad et al., 2005; Rauch et al., 2005). Based on our recent findings in female rats and the literature reviewed above, we hypothesized that estradiol and progesterone would facilitate the extinction of conditioned fear and/or the consolidation thereof, such that women with higher levels of these hormones would display lower conditioned responses (CRs) during subsequent extinction recall. Further, these hormonal differences would, in part, account for sex differences in conditioned fear extinction.

EXPERIMENTAL PROCEDURES

Participants

Fifty-four participants (36 women, 18 men) ages 18–30 were recruited from the local community via advertisement. Women were divided by hormonal levels into two groups of 18 (see below). All participants were right-handed, without endocrinologic, neurologic, or other medical conditions, and without Axis I mental disorders, including substance dependence or abuse, as determined by the Structured Clinical Interview for DSM-IV (First et al., 2002). No participant was using psychoactive or other potentially confounding drugs or medications. All female participants had regular menstrual cycles by history and had not been using oral contraceptives or hormone replacement for at least 3 months. After a complete description of the procedures, written informed consent was obtained from all participants in accordance with the requirements of the Partners Healthcare Human Research Committee.

Women were studied during the early follicular phase (days 3–5 of the cycle, $n=6$), late follicular phase (days 10–12, $n=10$), early luteal phase (days 18–20, $n=11$), or late luteal phase (days 25–27, $n=11$). Women were divided into low-estradiol group (LE) and high-estradiol (HE) groups, and separately into low-progesterone (LP) and high-progesterone (HP) groups based on median splits regardless of menstrual phase. Sixty-one percent of women in the LE group were part of the LP group, and 66% of women in the HE group were part of the HP group.

Conditioning and extinction procedure

The fear conditioning and extinction procedures were identical to those previously described (Milad et al., 2005, 2006a). Digital photographs of two different rooms constituted the visual contexts. Each context contained a lamp that was shown first in the off position (no color) and then switched on to one of two different colors (blue or red), which constituted the conditioned stimuli (CSs) (see Fig. 1). The selection of the CS+ color (followed by shock) and CS− color (no shock) and the contexts were pseudorandom and counterbalanced across participants. Contexts and CSs were displayed on a computer monitor three feet in front of participants who were seated upright in a chair. The unconditioned stimulus (US) was a 500 ms electric shock previously selected by the participant to be "highly annoying but not painful" delivered through electrodes attached to the second and third fingers of the right hand (Milad et al., 2005; Orr et al., 2000). The shock electrodes remained attached during each session of the experiment, but the US was administered only during the Conditioning session.

The experimental protocol was conducted over 2 days. On Day 1, the Habituation session consisted of eight trials in which the to-be CS+ and to-be CS− (four of each) were presented in a counterbalanced manner within both the to-be conditioning context and the to-be extinction context. The Conditioning session consisted of five CS+ and five CS− trials, presented within the conditioning context. The US occurred immediately following each CS+ offset (100% reinforcement). The Extinction session was divided into two sub-sessions: early and late, which were separated by an approximate one-minute rest period. Each sub-session consisted of five CS+ and five CS− trials, all presented within the extinction context. On day 2, the Recall session was identical to an Extinction sub-session given on day 1. The US was never presented on day 2. When questioned after completion of the study, all subjects were explicitly aware of the CS–US contingency.

Serological measurements

All subjects underwent the experimental protocol in the morning and were instructed not to eat after midnight prior to participation. Blood samples were drawn on Days 1 and 2 20–30 min prior to the experiment. Estradiol levels were assessed using an RIA kit (Roche Diagnostics, Indianapolis, IN, USA) with a sensitivity of 18.4 pmol/L and an intra-assay coefficient of variance (CV) of 1.6%–5.7%. Progesterone levels were determined using an RIA kit (Roche Diagnostics, Indianapolis, IN, USA) with a sensitivity of 0.095 nmol/L, and an intra-assay CV of 1.5%–2.7%.

Psychometric measures

On a day of psychiatric assessment prior to the experiment, each subject completed the Beck Anxiety and Beck Depression Inventories, Anxiety Sensitivity Index (ASI), STAI Trait (T) assessment,