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Stress differentially affects fear conditioning in men and women☆

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Summary Stress and fear conditioning processes are both important vulnerability factors in the development of psychiatric disorders. In behavioral studies considerable sex differences in fear learning have been observed after increases of the stress hormone cortisol. But neuroimaging experiments, which give insights into the neurobiological correlates of stress × sex interactions in fear conditioning, are lacking so far. In the current functional magnetic resonance imaging (fMRI) study, we tested whether a psychosocial stressor (Trier Social Stress Test) compared to a control condition influenced subsequent fear conditioning in 48 men and 48 women taking oral contraceptives (OCs). One of two pictures of a geometrical figure was always paired (conditioned stimulus, CS+) or never paired (CS−) with an electrical stimulation (unconditioned stimulus). BOLD responses as well as skin conductance responses were assessed. *Sex-independently*, stress enhanced the CS+/CS− differentiation in the hippocampus in early acquisition but attenuated conditioned responses in the medial frontal cortex in late acquisition. In early acquisition, stress reduced the CS+/CS− differentiation in the nucleus accumbens in men, but enhanced it in OC women. In late acquisition, the same pattern (reduction in men, enhancement in OC women) was found in the amygdala as well as in the anterior cingulate. Thus, psychosocial stress impaired the neuronal correlates of fear learning and expression in men, but facilitated them in OC women. A sex-specific modulation of fear conditioning after stress might contribute to the divergent prevalence of men and women in developing psychiatric disorders.

☆ This study was carried out at the Bender Institute of Neuroimaging, Justus Liebig University Giessen.

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1. Introduction

Stress hormones strongly act on emotional and cognitive processes and cause vivid remembrance of emotionally arousing events (Wolf, 2008). In the case of traumatic experiences, this can occasionally result in excessive fear and anxiety such as in posttraumatic stress disorder (PTSD). Fear conditioning is an emotional learning process critically contributing to the development of PTSD and other psychiatric disorders (e.g. phobias; Bonne et al., 2004). These disorders occur to a much higher degree in women (Kessler et al., 2005). However, the underlying neurobiological mechanisms of stress, which potentially influence fear conditioning in men and women differently, remain insufficiently understood. A better comprehension of this crucial stress-related and sex-dependent fear circuit might ultimately lead to improved treatments.

An environmental threat triggers the stress response activating the sympathetic nervous system (SNS) as well as the hypothalamus–pituitary–adrenal (HPA) axis. The SNS stimulates the adrenal glands to release (nor)epinephrine, which can be indirectly measured via salivary alpha-amylase (sAA; Nater and Rohleder, 2009). Activation of the HPA axis leads to a release of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone, and glucocorticoids such as cortisol, the major stress hormones in humans. Cortisol readily enters the brain and modulates cortical as well as subcortical structures involved in learning and memory, e.g. the amygdala, the hippocampus or the prefrontal cortex (for reviews: Wolf, 2009; Joels et al., 2011). Such learning and memory processes can be investigated using fear conditioning designs. Typically, conditioned responses (CRs) are found in the amygdala, anterior cingulate, hippocampus, and medial prefrontal cortex (e.g. Büchel et al., 1998; LeDoux, 2000; Mechias et al., 2010). Besides, the formation of relations between conditioned (CS) and unconditioned stimuli (UCS) was associated with activation of the nucleus accumbens (Klucken et al., 2009). A prolonged activation of this fear circuit along with the release of stress hormones during initial association is proposed to be related to the development of pathologic fears (for a review: Rodrigues et al., 2009).

A few psychophysiological studies in humans provided evidence that stress hormones affect fear conditioning in men and women differently, e.g. using psychosocial stress (Jackson et al., 2006; Zorawski et al., 2006) or correlational approaches (Zorawski et al., 2006). In these experiments, stress hormones enhanced CRs in males, but reduced them in females or did not exhibit any significant effect in females. Neuroimaging studies from our groups used a pharmacological administration of 30 mg hydrocortisone (cortisol) prior to fear conditioning (Stark et al., 2006; Merz et al., 2010, 2012b; Tabbert et al., 2010): A reversed picture emerged with cortisol attenuating CRs in the fear circuit (including the amygdala, anterior cingulate, hippocampus, and medial prefrontal cortex) in men and in free-cycling women, but elevating CRs at the neuronal level in women taking oral contraceptives (OCs). The contribution of OCs on fear conditioning processes is especially interesting in terms of their common usage, but no studies are available on their possible impact on mental health.

Taken together, stress effects on fear conditioning were tested so far in humans at the electrodermal level only. To translate neuroimaging findings with pharmacological cortisol concentrations (Stark et al., 2006; Merz et al., 2010, 2012b; Tabbert et al., 2010) to physiological stress-induced cortisol concentrations, we used a psychosocial stressor prior to differential fear conditioning. Thus, we mirrored real-life stress with its concurrent activation of the SNS (assessed indirectly by measurement of sAA) and the HPA axis (as indexed by salivary cortisol). We were particularly interested in men and OC women, because they exhibited the most contrasting fear learning pattern in previous pharmacological cortisol studies (Stark et al., 2006; Merz et al., 2012b). All participants were instructed to pay close attention to any regularities between CS and UCS to ensure complete contingency awareness developing very early in the experiment. Accordingly, we expected fear learning related activation (in the amygdala, hippocampus, and nucleus accumbens) during early acquisition and fear expression and regulation related activation during late acquisition (in the amygdala, anterior cingulate, and medial prefrontal cortex; cf. Sotres-Bayon and Quirk, 2010). Based on our previous pharmacological neuroimaging studies (Stark et al., 2006; Merz et al., 2010, 2012b; Tabbert et al., 2010), we predicted that psychosocial stress leads to reduced CRs in men, but heightened CRs in OC women at the electrodermal level as well as in the respective brain regions involved in fear conditioning.

2. Materials and methods

2.1. Participants

We recruited 105 persons to ensure a total sample size of 96 participants (48 men). Two women were excluded because they did not develop contingency awareness (see Section 2.4), two women and two men because of excessive head movements, one man canceled the scanning session, one woman fell asleep during the task, and one woman was left-handed, which was an a priori exclusion criterion (assessed by the Edinburgh Inventory of Handedness; Oldfield, 1971). Further exclusion criteria covered standard fMRI exclusion criteria, somatic diseases, in particular endocrine diseases, history of psychiatric or neurological treatment, and regular medication usage except OCs. Women were required to have been taking their birth control pill (only monophasic preparations with an ethinylestradiol and a gestagenic component) for at least the last three months; we tested them during pill intake. Inclusion criteria comprised age between 18 and 35 and body mass index (BMI) between 18 and 28 kg/m^2.

All participants had normal or corrected vision. They received a detailed explanation of the general procedure; the conditioning schedule was not explained until the end. All participants gave written informed consent and received 10 Euros per hour for their attendance. All procedures were in accordance with the Declaration of Helsinki and approved by the university's local ethical review board.

2.2. Stress protocol, negative affect, salivary cortisol, and alpha-amylase

Men and women were randomly assigned to the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) or a non-stressful