

Visuocortical Changes During Delay and Trace Aversive Conditioning: Evidence From Steady-State Visual Evoked Potentials

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The visual system is biased toward sensory cues that have been associated with danger or harm through temporal co-occurrence. An outstanding question about conditioning-induced changes in visuocortical processing is the extent to which they are driven primarily by top-down factors such as expectancy or by low-level factors such as the temporal proximity between conditioned stimuli and aversive outcomes. Here, the authors examined this question using 2 different differential aversive conditioning experiments: participants learned to associate a particular grating stimulus with an aversive noise that was presented either in close temporal proximity (delay conditioning experiment) or after a prolonged stimulus-free interval (trace conditioning experiment). In both experiments, the authors probed cue-related cortical responses by recording steady-state visual evoked potentials. Although behavioral ratings indicated that all participants successfully learned to discriminate between the grating patterns that predicted the presence versus absence of the aversive noise, selective amplification of population-level responses in visual cortex for the conditioned danger signal was observed only when the grating and the noise were temporally contiguous. Our findings are in line with notions purporting that changes in the electrocortical response of visual neurons induced by aversive conditioning are a product of Hebbian associations among sensory cell assemblies rather than being driven entirely by expectancy-based, declarative processes.

Keywords: fear conditioning, steady-state visual evoked potentials, electroencephalogram

Perceptual facilitation of sensory cues that have been associated with harm through prior experience constitutes an important first step in a cascade of changes that occur when organisms flexibly adapt their behavior to motivationally significant environmental contingencies (Bolles & Fanselow, 1980; Sporns, Alámásy, & Edelman, 2000). Fear conditioning protocols where a previously neutral cue (conditioned stimulus; CS+) is repeatedly paired with an aversive event (unconditioned stimulus; UCS) constitute a viable experimental model for examining the experience-dependent acquisition of threat-related biases in perception. The organizational role exerted by aversive learning experiences on cortical sensory systems is attested to by a large body of evidence demonstrating that a variety of nonhuman animals exhibit rapid conditioning-induced changes in the cue-related tuning profiles of sensory neurons (for a comprehensive review, see Weinberger, 2007). Similarly, in human studies stimuli associated with aversive

outcomes boost responses of sensory brain regions as indexed by electric (Keil et al., 2007; Stolarova et al., 2006), magnetic (Dolan et al., 2006; Kluge et al., 2011; Moratti, Miller, & Keil, 2006; Moratti & Keil, 2005, 2009; Weisz et al., 2007), and blood-flow (Morris et al., 1998; Padmala & Pessoa, 2008; Thiel, Friston, & Dolan, 2002) measures of neural activity. Short-term plasticity of sensory cortical networks has been suggested as a mechanism that may underlie these forms of experience-dependent activity changes (Miskovic & Keil, 2012; Keil et al., 2007). Functionally, perceptual amplification of threat-related stimuli enables their efficient detection and identification, ultimately facilitating successful avoidance of harm (Pessoa, 2008; Vuilleumier, 2005).

The degree of temporal contiguity between the CS+ and the UCS sets constraints on the strength of associative learning (Mackintosh, 1983) and may be expected to influence the experience-dependent adaptation of cortical sensory systems to threat-related environmental features. To date, most studies that have examined perceptual processing of conditioned threat cues have relied on classical delay conditioning where the CS+ and the UCS overlap in time and coterminate. Classical delay conditioning in the laboratory mimics a particular class of aversive learning situations in natural settings, where conditioned stimuli are continuous in time with harmful outcomes (e.g., a rustle of leaves is temporally contiguous with the predator's leap). However, other forms of aversive conditioning have evolved in which organisms must form an association that bridges events that are discontinuous in time (e.g., nausea that is induced several hours after ingesting a toxic food substance). One way to model the latter form of aversive learning involves the use of a trace conditioning procedure where a stimulus-free interval lasting hundreds of milliseconds or several

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seconds separates CS+ offset from UCS onset. Conditioning in a trace protocol presumably requires a mnemonic mechanism for bridging the temporal gap that separates the two sensory events (Pavlov, 1927). An outstanding question not addressed by previous work is whether the visuocortical facilitation of conditioned danger signals is maintained under this latter scenario, when the signal cue and the noxious event are temporally discontinuous. It should be noted that our focus in the present article lies specifically on threat cue processing in the visual system. It is possible, indeed likely, that the dynamics of visuocortical responses to aversive learning are not shared across other sensory systems and that each sensory system may be characterized by a unique response profile in relation to aversive learning and the temporal relations between sensory events.

Many theories of conditioning emphasize the role of expectancy and attention-related processes (Bolles & Fanselow, 1980; Pearce & Bouton, 2001). If the enhanced visuocortical processing of conditioned threat cues is driven primarily by top-down cognitive factors such as attention and expectancy in humans (e.g., Vuilleumier & Driver, 2007), then one hypothesis is that trace conditioning should yield intact CS+ selective facilitation, so long as participants have successfully learned that the CS+ serves as a reliable predictor of an aversive event. However, evidence from a previous study suggests that enhancement of visual cortex activity in response to a conditioned danger cue depends on associative strength, accumulated in a bottom-up fashion through previous experience of CS–UCS linkage, and not on attention-guided UCS expectancy (Moratti & Keil, 2009). That is, the expectation of receiving a particular UCS following CS onset was not directly linked to an increase of visuocortical responding to the conditioned threat cue. If the CS+ perceptual amplification emerges as the result not entirely of expectancy, but of Hebbian associative processes (e.g., Rockstroh & Elbert, 2010), whereby the connective strength of cortical cell assemblies representing the CS+ and the UCS is increased by their synchronous activation, then one might expect threat-related biases in the visual cortex to be decreased by temporally separating the CS+ and the UCS.

The aim of the current study was to probe the dynamics of visuocortical responses during delay and trace aversive conditioning to determine whether CS+ amplification is modulated by CS–UCS temporal contiguity. The conditioned stimuli consisted of flickering grating patterns and the UCS consisted of an aversive loud noise that was paired with the CS+, so as to overlap with the last second of CS+ presentation (delay preparation) or 3 s following CS+ offset (trace preparation). In both experiments, we used a measure of stimulus entrained oscillatory mass activity (steady state visual evoked potentials; ssVEPs) to quantify the degree of visuocortical enhancement. The ssVEP is an oscillatory response to stimuli that are periodically modulated in luminance or contrast, in which the frequency of the electrocortical response recorded from the scalp equals that of the driving stimulus (Müller, Teder-Salejarvi, & Hillyard, 1998; Regan, 1989). Because the driven oscillatory cortical response has a precisely defined frequency signature, the steady-state potential can be reliably separated from background EEG (i.e., those aspects of the ongoing neurophysiological time series that oscillate outside of the driving range) and quantified in the frequency domain (Wang, Clementz, & Keil, 2007). Amplitude fluctuations can then be used to index sustained sensory processing—enhanced ssVEP amplitude indicates height-

ened sensory activation in response to a stimulus, with sensitivity to both intrinsic, motivational factors (e.g., Keil et al., 2003; Moratti et al., 2006) as well as extrinsic, task-related processes (e.g., Andersen & Müller, 2010; Müller et al., 1998).

Generators of the ssVEP have been localized to the extended visual cortex (Müller, Teder, & Hillyard, 1997), with strong contributions from V1, but also from higher-order cortices (Di Russo et al., 2007). As a measure of sustained and repeated low-level visual excitation, ssVEPs reflect both an early (primary visual) component of visual processing, but also allow for slower (e.g., reentrant) processes to affect these repeated lower level excitations. Thus, changes in the ssVEP can reflect variability in low-level sensory processing (e.g., differences in brightness of the stimulus) as well as subsequent reentrant modulation of sensory activity by higher order processes (Keil et al., 2009; Silberstein, Ciorciari, & Pipingas, 1995). Given these properties, it can be informative to examine the envelope of the ssVEP over time, when comparing experimental conditions. For instance, one may often observe a time course in which the ssVEPs for two conditions show identical amplitude initially, with delayed discrimination developing as stimulation is continued. This can then be taken as evidence that physical properties between the two classes of experimental stimuli are the same and that the late difference is due to slower reentry processes into visual areas, modulating the primary sensory response.

Methods

Participants

A total of 21 participants for the delay and 24 participants for the trace conditioning experiments were recruited from undergraduate psychology courses at the University of Florida. Participants were excluded due to noisy electrophysiological data and/or no reliable ssVEP elicitation as measured by the circular T-square statistic (Victor & Mast, 1991), requesting to opt out of experiment because of distress caused by the UCS, or because of technical difficulties in visual stimulus delivery. After these exclusions, the final sample consisted of 16 participants in the delay condition (12 females) (M age = 19.63 years, SD = 1.75) and 19 participants in the trace condition (16 females) (M age = 19.11 years, SD = 1.20).

Stimuli

The conditioned stimuli were generated using the Psychophysics Toolbox (Brainard, 1997; Pelli, 1997) for MATLAB. They consisted of black-and-white sinusoidal gratings (Gaussian-windowed with maximal contrast at center) subtending a visual angle of $\sim 10^\circ$. The gratings were low in spatial frequency (2 cycles per degree) and designated as the CS+ (45° clockwise tilt from vertical) and CS– (45° anticlockwise tilt from vertical). The UCS was a 1.2-s white noise burst, generated in MATLAB and presented through free-field speakers surrounding the participant, set at 92-dB SPL.

Procedure

After providing written informed consent and initial screening to rule out photic epilepsy/seizures, participants were seated in a

sound-attenuated, dimly lit testing room where the electroencephalogram (EEG) sensor net was applied.

Participants were explicitly instructed that the habituation phase would not involve UCS presentation. The habituation phase involved 20 unreinforced presentations each of the CS+ and CS− gratings. Participants were then informed that they would sometimes hear “a loud, annoying sound” during the conditioning phase, but they were not informed of a specific relation between CSs and the UCS (uninstructed conditioning). The conditioning phase involved 20 presentations each of the CS+ (100% reinforcement ratio) and CS− trials. Trial order in the habituation and conditioning phases was pseudorandomized such that there were no more than three successive trials of each type. To gauge participants’ awareness of the CS–UCS contingency structure, the conditioning phase also involved ratings of the likelihood that the UCS would follow either the CS+ or the CS−. These ratings were initiated during the early (after the first presentation), middle (after the 10th presentation), and late (after the 20th presentation) CS+ and the CS− conditioning trials; ratings were completed by dragging a mouse pointer across the horizontal plane of the display panel to indicate that the CS–UCS pairing was not likely (left), uncertain (middle) or likely (right). The extinction phase was included for ethical clearance, and involved six unreinforced CS+ presentations. Data from the extinction phase are not reported here.

Nonreinforced conditioning trials were 5.76 s in length. The reinforced conditioning trials were 6.9 s in length, with the extra 1.2 s added to provide adequate time for the UCS to coterminate with visual stimulus presentation (delay conditioning experiment). The extra 1.2 s of EEG data were excluded from subsequent analyses to avoid contamination of the ssVEP with sound presentation. Procedures were identical in the trace conditioning experiment, with the exception that CS+ offset was followed by a 3-s trace interval (dark screen) before UCS onset.

Participants were seated at a distance of ~ 1.2 m from the monitor. The monitor had a 70 Hz vertical refresh rate with a 19-inch display. Steady-state VEPs were elicited by phase reversal of the gratings at a 14-Hz driving frequency. Because of technical failure, different monitors were used for stimulus display in the delay (Samsung LCD) and trace (Sony CRT) experiments. Although such differences in display monitors may influence ssVEP amplitudes (Wu, Lai, Zia, Wu, & Yao, 2008), the results reported here were conducted on normalized ssVEP data (see *Statistical Analyses* subsection). In addition, we found no main effect of experiment ($F < 1, p > .67$).

The experiment was programmed and controlled using the MATLAB Psychophysics Toolbox. All procedures were approved by the local institutional review board of the University of Florida and were in line with the Declaration of Helsinki.

EEG Data Recording

EEG was continuously recorded from 257 electrodes using an Electrical Geodesics™ high-density sensor net, digitized at a rate of 250 Hz, using the vertex sensor (Cz) as the recording reference, with online bandpass filters set at 0.1 Hz (high-pass) and 50 Hz (low-pass). Sensor impedances were kept below 60 k Ω .

EEG Data Reduction and Analyses

Offline EEG analyses were implemented using the ElectroMagnetEncaphalography toolbox for MATLAB (Peyk, De Cesarei, & Junghofer, 2011). Relative to stimulus onset, epochs were extracted from the raw EEG that included 400 ms pre- and 5760 ms postonset for all conditions. Data were filtered using a 20-Hz low-pass (45 dB/octave, 12th order Butterworth) and a 10-Hz high-pass (18 dB/octave, 4th order Butterworth). As outlined by Junghöfer, Elbert, Tucker, & Rockstroh (2000), statistical parameters were used to find and remove artifact-contaminated channels and trials. The original recording reference (Cz) was first used to detect recording artifacts, and then the data was average referenced to detect global artifacts. Subsequently, bad sensors within individual trials were identified and interpolated based on rejection criteria for amplitude, standard deviation, and gradient.

Time-varying ssVEP amplitude was extracted by means of Hilbert transform, performed on time-domain averaged data using in-house written MATLAB scripts. To this end, data were first filtered with a 20th-order Butterworth bandpass filter having a width of .5 Hz (48 dB/octave), around the center frequency of 14 Hz. Adequacy of the filters and absence of ringing was tested in each individual subject by visual inspection of the filtered and nonfiltered data. Then a phase-shifted version (the analytic signal) of the empirical signal was generated using the native Hilbert function implemented in MATLAB, and time-varying amplitude extracted as the modulus of the empirical and analytic signal.

Figure 1 depicts the visual evoked potential (averaged across conditions and participants) in the time domain, demonstrating the onset of the oscillatory visuocortical response at the fundamental frequency matching the driving stimulus. Also displayed, for illustrative purposes, is the time-varying Hilbert-derived amplitude in the frequency domain for the 14-Hz ssVEP response (averaged across conditions and participants) as it evolves during the dura-

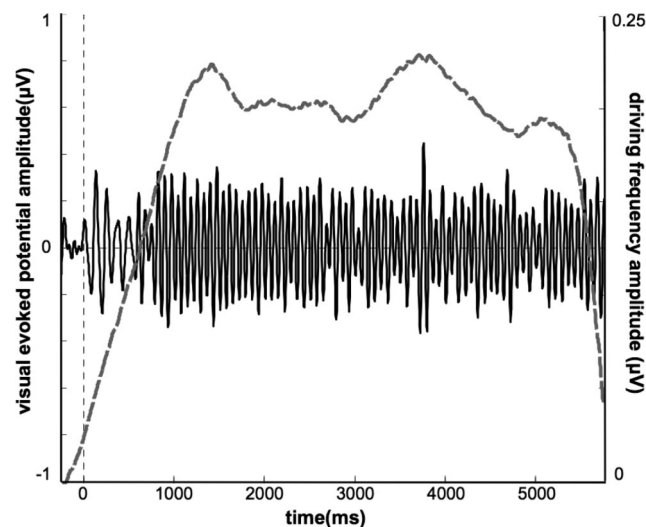


Figure 1. Grand mean ($N = 19$) steady-state visual evoked potentials (ssVEP) time series from the trace conditioning experiment at the Oz sensor site, averaged across all conditions. The dotted gray line depicts the evolutionary spectrum of the grand mean amplitude envelope of the 14 Hz driven response, derived by means of a Hilbert transform.

tion of the stimulus viewing epoch. The first second of the visual evoked response was not included in the main analyses to exclude initial nonstationary components of the brain response from the power spectrum.

Statistical Analyses

To test whether participants successfully learned the CS–UCS contingency structure, we entered participants' online ratings into a mixed-model analysis of variance (ANOVA) using Group (delay, trace) as the between-subjects factor with CS Type (CS+, CS–) and Time (early, middle, late portions of the conditioning block) as the within-subject factors. To test conditioning-induced changes in visuocortical responses to the CSs, we averaged the Electrical Geodesics Inc. (EGI) sensor corresponding to Oz in the International 10/20 System and 12 neighboring sensors (EGI sensors 120, 138, 119, 128, 118, 126, 139, 140, 141, 129, 109, 110). The ssVEP amplitudes were then normalized to lie between 0 and 1, using a min–max normalization procedure. The normalization was conducted separately for each individual and each 500-ms block of EEG data. The normalization was aimed at reducing large interindividual differences in ssVEP amplitude and variability due to switching between CRT/LCD displays. The 500-ms blocks were then averaged into early (1000–2500 ms), middle (2500–4000 ms), and late (4000–5500 ms) portions of the stimulus viewing period. The normalized data were entered into a mixed-model ANOVA with the factors of Group, CS Type, Learning Phase (Habituation, Conditioning), and Viewing Period (early, middle, late viewing). For the present purposes, a CS Type \times Learning Phase interaction was considered critical for inferring neural effects of aversive conditioning. For purposes of clarity, CS Type \times Viewing Period interactions are not discussed further here, as they do not illuminate questions of conditioning.

In cases where Mauchly's test indicated a violation of sphericity, we report the Greenhouse-Geisser corrected degrees of freedom and p values. An alpha level of 0.05 (two-tailed) was used for all statistical analyses.

Results

Learning About Threat: Behavioral Ratings of CS–UCS Contingency

Main effects of CS Type [$F(1, 33) = 79.62, p < .001$] and Time [$F(2, 66) = 17.22, p < .001$] were subsumed under a significant two-way CS Type \times Time [$F(1.66, 54.75) = 21.04, \epsilon = 0.83, p < .001, \eta_p^2 = .39$] interaction. There were no main or interaction effects involving Group ($F_s < 1, p_s > 0.46$), suggesting that the insertion of a gap between CS+ offset and UCS onset in the trace conditioning experiment did not interfere with participants successfully learning the contingency structure. As illustrated in Figure 2, the CS+ cue was rated as being more likely than the CS– to be paired with the UCS during the middle [$F(1, 37) = 145.00, p < .001, \eta_p^2 = .80$] and late [$F(1, 37) = 59.45, p < .001, \eta_p^2 = .62$] stages of the conditioning block, indicating that regardless of whether participants were trained in the delay or trace procedure, they showed evidence of discriminative learning, in that the CS+ selectively predicted an aversive event, whereas the CS– predicted its absence.

Visuocortical Amplification of Conditioned Cues: SsVEP Amplitude

Visuocortical facilitation of the conditioned threat cue differed as a function of participation in the delay versus trace conditioning experiment, as indicated by an omnibus Group \times CS Type \times Phase [$F(1, 33) = 4.37, p = .04, \eta_p^2 = .12$] interaction. To decompose this interaction, we conducted a series of follow-up repeated-measure ANOVAs separately for each conditioning group. Figure 3 depicts normalized ssVEP amplitudes evoked by the CS+ and the CS– gratings during the conditioning block, shown separately for the (a) delay and (b) trace conditioning preparations. Evidence of CS+ selective conditioning amplification was observed only in the delay group and this visual impres-

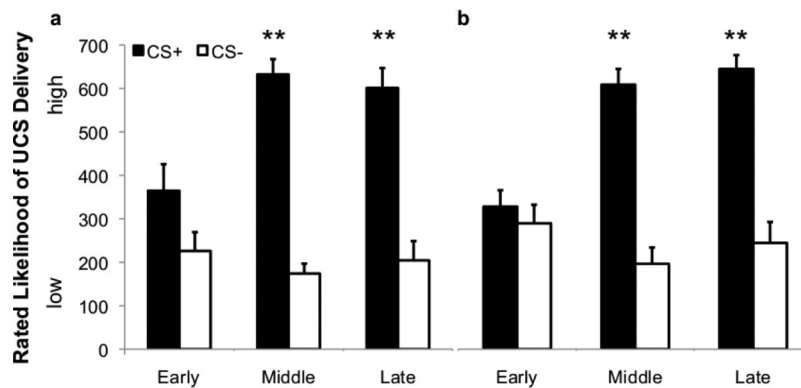


Figure 2. Online ratings of unconditioned stimulus (UCS) expectancy for the reinforced (CS+) and the nonreinforced (CS–) gratings in (a) delay and (b) trace conditioning experiments. Ratings were collected during the early, middle, and late phases of the conditioning block. The y-axis scale represents the horizontal dimensions of the display panel. Bars depict standard error. ** $p < 0.001$.

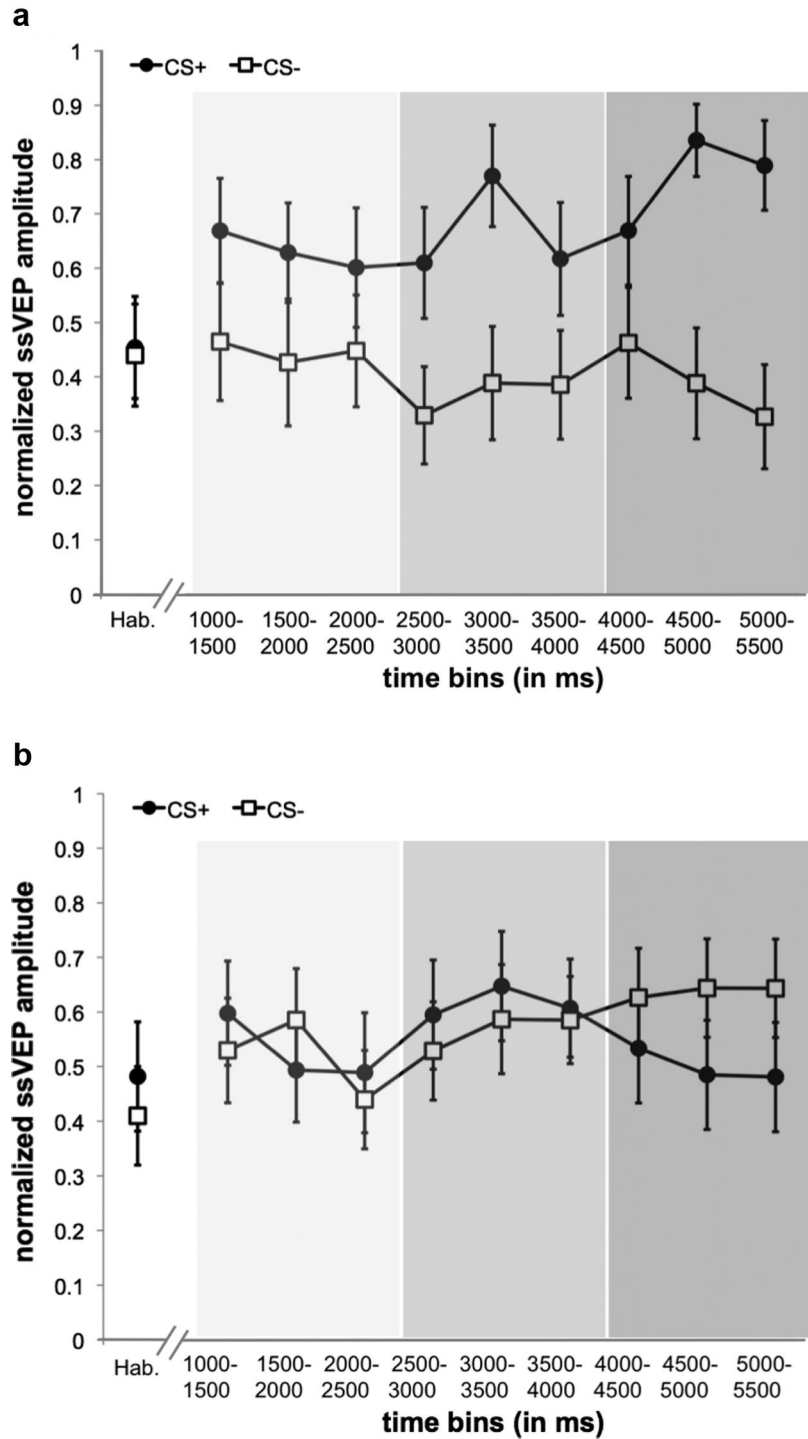


Figure 3. Time course (500 ms bins) of min-max normalized steady-state visual evoked potentials (ssVEP) amplitudes evoked by the reinforced (CS+) and the nonreinforced (CS-) gratings, shown separately for the (a) delay and (b) trace conditioning experiments. The ssVEP data submitted to statistical analyses were averaged into the early (light gray; 1000–2500 ms), middle (2500–4000 ms), and late (dark gray; 4000–5500 ms) portions of stimulus viewing. Bars depict standard error.

sion was confirmed by the statistical results.¹ The crucial CS Type \times Phase interaction was observed in the delay group [$F(1, 15) = 4.34, p = .05, \eta_p^2 = .22$], but was not evident in the trace group ($F < 1, \eta_p^2 = .04$).

A further breakdown of the EEG data indicated that there was an increase of normalized ssVEP amplitude evoked by the CS+ compared to the CS- cue during the conditioning phase of the delay experiment [$F(1, 15) = 7.28, p = .017, \eta_p^2 = .33$]. Although inspection of the means in Figure 3a might suggest that ssVEP enhancement peaked during the middle and late phases of stimulus viewing period, there was no reliable interaction involving Viewing Period ($ps > 0.30$).

There was no main effect of CS type during the habituation phase of the delay experiment ($F < 1, p > .86$). The normalized ssVEP amplitude evoked by the CS+ grating increased from the habituation ($M = 0.45, S.E.M. = 0.07$) to the conditioning ($M = 0.69, S.E.M. = 0.07$) phase [$F(1, 15) = 4.3, p = .056, \eta_p^2 = .22$] of the delay experiment, but remained unaffected across learning phase for the CS- grating ($F < 1, p > .72$) providing further evidence of threat-related visuocortical amplification.

Discussion

In the present study we used steady-state stimulation to investigate the engagement of visual cortical networks in response to flickering grating stimuli that were conditioned in close (delay conditioning) or distant (trace conditioning) temporal proximity to an aversive outcome. Behavioral results indicated that participants learned to successfully discriminate the cue that predicted the noxious event (CS+) from the one that did not (CS-) in both types of training regimens and at comparable rates. However, experience-dependent changes in cue-related cortical EEG potentials were sensitive to the degree of CS-UCS temporal contiguity. More specifically, the learned threat signal evoked stronger population-level responses within visual cortical regions compared to the nonreinforced (CS-) stimulus, but only when it was presented in close temporal proximity to the aversive event (i.e., the two sensory events overlapped and coterminated) during the learning phase. By contrast, selective facilitation of CS+ evoked visuocortical responses was no longer evident when a stimulus-free trace interval lasting 3 s separated the offset of the grating cue from the delivery of a loud, aversive noise.

To date, the majority of findings on the sensory dynamics of processing conditioned danger signals have stemmed from studies that used some variation of delay conditioning procedures. Our results here largely replicate previous work using steady-state driving of the visual cortex (Moratti et al., 2006; Moratti & Keil, 2005, 2009), where aversively conditioned cues are associated with enhanced population-level neuronal responses. By contrast, little is known about patterns of sustained sensory activation when the conditioned cues and aversive outcomes are discontinuous in time. One exception is a previous fMRI study (Knight, Cheng, Smith, Stein, & Helmstetter, 2004), where the blood-oxygen dependent level signal increased in the right middle occipital gyrus when participants viewed the CS+ compared to the CS-, the former being separated from the UCS by a 10-s duration trace interval. It is impossible to directly compare this blood-oxygen dependent level result with our evidence of absent CS+ enhancement in ssVEPs recorded during trace conditioning, though it is of

interest to note that in the fMRI study delay conditioning was associated with CS+ enhancement in more extended, bilateral regions of the occipital gyrus compared to a more regionally restricted effect of trace conditioning (Knight et al., 2004). In addition, the authors used a more intense UCS (cutaneous electric shock) than the one used in the present study.

More recently, several MEG studies (Brösckelmann et al., 2011; Steinberg et al., 2012) of affective associative learning have relied on atypical CS-UCS temporal pairing rules that cannot easily be classified either as classical delay or trace preparations. These studies found evidence of enhanced sensory responses to the CS+ occurring early in the processing stream when the CS+ followed and overlapped with a noxious odor (Steinberg et al., 2012), as well as when the CS+ temporally preceded the UCS (Brösckelmann et al., 2011). Importantly, these experiments did not involve a protracted temporal separation between the CS+ and its reinforcement, with some degree of CS-UCS overlap being present during the learning phase.

The absence of a visuocortical response bias for the threat cue in the trace conditioning experiment, despite participants having successfully learned the contingency rules linking the gratings to differential outcomes, suggests that the acquisition of a visuocortical bias for the CS+ is not completely driven by a declarative attention process associated with expectation of UCS delivery. One previous MEG study relied on the Perruchet (1985) experimental design to dissociate the relative contributions to visual cortex plasticity of top-down expectancy-related processes and those based on CS-UCS linkage established through cumulative bottom-up experience (Moratti & Keil, 2009). The main finding to emerge from that study was that conditioned changes in visual cortex activity were not predicted by subjective UCS expectancy following a CS encounter but were largely driven by the degree of accumulated CS-UCS associative strength. Taken together with other evidence that visuocortical enhancement for the CS+ cue represents a time-sensitive process, that peaks during intervals when CS-UCS convergence is most likely to occur (Moratti et al., 2006), it might be tentatively concluded that amplification for conditioned danger signals in the visual cortex is determined largely by such low-level parameters like the temporal proximity and cooccurrence of sensory cues.

In light of the evidence reviewed here that close CS-UCS temporal proximity is conducive to the development of a CS+ visuocortical response bias, an explanation for the effect that is formulated in terms of a fear network consolidated by Hebbian associative processes between sensory cortical cell assemblies may be empirically favored (e.g., Rockstroh & Elbert, 2010). One such interpretation of the findings is that when temporal proximity between the CS+ and UCS is high (as in the delay conditioning experiment), connective strength between the cortical sensory assemblies encoding the respective stimuli is strengthened, such that on future encounters with the CS+, the threat cue benefits from a boost in sensory activation that might enhance its perceptual representation. An expectation that a particular conditioned stim-

¹ Inspection of the raw, Hilbert-transformed data suggests that the null finding in the trace group was not due to a ceiling effect, as the ssVEP amplitude did increase in strength by about 15% from habituation to the conditioning phase, albeit nonspecifically.

ulus is highly likely to be followed by an aversive event appears therefore insufficient for observing threat-related visuocortical amplification without a close temporal relation between the cues that are being associated.

In situations where temporal proximity of the CS+ and the UCS is low (as in the trace conditioning experiment), the connective strength between the cortical sensory assemblies is weakened and isolated presentation of the CS+ does not necessarily result in a response gain within regions of the visual cortex.² One likely possibility is that during a trace conditioning protocol, representation of the CS–UCS linkage may reside in other, nonsensory brain regions (Perisse & Waddell, 2011): Evidence from human neuroimaging suggests that brain regions implicated in temporal coding and working memory processes (e.g., hippocampus, prefrontal cortex) are preferentially recruited during trace conditioning procedures (Sehlmeyer et al., 2009), as these regions might be crucial components of a more widespread network that constitutes a reverberatory mnemonic mechanism necessary for bridging the stimulus-free interval.

The present study examined whether the proximity of sensory events in time influences experience-dependent changes in visuocortical facilitation for threat-related stimuli. Our findings revealed that although participants could clearly learn to discriminate between the grating stimuli that predicted the presence versus absence of an aversive event, upregulation of activity in visuocortical regions was only observed for threat-related cues that temporally overlapped with an aversive outcome. It is important to emphasize that our findings do not argue against the role of expectancy in the emergence of a CS+ specific visuocortical response bias; rather, our findings suggest that expectancy is not a sufficient cause for the establishment of a neurophysiological visual response bias and that low-level parameters, such as temporal co-occurrence exert strong constraints on experience-dependent changes in the visual system for threat-related features.

The experiments presented here focused on visual sensory processing, using an electrophysiological measure that is almost exclusively sensitive to these changes, and other physiological measures of emotional engagement—such as startle blink modulation or heart rate changes—were not collected. The lack of information about peripheral system engagement limits our ability to discern the possible mechanisms mediating the pronounced visuocortical differences between delay and trace conditioning. A further limitation stems from the fact that the temporal distance in the trace design remained fixed and that manipulating the temporal gap between the CS+ and the US during trace conditioning may lead to systematically different results compared to the present findings. In the future, investigators may apply finer gradations of temporal discontinuity to demarcate the boundary regions for acquisition of threat-related biases within visual cortical networks. In addition, CS–UCS contiguity can also be manipulated along a spatial or combined spatiotemporal dimension to elucidate the critical parameters for aversive conditioning-related changes in the visuocortical system.

² A similar prediction might be derived from a standard operating procedures (SOP) account of conditioning, where associative strength between two stimuli is capable of being modulated only when both stimuli are simultaneously present in their active (A1) state, and not when one of these has decayed into an inactive (A2) state (Wagner, 1981) – as when the CS+ is no longer physically present during the stimulus-free trace interval.

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