Effects of Emotional Conditioning on Early Visual Processing: Temporal Dynamics Revealed by ERP Single-Trial Analysis

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Abstract: Studies using event-related potentials (ERPs) have shown that affectively arousing stimuli enhance attention and perception. In addition, simple neutral stimuli, when paired with emotionally engaging unconditioned stimuli (i.e., the CS+) in classical conditioning paradigms, were found to evoke increased sensory responses as learning progresses, compared to responses elicited by the same stimuli not paired with a noxious stimulus (CS−). To date the detailed trial-to-trial temporal dynamics of this sensory facilitation process is not known. Signal averaging required for the ERP analysis eliminates trial-to-trial information of temporal cortical dynamics. In the current study, a novel single-trial analysis method called Analysis of Single-trial ERP and Ongoing activity (ASEO) was adopted to study the detailed electrocortical dynamics of sensory processing during classical aversive conditioning. Focusing on the P1 component of the ERP evoked by simple grating patterns serving as CS+ and CS−, we found that over a session of conditioning trials, there were three phases of P1 amplitude changes for both CS+ and CS−: (1) an initial decrease phase, (2) a subsequent increase phase, and (3) a final habituating phase. Tests on the rates of P1 amplitude changes in each of the three phases between CS+ and CS− conditions revealed differential effects of CS+ and CS− for all three phases. No such effects were found for a session of control trials where the same grating patterns were paired with checkerboards. We interpret these results as providing evidence supporting the view that emotional experience can modulate early visual processing and dynamics of perceptual learning. *Hum Brain Mapp* 33:909–919, 2012. © 2011 Wiley Periodicals, Inc.

Key words: conditioning; emotional learning; single-trial analysis; ASEO; event-related potential

INTRODUCTION

Emotions, viewed in an evolutionary context, represent phylogenetically old action dispositions that facilitate sur-

vival of the organism and the species [Lang et al., 1998]. A key aspect of adaptively responding to stimuli that signify threats or rewards is the in-depth sensory analysis of such stimuli [Bradley et al., 2003]. In line with this view, neuroscience studies of emotional perception have suggested that processing of emotionally salient stimuli is facilitated at multiple stages of the visual pathway [Pizzagalli et al., 1999, 2003; Schupp et al., 2003, 2004; Vuilleumier et al., 2001]. In situations that require spatial orienting to affectively arousing stimuli, studies using event-related potentials (ERPs) have shown that early visual components C1 and P1 are enhanced by affective cues [Pourtois et al., 2004]. Specifically, experiments using differential classical conditioning have demonstrated increased early cortical

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responses over learning trials for the conditioned stimulus that predicts an unpleasant event (i.e., the CS+) across the conditioning blocks [Stolarova et al., 2006]. As suggested in Keil et al. [2007], a possible explanation for such early sensory enhancement are changes in the depth-of-processing needed for discrimination, as learning progresses: Experience with a given set of stimuli and spatial locations allows observers to predict certain aspects of the stimulus array, which then no longer require in-depth processing in each subsequent trial. In terms of neural mass activity, this predictability may lead to constantly increasing cortical facilitation for a specific set of features (e.g., the orientation of the CS+ grating), at increasingly early stages of visual analysis [Stolarova et al., 2006]. One prediction of this notion is that early trials should be characterized by slower in-depth processing, followed by a successive shift of discriminant neural engagement to fewer and earlier levels of visual cortex. Changes in sensory cortices as a function of learning have been observed in experimental animals [Lee et al., 2002; Schwabe and Obermayer, 2005; Tolia et al., 2005], particularly when using intense conditioning regimens [Elbert and Heim, 2001].

Although previous work has identified the effect of learning on the time scale of experimental trial blocks, it is important for both basic science and applied questions to obtain information on the time course of differential changes during emotional learning on a trial-to-trial basis. For instance, reports on the habituation of the amygdala during classical conditioning [Breiter et al., 1996; Büchel et al., 1998, 1999a; Whalen et al., 1998; Zald, 2003] suggest that re-entrant modulation of visual areas by amygdalo-fugal connections [Amaral and Price, 1984] decreases over time. Another question of interest is the time course and duration of early visual enhancement during the first trials, as it may represent a measure of the speed of acquisition of contingencies [Moratti and Keil, 2009]. Trial-by-trial dynamics of emotional learning are also an important aspect in translational research addressing the etiology, maintenance, and treatment of anxiety disorders [Lissek et al., 2008]. For instance, initial hyper-vigilance and subsequent perceptual avoidance of fear-related cues may represent a dysfunctional pattern of sensory processing of phobic cues in generalized anxiety disorder, or in depression [Mogg et al., 2000], and this pattern may change over trials, e.g., with attention training [Amir et al., 2009].

ERP measures obtained by means of ensemble averaging do not allow for a detailed picture of the learning dynamics in visual cortex because they require the pooling of a substantial number of trials and thus lack trial specificity. For instance, using ensemble averaging, Stolarova et al. [2006] examined electrocortical changes on the time scale of trial blocks, which obscures putative condition differences in terms of trial-to-trial dynamics. In this article, a novel single-trial ERP analysis method called Analysis of Single-trial ERP and Ongoing activity (ASEO) [Xu et al., 2009] is employed to study the emotional experience-

dependent modulation of the extrastriate cortex during a classical differential conditioning paradigm. Treating the visual P1 component elicited by the conditioned stimuli (CS+ and CS-) as an index of early sensory processing, we estimated the amplitude of P1 on a single-trial basis over conditioning and control blocks and compared the results across the two CSs. In contrast to previous reports by Keil et al. [2007] and Stolarova et al. [2006], where block-based averaging was used, the current work aimed at examining the evolving pattern of visual P1 amplitude within a single conditioning block. Given that the P1 has been shown to be modulated by classically conditioned cues in previous studies [Pizzagalli et al., 2003; Stolarova et al., 2006], we expected to observe differential temporal dynamics between the P1 amplitudes elicited by the conditioned stimuli predicting aversive (CS+) vs. neutral (CS-) pictures, respectively.

MATERIALS AND METHODS

Participants

Datasets from five participants (two males and three females, mean age: 22.4, SD = 1.8) were selected from an earlier study of classical emotional conditioning [Keil et al., 2007], in which 14 undergraduate students participated in exchange of class credit or a financial bonus of ~30 USD. To be selected for the single-trial analysis in the present study, datasets had to meet the following three criteria: (a) Reliable enhancement of the eye-blink startle response measured when viewing the CS+, compared to the CS-, which was taken to index successful acquisition of contingencies (see below), (b) reliable P1 ERP scalp topography among parietal-occipital sites, and (c) excellent signal-to-noise ratio of the recorded EEG signal as indexed by a 3-dB gain between the alpha band power and power in beta and gamma band. Eight subjects showing strong contingency acquisition were included according to the first criterion. Among the eight subjects, datasets from two subjects showing abnormal P1 topography during at least one experimental condition were excluded according to criterion b). We further excluded one subject's dataset showing long segments of excessive high frequency artifact from our study. The goal of the above subject inclusion criteria was to make sure that: (1) reliable P1 temporal dynamics could be extracted from each individual subject; and (2) the P1 effects seen in the present participants would be consistent with successful conditioning as indexed by startle reflex modulation. The data from five participants meeting all three criteria were processed using the single-trial analysis technique described below.

Stimuli

One hundred twenty pictures from the International Affective Picture System (IAPS; [Lang et al., 1997]) were

Figure 1.

Schematic of Paradigm and ERP. **(A)** The timeline of experiment. **(B)** Grand average ERPs for both hemispheres and for both conditions. Condition-induced differences in PI are not statistically significant (one-sided paired *t* test; left hemisphere: $P = 0.423$; right hemisphere: $P = 0.412$).

chosen based on their normative ratings of hedonic valence and emotional arousal as listed in the IAPS manual. The 60 unpleasant pictures contained mutilated bodies, attack scenes, and disgusting objects (mean valence: 2.2, $SD = 0.6$; mean arousal: 6.1, $SD = 0.8$). They served as the Unconditioned Stimuli (USs). The 60 neutral pictures served as control stimuli and contained landscapes, people, objects, and abstract patterns (mean valence: 5.9, $SD = 0.7$; mean arousal: 3.8, $SD = 0.9$). The differences between these two picture categories was maximized in terms of both emotional valence and their level of emotional arousal to facilitate differential conditioning despite the relative weakness of picture USs compared to sound or electric shock stimuli used in other works. Picture USs enabled us to use rapid presentation rates, resulting in high trial counts. The affective pictures were presented centrally on a 19-in. computer monitor with a refresh rate of 70 Hz and subtended a visual angle of 7.2° horizontally. In addition, four types of 8-by-8 checkerboards with different colors (black and bright red, black and dark red, black and bright green, and black and dark green) were designed such that they matched the size of affective pictures. These checkerboard patterns were used to replace the affective pictures during the control block with no contingencies (see below).

Two small black and white squares (visual angle horizontally: 2.2°) differing only in grating orientation (45° or 135°) were used as CS+ and CS-, respectively. They had a spatial frequency of 2.3 cycles per degree with 100% in contrast. The gratings were presented in either the upper left or right visual field (eccentricity of the inner border: 3.58°) prior to the affective pictures.

Paradigm

Two experimental blocks, a conditioning block and a control block, conducted in that order on the same day during the original experiment were analyzed in this study. The experimental timeline for the conditioning block is shown in Figure 1A. For each CS+ trial, the grating pattern designated as the CS+ appeared on the screen for a total of 600 ms. About 200 ms following the onset of the CS+, an affective picture was presented centrally for a duration of 400 ms. The inter-trial interval (ITI) varied randomly between 400 and 1,400 ms (Fig. 1A). For each neutral trial, the grating pattern designated as the CS- was paired with the neutral and low arousing pictures in the same manner as described for the CS+. Both trial types were presented in a randomized fashion within the block. The experimental block contained a total of 480 standard trials (grating followed by an IAPS stimulus), out of which four conditions were formed, by crossing the grating orientation (CS+ versus CS-) and visual hemifield (left versus right). There were 120 trials for each condition. The experimental time line for the control block was kept the same as the conditioning block except that the affective pictures were replaced with the checkerboards mentioned above. The experiment was designed such that there was no systematic association between the grating orientation and color of checkerboard to minimize contingency reinforcement. During the experiment, participants 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.

Data Acquisition

The EEG was recorded using an EGI 128-channel system. The vertex (Cz) was the recording reference. The sampling rate was 250 Hz and impedances were kept below 50 k Ω as recommended by the manufacturer. Data were subjected to 0.1 Hz high-pass and 100 Hz low-pass online filtering. Artifact-free epochs (196 ms pre- and 600 ms post-stimulus interval) were obtained using the SCADS procedure suggested by [Junghöfer et al., 2000]. This procedure creates distributions of statistical indices of data quality and lets researchers identify bad channels and trials, with the latter being discarded and the former being interpolated from the full channel set. In a subsequent step, data were re-referenced to average reference. The mean number of artifact-free trials per condition was 82.

ASEO Single-Trial Analysis

A recently developed single-trial analysis method, called Analysis of Single-trial ERP and Ongoing activity (ASEO) [Xu et al., 2009], was adopted to extract the visual single-trial ERP from the raw single trial EEG data. A detailed description of the method can be found in the above reference. Briefly, the recorded r th trial ($r = 1, 2, \dots, R$) of the EEG data is modeled by the variable signal plus ongoing activity (VSPOA) model [Chen et al., 2006; Xu et al., 2009]:

$$x_r(t) = \sum_{n=1}^N \beta_n s_n(t - \tau_n) + z_r(t)$$

where $s_n(t)$ ($n = 1, 2, \dots, N$) is the unknown ERP component waveform with N being the total number of such components, β_n and τ_n are the unknown amplitude scaling factor and the latency shift for the n th ERP component, and $z_r(t)$ is the ongoing activity which is further modeled as an AR random process. With proper initial conditions, the ASEO algorithm estimates the ERP component waveforms $\{s_n(t)\}$, the corresponding single-trial amplitude scaling factors $\{\beta_n\}$ and latency shifts $\{\tau_n\}$, and the AR coefficients of the ongoing activity in an iterative fashion. From these estimated quantities, the single-trial event-related potentials were reconstituted, forming the basis for estimating the amplitude of the P1 component. While implementing ASEO single-trial analysis, the number of components and the waveform for each component required by the algorithm were selected according to visual inspection of the ERP waveform for each subject. An example is illustrated in Figure 3C. The number of components used in this study varied from three to six. These initial components were selected according to computational considerations, and might not effectively represent the true underlying neural generating processes. Comparison of average ERPs from the raw single trial EEG time series and that from the single-trial ERPs estimated from the same raw single trial EEG time series was used to evaluate

the effectiveness of the ASEO procedure [Wang and Ding, in press; Wang et al., 2008].

The same analysis was applied to both the conditioning block and the control block. Because of the lateralized presentation of the CSs, the single-trial analysis was performed on the hemisphere contralateral to CS presentation at two lateral-posterior electrodes (channel 66 and 85 of the EGI 128-channel system) for every subject. These two electrodes, selected for exhibiting maximum ERP peak P1 amplitude, were located near sites P3 and P4 in the standard 10–20 system. Single-trial ERPs from these electrodes were estimated and reconstructed on a trial-by-trial basis for all four experimental conditions. The estimated single-trial ERPs were further subjected to a 10-trial moving averaging procedure to smooth the data and enhance signal-to-noise ratio, i.e., the 1st trial to the 10th trial were pooled together to form smoothing Bin 1 and the single-trial ERPs averaged to produce smoothed Trial 1, and the 2nd trial to the 11th trial were pooled together to form smoothing Bin 2 and the single-trial ERPs averaged to produce smoothed Trial 2, and so on. The window length for the trial-to-trial moving averaging was selected such that reliable P1 amplitude could be obtained without compromising much trial-to-trial specificity. The time interval of the contralateral P1 component was then defined for each condition and subject, separately, and the peak voltage within the time interval was selected as the P1 amplitude measurement.

Hemispheric conditions were further combined by averaging the results over the two electrodes, leaving for comparison two experimental conditions: CS+ and CS-. To examine the effect of emotional conditioning on the CS-evoked P1 component, P1 amplitudes for CS+ and CS- conditions were averaged across five subjects, and plotted against the smoothing bin index to reveal the temporal dynamics. The temporal functions of P1 amplitude data for CS+ and CS- were described by Multivariate Adaptive Regression Splines [MARS; Friedman, 1991], a segmented linear regression method. The parameters of these segmented linear functions were then subjected to statistical analysis.

Given the emphasis of our study on the P1 temporal dynamics, we took the following steps to ensure that the time across subjects and conditions were comparable when we performed the above hemispheric and cross-subject averaging. First, we ensured that among the subjects included in our analyses, the trials remained after artifact rejection were approximately evenly-distributed throughout the block without leaving any major discontinuity in time (Fig. 2A,B). Second, since the number of remaining trials tended to vary within different experimental conditions across subjects after artifact rejection, we further equalized the number of trials prior to the 10-trial moving average by deleting trials randomly from subjects with more remaining trials. The trial deletion indexes were sampled without replacement from a uniform distribution spanning the entire block. To further ensure that deleted

Figure 2.

Temporal distribution of trials analyzed in this study. **(A)** Trial raster plot for the conditioning block showing the remaining aversive (CS+) trials (vertical bars) after artifact rejection. LH: Left Hemifield and RH: Right Hemifield. The horizontal axis denotes the sequential trial index within the two experimental conditions examined in this plot: right hemifield and left hemifield CS+ trials. **(B)** Trial raster plot showing the remaining neutral (CS-) trials for the conditioning block. **(C)** Plot showing the relation between the smoothing bin index and the averaged within-condition trial index for the conditioning block. Note that a similar relation was observed also for the control block (not shown here).

trials covered the block evenly, we imposed an additional constraint such that no two consecutive trials were deleted. To demonstrate that the physical time remained comparable after the above procedure, we conducted for every condition within each subject a 10-trial moving average on the remaining within-condition trial indexes after trial equalization to estimate the approximate physical time associated with each smoothing bin used to reveal P1 temporal dynamics. We combined the hemispheric results and further performed a grand average across five subjects leaving for comparison the averaged physical time indexes

between CS+ and CS-. Here we used the within-condition trial indexes (i.e., the trial order within its own presentation stream for each condition) as a proxy of the actual physical time due to the fact that all four types of trials were evenly intermixed in our experiment design. Figure 2C showed the averaged trial index plotted against smoothing bin index for CS+ and CS-, respectively. The close similarity between CS+ and CS- as well as the quasi-linear pattern observed for both conditions suggest that the P1 dynamics between the two conditions can be compared to assess the effect of emotional conditioning on early visual processing.

RESULTS

Average ERP Analysis

The grand averaged ERPs time-locked to the onset of CS+ and CS- are shown in Figure 1B. A robust P1 response is seen over the parietal-occipital regions contralateral to the stimulated hemifield. Consistent with the previous report [Stolarova et al., 2006], no statistically significant difference between conditions was observed in grand averaged P1 amplitude (one-sided paired *t* test; left hemisphere: $P = 0.423$; right hemisphere: $P = 0.412$).

Single-Trial Estimation of ERPs

ASEO single-trial analysis was applied to extract the ERP on a trial-by-trial basis. The process is illustrated in Figure 3 for a representative subject. The raw single-trial EEG time series from the right parietal-occipital area are shown in Figure 3A. The ASEO estimated single-trial ERPs from the data in Figure 3A are shown in Figure 3B. Ensemble averages of the data in Figure 3A,B are compared in Figure 3C. The close similarity between the two averages is taken as evidence that the ASEO method adequately estimated the single-trial ERPs and did not introduce spurious artifacts. The ongoing activities, obtained by subtracting the single-trial ERPs in Figure 3B from the raw single-trial data in Figure 3A, are shown in Figure 3D. The relatively constant variance of the ongoing activity for the entire epoch (mean 54.921, SD = 11) is seen as further support for the validity of the single-trial estimation procedure [Truccolo et al., 2002; Wang and Ding, in press; Xu et al., 2009].

Temporal Dynamics of the P1 Component

To examine how the history of emotional conditioning affects early sensory processing, we first analyzed the temporal dynamics of the CS-evoked P1 amplitude for the conditioning block measured at sites contralateral to CS presentation across trials. The single-trial ERP data was smoothed by using a 10 trial moving average and each 10-trial ensemble was referred to a smoothing bin. Figure 4

Figure 3.

Illustration of ASEO single-trial analysis on a representative subject. **(A)** Raw single-trial EEG data from the right parietal-occipital channel under contralateral CS+ condition. **(B)** ASEO-estimated single-trial ERP time series. **(C)** Average of ASEO single-trial ERPs is compared with the AERP from raw data. The four components used for initiating the ASEO algorithm were indicated. **(D)** Single-trial ongoing activities obtained by subtracting single-trial ERPs in (C) from the raw single-trial EEG data in (A).

demonstrates the result for a typical subject for both CS+ and CS− conditions. The P1 amplitudes, averaged across two hemispheres and across all subjects, were plotted as a function of the bin index for both CS+ and CS− in Figure 5. Although the amplitude of P1 elicited by CS+ is generally larger than that elicited by CS−, the difference failed to reach significance at the level of $\alpha = 0.05$, agreeing with the grand averaged ERP results reported above. However, as time progressed along the CS+ stream or the CS− stream, the temporal dynamics of the P1 amplitude revealed three distinct phases for both CS+ and CS−: (a) an initial decrease phase, (b) a subsequent increase phase, and (c) a final habituating phase.

The data in Figure 5A were fit by piecewise linear functions using the MARS algorithm and the result is shown in Figure 5B. The durations of the initial decrease phase as measured in unit of bin indexes for CS+ and CS− were both brief and covered eight and six bins, respectively. According to the relationship between smoothing bin index and trial index shown in Figure 2C, this decrease phase covered about 26 CS+ trials and 18 CS− trials. The slopes for CS+ and CS− conditions during this phase were -0.028 and -0.16 . A t test on the slopes [Kleinbaum et al., 1988] revealed a significant condition related difference ($t(12) = 3.3174$, $P = 0.0061$) (Table I). For the subsequent increase phase, the CS+ trials spanned a shorter interval (from 9 to 17 as measured by the bin index), before reaching the maximum value than the CS− trials

(7 to 24). The estimated corresponding number of trials within each condition covered by this phase was 28 and 64 for CS+ and CS−. The slopes for the CS+ and CS− trials within this phase were 0.27 and 0.15 . A t test on the slopes within this phase between the two conditions revealed that the slope for CS+ trials was significantly larger than the slope for CS− trials ($t(20) = 3.1450$, $P = 0.0051$). The habituating phase for the CS+ trials covered a relatively longer period starting at about the 88th trial compared with the CS− trials which started around the 112th trial. The slopes for the CS+ and CS− trials within this phase were -0.064 and 0.0067 , respectively. As characterized by the slopes within this phase, the rate of habituation for CS+ trials was significantly larger than that for CS− trials (a slightly positive slope) ($t(64) = -8.3364$, $P < 10^{-4}$).

To demonstrate that the P1 amplitude dynamics is specific for the conditioning block, we also applied the same analysis to data from the control block and the result is shown in Figure 5C. In contrast to the conditioning block, no systematic changes in processing phase over trials were found during the control block. The average P1 amplitude is also significantly smaller than that of the conditioning block (one-sided t test, $P < 10^{-10}$). In addition, the relatively small variation of P1 amplitude across the entire block suggests that participants maintained a steady level of early electrocortical processing during the block. Fitting the P1 amplitude data by the MARS algorithm described above using three segments of linear functions revealed unsystematic effects between the CSs. A linear regression on P1 amplitude data across the block showed that the slopes of the linear functions were -0.0064 for CS+ and 0.0080 for CS−, respectively (Fig. 5D). Both slopes were considered to be close to zero.

Figure 4.

Smoothing of single-trial ERPs. Depicted are averaged single-trial ERP time series from a smoothing bin of 10 consecutive trials of the same condition. The P1 amplitude is estimated from the smoothed waveform and plotted as a function of time in Figure 5.

Figure 5.

Temporal dynamics of P1 amplitude. **(A)** The P1 amplitude as a function of smoothing bin index over the entire conditioning block for CS+ and CS−, respectively. Three distinct phases can be identified for both CS+ and CS−: an initial decrease phase, a subsequent increase phase, and a final habituating phase. **(B)** Piecewise linear regression using MARS. α 's and β 's indicate

the slopes of each segment for CS+ and CS− conditions. **(C)** P1 amplitude as a function of smoothing bin index over the entire control block for the two grating patterns served as CS+ and CS− in the conditioning block. **(D)** The linear regression analysis results for CS+ and CS− trials, respectively.

DISCUSSION

Emotionally salient stimuli can attract attentional resources involuntarily to affect visual processing [Müller et al., 2008]. Pourtois et al. [2004], incorporating an emotional cue within a spatial orienting task, found enhanced P1 time-locked to a bar that replaced a fearful face, compared to a bar that replaced a happy face. In addition, the C1 component time-locked to the face presentation had a

greater amplitude for fearful faces as compared to happy faces. In a classical conditioning paradigm, Keil et al. [2007] found gradual increase of early (60–90 ms) gamma band (>30 Hz) oscillations evoked by the CS+, across two consecutive experimental blocks. This was taken to suggest that an observer's learning history would continuously alter the processing of sensory information [Moratti and Keil, 2009]. In the current study, we examined this stimulus history dependent modulation in a classical delayed

TABLE I. Results from MARS piecewise linear regression analysis

	Segment 1*		Segment 2*		Segment 3**	
	Slope	SSE	Slope	SSE	Slope	SSE
CS+	−0.0281	0.3107	0.2657	1.1858	−0.0637	3.1112
CS−	−0.1614	0.0384	0.1494	0.4713	0.0067	4.3124
Slope difference	<i>t</i> -score	<i>P</i> -value	<i>t</i> -score	<i>P</i> -value	<i>t</i> -score	<i>P</i> -value
	3.3174	0.0061	3.1450	0.0051	−8.3364	8.24e-12

* $P < 0.01$; ** $P < 10^{-11}$

conditioning experiment on a more detailed time scale by means of single-trial ERP analysis [Xu et al., 2009]. By extracting the P1 component evoked by the conditioning stimuli on a trial-by-trial basis, we reported three results. First, three distinct phases of P1 amplitude as a function of time specific to emotional conditioning were found: (1) a short initial decrease phase, (2) a subsequent increase phase, and (3) a final habituating phase for both the CS+ and CS- trials within the block. Second, the P1 response to CS+ stimuli exhibited slower rate of decrease over the first phase, faster rate of increase over the second phase, and again faster rate of decrease over the third phase relative to that evoked by the CS- stimuli. Third, for the control block where the same grating patterns used as CS+ and CS- stimuli in the condition block were paired with checkerboards, no systematic temporal effects were found for the P1 amplitude.

Previous work has yielded mixed results with respect to P1 differences during classical conditioning [see Stolarova et al., 2006]. The present data are informative by revealing the intra-block P1 dynamics associated with learning of contingencies. One possible explanation for the initial P1 decline could be that during the early trials, the contingencies are not yet established and thus neural responses to both CSs may be subject to repetition suppression. Previous studies in human electrophysiology, neuroimaging, and primate single-cell recordings have shown that repeated stimulus presentation tends to elicit decreased neural responses over time in the visual cortex [Grill-Spector et al., 2006; Guo et al., 2007, 2008; Jiang et al., 2000, 2009; Liu et al., 2009; McMahon and Olson, 2007]. Research in classical conditioning has established that it frequently takes up to 5 trials of both the CS+ and the CS- for differential conditioning to take place, not only as reflected in psychophysiological measures, but also associated with selective enhancement in human visual cortex for the CS+ [e.g., Moratti and Keil, 2005]. Considering the fact that our experiment paradigm further added a potential confounding factor by adopting a lateralized CS presenting scheme, the number of trials within the initial decrease phase (26 for CS+, 18 for CS-) might indicate the average amount of trials required to obtain conditioning effect in early visual cortex during our experiment. The smaller reduction rate for CS+ as compared to that for CS- may be reflective of the fact that any suppression was accompanied by differential enhancement for the CS+. It is worth noting that repetition suppression was not apparent in the control block, which may reflect a floor effect, given that the control block was conducted subsequent to the conditioning block, and the overall P1 amplitude was small across the control block.

After the initial decrease phase, the P1 response to both CS+ and CS- underwent a subsequent increase phase, which is consistent with an increase in the predictive value of the CSs across these trials. At this stage, increased salience for both CSs appears to lead to increased attention bias and ultimately facilitated perceptual processing in

extrastriate cortex. The fact that the rate of P1 increase was greater for CS+ than for CS- suggests that the motivationally relevant predictive stimulus (i.e., the CS+) benefits more strongly from such facilitation. Hence, the difference between the slopes for CS+ and CS- during the increase phase further supports the view that experience established during classical conditioning affects the neural network organization underlying early visual processing [Keil et al., 2007].

Functional imaging studies of visual aversive conditioning [Adolphs, 2002; Büchel and Dolan, 2000; LaBar et al., 1998; LeDoux, 2003; Morris et al., 1998b] have highlighted the amygdala, higher-order sensory areas, and frontal cortices as key structures for providing re-entrant input into visual areas [Sabatinelli et al., 2009; Zald, 2003]. In addition, the amygdala has been hypothesized to mediate cortical plasticity during emotional learning [Hendler et al., 2003]. Given that the visual P1 component of the ERP originates in the extrastriate cortex [DiRusso et al., 2001], and that the amygdala projects to the extrastriate cortex in primates [Amaral and Price, 1984], the differential modulatory effects of the CSs on P1 may reflect a stronger involvement of the amygdala in re-entrant sensory modulation of the CS+ as opposed to the CS-.

The final phase for both CS+ and CS- after the increase phase was a habituating phase toward the end of the conditioning block. The P1 response to the CS+ declined gradually as time progressed whereas the P1 response to CS- ceased to further increase and remained at a constant level. A widely observed activation pattern of amygdala during emotional perception and conditioning is its initial activation followed by rapid habituation or even deactivation as experiment progresses [Breiter et al., 1996; Büchel et al., 1998, 1999a; Phillips et al., 2001; Zald, 2003]. Although no measurement from subcortical structures was available in our study, the habituation of the P1 amplitude across trials might reflect habituation of deep structures such as the amygdala, which may eventually cease to provide re-entry input into visual cortex.

From a network point of view, changes in connectivity as a function of learning and experience should be considered. In the domain of auditory classical conditioning, research has shown learning-induced plasticity in the receptive fields of the primary auditory areas in animals [Diamond and Weinberger, 1984; Quirk et al., 1997; Weinberger, 2004] and humans [Morris et al., 1998]. Such changes are accompanied by an increase in dopamine or acetylcholine release [Weinberger, 1995], possibly leading to long-term potentiation and the strengthening of neural connectivity [Fox and Wong, 2005]. Heightened neural connectivity has been shown during viewing of emotionally salient cues [Keil et al., 2003], and directional analyses of electrocortical data in humans have suggested re-entry can be observed from higher to lower tiers of visual cortex [Keil et al., 2009]. Because heightened connectivity is often associated with amplitude reduction of neural mass activity [Büchel et al., 1999b; Gruber et al., 2001], the response

habituation to the CSs in our experiment might thus reflect the strengthening of visual networks representing the CSs' locations and features after repeated contingency reinforcement. This is also in line with the notion that cortical modulations during learning occur at increasingly earlier stages as learning progresses, with early trials characterized by late modulation and late trials characterized by early modulation [Stolarova et al., 2006].

Regarding the observed slope difference between the CSs, our results agree with a previous study on the impact of emotion on repetition suppression [Ishai et al., 2004]. Using event-related fMRI to measure the rate of repetition suppression, Ishai et al. found that fearful faces which initially elicited stronger activation had a higher rate of repetition suppression compared with neutral faces. Although in our experiment the CSs acquired emotional salience through conditioning, the higher P1 increase rate during the second stage coupled with subsequent stronger suppression effects for CS+ compared to CS− during the third stage suggests that processing of emotional stimuli may become more shifted toward a highly efficient neural network.

In terms of attention mechanisms during conditioning, our P1 temporal dynamics during the conditioning block support the prediction error theory of attention during conditioning [Pearce and Hall, 1980], which states that subjects' level of attention tends to decrease if a stimulus fully predicted an event during previous trials and vice versa. Studies that measured participants' attentional response via EEG and eye tracking measurements [Wills, 2009; Wills et al., 2007] have lent support to this theory by providing evidence that increased attention is correlated with prediction error. In this regard, the increase phase and the habituating phase for the CSs in our study might reflect two distinct learning stages with the increase phase indicating high uncertainty level and habituating phase indicating the opposite case according to this theory. On a neurophysiological level, these theoretical notions are consistent with the perspective that processing simple, important, and predictable information may be accomplished by increasingly early stages of visual analysis as learning progresses [Keil, 2004]. Thus, initial P1 enhancement should be followed by P1 amplitude reduction (as found here), coupled with enhancement of the C1 component (60–90 ms post-stimulus). Such a pattern was observed for the CS+ when comparing blocks of an identical conditioning protocol [Stolarova et al., 2006].

In summary, by adopting a single-trial analysis method, our study revealed distinct phases of temporal dynamics of the visual P1 component within a classical emotional conditioning paradigm. The fact that the three distinct phases of P1 amplitude dynamics were only found in the conditioning block as opposed to the control block supports the notion that the subject's emotional processing experience can constantly modulate early visual processing through conditioning. By analyzing the differential effects within each processing phase between CS+ and CS− trials

we were able to examine possible neuronal mechanisms of conditioned response. Despite these positives, the present study also has limitations that should be addressed in future studies. First, owing to the stringent selection criteria (see Methods), the number of subjects is small ($n = 5$). Data from both hemispheres had to be combined to create a pool of sufficient trials. As a consequence, we were not able to test the hypotheses in regard to hemispheric lateralization of emotional processing [Alves et al., 2008]. Second, due to the limitation of scalp-EEG, we were not able to concurrently record activities from important subcortical structures such as amygdala. Thus, the linkage between the effects observed at the cortical level and the previously established subcortical conditioning effects remains to be further substantiated. Simultaneous EEG and fMRI recording is a promising technique to overcome this weakness.

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