

## ORIGINAL ARTICLE

# The impact of physical exercise on the consolidation of fear extinction memories

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**Abstract**

Based on the mechanisms of fear extinction, exposure therapy is the most common treatment for anxiety disorders. However, extinguished fear responses can reemerge even after successful treatment. Novel interventions enhancing exposure therapy efficacy are therefore critically needed. Physical exercise improves learning and memory and was also shown to enhance extinction processes. This study tested whether physical exercise following fear extinction training improves the consolidation of extinction memories. Sixty healthy men underwent a differential fearconditioning paradigm with fear acquisition training on day 1 and fear extinction training followed by an exercise or resting control intervention on day 2. On day 3, retrieval and reinstatement were tested including two additional but perceptually similar stimuli to explore the generalization of exercise effects. Exercise significantly increased heart rate, salivary alpha amylase, and cortisol, indicating successful exercise manipulation. Contrary to our expectations, exercise did not enhance but rather impaired extinction memory retrieval on the next day, evidenced by significantly stronger differential skin conductance responses (SCRs) and pupil dilation (PD). Importantly, although conditioned fear responses were successfully acquired, they did not fully extinguish, explaining why exercise might have boosted the consolidation of the original fear memory trace instead. Additionally, stronger differential SCRs and PD toward the novel stimuli suggest that the memory enhancing effects of exercise also generalized to perceptually similar stimuli. Together, these findings indicate that physical exercise can facilitate both the long-term retrievability and generalization of extinction memories, but presumably only when extinction was successful in the first place.

**KEYWORDS**

aerobic exercise, cardiovascular responses, consolidation, extinction learning, pupillometry, return of fear, stress hormones

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## 1 | INTRODUCTION

The effectiveness of extinction-based treatments for anxiety disorders such as exposure therapy is well documented (Hofmann & Smits, 2008; Norton & Price, 2007). However, a significant number of patients do not respond to treatment or experience relapse following initial symptom remission (Arch & Craske, 2009; Yonkers et al., 2003). The identification of novel strategies that improve the efficacy of exposure therapy and reduce relapse rates is thus of utmost importance (Pittig et al., 2016).

Extinction learning is considered as a key mechanism for the reduction of fear during exposure and is thus widely used as a laboratory model for exposure-based interventions (Vervliet, Craske, et al., 2013). It involves the repeated confrontation with a conditioned stimulus (CS; e.g., dog) in the absence of the unconditioned stimulus (UCS; e.g., dog bite), typically resulting in a decrement of conditioned fear responses. However, extinguished responses do not simply disappear but tend to reemerge through the mere passage of time (spontaneous recovery), following a change in context (renewal) or by unexpected exposure to a UCS (reinstatement; Bouton, 2014; Vervliet, Baeyens, et al., 2013). These recovery phenomena indicate that extinction does not erase the original fear memory trace, but rather constitutes a new learning process, in which a second inhibitory memory trace is acquired (Bouton, 2004). Which of the two then competing memories will be retrieved at a later point in time depends critically on the relative strength and retrieval availability of both memories. Fear memories are often robust, not bound to a specific context, and thus generalize more easily, whereas extinction memories are typically more transient, context-dependent, and thereby often fail to generalize over time, across contexts or variations of the feared stimulus not present at the time of extinction training (Laborda & Miller, 2012; Maren et al., 2013).

In recent years, this challenge has led many research groups to investigate various methods for enhancing the strength and long-term retrievability of extinction memories, ranging from cognitive behavioral modifications, over pharmacological adjuncts (Craske et al., 2018; Fitzgerald et al., 2014), to brain stimulation techniques (Marković et al., 2021). Growing knowledge on the modulating role of stress and glucocorticoids (GCs) in learning and memory has shown that stress hormones enhance the consolidation of extinction memories (Meir Drexler et al., 2019) and thus can act as a pharmacological adjuvant in extinction-based therapies as well (de Quervain et al., 2017). Yet, even with these promising findings, patients seeking care for anxiety disorders generally prefer psychosocial over pharmacological approaches (Arch, 2014). Furthermore, behavioral stress manipulations triggering a reliable neuroendocrine

stress response are typically associated with an increase in negative affect (Allen et al., 2014; Langer et al., 2020), questioning the acceptance and feasibility of incorporating acute stress protocols into therapeutic interventions.

Physical exercise can also activate the two major stress systems (Gatti & de Palo, 2011)—the sympathetic nervous system (SNS), resulting in the release of the catecholamines, noradrenaline and adrenaline, and the hypothalamus–pituitary–adrenocortical (HPA) axis stimulating the secretion of GCs (mainly cortisol in humans; Joëls & Baram, 2009). A certain intensity threshold yet needs to be exceeded to reliably elicit a HPA axis response, with the most robust cortisol increases observed in response to aerobic exercise at moderate-to-high intensity as opposed to light intensity or other types of exercise (Hill et al., 2008; Jacks et al., 2002; Loprinzi, Blough, et al., 2019). However, unlike psychosocial stressors, physical exercise typically increases positive affect and promotes health (Basso & Suzuki, 2017). Moreover, growing evidence for the role of exercise in modulating learning and memory (Blomstrand & Engvall, 2021; Loprinzi, Blough, et al., 2019; Roig et al., 2013) proposes particularly beneficial effects of exercise on emotional memory consolidation (Jentsch & Wolf, 2020; Loprinzi, Frith, et al., 2019). Yet, inconsistent findings (see, e.g., Loprinzi et al., 2020; Pace & Loprinzi, 2019, for null effects of exercise on intentional forgetting) also underscore that exercise effects on memory may critically depend on moderating factors, including the memory domain or cognitive task under study (Chang et al., 2012) or exercise characteristics such as type, duration, and intensity (Loprinzi et al., 2021). Importantly, work in rodents suggests that physical exercise can also enhance extinction processes (Keyan & Bryant, 2019b), rendering it a promising candidate for augmenting treatment success. For instance, it has been shown that acute exercise immediately before, during, or after fear extinction improves extinction memory consolidation and reduces the return of fear (ROF; Bouchet et al., 2017; Mika et al., 2015; Siette et al., 2014). However, exercising 6 hr after extinction learning—presumably after the consolidation window—did not affect extinction memory retrieval (Siette et al., 2014).

Studies exploring exercise effects on extinction learning in humans are scarce, but a pilot clinical study with patients suffering from posttraumatic stress disorder (PTSD) further revealed that engaging in an acute bout of exercise immediately before each exposure session resulted in greater symptom reductions when compared to exposure therapy without exercise (Powers et al., 2015). By contrast, pre-exposure exercise had only minor effects on treatment outcome in patients with panic disorder and agoraphobia (Bischoff et al., 2018) and even failed to yield any symptom improvement in adults with fear of

heights (Jacquart et al., 2017). This discrepancy between rodent experiments and clinical studies in humans may be attributed to differences in timing of exercise relative to fear extinction training. In fact, a literature review concluded that exercise prior to extinction training does not improve extinction retrieval, whereas exercise performed either during or immediately after extinction learning improves extinction memory retrieval and reduces subsequent ROF (Tanner et al., 2018). This implies that exercise specifically influences extinction memory consolidation. In line with this notion, aerobic exercise carried out during the consolidation window (i.e., immediately after fear extinction training) reduced spontaneous recovery in a nonclinical sample (Keyan & Bryant, 2019a) as well as reinstatement of fear (Crombie et al., 2021) and trauma-related distress and vividness in patients with PTSD (Voorendonk et al., 2021). Taken together, the few studies published to date suggest that a single bout of physical exercise can indeed not only facilitate extinction consolidation and reduce ROF but also underscore that the timing of exercise in relation to the specific phase of the extinction process could be crucial for its potential to modulate the long-term expression of extinction memories.

Little is also known about the mechanisms by which acute exercise exerts its extinction-facilitating effects and several signaling pathways sensitive to exercise could be involved, including changes in cardiovascular and neuroendocrine processes (e.g., increases in cortisol levels; Keyan & Bryant, 2019b). Furthermore, it is important to find strategies which overcome the stimulus specificity of extinction memory in order to maximize its long-term retrievability, and in consequence the efficacy of extinction-based interventions. However, to the best of our knowledge there has been no study to date exploring the potential role of exercise in generalizing extinction memories.

To address these issues, we tested whether a single bout of physical exercise following fear extinction training improves the consolidation and generalization of extinction memories on the next day. Firstly, we expected a single bout of physical exercise to activate both the SNS and the HPA axis, resulting in a significant increase in heart rate, salivary alpha-amylase (i.e., an index of SNS activity, Nater & Rohleder, 2009), and cortisol concentrations (Jentsch & Wolf, 2020; Skoluda et al., 2015). Secondly, exercise performed immediately after fear extinction training should facilitate extinction consolidation, resulting in enhanced extinction memory retrieval and reduced reinstatement of fear (Keyan & Bryant, 2019b; Tanner et al., 2018). In addition, we explored the potential role of exercise in generalizing extinction memories to novel stimuli.

## 2 | METHOD

### 2.1 | Participants

The required sample size was determined using G\*Power 3.1 (Faul et al., 2009), assuming a small- to moderate-sized effect of acute physical exercise on long-term memory, in particular on extinction memory recall as reported in meta-analyses by Roig et al. (2013) and Roquet and Monfils (2018); average effect sizes of  $d=0.52$  and  $g=0.40$ , respectively. Accordingly, the estimation of the sample size for a small-to-moderate effect size of  $f=0.23$  (Cohen, 1969), an assumed correlation of  $r=.5$  for repeated measurements, and a given significance level of  $\alpha=.05$  revealed a required sample size of 52 participants in order to achieve a power of  $1-\beta\geq .90$  to detect a significant CS $\times$ Group interaction.

To account for potential sample attrition due to the multiple-day design, 60 healthy male participants aged between 18 and 35 years ( $M=24.3$ ,  $SD=4.4$ ) with a normal BMI ranging between 19 and 27 kg/m<sup>2</sup> ( $M=23.0$ ,  $SD=2.2$ ) were recruited via online advertisements, social media, and flyers at the Ruhr University Bochum and surroundings to participate in this study. Exclusion criteria checked beforehand in a telephone interview encompassed chronic or acute mental, somatic or neurological diseases, and drug use including smoking, regular medication, or alcohol consumption. All participants were fluent in German and had normal or corrected-to-normal vision of not more than  $\pm 1.5$  diopters. We included only male participants as men and women differ in their cardiovascular and neuroendocrine responsiveness to physical and psychosocial stress (Dominelli & Molgat-Seon, 2022; Kudielka & Kirschbaum, 2005; O'Bryan et al., 2022). Moreover, sex differences have been reported in stress and physical exercise effects on emotional memory (Jentsch & Wolf, 2020; Merz & Wolf, 2017) and fear and extinction processes in particular (Merz et al., 2018) with more robust effects reported in males (Bouchet et al., 2017).

Participants were alternately assigned to either the exercise ( $N=30$ ) or control group ( $N=30$ ), which did not differ regarding their weekly physical activity ( $t(57)=.80$ ,  $p=.43$ ) assessed with the Godin–Shephard Leisure-Time Physical Activity Questionnaire (LTEQ; Godin, 2011) or sleep duration on all 3 testing days (all  $ps>.27$ ).

### 2.2 | Procedure

Participants were tested on 3 consecutive days with an interval of 24 hr ( $\pm 2$  hr) to allow memories to consolidate following each learning phase (Dudai, 2004) and to ensure that each participant was tested approximately at the same

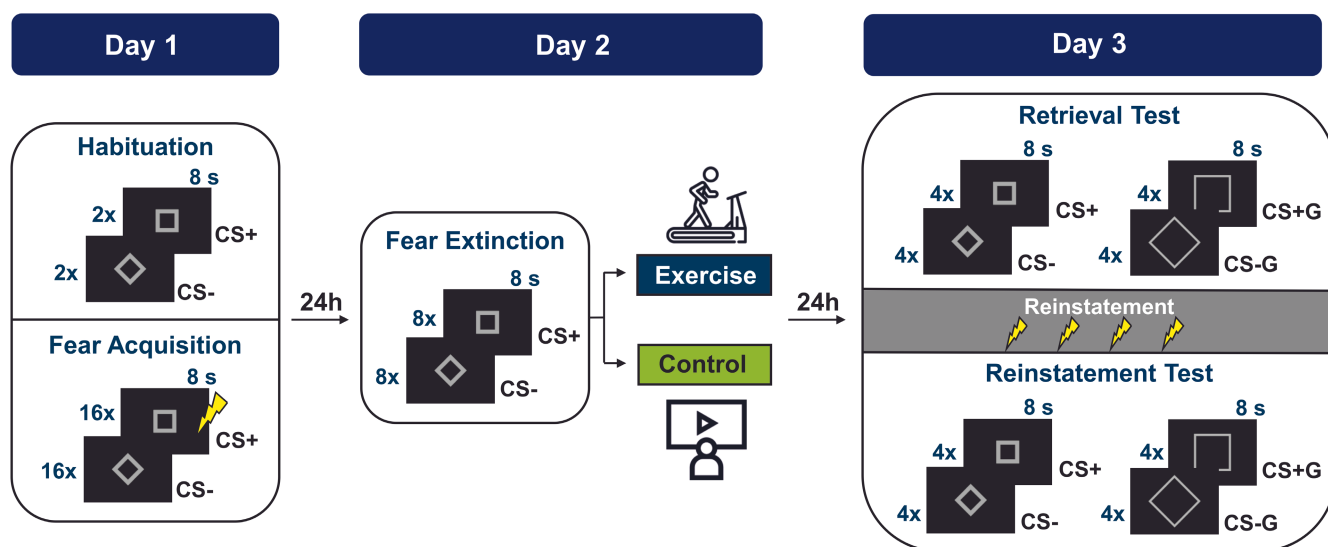
time on all 3 testing days (time difference between start of testing days 1 and 2:  $M=24.09$  h,  $SD=0.40$  h; time difference between start of testing days 2 and 3:  $M=23.49$  hr,  $SD=0.44$  hr). Sessions were scheduled between 1 and 6 p.m. to keep variations in the diurnal cortisol cycle at a minimum (Horrocks et al., 1990; Joëls & Baram, 2009). Furthermore, participants were asked to refrain from physical exercise, alcohol, drugs, and medication 24 hr before testing, as well as from caffeine, food, and beverages other than water 2 hr prior to testing.

The study protocol is summarized in Figure 1. In short, participants underwent a differential fear conditioning paradigm with fear acquisition training on day 1, fear extinction training followed by either a brief exercise or control intervention on day 2, and a retrieval and reinstatement test on day 3 with skin conductance responses (SCR) and pupil dilation (PD) as readouts of conditioned fear. To test whether the exercise effects also generalize to novel but perceptually similar stimuli, we incorporated two novel stimuli that were identical in shape to the original CS shown during fear acquisition and extinction training but displayed in a bigger size during the retrieval and reinstatement test. Participants provided written informed consent and were either reimbursed with 40€ or received course credit for their participation. All procedures were in accordance with the Declaration of Helsinki

and approved by the ethics committee of the Faculty of Psychology at the Ruhr University Bochum.

### 2.3 | Exercise and control intervention

A 20 min vigorous-intensity running task as described in Jentsch and Wolf (2020) was employed as the exercise intervention. It was carried out on a treadmill with a 10% slope and started out with a 1 min warm-up at walking pace, after which speed was increased stepwise by 0.5 m/s every 30 s until an individually set target heart rate ( $HR_{\text{target}}$ ) was reached (approx. after 4 min). Participants then trained for 15 min at their  $HR_{\text{target}}$  and concluded with a 1 min cool down. In order to reach an intensity threshold that reliably activates the HPA axis (Heijnen et al., 2016; Hill et al., 2008; Jacks et al., 2002),  $HR_{\text{target}}$  was defined as 85% of the participants' heart rate reserve and computed with the following formula:  $HR_{\text{target}} = [(HR_{\text{max}} - HR_{\text{rest}}) \times 85\%] + HR_{\text{rest}}$ , with the maximum age-predicted HR ( $HR_{\text{max}}$ ) calculated as 220 minus the participants' age (HRR method; American College of Sports Medicine, 2013). In addition, participants' exertion levels were monitored intermittently throughout the 20 min exercise intervention using the Borg rating of perceived exertion (Borg, 1998).



**FIGURE 1** Study protocol and experimental fear conditioning design. Participants underwent a habituation phase and fear acquisition training on day 1, fear extinction training followed by the exercise or control intervention on day 2, and a retrieval and reinstatement test on day 3. Pictures of a rhomb and a square served as the conditioned stimuli CS+ and CS- and were presented for 8 s against a black background. The UCS is depicted by the yellow flashes and was delivered in 10 of 16 CS+ trials (but never during CS- trials) during fear acquisition training. On day 3, the rhomb and the square were additionally shown in a larger size (generalized CS+ (CS+G) and CS- (CS-G)) to test whether exercise effects also generalize to novel but perceptually similar stimuli. During reinstatement, four unsignaled UCS were applied while a gray background was presented to the participants. During all phases, stimulation electrodes remained attached but did not provide electrical stimulation during fear extinction training, retrieval, and reinstatement test. Skin conductance and pupillary responses served as outcome measures of conditioned fear and were recorded during all experimental phases.

As realized before (cf. Coles & Tomporowski, 2008; Frith et al., 2017; Jentsch & Wolf, 2020), the resting control group was asked to sit quietly for 20 min while watching two documentaries. At the end of each video, participants provided valence and arousal ratings on a 9-point scale ranging from 1 = negative/calm to 9 = positive/aroused, showing that both videos were experienced as emotionally neutral ( $M = 7.07$ ,  $SD = 1.34$  and  $M = 6.60$ ,  $SD = 1.50$ ) and not arousing ( $M = 2.60$ ,  $SD = 1.61$  and  $M = 1.73$ ,  $SD = 0.91$ ).

## 2.4 | Cardiovascular, neuroendocrine, and subjective measures

### 2.4.1 | Heart rate

A Polar V800 watch (Polar® Electro, Finland) connected wireless to an elastic chest strap (Polar H10 Heart Rate Sensor, Polar® Electro, Finland) was used to record participants' HR at a sampling rate of 1000 Hz. Recordings were obtained during a 6 min resting period ( $HR_{rest}$ ), the 20 min exercise or control intervention ( $HR_{intervention}$ ), and a 6 min post-intervention period ( $HR_{post}$ ) on day 2. HR data were exported using device-specific software (Polar Flow; Polar® Electro, Finland) and further processed with Kubios HRV Premium 3.3.1 (Tarvainen et al., 2014) according to the guidelines of the Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology (1996). First, the sample length was adjusted to a total duration of 5 min for the baseline and post-intervention recordings and 15 min for the exercise/control intervention. After that, data were detrended (smoothn priors:  $\lambda = 500$ ) and abnormal or biologically implausible beats were detected using an automatic artifact correction algorithm that detects artifacts from a time series of differences between successive RR intervals using a time-varying threshold and then corrects corrupted beats using interpolation. HR was then calculated for each period.

### 2.4.2 | Salivary cortisol and alpha-amylase

Saliva samples were collected using Salivette sampling devices (Sarstedt, Nümbrecht, Germany) at multiple time points across the three experimental sessions and stored at  $-20^{\circ}\text{C}$  until assayed. Samples were taken prior to fear extinction training (baseline),  $-2$  min before the onset, as well as  $+1$  min,  $+10$  min, and  $+25$  min after the cessation of the exercise or control intervention on day 2. Further samples were collected before and after fear acquisition training on day 1 as well as before and after the retrieval and reinstatement test on day 3.

Free cortisol concentrations were determined on a Synergy2 plate reader (Biotek, Winooski, USA) using commercial enzyme-linked immunosorbent assays (IBL, Hamburg, Germany). In addition, a colorimetric test using 2-chloro-4-nitrophenyl- $\alpha$ -malto-trioiside (CNP-G3) as a substrate reagent was applied to assess alpha-amylase concentrations (SAA; Lorentz et al., 1999). Inter- and intra-assay variations of both analyses were below 10%.

### 2.4.3 | Affect ratings

Participants' affect was assessed concurrently with the collection of saliva samples at multiple time points using the Positive and Negative Affect Scale (PANAS; Watson et al., 1988). The PANAS is a self-report questionnaire consisting of two 10-item subscales quantifying positive (PA) and negative affect (NA) on a 5-point scale ranging from 1 (not at all) to 5 (very much).

## 2.5 | Fear conditioning paradigm

A modified version of the differential fear conditioning paradigm as described in Jentsch et al. (2020) was employed, consisting of three distinct phases: habituation followed by fear acquisition training on day 1, fear extinction training on day 2, and a retrieval and reinstatement test assessing extinction memory retrieval and return of fear on day 3, all of which were separated by 24 hr (see Figure 1). Pictures of two geometrical shapes (a rhomb and a square) that were gray-colored, identical in luminance, and presented for 8 s against a black background served as the conditioned stimuli CS+ and CS-. The UCS was a 100 ms transcutaneous electrical stimulation sent by a constant-voltage stimulator (STM200; BIOPAC Systems, Inc. Goleta, CA, USA) and delivered through two Ag/AgCl electrodes filled with isotonic (0.05 M NaCl) electrolyte medium (Synapse Conductive Electrode Cream; Kustomer Kinetics, Inc., Arcadia, CA). Electrodes were attached to the middle of the left shin, and UCS intensity was set individually to be "unpleasant but not painful" using a gradually increasing rating procedure.

During a short habituation phase, both stimuli were presented twice each without any electrical stimulation. Fear acquisition training then started (without any pause between the two phases), in which one stimulus (CS+) was repeatedly paired with an electrical stimulation (UCS; starting 7.9 s after CS+ onset) in 10 of 16 trials (62.5% partial reinforcement rate), whereas the second stimulus (CS-) was never followed by the UCS (total number of trials: 32, duration:  $\sim 12$  min). Fear extinction training on day 2 consisted of eight unreinforced presentations of the

CS+ intermixed with eight presentations of the CS− (total number of trials: 16, duration: ~8 min). Note that we chose to reduce the number of extinction compared to acquisition trials as it has been shown that return of fear phenomena are significantly reduced in paradigms with delayed extinction (Huff et al., 2009; Lonsdorf et al., 2017). Given that the aim of the current study was to evaluate the potential of physical exercise to facilitate extinction memory consolidation and to reduce reinstatement-induced return of fear, we thereby aimed to minimize potential floor effects (i.e., little return of fear in general). During the retrieval test on day 3, the CS+ and CS− were presented four times each without any electrical stimulation. In addition, both stimuli were shown in a larger size (generalized CS+ (CS+G) and generalized CS− (CS−G); identical in luminance to the original CS+ and CS−) four times each without any UCS to test whether exercise effects also generalize to novel but perceptually similar stimuli. After the retrieval test, reinstatement started with the application of four unsignaled UCS (100% acquisition intensity) separated by 5 s intervals (after 2, 7, 12, and 17 s; total duration: 20 s). To avoid incidental conditioning to the background shown during intertrial intervals (ITIs), a gray screen was presented during the UCS application period. After that, all four stimuli (CS+, CS−, CS+G, and CS−G) were again presented four times each without electrical stimulation during the reinstatement test. ITIs depicting a white fixation cross on a black screen were randomly jittered between 9.5 and 12 s (total trial duration: 20 s). After the retrieval test and before the reinstatement test, the ITI was shown for 10 s.

During all phases, stimulation electrodes remained attached but did not provide electrical stimulation during extinction, retrieval, and reinstatement test. For all phases, pseudo-randomized stimulus orders were used comprising the following restrictions: no more than two consecutive presentations of the same CS as well as an equal quantity of CS+ trials within the first and second half of the experiment for acquisition: five CS+ reinforced and three CS+ unreinforced trials; for extinction: four CS+ unreinforced trials and four CS− trials; for retrieval and reinstatement test: four blocks of four trials each comprising one presentation of each CS (cf. Jentsch et al., 2020; Merz et al., 2014). Additionally, stimulus presentation orders and CS allocation were matched between the exercise and control groups. The paradigm was realized using Matlab 2021a (Mathworks Inc., Sherborn, MA, USA).

### 2.5.1 | Contingency awareness

Prior to fear acquisition training, participants were instructed to pay attention to any possible association

between the occurrence of the geometrical shapes and the electrical stimulation. They were informed that if they would discover any relationship, it would remain stable in all experimental phases. This instruction was used to facilitate learning of contingencies and to avoid expectancy of a complete contingency reversal. However, participants were not informed about the actual CS-UCS contingencies or the absence of the UCS during extinction, retrieval, and reinstatement test. Immediately after fear acquisition training, participants rated the percentage occurrence (0%–100%) of the UCS after presentation of the two geometrical shapes (CS+ and CS−). To confirm contingency awareness, participants were at least required to correctly choose which of the two CSs never preceded the UCS in a forced-choice question.

### 2.5.2 | SCR data recording and analysis

SCRs were sampled at 1000 Hz with a commercial SCR coupler and amplifying system (MP150+GSR100C; BIOPAC Systems, Inc.; software: AcqKnowledge 4.2) using Ag/AgCl electrodes filled with the same isotonic electrolyte medium as for the electrical stimulation and fixed to the hypothenar surface of the left hand. Raw data were low-pass filtered with a cut-off frequency of 10 Hz. As previously described (Jentsch et al., 2020; Merz et al., 2014), SCRs were calculated as the trough-to-peak amplitude difference (in  $\mu\text{S}$ ) of the largest deflection and defined in two analysis windows (cf. Prokasy & Ebel, 1967): the maximum amplitude within a window of 1–4.99 s after CS onset was counted as the first-interval response (FIR) and within 4.5–8.5 s as the second-interval response (SIR). Raw SCRs were transformed with the natural logarithm to attain a normal distribution.

### 2.5.3 | Pupillometry

Testing took place in a sound-attenuated moderately lit room without daylight luminance and with light conditions kept constant across all measurements. Participants were seated in an adjustable chair in front of the computer screen with an eye-to-screen distance of 50 cm. To minimize head movements, participants were asked to place their chin and forehead on a headrest. Pupillary data were recorded with an EyeLink® Portable Duo eye tracker (SR Research Ltd., Ottawa, Canada) mounted on a tripod, placed below the screen, and connected to a Host PC (ThinkPad T470 W10DG, Lenovo Notebook). A high-speed USB camera including a near-infrared illuminator for dark pupil detection measured retinal and corneal reflections to obtain participants' pupil diameter

(in arbitrary units (AU)) with a binocular sampling rate of 250 Hz, a gaze tracking range of 32° horizontally and 25° vertically, and a gaze position accuracy of 0.15°. A standard 9-point calibration was carried out prior to data recording on each testing day to determine gaze position on the screen and to ensure correct tracking of the participants' eyes.

Preprocessing of the raw pupil size time series was performed in Matlab (version 2021a, MathWorks, Inc., Sherborn, MA, USA) based on routines reported in Kinner et al. (2017) and Jentsch et al. (2020): first eyeblinks (markers provided by EyeLink software, SR Research Ltd., Ottawa, Canada) and unnaturally sudden and large jumps in pupil diameter, which are typically caused by undetected blink artifacts or sudden changes in pupil position (e.g., dilation speed outliers, edge artifacts, trendline deviation outliers, and temporally isolated islands (guidelines and code adapted from Kret & Sjak-Shie, 2019)), were identified and removed. Recorded data were averaged across both eyes and gaps resulting from blink, and artifact removal were filled using cubic spline interpolation marking data 200 ms prior to and following the detected artifact interval as the anchor points for interpolation (Steinhauer et al., 2022; average percentage of interpolated data for all participants across all CS and phases:  $M=17.55\%$ ,  $SD=0.76\%$ ). Raw data were then smoothed with a 120 ms sliding window, low-pass filtered at 3 Hz and onsets of event-locked segments (CS+, CS-, UCS, and ITI) were marked for each trial. Trials containing more than 50% of interpolated data points were discarded (5.05% of all trials,  $SD=0.94\%$ ; see also Leuchs et al., 2019; Stemerding et al., 2022) and invalid trials were treated as missing data. Participants with more than 30% of invalid trials for one of the CSs were excluded from analyses of the respective learning phase.

For each participant and each trial, baseline pupil size was defined as the average pupil diameter recorded during the 300 ms prior to CS onset and subtracted from the pupil size during CS presentation to account for random fluctuations in pupil size over time (Mathôt et al., 2018).

As previous work has shown that pupil diameter discriminates most strongly between the CS+ and CS- in a time window immediately preceding the UCS (Finke et al., 2021; Jentsch et al., 2020; Leuchs et al., 2019; Reinhard & Lachnit, 2002), we determined mean pupil size for CS+ and CS- trials within the last 2 s before UCS onset (i.e., 6–8 s of CS presentation).

## 2.6 | Exclusion of participants

Cortisol and sAA data of one participant of the exercise group had to be excluded from day 2 cortisol and sAA

analyses due to an empty salivette. Two participants from the control group were excluded from all SCR and pupillary analyses because they failed to show contingency awareness after fear acquisition training (see Section 2.5.1), and one participant of the exercise group was excluded from SCR and pupillary analyses of the reinstatement test because of a technical failure to apply the reinstatement shocks. One additional participant from the exercise group had to be excluded from all SCR analyses due to unexceptionally low responding to the UCS (less than one-third of detectable responses in all UCS trials, cf. Jentsch et al., 2020; Kinner et al., 2018; Lonsdorf et al., 2017) and another six participants (five from the control and one from the exercise group) were excluded from SCR analyses due technical difficulties during data recording (e.g., broken connection to recording computer and poor data quality due to random noise) at least on 1 of the 3 days. For pupillary analyses, seven participants (three from the control group and four from the exercise group) were excluded at least on one of the three testing days due to technical difficulties or poor data quality (e.g., due to large percentage of invalid trials that were discarded and not interpolated). The final sample, thus, consisted of  $N=59$  for cortisol and sAA analyses, for SCR analyses  $N=57$  for fear acquisition, fear extinction, and the retrieval test, and  $N=55$  for the reinstatement test. For pupillary analyses, the final sample consisted of  $N=58$  for fear acquisition,  $N=55$  for fear extinction,  $N=53$  for retrieval, and  $N=52$  for the reinstatement test. For all remaining analyses, the full sample could be used.

## 2.7 | Statistical analyses

Statistical analyses were carried out using IBM SPSS Statistics for Windows (Version 22.0. Armonk, NY: IBM Corp.) and R implementation in RStudio (R Core Team, 2019; RStudio Team, 2019) with the significance level set to  $\alpha=.05$ . Data were checked for normality using Kolmogorov–Smirnov tests, which showed skewness of HR, cortisol, sAA, affect, and SCR data. These data were thus log-transformed before use in subsequent analyses.

For HR, salivary cortisol, sAA, and affect ratings, mixed-design analyses of variance (ANOVAs) including the between-subjects factor *group* (exercise vs. control) and the within-subjects factor *time* (cortisol, sAA, affect ratings: day 1: before and after fear acquisition training, day 2: at baseline, –2 min before, as well as +1 min, +10 min, and +25 min after the intervention, day 3: before and after the retrieval and reinstatement test; HR: day 2: before ( $HR_{rest}$ ), during ( $HR_{intervention}$ ), and after ( $HR_{post}$ ) the intervention) were conducted. Greenhouse–Geisser corrected *p* values were used if the assumption of

sphericity was violated and partial eta-square ( $\eta^2_p$ ) were reported as estimations of effect sizes. Significant results were followed by Bonferroni–Holm adjusted post hoc tests and Cohen's  $d$  was calculated as an estimate of effect size.

Because trial-level data are nested within participants, SCRs and PD were analyzed with linear mixed models using the R-package *lm4* (Bates et al., 2015) and the *lmer* function. We conducted separate models for fear acquisition, fear extinction, the retrieval, and reinstatement test including *subject* as a random factor in all models to account for clustering of individual subject effects (inclusion of this random factor improved the fit of all models as estimated with the intraclass correlation [ICC]: for fear acquisition: SCRs ICC = 0.49, PD ICC = 0.34; for fear extinction SCRs: ICC = 0.56, PD ICC = 0.28; for the retrieval test: SCRs ICC = 0.68, PD ICC = 0.27; and for the reinstatement test: SCRs ICC = 0.59, PD ICC = 0.27, justifying our decision to use multilevel models). To test for learning-related changes in conditioned responding during fear and extinction learning, we included the within-subjects factors *CS* (CS+ vs. CS-) and *block* (eight and four blocks comprising the mean across two trials of each CS for fear acquisition and fear extinction training, respectively), as well as the interaction term *CS* × *block* and the between-subjects factor *group* (exercise vs. control) as fixed effects in the model. Furthermore, we investigated (a) whether the exercise intervention modulated conditioned responses to the original CS+ and CS- during the retrieval and reinstatement test and (b) the generalization of this effect to novel but perceptually similar stimuli (i.e., CS+G and CS-G). We thus entered the within-subjects factors *CS* (CS+ vs. CS-), *generalization* (CSorig vs. CSgen), and *block* (two blocks comprising the mean across two trials of each CS), as well as the between-subject factor *group* (exercise vs. control) as fixed effects into the model, including also the interaction term among *CS*, *generalization*, *block*, and *group*. We used maximum-likelihood estimation and tested statistical significance of fixed effects with type II Wald Chi-square tests using the *Anova* function from the R-package *car* (Fox & Weisberg, 2019). Post hoc pairwise comparisons were conducted using  $t$  tests and adjusted  $p$  values using Bonferroni–Holm correction in the R-package *emmeans* (Lenth, 2020).

## 3 | RESULTS

### 3.1 | Manipulation check: Exercise and control intervention

#### 3.1.1 | Heart rate

Exercise significantly increased participants' HR when compared to controls (main effect *time*:  $F(1.5,$

$88.2) = 657.10; p < .001; \eta^2_p = 0.92$ , main effect *group*:  $F(1, 58) = 167.96; p < .001; \eta^2_p = 0.74$ , *time* × *group* interaction:  $F(1.5, 88.2) = 812.97; p < .001; \eta^2_p = 0.93$ ). Whereas HR did not differ at rest ( $t(58) = 0.98, p = .33, d = 0.26$ ), participants exhibited significantly higher HR during ( $t(58) = 35.16, p < .001, d = 9.23$ ) and after ( $t(58) = 10.89, p < .001, d = 2.86$ ) the exercise relative to the control intervention (see Figure 2a). Detailed ANOVA results are summarized in Table S1.

#### 3.1.2 | Salivary cortisol and alpha-amylase

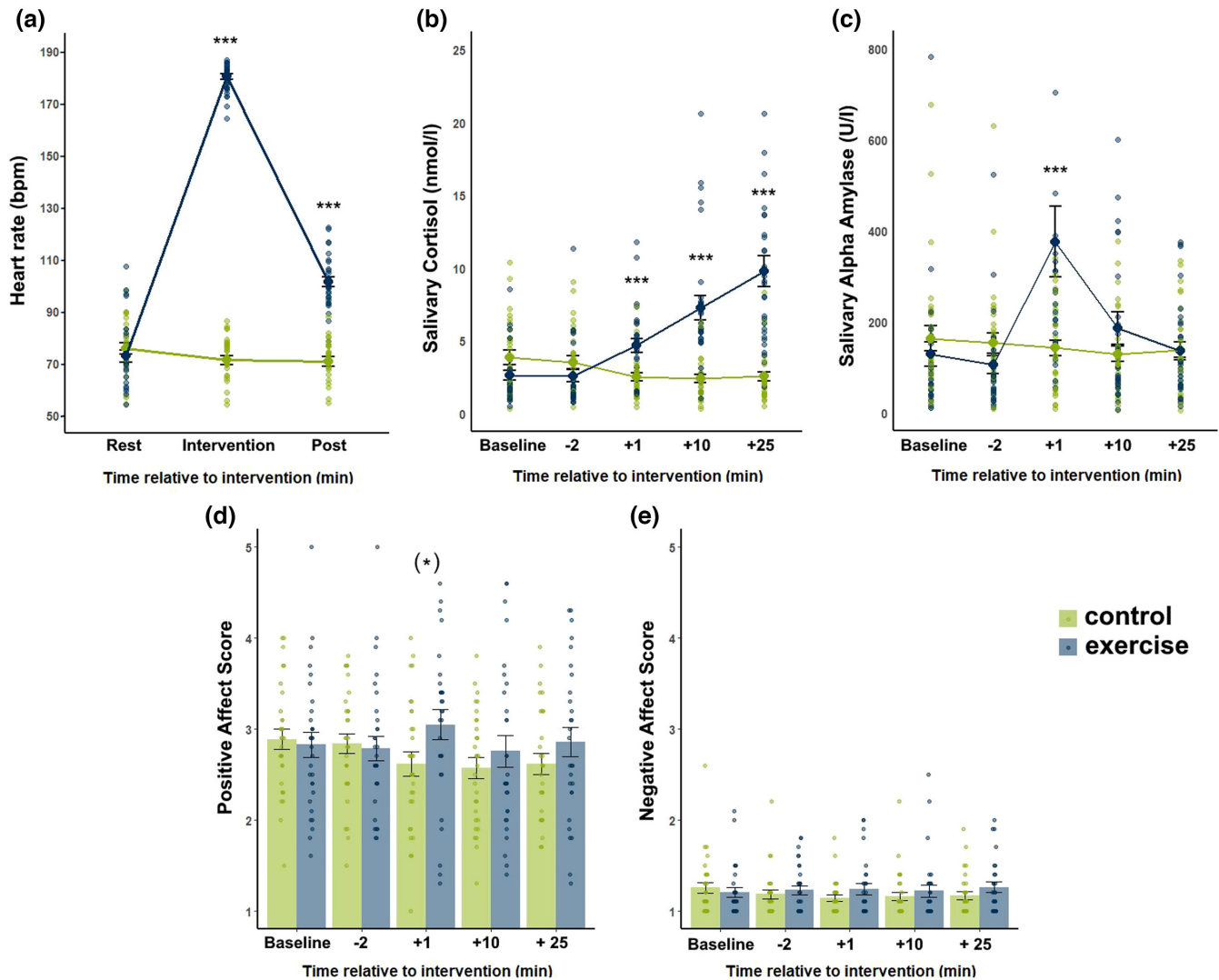
Acute exercise led to a significant increase in salivary cortisol (main effect of *time*:  $F(1.96, 111.89) = 21.95; p < .001, \eta^2_p = 0.28$ ; main effect of *group*:  $F(1, 57) = 11.71, p = .001; \eta^2_p = 0.17$ ; *time* × *group* interaction:  $F(1.96, 111.89) = 60.75, p < .001, \eta^2_p = 0.52$ ) and alpha-amylase (main effect of *time*:  $F(2.69, 153.45) = 24.75, p < .001, \eta^2_p = 0.30$ , *time* × *group* interaction:  $F(2.69, 153.45) = 29.52, p < .001, \eta^2_p = 0.34$ , Table S1). Cortisol concentrations were significantly elevated +1 min ( $t(58) = 4.21, p < .001, d = 1.11$ ), +10 min ( $t(58) = 6.35, p < .001, d = 1.67$ ), and +25 min ( $t(58) = 8.80, p < .001, d = 2.31$ ) after the exercise relative to the control intervention, whereas groups did not differ at baseline (i.e., before fear extinction training) or 2 min before the exercise manipulation (i.e., directly after fear extinction training, all  $ps > .10$ ; Figure 2b). Alpha-amylase was significantly increased immediately after the exercise but not control intervention (+1 min:  $t(58) = 3.86, p < .001, d = 1.01$ ), whereas no group differences occurred for any of the other time points on day 2 (all  $ps > .15$ , Figure 2c). Likewise, there were no group differences in salivary cortisol or alpha-amylase on day 1 (i.e., before or after fear acquisition training, all  $ps > .25$ ) or day 3 (i.e., before or after the retrieval and reinstatement test, all  $ps > .13$ ).

#### 3.1.3 | Affect ratings

For positive affect, a significant main effect of *time* ( $F(3.33, 176.85) = 4.93, p = .002, \eta^2_p = 0.09$ ) and *time* × *group* interaction ( $F(3.33, 176.85) = 5.48, p = .001, \eta^2_p = 0.09$ , Table S1) occurred. Post hoc  $t$  tests revealed that the exercise group tended to experience more positive affect immediately after the exercise relative to the control intervention ( $t(56) = 1.76, p = .08, d = 0.47$ ; Figure 2d). No other group differences in positive affect were found for any of the other time points on day 2, and neither on day 1 nor on day 3 (all  $ps > .24$ ).

For negative affect, no significant main or interaction effects with the factor *time* or *group* occurred for days 1 and 2 (all  $ps > .11$ ), suggesting that participants of both groups experienced relatively stable and low negative





**FIGURE 2** Physiological and affective responses to the exercise and control intervention. Mean ( $\pm$ SEM) and individual (a) heart rate (HR), (b) salivary cortisol, and (c) alpha-amylase (sAA) as well as (d) positive and (e) negative affect ratings at rest, during and post-intervention (for HR), and at baseline,  $-2$  min,  $+1$  min,  $+10$  min, and  $+25$  min relative to the exercise/control intervention (for salivary cortisol, sAA, and affect ratings). Acute exercise significantly increased HR, salivary cortisol, and sAA relative to the control intervention. Participants in the exercise group reported a slight increase in positive affect immediately after exercising when compared to the control group. Negative affect did not differ between groups. \*\*\* $p < .001$ , (\*) $p = .08$ .

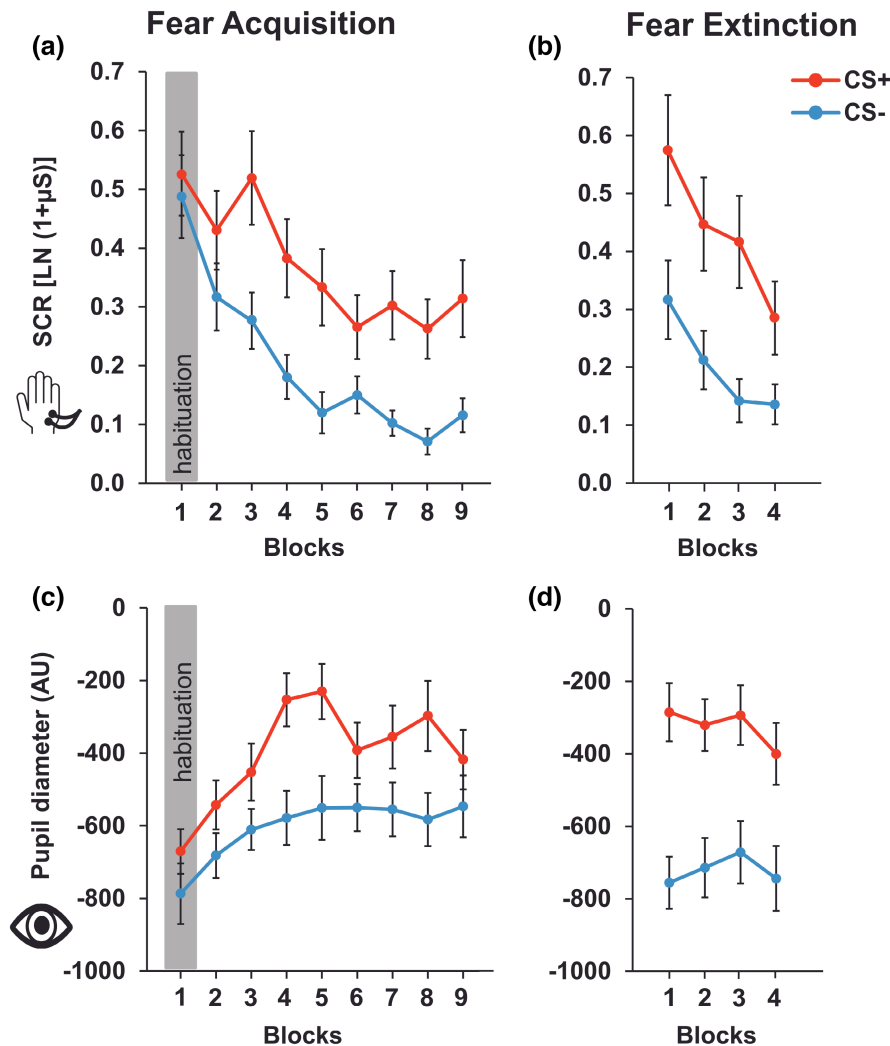
affect across both experimental testing days (see Figure 2e; Table S1). For day 3, a significant main effect of *time* ( $F(1, 58) = 29.60$ ,  $p < .001$ ,  $\eta^2_p = 0.34$ ) was found, indicating an increase in negative affect after the retrieval and reinstatement test in both groups.

## 3.2 | SCRs

### 3.2.1 | Fear acquisition

Fear acquisition was successful, as revealed by a significant effect of *CS* ( $\chi^2(1) = 109.15$ ,  $p < .001$ ) in the linear mixed-effects model, showing higher SCRs toward the

CS+ as compared to the CS- in the FIR (see Figure 3a). In addition, *block* was a significant predictor of SCRs ( $\chi^2(1) = 60.70$ ,  $p < .001$ ), reflecting a general decrease due to habituation processes, whereas the *CS*  $\times$  *block* interaction ( $\chi^2(1) = 0.13$ ,  $p = .72$ ) was not significant. As expected, *group* was not a significant predictor for the FIR ( $\chi^2(1) = 0.68$ ,  $p = .41$ ), indicating that there is no relationship between group and a change in conditioned responding. Analyses for the SIR yielded similar results (details regarding the analyses and results of the SIR for all experimental phases are provided in the supplements). Mean as well as individual SCRs and PDs during fear acquisition and fear extinction are illustrated in Figure S1.



**FIGURE 3** Fear acquisition and fear extinction. Mean ( $\pm$ SEM) skin conductance responses (SCRs; upper panels) and mean ( $\pm$ SEM) pupil diameter (in arbitrary units (AU); lower panels) are depicted for the CS+ and CS- during fear acquisition (left panels) and fear extinction training (right panels). Corresponding to the statistical analyses, each block comprised two CS trials. Note that data of the first two habituation trials (i.e., first block during fear acquisition marked with shaded areas) are also shown for illustrative purposes but not included in the analyses. During fear acquisition training, participants showed significantly higher SCRs (a) and larger pupil diameter (c) to the CS+ as compared to the CS-. During fear extinction training, differential SCR (b) and pupil diameter (d) decreased slightly, but still significantly differ between the CS+ and the CS-.

### 3.2.2 | Fear extinction

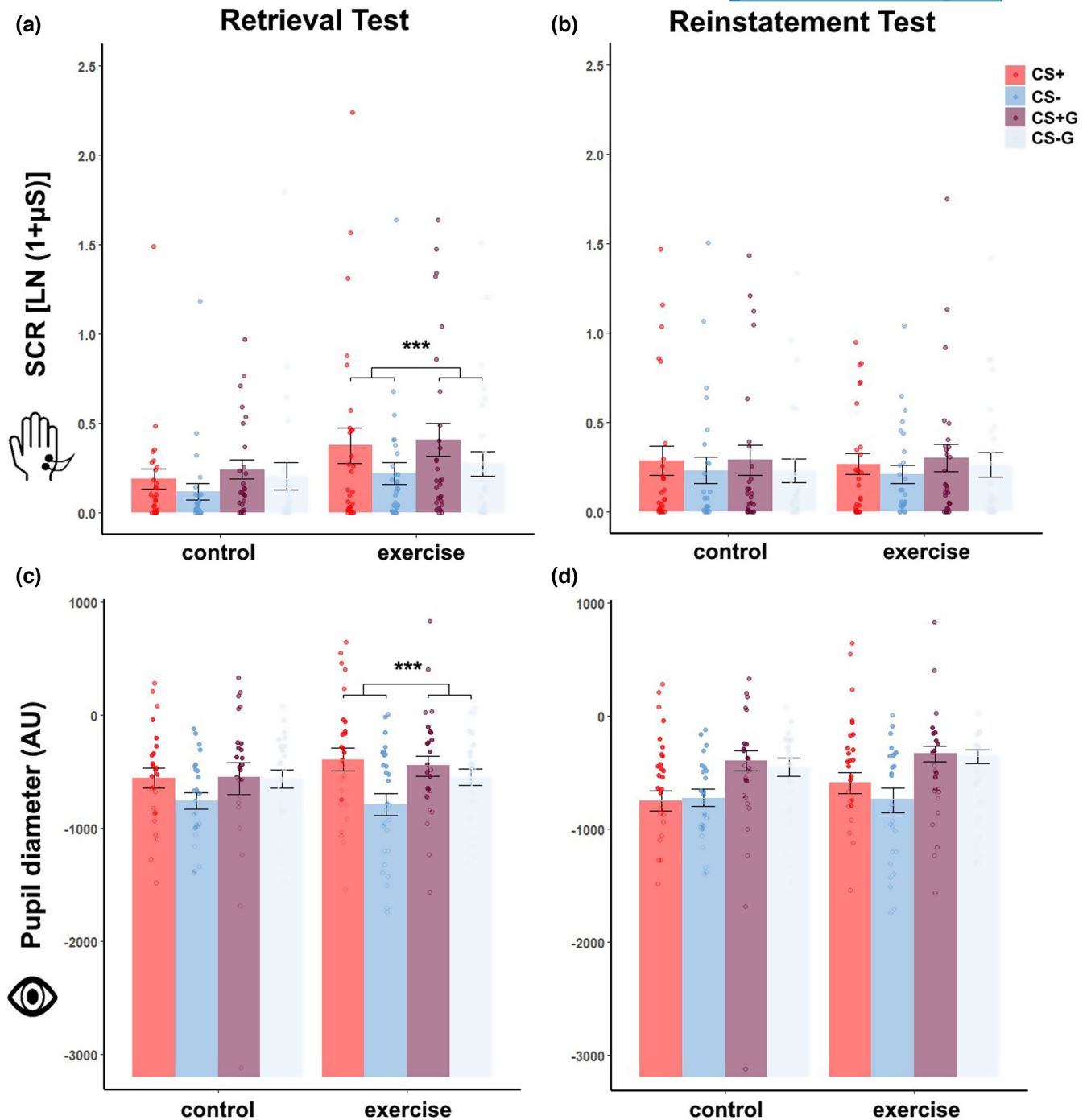
For fear extinction, again both *CS* ( $\chi^2(1)=62.95$ ,  $p < .001$ ) and *block* ( $\chi^2(1)=34.35$ ,  $p < .001$ ) but not the *CS*  $\times$  *block* interaction ( $\chi^2(1)=1.25$ ,  $p = .26$ ) significantly predicted conditioned SCRs in the FIR. As depicted in Figure 3b, differential SCRs decreased across blocks; however, an exploratory *t* test revealed that participants still showed a significant discrimination between CS+ and CS- at the end of extinction training (block 4:  $t(54)=2.91$ ,  $p < .01$ ), implying that fear extinction was not successful or at least incomplete. Similar to fear acquisition, *group* ( $\chi^2(1)=0.04$ ,  $p = .85$ ) did not significantly contribute to the model fit.

### 3.2.3 | Retrieval and reinstatement test

For the retrieval test, parameter estimates from the linear mixed model revealed a significant *CS*  $\times$  *group* interaction for the FIR ( $\chi^2(1)=4.87$ ,  $p = .03$ ). Post hoc pairwise comparisons indicated that the exercise group exhibited significantly higher SCRs to the CS+ as compared to the

CS- ( $t(399.5)=5.10$ ,  $p < .001$ ) reflecting stronger fear memory retrieval, whereas SCRs to the CS+ and CS- did not significantly differ in the control group ( $t(399.5)=1.82$ ,  $p = .28$ ; see Figure 4a). The three-way interaction between *CS*, *generalization*, and *group* was not significant ( $\chi^2(1)=0.01$ ,  $p = .92$ ), implying that the exercise effects on SCRs were not only specific to the original CS but also generalized to the perceptually similar CS (i.e., higher SCRs to the CS+G relative to the CS-G:  $t(399.5)=3.28$ ,  $p < .05$ ). Furthermore, *CS* ( $\chi^2(1)=25.20$ ,  $p < .001$ ) and *block* ( $\chi^2(1)=28.90$ ,  $p < .001$ ) were significant to the model fit, again showing higher SCRs to the CS+ as compared to the CS- and a general decrease in conditioned responding from the first to the second block of the retrieval test. In addition, we found a significant effect of *generalization* ( $\chi^2(1)=7.41$ ,  $p < .01$ ), indicating generally higher SCRs to the generalized as compared to the original CS in the FIR.

For the reinstatement test, *CS* significantly predicted SCRs, confirming higher SCRs to the CS+ as compared to the CS- in the FIR (Figure 4b). In addition, we found a significant *CS*  $\times$  *generalization*  $\times$  *block* interaction. Post hoc pairwise comparisons did not reveal any significant



**FIGURE 4** Exercise effects for the retrieval and reinstatement test. Mean ( $\pm$  SEM) and individual skin conductance responses (SCRs; upper panels) and mean ( $\pm$  SEM) and individual pupil diameter (PD; in arbitrary units (AU); lower panels) averaged over four trials of the retrieval (left panels) and reinstatement test (right panels) are depicted for the original CS+ and CS- (i.e., stimuli already shown during fear acquisition training on day 1 and fear extinction training on day 2) as well as for the generalized CS+G and CS-G (i.e., novel stimuli only shown during the retrieval and reinstatement test on day 3). Separate bar charts are outlined for the control and exercise group, respectively; the exercise intervention took place after fear extinction training on day 2. During the retrieval test, participants in the exercise group expressed significantly higher SCRs (a) and larger pupil diameter (c) in response to the CS+ relative to the CS-, reflecting stronger fear memory retrieval. This effect of exercise was not only specific to the original CS (CS+>CS-) but also generalized to the novel stimuli (CS+G>CS-G). For the reinstatement test (b, d), no significant exercise effects occurred. \*\*\* $p < .001$ , significant effects after Bonferroni-Holm corrected post hoc  $t$  tests.

differences between the factors (all  $ps > 1.0$ ). Descriptively however, CS+/CS- differentiation was stronger for the original than for the generalized stimuli in the first block, whereas being larger for the generalized than for the original stimuli in the second block.

### 3.3 | Pupillary responses

#### 3.3.1 | Fear acquisition

Consistent with SCR data, successful fear acquisition was reflected by a significant effect of CS on pupillary responses ( $\chi^2(1) = 47.69$ ,  $p < .001$ ), showing stronger pupil dilation toward the CS+ as compared to the CS- (see Figure 3c). *Block* ( $\chi^2(1) = 47.69$ ,  $p < .001$ ) also significantly contributed to the model fit, indicating a general increase in conditioned PD across blocks of fear acquisition training. Similar to SCRs, neither the *CS* × *block* interaction ( $\chi^2(1) = 0.00$ ,  $p = .96$ ) nor *group* ( $\chi^2(1) = 0.93$ ,  $p = .33$ ) were significant predictors for pupillary responses.

#### 3.3.2 | Fear extinction

For fear extinction, we only found a significant effect of CS ( $\chi^2(1) = 72.25$ ,  $p < .001$ ), but no effect of *block* ( $\chi^2(1) = 0.34$ ,  $p = .56$ ) or *CS* × *block* interaction ( $\chi^2(1) = 0.89$ ,  $p = .35$ ) on pupillary responses. As illustrated in Figure 3d and similar to SCR data, differential conditioned PD (CS+ > CS-) slightly decreased over time; however, PD still significantly differed between CS+ and CS- at the end of extinction training (block 4:  $t(54) = 3.54$ ,  $p < .01$ ), again indicating unsuccessful or incomplete fear extinction. As expected, and in accordance with SCR data, *group* ( $\chi^2(1) = 0.26$ ,  $p = .61$ ) was not a significant predictor of the model fit, confirming that the exercise and control groups did not differ in conditioned response during fear extinction.

#### 3.3.3 | Retrieval and reinstatement test

For the retrieval test, linear mixed-model analysis revealed a significant effect of CS ( $\chi^2(1) = 15.52$ ,  $p < .001$ ), reflecting higher PD to the CS+ relative to the CS-, and a significant effect of *generalization* ( $\chi^2(1) = 4.40$ ,  $p < .05$ ), indicating generally stronger PD to the generalized as compared to the original CS. A significant *CS* × *generalization* interaction ( $\chi^2(1) = 7.31$ ,  $p < .01$ ) furthermore revealed a significant CS+/CS- differentiation in pupillary responses for the original stimuli (CS+ > CS-,  $t(386) = 4.512$ ,  $p < .001$ ), but not for the generalized stimuli (CS+G = CS-G;  $t(386) = 0.81$ ,  $p = .84$ ). However,

participants' pupil diameter was significantly increased to both generalized CS when compared to the original CS- (CS+G > CS-:  $t(386) = 4.12$ ,  $p < .001$  and CS-G > CS-:  $t(386) = 3.31$ ,  $p < .01$ ). Consistent with SCRs, we also found a trend for a *CS* × *group* interaction ( $\chi^2(1) = 2.93$ ,  $p = .08$ ). Paralleling the analyses of SCR data, exploratory post hoc comparisons showed significantly higher PDs to the CS+ as compared to the CS- and thus stronger fear retrieval in the exercise group ( $t(386) = 3.96$ ,  $p < .001$ ) but no significant CS+/CS- differentiation in the control group ( $t(386) = 1.43$ ,  $p = .61$ ; see Figure 4c).

An interaction between *generalization* and *block* was also apparent as a trend ( $\chi^2(1) = 3.28$ ,  $p = .07$ ). Exploratory post hoc comparisons indicated that stronger pupillary responses to the generalized relative to the original CS occurred in the second block of the retrieval test ( $t(386) = 2.69$ ,  $p < .05$ ), whereas no such PD difference was evident for the first block ( $t(386) = 0.23$ ,  $p = 1.0$ ).

During the reinstatement test, pupillary responses were again significantly stronger to the generalized as compared to the original CS ( $\chi^2(1) = 55.50$ ,  $p < .001$ , see Figure 4d). Moreover, we found a *CS* × *group* × *block* interaction ( $\chi^2(1) = 3.83$ ,  $p = .05$ ). Exploratory post hoc comparisons did not reveal any significant differences between these factors (all  $ps > 1.0$ ). However, the control group exhibited descriptively larger PD to the CS+ relative to the CS- in the first block, whereas this pattern completely reversed in the second block revealing larger PD to the CS- relative to the CS+. By contrast, in the exercise group, a CS+ > CS- differentiation was evident in the second but not in the first block of the reinstatement test. There was no significant effect of *CS* ( $\chi^2(1) = 1.39$ ,  $p = .24$ ) and no further interactions with the predictor *group*.

## 4 | DISCUSSION

In this study, we investigated whether a single bout of physical exercise can enhance extinction memory consolidation in healthy men and further explored if these effects generalize to novel but perceptually similar stimuli. As expected, we found a significant increase in HR, salivary alpha-amylase, and cortisol in response to the exercise as compared to the control intervention. Yet, contrary to our main hypothesis, exercise did not enhance but rather impaired extinction memory retrieval on the following day, as reflected by significantly stronger differential SCRs and PD. This effect was not only specific to the original CS but also generalized to the two novel stimuli, indicated by significantly higher SCR and PD to the CS+G relative to the CS-G in the exercise relative to the control group. Although physical exercise enhanced fear recovery during the initial retrieval test, it did not further affect the reinstatement of fear.

Accumulating evidence from both animal and human studies suggests a beneficial impact of physical exercise on extinction processes (for a summary of recent findings, see Keyan & Bryant, 2019b). More specifically, it has been observed that aerobic exercise, especially performed immediately after extinction training, leads to improved extinction memory retrieval and less ROF (Roquet & Monfils, 2018; Tanner et al., 2018). This implies that an acute bout of physical exercise may trigger transient neurochemical processes that are able to support the consolidation of the newly acquired extinction memory. Based on these considerations, it might appear to be surprising at a first look that we did not find improved but rather impaired extinction memory retrieval in the present study. Yet, for the extinction consolidation-enhancing effects of exercise to occur, it is necessary to successfully acquire extinction memory in the first place. However, although we observed a slight decrease in differential conditioned fear responses across extinction, participants still exhibited higher SCR and PD to the CS+ relative to the CS− at the end of fear extinction training. It is therefore reasonable that exercise following unsuccessful or incomplete fear extinction might have boosted the consolidation of the intact and still dominant fear memory trace instead. This idea aligns with studies in rodents showing physical exercise to enhance contextual and cued fear conditioning (Baruch et al., 2004; Falls et al., 2010; Siette et al., 2014) as well as work in humans revealing an exercise-induced facilitation of emotional memory (re)consolidation (Jentsch & Wolf, 2020; Keyan & Bryant, 2017a, 2017b). It has to be noted that we decided to reduce the number of extinction trials because ROF phenomena have been shown to be significantly reduced in delayed extinction paradigms (Huff et al., 2009; Lonsdorf et al., 2017). Given that we aimed to investigate the potential role of physical exercise in mitigating ROF, we thereby sought to limit potential floor effects (i.e., too little ROF in general) masking true effects of our manipulation. Although existing literature on the extinction-enhancing effects of exercise provides promising evidence for its use in exposure-based treatments, our results yet reveal that exercise interventions may also bear a potential risk of curtailing therapeutic success when they are employed too early or in combination with an unsuccessful exposure session. Our findings thereby not only inform future researchers about critical methodological considerations but also have important implications for clinicians working with anxiety patients. Future studies systematically varying the number of trials during fear acquisition and extinction training are warranted to elucidate such procedural aspects which may influence exercise effects on extinction learning.

The potential role of physical exercise in promoting the generalization of extinction memories has not been

investigated so far. Here, we demonstrated for the first time that the memory-enhancing effects of exercise may also generalize to novel but perceptually similar stimuli that were not present at the time of extinction training. In particular, analyses of SCRs and PD revealed exercise not only to increase conditioned fear responses toward the original CS+ relative to the original CS−, reflecting stronger fear memory retrieval, but also to enhance differential fear responding toward the generalized CS during a retrieval test. Although the strength of this CS differentiation appeared to be similar for the original and generalized stimuli in SCR, a *CS* × *generalization* interaction furthermore indicated that differential pupillary responses were more pronounced for the original than for the generalized CS. Our results thereby contrast previous studies from the human stress literature showing post-extinction stress or pharmacological GC manipulations to rather diminish the generalization of extinction memories (Meir Drexler et al., 2019). Specifically, it has been proposed that elevated stress hormone levels after extinction training promote extinction consolidation, but in a context-bound manner, whereas stress/GCs prior to extinction facilitate memory consolidation in a context-independent manner, thereby making extinction memories more resistant to relapse following contextual changes. By contrast, a recent neuroimaging study from our lab using multiple sizes of one CS+ during extinction training to create a generalized extinction memory trace revealed that pre-extinction cortisol administration selectively reduced fear-related neural activation only for a standard but not for the generalized CS+ (Hagedorn et al., 2022). Contradictory findings may be attributed to methodological differences between studies, including procedural variations, such as triggering generalization processes already during extinction training or testing them only at a retrieval/ ROF test. Furthermore, distinct outcome measures despite sharing overlapping mechanisms are also known to diverge (Lonsdorf et al., 2017) and thus could be responsible for some heterogeneity. However, it is also reasonable that stimulus—as opposed to context specificity of extinction memories—is differently modulated by stress/exercise interventions. Future studies comparing multiple cues and multiple contexts in a single experimental design (e.g., as realized in Shibani et al., 2015) as well as their modulation by physical exercise should test this possibility. Likewise, it could be speculated that physical exercise compared to psychosocial stress, although initiating similar neuroendocrine and cardiovascular responses (Gatti & de Palo, 2011; Skoluda et al., 2015), differ in their modulatory properties to alter the generalization of extinction memories. For instance, it remains to be shown whether physical exercise also timing dependently enhance or reduce the context specificity of extinction memories as

psychosocial stress or GC manipulations do. A systematic comparison of these interventions regarding their potential to augment the retrieval and generalization of extinction memories together with an in-depth investigation of the specific mechanistic pathways responsible for their effects to occur will contribute to a better understanding of the factors necessary to create strong, relapse-resistant extinction memories.

Whereas physical exercise enhanced fear recovery upon initial exposure with the conditioned and generalized stimuli during the retrieval test, SCR and PD were not further modulated by our exercise intervention following reinstatement. Instead, we found generally stronger SCR and PD to the generalized as compared to the original stimuli during both the retrieval and reinstatement test, most probably reflecting attentional orienting in response to stimulus novelty (Boucsein et al., 2012; Steinhauer et al., 2022; Strauch et al., 2022).

Our study had some limitations which need to be considered when interpreting the results. First and foremost, we tested only male participants. Given the well-documented sex differences in physical and psychosocial stress responsivity (Dominelli & Molgat-Seon, 2022; Kudielka & Kirschbaum, 2005; O'Bryan et al., 2022) and converging evidence revealing sex-specific stress hormone effects on emotional memory (Jentsch & Wolf, 2020; Merz & Wolf, 2017) and fear-conditioning processes (Merz et al., 2018), our findings cannot be generalized to women. As anxiety and stress-related disorders occur twice as likely and with a higher severity in women compared to men (Cover et al., 2014), it will be of utmost importance to examine exercise effects on extinction processes in both sexes. However, besides sex differences per se, sex hormone variations over the course of the menstrual cycle or due to the intake of hormonal contraceptives have been shown to further modulate stress hormone effects on fear conditioning in women (Jentsch et al., 2022; Merz et al., 2018; Merz & Wolf, 2017). For future studies, it would be thus desirable to include subsamples of women in different phases of their menstrual cycle and hormonal contraceptive users for a comprehensive comparison with men. A better understanding of how physical exercise, stress, and sex hormones interact with fear and extinction processes will be crucial to develop effective therapeutic strategies that are specifically tailored to men and women (and perhaps also to different groups of women).

We also note that we recruited individuals who were in principle willing to participate in an experiment involving an exercise intervention. We thus cannot preclude the possibility of testing a biased sample of individuals regularly engaging in sport activities anyway or having a positive view of physical exercise and its potential benefits. Regular physical activity promotes neurogenesis (Cotman

& Berchtold, 2002), increases hippocampal volume, and improves cognition (Erickson et al., 2011). Moreover, aerobic fitness appears to be related to the magnitude and quality of acute exercise-induced effects on memory (Loprinzi et al., 2023; Pontifex et al., 2019; Roig et al., 2013). It is therefore possible that an individual's cardiorespiratory fitness may moderate the physiological response to and/or perception of an acute exercise bout and, in turn, influence its effects on extinction memory. In the current study, there was no difference in self-reported exercise frequency per week between participants of the exercise and control group, suggesting that the fitness level was not very likely to influence our results. However, future studies would benefit from employing a progressive exercise test (e.g., a  $VO_2$ max test) at baseline for the assessment of the participants' fitness level to better explore whether fitness may modulate acute exercise effects on extinction memories. Such a procedure would also enhance the accuracy in determining the individual exercise intensity as opposed to estimation methods that typically use the age-predicted maximal HR for the calculation of target intensities as employed in the present study. Moreover, even though the neutral videos shown during the control intervention did not elicit a strong affective response and thus served as an adequate and commonly used nonarousing control condition (Pontifex et al., 2019), future researchers should consider presenting the videos during both interventions or to employ an active control condition (e.g., light-intensity exercise) to minimize attentional and affective differences and to enhance comparability between groups. Finally, we note that the current findings can neither be generalized to other exercise types or intensities nor do they necessarily inform whether regular aerobic exercise would have similar effects on the retrieval and generalization of extinction memories.

In conclusion, the present study demonstrates that a single bout of physical exercise following extinction learning enhanced fear memory retrieval in healthy men. We thereby provide further evidence for physical exercise to not only facilitate memory consolidation but also reveal a potential risk of boosting the fear rather than the extinction memory trace if exercise is employed after incomplete or unsuccessful fear extinction. The generalization of the memory-enhancing exercise effects to novel stimuli furthermore suggests that acute physical exercise may provide a promising adjunctive behavioral strategy not only for enhancing the strength and long-term retrievability of extinction memories but also their transfer to other perceptually similar stimuli and contexts.

## AUTHOR CONTRIBUTIONS

**Valerie L. Jentsch:** Conceptualization; data curation; formal analysis; funding acquisition; investigation;

methodology; project administration; validation; visualization; writing – original draft. **Oliver T. Wolf:** Funding acquisition; resources; supervision; validation; writing – review and editing. **Tobias Otto:** Data curation; software. **Christian J. Merz:** Conceptualization; funding acquisition; methodology; supervision; validation; writing – review and editing.

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## CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data set analyzed during the current study is available at the Open Science Framework (OSF) under <https://osf.io/wtr75/>.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**TABLE S1** Mixed design ANOVAs for the physiological (heart rate, salivary cortisol, and alpha amylase) and affective responses (positive and negative affect) to the exercise and control intervention on day two.

**FIGURE S1** Fear acquisition and fear extinction. Mean ( $\pm$ SEM) and individual skin conductance responses (SCRs; upper panels) and mean ( $\pm$ SEM) and individual pupil diameter (in arbitrary units (AU); lower panels) are

depicted for the CS+ and CS− during fear acquisition (left panels) and fear extinction training (right panels). Corresponding to the statistical analyses, each block comprised two CS trials. Note that data of the first two habituation trials (i.e., first block during fear acquisition marked with shaded areas) are also shown for illustratory purposes but not included in the analyses. During fear acquisition training, participants showed significantly higher SCRs (a) and larger pupil diameter (c) to the CS+ as compared to the CS−. During fear extinction training, differential SCR (b) and pupil diameter (d) decreased

slightly, but still significantly differ between the CS+ and the CS−.

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