

Social Pavlovian conditioning: Short- and long-term effects and the role of anxiety and depressive symptoms

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Abstract

Today's stressors largely arise from social interactions rather than from physical threat. However, the dominant laboratory model of emotional learning relies on physical stimuli (e.g. electric shock) whereas adequate models of social conditioning are missing, possibly due to more subtle and multilayered biobehavioral responses to such stimuli. To fill this gap, we acquired a broad set of measures during conditioning to negative social unconditioned stimuli, also taking into account long-term maintenance of conditioning and inter-individual differences. Fifty-nine healthy participants underwent a classical conditioning task with videos of actors expressing disapproving (US-neg) or neutral (US-neu) statements. Static images of the corresponding actors with a neutral facial expression served as CS+ and CS−, predicting US-neg and US-neu, respectively. Autonomic and facial-muscular measures confirmed differential unconditioned responding whereas experiential CS ratings, event-related potentials, and evoked theta oscillations confirmed differential conditioned responding. Conditioning was maintained at 1 month and 1 year follow-ups on experiential ratings, especially in individuals with elevated anxiety and depressive symptoms, documenting the efficiency of social conditioning and its clinical relevance. This novel, ecologically improved conditioning paradigm uncovered a remarkably efficient multi-layered social learning mechanism that may represent a risk factor for anxiety and depression.

Key words: neuroscience; classical conditioning; major depression; anxiety disorders; emotion

Introduction

'If it is not learning, what is it?' (Wolpe, 1976, p. 67)— a stirring question in experimental psychology of human behavior, in social contexts, and with a clinical impact on human fear learning (Eelen and Vervliet, 2006). An evolutionary perspective portrays fear and learning as central to the development of human defense systems in response to threat and danger (Öhman and Mineka, 2001). Fear conditioning results from a classical conditioning process that converts an originally neutral stimulus into a defensive-response provoking signal (conditioned stimulus:

CS) through repeated pairing with an aversive unconditioned stimulus (US; Pavlov, 1927; De Houwer *et al.*, 2013). Fear conditioning is generally considered as adaptive and functional but exaggerated and extinction resistant responses are found in various anxiety disorders (see Lissek *et al.*, 2005; Duits *et al.*, 2015 for an overview). Prior research utilized physical threats (electric shocks, loud tones) as the primary US due to their evolutionary significance. While this is crucial when examining primary threat defense systems, it deviates from the most typical and prevalent aversive events experienced in modern societies:

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ego-threats and social stress resulting from negative evaluation by others. Its significance contrasts with the small number of scientific approaches to this stimulus type: some studies have paired aversive USs such as social or physical stressors with neutral facial expressions as CSs but only few have included truly social USs (i.e. social conditioning; Hermann et al., 2002; Lissek et al., 2008; Pejic et al., 2013; Wieser et al., 2014; Ahrens et al., 2015; Blechert et al., 2015b) possibly due to uncertainties about the sensitivity of various physiological indicators to this type of conditioning.

Negative social interactions leave traces on functionally distinct systems. Individuals will remember what happened (i.e. what has been said by whom) and archive valenced memory templates, which in turn, will prepare them for similar future encounters with that particular person. Preparation for social interactions will likely differ from preparation for physical noxious stimuli: experiential systems (valence, 'liking' of that person), arousal mediating systems (autonomic nervous systems) and communicative systems (e.g. facial responses) will accompany social approach or avoidance. Thus, studying social conditioning requires a careful selection of response markers to map immediate and delayed signatures of adaptive social responding. In the following, we review various conditioning markers that have been used in past Pavlovian conditioning designs and examine their efficacy for indexing aspects of social conditioning (experiential, autonomic, facial-muscular and neural).

The *experiential response system* is often neglected by neuroscientific conditioning research despite clear evidence for robust changes in the evaluation of conditioned stimuli due to the pairing with valenced USs (Blechert et al., 2008). In fact, such changes are known to evolve in parallel to preparatory psychophysiological conditioned responses (CRs) and have been studied intensely under the term *evaluative conditioning* (EC; reviewed by De Houwer et al., 2001). Its remarkable extinction resistance has been demonstrated (Vansteenwegen et al., 2006) and suggests the beneficial inclusion of mid- and long-term measures in social conditioning.

Traditionally, *autonomic response indices* have played a key role in Pavlovian conditioning research. Sympathetically innervated, electrodermal activity (EDA) is reported as being sensitive to both orienting to and anticipation of events that signal fear eliciting stimuli (Öhmann et al., 2000). Similarly, heart rate (HR), innervated by sympathetic and parasympathetic mechanisms, mediates orienting and anticipation along with defensive responding (Hamm and Vaitl, 1996; Bradley et al., 2001). However, these are 'costly' systems requiring significant energy resources that are most likely active during imminent or intense threat (Blechert and Wilhelm, 2014) which might be represented by unconditioned responding in social conditioning and only to a minor degree during anticipation.

Although differential *facial-muscular responding* has also been measured in conditioned shock anticipation (van Meurs et al., 2014), facial communicative action is of central importance in social situations: Social cues trigger coherent facial activity patterns of sympathy or antipathy due to their role in signaling (social) emotions and possibly intentions (Adolphs, 2010) and do so spontaneously and rapidly (Buck, 1984; Dimberg, 1990). Specifically, facial electromyography (EMG) activity of the *corrugator supercilii* muscle (related to frowning) is increased in response to negative faces (Dimberg, 1990). Thus facial EMG involvement in CS recognition and US anticipation is likely.

Importantly, being an amygdala-mediated process, Pavlovian conditioning in the social domain might also elicit

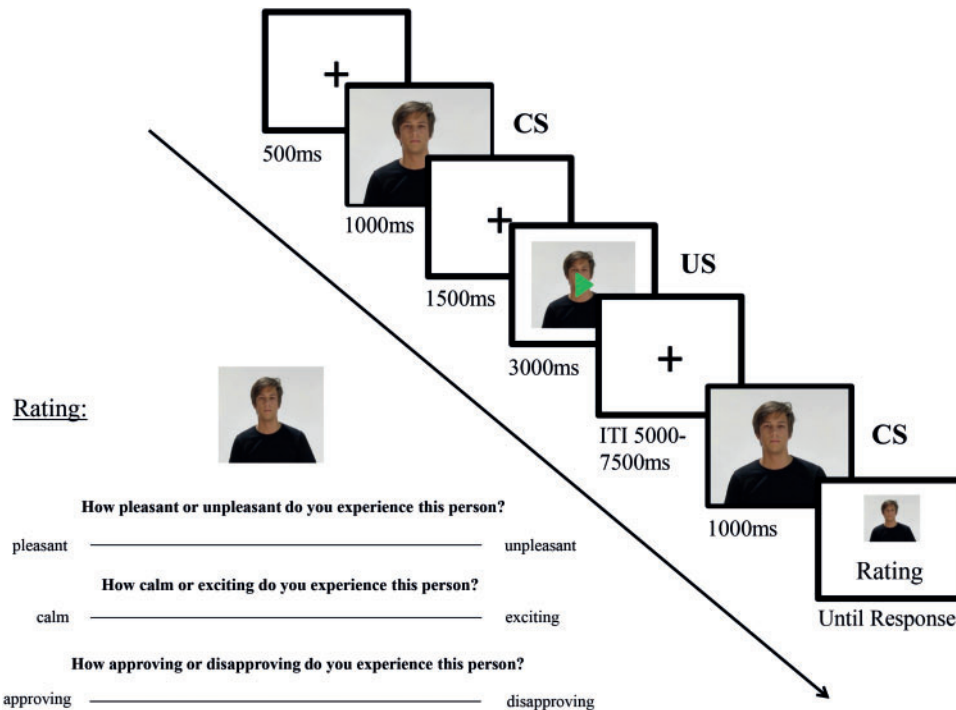
differential *neural responding*. Functionally, detection, recognition and memory (updating) of predictive conditioned and unconditioned social cues might involve different neural systems (Schupp et al., 2006). Our prior study found differential LPPs to social-evaluative videos (Wiggert et al., 2015b) but differential electrocortical CRs may occur much earlier: Previous research has documented differential responses specifically on mid-latency (P3, EPN) but also on early (P1, N1) and very early (C1) event-related potentials (ERPs; Montoya et al., 1996; Wong et al., 1997; Skrandies and Jedynek, 2000; Stolarova et al., 2006) as well as steady-state visual evoked potential (ssVEPs) effects (Ahrens et al., 2015). More fine grained functional interpretations, however, can be obtained by studying the neural oscillations that generate ERPs (Karakas et al., 2000; Basar, 2006). Previous research has linked alpha oscillations to inhibition and timing of cortical processing in support of an early access (~100 ms post stimulus onset) to the knowledge system (Klimesch et al., 2007; Klimesch, 2012). Within this framework, theta oscillations are pronounced ~300 ms after stimulus onset and associated with episodic memory processes (Klimesch, 1996; Klimesch et al., 1997, 2006). In addition, other research modeled threatening contexts more specifically and found alpha band activity to be modulated by threatening stimuli (Vagnoni et al., 2015) whereas anterior midcingulate cortex theta band activity is associated with the recall of conditioned fear (Mueller et al., 2014). However, theta oscillations are sometimes associated with arousal during anticipation of noxious stimuli (Pape and Pare, 2010) suggesting broader roles of the respective frequency bands. Thus, by assessing oscillatory measures of alpha and theta frequency bands more specificity in the interpretation of the ERP signal may be achieved.

The multi-layered documentation of immediate responding to anticipated and experienced negative social encounters builds important foundations for social neuroscience and learning. However, Pavlovian conditioning has also important clinical implications: It represents the primary basis of contemporary etiological accounts of anxiety disorders (Lissek et al., 2005, 2010, 2014; Duits et al., 2015) and recent research also shows associations with depressive disorders (Nissen et al., 2010; Kuhn et al., 2014). Anxiety and depressive symptoms characterize individuals with different anxiety disorders and sub-clinical precursor conditions. Thus, the present study included individual difference measures for trait anxiety and depressive symptoms to examine subclinical symptoms in the context of social learning. Such measures may be particularly interesting with regard to the long-term course of social conditioning and EC in particular due to its known extinction resistance.

The present research provides a social conditioning framework incorporating E.Vids comprising 3000 ms duration videos with negative, neutral and positive sentences (Table 1; Blechert et al., 2013; Reichenberger et al., 2015; Miedl et al., 2016). Here, we used still images of neutral faces of four different actors (2 female) as CSs which predicted negative/disapproving evaluations vs neutral statements (dynamic videos) of the same actors as USs. We assumed that the CS+ (the still image predicting negative-evaluative videos) would be experienced as more unpleasant, arousing and disapproving compared to the CS- (the still image predicting neutral videos). We also expected increased HR and EDA towards negative USs (i.e. US-neg: negative videos) than to neutral USs (i.e. US-neu: neutral videos) mediating energy and arousal during direct US confrontation. Furthermore, increased corrugator muscle activity was expected to both CS+ (relative to CS-) and US-neg (relative to

Table 1. Neutral, positive and negative sentences from the E.Vids video set

Neutral Sentences	Positive Sentences	Negative Sentences
'What time is it?'	'I'm proud of you'.	'You're ridiculous'.
'The bus is stopping'.	'You're looking good'.	'I hate you'.
'The traffic light changes to red'.	'One can really count on you'.	'I can't bear you'.
'I lost my keys'.	'I like you'.	'I'm disappointed in you'.
'I'm late'.	'You're very important to me'.	'You're embarrassing'.
'It's windy outside'.	'You've got it'.	'You're so stupid'.
'The train goes fast'.	'I'm happy you are here'.	'You're getting on my nerves'.
'It's 4 o'clock'.	'You are class'.	'You're weird'.

**Fig. 1.** Experimental task and ratings.

US-neu) to allow adaptive facial-communicative responding. Neurally, we expected increased mid- to long-latency ERPs selectively for CS+ and US-neg relative to CS- and US-neu. Alpha or theta CS-evoked oscillations might differentiate CSs depending on whether early stimulus categorization or differential episodic memory (or both) differ between CSs. Finally, anxiety and depressive symptoms might enhance arousal and valence ratings to the CS+ (relative to the CS-).

Methods

Participants

A sample of 59 participants (40 female) with an average age of 25.12 years ($SD = 3.36$) was recruited through online advertisement and in psychology classes. Participants reported no current mental or neurological disorders, no current use of prescriptive medication except contraceptives and no current alcohol or drug abuse. Eligible participants read and signed an informed consent form approved by the university ethics committee and received monetary compensation (10€) or course credit for participation.

Procedure

Prior to the laboratory session participants were asked to fill out an online assessment battery including well-established questionnaires for trait anxiety [State-Trait Anxiety Inventory (STAI)-German version; Laux et al., 1981; Cronbach's $\alpha = 0.86$] and depressive symptoms [State-Trait Depression Scale (STDS)—German version; Spaderna et al., 2002; Cronbach's $\alpha = 0.66$]. The laboratory assessment started with sensor application for central and peripheral physiological measurements followed by a 4-min quiet sitting baseline and a 3-min heartbeat perception phase (results not reported here). As illustrated in Figure 1, the pre-conditioning rating phase evaluated neutral still images of four actors (see Supplementary Data A) on arousal, valence and disapproval on an on-screen visual analog scale. No information about possible stimulus contingencies (CS-US pairings) was given. Conditioning comprised 64 trials: 32 CS+ trials (16 per actor) and 32 CS- trials (16 per actor) interspersed by an inter-trial interval varied randomly between 5000 and 7000 ms. Each actor expressed eight different sentences, each repeated once. Evaluative ratings of each actor's still image (same rating scale as described above) were obtained after each 5th trial. Stimuli were presented on a 23-inch LCD monitor with

a resolution of 1920 × 1080 pixel and 120 Hz refresh rate, using E-Prime 2.0 (Psychology Software Tools, Inc., Sharpsburg, PA, USA). Video volume (delivered via external active speakers) was constant across participants. After completion of the task and sensor removal, participants completed several questionnaires and were debriefed afterwards. Post-conditioning, another rating phase was completed. Similarly, follow-up measurements of subjective ratings were obtained 3 days, 1 month and 1 year after the laboratory session via an online assessment on identical scales as used in the laboratory.

Peripheral physiological recording

Psychophysiological data was recorded with a REFA 8-72 digital amplifier system (TMSi) with 24 bits/channel resolution at 400 Hz, streamed to disk and displayed on a PC monitor for online monitoring of data quality. Skin conductance level (SCL), electrocardiography (ECG), vertical electrooculography (i.e. EOG; above and below the right eye), and facial EMG measures were bipolar recordings using Ag/AgCl electrodes. SC electrodes were placed on the middle phalanx of index and middle fingers of the non-dominant hand. ECG was recorded at the initial point of the sternum and distal end of the left costal arch. Electrodes of the *musculus corrugator supercillii* activity were placed according to international guidelines (Fridlund and Cacioppo, 1986) near the left eye-brow.

Offline data inspection and manual artifact rejection for SCL, ECG and EMG was done in ANSLAB 2.6 (Blechert et al., 2015a). EMG preprocessing comprised a 28 Hz high-pass filter, 50 Hz notch filter, rectification, low pass filtering (15.92 Hz) and 50 ms moving average filter. Due to the relatively slow response characteristic of the non-EEG channels, CRs were defined as averages across the 1000 ms picture presentation and the subsequent 1500 ms fixation cross, relative to a 500 ms pre-stimulus baseline. The UR-time window included the 3000 ms of the video and the subsequent 5000 ms ITI due to continued and delayed responses, relative to a 1000 ms baseline (immediately preceding video onset, last 1000 ms of fixation interval). CR and UR responses were then averaged across trials, separately for CS+/UR-neg and CS-/UR-neu. These response patterns were analyzed in 500 ms segments.

EEG recording

EEG was recorded using an actively shielded 64-channel electrode cap (sintered Ag/AgCl electrodes, manufactured for TMSi, Twente Medical Systems International, EJ Oldenzaal, Netherlands). Each unipolar EEG channel was recorded against the average of all EEG channels. A wet band on the left wrist served as grounding for all channels. All scalp positions in the international 10–20 System were used, with additional sites 10% inferior to the standard electrodes (PO9, TP9, FT9, PO10, TP10 and FT10). EEG electrode impedances were kept below 30 kΩ for all electrodes.

Offline EEG data analysis. Offline analysis was done with Brain Vision Analyzer 2.0 software (Brain Products Inc., Gilching, Germany) and comprised the following steps: Low pass filtering at 40 Hz, high pass filtering at 0.05 Hz, 50 Hz notch filtering, semiautomatic eye-blink correction using independent component analysis, manual screening for remaining artifacts or bad channels, segmentation (from 200 ms pre-stimulus to 1000 ms CS response and 3000 ms US response post-stimulus onset), artifact correction (exclusion of epochs exceeding > 150 μV amplitude change or low activity) and baseline-correction (200

ms). Three participants had poor signal quality (less than 50% of epochs available) and were excluded from EEG analyses. The percentage of included epochs for remaining participants was high (CS+: 96.7%, CS-: 96.8%, US-neg: 95%, US-neu: 95.4%) and did not differ by CS-type nor US-type, $t_s < 0.50$, $p_s > 0.616$. Alpha (8–12 Hz) and theta (3–8 Hz) oscillation calculation incorporated filtering with an infinite impulse response (IIR) filter (slope of 48 dB) in the respective frequency bands before segmenting. Epochs were averaged over trials and parietal-occipital sensors (PO9, PO7, PO3, POz, PO4, O2, Oz and O1).

Data reduction and statistical analysis

Conditioning. Preliminary analyses showed that the factor participant-gender and stimulus-gender did not reach significance in any of the analyses, all $F_s < 1.66$, $p_s > 0.203$ and were therefore dropped from subsequent analyses (this is in line with a recent report of rather subtle gender differences in response to E.Vids; Wiggert et al., 2015a). Differential conditioning on self-report data was assessed through separate 2 (CS-type: CS+, CS-) × 11 (time: pre-rating, block 1–8, 3 days follow-up (FU), 1 month FU) analyses of variance (ANOVAs) with repeated measures on CS-type and time for arousal, valence and disapproval. Additionally, 1 year FU ratings were separately analyzed since only 70% of participant data was available: here t-test statistics are reported. Peripheral physiological markers were analyzed separately for CRs and URs in 500 ms segments. EEG analyses were conducted using electrode clusters separately for the CR (P280) and the UR (LPP): multiple t-tests used Bonferroni correction and Bias corrected accelerated (BCa) bootstrapped confidence intervals (CI) are displayed. For calculation of the P280 and oscillatory responses the mean amplitude between 200 and 320 ms on parietal-occipital sensors (PO9, PO7, PO3, POz, PO4, O2, Oz and O1) was used. The same time window and electrode cluster were used for computing the mean amplitude of the theta and alpha band pass filtered ERPs. The LPP calculation comprised the mean amplitude 700–1500 ms on centro-parietal sensors (C1, Cz, C2, CP1, CPz, CP2, P1, Pz and P2; Wiggert et al., 2015a,b). Significant main or interaction effects were followed by pairwise comparisons for repeated measure designs applying the Sidák correction (Mean difference = MD, significance levels, and 95% CIs are displayed for single comparison reports).

Anxiety and depressive symptoms. To investigate relationships with individual differences we defined a maintenance index (MI) of the EC effect. For each follow-up measure (3 days, 1 month and 1 year post-acquisition), we accounted for values during the second half of acquisition (statistically, residuals from a regression analysis predicting follow-up measures from acquisition values, separately for each CS-type, were saved). The resulting MI for the CS+ was then correlated with each individual difference measure (STAI, STDS), while partialling out the MI of the CS-. Thus, the resulting correlation accounts for conditioning strength (second half of acquisition) and reflects post-acquisition changes (maintenance) in a differential (accounting for CS- changes) manner. Here, we opted against a 'double' difference score approach, due to its known reliability problems (Lord, 1967; Rogosa and Willett, 1983; Wainer and Brown, 2004; see Supplementary Data B). Partial correlations were analyzed for depressive symptoms/trait anxiety and the MI of the EC effect for the three rating scales arousal, valence, and disapproval for CS+ accounting for CS-.

The alpha level was set to 0.05. Effect sizes are reported as partial eta squared (η^2_p). When the sphericity assumption was violated in ANOVAs, the Greenhouse–Geisser correction was

applied with nominal degrees of freedom and epsilon (ϵ) being reported.

Results

Experiential data

Arousal ratings. The 2 (CS-type) \times 11 (time: pre-rating, trial 1–8 and 3 days FU, 1 month FU) ANOVA revealed main effects of CS-type and time, $F_s > 11.00$, $P_s < 0.001$, $\eta^2_{ps} > 0.16$ and a significant two-way interaction of CS-type \times time, $F(10,570) = 14.16$, $P < 0.001$, $\eta^2_p = 0.20$, $\epsilon = 0.43$. CS-types did not differ prior to acquisition ($MD = -0.00$, $P = 0.969$). During acquisition CS+ was rated as more arousing than CS–, which remained significant after 3 days and 1 month ($MDs > 0.50$, $P_s < 0.001$; Figure 2a). Interestingly, CS+ was still rated higher than CS– after 1 year, $t(35) = 2.15$, $P = 0.039$, 95%CI [0.06, 0.68] (Figure 2a).

Valence ratings revealed similar main effects, $F_s > 28.63$, $P < 0.001$, $\eta^2_{ps} > 0.33$. The CS–type \times time interaction, $F(10,570) = 20.84$, $P < 0.001$, $\eta^2_p = 0.27$, $\epsilon = 0.40$ was also significant. CS-types did not differ in the pre-rating condition prior to acquisition ($MD = -0.04$, $P = 0.703$). As expected, the CS+ was experienced as more unpleasant than the CS–, during acquisition (block 1–8), after 3 days and after 1 month ($MDs > 0.47$, $P_s < 0.001$; Figure 2b). However, after 1 year differential conditioning had extinguished, $t(35) = 1.58$, $P = 0.124$.

Disapproval ratings showed main effects of CS-type and time ($F_s > 30.96$, $P_s < 0.001$) and an interaction, $F(10,570) = 29.38$, $P < 0.001$, $\eta^2_p = 0.34$, $\epsilon = 0.42$. Again, both CS-types did not differ before conditioning ($MD = -0.02$, $P = 0.890$) but CS+ actors were rated as more disapproving than CS– actors during acquisition, after 3 days and after 1 month ($MDs > 0.66$, $P_s < 0.001$; Figure 2c). Ratings were not different after 1 year, $t(35) = -0.84$, $P = 0.405$.

Individual differences of depressive symptoms and trait anxiety

Partial correlation analyses for the MI of arousal ratings and depressive symptoms revealed that higher scores of depressive symptoms went along with higher arousal ratings of the CS+ after 3 days, $r(53) = 0.28$, $P = 0.041$, and after 1 month, $r(52) = 0.30$, $P = 0.029$ (Figure 3), but not after 1 year, $r(33) = 0.15$, $P = 0.401$. Similar associations were found for trait anxiety: scores were positively correlated with MI after 1 month, $r(55) = 0.31$, $P = 0.020$ (Figure 3) but not after 3 days or 1 year, $r_s < 0.18$, $P_s > 0.175$. These effects were not found for individual differences and valence/disapproval ratings after 3 days, 1 month or 1 year, $r_s < 0.17$, $P_s > 0.209$.

Autonomic measures

SCL. The CR 2 (CS-type) \times 5 (time: 2500 ms) \times 16 (trials) ANOVA showed no main effects of CS-type or time, $F_s < 3.14$, $P_s > 0.08$. However, the UR 2 (US-type) \times 16 (time: 8000 ms) \times 16 (trials) ANOVA showed main effects of US-type and time $F_s > 8.51$, $P_s < 0.001$ modulated by the US-type \times time interaction, $F(15,855) = 15.01$, $P < 0.001$, $\eta^2_p = 0.21$, $\epsilon = 0.09$. SCLs for US-type did not differ during the first 2500 ms after video onset ($MDs < 0.002$, $P_s < 0.079$) but significantly increased for negative compared to neutral videos after 2500–8000 ms ($MDs > 0.009$, $P_s < 0.023$; Figure 2d; see Supplementary Data C).

HR. The same CR ANOVA showed a main effect of time, $F(4,224) = 41.99$, $P < 0.001$, $\eta^2_p = 0.43$, $\epsilon = 0.37$, but no main effect of CS-type or any interaction, $F_s < 0.66$, $P_s > 0.419$. The 2 (US-type) \times 6 (time: 3000 ms) \times 16 (trials) UR ANOVA showed main effects of

US-type and time, $F_s > 15.00$, $P < 0.001$, $\eta^2_{ps} > 0.21$ and a significant interaction, $F(4,280) = 4.22$, $P = 0.021$, $\eta^2_p = 0.07$, $\epsilon = 0.36$. For the first 500 ms, there was no significant difference of US-type ($MD = 0.08$, $P = 0.175$). However, from 1500 to 3000 ms HR decelerated significantly more for negative relative to neutral videos ($MDs > 0.34$, $P_s < 0.009$; Figure 2f).

EMG: corrugator supercillii muscle

The 2 (CS-type) \times 5 (time: 2500 ms) \times 16 (trials) CR ANOVA revealed a main effect of time, $F(4,232) = 4.86$, $P = 0.01$, $\eta^2_p = 0.08$, $\epsilon = 0.50$ but no main effect of CS-type or any interactions, $F_s < 0.7$, $P_s > 0.443$. The 2 (US-type) \times 6 (time: 3000 ms) \times 16 (trials) UR ANOVA showed main effects of US-type and time, $F_s > 15.10$, $P_s < 0.001$ and an interaction, $F(5,290) = 12.34$, $P < 0.001$, $\eta^2_p = 0.18$, $\epsilon = 0.47$. The first 1000 ms showed no significant difference of US-type ($MDs < 0.008$, $P_s > 0.87$). However, 2000–3000 ms the corrugator muscle showed a significant relaxation during negative relative to neutral videos ($MDs > 0.180$, $P_s < 0.003$, 95% CI [0.07, 0.41]; Figure 2e).

Event-related EEG

The P280 component discriminated between CS-types with larger positive amplitudes for CS+ ($M = 5.86$) than CS– ($M = 5.41$), $t(58) = 2.33$, $P = 0.023$, $d = 0.14$ (Figure 4). Evoked theta oscillations showed differences between CS+ and CS–, $t(58) = 2.61$, $P = 0.011$, $d = 0.17$, and demonstrated the same peak as for the event-related P280 (Figure 4). This was not shown for alpha oscillations, $t(58) = 1.66$, $p = 0.102$.

The UR showed a significant difference of US-type, $MD = 0.43$, 95% CI [0.27, 0.62], $t(55) = 4.61$, $P < 0.001$, $d = 0.61$, with larger LPPs (more relative positivity) for US-neg, $M = 2.18$, 95%CI [1.98, 2.40] relative to US-neu, $M = 1.75$, 95%CI [1.63, 1.88].

Discussion

Social cognition and social evaluation rely on efficient learning and memory systems to generate valenced representations of significant others from current and prior social experiences (Eagly and Chaiken, 1993). To the best of our knowledge, this is the first study to investigate social conditioning with purely social CSs and USs (i.e. high external validity) along with the short- and long-term course of a comprehensive dependent variable set indexing relevant response systems.

Results can be summarized as followed: subjective experience (arousal, valence and disapproval ratings) during acquisition clearly supported the expected differential conditioned responses between neutral actor faces paired with negative vs neutral social evaluations. These effects were still prominent after 1 month and for arousal even after 1 year of acquisition highlighting long-term effects of EC and resembling effects of resistance to extinction. Importantly, depressive symptoms and trait anxiety were positively correlated with long-term effects of EC. Regarding autonomic/facial responding, we found the expected differential URs in arousal/energy mobilizing autonomic measures (EDA, HR) as well as in facial communicative corrugator EMG activity. Differential CRs were not found on these measures. Electrocortical measures discriminated CS+ and CS– through a mid-latency P280 possibly generated by differential theta bursts and US-neg vs US-neu through an LPP.

The current study revealed URs of arousal/energy-modulating response systems: negative videos elicited the expected increased EDA and an initial HR deceleration probably reflecting an

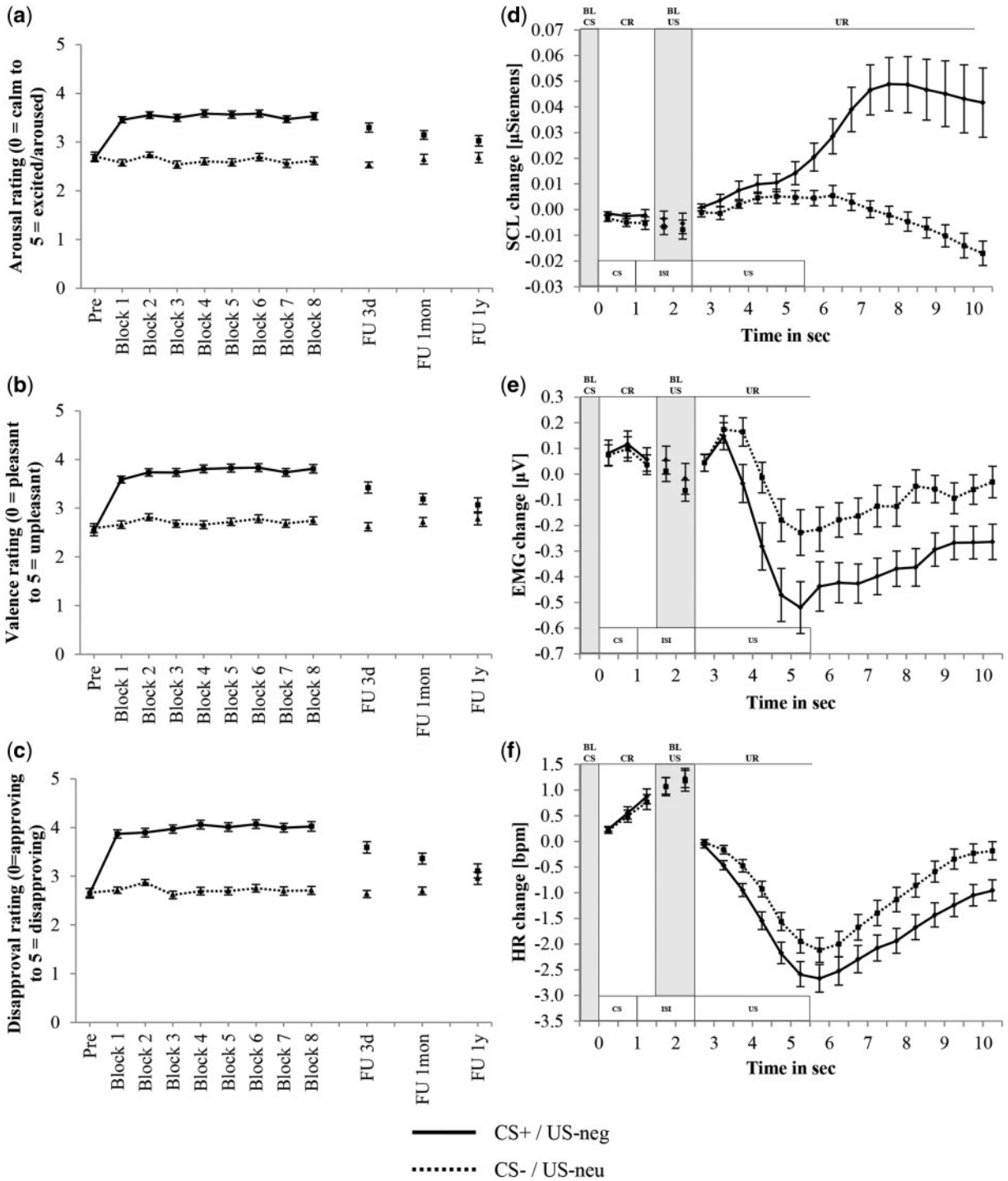


Fig. 2. Means and standard errors of self-report data for (a) arousal, (b) valence and (c) disapproval for conditioned stimuli (CS+ and CS-, respectively). All ratings show 12 time points (pre-rating; blocks 1-8; 3 days (3d) follow-up (FU); 1 month (1 mon) FU; 1 year (1y) FU); (d) displays skin conductance level (SCL) change in μ Siemens, (e) shows the corrugator electromyography (EMG) change in μ V and (f) shows heart rate (HR) change in beats per minute (bpm) for the CS+, CS-, US-neu and US-neg. Labels on the time axis of plots d, e and f indicate on/offsets of conditioned stimuli (CS), interstimulus intervals (ISI) and unconditioned stimuli (US); upper labels indicate baseline (BL) periods (grey windows) for the CSs and USs, conditioned response (CR) windows and unconditioned response (UR) windows.

orientating response to the personally relevant US (Bradley et al., 2001). Although we followed established fear conditioning routines in the timing and selection of conditions, our choice of a social US (in contrast to more conventional shock stimuli) likely introduced different mechanisms as reviewed above. A shock US prompts an immediate somatic response and anticipatory

preparation. Shock prediction becomes the main concern of any organism. Although some of the social stimuli used here could similarly signal an impending physical attack (i.e. 'I hate you') other stimuli do not hint at any physical encounter but appeal to social-evaluative/social exclusion/inclusion related cognitions and associated emotions (i.e. 'You're weird').

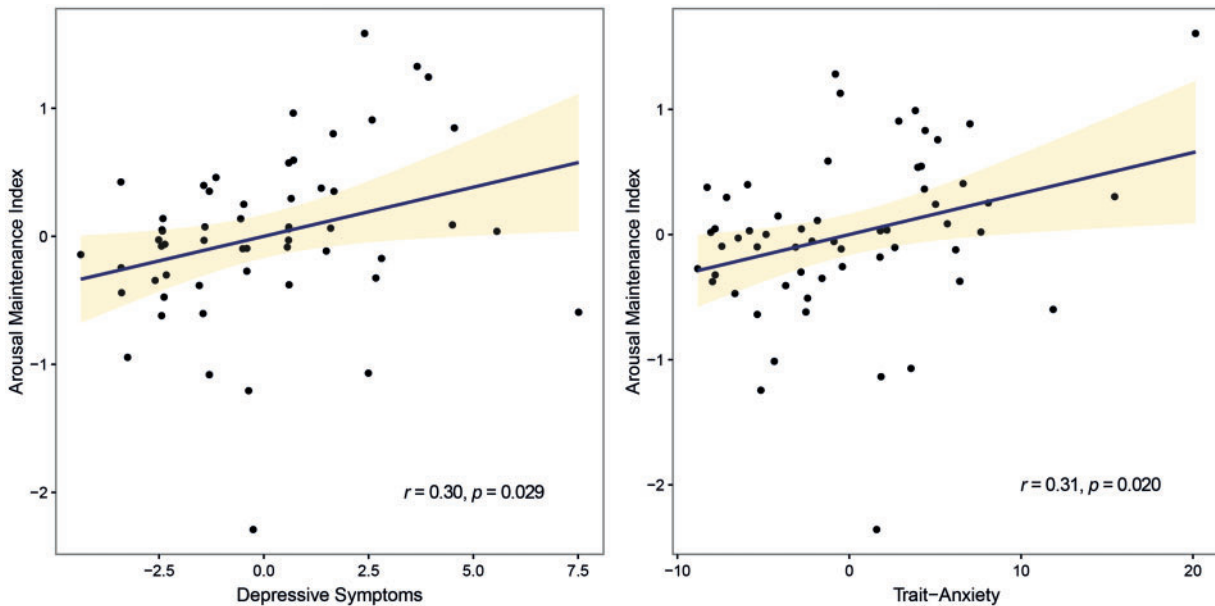


Fig. 3. Partial correlations between depressive symptoms (left), trait anxiety (right), and the arousal maintenance index (MI) for the CS+ after 1 month post acquisition accounting for CS. Yellow areas denote confidence intervals (95%). Note that graphs display residualized scores of depressive symptoms, trait anxiety and the arousal MI.

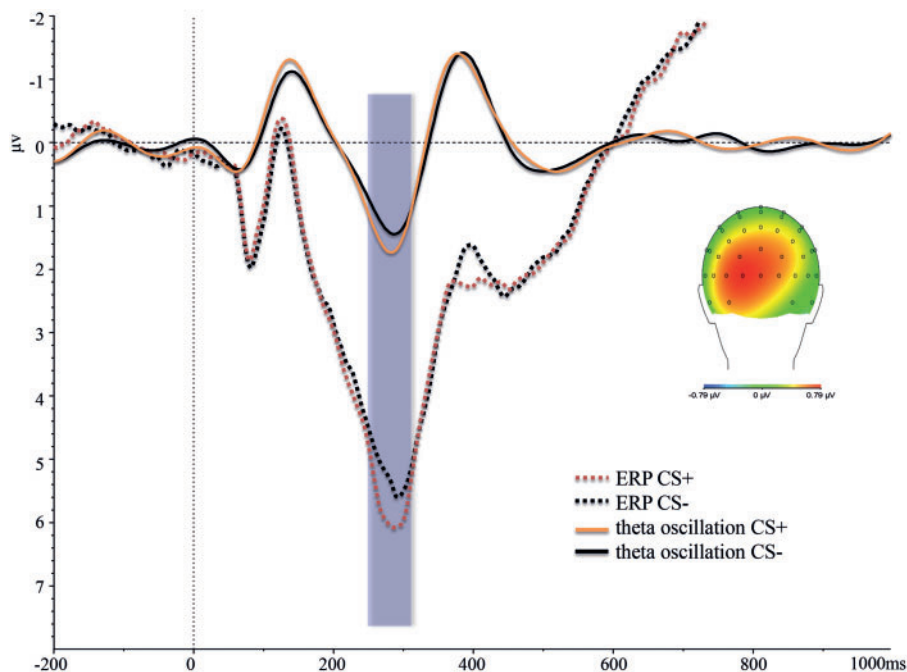


Fig. 4. Event-related potentials (dotted lines) for the selected sensor POz of the conditioned response (CR) in the time window 200–320 ms (peaking at 280 ms) over parietal–occipital sensors (averaged over Po9, Po7, Po3, Poz, Po4, O2, Oz and O1) evoked by pictures of neutral facial expressions paired with negative videos (CS+) or neutral videos (CS-). Topography plot illustrates a CS+ vs CS- difference waveform in the respective time window. Additionally, theta oscillatory responses on the selected POz scalp sensor to the CS+ (orange line) and CS- (black line) are depicted, peaking in the same time window (around 280 ms) as the positive event-related potential of the CS+ and CS-.

Decoding and interpretation of the communicative aspects of an opponent's facial cues and utterances likely involves different neural and peripheral systems compared to purely physical stimuli. The facial EMG finding may hint at the social-communicative functions of UR responding: the decrease of corrugator activity (less frowning) during negative vs. neutral videos is counterintuitive at first sight. However, it is consistent

with previous findings in an independent sample (Wiggert et al., 2015b). According to Bourgeois and Hess (2008), negative facial responses are withheld in many settings because of the risk of negative outcomes but might be expressed toward in-group members. Thus, the present context with no obvious shared group between participants and actors might have provoked this facial response tendency.

Neurally, UR responding on the LPP closely replicated video responses in our previous study (despite a different subset of E.Vids; Wiggert et al., 2015b). LPPs in affective picture viewing have been interpreted as reflecting arousal driven sustained attention to motivationally salient stimuli (Schupp et al., 2000, 2004). The latency of this component and its known sensitivity to deliberate, top-down emotion regulation (Hajcak and Nieuwenhuis, 2006) suggest that conscious responses drive this UR. Combined EEG-fMRI research found sources in occipitotemporal-parietal cortical areas underlying the LPP (Sabatinelli et al., 2013) regions that might be sensitive to distraction based downregulation (McRae et al., 2010). A differential CR was found at 280 ms over parietal-occipital sensors and thus 'half-way' between a P2 and a P3. The P2 is reported to peak between 150 and 280 ms at anterior and central scalp sites and to be involved in selective attention, feature detection processes and retrieval from short-term memory (Chapman et al., 1978; Hackley et al., 1990; Luck and Hillyard, 1994), however, little is known about the posterior P2 (Luck, 2005). The P3 peaks between 250 and 500 ms over frontal to parietal midline electrodes (Fz, Cz, Pz; Polich, 2007) and is involved in context updating when there is a need to revise current representations in working memory (Karis et al., 1984). The present experimental context makes the latter interpretation more likely: successively more sentence-based information about an actor needs to be incorporated into an 'actor-memory'. Research by Klimesch et al. (2006) demonstrated that episodic traces are first processed at parietal sites at approximately 300 ms (time window of the P2) relating to theta oscillations. The present result of the P280 ERP effect for the CS+ dovetails nicely with the theta oscillation indicating that episodic memory processes, crucial for personally experienced events to be retrieved later in time, may have occurred in the current task. In contrast, alpha oscillations did not play a major role in the P280 ERP effect indicating that access to the knowledge system was not as crucial in this task.

EC plays an important role in the context of social interactions: it refers to attitude formation towards objects or social cues such as facial expressions due to the contingency with other valenced stimuli (e.g. social evaluations; Jones et al., 2010). The current study showed that the negative valence of disapproving social messages (USs) transferred to the neutral facial expression of the same actor/actress (CSs) as evidenced by increased self-reported unpleasantness, arousal and disapproval. This is in line with prior conditioning research using socially relevant stimuli (Lissek et al., 2008; Blechert et al., 2015b). Notably, this effect was persistent for at least 1 month supporting the evidence that EC appears to be particularly resistant to extinction (Vansteenwegen et al., 2006). Most interestingly, self-reported arousal was higher for neutral faces representing the CS+ than for the CS- even after 1 year. This replicates results from prior follow-ups of valence ratings in classical conditioning (Schiller et al., 2010; Blechert et al., 2015b) but, to the best of our knowledge, has not been shown for arousal ratings and for social conditioning. Thus, once a negative evaluation towards a person is formed, it appears to be long lasting, which in turn, may affect future social interactions with the same person promoting interpersonal withdrawal, contempt or even aggression (c.f., Maner et al., 2007).

This interpretation dovetails with the fact that anxiety and depressive symptoms played an important role for this variable: the more anxious and depressed participants were, the more aroused they reported to be 1 month after acquisition. This is generally in line with previous research of anxiety disorders demonstrating delayed extinction as one of the key factors of the disorder (Blechert et al., 2007; Michael et al., 2007). While we

did not study extinction but acquisition retention, our data suggest the clinical relevance of post-acquisition processes (extinction, retention). The fact that symptom related modulations became evident with a delay (not seen during acquisition and only partially present 3-day post-acquisition) suggests an involvement of memory recall: repeated CS presentations and ratings involve repeated recall from memory along with the chances of biases in this process and reconsolidation updating. In fact, memory biases have been repeatedly reported both in the anxiety and the depression literature (Coles and Heimberg, 2002; Watkins, 2002). Interestingly, the role of depression for fear acquisition and extinction has only recently become an active research area (Nissen et al., 2010; Kuhn et al., 2014; Otto et al., 2014), however, with different theoretical assumptions and models. Our results suggest that this is an interesting area for future inquiries.

Despite the strengthening multimodal approach, the current study faces some limitations. First, the fact that the US has no discrete onset makes the computation of a single-event-related potential (i.e. LPP) in response to 'the US' tricky. For example, is a positive deflection at 700 ms an LPP to the video onset or a P300 to the onset of the second word or a P100 to the third word or a mix of everything? It could be argued that the broad LPP acts as a low-pass filter that includes responses to all the elements of the US. With this regard the consistency of a broad LPP deflection across studies is reassuring (Wiggert et al., 2015b). Second, with a CS-US stimulus onset asynchrony of 2.5 s, the electrodermal and cardiac CRs probably did not have sufficient time to unfold and may also influence the following URs. To exclude this possibility future studies should use longer CS duration or CS-US trace intervals or, introduce 'catch trials' (CS presentation with US omission during acquisition) to follow-up on this important issue. Third, the different nature of physical and social USs (electrocutaneous, painful, noxious, time limited vs complex audiovisual, non-painful and social) might be independent of their intensity (social stimuli are not just weaker USs). Future research crossing US intensity with US type (i.e. high vs low intense social USs, high vs low intensity shock) will be relevant here and research has already started to compare different US classes (Delgado et al., 2006; Miedl et al., 2014). Forth, paradigmatic differences (CS- predicting US-neu to control for sensory stimulation during the UR) as well as high similarity between CS and US (actor identity to increase external validity) could have affected our results and led to differences from typical shock-conditioning studies (no stimulus following CS- and no similarity between CS and US), i.e. faster acquisition speed and longer retention. Again future studies would have to independently vary stimulus type and task parameters to confirm this.

The present results of social learning with naturalistic stimuli encourage research along several lines: Primary systems can be mapped on distinct functions of detection, recognition, evaluation, memory and arousal. Long-term memory mechanisms become involved and are associated with clinically significant individual differences. Many questions that have been addressed in the past century in Pavlovian conditioning research (e.g. extinction, reinstatement, renewal, counter conditioning) are now applicable to social neuroscience modeling social environments. Thus, if it is not learning that mostly influences our daily social interactions, then what is it?

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Supplementary data

Supplementary data are available at SCAN online.

Conflict of interest. None declared.

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