Classical conditioning without awareness: An electrophysiological investigation

A Thesis

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Abstract

Evaluative conditioning (EC) is a type of classical or Pavlovian conditioning referring to the process of attitude formation where a previously neutral stimulus gains a positive or negative valence from pairings with a positively or negatively valenced unconditioned stimulus. One controversy in the field of EC research that has not been resolved is whether or not this type of conditioning can occur without contingency awareness. To add to this debate, we utilized both the acoustic startle response and the late positive event-related potential (LPP) component as measures of conditioning in addition to typical self-report measures. Sixteen participants performed a shape discrimination task while continuous EEG was recorded. Half of the visual displays they saw were paired with a 99-dB white noise burst on 50% of those trials, while the other half were never paired with the aversive sound. No participants were able to explicitly report the CS-US contingency and we found no differences on any of these measures for paired versus unpaired stimuli, suggesting that either contingency awareness is necessary for successful acquisition of a conditioned response (CR) or that our methods were insufficient for successful unconscious conditioning.

Chapter 1: Introduction

1.1 Classical Conditioning

From flatworms to capuchin monkeys to cuttlefish to humans, organisms spanning various phyla share a basic capacity for associative learning, or the ability to form an association between stimuli and responses (Baxter & Kimmel, 1963; Beran, Parrish, Perdue, & Washburn, 2014; Cole & Adamo, 2005; De Houwer, Thomas, & Baeyens, 2001; Hofmann, De Houwer, Perugini, Baeyens, & Crombez, 2010). The prevalence of this learning process across species differing in habitats and physiologies has led to a staggering number of questions driving the investigative efforts of behaviorists. Even though the basic principles are well understood, there are many basic questions that are still unanswered.

In the field of behavioral psychology, two broad categories of associative learning (operant conditioning and classical conditioning) describe distinct processes that differ in the way that stimuli and responses relate to one another. In classical conditioning, an unconditioned stimulus (US) that elicits an unconditioned response (UR) is paired with a neutral stimulus that becomes a conditioned stimulus (CS) if through the pairings it begins to elicit the newly conditioned response (CR) even without the presence of the US. For example, if a loud sound (an unconditioned stimulus) that elicits a reflexive muscle twitch (the unconditioned response) is paired enough with a picture of a square (the neutral stimulus) and the square begins to elicit a muscle twitch (a conditioned response) even when the sound is not present, then the square has become a conditioned stimulus. While general principles of classical or Pavlovian conditioning have been well documented and explored over decades of research (Pierce & Cheney, 2013), the advent of modern imaging and recording technologies has permitted novel questions about some possible neurobiological mechanisms behind associative learning processes. One such question concerns identifying the necessary and sufficient requirements for classical conditioning to occur.

1.1.1 Awareness Debate

The functional role of contingency awareness in classical conditioning is a matter of debate. Contingency awareness has been defined as an organism's ability to "read back its memory records of response output and stimulus input and to select that behavior emitted just prior to the reception of the rewarding stimulus" (Watson, 1966, p. 124). To put it more simply, contingency awareness is an explicit knowledge of the relationship between CS and US such that the relationship can be reported. Some argue that the establishment of a conditioned response is independent of conscious contingency awareness (Baeyens, Eelen, & van den Bergh, 1990; Walther & Nagengast, 2006) and others argue that learning is fundamentally dependent on it (Pleyers, Corneille, Luminet, & Yzerbyt, 2007).

Three possible models for the role of awareness in classical conditioning have been proposed in the literature by Lovibond & Shanks (2002): two versions of a single process model, and a dual process model (Figure 1.1). These models provide different theoretical mechanisms for the production of conscious contingency awareness and the conditioned response. The implications of single versus dual process models have been described as reflecting the relationship of a hippocampal cortical system associated with conscious contingency awareness, and a reflexive subcortical system that does not rely on awareness, suggesting that experimenters should strive to select measures representing both systems to better understand their relationship to one another (Clark, 1998).

A mediating link in these models is a propositional learning process. This learning process assumes the generation and evaluation of a proposition with a truth value, that is moderated by nonautomatic processes such as awareness, cognitive resources, time, and goals (De Houwer, 2009). For example, an organism may form a belief about the relationship between two stimuli (the proposition) through exposure to stimulus pairings. This belief is then evaluated for correctness if there are subsequent opportunities to observe the pairings between the stimuli. Factors moderating the truth value evaluation include verbal knowledge of the contingency (perhaps in the form of instruction), other cognitive tasks that must be performed that may act as interference, the amount of time that is available, and any goals regarding the proposition that the organism has.

A strong version of the single process model suggests that exposure to the CS-US contingency triggers a propositional learning process, causing conscious awareness, which directly elicits the production of the conditioned response. A weak version of the single process model similarly suggests that exposure to the CS-US contingency causes a propositional learning process to occur, but this process is the direct cause of both conscious awareness of the contingency and production of the CR. In other words, consciousness does not directly produce the CR, as theorized above, instead in this model, conscious awareness and the conditioned response are both products of the propositional learning process. These single process models propose that there is only one mechanism involved in producing conscious awareness and a conditioned response, but only the first suggests that awareness is a prerequisite for conditioning while the second treats awareness and a CR both as byproducts of the same mechanism. In other words, the strong single process model states that if conscious awareness is disrupted, then there will be no CR, whereas under the weak model the mediating propositional learning mechanism produces both awareness and the response, suggesting that blocking awareness would not necessarily disrupt the response.

Contrary to single process models, dual process models propose that there are two distinct learning processes, a propositional process that produces conscious contingency awareness, and a non-propositional process that produces the conditioned response. Theories on propositional learning describe a process where there is a formation and analysis of a proposition about the relationship between stimulus and response, which may be dissociated from the response itself (De Houwer, 2009). Essentially, in the single process models the acquisition of a CR is dependent on propositional learning, whereas in the dual model the production of a CR is independent of the propositional learning process. The distinction of the propositional learning process as an intermediary stage in each model is due to the different potential mechanisms of producing contingency awareness and a conditioned response.

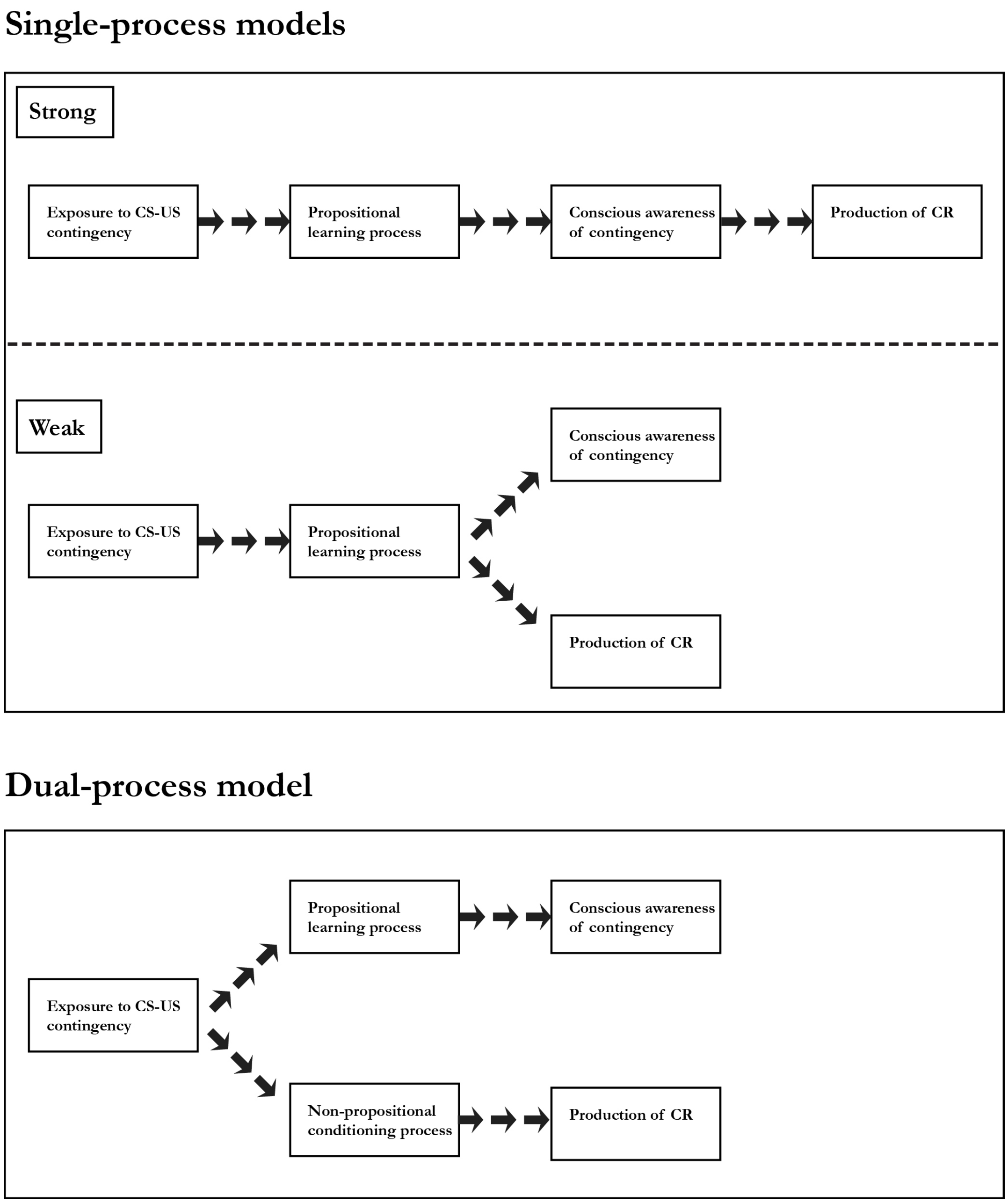

Figure 1.1 Three Models of the Role of Awareness in Classical Conditioning
The strong version of a single process model, the weak version of a single process model, and the dual process model are depicted in this figure. Adapted from Lovibond & Shanks (2002) The role of awareness in Pavlovian conditioning: empirical evidence and theoretical implications

Several lines of research have indirectly supported a single process model. Utilizing delay and trace conditioning paradigms with two neutral tones as their CSs and a 100 ms airpuff as their US, Lovibond, Liu, Weidemann & Mitchell (2011) measured conditioning and awareness in contingency aware and unaware groups by instructing one group to pay attention to the CSs and USs, and the other to pay attention to the distractor task. In a delay conditioning paradigm, a CS is presented before a US, and the two offset simultaneously, while in trace conditioning, a variation on this basic paradigm, the CS is followed by a short inter-stimulus interval (ISI) typically lasting 500 to 1000 ms before the presentation of the US (Figure 1.2). These authors found that in both delay and trace conditioning paradigms, only participants who were aware of the contingency demonstrated a conditioning effect (an eyeblink occurring after CS onset), which they believe suggests a single coordinated system involving contingency awareness and the acquisition of a conditioned response. Weidemann & Antees (2012) reported similar findings even with more rigorous awareness manipulations to argue that accounts of conditioning without awareness are due to insensitive post-questionnaire measures. These authors used a backwards masking task to slow the rate of acquiring contingency awareness, allowing them to implement an online awareness assessment by asking participants to respond when they believed the US would be delivered on each trial.

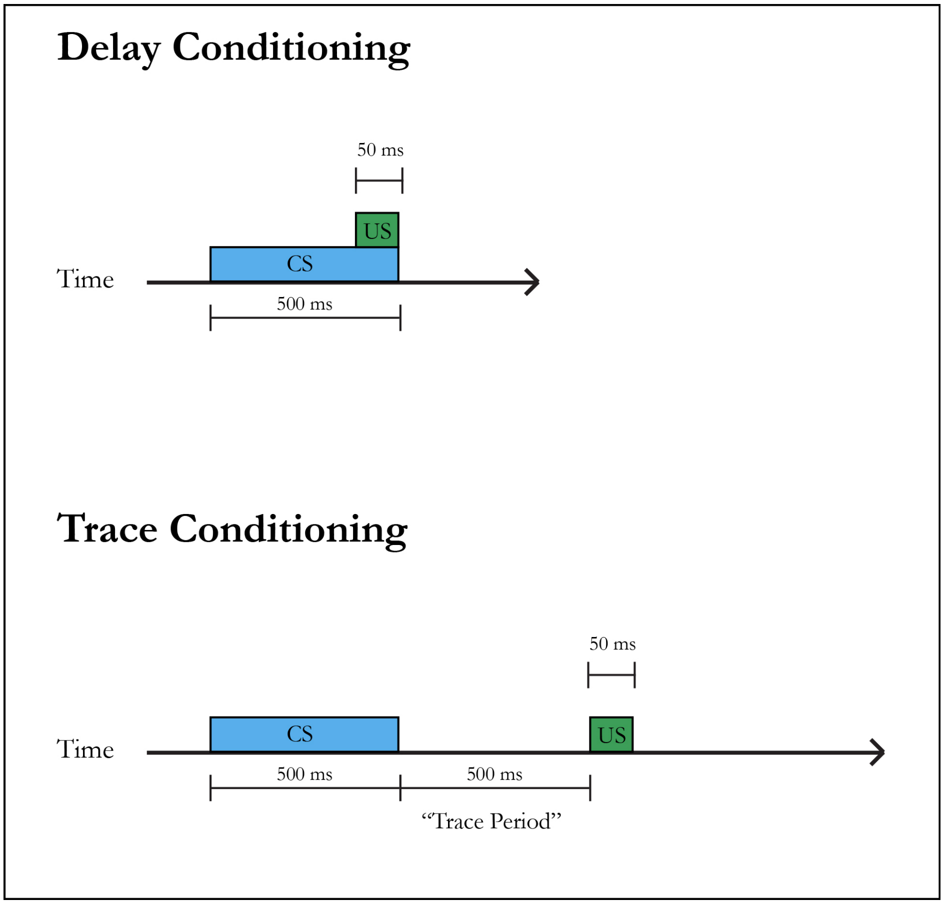

Figure 1.2 Diagram of delay and trace conditioning paradigms
Adapted from Clark (1998) Classical conditioning and brain systems: The role of awareness

Recent work utilizing a new design provided a more systematic investigation of the role of awareness, strongly suggesting that the acquisition of a CR occurs only with contingency awareness, and not in unaware participants (Weidemann, Satkunarajah, & Lovibond, 2016). These authors interleaved two separate reaction time tasks, where an airpuff US was salient to one task, and potential CSs (different colored geometric shapes) were salient to the other. This method ensured that all participants were attending to both the CS and USs. To more directly assess the role of awareness, half of their subjects were explicitly informed of the CS-US contingency while the other half were only told that the two tasks were related without any other details. The majority of informed participants were categorized as contingency aware (were able to report a predictive relationship between CSs and US) while participants in the uninformed group were largely unable to describe this relationship. Even more compelling, the awareness status (aware/unaware) was a better predictor of CR acquisition than whether or not participants were given contingency information. The unaware participants in the informed group did not display a conditioned response, while the aware participants in the uninformed group did.

In contrast to these findings, some laboratories report that contingency awareness and some types of conditioning effects are uncorrelated under a delay eyeblink conditioning paradigm, providing support for a dual process model. Clark and Squire (1999) found that contingency awareness was not necessary for successful conditioning in a delay conditioning paradigm, but was essential for successful trace conditioning. In this experiment, they manipulated awareness of the CS-US contingencies by giving two groups a distractor task and informing two groups of the relationship between CS and US. Awareness was assessed with a series of true or false statements about the relationship between the CS and US. Only contingency aware participants demonstrated the CR under the trace conditioning paradigm. Smith, Clark, Manns, & Squire (2005) reported similar results to Clark and Squire (1999) such that aware and unaware participants demonstrated comparable levels of differential eyeblink conditioning.

Lovibond and Shanks propose an important distinction between contingency awareness and expectancy awareness. While contingency awareness is the explicit knowledge that a CS predicts a particular US, expectancy awareness is when the presentation of a specific CS produces awareness that a specific US will follow even if participants are unable to express the exact contingency. In a hypothetical experimental paradigm where a CS is paired with a US 75% of the time and a neutral stimulus is never paired with the US, when presented with the CS and the neutral stimulus and asked to predict the likelihood of a US occurring, participants may be able to guess that there is higher likelihood for the US to follow the CS than the neutral stimulus without being able to say explicitly “the CS predicts the US”.

1.1.2 Evaluative Conditioning and Subjective Report

Evaluative conditioning is a subset of classical conditioning that refers to the process where a previously neutral stimulus becomes a conditioned stimulus (CS) through a history of repeated parings with positively or negatively valenced unconditioned stimuli (De Houwer et al., 2001; Jones, Olson, & Fazio, 2010). In this process, the CS gains the valence of the unconditioned stimulus (US) through repeated pairings. Unlike in typical classical conditioning paradigms, evaluative conditioning effects can be observed when there are a high number of repeated pairings of CS and US irrespective of a probabilistic relationship. Typical attempts at other types of classical conditioning are successful only if there is a high probability that the US will occur with the CS regardless of the absolute number of simultaneous appearances (Baeyens, Hermans, & Eelen, 1993). The fewer CS-US pairings relative to the total number of trials required for successful acquisition of a CR makes evaluative conditioning a particularly useful tool to explore the role of awareness in classical conditioning simply because the lower number of pairings reduces the likelihood for participants to become explicitly aware of the contingency. For example, consider two hypothetical studies that both require two hundred trials per condition (which most typical electroencephalography studies do). Both experiments have a finite set of 10 neutral stimuli, half of which will sometimes be paired with an unconditioned stimulus and half that never will. I will refer to the first half as the potential CSs, and the second set as the neutral stimuli. Under evaluative conditioning methods, you could pair 50% of the potential CS trials with the US for a total of 50 required CS-US trials. In typical classical conditioning, you would have to pair enough trials for the CS and US to have a high probability of occurring together, which might require 90% of trials to paired, or 90 trials.

Within the evaluative conditioning literature, there are several controversies regarding the robustness and reproducibility of EC effects, including the aforementioned role of attention and awareness. While some authors report successful evaluative conditioning without contingency awareness (Baeyens et al., 1993; Field, 2000) and that the level of awareness is not correlated with size of evaluative conditioning effect (Baeyens, Eelen, Crombez, & Van den Bergh, 1992) there are a large number of studies that do not support these findings (Kattner, 2012; Pleyers et al., 2007). Thus far, research has relied heavily on behavioral measures to provide answers in this debate, but recent experimental work using electrophysiology may provide additional insight into evaluative conditioning mechanism.

1.1.3 Neural Correlates of Conditioning

Electroencephalography (EEG) is a method for recording neural activity from post-synaptic electrical potentials that are summed within local regions of the brain and observable at scalp electrode sites. Event-related potentials (ERPs) are components of electroencephalogram activity representative of brain activity elicited by particular events such as the presentation of a visual or auditory stimulus. Averaging together many trials that are time-locked to the onset of stimuli allows us to isolate these ERP components by removing the non-specific neural background noise ultimately revealing a waveform that captures the neural activity related to stimulus processing (Luck, 2014).

The late positive potential (LPP) is an ERP component that is found in EEG recordings and is thought to be related to local attentional bias to emotional stimuli (Gable & Harmon-Jones, 2010; Hajcak, Dunning, & Foti, 2009). This component begins roughly 350 ms after the presentation of emotional stimuli, reaching its peak around 1 second after stimulus onset (Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000; Hajcak et al., 2009) continuing even after stimulus offset (Hajcak & Olvet, 2008). A larger LPP is elicited for both positively and negatively valenced stimuli compared to neutral stimuli (Cuthbert et al., 2000), and though the amplitude decreases with repeated exposure to the same stimuli, the LPP does not completely habituate to repeated stimulus exposure (Codispoti, Ferrari, & Bradley, 2006, 2007; Olofsson & Polich, 2007), making this component a useful index of emotional arousal in evaluative conditioning paradigms. The magnitude of the late positive potential is moderated by stimulus intensity, such that higher intensity stimuli elicit larger LPPs compared to lower intensity stimuli (Cuthbert et al., 2000), but is not affected by physical stimulus properties such as size (De Cesarei & Codispoti, 2006) or other perceptual stimulus characteristics (Bradley, Hamby, Löw, & Lang, 2007).

1.1.4 Conditioning Startle Response

The mammalian startle response is reflexive muscle activity triggered by surprising or aversive stimuli (Landis & Hunt, 1939) that is elicited by acoustic (Davis, Gendelman, Tischler, & Gendelman, 1982), tactile (Taylor, Castro, & Printz, 1991), and vestibular (Gruner, 1989) stimulation, and is potentiated by synchronous presentation of stimuli from two or more of these modalities (Yeomans, Li, Scott, & Frankland, 2002). The acoustic startle response (ASR) has been used as a measure of emotional valence because it occurs independently from attention to stimuli (Vrana, Spence, & Lang, 1988). In particular, the use of white noise bursts demonstrates greater conditioning effects on self-report ratings, skin conductance responses, and evoked heart period changes compared to electric shock in a conditioning paradigm involving hundreds of trials, making this an appropriate unconditioned stimulus for an ERP study requiring data from many trials to create a comprehensible average (Sperl, Panitz, Hermann, & Mueller, 2016).

Several characteristics of the ASR work together to make it a useful index of conditioning. Like other conditioned responses, the startle is subject to habituation, such that repeated exposure to the unconditioned stimulus will eventually produce a diminished startle response (Kretschmer & Koch, 1998). Significantly, the introduction of a predictive stimulus dulls the habituation effect, where the startle amplitude becomes larger when participants can anticipate the upcoming white noise burst or shock (Grillon, Ameli, Woods, Merikangas, & Davis, 1991), making the amplitude of the ASR a good index of learning in our conditioning paradigm.

1.2 Research Question and Hypotheses

Our primary research question is generally the problem of whether or not evaluative conditioning can occur without conscious awareness of the contingency between the unconditioned stimulus and conditioned stimulus. In addition to this, the present study was also an attempt at the broader goal of developing appropriate methods for investigating neural correlates of conditioning.

Our independent variables were the relationship between previously neutral stimuli and an aversive white noise burst (paired or unpaired), and the amount of exposure to the CS-US contingency (Block 1 – no exposure, block 2 – some exposure block 3 – more exposure, and block 4 – most exposure). We selected a range of measures to most effectively fulfill our exploratory goals. Dependent measures of conditioning included post-session self-reported ratings of pleasure and arousal when presented with certain visual stimuli, expectancy awareness (ability to predict which visual stimuli were more likely to be accompanied by the US), the acoustic startle response amplitude, and the LPP amplitude.

Lovibond and Shanks's models for the role of awareness in evaluative conditioning provide different predictions regarding each of the measures we selected for this study. The strong single process model is the only model that positions contingency awareness as a prerequisite for the acquisition of a conditioned response, as opposed to the weak single process and dual process models, which proposes that they are independent and dissociable.

We created 24 visual stimuli composed of white line segments on a black screen. The participants were asked to respond to the figure in the foreground of the stimuli, while the CS was one of two background line segment orientations. Participants were told that our unconditioned stimulus, a 99-dB white noise burst that accompanied half of the CSs was not part of the task and was just a distractor to be ignored. We designed this task to make it intentionally difficult for participants to become aware of the CS-US contingency so that we could examine conditioning effects of stimuli paired with a US compared to stimuli that were never paired with a US in the absence of contingency awareness. Participants were classified as aware if they were able to report that one of the background orientations was more likely to be paired with the US than the other, and unaware if they did not provide this answer.

Under the single process models, unaware participants should never demonstrate a conditioned response, so we would expect to see the typical habituation of the startle response across blocks, and no difference between LPP magnitude for paired versus unpaired stimuli in any of the conditions.

On the other hand, the dual process model predicts that unaware participants will still demonstrate a conditioned response that may be particularly indexed by the ASR and LPP measures. The startle can deviate from its typical habituation pattern if a predictive stimulus is introduced, so if there is successful conditioning we would expect to see comparable startle amplitudes across all blocks. A greater LPP positivity for paired compared to unpaired stimuli would also provide support for this model.

Chapter 2: Methods

2.1 Participants

We recruited participants between the ages 18 and 30 with normal or corrected-to-normal vision and no history of neurological disorder or damage from Psychology 121 labs and with flyers posted to Reed Facebook groups. Five people (3 female, mean age 20, SD = 1.67332) participated in the initial pilot study and were compensated for their time with 2 psychology lottery tickets, each roughly equivalent to a 1 in 50 chance to win $50. Sixteen people (12 female, mean age 19, SD = 1.414214) participated in the experiment proper, and were compensated for up to three hours of participation with 6 psychology lottery tickets. We obtained approval from the Institutional Review Board (IRB) before beginning data collection, and all participants provided informed consent prior to participation.

2.2 Stimuli

One of eight different visual displays were presented to participants for 700 ms during each trial, followed by an interstimulus interval (ISI) of 700 to 900 ms. These displays were composed of white line segments of differing line orientations such that a central rectangle (either taller or wider) was formed from either vertical (90°) or horizontal (0°) lines, and superimposed over a background that was formed from either 45° or 135° lines. Half of these paired trials were accompanied by a 99-dB white noise burst beginning 650 ms after the onset of the visual stimulus and lasting 50 ms so that the offsets of the visual and auditory stimuli were simultaneous. The stimuli pairings were counterbalanced between participants so that either the displays with the 45° or 135° background line orientations would always be the conditioned stimuli (CS) paired with the aversive tone on 50% of CS trials. Visual stimuli were created in Adobe Illustrator

CC (Adobe Systems Incorporated, San Jose, CA) and auditory stimuli (99-dB tone burst and a placeholder 50-ms of silence) were created using Audacity.

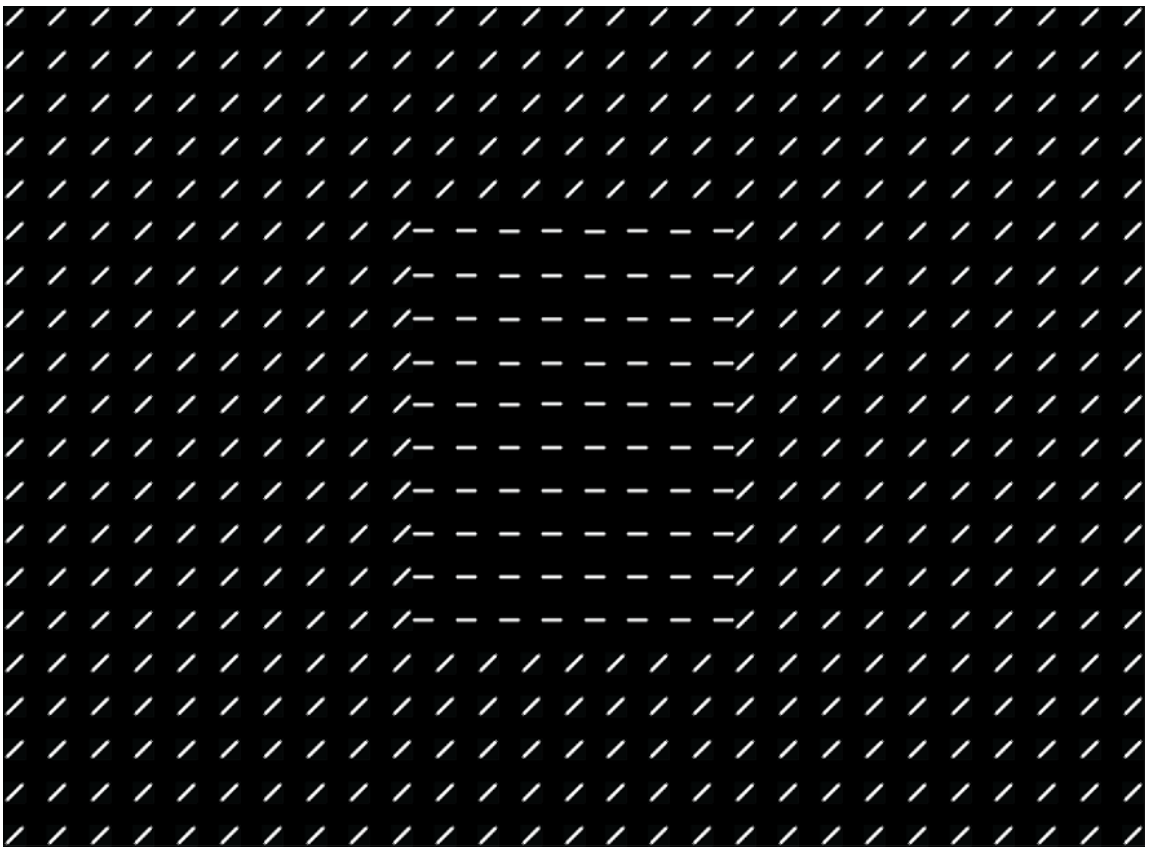

Figure 2.1 Example Stimulus
One of the 24 stimuli presented on experimental trials. All stimuli were created by combining the variations on background segment orientation (45° or 135°), foreground segment orientation (0° or 90°), foreground shape (tall or wide), and foreground location (centered, shifted left/down, shifted right/up).

2.3 Procedure

The goals of this study required participants to be unaware of the contingency so we recruited 5 people to perform the computerized task without recording EEG in a pilot study to determine if participants were able to report the CS-US contingency. Only one of these participants reported that the tone was paired with a certain background orientation, and when asked to specify which, responded with the unpaired background.

All participants recruited for the experiment proper were asked to attend a single 3-hour electroencephalography (EEG) and electromyography (EMG) recording session, which was followed by the administration of a computerized post-session questionnaire.

2.3.1 EEG and EMG Recording

During the EEG and EMG recording session, a 32-channel electrode cap was fitted to each participant (Figure 2.3). The ground electrode was CP2 and the reference was the right mastoid. Two EMG electrodes were placed on and near the left lower eyelid roughly 12 mm apart to record the acoustic startle response. The remaining channels were surface electrodes including the vertical electrooculogram electrode (VEOG) used to identify eyeblink artifacts, left and right horizontal electrooculogram electrodes (HEOG L/R) used to detect eye movements and the left mastoid used in post-recording to re-reference to the right mastoid. Electrophysiological data were recorded using BrainVision Recorder (Brain Products, Germany).

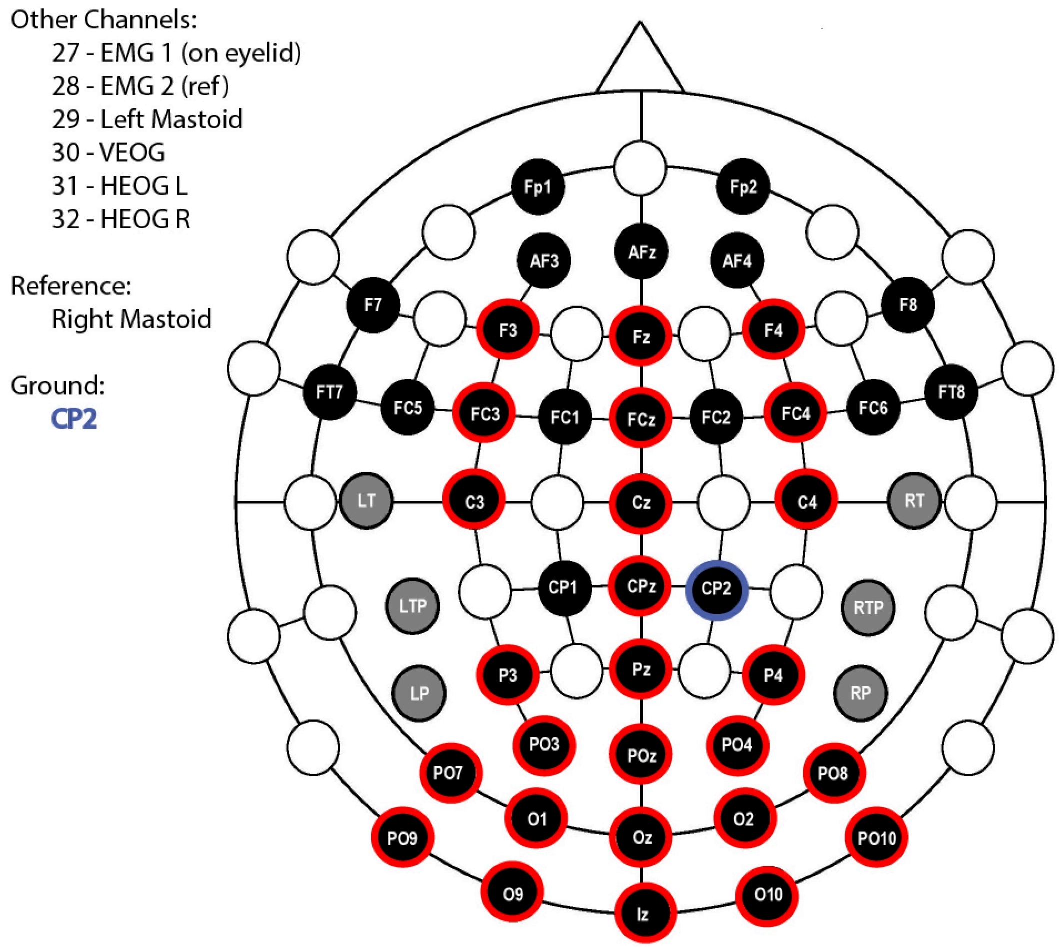

Figure 2.3 Electrode Map

The 26 electrodes used in this experiment are shown in red while the ground electrode is shown in blue. The remaining channels were free electrodes attached directly to the skin, including 2 EMG electrodes, VEOG, HEOG L/HEOG R, and left and right mastoid electrodes.

2.3.2 Computerized Task

Experimental programs for stimulus delivery were written using Presentation (Neurobehavioral Systems, Berkeley, CA). Participants performed the computerized task in a sound attenuated booth while wearing a 32-channel electrode cap. For half of the subjects, the CS+ were stimuli with background orientations of 45° line segments, and for the other half the CS+ were stimuli with background orientations of 135° line segments. The experimental session consisted of four blocks separated by long breaks, and two short self-paced breaks in the middle of each block. On the first block, the visual stimuli were presented without the tone-bursts to record baseline brain activity to the displays. During the following 3 blocks, participants were presented with one of the 24 possible visual displays on each trial, and the US, a 99-dB tone burst accompanied half of all CS+ trials. During all blocks, participants were instructed to respond with one key press if the central rectangle was wider than it was tall and another key press if it was taller than it was wide. Blocks consisted of 432 trials each, so each participant completed 1,728 trials total during the recording session.

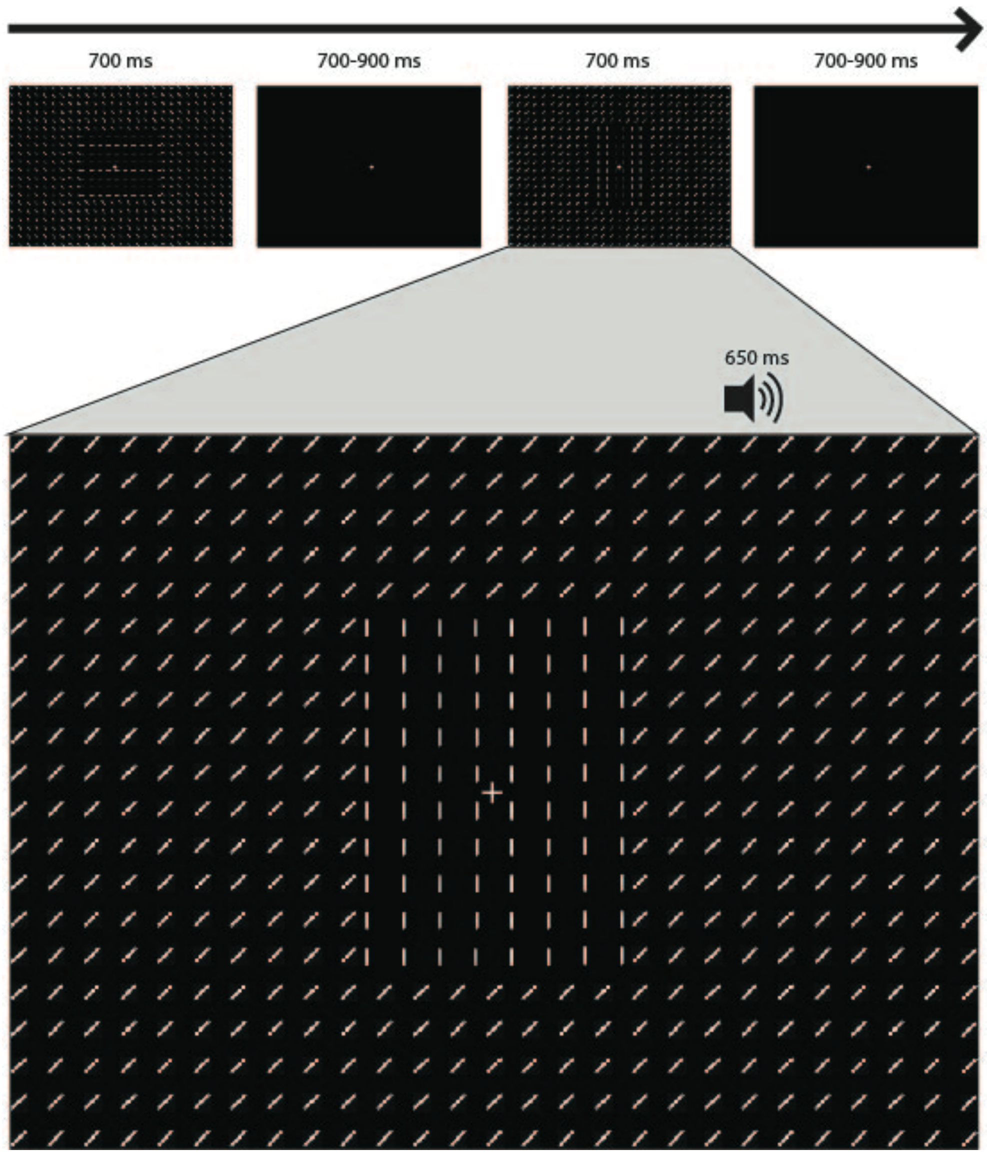

Figure 2.2 Diagram of the Conditioning Task
During each trial one of 24 possible visual stimuli was displayed for 700 ms. In blocks 2, 3, and 4 half of the CS+ trials were accompanied by the onset of a 50 ms 99-dB white noise burst at 650 ms so that visual and auditory stimuli terminated simultaneously.

2.3.3 Post Questionnaire

Following the electrophysiological portion of the session, all participants responded to a questionnaire gauging conditioning in four dimensions: Pleasure, arousal, expectancy awareness, and contingency awareness. Pleasure and arousal were measured using the self-assessment manikin (SAM) (Bradley & Lang, 1994) for each quality (Figure 2.4, 2.5) where participants rated the background and foreground stimulus components separately on both the pleasure and arousal manikins to ensure participants were responding to the background orientations and not to another stimulus feature (Figure 2.6). Anchoring terms on either end of both self-assessment manikins were employed to guide participants in their ratings. The highest pleasure rating was anchored by the terms: happy, pleased, satisfied, contented, and hopeful, while the lowest pleasure rating was anchored by the terms: unhappy, annoyed, unsatisfied, melancholic, and bored. The highest arousal rating was anchored by the terms: stimulated, excited, frenzied, jittery, wide-awake, and aroused, while the lowest arousal rating was anchored by the terms: relaxed, calm, sluggish, dull, sleepy, and unaroused. We measured expectancy awareness by presenting the visual stimuli from the task and asking participants to predict the likelihood of a sound-burst for each display. The final question in the post-experiment questionnaire asked participants to if they noticed a relationship between the visual displays and the sound burst, and if so to describe this relationship (For the full questionnaire, see Appendix B).

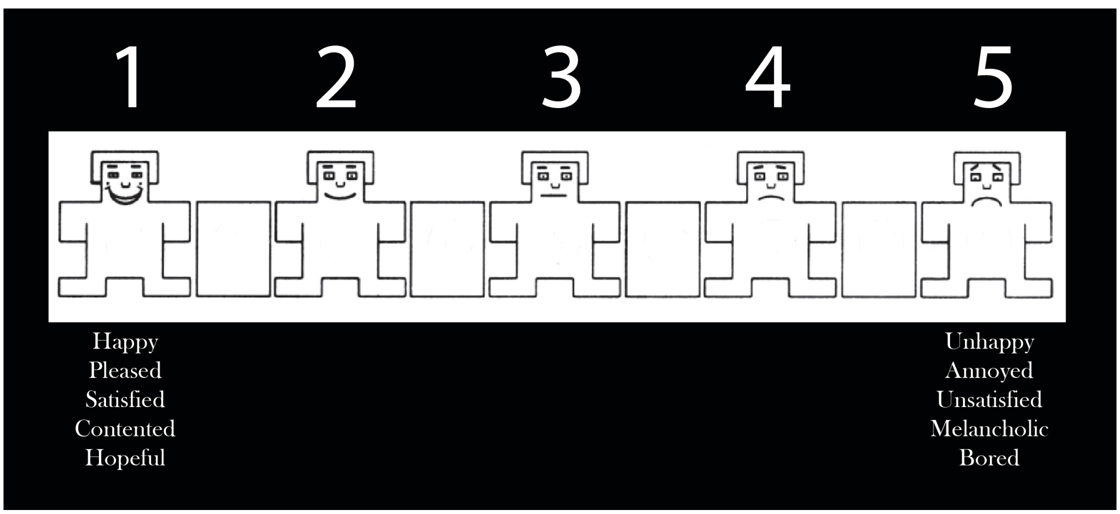

Figure 2.4 Pleasure Self-Assessment Manikin
The pleasure scale of the Self-Assessment Manikin reproduced and adapted from Bradley & Lang (1994) Measuring emotion: The Self-Assessment Manikin and the Semantic Differential. The figure was changed to include the anchoring terms on either end of the scale, as well as numbers to facilitate participant rating responses.

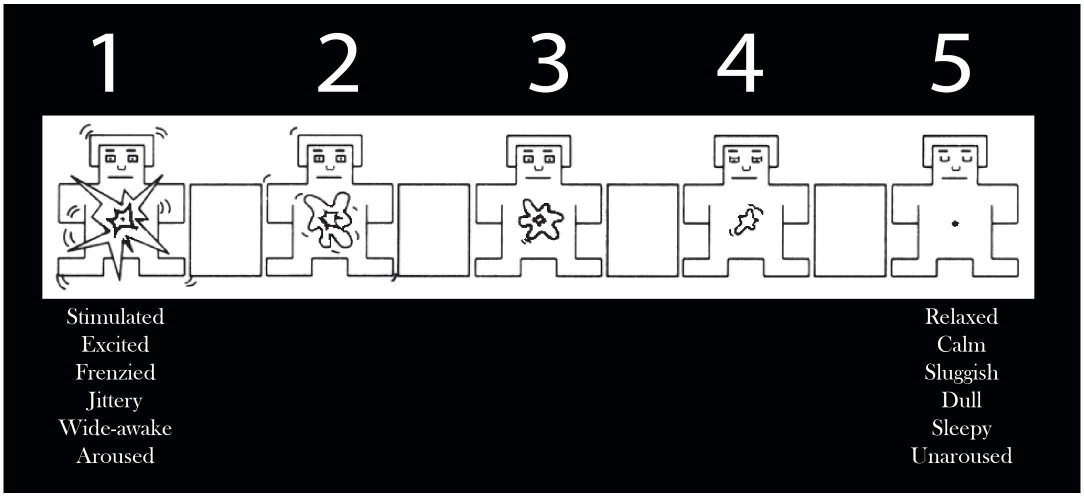

Figure 2.5 Arousal Self-Assessment Manikin

The arousal scale of the Self-Assessment Manikin reproduced and adapted from Bradley & Lang (1994) Measuring emotion: The Self-Assessment Manikin and the Semantic Differential. The figure was changed to include the anchoring terms on either end of the scale, as well as numbers to facilitate participant rating responses.

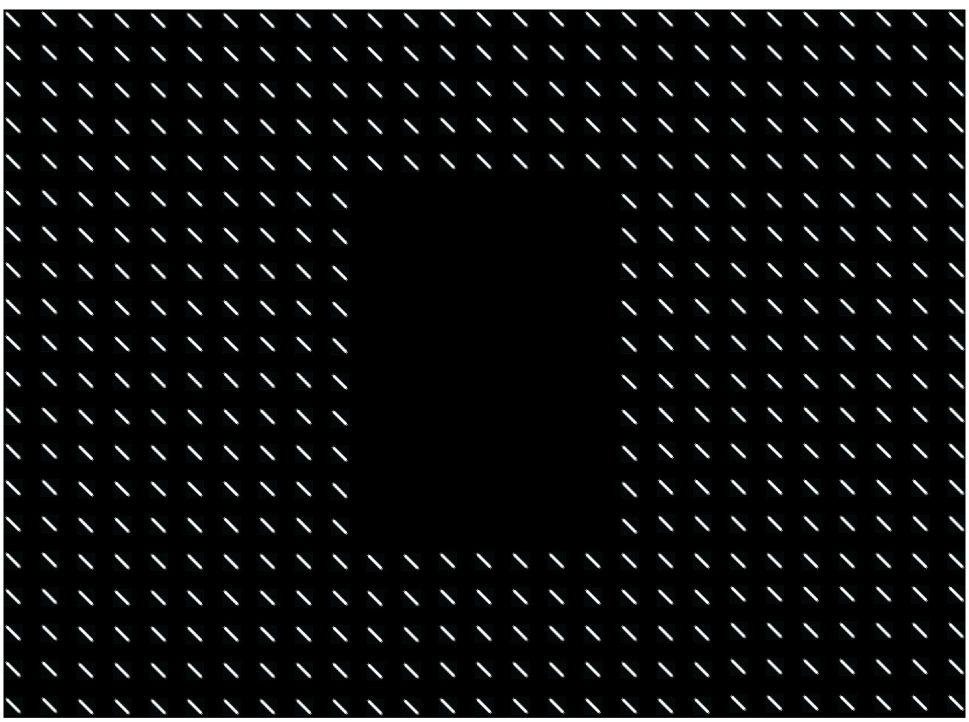

Figure 2.6 Example Rating Stimuli

To better directly assess self-report of conditioning, the participants rated background and foreground figures separately. Eight deconstructed displays were presented in this section of the self-report questionnaire.

2.4 Data Analysis

2.4.1 ERP Pre-processing and analysis

The raw EEG data were processed using BrainVision Analyzer 2 (Brain Products, Germany). Bad channels were topographically interpolated (*Mean* = 1.2% of channels), then the data were segmented into trials by experimental block (segmentation window began -200 ms before stimulus onset, and ended 1000 ms after stimulus onset producing segments 1200 ms in duration). Following segmentation of trials by condition, a 25 Hz high cutoff filter (24 dB/octave roll-off) was applied before the data were re-referenced to the average of the mastoids, and then the horizontal electrooculogram. Semiautomatic artifact rejection excluded an average of 20.5% of trials, or 44.7 segments per condition for a remaining 79.5% or 171.3 trials. The EMG data were segmented into trials by experimental block (time window = -200-1000 ms relative to the sound onset), band-pass filtered (28-512 Hz; 24 dB/octave roll-off), rectified, low-pass filtered (30 Hz; 24 dB/octave) and baseline corrected. For each conditioning block, mean startle response magnitude was measured in the 20-120 ms time window after stimulus onset at time zero. The scalp location of the LPP changes from posterior to central electrodes over course of affective processing (Hajcak, Weinberg, MacNamara, & Foti, 2012), so for the time window 350-600 ms, electrodes P3, Pz, P4, PO3 and PO4 were pooled based on prior methods for this component (Van Dongen, Van Strien, & Dijkstra, 2016). Startle and LPP results were analyzed using STATA Data Analysis and Statistical Software (StataCorp LP, College Station, Texas). Paired *t*-tests for mean startle amplitude across blocks 2, 3, and 4 and mean LPP amplitude within each block were employed to test for physiological evidence of conditioning

Chapter 3: Results

3.1 Questionnaire Results

3.1.1 Pleasure Ratings

Participants reported similar pleasure ratings for paired versus unpaired stimuli on the Pleasure Self-Assessment Manikin. A dependent means *t*-test revealed no significant differences between groups (paired mean = 2.53, unpaired mean = 2.57, $t(16) = 0.527$, $p = 0.6053$).

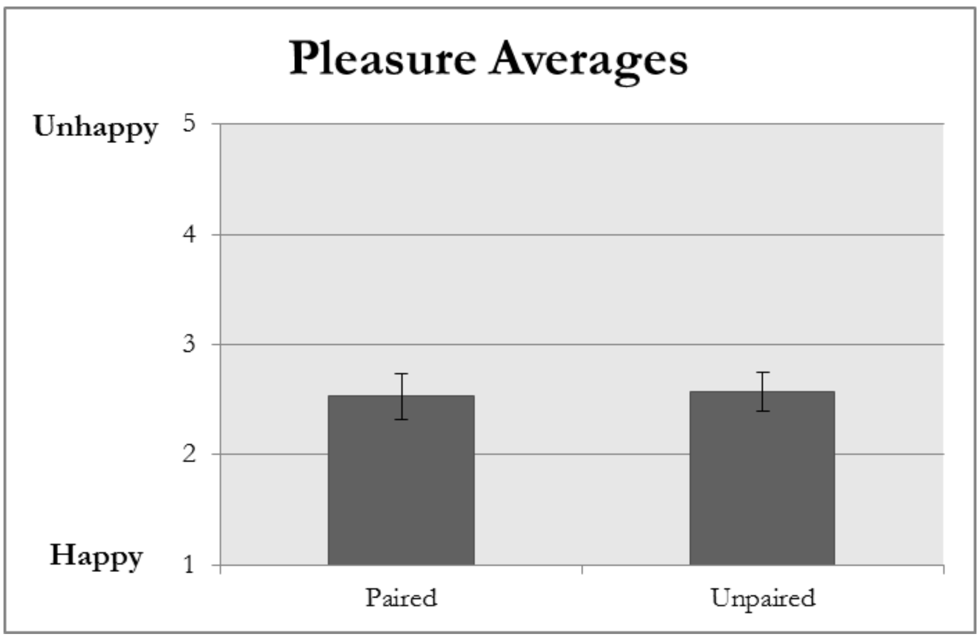

Figure 3.1 Average pleasure ratings
Ratings (mean ± SEM) of paired and unpaired stimuli on the Pleasure SAM across all participants.

3.1.2 Arousal Ratings

Participants reported no difference in arousal for paired versus unpaired stimuli on the Arousal Self-Assessment Manikin. A dependent means t-test revealed no significant differences between groups (paired mean = 3.35, unpaired mean = 3.15, t(16) = 0.672, p = 0.5112).

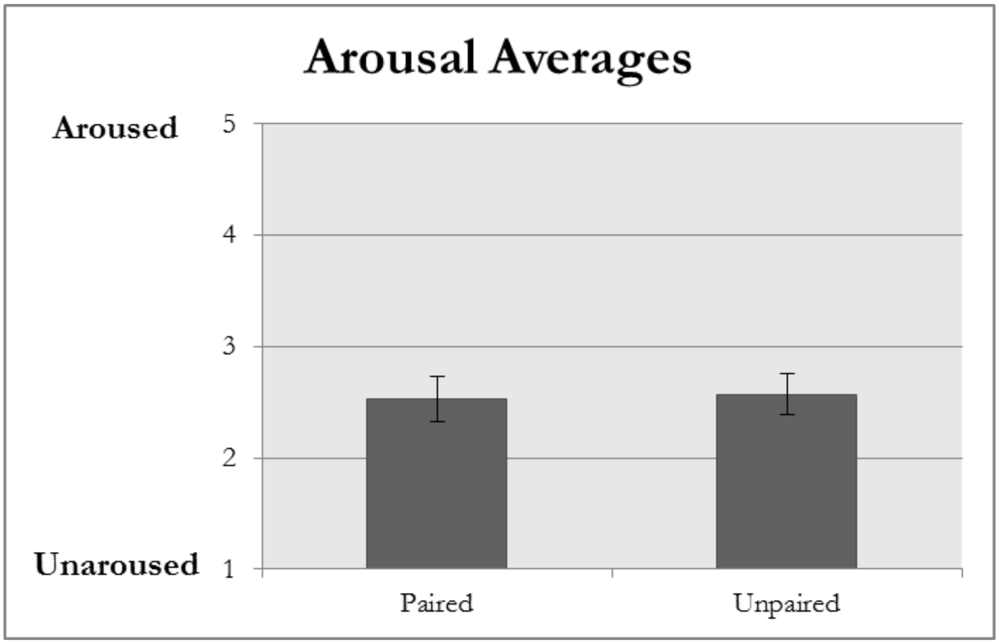

Figure 3.2 Average arousal ratings
Ratings (mean ± SEM) of paired and unpaired stimuli on the A SAM across all participants.

3.1.3 Expectancy Awareness Results

Participants did not demonstrate expectancy awareness for the likelihood of the US when presented with the experimental stimuli. A dependent means t-test revealed no significant differences between predictions for paired versus unpaired stimuli (paired mean = 40%, unpaired mean = 42%, t(16) = 0.762, p = 0.457).

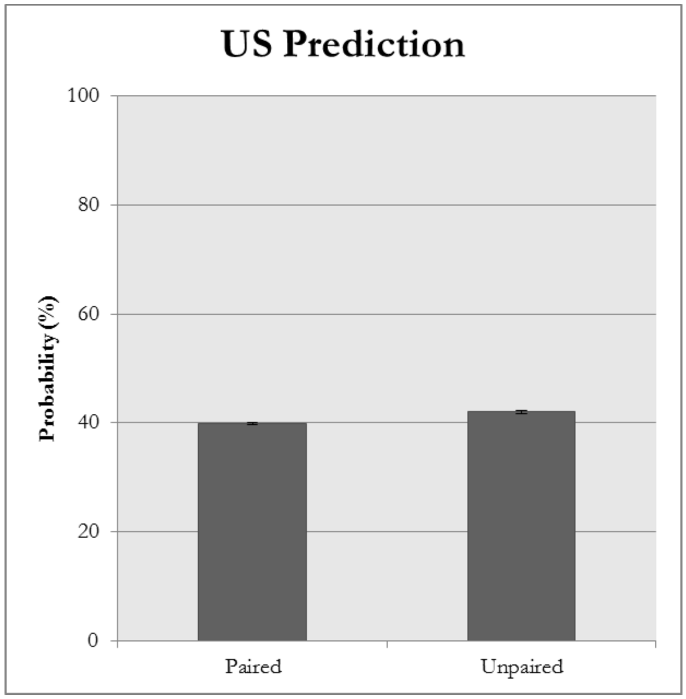

Figure 3.3 Average tone predictions for paired and unpaired stimuli
Tone probability prediction (mean ± SEM) across all participants.

3.1.4 Contingency Awareness Results

Participants were categorized as contingency aware if they were able to explicitly report the relationship between the paired background and the tone burst. None of the 16 participants accurately identified the CS-US contingency (ie., none were able to say that the tone accompanied the paired background more than the unpaired background), and several people even described detailed but inaccurate theories about the relationship between the visual displays and the white noise.

3.2 Electrophysiological Results

3.2.1 EMG Results

Due to programming error, data from only 11 participants were included in EMG analyses. The acoustic startle response (ASR) data followed the trend of habituation to the US typically found in the literature, with the largest amplitude in block 2 and decreasing in blocks 3 and 4. This suggests that there was no conditioning effect on the startle response. A conditioning effect would have attenuated or even neutralized the normal decrease of startle magnitude across blocks. Mean startle amplitude was calculated in microvolts for block 2 (M = 1.838, SD = 2.474), block 3 (M = 0.937, SD = 1.098), and block 4 (M = 0.771, SD = 0.883). Paired t-tests revealed a significant difference between mean ASR amplitude between blocks 2 and 4 ($t(10) = 1.934$, p = 0.0409), a near significant difference between blocks 2 and 3 ($t(10) = 1.778$, p = 0.0529), and no difference between blocks 3 and 4 ($t(10) = 1.6227$, p = 0.0679).

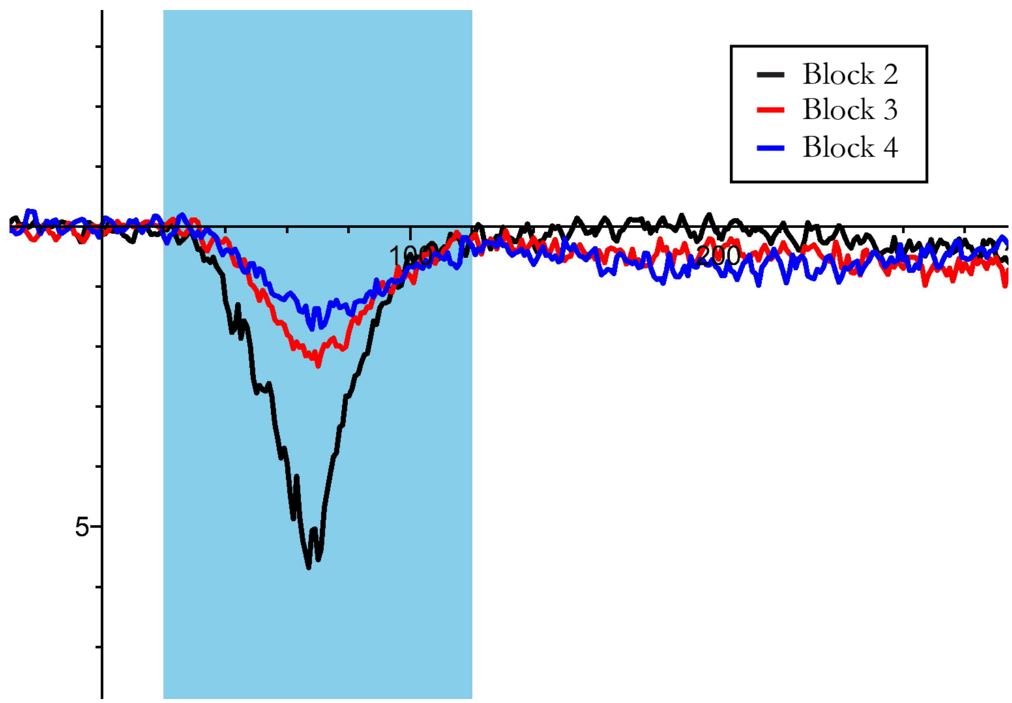

Figure 3.4 Startle response across blocks
Average startle response for each conditioning block time-locked to the onset of the onset of the auditory stimulus. The mean amplitude for each block was calculated for the highlighted region (20 – 120 ms). Time is plotted in milliseconds and amplitude is plotted in microvolts.

3.2.2 EEG Results

The mean LPP amplitude in the 350-600 ms window for unpaired and paired stimuli in block 1 (Figure 3.5, Panel A), block 2 (Figure 3.5, Panel B), block 3 (Figure

3.5, Panel C), and block 4 (Figure 3.5, Panel D) were virtually identical (Table 3.1). Dependent means t-tests for block 1 (t(16) = 0.112, p = 0.913), block 2 (t(16) = 1.330, p = 0.203), block 3 (t(16) = 0.194, p = 0.849), and block 4 (t(16) = 0.321, p = 0.752) revealed no significant differences between ERPs elicited by unpaired and paired stimuli.

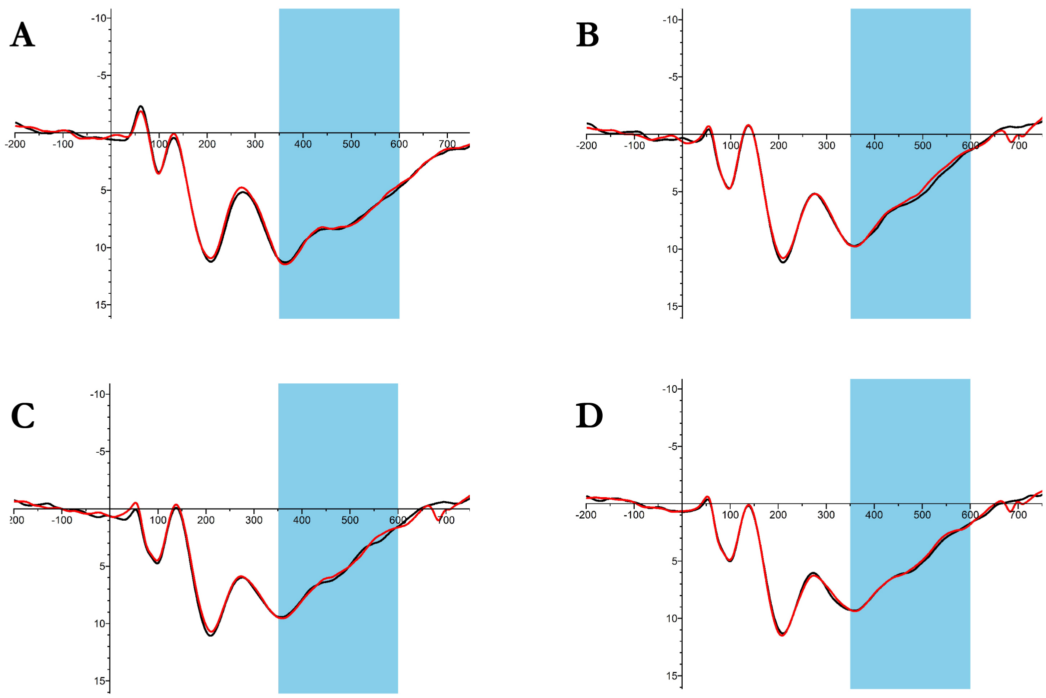

Figure 3.5 Late positive potentials for paired and unpaired stimuli across blocks
For all four panels, the unpaired potentials are displayed in black and the paired potentials are displayed in red. The highlighted regions from 350-600 ms indicate the typical time course of the early late positive potential. A: Block 1 B: Block 2 C: Block 3 D: Block 4

Table 3.1 Mean LPP Amplitude

Block	**1**		**2**		**3**		**4**	
	Unpaired	Paired	Unpaired	Paired	Unpaired	Paired	Unpaired	Paired
Mean	6.168	5.771	5.725	5.638	6.486	5.601	5.556	5.598
SD	2.547	2.604	2.460	2.330	2.459	2.309	2.212	2.305

Chapter 4: Discussion

4.1 Contingency Awareness and Conditioning

The self-report results on the pleasure and arousal self-assessment manikins showed no difference between ratings for paired and unpaired backgrounds. Participants were also unable to predict which visual stimuli were more likely to be accompanied by the displays, reporting similar likelihoods for paired and unpaired displays. Consistent with research on the habituation of the startle response, the magnitude of the muscle twitch significantly decreased between blocks 2 and 4. Additionally, no participants were able to report the exact CS-US contingency in place during their conditioning session. The LPP component of the EEG also did not show any differential amplitude changes over the course of conditioning. Taken together, these null findings indicate that the conditioning paradigm we used was unsuccessful in producing any kind of CR, which may have been because contingency awareness is necessary for learning, or because of methodological shortcomings of the current design. If these results reflect the role of contingency awareness in evaluative conditioning, then they support a single process model of CR acquisition, suggesting that there is not a separate non-propositional conditioning process involved in eliciting a conditioned response that the dual process model suggests. This would indicate that a propositional learning process, where a truth statement is generated and evaluated, is crucial for the production of a conditioned response.

4.2 Implications and Future Directions

It is possible that the participants were not able to acquire a conditioned response on any of our measures because the task so successfully distracted attention away from the orientation of the background line segments, potentially making them insufficiently salient for a CS-US relationship to be formed. Follow up studies may be beneficial in determining whether the results of this experiment are due to inappropriate conditioning methods, or the role of contingency awareness in CR acquisition. A first step would be to inform some participants of the CS-US contingency from the outset and compare the results from these contingency aware participants to the present study's contingency unaware subjects. If a difference in mean LPP was apparent for paired and unpaired stimuli in the later blocks (or an attenuated habituation of the startle response), we could conclude that the role of awareness was playing a key role in establishing these physiological correlates of a CR. If there was still no difference in LPP (or startle habituation), then we would need to adjust our methods further to achieve successful conditioning in aware participants. Additionally, the LPP is a component that is strongest in posterior electrodes in an early (350 – 600 ms) time window and in central electrodes in a later (600 – 900 ms) time window (Hajcak et al., 2012). Our experimental design presented the white noise 650 ms after the onset of the visual stimulus in order to maximize our trial numbers, but in doing so prevented us from examining the late LPP (i.e. we could not analyze data from 650 ms on due to contamination of the LPP by the neural response to the auditory stimulus). Future iterations of an evaluative conditioning EEG study may want to change the duration of the visual stimuli to facilitate analysis of this later potential. More closely adapting a conditioning paradigm from a successful prior experiment may also be useful, though there are many difficulties in attempting to create methods that are also productive for EEG recording. Many of the previous studies in the literature cannot be replicated in an EEG experiment because of stimulus properties and inadequate trial numbers that would produce noisy ERP data.

One common measure utilized by the classic EC literature as well as our electrophysiological study is the post-conditioning self-report. One issue with preserving the Self-Assessment Manikin (SAM) as a metric of conditioning is due to the difference in stimuli between typical evaluative conditioning images and the visual displays we designed. In many typical EC studies, participants are shown pictures from the International Affective Picture System (IAPS), which includes highly unpleasant photos such as mutilated limbs. It is unlikely that our one-hour conditioning session would have produced the same magnitude of emotional response for our previously neutral visual stimuli. Due to the extremity of the intensity of IAPS stimuli that the SAM is typically used to assess, it is likely that this self-report measure is not sufficiently sensitive for detecting a change in stimulus valence under our conditioning paradigm. As the behavioral results are the only measure that can be compared to the majority of the existing literature, it would be productive to follow-up using a more appropriate self-assessment scale to preserve the ability to make comparisons to the previous research on this conditioning measure, and so that it may still be used to complement the physiological measures for a more complete picture of the conditioning process.

4.3 The Next Study

The next step in disentangling the role of methodological shortcomings and contingency awareness in our current results is to conduct the exact same study, but inform participants of the CS-US relationship prior to the conditioning session. If the results we obtained reflect the essential role of contingency awareness in learning, then we would expect to see a difference for paired and unpaired stimuli on each of our measures. On the Pleasure and Arousal SAMs, paired stimuli should be rated less pleasurable and more arousing than unpaired stimuli. The amplitude of the LPP should be more positive for paired compared to unpaired stimuli in the later blocks compared to the baseline ERPs. We would also expect an attenuation of the typical ASR habituation such that the mean startle amplitude across the last three blocks would be comparable to one another because of the predictive value of the CS. If we obtained null findings due to insufficient conditioning methods, then we would expect to see the same pattern even if participants were informed of the contingencies prior to the session (with the exception of the self-report measure, as these results are often susceptible to demand characteristics).

If the methodological issues are in fact what influenced the results of this study, the next novel experiment should make an attempt to adapt a method that has been proven to be successful in producing a conditioned response for an EEG study. One key factor that may have made our task inappropriate for conditioning is attention, as the property of the stimuli that was paired with the US was not what participants were asked to attend to. It is possible that subjects in our study did not receive enough information to establish a CR because there were not enough attentional resources devoted to the background of the visual displays. One promising path would be to modify the methods Weidemann, Satkunarajah, and Lovibond used in their 2016 experiment, where two distinct reaction time tasks are interleaved and the CS-US relationship could be easily manipulated to promote or reduce the likelihood of contingency awareness. Following this design would also allow for an investigation of the role of attention.

The evaluative conditioning literature is also still relatively devoid of experiments investigating neural correlates of conditioning and there are many other ERP components that may also be relevant to this question. Another step could be to do an exploratory pilot study with groups of informed and uniformed participants to see what differences between aware and unaware conditions may arise in the ERPs. If that was successful in highlighting components of interest, different methodological manipulations could be made in order to further explore those components and to illuminate which aspects of the conditioning process they were indexing. This field of research still has so many promising opportunities that have yet to be explored, and there are countless studies that could answer some fundamental questions.

Appendix A: Consent Forms

REED COLLEGE CONSENT
PARTICIPATION IN A RESEARCH PROJECT

Visual shape perception: An electrophysiological investigation of difficult visual discriminations

Invitation to Participate and Description of Project

You are invited to participate in a study designed to gather information about visual shape perception at Reed College conducted by Jasmine Huang. The results of the study will be used to better understand neural activity correlated with making difficult visual discriminations. Your participation in this study will require approximately 60 minutes of your time. You will be given 2 lottery tickets for your participation, and you will not lose this incentive if you withdraw from the study.

Description of the Procedures

If you decide to participate in this study, you will be asked to perform a visual discrimination task, where you will report whether a rectangular shape in the center of the screen is taller or wider while EEG is recorded. Additionally, during some trials to make the task more difficult you will hear a 99-dB burst of white noise for 50 ms ($1/20^{th}$ of a second); this noise is not of sufficient intensity to cause any hearing damage, but it is loud and may be startling.

Your name and this consent form will be stored separately from the data, and the data will never be presented or published along with any identifying information that could be linked back to you.

Your participation in this study is entirely voluntary. You may refuse to answer individual questions or to engage in individual activities. You may also discontinue participation in this study at any time, without loss of lottery tickets. We will be happy to answer any questions about the study or the information contained in this form.

Authorization:

Do you agree to participate in this study (please circle one)? Yes No

I, ______________________________, have read this form and decided that I will participate in the project described above. Its general purposes, the nature of my involvement, and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I am 18 years of age or older.

____ __________________

Signature **Date**

If you have any further questions, please address them to Michael Pitts (mpitts@reed.edu) or the Co-Chairs of the Reed College Institutional Review Board: Tim Hackenberg (hack@reed.edu) and Jon Rork (jrork@reed.edu).

REED COLLEGE CONSENT
PARTICIPATION IN A RESEARCH PROJECT
Visual shape perception: An electrophysiological investigation of difficult visual discriminations

Invitation to Participate and Description of Project

You are invited to participate in a study designed to gather information about visual shape perception at Reed College conducted by Jasmine Huang. The results of the study will be used to better understand neural activity correlated with making difficult visual discriminations. Your participation in this study will require approximately 180 minutes of your time. You will be given 6 lottery tickets for your participation, and you will not lose this incentive if you withdraw from the study.

Description of the Procedures

If you decide to participate in this study, you will be asked to perform a visual discrimination task, where you will report whether a rectangular shape in the center of the screen is taller or wider while EEG is recorded. Additionally, during some trials to make the task more difficult you will hear a 99-dB burst of white noise for 50 ms ($1/20^{th}$ of a second); this noise is not of sufficient intensity to cause any hearing damage, but it is loud and may be startling.

EEG involves passively recording electrical activity from various locations on the scalp via small metal disks mounted in a cloth cap. This method allows us to access precise temporal information about the brain activity levels associated with different cognitive processes. There are no risks associated with this procedure. The electrodes record electrical activity produced by your brain, and at no point will we send any electricity. In order to ensure that the EEG recording is of good quality, it may be necessary to abrade some skin under the electrodes with blunt-tipped needles. The capping process will not cause more than minor and short-lasting irritation. The data collected in this experiment will be kept confidential. Your name and this consent form will be stored separately from the data, and the data will never be presented or published along with any identifying information that could be linked back to you.

Your participation in this study is entirely voluntary. You may refuse to answer individual questions or to engage in individual activities. You may also discontinue participation in this study at any time, without loss of lottery tickets. We will be happy to answer any questions about the study or the information contained in this form.

Authorization:

Do you agree to participate in this study (please circle one)? Yes No

I, ________________________________, have read this form and decided that I will participate in the project described above. Its general purposes, the nature of my involvement, and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I am 18 years of age or older.

____ __________________

Signature **Date**

If you have any further questions, please address them to Michael Pitts (mpitts@reed.edu) or the Co-Chairs of the Reed College Institutional Review Board: Tim Hackenberg (hack@reed.edu) and Jon Rork (jrork@reed.edu).

Appendix B: Post Session Questionnaire

In this portion of the experiment you will be rating components of the stimuli from the experimental blocks on an affective scale. You will be presented with a display followed by a scale that will be anchored with affective terms on both ends. You will see an example of the scale, press the space bar when you are familiar with it. Press the spacebar to begin.

You will be able to view the stimuli until you are ready to rate them. Press the spacebar to switch from the stimulus to the rating scale. Press the spacebar to begin.
[Participants are presented with the deconstructed stimuli and the Pleasure SAM]

Now you will be asked to rate 6 more displays on a different scale. Press the spacebar to begin.
[Participants are presented with the deconstructed stimuli and the Arousal SAM]

Now you will be asked to predict the likelihood of a sound burst if you saw the following displays. Press the spacebar to begin.

On a scale from 0 to 100 percent, please indicate how likely it is the previous stimulus would be followed by a noise burst by pressing 0 for 0%, 1 for 10%, 2 for 20%, 3 for 30%, 4 for 40%, 5 for 50%, 6 for 60%, 7 for 70%, 8 for 80%, 9 for 90%, 'Enter' for 100%. If you are completely unsure, please press the U key, otherwise take your best guess.
[Participants are presented with all 8 stimuli where the foregrounds are centered, between each, they are shown the button-press instructions again]

Did you notice a relationship between the visual stimuli and the noise bursts? If so, please describe what this relationship is on the blank piece of paper provided.
Let the experimenter know when you're finished

Appendix C: Debrief

REED COLLEGE DEBRIEF

Visual shape perception: An electrophysiological investigation of difficult visual discriminations

Thank you for participating in my study!

I would like to discuss the research question of my thesis in more detail.

In some cases, research methods may require that participants in research studies not be given complete information about the research until after the study is completed, but following the completion of data collection we would like to fully debrief you about the true purpose of the study.

My thesis is actually an investigation of evaluative conditioning, a type of classical conditioning that refers to the process of attitude formation where a previously neutral stimulus (the orientation of the background lines) is paired with a valenced unconditioned stimulus (the aversive sound burst) and gains the valence of the unconditioned stimulus.

An unresolved debate in this field of research is whether conditioning can occur without contingency awareness (the participant's explicit ability to report the relationship between the CS and the US). The purpose of this study is to contribute to this debate by incorporating an electrophysiological measure in addition to the traditional self-report questionnaires to provide another quantitative measure that can be used to support subjects' reports of conditioning effects.

If other people knew the true purpose of the study, they would not be able to participate because it would be impossible to get conditioning without awareness, so I ask that you do not share the information we just discussed.

If you have any other questions or comments about the study, you can ask me now, or email me at huangj@reed.edu anytime!

Thanks again for participating!

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