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Event-related potential components as measures of aversive conditioning in humans

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Abstract

For more than sixty years, the gold standard for assessing aversive conditioning in humans has been the skin conductance response (SCR), which arises from the activation of the peripheral nervous system. Although the SCR has been proven useful, it has some properties that impact the kinds of questions it can be used to answer. In particular, the SCR is slow, reaching a peak 4–5 seconds after stimulus onset, and it decreases in amplitude after a few trials (habituation). The present study asked whether the late positive potential (LPP) of the event-related potential (ERP) waveform could be a useful complementary method for assessing aversive conditioning in humans. The SCR and LPP were measured in an aversive conditioning paradigm consisting of three blocks in which one color was paired with a loud noise (CS+) and other colors were not paired with the noise (CS−). Participants also reported the perceived likelihood of being exposed to the noise for each color. Both SCR and LPP were significantly larger on CS+ trials than on CS− trials. However, SCR decreased steeply after the first conditioning block whereas LPP and self-reports were stable over blocks. These results indicate that the LPP can be used to assess aversive conditioning and has several useful properties: 1) it is a direct response of the central nervous system; 2) it is fast, with an onset latency of 300 ms; 3) it does not habituate over time.

Keywords

aversive conditioning; event related potentials (ERPs); late positive potential (LPP); stimulus-preceding negativity (SPN); skin conductance response (SCR)

Aversive conditioning is a commonly used paradigm in both humans and rodents for assessing the learning of associations between neutral stimuli and aversive stimuli (Maren, 2001). In this paradigm, a neutral stimulus is paired with an aversive stimulus such as a shock or loud sound (the *unconditioned stimulus* or *US*), which by itself produces an automatic response such as an eyeblink (the *unconditioned response* or *UR*). After multiple pairings, the neutral stimulus elicits a physiological and/or behavioral response that is typically similar or identical to the UR. At this point, the formerly neutral stimulus is now called a conditioned stimulus (CS+), and the response that it elicits is called a conditioned response (CR). A CR is not elicited by a stimulus that has been paired with the absence of the US (CS−).

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The psychophysiological study of aversive conditioning in humans has frequently used peripheral nervous system (PNS) responses—such as the skin conductance response (SCR) and the startle reflex—as the UR and CR. Although the SCR has proven useful for assessing aversive conditioning effects, showing greater response (i.e. increased amplitude) for the CS + relative to the CS−, it has a major limitation: it can show dramatic habituation effects after a few trials (Codispoti, Ferrari, & Bradley, 2006). It would therefore be useful to have a tool for measuring aversive conditioning that does not habituate rapidly. Moreover, the SCR is a slowly varying PNS response that takes several seconds to develop following the CS+ (Codispoti & De Cesarei, 2007), and it would be useful to have a measure that reflects rapid discrimination between CS+ and CS− in the central nervous system (CNS).

Event-related potentials (ERPs) are rapidly varying responses of the CNS and may therefore provide useful complementary measures of aversive conditioning. Various ERP components have been used to study emotional processing, including the late positive potential (LPP) and the stimulus preceding negativity (SPN). The LPP is a positive voltage deflection that onsets at approximately 300 ms after stimulus onset, with a maximum peak at midline central and parietal electrodes, that is larger for both pleasant and unpleasant stimuli relative to neutral stimuli. The LPP typically lasts several hundred milliseconds and is associated with subjective measures of arousal (Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000). Moreover, the LPP is stable over time (Liu, Huang, McGinnis-Deweese, Keil, & Ding, 2012), and although overall LPP amplitude may decline across trial blocks, the ability of the LPP to differentiate between emotional and neutral stimuli remains intact (Codispoti, Ferrari, & Bradley, 2007).

The SPN is a negative potential at midline fronto-central and parietal electrodes that has been related to the anticipation of an incoming stimulus (C. H. Brunia & Damen, 1988). It has been shown to be sensitive to affective modulation, with greater amplitude for arousing unpleasant or pleasant stimuli relative to neutral stimuli (Poli, Sarlo, Bortoletto, Buodo, & Palomba, 2007). The SPN is typically longer in duration than the LPP.

Previous research suggests that LPP-like ERP components are impacted by aversive conditioning (Pizzagalli, Greischar, & Davidson, 2003; Skrandies & Jedynak, 2000). In threat anticipation paradigms, for example, a cue stimulus predicts whether a subsequent stimulus will be a threat image or a neutral image (Grant, Judah, White, & Mills, 2015; Michalowski, Pane-Farre, Low, & Hamm, 2015). This is analogous to an aversive conditioning paradigm, except that threat images are not intrinsically aversive unconditioned stimuli in the same way that shocks and loud noises are. Studies of threat anticipation have found three key effects. First, the final image produces a larger LPP if it is a threat image than if it is a neutral image (much like an unconditioned response to an unconditioned stimulus). Second, a cue predicting a threat image may produce a larger LPP than a cue predicting a neutral image. Third, when the cue is associated with a threat image, a frontocentral negative potential occurs late in the response to the cue, just prior to the appearance of the threatening images. This appears to be the stimulus-preceding negativity (SPN), which has been associated with anticipation of emotional stimuli, including aversive noise (Kotani, Hiraku, Suda, & Aihara, 2001).

Other studies have used unambiguous classical conditioning paradigms in which one conditioned stimulus (CS+) is paired with the presence of an aversive stimulus (a loud sound or a shock) whereas another conditioned stimulus (CS−) is paired with the absence of the aversive stimulus. For example, Kastner et al. (2016) used odorants as CS+ and CS−, and they found a larger LPP for a visual flash when it was presented near the time of the CS+ than when it was presented near the time of the CS− (Kastner, Flohr, Pauli, & Wieser, 2016). Nelson et al. (2015) used visual stimuli as the CS+ and CS− and found that an LPP-like component in a principal component analysis was larger for the CS+ than for the CS− (Nelson, Weinberg, Pawluk, Gawlowska, & Proudfit, 2015). However, neither of these studies examined the SPN. To the best of our knowledge, only one study has used the SPN to test for aversive conditioning effects: using a trace-conditioning paradigm, Hellwig et al, (2008) found greater SPN amplitude for a CS+ than for a CS− (Hellwig et al., 2008). Thus, considering all the previous evidence, there is good reason to believe that the LPP, and possibly the SPN, can be used as measures of aversive conditioning.

The aim of the present study was therefore to assess the use of the LPP and SPN as measures of aversive conditioning. We hypothesized that the LPP, and possibly the SPN, would show less habituation over trial blocks compared to the SCR, thus providing a more stable measure of conditioning over time. We used a simple delay conditioning paradigm, in which circles of one color were paired with a loud noise (CS+) and circles of other colors were paired with the absence of this noise (CS−). Three separate blocks of conditioning were tested in each participant to evaluate habituation. In addition, participants provided a subjective report of the threat status of each color at the end of each conditioning block so that we could assess whether our measures showed the same pattern over blocks as the subjective appraisal of threat. We also asked whether an SPN would be observed on CS+ trials as sign of anticipation of the aversive noise. We made two key predictions. First, we predicted that the LPP (and possibly the SPN) would show greater amplitude for the CS+ compared to the CS−. Second, we predicted that this effect would not decrease in amplitude across aversive conditioning blocks.

Method

Participants

Seventy volunteers from the UC Davis community with no history of neurological or psychiatric conditions participated in this experiment (49 females). All participants were screened with a standard questionnaire for color blindness and visual acuity and all of them reported normal color perception and normal or corrected-to-normal visual acuity. The age ranged between 18–29 years with a mean of 21 years. They were originally recruited in two groups of 35 participants for two separate studies, but the analyses presented below were collapsed across all 70 participants. For the block-by-block LPP, SPN and SCR analyses we excluded one participant because of technical problems with the EEG recording hardware. For the exploratory analysis of the extinction phase, sixty-three participants were included because some subjects' recordings showed EEG artifacts during this phase that could not be corrected via ICA.

Experiment setup

The experiment consisted of three phases: 1) habituation, 2) aversive conditioning, and 3) extinction. In all three phases, participants were seated 100 cm from an HP ZR244Ow LCD monitor with a black background and a continuously visible fixation point at the center. The monitor delay (8 ms) was measured with a photodiode and the stimulus event codes were corrected accordingly prior to data analysis. In each phase, the participants passively viewed a sequence of trials in which a circle (1.3°) was presented in the middle of the monitor. Stimulus duration was 4 s, and the stimuli were separated by an inter-trial interval (ITI) that varied randomly between 10 and 12 s.

Habituation Phase—In the habituation phase, the stimulus was a gray (15 cd /cm^2) circle on every trial. Eight trials were presented. Participants were told simply to relax and pay attention to the stimuli. Note that the habituation phase is a common procedure that serves to prevent novelty-related EEG and SCR responses from contaminating the data during the conditioning phase. Also, the habituation phase helps ensure that participants are comfortable and understand the experimental procedures (Lonsdorf et al., 2017).

Aversive Conditioning Phase—The aversive conditioning phase was repeated three times in separate blocks of trials. In this phase, the circles were blue, green or yellow with luminance of 15 cd /cm^2. For each participant, one color was randomly chosen as the conditioned stimulus (CS+), whereas the other two colors (CS−) were never paired with the unconditioned stimulus. The unconditioned stimulus (US) consisted of a 100 dB white noise burst presented through two speakers at a distance of 150 cm. The USd duration was 1500 ms, which is common for studies using noise as the unconditioned stimulus (Kastner et al., 2016). The US was presented on 50% of CS+ trials. As illustrated in Figure 1, the US began 2500 ms after CS+ onset and co-terminated with the CS+. There were 32 trials in each block: 16 CS−, 8 CS+ without US (CS+US−), and 8 CS+ paired with US (CS+US+). The stimuli were presented in pseudorandom order with the constraints that the first three CS+ stimuli were always paired with the noise and that there could be no more than two trials of the same color in a row. Participants were instructed to attend to the colored circles and to figure out if there was a relationship between a specific color and the presentation of the loud noise. They were told that only one color would be associated with the noise. As in the habituation phase, participants were not asked to make any motor responses to the stimuli in this phase.

After each block of the aversive conditioning phase, the participants used a 5-point scale to report their assessment of the likelihood of hearing a loud noise following each color (1 = “sure no noise”, 3 = “unsure”, 5 = “sure noise”).

Each block of the conditioning phase was separated by approximately 40 minutes. In the period between blocks, participants performed a simple visual search task in which they were instructed to attend to a color target and to report whether the target had a gap on top or bottom. The blue, green, and yellow colors used in the aversive conditioning phase were used as both targets and distractors in this visual search task (in separate blocks), but the stimuli were presented peripherally, and the US was never presented (Bacigalupo, 2016).

Note that the results from the visual search task will be the subject of a future report. Because of the visual task that was interposed between conditioning blocks, some extinction may have occurred between blocks. This design therefore stresses the stability of the conditioning.

Extinction—The extinction phase was similar to the habituation phase, with the exception that the stimuli were blue, green or yellow, and 10 trials per color were presented in random order, with the constraint that a given color could appear no more than twice in succession (30 trials total). The US was never presented during the extinction phase. Participants were told to relax andatte6nd to the colored circles that were presented on the computer screen. They were told that no noises would be presented.

Psychophysiological Recording and Analysis

The EEG was recorded using a Brain Products ActiCHamp system with electrodes located over the left and right mastoid processes and at 27 standard scalp locations (Fp1, Fp2, F3, F4, F7, F8, C3, C4, P3, P4, P5, P6, P7, P8, P9, P10, PO3, PO4, PO7, PO8, O1, O2, Fz, Cz, Pz, Poz, Oz). The horizontal and vertical electrooculogram (EOG) was measured from electrodes located approximately 1 cm lateral to the outer canthus of each eye and from an electrode placed below the right eye. The skin conductance response was recorded simultaneously by this same system, using Brain Products bipolar GSR electrodes placed on the distal phalanges of the second and third digits (Scerbo, Freedman, Raine, Dawson, & Venables, 1992).

All signals were recorded in single-ended mode and digitized at 1000 Hz with a cascaded integrator-comb antialiasing filter with a half-power cutoff at 260 Hz. The electrode impedances were kept below 80 KΩ. All data analyses were performed using EEGLAB Toolbox (Delorme & Makeig, 2004) and ERPLAB Toolbox (Lopez-Calderon & Luck, 2014), which are open-source Matlab packages for EEG/ERP analysis. The signals were re-sampled offline to 250 Hz (after application of an antialiasing filter). The EEG signals were referenced to the average of the two mastoid electrodes and high-pass filtered using a non-causal Butterworth filter (half-amplitude cutoff = 0.1 Hz, slope = 12 dB/octave).

Averaged SCR waveforms were computed with a 14 s epoch, starting 1 s before the stimulus onset. The SCR was measured as the mean amplitude from 4.5–6.5 s post-stimulus onset using a 1 s pre-stimulus baseline. Aversive conditioning was assessed by computing the difference in SCR amplitude between trials in which the US was not presented (CS+US− trials) and CS− trials. The measurement time window was determined after examining the data of the first 35 participants (group 1) and was used as an a priori window for the second 35 participants (group 2). Because the pattern of statistical significance was identical across the two groups, we are showing the data collapsed across them.

To correct the EEG for eye blinks and horizontal eye movements, independent component analysis (ICA) was conducted using EEGLAB's BINICA routine. The criterion for component rejection was the consistency between the shape, timing and spatial location of a given component compared to the single-trial EOG data. For eye blinks, one or two

components were identified per participant whereas for horizontal eye movements, one component was selected for correction in each participant.

The artifact correction process was supplemented with artifact rejection to eliminate trials with clearly artifactual voltage deflections. Specifically, trials were excluded if the peak-to-peak voltage within the EEG epoch was greater than 300 μV in any 200-ms window in any channel. An average of 0.69% of trials was rejected in the participants (range = 0–15.6%). One volunteer completed only two of the three conditioning blocks and was therefore not considered for the block-by-block SCR/ERP analysis, but was included in all other analyses.

For the late positive potential (LPP) analysis, averaged ERP waveforms were computed with a 1000-ms epoch, starting 200 ms before stimulus onset, whereas for the stimulus-preceding negativity (SPN) longer 4500 ms epochs were computed, starting 500 ms before stimulus onset. A low-pass filter (half-amplitude cutoff = 30 Hz, slope = 12 dB/octave) was used for plotting purposes after averaging. The LPP was measured as the mean amplitude at the Pz electrode site in the time window between 350–650 ms (Liu et al., 2012; Weinberg, Hilgard, Bartholow, & Hajcak, 2012). The SPN was measured as the mean amplitude between 2000–2500 ms after stimulus onset (i.e. 500 ms prior to the time of US presentation) from the Cz electrode site. ERP waveforms were measured using pre-stimulus baseline. Aversive conditioning was assessed by computing the difference in LPP amplitudes between CS+ trials in which the US was not presented (CS+US− trials) and CS− trials. As with the SCR, the measurement time window for LPP was determined after evaluating group 1 data and used as an a priori window for group 2. Again, the results from group 1 were replicated in group 2, so the data are shown collapsed across all participants.

Statistical Analysis

Statistical analyses were performed using R statistical software version 3.3.2. The SCR and ERP data were analyzed using two-way repeated measures ANOVAs with factors of condition (CS+US−/CS−) and block (1st, 2nd, 3rd). Non-parametric statistics were used for analysis of the self-reports (Wilcoxon signed rank and Kruskal-Wallis tests). Correlations were computed using Spearman and Pearson coefficients. The alpha threshold for statistical significance was 0.05.

Results

Participant self-reports

As shown in Figures 2a–c, the participants reported a greater likelihood of being exposed to a loud noise following the CS+ color compared to the CS– colors in all experimental blocks. The median scores were 4 for CS+ and 1 for CS−. Paired Wilcoxon signed rank tests yielded significant differences between CS+ and CS− for block 1 ($v = 2485$, $p < 0.001$), block 2 ($v = 2485$, $p < 0.001$) and block 3 ($v = 2485$, $p < 0.001$). The perceived likelihood of being exposed to a loud noise was very similar across blocks for both the CS+ and CS− stimuli. Kruskal-Wallis tests indicated no significant effect of block for either CS+ ($X^2_{(2)} = 1.77$, $p = 0.41$) or CS− ($X^2_{(2)} = 2.03$, $p = 0.36$).

Skin Conductance Response (SCR)

As shown in Figure 3a, the SCR waveform started increasing in amplitude approximately 2.5 seconds after stimulus onset for the CS+US− and CS+US+ trials, but not for the CS− trials. Moreover, the SCR waveform in the CS+US+ condition consisted of two sub-components: the first one — starting at around 2.5 sec — was presumably elicited by the CS +; the second — starting at approximately 4.5 sec — was presumably elicited by the noise burst (US). Both subcomponents decreased in amplitude over blocks. In support of these observations, a two-way repeated measures ANOVA with factors of condition (CS+US−/CS −) and block yielded significant main effects of condition ($F_{(1, 68)} = 32.94$, $p < 0.001$) and block ($F_{(2, 136)} = 11$, $p < 0.001$) and a significant condition by block interaction ($F_{(2, 136)} = 12.86$, $p < 0.001$). To test for differences in SCR for each specific condition across blocks, separate one-way ANOVAs for the CS+US− and CS− trials including block as factor were performed. These analyses showed that the SCR declined significantly over blocks for the CS+US− ($F_{(2, 136)} = 12.74$, $p < 0.001$) but not for the CS− ($F_{(2, 136)} = 1.86$, $p = 0.15$) (Figures 3b–e). Thus, SCR amplitude was greater for CS+US− compared to CS− trials — demonstrating aversive conditioning effects — and the SCR for CS+US− trials habituated after the first block. The same pattern of significance was observed for each individual group of 35 participants[1].

Late positive potential (LPP)

The ERP waveforms from the Pz electrode site are shown in Figure 4 (and SCR waveforms are shown in this same format in Supplementary Figure S1). The ERP waveforms began with a P2 wave peaking around 250 ms, followed by an N2 wave peaking around 300 ms, and then a LPP that extended for several hundred milliseconds. Figure 5 shows that the LPP was focused over midline parietal cortex. Like the SCR, the LPP was larger on CS+US− trials than on CS− trials. Unlike the SCR, this effect did not decline across the three blocks of conditioning trials (see especially the CS+US− minus CS− difference waves shown in Figure 4e).

A two-way repeated measures ANOVA with factors of condition (CS+US−/CS−) and block yielded a significant main effect for condition ($F_{(1, 68)} = 25.29$, $p < 0.001$) but not a significant effect for block ($F_{(2, 136)} = 1.13$, $p = 0.32$). The condition by block interaction was not significant ($F_{(2, 136)} = 0.5$, $p = 0.6$). Separate one-way ANOVAs for CS+US− and CS− trials with block as the factor did not yield significant effects of block (CS+US−: $F_{(2, 136)} = 1.24$, $p = 0.29$; CS−: $F_{(2, 136)} = 0.18$, $p = 0.83$). These analyses show that the LPP amplitude was greater for CS+US− compared to CS− trials — demonstrating aversive conditioning effects — and that this difference did not habituate over blocks. The same pattern of significance was observed for each individual group of 35 participants[2, 3]. Figure

[1]We also conducted separate analyses on each group of 35 participants. Two-way repeated measures ANOVAs with factors of condition (CS+/CS−) and block yielded a significant main effect of condition for Group 1 ($F_{(1, 33)} = 11.62$, $p < 0.01$) and Group 2 ($F_{(1, 34)} = 21.81$, $p < 0.001$). The main effect for block was also statistically significant for Group 1 ($F_{(2, 66)} = 4.18$, $p < 0.05$) and Group 2 ($F_{(2, 68)} = 6.79$, $p < 0.01$) as was also the interaction between condition and block for Group 1 ($F_{(2, 66)} = 5.05$, $p < 0.01$) and Group 2 ($F_{(2, 68)} = 7.7$, $p < 0.001$). Separate one-way ANOVAs for the CS+ and CS− trials with block as the factor yielded statistical significance for the CS+ in both Group 1 ($F_{(2, 66)} = 5$, $p < 0.01$) and Group 2 ($F_{(2, 68)} = 7.64$, $p < 0.01$) but not for the CS− (Group 1: $F_{(2, 66)} = 0.38$, $p < 0.68$; Group 2: $F_{(2, 68)} = 1.77$, $p < 0.18$). Thus, both groups exhibited the same pattern observed in the combined analysis.

6 summarizes the effects of block on the difference between the CS+ and CS− response for the self-reports, the SCR, and the LPP. Both the selfreports and the LPP showed a similar pattern over time, with no significant changes across blocks. By contrast, the SCR conditioning effect (Figures 3e and 6a) habituated rapidly after the first block. The SCR response following the US also habituated rapidly (Figure 3b).

Correlations Among Measures

We conducted a set of exploratory correlational analyses to examine associations among the LPP, SCR, and self-report measures. To focus on conditioning effects and reduce the number of statistical tests (and therefore the familywise Type I error rate), these correlations were performed on difference scores (CS+US− minus CS−). Given that the LPP and SCR conditioning effects exhibited very different patterns of change over blocks, they likely reflect different underlying processes, and one might expect that they would be uncorrelated. To examine this, we averaged the data across blocks and computed Pearson correlations between LPP amplitude and SCR amplitude. We found that the correlation was not significant ($r = -0.08$, $p = 0.49$). We also asked whether either of these measures was correlated with the self-report score using Spearman rank-order correlations. The self-report score was not significantly correlated with either LPP amplitude ($r = -0.008$, $p = 0.94$) or SCR amplitude ($r = 0.0009$, $p = 0.99$). The lack of correlations with the self-report measure may simply reflect variability among participants in how they mapped their internal representations of the probability of the US to the 5-point response scale.

Slow Potentials

We also conducted a set of exploratory analyses examining ERPs over the entire 4-second duration of the conditioned stimuli, with a 500-ms prestimulus interval. As shown in Supplementary Figure S2, the LPP effect (difference between CS+US− and CS− trials) ended within 1000 ms of CS onset. However, this effect was followed by a late, slow negativity that was greater on CS+US− trials than on CS− trials. As shown in Supplementary Figure S3, this late, slow negativity was frontocentrally distributed. However, the difference in amplitude between CS+US− and CS− trials was only marginally significant (see Supplementary Figure S3 for details).

Extinction Phase

We also conducted a set of exploratory analyses examining the extinction phase, in which each of the three stimulus colors was presented 10 times (in random order) without a US.

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[2]We also conducted separate analyses on each group of 35 participants. Two-way repeated measures ANOVA with factors of condition (CS+/CS−) and block yielded a significant main effect of condition for Group 1 ($F_{(1, 33)} = 53.17$, $p < 0.001$) and Group 2 ($F_{(1, 34)} = 6.22$, $p < 0.05$) but not a significant effect of block neither for Group 1 ($F_{(2, 66)} = 0.29$, $p = 0.75$) nor Group 2 ($F_{(2, 68)} = 2.36$, $p = 0.1$). The condition by block interaction was not significant for both Group 1 ($F_{(2, 66)} = 1.84$, $p = 0.16$) and Group 2 ($F_{(2, 68)} = 0.08$, $p = 0.92$). Separate one-way ANOVAs for CS+ and CS− trials with block as a factor did not yield significant effects of block in Group 1 (CS+: $F_{(2, 66)} = 0.59$, $p = 0.55$; CS−: $F_{(2, 66)} = 1.47$, $p = 0.24$) and Group 2 (CS+: $F_{(2, 68)} = 0.69$, $p = 0.5$; CS−: $F_{(2, 68)} = 2.77$, $p = 0.07$). Thus, both groups exhibited the same pattern observed in the combined analysis.

[3]To test for differences between the three CS colors used in this study, we performed a two-way between-subjects ANOVA for the LPP data with factors of Condition (CS+US− and CS−) and Color (Blue, Green and Yellow). The results of this analysis yielded a significant main effect of Condition ($F_{(1, 132)} = 9.32$; $p < 0.01$), but no significant main effect of Color ($F_{(2, 132)} = 1.2$; $p = 0.3$) or Condition x Color interaction ($F_{(2, 132)} = 0.3$; $p = 0.7$). Thus, there was no evidence for differences among colors in the aversive conditioning effect.

We examined the SCR and ERP activity over the entire 4-second trial duration, with a 500-ms pre-stimulus interval. Supplementary Figure S4 shows the SCR and ERP difference waveforms (CS+US− minus CS−) during this phase overlaid with the difference waveforms from the third block of conditioning. The conditioning effect was virtually eliminated for the SCR and the ERP slow wave during the extinction block, and the LPP was reduced but not eliminated. This extinction effect was statistically significant for the SCR, marginally significant for the LPP, and did not approach significance for the LPP (see Supplementary Figure S4 for details). This may indicate that LPP activity for the CS+ declined gradually over the course of the extinction phase, leading to a relatively small reduction when averaged over all 10 extinction trials. However, it should be noted that these analyses were exploratory and were based on a relatively small number of extinction trials.

Discussion

Replicating prior research, we found that the SCR was significantly larger on CS+US− trials than on CS− trials. However, this effect habituated very rapidly and was quite small after the first aversive conditioning block. We also found that the LPP was larger on CS+US− trials than on CS− trials, thus showing that it can be used as an index of aversive learning (see also Nelson et al., 2015). Unlike the SCR, however, the LPP conditioning effect was stable over blocks, as was the subjective self-report measure. This provides preliminary evidence that the LPP may be more closely related to conscious awareness of threat than is the SCR. When combined with the fact that the LPP is a direct and immediate measure of neural activity in the brain, the present findings indicate that the LPP is a useful complement to the SCR for assessing aversive conditioning. Thus, researchers may find it valuable to use the LPP in combination with the SCR in future research. However, additional research is needed to further explore the strengths and weaknesses of these two measures.

We also found evidence of a much slower, later negativity with a more frontocentral distribution, although the conditioning effect was only marginally significant for this negativity. This is likely the stimulus-preceding negativity (SPN), which is the latter portion of the contingent negative variation that is observed in the period between a warning stimulus and a target stimulus (Brunia, van Boxtel, & Böcker, 2012). This may also turn out to be a useful measure of aversive conditioning, although it appears to be less robust than the LPP and SCR.

The LPP findings from the present study could have a positive impact on future studies of anxiety and trauma. For example, previous reports have used the SCR as a method to assess extinction of aversive conditioning, suggesting that aversive-related memories could be erased through memory re-consolidation mechanisms (Agren et al., 2012; Schiller et al., 2010). However, the shortcomings of the SCR as a method for assessing aversive conditioning could limit the conclusions that can be drawn from studies such as these. For example, changes in the SCR across conditions could reflect changes in the coupling between the CNS response and the PNS response rather than a change in the actual aversive response within the CNS. Similarly, the fast habituation of the SCR makes it difficult to assess conditioning after the first few trials, making it difficult to assess the long-term effects of extinction on aversive responses. If the LPP were assessed in addition to the SCR, it

would be possible to obtain a fuller picture of the emotional responses to conditioned stimuli in studies of this nature.

Another widely used peripheral method for assessing aversive conditioning in humans is the startle response, in which eyeblink amplitude is measured in response to a sudden stimulus. The evidence suggests that the SCR and the startle reflex may reflect different aspects of aversive conditioning, with the SCR reflecting orienting and arousal (Codispoti et al., 2006; Hamm & Vaitl, 1996) and the startle reflex being related to aversive learning and stimulus valence (Grillon & Baas, 2003; Hamm & Vaitl, 1996). However, unlike the LPP and SCR, research suggests that the startle response is sensitive to valence rather than arousal (Bradley, Cuthbert, & Lang, 1990). Thus, to have a deeper understanding of aversive conditioning in humans, future research could combine the SCR, the LPP and the startle response.

However, the use of startle measures may complicate the interpretation of aversive conditioning studies because the stimuli used to elicit the startle response are often aversive. For example, most studies use loud noises to elicit the startle response (Grillon & Baas, 2003), and these loud noises are similar to the stimulus used as the US in the present study. Indeed, loud noise stimuli have been shown to produce more reliable and stable aversive conditioning than electric shocks (Sperl, Panitz, Hermann, & Mueller, 2016), which is one reason why we chose to use noise bursts rather than shocks as the US in the present study. If an aversive conditioning paradigm uses a stimulus such as a shock as the US and also includes noise bursts as the startle probes, the presence of two types of aversive stimuli may impact the conditioning results. In particular, the startle-eliciting stimuli are typically unpredictable, and the presence of an unpredictable aversive stimulus within a conditioning paradigm may increase the participants' anxiety, which may in turn modulate the conditioning. Thus, studies that use startle responses to assess conditioning must be careful to ensure that the startle-eliciting stimuli are not themselves aversive.

Although the LPP and the SPN may have some practical advantages over the SCR as measures of aversive conditioning, the value of these different measures in a given study also depends on exactly what internal neural and psychological processes they reflect. Much more research is necessary to determine the processes reflected by the LPP in the context of aversive conditioning, but we can speculate on the basis of LPP findings from other experimental paradigms (see review by Hajcak et al., 2012). The early part of the LPP tends to have the same parietally maximal scalp distribution as the P3b component and may in fact be the same component, whereas the later part of the LPP is more centrally distributed (Weinberg & Hajcak, 2010). The LPP conditioning effect in the present study had a distinctly parietal maximum (see Figure 5c) and may therefore be identical to the P3b component. If this is correct, then the LPP likely reflects conscious aspects of conditioning and a greater allocation of post-perceptual processing resources to the CS+ than to the CS− (see Polich, 2012). However, studies examining correlations between LPP amplitude and the BOLD signal during picture viewing have provided a more nuanced view, showing that LPP amplitude is correlated with activity in many different cortical regions, but with somewhat different patterns of correlation depending on the valence of the stimulus (Liu et al., 2012; Sabatinelli, Lang, Keil, & Bradley, 2007). More research is needed to determine exactly which aspects of aversive conditioning are reflected by the LPP.

To summarize, the results of this study suggest that the LPP has several useful properties for assessing aversive conditioning in humans: 1) it is a direct response of the CNS; 2) it is fast, beginning approximately 300 ms post-stimulus onset; 3) it exhibits little or no habituation; and 4) it appears to show the same pattern as the participants' subjective experience. However, further research is needed to provide a more detailed assessment of the relationship to subjective experience and to determine whether the LPP is suitable for a broad range of experimental paradigms. In addition, further research is needed to determine whether the marginally significant SPN effects observed here are replicable and sufficiently robust to be used to assess conditioning.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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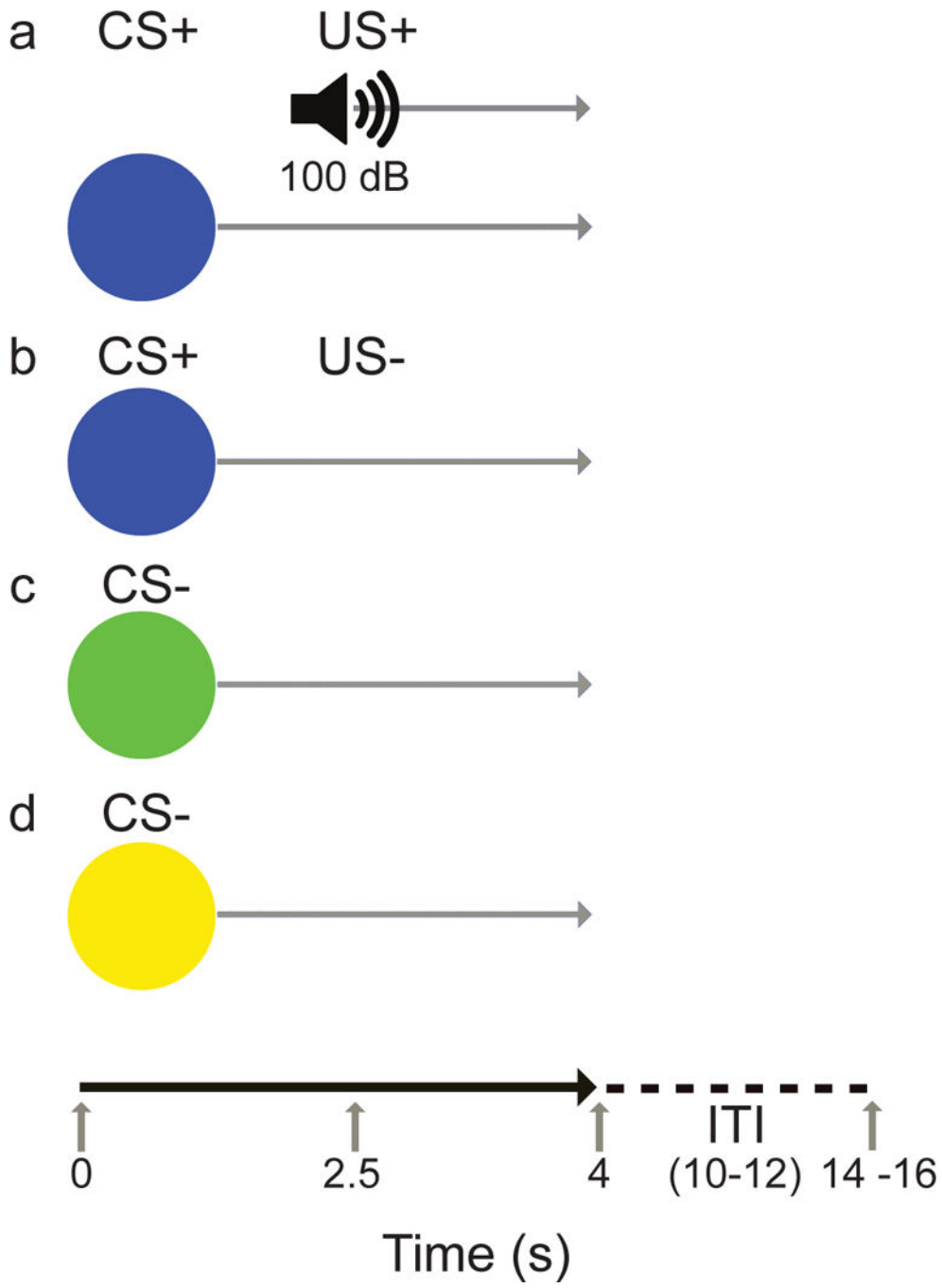

Figure 1.

Aversive conditioning procedure. For each participant, one color was randomly chosen as the conditioned stimulus (CS+; a and b), whereas the other two colors (CS−) were never paired with the unconditioned stimulus (c and d). The unconditioned stimulus (US) consisted of a 1500 ms 100 dB white noise burst. The US was presented on 50% of CS+ trials (denoted CS+US+ trials), beginning 2500 ms after CS+ onset and co-terminating with the CS+ (a). No US was presented on the other 50% of CS+ trials (denoted CS+US− trials) (b).

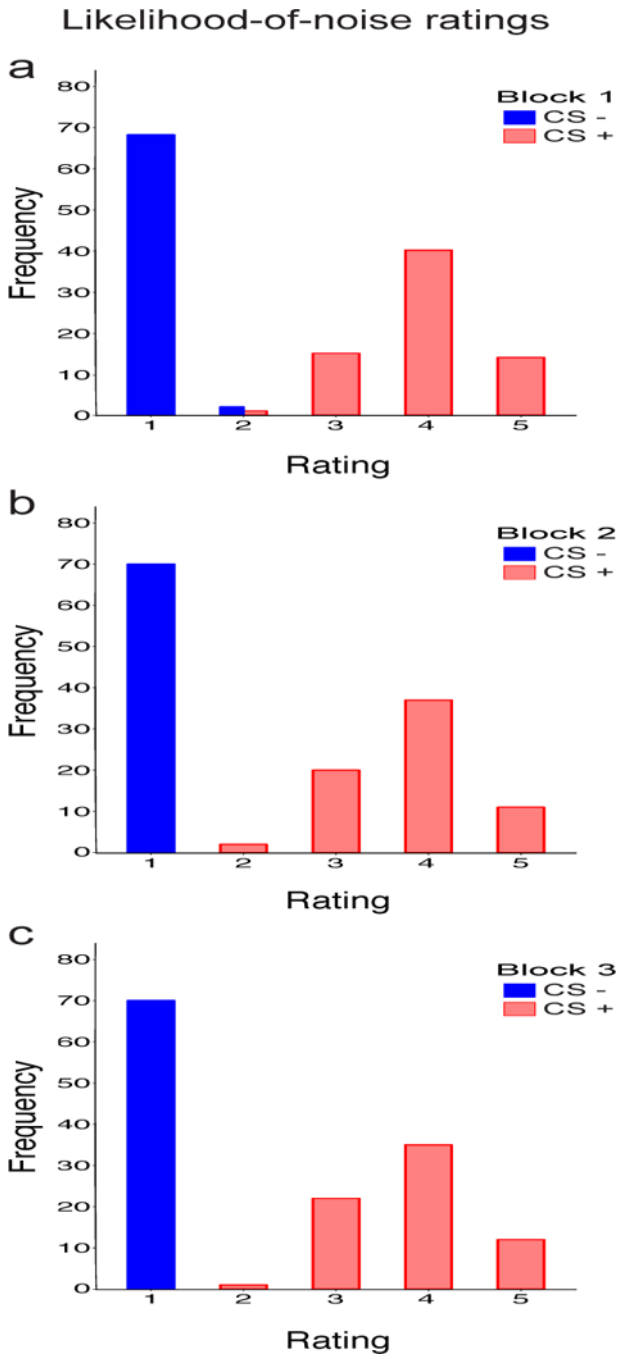

Figure 2.

Frequency distribution of the subjective rating of the likelihood-of-noise for the CS+ and CS − colors, assessed at the end of each conditioning block, and shown for Block 1 (a), Block 2 (b), and Block 3 (c). Each bar shows the number of participants who gave a given rating during that block. For example, almost all 70 participants gave a rating of “1” for the CS− in each block, so the bar for CS− has a value of nearly 70 for every block. For the CS+, most participants gave a rating of “4”, but a substantial number gave ratings of “3” or “5”.

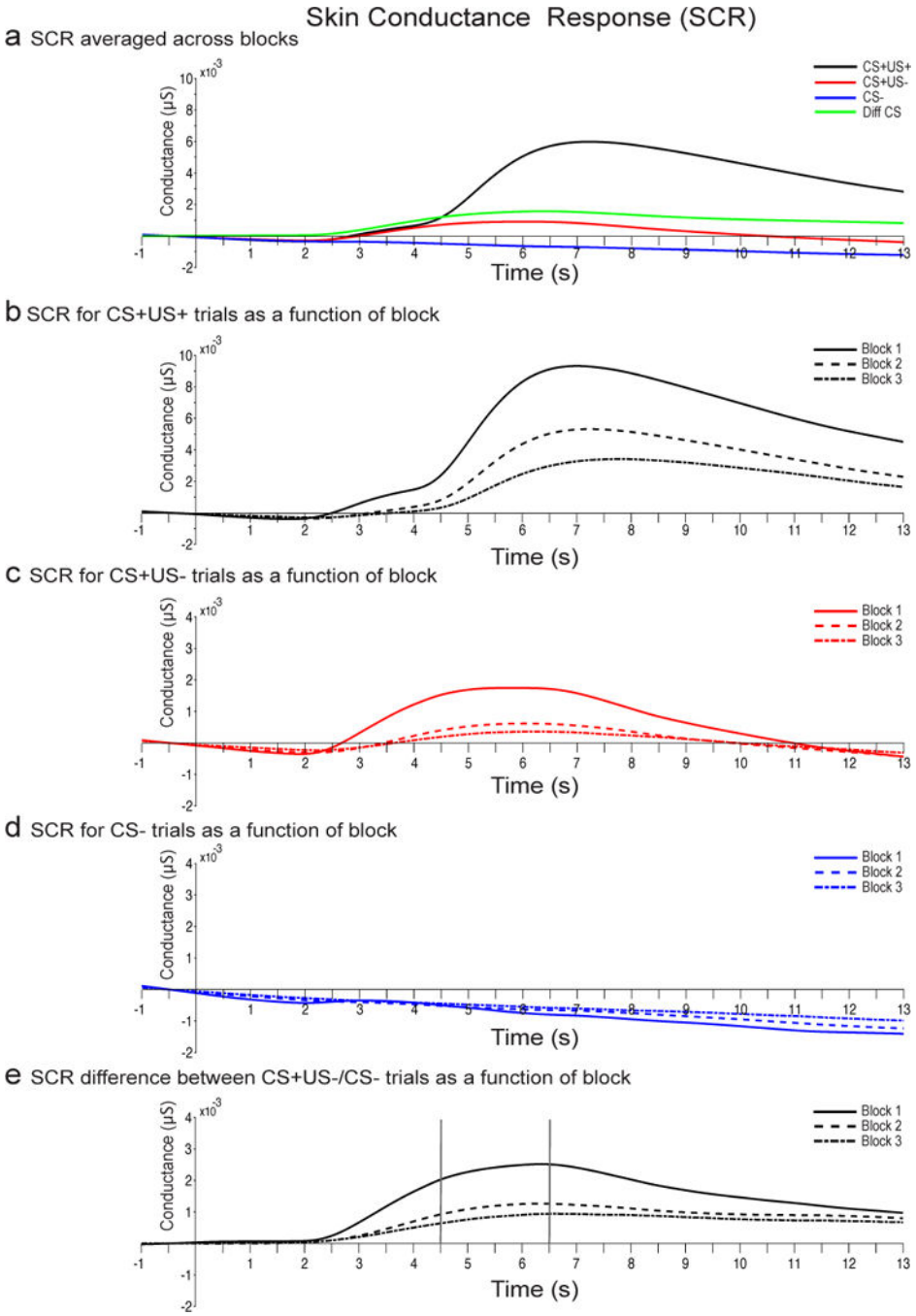

Figure 3.

Grand average skin conductance response (SCR) results. a) SCR for each trial type, collapsed across blocks. b) SCR on CS+US+ trials, shown separately for each block. c) SCR for CS+US− trials, shown separately for each block. d) SCR for CS− trials, shown separately for each block. e) Effect of conditioning on the SCR (CS+US− minus CS− difference wave), shown separately for each block. The SCR measurement window (4.5–6.5 s after CS onset) is shown by the vertical bars.

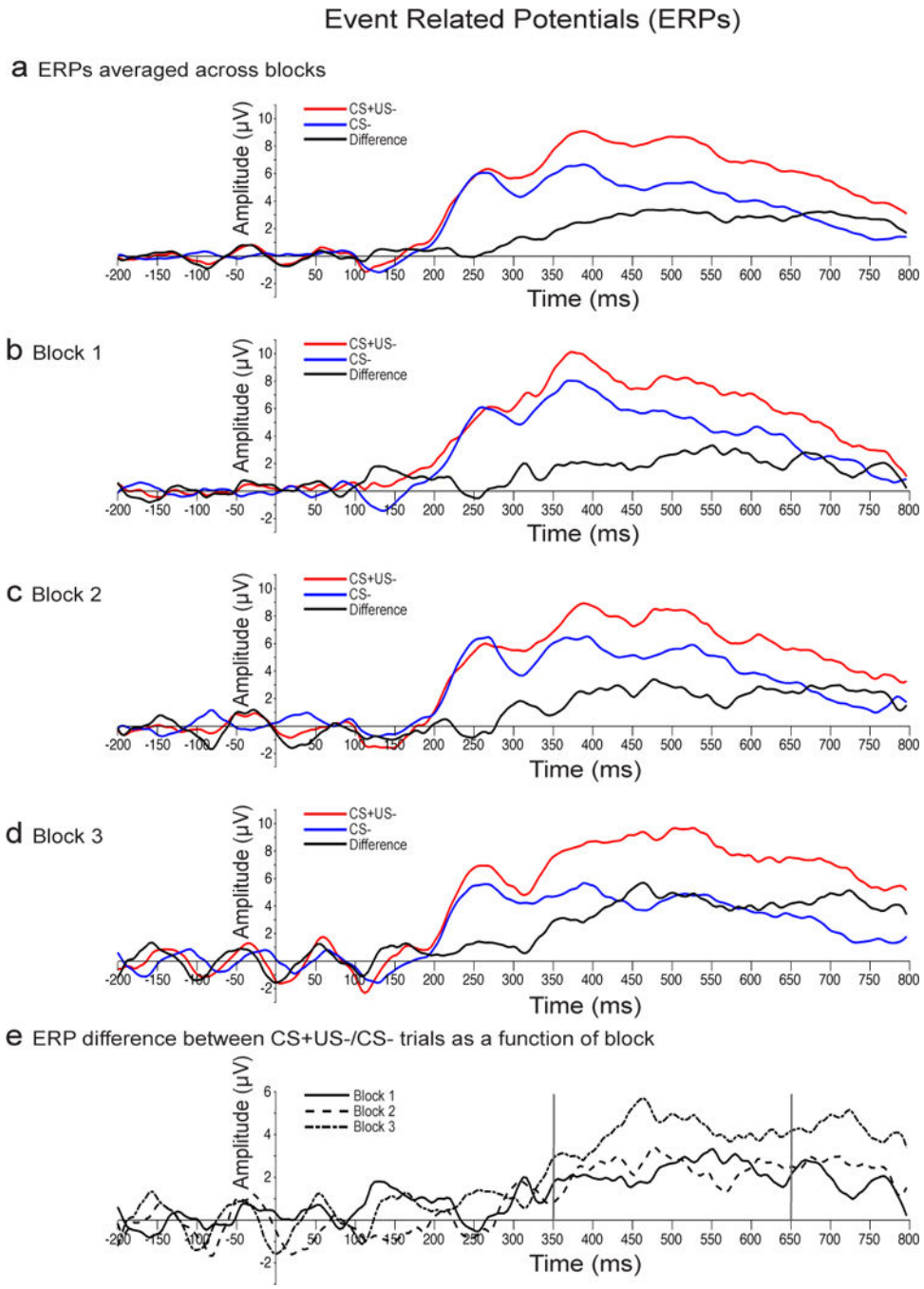

Figure 4.

Grand average event-related potential waveforms at the Pz electrode site, time-locked to the onset of the conditioned stimulus (CS). The data are shown averaged across blocks (a), for block 1 only (b), for block 2 only (c), and for block 3 only (d). The conditioning effect (difference between CS+ and CS− trials) is also shown for each block (e). The measurement window for the late positive potential (350–650 ms after CS onset) is shown by the vertical lines.

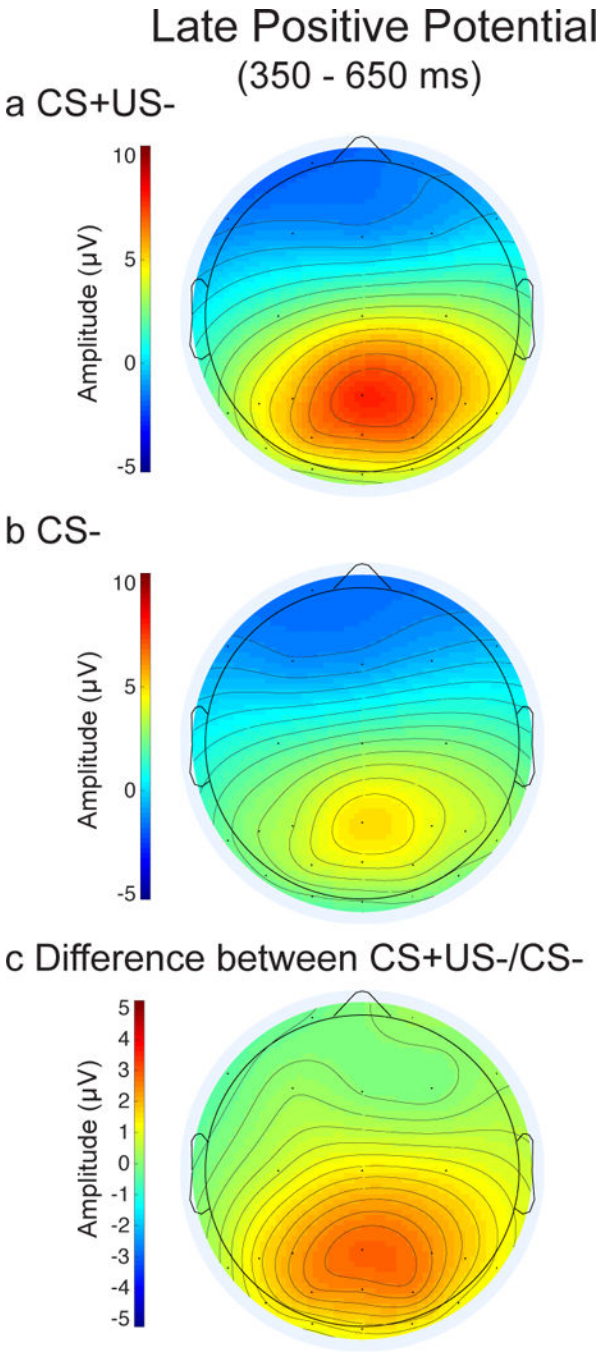

Figure 5.

Grand average topographic maps of the late positive potential (LPP) measured from 350–650 ms for CS+US− trials (a), for CS− trials (b), and for the CS+US− minus CS− difference (c). The scalp distribution of the LPP was greatest over midline posterior parietal scalp sites for all conditions.

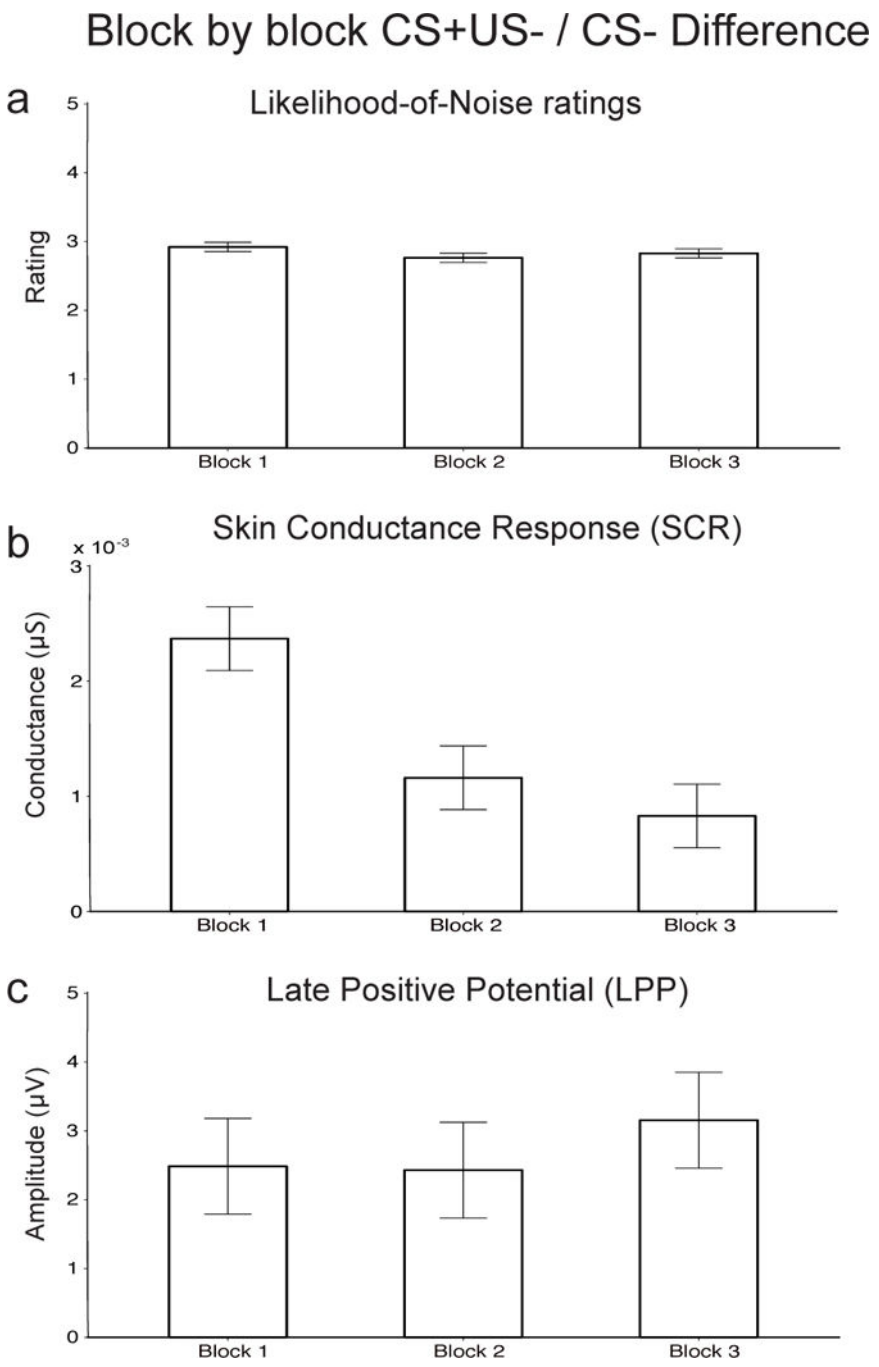

Figure 6.

Conditioning effect (CS+ minus CS−) for the subjective ratings of noise burst probability (a), for the skin conductance response (SCR; b), and for the late positive potential (LPP; c) across blocks. Error bars reflect the within-subjects standard error of the mean (O'Brien & Cousineau, 2014).