

COGNITIVE NEUROSCIENCE

When neutral turns significant: brain dynamics of rapidly formed associations between neutral stimuli and emotional contexts

Carlos Ventura-Bort,^{1,2} Andreas Löw,³ Julia Wendt,¹ Florin Dolcos,⁴ Alfons O. Hamm¹ and Mathias Weymar¹¹Department of Biological and Clinical Psychology, University of Greifswald, Franz-Mehring-Str. 47, 17487 Greifswald, Germany²Department of Basic and Clinical Psychology, and Psychobiology, Universitat Jaume I, Castellón, Spain³Department of Humanities and Social Sciences, Helmut-Schmidt-University/University of the Federal Armed Forces Hamburg, Hamburg, Germany⁴Psychology Department, Neuroscience Program, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Champaign, IL, USA**Keywords:** associative learning, binding, context, emotion, event-related potentials

Edited by Gregor Thut

Received 29 March 2016, revised 17 June 2016, accepted 22 June 2016

Abstract

The ability to associate neutral stimuli with motivationally relevant outcomes is an important survival strategy. In this study, we used event-related potentials (ERPs) to investigate brain dynamics of associative emotional learning when participants were confronted with multiple heterogeneous information. Participants viewed 144 different objects in the context of 144 different emotional and neutral background scenes. During each trial, neutral objects were shown in isolation and then paired with the background scene. All pairings were presented twice to compare ERPs in response to neutral objects before and after single association. After single pairing, neutral objects previously encoded in the context of emotional scenes evoked a larger P100 over occipital electrodes compared to objects that were previously paired with neutral scenes. Likewise, larger late positive potentials (LPPs) were observed over parieto-occipital electrodes (450–750 ms) for objects previously associated with emotional relative to neutral contexts. The LPP – but not P100 – enhancement was also related to subjective object/context binding. Taken together, our ERP data provide evidence for fast emotional associative learning, as reflected by heightened perceptual and sustained elaborative processing for neutral information previously encountered in emotional contexts. These findings could assist in understanding binding mechanisms in stress and anxiety, as well as in addiction and eating-related disorders.

Introduction

One important survival strategy is to perceive fluctuating changes that occur in contiguous environments in order to readjust the momentary motivational relevance of incoming information. This ability allows developing flexible and adaptive responses based on the history of contingencies encountered by the individual (Miskovic & Keil, 2012). In this sense, it has been observed that a previously neutral stimulus (conditioned stimulus; CS+) continuously associated with an aversive event (unconditioned stimulus; UCS) acquires motivational relevance, compared to a neutral stimulus (CS–) unpaired with a UCS or associated with a non-emotional UCS, a process called associative learning. Traditionally, a large number of pairings between few and/or simple CS+ and a strongly aversive

UCS have been used in learning paradigms to generate strong associations (Lissek *et al.*, 2006). However, rather than single, unambiguous and/or isolated CS/UCS pairings, we are constantly confronted with multiple different events that imply associations between neutral and moderately relevant stimuli. Thus, the use of paradigms involving ‘weak’ ambiguous situations (e.g. less salient UCS, multiple complex pairings and/or few contingencies CS/UCS) would provide a better understanding of the underpinnings of associative learning (Lissek *et al.*, 2006; Beckers *et al.*, 2013; Steinberg *et al.*, 2013b; Hur *et al.*, in press). In the present study, we investigated the role of UCS heterogeneity on the formation of associations using electrophysiological correlates of associative learning for multiple neutral events paired with multiple emotional contingencies (emotional scenes).

Recent studies from Junghöfer and colleagues (e.g. Pastor *et al.*, 2015; see Steinberg *et al.*, 2013b, for review) used the so-called

Correspondence: Carlos Ventura-Bort and Mathias Weymar, as above.

E-mails: carlos.ventura-bort@uni-greifswald.de and mathias.weymar@uni-greifswald.de

MultiCS conditioning, in which multiple CSs+ (e.g. pictures of different faces) were associated with emotionally relevant UCSs (e.g. aversive and appetitive sounds, electric shocks), while other CSs– remained unpaired or were associated with neutral events. Brain activation was measured using electro- and magnetoencephalography (EEG, MEG) during these conditioning procedures. After multiple pairings, CSs+ compared to CSs– evoked enhanced neural activity at prefrontal and sensory cortical regions during earlier (< 300 ms; Bröckelmann *et al.*, 2011; Steinberg *et al.*, 2012, 2013a; Rehbein *et al.*, 2014, 2015) and later stages of processing (> 300 ms; Pastor *et al.*, 2015), irrespective of contingency awareness. These results suggest the existence of a rather automatic learning mechanism that rapidly transfers the emotional properties of the UCS to CSs, leading to a facilitated perceptual and a more elaborated processing of the CS+.

Nevertheless, these studies have only used highly salient UCS. Therefore, it is unclear whether such associative learning processes also occur in the presence of less intense emotional events – i.e., reproducing daily interactions – or whether the formation of associations is exclusively facilitated in survival-specific contexts (Öhman & Mineka, 2001). It is also unclear whether the acquired motivational significance leading to neural response enhancement for emotion-associated stimuli occurs rapidly after one single pairing (e.g. Morel *et al.*, 2012; Rehbein *et al.*, 2014), or whether more than one repetition is needed to form such associations (e.g. Steinberg *et al.*, 2012). While most of the electrophysiological conditioning studies have used aversive cues as UCS (see Miskovic & Keil, 2012, for review), it has recently been observed that pleasant information can also serve as effective, intrinsically motivating UCSs (Schacht *et al.*, 2012; Steinberg *et al.*, 2013a; Blechert *et al.*, 2016; see Martin-Soelch *et al.*, 2007, for neuroimaging findings). Both aversive and appetitive conditioning processes likely not only contribute to various disorders, such as trauma- and stress-related disorders, but also to substance abuse and eating-related disorders (e.g. Martin-Soelch *et al.*, 2007; Pape & Pare, 2010). Thus, more evidence regarding the effect of valence on associative conditioning is needed.

In the present study, we therefore investigated brain dynamics of associative emotional learning when participants viewed neutral objects in the context of different emotionally arousing (both

pleasant and unpleasant) and neutral background scenes. Object and scene presentation occurred always in the same order; first objects were presented in isolation (CS) and then a picture scene was added as background (see Fig. 1). Pairings were presented in two consecutive blocks, allowing to compare the processing of CS+ objects – paired with emotional scenes – and CS– objects – paired with neutral scenes, before (first block) and after single pairing (second block). Based on previous EEG and MEG conditioning studies (see Miskovic & Keil, 2012, for review), we predicted enhanced processing of neutral cues previously paired with emotional contexts, irrespective of valence, relative to cues previously paired with neutral contexts at different stages of processing. Because both perceptual and sustained elaborative processing have been found to be enhanced for stimuli associated with CS+, we predicted enhanced positivity for the CS+ compared with the CS– at (a) earlier (P100) and (b) later stages of processing [late positive potential (LPP)].

Materials and methods

Participants

Thirty-one students (27 women, 4 men; mean age = 22.7 years; three left-handed) from the University of Greifswald participated in the study for course credits or financial compensation. All participants had normal or corrected-to-normal vision, and provided written informed consent for a protocol approved by the Review Board of the German Psychological Society (DGPs). Data from six participants were excluded due to software problems (one participant) or excessive artifacts in the EEG data (five participants).

Stimulus materials

Overall, 288 neutral objects (CSs) together with 96 emotionally arousing (UCSs) and 48 neutral background scenes were used as stimulus material. Neutral objects were selected from the following two different standardized sets: The Bank of Standardized Stimuli (BOSS); Brodeur *et al.*, 2010 and the ecological adaptation of Snodgrass and Vanderwart (Moreno-Martínez & Montoro, 2012). In order to assure variability between multiple CSs, objects were

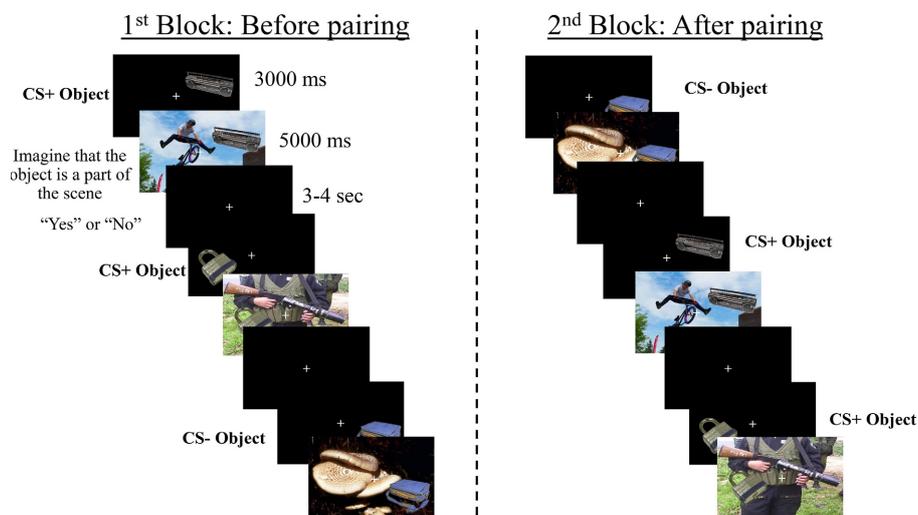


FIG. 1. Schematic view of the stimulus presentation during the first and the second associative learning blocks. When an object was seen during the first block, the object/background association has not yet taken place, and when the object was seen in the second block, object and background scene have been associated once. [Correction added on 19 Aug 2016, after original online publication: Figure 1 has been corrected.]

selected to belong to different semantic categories (e.g. office supplies, electronics, household objects). Objects were grouped in six different sets of 48 items each, carefully matched in terms of semantic category, familiarity, object agreement and manipulability according to the normative ratings of the standard samples (see BOSS and ecological adaptation of Snodgrass and Vanderwart norms). Each participant viewed three (out of six) different sets, consisting of a total of 144 neutral objects. Half of the neutral objects (144) were used in a recognition memory task that occurred 1 week later (published elsewhere, see Ventura-Bort *et al.*, 2016). One hundred and forty-four background scenes – 48 pleasant, 48 neutral and 48 unpleasant – were selected from the International Affective Picture System (IAPS; Lang *et al.*, 2008) as UCS. To ensure the heterogeneity between the UCSs, the pictures were carefully selected to include different contents. Specifically, the pleasant category comprised pictures of erotica, adventure and nurturance contents; the neutral category contained pictures of buildings, landscapes and neutral human faces and the unpleasant category included pictures showing mutilation, attack and disgust/accident. Mean (SD) valence and arousal ratings were 6.94 (0.63) and 5.84 (0.77) for pleasant, 5.27 (0.82) and 3.51 (0.57) for neutral and 2.73 (0.85) and 5.93 (0.57) for unpleasant pictures. Pleasant and unpleasant pictures did not differ in emotional arousal ($F < 1$). All pictures were matched for complexity, brightness and contrast (all P s > 0.23). Object sets were counterbalanced across participants, so that each set was paired with each emotional picture category equally often.

Procedure

The experiment took place in a sound-attenuated, dimly lit room. After electrodes were attached, participants were seated in a reclining chair and were instructed to avoid eye blinks and body movements during event-related potential (ERP) measurement. The session consisted of two blocks in which 144 different neutral CS objects were presented superimposed on 144 different background scenes. Each CS was first presented alone for 3000 ms in one of the four quadrants of a blank screen. The four positions of the CS were selected randomly and with the same probability for each background scene category. Then, while the CS remained on the screen, the UCS was added as background. Each CS/UCS pairing was presented for 5000 ms. Inter-trial intervals (ITIs) were 3000, 3500 or 4000 ms. One of the 48 objects set (CS+) was paired with unpleasant UCSs, one set (CS+) with pleasant UCSs, and one set (CS-) with neutral scenes (i.e., no motivationally relevant UCSs were presented). Sets were counterbalanced across participants. To facilitate CS/UCS associative binding, participants were instructed to imagine that the object was a part of the scene and to indicate after CS/UCS offset whether the imagination was successful or not by pressing a 'yes' or a 'no' button on a response pad (see Fig. 1). Finally, all 96 CS/UCS pairings and all 48 CSs and UCSs/neutral pairs were presented in a first block and, after 1 min break, the same 144 CSs and UCSs/neutral scenes were repeated in a second block, but in a different randomized order.

Apparatus and data analysis

Electroencephalography signals were recorded continuously from 257 electrodes using an Electrical Geodesics (EGI) HydroCel high-density EEG system with NETSTATION software (4.3.1) and a NA 300 amplifier on a Macintosh computer. The EEG recording was digitized at a rate of 250 Hz, using vertex sensor (Cz) as recording reference. Scalp impedance for each sensor was kept below 30 k Ω , as recommended

by the manufacturer guidelines. All channels were band-pass filtered online from 0.1 to 100 Hz. Offline reduction was performed using Electro-Magnetic-EncephaloGraphy Software (EMEGS; Peyk *et al.*, 2011) and included low-pass filtering at 40 Hz, artifact detection, sensor interpolation, baseline correction and conversion to the average reference (Junghöfer *et al.*, 2000). Stimulus-synchronized epochs were extracted from 100 ms prior to 1200 ms after onset of the object and baseline corrected (100 ms prior to stimulus onset). Each extracted epoch was corrected for eye movement and blink artifacts using the MATLAB-based toolbox BIOSIG (Vidaurre *et al.*, 2011).

For each participant, separated ERP averages were computed for each sensor and condition. Based on previous research (Pizzagalli *et al.*, 2003; Liu *et al.*, 2012b; Schacht *et al.*, 2012; Pastor *et al.*, 2015), two ERP components were analyzed: the P100 component, as an index of early perceptual processing (Pizzagalli *et al.*, 2003; Liu *et al.*, 2012b; Schacht *et al.*, 2012), and the LPP, considered as an index of motivationally driven, sustained attentional processing (Schupp *et al.*, 2000; Pizzagalli *et al.*, 2003; Heim & Keil, 2006; Ferrari *et al.*, 2008; Bradley, 2009; Pastor *et al.*, 2015). For the P100, ERP mean amplitudes were averaged over lateral occipital brain regions that encompassed the EGI sensors 95, 96, 104, 105, 106, 107, 113, 114, 115, 122 (left) and 159, 160, 167, 168, 169, 170, 176, 177, 178, 189 (right; see Fig. 2A, inset). To determine the time window, we identified the maximum P1 peak latency (162 ms) based on the overall grand average across conditions and defined a symmetrical time epoch from 140 to 184 ms for subsequent analysis (see Appendix 1). For the LPP, mean ERP amplitudes were analyzed over a cluster of parieto-occipital sensors (EGI sensors: 85, 86, 87, 88, 89, 96, 97, 98, 99, 100, 101, 106, 107, 108, 109, 110, 118, 119, 127, 128, 129, 130, 140, 141, 142, 151, 152, 153, 160, 161, 162, 169, 170 and 171; see Fig. 3A, inset). Due to the difficulty to extract a maximum peak for the LPP (Schupp *et al.*, 2000), a 450–750 ms time window was selected for the LPP based on previous literature and visual inspection of the waveforms.

To investigate the emotion's role in subjective object/context binding, we also compared response rates of successful object/context imaginations. Response rates were analyzed using a repeated measures ANOVA including the within-subjects factors Block (first block vs. second block) and Category (pleasant vs. neutral vs. unpleasant).

To determine associative learning, we compared ERPs in response to CS objects in the first block, relative to ERP responses to CS objects in the second block. Therefore, mean ERP data were analyzed using a repeated measures ANOVA including the within-subjects factors Block (first block vs. second block) and Category (pleasant vs. neutral vs. unpleasant). If no significant Block \times Category interaction was observed, additional exploratory analyses were performed collapsing both emotional picture categories together into one emotional category. Then, a repeated measures ANOVA was conducted including the within-subjects factors Block and Emotion (neutral vs. emotional). The factor Laterality (left vs. right) was added as a further within-subjects factor for the P100 component.

Finally, a correlation analysis was performed to determine possible relations between ERP and behavioral data. Therefore, subjective object/context imagination (CS/UCS; CS/neutral scene) was correlated with the mean amplitudes of the P100 and LPP.

Results

Behavioral data

Analyses revealed a main effect of Block ($F_{1,24} = 4.29$, $P = 0.049$, $\eta_p^2 = 0.15$) and Category ($F_{2,48} = 16.46$, $P < 0.0001$, $\eta_p^2 = 0.4$),

but there was no Block \times Category interaction ($F_{2,48} = 1.12$, $P = .33$, $\eta_p^2 = 0.04$). *Post hoc* comparisons revealed that subjective object/context imagination was less successful in aversive CS+ (mean = 0.74) compared to appetitive CS+ (mean = 0.86), $t(24) = 5.79$, $P < 0.0001$, $d = 1.15$, and CS- (mean = 0.88), $t(24) = 5.12$, $P < 0.0001$, $d = 1.02$. No differences were observed between appetitive CS+ and CS-, $t(24) = 1.37$, $P < 0.18$, $d = 0.27$.

ERP data

P100 (140–184 ms)

Figure 2A illustrates grand-averaged ERPs collapsed across the occipital cluster for objects paired with emotional backgrounds (CS+ objects) and objects paired with neutral backgrounds (CS- objects) during the first and the second blocks.

A main effect of Block ($F_{1,24} = 5.71$, $P = 0.03$, $\eta_p^2 = 0.19$) and Category were observed, $F_{2,48} = 5.35$, $P = 0.008$, $\eta_p^2 = 0.19$. Although the interaction Block \times Category did not reach significance ($F_{2,48} = 1.99$, $P = 0.15$, $\eta_p^2 = 0.08$), a significant quadratic trend was observed ($F_{1,24} = 4.59$, $P = 0.043$, $\eta_p^2 = 0.16$), suggesting that the activity for appetitive and aversive CS+ increased in the second block compared to CS- objects. Neither a main effect of Laterality ($F < 1$, $P > 0.36$) nor other interaction effects were found ($F_s < 1$, $P_s > 0.42$). *Post hoc* comparisons revealed that both CS+ objects, relative to CS- objects prompted a larger P100 in the second block (objects paired with pleasant scenes vs. objects paired with neutral scenes, $t(24) = 3.04$, $P = 0.006$, $d = 0.61$; objects paired with unpleasant scenes vs. objects paired with neutral scenes, $t(24) = 3.31$, $P = 0.003$, $d = 0.66$; see Fig. 2B), but not in the first block ($t_s < 1$, $P_s > 0.45$). No differences were observed between the appetitive and aversive CS+ objects during the second block ($t < 1$, $P > 0.61$).

When pleasant and unpleasant objects were collapsed into an Emotional category, statistical analyses showed a main effect of Emotion, indicating a larger P100 for those objects associated with emotional background scenes (CS+) compared to objects paired with neutral scenes (CS-; Emotion: $F_{1,24} = 8.16$, $P = 0.009$, $\eta_p^2 = 0.25$). Critically for demonstrating an associative learning process, this effect was driven by CS+/CS- differences during the second block, supported by an interaction between Block and Emotion ($F_{1,24} = 4.58$, $P = 0.043$, $\eta_p^2 = 0.16$). Neither the main effect of Block ($F_{1,24} = 2.47$, $P = 0.13$, $\eta_p^2 = 0.1$) nor Laterality ($F < 1$, $P > 0.5$) reached significance. No other interaction effects were found ($F_s < 1$, $P_s > 0.44$). Follow-up comparisons confirmed that CS+ items prompted a larger positivity in the second compared to the first block ($t[24] = 3.04$, $P = 0.006$, $d = 0.61$). Moreover, CS+ objects presented in the second block also elicited an enhanced P100 in comparison to CS- objects presented in the first ($t[24] = 2.95$, $P = 0.007$, $d = 0.6$), and in the second block ($t[24] = 3.77$, $P = 0.001$, $d = 0.75$; see Fig. 2C). No ERP differences were found between blocks for CS- objects, or between CS- and CS+ objects during the first block ($t_s < 1$, $P_s > 0.34$).

In sum, we found that single pairing of neutral information with emotional experience modulates subsequent early visual processing when encountering this stimulus again.

LPP (450–750 ms)

Figure 3A illustrates grand average ERPs extracted from parieto-occipital regions for CS+ and CS- objects during the first and the second blocks.

Analyses showed a main effect of Block ($F_{1,24} = 7.92$, $P = 0.01$, $\eta_p^2 = 0.25$) and Category ($F_{2,48} = 4.88$, $P = 0.02$, $\eta_p^2 = 0.18$). Moreover, the interaction Block \times Category approached

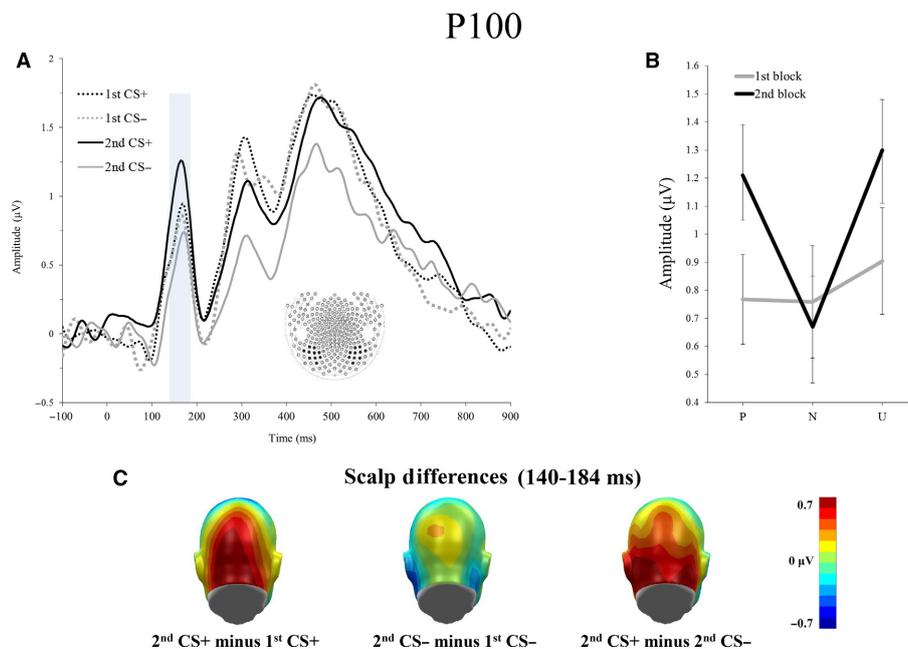


FIG. 2. Objects encoded in emotional contexts modulate early visual processing (P100). (A) Grand average ERPs prompted by CS+ objects (black lines) and CS- objects (gray lines) presented during the first (dotted lines) and the second block (thick lines). ERPs were averaged across channels within an occipital cluster (see inset). (B) Mean (SE) ERPs collapsed across lateralized clusters during the 140–184 ms for CS+ objects previously paired with pleasant images (P), CS- objects paired with neutral images (N) and CS+ objects associated with unpleasant images (U). (C) Display of the scalp topographies for the ERP differences during the 140–184 ms time window between first and second blocks for CS+ (left) and CS- (middle) objects, and between CS+ and CS- objects during the second block (right).

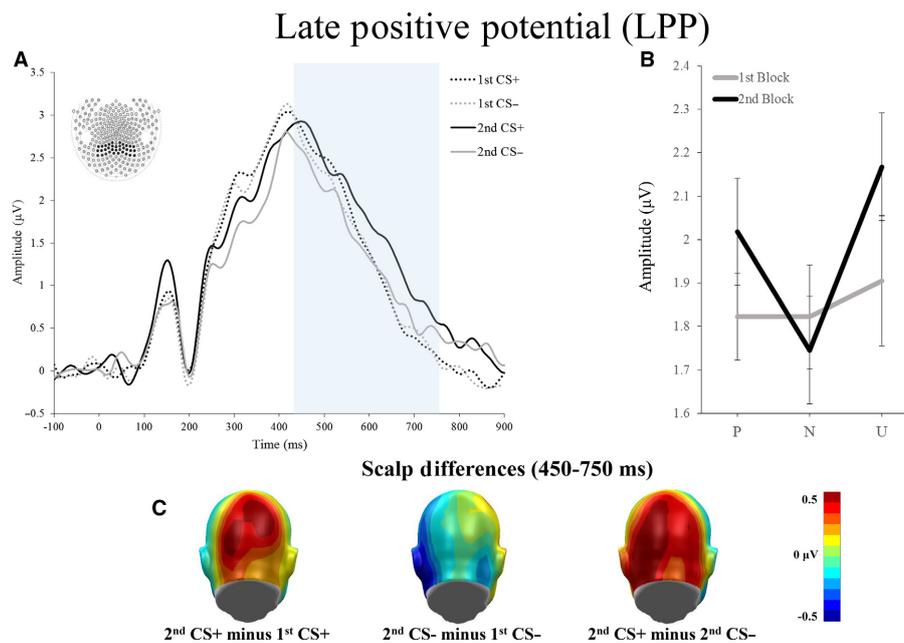


FIG. 3. Objects from emotional contexts prompt larger late positive potentials (LPPs) indicating motivational relevance. (A) Grand average ERPs prompted by CS+ objects (black lines) and CS− Objects (gray lines) presented during the first (dotted lines) and the second block (thick lines). ERPs were averaged across channels within a parieto-occipital cluster (see inset). (B) Mean (SE) ERPs collapsed across electrodes during the 450–750 ms for CS+ objects from pleasant contexts (P), CS− objects from neutral contexts (N) and CS+ objects from unpleasant contexts (U). (C) Display of the scalp topographies for the ERP differences during the 450–750 ms time window between first and second blocks for CS+ (left) and CS− (middle) objects, and between CS+ and CS− objects during the second block (right).

significance, $F_{2,48} = 2.51$, $P = 0.09$, $\eta_p^2 = 0.1$, showing a significant quadratic trend ($F_{1,24} = 4.74$, $P = 0.039$, $\eta_p^2 = 0.17$) that suggests that the activity for appetitive and aversive CS+ objects increased in the second block compared to CS− objects. *Post hoc* comparisons showed that both objects bound with pleasant and unpleasant relative to neutral background scenes elicited an enhanced LPP after the first pairing (objects paired with pleasant scenes vs. objects paired with neutral scenes, $t[24] = 2.51$, $P = 0.02$, $d = 0.5$; objects paired with unpleasant scenes vs. objects paired with neutral scenes, $t[24] = 3.31$, $P = 0.003$, $d = 0.66$; see Fig. 3B), but not before the pairing ($ts < 1$, $Ps > 0.45$). No differences were observed between objects associated with unpleasant and pleasant scenes during the second block, $t(24) = 1.27$, $P = 0.23$, $d = 0.25$.

After collapsing pleasant and unpleasant CS+ into an Emotional category, a trend main effect for the factor Block ($F_{1,24} = 3.73$, $P = 0.07$, $\eta_p^2 = 0.13$), and a main effect of the factor Emotion ($F_{1,24} = 7.96$, $P = 0.009$, $\eta_p^2 = 0.25$) were observed. As for the P100, larger ERP positivity was present for CS+, compared to CS− after single pairing with UCS as indicated by the Block \times Emotion interaction ($F_{1,24} = 4.74$, $P = 0.04$, $\eta_p^2 = 0.17$). Single comparisons revealed that CS+ items evoked a significantly larger ERP activity in the second compared to the first block, $t(24) = 3.75$, $P = 0.001$, $d = 0.75$. Larger ERP positivity was also observed for CS+ relative to CS− objects during both the first, $t(24) = 3.35$, $P = 0.003$, $d = 0.66$, and the second block, $t(24) = 3.36$, $P = 0.007$, $d = 0.67$ (see Fig. 3C). No differences were found between CS+ and CS− objects during the first block, or between the first and the second blocks for CS− objects ($ts < 1$, $Ps > 0.69$).

To summarize, neutral information from emotional, but not neutral, contexts prompt elaborative processing as indicated by enhanced late positive potentials.

Correlation between subjective object/context association (CS/UCS) and ERPs

Correlational analyses showed a positive relation between successful object/context imagination rates during the first block and LPP activity during the second block for both objects bound with neutral ($r_s = 0.42$, $P = 0.04$) and emotional ($r_s = 0.64$, $P = 0.0005$) contexts. Furthermore, a significant correlation between LPP amplitudes for objects bound with emotional contexts and successful object/context imagination rates in the second block was found ($r_s = 0.42$, $P = 0.03$). This relation was not found for objects paired with neutral backgrounds ($r_s = 0.07$, $P = 0.71$). No significant correlations were found between behavioral performance and P100 amplitudes ($r_s < 0.24$, $P < 24$).

In sum, successful subjective object/context binding was related to larger LPP – but not P100 – amplitudes. LPP amplitudes were associated with better subjective object/context association when the context was emotional.

Discussion

In the present study, brain potentials were used to investigate neural processing when multiple neutral objects were associated with either emotional or neutral background scenes. Our results showed that, (a) after a single pairing with an emotional event, neutral CS+ objects compared to objects paired with neutral events (CS−) evoked an enhanced positivity (P100) at occipital regions in the 140–184 ms time window. These differences in ERP positivity can be interpreted as a result of an associative learning process because they were not present prior to the initial pairing (first block). Likewise, (b) objects paired with emotionally arousing scenes, compared to objects paired with neutral scenes, elicited a larger LPP at

parieto-occipital electrodes between 450 and 750 ms after stimulus onset. The LPP enhancement was also related to better subjective context/item binding. Our data provide evidence for rapid item/context learning (i.e. after one pairing), as reflected by enhanced perceptual and sustained elaborated processing for items from emotional contexts.

The P100 component is assumed to indicate amplification of sensory visual information, reflecting perceptual processing in the extrastriate visual cortex (Clark *et al.*, 1995; Di Russo *et al.*, 2002). Some studies have observed enhanced P100 amplitudes for emotional compared to neutral visual stimuli (e.g. Carretié *et al.*, 2004; Pourtois *et al.*, 2005; Rellecke *et al.*, 2011), suggesting that perceptual attentional resources may be allocated toward emotionally arousing events, via amygdala projections (Rotshtein *et al.*, 2010). Learning studies also showed that conditioned visual stimuli associated with positive (Schacht *et al.*, 2012) or negative UCS evoke more pronounced P100 ERP (Pizzagalli *et al.*, 2003; Fritsch & Kuchinke, 2013) and electromagnetic field activation (Dolan *et al.*, 2006; Steinberg *et al.*, 2012, 2013a), reflecting activity in visual-related areas (Pizzagalli *et al.*, 2003; Dolan *et al.*, 2006). Enhanced perceptual activation for emotionally conditioned events has been attributed to the learning history of the individual that tunes the sensitivity of sensory neural populations to specific emotional features by means of re-shaping the processing of incoming sensory information (Miskovic & Keil, 2012; Steinberg *et al.*, 2013b). Some studies suggest that the short-term neural reorganization in sensory-related regions occurs after several associations (Stolarova *et al.*, 2006; Keil *et al.*, 2007; Hintze *et al.*, 2014). Recent research using MultiCS paradigms, however, found that CS+ could also produce changes in visual- and auditory-related areas in a very fast fashion – i.e. after few or single associations (Bröckelmann *et al.*, 2011; Steinberg *et al.*, 2012; Rehbein *et al.*, 2014). This study extends these findings showing that one single association between neutral objects and relatively weak UCSs (images) is enough to readjust the visual processing of relevant information based on a rapid update of its motivational value, evoking enhanced P100 amplitudes in response to neutral cues bound to or associated with emotional contexts.

In addition, CS+ objects also evoked a larger positive-going waveform over parieto-occipital electrodes between 450 and 750 ms, relative to the CS– objects. This later component corresponds to the LPP and extends previous findings using aversive (Pizzagalli *et al.*, 2003) and appetitive UCSs (Schacht *et al.*, 2012; Blechert *et al.*, 2016), and multiple CS/UCS pairings (Pastor *et al.*, 2015). Enhanced LPP during encoding of motivationally significant – appetitive and aversive – stimuli is a hallmark finding in affective neuroscience (Cuthbert *et al.*, 2000; Schupp *et al.*, 2000; Foti *et al.*, 2009). In the current study, we found similar ERP positivity for neutral objects paired with emotional scenes as consistently found for emotional images (see Appendix 2). While enhanced LPPs to emotional scenes have been reliably found over central-parietal areas (Cuthbert *et al.*, 2000), which may represent aggregated activity from ventral, dorsal and occipital visual cortex (e.g. Sabatinelli *et al.*, 2013), the current study showed a more posterior distribution of the enhanced LPP, which is likely due to the involvement of occipitotemporal regions in contextual object-processing (Bar, 2004). Because LPPs are related to initial orienting, sustained perceptual processing, and motivated actions (Bradley, 2009; Lang & Bradley, 2010), our data suggest that objects associated with both appetitive and aversive scenes received sustained elaborated processing due to the acquired motivational relevance.

In a recent study, Mueller & Pizzagalli (2016) demonstrated that rapid perceptual enhancement for fear-conditioned events could occur when conditioning acquisition took place in a remote past. These authors observed that CS+ faces produced enhanced early ERP activity 1 year after the CS/UCS association, suggesting that associative learning processes can produce long-lasting changes, even when participants were fully unaware of the CS/UCS contingency. In another study, Dolcos & Cabeza (2002) observed that enhanced elaborated processing during encoding, as indexed by LPP amplitude, predicted better long-term memory performance for emotional information. Consistent with this, we have recently observed that objects paired with emotional, relative to neutral contexts, elicited enhanced electrophysiological correlates implicated in mnemonic retrieval processing (Ventura-Bort *et al.*, 2016). The present study, thus, indicate that faster enhanced perceptual and elaborative processes of neutral information bound to emotional compared to neutral contexts might also promote memory storage (Dolcos & Cabeza, 2002; Weymar *et al.*, 2012; Ventura-Bort *et al.*, 2016).

Human brain imaging studies (Vuilleumier *et al.*, 2004; Vuilleumier & Driver, 2007; Wendt *et al.*, 2011) have observed that the amygdala plays an important role in the processing of stimulus salience (e.g. Adolphs, 2010; Weymar & Schwabe, 2016). Moreover, there are strong associations between high cortical areas and the amygdala (e.g. Sabatinelli *et al.*, 2005, 2014; Liu *et al.*, 2012a), suggesting that the affective evaluation of visual stimulation results from re-entrant projections between subcortical and cortical brain regions (Vuilleumier, 2005; Pessoa & Adolphs, 2010). It has been proposed that the amygdala is responsible of identifying the conditioned stimuli as emotionally relevant and providing ongoing feedback to higher cortical brain regions such as inferotemporal, occipital and prefrontal cortex (Keil *et al.*, 2009; Sabatinelli *et al.*, 2009, 2014; Liu *et al.*, 2012a) that give rise to a cascade of perceptual, attentional and action preparation processes (Lang & Bradley, 2010). Substantiating this view, using simultaneous EEG-fMRI recording, Liu and colleagues (2012a) demonstrated that the amygdala activity modulates the LPP amplitude for emotional stimuli. In the present study, we observed that the encoding of conditioned stimuli was modulated after one pairing with an emotional background scene, which can be mediated by such re-entrant influences from the amygdala.

Taken together, the present findings provide evidence for fast emotional learning when emotional contexts and neutral cues are present. Neutral objects paired with emotional relative to neutral scenes rapidly acquired motivational relevance, engaging heightened perceptual and attentional processes, as indexed by enhanced P100 and LPP amplitudes. On the one hand, the ability to rapidly processing changes for stimuli that signal motivationally significant events may facilitate the detection of relevant information, and the execution of fast motivational responses (Lang *et al.*, 1997), when encountered in the future. On the other hand, however, such cues might also become maladaptive when bound to traumatic memories, by their ability not only to grab attention but also to activate the traumatic memory including exaggerated fear responses. In the same vein, appetitive cues linked to addiction- and food-related contexts are able to prompt impulsive consuming behaviors.

Conflict of interest

The authors declare no competing financial interests.

Supporting Information

Additional supporting information can be found in the online version of this article:

Appendix 1. Short note on P100 latency in the current data.

Appendix 2. ERP analyses of emotional and neutral background processing.

Acknowledgements

This research was supported by a grant from the German Research Foundation (DFG, WE 4801/3-1) to Mathias Weymar at the University of Greifswald.

Abbreviations

CS, conditioned stimulus; EEG, electroencephalography; ERPs, event-related potentials; LPP, late positive potential; UCS, unconditioned stimulus.

References

- Adolphs, R. (2010) What does the amygdala contribute to social cognition? *Ann. N. Y. Acad. Sci.*, **1191**, 42–61.
- Bar, M. (2004) Visual objects in context. *Nat. Rev. Neurosci.*, **5**, 617–629.
- Beckers, T., Krypotos, A.-M., Boddez, Y., Eftting, M. & Kindt, M. (2013) What's wrong with fear conditioning? *Biol. Psychol.*, **92**, 90–96.
- Blechert, J., Testa, G., Georgii, C., Klimesch, W. & Wilhelm, F.H. (2016) The Pavlovian craver: neural and experiential correlates of single trial naturalistic food conditioning in humans. *Physiol. Behav.*, **158**, 18–25.
- Bradley, M.M. (2009) Natural selective attention: orienting and emotion. *Psychophysiology*, **46**, 1–11.
- Bröckelmann, A.-K., Steinberg, C., Elling, L., Zwanzger, P., Pantev, C. & Junghöfer, M. (2011) Emotion-associated tones attract enhanced attention at early auditory processing: magnetoencephalographic correlates. *J. Neurosci.*, **31**, 7801–7810.
- Brodeur, M.B., Dionne-Dostie, E., Montreuil, T. & Lepage, M. (2010) The bank of standardized stimuli (BOSS), a new set of 480 normative photos of objects to be used as visual stimuli in cognitive research. *PLoS One*, **5**, e10773.
- Busch, N.A., Debener, S., Kranczoch, C., Engel, A.K. & Herrmann, C.S. (2004) Size matters: effects of stimulus size, duration and eccentricity on the visual gamma-band response. *Clin. Neurophysiol.*, **115**, 1810–1820.
- Caretta, L., Tapia, M., Mercado, F., Albert, J., López-Martín, S. & De La Serna, J.M. (2004) Voltage-based versus factor score-based source localization analyses of electrophysiological brain activity: a comparison. *Brain Topogr.*, **17**, 109–115.
- Clark, V., Fan, S. & Hillyard, S. (1995) Identification of early visual evoked potential generators by retinotopic and topographic analyses. *Hum. Brain Mapp.*, **187**, 170–187.
- Cuthbert, B.N., Schupp, H.T., Bradley, M.M., Birbaumer, N. & Lang, P.J. (2000) Brain potentials in affective picture processing: covariation with autonomic arousal and affective report. *Biol. Psychol.*, **52**, 95–111.
- Di Russo, F., Martinez, A., Sereno, M.I., Pitzalis, S. & Hillyard, S.A. (2002) Cortical sources of the early components of the visual evoked potential. *Hum. Brain Mapp.*, **15**, 95–111.
- Dolan, R.J., Heinze, H.J., Hurlmann, R. & Hinrichs, H. (2006) Magnetoencephalography (MEG) determined temporal modulation of visual and auditory sensory processing in the context of classical conditioning to faces. *NeuroImage*, **32**, 778–789.
- Dolcos, F. & Cabeza, R. (2002) Event-related potentials of emotional memory: encoding pleasant, unpleasant, and neutral pictures. *Cogn. Affect. Behav. Ne.*, **2**, 252–263.
- Ferrari, V., Codispoti, M., Cardinale, R. & Bradley, M.M. (2008) Directed and motivated attention during processing of natural scenes. *J. Cognitive Neurosci.*, **20**, 1753–1761.
- Foti, D., Hajcak, G. & Dien, J. (2009) Differentiating neural responses to emotional pictures: evidence from temporal-spatial PCA. *Psychophysiology*, **46**, 521–530.
- Fritsch, N. & Kuchinke, L. (2013) Acquired affective associations induce emotion effects in word recognition: an ERP study. *Brain Lang.*, **124**, 75–83.
- Heim, S. & Keil, A. (2006) Effects of classical conditioning on identification and cortical processing of speech syllables. *Exp. Brain Res.*, **175**, 411–424.
- Hintze, P., Junghöfer, M. & Bruchmann, M. (2014) Evidence for rapid prefrontal emotional evaluation from visual evoked responses to conditioned gratings. *Biol. Psychol.*, **99C**, 125–136.
- Hur, J., Jordan, A.D., Berenbaum, H. & Dolcos, F. (in press) Emotion-attention interactions in fear conditioning: moderation by executive load, neuroticism, and awareness. *Biol. Psychol.*
- Isik, L., Meyers, E.M., Leibo, J.Z. & Poggio, T. (2014) The dynamics of invariant object recognition in the human visual system. *J. Neurophysiol.*, **111**, 91–102.
- Junghöfer, M., Elbert, T., Tucker, D.M. & Rockstroh, B. (2000) Statistical control of artifacts in dense array EEG/MEG studies. *Psychophysiology*, **37**, 523–532.
- Keil, A., Stolarova, M., Moratti, S. & Ray, W.J. (2007) Adaptation in human visual cortex as a mechanism for rapid discrimination of aversive stimuli. *NeuroImage*, **36**, 472–479.
- Keil, A., Sabatinelli, D., Ding, M., Lang, P.J., Ihssen, N. & Heim, S. (2009) Re-entrant projections modulate visual cortex in affective perception: evidence from granger causality analysis. *Hum. Brain Mapp.*, **540**, 532–540.
- Lang, P.J. & Bradley, M.M. (2010) Emotion and the motivational brain. *Biol. Psychol.*, **84**, 437–450.
- Lang, P.J., Bradley, M.M. & Cuthbert, B.N. (1997) Motivated attention: affect, activation and action. In Lang, P.J., Simons, R.F. & Balaban, M.T. (Eds), *Attention and Orienting Sensory and Motivational Processes*. Lawrence Erlbaum Associates, Hillsdale, NJ, pp. 97–135.
- Lang, P.J., Bradley, M.M. & Cuthbert, B.N. (2008) *International affective picture system (IAPS): affective ratings of pictures and instruction manual* (Technical Report No. A-8). University of Florida, Gainesville.
- Lissek, S., Pine, D.S. & Grillon, C. (2006) The strong situation: a potential impediment to studying the psychobiology and pharmacology of anxiety disorders. *Biol. Psychol.*, **72**, 265–270.
- Liu, Y., Huang, H., McGinnis-Deweese, M., Keil, A. & Ding, M. (2012a) Neural substrate of the late positive potential in emotional processing. *J. Neurosci.*, **32**, 14563–14572.
- Liu, Y., Keil, A. & Ding, M. (2012b) Effects of emotional conditioning on early visual processing: temporal dynamics revealed by ERP single-trial analysis. *Hum. Brain Mapp.*, **33**, 909–919.
- Martin-Soelch, C., Linthicum, J. & Ernst, M. (2007) Appetitive conditioning: neural bases and implications for psychopathology. *Neurosci. Biobehav. R.*, **31**, 426–440.
- Miskovic, V. & Keil, A. (2012) Acquired fears reflected in cortical sensory processing: a review of electrophysiological studies of human classical conditioning. *Psychophysiology*, **49**, 1230–1241.
- Morel, S., Beaucousin, V., Perrin, M. & George, N. (2012) Very early modulation of brain responses to neutral faces by a single prior association with an emotional context: evidence from MEG. *NeuroImage*, **61**, 1461–1470.
- Moreno-Martínez, F.J. & Montoro, P.R. (2012) An ecological alternative to Snodgrass & Vanderwart: 360 high quality colour images with norms for seven psycholinguistic variables. *PLoS One*, **7**, 34–42.
- Mueller, E.M. & Pizzagalli, D.A. (2016) One year old fear memories rapidly activate human fusiform gyrus. *Soc. Cogn. Affect. Neur.*, **11**, 308–316.
- Öhman, A. & Mineka, S. (2001) Fears, phobias, and preparedness: toward an evolved module of fear and fear learning. *Psychol. Rev.*, **108**, 483–522.
- Pape, H.C. & Pare, D. (2010) Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiol. Rev.*, **90**, 419–463.
- Pastor, M.C., Rehbein, M.A., Junghöfer, M., Poy, R., López, R. & Moltó, J. (2015) Facing challenges in differential classical conditioning research: benefits of a hybrid design for simultaneous electrodermal and electroencephalographic recording. *Front. Hum. Neurosci.*, **9**, 336.
- Pessoa, L. & Adolphs, R. (2010) Emotion processing and the amygdala: from a “low road” to “many roads” of evaluating biological significance. *Nat. Rev. Neurosci.*, **11**, 773–783.
- Peyk, P., De Cesarei, A. & Junghöfer, M. (2011) Electro Magneto Encephalography Software: overview and integration with other EEG/MEG toolboxes. *Comput. Intell. Neurosci.*, **2011**, 861705.
- Pizzagalli, D.A., Greischar, L.L. & Davidson, R.J. (2003) Spatio-temporal dynamics of brain mechanisms in aversive classical conditioning: high-density event-related potential and brain electrical tomography analyses. *Neuropsychologia*, **41**, 184–194.

- Pourtois, G., Dan, E.S., Grandjean, D., Sander, D. & Vuilleumier, P. (2005) Enhanced extrastriate visual response to bandpass spatial frequency filtered fearful faces: time course and topographic evoked-potentials mapping. *Hum. Brain Mapp.*, **26**, 65–79.
- Rehbein, M.A., Steinberg, C., Wessing, I., Pastor, M.C., Zwitserlood, P., Keuper, K. & Junghöfer, M. (2014) Rapid plasticity in the prefrontal cortex during affective associative learning. *PLoS One*, **9**, e110720.
- Rehbein, M., Wessing, I., Zwitserlood, P., Steinberg, C., Eden, A.S., Dobel, C. & Junghöfer, M. (2015) Cortex activation towards aversively paired faces and enhanced contingency detection are observed in highly trait-anxious women under challenging conditions. *Front. Behav. Neurosci.*, **9**, 1–19.
- Rellecke, J., Palazova, M., Sommer, W. & Schacht, A. (2011) Brain and cognition on the automaticity of emotion processing in words and faces: event-related brain potentials evidence from a superficial task. *Brain Cognition*, **77**, 23–32.
- Rotshtein, P., Richardson, M.P., Winston, J.S., Kiebel, S.J., Vuilleumier, P., Eimer, M. & ... Dolan, R.J. (2010) Amygdala damage affects event-related potentials for fearful faces at specific time windows. *Hum. Brain Mapp.*, **31**, 1089–1105.
- Sabatinelli, D., Bradley, M.M., Fitzsimmons, J.R. & Lang, P.J. (2005) Parallel amygdala and inferotemporal activation reflect emotional intensity and fear relevance. *NeuroImage*, **24**, 1265–1270.
- Sabatinelli, D., Lang, P.J., Bradley, M.M., Costa, V.D. & Keil, A. (2009) The timing of emotional discrimination in human amygdala and ventral visual cortex. *J. Neurosci.*, **29**, 14864–14868.
- Sabatinelli, D., Keil, A., Frank, D.W. & Lang, P.J. (2013) Emotional perception: correspondence of early and late event-related potentials with cortical and subcortical functional MRI. *Biol. Psychol.*, **92**, 513–519.
- Sabatinelli, D., Frank, D.W., Wanger, T.J., Dhamala, M., Adhikari, B.M. & Li, X. (2014) The timing and directional connectivity of human frontoparietal and ventral visual attention networks in emotional scene perception. *Neuroscience*, **277**, 229–238.
- Schacht, A., Adler, N., Chen, P., Guo, T. & Sommer, W. (2012) Association with positive outcome induces early effects in event-related brain potentials. *Biol. Psychol.*, **89**, 130–136.
- Schupp, H.T., Cuthbert, B.N., Bradley, M.M., Cacioppo, J.T., Ito, T. & Lang, P.J. (2000) Affective picture processing: the late positive potential is modulated by motivational relevance. *Psychophysiology*, **37**, 257–261.
- Schupp, H.T., Junghöfer, M., Weike, A.I. & Hamm, A.O. (2003) Emotional facilitation of sensory processing in the visual cortex. *Psychol. Sci.*, **14**, 7–13. Retrieved from <http://www.hubmed.org/display.cgi?uids=12564747>.
- Steinberg, C., Dobel, C., Schupp, H.T., Kissler, J., Elling, L., Pantev, C. & Junghöfer, M. (2012) Rapid and highly resolving: affective evaluation of olfactorily conditioned faces. *J. Cognitive Neurosci.*, **24**, 17–27.
- Steinberg, C., Bröckelmann, A., Dobel, C. & Elling, L. (2013a) Preferential responses to extinguished face stimuli are preserved in frontal and occipito-temporal cortex at initial but not later stages of processing. *Psychophysiology*, **50**, 230–239.
- Steinberg, C., Bröckelmann, A.-K., Rehbein, M., Dobel, C. & Junghöfer, M. (2013b) Rapid and highly resolving associative affective learning: convergent electro- and magnetoencephalographic evidence from vision and audition. *Biol. Psychol.*, **92**, 526–540.
- Stolarova, M., Keil, A. & Moratti, S. (2006) Modulation of the C1 visual event-related component by conditioned stimuli: evidence for sensory plasticity in early affective perception. *Cereb. Cortex*, **16**, 876–887.
- Ventura-Bort, C., Löw, A., Wendt, J., Moltó, J., Poy, R., Dolcos, F., Hamm, A. & Weymar, M. (2016) Binding neutral information to emotional contexts: brain dynamics of recognition after a long retention interval. *Cogn. Affect. Behav. Ne.*, **16**, 234–247.
- Vidaurre, C., Sander, T.H. & Schlögl, A. (2011) BioSig: the free and open source software library for biomedical signal processing. *Comput. Intell. Neurosci.*, **2011**, 935364.
- Vuilleumier, P. (2005) How brains beware: neural mechanisms of emotional attention. *Trends Cogn. Sci.*, **9**, 585–594.
- Vuilleumier, P. & Driver, J. (2007) Modulation of visual processing by attention and emotion: windows on causal interactions between human brain regions. *Philos. T. Roy. Soc. B*, **362**, 837–855.
- Vuilleumier, P., Richardson, M.P., Armony, J.L., Driver, J. & Dolan, R.J. (2004) Distant influences of amygdala lesion on visual cortical activation during emotional face processing. *Nat. Neurosci.*, **7**, 1271–1278.
- Wendt, J., Weike, A.I., Lotze, M. & Hamm, A.O. (2011) The functional connectivity between amygdala and extrastriate visual cortex activity during emotional picture processing depends on stimulus novelty. *Biol. Psychol.*, **86**, 203–209.
- Weymar, M. & Schwabe, L. (2016) Amygdala and emotion: the bright side of it. *Front. Neurosci.*, **10**, 224.
- Weymar, M., Schwabe, L., Löw, A. & Hamm, A.O. (2012) Stress sensitizes the brain: increased processing of unpleasant pictures after exposure to acute stress. *J. Cognitive Neurosci.*, **24**, 1511–1518.