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In search of the behavioral effects of fear: A paradigm to assess conditioned suppression in humans

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Abstract

Conditioned fear can substantially reduce the likelihood that an individual will engage in reward-related behavior––a phenomenon coined conditioned suppression. Despite the unmistakable relevance of conditioned suppression for excessive fears and their adverse consequences, the phenomenon has primarily been observed in animal models and is not yet well understood. Here, we aimed to develop a conditioned suppression paradigm that enables a robust quantification of the effect of Pavlovian fear on subsequent reward-related behavior in humans and assess its potential relation to physiological measures of fear. In phase 1, an instrumental response was incentivized with monetary rewards. In phase 2, one of two conditioned stimuli (CS+) was reinforced with an aversive unconditioned stimulus (US, i.e., electric stimulus). During Pavlovian fear learning we assessed differential skin conductance (SCR) and fear- potentiated startle responses (FPS). Lastly, we tested the effect of the fear conditioned CS+ on the response rate of the instrumental response in a transfer phase. Despite strong Pavlovian fear conditioning, as indicated by large effect sizes in differential SCR and FPS, we did not find any evidence for conditioned suppression: that is, there was no significant reduction of instrumental responding in the presence of the CS+ compared to a new control stimulus. This lack of conditioned suppression is in line with previous studies that reported difficulties inducing conditioned suppression and points toward a general challenge in investigating conditioned suppression in humans. Implications and directions for future research on the highly relevant behavioral effects of fear and anxiety are discussed.

KEYWORDS

anxiety, anxiety sensitivity, aversive, Pavlovian-to-instrumental transfer, PIT, trait anxiety

1 | INTRODUCTION

To optimize our chances for survival and prepare for situation-appropriate action, fear, and anxiety can interfere with ongoing behavior, such as going to work or engaging in social interactions. In face of actual threat, behavioral effects of fear are adaptive, but they can cause great harm when the actual threat is low and fear excessive. When instrumental behavior is repeatedly or constantly disrupted, even though the costs of interrupting the current action

are high, an individual will experience less frequent rewards. The fear-induced reduction of rewarding activities could be a precursor for developing comorbid depression, or as stated in Hippocrates Epidemics, long-lasting fright may turn into melancholy (Hippocrates, 1868). In line with this thinking, the majority of comorbid mood and anxiety disorders are characterized by anxiety disorder symptoms preceding symptoms of depression (e.g., Cole et al., 1998; Kaufman & Charney, 2000; Moitra et al., 2008; Starr & Davila, 2012; Wittchen et al., 2000). Recent years have seen a renewed interest in other, more direct behavioral consequences of conditioned fear, such as active and passive avoidance or escape (Dymond, 2019; Krypotos et al., 2015; LeDoux et al., 2017; Pittig et al., 2020). The more indirect, or subsequent, effects of fear on reward-related behavior are, however, largely understudied in humans. One reason for this apparent gap in the literature may be the lack of a suitable paradigm to examine this phenomenon in humans.

Pavlovian-to-Instrumental transfer (PIT) paradigms have long been employed in research on appetitive learning (Cartoni et al., 2016; Holmes et al., 2010) but also allow us to investigate the effect of Pavlovian fear on reward-related behavior (LeDoux et al., 2017). Transfer paradigms typically consist of three phases: (1) a Pavlovian phase, in which an initially neutral stimulus (CS) predicts the occurrence of either an appetitive (e.g., food) or an aversive (e.g., shock) unconditioned stimulus (US), (2) an instrumental phase, in which a behavior is reinforced by the omission of an aversive or the delivery of a rewarding stimulus, and (3) a transfer phase, in which the behavioral response can be exerted again in the presence of the Pavlovian CS. If the presence of a Pavlovian CS increases the exertion of the instrumental response one speaks of "conditioned faciliation", when it decreases the exertion of the instrumental responses of "conditioned suppression". When confronted with an aversively conditioned CS, animals increase avoidance responses that were trained with the same (outcome-specific PIT) or a different (general PIT) US than the Pavlovian CS itself (Hendersen et al., 1980; LoLordo, 1967; Rescorla & LoLordo, 1965; Solomon & Turner, 1962) and suppress responding associated with a rewarding outcome (Estes & Skinner, 1941). Whereas transfer paradigms have long been employed to study conditioned suppression in animals, a robust paradigm to investigate fear-induced suppression in humans is still lacking.

The small number of previous studies on conditioned suppression in humans were not consistently successful in showing a PIT effect (for review see Gerlicher & Kindt, 2020). Commonly, aversive USs such as bitter juice, a small monetary loss, or a loss of points in a computer game were employed that bear little ecological validity for the study of fear, or the instrumental response was merely instructed (Di Giusto et al., 1974; Hebart & Gläscher, 2015; Trick et al., 2011). This makes comparisons to real-life reward behavior acquired by actual experience and reinforced by appetitive stimuli difficult. In a recent study these two issues were addressed by investigating the effect of a Pavlovian CS paired with an electric stimulus on an acquired instrumental response reinforced with a primary food reward (i.e., chocolate; Xia et al., 2019). Though different from classic conditioned suppression paradigms in animals, a response conflict (Go/noGo task) was used to assess the motivational effects of fear conditioning on behavior. Participants were asked to either withhold responding (No Go-conditions), or direct a coin toward ("approach") or away ("withdraw") from a target (Go-conditions) while a previously aversively conditioned CS (CS+) or a control stimulus that was never paired with the US (CS-) were presented in the background. In the presence of the CS+ compared to the CS-, a facilitation of withdrawal-responses was observed. However, no conditioned suppression of approach-responses was observed. Critically, participants only received the reward when the number of button presses resulted in a coin hitting a spatial target window. This may have limited the range of successful responses and potentially made response-rates less sensitive to more subtle effects of conditioned suppression. Furthermore, the paradigm did not include a baseline condition. As the CS- has been a reliable predictor of the absence of the US it can become an inhibitory or safety stimulus with its own effects on behavior (Rescorla, 1969). The lack of a baseline condition can thus make it difficult to differentiate effects of an aversively trained CS+ from potential effects of the CS-.

Here, we aimed to test a paradigm that more closely resembles PIT paradigms developed for animals and addressed potential caveats of previous PIT studies in humans. Hence, we employed an electric stimulus as Pavlovian US, did not employ any response conflict (Go/NoGo), allowed participants to respond freely and as often they wanted, and included a baseline condition to discern effects of the aversively conditioned Pavlovian CS+ from the effect of a potentially inhibitory CS-. We employed a PIT paradigm comprising three phases (see Figure 1). In an instrumental learning phase (phase 1), participants were asked to learn the sequence in which three keyboard buttons needed to be pressed in order to receive a monetary reward (€0.50). Correctly entered sequences were reinforced with a cash-sound and the presentation of a 50 Cent coin on the screen until each participant had earned €8.00. To ensure that the instrumental behavior was sufficiently incentivized, the reward was paid out in cash directly after phase 1. In the subsequent Pavlovian fear conditioning phase (phase 2), participants were presented

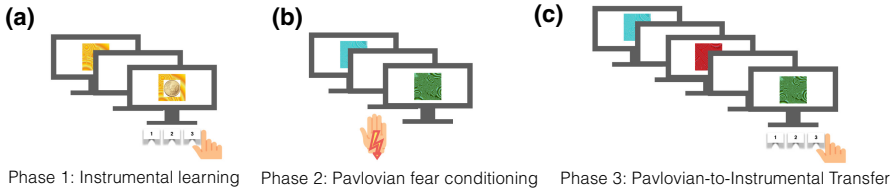

FIGURE 1 Overview of the experimental design. The experiment consisted of three phases: (a) an instrumental learning phase in which participants learned which sequence of three button presses was reinforced with a monetary reward of 50 cent. The correct sequence was only reinforced when an image was presented on the screen. The earned amount was paid out directly after phase 1. (b) in the second phase, the Pavlovian fear conditioning phase, participants were presented with two new images, one of which was reinforced by an electric stimulus in 50% of the trials (CS+), the other one was never reinforced (CS-). (c) in the last phase, participants could perform the acquired response sequence again while either a new control stimulus, the aversively conditioned CS+ or the CS- were presented on screen. The Pavlovian-to-instrumental test phase took place in extinction: Neither the instrumental response nor the CS+ were reinforced

with two conditioned stimuli (CS), one of which (CS+) was reinforced with an uncomfortable electric stimulus whereas the other was never reinforced and served as a control stimulus (CS-). The success of Pavlovian conditioning was assessed as differential (CS+ > CS-) skin conductance (SCR) and fear-potentiated startle response (FPS). In the third phase, participants could exert the instrumental response again while either the CS+, CS-, or a new control stimulus (that had not been seen before) were presented on the screen. In order to exclude that response suppression is caused by the actual delivery of the US instead of the acquired CS-US association, the transfer test was conducted in extinction, that is, correct button presses were not reinforced by a monetary reward and CS+ presentations were not followed by the US anymore. We hypothesized that the presentation of the fear conditioned CS+ compared to a new control stimulus would result in a reduction of instrumental responding (i.e., general PIT effect or *conditioned suppression*). Furthermore, if the CS- acquired any safety properties during Pavlovian learning, it may act not only as an aversive inhibitor but also as an appetitive excitor (Konorski, 1967) and its presentation might increase instrumental reward responding compared to the presentation of the new control stimulus (i.e., general PIT effect or *conditioned facilitation*). Lastly, we were interested in whether the strength of Pavlovian fear conditioning could serve as a predictor of conditioned suppression and assessed whether individual differences in differential (CS+ > CS-) SCR and FPS were associated with differences in later transfer effects.

2 | METHOD AND MATERIALS

2.1 | Participants

Based on an effect size of Cohen's $d = .40$ reported by a previous aversive transfer study in human participants (Xia et al., 2019), a power of $1\text{-}\beta = .80$, and an alpha error probability of $\alpha = .05$, we estimated a required sample size of $N = 52$ using G*Power (Faul et al., 2007). A pilot study in which we observed conditioned suppression with an effect size of $d_z = .78$ confirmed that this sample size would be sufficient to achieve a power of $1\text{-}\beta = .99$ (see Figure S1). Before inclusion into the experiment, participants were screened for color-blindness. In total, $N = 60$ participants were recruited for the experiment. Eight participants needed to be excluded ($N = 1$ quit the experiment due to the aversiveness of the startle-probe, $N = 2$ had participated in another fear conditioning study the same week, $N = 5$ did not learn the correct order of button presses in the instrumental phase), leaving $N = 52$ participants (mean 21.07 ± 2.59 years, range: 18–28 years; 37 female, 13 male, 1 other) for statistical analysis. The study was approved by the Ethics Review Board of the Department of Psychology of the University of Amsterdam and all participants signed written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

2.2 | Questionnaires

For sample characterization, we collected questionnaire data of the trait-version of the Spielberger State–Trait-Anxiety Inventory (STAI-T; Spielberger, 1983) and the Anxiety Sensitivity Index (ASI-3; Taylor et al., 2007). Questionnaire data of one participant was missing due to a technical error during data collection.

2.3 | Stimuli

Four differently colored images (blue, yellow, green, or red fractal, see Figure 1) were presented in the center of the screen and served either as instrumental stimulus, CS during Pavlovian learning, or new transfer stimulus during the PIT phase. Assignment of the four images to

instrumental stimulus, CS+, CS-, or transfer stimulus was randomized between participants.

2.4 | Unconditioned stimuli (US)

An electric stimulus delivered to the wrist of the left hand via two 20 by 25 mm Ag/AgACl electrodes with fixed inter-electrodes mid-distances of 45 mm served as US. Conductive-gel was applied to the electric stimulus electrode (Signa Gel, Parker Laboratories Inc.). The electric stimulus itself consisted of a train of three square-wave pulses with a duration of 2 ms and an inter-pulse interval of 100 ms and 200 ms. The delivery of the US was controlled by a Digitimer DS7A constant current stimulator (Digitimer, Weybridge). Before the start of the experiment the intensity of the electric stimulus was calibrated individually to a level judged as "maximally uncomfortable, but not yet painful" by the participant on a rating scale (i.e., 0 = "I do not feel anything", 5 = "medium uncomfortable", 10 = "already painful"). Subjective intensity ratings had a mean ± *SD* of 9.14 ± .74 (range: 9.00–9.90) and the actual intensity of the US ranged from 4.40–61.00 mA with a mean ± *SD* of 26.76 ± 14.6 mA.

2.5 | Fear-potentiated startle response (FPS)

In order to elicit startle responses, a loud noise (40 ms; 104 dB) was presented binaurally via headphones (Model MD-4600; Compact Disk Digital Audio, Monacor). We recorded electromyographic (EMG) activity using 7 mm Ag/AgCl electrodes filled with conductive gel and positioned approximately 1 cm below the pupil and 1 cm below the lateral canthus, the outer corner of the eye (Fridlund & Cacioppo, 1986). A ground electrode was placed on an electrically neutral site on the forehead. The EMG signal was amplified and digitized at 1000 Hz. The signal was analyzed offline using Psycho-Physiological Modeling (PsPM 5.0.0; Bach et al., 2018) in Matlab 2020a (Mathworks ®, Natrick, Massachusetts, USA). In PsPM, the signal was rectified and band-pass filtered (cut-off: 50 Hz and 470 Hz, 4th order Butterworth filter). Furthermore, a notch filter was applied to remove 50 Hz harmonics. The resulting signal was smoothed using a low-pass filter (cut-off: 53.05 Hz, 4th order Butterworth filter) and the data were down-sampled to 500 Hz (Khemka et al., 2017). To estimate trial-by-trial startle responses we employed a single-trial general linear model (GLM) with one regressor for each startle-probe onset. The single-trial regressors were convolved with a canonical startle response function with a flexible response onset latency of 0–100 ms. Single-trial parameter estimates were Z-transformed across stimuli (CS+, CS-, NA; excluding habituation trials) and within each participant for statistical analysis.

2.6 | Skin conductance response (SCR)

Electrodermal activity (EDA) was recorded from the middle phalanges of the index and middle finger of the left hand using two AG/AgCl Electrodes of 20 by 16 mm. The signal was recorded using a sine-shaped excitation voltage (5 V) of 50 Hz derived from the mains frequency and was digitized at 1000 Hz through a 16-bit AD-converter. The EDA signal was analyzed using PsPM 5.0.0. Specifically, we employed a single-trial GLM (Bach et al., 2009, 2010) with one regressor for each CS onset and one regressor for each US delivery and a canonical SCR function with time-derivative and fixed response latency. For statistical analysis, single-trial parameter estimates were Z-transformed across stimuli (CS+, CS-) and within each participant.

2.7 | Procedure

2.7.1 | Instrumental phase

Upon arrival, participants filled in informed consent and questionnaires. Subsequently, the intensity of the US was calibrated. Before the start of the experiment, participants were informed that the experiment would consist of three phases and that they would be presented with a steady background noise throughout all three phases to shield them from environmental noise. Participants were then verbally instructed that they could earn money in the first phase by pressing three buttons in a specific order. The buttons (1, 2, 3 on the number pad) were marked with red dots on the keyboard. Whenever participants would press the buttons in the right order, they would be rewarded with 50 Cent, signaled by the presentation of a 50 Cent coin on the screen and a cash sound. The actual money earned throughout the phase would be paid out directly after the phase. Participants were instructed that they could only press the buttons to earn money when the fixation cross was not on the screen. They were told that they could earn as much money as they wanted by pressing the buttons in the correct order as often as possible, but that not every correct order would yield a reward. The first phase started with a written repetition of the instructions. During the instrumental phase, either one of the four images ("instrumental stimulus") or the fixation cross were presented in the center of the screen. The instrumental stimulus was presented for 8000 ms.

During inter-trial intervals (ITI) the fixation cross was presented in the center of the screen. The duration of ITIs was randomized between 15 and 20 s, with a mean of 17.5 s. As in previous research (Weber et al., 2016), a variable-ratio schedule was faded in. That is, in the beginning a fixed-ratio reinforcement schedule was used in order to facilitate learning (i.e., 1, 1, 1, 1, 2, 4, 8, 16), subsequently, on average every 15th (range: 5–25) correct order was reinforced with 50 Cent. The first phase ended as soon as the participant had earned 8 €, which was on average after 32.06 ± 12.96 trials (range: 13–83). After the end of the first phase, the experimenter entered the room, paid out the reward directly to the participant and asked the participant to report the order of button presses that yielded the reward. Participants who could not report the sequence correctly were excluded from the experiment.

2.7.2 | Pavlovian phase

Before the start of the Pavlovian phase, participants were instructed that they would see two different images and would occasionally receive an electric stimulus. Their task was to pay attention to any relationship between the images and the electric stimulus. Furthermore, they were instructed that they may hear loud noises during this phase. The Pavlovian phase started with the presentation of 10 NA trials for startle response habituation. Subsequently, 10 CS+, 10 CS-, 10 noise alone (NA) trials were presented. Each CS was presented for 8000 ms in total. Trial order was randomized in such a way that no more than two trials of the same type were presented after each other. The startle probe (104 dB, 40 ms) was delivered 7150 ms after stimulus onset and the train of three USs started 500 ms later in case of reinforced CS+. The CS+ was reinforced in 50% of trials. Reinforcement was randomized in such a way that not more than two succeeding CS+ presentations could be reinforced or unreinforced. During ITIs a fixation cross was presented in the center of the screen. The duration of the ITI was randomized between 15 and 20 s with a mean of 17.5 s.

2.7.3 | PIT phase

Before the start of the transfer phase, participants were instructed that they could again earn money by pressing the buttons in the same order as in phase 1. During the transfer phase the CS+, CS-, and a new stimulus were presented for six trials each. Each trial lasted 8000 ms. During ITIs a fixation cross was presented in the center of the screen and the duration of the ITI was randomized between 15 and 20 s with a mean of 17.5 s. In contrast to the instrumental and Pavlovian learning phase, the transfer phase was conducted in extinction. That is, participants did not receive any monetary reward and no further USs were administered.

2.8 | Statistical analysis

Statistical analyses were conducted using RStudio (v1.1456, RStudio Team, 2020). To test whether participants acquired the correct order of button presses in the instrumental phase, we compared the number of correct responses averaged across the first two trials to the last two trials of the instrumental phase using a nonparametric Wilcoxon signed-rank test. The success of Pavlovian learning was assessed by comparing conditioned responses (i.e., FPS, SCR) averaged across the first and last two trials of the Pavlovian phase using repeated-measures ANOVA with stimulus (CS+, CS-) and trial (first 2, last 2) as within-subject factors and Type-III sum of squares (ez-package v4.4.0; Lawrence, 2016). In pilot data with $N = 5$ participants, we had observed that conditioned suppression of the number of correct responses was strongest on the first trial of the PIT phase (see Figure S1). For this reason, we tested specifically whether conditioned suppression occurred on the first trial of the PIT phase by comparing the number of correct responses during the new control stimulus to the number of correct responses during CS+ using a paired sample t test. To assess potential effects of conditioned facilitation by the CS-, we also compared the number of correct responses during CS- and control stimulus on the first trial (paired sample t test). Results were considered significant when $p < .05$ (two-sided tests).

3 | RESULTS

The present sample had a trait anxiety (STAI-T, Spielberger, 1983) score of mean ± *SD* 43.31 ± 8.34 (range: 27–62) and an anxiety sensitivity score (ASI-3; Taylor et al., 2007) of mean ± *SD* 17.82 ± 11.96 (range: 2–59).

3.1 | Phase 1—Instrumental phase

All participants acquired the correct order of button presses as indicated by a significant increase of correct responses from the first to the last two trials of the instrumental phase (52 of 52 participants rank last two trials > first two trials; Wilcoxon Signed-Rank Test, $Z = -6.27$, $p < .001$; see Figure 2).

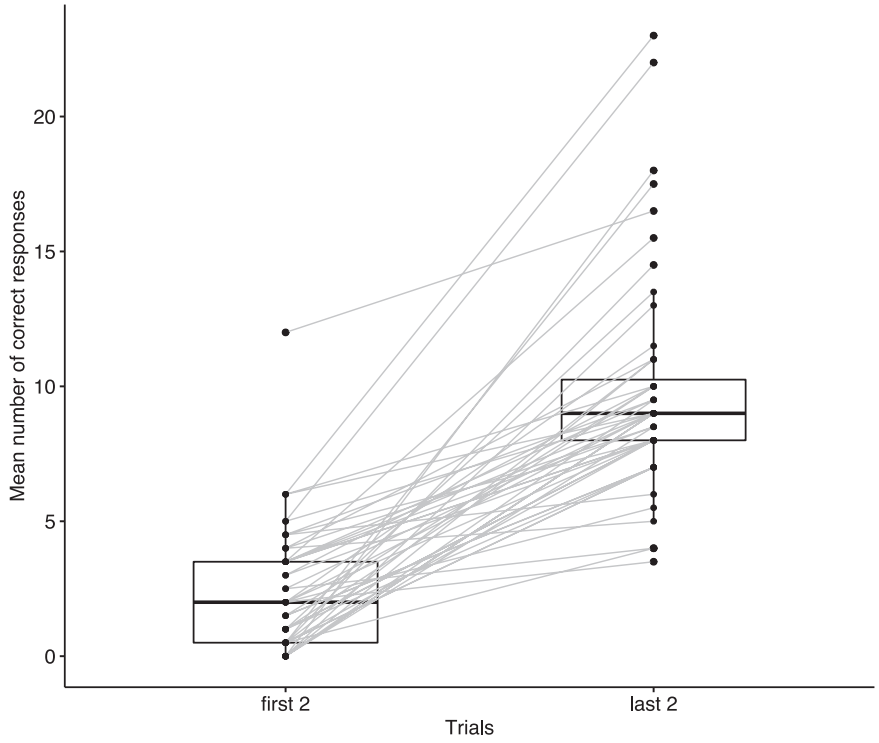

FIGURE 2 Phase 1—instrumental learning. As to be expected after the exclusion of participants who did not learn the correct order, all participants showed a higher number of correct responses in the last two compared to the first two trials of the instrumental learning phase indicating a successful acquisition of the instrumental response

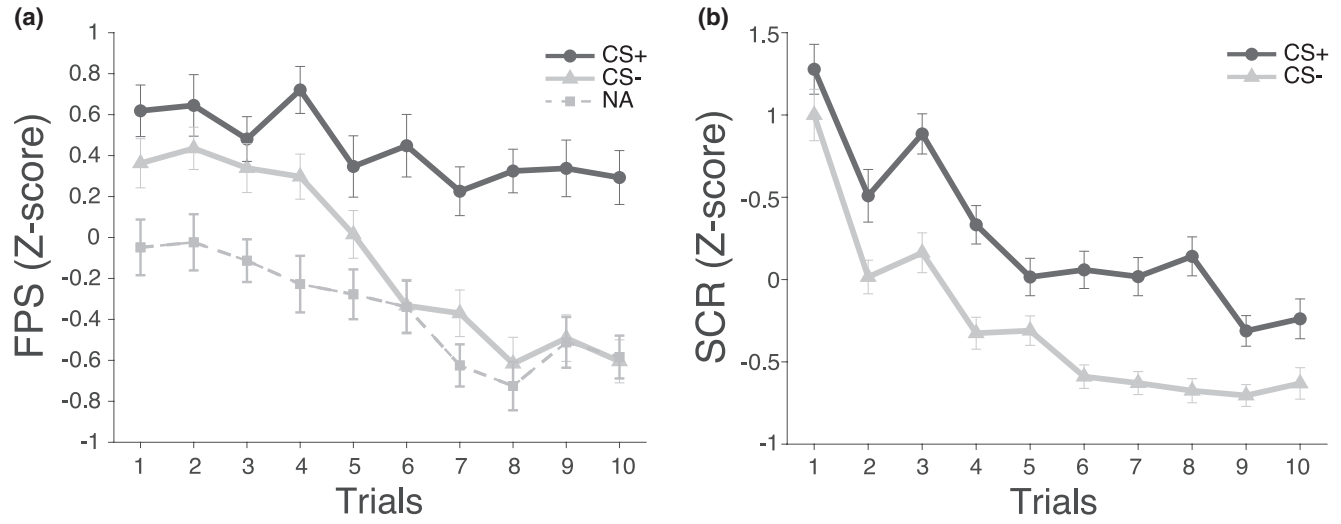

FIGURE 3 Phase 2—Pavlovian learning. Pavlovian learning was successful as indicated by (a) a significant increase in differential (CS+ > CS-) fear-potentiated startle responses from the beginning (first 2 trials) to the end (last 2 trials) of Pavlovian learning, and by (b) significantly greater skin conductance responses to the CS+ than the CS- both at the beginning (first 2 trials) and the end (last 2 trials) of the Pavlovian phase. Error bars depict standard error of the mean (*SEM*)

3.2 | Phase 2—Pavlovian phase

Pavlovian fear conditioning was successful as indicated by a significant increase of differential (CS+ > CS-) startle responses from the beginning to the end of the Pavlovian phase (stimulus: $F_{1,51} = 30.89$, $p < .001$, $\eta_p^2 = .38$; trial: $F_{1,51} = 40.00$, $p < .001$, $\eta_p^2 = .44$; stimulus* trial: $F_{1,51} = 10.70$, $p = .002$, $\eta_p^2 = .17$; Figure 3). In detail, there was no significant difference between FPS to CS+ and CS- during the first two trials ($t_{51} = 1.94$, $p = .06$), but during the last two trials of conditioning ($t_{51} = 7.14$, $p < .001$, $d = .99$). Skin conductance responses also reflected successful acquisition of CS-US contingencies over the course of the Pavlovian phase (stimulus: $F_{1,51} = 130.63$, $p < .001$, $\eta_p^2 = .72$; trial: $F_{1,51} = 23.03$, $p < .001$, $\eta_p^2 = .31$; stimulus*trial: $F_{1,51} = .00$, $p = .99$, Figure 3). SCRs were significantly greater to CS+ than CS- during the first two trials already ($t_{51} = 7.37$, $p < .001$, $d = 1.02$) and this stimulus effect was sustained up to the last two trials of

conditioning ($t_{51} = 8.47$, $p < .001$, $d = 1.31$). Note, for both FPS and SCR effect sizes reflecting fear acquisition were large (FPS: $d = .99$, SCR: $d = 1.31$).

3.3 | Phase 3—Pavlovian-to-Instrumental transfer (PIT)

In the transfer phase, we compared the number of correct responses in the presence of the CS+ to a new baseline stimulus. In contrast to our hypothesis, we did not observe any significant difference between the number of correct responses during the CS+ compared to the new stimulus on the first trial ($Z = -.74$, $p = .46$, see Figure 4a). That is, the presence of the CS+ did not induce any suppression of instrumental responding in our experiment. There was also no significant difference between the number of correct responses during the CS- compared to the presentation of the new stimulus ($Z = -.71$, $p = .48$). This result suggests that the presentation of the CS- as a potential safety stimulus did also not evoke any conditioned facilitation. The results did not change when we compared responses averaged across all six trials of the transfer phase (CS+ vs. new: $Z = -.30$, $p = .76$; CS- vs. new: $Z = -1.21$, $p = .22$). Furthermore, we assessed whether conditioned suppression may have been stronger in participants with high trait anxiety or high anxiety sensitivity. Even though there was indeed a positive relationship between trait anxiety and conditioned suppression, that is, the number of correct responses in the presence of the new stimulus compared to the CS+; this relationship as well as the relationship between anxiety sensitivity and conditioned suppression were, however, not significant (STAI-T: $rho = .20$, $p = .16$; ASI: $rho = .02$, $p = .90$). In additional exploratory analyses, we asked whether conditioned suppression was expressed as slower responding. Time between succeeding button presses did, however, not differ significantly between the CS+ (mean: 246.17 ± 77.08 ms, $t_{51} = 1.04$, $p = .30$) or the CS- (mean: 239.17 ± 72.53 ms, $t_{51} = -.21$, $p = .83$) compared to the new stimulus (mean: 240.55 ± 77.30 ms), even when looking at the first 1000 ms of the first trial only (see Figure S2). In other words, despite strong Pavlovian fear conditioning, neither the number of correct responses nor the time between succeeding button presses provided any evidence for conditioned suppression in the present study.

We furthermore aimed to assess whether the strength of Pavlovian learning would predict the amount of conditioned suppression in the PIT phase. However, the relationship between differential (CS+ > CS-) FPS at the end of conditioning and conditioned suppression as assessed by the number of correct responses in the presence of CS- compared to the CS+ was not significant ($rho = .02$, $p = .88$). The result did not change when assessing conditioned suppression as time between succeeding responses ($r = -.02$, $p = .90$). In line with previous reports (Xia et al., 2019), there was also no significant relationship between differential SCR at the end of conditioning and either measure of conditioned suppression (number correct responses: $rho = .04$, $p = .77$; time between responses: $r = .03$, $p = .84$).

4 | DISCUSSION

In the present study we aimed to develop a Pavlovian-to-Instrumental transfer paradigm to assess conditioned

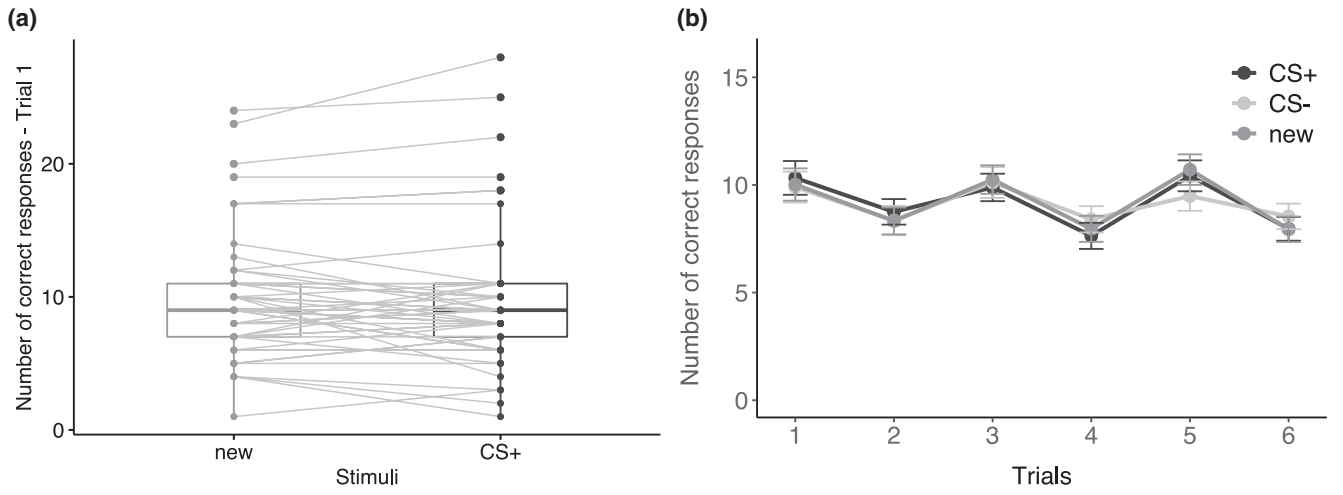

FIGURE 4 Phase 3—Number of correct responses during Pavlovian-to-instrumental transfer. (a) in contrast to our hypothesis, there was no suppression of the number of correct responses in the presence of the CS+ compared to the new stimulus on the first trial of the PIT phase. (b) Number of correct responses across the six trials of the PIT phase did not differ significantly between the CS+, CS- and new stimulus (error bars depict standard error of the mean, *SEM*)

suppression after Pavlovian fear conditioning in human participants. In contrast to previous studies, we used a primary aversive reinforcer during Pavlovian learning (i.e., an electric stimulus) and ensured that participants learned the instrumental response that resulted in a small monetary reward (secondary reinforcer) by trial and error. Furthermore, we employed a control stimulus in the transfer test in order to be able to dissociate effects from the aversively conditioned CS+ from potential effects of the CS-. In the instrumental phase of the experiment, the majority of recruited participants (52 out of 57) acquired the correct instrumental response within 32 trials. Participants who did not acquire the response were excluded from the experiment. Subsequent Pavlovian fear conditioning as assessed by SCR and FPS was successful as reflected in large effect sizes. However, despite successful instrumental and Pavlovian learning, the presentation of the aversively conditioned CS+ did not affect instrumental responding in the transfer phase. Instead participants showed a comparable number of correct responses during the CS+ and the new control stimulus. There was also no effect of the CS- on the response rate and we did not observe any relationship between individual differences in trait anxiety, anxiety sensitivity, or measures of Pavlovian conditioning with conditioned suppression. Exploratory analyses of the time between succeeding responses in the first trial or even only the first 1000 ms of the first trial did not change the results. This indicates that the lack of a suppression effect cannot be explained by subtle suppression effects that extinguish quickly and therefore would only be detectable in the very beginning of the transfer phase.

Given the strong effect sizes in both measures of aversive Pavlovian conditioning (i.e., SCR and FPS) we can rule out that conditioning was not successful. It is, however, conceivable that fear conditioning in human participants is not as impactful as in animals and employing a stronger US intensity would be necessary to induce conditioned suppression in the present human paradigm. This notion is indeed corroborated by animal research where conditioned suppression has been shown to be a function of US intensity, with stronger USs inducing stronger suppression (Annau & Kamin, 1961). Simply increasing the intensity of the here-employed electric stimulus would be unethical, but a multimodal aversive stimulus as US could be an option to further enhance the motivational effects of conditioning. A multimodal US could be composed of an image and a corresponding sound (e.g., sound of snapping celery––image of badly broken leg) and thereby emulate a real-life, multimodal experience that amplifies the impact of a stimulus (de Vries et al., 2021). These stimuli do not pose a direct threat to the participant, but have been shown to be perceived as highly aversive and evoke a very strong physiological defensive responses (de Vries et al., 2021). In the present study, we merely assessed whether a Pavlovian CS would elicit conditioned suppression. It bears mentioning that Pavlovian stimuli also facilitate avoidance responses after aversive conditioning––a finding that has been reported more consistently than conditioned suppression (e.g., Garofalo & Robbins, 2017; Xia et al., 2019). As an additional manipulation check for the success of fear conditioning, the paradigm could be extended with conditions to assess the facilitation of avoidance responses. However, it has been shown that the same Pavlovian CS that reliably induces conditioned facilitation does not necessarily elicit conditioned suppression in the same participant (Xia et al., 2019), suggesting that the factors that modulate the induction of conditioned suppression differ from those that induce conditioned facilitation (i.e., avoidance behavior).

Another critical factor determining the strength of conditioned suppression in animals is the motivational drive to perform the instrumental response (Millenson & de Villiers, 1972). It is therefore likely that also conditioned suppression in humans is substantially modulated by the value of the instrumental reward, with instrumental responses reinforced by outcomes with relatively low reward value being more easily suppressed than those reinforced with outcomes with high reward value. In line with this idea, studies employing instrumental reinforcers such as points in a computer game or art slides have previously reported to observe conditioned suppression (Di Giusto et al., 1974; Di Giusto & Bond, 1978; Punch et al., 1976). However, when the incentive to perform the instrumental action was increased by employing primary appetitive reinforcers such as chocolate (Xia et al., 2019) or secondary appetitive reinforcers such as money (present study, Hebart & Gläscher, 2015), studies failed to find conditioned suppression in humans. Future research should address to what extent conditioned suppression after human fear conditioning is modulated by the aversiveness of the Pavlovian US and the reward value of the instrumental response, and/or their interaction.

A paradigm to assess conditioned suppression in humans could foster research into the causes of the detrimental behavioral consequences of fear, their neurobiological mechanisms and ways to overcome them. As an example, further exploring individual differences (e.g., reward sensitivity) as well as general factors (e.g., reward value) in modulating, amplifying, and reducing conditioned suppression of reward behavior could bring about new treatment approaches. A robust paradigm of conditioned suppression could in itself also be useful in translating insights from basic to clinical science, for instance by directly investigating the behavioral effect of new interventions instead of solely using physiological

read-outs. So far, the attempts of developing a paradigm of fear-induced conditioned suppression in humans have been unsuccessful, but additional research to further explore this phenomenon may be worthwhile.

Summarizing, the present study aimed to establish a Pavlovian-to-instrumental transfer paradigm to assess conditioned suppression after Pavlovian fear conditioning in humans. Despite strong fear conditioning we did not observe an effect of the aversively trained CS on instrumental reward behavior. Future studies may explore whether reducing the incentive to perform the instrumental response and/or increasing the intensity of the Pavlovian US may facilitate the induction of conditioned suppression in humans.

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AUTHOR CONTRIBUTIONS

Anna M. V. Gerlicher: Conceptualization; data curation; formal analysis; investigation; visualization; writing – original draft. **Vivian N. Metselaar:** Conceptualization; data curation; investigation; writing – review and editing. **Merel Kindt:** Conceptualization; funding acquisition; methodology; supervision; writing – review and editing.

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SUPPORTING INFORMATION

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Figure S1 Pilot data of $N = 11$ participants suggested that conditioned suppression is strongest during the first trial of the PIT phase. That is, participants made significantly less correct responses during the presentation of the CS+ than the new stimulus on trial 1 ($Z = -2.35$, $p = .02$, $dz = .78$), but not the remaining five trials (all p's $> .05$). In order to be able to assess the speed of extinction of potential PIT effects, all six trials were retained for the main experiment, but the effect of conditioned suppression only tested on the first trial. During the pilot, the instrumental stimulus was also presented in the PIT phase. This stimulus was not included in the main experiment anymore as it did not serve to test any specific hypotheses

Figure S2 Time between succeeding responses averaged over time-bins of 1000 ms. There was no significant effect of the presentation of the CS+ compared to the new control stimulus on the time between succeeding responses in the beginning of the first CS+ and new stimulus trial (0–1000 ms; $t_{30} = .76$, $p = .46$). As not every participant pressed the buttons more than once within the first 1000 ms of the CS+ ($N = 38$) or the new stimulus presentation ($N = 40$) only $N = 31$ participants contributed to these data

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