Effects of acute stress on exposure therapy outcome and generalization in woman: considering the modulatory role of hormonal contraceptive use

Short Title: The impact of stress on exposure therapy outcome

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Abstract

The administration of glucocorticoids (GC) as an adjunct to exposure might represent a promising strategy to improve exposure therapy outcome in anxiety disorders (AD). The beneficial effects, however, might be sex-dependent and/or further modulated by hormonal factors (e.g., contraceptive usage in women). In the present study, we investigated whether acute stress before exposure therapy affects its efficacy in women using oral contraceptives (OC) relative to free-cycling (FC) women. In addition, possible effects of stress on generalization of therapy effects towards untreated stimuli were examined. Women with fears of spiders and cockroaches were randomly assigned to a stress (n=24) or no-stress (n=24) group prior to a standardized one-session exposure. Acute stress did not influence exposure-induced reduction in fear and avoidance of the treated stimuli (spiders). However, stress led to a less pronounced beneficial exposure outcome for treated stimuli in OC women relative to FC women. This effect occurred on the level of subjective fear and self-report questionnaires at post-treatment (24 hours after exposure) and follow-up (4 weeks after exposure). No effects of stress on generalization of therapy effects towards untreated stimuli (cockroaches) were found. Our findings suggest that OC usage diminishes the beneficial effects of stress on exposure therapy outcome seen in FC women. We present first clinical findings regarding the interaction of stress (and possibly GCs) and OC in exposure therapy of AD. OC intake in women constitutes a crucial factor to be considered in augmentation studies using stress and GCs.

Introduction

Anxiety disorders are highly prevalent, but treatment options are still limited. Exposure therapy can lead to symptom relief, although substantial variation in treatment efficacy is observed across patients [1]. Attempts to increase the efficacy of exposure therapy include the adjunct administration of glucocorticoids (GCs) [2]. GC administration in combination with exposure promotes therapeutic outcome in height, and spider phobia [3, 4]. Such beneficial effects, however, depend on further factors such as the timing [5] and the frequency of GC administration [6].

The mechanism underlying the therapeutic effectiveness of GCs as an adjunct to exposure is not yet fully explored [2, 7]. On the one hand, fear extinction constitutes the central mechanism of exposure therapy [1]. On the other hand, GCs alter fear extinction memories via modulation of hypothalamus-pituitary-adrenocortical (HPA) axis activity [8]. In a similar vein, experimentally induced stress activates the HPA and alters both the acquisition and consolidation of fear extinction memories [9]. However, findings from respective studies in humans assessing the impact of stress and GCs on fear extinction processes have yielded mixed results [8].

We have introduced the STaR (Stress Timing and Relapse) model [summarized in 8] to account for these complex findings in the fear conditioning literature. The STaR model further provides a valuable tool to guide and promote clinical studies examining the effects of GCs and stress during exposure-based therapies. The present study incorporates the general propositions of the STaR model and aimed to investigate the effects of stress - administered with the socially evaluated cold-pressor test (SECPT; [10]) prior to exposure - on exposure therapy efficacy.

Pre-extinction stress can promote subsequent fear extinction learning and extinction retrieval [11, 12, 13] in a sex-dependent manner. Fear extinction is diminished in women who take oral contraceptives (OCs) relative to FC woman; [14, 15]). This suggests modulatory effects of OCs and naturally occurring changes in estrogens and gestagens on fear extinction learning [14-17]. However, the link between contraceptive use, sex hormone status, and exposure therapy efficacy is less well explored. This is surprising, given that women are not only more likely to develop an anxiety disorder [18, 19], but also exhibit greater severity and disability [20] relative to men. Nevertheless, we [21] and others [22] demonstrated that the responsiveness to exposure therapy is less pronounced in female spider-fearful participants taking OCs. It remains to be explored whether stress exerts a differential effect on exposure therapy efficacy in women based on OC use.

Fear extinction learning is a highly context-dependent process [23]. Critically, stress disrupts the contextualization of fear extinction learning [8, 11, 12]. These findings bear specific implications regarding the possible effects of GCs and/or stress on context-dependent generalization of exposure therapy. Insufficient generalization of therapy effects towards unfamiliar contexts might represent a source for fear relapse after successful treatment [24-26]. Interestingly, we have shown that GC administration after exposure has a detrimental effect on generalization of exposure therapy effects across different contexts [5]. Failure to generalize therapy effects to fearful stimuli, which are perceptually and/or conceptually similar to the treated feared stimuli, might equally enhance the probability for relapse [26]. Clinical research on the generalization of exposure therapy effects towards untreated stimuli (see [25]) as well as studies investigating the effects of augmentation strategies on such stimulus-dependent generalization during exposure, however, are rare [27, 28]. To date, no study has investigated whether GCs or stress might affect the generalization of exposure therapy effects towards untreated stimuli.

THE IMPACT OF STRESS ON EXPOSURE THERAPY OUTCOME

To summarize, in the present study we investigated the effects of acute stress on exposure therapy outcome and generalization in women with specific fears of spiders and cockroaches. The study was conducted exclusively in women (either taking OCs or not) to provide further insights into the modulatory effects of OC usage on exposure treatment efficacy. Exposure therapy outcome was operationalized as exposure-induced reductions in fear towards treated stimuli (i.e., spiders) and related symptom relief. Generalization of exposure therapy effects was defined as exposure-induced changes in fear and symptom relief with respect to highly related but untreated stimuli (i.e., cockroaches; adapted from [25]). To ensure comparability, exposure-induced changes towards treated and untreated stimuli were tested by applying identical behavioral (i.e., specific behavioral approach tests (BATs) for spiders and cockroaches) and subjective (i.e., spider and cockroach fear-related questionnaires) measures.

Materials and Methods

Participants

The study was preregistered on ClinicalTrials.gov (Identifier: NCT03505437). Female participants with a fear of spiders and cockroaches were recruited via diverse advertisements (e.g., social media groups and online bulletin boards). Participants with mental disorders - other than a specific fear for spiders and cockroaches - were not invited to the study. Other exclusion criteria involved the presence of neurological and/or endocrine disorders, pregnancy, current pharmacological treatment, and current smoking (> than five cigarettes per month). Finally, participants with a body mass index (BMI) greater than 27 or smaller than 19 kg/m² and shift workers were excluded. Amendments to initial inclusion criteria were conducted to study the stress effects on exposure-based anxiety reductions toward spiders and cockroaches in both, females using OC and FC females. All participants were instructed to abstain from eating, drinking, or smoking and not to engage in any kind of excessive exercise for approximately 90 minutes prior to each of the three sessions.

A total of 104 individuals took part in a pre-experimental telephone screening to check for inclusion/exclusion criteria. Additionally, the fear of spiders questionnaire (FSQ) and its exact adaptation with cockroaches were administered to ascertain the fear of spiders and cockroaches. After this screening, 77 individuals remained eligible for participation. A sum of 29 individuals dropped out of the study for the following reasons: no longer interested in participation ($N = 13$), unable to attend ($N = 4$) or not showing up for their appointment ($N = 12$).

The final sample, therefore, included 48 women who were assigned to either the stress or the no-stress group. The proportion of free-cycling (FC) women and women who were taking oral contraceptives (OC) was matched across the stress and no-stress group. In particular, 15 FC women and 9 OC women were included in the stress group, 14 FC women and 10 OC women in the no-stress group. All FC women had a regular menstrual cycle and were tested only in the follicular phase of their menstrual cycle (i.e., between the 3rd and 9th day after menstruation onset). Additional measures were taken to ensure comparable levels of endogenous sex hormones between FC women and OC women. Briefly, all OC women were tested during their OC intake phase wherein endogenous sex hormone levels are assumed to be comparable to those of FC women in the follicular phase of their menstrual cycle (see [29-30]).

All experimental procedures were approved by the ethics committee of the Faculty of Psychology of the Ruhr University Bochum and carried out in accordance with the Declaration of Helsinki. All participants provided written informed consent and received a compensation of 60€ for completion of the study.

Materials and Measures

Control Variables

Possible differences in anxiety and depression levels between the stress and no stress group were assessed with the Beck Depression Inventory-II (BDI-II; [31]) and both versions of the State-Trait Anxiety Inventory (STAI-T and STAI-S; [32]).

Spider and Cockroach Fear-related Questionnaires

Different aspects related to the fear of spiders and cockroaches were assessed by using a set of questionnaires. We used (1) the German version of the Fear of Spiders Questionnaire (FSQ; [33]) which includes 18 items that are scored on a 7-point Likert scale, (2) the Spider Phobia Questionnaire (SPQ; [34]) which comprises 31 dichotomously coded (i.e., true vs.

false) items, and (3) the Spider Beliefs Questionnaire (SBQ; [35]) which measures dysfunctional beliefs on 48 items according to a scale from 0 (“not at all”) to 100 (“completely”). For fear of cockroaches, the Fear of Cockroaches Questionnaire (FCQ; [36]), which is an exact adaptation of the FSQ but only relating to cockroaches, and a modified version of the SBQ to reflect beliefs about cockroaches (see [37]) were applied. Higher scores reflect a higher level of fear in each of the questionnaires.

Behavioral Approach Test (BAT)

Both fear and avoidance of a spider and a cockroach were assessed with separate and independent BATs prior to the commencement of the first session, at post-treatment, as well as during the follow-up (adapted from [25]). To this end, the fearful stimulus (spiders: *Tegenaria domestica*, 1cm; Cockroaches: *Blaptica dubia*, 4cm) was placed in a transparent plastic container at the far end of a 3m x 3m room. Participants were instructed to enter the room, approach the fearful stimulus as fast as possible, and ultimately place it in a jar in another container which was located 1.5m next to the first container. Participants were instructed to continue their approach behavior until they experienced fear that would be intolerable for continuation.

Both the spider and cockroach BATs were scaled in approach distance which comprised fifteen different steps according to a modified and expanded version of the BAT used by Lass-Hennemann and Michael [38]. The steps ranged from 0 (=refusal to enter the test room) to 14 (=successfully placing the stimulus into a target jar that was positioned in another container).

Subjective Units of Distress Scale (SUDs)

The participant’s subjective fear at the initial and final approach distance during the BAT was recorded (cf. [5]). The SUDs with scores from 0 (=no fear) to 100 (=excessive fear) served as the primary measure of subjective fear (reported verbally) during the BAT and the exposure.

Salivary Cortisol

Salivary cortisol concentrations were measured as an approximation for HPA axis activity [39]. Samples were taken with saliva sampling tubes (Sarstedt, Nümbrecht, Germany) on 11 different time points across the course of the experiment (please refer to Table 1 for a precise overview). Chemiluminescence immunoassay with high sensitivity (IBL International, Hamburg, Germany) was used to measure salivary cortisol concentrations. The intra- and inter-assay coefficients of variance for cortisol were both below 8.0%.

Socially Evaluated Cold-Pressor Task (SECPT)

Participants were exposed to either the stress or the control condition of the SECPT (adapted from [10]). Participants in the stress group were instructed to place their dominant hand, including their forearm, into a small tub which contained ice water with a temperature between 0° and 3°C for a duration of three minutes. An additional experimenter was present during the procedure, observing and videotaping the participant during the procedure. If participants were not able to tolerate the cold water for the duration of three minutes, they were instructed to place their arm above the water in the repository. Participants in the control group had to place their dominant hand into warm water with a temperature of 36°C to 37°C; they were neither filmed nor observed. To assess activation of the sympathetic nervous system, blood pressure (i.e., systolic and diastolic (mmHg)) was assessed at nine times in total. These referred to three times before (baseline), during (peak), and after (post) the procedure. Mean values were computed for each assessment time. After the procedure, participants had to indicate on a scale from 0 (i.e., “not at all”) to 100 (i.e., “very much”) how difficult, unpleasant, stressful, and painful the experience was felt (ratings adapted from [10]).

Exposure Treatment

The exposure session consisted of the first eight steps of a standardized fourteen-step fear hierarchy (steps increased in difficulty; see [40]). The experimenter assessed individual levels of fear (SUDs) by questioning the participant during the performance of each step.

Each step needed to be performed until the participant reached a SUDs rating of 30 or lower. The exposure session was terminated after 45 minutes or once the participant had completed all steps.

Experimental Design and Procedure

The study comprised three experimental sessions (see Figure 1), all of which were conducted in the afternoon (1 to 5 p.m.) to reduce daytime-related variability in stress hormone secretion. The first session started with two BATs (first spider BAT, then cockroach BAT) and the completion of the BDI-II, STAI, as well as the spider and cockroach-fear related questionnaires (i.e., SPQ, FSQ/FCQ, SBQ/CBQ). After that, participants had to complete either the cold water (i.e., stress group) or the warm water (i.e., no-stress group) condition of the SECPT. Thereafter, participants received a brief psychoeducation about specific phobia and subsequently engaged in the in-vivo exposure, which commenced exactly 20 minutes after participants had immersed their hand into the water. The second session took place 24 hours later and involved a spider BAT and both the spider and the cockroach fear-related questionnaires. Session 3 took place four weeks later and comprised both BATs as well as the completion of the spider and the cockroach fear-related questionnaires. At the end of session three, participants were debriefed and received their compensation.

Statistical Analyses

Statistical analyses were carried out in IBM SPSS Statistics for Macintosh, Version 25.0 (Armonk, NY: IBM Corp.). Pre-exposure participant characteristics and stress ratings were compared using a series of two-way ANOVAs with the between-subjects factors *stress* (vs. no-stress) and *OC* (vs. no-OC). Blood pressure as well as salivary cortisol were analyzed with mixed ANOVAs with the within-subjects factor *time* (salivary cortisol: baseline vs. 25 min after SECT; blood pressure: baseline vs. during vs. after the SECPT) and the between-

subjects factors *stress* and *OC*. Exposure-induced changes in outcome measures (i.e., BAT score, subjective fear during the BAT, questionnaires) were also analyzed with mixed ANOVAs with three levels of the within-subjects factor *time* (pre, post, follow-up). Note that analyses of the BAT score and subjective fear at the initial and final approach distances comprised only two levels (pre and follow-up) when cockroaches were considered. A result was considered significant at $p < .05$.

Results

Pre-exposure Participant Characteristics

As displayed in Table 2, all experimental groups were comparable in age, BMI, and their scores on the BDI-II and the STAI-T before exposure (all main and interaction effects, $F_{(1,44)} < 1.66$, $p > .2$). Most importantly, groups displayed no pre-treatment differences in the spider- and cockroach-fear related questionnaires (all main and interaction effects: $F_{(1,44)} < 2.34$, $p > .13$) as well as in subjective fear at the initial approach distance to the spider and the cockroach during the pre-treatment BAT (all main and interaction effects: $F_{(1,44)} < 1.07$, $p > .3$). However, as indicated by a significant main effect for OC (all $F_{(1,44)} > 12.01$, all $p = .001$; all other effects: $p > .52$), we found that OC women showed less approach behavior during the pre-treatment BAT with the spider ($M = 4.79$, $SD = 1.40$) and the cockroach ($M = 5.00$, $SD = 1.92$) relative to FC women (spider: $M = 6.69$, $SD = 2.04$; cockroach: $M = 7.17$, $SD = 2.07$).

Manipulation Check: Stress response

For two participants, cortisol concentrations were undetectable, resulting in a sample size of 23 participants in both the stress and no-stress group. Stress induction was successful as indicated by a significant time x stress interaction in terms of cortisol concentrations ($F_{(1,42)} = 7.57$, $p = .009$; all other main and interaction effects: $p > .28$). Analysis of simple effects showed that cortisol concentrations were equal in both the stress and no stress group at

baseline (i.e., $p=.452$). Twenty-five minutes after stress induction, we observed a difference in cortisol concentrations between the stress and no-stress group ($p=.047$) with an increase in cortisol concentrations in the stress ($p=.015$) but not in the no-stress group ($p=.177$), cf. Table 2.

Assessments of blood pressure (cf. Table 2) also indicated successful stress induction (*systolic blood pressure*: main effect for time, $F_{(2,88)}=38.83$, $p<.001$, main effect for stress, $F_{(1,44)}=8.8$, $p=.005$, and a time x stress interaction, $F_{(2,88)}=35.38$, $p<.001$, all other main and interaction effects: $p>.18$; *diastolic blood pressure*: main effect for time, $F_{(2,86)}=54.01$, $p<.001$, main effect for stress, $F_{(1,43)}=6.90$, $p=.012$, and a significant time x stress interaction, $F_{(2,88)}=22.35$, $p<.001$; all other main and interaction effects: $p>.08$). Breaking down the significant time x stress interaction indicated that there was no difference between the stress and no-stress group prior to and after the SECPT with regard to both systolic (baseline: $p>.67$; post: $p>.06$) and diastolic blood pressure (baseline: $p>.48$; post: $p=.28$). Yet, as expected, the stress group had significantly higher systolic and diastolic blood pressure relative to the no-stress group during the SECPT (all $p<.001$).

In line, assessments of the subjective stress scale (cf. Table 2 for descriptive statistics) showed that the stress group rated the SECPT experience as more difficult, stressful, painful, and unpleasant than the no-stress group (main effect for stress: $F_{(1,43)}>129.61$, $p<.001$; all other effects: $p>.42$).

Effects of Stress and OC on Exposure Success: Spider BAT

As indicated by a significant main effect for time ($F_{(1.56, 68.56)}=97.33$, $p<.001$) regarding the BAT score, exposure was effective in enhancing participants' approach behavior towards spiders. Furthermore, OC women showed less approach behavior during the BATs on all three assessments (main effect for OC: $F_{(1,44)}=13.77$, $p=.001$). However, we did not observe any main effect for stress or any interaction effects for the BAT score (all $p>.4$). Interestingly,

at the initial approach distance of the BAT, we found that the main effect for time ($F_{(2,88)}=79.63, p<.001$) was qualified by a significant three-way time x stress x OC interaction ($F_{(2,88)}=3.14, p=.048$). As shown in Figure 2, analyses of simple effects showed that OC women reported more fear at post-treatment ($p=.031$) and follow-up ($p=.026$) relative to FC women in the stress group. This effect did not emerge in the no-stress group (post: $p=.879$, follow-up: $p=.938$). No other main or interaction effects were observed for the omnibus tests (all $p>.24$).

Exposure also promoted fear reduction at the final approach distance of the BAT over the assessments (main effect for time: $F_{(2,88)}=7.92, p=.001$). This improvement was equally evident in all experimental groups (i.e., no other main or interaction effects; all $p>.23$).

Spider Fear-Related Questionnaires

All analyses revealed a significant main effect for time (all $F_{(2,88)}>37.88, p<.001$), demonstrating a beneficial effect of exposure on reduction of spider fear on all questionnaires (Figure 3). For the FSQ, we further observed a main effect for OC ($F_{(1,44)}=5.55, p=.023$), which indicated higher scores in OC women. Of note, regarding the SBQ, we also attained a two-way stress x OC ($F_{(1,44)}=4.16, p=.047$) and a three-way time x stress x OC interaction ($F_{(2,88)}=3.83, p=.025$). Similar to the results for fear at the initial approach distance, breaking down the latter interaction with simple effects showed that OC women in the stress group had higher scores on the SBQ at post-treatment ($p=.009$) and follow-up ($p=.004$) relative to FC women (Figure 3). This was not the case in the no-stress group. No other main or interaction effects were observed for the three omnibus tests (all $p>.056$).

Effects of OC and Stress and Exposure Generalization: *Cockroach BAT*

The analysis of approach distance from pre-treatment to follow-up revealed a significantly higher approach distance at follow-up (main effect for time: $F_{(1,44)}=54.19, p<.001$), underscoring a generalization of treatment gains. The same analysis yielded a main

effect for OC ($F_{(1,44)}=10.6$, $p=.006$), but no main effects for stress or any interactions were observed (all $p>.48$). Fear at the final approach distance of the BAT did not reduce over time ($F_{(1,43)}=3.25$, $p=.078$) and there were no main effects for OC, stress, or any interaction (all $p>.5$). However, a significant reduction in fear at the initial approach distance emerged from pre-treatment to follow-up (main effect for time: $F_{(1,42)}=50.1$, $p<.001$; Figure 2), which was not subjected to any group differences (i.e., all other main and interaction effects, $p>.25$)

Cockroach Fear-Related Questionnaires

For the cockroach related FCQ as well as for the CBQ, we found significant main effects for time (both $F_{(2,88)}>17.43$, $p<.001$) over the three assessments (Figure 3), indicating that the untreated fear of cockroaches was significantly reduced across the experiment. There were no other main or interaction effects across both measures (all $p>.08$).

Discussion

We have investigated the effects of acute stress on exposure therapy outcome and generalization in female participants with a fear of spiders and cockroaches. Acute stress, administered via the SECPT prior to exposure, did not influence exposure-induced reduction in fear and avoidance for the treated stimuli (spiders). Furthermore, no effects on generalization of exposure effects towards untreated stimuli (cockroaches) were evident. However and importantly, when taking OC status into account, effects of stress on exposure therapy outcome for the treated stimuli were found. More specifically, stress led to a less pronounced exposure outcome for treated stimuli in women taking OC relative to FC women highlighting the importance to consider sex hormones as potential moderator in stress effects on exposure success.

The present study extends previous augmentation studies using GCs as an adjunct to exposure therapy. So far, it was demonstrated that adding GCs (leading to supraphysiological cortisol levels) prior to exposure with specific fear stimuli and situations is more effective in reducing respective fear and avoidance relative to placebo [3, 41]. Similarly, a higher endogenous cortisol level during exposure is more effective in reducing fear of spiders than exposure with lower levels of endogenous cortisol [38]. In contrast to these studies, the present results suggest that acute stress (and the accompanying physiological cortisol

increase) did not promote exposure-induced reduction of fear and avoidance towards the treated stimuli in female participants overall, but specific in OC women.

A growing body of research related to the effects of stress and GCs on fear-related memories [2, 42] exists, providing a theoretical framework for examining the effects of stress on exposure therapy efficacy. Exposure therapy might constitute an intervention during which fear-related memories are being activated and extinguished [7]. Among other studies, investigations showing a functional link between fear extinction learning and exposure therapy outcome support this assumption (e.g. [43, 44]). GCs might facilitate extinction learning during exposure, or alternatively, inhibit the activation of full-blown fear-related memories in the presence of fear-provoking stimuli [7]. According to this model, GCs and/or stress potentially support new inhibitory learning during exposure therapy [24].

Fear conditioning studies suggest that stress before extinction training leads to a stronger extinction memory [8, 12]. However, the effects of stress on extinction memories are further dependent on sex and hormonal factors [8, 29, 30]. In particular, fear extinction performance differs between men and women, but fear extinction memories in women seem to further depend on changes in menstrual cycle phase and/or contraceptive use (for a review, [15]). Translating these findings to the exposure therapy setting, one might expect that both GCs and stress may influence exposure outcome depending on the sex of participants and/or the use of hormonal contraceptives. These factors have not been assessed systematically in previous therapy studies [3, 4] or the studies were performed exclusively with women taking OC [38]. However, we and others showed that OC use diminishes the exposure-induced benefit in female participants with spider phobia [20, 21].

In the present study, effects of acute stress on exposure therapy outcome towards treated stimuli was less pronounced in women taking OC. In particular, in the stress group, OC women showed less exposure-induced fear reduction towards treated stimuli, both at post-treatment and follow-up, relative to FC women. The latter was evidenced on different

outcome measures (i.e., subjective fear during the spider-BAT and self-reported spider fear). This is partially in line with previous observations [21, 22], although we found this effect only in the stress-exposed group. The latter might be due to differences in the therapy protocol or sample characteristics used in the respective studies. Nevertheless, the detrimental effect of OC use on exposure-efficacy is in line with findings from basic research showing that OC usage diminishes cortisol effects on emotional learning in women [29, 30]. Based on the current state of research one might conclude that GC administration and/or stress facilitates inhibitory learning during exposure in FC women, but to a lesser degree in women taking OCs [45].

Notably, our findings that stress affects exposure-induced changes in fear towards treated stimuli depending on OC status were restricted to subjective outcome measures. The absence of significant findings on the behavioral level, however, is in line with reports from existing studies using GC as an add-on to exposure [3]. One might conclude that stress and GCs affect mainly aberrant fear-processing underlying subjective fear [46]. Alternatively, the absence of stress effects on approach behavior in the spider-related BAT from pre- to post-treatment was due to a ceiling effect (see also [5]). In particular, FC woman showed a relatively high increase in approach distance in the spider-related BAT from pre- to post-treatment. This might have prevented the detection of any additional effects of stress on the level of BATs.

Another important aim of this study was to investigate possible effects of stress on generalization of exposure-therapy effects towards untreated stimuli [25]. Studies investigating processes governing generalization of exposure therapy effects towards untreated stimuli are highly clinically relevant, yet extremely scarce [47]. We developed novel approaches to the study of stimulus-dependent generalization effects in the exposure therapy setting [5, 25]. We further showed that exposure therapy for spider fear is associated with not only a reduction of fear-related responses towards spiders, but also a reduction of

fear towards untreated stimuli (cockroaches), which share common fear-evoking characteristics with spiders [25]. Interestingly, in the present study we replicated this finding by showing a generalization of therapy-effects across different fear-evoking stimuli. All participants showed increased approach behavior towards both treated and untreated stimuli after exposure, irrespective of group and/or OC status. However, we did not find main effects of stress and/or stress x OC status interaction for generalization of therapy-effects. We conclude that stress and/or OC status did not influence generalization of therapy-effects towards untreated stimuli.

Findings from basic research suggest that stress promotes generalization of extinction memories across different contexts [11] and abolishes context-dependent fear renewal [12]. Stress, thus, might be a promising strategy to support the generalization of therapeutic effects from the treatment context to other contexts by counteracting relapse phenomena after extinction training [23, 48]. Our findings suggest that this conclusion does not apply for the generalization of therapeutic effects from the treated to other untreated stimuli. A parsimonious explanation might be that the latter process differs from the process underlying context-dependency in fear extinction. The transfer of therapeutic effects from the treated to untreated stimulus might be related to stimulus-based extinction generalization [49]. The contribution of stress and GCs to stimulus-based extinction generalization is less well explored [50]. Thus, in order to understand how stress and GCs affect generalization of therapy-effects across different exemplars of the treated stimulus [25-27] or across treated and untreated stimuli [24], further mechanistic studies on stimulus-based extinction generalization would be valuable.

The proportion of FC women and women using OCs was only matched and not equally distributed across the stress and no-stress group, leading to quite small sample sizes. This somehow limits the interpretation of our results. Direct comparisons between results from the present study and findings from pharmacological studies using GCs are missing.

Notably, application of stress as an adjunct to exposure therapy might be a promising alternative to GC administration since the implementation of pharmacological enhancement in routine clinical care (in particular in outpatient centers) may be difficult. Nevertheless, our data highlight the importance of considering OC status when interpreting the effects GCs and stress on exposure therapy efficacy. A growing body of research suggest an important role of OC status when examining the impact of stress- and GCs on fear extinction [45]. We present here first clinical findings regarding the interaction of stress/GCs and OC in exposure therapy of AD.

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Conflict of interest

The authors declare no conflict of interest.

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Figure Legends

Figure 1. Outline of the experimental design and main outcome measures. Two BATs (spider and cockroach) and spider and cockroach-fear related questionnaires were presented at pretreatment. Thereafter, participants received the cold water (i.e., stress group) or the warm water (i.e., no-stress group) condition of the SECPT. All participants subsequently engaged in the in-vivo exposure, which commenced exactly 20 minutes later. At post-treatment (24 hours later) the spider BAT and both the spider and the cockroach fear-related questionnaires were presented. At follow-up (four weeks later) both BATs as well as the spider and the cockroach fear-related questionnaires were presented again.

Figure 2. Mean self-reported fear (SUDS) at the initial approach distance: a.-b.) SUDS during the spider related BAT from pre- to post-treatment and follow-up for the stress and no-stress group; c.-d.) Mean self-reported fear (SUDS) at the initial approach distance of the cockroach related BAT from pretreatment to follow-up for the stress and no-stress group. Abbreviations: FC, free-cycling; OC, oral contraceptive.

Figure 3. Pre-, post-treatment and follow-up assessment scores on spider and cockroach fear-related questionnaires. a.-d.) Mean FSQ and FCQ scores in the stress and no-stress group; e.-h.) Mean SBQ and CBQ scores in the stress and no-stress group; i.-j.) Mean SPQ scores in the stress and no-stress group. Abbreviations: FSQ, Fear of Spiders Questionnaire; SPQ, Spider Phobia Questionnaire; SBQ, Spider Beliefs Questionnaire; FCQ, Fear of Cockroaches Questionnaire; CBQ, Cockroach Beliefs Questionnaire; FC, free-cycling; OC, oral contraceptive.

Figure 1. Outline of the experimental design and main outcome measures.

Spider

Cockroach

Stress

No-stress

Table 1. Times of salivary cortisol measurements.

Sample	Experimental Session	Salivary Control Sample Times
1	Session 1	Start of session 1
2	Session 1	25min after BAT onset
3	Session 1	Arrival at the stress laboratory
4	Session 1	After questionnaire completion / Before stress induction
5	Session 1	25min after stress induction
6	Session 1	25min after exposure treatment session start
7	Session 1	End of exposure treatment
8	Session 2	Start of session 2
9	Session 2	25min after BAT onset
10	Session 3	Start of session 3
11	Session 3	25min after BAT onset

Note. The most relevant cortisol measures in this paper were sample number 4 and sample number 5. These correspond to Baseline (Sample 4) and Post-Stress induction (Sample 5).

Table 2 Overview of participant characteristics, salivary cortisol, blood pressure and stress ratings.

	No stress						Stress					
	FC women (n=15)		OC women (n=9)		Total (n =24)		FC women (n=14)		OC women (n=10)		Total (n =24)	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
<i>Demographics</i>												
Age (years)	22.73	3.54	24.67	3.08	23.46	3.44	23.93	2.84	24.50	3.66	24.17	3.14
BMI (kg/m ²)	22.03	2.70	21.87	2.10	21.97	2.45	22.71	3.20	21.21	2.01	22.09	2.81
STAI-T	40.07	7.54	37.33	8.11	39.04	7.70	41.00	11.52	40.90	10.09	40.96	10.72
STAI-S	46.80	10.04	43.89	7.54	45.71	9.12	44.07	14.32	50.30	9.23	46.67	12.61
BDI	9.40	7.08	5.56	5.79	7.96	6.77	9.77	8.25	10.00	5.44	9.87	7.02
<i>Salivary cortisol (nmol/l)</i>												
Baseline	3.85	2.58	2.92	1.39	3.48	2.21	3.08	2.15	2.76	1.33	2.94	1.81
25 min after	2.80	1.85	2.30	1.10	2.60	1.59	4.39	3.00	4.48	5.10	4.43*	3.94
<i>stress</i>												
<i>Blood pressure</i>												
<i>Systolic</i>												
(mmHg)												
Baseline	104.53	8.43	110.63	12.28	106.82	10.24	106.38	8.24	111.17	10.74	108.38	9.45
Peak	105.64	9.40	108.37	10.16	106.67	9.56	125.05	15.47	129.87	19.41	127.06**	16.99
Post	103.16	9.70	107.15	9.71	104.65	9.69	109.60	9.22	112.23	12.30	110.69	10.44
<i>Diastolic</i>												
(mmHg)												
Baseline	59.84	5.77	67.44	9.37	62.69	8.06	63.57	7.71	66.83	7.43	64.85	7.60
Peak	65.18	8.10	69.56	8.35	66.82	8.30	79.81	12.76	83.44	11.11	81.23**	12.02
Post	62.20	7.53	66.78	8.01	63.92	7.87	66.69	7.85	67.89	11.25	67.16	9.10
<i>Stress ratings</i>												
Difficulty	1.33	3.52	2.50	7.07	1.74	4.91	78.57	21.43	82.00	20.44	80.00**	20.64
Unpleasant	7.33	18.70	1.25	3.54	5.22	15.34	82.86	20.16	79.00	29.61	81.25**	24.01
Stressful	2.00	7.75	2.50	7.07	2.17	7.36	67.14	26.73	70.00	24.94	68.33**	25.48
Painful	0.00	0.00	0.00	0.00	0.00	0.00	85.00	22.10	82.00	23.94	83.75**	22.42

* $p < .05$ ** $p < .001$ compared to the no-stress group