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Delayed effects of cortisol enhance fear memory of trace conditioning

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Summary Corticosteroids induce rapid non-genomic effects followed by slower genomic effects that are thought to modulate cognitive function in opposite and complementary ways. It is presently unknown how these time-dependent effects of cortisol affect fear memory of delay and trace conditioning. This distinction is of special interest because the neural substrates underlying these types of conditioning may be differently affected by time-dependent cortisol effects. Delay conditioning is predominantly amygdala-dependent, while trace conditioning additionally requires the hippocampus. Here, we manipulated the timing of cortisol action during acquisition of delay and trace fear conditioning, by randomly assigning 63 men to one of three possible groups: (1) receiving 10 mg hydrocortisone 240 min (slow cort) or (2) 60 min (rapid cort) before delay and trace acquisition, or (3) placebo at both times, in a double-blind design. The next day, we tested memory for trace and delay conditioning. Fear potentiated startle responses, skin conductance responses and unconditioned stimulus expectancy scores were measured throughout the experiment. The fear potentiated startle data show that cortisol intake *240 min before* actual fear acquisition (slow cort) uniquely strengthened subsequent trace conditioned memory. No effects of cortisol delivery 60 min prior to fear acquisition were found on any measure of fear memory. Our findings emphasize that slow, presumably genomic, but not more rapid effects of corticosteroids enhance hippocampal-dependent fear memories. On a broader level, our findings underline that basic experimental research and

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clinically relevant pharmacological treatments employing corticosteroids should acknowledge the timing of corticosteroid administration relative to the learning phase, or therapeutic intervention.

1. Introduction

Corticosteroids are released after stress and modulate learning and memory processes via mineralocorticoid and glucocorticoid receptors. These receptors are abundantly expressed in limbic brain areas, like the hippocampus (Reul and de Kloet, 1985). In humans, effects of cortisol on memory have predominantly been investigated for non-associative and distinct emotional stimuli (De Quervain et al., 2009).

Stress is considered to be an important vulnerability factor for anxiety disorders (Korte, 2001). It is generally assumed that anxiety disorders originate from a learned association between a previously neutral or ambiguous event (conditioned stimulus; CS) and an anticipated aversive event (unconditioned stimulus; US) (Mineka and Oehlberg, 2008). Thus, associative fear learning (i.e., discriminative fear conditioning) seems a suitable experimental model to delineate the mechanism by which corticosteroids contribute to the development of anxiety disorders.

Earlier animal and human studies have shown that stress and/or corticosteroids can indeed alter associative fear learning (Rodrigues et al., 2009; Wolf et al., 2012). However, the effects of stress hormones on fear acquisition seem equivocal, even when differences in the employed paradigms, dependent variables, and sex are taken into account: Studies investigating relationships between cortisol and *delay* fear conditioning as measured by skin conductance reported enhanced (Jackson et al., 2006; Zorawski et al., 2005, 2006), impaired (Van Ast et al., 2012; Stark et al., 2006; Wolf et al., 2009), or unaltered acquisition (Merz et al., 2012a, 2013a) in men. In women, both impairing effects (Merz et al., 2013a; Wolf et al., 2009) and no effect of cortisol on fear acquisition (Van Ast et al., 2012; Merz et al., 2012a; Stark et al., 2006; Tabbert et al., 2010) have been reported. Only two studies investigated the relationship between post acquisition cortisol and fear retention, but these studies did not reveal any relationships between cortisol and retention in either men or women (Zorawski et al., 2005, 2006). On the neural level, cortisol seemed not to influence instructed fear conditioning (Merz et al., 2012a, 2013a) neither in men nor in women, but when fear learning was involved (either in a learned aware or an unaware sample), men displayed reduced neuronal fear responses after cortisol application, whereas women taking oral contraceptives exhibit enhanced fear responses on the neuronal level (Merz et al., 2010, 2012b, 2013b; Stark et al., 2006; Tabbert et al., 2010).

Studies investigating the relationship between cortisol and *trace* fear conditioning (or occasion setting) in men reported impairing (Wolf et al., 2012) or enhancing (Kuehl et al., 2010) effects on fear acquisition as measured by % eyeblink responses, enhancing effects as measured by fear-potentiated startle (Van Ast et al., 2012), or no effects as measured by skin conductance (Van Ast et al., 2012). One study suggested impairing effects of cortisol on trace conditioning, but here sex effects were not investigated (Nees et al., 2008). Taken together, the high variety of paradigms (e.g., delay or trace conditioning), dependent measurements (e.g., skin conductance responses vs. startle responses), and participants (men vs. women) used across studies, precludes valid comparisons. In addition, the majority of these studies only focused on fear acquisition, that is, they did not examine long-term memory aspects that are exquisitely sensitive to corticosteroids. Revealing the effects of cortisol on retention of fear might be very relevant to understanding maintenance of fear in anxiety disorders, as opposed to cortisol effects on fear learning or mere fear expression (i.e., in instructed conditioning paradigms). For these reasons, we aimed to target the expression of fear memory as assessed 1 day after fear acquisition.

Corticosteroids are known to affect neurobiological processes in a time-dependent manner (Diamond et al., 2007; Joëls et al., 2006); this may have added to seemingly inconsistent cortisol effects across studies. Shortly after stress, corticosteroids interact with noradrenaline to synergistically promote rapid increases in neuronal activity (Karst et al., 2005, 2010). This effect is most pronouncedly sustained in the basolateral amygdala (BLA) through a nongenomic pathway (Karst et al., 2005, 2010). In humans, such rapid corticosteroid effects have indeed been described for emotion- and arousal-related brain areas, such as the amygdala (Van Marle et al., 2010). They promote habitual, reflex-like behaviour (Schwabe et al., 2010) and attention (Vedhara et al., 2000), at the expense of goal-directed behaviour (Schwabe et al., 2010) and higher cognitive functioning (Elzinga and Roelofs, 2005). Indeed, shortly after stress a vigilance network including the amygdala is activated, as demonstrated with fMRI in humans (see Hermans et al., 2011). During memory encoding after stress, hippocampal and prefrontal cortex activity is generally suppressed (e.g. Qin et al., 2009; Van Stegeren et al., 2010). All in all, most observations in animals and humans agree that rapid cortisol effects — presumably through nongenomic pathways and in interaction with arousal-evoked central adrenergic release — enhance amygdala activity while reducing hippocampal and PFC activity. This may help the organism to focus and subsequently remember the most significant aspects of an event (Roozendaal et al., 2006), at the cost of the more complex, cognitive aspects (see also Joëls et al., 2011; Karst et al., 2010).

By contrast, some hours after stress, slower long-lasting genomic corticosteroid actions develop (De Kloet et al., 2005; Wiegert et al., 2005). Such delayed effects of cortisol on the brain are thought to restore homeostasis following stressful periods (Diamond et al., 2007; Joëls et al., 2006). Although slower genomic effects have not been investigated in fear conditioning studies, other studies indicated that genomic effects promote consolidation (Barsegyan et al., 2010), cognitive self-control (Oitzl et al., 2001), enhance working memory (Henckens et al., 2011), promote sustained attentional processing (Henckens et al., 2012), and