Fear memory uncovered: Prediction error as the key to memory plasticity
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Citation for published version (APA):
Sevenster, D. (2014). Fear memory uncovered: Prediction error as the key to memory plasticity

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Chapter 2

Fear conditioning of SCR but not the startle reflex requires conscious discrimination of threat and safety

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ABSTRACT – There is conflicting evidence as to whether awareness is required for conditioning of the skin conductance response (SCR). Recently, Schultz and Helmstetter (2010) reported SCR conditioning in contingency unaware participants by using difficult to discriminate stimuli. These findings are in stark contrast with other observations in human fear conditioning research, showing that SCR predominantly reflects the cognitive component of fear learning. Therefore, we repeated the study by Schultz and Helmstetter and additionally measured conditioning of the startle response, which seems to be less sensitive to declarative knowledge. Conditioning of SCR took place in aware (n = 16) but not in unaware participants (n = 18), while startle conditioning occurred irrespective of awareness. Thus, SCR but not startle conditioning depends on conscious discriminative fear learning. Although valuable in conditioning research, SCR is not a specific index of fear.
BACKGROUND

There is a long-standing debate whether fear learning can occur without awareness of the relationship between the conditioned stimulus (CS) and the unconditioned stimulus (US). Some studies showed that conditioned skin conductance response (SCR) can take place in absence of contingency awareness (Bechara et al., 1995; Esteves, Parra, Dimberg, & Öhman, 1994; Knight, Nguyen, & Bandettini, 2003, 2006). However, other studies showed that SCR conditioning can only be observed in parallel with the conscious expectancy of the CS to be followed by the US (Dawson & Biferno, 1973; Dawson & Furedy, 1976; Hamm & Vaitl, 1996; Hamm & Weike, 2005; Lovibond & Shanks, 2002; Purkis & Lipp, 2001; Weike, Schupp, & Hamm, 2007). Importantly, under conditions that prevent both awareness and conditioned SCR responding, conditioned startle potentiation could still be observed (Hamm & Vaitl, 1996; Weike et al., 2005, 2007).

The fear potentiated startle response is an automatic defensive reflex and therefore generally accepted to reflect the emotional component of fear learning (LeDoux, 2003; Walker & Davis, 2002). In contrast, the nature of SCR responding is under debate. In addition to the studies that show that SCR conditioning requires contingency learning (Dawson & Biferno, 1973; Dawson & Furedy, 1976; Hamm & Vaitl, 1996; Hamm & Weike, 2005; Lovibond & Shanks, 2002; Purkis & Lipp, 2001; Weike et al., 2005, 2007), both pharmacological and cognitive manipulations revealed that SCR is strongly related to expectancy learning. That is, propranolol administered before or after fear memory reactivation left both expectancy and SCR responding intact but reduced the startle response (Sevenster, Beckers, & Kindt, 2012a; Soeter & Kindt, 2010, 2011a). Moreover, the simple instruction that the CS would no longer be followed by the US eliminated both differential US-expectancy ratings and SCR (Hugdahl & Öhman, 1977; Hugdahl, 1978; Lipp & Edwards, 2002; Sevenster, Beckers, & Kindt, 2012b) but not the startle fear response (Sevenster et al., 2012b). Hence, SCR responding appears to mirror expectancy beliefs (see also Lovibond, 2004), whereas the startle response can act independent from cognitive knowledge. These findings suggest that 1) the emotional and cognitive expression of fear conditioning are subserved by different underlying systems, supporting a dual processing system of fear learning (Baeyens et al., 1995; Hamm & Weike, 2005) and 2) like US-expectancies, SCR reflects the cognitive level of fear conditioning whereas fear potentiated startle responding reflects the emotional component of fear conditioning. In sum, there is
conflicting evidence on the relation between contingency learning and SCR. One line of evidence shows that SCR does not require expectancy learning (Bechara et al., 1995; Esteves et al., 1994; Knight et al., 2003, 2006), while other evidence shows that SCR conditioning requires conscious knowledge of the contingencies (Dawson & Biferno, 1973; Dawson & Furedy, 1976; Hamm & Vaitl, 1996; Hamm & Weike, 2005; Lovibond & Shanks, 2002; Lovibond, 2004; Purkis & Lipp, 2001; Weike et al., 2005, 2007). Most evidence that SCR dissociates from expectancy learning comes from studies using a distractor task or subliminal presentation of the stimuli. By using these tasks it is hard to ascertain whether participants are completely unaware of the contingency.

Recent findings are in line with previous studies showing that SCR does not depend on expectancy learning but counteract the observed correspondence between contingency learning and SCR responding. Schultz and Helmstetter (2010) manipulated contingency awareness by constructing pairs of stimuli (CS+ and CS-) that were either easy or difficult to discriminate (see Fig. 1). US-expectancy and SCR were registered concurrently during the conditioning session. Differential US-expectancy ratings were observed in the easy but not in the difficult condition, whereas differential SCR was observed in both the easy and difficult discrimination condition. These findings indeed suggest that SCR conditioning does not depend on contingency awareness.

The dissociation between expectancy learning and SCR conditioning observed by Schultz and Helmstetter (2010) is remarkable in the light of the strong convergence between expectancy learning and SCR conditioning that is observed in other labs (Hamm & Vaitl, 1996; Lovibond, 2003; Sevenster et al., 2012b; Sevenster, Beckers, & Kindt, 2013; Soeter & Kindt, 2010; Weike et al., 2005, 2007). One explanation may be that the manipulations employed in these previous studies are simply not appropriate for dissociating the two conditioned responses. Alternatively, studies that do not find SCR conditioning among unaware participants often use post-conditioning questionnaires (Hamm & Vaitl, 1996; Weike et al., 2005, 2007). Retrospective ratings of awareness are susceptible to forgetting or interference and may be insensitive to subtle discrimination of the CSs (Knight, Nguyen, & Bandettini, 2003; Smith, Clark, Manns, & Squire, 2005). The concurrent measurements of US-expectancy ratings and psychophysiological conditioned responding — a strong feature of the study by Schultz and Helmstetter (2010) — clearly overcomes this limitation. However, a shortcoming of the study by Schultz and Helmstetter (2010) is that more than half of the participants who are
meant to be unaware of the learned association (difficult discrimination condition) actually demonstrated a certain degree of awareness of the CS-US contingencies. That is, a more tolerant criterion for contingency awareness revealed that more than half of the remaining, supposedly unaware participants (6 out of 10) approach contingency awareness in the difficult discrimination condition. The follow-up analyses showed that the six aware and the four unaware participants did not differ in SCR conditioning; it was therefore concluded that the results are not confounded by the participants’ contingency awareness. However, the small sample sizes (n = 4 vs. n = 6) make the observation of a significant difference between those two groups unlikely.

To further investigate the role of awareness in conditioned responding, we repeated the study by Schultz and Helmstetter (2010). We increased sample sizes to allow investigation of differential SCR in participants who unintentionally acquired a certain degree of awareness and participants who were strictly unaware within the difficult condition. We expected differential SCR both in the easy condition and in participants who showed some contingency awareness in the difficult condition. Given the previously observed dissociation between the cognitive (US-expectancy learning) and the emotional component (fear potentiated startle) of associative fear learning, we additionally tested whether conditioning of the startle response would occur irrespective of contingency awareness. Finally, following a recent replication study where trial sequence in fact explained unaware SCR conditioning (Singh, Dawson, Schell, Courtney, & Payne, 2013), we additionally explored the effect of predictable stimulus presentation on SCR and startle conditioning.

**MATERIALS AND METHODS**

**Participants**
Thirty-seven (20 male; 17 female) healthy undergraduate students participated in the study, ranging in age between 18 and 27 years (M = 21.73, SD = 2.31). Participants received either partial course credit or a small amount of money for their participation. All participants gave informed consent and were notified that they could withdraw from participation at any time. Participants were medically screened to assure they were free from a physical (heart disease or epilepsy) or psychiatric disorder. The Ethics Committee of the University of Amsterdam approved the study. Participants (n = 37) were randomly assigned to either the easy (n = 18) or the difficult condition (n = 19).
Two unaware participants in the easy condition and one aware participant in the difficult condition were excluded from analysis, resulting in $n = 16$ in the easy and $n = 18$ participants in the difficult condition.

**Apparatus**

**Stimuli.** The conditioned stimuli (CSs) were adapted from Schultz and Helmstetter (2010) and consisted of two pairs of complex sine wave gratings, composed of high and low frequency components (Fig. 1). The high frequency component was equal for all stimuli. The low frequency components were adjusted so as to create conditions in which the two stimuli would be easy (easy condition) or difficult (difficult condition) to discriminate. Electrical stimulation was delivered through a pair of Ag electrodes of 20 by 25 mm with a fixed inter-electrode mid-distance of 45 mm. Shock deliverance was controlled by a Digitimer DS7A constant current stimulator (Hertfordshire, UK). Between the electrodes and the skin a conductive gel (Signa, Parker) was applied.

**Fear potentiated startle (FPS).** Startle response was measured through electromyography (EMG) of the right orbicularis oculi muscle. Two 6 mm sintered Ag/AgCl electrodes filled with a conductive gel (Signa, Parker) were positioned approximately 1 cm under the pupil and 1 cm below the lateral canthus, respectively; a ground electrode was placed on the forehead, 1 cm below hairline (Blumenthal et al., 2005). The startle probe was a 40 ms duration noise burst (104 dB) with a rise/fall time shorter than 1 ms, which was presented binaurally through headphones (Sennheiser, model HD 25-1 II). The EMG signal was amplified in two stages. The input stage had an input resistance of 10 MΩ, a frequency response of DC-1500 Hz and an amplification factor of 200. A 50-Hz
notch filter was used to reduce interference of the mains noise. The second stage amplified the signal with a variable amplification factor of 0–100 x. The raw EMG signal was sampled at 1000 Hz and band-pass filtered (28–500 Hz, Butterworth, 4th order (Blumenthal et al., 2005)) to obtain the cleanest possible data without affecting response amplitude. After rectifying and contour following (time constant = 10 ms) the peak amplitude was found by analysing the first derivative of the resulting signal in a 30–150 ms interval following probe onset.

**Skin conductance response.** Electrodermal activity was measured using an input device with a sine-shaped excitation voltage (1 V peak-peak) of 50 Hz, which was derived from the mains frequency. Two Ag/AgCl electrodes of 20 by 16 mm were attached with adhesive tape to the medial phalanges of the first and third fingers of the non-preferred hand. The SCL signal from the input device was converted to 0.2V/uS by a current to voltage converter. Startle response and electrodermal activity were recorded with the software program VSSRP98 at 1000 Hz. Phasic skin conductance response to the CS was calculated by subtracting the baseline (mean skin conductance level during the 2 s period before stimulus onset) from the maximum score. Maximum score was defined as the largest 0.5 s interval mean SCL, during the 0 to 7 s window after CS onset (entire-interval response, EIR) (Pineles, Orr, & Orr, 2009) before the onset of the startle probe. This is a well-established approach of examining electrodermal reactivity and has been used extensively in human psychophysiological research (Milad, Orr, Pitman, & Rauch, 2005; Orr et al., 2000; Pineles et al., 2009; Raes, Houwer, Verschuere, & Raedt, 2011).

**Online US-expectancy ratings.** US-expectancy was measured continuously (5 samples/s), on an 11-point scale ranging from ‘certainly no electric stimulus’ (-5) through ‘uncertain’ (0) to ‘certainly an electric stimulus’ (5). The scale was placed at the bottom of the screen. Participants rated US-expectancy levels by shifting the cursor on the scale with use of the mouse. Subjects were not informed about CS-US contingencies and were instructed to update their US-expectancy throughout the experiment. Continuous US-expectancy ratings during the last 4 s of CS presentation were averaged for each CS presentation. Ratings were converted to a 0 to 100 scale.

**Subjective assessments.** Evaluation of the US was assessed on an 11-point scale ranging from ‘unpleasant’ (-5) to ‘pleasant’ (5). General level of anxiety was measured with the Trait Anxiety Inventory (STAI-T; Spielberger, Gorsuch, & Lushene, 1970) to control for general level of anxiety.
Procedure
After giving informed consent participants were seated in front of a computer screen in a sound-attenuated room. The EMG, SCR and shock electrodes were attached and US-intensity level was determined by gradually increasing shock intensity (starting at 1 mA) until subjects indicated the shock to be ‘uncomfortable though not painful’. The experiment started with ten startle habituation trials to stabilize baseline startle reactivity. To assess baseline startle responding during the experimental phase, startle probes alone (Noise Alone; NA) were presented in addition to the CS presentations. Throughout the entire conditioning phase participants continuously rated their US-expectancy.

Fear conditioning. The testing procedure was adapted from Schultz and Helmstetter (2010). In the current study NA trials were presented in addition to the CS presentations. Stimuli (CS+, CS-, NA) were presented randomly in a block of three trials. The CSs consisted of 2 different images depicting complex sine wave gratings. Both CSs were presented 8 times, with a duration of 8 s. The experiment consisted of 8 consecutive blocks. One of the images (CS+) was paired with a mild shock to the wrist (US) on all the eight trials, whereas the other picture was never paired with a shock (CS-). A startle probe (40 ms; 104 dB) was delivered 7 s after CS onset, followed by the US after another 500 ms. The US consisted of an electrical stimulus (2 ms). Note that delivery of neither the startle probe nor the US interfered with measurement of SCR as maximum SCR score was determined during 7 s following stimulus onset before the startle probe and the US onset. Intertrial intervals (ITI) varied from 15 s to 25 s with an average of 20 s. The stimuli that were easy to discriminate served as CSs in the easy condition and the stimuli that were difficult to discriminate served as CSs in the difficult condition (Fig. 1). Participants did not receive information about the CS-US relationship. They were instructed to continuously place the cursor of the mouse on the position on the scale corresponding to their US-expectancy, ranging from ‘certainly no electric stimulus’ (-5) through ‘uncertain’ (0) to ‘certainly an electric stimulus’ (5). After conclusion of the experimental phase, participants filled in the trait anxiety (STAI-T) questionnaire and rated US-pleasantness.

Awareness
Continuous US-expectancy ratings during the last 4 s of CS duration were averaged for each CS presentation. Awareness was defined according to the two criteria set by Schultz and Helmstetter (2010). First, participants were classified as
Data Analysis

US-intensity, US-evaluation and STAI-T scores were subjected to ANOVAs with condition (easy vs. difficult) as between-subjects factor. Startle and skin conductance response outliers were defined by means of within-participants Z scores (Z > 3) and replaced by linear trend at point. Skin conductance responses were mean corrected, to equalize the opportunity for each subject to contribute to the group mean (Lovibond, Siddle, & Bond, 1988). The mean value used for correction was based on the eight conditioning trials. US-expectancy, skin conductance and startle potentiation data were averaged over all eight conditioning trials, resulting in a single mean response to the CS+ and a single mean response to the CS-. US-expectancy ratings, startle responses and electrodermal activity were then subjected to a mixed analysis of variance for repeated measures (ANOVA) with condition (easy vs. difficult) as between-subjects factor and stimulus (CS+ vs. CS-) and trial sequence (alternating vs. non-alternating) as within-subjects factor. Planned comparisons (early vs. late conditioning) were performed for each condition separately. The alpha level was set at .05 for statistical analyses.

RESULTS

Easy vs. difficult

First, we analysed the data according to Schultz and Helmstetter (2010). Awareness was defined as five consecutive CS presentations during which US-expectancy ratings to the CS+ were higher than 75 and US-expectancy ratings to the CS- were lower than 25. Based on this criterion two unaware participants in the easy condition and one aware participant in the difficult condition were excluded from further analysis, resulting in n = 16 in the easy and n = 18 participants in the difficult condition. The individually set shock intensity ranged from 6 to 40 mA (M =
There was no difference in US-intensity, US-evaluation and reported trait anxiety (Fs < 1) between the aware and unaware participants.

**US-expectancy ratings.** Analysis revealed a difference between conditions in differential US-expectancy (stimulus x condition; F(1, 32) = 173.43, p < .001, η²p = .84). US-expectancy ratings to the CS+ were higher compared to the CS- in the easy condition (main effect stimulus; F(1, 15) = 311.99, p < .001, η²p = .95), while this difference was absent in the difficult condition (main effect stimulus; F(1, 17) < 1) (Fig. 2A).

**Skin conductance response.** We observed a near-significant difference in SCR conditioning between the easy and difficult condition (stimulus x condition; F(1, 32) = 2.99, p < .094, η²p = .09) (Fig. 2B). In line with the US-expectancies, we found higher SCR to the CS+ compared to the CS- in the easy condition (main effect stimulus; F(1, 15) = 5.55, p < .032, η²p = .27), while we did not observe such differential responding in the difficult condition (main effect stimulus; F(1, 17) < 1). Thus in
contrast to Schultz and Helmstetter (2010), we found that contingency awareness is a prerequisite for SCR conditioning.

**Startle fear response.** The easy and difficult condition differed neither on startle habituation (trials 1 to 10; stimulus x condition; $F_{(4.78, 152.99)} < 1$) nor on startle responding during the ITI (trials 1 to 8; stimulus x condition; $F_{(5.03, 161)} < 1.12$). Even though the easy and difficult condition did not differ in conditioning of differential startle fear response (stimulus x condition; $F_{(1, 32)} < 1$), we observed general startle conditioning evidenced by stronger startle potentiation to the CS+ compared to the CS− (main effect stimulus; $F_{(1, 32)} = 5.84$, $p < .022$, $\eta_p^2 = .15$) (Fig. 2C). Follow-up analyses showed that conditioning of the startle was present in the difficult condition (main effect stimulus; $F_{(1, 17)} = 4.78$, $p < .043$, $\eta_p^2 = .22$) but not in the easy condition (main effect stimulus; $F_{(1, 15)} < 1.66$). Note that while startle conditioning was absent on the first trial (main effect stimulus; $F_{(1, 15)} < 1$), there was a significant effect of differential responding during the second to the fifth trial of early conditioning in the easy condition (trials 2 to 5; main effect stimulus; $F_{(1, 15)} = 4.61$, $p < .05$, $\eta_p^2 = .24$). This effect was collapsed at the end of conditioning (trials 6-8: main effect stimulus; $F_{(1, 15)} < 1$).

**Easy vs. difficult: Trial sequence**
A recent study showed that stimulus presentation sequence could account for unaware differential SCR (Singh et al., 2013). Thus, what initially appeared to be unaware differential SCR conditioning could also be explained by expectancy “learning”, arising from a predictable trial sequence. In the current study we presented the stimuli (CS+, CS−, NA) randomly within a block, for 8 consecutive blocks. Similar to the Schultz and Helmstetter study, this presentation scheme increases the probability of shock deliverance when a preceding trial is not reinforced. In contrast, when a preceding trial is reinforced the probability of shock deliverance decreases. Therefore, we re-analysed the data with trial sequence (alternating vs. non-alternating) as an additional within subjects factor. Following Singh et al. (2013) we separately averaged conditioned responses (US-expectancy, SCR, startle response) for CS+ and CS− trials that were preceded by an opposite CS type (e.g., CS− followed by CS+; alternating trial) or a similar CS type (e.g., CS+ followed by CS+; non-alternating trial). The first two trials were not included in the analyses, since trial sequence based expectancies could not yet have been inferred for these trials (Singh et al., 2013).
US-expectancy ratings. Differential ratings were greater in the easy condition compared to the difficult condition on both alternating (stimulus x condition; $F_{(1, 32)} = 83.82, p < .001, \eta^2_p = .72$) and non-alternating trials (stimulus x condition; $F_{(1, 28)} = 54.36, p < .001, \eta^2_p = .66$) (Fig. 3A). Both an alternating (main effect stimulus; $F_{(1, 15)} = 350.20, p < .001, \eta^2_p = .96$) and non-alternating trials (main effect stimulus; $F_{(1, 12)} = 78.71, p < .001, \eta^2_p = .87$) responding to the CS+ was higher compared to the CS− in the easy condition. Participants in the difficult condition capitalized on trial sequence, evidenced by higher ratings to the CS+ compared to the CS− on alternating trials (main effect stimulus; $F_{(1, 17)} = 6.56, p < .020, \eta^2_p = .28$), while on non-alternating trials this pattern was reversed with higher responding to the CS− compared to the CS+ (main effect stimulus; $F_{(1, 16)} = 6.06, p < .026, \eta^2_p = .28$). Thus, in line with previous findings (Singh et al., 2013), we found that trial sequence can result in US-expectancy learning when stimuli are in fact difficult to discriminate.

Skin conductance response. Differential responding was greater in the easy condition compared to the difficult condition on the alternating

![Graph A](image)

**Figure 3.** US-expectancy ratings (A), skin conductance responses (B) and startle responses (C) to the CS+ and CS− on alternating and non-alternating trials for the Easy ($n = 16$) and Difficult ($n = 18$) conditions. Error bars represent SEM.
trials (stimulus x condition; $F_{(1, 32)} = 7.38, p < .011, \eta_p^2 = .19$) (Fig. 3B). Indeed, responding to the CS+ was higher compared to the CS- in the easy condition on alternating trials (main effect stimulus; $F_{(1, 15)} = 11.00, p < .001, \eta_p^2 = .42$). Contrary to the previous findings (Singh et al., 2013), this effect was not observed in the difficult condition (main effect stimulus; $F_{(1, 17)} < 1$). The easy and difficult condition did not differ on SCR on non-alternating trials (stimulus x condition; $F_{(1, 28)} < 1$). Follow-up analyses confirmed that differential SCR was absent in both conditions on non-alternating trials (main effect stimulus; $F_s < 1.05$).

Startle fear response. The easy and difficult condition did not differ on startle response on both alternating and non-alternating trials (stimulus x condition; $F_s < 1$). In sum, we replicated the US-expectancy results of Singh et al. (2013). Predictable trial sequence did facilitate expectancy learning in spite of difficult to discriminate stimuli. However, trial sequence only facilitated differential SCR in the easy but not in the difficult condition. Differential startle responding was not affected by trial sequence.

DISCUSSION

The aim of the present study was to investigate whether SCR and startle conditioning correspond with contingency awareness. We observed differential SCR conditioning in the easy but not in the difficult condition. Moreover, while SCR mirrored the US-expectancy ratings, differential conditioning of the fear potentiated startle was observed in spite of difficult to discriminate conditioned stimuli. While Schultz and Helmstetter (2010) found more electrodermal responding to the CS+ compared to the CS-, irrespective of the difficulty to perceptually discriminate these stimuli, we did not replicate these findings. More than half of the participants in the difficult condition showed contingency awareness according to the tolerant criterion in their study, but only four out of the 19 participants in the difficult condition met the same awareness criterion in the current study. Therefore, SCR conditioning in the study by Schultz and Helmstetter (2010) can arguably be attributed to the relatively large sample of participants who unintentionally acquired some level of contingency awareness in the difficult condition.

An alternative explanation for the findings by Schultz and Helmstetter (2010) is that participants classified as aware in the difficult condition could have capitalized on the trial sequence to predict the occurrence of the US, as was convincingly argued in a recent replication study (Singh et al., 2013). When presentation of the CS+ and CS- followed an alternating sequence, participants
not only showed higher US-expectancy to the CS+ compared to the CS-, but also differential SCR. Thus, although incapable of discriminating the two CSs, predictable trial sequence may have resulted in unintended contingency awareness and subsequent differential SCR conditioning. We partly replicated this effect, by showing that expectancy “learning” occurred on alternating but not on non-alternating trials when stimuli were difficult to discriminate. However, we did not observe that this artificial expectancy learning resulted in differential SCR in the difficult condition. Apparently, in the current study participants in the difficult condition did not benefit from a predictable trial sequence. Our design differed from Singh et al. (2013) in that their conditioning procedure was extended with two more blocks of CS presentations. These additional trials might have been essential for the participants to infer rules about the trial sequence. Surprisingly, we found that differential SCR was affected by trial sequence in aware participants. This shows that awareness may be required but not necessarily sufficient for differential SCR to occur, since SCR was absent for the non-alternating trials in participants who were contingency aware. This stresses the urge to control for trial sequence, especially when using SCR as a measure of conditioned responding. Notably, differential startle responding occurred irrespective of condition and trial sequence.

While the easy and difficult conditions did not differ in startle responding we unexpectedly did not observe startle conditioning in the easy condition. Thus, startle responding to the CS+ was not higher than startle responding to the CS- when analysing the 8 conditioning trials together. However, participants in the easy condition showed startle conditioning at the beginning of the fear acquisition phase but this effect was no longer present by the end of conditioning. Visual inspection of the graphs suggests a similar pattern of conditioning over trials for the startle response in the difficult condition and SCR in the easy condition. The observation of a transient physiological conditioning effect contrasts with our previous studies, in which we did observe SCR and startle conditioning during late acquisition (Kindt et al., 2009; Sevenster et al., 2012a; Soeter & Kindt, 2010). In these studies we used fear-relevant stimuli, while in the current study CSs consisted of neutral pictures. The absence of differential psychophysiological responding at the end of conditioning in the current study might be explained by faster habituation to neutral CSs than to fear-relevant stimuli. Note that in the current paradigm the use of neutral pictures is preferred over fear-relevant stimuli as these stimuli have
an innate prepotency for eliciting fear responses (Lovibond, Hanna, Siddle, & Bond, 1994), and might therefore facilitate unaware conditioning.

In line with previous evidence, the current study shows that SCR closely mirrors expectancy learning and thus dissociates from the startle response. Whereas the startle reflex is potentiated in response to negative valence, SCR increases with arousal and is independent of emotional valence (Bradley & Vrana, 1993; Lang, 1995). Indeed, we previously showed that the startle response but not SCR remained potentiated in response to the feared stimulus even when the US could not be delivered (Sevenster et al., 2012a) or when participants were instructed that the CS would no longer be followed by the US (Sevenster et al., 2012b). Thus, the CS maintains its negative valence when there is no threat of shock, as reflected by preserved startle potentiation. In contrast, absence of possible US deliverance eliminates anticipatory arousal, indicated by reduced electrodertal responding. Here, we show that transfer of valence from the CS to the US can occur independent of contingency awareness, whereas anticipatory arousal requires knowledge of the CS-US relationship. The dissociation between arousal (SCR) and valence (startle response) raises important questions concerning the function of unaware startle conditioning. Remarkably, the unaware startle conditioning effect seems to be of a transient nature, since it is no longer observed during an extinction phase immediately following conditioning (Weike et al., 2007). Imaging research already showed that conditioning-related neural responses can take place in absence of awareness and SCR conditioning (Tabbert et al., 2011). It would be interesting to see whether evidence for an intact memory representation of unaware conditioning can still be observed at a later retention test. If there is no indication that the immediately developed unaware conditioned responding outlasts the conditioning phase — either behaviourally or neurally — the question is whether there is any adaptive value in the online but transient development of automatic conditioned responding.

The current findings confirm the notion that fear learning involves at least two processes. The US-expectancy ratings and the closely associated SCR seem to reflect the propositional level of associative fear learning, whereas the startle response may be better explained in terms of emotional learning and seems a more automatic, low-level index of fear. The finding that the emotional and cognitive components of fear learning rely on different neural circuits further challenges the single-process account. That is, declarative knowledge of the CS-US contingencies relies among other brain areas on the hippocampal complex
In contrast, the startle response is an amygdala-initiated response and therefore considered to reflect the brain's subcortical defense system (LeDoux, 2003; Walker & Davis, 2002) (for a more elaborate discussion on the single vs. dual process account see Sevenster et al., 2012b).

Note that while we consider SCR not an optimal correlate for fear, we do not argue that SCR is not a suitable measure in human fear conditioning research. During standard fear conditioning and extinction procedures, the measures corresponding to different response systems generally converge into similar learning patterns (Sevenster et al., 2012a, 2012b; Soeter & Kindt, 2010, 2011a; Vansteenwegen et al., 2005; Vervliet, Vansteenwegen, & Hermans, 2010). Only specific manipulations, either pharmacological or behavioural, may reveal the differences between these conditioned responses (Sevenster et al., 2012a, 2012b, 2013; Soeter & Kindt, 2010, 2011a). In human fear conditioning research, multiple indices of the behavioural expression of fear (e.g., US expectancies, distress ratings, SCR, startle potentiation) are usually obtained for reasons of cross-validation. Given that these measures of fear learning do not necessarily converge (Beckers, Krypotos, Boddez, Eftling, & Kindt, 2013; Hamm & Weike, 2005; Kindt et al., 2009; Sevenster et al., 2012a, 2012b, 2013; Soeter & Kindt, 2010), future research should incorporate this apparent divergence by predicting a priori (differential) effects for the cognitive and emotional expression of fear learning.