

## Modulation of the C1 Visual Event-related Component by Conditioned Stimuli: Evidence for Sensory Plasticity in Early Affective Perception

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**Previous research has demonstrated optimized processing of motivationally significant stimuli early in perception. In the present study, the time course and underlying mechanisms for such fast differentiation are of interest. We investigated the involvement of the primary visual cortex in affective evaluation of conditioned stimuli (CSs). In order to elicit learning within the visual system we chose affective pictures as unconditioned stimuli and used laterally presented gratings as CSs. Using high-density electroencephalography, we demonstrated modulation of the C1 visual event-related component for threat-related stimuli versus neutral stimuli, which increased with continuing acquisition of affective meaning. The differentiation between aversive and neutral visual stimuli occurred as early as 65–90 ms after stimulus onset and suggested involvement of the primary visual areas in affective evaluation. As an underlying mechanism, we discuss short-term reorganization in visual cortex, enabling sensory amplification of specific visual features that are related to motivationally relevant information.**

**Keywords:** conditioning, emotion, event-related potentials (ERP), human, visual

### Introduction

The perception of motivationally relevant visual stimuli and their efficient processing, which enable the organism to respond in a fast and appropriate way, are crucial human assets. It has been suggested that there is an attentional bias mechanism, which enhances early perception and cognitive processing of highly arousing or threat-related stimuli. Broad evidence for this hypothesis comes from a variety of behavioral, electrophysiological and imaging studies with healthy participants as well as clinical populations (Hartikainen *et al.*, 2000; Carretie *et al.*, 2001; Ohman *et al.*, 2001b; Vuilleumier *et al.*, 2001; Derryberry and Reed, 2002; Smith *et al.*, 2003; Bishop *et al.*, 2004). The tendency for faster orientation towards threat-related stimuli seems to be stronger in anxiety patients and specifically for phobic stimuli (Ohman *et al.*, 2001a; Fox, 2002; Mogg *et al.*, 2004). Previous research has shown optimized processing of affective pictures, sounds, words, emotional faces and aversive smells (Pizzagalli *et al.*, 1999; Bradley and Lang, 2000; Anderson and Phelps, 2001; Bradley *et al.*, 2001; Bensafi *et al.*, 2002; Lewis *et al.*, 2003; Keil and Ihssen, 2004). Conditioned stimuli, often used in experimental settings to study basic learning processes and the acquisition of affective or motivational connotation, have also been reported to elicit faster behavioral responses and enhanced electrophysiological activity in humans (Hermann *et al.*, 2000; Pizzagalli *et al.*, 2003). In the domain of auditory classical conditioning, research has repeatedly shown learning-induced plasticity in the receptive fields of the primary auditory areas in animals (Diamond and Weinberger, 1984; Recanzone

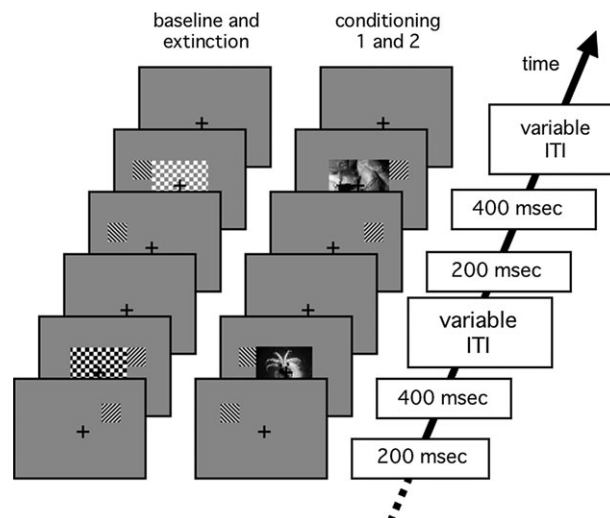
*et al.*, 1993; Scheich *et al.*, 1993; Weinberger, 1998, 2004) and humans (Morris *et al.*, 1998). As a possible underlying mechanism for such fast, experience-dependent cortical reorganization, an increase in dopamine (Bao *et al.*, 2001) or acetylcholine release (Weinberger *et al.*, 1995) have been proposed, possibly leading to long-term potentiation and strengthening neural connectivity. The limbic system, specifically the amygdala, has been thought to mediate these processes (LeDoux *et al.*, 1988). Support for this view comes from neuroanatomical and neuroimaging studies showing strong connections between the amygdaloid regions and primary auditory cortices (LeDoux *et al.*, 1985).

Experience-related changes of visual receptive fields, which are similar to the learning-induced cortical reorganization in primary auditory areas, have repeatedly been reported (Kapadia *et al.*, 1994; Das and Gilbert, 1995). More recently, the interaction between early cortical reorganization and attention has been studied in detail (Gilbert *et al.*, 2000). For instance, Lee and colleagues have demonstrated that increasing behavioral relevance of complex visual stimuli led to preferential processing of these stimuli in V1 and V2 neurons of macaque monkeys (Lee *et al.*, 2002). Studies on early discrimination of aversive visual stimuli in humans also aim to clarify the relationship between motivational significance, attention and early cortical plasticity. A recent line of evidence suggests that this differentiation involves the occipito-temporal cortices (Pizzagalli *et al.*, 2003). Analyses of the topographic distribution of electrophysiological brain responses, specifically of the P3 component, point in the same direction (Baas *et al.*, 2002; Schupp *et al.*, 2004). Affective modulation of earlier ERP responses, e.g. the N1 and the P1 visual components, have also been reported (Keil *et al.*, 2002; Schupp *et al.*, 2003; Delplanque *et al.*, 2004), giving rise to the hypothesis that affective discrimination might be mediated by sensory gain mechanisms as proposed for selective attention (Hillyard *et al.*, 1998; Martinez *et al.*, 2001). In line with that notion, there is growing evidence for increased activation of primary visual areas when emotional stimuli are viewed (Lang *et al.*, 1998; Bradley *et al.*, 2003; Pourtois *et al.*, 2004; Sabatinelli *et al.*, 2004). Taken together with anatomical studies in primates, which have found projections from the amygdala and other limbic regions directly to primary visual areas (Amaral *et al.*, 2003), these observations raise the question of how visual cortices are involved in affective stimulus processing. As outlined above, visual cortical activity might be mediated by the amygdaloid complex, leading to an increase of attention/awareness for affectively arousing stimuli, as has been suggested, for example, by Anderson and Phelps (2001). Consequently, acquisition of motivational significance through learning may involve increasing amplification of threat-relevant

features in primary visual cortices across time. Such tuning of early vision may rely on short-term plastic changes, which are expected to increase during exposition to reinforcement contingencies.

One way to investigate this possibility is to turn to the earliest recordable event-related response of the visual cortex and examine its sensitivity for affective content across trials. Extensive research in the domain of visual event-related potentials (VEPs) has demonstrated the existence of an early negative deflection, peaking at 60–90 ms, and labeled the C1-component, which reflects the initial response of the primary visual cortex to a stimulus (Di Russo *et al.*, 2003). Taking into account the topography of the C1, specifically its retinotopic properties, demonstrated for example by Hillyard and colleagues (e.g. Hillyard and Anllo-Vento, 1998), together with evidence from imaging studies and source analysis approaches, researchers have argued that its neural generators are probably located in deep structures of the primary visual cortex, specifically the calcarine sulcus (Di Russo *et al.*, 2002; Pourtois *et al.*, 2004). It has also been repeatedly demonstrated that the C1, as opposed to, for example, the P1 and N1 components, is not modulated by spatial or feature-based attention tasks when simple neutral visual stimuli, such as gratings or checkerboards, are used (Gomez Gonzalez *et al.*, 1994; Martinez *et al.*, 1999; Fu *et al.*, 2001; Di Russo *et al.*, 2003). In a recent study, Pourtois *et al.* (2004) made use of a spatial attention paradigm adapted for the study of affective modulation. The authors investigated the effects of emotional cues on selective spatial attention in healthy participants, employing high-density electroencephalography (EEG) and source localization techniques. They reported C1 modulation when the initial VEPs to fearful versus happy faces were analyzed, with fearful faces eliciting greater C1 amplitude than happy ones. The authors concluded that the emotional relevance of the stimuli had led to an increased activation within the primary visual cortex, possibly due to an interaction with subcortical structures responsible for the detection of threat-related stimuli in the environment.

In the present study, we sought to replicate and extend these findings by investigating the affective modulations of the C1 visual component across time. Further, we aimed to provide additional evidence for the involvement of early visual processing in the evaluation of affective stimuli and to investigate the acquisition of affective meaning. To this end, we attempted to induce learning within the visual system, employing pictures from the International Affective Pictures System (IAPS) as unconditioned stimuli (UCS) in a classical conditioning paradigm. Building on previous reports discussing the VEP with regard to the C1 component (Kenemans *et al.*, 2000; Fu *et al.*, 2001), we chose small, high-contrast, eccentrically presented gratings to serve as conditioned stimuli eliciting the ERP component of interest. The electrophysiological brain potentials elicited by these originally neutral gratings were recorded during four experimental blocks, using a high-density electrode montage. In the baseline condition, no affective stimuli were presented. The gratings were shown along with affectively neutral checkerboards instead. During the two consecutive conditioning blocks one grating was paired with highly arousing unpleasant pictures, the other one with neutral ones. The extinction condition was identical to the baseline block, with affective pictures being replaced again by checkerboards (Fig. 1). This experimental protocol allowed for an evaluation of the early differentiation between two originally neutral



**Figure 1.** Schematic outline of the experimental design. Small, high-contrast, eccentrically presented gratings served as conditioned stimuli (CSs). In the baseline block the gratings were shown along with neutral checkerboards. During the two consecutive conditioning blocks one grating was paired with highly arousing unpleasant pictures as unconditioned stimuli (UCSs), the other one with neutral ones. The extinction condition was identical to the baseline block, with affective pictures being replaced by checkerboards. Each trial consisted of 200 ms grating presentation, 400 ms UCS and CS presentation and a variable intertrial interval (ITI).

stimuli, both gaining affective meaning through experimentally controlled learning and losing it again in an extinction procedure. Thus, general enhancement of early visual processing for both CSs compared to baseline and extinction was expected. In addition, we expected that with time successful conditioning will lead to differentiation between the CS+ and the CS-, as the CS+ will become associated with an unpleasant event, while the CS- will signal the absence of threat. Again, this discrimination should be highest in the conditioning blocks, reflecting greater sensitivity of visual cortex to CS+ features.

As a measure of emotional learning, we recorded participants' startle responses, elicited by a noise probe in selected trials, while the gratings were presented. Both the CS- and the CS+ can be assumed to be affectively more arousing during conditioning than during baseline and extinction. Hence, during conditioning an increase in startle magnitude for the CS+ and a decrease for the CS- compared to the startle response elicited in the inter-trial intervals (ITIs), as well as to the ones elicited during baseline and extinction, were predicted.

## Materials and Methods

### Participants

Twenty-three volunteers consented to participate in this experiment and either received course credit or a financial incentive of €20 (roughly US\$25 at the time of testing). One subject withdrew from the study prior to the completion of the protocol and these data were not included in the analysis. An additional four data sets were excluded from further processing due to equipment errors. The final data set included 18 participants (9 male, 14 right-handed) with normal or corrected-to-normal vision, age range 19–33 years, of a mean age of 25.6.

### Stimuli

One hundred and twenty pictures were selected from the IAPS based on their normative valence and arousal ratings (Lang *et al.*, 1999). The 60 unpleasant/arousing pictures showed mutilated bodies, attack scenes

and disgusting objects (mean valence: 2.2, SD = 0.6; mean arousal: 6.1, SD = 0.8), the 60 neutral photographs depicted landscapes, people, objects and abstract patterns (mean valence: 5.9, SD = 0.7; mean arousal: 3.8, SD = 0.9). In addition, checkerboards of four different colors (black-bright red, black-dark red, black-bright green and black-dark green) were generated to match the affective pictures in size. The affective pictures and the checkerboard patterns were presented centrally on a 19 inch computer monitor with a refresh rate of 70 Hz and subtended a visual angle of 7.2° horizontally.

Two small black and white squares (horizontal visual angle: 2.2°), differing only in grating orientation (45° or 135°), were used as CS+ and CS-. They had a spatial frequency of 2.3 cycles per degree with 100% contrast. The gratings were presented in the upper left or right visual field (eccentricity of the inner border: 3.58°). White noise bursts (50 ms; 90 dB) were used as auditory startle probes and were delivered binaurally through headphones.

### Procedure

The experimental design consisted of four recording blocks: a baseline block, two consecutive conditioning blocks and a final extinction block. During the conditioning blocks the grating pattern randomly designated as CS+ was presented together with the unpleasant/arousing photographs, used here as unconditioned stimuli (UCS). The other grating pattern (CS-) was paired with the neutral, low arousing pictures; the presentation order was randomized. During the baseline and the extinction blocks, the affective pictures were replaced by the checkerboard patterns described above. They were presented without any systematic relationship between the grating pattern and checkerboard color. The timing and presentation parameters of the affective stimuli and the checkerboards were otherwise identical (see Fig. 1).

A total of 480 standard trials (grating followed by a checkerboard during baseline and extinction, and by an IAPS stimulus during conditioning) were presented in each of the four recording blocks, resulting in 120 trials per condition: grating orientation (45° or 135°) and visual hemifield (left or right) combination. In order to maintain vigilance during baseline and extinction, an additional 60 target trials were included, during which participants were required to respond with a speeded button press to a checkerboard of a certain color. Subjects sat at a distance of 80 cm from the computer screen. They were asked to maintain fixation of a white cross in the middle of the screen present at all times throughout recording. A chin rest was used to ensure consistency of head position and to minimize head movements.

In both the standard and the target trials, the grating patterns (CS+ and CS-) were shown for 600 ms; 200 ms after grating onset, an affective picture or a checkerboard appeared in the center of the screen, the grating square remained attached to the upper left or right corner of the centrally presented stimulus for the rest of the trial (400 ms). The inter-trial interval (ITI) varied randomly between 400 and 1400 ms. The 120 affective pictures (60 unpleasant, 60 neutral) were repeated randomly eight times across the two conditioning blocks, allowing for a total of 240 trials per affective category and conditioning block.

To record subjects' startle responses, 54 startle trials per block were included. Eighteen startle probes were presented during randomly selected ITIs, 36 were delivered along with the conditioned stimuli (18 with the CS+ and 18 with the CS-) at varying times after the grating onset (700, 800 or 900 ms). During the startle trials the gratings were shown for a total of 1500 ms and were followed by a prolonged variable ITI (650–1850 ms). To prevent learning of an association between the acoustic startle and the longer grating presentations, 60 trials with prolonged grating presentation (1500 ms) but without a startle probe were intermixed in each recording block.

The experiment was conducted in two sessions on two consecutive days at the same time of the day with each participant. On day one, the protocol included informed consent, handedness and personal information questionnaires, as well as collection of EEG and startle responses during the baseline block. Two parallel versions of a mood questionnaire MDBF (Steyer *et al.*, 1997) were administered at the beginning and at the end of this first testing session. On day two, the protocol included recording of the two conditioning blocks and the extinction block. There were breaks between the different blocks as well as in the middle of the extinction block, approximately every 15–20 min. Parallel

versions of the MDBF were administered at the beginning, middle and end of the second session. In addition, during the pause between the second conditioning block and the extinction block, subjects were asked to rate four grating patterns on a scale from 1 (most unpleasant) to 4 (most pleasant). Two of the grating patterns were identical with the ones used as CS+ and CS- (45° and 135° grating orientation), the other two were new in the context of this experiment and had horizontal (180°) and vertical (90°) orientations. At the end of the extinction block participants were asked if they were aware of seeing two different grating patterns, and if they had noticed any connection between the kinds of pictures they saw and the gratings presented with them. Finally, all participants viewed the 120 affective pictures used in the experiment in pseudo-randomized order and rated them on the dimensions affective valence and arousal, using a paper and pencil version of the self-assessment manikin (Bradley and Lang, 1994). No time constraints were imposed during the rating and the subjects' viewing time for each picture was recorded.

### Electrophysiological Data Collection, Data Reduction and Analyses

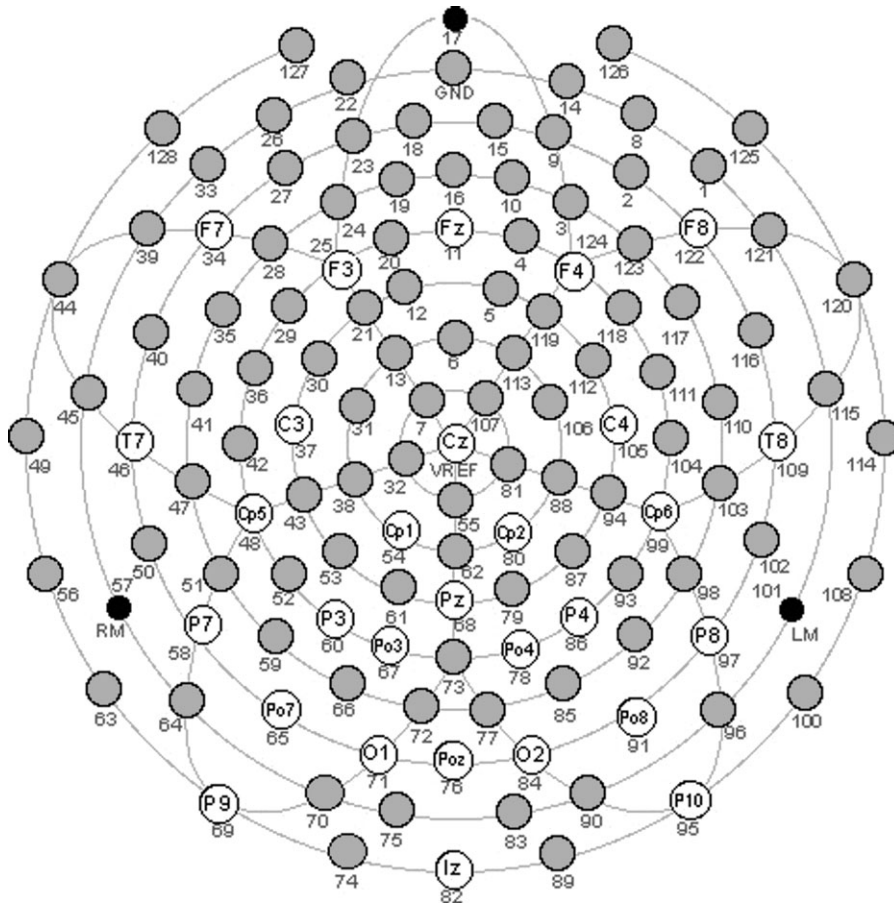
#### Event-related Potentials

The EEG was recorded using an Electrical Geodesics, Inc. 128-channel system, the vertex (Cz) was the recording reference (for the electrode array, see Fig. 2). The sampling rate was set at 250 Hz and impedances were kept below 50 k $\Omega$  as recommended by the manufacturer. Data were subjected to a 0.1 Hz high-pass and a 100 Hz low-pass online filters. Artifact-free epochs (196 ms pre- and 600 ms post-stimulus interval) were obtained using the SCADS procedure suggested by Junghöfer *et al.* (2000). In a subsequent step, data were re-referenced to average reference and additional filtering was applied (high pass: 1–3 Hz). The mean number of artifact-free trials per condition was 76 for block 1, 80 for block 2, 80 for block 3 and 81 for block 4.

Given the deep location of C1 sources, as well as the high amount of anatomical variability of primary visual cortex (Van Essen *et al.*, 1998), we decided to rely on voltage data, rather than conducting source-space analyses. Indeed, inspection of the present data on the single-subject level suggests low reliability of topography, but highly consistent timing of the early electrocortical response across individuals. In line with previous reports and the deep location of the C1 generators, a wide spread posterior negativity was observed for each participant in the C1 time range. Artifact-free epochs of 196 ms pre- and 600 ms post-stimulus were averaged separately for each subject, condition and recording block. The mean voltage of a 150 ms segment preceding the onset of the grating stimulus was used for baseline subtraction. Visual inspection of the grand means and the individual averages for each condition revealed six components of interest, and the corresponding time windows were identified. The first three time windows were used to evaluate the ERP response following the grating stimulus (CS- or CS+, presented in the left or right hemifield) and included the following components: C1 (65–90 ms), P1 (120–150 ms) and N1 (160–196 ms). Three additional time windows were used to analyze the ERP deflections that were related to the presentation of checkerboards (baseline and extinction blocks) or affective pictures (conditioning blocks), which occurred at 200 ms after the onset of the grating stimulus. These time ranges will be referred to as bP1 (310–340 ms), bN1 (360–420) and P3 (520–600 ms).

For the purpose of statistical analyses, regional means for the ERP-amplitude in the six selected windows were created, using four groups of six electrodes each: left anterior, left posterior, right anterior and right posterior (see Fig. 2). This configuration allowed the evaluation of hemisphere effects due to hemifield stimulation, as well as analyses of anterior-posterior shifts in topography. The mean values of the four electrode groups for each component were subjected to an omnibus repeated-measures ANOVA with the factors BLOCK (baseline, conditioning 1, conditioning 2 and extinction), CONDITION (CS+, CS-), HEMIFIELD (right, left), HEMISPHERE (left, right), CAUDALITY (anterior, posterior) and SITE (6 electrodes as shown in Fig. 2). Follow-up ANOVAs for each block were conducted where appropriate. In particular, significant effects involving CONDITION in the C1 time range were followed by ANOVAs on an average over parietal electrode sites (immediate neighbors of P3/P4), where the C1 was most pronounced





**Figure 2.** Layout of the electrode array. The electrodes, labeled here according to the international 10–20 system, were grouped for the purpose of statistical analyses. The electrodes posterior to Pz comprise the posterior groups, and the midline divided left and right electrodes.

(see results). To correct for violation of the sphericity assumption, Greenhouse–Geisser correction was applied to all repeated-measures ANOVAs. In the following, we report uncorrected degrees of freedom together with corrected *P*-values, where appropriate.

#### Startle Response

Startle responses were extracted from the vertical EOG recordings of the right eye by subtracting the data recorded at the upper-eye electrode (no. 8) from the lower-eye electrode (no. 126) (see Fig. 2). The 54 startle trials (18 ITIs, 18 CS+ and 18 CS-) obtained per block were edited individually for each participant; artifact-contaminated trials were omitted. The complete data sets of two participants were excluded due to the absence of scoreable startle responses. The startle magnitude was obtained by subtracting the peak amplitude within a 20–120 ms window from the baseline. The absolute values were then standardized within subjects in order to decrease variability, caused by differences in absolute size of the startle response across participants, and were expressed in *z*-scores. For each recording block, a repeated-measures ANOVA was conducted to test for condition differences in the magnitude of the startle response. Again the Greenhouse–Geisser method was applied where appropriate.

## Results

### Behavioral Data

#### Mood Questionnaire (MDBF)

The MDBF questionnaire (Steyer *et al.*, 1997) was used to assess the participants' mood changes during the different phases of

this experiment. It included scales for the following dimensions: feeling well versus not well, alert versus tired, and calm versus aroused. The participants were asked to complete one of two parallel versions of this questionnaire at five time points during the experiment. A repeated-measures ANOVA with TIME POINT and DIMENSION as within-subject factors and GENDER as a between-subject factor revealed main effects of TIME POINT ( $F = 4.5$ ,  $P < 0.01$ ) and DIMENSION ( $F = 9.4$ ,  $P < 0.01$ ), as well as a significant TIME POINT  $\times$  DIMENSION interaction ( $F = 2.5$ ,  $P < 0.05$ ). While participants demonstrated similar levels of well-being, alertness and calmness at the beginning of the recording sessions on day 1 and day 2, the reported values for all three scales decreased when measured after the conditioning blocks compared to the baseline block. At the end of the experiment, after recording the extinction block, well-being and calmness increased again, while the reported alertness continued to decrease.

#### SAM Ratings and Viewing Time

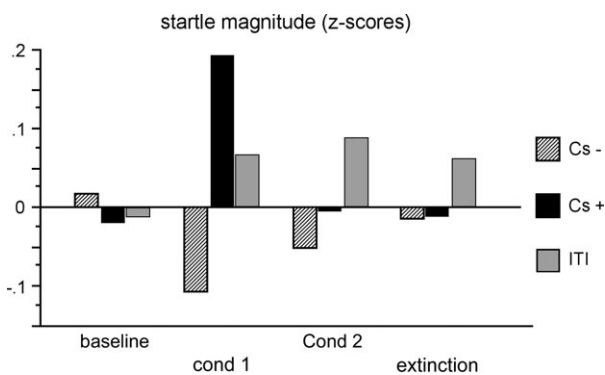
As expected, participants rated the unpleasant pictures (valence: mean = 2.5, SD = 0.8; arousal: mean = 6.6, SD = 0.9) lower in valence [ $t(59) = -26.3$ ,  $P < 0.001$ ] and higher in arousal [ $t(59) = 21.3$ ,  $P < 0.001$ ] than the neutral pictures (valence: mean = 6.4, SD = 1.0; arousal: mean = 3.0, SD = 0.9). The analysis of the viewing times during the rating procedure revealed no significant differences between the neutral and the unpleasant pictures.

### Awareness and Grating Ratings

When asked at the end of the EEG recordings, 16 out of 18 participants (88.9%) were not aware of any systematic relationship between the gratings and the pictures they saw during the conditioning blocks, nine participants reported not to have realized that two different kinds of gratings (45° and 135°) were shown. Nevertheless, 15 participants (83.3%) rated the stimulus used as CS+ as less pleasant than the one used as CS- (Wilcoxon-test:  $P < 0.05$ ). No differences were found in any of the other comparisons, including the two novel gratings (90° and 180° gratings) used as distracters. An additional 18 age- and gender-matched controls were recruited to assess *a priori* differences in the affective valence of the four gratings. The analyses revealed no differences between the two grating patterns used as CS+ and CS- in the present study; however, the two distracter-gratings (not used in the reported experiment, 90° and 180°) were rated as less pleasant ( $P < 0.05$ ) than the 135°-grating.

### Startle Responses

No condition differences in magnitude of the startle responses were found for the baseline and the extinction blocks. A reliable modulation was found during the first conditioning block, where participants demonstrated a weaker startle response to the CS- than to the CS+ [ $F(2,16) = 3.5$ ,  $P < 0.05$ ]. This differentiation did not reach significance during the second conditioning block, but pointed in the same direction, while amplitudes generally decreased across blocks (Fig. 3).



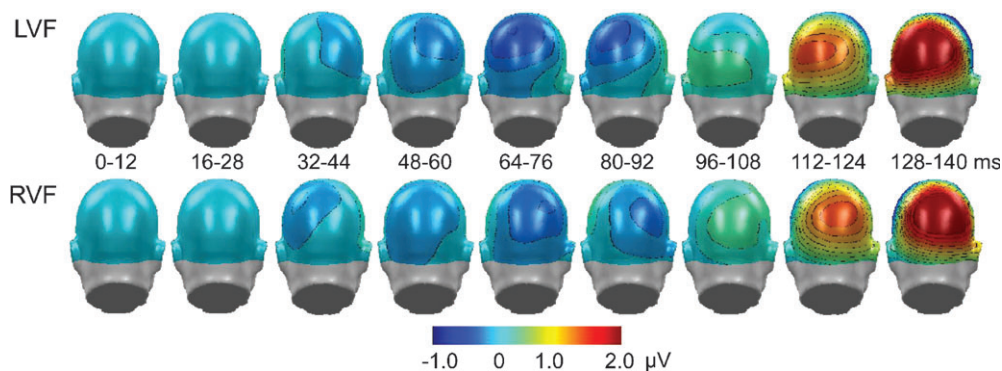
**Figure 3.** Changes in startle magnitude (expressed in z-scores) across recording blocks and conditions.

### ERP Response to the Grating Stimuli

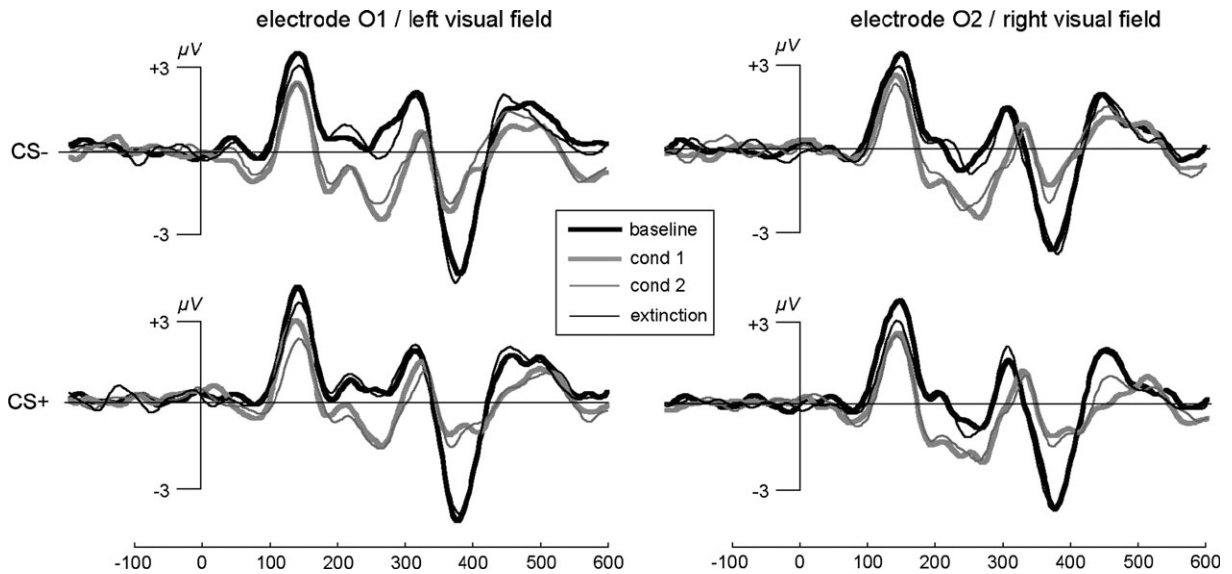
Figure 4 shows the time course of topographical distribution across all blocks and CS conditions, for stimuli being present in the left (top) or right visual field (bottom). Using the average reference, the distribution of the C1 component started with a negativity contralateral to the stimulus location at ~60 ms after stimulus onset. Consistent with a deep calcarine generator, this negativity was widely distributed and shifted towards the ipsilateral hemisphere while increasing in amplitude and giving rise to contralateral P1 at ~80–90 ms and a contralateral N1 ~140 ms after stimulus onset. Statistical analyses were conducted for mean voltages in these time segments, with the C1 time segment encompassing both the initial contralateral negativity as well the ipsilateral part, to increase signal-to-noise of the mean voltage. ERP waveforms at representative electrodes are depicted for each experimental condition in Figure 5.

#### C1: 65–90 ms

The omnibus ANOVA with BLOCK (baseline, conditioning 1, conditioning 2 and extinction), CONDITION (CS+, CS-), HEMIFIELD (right, left), HEMISPHERE (left, right), CAUDALITY (anterior, posterior) and SITE as within-subject factors revealed a main effect of BLOCK [ $F(3,17) = 5.9$ ,  $P < 0.01$ ] with a significantly more negative C1 amplitude during the two conditioning blocks than during baseline and extinction as well as an effect of CAUDALITY [ $F(1,17) = 8.4$ ,  $P < 0.05$ ], confirming the expected C1 distribution with negativity at posterior sites and positivity at anterior ones. Across all blocks there was an interaction of HEMIFIELD × HEMISPHERE [ $F(1,17) = 6.6$ ,  $P < 0.05$ ], the grating stimuli elicited overall stronger negativity at ipsilateral, compared to contralateral sites. This negativity was greater for left than for right hemifield presentation. In addition, a three-way interaction of BLOCK × HEMIFIELD × HEMISPHERE was observed [ $F(3,17) = 3.0$ ,  $P < 0.05$ ], showing that the difference between left and right hemifield presentation was greatest during the baseline block. Summarizing the results of the C1 omnibus ANOVA, a clear effect of experimental context emerged across the four recording blocks: the C1 for all conditions was more negative during the two conditioning blocks than during baseline and extinction. In addition, interaction between presentation site and response site was observed, with a stronger response measured ipsilaterally to the presentation hemifield. All interactions including the factor



**Figure 4.** Grand mean ( $n = 18$ ) spline interpolated topography of the mean voltage amplitude across all blocks and CS conditions, for stimuli being present in the left (top) or right visual field (bottom). The distribution of the C1 component started with a negativity contralateral to the stimulus location at ~60 ms after stimulus onset. Consistent with a deep calcarine generator, this negativity was widely distributed and shifted towards the ipsilateral hemisphere while increasing in amplitude and giving rise to contralateral P1 at ~80–90 ms and a contralateral N1 ~140 ms after stimulus-onset.

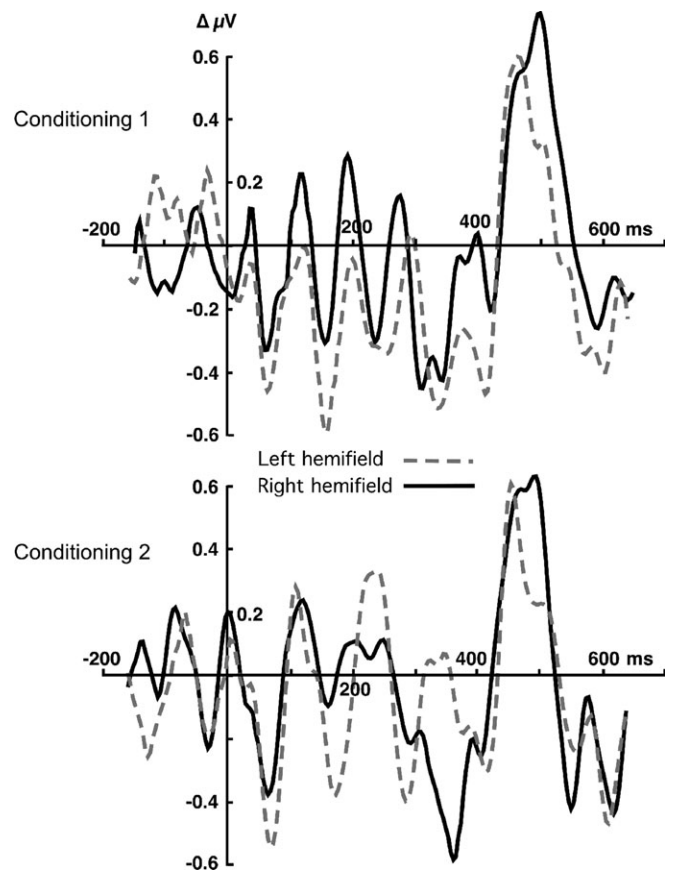


**Figure 5.** Grand mean ( $n = 18$ ) event-related potentials at O1 (left hemifield presentation) and O2 (right hemifield presentation) for each condition across the recording blocks.

CONDITION were followed up using separate ANOVAs for each block.

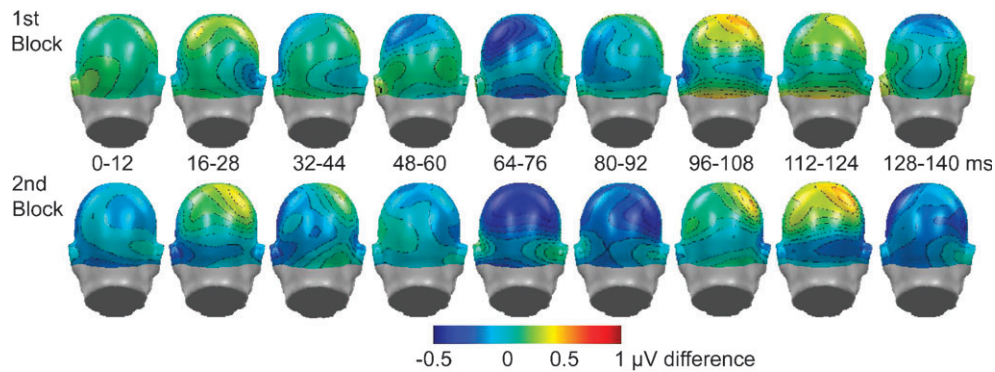
For the baseline block, a HEMIFIELD  $\times$  HEMISPHERE interaction [ $F(1,17) = 18.1, P < 0.01$ ] was observed, confirming the above-reported effect found in the omnibus ANOVA. Regarding the first conditioning block, a main effect of CAUDALITY [ $F(1,17) = 14.3, P < 0.01$ ] was found, reflecting the topography of the C1 component with strong negativity at posterior and weak positivity at anterior sites. In addition, a CONDITION  $\times$  HEMIFIELD  $\times$  HEMISPHERE  $\times$  CAUDALITY interaction [ $F(1,17) = 6.0, P < 0.05$ ] was observed, showing that the CS+ elicited a more negative response at left and right posterior sites than the CS-, when presented in the right hemifield. When shown in the left hemifield, the CS- tended to elicit less overall positivity than the CS+ across anterior and posterior ipsilateral sites. ANOVA on parietal electrodes showed that negativity was greater for the CS+ in both hemifield conditions [ $F(1,17) = 5.1, P < 0.05$ ]. During the second conditioning block an effect of CAUDALITY [ $F(1,17) = 12.5, P < 0.01$ ] similar to the one reported above was observed. In addition a CONDITION  $\times$  HEMIFIELD interaction [ $F(1,17) = 8.0, P < 0.01$ ] emerged, showing that the CS+ elicited a larger C1 when presented in the right hemifield, while, when presented in the left hemifield, the CS- elicited larger negativity/less positivity across all electrode sites. As opposed to the first conditioning block, this effect held true for both hemispheres. Focusing on parietal sensors during the two conditioning blocks showed a consistent pattern of enhanced posterior negativity for the CS+, compared to CS- [ $F(1,17) = 4.7, P < 0.05$ , see Fig. 6]. Comparing parietal C1 amplitude across the two conditioning blocks did not yield any significant main effects or interactions involving BLOCK however ( $F_s < 2.6$ ). No effects involving condition were found for the extinction block.

During baseline and extinction no condition effects for the C1 were found. Differences between the early electrophysiological response following the CS+ and the CS- emerged during the first conditioning block, where the overall topographic pattern differed across the two hemispheres and depended on the presentation site of the conditioned stimuli. In contrast,



**Figure 6.** Grand mean ( $n = 18$ ) waveforms for the difference between CS+ and CS-, calculated for a group of parietal electrode sites (immediate neighbors of P3/P4, see Fig. 2), where the C1 was most pronounced. The same parietal region was used for statistical analysis (see Materials and Methods). Greater negativity of the difference waveform indicates greater negativity for CS+ compared to CS-. Data are shown for the first (top panel) and second (bottom panel) conditioning block, as a function of left (dashed) and right (solid) visual field presentation of the CS.





**Figure 7.** Grand mean difference topographies ( $n = 18$ ) for CS+ minus CS-, averaged across presentation sites. Greater C1 negativity for the CS+ can be seen at ~70 ms. This difference in negativity was more widespread in the second conditioning block (bottom panel).

parietal negativity, where C1 negativity was most pronounced, showed enhancement for CS+, compared to CS-. During the second conditioning block, the influence of presentation site remained significant; the effects of condition, however, spread across both hemispheres alike. These effects are summarized in Figure 7, showing the time course of difference topographies for CS+ minus CS- across presentation sites. While differences are small, greater and more widespread negativity for the CS+ can be seen at ~70 ms in the second conditioning block. However, it must be noted that this difference did not reach significance. Thus, early amplification did not habituate over the time range examined here, and did build up within the first conditioning block.

#### P1: 120–150 ms

The omnibus ANOVA revealed main effects of BLOCK [ $F(3,51) = 5.6, P < 0.01$ ] and CAUDALITY [ $F(1,17) = 36.7, P < 0.01$ ], as well as a BLOCK  $\times$  CAUDALITY interaction [ $F(3,51) = 4.6, P < 0.01$ ]. As expected, the P1 distribution was characterized by posterior positivity and weaker anterior negativity. At posterior sites, the P1 amplitude was greater during the baseline and extinction blocks, as compared to the two conditioning blocks. There were no differences at anterior sites. A HEMIFIELD  $\times$  HEMISPHERE interaction [ $F(1,17) = 21.9, P < 0.01$ ] was also found, and the eccentric grating presentation elicited a stronger P1 response in the ipsilateral hemisphere than in the contralateral one. No interactions with condition were observed, and thus no follow-up ANOVAs were conducted.

#### N1: 160–200 ms

For the N1 component a main effect of BLOCK [ $F(3,51) = 4.6, P < 0.01$ ] and a BLOCK  $\times$  CAUDALITY interaction [ $F(3,51) = 8.1, P < 0.01$ ] were observed. At posterior sites, the N1 was more negative for the two conditioning blocks than for the baseline and the extinction block. There was also a HEMIFIELD  $\times$  HEMISPHERE interaction [ $F(1,17) = 10.1, P < 0.01$ ], the grating stimuli elicited a greater negative response in the contralateral hemisphere compared to the ipsilateral one.

#### ERPs in Response to the Unconditioned Stimuli (UCS)

At 200 ms after onset of the grating stimuli (CS+ and CS-), a centrally presented checkerboard (in the baseline and the extinction blocks) or an affective picture (conditioning 1 and 2) appeared on the screen for 400 ms. The following three time

windows were used to evaluate the ERP response to these stimuli.

#### bP1: 310–340 ms

For the P1 component following the checkerboards or affective picture presentation (called here bP1), a main effect of BLOCK [ $F(3,51) = 4.2, P < 0.01$ ] was found, showing that the amplitude of the bP1 was smaller following the affective pictures during the two conditioning blocks than following the checkerboards in the baseline and in the extinction blocks. The main effect of CAUDALITY [ $F(1,17) = 7.7, P < 0.05$ ] was due to the expected greater positivity at posterior sites than at anterior ones. A HEMIFIELD  $\times$  HEMISPHERE interaction [ $F(1,17) = 13.3, P < 0.01$ ] was also observed: when the grating stimulus (CS) was presented in the right hemifield, the centrally presented UCS elicited a lateralized response with stronger left hemisphere positivity and vice versa: CS presentation in the left hemifield led to a stronger right hemisphere positivity in response to the UCS. Interactions with the factor CONDITION were followed up with separate ANOVAs for each block. As the UCS differed for baseline and extinction versus the conditioning blocks, they were not directly comparable.

For the baseline block, a main effect of CAUDALITY [ $F(1,17) = 5.6, P < 0.05$ ] illustrated the bP1 surface distribution with posterior positivity. The above-reported HEMIFIELD  $\times$  HEMISPHERE interaction [ $F(1,17) = 7.0, P < 0.05$ ] was also present. For the first conditioning block, analyses of the bP1 amplitude revealed the expected CAUDALITY effect [ $F(1,17) = 7.5, P < 0.05$ ] and stronger HEMIFIELD  $\times$  HEMISPHERE [ $F(1,17) = 20.1, P < 0.001$ ] interaction than in the baseline block. In addition, CONDITION  $\times$  HEMISPHERE [ $F(1,17) = 4.3, P < 0.05$ ] and CONDITION  $\times$  CAUDALITY [ $F(1,17) = 5.2, P < 0.05$ ] interactions were observed. The unpleasant pictures elicited a significantly bigger bP1 response at posterior, right hemispheric sensors than did the neutral pictures. A three-way CONDITION  $\times$  HEMIFIELD  $\times$  CAUDALITY interaction [ $F(1,17) = 10.5, P < 0.01$ ] showed that the condition differences at posterior sites were biggest when the CSs were presented in the left hemifield. During the second conditioning block, the same effects and interactions as in the first one were observed with an additional significant CONDITION  $\times$  HEMIFIELD interaction [ $F(1,17) = 6.7, P < 0.05$ ], showing again that the unpleasant pictures elicited a larger bP1 amplitude when the CS+ preceding them was shown in the left visual hemifield. In the extinction block a main effect of CAUDALITY [ $F(1,17) = 7.6,$

$P < 0.05$ ] with greater positive amplitude at posterior leads was observed. Also, a HEMIFIELD  $\times$  HEMISPHERE  $\times$  CAUDALITY interaction [ $F(1,17) = 5.1, P < 0.05$ ], showing that the P1 amplitude as response to the UCS at right posterior leads was greater when the CSs were presented in the left hemifield than in the right one.

For the bP1 component following the centrally presented checkerboards during baseline and extinction, and affective pictures during the two conditioning blocks, we observed effects of presentation site of the preceding grating stimuli. These effects were stronger during the two conditioning blocks than during baseline and extinction. In addition, effects of affective content during the conditioning blocks were found: the unpleasant pictures elicited greater bP1 than the neutral ones. This effect was strongest at right hemispheric posterior sites when the CS+ preceding the unpleasant picture was presented in the left hemifield.

#### *bN1: 360–420 ms*

The omnibus ANOVA revealed the main effects of BLOCK [ $F(3,51) = 28.9, P < 0.001$ ] and CAUDALITY [ $F(1,17) = 33.0, P < 0.001$ ], as well as a BLOCK  $\times$  CAUDALITY interaction [ $F(3,51) = 18.6, P < 0.001$ ]. The bN1 amplitude in response to the UCS was significantly more negative during baseline and extinction than during the two conditioning blocks, and had the expected surface distribution with negativity at posterior and positivity at anterior sensors. The block differences applied to the posterior negativity, but not to the anterior positivity of the bN1 component. In addition, a HEMIFIELD  $\times$  HEMISPHERE interaction [ $F(1,17) = 42.0, P < 0.001$ ] showed that the bN1 component was bigger at left hemispheric sensors than at right hemispheric ones when the CSs were presented in the left visual field. The interactions with CONDITION were followed up with separate ANOVAs for each block.

For the baseline block, the above-reported effect of CAUDALITY [ $F(1,17) = 37.7, P < 0.001$ ] and the HEMIFIELD  $\times$  HEMISPHERE [ $F(1,17) = 24.1, P < 0.001$ ] interactions were confirmed. Beside the CAUDALITY [ $F(1,17) = 6.3, P < 0.05$ ] and the HEMIFIELD  $\times$  HEMISPHERE [ $F(1,17) = 4.2, P < 0.05$ ] effects explained above, a CONDITION  $\times$  HEMIFIELD  $\times$  CAUDALITY [ $F(1,17) = 5.0, P < 0.05$ ] interaction was found in the first conditioning block and showed that the bN1 to the unpleasant pictures (UCS) was more negative than to the neutral ones at posterior leads when the CSs were presented in the right visual hemifield, whereas when the CSs were presented in the left visual field no significant differences were found. During the second conditioning block a CAUDALITY effect [ $F(1,17) = 9.7, P < 0.01$ ] was observed. Similar effect of CAUDALITY [ $F(1,17) = 56.8, P < 0.001$ ] and HEMIFIELD  $\times$  HEMISPHERE [ $F(1,17) = 20.1, P < 0.001$ ] interaction as in the baseline block were found during extinction.

Similar to the effects found for the bP1 response, for the bN1 component we observed effects of presentation site of the preceding grating stimuli in all four recording blocks. In the first conditioning blocks, we also found effects of affective content, with unpleasant pictures eliciting greater bN1 than neutral ones.

#### *P3: 520 – 600 ms*

The omnibus ANOVA revealed a main effect of BLOCK [ $F(3, 51) = 12.3, P < 0.001$ ], as well as BLOCK  $\times$  CAUDALITY [ $F(3,51) = 5.3, P < 0.01$ ] and BLOCK  $\times$  HEMISPHERE [ $F(3,51) = 6.0, P < 0.01$ ] interactions. The P3 showed the expected distribution

with posterior positivity. It was more positive in the baseline and in the extinction blocks when checkerboards were presented than during the two conditioning blocks, following presentation of affective pictures. The block differences were more pronounced over the left than over the right hemisphere. A HEMIFIELD  $\times$  HEMISPHERE interaction [ $F(1,17) = 8.8, P < 0.01$ ] was also found: the P3 to the UCS had greater positive amplitudes at left hemispheric sensors than at right ones, when the CS preceding the UCS was presented in the left visual hemifield.

A main effect of CONDITION, as well as all interactions with condition, were followed up with separate ANOVAs for each block, as the UCSs were checkerboards during baseline and extinction and affective pictures during conditioning. No significant main effects or interactions were found in the baseline block. In the first conditioning block a main effect of HEMISPHERE [ $F(1,17) = 8.3, P < 0.05$ ] and a CONDITION  $\times$  HEMISPHERE [ $F(1,17) = 6.6, P < 0.05$ ] interaction were found. The P3 was more positive at left hemispheric sites; however, only over the right hemisphere were significant differences found between the unpleasant and the neutral pictures, with the unpleasant stimuli eliciting more positive P3 than the neutral ones. During the second conditioning block we observed the reported above main effect of CAUDALITY [ $F(1,17) = 10.1, P < 0.01$ ] and HEMIFIELD  $\times$  HEMISPHERE interaction [ $F(1,17) = 5.6, P < 0.05$ ], as well as a main effect of CONDITION [ $F(1,17) = 7.3, P < 0.05$ ] and a CONDITION  $\times$  CAUDALITY [ $F(1,17) = 6.1, P < 0.05$ ] interaction. At posterior sites the unpleasant pictures elicited a more positive response than the neutral ones, over both hemispheres. No condition effects were found for the extinction block. A main effect of HEMISPHERE [ $F(1,17) = 9.3, P < 0.01$ ] and a HEMIFIELD  $\times$  HEMISPHERE interaction [ $F(1,17) = 9.1, P < 0.01$ ] showed that the response to the checkerboards was generally more positive over left hemispheric sites, this effect was strongest when the grating preceding the centrally presented checkerboard appeared in the left visual hemifield.

For the P3 component effects of presentation site were observed, similar to those reported for the bP1 and the bN1 components. We also found a greater P3 for the unpleasant pictures than for the neutral ones, in the first conditioning block predominantly over the right hemisphere, in the second one over both hemispheres alike.

## Discussion

We set out to examine the modulation of early visual ERPs as a result of affective learning, hoping that this would let us gain a better understanding of the processes and structures involved in rapid affective evaluation of visual stimuli. We focused on the earliest visual component (C1), which is thought to be generated in the primary visual cortex and is known to peak at ~65–90 ms (Martinez *et al.*, 1999). The experimental manipulation was designed to elicit a measurable C1 and to allow for an evaluation of its changes, both between two originally neutral stimuli, gaining divergent affective meanings through classical conditioning and losing it again in an extinction procedure, as well as within the same stimulus across a learning continuum from a baseline measure through two consecutive conditioning blocks to an extinction block. As we aimed to study conditioning within the visual modality, we used affective pictures as unconditioned stimuli.



The behavioral results indicated that the conditioning protocol was effective, even though pictures can be regarded as relatively weak unconditioned stimuli. After conditioning, participants were more likely to rate the originally neutral grating stimulus used as CS+ as less pleasant than the one used as CS-. As expected, they also rated the unpleasant pictures as more arousing and more unpleasant than the neutral ones. The majority of participants were not aware of the contingencies associated with conditioning. In addition to the behavioral ratings, we recorded participants' startle responses and ERP responses in each of the experimental blocks (Fig. 3). The startle responses were used to directly assess the success of conditioning by means of the fear-potentiated startle procedure (Hamm and Vaitl, 1996; Koch, 1999). During baseline and extinction, no differentiation between the startle responses to the CS+ and CS- presentation was observed. In the first conditioning block, the startle elicited during CS- presentation was significantly weaker than that elicited during CS+ presentation (Fig. 3). In the second conditioning block, the pattern of discrimination remained similar, although the magnitude of the startle response across all three conditions (CS+, CS- and ITI) decreased and the difference between CS+ and CS- conditions failed to reach significance. This may be due to several factors, including habituation effects, small group size, or the fact that we indirectly monitored the startle responses using vertical EOG, rather than using electrodes directly attached over the musculus orbicularis oculi. Nevertheless, we regard the startle results as evidence that conditioning within the visual modality occurred, even in the absence of awareness concerning the experimental manipulation.

With regard to the C1 ERP component, which was of critical interest in our study, we observed the typical C1 response having widespread distribution over the occipito-parietal part of the scalp. As shown, for example, by Di Russo *et al.* (2002), the C1 component originates in the striate cortex (area 17 in the primary visual cortex). While the primary cortical response was stronger and earlier contralateral to the locus of stimulus presentation, negativity moved to ipsilateral sites, giving rise to contralateral positivity of the P1 component. Overall, the morphology and the topography of the C1 visual ERP component elicited in this study (Figs 4 and 5) were very similar to those reported in previous studies on early visual selective attention and perceptual processing (Gomez Gonzalez *et al.*, 1994; Hillyard and Anllo-Vento, 1998; Di Russo *et al.*, 2003), where it has been convincingly demonstrated that the C1 is not affected by spatial- and feature-based attention when simple neutral stimuli are used. We did, however, find reliable modulations based on the acquired affective content of the conditioned stimuli. Importantly, the C1 was more negative during all the conditioning blocks, indicating that providing a motivationally relevant context enhanced early visual processing. Theoretically, this may relate to the fact that both CS- and CS+ obtain predictive value by contingent presentation with different UCS stimuli and thus both gain affective relevance. Similar findings have been obtained with pleasant and unpleasant affective stimuli, which were reported to elicit greater visual N1/P2/P3 responses, compared to affectively neutral stimuli (e.g. Keil *et al.*, 2002).

While there were no differences between the C1 components elicited by the two grating patterns (i.e. CS+ and CS-) during the baseline and the extinction blocks, differentiation of the CS+ and the CS- was found in the two conditioning blocks

(see Figs 6 and 7). The CS+ elicited a more negative C1 deflection than the CS- at parietal electrode sites throughout the conditioning blocks. There was small and indirect evidence of increasing differentiation between CS+ and CS- from the first to the second conditioning block, as the effects were more widespread across recording sites. Directly testing this difference at parietal sites however did not show an interaction between BLOCK (conditioning 1 and 2) and CONDITION (CS+ and CS-). This in turn suggests that cortical facilitation for the most relevant stimulus appeared early in the first conditioning block and did not habituate during the experiment.

The observed strong main effect of CONDITION provided evidence that learned affective arousal associated with originally neutral stimuli can lead to a modulation of the earliest measurable electrophysiological response as indicated by the C1. These results are in agreement with three recent studies that report similar modulations of early visual responses using faces as stimuli. For instance, Eger *et al.* (2003) have demonstrated that an early VEP component with a latency of 80-90 ms is sensitive to the emotional content of facial expression. Pourtois *et al.* (2004) showed modulation of the C1 component, with fearful faces eliciting a more negative response than happy faces. In a similar vein, early MEG response, possibly originating in the calcarine fissure, may be sensitive to emotional facial expressions (Halgren *et al.*, 2000). As Pourtois *et al.* (2004) point out, the lack of a greater number of previous reports on C1 modulations depending on valence or arousal might be partially due to the fact that emotional stimuli have often been presented centrally, or along the horizontal meridian, which cancels out this relatively small component having a retinotopic topography.

In this study, we used eccentric presentation in the upper visual field and high-contrast black and white stimuli. This enabled us to elicit a stable C1 component and analyze its changes along a learning continuum. We did not find modulation of the C1 component during baseline and extinction in this study, which, together with the consistent previous reports showing no modulation of the C1 with spatial attention tasks (and with the recent findings of C1 modulations depending on emotional facial expressions), suggests that the learned affective meaning of the stimuli can influence early visual processing. In terms of the time course of C1 modulation during conditioning, we did not observe a CS+-related increase of amplitude across conditioning blocks. This suggests that the process mediating enhanced sensitivity of visual cortex as indexed by the C1 ERP component developed during the first block, which encompassed 240 trials. Additional support for this notion comes from the startle data, showing potentiation for the CS+ specifically during the first conditioning block. As reported above, there was pronounced variability in the topographical distribution of C1 across participants and conditions. Across the full electrode set, condition differences varied with the presentation site of the CS and changed in topography across the two conditioning blocks, representing two factors that might have contributed to the fact that no BLOCK interactions with CONDITION were found in the omnibus ANOVA. It is possible that the presence of affective pictures, some of them highly arousing and unpleasant, contributed to these lateralization differences (see below for a more detailed discussion regarding the context effects on all recorded components).

In terms of the P1 and the N1 ERP components elicited by the grating stimuli, previous studies report reliable selective

attention modulations of these components (Gomez Gonzalez *et al.*, 1994; Hillyard *et al.*, 1998). Valence and arousal modulations have also been repeatedly observed (Keil *et al.*, 2002; Schupp *et al.*, 2003; Delplanque *et al.*, 2004). Although we were able to measure the P1 and N1 visual components showing the expected topography, morphology and reliable effects of presentation site, we did not find condition-dependent differentiation between the CS+ and the CS- in any of the recording blocks. As mentioned above, our stimuli and presentation parameters were chosen specifically to elicit the C1 component and were thus not ideally suited to measure modulations of the P1 and N1 components. Studies showing arousal modulations of the P1 and N1 components mostly use very salient, relatively big and often colorful, centrally or parafoveally presented affective pictures (Keil *et al.*, 2002; Schupp *et al.*, 2003). In contrast, our conditioned stimuli were small black and white gratings and were presented in the periphery of the visual field.

In addition to the ERP response following the CS+ and CS-, we also examined the electrophysiological brain response following the centrally presented UCSs (neutral checkerboards during baseline and extinction and affective pictures in the two conditioning blocks). As expected, no differentiation was found between the checkerboard patterns in the baseline and in the extinction block. In the two conditioning blocks, we observed bigger bP1, bN1 and P3 components for the unpleasant pictures, compared to the neutral ones, replicating previous studies on affective processing (Palomba *et al.*, 1997; Lang *et al.*, 1998; Keil *et al.*, 2002; Schupp *et al.*, 2003). These effects were ipsilateral to the presentation site of the preceding grating stimulus. This was surprising, given that the UCSs were presented centrally, with participants fixating on a small cross in the middle of the stimuli while no evidence for lateralized eye movements was found in trials retained for averaging. It appears that the CSs were implicitly attracting participants' attention. During the two conditioning blocks, however, the general hemifield effects were stronger than during baseline and extinction, suggesting increasing attraction of attention by the CSs when learning was taking place or in the context of affective stimuli presentation. In addition, we found lateralized condition differences for the bP1 and bN1 components. The differentiation between unpleasant and neutral pictures at the bP1 was greatest when the gratings preceding them were presented in the left hemifield. The opposite applied to the bN1 component showing differences between the affective categories specifically when the CSs were presented in the right hemifield. P3 amplitudes were independent from the presentation site of the gratings, although they were found predominantly over the right hemisphere.

In addition to the condition-specific differentiations, we observed stable effects of recording context for all analyzed ERP components. When comparing the same condition across the four experimental blocks, the elicited responses during baseline and extinction were very similar to each other in morphology and topography (see Fig. 5), although recorded on two consecutive days. The same similarity was found for the responses elicited during the two conditioning blocks. When presentation of affective pictures (as opposed to checkerboards) was expected, the electrophysiological response to the grating stimuli (CS+ and CS-) preceding those pictures was characterized by enhanced C1 and N1 amplitudes and reduced P1 amplitude. Context effects, specifically for the early components elicited by the grating stimuli, provide evidence that the observed condition differences may not be attributed to effects

of fatigue or habituation. They also suggest that the extinction procedure was effective. In addition, they might indicate a general arousal or attention induced activation during the conditioning blocks. Such a process was suggested, for example, in a recent functional magnetic resonance imaging study (Downar *et al.*, 2001) for behaviorally relevant context features. This, however, needs to be investigated further in future experiments.

With the present study we provide direct evidence for a modulation of the C1 visual component by conditioned stimuli. The aversive conditioning occurred within the visual system and might have led to a short-term reorganization of the early sensory processing, allowing for a differentiation between CS+ and CS- 65–90 ms after stimulus onset. Conditioned stimuli can be considered threat-related and thus belong to a class of stimuli likely to capture attention automatically (LeDoux, 2003; Koster *et al.*, 2004). It has been previously shown that perception for motivationally or emotionally relevant stimuli is associated with increased cortical activity (Keil *et al.*, 2002; Phan *et al.*, 2002; Schupp *et al.*, 2003; Smith *et al.*, 2003; Krolak-Salmon *et al.*, 2004). As a possible mechanism, it has been hypothesized that the increased activation in perceptual cortices might be due to re-entrant feedback, linking subcortical and higher cortical structures with perceptual areas (Martinez *et al.*, 2001; Vuilleumier and Schwartz, 2001; Amaral *et al.*, 2003). While this is likely in the initial stages of contingency acquisition, we think that, with increasing consolidation, a direct involvement of the primary visual areas in arousal or valence differentiation is possible. We demonstrate that conditioned stimuli can modulate the initial sensory component in the human visual cortex. Even though this component is not modulated by spatial attention tasks, it appears sensitive to affective connotation of visual stimuli. Because of its short latency, it is unlikely to be directly influenced by cortical or subcortical feedback occurring on a single-trial time scale. This points to the important role of sensory plasticity in early attention for emotional stimuli. Our findings suggest that early sensory processing can also reflect affective information or motivational relevance. Whether the involved neural networks have come to expect affective components or simply tag the importance of the incoming information is an important question for future studies.

## Notes

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## References

- Amaral DG, Behnia H, Kelly JL (2003) Topographic organization of projections from the amygdala to the visual cortex in the macaque monkey. *Neuroscience* 118:1099–1120.
- Anderson AK, Phelps EA (2001) Lesions of the human amygdala impair enhanced perception of emotionally salient events. *Nature* 411:305–309.

- Baas JM, Kenemans JL, Bocker KB, Verbaten MN (2002) Threat-induced cortical processing and startle potentiation. *Neuroreport* 13:133-137.
- Bao S, Chan VT, Merzenich MM (2001) Cortical remodelling induced by activity of ventral tegmental dopamine neurons. *Nature* 412:79-83.
- Bensafi M, Rouby C, Farget V, Bertrand B, Vigouroux M, Holley A (2002) Influence of affective and cognitive judgments on autonomic parameters during inhalation of pleasant and unpleasant odors in humans. *Neurosci Lett* 319: 162-166.
- Bishop S, Duncan J, Brett M, Lawrence AD (2004) Prefrontal cortical function and anxiety: controlling attention to threat-related stimuli. *Nat Neurosci* 7:184-188.
- Bradley MM, Lang PJ (1994) Measuring emotion: the Self-Assessment Manikin and the Semantic Differential. *J Behav Ther Exp Psychiatry* 25:49-59.
- Bradley MM, Lang PJ (2000) Affective reactions to acoustic stimuli. *Psychophysiology* 37:204-215.
- Bradley MM, Codispoti M, Cuthbert BN, Lang PJ (2001) Emotion and motivation I: defensive and appetitive reactions in picture processing. *Emotion* 1:276-298.
- Bradley MM, Sabatinelli D, Lang PJ, Fitzsimmons JR, King W, Desai P (2003) Activation of the visual cortex in motivated attention. *Behav Neurosci* 117:369-380.
- Carrette L, Mercado F, Tapia M, Hinojosa JA (2001) Emotion, attention, and the 'negativity bias', studied through event-related potentials. *Int J Psychophysiol* 41:75-85.
- Das A, Gilbert CD (1995) Receptive field expansion in adult visual cortex is linked to dynamic changes in strength of cortical connections. *J Neurophysiol* 74:779-792.
- Delplanque S, Lavoie ME, Hot P, Silvert L, Sequeira H (2004) Modulation of cognitive processing by emotional valence studied through event-related potentials in humans. *Neurosci Lett* 356:1-4.
- Derryberry D, Reed MA (2002) Anxiety-related attentional biases and their regulation by attentional control. *J Abnorm Psychol* 111:225-236.
- Di Russo F, Martinez A, Sereno MI, Pitzalis S, Hillyard SA (2002) Cortical sources of the early components of the visual evoked potential. *Hum Brain Mapp* 15:95-111.
- Di Russo F, Martinez A, Hillyard SA (2003) Source analysis of event-related cortical activity during visuo-spatial attention. *Cereb Cortex* 13:486-499.
- Diamond DM, Weinberger NM (1984) Physiological plasticity of single neurons in auditory cortex of the cat during acquisition of the pupillary conditioned response: II. Secondary field (AII). *Behav Neurosci* 98:189-210.
- Downar J, Crawley AP, Mikulis DJ, Davis KD (2001) The effect of task relevance on the cortical response to changes in visual and auditory stimuli: an event-related fMRI study. *Neuroimage* 14:1256-1267.
- Eger E, Jedynak A, Iwaki T, Skrandies W (2003) Rapid extraction of emotional expression: evidence from evoked potential fields during brief presentation of face stimuli. *Neuropsychologia* 41:808-817.
- Fox E (2002) Processing emotional facial expressions: the role of anxiety and awareness. *Cogn Affect Behav Neurosci* 2:52-63.
- Fu S, Fan S, Chen L, Zhuo Y (2001) The attentional effects of peripheral cueing as revealed by two event-related potential studies. *Clin Neurophysiol* 112:172-185.
- Gilbert C, Ito M, Kapadia M, Westheimer G (2000) Interactions between attention, context and learning in primary visual cortex. *Vision Res* 40:1217-1226.
- Gomez Gonzalez CM, Clark VP, Fan S, Luck SJ, Hillyard SA (1994) Sources of attention-sensitive visual event-related potentials. *Brain Topogr* 7:41-51.
- Halgren E, Raji T, Marinkovic K, Jousmaki V, Hari R (2000) Cognitive response profile of the human fusiform face area as determined by MEG. *Cereb Cortex* 10:69-81.
- Hamm AO, Vaitl D (1996) Affective learning: awareness and aversion. *Psychophysiology* 33:698-710.
- Hartikainen KM, Ogawa KH, Knight RT (2000) Transient interference of right hemispheric function due to automatic emotional processing. *Neuropsychologia* 38:1576-1580.
- Hermann C, Ziegler S, Birbaumer N, Flor H (2000) Pavlovian aversive and appetitive odor conditioning in humans: subjective, peripheral, and electrocortical changes. *Exp Brain Res* 132:203-215.
- Hillyard SA, Anllo-Vento L (1998) Event-related brain potentials in the study of visual selective attention. *Proc Natl Acad Sci USA* 95:781-787.
- Hillyard SA, Vogel EK, Luck SJ (1998) Sensory gain control (amplification) as a mechanism of selective attention: electrophysiological and neuroimaging evidence. *Philos Trans R Soc Lond B Biol Sci* 353:1257-1270.
- Junghöfer M, Elbert T, Tucker DM, Rockstroh B (2000) Statistical control of artifacts in dense array EEG/MEG studies. *Psychophysiology* 37:523-532.
- Kapadia MK, Gilbert CD, Westheimer G (1994) A quantitative measure for short-term cortical plasticity in human vision. *J Neurosci* 14:451-457.
- Keil A, Ihssen N (2004) Identification facilitation for emotionally arousing verbs during the attentional blink. *Emotion* 4:23-35.
- Keil A, Bradley MM, Hauk O, Rockstroh B, Elbert T, Lang PJ (2002) Large-scale neural correlates of affective picture processing. *Psychophysiology* 39:641-649.
- Kenemans JL, Baas JM, Mangun GR, Lijffijt M, Verbaten MN (2000) On the processing of spatial frequencies as revealed by evoked-potential source modeling. *Clin Neurophysiol* 111:1113-1123.
- Koch M (1999) The neurobiology of startle. *Prog Neurobiol* 59:107-128.
- Koster EH, Crombez G, Van Damme S, Verschuere B, De Houwer J (2004) Does imminent threat capture and hold attention? *Emotion* 4:312-317.
- Krolak-Salmon P, Henaff MA, Vighetto A, Bertrand O, Mauguiere F (2004) Early amygdala reaction to fear spreading in occipital, temporal, and frontal cortex: a depth electrode ERP study in human. *Neuron* 42:665-676.
- Lang PJ, Bradley MM, Cuthbert BN (1998) Emotion, motivation, and anxiety: brain mechanisms and psychophysiology. *Biol Psychiatry* 44:1248-1263.
- Lang PJ, Bradley MM, Cuthbert BN (1999) International Affective Pictures System (IAPS): Instruction manual and affective ratings. Technical Report A-4. Gainesville, FL: The Center for Research in Psychophysiology, University of Florida.
- LeDoux J (2003) The emotional brain, fear, and the amygdala. *Cell Mol Neurobiol* 23:727-738.
- LeDoux JE, Sakaguchi A, Iwata J, Reis DJ (1985) Auditory emotional memories: establishment by projections from the medial geniculate nucleus to the posterior neostriatum and/or dorsal amygdala. *Ann NY Acad Sci* 444:463-464.
- LeDoux JE, Iwata J, Cicchetti P, Reis DJ (1988) Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *J Neurosci* 8:2517-2529.
- Lee TS, Yang CF, Romero RD, Mumford D (2002) Neural activity in early visual cortex reflects behavioral experience and higher-order perceptual saliency. *Nat Neurosci* 5:589-597.
- Lewis S, Thoma RJ, Lanoue MD, Miller GA, Heller W, Edgar C, Huang M, Weisend MP, Irwin J, Paulson K, Canive JM (2003) Visual processing of facial affect. *Neuroreport* 14:1841-1845.
- Martinez A, Anllo-Vento L, Sereno MI, Frank LR, Buxton RB, Dubowitz DJ, Wong EC, Hinrichs H, Heinze HJ, Hillyard SA (1999) Involvement of striate and extrastriate visual cortical areas in spatial attention. *Nat Neurosci* 2:364-369.
- Martinez A, DiRusso F, Anllo-Vento L, Sereno MI, Buxton RB, Hillyard SA (2001) Putting spatial attention on the map: timing and localization of stimulus selection processes in striate and extrastriate visual areas. *Vision Res* 41:1437-1457.
- Mogg K, Philippot P, Bradley BP (2004) Selective attention to angry faces in clinical social phobia. *J Abnorm Psychol* 113:160-165.
- Morris JS, Friston KJ, Dolan RJ (1998) Experience-dependent modulation of tonotopic neural responses in human auditory cortex. *Proc R Soc Lond B Biol Sci* 265:649-657.
- Ohman A, Flykt A, Esteves F (2001a) Emotion drives attention: detecting the snake in the grass. *J Exp Psychol Gen* 130:466-478.
- Ohman A, Lundqvist D, Esteves F (2001b) The face in the crowd revisited: a threat advantage with schematic stimuli. *J Pers Soc Psychol* 80:381-396.



- Palomba D, Angrilli A, Mini A (1997) Visual evoked potentials, heart rate responses and memory to emotional pictorial stimuli. *Int J Psychophysiol* 27:55-67.
- Phan KL, Wager T, Taylor SF, Liberzon I (2002) Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *Neuroimage* 16:331-348.
- Pizzagalli D, Regard M, Lehmann D (1999) Rapid emotional face processing in the human right and left brain hemispheres: an ERP study. *Neuroreport* 10:2691-2698.
- Pizzagalli DA, Greischar LL, Davidson RJ (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, Grandjean D, Sander D, Vuilleumier P (2004) Electrophysiological correlates of rapid spatial orienting towards fearful faces. *Cereb Cortex* 14:619-633.
- Recanzone GH, Schreiner CE, Merzenich MM (1993) Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. *J Neurosci* 13:87-103.
- Sabatinelli D, Flaisch T, Bradley MM, Fitzsimmons JR, Lang PJ (2004) Affective picture perception: gender differences in visual cortex? *Neuroreport* 15:1109-1112.
- Scheich H, Heil P, Langner G (1993) Functional organization of auditory cortex in the mongolian gerbil (*Meriones unguiculatus*). II. Tonotopic 2-deoxyglucose. *Eur J Neurosci* 5:898-914.
- Schupp HT, Junghofer M, Weike AI, Hamm AO (2003) Emotional facilitation of sensory processing in the visual cortex. *Psychol Sci* 14:7-13.
- Schupp HT, Junghofer M, Weike AI, Hamm AO (2004) The selective processing of briefly presented affective pictures: an ERP analysis. *Psychophysiology* 41:441-449.
- Smith NK, Cacioppo JT, Larsen JT, Chartrand TL (2003) May I have your attention, please: electrocortical responses to positive and negative stimuli. *Neuropsychologia* 41:171-183.
- Steyer R, Schwenkmezger P, Notz P, Eid M (1997) Der Mehrdimensionale Befindlichkeitsfragebogen (MDBF). Göttingen: Hogrefe Verlag GmbH.
- Van Essen DC, Drury HA, Joshi S, Miller MI (1998) Functional and structural mapping of human cerebral cortex: solutions are in the surfaces. *Proc Natl Acad Sci USA* 95:788-795.
- Vuilleumier P, Schwartz S (2001) Beware and be aware: capture of spatial attention by fear-related stimuli in neglect. *Neuroreport* 12:1119-1122.
- Vuilleumier P, Armony JL, Driver J, Dolan RJ (2001) Effects of attention and emotion on face processing in the human brain: an event-related fMRI study. *Neuron* 30:829-841.
- Weinberger NM (1998) Physiological memory in primary auditory cortex: characteristics and mechanisms. *Neurobiol Learn Mem* 70:226-251.
- Weinberger NM (2004) Specific long-term memory traces in primary auditory cortex. *Nat Rev Neurosci* 5:279-290.
- Weinberger NM, Javid R, Lapan B (1995) Heterosynaptic long-term facilitation of sensory-evoked responses in the auditory cortex by stimulation of the magnocellular medial geniculate body in guinea pigs. *Behav Neurosci* 109:10-17.