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# Differential impact of the first and second wave of a stress response on subsequent fear conditioning in healthy men

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A B S T R A C T

Stress is a process of multiple neuroendocrine changes over time. We examined effects of the first-wave and second-wave stress response on acquisition and immediate extinction of differential fear conditioning, assessed by skin conductance responses. In Experiment 1, we placed acquisition either close to the (second-wave) salivary cortisol peak, induced by a psychosocial stressor (experimental group, EG), or after non-stressful pretreatment (control group, CG). Contrary to predictions, groups did not differ in differential responding. In the EG only, mean differential responding was negatively correlated with cortisol increases. In Experiment 2, we placed conditioning near the first-wave stress response, induced by a cold pressor test (CPT), or after a warm-water condition (CG). CPT-stress increased extinction resistance. Moreover, acquisition performance after CPT was positively correlated with first-wave blood pressure increases. Data suggest that mediators of the first-wave stress response enhance fear maintenance whereas second-wave cortisol responsivity to stress might attenuate fear learning.

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## 1. Introduction

Stress is a process that involves a cascade of neuroendocrine changes over the course of time. These changes can be subdivided into a first-wave and a second-wave response (Sapolsky, Romero, & Munck, 2000). The *first-wave stress response* involves (among others) increased release of noradrenaline (NA), and corticotropin releasing hormone (CRH) in the brain, as well as increased sympathetic tone with secretion of adrenaline and NA in the periphery. The *second-wave stress response* involves (among others) increased peripheral glucocorticoid (GC) secretion as a result of the activation of the hypothalamus–pituitary–adrenocortical (HPA) axis (Joëls & Baram, 2009; Sapolsky et al., 2000).

Theoretical models have suggested that the effects of stress on emotional learning and memory are determined by the temporal distance between stress exposure and learning (Diamond, Campbell, Park, Halonen, & Zoladz, 2007; Joëls, Pu, Wiegert, Oitzl, & Krugers, 2006). These stress effects involve a dynamic interplay of NA and GC over time (Joëls, Fernandez, & Roozendaal, 2011), acting especially in the basolateral amygdala (Roozendaal, Okuda, Van der Zee, & McGaugh, 2006; Roozendaal, McEwen, & Chattarji, 2009).

Recently, Schwabe, Joëls, Roozendaal, Wolf, and Oitzl (2012) proposed a model integrating the timing perspective (Joëls et al., 2011) with the underlying neural mechanisms (Roozendaal et al., 2009). The Schwabe et al. (2012) model makes the following predictions: If stress is experienced immediately before learning, mediators of the first-wave stress response should promote encoding of emotional material by a synergistic action of NA and fast non-genomic GC effects. Further, mediators of the second-wave stress response – via slower genomic GC effects – should enhance the consolidation of the material learned under stress (Schwabe et al., 2012). If however, stress is experienced long before learning, the first-wave stress response has subsided, but second-wave stress mediators (such as GCs) with their genomic effects are still active during encoding. In this case, stress should suppress the encoding of *new* material (Schwabe et al., 2012). Evidence supporting these predictions is found from the neurofunctional to the behavioral level in animals (Henckens, van Wingen, Joëls, & Fernandez, 2010; Liebmann, Karst, & Joëls, 2009; Pu, Krugers, & Joëls, 2009; Roozendaal et al., 2006, 2009), and humans (Lovallo, Robinson, Glahn, & Fox, 2010; Zoladz et al., 2011).

The model (Schwabe et al., 2012) however, is based on data from inhibitory (passive) avoidance and object recognition tasks in animals, and mainly on declarative memory data in humans. This raises the question, if the predictions are valid for other forms of emotional learning and memory, such as classical fear conditioning. Classical fear conditioning is an influential experimental model to study emotional learning and memory, because it can be studied

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in animals and humans, and because its neural and molecular mechanisms are well understood (e.g., Pape & Pare, 2010). In fear conditioning, an initially neutral stimulus (cue or context) is paired with an aversive unconditioned stimulus (US), capable of eliciting a fear response. After one or more pairings during the acquisition phase, the neutral stimulus becomes a conditioned stimulus (CS), now capable of eliciting conditioned fear responses. In extinction, the CS is repeatedly presented without the US, and conditioned responses decline.

Examining the specific effect of prior stress on subsequent classical fear conditioning is also of clinical relevance. Severe (traumatic) stress can lead to the development of posttraumatic stress disorder (PTSD): a debilitating disorder, characterized by symptoms of re-experiencing, avoidance, numbing and increased arousal. Stress-enhanced classical fear conditioning is used as an animal model of PTSD (e.g., Rau, DeCola, & Fanselow, 2005). Fear conditioning is also proposed as the mechanism behind re-experiencing symptoms in clinical models of PTSD (Ehlers & Clark, 2000) and impaired fear acquisition and extinction are assumed to contribute to PTSD etiology (Mineka & Zinbarg, 2006; Mineka & Oehlberg, 2008). Moreover, PTSD has been associated with enhanced conditionability (Orr et al., 2000), a higher resistance to extinction (Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Orr et al., 2000; see also Lissek et al., 2005), and a poorer recall of extinction memory for classically conditioned fear (Milad et al., 2008).

Animal fear conditioning is modified by stress: Acute (Rau & Fanselow, 2009) and chronic stress (Conrad, LeDoux, Magarinos, & McEwen, 1999) prior to learning facilitated subsequent (+24 h or more) acquisition. Acute stress also enhanced extinction resistance 24 h after stress (Izquierdo, Wellman, & Holmes, 2006; Rau & Fanselow, 2009). Stress immediately after acquisition facilitated the consolidation of fear memory, tested 24 h later (Hui, Hui, Roozendaal, McGaugh, & Weinberger, 2006; see Rodrigues, LeDoux, & Sapolsky, 2009 for a review). Considering the mediators, there is accumulating evidence (reviewed in Rodrigues et al., 2009) that NA facilitates fear acquisition and/or short term memory via β-adrenergic receptors in the amygdala, while GCs facilitate the consolidation, but not the acquisition of fear, thus supporting the differential role of mediators of the first vs. second-wave stress response.

So far, no human study has *directly* addressed the role of timing of stress relative to encoding in fear conditioning by using experimentally-induced stress. Instead, studies using stress-hormone administration were conducted and are reviewed below with regard to their correspondence with the model of Schwabe et al. (2012). Dependent measures range from skin conductance responses (SCR) and level (SCL), fear potentiated startle (FPS), and blood oxygenation level dependent contrast (BOLD) in functional imaging, to subjective ratings of US expectancy (US-expectancy).

When mediators of the first-wave stress response (as NA) are active, we would expect fear encoding to be enhanced. Two studies (Soeter & Kindt, 2011, 2012) examined cued fear conditioning and delayed extinction after 20 mg yohimbine (α2-adrenoceptor antagonist, stimulating central NA activity) vs. placebo 30 min prior to acquisition. Both showed a slower extinction process 48 h later, indicated by FPS. The authors conclude that NA increases resistance to delayed extinction by strengthening the original fear association. Grillon, Cordova, Morgan, Charney, and Davis (2004) blocked β-adrenergic receptors with 40 mg propranolol 60 min prior to acquisition which resulted in impaired contextual fear responses (SCL, subjective arousal) when participants returned to the conditioning context one week after acquisition. Acquisition and retention of cued fear conditioning (SCR, FPS) were unaffected (Grillon et al., 2004). Since there are no results with noradrenergic manipulation *after* acquisition, it is unclear if NA strengthened

encoding or consolidation, or both, in these experiments (Grillon et al., 2004; Soeter & Kindt, 2011, 2012). Propranolol (40 mg) 80 min before extinction of cued conditioning (Bos, Beckers, & Kindt, 2012) impaired extinction in US-expectancy only, while leaving SCR and FPS unaffected.

The second prediction based on Schwabe et al. (2012) is that encoding is suppressed when mediators of the second-wave stress response are active. Correspondingly, functional imaging studies have shown that increase of GC (30 mg hydrocortisone) 15 min (Stark et al., 2006), and 45 min (Merz et al., 2010) prior to cued fear conditioning, impairs differential BOLD-responses during acquisition in healthy men. The same was shown for SCR in one (Stark et al., 2006), but not in later studies (Merz et al., 2010, 2011). The GC effect on conditioned BOLD-responses was not found, when participants were instructed about the CS–US contingency before training (Merz et al., 2011). In women, hydrocortisone improved conditioned BOLD responses (Merz et al., 2010; Stark et al., 2006; Tabbert et al., 2010).

So far, we reported effects of single mediators of the stress response from pharmacological manipulations. This is important, but no substitute for experimentally-induced stress. As stress responses are typically defined by the interplay of multiple mediators, and as GC effects in the amygdala were shown to depend on arousal induced NA-signaling (Roozendaal et al., 2006), single pharmacological manipulations are insufficient. To date there are only three published studies in humans examining the effects of experimental stress induction on fear conditioning and extinction (Bentz et al., 2013; Jackson, Payne, Nadel, & Jacobs, 2006; Zorawski, Blanding, Kuhn, & LaBar, 2006), all using cue conditioning. Exposure to a psychosocial stressor 60 min before conditioning (Jackson et al., 2006) resulted in better acquisition and higher extinction resistance in healthy men, but not in women. However, GC levels of men in this experiment (Jackson et al., 2006) were back to baseline by the time conditioning started, and GCs were not elevated at all in women. Using a three-day protocol, Bentz et al. (2013) placed a physical stressor (cold pressor test, CPT) 20 min prior to delayed extinction on day 2. Here stress impaired retrieval of conditioned fear (measured by US-expectancy) in men, but not in women. However, the conditioning protocol failed to produce fear learning in physiological measures (SCR, heart rate) in any group or sex. Finally, Zorawski et al. (2006) placed a psychosocial stressor after acquisition (day 1) to examine GC effects on extinction trials (i.e., consolidation) on day 2. In men, but not in women, a positive correlation between post-acquisition GC levels on day 1 and fear acquisition (SCR) was found, but a control group was missing, and the same pattern of correlations was found in a previous study without stress (Zorawski, Cook, Kuhn, & LaBar, 2005).

To summarize: Pharmacological studies (Bos et al., 2012; Grillon et al., 2004; Merz et al., 2010, 2011; Soeter & Kindt, 2011, 2012; Stark et al., 2006; Tabbert et al., 2010) have begun to clarify the importance of single stress mediators for human fear conditioning, and results are largely consistent with the predictions of the model by Schwabe et al. (2012). Studies examining the effects of experimental stress induction on fear conditioning in humans are less numerous (Bentz et al., 2013; Jackson et al., 2006; Zorawski et al., 2006), and results are less consistent. Thus, the temporal localization of the learning task, relative to experimental stress induction, should now be explicitly addressed in human fear conditioning. In two experiments, we want to examine the impact of the first-wave vs. second-wave of the stress response on fear conditioning and immediate extinction. Due to reported sex differences in fear conditioning in humans (Bentz et al., 2013; Jackson et al., 2006; Merz et al., 2010; Stark et al., 2006; Zorawski et al., 2005, 2006) and animals (reviewed in Dalla & Shors, 2009), we restricted our experiments to male participants.