



# Affect, attention, or anticipatory arousal? Human blink startle modulation in forward and backward affective conditioning

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## ARTICLE INFO

### Article history:

Received 2 October 2007  
Received in revised form 10 February 2008  
Accepted 15 February 2008  
Available online 29 February 2008

### Keywords:

Blink reflex  
Startle modulation  
Affective learning  
Backward conditioning  
Attention

## ABSTRACT

Affect modulates the blink startle reflex in the picture-viewing paradigm, however, the process responsible for reflex modulation during conditional stimuli (CSs) that have acquired valence through affective conditioning remains unclear. In Experiment 1, neutral shapes (CSs) and valenced or neutral pictures (USs) were paired in a forward (CS→US) manner. Pleasantness ratings supported affective learning of positive and negative valence. Post-acquisition, blink reflexes were larger during the pleasant and unpleasant CSs than during the neutral CS. Rather than affect, attention or anticipatory arousal were suggested as sources of startle modulation. Experiment 2 confirmed that affective learning in the picture–picture paradigm was not affected by whether the CS preceded the US. Pleasantness ratings and affective priming revealed similar extents of affective learning following forward, backward or simultaneous pairings of CSs and USs. Experiment 3 utilized a backward conditioning procedure (US→CS) to minimize effects of US anticipation. Again, blink reflexes were larger during CSs paired with valenced USs regardless of US valence implicating attention rather than anticipatory arousal or affect as the process modulating startle in this paradigm.

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## 1. Introduction

Modulation of the eyeblink startle reflex during processing of a foreground stimulus varies depending on the lead interval at which the startle eliciting probe is presented. The lead interval is the time between the onset of a foreground stimulus and the presentation of the startle eliciting stimulus. Evidence for affective modulation of the eyeblink startle reflex at long lead intervals (e.g., 2 s +) has been demonstrated during the perception of emotional pictures (Lang et al., 1990), films, odours and sounds (Bradley et al., 1999) and in aversive Pavlovian conditioning (e.g., Hamm et al., 1993; Lipp et al., 1998). Eyeblink startle magnitude is modulated in a linear fashion according to foreground stimulus valence. For instance, the magnitude of the startle eyeblink response is smallest during highly arousing pleasant pictures and largest during highly arousing unpleasant pictures (Cuthbert et al., 1996). In aversive conditioning experiments eyeblink startle responses elicited during a conditional stimulus (CS+) that precedes an aversive unconditional stimulus (US; e.g., an electrocutaneous stimulus) are facilitated in comparison to responses elicited during another conditional stimulus (CS-) presented alone (Hamm et al., 1993; Lipp et al., 1998).

Demonstration of potentiated startle during a CS+ compared to during a CS- has not been restricted to conditioning procedures using an aversive US. In a Pavlovian conditioning experiment using a non-aversive, but arousing reaction time task as the US, significant startle

facilitation occurred during the cue (CS+) that signaled the reaction time task (Lipp et al., 2000). Furthermore, investigation of eyeblink startle modulation during the anticipation of emotional pictures revealed that blink startle responses were similarly facilitated during cues predicting pleasant and unpleasant pictures relative to cues predicting neutral pictures (Sabatinelli et al., 2001; Lipp et al., 2001; Dichter et al., 2002). These findings suggest that startle facilitation during a CS that predicts the occurrence of a salient US is not necessarily a reflection of the valence (unpleasantness) of the US or the CS, but may reflect another psychological process associated with the impending US, such as anticipatory arousal (Sabatinelli et al., 2001), or attention (Lipp et al., 2002, 2001).

Mallan and Lipp (2007) trained two groups of participants in a differential picture–picture affective learning<sup>1</sup> procedure with either a pleasant or unpleasant US. The CS+ acquired positive or negative affective value as confirmed by ratings and affective priming post-acquisition. Blink modulation was assessed during subsequent extinction to minimize potential confounds due to US anticipation. It was predicted that blinks during the CS+ paired with an aversive US would be larger than those during the CS- whereas the inverse would emerge for the CS+ paired with the pleasant US. In variance with this prediction, eyeblink startle magnitude was larger during the CS+ than during the CS- in both groups, regardless of US valence. This outcome was similar to previous studies (e.g., Lipp et al.,

<sup>1</sup> The term affective learning/conditioning is used throughout this paper as a general definition of the learning of likes and dislikes. The term evaluative conditioning is used when referring to a specific theory, namely dual process theory (Baeyens et al., 1995) that considers affective learning as qualitatively distinct from Pavlovian conditioning.

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2001; Sabatinelli et al., 2001; Dichter et al., 2002) and indicates that potentiation of eyeblink startle during CSs in human conditioning does not necessarily reflect on the valence of the CSs.

We suggested that attention may be the underlying process modulating startle in the picture–picture affective learning context based on previous demonstrations of modality non-specific potentiated startle to attended stimuli (Lipp et al., 2003; Lipp and Hardwick, 2003; Lipp et al., 2002) and evidence that greater attention is allocated to CS+ compared to CS- (Dawson et al., 1982). However, despite assessing startle during extinction in an attempt to reduce US anticipation, our previous study did not exclude the possibility that anticipatory arousal, rather than attention, may determine startle magnitude in these paradigms (see Sabatinelli et al., 2001). Furthermore, the extent to which the findings by Mallan and Lipp (2007) can be generalized to other affective conditioning procedures remains unknown. The majority of affective learning studies do not use a differential conditioning procedure. Rather than a single CS–US pair, 'traditional' affective learning designs involve multiple CS–US pairings with positive and negative USs (e.g., Levey and Martin, 1975). Furthermore, startle modulation was only assessed in the extinction phase so it remains uncertain whether a similar pattern of results would be found during acquisition.

The purpose of the present study was to extend our previous findings (Mallan and Lipp, 2007) in a number of ways in order to (a) test the generality of the observation that startle was not modulated by valence in a differential conditioning picture–picture preparation, and (b) to disentangle potential effects of anticipation and attention on startle modulation in the conditioning task. Blink startle modulation during three CSs that cued a pleasant, neutral or unpleasant US was assessed across acquisition and extinction training in Experiment 1. Experiment 2 assessed whether the temporal arrangement of CSs and USs (i.e., forward, backward, or simultaneous picture pairings) would impact on affective learning in order to provide a procedure in which the effects of anticipatory arousal and emotional learning can be separated. The backward conditioning procedure was then employed in Experiment 3 to assess startle modulation during affective learning in the absence of any effect of US anticipation. In all three experiments a picture–picture affective (evaluative) learning paradigm was used (e.g., Levey and Martin, 1975; De Houwer et al., 2000). Four neutral novel shapes were counterbalanced as CSs and during acquisition were paired with pleasant or unpleasant pictures (USs) or presented with neutral pictures (Experiments 1 and 3) or alone (Experiment 2). The three shapes (CSs) were then presented alone in an extinction phase. Affective learning as indexed by changes in CS valence was assessed with pleasantness ratings (and affective priming in Experiment 2 only) pre-acquisition, post-acquisition and post-extinction. Eyeblink startle reflexes were elicited during all three CSs and inter-trial intervals throughout acquisition and extinction phases in Experiments 1 and 3.

## 2. Experiment 1

The two key hypotheses of Experiment 1 were (1) that evidence for affective learning of positive and negative valence to the CSpos and CSneg, respectively, would be reflected in post-acquisition CS pleasantness ratings and, (2) that if emotional valence modulated eyeblink startle response magnitude during pleasant and unpleasant CSs then startle reflexes elicited throughout acquisition and extinction would be largest during the CSneg and smallest during the CSpos.

### 2.1. Method

#### 2.1.1. Participants

Twenty-four first year students (13 female, 11 male; mean age=20.33 years and range=17–41 years) from the University of Queensland voluntarily participated in exchange for credit towards a psychology course. Data from one participant were excluded from the pleasantness

ratings analysis due to failure to complete the rating tasks. Eyeblink response data were excluded from the acquisition phase analysis for two participants and from the extinction phase analysis for one participant because over one third of trials were scored as missing or as a zero response. The total number of complete data sets was 21. All participants provided informed consent and the experimental procedures had been cleared by the University of Queensland Ethics Review Board.

#### 2.1.2. Apparatus

Four two-dimensional novel shapes presented as black outlines on a white background, 600×450 pixels in size, were used as CSs. During the rating tasks and acquisition and extinction training, the shapes were presented in the centre of a 17 inch CRT (Samsung Multisync) computer screen.

Pictures that served as USs were selected from the IAPS picture set (CSEA, 1999) based on valence and arousal rating norms (Lang et al., 1999) and reformatted using Microsoft Picture Manager to be 1200×900 pixels in size with a colour depth of 256 colours. The USs consisted of four highly pleasant and highly arousing pictures (for males IAPS reference numbers: 4660; 7330; 8190, and 4250, and for females IAPS reference numbers: 7330; 2070; 4572, and 1440), four highly unpleasant and highly arousing pictures (IAPS reference numbers: 1050; 1300; 6560, and 6313), and four neutral and low arousing pictures (IAPS reference numbers: 7000; 7010; 7175, and 7217).

During acquisition one of the four shapes was presented paired with the pleasant pictures (CSpos→pleasant US), a second was paired with the unpleasant pictures (CSneg→unpleasant US), and a third was paired with the neutral pictures (CSneut→neutral US). During extinction the three shapes were presented alone and the pleasant, unpleasant or neutral pictures were not presented. The fourth shape (control) was not presented during either phase. All four shapes were counterbalanced between participants as CSpos, CSneg, CSneut and the control stimulus.

During the rating tasks each of the four shapes was presented individually and rated on the dimension of pleasantness using a 9-point scale where 1=unpleasant and 9=pleasant. The keyboard keys 1–9 were used to record a pleasantness rating for each shape.

DMDX programs (for details see Forster and Forster, 2003) run on a Pentium 4 IBM compatible computer with dual monitors controlled the stimulus sequences, durations, inter-stimulus intervals and inter-trial intervals for acquisition and extinction phases and for the rating tasks. The trial sequences in the three rating tasks were randomised by the DMDX program.

Obicularis oculi EMG was measured using two Ag/AgCl electrodes filled with electrode gel and fitted with adhesive collars. An electrode was placed on the skin directly underneath the participant's left eye and another electrode was placed approximately 1 cm to the left below the corner of the left eye. A reference electrode was placed on the centre of the forehead. All three electrodes were connected to a BIOPAC EMG 100 C amplifier (low pass filter of 500 Hz and high pass filter of 10 Hz). Responses were recorded with AcqKnowledge373 at a sampling rate of 1000 Hz. During acquisition and extinction a noise generator produced 43 ms acoustic probes – 105 dB burst of white noise with an instantaneous rise time – that were presented through Sennheiser (HD 25-1; 70 Ω) headphones. Probes were presented 3.5 s or 4.5 s after onset of a CS and during inter-trial intervals 12 s post-CS offset. Six probes were presented per CS during acquisition and four probes were presented per CS during extinction. Twelve probes were presented during inter-trial intervals in acquisition and extinction.

#### 2.1.3. Procedure

Participants read an information sheet that detailed the nature of the tasks and the stimuli in the experiment. Participants were informed that their participation was voluntary, that they could leave at any time without penalty and that all data collected were anonymous and confidential. The participant's age and gender were recorded. The

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