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In Dubio Pro Defensio: Initial Activation of Conditioned Fear Is Not Cue Specific

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This study explored the time course of conditioned fear response expression. Two neutral male facial expressions served as conditioned stimuli (CS) in a differential trace conditioning that involved either an aversive ($n = 14$) or a nonaversive ($n = 12$) unconditioned stimulus (UCS) in a between-subjects design. Skin conductance response (SCR) to the CSs and startle response magnitudes to acoustic probes presented at early (250 ms) or late (1,750 ms) probe times after CS onset were measured. As expected, conditioned SCR discrimination was observed in both aversive and nonaversive learning, whereas the conditioned potentiation of the startle response was only observed for the aversive UCS condition. Interestingly, conditioned startle discrimination was specific for the later probe time. In contrast, conditioned fear potentiation of the startle response at the early probe time was equally pronounced for CS+ and CS−. These findings suggest that fear-eliciting neural structures are rapidly activated in fear learning, whereas the expression of inhibitory conditioning requires more time, presumably reflecting the involvement of cortical top–down control processes.

Keywords: aversive learning, fear-potentiated startle, differential trace conditioning, temporal specificity

Classical conditioning is considered a central mechanism in the development of pathological states of fear and anxiety (e.g., Bouton, Mineka, & Barlow, 2001). Accordingly, studying the learning and unlearning of classically conditioned fear responses may promote important clinical implications (cf. Davis, 2002). A valuable tool in the study of the human fear response is the potentiation of the startle blink reflex. It is well established in animal and human research that the startle response, a defensive brain stem reflex, is potentiated when elicited in the presence of a fear-eliciting stimulus, and the neural mechanisms of fear-potentiated startle have been detailed in animal research (for an overview, see Davis, 2006). The major advantages of the startle probe methodology in the study of fear learning include the specificity to index the affective impact of a conditioned stimulus. Although other physiological measures like conditioned skin conductance responses can be observed during both aversive and nonaversive learning, conditioned startle potentiation is specifically pronounced in aversive conditioning (Hamm & Vaitl, 1996). Moreover, the startle blink reflex is a short-latency brain stem reflex, thus enabling the study of the temporal dynamics of the fear response. Accordingly, the startle probe methodology was used in the present study as a primary measure with which to index the time course of fear activation in a differential fear-conditioning paradigm.

The temporal specificity of the conditioned fear-potentiated startle has been successfully explored in animal research, revealing an increase in fear potentiation with increasing temporal proximity of the startle-eliciting probe stimulus to the aversive unconditioned stimulus (Burman & Gewirtz, 2004; Davis, Schlesinger, & Sorenson, 1989; Siegel, 1967). However, whether a similar temporal specificity of the fear response expression can be also observed in humans has been much less extensively studied. A recent conditioning study in humans revealed a slightly more pronounced startle potentiation to those startle probes, which preceded the onset of the unconditioned stimulus (UCS) more closely (Weike, Schupp, & Hamm, 2007). However, this study did not specifically aim to explore the temporal course of the conditioned fear response and thus included only startle probe times close to the onset of the UCS. Another source of data about the temporal specificity of fear-potentiated startle in humans is research using the instructed fear paradigm (e.g., Grillon, Ameli, Foot, & Davis, 1993; Grillon, Ameli, Goddard, Woods, & Davis, 1994; Grillon, Ameli, Merikangas, Woods, & Davis, 1993). In these studies, two different lights signaled threat and safe conditions, respectively. Participants were instructed that they might receive an aversive (but not painful) electric shock stimulus during the last 10 s of the threat condition (temporal information was provided by a digital timer), and no shock stimuli would be delivered during the safe condition. In addition to the expected potentiation of the startle blink responses during the threat condition as compared with the safe condition, the amount of startle potentiation varied as a function of temporal proximity to the anticipated aversive event. Specifically, delivering startle probes at different times throughout each condi-

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tion revealed that the amount of startle potentiation was maximal during the final 10 s of the threat condition, that is, when aversive shocks were anticipated (but not actually presented). In the present study, we explored whether a similar temporal specificity of fear-potentiated startle can be observed in classical conditioning without explicitly providing information about timing and contingencies between conditioned and unconditioned stimuli.

Eliciting the startle reflex shortly after the presentation of a fear cue enables the assessment of the speed of defensive fear activation (e.g., Globisch, Hamm, Esteves, & Öhman, 1999). However, another modulatory phenomenon has to be taken into account at those very early probe times. The presentation of any stimulus shortly before the acoustic startle probe stimulus (<500 ms) results in a relative inhibition of the startle response magnitudes, the so-called prepulse inhibition (PPI; cf. Graham, 1975). The PPI of the startle response is thought to reflect a sensorimotor gating mechanism preventing the early processing of the first (prepulse) stimulus from disruption by the processing of the startle stimulus. As revealed by animal research, the PPI effect is conveyed by neural circuits within the brain stem that impinge onto the nucleus reticularis pontis caudalis, the sensorimotor relay center of the primary startle pathway (cf. Koch, 1999). More important, the fear-potentiated startle effect is also mediated by modulatory influences onto the reticularis pontis caudalis via direct and indirect excitatory connections from the amygdaloid complex (cf. Davis, 2000; Koch, 1999). Thus both inhibitory and excitatory connections impinge onto the reticularis pontis caudalis, suggesting that a lack of PPI of the startle response at early probe times following a fear-eliciting stimulus does not index a deficit in sensorimotor gating but rather reflects the coactivation of both PPI-related inhibitory and fear-related excitatory influences on the primary startle pathway.

In line with these considerations about the neural mechanisms of startle modulation, participants with phobic fears of either spiders or snakes exhibited a pronounced potentiation of the startle response across a variety of startle probe times, including the lack of PPI at the early probe time of 300 ms, when confronted with pictures of their feared cues (Globisch et al., 1999). Moreover, this fast recruitment of fear was independent of the duration of the fear-eliciting stimuli; that is, the observed fear-potentiated startle patterns were comparable between short- (150-ms) and long-lasting (6,000-ms) picture presentations. These findings suggest that the fear system is capable of rapidly responding to threatening cues. Interestingly, a recent brain imaging study revealed that the blood oxygen level–dependent response in the amygdala to phobic visual cues was not more pronounced in spider-phobic participants as compared with nonphobic participants, but was more rapidly recruited in the phobic participants (Larson et al., 2006). Furthermore, the activation of the subcortical defensive networks might not necessarily depend on cortical sensory processing. For example, a case study of a patient suffering from bilateral cortical blindness revealed the acquisition of a fear-conditioned startle potentiation to a visual cue despite the lack of sensory encoding of the conditioned stimulus in the primary visual cortex (Hamm et al., 2003). These and other studies have provided accumulating evidence for the rapid and highly automatic nature of fear activation, a characteristic of the so-called fear module of the brain as suggested by Öhman and Mineka (2001).

Besides conditioned startle potentiation skin conductance response (SCR) magnitudes to the conditioned stimulus (CS) have traditionally been used to study human fear conditioning (for an overview, see Öhman, 1983). Accordingly, conditioned SCR discrimination, that is, larger SCRs to the CS+ predicting the upcoming UCS compared with the CS− signaling the absence of the UCS, is a robust finding in fear conditioning. However, SCR discrimination is not specific to aversive learning and can also be obtained for nonaversive UCS conditions (e.g., Hamm & Vaitl, 1996). Moreover, the acquisition of conditioned SCR discrimination is specifically pronounced in those participants who also acquired declarative knowledge about the CS–UCS contingencies (cf. Hamm & Weike, 2005), which is in line with models that link SCR conditioning to increased orienting to the reinforced CS (cf. Öhman, Hamm, & Hugdahl, 2000). SCR conditioning can also be obtained on a more automatic level if the detection of the conditioned stimulus is rendered more difficult. For instance, Knight, Nguyen, and Bandettini (2003, 2006) found conditioned SCR discrimination to tone CSs that were presented at perithreshold intensities, but only in a delay procedure, not a trace conditioning procedure, that is, when a temporal gap had to be bridged between CS offset and UCS onset (Knight et al., 2006). Interestingly, when using fear-relevant CSs (e.g., pictures of snakes or angry faces) that are backwardly masked, conditioned SCR discrimination can be obtained in a trace conditioning paradigm as well (Esteves, Parra, Dimberg, & Öhman, 1994; Öhman & Soares, 1998).

The present study was specifically aimed at exploring the temporal course of cue-specific fear activation in differential fear conditioning. Toward this end, conditioned startle potentiation served as the primary dependent measure because the short latency of the blink reflex enables determination of the onset of defensive activation and the speed of conditioned discrimination between reinforced and nonreinforced stimuli. The conditioned stimuli were presented for only 30 ms to promote a more automatic level of learning. The UCS was presented 2,000 ms following the onset of the CS+ (thus resulting in a trace conditioning paradigm) because a shorter interstimulus interval would have obscured the analysis of the temporal course of conditioned fear activation and the use of a delay-conditioning procedure would have resulted in a more elaborated processing of the CSs. To ensure that the present findings specifically related to fear conditioning, a separate group of participants was tested in a nonaversive conditioning paradigm (cf. Hamm & Vaitl, 1996). Conditioned SCR discrimination was investigated as an index of acquired changes in stimulus significance. We expected conditioned SCR discrimination during both aversive and nonaversive learning, and we assumed conditioned startle potentiation was specific to the aversive UCS condition.

Method

Participants

Thirty-seven psychology students (28 women and 9 men) of the University of Greifswald (Greifswald, Germany) participated in the present study for course credit. Five participants had to be excluded from data analyses because of malfunctions of the technical equipment. The remaining 32 students (24 women and 8 men) were randomly assigned to two experimental groups, that is, there were 16 participants each in the aversive conditioning group and the nonaversive conditioning group, respectively. The participants' mean age was 22.9 years (±0.54 *SE*), which did not differ between the aversive and nonaversive conditioning groups ($F <$

1). All participants signed an informed consent form before the study, which was approved by the Ethics Committee of the University of Greifswald.

Stimulus Materials

Eighteen pictures depicting pleasant, unpleasant, or neutral scenes and objects were selected from the International Affective Pictures System (IAPS; Lang, Bradley, & Cuthbert, 2005). Two male faces with neutral expressions (IAPS Numbers 2200 and 2210) from the neutral picture category served as the conditioned stimuli. The pictures were projected onto a screen approximately 2 m in front of the participants in a visible size of 38 cm × 57 cm with a duration of 30 ms. Four seconds before each picture presentation, a red fixation dot was presented for 3 s (see Figure 1). Picture presentation times were controlled by a tachistoscopic shutter (G1166, Gerbrands Corporation, Arlington, MA), which was situated together with the slide projector (Kodak Ektapro 5000) in a room adjacent to the sound-shielded experimental room.

The aversive UCS consisted of an electric stimulation (500-Hz monopolar DC pulse) to the participant's left forearm in a 10-ms train of single pulses (1 ms). The nonaversive UCS consisted of a 300-ms train of single electric pulses (50-Hz monopolar DC) to the participant's left forearm, which served as an imperative stimulus for a reaction time (RT) task. Feedback of RT task performance was realized by red and green LEDs, which were situated 2 m in front of the participants below the projection screen. The electric UCSs were generated by a commercial stimulator (Grass Instruments S48K; Grass Instruments, Quincy, MA), isolated (SIU5), and transmitted via a constant current unit (CCU1) to a bipolar electrode (F-E10S2). The intensity of the nonaversive UCS was kept at a constant level of 1.2 mA, whereas the intensity of the aversive UCS was individually adjusted to a level that was experienced as highly annoying, but not painful, resulting in a mean physical intensity of the aversive UCS of 4.9 mA (±0.5 *SE*).

A 50-ms burst of white noise with an intensity of 95 dB[A] (rise–fall < 1 ms) was generated by a Coulbourn S81-02 (Coulbourn Instruments, Allentown, PA) and presented binaurally over headphones (Sony MDR-CD 170) to serve as a startle-eliciting stimulus.

Physiological Recordings

Skin conductance was recorded from the hypothenar eminence of the palmar surface of the participant's right hand. A Coulbourn S71-22 skin conductance coupler (Coulbourn Instruments, Allentown, PA) provided a constant 0.5 V across two Ag/AgCl standard electrodes (8 mm diameter; Marquette Hellige, Freiburg, Germany) filled with a 0.05 molar sodium chloride electrolyte medium. The signal was processed with a resolution of 0.01 μS and sampled with a rate of 10 Hz.

The electromyographic (EMG) activity over the left orbicularis oculi muscle was recorded to measure the eyeblink component of the startle response. Ag/AgCl miniature surface electrodes (Sensormedic, Yorba Linda, CA) filled with electrolyte (Marquette Hellige) were attached beneath the lower eyelid using adhesive rings (Marquette Hellige). The raw EMG signal was amplified and filtered through a 30- to 1000-Hz bandpass, using a Coulbourn S75-01 bioamplifier (Coulbourn Instruments, Allentown, PA). Digital sampling with a rate of 1000 Hz started 100 ms before the onset of the acoustic startle stimulus until 400 ms after. The EMG signal was filtered offline through a 60-Hz highpass filter and was rectified and integrated (time constant = 10 ms) using a digital filter. Data acquisition and stimulus presentations were synchronized using an IBM-compatible computer.

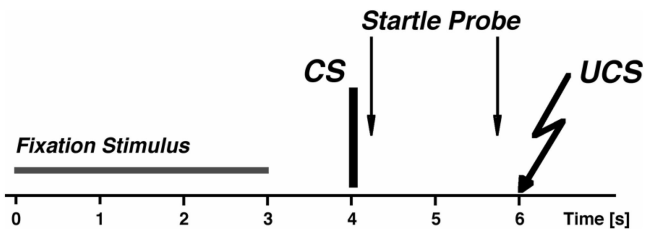

Figure 1. Timing of the presentations of the conditioned stimulus (CS) and the unconditioned stimulus (UCS) during the conditioning. Acoustic startle probes followed CS onset in two thirds of the trials by either 250 or 1,750 ms. The UCS consisted of an aversive electric stimulation during aversive conditioning and a nonaversive electric stimulus (serving as an imperative stimulus for a reaction time task) in the nonaversive conditioning group.

Procedure

Participants first read and signed the informed consent form before being seated in a reclining chair in an upright position with both arms placed comfortably on enlarged armrests. After attaching the physiological sensors and the stimulating electrode, participants underwent five consecutive experimental phases.

Preconditioning. Each of six pleasant, unpleasant, and neutral pictures were presented for 30 ms, with each presentation being preceded by a red fixation dot (see Figure 1). The pictures were presented in a random order with the restriction that the designated conditioned stimuli (CS+ and CS−) included in the neutral picture category were always presented last (counterbalanced across participants). Before the picture presentation, 10 acoustic startle stimuli were delivered for an initial habituation of the startle blinks. Two startle probes were delivered at either 250 or 1,750 ms after picture onset for each picture category. The designated CSs were always followed by an acoustic startle probe presentation; that is, for half of the participants the designated CS+ was followed by a startle probe 250 ms after picture onset and the designated CS− was followed by the startle probe 1,750 ms after picture onset, whereas this timing was reversed for the other half of the participants. Furthermore, six acoustic startle probes were administered in the intertrial intervals (ITI), which varied between 15 s and 22 s. The participants were told to attend to the pictures and to ignore the acoustic stimuli being occasionally presented via headphones.

UCS work-up. Following the preconditioning phase, the intensity of the electric UCS in the aversive conditioning group was individually adjusted within five warned presentations of the UCS to a level that was experienced as highly annoying, but not painful. In the nonaversive conditioning group, five practice trials of the simple reaction time task were realized. Participants were instructed to press a button with the left thumb as fast as possible following the mild electrotactile UCS (imperative stimulus). Performance feedback was given by red and green LEDs, which were fixed below the projection screen. One to three green LEDs indicated good, better, or best performance level, respectively, which

were rewarded with small amounts of money, and a red LED indicated an invalid or too slow response.

Conditioning. The conditioning phase started with a series of five acoustic startle probes. The differential trace conditioning consisted of nine presentations of each CS, that is, one male neutral face (CS+) was always followed by the UCS 2 s later, whereas the other male neutral face (CS−) was never paired with the UCS. The CSs were presented for 30 ms, preceded by a fixation stimulus (red dot) 4 s earlier (see Figure 1). The order of the CS presentations was random, with the restriction that there could be no more than two consecutive presentations of the same picture. Eight different stimulus orders were realized, and the participants of each conditioning group were equally assigned to these orders. In the aversive conditioning group, the CS+ was always followed by the aversive electric UCS, whereas in the nonaversive conditioning group, the CS+ was always followed by the mild electrotactile UCS (serving as imperative stimulus for the RT task). Three presentations of each CS were followed by an acoustic startle probe 250 ms after CS onset (early probe time), and three further CS presentations were followed by an acoustic startle probe 1,750 ms after CS onset (late probe time), leaving three presentations per CS without further acoustic stimulation. Moreover, six acoustic startle probes were presented during the ITIs that varied between 18 and 24 s. Participants were instructed that a series of pictures and some electric pulses would be presented and that the acoustic stimuli being presented occasionally via headphones could be ignored. No information on the contingencies between the pictures and the electric pulses was provided.

Postconditioning. The postconditioning phase immediately followed the conditioning phase; that is, there was neither a break nor any signal that indicated that no further UCSs would be presented. During the postconditioning phase, each CS was presented 12 times in a random order with the restriction of no more than two presentations of either CS in a row. There were no further presentations of the UCS. Four presentations of each CS were followed by an acoustic startle probe 250 or 1,750 ms after picture onset, leaving four presentations per CS without further acoustic stimulation. Moreover, eight acoustic startle probes were presented during the ITIs that varied between 18 and 24 s.

Postexperimental interview. After the postconditioning phase, a postexperimental interview was carried out to determine whether the participants were aware of the CS–UCS contingencies (cf. Bechara et al., 1995; Dawson & Schell, 1987). Furthermore, the participants were asked to evaluate retrospectively the valence and arousal of the electric UCS by means of the Self-Assessment-Manikin (Lang, 1980), which involves a 9-point scale ranging from 1 (*very unpleasant* or *very calm,* respectively) to 9 (*very pleasant* or *highly arousing,* respectively).

Data Reduction and Response Definition

The magnitude of the SCR to the pictorial stimuli was scored as the largest increase in skin conductance between 0.9 and 4.0 s after stimulus onset (first interval response; Prokasy & Kumpfer, 1973), using a computer program (Globisch, Hamm, Schneider, & Vaitl, 1993). Only those picture presentations that were not followed by an acoustic startle probe were considered for SCR evaluation to avoid contaminations with SCRs to the acoustic startle stimuli. The mean onset latency of the SCRs to the pictorial stimuli was 1.8 s ($SE = 0.1$), and thus these SCRs could be discriminated clearly from the unconditioned responses, which were scored as the largest increase in skin conductance between 0.9 and 4.0 s after the onset of the electrical stimulus, resulting in a mean onset latency of the SCRs to the UCS of 1.6 s ($SE = 0.1$).[1] Trials in which no response could be detected or with a response magnitude of less than 0.05 μS were considered as zero responses. Of the trials, 1% were rejected because of respiration or recording artifacts, as identified by large SCRs before the designated latency window or poor signal quality, respectively. Number of discarded trials neither exceeded five trials per participant nor differed between both conditioning groups ($F < 1$). Missing values were replaced individually for each participant by the mean SCR magnitude to all picture trials or the mean unconditioned response magnitude averaged across all UCS presentations, respectively. Logarithms of all values were computed to normalize the distribution (Venables & Christie, 1980). To reduce interindividual variability that was not related to the task, the log values were range corrected by dividing each individual score by the participant's maximum response (Lykken & Venables, 1971).

The magnitude of the startle eyeblink was scored offline using a computer program (Globisch et al., 1993) that identified latency of blink onset in milliseconds and peak amplitude in microvolts. Blinks with onset latencies between 20 and 100 ms after probe onset and peak latencies within 150 ms were scored as valid startle responses. No detectable eyeblinks were scored as zero responses. Fewer than 1% of the trials had to be rejected because of excessive baseline activity or recording artifacts. Number of rejected trials varied between zero and four per participant, and both conditioning groups did not differ in the number of missing trials ($F < 1$). Before the statistical data analyses, all missing values were replaced individually for each participant by the overall mean blink response magnitude of the participant. The raw blink magnitude to startle probes during the intertrial interval did not differ between the aversive and the nonaversive conditioning groups (Ms = 46.6 and 56.8, SEs = 11.3 and 15.0, for the aversive and nonaversive conditioning group, respectively; $F < 1$). Thus, to ensure that each participant contributed equally to the group's mean, startle blink magnitude data were standardized to T scores [$50 + (z \times 10)$].

Data Analysis

SCRs to the conditioned stimuli and startle blink responses elicited at early and late startle probe times following the presentation of the conditioned stimuli were analyzed separately for the different phases of the experiment. Unless otherwise noted, all statistical tests used the .05 level of statistical significance.

Preconditioning. The evaluation of the SCRs to the designated CS+ versus CS− was rendered impossible during the preconditioning phase because each CS was followed by either an early or a late startle probe, respectively. With regard to the startle data,

[1] Analyses of the onset latencies of the CS-elicited SCRs revealed a potential onset latency overlap to the UCS-elicited SCRs for 21 trials (out of 448). Of these 21 trials, 7 involved a CS− presentation during the acquisition phase, whereas during the extinction phase 6 CS− trials and 8 CS+ trials involved an SCR onset latency of more than 2.8 s. A potential onset latency overlap to UCS-elicited SCRs is rendered impossible, because no UCSs were presented in these trials. Moreover, a possible contamination with SCRs elicited by the UCS omission during the extinction phase is at least kept constant across SCRs elicited by CS+ and CS−.

testing the differences between both to-be-conditioned stimuli (CS+ vs. CS−) at early and late probe times involved a between-subjects comparison, whereas the general startle modulation involved a within-subjects comparison between CS and ITI. The computed mixed-model analysis of variance (ANOVA) was completed by the between-subjects factor group (designated aversive vs. nonaversive UCS).

Conditioning and postconditioning. For data analyses of the conditioning and postconditioning phases of the experiment, we calculated mixed-model ANOVAs for SCR and startle blink magnitudes involving the between-subjects factor group (aversive UCS vs. nonaversive UCS) and the within-subjects factors conditioning (CS+ vs. CS− for skin conductance responses; CS+ vs. CS− vs. ITI for startle blink responses) and trial block (first vs. second). For the conditioning phase, the first trial of each CS was considered the first trial block, whereas the averaged second and third CS trials made up the second trial block. For the postconditioning phase, two CS trials each were blocked to constitute the first and second trial block. The blink responses to the ITI startle probes were blocked by three or four trials for the conditioning and the postconditioning phases, respectively.

Results

Manipulation Check

Because the present experiment involved a differential trace conditioning procedure with neutral CSs, the evaluation of the participants' acquisition of declarative knowledge about the CS–UCS contingencies is an important prerequisite to assess fear acquisition (cf. Knight et al., 2006; Weike et al., 2007). As revealed by the postexperimental interview, 2 participants in the aversive conditioning group and 4 participants in the nonaversive conditioning group were not aware of the CS–UCS contingencies, that is, they were not able to correctly recognize the CS+. Although the (expected) low rate of unaware participants did not allow for statistical comparisons between aware and unaware participants, the descriptive data analyses confirmed previous findings that contingency awareness is a prerequisite for the acquisition of a fear-conditioned startle potentiation in a trace conditioning paradigm (Weike et al., 2007). Specifically, startle response modulations in the unaware participants of the aversive conditioning during the second acquisition block were negligible (CS+ − ITI: $\Delta T = -4.78$, $SE = 3.25$) and similar to those of the unaware participants of the nonaversive conditioning group, for whom no effects of conditioned startle potentiation had been expected (CS+ − ITI: $\Delta T = -4.62$, $SE = 2.23$). Furthermore, replicating previous findings (Hamm & Vaitl, 1996; Knight et al., 2006; Weike et al., 2007), no conditioned SCR discrimination could be observed in the unaware participants irrespective of the conditioning group (mean differences CS+ − CS−: Δlog[1 + first interval response(μS)] = 0.02 and −0.13, *SE*s = 0.02 and 0.10 for aversive and nonaversive UCS conditions, respectively). Thus, the unaware participants were excluded from further data analyses, and the data from 14 participants (13 women and 1 man) of the aversive conditioning group and 12 participants (7 women and 5 men) of the nonaversive conditioning group were analyzed to explore the time course of conditioned fear.[2] As expected, the UCS was rated as more unpleasant ($M = 3.0$, range = 2–4 vs. $M = 5.3$, range = 3–8) and more arousing ($M = 7.3$, range = 6–9 vs. $M = 5.3$, range = 3–7) in the aversive conditioning group as compared with the nonaversive conditioning group,[3] Fs(1, 24) = 19.8 and 18.0, $p < .001$, for valence and arousal rating, respectively, during the postexperimental interview.

SCR Magnitudes

Conditioning. As illustrated by Figure 2, presentation of the CS+ resulted in increasingly larger SCR magnitudes than did presentation of the CS− during the acquisition phase, resulting in a significant interaction of Conditioning × Trial Block, $F(1, 24) = 5.37$, $p = .029$. As expected, the CS+ elicited larger SCR magnitudes than the CS− during the second trial block, $F(1, 24) = 5.22$, $p = .032$, but not during the first ($F < 1$). These effects of conditioned SCR discrimination were equally pronounced in the aversive and nonaversive conditioning groups (all interaction effects, $F < 1$).

Postconditioning. As expected, the CS+ continued to elicit larger SCR magnitudes than the CS− during the postconditioning phase of the experiment, $F(1, 24) = 6.29$, $p = .019$. Although the Conditioning × Trial Block interaction in the main analysis fell short of statistical significance, $F(1, 24) = 1.53$, $p = .228$, Figure 2 illustrates the pronounced conditioned SCR discrimination observed during the first block, $F(1, 24) = 9.09$, $p = .006$, which apparently diminished in the second block, $F(1, 24) = 1.47$, $p = .237$. Similar to the conditioning phase, no differences between the aversive and nonaversive UCS conditions were observed with regard to SCR discrimination (all Fs < 1).

Startle Blink Responses Elicited at the Early Probe Time

Preconditioning. Startle blink responses elicited 250 ms after the presentation of the designated CSs were significantly smaller

[2] Because of the small number of male participants, the volunteers' sex could not be considered as an additional experimental factor in the statistical analyses. However, descriptive analyses revealed that the patterns of responses were comparable between male and female participants in each conditioning group. Moreover, data analyses within the female participants confirmed the findings obtained in the whole-group analyses.

[3] Accordingly, the valence and arousal ratings of the UCS covaried overall with the amount of conditioned startle potentiation during the second acquisition block for both the early startle probe time ($r_s = -.61$, $p = .001$, and $r_s = .42$, $p = .032$, for valence and arousal, respectively) and the late startle probe time ($r_s = -.53$, $p = .006$, and $r_s = .48$, $p = .014$, respectively). However, when analyzing these covariations separately for the aversive conditioning group and the nonaversive conditioning group, no significant correlations between conditioned startle potentiation and the rated valence or arousal of the UCS were observed in the nonaversive conditioning group (all ps $> .170$). For the aversive conditioning group, though, the computed Spearman correlation coefficients still indicated some covariation between the rated valence or arousal of the UCS and the amount of conditioned startle potentiation for the early startle probe time ($r_s = -.61$, $p = .021$, and $r_s = .47$, $p = .087$, respectively), but not for the late startle probe time (both ps $> .250$). These patterns of correlations suggest that different learning processes in terms of conditioned startle potentiation were involved in aversive conditioning as compared with nonaversive conditioning despite the small overlaps in rated UCS valence and arousal. Moreover, no significant correlations were observed between the amount of conditioned SCR discrimination and the rated valence or arousal of the UCS, respectively, neither in the overall nor in the separate group analyses (all ps $> .125$).

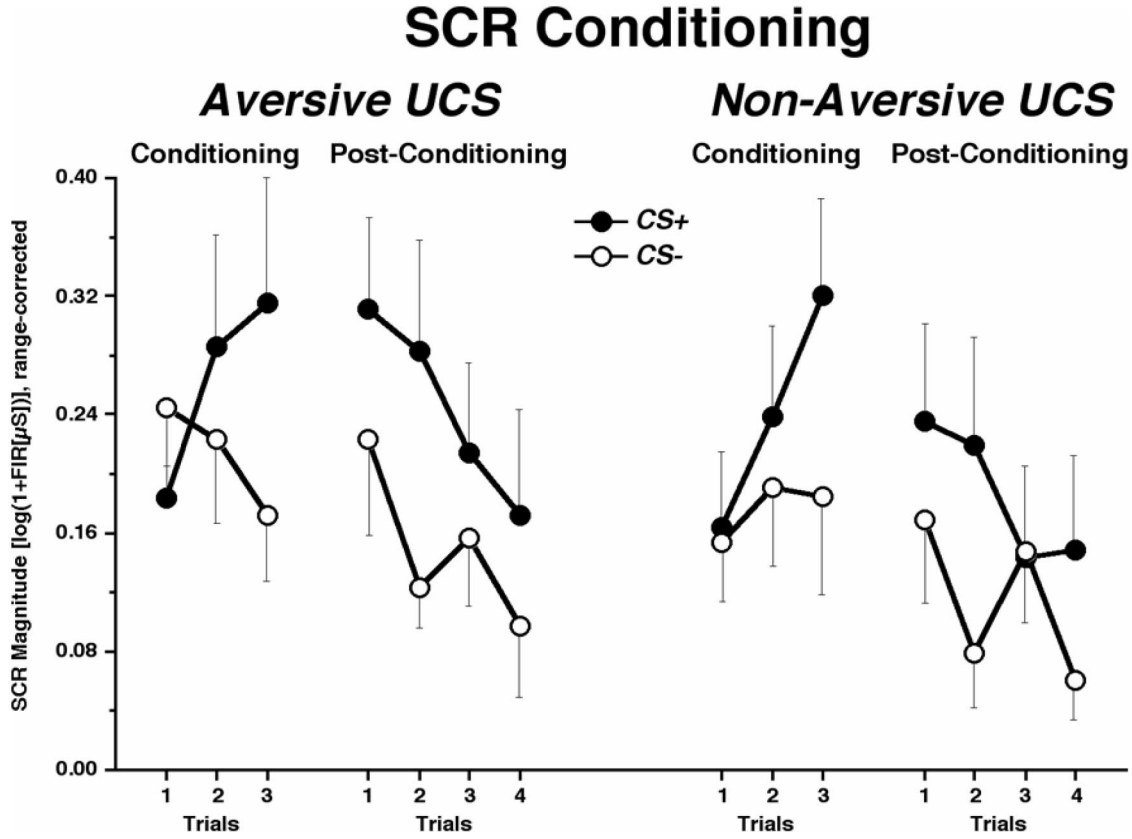

Figure 2. *M* (±*SE*) skin conductance response (SCR) magnitudes elicited by the CS+ and CS− in the aversive and the nonaversive conditioning groups, respectively. Evaluation of SCR magnitudes to the CSs before the conditioning was rendered impossible because both CS presentations were followed by the presentations of acoustic startle probes.

than those elicited during the ITI, $F(1, 22) = 20.26$, $p < .001$, and did not differ between CS+ and CS− ($F < 1$). As expected before the conditioning phase, this PPI effect at the early probe time was observed in both the aversive conditioning group, $F(1, 12) = 6.60$, $p = .025$, and the nonaversive conditioning group, $F(1, 10) = 21.43$, $p = .001$ (see Figure 3).

Conditioning. During the conditioning phase, startle blink responses elicited at the early probe time after the CSs remained inhibited as compared with those elicited during the ITI in the nonaversive conditioning group, $F(2, 22) = 20.91$, $p < .001$, $\varepsilon = .72$. By contrast, in the aversive conditioning group this startle inhibition effect was no longer observed during the conditioning phase ($F < 1$, $\varepsilon = .78$), resulting in a significant Conditioning × Group interaction in the main analysis, $F(2, 48) = 4.71$, $p = .022$, $\varepsilon = .78$. As illustrated in the upper left panel of Figure 3, startle blink responses elicited in the ITI during the aversive conditioning did not differ from those elicited shortly after the CS+, $F(1, 13) = 1.73$, $p = .211$, or the CS−, $F < 1$, respectively. By contrast, during the nonaversive conditioning (see lower left panel of Figure 3), startle responses elicited shortly after the CS+ or the CS− were both substantially inhibited compared with the startle blink magnitudes observed in the ITI, Fs(1, 11) = 53.08 and 16.05, ps $< .003$, for CS+ and CS−, respectively, thus reflecting the typical PPI effect. Moreover, startle blinks elicited after the CS+ did not differ from those elicited after the CS−, neither during aversive conditioning ($F < 1$) nor during the nonaversive conditioning, $F(1, 11) = 1.31$, $p = .310$.

Postconditioning. As observed during the conditioning phase, the presentation of startle probes shortly after the CS+ and CS− still did not result in any substantial startle inhibition compared with the ITI in the aversive conditioning group (both Fs < 1). Moreover, the startle responses to the early probe stimuli following the CS+ or the CS− in the nonaversive conditioning group remained inhibited as compared with the ITI probes, Fs(1, 11) = 8.19 and 16.68, ps $< .016$, for CS+ and CS−, respectively, although the Conditioning × Group interaction reached only trend-level significance in the main analysis, $F(2, 48) = 2.99$, $p = .072$, $\varepsilon = .81$. However, Figure 3 illustrates that the pattern of different startle modulation effects in both conditioning groups was consistently observed during the postconditioning phase. In the aversive conditioning group, the lack of startle inhibition at the early probe time was stable across the first and second extinction blocks (both Fs < 1, εs = .68 and .74, for the first and second trial block, respectively). In a similar vein, the substantial PPI of the startle response observed in the nonaversive conditioning group was also observed during both the first and the second trial blocks, Fs(2, 22) = 8.52 and 6.32, ps $< .018$, εs = .93 and .69. Again, no differences were observed between the CS+ and CS− in any group (all Fs < 1).

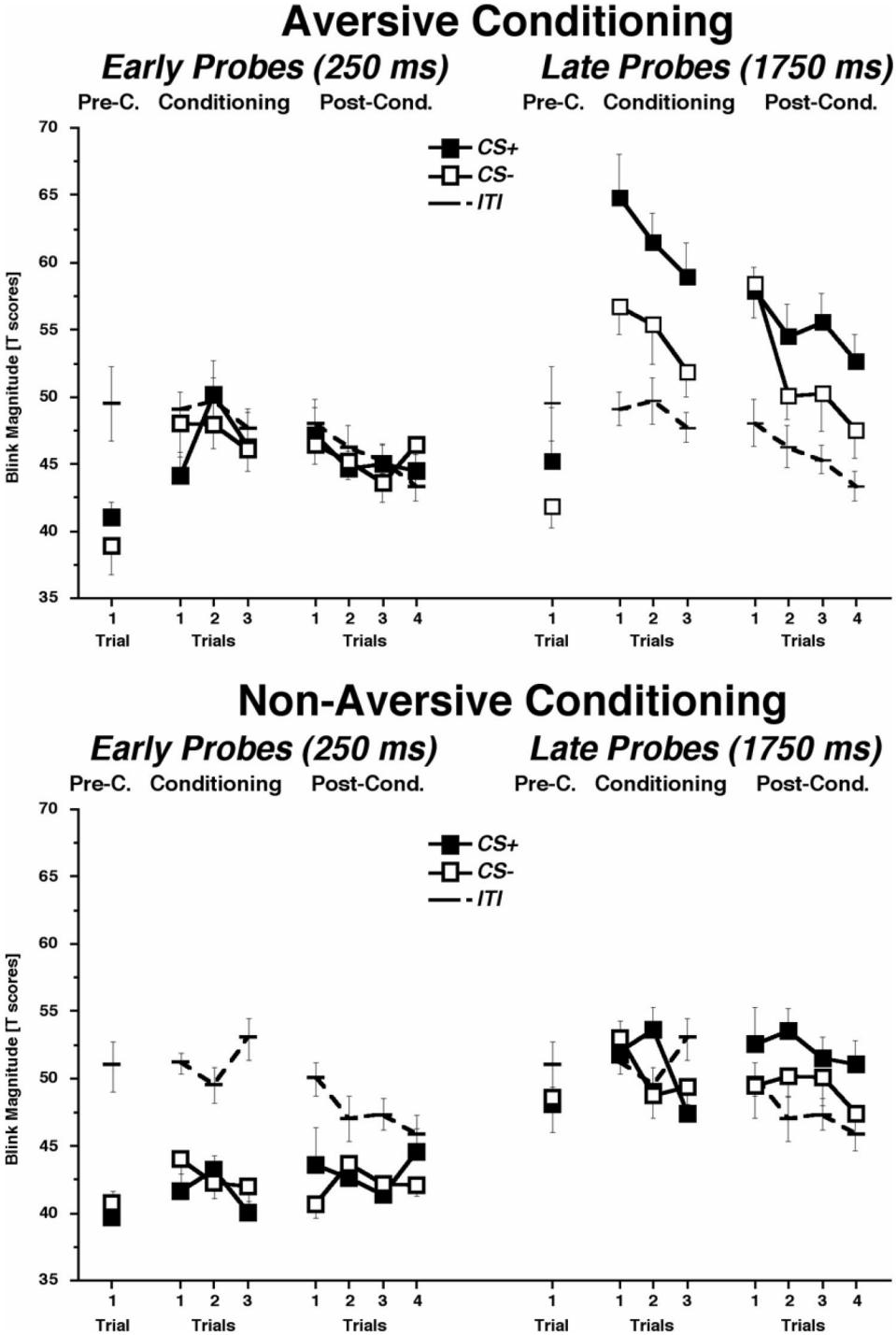

Figure 3. *M* (±*SE*) startle blink response magnitudes in the aversive conditioning group (upper panel) and the nonaversive conditioning group (lower panel). Startle responses were elicited during the intertrial interval (ITI) or following the presentation of the CS+ or the CS−. The CS-related startle blinks were elicited either at 250 ms (early probes; left panels) or at 1,750 ms (late probes; right panels) following CS onset. Pre-C. = preconditioning; Post-C. = postconditioning.

Startle Blink Responses Elicited at the Late Probe Time

Preconditioning. Startle blink responses elicited 1,750 ms after the presentation of the designated CSs were slightly smaller than those elicited during the ITI, $F(1, 22) = 5.73$, $p = .026$. However, this inhibitory effect was not substantiated when analyzed separately for both conditioning groups, $F(1, 12) = 3.76$, $p = .076$ (aversive conditioning), and $F(1, 10) = 2.66$, $p = .134$ (nonaversive conditioning), respectively. Moreover, startle blink

responses elicited at the late probe time did not differ between CS+ and CS− presentations before the conditioning ($F < 1$), neither in the aversive conditioning group nor in the nonaversive conditioning group (both Fs < 1; see Figure 3).

Conditioning. Unlike during the preconditioning phase, startle blinks to the late probes following the presentation of the CSs in the conditioning phase were generally potentiated compared with those elicited during the ITI, $F(2, 48) = 12.31$, $p < .001$, $\varepsilon = .72$. As expected, this cue-related startle potentiation was specifically observed in the aversive conditioning group, $F(2, 26) = 20.73$, $p < .001$, $\varepsilon = .69$, whereas startle responses to the late probes did not differ from those to the ITI startle probes in the nonaversive conditioning group ($F < 1$, $\varepsilon = .78$), resulting in a significant interaction of Conditioning × Group in the main analysis, $F(2, 48) = 12.29$, $p < .001$, $\varepsilon = .72$. As illustrated in the upper right panel of Figure 3, conditioning involving an aversive UCS resulted in potentiated startle responses at the late probe time following both CS+ and CS− presentations relative to the ITI, Fs(1, 13) = 26.93 and 22.38, ps $< .001$, for CS+ and CS−, respectively. As expected for aversive conditioning, startle responses were also potentiated when elicited after CS+ presentations compared with the CS−, $F(1, 13) = 11.23$, $p = .005$, reflecting a pronounced conditioned startle discrimination. By contrast, in the nonaversive conditioning group, startle responses did not differ between CS-related later probe times and the ITI or between CS+ and CS− presentations (all Fs < 1; see lower right panel of Figure 3).

Postconditioning. Similar to the conditioning phase, startle responses elicited at the late probe time were potentiated relative to ITI startle blinks during postconditioning in the aversive conditioning group, $F(2, 26) = 16.32$, $p = .001$, $\varepsilon = .60$, but not in the nonaversive conditioning group, $F(2, 22) = 2.48$, $p = .113$, $\varepsilon = .91$, although the Conditioning × Group interaction fell short of statistical significance in the main analysis, $F(2, 48) = 1.97$, $p = .154$, $\varepsilon = .94$ (see Figure 3). As expected for the aversive UCS conditions, startle blink responses remained to be potentiated during the postconditioning phase when elicited 1,750 ms after the CS+ relative to the CS−, $F(1, 13) = 22.79$, $p < .001$, or compared with the ITI, $F(1, 13) = 20.74$, $p = .001$. Moreover, the startle responses elicited after the CS− were still potentiated compared with the ITI in the aversive conditioning group, $F(1, 13) = 9.79$, $p = .008$. By contrast, startle response magnitudes at the late probe time in the nonaversive conditioning group did not differ between the CS− and the CS+, $F(1, 11) = 1.46$, $p = .253$, or the ITI, $F < 1$, respectively. However, startle blink responses elicited at the late probe time following the CS+ in the postconditioning phase in the nonaversive conditioning group were slightly larger than those elicited during the ITI, $F(1, 11) = 5.30$, $p = .042$. Nonetheless, the CS+-related startle potentiation compared with the ITI observed in the aversive conditioning group remained more pronounced than the startle modulation observed in the nonaversive conditioning group even during the last postconditioning block, $F(1, 24) = 3.66$, $p = .068$ (see Figure 3).

Time Course of Conditioned Fear Potentiation

The startle potentiation effects observed for the aversive conditioning group were followed up in a conclusive data analysis involving difference scores (CS minus ITI) to explore the time course of the startle potentiation. Mixed-model ANOVAs included the within-subjects factors stimulus (ΔCS+ vs. ΔCS−) and probe time (early vs. late probe time) and the between-subjects factor group (aversive and nonaversive conditioning). The CS-related startle response modulation in the aversive conditioning group turned out to be a pronounced and general potentiation at both startle probe times when contrasted with the nonaversive UCS conditions, as reflected in a significant main effect of group, $F(1, 24) = 17.75$, $p = .001$. Figure 4 illustrates this general startle potentiation in aversive UCS conditions compared with nonaversive UCS conditions by difference scores between both groups. Moreover, the conditioned startle potentiation during aversive conditioning was equally pronounced for both CS+ and CS− at the early probe time ($F < 1$), and a cue-specific (CS+-related) fear potentiation was not observed until the later probe time, $F(1, 13) = 13.92$, $p = .003$, accordingly resulting in a significant interaction of Stimulus × Probe Time, $F(1, 13) = 10.48$, $p = .006$.

Discussion

The present study explored the time course of a conditioned fear response using a differential trace conditioning paradigm with aversive and nonaversive UCS conditions, respectively. In replication of previous findings (e.g., Hamm & Vaitl, 1996), results revealed comparable electrodermal differential conditioning with both aversive and nonaversive UCS conditions, whereas a conditioned startle blink discrimination was only observed for aversive conditioning. Specifically, the aversive conditioning group exhibited a pronounced startle potentiation during both the conditioning and the postconditioning phases of the experiment when startle probes were presented at the later probe time (1,750 ms) after the CS+ relative to both the CS− and the ITI, respectively. By contrast, the nonaversive conditioning group did not exhibit a consistent startle potentiation at the later startle probe time. Startle blink responses in the nonaversive conditioning group did not differ between the CS+ or CS− trials, although a slightly increased CS+-related startle response compared with ITI levels was observed during the postconditioning phase. Previous findings of startle facilitation in nonaversive conditioning suggested that such effects might be driven by selective attention directed to the CS+ (Lipp, 2002). However, attention effects would be expected to be present during the conditioning phase as well, whereas the CS+-related startle blink facilitation in nonaversive UCS conditions in the present study was restricted to the postconditioning phase. Thus, the presently observed increased startle response might also suggest an increased generalized motor preparation (cf. Brunia, 1993) following the CS+ because the UCS (i.e., the imperative stimulus of the RT task) was omitted during the postconditioning phase. However, it is important to note that the CS+-related startle blink potentiation during the postconditioning phase in the nonaversive conditioning group was much smaller than the pronounced fear-potentiated startle observed in the aversive conditioning group, suggesting that the affective modulation of the startle response is much more potent in the present experimental design than any effect of motor preparation or selective attention.

The conditioned startle potentiation observed in the aversive conditioning group was rather rapidly expressed; that is, it was not only observed at the later but also at the early startle probe time. Although before the conditioning phase, startle blinks elicited 250 ms after CS onset were clearly inhibited compared with those elicited during the ITI, the CS-related blink magnitudes to the early

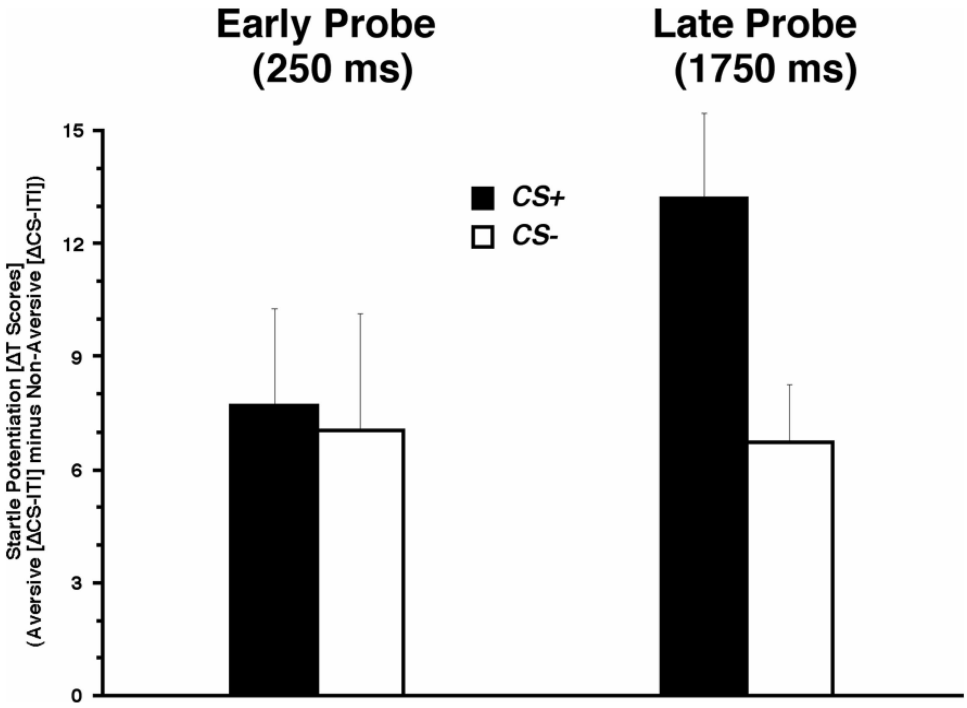

Figure 4. *M* (±*SE*) amount of fear-conditioned startle potentiation observed during conditioning at early (250 ms) and late (1,750 ms) probe times following the conditioned stimulus (CS) onset, respectively. For illustration purposes, the startle modulation effects (CS − ITI) in the nonaversive conditioning group were subtracted from those observed in the aversive conditioning group.

probes were no longer inhibited during the conditioning and the postconditioning phases in the aversive learning group but were instead of equal magnitude compared with the ITI. Specifically, the PPI effect, which is normally observed at short stimulus onset asynchronies between a lead stimulus and the startle-eliciting probe (and was accordingly observed in the nonaversive conditioning group throughout the whole experiment[4]), was no longer observed when the CS+ was paired with an aversive UCS. With regard to the fear-conditioned startle potentiation observed for the later probe time, this pattern of results suggests that the CS-induced potentiation of the startle response was already activated 250 ms after the CS onset and thus counteracted the PPI effect (cf. Globisch et al., 1999).

More important, the fear-conditioned startle potentiation observed in the present study differed between early and late probe times in terms of cue specificity. Although startle blink responses elicited 1,750 ms after the onset of the CS+ were potentiated compared with both the CS− and the ITI, startle magnitudes at 250 ms after the CS+ were not different from those elicited 250 ms after the CS− onset; that is, both CSs elicited comparable amounts of relative startle potentiation in the aversive conditioning group. This lack of conditioned startle discrimination at the early probe time suggests that not only the CS+ but also the CS− acquired some aversive motivational properties, which is substantiated by the finding that startle blink responses elicited at the later probe time not only after the CS+ but also after the CS− presentations were larger than those elicited during the ITI. This relative startle potentiation related to the CS− might reflect some kind of generalization of a cue-related conditioned fear response because both CSs depicted neutral male facial expressions. Although such a generalization is not typically observed in differential fear-conditioning studies (cf. Hamm & Vaitl, 1996; Weike et al., 2007), it might have been promoted in the present study by the short presentation times of the CSs (30 ms), possibly resulting in a less elaborated sensory encoding of the facial displays. However, despite the limited presentation times, both CSs were clearly visible and were clearly identified as two different stimuli even in those participants who were not able to correctly identify the CS–UCS contingencies in the postexperimental interview (and were therefore excluded from further data analyses). Thus, other factors need to be considered in the evaluation of the presently observed CS−-related startle potentiation effects.

Of most importance, the present findings suggest that the typically observed inhibitory conditioning, which is associated with the CS− because it is signaling the nonoccurrence of the aversive UCS, was less pronounced in the present study, thus resulting in the relative startle potentiation related to the CS− at both early and late probe times. From this point of view, the present findings do

[4] The PPI effects observed within the nonaversive conditioning group did not differ between the CS+ and the CS−, although the increase of PPI by selective attention drawn to the prepulse is a ubiquitous finding in the literature (cf. Filion, Dawson, & Schell, 1993). However, to reveal attentional modulation of PPI, it is necessary to constantly devote the attention to the prepulse while the startle response is elicited, which was not the case in the present experimental design because the CS presentation was terminated before the presentation of the startle probe stimulus.

nicely relate to previous studies specifically aimed at disentangling conditioned fear potentiation and inhibition, respectively (Jovanovic et al., 2005, 2006). In these studies, a conditional discrimination procedure (AX+/BX−) was used to assess excitatory conditioning (conditioned fear as indexed by fear-potentiated startle during AX trials) and the inhibitory conditioning as indexed by the conditioned inhibition of fear-potentiated startle (BX trials compared with AX trials). Results consistently revealed that both conditioned excitation and inhibition of fear were successfully acquired and that the conditioned inhibition could be transferred to the fear-eliciting stimulus in a summation test (AB). Moreover, the conditioned inhibition was more pronounced than the effect of external inhibition by a novel stimulus (AC). The inhibitory learning, though, was restricted to those participants who were aware of the stimulus contingencies, suggesting that associative excitatory and inhibitory learning involves different processes (cf. Davis, Falls, & Gewirtz, 2000). Interestingly, aside from the pronounced conditioned startle discrimination (AX > BX), startle responses during the conditioned inhibitory stimulus were nonetheless potentiated compared with startle responses elicited in the intertrial interval, which is obviously in line with the present findings. Jovanovic et al. (2006) argued that a paradigm exclusively involving cue-related UCS presentations promotes a general cue-related startle potentiation in comparison to paradigms consisting of UCS presentations in the absence of any specific cues. Thus, the presently observed weak inhibitory conditioning to CS−-related blink responses might be due to the cue-specific learning paradigm.

Although the aforementioned studies by Jovanovic et al. (2005, 2006) focused on the fear-potentiated startle response, another previous study aimed at exploring the conditioned inhibition of fear in participants with high or low trait anxiety measured both fear-potentiated startle and SCRs (Grillon & Ameli, 2001). Although the high-anxious group failed to exhibit a substantial conditioned inhibition of their startle responses, the conditioned startle discrimination acquired by the low-anxious participants was not observed until the last (fourth) trial block during the conditioning training, suggesting that the acquisition of conditioned inhibition needs some time to be expressed. Interestingly, the conditioned SCR discrimination, which is considered to relate to the more cognitive learning of CS–UCS contingencies (Hamm & Weike, 2005), was already observed during the second trial block; that is, the acquisition of conditioned inhibition of startle potentiation took more time to be expressed. Taken together, these findings suggest that the cognitive learning about the CS–UCS contingencies might be a prerequisite (cf. Jovanovic et al., 2006) but is not sufficient for the suppression of fear-conditioned startle potentiation. Accordingly, the present study revealed that the CS-related startle potentiation at the early probe time was equally pronounced for both CS+ and CS−, although all participants were clearly aware of the CS–UCS contingencies.

It is important to note that the present findings were obtained in a differential trace conditioning paradigm, which involves not only two distinct processes of excitatory and inhibitory learning but also the necessity of bridging a temporal gap between the CS and UCS presentations. Trace conditioning procedures are considered to involve more complex higher order processing as compared with delay conditioning procedures (cf. Clark & Squire, 1998, 1999). Accordingly and in replication of previous findings, the acquisition of conditioned fear responding was only obtained for those participants who were aware of the CS–UCS contingencies (e.g., Knight et al., 2006; Weike et al., 2007). However, the observation of conditioned startle potentiation for the CS− (i.e., the safety signal) during both early and late startle probe times despite the fact that the participants were aware of the differential reinforcement schedule underscores the ease of fear acquisition and the concurrent unease of gaining conscious control over this excitatory learning (cf. Öhman & Mineka, 2001). Thus, similar findings of fear-conditioned startle potentiation with regard to early fear activation may be expected for differential delay conditioning, which is considered to involve less complex processes of associative memory formation compared with trace paradigms. However, whether similar effects with regard to startle discrimination can be obtained in delay conditioning procedures needs to be explored in future research.

The present findings of a less efficient conditioned startle inhibition compared with the findings of previous fear-conditioning studies imply that inhibitory compared with excitatory conditioning needs some more time to be expressed, not only in terms of the number of training trials (cf. Grillon & Ameli, 2001), but also in terms of the time course within each trial. Specifically, the presently observed conditioned startle discrimination was not evident until the late probe time of 1,750 ms, which is still a rather early probe time compared with previous fear-conditioning studies using probe times of at least 4,000 ms following CS onset (e.g., Hamm & Vaitl, 1996; Weike et al., 2007). However, because the present study used an interstimulus interval (ISI) of 2,000 ms between the onset of the CS and the UCS, we cannot rule out that startle discrimination effects might even be obtained earlier when using shorter ISIs. However, it is important to note that a general (cue-unspecific) startle potentiation was already active at the early startle probe time (resulting in a lack of PPI), although the participants were able to correctly identify the safety signal in the postexperimental interview. Moreover, the notion of early fear activations independent of the actual ISI between CS and UCS onsets is in line with findings obtained in animal research, which revealed that fear-conditioned startle potentiation can be observed early in both short and long ISI conditioning (Burman & Gewirtz, 2004).

The temporal course of conditioned startle discrimination observed in the present study nicely relates to the time-dependent discrimination between feared (phobic) and nonfeared (but fear-relevant) stimuli observed in a brain imaging (positron emission tomography) study exploring the brain activation patterns in either spider- or snake-phobic participants in response to masked and unmasked presentations of spider and snake pictures, respectively (Carlsson et al., 2004). Although the masked presentation of the unpleasant stimuli resulted in amygdala activations to both phobia- and fear-relevant (but nonfeared) stimuli, amygdala activations were restricted to the phobia-relevant (feared) stimuli when presentation durations allowed for a more elaborate processing in the unmasked presentation condition. Furthermore, the unmasked presentation of phobic stimuli resulted in brain activations of further neural structures thought to be involved in emotional processing (e.g., the insula). Interestingly, the unmasked presentation of the fear-relevant (but nonfeared) stimuli elicited brain activations of prefrontal regions, that is, those neural structures thought to represent cortical (voluntary) top–down control of stimulus processing. From this point of view, the findings of the present study suggest that similar mechanisms might be active during differential fear conditioning.

The pronounced and generalized fear potentiation of the startle response observed in aversive learning indicates a rather rapid recruitment of fear-activating neural structures. A likely candidate structure for such an affectively driven, bottom–up processing of fear activation is the amygdaloid complex, as revealed by a number of animal and human studies (cf. Davis & Lang, 2003; Hamm & Weike, 2005; Weike et al., 2005). Moreover, the present observation that the conditioned startle discrimination, that is, the relative inhibition of fear-potentiated startle following the CS−, was observed only at the later probe time implicates a more time-consuming (more complex) information processing, presumably including some top–down (cortical) inhibitory control of the CS-elicited (subcortical) fear response activation. In consideration of the clinical implications, the present findings are quite in line with the existing literature. For example, patients suffering from clinically relevant anxiety disorders do not necessarily show an exaggerated fear potentiation of the startle response but instead exhibit a diminished inhibition of startle related to safety signals (e.g., Grillon & Morgan, 1999; for an overview, see Lissek et al., 2005), suggesting deficient cortical control over the subcortical fear circuits. Öhman and Mineka (2001) have suggested that these subcortical fear circuits constitute the fear module of the brain, which is characterized by its encapsulation from higher cognitive influences, that is, the relative independence from and resistance to conscious cognitive control once it is activated. Moreover, the fear module is preferentially activated automatically by fear-relevant stimuli and is considered an evolutionarily shaped behavioral system, specifically suited to rapidly detect and process threatening stimuli. Therefore, defensive activation appears to be the default option in the context of aversive stimulation before the organism learns to identify cues signaling the occurrence and nonoccurrence of the actual threat, that is, promoting cue-specific conditioned inhibition of the initial defensive activation.

References

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. R. (1995, August 25). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science, 269,* 1115–1118.

Bouton, M. E., Mineka, S., & Barlow, D. H. (2001). A modern learning theory perspective on the etiology of panic disorder. *Psychological Review, 108,* 4–32.

Brunia, C. H. M. (1993). Waiting in readiness: Gating in attention and motor preparation. *Psychophysiology, 30,* 327–339.

Burman, M. A., & Gewirtz, J. C. (2004). Timing of fear expression in trace and delay conditioning measured by fear-potentiated startle in rats. *Learning & Memory, 11,* 205–212.

Carlsson, K., Petersson, K. M., Lundqvist, D., Karlsson, A., Ingvar, M., & Öhman, A. (2004). Fear and the amygdala: Manipulation of awareness generates differential cerebral responses to phobic and fear-relevant (but nonfeared) stimuli. *Emotion, 4,* 340–353.

Clark, R. E., & Squire, L. R. (1998, April 3). Classical conditioning and brain systems: The role of awareness. *Science, 280,* 77–81.

Clark, R. E., & Squire, L. R. (1999). Human eyeblink classical conditioning: Effects of manipulating awareness of the stimulus contingencies. *Psychological Science, 10,* 14–18.

Davis, M. (2000). The role of the amygdala in conditioned and unconditioned fear and anxiety. In J. P. Aggleton (Ed.), *The amygdala* (2nd ed., pp. 213–287). Oxford, England: Oxford University Press.

Davis, M. (2002). Role of NMDA receptors and MAP kinase in the amygdala in extinction of fear: Clinical implications for exposure therapy. *European Journal of Neuroscience, 16,* 395–398.

Davis, M. (2006). Neural systems involved in fear and anxiety measured with fear-potentiated startle. *American Psychologist, 61,* 741–756.

Davis, M., Falls, W. A., & Gewirtz, J. (2000). Neural systems involved in fear inhibition: Extinction and conditioned inhibition. In M. S. Myslobodsky & I. Weiner (Eds.), *Contemporary issues in modeling psychopathology* (pp. 113–141). Boston: Kluwer Academic.

Davis, M., & Lang, P. J. (2003). Emotion. In M. Gallagher, R. J. Nelson, & I. B. Weiner (Eds.), *Handbook of psychology* (Vol. 3, pp. 405–439). New York: Wiley.

Davis, M., Schlesinger, L. S., & Sorenson, C. A. (1989). Temporal specificity of fear conditioning: Effects of different conditioned stimulus-unconditioned stimulus intervals on the fear-potentiated startle effect. *Journal of Experimental Psychology: Animal Behavior Processes, 15,* 295–310.

Dawson, M. E., & Schell, A. (1987). Human autonomic and skeletal classical conditioning: The role of conscious cognitive factors. In G. Davey (Ed.), *Cognitive processes and Pavlovian conditioning in humans* (pp. 27–55). New York: Wiley.

Esteves, F., Parra, C., Dimberg, U., & Öhman, A. (1994). Nonconscious associative learning: Pavlovian conditioning of skin conductance responses to masked fear-relevant facial stimuli. *Psychophysiology, 31,* 375–385.

Filion, D. L., Dawson, M. E., & Schell, A. M. (1993). Modification of the acoustic startle-reflex eyeblink: A tool for investigating early and late attentional processes. *Biological Psychology, 35,* 185–200.

Globisch, J., Hamm, A. O., Esteves, F., & Öhman, A. (1999). Fear appears fast: Temporal course of startle reflex potentiation in animal fearful subjects. *Psychophysiology, 36,* 66–75.

Globisch, J., Hamm, A. O., Schneider, R., & Vaitl, D. (1993). A computer program for scoring reflex eyeblink and electrodermal responses written in PASCAL. *Psychophysiology, 30* (Suppl. 1), S30.

Graham, F. (1975). The more or less startling effects of weak prestimulation. *Psychophysiology, 12,* 238–248.

Grillon, C., & Ameli, R. (2001). Conditioned inhibition of fear-potentiated startle and skin conductance in humans. *Psychophysiology, 38,* 807–815.

Grillon, C., Ameli, R., Foot, M., & Davis, M. (1993). Fear-potentiated startle: Relationship to the level of state/trait anxiety in healthy subjects. *Biological Psychiatry, 33,* 566–574.

Grillon, C., Ameli, R., Goddard, A., Woods, S. W., & Davis, M. (1994). Baseline and fear-potentiated startle in panic disorder patients. *Biological Psychiatry, 35,* 431–439.

Grillon, C., Ameli, R., Merikangas, K., Woods, S. W., & Davis, M. (1993). Measuring the time course of anticipatory anxiety using the fear-potentiated startle reflex. *Psychophysiology, 30,* 340–346.

Grillon, C., & Morgan, C. A. (1999). Fear-potentiated startle conditioning to explicit and contextual cues in Gulf War veterans with posttraumatic stress disorder. *Journal of Abnormal Psychology, 108,* 134–142.

Hamm, A. O., & Vaitl, D. (1996). Affective learning: Awareness and aversion. *Psychophysiology, 33,* 698–710.

Hamm, A. O., & Weike, A. I. (2005). The neuropsychology of fear learning and fear regulation. *International Journal of Psychophysiology, 57,* 5–14.

Hamm, A. O., Weike, A. I., Schupp, H. T., Treig, T., Dressel, A., & Kessler, C. (2003). Affective blindsight: Intact fear conditioning to a visual cue in a cortically blind patient. *Brain, 126,* 267–275.

Jovanovic, T., Keyes, M., Fiallos, A., Myers, K. M., Davis, M., & Duncan, E. J. (2005). Fear potentiation and fear inhibition in a human fear-potentiated startle paradigm. *Biological Psychiatry, 57,* 1559–1564.

Jovanovic, T., Norrholm, S. D., Keyes, M., Fiallos, A., Jovanovic, S., Myers, K. M., et al. (2006). Contingency awareness and fear inhibition in a human fear-potentiated startle paradigm. *Behavioral Neuroscience, 120,* 995–1004.

Knight, D. C., Nguyen, H. T., & Bandettini, P. A. (2003). Expression of conditional fear with and without awareness. *Proceedings of the Na-*

tional Academy of Sciences of the United States of America, 100, 15280–15283.

Knight, D. C., Nguyen, H. T., & Bandettini, P. A. (2006). The role of awareness in delay and trace fear conditioning in humans. *Cognitive, Affective, & Behavioral Neuroscience, 6,* 157–162.

Koch, M. (1999). The neurobiology of startle. *Progress in Neurobiology, 59,* 107–128.

Lang, P. J. (1980). Behavioral treatment and the bio-behavioral assessment: Computer applications. In J. B. Sidowski, J. H. Johnson, & T. A. Williams (Eds.), *Technology in mental health care delivery systems* (pp. 119–137). Norwood, NJ: Ablex.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2005). *International affective picture system (IAPS): Digitized photographs, instruction manual and affective ratings* (Tech. Rep. No. A-6). Gainesville, FL: University of Florida.

Larson, C. L., Schaefer, H. S., Siegle, G. J., Jackson, C. A. B., Anderle, M. J., & Davidson, R. J. (2006). Fear is fast in phobic individuals: Amygdala activation in response to fear-relevant stimuli. *Biological Psychiatry, 60,* 410–417.

Lipp, O. V. (2002). Anticipation of a non-aversive reaction time task facilitates the blink startle reflex. *Biological Psychology, 59,* 147–162.

Lissek, S., Powers, A. S., McClure, E. B., Phelps, E. A., Woldehawariat, G., Grillon, C., et al. (2005). Classical fear conditioning in the anxiety disorders: A meta-analysis. *Behaviour Research and Therapy, 43,* 1391–1424.

Lykken, D. T., & Venables, P. H. (1971). Direct measurement of skin conductance: A proposal for standardization. *Psychophysiology, 8,* 656–672.

Öhman, A. (1983). The orienting response during Pavlovian conditioning. In D. Siddle (Ed.), *Orienting and habituation: Perspectives in human research* (pp. 315–369). Chichester, England: Wiley.

Öhman, A., Hamm, A., & Hugdahl, K. (2000). Cognition and the autonomic nervous system. In J. T. Cacioppo, L. G. Tassinary, & G. G. Berntson (Eds.), *Handbook of psychophysiology* (2nd ed., pp. 533–575). Cambridge, England: Cambridge University Press.

Öhman, A., & Mineka, S. (2001). Fears, phobias, and preparedness: Toward an evolved module of fear and fear learning. *Psychological Review, 108,* 483–522.

Öhman, A., & Soares, J. J. F. (1998). Emotional conditioning to masked stimuli: Expectancies for aversive outcomes following nonrecognized fear-relevant stimuli. *Journal of Experimental Psychology: General, 127,* 69–82.

Prokasy, W. F., & Kumpfer, K. L. (1973). Classical conditioning. In W. F. Prokasy & D. C. Raskin (Eds.), *Electrodermal activity in psychophysiological research* (pp. 157–202). New York: Academic Press.

Siegel, A. (1967). Stimulus generalization of a classically conditioned response along a temporal dimension. *Journal of Comparative and Physiological Psychology, 64,* 461–466.

Venables, P. H., & Christie, M. J. (1980). Electrodermal activity. In I. Martin & P. H. Venables (Eds.), *Techniques in psychophysiology* (pp. 3–67). Chichester, England: Wiley.

Weike, A. I., Hamm, A. O., Schupp, H. T., Runge, U., Schroeder, H. W. S., & Kessler, C. (2005). Fear conditioning following unilateral temporal lobectomy in humans: Dissociation of conditioned startle potentiation and autonomic learning. *Journal of Neuroscience, 25,* 11117–11124.

Weike, A. I., Schupp, H. T., & Hamm, A. O. (2007). Fear acquisition requires awareness in trace but not delay conditioning. *Psychophysiology, 44,* 170–180.