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Oral contraceptive usage alters the effects of cortisol on implicit fear learning ☆

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ABSTRACT

An important feature of the human defense system comprises fear learning, which stress hormones can crucially modulate. However, stress hormones might influence men and women differently, in part because of interactions with sex hormones. In women, distinct stages of the menstrual cycle or the intake of oral contraceptives (OC) affect sex hormone levels. In this study, we used a differential fear conditioning paradigm with electrical stimulation as unconditioned stimulus (UCS) following one neutral stimulus (conditioned stimulus, CS+), but not another (CS−).To investigate implicit fear learning, participants were distracted from detecting the contingencies between CS and UCS. To address interaction effects of sex and stress hormones, 32 men, 30 women in the early follicular phase of the menstrual cycle (FO), 30 women in the luteal phase (LU), and 30 OC women received either 30 mg cortisol or a placebo. In the contrast CS+ minus CS−, an interaction between cortisol administration and sex hormone status emerged in the anterior parahippocampal gyrus and the hippocampus. Cortisol reduced fear learning in men, FO, and LU women, but enhanced it in OC women. Additionally, cortisol attenuated differential amygdala activation in the entire group. These results demonstrate that OC usage substantially modifies cortisol effects on emotional learning in women, particularly in memory-related medial temporal lobe regions. Further, a high dose of cortisol reduces amygdala differentiation pointing to a lowered learning ability of the defense system under high cortisol concentrations, irrespective of current sex hormone availability.

Introduction

Essential features of the human defense system include detecting threats and initiating adequate responses to cope with them. Even if one does not consciously perceive a potential danger, a subcortical fear circuit centered around the amygdala might be activated automatically (LeDoux, 2003). Fear learning is highly adaptive, because it supports recollection of potential dangers and promotes adequate future behavior.

In humans, fear learning can be investigated in the laboratory using differential fear conditioning designs. They typically reveal fear conditioned responses (CRs) at the electrodermal level or in the neuronal fear circuit including the amygdala, the anterior parahippocampal gyrus, the hippocampus, the insula, and the orbitofrontal cortex (Knight et al., 2004a,b; LeDoux, 2000; Mechias et al., 2010; Rolls, 1999). However, a dissociation between electrodermal and neuronal fear responses can be found using neutral, supraliminally presented conditioned stimuli (CS; e.g. Tabbert et al., 2006, 2011). In particular, persons that cannot explicitly report any association between CS and the unconditioned stimulus (UCS) did not exhibit CRs at the electrodermal level, but in the neuronal fear circuit (e.g. in the amygdala). A prolonged or exaggerated activation of the fear module during initial conditioning, even if not accessible to one's awareness, might be associated with the development of pathologic fears (for reviews: Etkin and Wager, 2007; Öhman and Mineka, 2001; Shin and Liberzon, 2010).

An environmental threat triggers this fear module, which initiates a stress response resulting in the release of (nor)epinephrine and cortisol, the major stress hormone in humans, from the adrenal glands. Then, cortisol influences several cortical and subcortical structures such as the amygdala or the hippocampus (for reviews: Rodrigues et al., 2009; Wolf, 2008). Stress and stress hormones have been implicated in the pathogenesis of several psychiatric disorders, in particular of anxiety disorders (for reviews: Korte, 2001; Wolf, 2008). Besides, prominent sex differences in the prevalence of anxiety disorders exist with a more frequent occurrence in women (Kessler et al., 2005). Neurobiological explanations of these discrepant prevalence

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rates encompass the involvement of sex hormones (Solomon and Herman, 2009; Toufexis et al., 2006).

Sex hormones such as estradiol and progesterone affect the brain and the periphery through activational and organizational effects. Organizational effects refer to long-term influences of sex hormones on physiology and morphology during development, whereas activational effects relate to circulating sex hormones inducing physiological and morphological changes through the whole lifespan (Gillies and McArthur, 2010). Activational effects can be explored in women during different stages of the menstrual cycle. Low sex hormone levels characterize the early follicular phase (FO), whereas these concentrations increase in the luteal phase (LU), peak levels can be observed during ovulation. Several studies implicate that sex hormones alter fear processing, e.g. using estrogen administration or comparing different cycle stages in female rodents (Gupta et al., 2001; Morgan and Pfaff, 2001; for a review: Morgan et al., 2004) and humans (Milad et al., 2006; Zeidan et al., 2011) or investigating women with low or high estradiol levels (Milad et al., 2010).

However, a considerable percentage of women are using oral contraceptives (OCs). Despite this crucial relevance, reports on specific OC effects in emotional learning tasks are sparse. OCs contain exogenous sex hormones such as ethinylestradiol, which acts centrally and peripherally and continuously suppresses endogenous sex hormones. Therefore, the combined investigation of OC women with free-cycling women exhibiting low (FO) or high (LU) endogenous sex hormone concentrations ignores the evidence concerning activational effects.

The combined examination of the effects of stress and sex hormones (especially concerning free-cycling and OC taking women) on fear learning is important to elucidate basal modulatory influences on the human defense system. Previous experiments from our group observed that cortisol had opposing effects on the neuronal correlates of fear learning in men and women (Merz et al., 2010; Stark et al., 2006; Tabbert et al., 2010). In these studies, the mediating role of sex hormones on this effect could not be investigated due to small groups of women and/or mixed sex hormone status. Thus, in this experiment, men, FO, LU, and OC women were tested receiving either cortisol or placebo prior to a classical fear conditioning paradigm. Because we were particularly interested in implicit fear learning, distractors were introduced to prevent participants from detecting the relationship between the CS and UCS (cf. Merz et al., 2010; Tabbert et al., 2006, 2010, 2011). Inferring from these prior studies, we hypothesized no conditioning signs on the electrodermal, but on the neuronal level in structures involved in fear learning (e.g. anterior parahippocampal gyrus, hippocampus). Further, our prior results concerning implicit fear learning predict that cortisol should reduce fear CRs especially in the amygdala (cf. Merz et al., 2010). Based on previous conditioning studies using functional magnetic resonance imaging (fMRI; Merz et al., 2010; Stark et al., 2006; Tabbert et al., 2010), we hypothesized that cortisol would lead to higher CRs in OC women, but to reduced CRs in men. Notably, the additional investigation of FO and LU women is highly interesting for the interpretation of the obtained results. This approach will reveal if cortisol effects in OC women are due to OC intake or due to lowered endogenous sex hormones. So, for the first time, we examined activational and organizational effects of sex hormones on cortisol-modulated subcortical fear learning.

Material and methods

General background

The present experiment is part of a larger project investigating different groups, which were either instructed about the CS-UCS contingencies in advance or not (i.e. instructed vs. unaware fear conditioning; see Tabbert et al., 2011). In this report, we focus on the latter participants; they were not informed about a relationship between CS and UCS in advance. Adding distractors into the experimental design (a numerical two-back task and a distractor stimulus; cf. Merz et al., 2010) hampered contingency learning in the course of the experiment. Those subjects, who nevertheless noticed the correct CS-UCS contingencies, were excluded from the present analyses because of the impact of contingency awareness on various correlates of fear conditioning (e.g. Hamm and Vaitl, 1996; Klucken et al., 2009; Tabbert et al., 2006; see Tabbert et al., 2010, 2011 for the exact results).

An analysis of a subsample ($n = 42$ from the placebo group) has been published previously together with two additional groups (learned and instructed aware participants; Tabbert et al., 2011). This prior data analysis was concerned with the differential effect of contingency awareness on fear acquisition, not with the influence of cortisol or sex hormone status. A first report on the effects of cortisol and sex has also been published based on an overlapping small subsample ($n = 39$; Merz et al., 2010). The detailed impact of sex hormone status could not be analyzed there because of low cell frequency in the women groups ($n \leq 5$) leading to a joint examination of LU and OC women. Now, in the available complete large sample, we are able to investigate cortisol effects in men as well as in three different groups of women.

Participants

In total, 122 participants (117 undergraduate and five graduate students) completed the study. To assess different sex hormone statuses, we invited 32 men, 60 free-cycling women, and 30 OC taking women. Free-cycling women did not take any kind of contraceptives. They reported to have a regular menstrual cycle; one half was invited in the early follicular phase (FO; 3rd to 8th day after the onset of their last menstruation) and the other half in the luteal phase (LU; 3rd to 9th day before the onset of their next menstruation) of the individual menstrual cycle. OC women were required to have been taking their birth control pill (only monophasic preparations with an ethinylestradiol (0.02–0.035 mg) and a gestagenic component) for at least the last three months and we tested them during the pill intake phase.

None of the participants was taking regular medication except OCs or had a history of psychiatric or neurological treatment. Exclusion criteria covered somatic diseases, in particular endocrine diseases, which can influence hormone concentrations, as well as standard fMRI exclusion criteria (e.g. implants, previous brain surgery or intrauterine devices). Inclusion criteria comprised an age between 18 and 35 and a body mass index (BMI) between 18 and 28 kg/m^2. The mean age for the eight groups ranged from 21.3 to 24.8 years and the mean BMI from 21.1 to 22.8 kg/m^2. Further, only right-handed persons were included as assessed by the Edinburgh Inventory of Handedness (Oldfield, 1971).

All participants had normal or corrected vision. We instructed them to refrain from smoking, food intake, and drinking anything but water for at least two hours before the start of the experiment. Each session began between 2 and 5 p.m. to guarantee low and relatively stable endogenous cortisol levels. At first, participants got a detailed explanation of the general procedure (naturally, the conditioning schedule was not explained until the end). The cover story concealing the conditioning procedure included the investigation of the impact of cortisol and several distractors (including an electrical stimulation and visual stimuli) on memory performance. Participants were instructed to pay close attention to all stimuli and to complete the implemented two-back task. All participants gave written informed consent and received at least 25 Euros for their attendance. The ethics committee of the German Psychological Society approved this study.

Conditioned stimuli (CS), unconditioned stimulus (UCS), and experimental procedure

Three pictures of geometric figures (a rhomb, a square, and a triangle) served as CS+, CS−, and as distractor stimulus (non-CS; always the triangle). All figures had identical luminance, were gray-colored, and