Biological Psychology 166 (2021) 108210

Contents lists available at ScienceDirect

Biological Psychology

journal homepage: www.elsevier.com/locate/biopsycho

Exposure to acute stress affects the retrieval of out-group related bias in healthy men

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A R T I C L E I N F O

Keywords:
Extinction learning
Fear conditioning
Racial bias
Retrieval
SECPT
Stress hormones

A B S T R A C T

Individuals have a tendency to show enhanced vigilance to groups of which they themselves are not a member. Stress can up-regulate hypervigilance towards threatening stimuli and was shown to promote the reinstatement of out-group related biases in a previous study conducted in women only. The current study examines how exposure to acute stress affects the retrieval of out-group related extinction biases in male participants. Results showed that men exerted a specific out-group related bias at the beginning of extinction training indexed by higher skin conductance responses (SCRs) towards out-group faces, while stress led to a return of this extinguished out-group bias. Specifically, the stress group showed higher SCRs towards out-group faces during retrieval compared to the control group and the bias index was negatively related to post-stress cardiovascular recovery. These results indicate the important interaction between stress and intergroup bias in fear conditioning, along with a potential modulation of sex.

1. Introduction

In the perspective of preparedness theory, fear-relevant stimuli such as spiders and snakes are more readily associated with aversive events compared to the fear-irrelevant counterparts (e.g., flowers or birds). Specifically, humans showed rapid fear acquisition (i.e., one trial learning) and high resistance to extinction for these prepared stimuli, presumably due to their underlying evolutionary significance (Ohman and Mineka, 2001, Seligman, 1971).

A social analog of prepared stimuli proposed by Olsson and colleagues (2005) can be seen in racial out-group faces, which are also considered to serve as a signal of built-in threat but in a social aspect. Out-group faces were found to resemble response patterns observed for fear-relevant stimuli, showing an amplified response during fear conditioning paradigms (Navarrete et al., 2009, Navarrete et al. 2012, Olsson, Ebert, Banaji, & Phelps, 2005, Golkar, Castro, & Olsson, 2015, O'Donnell, Neumann, Duffy, & Paolini, 2019). An influential example was the observation of impaired extinction learning in Caucasian participants when black faces were used as conditioned stimuli (CS) and in African-Americans when white faces served as CS, indexed by enhanced CS+/CS- differences for out-group faces during extinction (Olsson et al. 2005). Indeed, people have a deeply-rooted tendency to attune to their group membership status (Brewer, 1999) and show vigilance to groups of which they themselves are not a member (Riek, Mania, & Gaertner, 2006). This out-group prejudice/bias can be demonstrated in both explicit attitudes like low trust and also in more implicit measures like reaction times from implicit association tests (Payne, 2001, Vorauer, 2012, Kubota, Peiso, Marcum, & Cloutier, 2017, Cottrell and Neuberg, 2005). In ancestral environments, higher levels of caution and hostility for out-groups helped humans to better protect themselves and their offspring from outside dangers (Kaya, 2015), while in modern society, this out-group prejudice, especially involving racial aspects, may be implicated in xenophobia and racial discrimination (Faulkner, Schaller, Park, & Duncan, 2004, Kelly, Faucher, & Machery, 2010).

A very important factor that may regulate this out-group bias is stress. Stress causes a coordinated response of the hypothalamic-pituitary-adrenocortical (HPA) axis and the sympathetic nervous system, which interact with each other under the modulation of the central nervous system (Chrousos, 2009). Indeed, prior research indicated acute stress to reduce the perceived trustworthiness for out-group faces (Salam, Rainford, van Vugt, & Ronay, 2017). Additionally, HPA axis activity indexed by the stress hormone cortisol was positively related to

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https://doi.org/10.1016/j.biopsycho.2021.108210
Received 22 July 2021; Received in revised form 14 October 2021; Accepted 15 October 2021
Available online 22 October 2021

subjective prejudice when anticipating to interact with out-group members (Bijleveld, Scheepers, & Ellemers, 2012). The amplification effect of stress on out-group prejudice may be related to the general effects of stress on promoting vigilance, at the cost of the executive network (Hermans, Henckens, Joels, & Fernandez, 2014), or in other words inhibiting our control system and shifting our behavior to a more habitual and automatic manner (Schwabe and Wolf, 2013). Particularly in the fear conditioning paradigm, pre-retrieval stress can substantially impair the recall of the inhibitory extinction memory trace and promote the relapse of initial fear (Meir Drexler, Merz, Jentsch, & Wolf, 2019). This may extend to the implementation of out-group faces as CSs manifesting as the potential detrimental stress effect on the extraction of safety signals from foreign ethnic groups.

Indeed, we previously observed that stress increased fear conditioned responses towards out-group faces during reinstatement, particularly for the out-group CS- expected to serve as safety signal (Merz, Eichholtz, & Wolf, 2020). This finding illustrates the impact of stress on retrieval of security experiences linked to out-group members, thus posing challenges to overcome out-group prejudice under stress.

However, our previous study (Merz et al., 2020) only included women, so, the relevant conclusion may not be easily generalized to men, especially given the potentially complex interactions between stress and sex hormones in emotional learning processes (Merz and Wolf, 2017). More importantly, with regard to the out-group response bias, men and women may have inconsistent manifestations due to differentiated evolutionary roots (Navarrete, McDonald, Molina, & Sidanius, 2010). Specifically, according to the assumption derived from the male warrior hypothesis (McDonald, Navarrete, & Van Vugt, 2012), men have historically been the primary agents of intergroup counter-attack in humans. Thus, men's persistence of a conditioned fear response towards out-group members might be associated with aggression and dominance, while women's bias against out-group men may be more related to fear and avoiding sexual coercion from out-group men (Navarrete et al. 2010, McDonald et al. 2012). Accordingly, the sex-dependent impact of stress on out-group biases may also diverge, such as fight-or-flight vs. tend-and-befriend behavior (Taylor *et al.* 2000). Nevertheless, the influence of stress on fear and extinction retrieval of out-group faces in men is yet unknown. It is thus of major interest to investigate this stress-amplifying effect on out-group fear recovery in a sample of men to further elucidate the underlying mechanisms regarding the stress x racial bias interaction and potential sex differences.

In the current study, we employed a similar experimental design as before (Merz et al., 2020) but in a male sample, in which Caucasian participants acquired and extinguished fear associations for both in-group (Caucasian) and out-group (Moroccan) faces via a typical differential fear conditioning paradigm on the first day. On the second day, exposure to a stress or control condition preceded the retrieval test.

According to our previous results in women (Merz et al. 2020) and findings revealing stress to reduce trustworthiness and enhanced subjective prejudice for out-group members basically originate from male participants (Bijleveld et al. 2012, Salam et al. 2017), we hypothesize that in the current study stress can generally amplify the fear response for out-group faces during retrieval in healthy men. On the other hand, due to possible differences in the manifestation of the out-group bias between men and women (Navarrete et al. 2010), we hypothesize that out-group-related fear conditioning processes in men do not necessarily converge with the pattern observed in women, and may exhibit a unique stress-related manifestation.

2. Materials and methods

2.1. Participants

Forty-four healthy, medication-free, Caucasian (German) male university students were recruited to take part in a two-day experiment. Individuals who reported 1) chronic or acute illnesses, 2) regular intake of medicine, 3) current medical or psychotherapeutic treatment, 4) drug use including smoking (more than five cigarettes/month), 5) body mass index (BMI) < 18 kg/m^2 or > 27 kg/m^2, 6) age < 18 years or > 35 years, 7) no German descent until grandparents, 8) working in shift work or vaccination during the last two weeks prior to the experiment, 9) traveling to a country with a time difference > 5 h or 10) blood donation in the last month were excluded via a pre-experimental telephone interview.

Participants were required to abstain from alcohol the day before the experiment and between the two experimental sessions. Besides, eating or drinking anything but water 90 min or any extra physical exercise should be avoided before the beginning of the experimental sessions. Participants could either receive partial course credits or 25 Euros as compensation. All procedures complied with the Declaration of Helsinki and were approved by the ethics committee of the Faculty of Psychology at the Ruhr University Bochum.

Four participants were excluded from all analyses because of quitting day 2 of the experiment ($n = 3$) or malfunctioning of the electrical stimulation ($n = 1$), leaving a final sample of 40 men.[1] The sample size of at least 17 per group for a repeated-measures analysis of variance (ANOVA) was derived using G*Power 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007), assuming the effect size from a meta-analysis regarding stress hormone effects on memory retrieval ($d = -0.49$; Het, Ramlow, and Wolf (2005)) to achieve a 95% power to detect a significant CS × in-/out-group face × stress interaction during retrieval with an α-level of 0.05. In the current study, 20 participants were assigned into the stress and 20 into the control group. There were no significant differences in age (Control: 24.15 ± 3.34 years, Stress: 23.30 ± 3.74 years, $t_{(38)} = 0.13$, $p = 0.894$) or BMI (Control: 22.92 ± 2.06 kg/m^2, Stress: 23.23 ± 2,33 kg/m^2, $t_{(38)} = 0.46$, $p = 0.651$) between the two groups.

We collected participants' previous experience with people of different cultures (including Germans, Russians, Arabs, Chinese, and Black African), as well as the characteristics of their ethnic environment for the designated in-/out-group (including the number of German and Arab neighbors, colleagues, close friends, romantic appointments and partnerships). For all these quantitative indicators, we conducted a repeated-measures ANOVA with the within-subjects factor group belongingness (in-group / out-group) and the between-subjects factor group (stress / control). In our group of German participants, the German (in-group) condition was significantly higher than the Arab (out-group) condition (all main effects of group belongingness: $p_s \leq 0.044$). There were no significant differences regarding these ethnic environment characteristics between the stress and the control group (all main and interaction effects with group: $p_s \geq 0.198$). Additionally, participants underwent a racial identification task regarding the used CS faces. All participants successfully judged the in-group faces (German faces) as white (Caucasian) and the out-group faces (Moroccan face) as non-Caucasian.

2.2. Materials and stimulus presentations for fear conditioning

The stimulus materials and response collection equipment are consistent with our previous report (Merz et al., 2020). Specifically, four neutral male facial pictures (with comparable valence ratings, 3.21 ~ 3.65) were used as conditioned stimuli (CS) from the Radboud Faces Database (Langner *et al.* 2010), in which two Caucasian faces represented the in-group (picture codes: Rafd090_23, Rafd090_33), while the other two faces were Moroccan faces representing the out-group for the

[1] Due to a technical error, SCRs data for one specific participant were not recorded during fear acquisition training. This individual was not included in SCR analysis of fear acquisition training. The other variables of the individual were complete, including all core indicators, so the participant was still included in all remaining analyses.

participants (picture codes: Rafd090_69, Rafd090_70). Here, male faces were selected because the out-group bias was typically observed when male faces were used as target faces (Navarrete et al. 2009, Navarrete et al. 2010).

During fear acquisition training, each of the four CS faces was presented eight times. Both CS+ (in-group and out-group) were followed by an unconditioned stimulus (UCS) in five out of eight trials (62.5% reinforcement rate), whereas the in-group and out-group CS- were presented eight times alone with no UCS shock accompanied. During subsequent extinction training as well as day 2 re-extinction, all CS were shown eight times without any pairing with the UCS.

The stimulus orders were pseudo-randomized, in which the first and last CS+ trials (both in-group and out-group CS+) were reinforced during fear acquisition training, and no more than two consecutive presentations of a CS+ or CS- were allowed in general. To ensure fear learning to start as early as possible, the first trial was always an in-group or out-group CS+ and the second trial was always an in-group or out-group CS- during fear acquisition training. Stimulus presentation orders and CS allocation were matched between the stress and the control group. Allocation of the in-group and out-group CS+ and CS- was counterbalanced and also matched between the stress and the control group.

Each CS picture was presented for 6 s on a 19-inch computer screen positioned approximately 50 cm in front of the participants. A black screen with a white fixation cross (located at the level of the eyes of the CS faces) was shown with 15 s inter-trial intervals. Visual stimuli were presented using Presentation® software (version 18.0, Neurobehavioral Systems, Inc., Berkeley, CA, www.neurobs.com).

A transcutaneous electrical stimulation served as UCS occurring 5.9 s after the onset of the in-group and out-group CS+. A constant voltage stimulator (STM200; BIOPAC Systems, Inc.) delivered 100 ms electrical stimulation via two Ag/AgCl electrodes filled with isotonic electrolyte medium (Synapse Conductive Electrode Cream, Kustomer Kinetics, Inc., Arcadia, CA) fixed to the middle of the left shin. The intensity of the electrical stimulation was individualized to be "unpleasant but not painful" via a gradually increasing rating procedure. Stimulation electrodes remained attached during all phases of the fear conditioning procedure, but only delivered electrical stimulation during fear acquisition training.

2.3. Ratings and skin conductance responses

Valence and arousal ratings for CS pictures were collected at different time points (day 1: baseline, after fear acquisition training, after extinction training, day 2: baseline, after re-extinction) to reflect evaluative conditioning dynamics using the Self-Assessment Manikin (Bradley and Lang, 1994). Specifically, the valence rating asks about the degree to which individuals feel pleasant with the targeted face within a scale from 1 (very unpleasant) to 9 (very pleasant). The arousal rating asks about the degree to which the face makes the individual being aroused within a scale from 1 (calm and relaxed) to 9 (very excited).

Skin conductance responses (SCRs) were collected during all fear conditioning phases using Ag/AgCl electrodes filled with isotonic electrolyte medium (Synapse Conductive Electrode Cream) attached to the participants' hypothenar on the non-dominant hand. Signals were recorded with a sampling rate of 1000 Hz and a low pass filter of 10 Hz via a commercial SCR coupler and amplifying system (MP150 + GSR100C, BIOPAC Systems, Inc; software: Acqknowledge 4.2). For offline analyses, a high-pass filter with a cut-off frequency of 0.05 Hz was applied to raw SCR data. The conditioned SCRs were defined as the trough-to-peak amplitude of the largest deflection (minimum amplitude threshold: 0.01μS) starting within a window of 1–6.5 s after CS onset (Lonsdorf et al. 2017, Merz et al. 2020). The natural logarithm was applied to SCR data (LN(1 +μS)) in order to attain a normal distribution. Due to a technical failure, SCR data from one participant in the stress group are missing for fear acquisition training only.

2.4. Stress manipulation and measurements

Participants were randomly assigned to either the Socially Evaluated Cold-Pressor Test (SECPT; $n = 20$) or a non-stressful control procedure ($n = 20$; Schwabe, Haddad, and Schächinger (2008)). In the SECPT, participants were required to immerse their right hand and wrist in a basin filled with ice-cold water (0–2 °C) for a maximum time of 3 min. In the meanwhile, video recording and monitoring was conducted by an unknown, reserved acting female experimenter. Control group participants immersed their right hand into warm water (36–37 °C) for 3 min without videotaping and monitoring.

Salivary cortisol concentrations as measures of HPA axis activity were sampled at three time-points: 5 min before the start of the stress or control procedure (baseline), + 10 min, and + 25 min after the end of the stress procedure, to confirm stress induction. Saliva samples were collected using Salivette sampling devices (Sarstedt, Nümbrecht, Germany) and stored at − 20 °C until analyses. Free cortisol concentrations were analyzed on a Synergy2 plate reader (Biotek, USA) using commercial enzyme-linked immunosorbent assays (ELISAs; free cortisol in saliva; Demeditec, Germany) according to the manufacturer's instructions. Intra- and inter-assay variability were both less than 10%.

Systolic and diastolic blood pressure was collected as indicator of SNS activity via the Dinamap vital signs monitor (Criticon, Tampa, FL; cuff placed on the left upper arm). The blood pressure before, during, and after (5 min later) hand immersion into ice-cold or warm water was recorded three times at each measurement point and the respective mean was used for the analyses. Besides, participants were required to report their subjective stress, pain and unpleasantness after the stress and control manipulation with a scale ranging from 0 (not at all) to 100 (very much; ratings adopted from Schwabe et al. (2008)).

2.5. General procedure and timeline

In order to control for the circadian cortisol rhythm, experimental sessions started between 12:45 p.m. and 5 p.m. on two consecutive days.

On day 1, participants were first informed about the details of the experiment (application of electrical stimulations, SCR measurement, stress induction, saliva sampling) and could ask further questions. Then, they provided written informed consent, filled out demographic questionnaires and rated CS valence and arousal. After that, participants were instructed that throughout the experiment, if a face is safe, then this face will always be safe. If the face is coupled with the electrical stimulation, it may or may not happen again after this particular face is presented.

Electrodes for SCR recordings and electrical stimulation were attached and the intensity of the electrical stimulation was determined. After that, fear acquisition training started with in-group and out-group CS+ partially followed by the UCS, whereas the in-group and out-group CS- were never followed by the UCS. Between fear acquisition and extinction training, CS valence and arousal were rated again. During subsequent extinction training, all CSs were no longer paired with the UCS. At the end of day 1, CS valence and arousal ratings were collected again.

On day 2, about 24 h later (M = 24 h 12 min, SD =1 h 17 min), participants rated CS valence and arousal again and were then exposed either to the stress or control procedure. Salivary cortisol and blood pressure data were collected as described above to confirm the stress manipulation. Approximately 10 min after stress offset, re-extinction started with presentations of all four CS again not coupled with the UCS. In particular, the first CS block (i.e., the first CS+ in-group face, CS+ out-group face, CS- in-group face and CS- out-group face) of re-extinction was considered to represent retrieval of the fear conditioned and extinguished associations. The SCRs were recorded during the whole re-extinction phase, and the final CS valence and arousal ratings were performed after the end of re-extinction (see Fig. 1 for the general experimental procedure).

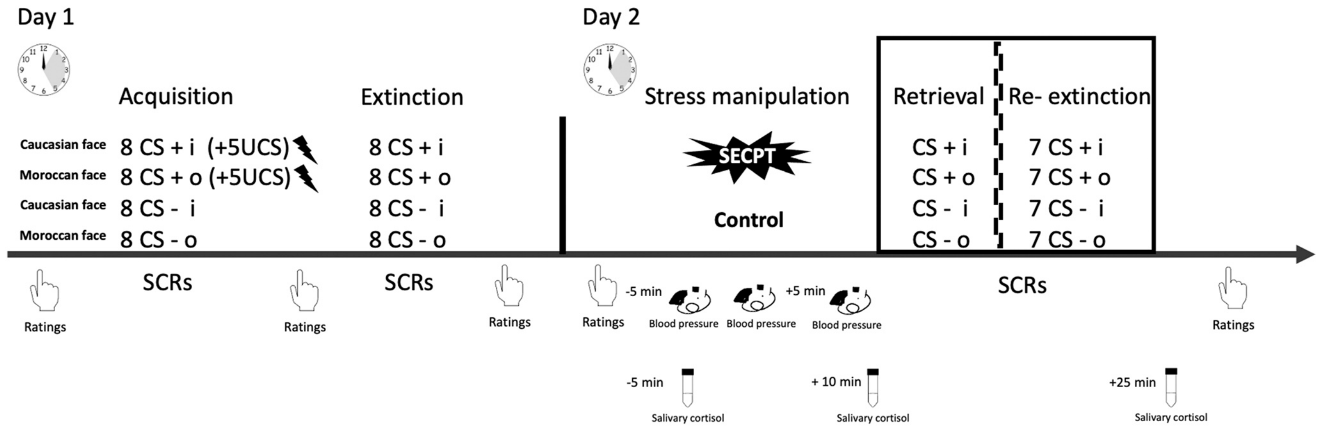

Fig. 1. General experimental procedure. The study consisted of two experimental days. On day 1, fear acquisition and extinction training took place. CS type and in-group (i)/out-group (o) faces were within-subjects factors. Acute stress manipulation was performed on day 2, systolic and diastolic blood pressure as well as saliva cortisol concentrations were measured at different time points before and after stress induction. The first trial of each CS of the subsequent re-extinction phase was the main focus of the analyses determining retrieval of fear conditioned and extinguished associations. Skin conductance responses (SCRs) were recorded during all stages of the fear conditioning protocol, and CS valence and arousal ratings were performed between the different conditioning phases. For timelines of stress measurements, "– / + " means the time before and after stress onset.

There was no difference for the time points of key experimental sessions between the stress and the control group, including the start time on day 1, day 2, the difference in start times and time points of saliva sampling (all $p_s \geq 0.389$).

2.6. Data analyses

Statistical analyses for CS ratings were conducted separately for valence and arousal via repeated-measures ANOVA with time (baseline / after fear acquisition training / after extinction training/ baseline day 2 / after retrieval day 2), CS type (CS+ / CS-), CS face (in-group / out-group) as within-subjects factors and group (stress / control) as between-subjects factor. Statistical comparisons of SCRs were conducted separately for each phase (fear acquisition training, extinction training, re-extinction) via repeated-measures ANOVA with trial (8 trials), CS type, CS face as within-subjects factors and group as between-subjects factor. For stress-related measures, repeated-measures ANOVA were conducted separately for cortisol, systolic and diastolic blood pressure, including the repeated measurement factor time (cortisol: baseline / +10 min / +25 min; blood pressure: pre / during / post) and the between-subjects factor group.

Since the main aim of the current study was to test the impact of stress on the retrieval of out-group related bias, we particularly focused on the first trial of the re-extinction phase to be interpreted as pure retrieval compared to re-extinction processes evolving with more trials. Thus, the repeated measures ANOVA was also conducted with CS type, CS face and group as factors for the first re-extinction trial of each CS.

For an index of out-group related bias, it should be noted that the traditionally proposed out-group bias index of enhanced response differences between CS+ and CS- for out-group faces has been seriously challenged (Koenig *et al.* 2017, Dang, Xiao, & Mao, 2015) given the difference score may derive from a larger out-group CS+ response (the pure out-group bias) or a reduced out-group CS- response (indeed the opposite aspect of the out-group response bias). Therefore, we employed both CS type (CS+ / CS-) and CS face (in-group / out-group) as two factors in our analyses. Additionally, we calculated the out-group bias index via the direct difference between the in- and out-group faces (the response towards out-group faces minus the response towards in-group faces) to better clarify the influence of stress on the out-group related bias.

Pearson correlation analyses were performed for the control and stress group respectively to examine the relationship between the expression of the out-group related bias and dynamics of stress markers. The indicator of stress markers refers to the difference value between two adjacent time points of saliva cortisol and blood pressure measurements.

All statistical analyses were performed in IBM SPSS Statistics for Mac (Version 22.0. Armonk, NY: IBM Corp.) with the statistical significance level set to $\alpha = 0.05$. Greenhouse-Geisser corrected *p*-values were used if assumptions of sphericity were violated (with unadjusted degrees of freedom and epsilon values ($\mathcal{E}$) reported). Significant main and interaction effects for the overarching ANOVA were reported. Decomposed ANOVAs and the post-hoc *t*-tests were conducted to clarify the specific implications of potential interactive effects.

3. Results

3.1. Ratings

For valence ratings, repeated ANOVA revealed a main effect of time ($F_{(4,\ 152)}= 5.24$, $p = 0.003$, $\eta_p^2 = 0.12$, $\mathcal{E}= 0.656$), CS type ($F_{(1,38)}= 26.82$, $p < 0.001$, $\eta_p^2 = 0.41$) and a CS type × time interaction ($F_{(4,152)}= 9.59$, $p < 0.001$, $\eta_p^2 = 0.20$, $\mathcal{E}= 0.656$; cf. Fig. 2a). Post hoc decomposed ANOVA tests for each time point with Bonferroni correction showed no significant differences in valence ratings between CS+ and CS- during baseline ($p = 0.055$), while after fear acquisition training, the valence rating of the CS+ was significantly lower compared to the CS- ($p < 0.001$). This difference remained after extinction training as well as before and after re-extinction on day 2 ($p_s \leq 0.005$). Overall, the main effect of CS face was not significant ($F_{(1,38)}= 2.27$, $p = 0.140$, $\eta_p^2 = 0.06$), and no group related effects were observed ($p_s > 0.124$).

For arousal ratings, a main effect of time ($F_{(4,152)}= 11.19$, $p < 0.001$, $\eta_p^2 = 0.23$, $\mathcal{E}= 0.712$), CS type ($F_{(1,38)}= 24.45$, $p < 0.001$, $\eta_p^2 = 0.39$) and a CS type × time interaction emerged ($F_{(4,\ 252)}= 8.86$, $p < 0.001$, $\eta_p^2 = 0.19$, $\mathcal{E}= 0.764$). Post hoc decomposed ANOVA for each time point with Bonferroni correction revealed no significant differences in arousal ratings between CS+ and CS- during baseline ($p = 0.785$), but arousal ratings were significantly higher for the CS+ compared to the CS- after fear acquisition and extinction training ($p_s < 0.001$) as well as before and after re-extinction on day 2 ($p_s \leq 0.005$). In addition, a significant main effect of CS face occurred ($F_{(1,38)}= 5.45$, $p = 0.025$, $\eta_p^2 = 0.13$) revealing higher arousal ratings for out-group faces compared to in-group faces in a general manner, indicating the out-group related face bias indexed by arousal ratings (cf. Fig. 2b).

Surprisingly, the current data also showed a significant main effect of group ($F_{(1,38)}= 8.64$, $p = 0.006$, $\eta_p^2 = 0.19$): arousal ratings from the

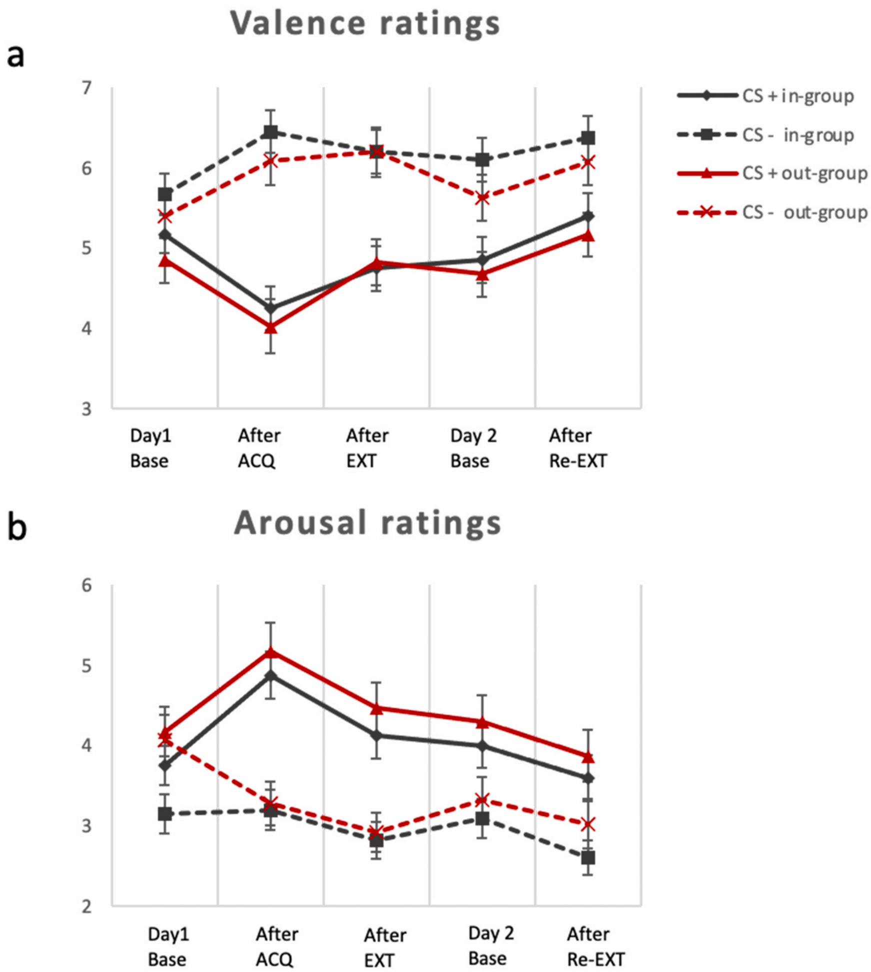

Fig. 2. Mean valence and arousal ratings for in-group and out-group CS+ and CS- over the course of the fear conditioning design. After fear acquisition training (ACQ), participants rated the valence of the CS+ significantly lower compared to the CS- (a), and arousal of the CS+ significantly higher compared to the CS- (b), which was maintained both after extinction training (EXT) as well as before (Day 2 Base) and after re-extinction (Re-EXT) on day 2. No differences emerged for the baseline on day 1 (Day 1 Base). The overall arousal ratings of the out-group face (red lines) were generally higher compared to the in-group face (gray lines), showing an evaluative out-group related bias indexed by arousal ratings (b). Error bars represent standard errors of the mean.

stress group were generally higher compared to the control group, but with no related interaction effects, indicating some pre-manipulation rating bias. Since this main effect of group was not modulated by other conditions (time, CS type or CS face), it did not impact the main findings on successful fear acquisition or out-group related bias indexed by the arousal ratings.

3.2. Day 1 SCRs: fear acquisition and extinction training

For fear acquisition training, repeated-measures ANOVA revealed a main effect of CS type ($F_{(1,37)}$= 67.58, $p < 0.001$, $\eta_p^2 = 0.65$), trial ($F_{(7,259)} = 6.00$, $p < 0.001$, $\eta_p^2 = 0.14$, $\mathcal{E}$= 0.619) and a significant CS type × trial interaction ($F_{(7,259)}$= 2.65, $p = 0.027$, $\eta_p^2 = 0.07$, $\mathcal{E}$= 0.671; cf. Fig. 3a) indicating successful fear acquisition. Differences between CS+ and CS- already occurring at the very first trial seem to be related to habituation of the SCRs due to the stimulus sequence, in which the first trial was always a CS+ and the second trial was always a CS-. No effects related to CS face were observed ($p > 0.162$) revealing the out-group related bias to be absent during fear acquisition training.

For extinction training, a main effect of CS type ($F_{(1,38)}$= 24.79, $p < 0.001$, $\eta_p^2 = 0.40$), trial ($F_{(7,266)}$= 8.13, $p < 0.001$, $\eta_p^2 = 0.18$, $\mathcal{E}$= 0.643) and a CS type × trial interaction occurred ($F_{(7,266)}$= 3.70, $p = 0.004$, $\eta_p^2 = 0.09$, $\mathcal{E}$= 0.691; cf. Fig. 3b) indicating successful extinction with decreasing CS+ /CS- differentiation over time.

Importantly, a significant CS face × trial interaction emerged ($F_{(7,266)}$= 2.80; $p = 0.021$, $\eta_p^2 = 0.07$, $\mathcal{E}$= 0.663), which could be traced back to a significant difference during the first trial only ($F_{(1,38)}$= 6.12, $p = 0.018$, $\eta_p^2 = 0.14$): SCRs for out-group faces were significantly higher than for in-group faces. Although the CS type × CS face interaction was not statistically significant ($F_{(1,38)}$= 2.73; $p = 0.107$, $\eta_p^2 = 0.07$), we noted that the in/out-group difference was mainly driven by the CS-, i.e., the CS- out-group face showed higher SCRs than the CS- in-group face ($t_{(39)}$= 2.71, $p = 0.010$, d= 0.87), while the difference between CS+ out-group and CS+ in-group faces was not obvious ($t_{(39)}$= 0.94, $p = 0.354$, d= 0.30). These findings indicated an out-group related bias to exist at the very beginning of extinction training, particularly restricted to enhanced SCRs towards CS- out-group faces.

3.3. Day 2 stress manipulation: blood pressure and cortisol

For the diastolic blood pressure, repeated-measures ANOVA revealed a main effect of time ($F_{(2,76)}$= 24.73, $p < 0.001$, $\eta_p^2 = 0.39$), group ($F_{(1,38)}$= 4.22; $p = 0.047$, $\eta_p^2 = 0.10$), and a time × group interaction ($F_{(2,76)}$= 27.53, $p < 0.001$, $\eta_p^2 = 0.42$; cf. Fig. 4a). Post-hoc t-tests showed that diastolic blood pressure was significantly higher in the stress than in the control group during water immersion ($t_{(38)}$= 5.23, $p < 0.001$, d= 1.65), whereas no differences occurred at baseline ($p = 0.885$) or post immersion ($p = 0.481$). The same pattern was observed for systolic blood pressure: a main effect of time ($F_{(2,76)}$= 37.90, $p < 0.001$, $\eta_p^2 = 0.50$), group ($F_{(1,38)}$= 4.57; $p = 0.039$, $\eta_p^2 = 0.11$), and a time × group interaction emerged ($F_{(2,76)}$= 30.19, $p < 0.001$, $\eta_p^2 = 0.44$; cf. Fig. 4b). Post-hoc t-tests indicated that systolic blood pressure was significantly higher in the stress compared to the control group during water immersion ($t_{(38)}$= 4.97, $p < 0.001$, d= 1.57), but not at baseline ($p = 0.629$) or post immersion ($p = 0.322$).

For cortisol concentrations, a main effect of time ($F_{(2,76)}$= 7.60, $p = 0.004$, $\eta_p^2 = 0.17$, $\mathcal{E}$= 0.672) and a significant time × group interaction emerged ($F_{(2,76)}$= 24.15, $p < 0.001$, $\eta_p^2 = 0.39$, $\mathcal{E}$= 0.672; cf.

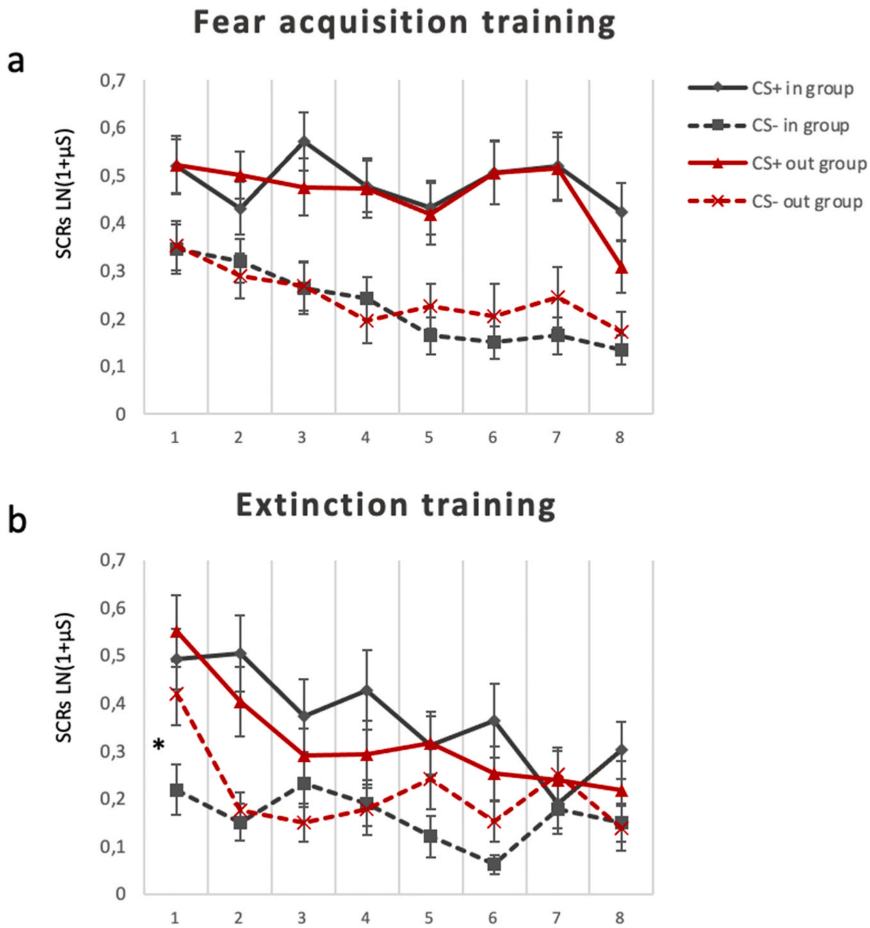

Fig. 3. SCRs during fear acquisition and extinction training on day 1. Fear acquisition was successful with increasing CS+ /CS- differentiation over the course of fear acquisition training (a). Extinction was also successful with decreasing CS+ /CS- differentiation over time. (b). During the first trial of extinction training, out-group faces (red lines) showed higher SCRs than in-group faces (gray lines), especially for the out-group CS- (the red dotted line), indicating some out-group related bias during initial extinction. Error bars represent standard errors of the mean, * $p < 0.05$.

Fig. 4c). Post-hoc *t*-tests showed that the control group showed unexpectedly higher cortisol concentrations than the control group at baseline ($t_{(38)}$= 2.23, $p = 0.025$, d= 0.70). But cortisol concentrations of the stress group gradually increased; 25 min after stress induction, cortisol concentrations were significantly higher in the stress compared to the control group ($t_{(38)}$= 3.79, $p = 0.001$, d= 1.20) indicating a successful stress induction based on cortisol concentrations.

3.4. Day 2 SCRs: the impact of stress on retrieval

For the whole re-extinction phase, repeated-measures ANOVA revealed a main effect of CS face ($F_{(1,38)}$= 4.67, $p = 0.037$, $\eta_p^2 = 0.11$; SCRs for in-group faces were higher than for out-group faces), CS type ($F_{(1,38)}$= 26.97, $p < 0.001$, $\eta_p^2 = 0.42$), trial ($F_{(7,266)}$= 7.25, $p < 0.001$, $\eta_p^2 = 0.16$, $\mathcal{E}$= 0.583) and a significant CS type × trial interaction ($F_{(7,266)}$= 3.86, $p = 0.003$, $\eta_p^2 = 0.09$, $\mathcal{E}$= 0.702).

Focusing on the first trial during re-extinction to capture retrieval more purely, a significant main effect of CS type ($F_{(1,38)}$= 18.76, $p < 0.001$, $\eta_p^2 = 0.33$) and more importantly, a significant CS face × group interaction occurred ($F_{(1,38)}$= 5.75, $p = 0.022$, $\eta_p^2 = 0.13$; cf. Fig. 5a). To decompose this interaction, we calculated the out-group related bias index via the difference scores (SCRs for out-group faces minus SCRs for in-group faces). Results revealed that the stress group showed a generally higher out-group related bias compared to the control group ($p = 0.022$) regardless of CS type (cf. Fig. 5b). Here, we should note that during retrieval, the control group no longer showed an out-group related bias, but higher SCRs for in-group faces (i.e., the out-group related bias score was negative). In contrast, the stress group still exerted the out-group related bias as generally found during extinction training on the first day.

Further correlation analyses showed that changes in cardiac stress markers were associated with the out-group related bias during retrieval. For the stress group, blood pressure changes (both systolic and diastolic) from the immersion to the post-immersion period were negatively related to the out-group related bias index (diastolic: $r_{(20)}$= −0.57, $p = 0.008$; systolic: $r_{(20)}$= −0.51, $p = 0.022$; cf. Fig. 5c). The greater (faster) the blood pressure recovery from stress to post-stress, the smaller the out-group related bias. This correlation could not be observed in the control group (diastolic: $r_{(20)}$= 0.11, $p = 0.653$; systolic: $r_{(20)}$= −0.14, $p = 0.563$; cf. Fig. 5c). The correlation coefficients were significantly differed between the two groups in diastolic pressure ($z = 2.21$, $p = 0.014$, but not for systolic pressure, $z = 1.23$, $p = 0.109$). No significant correlations referring to cortisol dynamics were revealed.

4. Discussion

The current study extended previous results on the out-group related bias during fear conditioning, revealing elevated arousal ratings and also an initial safety deficit at the beginning of extinction training for conditioned out-group faces (indexed by higher SCRs towards out-group faces, particularly the out-group CS-). More importantly, we demonstrate stress to lead to a recovery of the out-group related bias: higher SCRs towards out-group faces during retrieval was only observed in the stress group (but reversed in the control group), which was basically consistent with our previous findings from female participants (Merz et al. 2020) and also compatible with literature proving that stress magnifies the general out-group related racial bias (Bijleveld et al. 2012, Salam et al. 2017). Interestingly, we further found that the reduction of the out-group related bias index was related to post-stress cardiac

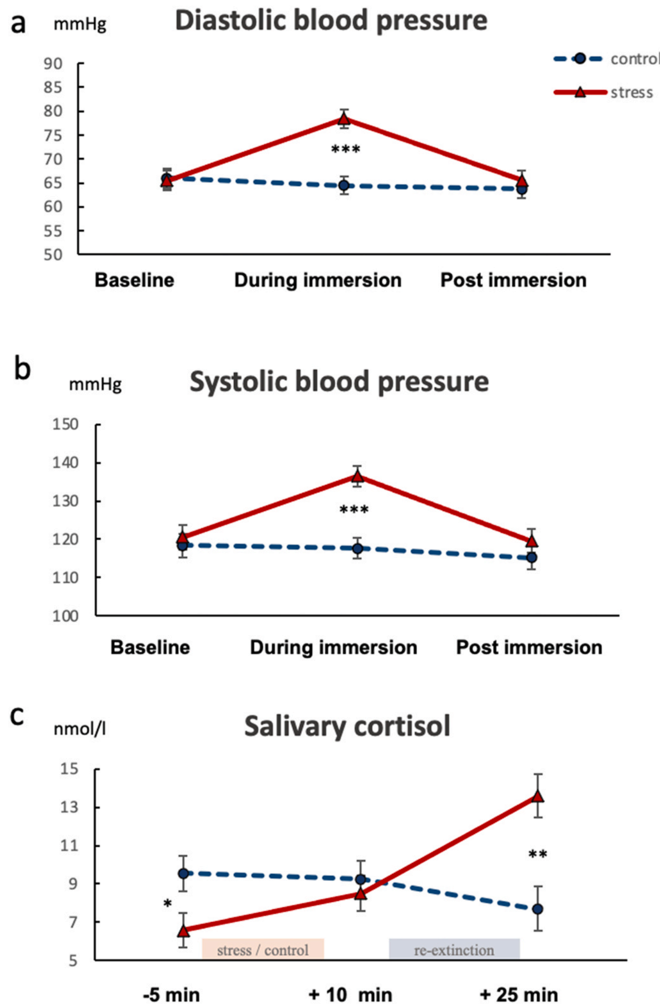

Fig. 4. Changes of diastolic and systolic blood pressure as well as salivary cortisol concentrations before and after the stress manipulation. Stress significantly increased diastolic and systolic blood pressure during the cold-water immersion, which recovered to baseline post immersion (a, b). Salivary cortisol concentrations increased in the stress group, but not in the control group; significant group differences occurred 25 min after stress onset (c), indicating a successful stress induction. Error bars represent standard errors of the mean, * ** $p < 0.001$, * * $p < 0.010$, * $p < 0.05$.

recovery (seen in systolic and diastolic blood pressure), suggesting the potential modulating impact of stress recovery on shifting the out-group related bias.

In the current data, participants' arousal ratings of out-group faces were significantly elevated compared to in-group faces and constantly maintained throughout the whole fear conditioning experiment. This increased degree of arousal or, in other words, subjective salience of out-group stimuli probably derives from its attentional novelty. Low-familiar foreign faces may automatically activate a certain vigilance system in a general sense (Olsson and Phelps, 2007, Vorauer, 2012). However, this bias does not necessarily represent a negative affective tendency (Lang, Greenwald, Bradley, & Hamm, 1993), since no rating differences between out-group and in-group faces were observed in the valence aspect.

Nevertheless, ratings are usually affected by cultural norms. The out-group related bias might be more precisely reflected by implicit and physiological measures. In the current study, there were significantly higher SCRs for the out-group CSs relative to in-group counterparts, especially the out-group CS-, in the first trial of extinction training. This pattern might indicate that participants tended to remain vigilant towards out-group faces after fear acquisition training and showed higher SCRs towards out-group faces at the beginning of extinction training. In particular, the increased response towards the out-group CS- implies that for signals supposed to be safe, it might be more difficult to remember their non-threatening characteristics because of the attribute "out-group". Indeed, safety learning can be more difficult for out-group relative to in-group stimuli (Golkar et al. 2015, Molapour, Golkar, Navarrete, Haaker, & Olsson, 2015), with amygdala and anterior insula activations assumed to modulate these biased learning processes (Molapour et al. 2015). Alternatively or additionally, the first trial of extinction training reflects fear retrieval. The general initial over-reaction towards out-group faces may indicate some retrieval facilitation for the out-group danger signals, which may be related to its evolutionary function of a quick start for the threat scenario; while the generalization of the amplified response to the safety signal should be its maladaptive manifestation (McDonald et al. 2012, Kaya, 2015).

Notably, our current SCR findings did not completely mirror the bias patterns in the original report, in which an increased CS+ /CS- difference for out-group faces during extinction training was revealed (Olsson et al. 2005). In contrast, the enhanced SCRs of the out-group CS- in the current data hinder the widening of this CS+ /CS- discrimination. Recent reports argued that the difference score of "out-group CS+ minus out-group CS-" cannot characterize the out-group related bias fully, given that the difference score can be amplified due to increased responding to the reinforced out-group face (CS+) but also due to decreased responding to the non-reinforced out-group face (CS-; Koenig et al. 2017, Dang et al. 2015). The latter effect might even represent a better safety signal learning for out-group faces. Thus, the absence of larger CS+ /CS- differences in out-group faces with an enhanced out-group CS- response could not cancel the existence of the out-group related bias; instead, it can be interpreted as to its unique manifestation in the current sample. Another reason for the absent increase of CS+ /CS- differentiation for out-group faces might be related to our selection of stimuli. Specifically, we used Moroccan (Arab) faces as out-group stimuli due to reasons of ecological validity and practical implications in our sample of German participants. Prior evidence revealed that Caucasian Europeans may not necessarily exhibit a bias towards Arab faces as towards Black faces (Golkar, Björnstjerna & Olsson, 2015). Since both multi-ethnic experiences on the individual level and environmental factors like regional ethnic characteristics can deeply modulate the bias to a specific out-group (Golkar, et al., 2015; Judd, Park, Yzerbyt, Gordijn, & Muller, 2005), the pattern of the out-group related bias in independent samples may show its own features.

The main goal of our present study was to examine the effect of acute stress on the out-group related bias. We found that stress led to a return of extinguished out-group related fear during retrieval on the second day. Specifically, at the beginning of re-extinction, the stress group exhibited larger out-group over in-group SCRs compared to the control group, which seemed to follow the response pattern observed at the beginning of extinction training on day 1. In contrast, the non-stressed group no longer showed increased SCRs towards out-group faces. Quite a few studies already showed that acute stress reduces the retrieval of extinction memory or safety-related processes (Raio, Brignoni-Perez, Goldman, & Phelps, 2014, Deschaux et al. 2013) and according to the STaR (Stress Timing affects Relapse) model, pre-retrieval stress should substantially impair extinction retrieval and promote relapse (Meir Drexler, Merz, Jentsch, & Wolf, 2019). Our data fit well to the model's predictions and extends it to areas where socially threatening elements were involved; that is, acute stress can impede the expression of learned safety signals associated with out-group faces. This modulation effect of stress may depend on the prefrontal cortex-amygdala circuit (Akirav and Maroun, 2007): Specifically, stress hormones decreased activation in fear-inhibitory regions like the ventromedial prefrontal cortex (Kinner, Merz, Lissek, & Wolf, 2016, Hagedorn, Wolf, & Merz, 2021), while amplifying the amygdala signaling during retrieval of conditioned responding (Kinner, Wolf, &

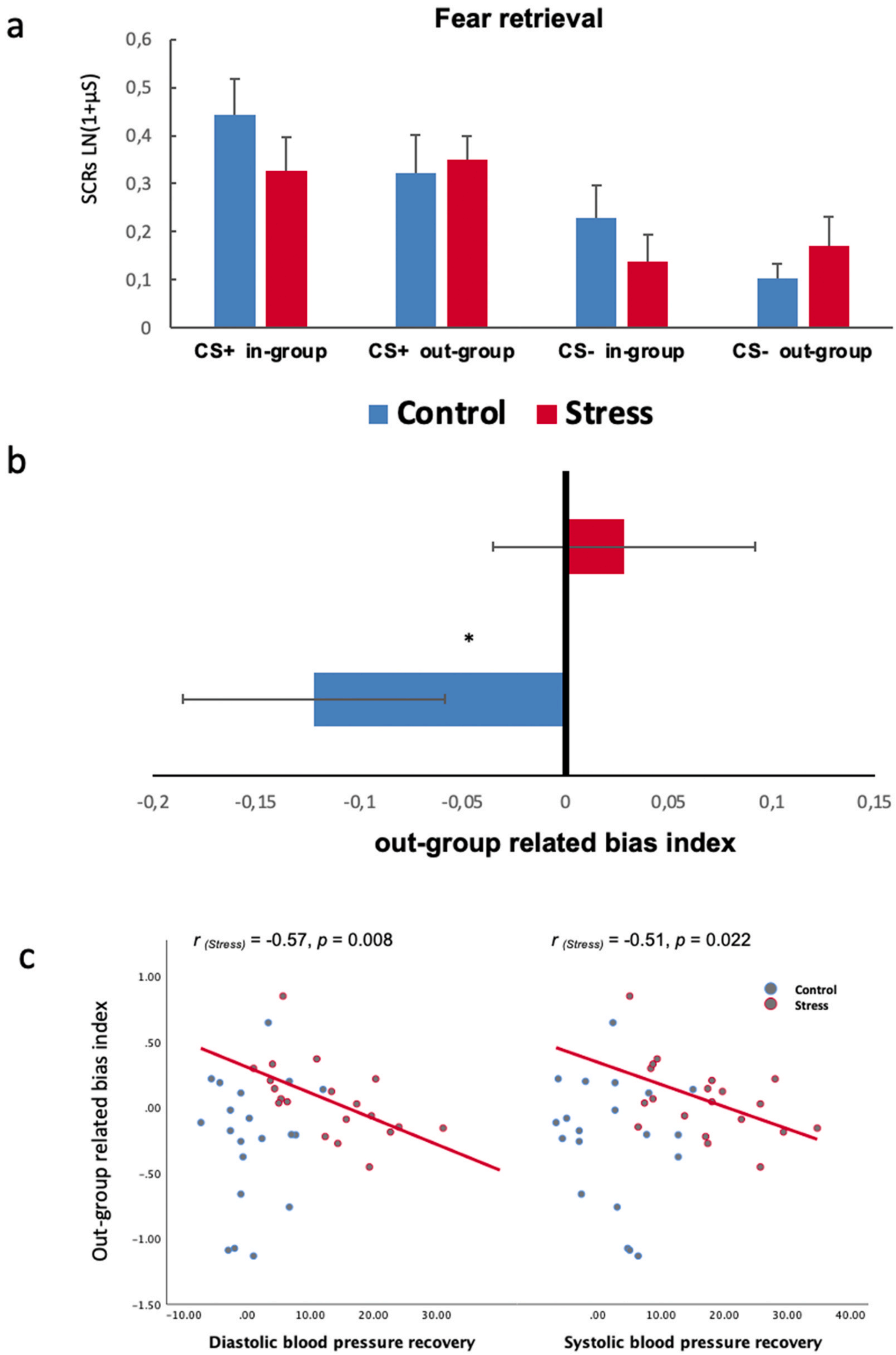

Fig. 5. The impact of stress on retrieval of out-group related fear conditioned stimuli. (a) SCRs during retrieval for in-group and out-group CS+ and CS- after exposure to stress or the control condition, error bars represent standard errors of the mean. (b) Stress modulates the out-group related bias index (i.e., SCRs towards out-group minus in-group faces): the control group showed a negative value, while the stress group showed a zero to positive bias trend. (c) The blood pressure recovery index (during stress minus post-stress) negatively correlated with the out-group related bias in the stress group (red points), but no correlation occurred in the control group (blue points). In addition, the scatterplot shows once more that the out-group related bias in the stress group is significantly higher than in the control group.

Merz, 2018). More importantly, the prefrontal-amygdala circuit is not only critical for fear conditioning processes, but also for the automatic exhibition of racial bias (Wheeler and Fiske, 2005) and its deliberate control (Kubota, Banaji, & Phelps, 2012, Amodio, 2014). It is possible that stress impairs our goal-oriented attempts to retrieve learned safety signals relevant for out-group face processing, which are mainly regulated by the neocortex, in favor of more salience-biased response patterns dominated by subcortical structures. These notions would be in line with the general function of stress in shifting cognitively demanding and flexible behavior to more automated response and habitual behavior (Schwabe and Wolf, 2013, Hermans et al. 2014).

Furthermore, the current data also showed a negative correlation between the retrieval of the out-group related bias and the cardiac indicators of stress recovery. Response patterns in individuals, whose blood pressure recovered more slowly from stress, were more consistent with the bias pattern observed during initial extinction training, showing relatively higher SCRs to out-group faces. Cardiovascular stress recovery processes might be more sensitive for associations with stress-induced maladaptive responses. For example, blunted recovery of blood pressure after acute stress has long been closely related to perseverative cognition such as rumination (Brosschot, Gerin, & Thayer, 2006, Brosschot, Pieper, & Thayer, 2005). Additionally, prolonged post-stress cardiac recovery was associated with implicit perseverative cognition operationalized as automatic vigilance in a lexical decision task (Verkuil, Brosschot, de Beurs, & Thayer, 2009). These findings seem to echo our results well and indicate that the dynamic process of cardiac stress recovery may influence its cognitive consequences. Nevertheless, we were not able to detect correlations between changes in cortisol concentrations and the out-group related bias, which may be related to the low number of cortisol measurements on day two (only three samples). Previous research showed that police officers exhibiting larger cortisol increases to stress subsequently made more racial biased decisions like to shoot armed black targets more relative to armed white targets in a laboratory setting (Akinola and Mendes, 2012), implying that increased stress hormones serve as a potential risk factor to stimulate social racial prejudices. The current results of the correlation between stress recovery and out-group related bias may provide at least hints regarding another direction of this stress-bias interaction, that is the potential protective role of stress coping in xenophobia reduction, while this suggestion obviously needs more empirical verification.

Basically, the amplifying effect of stress on the out-group related bias seems to occur in both the current sample of men and previous data in women (Merz et al. 2020), but a closer inspection still reveals some subtle sex differences in this stress-related modulation. Specifically in women, initial retrieval was not affected by stress and the magnification of stress-enhancing out-group related bias was observed not until the reinstatement test (Merz et al. 2020). However, in men, the impact of stress was already noticeable during retrieval, indicating the recovery of the out-group racial bias in male participants might be susceptible to stress in a different way than in female participants. Some evidence suggests that low levels of estrogens can be linked to deficient extinction learning (Graham and Milad, 2013), and (endogenously or exogenously) increased estrogen concentrations might weaken stress hormone effects on retrieval processes (Merz and Wolf, 2017, Stockhorst and Antov, 2015). This may partly explain that the stress-induced recovery of the out-group related bias can be more easily observed in men during retrieval without an additional reinstatement manipulation (compared to women, Merz et al., 2020). Since stress hormones altered PFC-amygdala functioning during retrieval in men but not (or in the opposite direction) in women taking oral contraceptives (Kinner et al. 2016, Kinner et al. 2018), our findings further illustrate the complex interaction between stress and sex hormones, which may affect the microscopic aspects of fear learning ultimately leading to sex (hormone)-dependent behavior patterns in response to acute stress (Taylor et al. 2000).

It is necessary to note that in this study, the control group no longer showed higher responses for out-group faces during retrieval, but rather an "in-group related bias", in which SCRs towards in-group faces were relatively higher than out-group faces. After extinction training, the control group might exhibit an increased "tend and befriend" response pattern to their in-group members (Taylor et al. 2000), or alternatively, an attention enhancement due to potential intragroup competitions (same sex and race of stimuli implies a potentially closer competitive relationship in the common living environment for seeking limited resources; Festinger, 1954, Suls and Miller, 1977). However, regardless of whether the increased physiological attention to in-group stimuli comes from friendliness or competition, this kind of social affinity or comparison is more implicated with empirical identity assessment and dependent on model-based neural circuits such as medial frontal regions (Moore, Merchant, Kahn, & Pfeifer, 2014, Swencionis and Fiske, 2014). Thus, the alternative interpretation of our data that stress reversed this in-group related bias to an out-group related bias during retrieval might include the detrimental effect of stress on flexible mentalizing processes, which have been suggested via various decision-making paradigms (Reyes, Silva, Jaramillo, Rehbein, & Sackur, 2015, Leder, Hausser, & Mojzisch, 2013).

Despite these enlightening results regarding acute stress and the out-group related bias reoccurrence in men, several limitations should be stated to inspire future studies. Firstly, SCRs, even as the classic indicator of conditioned fear, may not specifically reflect underlying attention and emotional processing, which limits the deeper discussions on the functional mechanisms of the out-group related bias. Pupil diameter, fear-potentiated startle, functional magnetic resonance imaging or electroencephalography should be applied in future studies to better examine the underlying mechanisms of the interaction between stress and intergroup bias processing. Second, sex differences regarding stress effects on the out-group related bias were not directly compared here, given that our two studies were conducted separately in male and female participants with additional slight variations in experimental design (e.g., timing of stress relative to retrieval, added reinstatement). Thus, studies including men and women simultaneously along with more functional-specific measures of fear and stress should be expected in the future. Third, as mentioned above, individual experiences, social and cultural factors will profoundly modulate the appearance and manifestation of the out-group related bias, if the in-/out-group condition is naturally manipulated by embedded race or ethnic features. Controlling the ethnic environment on the individual level (e.g., growing up in a heterogeneous vs. homogeneous environment) should be helpful for more elaborated investigations in the future (cf. Golkar, et al., 2015). Another alternative includes the creation of an in-group and out-group via manual/artificial manipulation associated with more "pure" disclosure of an intergroup bias and its interaction with stress (Schweda, Faber, Crockett, & Kalenscher, 2019).

In summary, the current data revealed a unique out-group related bias in men: higher arousal ratings for out-group faces as well as difficulties in safety-signaling at the beginning of extinction training regarding out-group faces. Importantly, acute stress led to the return of the out-group related bias during retrieval, providing evidence on critical interaction effects between stress and out-group biases relevant for the understanding of (re)emergence of xenophobia and racial prejudices. The absence of out-group related bias during retrieval in the control group and the relationship between the out-group related bias and blood pressure recovery suggest that stress coping strategies during intergroup interactions may offer a useful function to reduce related social biases.

Role of the Funding Source

This work was supported by the Deutsche Forschungsgemeinschaft (DFG; German Research Foundation) within the SFB 1280 Extinction Learning (grant no. 316803389 - SFB1280; project A09 [OTW, CJM]) and an additional DFG grant (ME 3831/5–1 to CJM). The DFG had no role in study design, collection, analysis and interpretation of data, writing of the manuscript, or in the decision to submit the paper for publication.

CRediT authorship contribution statement

Dong-ni Pan: Formal analysis, Writing – original draft, Visualization; Oliver T. Wolf: Conceptualization, Writing – review & editing, Supervision; Christian J. Merz: Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

None.

Data Availability Statement

The datasets generated and analyzed during the current study are available on the Open Science Framework: https://osf.io/x2av9/?view_only=de3e098665e04c91827bb3c449f72ce2.

Acknowledgments

We gratefully acknowledge the help of Annika Eichholtz, Maike Strothmann and Tanja C. Hamacher-Dang during data collection and recruitment of participants.

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