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Stress hormones are associated with the neuronal correlates of instructed fear conditioning☆

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ABSTRACT

The effects of sex and stress hormones on classical fear conditioning have been subject of recent experimental studies. A correlation approach between basal cortisol concentrations and neuronal activation in fear-related structures seems to be a promising alternative approach in order to foster our understanding of how cortisol influences emotional learning. In this functional magnetic resonance imaging study, participants with varying sex hormone status (20 men, 15 women taking oral contraceptives, 15 women tested in the luteal phase) underwent an instructed fear conditioning protocol with geometrical figures as conditioned stimuli and an electrical stimulation as unconditioned stimulus. Salivary cortisol concentrations were measured and afterwards correlated with fear conditioned brain responses. Results revealed a positive correlation between basal cortisol levels and differential activation in the amygdala in men and OC women only. These results suggest that elevated endogenous cortisol levels are associated with enhanced fear anticipation depending on current sex hormone availability.

1. Introduction

Classical conditioning is thought to represent a central mechanism in the development of anxiety disorders (Bangasser and Shors, 2010; Hofmann, 2008; Mineka and Oehlberg, 2008). Stress influences emotional learning and is a potent modulator of psychiatric diseases, in particular concerning anxiety disorders such as post-traumatic stress disorder (de Quervain et al., 2009; Holsboer and Ising, 2010; Wolf, 2008). More detailed knowledge of the neuroendocrine modulation of emotional learning might have valuable implications for the prevention and treatment of anxiety disorders (Bentz et al., 2010). In particular, women are more likely to develop an anxiety disorder (Kessler et al., 2005; McLean et al., 2011), which could suggest an enhanced susceptibility to stress. However, the precise influence of sex and stress hormones on fear conditioning is still not fully understood.

Amongst others, stress induces an activation of the hypothalamus-pituitary-adrenal (HPA) axis (release of glucocorticoids (GCs): corticosterone in rodents; cortisol in humans). Elevated GCs in turn reduce HPA activity via negative feedback. The HPA axis can be inhibited by the orbitofrontal cortex (OFC) and the hippocampus (including the parahippocampal gyrus), but can be activated by the amygdala (Dedovic et al., 2009; Diorio et al., 1993; Herman et al., 2003, 2005; Liberzon et al., 2007; Oei et al., 2007; Prüssner et al., 2008).

These critical brain structures overlap with the neuronal correlates of emotional learning studied in classical fear conditioning paradigms (Cheng et al., 2006; Knight et al., 1999; LeDoux, 2000; Mechias et al., 2010; Rolls, 1999; Sehlmeyer et al., 2009). Differential fear conditioning includes a stimulus paired with an aversive event (unconditioned stimulus, UCS), which becomes a conditioned stimulus (CS+), whereas another stimulus is never paired (CS−). The fear conditioning protocol can be assumed as successful if higher responses, e.g. skin conductance responses (SCRs) or neuronal activation, towards the CS+ compared to the CS− are observed.

As the most prominent structure in the fear circuit, the amygdala is crucial for fear learning and expression (LeDoux, 2000; Maren, 2005). Importantly, research on fear extinction and emotion regulation revealed that amygdala activation is modulated by the medial prefrontal cortex (PFC), largely by inhibitory projections; however, also excitatory projections exist (Kalisch et al., 2006; Milad et al., 2007; Paré et al., 2004; Phelps et al., 2004; for reviews see Delgado et al., 2006; Ochsner and Gross, 2005).

Stress and the accompanying occupation of glucocorticoid receptors in the PFC might change this top–down control. More

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precisely, rodent studies revealed that elevated GC concentrations impair prefrontal function and the PFC is hence no longer able to inhibit the amygdala (e.g. Akirav and Maroun, 2007; Izquierdo et al., 2006). In human studies, the modulating role of stress hormones on the top–down control of the amygdala by the medial PFC is still rather unclear (e.g. Ahs et al., 2006; Kern et al., 2008; Urry et al., 2006; Wang et al., 2005). Besides, the influence of sex and stress hormones on this interplay has been widely neglected; further research on this topic is thus highly relevant.

Rodent studies using eye-blink conditioning (Dalla and Shors, 2009; Shors, 2004) have revealed that stress hormones have sex-dependent effects on conditioned responses (CRs). Stress led to higher CRs in males, but impaired CRs in females. In human fear conditioning, high endogenous or stress-induced cortisol levels are associated with enhanced fear conditioned SCRs in men, but not in women (Jackson et al., 2006; Zorawski et al., 2005, 2006). Neuroimaging studies using a high cortisol dosage (30 mg; Merz et al., 2010; Stark et al., 2006; Tabbert et al., 2010), however, revealed reduced CRs in men after GC application, but enhanced CRs in women in several brain structures.

One possible explanation of these divergent results could be the exact cortisol concentration during fear conditioning. Basal endogenous or stress-induced cortisol levels might exert effects quite different to those induced by exogenous GC application often leading to supraphysiological hormone concentrations. An inverted U-shaped curve concerning cortisol and memory processes, but also a more linear relationship has been proposed (de Kloet et al., 1999; Lupien et al., 2007; Sandi and Pinelo-Nava, 2007). In males but not in females, linear associations between stress hormones and CRs have been reported (Jackson et al., 2006; Wood et al., 2001; Zorawski et al., 2005, 2006).

The differing fear learning patterns in men and women in response to elevated cortisol levels could be due to the influence of circulating sex hormones on brain activation. The impact of menstrual cycle phase and stress hormones on emotional learning has already been studied in rodents (Shors et al., 1998; Wood and Shors, 1998; Wood et al., 2001). These studies indicate enhanced conditioning performance in females when estradiol levels are high; heightened stress hormones abolished this enhancement. No experiment on this topic exists in humans so far, in particular concerning a correlation approach between basal cortisol concentrations and fear conditioned neuronal activation.

In the present study, we conducted a differential conditioning experiment with an electrical stimulation as UCS. All participants were instructed about the CS-UCS-contingencies before the experiment. Thus, we most likely measured fear expression rather than fear learning. The present sample has already been compared with a group receiving 30 mg cortisol prior to fear conditioning (Merz et al., in press) revealing no effects of exogenous cortisol on CRs. In the present report, we were interested in endogenous cortisol and its correlation with differential neuronal activation.

Based on previous human studies investigating endogenous (basal or stress-induced) cortisol levels (Jackson et al., 2006; Zorawski et al., 2005, 2006), we expected positive correlations between cortisol concentrations and differential amygdala activation, in particular in men. The CS+/CS− differentiation in the PFC should also be associated with endogenous cortisol either positively or negatively depending on the particular subregion (medial PFC vs. OFC). We had additional specific hypotheses concerning the (para)hippocampal complex and the insula. Heightened cortisol levels consistently influence these structures sex-dependently, as has been shown before (Merz et al., 2010; Tabbert et al., 2010). All these hypotheses are independent of each other. Because of the inconsistent results in the literature regarding women, we exploratively investigated two groups of women with different hormonal statuses. We were particularly interested in two groups of women, which are most different from each other in terms of sex hormone status. More specifically, we tested women in the luteal phase of the menstrual cycle (LU; high endogenous estradiol and progesterone levels) and women taking oral contraceptives (OC; low endogenous estradiol and progesterone levels because of pill intake; cf. Buffet et al., 1998; Kirschbaum et al., 1999).

2. Materials and methods

2.1. General background

The data presented are part of a larger ongoing project investigating the effects of contingency awareness, stress, and sex hormones on fear conditioning. Participants received either 30 mg cortisol (hydrocortisone; Hoechst) or placebo (tablettose and magnesium) orally about 45 min before the fear conditioning protocol. In the present data analysis, only participants, who were informed about the relationship between CS and UCS in advance of the experiment (i.e. instructed fear conditioning; see Tabbert et al., 2011), were included. Further, only participants receiving placebo were included to explore the impact of endogenous cortisol levels on fear CRs. The effects of the exogenous cortisol administration resulting in supraphysiological cortisol levels as well as results of the extinction phase will be reported elsewhere (Merz et al., in press). A group analysis of the same sample has been published previously together with two additional groups (unaware and learned aware participants; cf. Tabbert et al., 2011). This prior analysis was concerned with the differential impact of contingency awareness on fear acquisition, not with sex hormone status or the relation between cortisol concentrations and fear responses.

2.2. Subjects

In total, 50 participants completed the study; 44 were undergraduate students, the remaining six had already graduated. To assess different sex hormone statuses in women, we invited 15 free-cycling women and 15 OC taking women. We also investigated 20 men. Free-cycling women reported to have a regular menstrual cycle and were invited in the luteal phase (LU) of their individual menstrual cycle (3rd to 9th day before the onset of their next menstruation; Buffet et al., 1998). OC women were required to have been taking their birth control pill (only monophasic preparations with an ethinylestradiol component) for at least the last three months. They were tested 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 were, in addition to somatic diseases, in particular endocrine diseases, which can influence hormone concentrations. Inclusion criteria were an age between 18 and 35 and a body mass index (BMI) between 18 and 28 kg/m^2. The mean age for the three sex hormone status groups (men: 24.15 ± 3.08 (standard deviation); LU women: 25.27 ± 3.69; OC women: 23.60 ± 2.13) as well as the mean BMI (men: 23.04 ± 1.94; LU women: 22.79 ± 1.59; OC women: 21.57 ± 1.85) were comparable (both $ps > .05$).

All participants were right-handed as assessed by the Edinburgh Inventory of Handedness (Oldfield, 1971) and had normal or corrected vision. They were instructed to refrain from smoking, food intake, and drinking anything but water for at least two hours before the experiment. Each experimental session was scheduled to begin between 2 and 5 p.m. to guarantee low and relatively stable endogenous cortisol concentrations. At first, participants received a detailed explanation of the procedure in general. All participants gave written informed consent and received at least 25 Euros for their attendance. The study was approved by the ethics committee of the German Psychological Society.

2.3. Conditioned visual stimuli, 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 were gray-colored, had identical luminance, and were presented against a black background for 8 s. Using an LCD projector (EPSON EMP-7250), stimuli were projected onto a screen at the end of the scanner (visual field = 18°) and were viewed through a mirror mounted on the head coil. A custom-made impulse-generator (833 Hz) provided transcutaneous electrical stimulation (UCS) for 100 ms through two Ag/AgCl electrodes (1 mm^2 surface each). Electrodes were fixed to the middle of the left shin and stimulus intensity was set individually using a gradually increasing rating procedure to achieve a level of sensation, which was “unpleasant but not painful”. The onset of the UCS presentation started 7.9 s after CS+ onset (100% reinforcement; delay conditioning). The CS− and the non-CS were never paired with the UCS. Non-UCS was defined as the UCS omission 7.9 s after the onset of the CS−.

The conditioning experiment consisted of an instructed fear phase, an extinction phase, and an implemented two-back task (cf. Merz et al., 2010 and Tabbert et al., 2010 for further details). Twenty trials of CS+ as well as CS− and ten trials of non-CS were presented in the instructed fear phase. Inter-trial intervals (ITI) between the numbers of the two-back task and the geometrical figures lasted 5 s and were randomly jittered between 0 and 2.5 s (i.e. ITI of 5–7.5 s). For each participant, pseudo-randomized stimulus orders were used (cf. Merz et al., 2010).