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Temporal dynamics of conditioned skin conductance and pupillary responses during fear acquisition and extinction

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ABSTRACT

Fear acquisition manifests in the development of conditioned fear responses (CRs), whereas a decrement in CRs as a consequence of unconditioned stimulus (UCS) omission is referred to as extinction learning. Time windows for CR scoring in physiological readouts are subject to discussion, especially regarding the subdivision of skin conductance responses (SCRs) into first- (FIR) and second-interval responses (SIR). However, distinct temporal CR trajectories within or across measures may reflect specific characteristics of the underlying associative processes. In this study, 41 participants underwent fear acquisition and extinction, while SCRs and pupillary responses were recorded and separated into different time bins to explore the temporal dynamics of CRs across both learning phases. For SCRs, we observed a shift from early (FIR) to late (SIR) time intervals during fear acquisition most likely reflecting subsequent learning processes, in which CS-UCS associations and their relative timing are formed. During extinction, only the FIR exhibited a CR decline and was thus able to track the learning progress. These results indicate that conditioned SCRs follow a dynamic temporal pattern that may be related to different learning dimensions. By contrast, pupillary CRs were generally better captured by a late pupillary response component, suggesting a rather stable temporal CR pattern for the pupil in both learning phases. Our findings underscore the importance of specifying CR quantification for different physiological readouts when evaluating learning performance in the context of fear acquisition and extinction and may motivate further investigation of time-specific CR patterns and their relation to specific associative dimensions.

1. Introduction

Fear conditioning is among the most fundamental and prevailing experimental procedures to investigate the neurobiological mechanisms of aversive learning across species (Maren, 2001) and provides a useful etiological model for pathological fear (Mineka and Oehlberg, 2008). During differential fear acquisition training, an initially neutral stimulus (the to-be conditioned stimulus; CS+) is presented with an aversive unconditioned stimulus (UCS; e.g. electrical stimulation), whereas another stimulus (CS-) is never paired with the UCS. This procedure promotes associative learning and manifests in the development of conditioned responses (CRs; i.e. differential fear responses with higher responses to the CS+ compared to the CS-). During extinction training, the CS+ is presented repeatedly in the absence of the UCS, typically leading to a decline of CRs, formally referred to as extinction learning (Graham and Milad, 2011). CRs can be indexed in various ways, including ratings of fear and UCS expectancy or measures of physiological arousal, such as skin conductance responses (SCRs), pupil dilation or fear potentiated startle (see Lonsdorf et al., 2017 for an overview).

In humans, SCRs represent the most commonly employed outcome measure in fear conditioning research (Lonsdorf et al., 2017). SCRs reflect sympathetic nervous system activation and are sensitive to emotional arousal in the presence of salient or novel stimuli, particularly in response to affective stimuli (Dawson et al., 2007; Wallin, 1981). As a readout of CRs, SCRs thus provide a means to assess whether fear and extinction learning were successful or not. Problematically however, there has been substantial variance in the definition of time windows used for CR scoring, both within and across labs (Lonsdorf et al., 2017). These discrepancies have sparked an ongoing debate on the validity of subdividing SCRs into a first- (FIR) and second-interval response (SIR; Luck and Lipp, 2016; Öhman, 1972, 1974; Pineles et al., 2009; Prokasy, 1977; Prokasy and Ebel, 1967), a distinction first proposed in the 1960s (Prokasy and Ebel, 1967; Stewart et al., 1961). These studies showed that more than one conditioned SCR could be recorded during a given trial, in case the CS-UCS interval is sufficiently

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long (usually 6 s or more). In particular, it has been demonstrated that one response usually occurred immediately after the CS, while another occurred just prior to the UCS. The first response (i.e. the FIR) was further argued to be more sensitive to orienting behavior, which is triggered by novelty and habituates over time, whereas the SIR was assumed to reflect UCS expectancy and thus interpreted as a purer measure of CS-UCS contingency (Öhman, 1972, 1974; Wolter and Lachnit, 1993). Based on these assumptions, the SIR should thus increase during fear acquisition and again decrease during extinction, while the FIR should rather show a pattern of declining responses during both fear acquisition and extinction.

Given that the FIR and SIR follow these distinct temporal trajectories, they may also capture specific characteristics of the underlying learning process and should thus be more suitable to depict either fear or extinction learning, respectively. Contrary to this presumption however, there is also evidence suggesting that the FIR and SIR are positively correlated and equally suitable for detecting conditioning effects (Pineles et al., 2009). Yet, many conditioning studies reporting both the FIR and the SIR used 100% reinforcement schedules (Cook, 1969; Merz et al., 2012; Öhman et al., 1978), instructed fear paradigms (e.g. Tabbert et al., 2011; Tabbert et al., 2006) and/or only a small number of trials (e.g. Pineles et al., 2009; Pohlack et al., 2012), resulting in rapid acquisition and extinction of CRs. Beyond that, performance-based exclusion of participants is often applied but can be problematic, especially when applied to different intervals or outcome measures. For instance, a failure of the FIR to show successful fear or extinction learning does not permit the inference that the SIR would not indicate successful learning (Lonsdorf et al., 2017). Given that changes over time are of particular importance in learning paradigms, a first aim of the current study was therefore to explore the temporal dynamics of the two SCR components across fear acquisition and extinction.

In contrast to SCRs, pupillary responses have been employed less frequently in the field. Yet, several studies have demonstrated that anticipation of an aversive event during presentation of the CS+ as compared to the CS- was associated with greater pupil dilation (de Voogd et al., 2016; Leuchs et al., 2017; Morriss et al., 2015; Reinhard and Lachnit, 2002; Visser et al., 2015). This corroborates with past work showing that the pupil dilates in response to emotionally arousing stimuli (Bradley et al., 2008; Hermans et al., 2013; Kret et al., 2013; Partala and Surakka, 2003) and covaries with other autonomic measures of arousal such as SCRs (Bradley et al., 2008). However, similar to SCR quantification, methods for analyzing pupillary responses vary across studies, ranging from calculating the mean pupil diameter (Morriss et al., 2015; Reinhard and Lachnit, 2002; Reinhard et al., 2006) and peak scoring within a predefined time window (Leuchs et al., 2017; Visser et al., 2016; Visser et al., 2015; Visser et al., 2013) to computational modeling approaches (Korn and Bach, 2016; Korn et al., 2017).

Previous work has shown that differences in pupil dilation to the CS+ and CS- are most pronounced in a time window immediately preceding the UCS (Reinhard and Lachnit, 2002). Yet, there is also some evidence suggesting that the initial reflexive constriction of the pupil in response to light is attenuated when anticipating an aversive event (Bitsios et al., 1996, 2004), such as during the presentation of the CS+ (Reinhard et al., 2006). Along with the considerations made for SCRs, it is thus reasonable that learning-dependent changes in CRs might also be captured differentially by distinct temporal pupillary response components. We therefore sought to track the pupillary response function as a whole by defining continuous time bins that potentially could aid in detecting progressive temporal shifts in CRs over the course of learning. Taken together, the goal of the current study was to investigate the temporal dynamics of CR patterns during fear and extinction learning using SCRs and pupil dilation. Since distinct temporal CR trajectories within or across measures may reflect specific learning dimensions, CRs were separated into different time intervals, tracked throughout learning and compared across both measures.

2. Method

2.1. Participants and general procedure

The required sample size was derived from G*Power 3.1 (Faul et al., 2009) for a repeated-measures analysis of variance (ANOVA), assuming a medium-sized effect to detect a significant stimulus by time interaction in SCRs and pupillary responses during fear acquisition and extinction ($f = 0.25$) as reported in previous studies (de Haan et al., 2018; Leuchs et al., 2019; Luck and Lipp, 2016). Accordingly, in order to detect a significant interaction comprising three within-subjects factors (i.e. CS, block and interval, cf. 2.6) with a 1-$\beta \geq 0.99$ power, an α-level of 0.05 and an assumed correlation of $r = 0.20$ for repeated measurements, a sample size of 31 participants was required.

Forty-one healthy participants (20 females) aged 18–35 years were recruited at the Ruhr University Bochum to participate in this study. Exclusion criteria checked beforehand in a telephone interview covered chronic or acute illnesses, any history of psychiatric or neurological treatment, drug use, and regular medication. All participants were right-handed, fluent in German, and had normal vision. Women were required to have been taking oral contraceptives (only monophasic preparations with an ethinylestradiol and a gestagenic component) for at least three months and were tested during the active pill phase to eliminate potential influences due to changes in circulating sex hormones across the menstrual cycle (Merz et al., 2018).

Three participants were excluded from all analyses because they failed to show contingency awareness after fear acquisition training (see Section 2.3). One additional participant had to be excluded from SCR analyses due to unexceptionally low responding to the UCS (less than one third detectable responses in all UCS trials; cf. Kinner et al., 2018; see also Lonsdorf et al., 2017) and another three participants were excluded from the analyses of pupillary responses due to technical difficulties during data recording. The final sample, thus, consisted of $N = 37$ (18 females; age: $M = 22.76$, $SD = 3.63$) for the SCR analyses and $N = 35$ (18 females; age $M = 22.83$, $SD = 3.74$) for analyses of pupillary responses.

Participants provided written informed consent before the start of the experiment and received a monetary compensation of 8€ 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.

2.2. Fear conditioning

A modified version of the differential fear conditioning paradigm as described in Merz et al. (2014) was applied, consisting of fear acquisition and subsequent fear extinction training. In this task, pictures of two geometrical shapes (a rhomb and a square) served as the CS+ and CS- (counterbalanced stimulus allocation); both stimuli were gray in color, identical in luminance and presented for 8 s against a black background. During fear acquisition training, one stimulus (CS+) was followed by an aversive electrical stimulation (UCS; 100 ms, starting 7.9 s after CS+ onset) in 10 out of 16 trials (62.5% partial reinforcement rate), whereas the second stimulus (CS-) was never paired with the UCS.

During extinction training, stimulation electrodes remained attached, but did not provide electrical stimulation. A total of 16 CS+ and CS- trials were presented during fear acquisition and extinction training (total time for each session ~ 10 min), during extinction training, 16 unreinforced presentations of the CS+ were intermixed with 16 presentations of the CS-. Inter-trial intervals (ITI) depicting a black screen were randomly jittered between 9.5 and 12 s. For each participant, pseudo-randomized stimulus orders were used (cf. Merz et al., 2014). The experiment was realized using the Presentation software package (Neurobehavioral Systems, Albany, CA).

2.3. Contingency awareness

Prior to the start of the fear conditioning paradigm, participants were instructed to pay attention to any possible regularity in the occurrence of the geometrical shapes and the electrical stimulation. They were informed that if they should discover such a relationship, it would remain stable in all experimental phases. This instruction was used to facilitate learning of contingencies and to avoid that participants expect a complete reversal of contingencies during extinction training. However, participants were not informed about the actual CS-UCS contingencies or the absence of the UCS during extinction training.

Immediately after fear acquisition training, participants rated the percentage occurrence (0–100%) of the UCS after presentation of the two geometrical shapes (i.e. CS+ and CS-). To confirm contingency awareness, a forced choice questionnaire further required participants to choose which of the two CSs never preceded the UCS.

2.4. Electrical stimulation, SCR data recording and analysis

A constant-voltage stimulator (STM200; BIOPAC Systems, Inc. Goleta, CA, USA) was used to deliver transcutaneous electrical stimulation (UCS; 100 ms) 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 stimulus intensity was set individually to be “unpleasant but not painful” using a gradually increasing rating procedure.

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. Electrodes were placed to the hypothenar surface of the non-dominant hand. Raw data were low-pass filtered with a cut-off frequency of 10 Hz. As previously (Merz et al., 2014; Merz et al., 2013), SCRs were calculated as the trough-to-peak amplitude difference (in μS) of the largest deflection and defined in two analysis windows (cf. Prokasy and 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 5–8.5 s as the second-interval response (SIR). Data were transformed with the natural logarithm to attain a normal distribution.

2.5. Pupillometry

Testing took place in a sound-attenuated moderately lit room and 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 put on the eye-tracking glasses and to place their chin and forehead on a headrest.

Pupillary data were recorded with iView eye-tracking glasses (iViewETG 2.0, SensoMotoric Instruments, Germany) connected to an SMI-ETG recording device (Lenovo X230-Notebook) compatible to the iViewETG software. A high-definition scene camera including an infrared-sensitive eye camera for dark pupil detection measured retinal and corneal reflections to obtain participants' pupil diameter of the left and right eye. Pupillary data were recorded at a binocular sampling rate of 30 Hz, a gaze tracking range of 80° horizontal and 60° vertical visual angle, and a gaze position accuracy of 0.5°. A one-point calibration was carried out to ensure that the participants' eyes were correctly tracked by the iView System.

Preprocessing of the raw pupil size time series was performed in Matlab (version 2012a, MathWorks, Inc., Sherborn, MA, USA) based on routines reported in Kinner et al. (2017): recorded data were averaged across both eyes, smoothed with a finite impulse response filter at 6 Hz, and onsets of event-locked segments (CS+, CS-, UCS, ITI) were marked for each trial. Trials with major blinks ($>$ 100 ms) were discarded and smaller artifacts were corrected by linear interpolation (Beatty and Lucero-Wagoner, 2000; Kret and Sjak-Shie, 2018). 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 (Mathot et al., 2018).

Mean pupil size for CS+ and CS- trials were determined within a time window from 0 to 8 s after CS onset in bins of 2 s (0–2 s, 2–4 s, 4–6 s, and 6–8 s) (cf. Koenig et al., 2017). Note that the temporal resolution of pupillary responses is generally higher compared to SCRs, leading to faster response onsets of 0.1–0.2 s for the pupil (Beatty and Lucero-Wagoner, 2000; Kinner et al., 2017) vs. 1–4 s for SCRs (Boucsein et al., 2012). We have thus decided to choose more fine-grained time bins to describe the pupillary response.

2.6. Statistical analyses

All statistical analyses were carried out using IBM SPSS Statistics for Windows (Version 22.0. Armonk, NY: IBM Corp.) with the significance level set to $\alpha = 0.05$. Greenhouse-Geisser corrected p-values were used if the assumption of sphericity was violated and partial eta-square (η_p^2) were reported as estimations of effect sizes. SCRs and pupillary responses were analyzed separately for fear acquisition and extinction by conducting repeated measures ANOVAs with the within-subjects factor CS (CS+ vs. CS-), block (four blocks each comprising the mean across four trials of each CS; 1–4 vs. 5–8 vs. 9–12 vs. 13–16), and interval (for SCRs: FIR vs. SIR; for pupillary responses: four 2 s time bins; 0–2 s vs. 2–4 s vs. 4–6 s vs. 6–8 s). Significant results of the ANOVAs were followed by Bonferroni-adjusted post-hoc tests ($p = 0.05/4 = 0.0125$; when tested separately for each of the four blocks) and Cohen's d was calculated as an estimate of effect size. In order to explore whether early and late time intervals of conditioned SCRs and pupillary responses were related to each other during fear acquisition and extinction, we first calculated the mean differential response (CS+ minus CS-) across the four blocks of both phases for the FIR and SIR of SCRs as well as for the early (0–2 s) and late (6–8 s) time interval of the pupillary response. Mean differential CRs for each time interval were then correlated between both outcome measures using Pearson product-moment correlations, adjusted for multiple testing ($p = 0.05/4$, when correlating early and late time intervals of both measures, respectively).

3. Results

3.1. SCRs

3.1.1. Fear acquisition

Fear acquisition was successful as indicated by significantly higher SCRs towards the CS+ compared to the CS- (main effect CS: $F_{(1,36)} = 59.77$; $p < .001$; $\eta_p^2 = 0.62$). As illustrated in Fig. 1A, a significant three-way interaction between CS, block and interval ($F_{(3,108)} = 9.50$; $p < .001$; $\eta_p^2 = 0.21$) further revealed that differential responding as measured by the SIR increased over the course of fear acquisition (CS × block interaction: $F_{(3,108)} = 3.00$, $p < .001$, $\eta_p^2 = 0.08$; 1st block: $t_{(36)} = 1.53$, $p = .134$; 2nd block: $t_{(36)} = 5.55$, $p < .001$, $d = 0.91$; 3rd block: $t_{(36)} = 4.27$, $p < .001$, $d = 0.70$; 4th block: $t_{(36)} = 4.01$, $p < .001$, $d = 0.66$), whereas it remained stable or rather decreased for the FIR (CS × block interaction: $F_{(3,108)} = 8.12$, $p < .001$, $\eta_p^2 = 0.18$; 1st block: $t_{(36)} = 8.11$, $p < .001$; $d = 1.33$; 2nd block: $t_{(36)} = 4.22$, $p < .001$, $d = 0.69$; 3rd block: $t_{(36)} = 4.37$, $p < .001$, $d = 0.68$; 4th block: $t_{(36)} = 3.62$, $p < .01$, $d = 0.59$). Generally, SCRs decreased from the first to the last block (main effect block: $F_{(3,108)} = 28.49$; $p < .001$; $\eta_p^2 = 0.44$), reflecting habituation processes.

3.1.2. Fear extinction

Successful extinction was reflected by a decreasing differentiation between the CS+ and CS- over time (CS × block interaction:

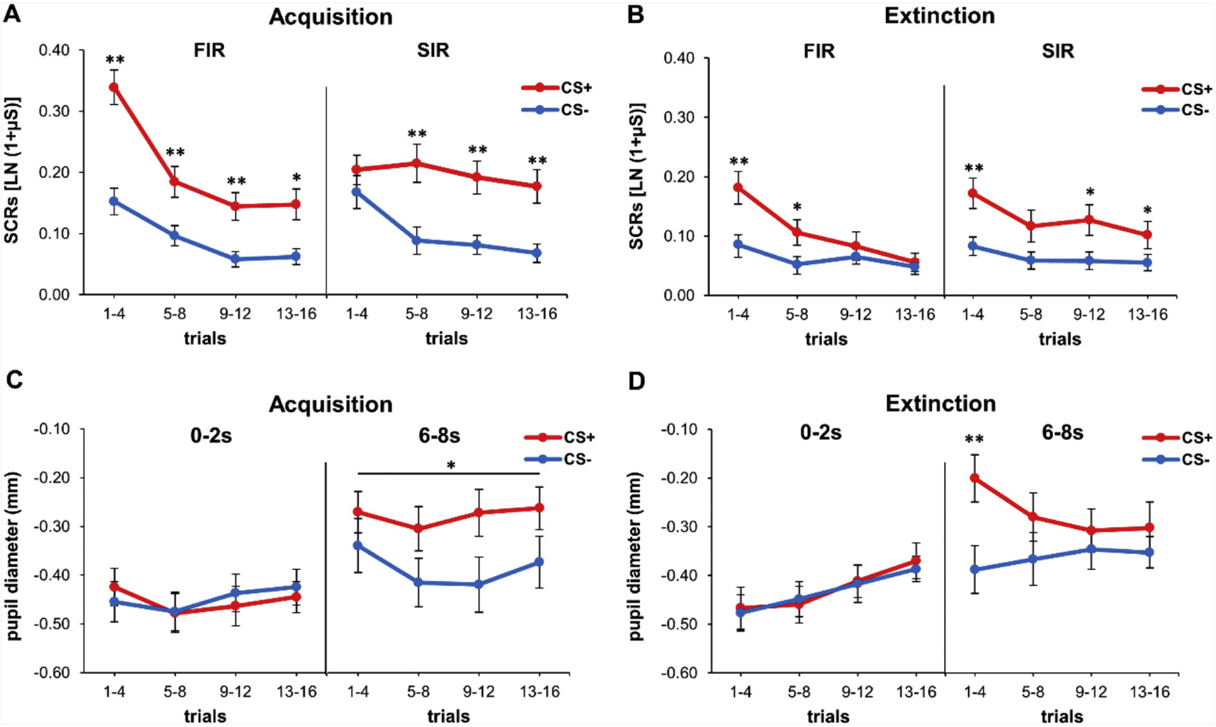

Fig. 1. Mean (± SEM) skin conductance responses (SCRs; upper row; A, B) and pupil diameter (lower row, C, D) for the CS+ and the CS- over the course of fear acquisition (left) and extinction (right). Corresponding to the statistical analyses, each block comprised four CS trials. SCRs are depicted separately for the first- (FIR) and second-interval response (SIR). Pupil diameter is shown for the first (0–2 s) and last (6–8 s) time interval, respectively. $^{**}p < .001$, $^{*}p < .01$, post-hoc Bonferroni-corrected *t*-tests.

For SCRs, the SIR significantly increased, but the FIR decreased during fear acquisition (A). In contrast, during fear extinction, only the FIR showed a decrease of differential CRs (B). For pupillary responses, only the late time bin (6–8 s) revealed an increase and decrease in CS differentiation during fear acquisition (C) and extinction (D), respectively.

$F_{(2.5,90.3)} = 4.39$; $p < .05$; $\eta_p^2 = 0.11$; main effect CS: $F_{(1,36)} = 26.59$; $p < .001$; $\eta_p^2 = 0.43$, main effect block: $F_{(3,108)} = 17.47$; $p < .001$; $\eta_p^2 = 0.33$; see Fig. 1B). The CS × interval × block interaction did not reach significance ($F_{(3,108)} = 1.72$; $p = .17$; $\eta_p^2 = 0.05$). However, planned ANOVAs conducted separately for the FIR and SIR indicated that successful extinction only occurred in the FIR (CS × block interaction: $F_{(2.4,87.9)} = 4.86$, $p < .01$, $\eta_p^2 = 0.12$; 1st block: $t_{(36)} = 4.28$, $p < .001$, $d = 0.70$; 2nd block $t_{(36)} = 2.94$, $p < .01$, $d = 0.48$; 3rd block $t_{(36)} = 0.83$, $p = .41$; 4th block: $t_{(36)} = 0.41$, $p = .68$), whereas the SIR still showed a significant differentiation between CS+ and CS- at the end of extinction (main effect CS: $F_{(1,108)} = 15.15$, $p < .001$, $\eta_p^2 = 0.30$; CS × block interaction: $F_{(3,108)} = 1.32$, $p = .27$, $\eta_p^2 = 0.03$; 1st block: $t_{(36)} = 4.27$, $p < .001$, $d = 0.70$; 2nd block: $t_{(36)} = 2.39$, $p < .05$, $d = 0.39$; 3rd block: $t_{(36)} = 2.87$, $p < .01$, $d = 0.47$; 4th block: $t_{(36)} = 2.82$, $p < .01$, $d = 0.046$).

3.2. Pupillary responses

3.2.1. Fear acquisition

Consistent with SCR data, successful fear acquisition was reflected by significantly stronger pupil dilations in response to the CS+ as compared to the CS- (main effect CS: $F_{(1,33)} = 5.15$; $p < .05$; $\eta_p^2 = 0.14$). Furthermore, ANOVA yielded a significant main effect of interval ($F_{(1.6,54.1)} = 20.89$; $p < .001$; $\eta_p^2 = 0.39$), a significant CS × interval interaction ($F_{(2.3,76.5)} = 12.21$; $p < .001$; $\eta_p^2 = 0.27$) and a CS × interval × block interaction ($F_{(5.0,165.8)} = 2.61$; $p < .05$; $\eta_p^2 = 0.73$). Following up on this, separate ANOVAs for each time bin revealed a significant main effect of CS ($F_{(1,33)} = 12.10$; $p < .01$; $\eta_p^2 = 0.27$) only for the last time bin (i.e. 6–8 s). Pupil dilation was significantly larger to the CS+ than to the CS- specifically within the 2 s time bin preceding the UCS which, however, did not significantly change over the course of fear acquisition (CS × block interaction: $F_{(3,99)} = 0.61$; $p = .61$; $\eta_p^2 = 0.2$; for other time bins, all *ps* > .05; Fig. 1C).

3.2.2. Fear extinction

For fear extinction, significant main effects of CS ($F_{(1,33)} = 8.91$; $p < .01$; $\eta_p^2 = 0.21$) and interval ($F_{(1.9,62.6)} = 21.23$; $p < .001$; $\eta_p^2 = 0.39$) as well as significant interactions of CS × interval ($F_{(2.0,64.9)} = 6.96$; $p < .01$; $\eta_p^2 = 0.17$), interval × block ($F_{(5.4,176.7)} = 4.53$; $p < .001$; $\eta_p^2 = 0.12$) and a three-way interaction of CS × interval × block ($F_{(5.8,191.3)} = 2.20$; $p < .05$; $\eta_p^2 = 0.06$) were observed. As depicted in Fig. 1D, a decrement in differential responding from the first to the last block of extinction occurred only for the late (i.e. the 6–8 s time bin; CS × block interaction: $F_{(3,99)} = 2.38$; $p = .07$; $\eta_p^2 = 0.7$; 1st block: $t_{(33)} = 4.59$, $p < .001$, $d = 0.79$; 2nd block: $t_{(33)} = 1.94$, $p = .061$; 3rd block: $t_{(33)} = 0.55$, $p = .59$; 4th block: $t_{(33)} = 1.16$, $p = .25$), but not for the early pupillary time bin (i.e. the 0–2 s time bin; CS × block interaction: $F_{(3,99)} = 0.47$; $p = .70$; $\eta_p^2 = 0.1$; 1st block: $t_{(33)} = 0.39$, $p = .70$; 2nd block: $t_{(33)} = 0.50$, $p = .62$; 3rd block: $t_{(33)} = 0.16$, $p = .88$; 4th block: $t_{(33)} = 0.91$, $p = .37$).[1]

3.3. Relationship between conditioned SCRs and pupillary responses

During fear acquisition, the late time interval of differential SCRs

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[1] Additional analyses were conducted for both SCRs and pupillary responses including the factor sex as a between-subjects factor. Results for both outcome measures were highly similar to the original analyses regarding fear acquisition and fear extinction. Furthermore, no main or interaction effects with the factor sex occurred for neither of these analyses.

(SIR) was positively related to the late time bin (6–8 s) of differential pupillary responses ($r = 0.481$, $p = .004$), while the positive correlation between the differential FIR and the early differential pupillary response (0–2 s) just failed to reach significance ($r = 0.404$, $p = .018$). For fear extinction, we did not find any significant correlations between the two outcome measures.

4. Discussion

This study follows up on the debate about the variety and general validity of predefining response time windows for CR quantification in fear conditioning research (Luck and Lipp, 2016; Öhman, 1972, 1974; Pineles et al., 2009; Prokasy, 1977; Prokasy and Ebel, 1967). Time windows used for the scoring of conditioned skin conductance and pupillary responses vary largely between but also within labs (Lonsdorf et al., 2017), making it difficult to compare and integrate results from different studies. Given that CR latencies may change over the course of fear and extinction learning (Sjouwerman and Lonsdorf, 2018), it is reasonable that the optimal time interval for detecting such responses also vary as a function of learning progress or learning phase. In the present study, we provide first evidence that conditioned SCRs may shift from early to late time intervals across fear learning, whereas extinction learning appeared to be better tracked by the early interval. In contrast, pupillary CRs were generally better captured by a late pupillary response component, suggesting a rather stable temporal CR pattern for the pupil in both learning phases.

In particular, analyses with the two predefined SCR intervals revealed that differential responding as measured by the SIR significantly increased during fear acquisition training, indicating successful fear learning. In contrast, for the FIR, CS differentiation remained rather stable or even decreased when fear learning proceeded (see Fig. 1A). This corroborates with a study reporting significant SCR differences between CS+ and CS- at stimulus-offset but not at stimulus-onset at the end of fear acquisition (Spoormaker et al., 2011). The different temporal trajectories of the FIR and SIR found in the current study support the idea that both intervals may capture distinct characteristics of the underlying fear learning process. It has been previously argued that the SIR reflects CS-UCS contingencies evolving over time, while the FIR rather signals orienting behavior occurring primarily at the beginning of learning and then habituating over time (Öhman, 1972, 1974; Wolter and Lachnit, 1993). Yet, based on these considerations, the SIR should be generally better suited to detect learning-related changes during both, fear acquisition and extinction. However, here we demonstrated that during extinction training only the FIR showed a decreasing pattern of CRs, indicating successful extinction, while the SIR still revealed a significant differentiation between the CS+ and CS- at the end of extinction training (see Fig. 1B). These results support the idea that the FIR is not solely reflecting orienting or novelty responses (Öhman, 1972, 1974) but also linked to associative processes.

Prior research has shown that successful Pavlovian conditioning is not only dependent on learning the cue that predicts threat but also the temporal relationship between the cue and the threat (Balsam et al., 2010; Gallistel and Gibbon, 2000; Kirkpatrick and Balsam, 2016). An alternative explanation for the temporal shift from FIR to SIR during fear acquisition might therefore be the existence of two slightly staggered but intertwined learning processes – one about the association between the CSs and the UCS and one about the relative timing between them. As such, participants may first learn the CS-UCS association (FIR), but require some additional time to learn when exactly the UCS is delivered (SIR), especially in experimental designs using variable ITIs and thus a longer stimulus onset asynchrony. Consistent with this idea, neuroimaging data suggest that temporal intervals between events are encoded independently from the CS-UCS representation during associative fear learning (Harnett et al., 2016; Knight et al., 2004). More specifically, it has been shown that learning-related activity in the hippocampus, dorsolateral prefrontal cortex and amygdala was particularly associated with the processing and formation of the CS-UCS time interval (Díaz-Mataix et al., 2014; Harnett et al., 2016; Knight et al., 2004). Experimental data further suggest that individuals even learn about the duration of cues when the UCS is omitted during extinction (Balsam et al., 2010). For instance, when animals were conditioned using a fixed CS-UCS interval and then extinguished with CSs of longer or shorter durations, they showed faster extinction (Prenoveau et al., 2013), but also a stronger recovery of conditioned responding when re-exposed to the original CS duration (Drew et al., 2004; Haselgrove and Pearce, 2003). Assuming that the SIR reflects the learning of temporal associations between the CSs and UCS or CS and UCS omission – as during the secondary learning process of extinction – this might also explain why the SIR still exhibits a significant CS differentiation at the end of extinction training. Our results, together with the evidence reviewed here thus indicate that temporal CS-UCS intervals seem to be encoded distinctively in the brain, and critically determine behavioral output (i.e. anticipatory CRs) during fear and extinction learning. Thus, especially for studies using longer CS durations, which allow for a separation into FIR and SIR, different scoring windows may be recommended for the analyses of fear and extinction processes in order to enhance the sensitivity to track learning-related changes. Accordingly, Luck and Lipp (2016) demonstrated that using a single window scoring technique failed to detect effects of instructed extinction, leading to the loss of important information and therefore concluded that multiple latency windows (i.e. using both FIR and SIR) seem advisable.

Alternatively, it seems also plausible that temporal processing is inherent to the associative learning process, with one conditioned response gradually shifting in latency over the course of training. In line with this presumption, it has been recently shown that SCR latencies towards the CS+ and CS- increased over the course of fear acquisition (Sjouwerman and Lonsdorf, 2018). Yet, this study included only fear acquisition training and it remains unclear, whether SCR latencies would remain stable or change again across extinction training. Future research is warranted to delineate, whether there are two distinct responses changing their amplitude or a single CR gradually shifting in latency over time. To answer these research questions we need to find more adept ways to characterize associative and temporal phenomena of learning, for example by analyzing changes in behavior within a single trial (Buhusi, 2014; Reyes and Buhusi, 2014). In this context, computational approaches might provide a promising vantage point for evaluating precise trial-by-trial dynamics of associative learning, while taking into account both response entities – amplitude and latency.

In addition to SCRs, we also tracked pupillary responses during fear acquisition and extinction training. For fear acquisition training, an increase in CS differentiation was only observed for the late pupillary response component (see Fig. 1C). This lines up with previous work, demonstrating that pupil dilations discriminate most strongly between CS+ and CS- in close proximity to the expected UCS onset (Leuchs et al., 2017; Reinhard and Lachnit, 2002; Visser et al., 2015). By contrast, we did not find any evidence for the fear-inhibited light reflex (i.e. attenuated pupil constriction to the CS+ as compared to the CS-) in the early pupillary time bin as reported previously (Reinhard et al., 2006; but see, Leuchs et al., 2017). Also during extinction training, a decrement in differential CRs occurred only for the late but not for the early pupillary time bin. Thus, in contrast to the temporal shift we found for conditioned SCRs, the pupil appeared to show a relatively stable temporal response pattern across both learning phases. This in turn implies that the learning dynamics of both fear acquisition and extinction are best captured by a late pupil dilation component and should thus be chosen as the primary read-out interval for the analyses of pupillary CRs in future fear conditioning studies.

SCRs and pupillary responses originate from distinct sensory processing pathways and generally differ with regard to response speed (Beatty and Lucero-Wagoner, 2000; Boucsein et al., 2012; Kinner et al., 2017). It is thus reasonable that both measures also follow a distinct temporal trajectory of CR latencies across fear and extinction learning. Accordingly, it has long been argued that different physiological readout measures of CRs, despite commonly discriminating between CS + and CS-, may partly tap into different dimensions of the respective learning process and hence do not necessarily converge (Lonsdorf et al., 2017; Lonsdorf and Merz, 2017). For instance, Tzovara et al. (2018) provided systematic evidence from psychophysiological modeling and computational learning theory that conditioned SCRs may reflect a mixture of expected outcome and uncertainty, while conditioned pupillary responses track expected outcome alone. This corroborates with experimental data showing that pupil dilations increased towards the CS+ throughout fear acquisition, most closely mirroring UCS expectancy ratings in comparison to SCRs or startle responses (Leuchs et al., 2019). Besides responding to arousal, pupil dilation has been also linked to a broad range of cognitive processes (Andreassi, 2000; Beatty and Lucero-Wagoner, 2000; Sirois and Brisson, 2014), including attention (Laeng et al., 2012), memory (Goldinger and Papesh, 2012) and cognitive load (Just et al., 2003; Wel and Steenbergen, 2018). It is therefore reasonable, that changes in pupil size reflect additional higher-order cognitive processes such as threat appraisal or explicit UCS expectancy (Leuchs et al., 2019) during associative learning, which might be also characterized by a different temporal pattern. A promising avenue for future research is thus to determine whether other physiological outcome measures, such as fear potentiated startle or heart rate responses would show similar temporal CR trajectories as either conditioned SCRs or pupillary responses, which helps characterizing the progression in either fear or extinction learning.

However, even though different readouts may be differentially sensitive to distinct facets or subcomponents of the underlying learning process, they also share overlapping mechanisms inherent to the associative learning system (Lonsdorf et al., 2017; Lonsdorf and Richter, 2017). In line with this idea, we found late conditioned SCRs (i.e. the SIR) and late pupillary responses (i.e. the 6–8 s time bin) to be positively correlated during fear acquisition. To date, little is known about the relationship between different outcome measures, and their differential sensitivity to specific subprocesses of fear conditioning is controversially discussed. More studies including multiple indices of fear and extinction learning are warranted in order to pinpoint the distinct and common mechanisms that are reflected by each of these measures.

Taken together, the present study provides first evidence for a temporal CR shift from early to late SCR time intervals over the course of fear acquisition, which might be reflective of two separate processes, in which associations between CSs and UCS and their relative timing are subsequently formed. During extinction, only the FIR was able to track the decline of CRs and thus the progress in learning, whereas the SIR still differentiated between the CSs. These distinct learning curves indicate that conditioned SCRs follow a dynamic temporal pattern that may be related to different dimensions of the underlying learning process. By contrast, pupillary CRs were generally better characterized by a late pupillary response component, suggesting a rather stable temporal CR pattern for the pupil in both learning phases. Our findings are helpful in specifying CR response quantification for different physiological read-out measures and/or learning processes during fear conditioning and may motivate future researchers to investigate time-specific CR patterns and their relation to specific learning dimensions.

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