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# No Time-Dependent Effects of Psychosocial Stress on Fear Contextualization and Generalization: A Randomized-Controlled Study With Healthy Participants

**Milou S. C. Sep[1,2], Rosalie Gorter[1], Vanessa A. van Ast[3], Marian Joëls[2,4], and Elbert Geuze[1,5]**

## Abstract

The formation of context-dependent fear memories (fear contextualization) can aid the recognition of danger in new, similar, situations. Overgeneralization of fear is often seen as hallmark of anxiety and trauma-related disorders. In this randomized-controlled study, we investigated whether exposure to a psychosocial stressor influences retention of fear contextualization and generalization in a time-dependent manner. The Trier Social Stress Test was used to induce psychosocial stress. Healthy male participants (n = 117) were randomly divided into three experimental groups that were subjected to the acquisition phase of the Fear Generalization Task: (1) without stress, (2) immediately after acute stress, or (3) 2 h after acute stress. In this task, a male with neutral facial expression (conditioned stimuli) was depicted in two different contexts that modulated the conditioned stimuli–unconditioned stimuli (=shock) association (threat, safe). Salivary alpha-amylase and cortisol levels were measured throughout the experiment. After a 24-h delay, context-dependency of fear memory was investigated with an unannounced memory test consisting of the threat and safe contexts alternated with a novel context (the generalization context). Multilevel analyses revealed that participants showed increased fear-potentiated startle responses to the conditioned stimuli in the threat compared to the safe context, at the end of the acquisition phase, indicating adequate fear contextualization. Directly after acquisition, there were no time-dependent effects of psychosocial stress on fear contextualization. Context-dependency of fear memories was retained 24 h later, as fear-potentiated startle responding was modulated by context (threat > safe or novel). At that time, the context-dependency of fear memories was also not influenced by the early or late effects of the endogenous stress response during acquisition. These results with experimental stress deviate in some aspects from those earlier obtained with exogenous hydrocortisone administration, suggesting a distinct role for stress mediators other than cortisol.

## Keywords

context, fear conditioning, fear generalization, stress, sympathetic nervous system, hypothalamic–pituitary–adrenal-axis

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## Introduction

Fear can aid threat detection in the environment. It is well-known, from years of research on classical Pavlovian *fear conditioning*, that both animals and humans quickly learn to associate negative experiences (or unconditioned stimuli; US) with their preceding signals (or conditioned stimuli; CS).[1–3] However, the actual threat emanating from a danger signal is often determined by the environment (or context) in which it occurs (c.f. a lion in the zoo vs. a lion in the wild).

[1]Brain Research and Innovation Centre, Ministry of Defence, Utrecht, the Netherlands
[2]Department of Translational Neuroscience, UMC Utrecht Brain Center, Utrecht University, Utrecht, the Netherlands
[3]Department of Clinical Psychology, University of Amsterdam, Amsterdam, the Netherlands
[4]University of Groningen, University Medical Center Groningen, Groningen, the Netherlands
[5]Department of Psychiatry, UMC Utrecht Brain Center, Utrecht University, Utrecht, the Netherlands

**Corresponding Author:**
Milou S. C. Sep, Universiteitsweg 100, 3584 CG Utrecht, the Netherlands.
Email: m.s.c.sep-2@umcutrecht.nl

In other words, the contextual information can change the predictive value of a CS for the US, by acting as a so-called *occasion setter*.[4–7] Therefore, simple learning of CS–US associations alone is likely insufficient for adaptive responding to continuously changing environments.

Through the process of *fear contextualization*, CS–US associations can be enriched with implicit contextual information to form context-dependent fear memories.[5,8,9] A recent review underlined that the interaction between the hippocampus and amygdala underlies the consolidation and storage of these memories.[10] Recollection of context-dependent fear memories in novel, potentially dangerous, situations, can inform behavior through the mechanism of *fear generalization* (i.e., the transfer of fear to stimuli with perceptual similarities to the original stimulus[11,12]). The generalization of fearful information appears to be mediated by the medial prefrontal cortex.[13]

While fear generalization is highly adaptive, overgeneralization of fear to safe stimuli or environments can predispose to pathological conditions,[5,14–17] like post-traumatic stress disorder,[18] generalized anxiety disorder,[19] and panic disorder.[20] Fear overgeneralization is often referred to as the hallmark of anxiety and trauma-related disorders;[9,13] it has also been suggested that the context-dependency of fear (memories and their extinction) influences the treatability of pathological fear and anxiety.[10,21,22]

Currently, it is not fully understood why and how adaptive fear contextualization changes into overgeneralization in some individuals, but not in others. The identification of factors that modulate these processes could help to understand individual differences in vulnerability to anxiety disorders. A potentially interesting modulator is the (acute) stress response, as it is the main physiological reaction to threatening events, and known to influence learning and memory processes.[23–26] Upon confrontation with a stressor the autonomic nervous system (ANS) is activated, leading to catecholamines release. Shortly thereafter, the hypothalamic–pituitary–adrenal (HPA)-axis becomes active, resulting in enhanced corticosteroid levels for 1 to 2 h. Corticosteroids can bind to receptors in limbic brain areas and induce non-genomic (immediate) and genomic effects, the latter with a delay of >1 h.[23,27,28] Due to their distinct effects on in brain, the rapid ANS and rapid non-genomic corticosteroid effects are considered the immediate effects of stress (generally within 30 min post-stressor), while the genomic corticosteroid effects are considered the delayed effects of stress (>1 h post-stressor).[29,30] Interestingly, we have shown that acute stress and glucocorticoid exposure can influence the context-dependency of neutral and emotional memories, in a time-dependent manner.[25,31] Thus, it was found that the contextualization of information is reduced directly after a peak in cortisol—as in the case of fear memories—while the opposite is seen >1 h after the cortisol peak.

With respect to fear contextualization and context-dependent fear expression, most clinical and preclinical studies have focused on the immediate effects of stress, leaving the delayed effects largely unexplored. In mice, glucocorticoid injections into the hippocampus immediately after fear conditioning (i.e., fear consolidation), increased later fear generalization.[32] In humans, it was found that stressed participants are unable to use situational cues (occasion setters) in a fear conditioning task (10-min post-stress),[33] pointing toward reduced fear contextualization. In agreement, two recent studies in humans showed that the immediate effects of acute stress impair contextual fear conditioning (up to 30-min post-stress) in men and women.[34,35] A previous study with exogenous hydrocortisone administration immediately before acquisition reported impairing effects on context-dependent fear expression in women but enhancing effects on contextualization in men.[8] These sex differences could originate from the interaction between stress and sex hormones in fear acquisition and generalization processes.[36] For example, brain areas involved in the fear neurocircuitry (including amygdala, ventromedial prefrontal cortex, hippocampus), express sex-specific steroid receptors,[37] which allows sex hormones, like estrogens, to modulate responsiveness of the circuitry in fear learning and extinction processes.[38,39] Noteworthy, it has also been shown that the immediate effects of stress and cortisol prior to extinction learning reduce the context-dependency of extinction memories.[40] Mostly based on findings about the immediate effects of stress, it has been hypothesized that stress promotes the overgeneralization of fear, via its effects on pattern separation in the hippocampus.[41,42] Interestingly, the delayed effects of exogenous hydrocortisone were found to enhance hippocampal-dependent fear memories in another paradigm (i.e., trace-conditioning).[43]

The aim of the current project was to investigate the immediate and particularly the currently unknown delayed effects of the endogenous stress response on (i) fear contextualization during acquisition and (ii) subsequent context-dependency or generalization of fear memories during retention. In this project, the immediate effects were investigated 30 min post-stressor offset and the delayed effects 160 min post-stressor offset.[30] Based on the earlier findings, we hypothesized that contextualization of fear is suppressed immediately after stress exposure and enhanced >2.5 h later. Only male participants were included because sex-differences in fear-related processes[36,38,39] and acute stress-reactivity[44] are substantial and previous studies with exogenous hydrocortisone were conducted in males.

## Methods

### Participants

One hundred seventeen healthy male participants were included in this study (age: M (SD) = 24.9 (6.7), range = 18.1–49.3, also see Online Appendix A.1). Sample size was based on a priori power calculations with G*power ($\alpha = .05$, power = .80),[45] using previously reported effect sizes of the delayed (d = .621) and immediate (d = .561) of cortisol on the context-dependency of emotional memories.[31] All participants gave written informed consent and had (1) normal or corrected-to-normal vision, (2) normal uncorrected hearing, (3) a body mass index between 18.5 and 30, and (4) were fluent in the Dutch language. Participants did not (1) use medication known to influence central nervous system or endocrine systems, (2) have speech impairments, (3) have a (history of) psychiatric, neurological, somatic, or endocrine diseases, (4) were not color blind. Additional acute exclusion criteria were checked upon arrival at the institute. If participants had (1) an acute illness, fever, or a severe cold, (2) insufficient sleep during the previous night, (3) smoked within the last 2 h, (4) drank anything other than water or ate within the last 2 h, (5) ingested coffee or any caffeine-containing drink within the last 4 h, (6) used alcohol within the last 24 h, (7) had physical exercise within the last 12 h, or (8) used any recreational drugs within the last three days; appointments were rescheduled. Inclusion/exclusion criteria were checked via a screening questionnaire.

### Stress and Control Manipulations and Measures of Stress (Re)Activity

The Trier Social Stress Test (TSST)[46] was used as stress manipulation (15 min) and the placebo version of the TSST was used as control manipulation.[47] During the TSST[46] participants—after a 3-min preparation period—had to perform a free speech simulating a job interview (5 min), followed by a mental arithmetic task (3 min), in front of a nonresponsive jury while being video- and audio-taped. Participants received verbal instructions ~2 min before the preparation period; and in-between the speech and arithmetic task, there was a ~2-min delay for saliva and questionnaire collection. Altogether, this added up to a 15-min procedure. The placebo-TSST[47] mimics the physical (e.g., standing, speaking) and cognitive load of the TSST, without the uncontrollable social evaluation threat of the TSST. During the placebo-TSST, the participant was alone in a room, while performing a speech and arithmetic task. Timing of the several task elements was exactly the same as the TSST. Salivary alpha-amylase (sAA) and cortisol are frequently used measures of sympathetic nervous system (SNS) and HPA activity, respectively.[48,49] Salivettes® (Sarstedt, Nümbrecht, Germany) were used to collect saliva samples at 14 timepoints during the experimental protocol to measure stress (re)activity (Figure 3; also see Online Appendix A.2). Samples were collected at T-210, T-165, T-160, T-145, T-130, T-100, T-70, T-40, T-30, T-35, T-30, T0, and T30 relative to fear acquisition onset; and at T-30 and T0 with respect to fear generalization onset.

### Fear Contextualization and Generalization

Fear contextualization during acquisition and the tendency to generalize context-dependent fear memories to non-threatening contexts were assessed using the Fear Generalization Task (FGT; Figures 1 and 2) (freely modeled after Mühlberger et al.[50] and programmed in Presentation Version 18.1; Neurobehavioral Systems, Inc, RRID:SCR_002521). The FGT is discussed in brief below (also see Online Appendix A.3).

*Stimuli.* The unconditioned stimulus was an electric pulse (200 μs, 100–400 V), generated by constant current stimulator (DS7A, Digitimer Ltd., Letchworth Garden City, UK). US intensity was calibrated using a previously described shock workup procedure, in which each participant selected a "highly uncomfortable, but not painful" intensity (M: 27.98, SD: 22.93; range: 3–99.9 mA).[51–53] The CS consisted of an image of a Caucasian male in a suit, with a neutral facial expression (CUE). This CUE image, with high similarity to our participants (Caucasian, male), was selected because early perception might be different for ingroup/outgroup faces[54] and fear responses can be stronger toward male (compared to female) cues.[55] Three images of different office rooms were counterbalanced across participants to serve as threat (CTX+), safe (CTX−), and new (G-CTX) context (Figure 1). A startle probe (40 ms burst of white noise of 104 dB delivered via headphones) was used to evoke (fear-potentiated) startle responses (FPS). In total, 91 startle probes (different types: see Task Performance section) and 10 US were delivered during the FGT.

*Task Phases.* The FGT consists of an acquisition and a surprise test phase, separated by a 24-h delay (Figure 1; Online Appendix A.3.2). To reduce initial startle reactivity, both phases began with nine noise-alone habituation trials (NAh-probe; Figure 2(a)). The acquisition phase consisted of 24 trials (four-trial blocks). Half of the trials commenced with the presentation of the CTX+ (i.e., threat trials), the other half with the presentation of the CTX− (i.e., safe trials). The CUE appeared randomly after 6 to 9 s as partial overlay of the CTX. In 10 of the 12 threat trials, the US was presented 0.5 s before the

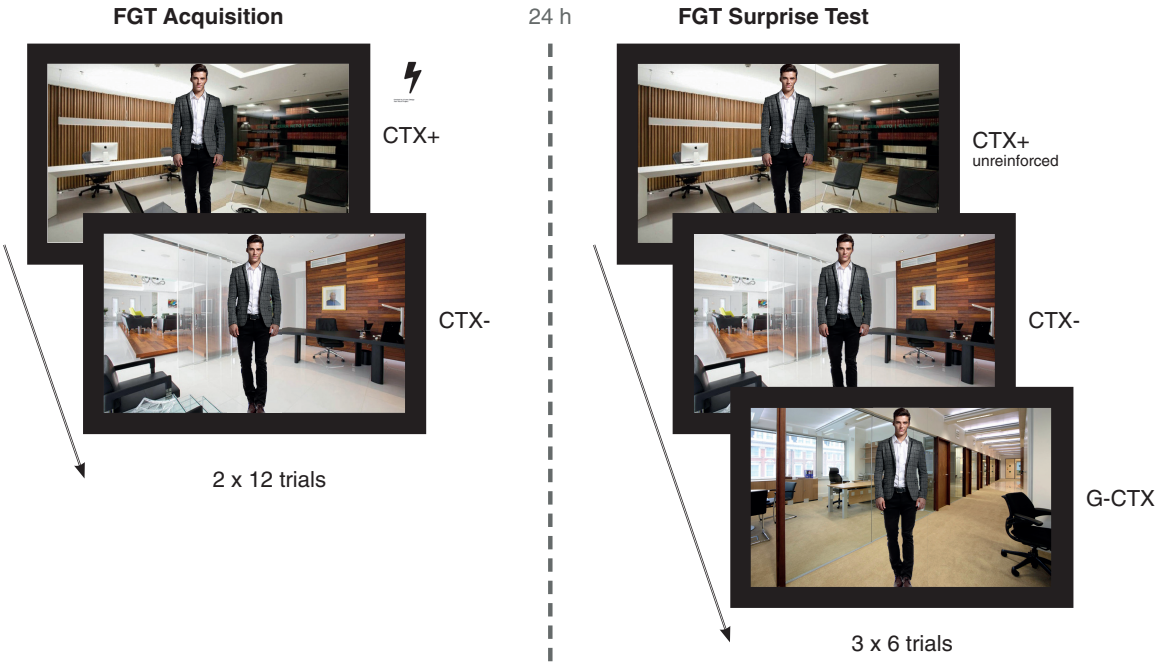


**Figure 1.** The Fear Generalization Task. Twelve threat (CTX+) and 12 safe (CTX−) trials were shown in the acquisition phase of the FGT task on day 1. Twenty-four hours later, participants were surprised with the unannounced test phase of the FGT. In this phase, they viewed six unreinforced threat trials (CTX+ unreinforced), six safe trials (CTX−), and six new trials (G-CTX). FGT: Fear Generalization Task.

CUE off-set (i.e., 83% reinforcement; NB: third and seventh threat trials were unreinforced). The timing of a single acquisition trial is depicted in Figure 2(b). The surprise test phase, 24 h later, contained six unreinforced threat trials (CTX+), six safe trials (CTX−), and six new trials (G-CTX) in semi-random order in six-trial blocks. The timing of a single surprise test trial is depicted in Figure 2(c), importantly no US was delivered in this task phase. During both phases, one startle probe per block was presented during the inter-trial interval (ITI-probe).

*Task Performance.* FPS eyeblink responses to the NAh, CTX, CUE, and ITI startle probes were used to measure FGT performance (see Online Appendix A.3.3 for preprocessing details). In this task, the NAh and ITI probes represent initial and baseline startle responsiveness, the CUE probes reflect fear for a stimulus in the environment (i.e., cue in context) and the CTX probes symbolize fear for the environment itself (i.e., context). FPS responses to the environments (CTX probes) and ambiguous stimulus–environment combinations (CUE probes in G-CTX trials) serve as a marker for fear generalization.

## Experimental Design and Procedure

This study was part of a larger project that also investigated the time-dependent effects of psychological stress on the contextualization of neutral and emotional memories (and for which participants perform another behavioral task). Details about this project and task have been published elsewhere[25] (also see Online Appendix A.4). In this randomized-controlled, single-blind study design, participants were randomly allocated to one of the three experimental groups using an a priori generated list from the random sequence generator of www.random.org. Each experimental group was subjected to two interventions (TSST or placebo-TSST) in a different sequence (Figure 3): (i) delayed-stress group (n = 35): TSST1-placeboTSST2; (ii) immediate-stress group (n = 42): placeboTSST1-TSST2; and (iii) no-stress group (n = 40): placeboTSST1-placeboTSST2. Note the first and second interventions ended, respectively, 160 and 30 min before fear acquisition. Prior and during study participation, all participants were blind to the study aims and experimental groups. This study was approved by the Medical Ethics Committee of the University Medical Center Utrecht and conducted in accordance with the ICH Guidelines for "Good Clinical Practice" and the Declaration of Helsinki.[56]

## Statistical Analysis

Additional information can be found in Online Appendix A.5, and data and code are available via Open Science Framework (https://osf.io/xbt5k/).

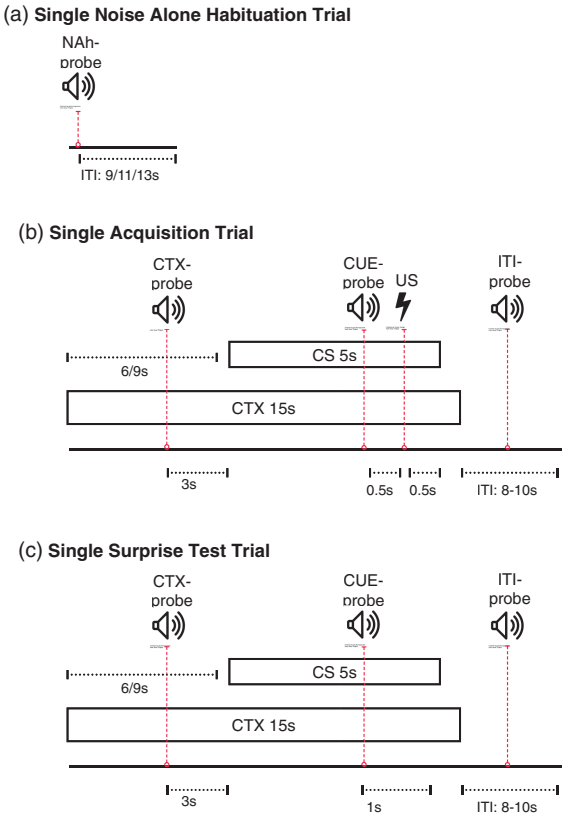


**Figure 2.** Timing of individual trials in the FGT. During the pre-acquisition and pre-test NAh trials, nine NAh-probes were delivered with 9, 11, or 13 s ITI (a). During an acquisition (b) and surprise test (c) phase trial, contexts were shown for 15 s. After 6 or 9 s after context onset, a cue was presented for 5 s. The CTX-probe was delivered 3 s before cue onset and the CUE-probe was delivered 1 s before cue offset. ITI was 8 to 10 s. During the acquisition threat trials (CTX+), an US was presented 0.5 s before cue-offset. NAh: noise-alone habituation; ITI: inter-trial interval; CS: conditioned stimuli.

The effects of the control and stress manipulation were checked with linear mixed models (LMMs) fitted to the sAA and cortisol data, to investigate hormone levels over the course of the experiment. Visual inspection of residual plots did not reveal any obvious deviations from normality of the residuals and homoscedasticity, after log-transformation of the sAA and cortisol values. Group, time, and their interaction were entered as fixed effects with the intercept, and intercepts for participants were entered as random effects in both LMMs. Endocrine levels of experimental groups were compared at each timepoint using Tukey adjusted post hoc pairwise comparisons.

Multiple imputations were used to deal with missing FPS responses (in total 10.4% of the trials, which is common for FPS measures[57]). For all FPS analyses, LMM assumptions were checked and satisfied within each imputed data set, after log-transformation of FPS responses. LMM analyses were also performed within each imputed dataset. For the NAh trials, preceding both task phase, LMMs with Group and Trialnumber as fixed effects and random intercepts for all participants were fitted to ln(FPS). For analysis of the habituation phase, Estimated Marginal Means (EMMs) were calculated for the three experimental groups if the analyses revealed significant (main or interaction) effects of Group on NAh-probes. The influence of experimental group on mean ln(FPS) responses to ITI probes during the acquisition and test phase was analyzed using linear models with Group as fixed effect. To analyze fear contextualization during acquisition and generalization during retention, LMMs were fitted to ln(FPS) responses to CUE- and CTX-probes during (1) the acquisition and (2) surprise test phase. In these models Group, Trialnumber and Trialtype (Threat, Safe, or New) and their interactions were entered as fixed effects with the intercept, the random effects contained intercepts for all participants. If this overall analysis revealed a significant (main or interaction) effects of factor "Trialnumber," together with a significant (main or interaction) effect of Group or Trialtype, mean ln(FPS) levels during the early, mid, and late task-epochs of the acquisition and surprise test phase were calculated. Subsequently, LMMs with fixed effects Group and Trialtype and random intercepts for all participants were fitted to these means. To follow-up significant (main or interaction) effects of Group within a specific task-epoch, the mean FPS of each experimental group within that epoch was estimated by the EMMs.

## Results

### *Manipulation Check: Stress (Re)Activity*

LMMs fitted to salivary ln(sAA) showed a significant group × time interaction ($\chi^2(26) = 215.550$, $p < .001$) and a main effect of time ($\chi^2(13) = 415.682$, $p < .001$; Online Table B.1.1). The TSST reliably increased sAA levels during the intervention, indicated by Tukey adjusted post hoc pairwise comparisons of the experimental groups at each timepoint (Figure 3(a), Online Table B.1.2). At T2, individuals who performed the TSST (delayed-stress group) had higher ln(sAA) levels than participants who performed the placebo treatment in the immediate-stress group ($t(171.513)=2.554$, $p = .031$, $d = .390$) (but not the control group). The second (placebo-)TSST (=TSST2) had similar effects. The participants who performed the TSST at this timepoint (immediate-stress group) had higher ln(sAA) levels than the individuals who performed the placebo-TSST (delayed-stress group (T10: $t(171.513)=-2.912$, $p = .011$, $d=-0.445$) and no-stress group (T9: $t(172.356)=3.670$, $p = .001$, $d = .0.559$; T10: $t(171.513)=3.528$, $p = .002$, $d = .539$). The sAA

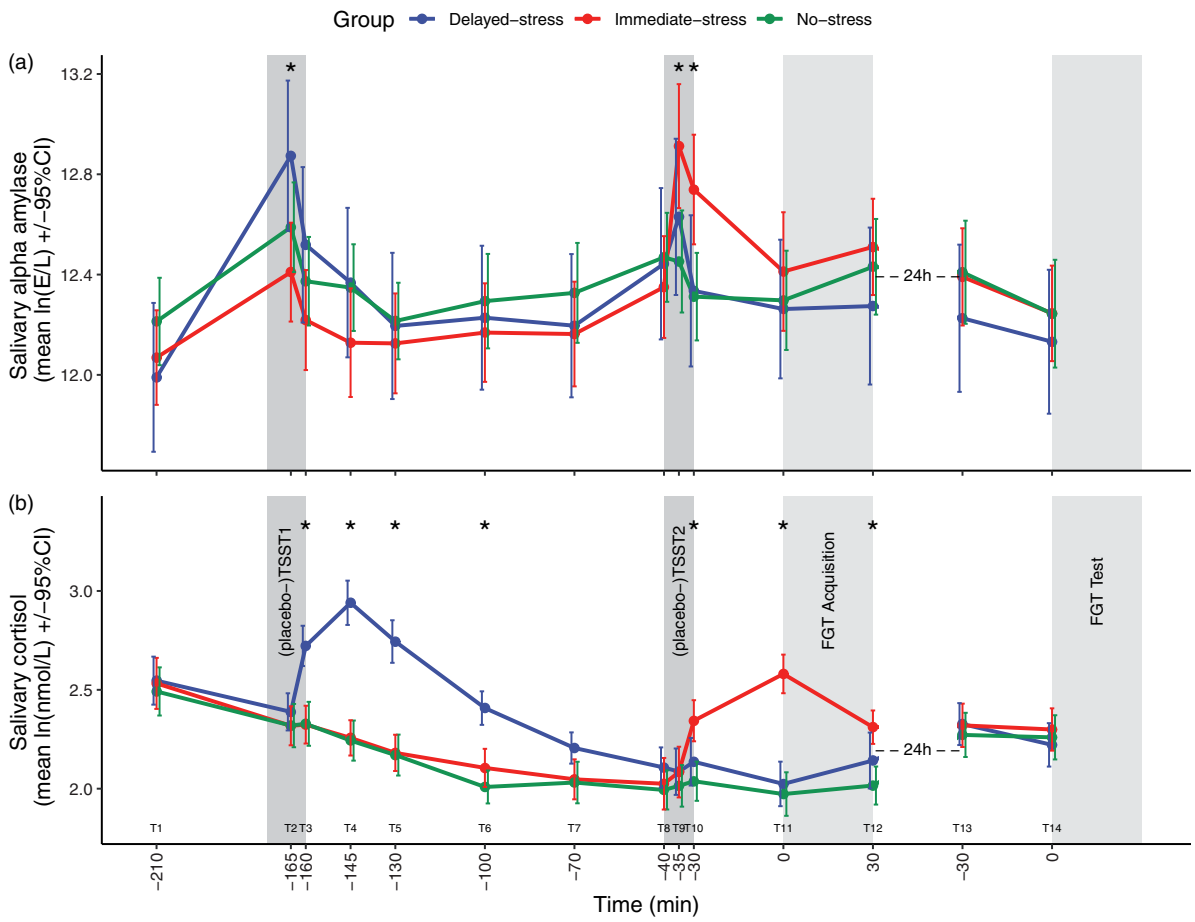


**Figure 3.** The experimental timeline with salivary alpha-amylase and cortisol levels. Mean salivary alpha-amylase (a) and cortisol (b) are shown per experimental group, error bars represent 95% confidence intervals. Natural logarithms were used to transform the endocrine data. Samples T1–T12 were collected at day 1 and samples T13 and T14 were collected at day 2. Eight minutes before T2 (i.e., 173 min before encoding), participants were exposed to the (placebo-)TSST1, at T8 (i.e., 40 min before encoding) participants performed the (placebo-)TSST2. Significant Tukey adjusted post hoc pairwise comparisons between experimental groups ($p < .05$) are indicated. FGT: Fear Generalization Task; TSST: Trier Social Stress Test; CI: confidence interval.

levels did not differ between experimental groups at other timepoints (Figure 3(a), Online Table B.1.2).

LMMs fitted to salivary ln(cortisol) revealed a significant group × time interaction ($\chi^2(26)=473.831$, $p < .001$), and main effects of time ($\chi^2(13)=372.543$, $p < .001$) and group ($\chi^2(13)=20.325$, $p < .001$; Online Table B.1.3). The TSST led to an increase in cortisol levels after the intervention, indicated by Tukey adjusted post hoc pairwise comparisons of the experimental groups at each timepoint (Figure 3(b), Online Table B.1.4). Cortisol levels in the delayed-stress group were significantly higher for 1 h immediately after the TSST1 (T3-T6) than after the placebo-TSST1 in the immediate and no-stress groups (all $p < .001$; Online Table B.1.4). Exposure to the TSST2 also elevated cortisol levels (in the immediate-stress group), compared to exposure to the placebo-TSST2 (in the delayed-stress and no-stress groups (T10-T12, all $p < .05$; Online Table B.1.4).

## *Fear Acquisition*

*Noise Alone Trials.* FPS responses to the pre-acquisition NAh trials are depicted in Figure 4(a). LMMs fitted to these ln(FPS) revealed a main effect of Trialnumber ($Dm = 3.673$, $rm = 3.232$, $df1 = 8$, $df2 = 1353.484$, $p < .001$) and no interaction effects (Online Table B.2.1). The FPS responses to ITI probes are shown in Figure 4(b) and (c). LMM fitted to the mean ln(FPS) to ITI-probes revealed no main effect of group ($Dm = 2.687$, $rm = .043$, $df1 = 2$, $df2 = 111383.053$, $p = .068$).

*Contextualization of Cued Fear.* FPS responses to CUE-probes during acquisition are depicted in Figure 4(b). LMMs fitted to ln(FPS) revealed a significant Trialnumber × Trialtype (Threat vs. Safe) interaction ($Dm = 5.353$, $rm = .135$, $df1 = 11$, $df2 = 76891.243$, $p < .001$), as well as a main effect of Trialnumber

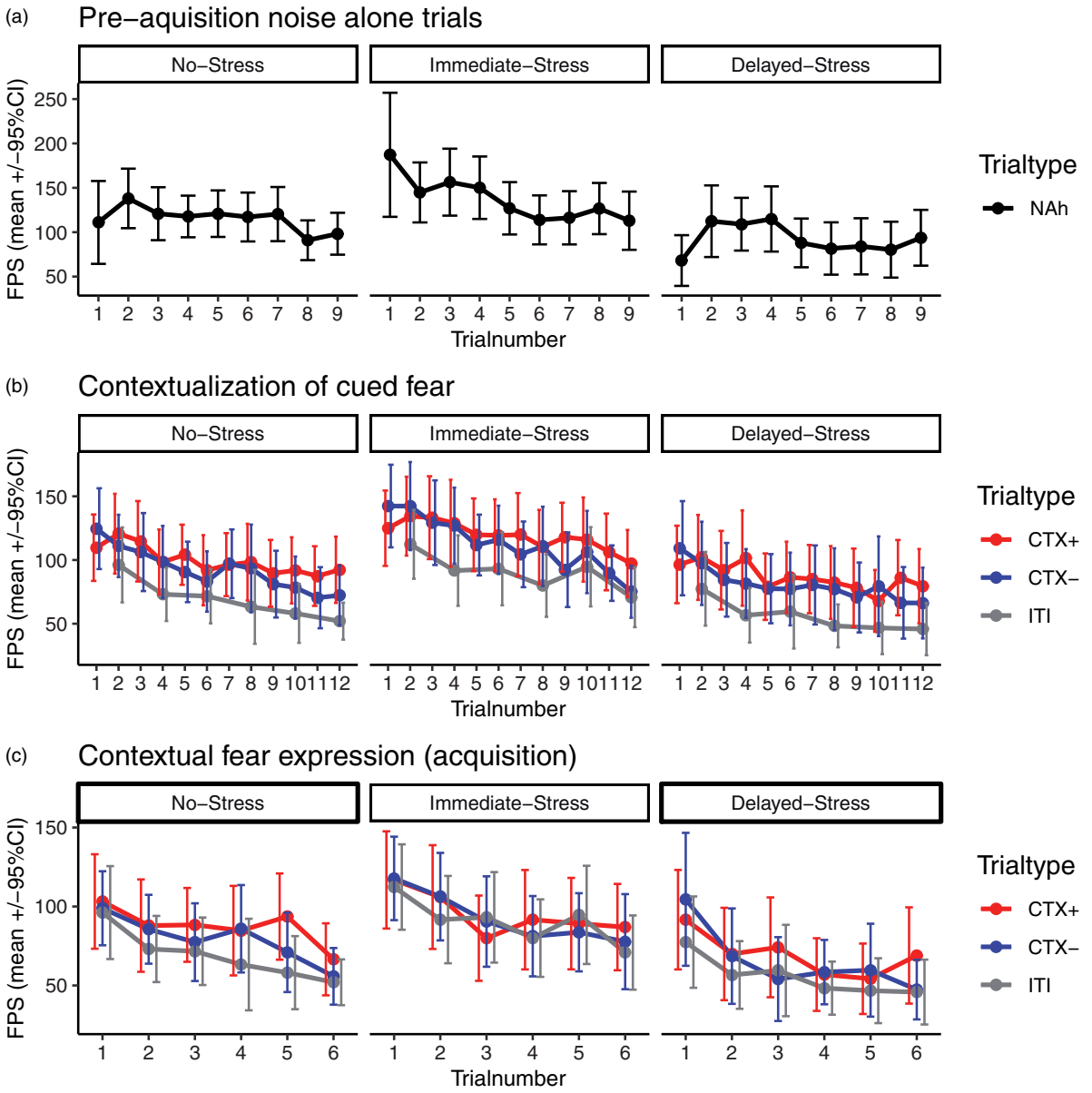


**Figure 4.** Fear contextualization. FPS responses to the pre-acquisition NAh-probes (a), CUE-probes (b), and CTX-probes (c) from the acquisition phase of the FGT, per experimental group. Response to the ITI-probes is depicted in (b) and (c). Error bars represent 95% confidence intervals. At the end of the acquisition phase, FPS responses to CTX+ trials were higher than responses to the CTX− trials. NAh: noise-alone habituation; ITI: inter-trial interval; CI: confidence interval; FPS: fear-potentiated startle.

(Dm = 28.621, rm = .133, df1 = 11, df2 = 78745.138, p < .001) and Trialtype (Dm = 20.509, rm = .120, df1 = 1, df2 = 8040.907, p < .001). To decompose this two-way interaction, mean ln(FPS) in the early, mid, and late epochs were analyzed separately. In the early (Trial1–4) and mid (Trial5–8) epochs, there were no significant effects. In the late epoch (Trial9–12), there was a significant effect of Trialtype (Dm = 4.455, rm = .356, df1 = 1, df2 = 1339.001, p = .035), indicating successful contextualization of cued fear (Threat: pooled-EMM (95% confidence interval (CI)) = 4.050 (3.416–4.685); Safe: pooled-EMM (95% CI) = 3.828 (3.190–4.466). The contextualization of cued fear was not influenced by experimental group (Dm = 2.008, rm = .003, df1 = 2, df2 = 26224040.478, p = .134). Statistics for all analyses are shown in Online Table B.2.1, all pooled-EMMs (95% CI) are shown in Online Table B.2.3.

*Contextual Fear Expression During Acquisition.* Figure 4(c) shows FPS responses to CTX-probes during acquisition. LMMs fitted to ln(FPS) showed a main effect of Trialnumber (Dm = 50.493, rm = .113, df1 = 5,

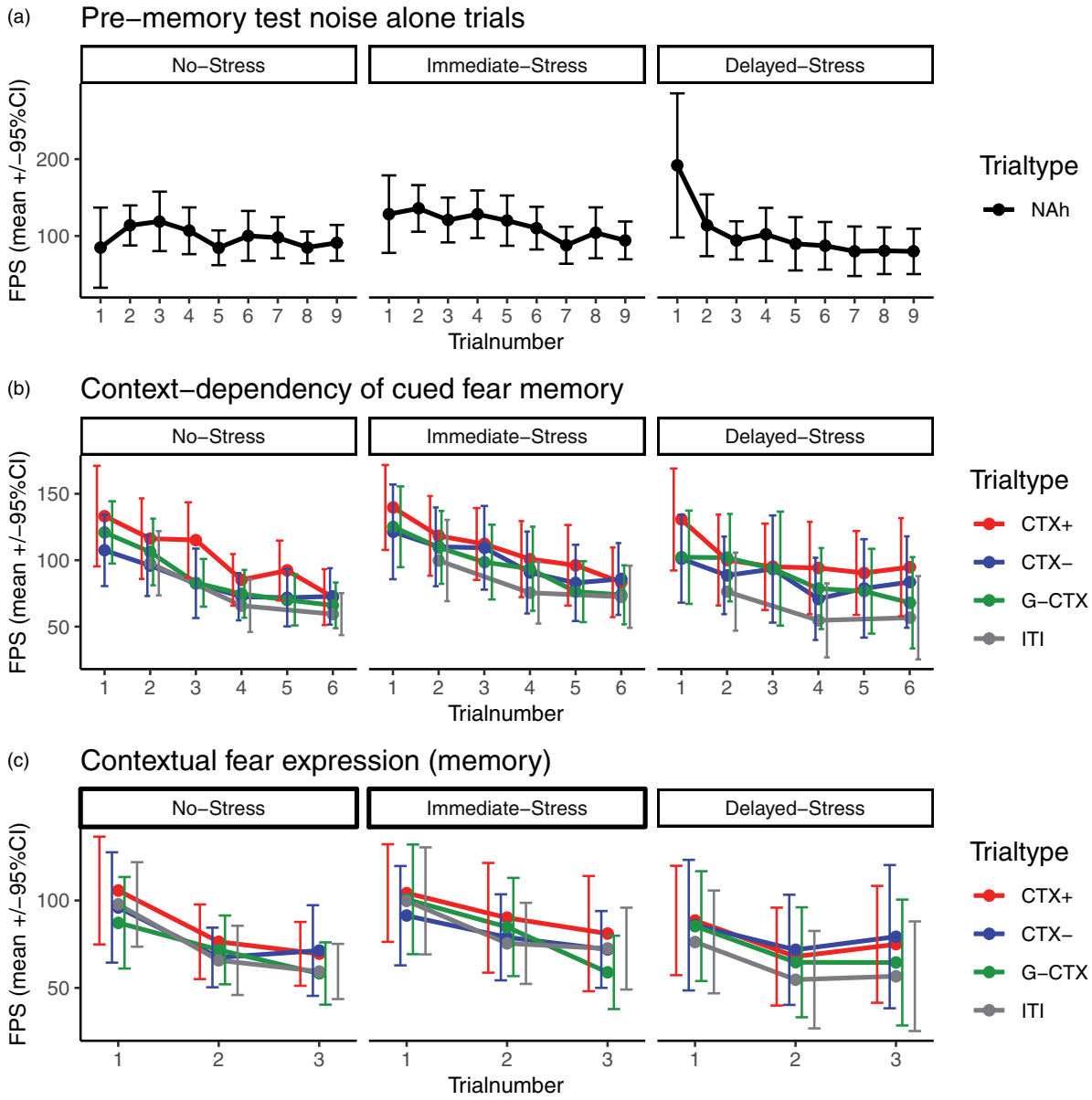


**Figure 5.** Context-dependent fear memory. FPS responses to the pre-test NAh-probes (a), CUE-probes (b), and CTX-probes (c) from the surprise test phase of the FGT, per experimental group. Response to the ITI-probes is depicted in (b) and (c). Error bars represent 95% confidence intervals. FPS responses were the highest in CTX+ trials. NAh: noise-alone habituation; ITI: inter-trial interval; CI: confidence interval; FPS: fear-potentiated startle.

df2 = 47360.576, p < .001) and Trialtype (Dm = 7.310, rm = .134, df1 = 1, df2 = 6582.796, p = .007), there were no interaction effects. Ln(FPS) responses were higher in the threat context (pooled-EMM (95% CI) = 3.885 (3.231–4.540) than for the safe context (pooled-EMM (95% CI) = 3.775 (3.120–4.430). In the follow-up analyses, there were no significant main or interaction effects of group or Trialtype on the early (Trial1–2), mid (Trial3–4), or late (Trial5–6) task-epoch. Contextual fear expression during acquisition was not influenced by experimental group (Dm = 2.402, rm = .005, df1 = 2, df2 = 8324193.439, p = .091). Statistics for all analyses are shown in Online Table B.2.1, all pooled-EMMs (95% CI) are shown in Online Table B.2.3.

## Fear Memory

*Noise Alone Trials.* The FPS responses to the pre-memory test NAh trials are depicted in Figure 5(a). LMMs fitted to these ln(FPS) revealed no significant effects (Online Table B.2.2). FPS responses to ITI-probes are shown in Figure 5(b) and (c). The LMM fitted to the mean

ln(FPS) to ITI-probes showed a marginal significant effect of group (Dm = 2.849, rm = .007, df1 = 2, df2 = 3647519.031, p = .058). Follow-up inspection of the pooled-EMMs suggest that the ln(FPS) responses to ITI probes in the delayed-stress group (pooled-EMM (95% CI) = 3.582 (2.751–4.414) were lower than in the immediate-stress (pooled-EMM (95% CI) = 3.982 (3.187–4.777) and no-stress group (pooled-EMM (95% CI) = 3.992 (3.187–4.798).

*Context-Dependency of Cued Fear Memories.* FPS responses to CUE-probes during the test phase are shown in Figure 5(b). LMMs fitted to ln(FPS) showed a main effect of Trialnumber (Dm = 81.029, rm = .110, df1 = 5, df2 = 49364.528, p < .001) and Trialtype (Threat, Safe, New) (Dm = 27.175, rm = .120, df1 = 2, df2 = 16718.773, p < .001). Follow-up analyses showed main effects of Trialtype in the early (Trial1–2) (Dm = 5.075, rm = .321, df1 = 2, df2 = 3239.173, p = .006), mid (Trial3–4) (Dm = 6.787, rm = .269, df1 = 2, df2 = 4262.980, p = .001), and late (Trial5–6) (Dm = 4.141, rm = .318, df1 = 2, df2 = 3278.485, p = .016) task-epochs. Pooled-EMMs indicate that ln(FPS) responses were the highest to CUE-probes in the threat context in the early, mid, and late epochs, pointing toward context-dependent fear memories (Online Table B.2.3). However, the context dependency of fear memories was not influenced by experimental group (Dm = .473, rm = .002, df1 = 2, df2 = 67494641.497, p = .623). Statistics for all analyses are shown in Online Table B.2.2, all pooled-EMMs (95% CI) are shown in Online Table B.2.3.

*Contextual Fear Expression During Memory Test.* Figure 5(c) shows FPS responses to CTX-probes during the test phase. LMMs fitted to ln(FPS) showed a main effect of Trialnumber (Dm = 43.240, rm = .118, df1 = 2, df2 = 17094.434, p < .001) and no interaction effects. Contextual fear expression during the memory test was not influenced by experimental group (Dm = .888, rm = .004, df1 = 2, df2 = 10882635.914, p = .412). Statistics for all analyses are shown in Online Table B.2.2.

## Discussion

In the current randomized-controlled study, we investigated the time-dependent effect of the endogenous stress response on fear contextualization and subsequent context-dependency of fear memories in healthy males.

### *Stress Induction*

Independent of the order in which participants performed the control and stress manipulation tests, the TSST consistently increased sAA and cortisol levels, while the placebo-TSST did not. The analyses confirm that participants in the immediate-stress group had higher cortisol levels during fear acquisition than participants in the delayed and no-stress groups. Cortisol levels in the delayed group were increased approximately 2 h prior to acquisition but, at the time of acquisition, comparable to the no-stress group. Notably, sAA levels of the experimental groups did not differ during fear acquisition, although sAA levels had been elevated approximately 30 min or 2 h prior to learning in the immediate and delayed group, respectively. No differences in hormone levels between the experimental groups were found when the context-dependency of fear memories was tested. This confirms that we indeed investigated the time-dependent effects of psychosocial stress on fear contextualization and the subsequent context-dependency of these fear memories.

### *Context-Dependent Expression of Fear*

In line with our expectations, participants displayed context-dependent expression of fear to the CS at the end of the acquisition phase, indicating adequate fear contextualization. The FPS response, as expression of fear, was also increased in the threatening context (compared to the safe context) in absence of the CS, which suggests that fear (for the US) (partially) generalized to the threatening environment itself. Adequate fear contextualization led to context-dependent fear memories for the CS in the threat context, measured 24 h later.

### *No Time-Dependent Effect of Psychosocial Stress on Fear Contextualization*

In contrast to our expectations, we found no immediate or delayed effects of psychosocial stress on fear expression, fear contextualization, or subsequent generalization of fear memories. The absence of an *acute effect* of psychosocial stress on fear contextualization in our study contrasts with previous studies that described impairing effects.[8,32–34,41] Possibly, we were not able to demonstrate the fast and immediate effect of acute stress in the current study, due to the relatively long delay between the acute stressor and fear acquisition (Figure 3). In the present study, fear contextualization was measured between 40 and 70 min after acute stress exposure onset (in the immediate-stress group), which is later than in earlier published studies[8,33–35] and slightly overlaps with potential delayed effects. This is a limitation of our design (see "No Time-Dependent Effect of Psychosocial Stress on Fear Contextualization" section) which was optimized to investigate the delayed effects of stress, since these (as opposed to immediate actions) are heavily understudied. In line with this reasoning,

Antov et al. investigated fear (but not context-dependency) and found no effects of acute stress >50 min prior to acquisition in healthy males, while stress 10 min before a cued fear condition task enhanced fear maintenance in their study.[58,59] It has been suggested that cued fear learning is enhanced after acute stress via noradrenergic enhancement of amygdala functioning.[60] Our analyses show that sAA levels (an indicator of SNS activity in this study) were not heightened at the time of encoding in the immediate-stress group. The absence of immediate effects in our study could imply that SNS activity, rather than HPA-axis activity, following acute stress modulates fear contextualization in previous studies. This would be in line with the findings of Antov et al. in a cued-fear conditioning paradigm,[58] but contrasts with earlier studies that identified a direct relation between context-dependency of emotional information and cortisol-responses following hydrocortisone administration[8,31] or psychosocial stress.[35] Importantly, these studies used different tasks to measure context-dependency of emotional information, including an episodic memory task[31] and fear conditioning tasks with skin conductance responses (SCR)[8,35,58] and FPS responses[8] as outcome measures. Since emotional episodic memory involves a different neurocircuit than fear conditioning[61] and SCR reflects different dimensions of fear learning than FPS responses,[62] it is likely that these tasks are also differentially affected by stress or cortisol. In addition, although the cortisol levels of the immediate-stress group were still increased during encoding (allowing immediate cortisol effects to occur), we cannot rule out interference of the delayed effects, which develop approximately 1 h after acute stress.[23,28,30] Another explanation for the discrepancy between the effect of hydrocortisone and psychosocial stress might be that a psychosocial stressor is a learning event in itself (whereas hydrocortisone administration is not).[63,64] As a consequence, processing of (emotional) characteristics from this event could interfere with subsequent learning experiences.[63,64] Our contradictory findings can also be modulated by methodological factors. For example, there could be important sex differences in the effects of acute stress on fear contextualization. We only investigated males in our study, yet are aware of the importance of sex differences. One study found impairing effects of acute exogenous hydrocortisone on context-dependent fear expression (as indicator for fear contextualization) in women, but enhancing effects in men.[8] It has also been shown that the effect of exogenous cortisol on hippocampal responses during differential fear conditioning is different in men and women. When women are using (oral) contraceptives, cortisol enhances differential fear conditioning, while it reduces differential conditioning in men (and free-cycling women).[65] The influence of hippocampal processing in fear contextualization[10] could contribute to a different relation between stress and fear contextualization in men and women. Besides, the acute effects of stress might differentially affect psychological (self-report) and physiological responses to fear. For example, one study found impairing effects of acute stress on self-reported US-expectancy, fear and valence ratings, but no effects on physiological measures in a contextual fear conditioning paradigm.[34]

Despite the fact that our design was optimized to observe potential delayed stress effects, we observed no effect of stress in this experimental group either. This differs from previously reported delayed effect of *exogenous* hydrocortisone administration on trace conditioning, which, similar to fear contextualization, involves hippocampal activity.[43] It also contrasts with the enhancing late effect of stress on memory contextualization observed in a different paradigm.[25,31] Interestingly, in the latter task the delayed effects of cortisol released during an *endogenous* stress response improved the contextualization of *neutral* information[25] whereas *exogenously* administered cortisol improved contextualization of *emotional* information.[31] This suggests that exposure to stress, which not only releases cortisol but also many other stress mediators, may preferentially affect neutral rather than fear-related contextual information, which would explain the lack of effects in the current study. It has been found before that catecholamines, including noradrenaline, can affect cognitive performance in different directions than corticosteroids.[66,67] More specifically, rodent and human studies have shown that immediately after stress, monoamines (and rapid corticosteroid effects via the MR-receptor) facilitate emotional processing by stimulating the amygdala/striatal circuits, at the cost of hippocampal and prefrontal circuits.[29,30] Conversely, corticosteroid actions via the GR-receptor, that develop after >1 h, promote activity in the hippocampal and prefrontal circuits, and facilitate (contextual) memory and reward-based decision making.[29,30]

## Strengths and Limitations

The current study employed a robust design, with a reliable stress induction and confirmation that participants acquired the behavioral task. Moreover, the study was well-powered, which enables us to draw clear conclusions. Yet, there are also several limitations. As mentioned earlier, fear contextualization was measured 40 and 70 min after acute stress exposure onset, which may have been too long a delay to study rapid-onset stress effects alone. Moreover, as this study was part of a larger project, the participants performed another behavioral task before the acquisition and test phase of the FGT. Although tasks combinations have been used before,[31,43]

we cannot completely rule out interference effects. Although most demographics of participants in the experimental groups did not differ, recreational drug use was more prevalent in the no-stress group compared to the immediate-stress group (Online Appendix A.1). In addition, we included only males in our dataset, which precludes our study from making any inferences with respect to gender, as stress and fear-related processes are substantially affected by sex.[36,65,68] Future studies should employ a sufficient number of both male and female participants to shed more light on how gender influences fear contextualization. It is important to emphasize that etiological models of anxiety disorders point out that sensitivity to stressful events varies for the two sexes, and has been associated with higher prevalence of mood and anxiety disorders in women.[69–71] Because of time-constraints, we did not include an unambiguously safe CUE (i.e. a specific CS-image cue), thus slight generalization between the present cue in the different contexts may have taken place. Furthermore, in the current study we used the TSST, a commonly used and robust method for stress induction. However, the TSST was not part of the "to-be-remembered" material, which is the case in real-life situations. Perhaps our results would have been different if a mode of stress induction was used which more accurately reflects experiences in real-life. Finally, since the effect of stress (and cortisol) levels on hippocampus-dependent learning follows an inverted-U-shaped curve,[72–74] it is highly likely that similar dose-dependent effects exists with respect to fear contextualization. It must be noted that methodological factors can influence cortisol levels. For example it is known that 10 mg hydrocortisone administration can lead to higher cortisol concentrations than a psychosocial stress manipulation.[26] Moreover, it has been shown that another stress protocol (the Socially Evaluated Cold Pressor Test) can lead to lower cortisol levels than the TSST that was used in the current experiment.[75] In addition, the TSST can result in different cortisol levels in males, free cycling females and females taking oral contraceptives.[65] Future studies on the dose-dependent relation between stress and fear contextualization might be of particular relevance with respect to the generalizability of our finding to conditions of heightened baseline cortisol levels, like depression or Cushing's syndrome.[76,77]

### *Implications*

As arousal facilitates the encoding of salient details from a stressful experience,[60,78] a healthy endogenous stress response might time-dependently boost the context-dependent encoding of neutral information, thereby leading to a comprehensive memory representation of the event. Interestingly, it has recently been observed that stress indeed enhances memory for both negative and neutral material from the context in which the stressor occurred.[79] The balance between salient and neutral detail encoding might be disturbed by abnormalities in the endogenous stress response; something that may have remained unnoticed in the healthy individuals included in the current investigation but could become apparent when examining a population at risk for psychopathology. Moreover, it is known that early life stress influences HPA-axis functioning, thereby predisposing to stress-related disorders.[80] Our findings might also imply a role for other characteristics than the stress response (alone) in determining why some people (over) generalize fear and others do not. This would agree with a recent meta-analysis in which we showed that the personality characteristic trait anxiety increases fear generalization in healthy humans.[81] Future strategies for prevention and therapy for anxiety and trauma-related disorders could benefit from more mechanistic insight into the factors that influence fear contextualization and context-dependent fear memories.

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## ORCID iDs

Milou S. C. Sep https://orcid.org/0000-0003-4715-7593
Elbert Geuze https://orcid.org/0000-0003-3479-2379

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