

# STIMULUS PROBABILITY EFFECTS IN EMOTIONAL SCENE PERCEPTION

by

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(Under the Direction of Dean Sabatinelli)

## ABSTRACT

Psychophysiological studies of emotional perception often use stimulus sets containing a greater proportion of emotionally arousing, relative to non-arousing stimuli. However, there is evidence suggesting that participants may learn to anticipate more common stimuli, and this differential expectation may bias reactivity at presentation. Here we manipulated emotional and neutral stimulus set proportions across two studies to investigate the potential role of emotional stimulus probability on central and peripheral indices of emotional scene perception. In study 1, (n=72) viewed a series of 168 scenes containing an equal number of emotional and neutral stimuli, while study 2 (n=72) included twice as many emotional as neutral stimuli. This manipulation did not yield a significant interaction across the 2 studies. This result suggests that commonly used stimulus proportions over-representing emotional stimuli do not result in expectation biases. Future work may investigate more extreme stimulus proportions to identify the threshold at which expectations influence reactivity.

INDEX WORDS: Emotion, Anticipation, Late Positive Potential, Early Posterior Negativity, Heart Rate, Skin Conductance

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## CHAPTER 1

### INTRODUCTION

Emotional perception is the process of evaluating external stimuli, viscerally reacting to them, and includes behavioral responses that maintain or terminate stimulation [Lang 1993]. Perceiving some objects as emotional, and some objects as ordinary allows us to focus on the features of the environment that promote survival, and avoid those that signify danger [Bradley 2000]. This aspect of perception has adaptive value, in that the activation of emotion and perceptual systems allows the organism to respond to a diverse set of environmental stimuli based on learned associations with reward or punishment. This process enhances the processing of features of emotional stimuli and their environmental context, enabling the organism to carry out an appropriate response.

The contextual information of a visual scene has important implications for our response to it. For instance, Susskind (2008) demonstrated that viewing a person displaying a fearful face causes an increase in visual-field size, saccadic velocity, and nasal inspiratory capacity in the observer. In contrast, viewing a person displaying an expression of disgust causes a directly opposite response pattern. These behavioral response profiles were suggested to be evolutionarily adaptive responses that would lead to a higher degree of scanning in the case of threat, and a reduction in inspiration in the case of disgust (perhaps to avoid noxious fumes). Similarly, perception of unpleasant images in a mixed-valence stimulus set leads to greater heart rate deceleration, compared to neutral or pleasant images [Bradley 2001a]. This response profile has been interpreted as an index of heightened sensory intake during the evaluation of the

emotional stimulus [Bradley 2001a]. Pastor (2008) further examined this by using an experimental design in which images were shown in blocks containing images of similar picture content, or mixed content. This experimental design had a differential impact on cortical measures of emotional perception and peripheral measures. When presenting images in blocks that contain only unpleasant images, cardiac deceleration is attenuated, suggesting that there is some associative information learned during picture presentation [Pastor 2008]. In contrast, this manipulation had no effect on heart rate, or event-related potentials commonly used to index emotional perception: the early posterior negativity and late positive potential. These studies suggest that contextual information during the perception of emotional events may modulate somatic, autonomic, and cortical processes. Examination of these responses, and how they change according to stimulus characteristics, task demands, and brain states can help us to gain an understanding of the underlying neural mechanisms involved in emotional perception.

Previous studies of visual perception have implicated the occipital and inferior temporal regions as important regions for object recognition [Mishkin & Ungerleider 1983; Goodale & Milner 1992]. Several neural pathways emanate from the occipitotemporal cortices, including projections to the medial temporal, amygdala, hippocampus, and orbitofrontal regions. These diverse projections are thought to be involved in the perception of emotional properties of a stimulus, emotional regulation, and enhancement of memory during emotional events, and the orienting of attention toward emotionally intense stimuli [Kravitz 2013]. Furthermore, the amygdala projects back onto nearly every region in the occipitotemporal pathway [Amaral 2003; Freese & Amaral 2005]. The view that re-entrant projections from the amygdala to the visual cortex play a role in emotional perception is supported by recent work. In the Sabatinelli et. al (2009), BOLD signal discriminated between emotional stimuli and neutral stimuli in the

amygdala and inferior temporal cortex prior to emotional discrimination in the medial occipital region, suggesting that higher order cortical and subcortical regions distinguish emotional content prior to occipital regions. Additionally, Keil et al (2009) used Granger causality techniques to show that anterior cortical regions influence visual cortical encephalographic (EEG) activity. This research was extended in Sabatinelli et al (2014), which demonstrated that Granger causality techniques could be applied to fast-sampled functional magnetic resonance imaging (fMRI) of affective picture viewing. This study identified bidirectional connectivity between the amygdala and fusiform gyrus, and bidirectional connectivity between the frontal eye fields and intraparietal sulcus. Further, this study demonstrated that the more anterior regions (amygdala and frontal eye fields) discriminate between emotional image content earlier than the posterior regions.

The extent to which re-entrant projections influence emotional perception, and the time-course of this proposed modulation is not currently known. Since top-down connectivity has been established, it is possible that visual areas are regulated at multiple stages of emotional perception. Neuroimaging and electrophysiological work have suggested that there may be preparatory activity in the cortex in anticipation of a future stimulus [Woldorff 2007; Altamura 2014; Los 2005]. However, the extent to which anticipation modulates the prioritized perception of emotional events is unclear. In this work, we manipulated the proportion of arousing to non-arousing images presented to participants, in order to establish implicit expectations of future stimuli. Then, we examined the degree to which indices of emotional perception were modulated by this manipulation.

## CHAPTER 2

### LITERATURE REVIEW

#### ERPs in affective processing: the early posterior negativity

Psychophysiological studies have demonstrated that human behavior and parameters of presentation stimuli can modulate components of event-related potentials (ERPs). For instance, ERP research has shed light on the neural correlates of visuo-spatial attention [Mangun 1991], error detection [Holroyd 2002], stimulus form and color [Hillyard & Anllo-Vento 1998], stimulus novelty, attention, and orienting [Naaanen 1979; Bradley 2009]. Most relevant to the present research, prior investigations have demonstrated reliable modulation of early and late components of onset ERPs during affective picture processing [Cuthbert et al. 2000; Keil et al. 2002; Schupp 2003b; Bradley et al. 2007].

The early posterior negativity (EPN) is a relative scalp negativity located over bilateral occipital cortex, evident during a 150-300ms window after stimulus onset. The EPN is the first cortical ERP component to selectively process the emotional content of stimuli during passive viewing paradigms. Junghofer and colleagues (2001) used a rapid serial visual presentation paradigm where images were presented in rapid succession (3-5Hz) to show that affective discrimination occurs early on in visual perception. Prior studies using this presentation paradigm suggested that it relies on short-term memory – participants quickly forget the content of images they perceive. This study showed that the EPN is able to discriminate between emotional and neutral images, independent of sensory characteristics of the stimuli such as color, spatial frequency, and complexity, during a task that has a high visuo-perceptual load.

Moreover, stimuli rated high in arousal generated higher EPN amplitude between 150ms and 260ms, compared to images rated low in arousal [Junghofer 2001]. Further studies have demonstrated emotional modulation of the EPN by different types of affective stimuli. The EPN is enhanced after presenting threatening face stimuli [Schupp 2004b], affective auditory stimuli [Schupp 2003c], and is most sensitive to highly arousing images such as erotica and mutilations [Schupp 2004c]. While the physical features of stimuli do not appear to modulate the EPN, reducing the size of the image has been shown to attenuate EPN amplitude [De Cesarei 2006]. Subsequent parametric work has indicated that the EPN is resistant to habituation after repetitive picture presentation across experimental blocks, but shows habituation within blocks [Codispoti 2007]. Additionally, the presentation of emotional content in the periphery (beyond 15° eccentricity) leads to the absence of emotion modulation in the EPN [De Cesarei 2009].

Since the EPN occurs relatively quickly and is topographically located over the bilateral occipitotemporal cortices, several researchers have questioned whether this phase of emotional discrimination is an obligatory process independent of the attention of the viewer [Schupp 2003a]. One way to assess this is to test whether participants retain EPN emotional discrimination while performing an explicit non-emotional attention task. The evidence for the interaction of attention and the EPN has grown over time through refinement of experimental design. Initially, Schupp and colleagues (2003a) asked participants to count the number of checkerboard images with a white central square, which were intermixed with emotional images. In this paradigm, the EPN was able to discriminate between emotional images and non-emotional images despite the allocation of attention to the non-emotional task. In contrast, when Schupp and colleagues (2007a) asked participants to count the number of target images that contain superimposed horizontal or vertical bars, the EPN's emotional discrimination effect

disappeared. In particular, the experimenters showed that the EPN did not discriminate between non-target emotional images in a stimulus set that contained 50% target images. Further examination using a counterbalanced attentional task in which participants were asked to count the number of target images from a specific category demonstrated that the emotional and attentional modulation of the EPN have additive effects [Schupp 2007b].

#### ERPs in affective processing: the late positive potential

The late positive potential (LPP) is a long lasting scalp positivity found over the centroparietal cortex beginning roughly 400ms after scene stimulus onset. Many early studies of the emotional modulation of the LPP used affective oddball paradigms. These studies demonstrated that rare target stimuli produce elevated P300 amplitude, which is stronger for target stimuli that have greater 'affective distance' from non-target (e.g. unpleasant targets in a pleasant series compared to neutral series). Oddball paradigms use brief exposure of stimuli, with short ITIs, and the choice to use this design left it unclear whether the observed modulation was due to stimulus rarity or emotional content. Schupp and colleagues (2000) sought to determine if LPP modulation occurred with a picture set composed of equal numbers of neutral, pleasant, and unpleasant images. In this case, non-arousing images were more rare than arousing images, allowing them to conclude that emotional content of the images was modulating the LPP, since any potential oddball effect would work in the opposite direction as the emotional modulation. This work was supported by Cuthbert and colleagues (2000), who demonstrated that the LPP has higher amplitude and is longer lasting when viewing natural scenes that are rated as emotionally intense, whether pleasant or unpleasant. Additionally, the LPP is stronger in response to highly arousing emotional images compared to less arousing emotional images, and does not change in its scalp topography when modulated by emotional content [Cuthbert 2000; Schupp 2004a].

Several studies have examined whether the physical dimensions of stimuli modulate LPP amplitude. One possible explanation for stronger LPP responses is that emotional scenes tend to be more complex, and that there may be enhanced processing requirements for visually complex scenes. Bradley et. al. used pleasant, neutral, and unpleasant pictures that had a relatively simple figure-ground composition, or were natural scenes with no central object. The natural scenes were established as having higher spatial frequency, and lower contrast, when compared to the figure-ground images. This study demonstrated that the LPP is primarily sensitive to the emotional intensity of viewed images, rather than image contrast, spatial frequency, or complexity. In addition, the dimensional size of the stimulus does not impact LPP amplitude [De Cesarei & Codispoti 2006], nor does color [Codispoti 2012]. The length of stimulus presentation that is sufficient to produce emotion modulation of the LPP has also been investigated. Codispoti and colleagues (2009) used visual masking to show that roughly 80ms is required to elicit emotional modulation. Then, once this threshold has been met, there is no increase in emotional reactivity as a result of longer stimulus exposure. In contrast to the EPN, the LPP shows slight habituation following repeated picture presentations across blocks, and within blocks, suggesting that the LPP and EPN reflect distinct stages of picture processing [Codispoti 2007]. Yet similar to the EPN, presentation of emotional images in the periphery leads to an absence of emotional modulation of the LPP [De Cesarei 2009]. Further investigations of task and stimulus parameters that impact ERP emotional modulation may shed light on how attention, mood, or self-regulation may impact emotional perception.

The sustained activity of the LPP is thought to reflect increased attentive processing, and may reflect activation of motivational systems in the brain [Bradley 2000]. Early studies of the LPP likened it to an extended P300, which has commonly been used as an index of attention and

postsensory stimulus evaluation [Johnson 1988; Schupp 2003a]. Similar to the EPN, the emotion modulation of the LPP is additive with attentional modulation [Schupp 2007b]. Further, sustained LPP activity may be sensitive to voluntary cognitive control – participants who are instructed to focus on a neutral portion of an unpleasant image show a reduced level of LPP activity [Hajcak 2009].

Neuroimaging studies have suggested that the LPP is primarily generated from the lateral occipital, intraparietal, and inferior temporal visual cortices [Keil 2002; Sabatinelli 2007; Liu 2012; Sabatinelli 2013], prefrontal cortex [Moratti 2011; Liu 2012] and amygdala [Liu 2012]. This is evidence that defensive- and approach-oriented motivational systems may be involved in the underlying LPP activity. Liu and colleagues (2012) used a combined EEG/fMRI paradigm to show differential activity in brain regions reacting to pleasant and unpleasant images. For pleasant images, LPP amplitude correlated with trial-by-trial BOLD activity of the occipitotemporal junction, medial prefrontal cortex, amygdala, and precuneus. For unpleasant images, LPP amplitude correlated with BOLD activity in the ventrolateral prefrontal cortex, insula, and posterior cingulate cortex. This indicates that the widespread cortical regions contribute to the LPP, and that many regions are valence-modulated. Given this, it is plausible that LPP amplitude could vary according to the sensitivity of these regions to other factors. Taken together, these studies suggest that the LPP is affected by the emotional arousal of viewed scenes, and plays a part in sustained attention to the emotional stimuli.

Overall, the EPN and LPP represent distinct aspects of the perceptual process, though they share some commonalities. The EPN is more sensitive to the physical dimensions of stimuli, and less susceptible to habituation – a trait that is thought to be unique to obligatory processes [Codispoti 2007]. It is evident that the EPN can be modulated by attention and

cognitive control, implying that it reflects more than simply bottom-up stimulus processing. One theory suggests that the EPN reflects 'natural selective attention' – where early stimulus processing (150-300ms) tags emotional stimuli so that their processing is prioritized [Schupp 2004a]. In contrast, the LPP is insensitive to the physical dimensions of the stimuli or stimulus duration. Rather, it is primarily responsive to the emotional content of the stimulus with an emphasis on stimulus arousal [Cuthbert 2000]. The LPP is localized further anterior, and appears to recruit widespread brain networks to aid in the perceptual processing of emotional stimuli [Liu 2012; Sabatinelli 2013]. The LPP occurs in a later window (450-900ms), in line with the notion that it is involved in heightened processing of stimuli that have been prioritized, but may also reflect top-down involvement of brain regions in emotion perception. For instance, it may reflect the recruitment or guidance of attentional resources [Schupp 2003a], stimulus evaluation [Schupp 2007b], or activation of the brain's motivational circuits, which prepare the organism for defensive or appetitive action [Bradley 2000].

#### Peripheral physiological indices of emotional arousal

The autonomic nervous system initiates several physiological changes when encountering an arousing stimulus, whether that stimulus is pleasant or unpleasant. For instance, a threatening situation may result in heightened heart rate and respiration [Graham 1966; Gomez 2008]. These physiological changes are thought to be part of an orienting response toward the stimuli, and the mobilization of metabolic and motor resources to ready the organism for action [Fujumura 2013; Gomez 2008]. Presentation of emotionally arousing images has been shown to induce similar category-specific changes in heart rate [Codispoti 2001; Bradley 2001a]. Using fMRI, Critchley & colleagues (2002) showed that changes in heart rate could be predicted by activity in the amygdala, insula, anterior cingulate, and brainstem. Furthermore, the amygdala is known to

project directly to the dorsal vagal nucleus, which is known to innervate portions of the lungs [Veening 1984].

Interestingly, several studies indicate that heart rate and respiration work in concert during physiological responses to emotional stimuli. In particular, an initial heart rate deceleration occurs when we attend to stimuli and visually process them [Lacey & Lacey 1970]. This deceleration is thought to co-occur with extended exhalation while orienting to the stimulus [Eckberg 1983; Graham 1966], and is modulated by the emotional content of the stimulus [Bradley 2001a; Codispoti 2001]. The deceleration phase is followed by an acceleration of heart rate that occurs with inspiration [Eckberg 1983], and is accompanied by an increase in the overall volume of ventilation per minute [Gomez 2008]. These physiological changes prepare the organism to respond to salient stimuli in the environment.

Skin conductance responses (SCRs) are elicited on the palms and soles during emotional stimulation. SCRs are generated from regulation of sweat gland activity by the autonomic nervous system. This regulation results in measurable potential differences in the conductance of applied current on the skin's surface [Critchley 2002]. Typical SCRs peak between 1-4s after stimulus presentation and diminish by 10s after stimulus presentation [Boucsein 2012]. Skin conductance reactions to emotional stimuli are thought to reflect a variable level of emotional reactivity. Highly arousing stimuli are thought to activate a strong sympathetic SCR component, which causes abruptly heightened electrodermal responses [Bradley 2001a].

Both central and peripheral measures are able to distinguish between emotional and non-emotional, yet there are distinct systems involved in each emotional index. Each measure

provides useful information about which physiological systems may be susceptible to experimental manipulation.

### ERP evidence for cue-related preparatory activation

Warning cues are able to elicit changes in ERP amplitude, as well as brain activity. It is unclear whether participants form expectations regarding upcoming stimuli, and whether this supposed foreknowledge could bias ERP reactivity. Psychophysiological studies of emotional perception often use a majority of arousing stimuli, compared to neutral stimuli. If participants learn that the majority of scenes are evocative, they may begin to anticipate the content of the upcoming image, and this anticipatory behavior may modulate reactivity at stimulus onset. This preparatory behavior may occur via explicit or implicit top-down modulation of the visual system, and may affect emotion-modulated ERP components. The following studies shed light on the possibility that anticipation and expectations of the future may mediate perception.

A well-studied slow-wave ERP known as the contingent negative variation (CNV) occurs between the presentation of a fixed-foreperiod warning cue and a target stimulus. The CNV can be thought of as a measure of non-specific preparation, reflecting a general preparedness for action in response to an anticipated event [Los 2005]. The CNV has two main components, an early orienting positive frontal component that appears in response to the warning cue, and a late, centro-parietal negative shift that occurs just prior to a predictable time-delayed target stimulus [Rohrbaugh 1976]. The latter portion of the CNV was initially thought to reflect the 'readiness potential', a measure of activity in the motor cortex leading up to muscle movement [Rohrbaugh 1983]. However, subsequent work has established that the late CNV has a non-motor component that can be observed in the absence of overt movement [Brunia 1988].

Several studies have observed the late CNV in response to forewarned erotic images [Simons, Öhman, & Lang 1979], negatively affective images [Klorman & Ryan 1980; Peng 2012], and expectation of reward [Fuentemilla 2013; Carlson 2011]. Neuroimaging research has extended our knowledge of the brain regions involved in cue-related anticipatory activation. Woldorff and colleagues (2007) found evidence of preparatory brain activity with a combined EEG/fMRI study using a spatial attention task using visually cues. After presenting participants with a directional cue, a target was shown in the corresponding visual field. This task led to enhanced post-cue (pre-target) BOLD signal in the medial frontal lobe, which was followed shortly thereafter by activity in the medial parietal lobe. When looking at the ERP data, the researchers found heightened activation of the contralateral primary visual cortex. These data suggest that expectation processes can cause a specific task-related preparatory bias in visual cortices.

Taken together, these studies show that the late CNV reflects anticipation of motivationally relevant upcoming stimuli. While the motivational content of the stimulus affects the amplitude of the late CNV, it is unclear whether this type of non-specific preparation could extend past the stimulus presentation and affect post-stimulus ERPs. Furthermore, CNV studies rely on using fixed inter-trial intervals, which is not common practice in most studies of emotional perception. The extent to which preparatory activity exists in the absence of explicit cues and its potential impact on emotional scene perception has not been studied.

#### Expectation bias through statistical learning

In humans, there are several studies that suggest that learning occurs during stimulus presentation, which may induce anticipatory processes. Two neuroimaging studies demonstrate

that perceptual anticipation can occur through the implicit recognition of patterns within the stimulus set presentation.

Turk-Browne and colleagues (2010) asked participants to discriminate between faces and scenes using a design in which face stimuli were paired with scenes, presented in succession. When comparing the first stimuli in paired sets to unpaired stimuli, an elevation in hippocampal BOLD response was found, suggesting its involvement in the processing of anticipated stimuli. This activity was negatively correlated with activity in primary visual cortex and positively correlated with inferior parietal lobule activity. This suggests that participants implicitly learn to anticipate stimuli by recognizing regularities in the experimental procedure, and modulate activity in subcortical and visual cortical structures as a result of learning.

In the second study, Altamura and colleagues (2014) used magnetoencephalography (MEG) to examine implicit statistical learning using a size discrimination task, similar to the Stroop task. Participants viewed side-by-side images of animals and were asked to report which animal (left or right) was larger in real life. For this experiment, the proportion of larger size-congruent animals appearing on the left was skewed to make up 60% of the overall trials. The authors predicted that modulation of brain regions associated with the proportionally biased trial type would be reflected by neural synchronization during peri-stimulus presentation. Analysis of the first quarter of trials using a -500ms to 0ms window revealed higher alpha event-related desynchronization in the anterior cingulate, caudate, middle temporal gyrus, and inferior frontal gyrus, which was interpreted to be associated with anticipation of motor events. Additionally, in the third and fourth quarters of trials, elevated alpha event-related synchrony in the occipitotemporal cortex and putamen were observed between -400ms and 0ms, and interpreted to reflect the perceptual anticipation of future stimuli. This design is distinct from typical statistical

learning studies in that regularities in the ordering of stimuli are not the property learned during stimulus presentation. Instead, it showed that humans may ascertain knowledge of imbalances in the overall frequency of experimental stimuli, and that this may lead to preparatory activation in the cortex as a result.

### Experimental approach

The prioritized perception of emotional stimuli is reflected in cortical, and autonomic measures. Electrophysiological techniques allow us to capture the rapid discrimination of emotional stimuli by the EPN (~150-300ms) and LPP (~400-900ms). These brain potentials reflect engagement of the primary and extended visual cortex, and may be regulated by limbic, and frontal regions [Sabatinelli 2007; Sabatinelli 2013; Liu 2012]. Heart rate and skin conductance have also been shown to distinguish between emotional and non-emotional stimuli. These reactions are delayed, reflecting the engagement of the autonomic nervous system, and are likely to be generated and regulated by many cortical regions [Critchley 2002; Tranel 1994; Nagai 2004]. Anticipatory processes may also regulate these measures of emotional modulation. Previous work has shown that explicit cues are able to generate preparatory activity reflected by ERP indices [Rohrbaugh 1976] as well as BOLD activation the visual cortex [Woldorff 2007]. Furthermore, participants are able to make unconscious associations between stimuli, which can modulate the activity of the visual cortices [Turke-Browne 2010]. Moreover, the use of stimulus proportionality as an implicitly manipulated variable can induce pre-stimulus desynchronization in the occipitotemporal cortex and putamen [Altamura 2014].

The primary aim of this work was to determine whether stimulus set proportions affects the emotional modulation of the EPN, LPP, heart rate, and skin conductance. In one study, we used a 50/50 ratio of arousing to neutral stimuli, and in the second we used a 66/33 ratio. As

participants become familiar with the images in study two, they may implicitly recognize that the occurrence of highly arousing content is greater than the occurrence of neutral images. This may affect processes involved in emotional perception. If emotional modulation of ERP and autonomic measures is sensitive to the stimulus set proportions, it would warrant revisiting the results garnered from prior experimentation. In contrast, if the emotional modulation of these measures is not sensitive to stimulus set proportions, we can continue to examine emotional perception without needing to balance the design with respect to stimulus proportions.

A secondary aim of this study is to establish the validity of a new picture set for use in further research, and characterize any relationships between sex, presentation block, valence & arousal ratings with electrophysiological and peripheral measures of emotion-modulated reactivity. Knowledge of the sensitivity of these responses to non-affective parametric manipulations will advance our understanding of the perceptual process, and aid in the design of experiments with higher validity.

## CHAPTER 3

### METHODS

#### Experimental design

Undergraduate psychology students at the University of Georgia were randomly assigned to one of two studies, and rewarded with course credit for their participation. In study 1, a gender-balanced group of 72 participants (36 male, 36 female; age:  $M=19.5$ ;  $SD=3.4$ ) viewed a set of 168 scenes including 84 emotional and 84 neutral stimuli. In study 2, a gender-balanced group of 72 participants (36 male, 36 female; age:  $M=19.7$ ;  $SD=2.5$ ) viewed a set of 126 scenes including 84 emotional and 42 neutral stimuli. The stimulus set from Study 1 included eight image categories: erotic couples, happy animals, neutral people, neutral animals, neutral faces, neutral textures, threatening people, and threatening animals. The stimulus set from Study 2 was derived from the stimulus set from Study 1, with neutral faces and textures removed.

#### Stimulus description

The image categories from Study 2 were chosen so that we would have one set of positive, neutral, and negative valence images for both humans and animals to compare between studies. In order to increase the proportion of neutral pictures in Study 1, we included neutral faces and neutral textures. The neutral texture category contains patterns that can be found in natural and manmade environments (e.g. a brick wall, the skin of a salamander). Images for the neutral faces category were created by cropping the peripheral features of previously used experimental face images to yield an oblong representation of each face. In order to maintain a

sufficient level of image complexity, these representations were superimposed on a random neutral texture background (Appendix A contains thumbnails of the experimental scene stimuli).

Images for each category were gathered from Internet databases, web searches, and the International Affective Picture Set (IAPS; Lang 2008). Several thousand un-copyrighted photographs were collected according to these criteria: 1024x768 pixel size, landscape orientation, scene content, and visual complexity. From these images, 168 (21 per category) were chosen for the experiment. Furthermore, scenes with a central focus were selected in order to minimize scanning eye movements. A diverse set of exemplars for each category was selected (e.g. no more than 2 of any species were included in animal categories), and each category was then balanced to be statistically equivalent in terms of luminance and complexity (t-test;  $p > 0.20$ ). The average brightness per pixel on a scale of 1-255 as determined by the GNU Image Manipulation Program (GIMP, <http://www.gimp.org/>) was used to measure luminance. Images that were outliers in terms of luminance were adjusted by a uniform increase or decrease in brightness using GIMP. Complexity was measured by 90% quality joint photographic experts group (JPEG) file size. Contrary to luminance, complexity cannot be increased for an image – only reduced. Thus, exceedingly low JPEG complexity was used as a criterion for excluding images. To balance complexity between categories, outlier images with high complexity were decreased through application of a 1x1 or 3x3 pixel run-length encoding Gaussian blur, which has a negligible effect on perceived scene sharpness.

### Experimental procedure

All volunteers consented to participate after being read a description of the study. Each image viewing session was conducted in a small, sound-attenuated room with ambient light. Volunteers were seated in a plastic chair and viewed a 32-inch monitor from 60 inches away,

yielding a viewing angle of 26°. Participants were instructed to fixate on a central point during three 10-13 minute image-viewing blocks. To minimize signal noise from eye movements and muscle tension, participants were instructed to remain relaxed and keep blinking to a minimum. Then, the participants were shown three blocks of randomized, category-balanced images. Images within each block were pseudo-random; randomly ordered images from each category (6 or 8 scenes) were generated so that each category would be distributed evenly across the block, and that no more than 2 successive presentations of a stimulus category occurred. Custom Matlab code was used to present each image for two seconds, with a variable inter-trial interval of 10-14 seconds. Between blocks, volunteers were allowed a short break or glass of water upon request. Additionally, EEG channels that began to show noisy signal during the picture-viewing block were refilled, and any disconnected external electrodes were reapplied. After the third block, participants provided arousal and pleasantness ratings for each viewed image using the Self-Assessment Mannikin (SAM; Bradley & Lang 1994). After the participant finished, they were debriefed about the purpose of the experiment.

#### Data collection

Electrocortical activity was sampled at 512Hz using a 64 channel ActiveTwo EEG system (Biosemi Instrumentations; Amsterdam, Netherlands) and SignaGel electrode gel (Parker Laboratories). Skin conductance was measured by placing auxiliary Ag/AgCl electrodes on the hypothenar eminence of the subject's left hand. Electrocardiographic (ECG) data were recorded by placing auxiliary Ag/AgCl electrodes on the volunteer's forearms. Data quality was monitored during acquisition using Biosemi's ActiView software. Stimulus presentation was controlled by a Macintosh workstation running Psychtoolbox [Brainard 1997], a Matlab-based open source software package.

Participants were instructed to look through a binder containing all of the images viewed during the experiment and provide ratings of pleasantness and arousal on a scale of 1-9 using pencil and paper. Images in the binder were presented in a unique pseudo-random order that was generated in a similar manner to the picture-viewing portion of the experiment.

#### Data reduction

Event-related potential data were reduced using the Electro-magneto-encephalography-software [EMEGS; Peyk & Junghofer 2011]. The data were lowpass filtered (18th order Butterworth, pass frequency=30Hz, attenuation=3dB; stop frequency=40Hz, attenuation=45dB) and highpass filtered (4<sup>th</sup> order Butterworth, stop frequency=0.05Hz, attenuation=1dB; pass frequency=0.1Hz, attenuation=18dB). ERP trial data were extracted using a window of 200ms prior to picture onset and 1000ms after picture onset, and baseline corrected to a 100ms pre-stimulus window. Trials containing artifacts were detected and removed from the analysis using EMEGS, and data from bad channels were removed and interpolated from surrounding channels using spherical spline interpolation.

Ratings for pleasantness and arousal were rescaled to yield values of 0-8, where a rating of 8 indicates maximal pleasantness or arousal. These ratings were averaged for each picture to assess whether any images were outliers. One picture from each category was removed based on ratings (data not shown). For example, a relatively arousing ‘neutral face’ image and a relatively pleasantly rated ‘threatening animal’ image were excluded from the analysis. Rating data for one participant were excluded from the analysis for technical reasons. Accordingly, ERP trial data associated with incomplete ratings were excluded from analyses of trial data.

Skin conductance was obtained in Ohm units using an ActiveTwo system with a 512Hz coupler. These data were converted to microsiemens ( $\mu\text{S}$ ) by taking the reciprocal, and

multiplying by one million. Next, the signal was lowpass filtered (1<sup>st</sup> order Butterworth cutoff=5Hz) and highpass filtered (1<sup>st</sup> order Butterworth; cutoff =0.0159Hz) using Matlab's digital filter '*filtfilt*'; these specifications were designed to correspond to an RC circuit time constant of 10s [Bach 2009]. Data for each trial was extracted using a window of 2 seconds prior to stimulus onset to 11 seconds after. These data were deviated from a half-second pre-stimulus baseline and then averaged into 250ms bins. Participants with excessive artifacts were excluded (14). Data reduction and analyses were performed in Matlab using the open-source program EEGLAB, and custom-made code.

Heart rate QRS complexes were identified using an algorithm based on Pan & Tompkin (1985). Data for each trial was extracted using a window of 4 seconds prior to stimulus onset to 13 seconds after. The calculated inter-beat-intervals were then used to calculate beats per minute (BPM) for 500ms bins, according to the method described in Graham (1978). Trial waveforms were then deviated from a one second pre-stimulus average, yielding change in heart rate over time in BPM. Criteria for rejecting trials were used in order to minimize the impact of transient artifacts on the heart rate data. Trials identified as having one or more bins with heart rate exceeding 120 BPM or three bins with lower than 40 BPM were excluded. This resulted in an average of 10.1 (+/- 20.5 std) trials being excluded per subject.

#### Data analysis

The trial data for all subjects were averaged by category and valence to obtain waveforms of ERP components across the scalp. Next, channels exhibiting characteristic early posterior negativity (P7, P8, P9, P10, PO7, PO8) and late positive potentials (Pz, CPz, CP1, CP2, C1, C2) were identified. A single measure of EPN and LPP per timeseries was calculated by averaging ERP amplitude within a post-stimulus window (EPN: 150-300ms, LPP: 450-900ms), and across

the six channels identified for their respective ERP. Topography of the EPN and LPP, and example waveforms can be found in Appendix B. Then each participant's trial ERP measures were averaged according to their block and category. These data were analyzed using four-way ANOVAs [sex, study, block, category] using the statistical program 'R'. Post-hoc Tukey tests were employed to analyze group differences for main effects and interactions discovered. In a separate sub-analysis, participant's trial ERP measures were compared to pictures ratings on a picture-by-picture basis.

Ratings data were used to create category averages for each participant. These values were analyzed using a three-way ANOVA [study, sex, category], with post-hoc Tukey tests examining main effects and interactions. We also investigated whether the self-reported rating of picture categories correlates to LPP amplitude. For this analysis, participants were separated into quartiles based on how highly they rated pictures on the pleasantness scale. LPP measures were calculated for each participant by averaging trial data for each category. These data were then combined for each quartile of participants, and compared against rated pleasantness to determine whether these factors are related.

Skin conductance data for each participant was averaged according to category, resulting in six waveforms for each participant. These waveforms were averaged across participants to generate time courses for categories within each study (Figure 17). Additionally, single values that represent the magnitude of the skin conductance response were calculated from these waveforms. An average of the 2-6s post-stimulus window yielded a single skin conductance score per category for each subject. These values were analyzed using a three-way ANOVA including study, sex, and category.

Heart rate data for each participant was averaged according to category, resulting in six waveforms for each participant. These waveforms were averaged across participants to generate time courses for categories within each study (Figure 18). Similar to skin conductance, single values representing the heart rate response were calculated using category waveforms. An average of the 1-3s post-stimulus window yielded a single heart rate value per category for each subject. This time window captured the deceleration phase of the heart rate response.

## CHAPTER 4

### RESULTS

#### Electrophysiological results: LPP & EPN

A four-way ANOVA was conducted to compare the effect of study, sex, picture category, and block on LPP amplitude. An identical ANOVA investigated the effect of these factors on EPN amplitude. Three-way ANOVAs examined the effect of study, sex, and category on picture ratings.

These data emphasize the consistent, replicable modulation of the LPP by category and sex. A main effect of Category on LPP amplitude,  $F(5,2515) = 174.31, p < .001$ , was found. A post-hoc Tukey test determined that all between-group comparisons were significant ( $p < .05$ ), except for between-group comparisons of animal threat, human threat, and happy animals (Figure 1, right).

ANOVA results indicate modulation of EPN amplitude by category and sex. A main effect of Category on EPN amplitude,  $F(5,2515) = 35.87, p < .001$ , was found. A post-hoc Tukey test determined that all between-group comparisons were significant ( $p < .05$ ), except for between-group comparisons of animal threat, human threat, and neutral animals (Figure 1, left).

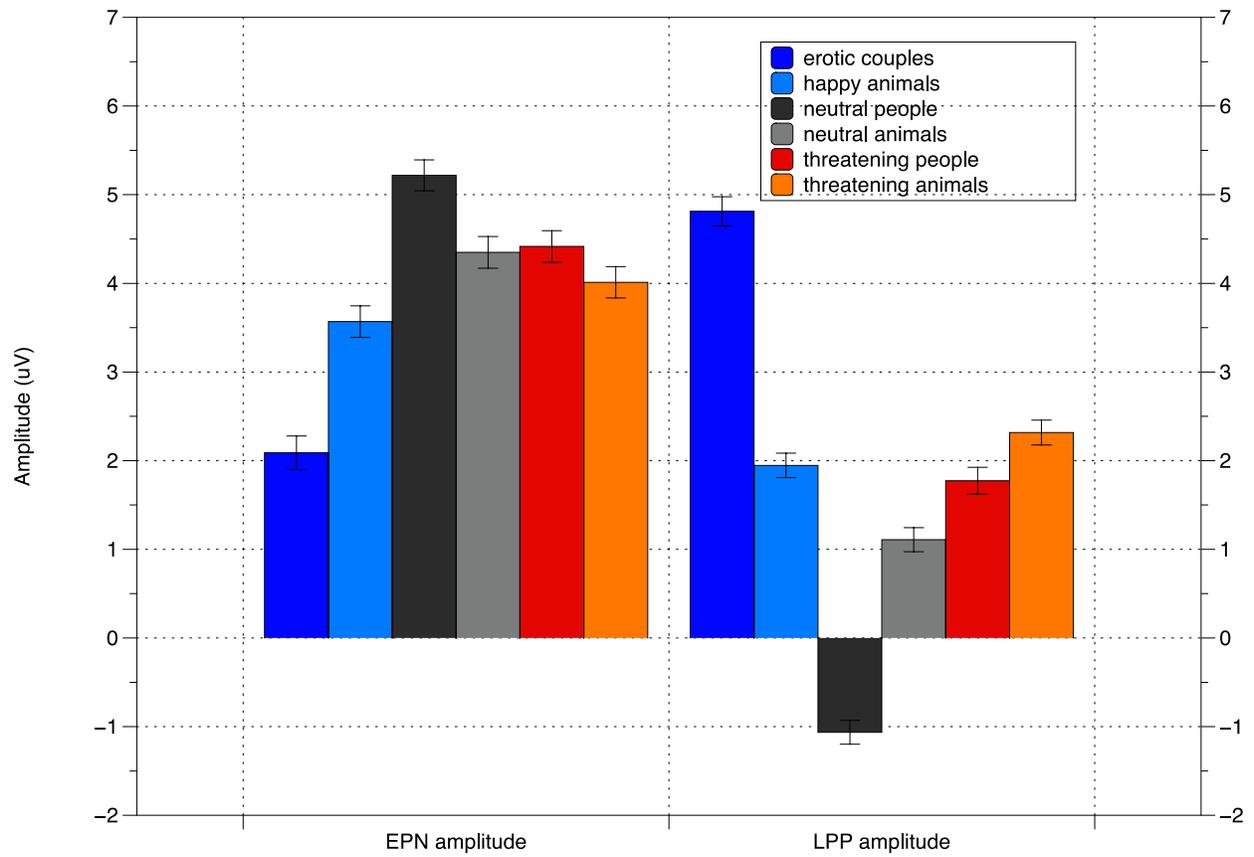


Figure 1. Experiment-wide EPN and LPP amplitude. Average event-related potential ( $\mu\text{V}$ ) for each image category.

A significant main effect of sex was found,  $F(1,2515) = 16.00$ ,  $p < .001$ , indicating that women have higher overall LPP amplitude in response to viewed images for most categories (Figure 2). Additionally, these data also show a main effect of block on LPP amplitude [ $F(2,2515) = 9.62$ ,  $p < .001$ ].

As Figure 3 illustrates, we did not find a main effect of Study, or a Study x Category interaction for LPP amplitude.

However, overall LPP amplitude in block one is reduced relative to blocks two and three (Tukey test;  $p < .01$ ). We did not find a Block x Category interaction (Figure 4).

However, we did find a Study x Sex interaction,  $F(1,2515) = 11.57$ ,  $p < .001$  in LPP amplitude. Post-hoc Tukey tests (Figure 5) indicate that men had lower overall LPP amplitude in Study 1 when compared to women from Study 1, and both sexes from Study 2 ( $p < .05$ ).

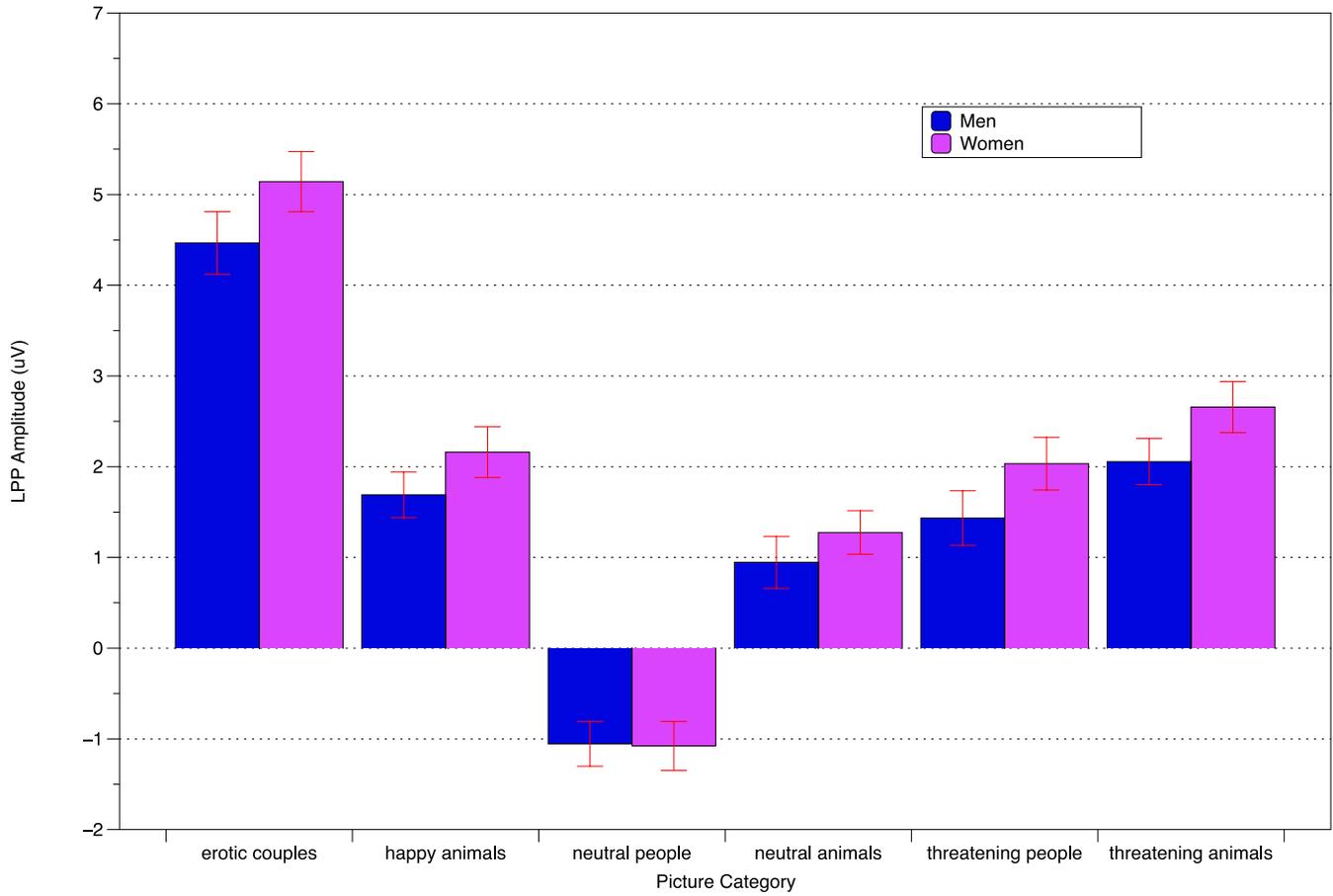


Figure 2. LPP amplitude by sex. Average event-related potential ( $\mu\text{V}$ ) for each image category, separated by sex.

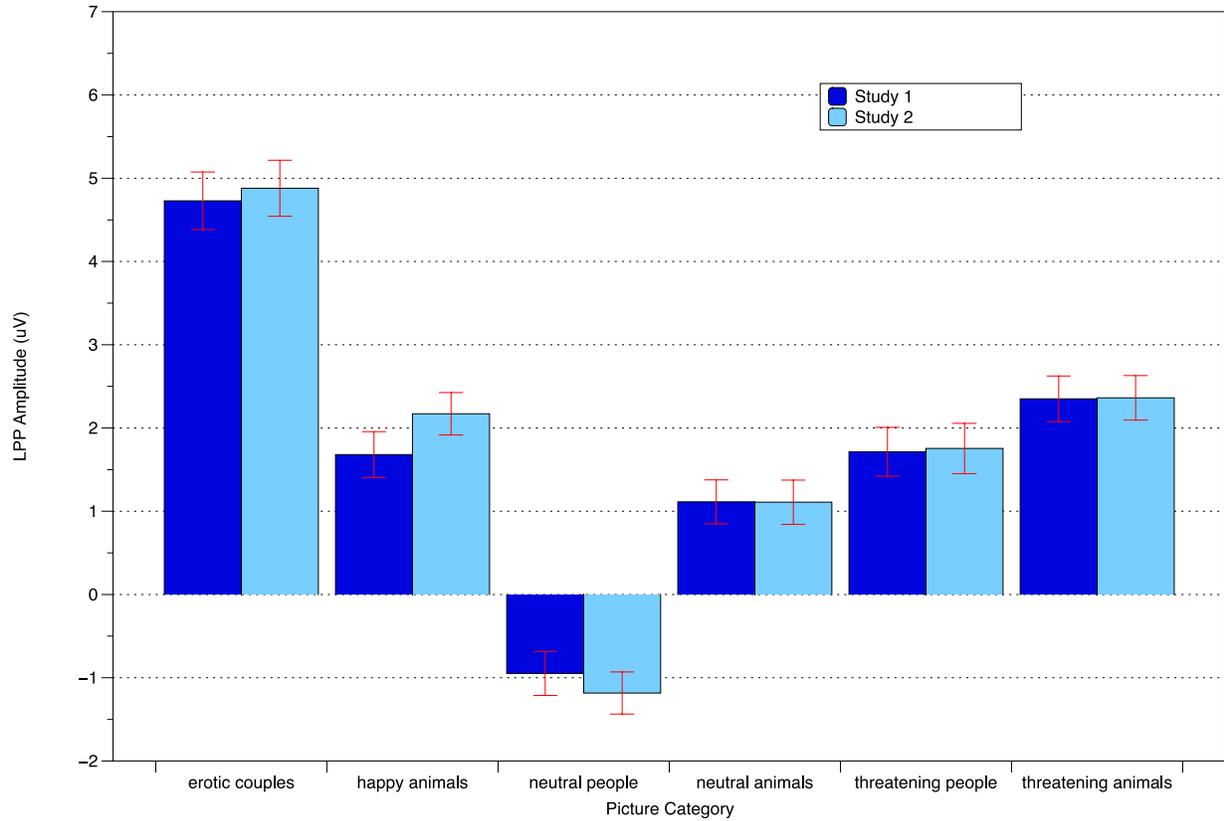


Figure 3. LPP amplitude by study. Average event-related potential ( $\mu\text{V}$ ) for each image category separated by study.

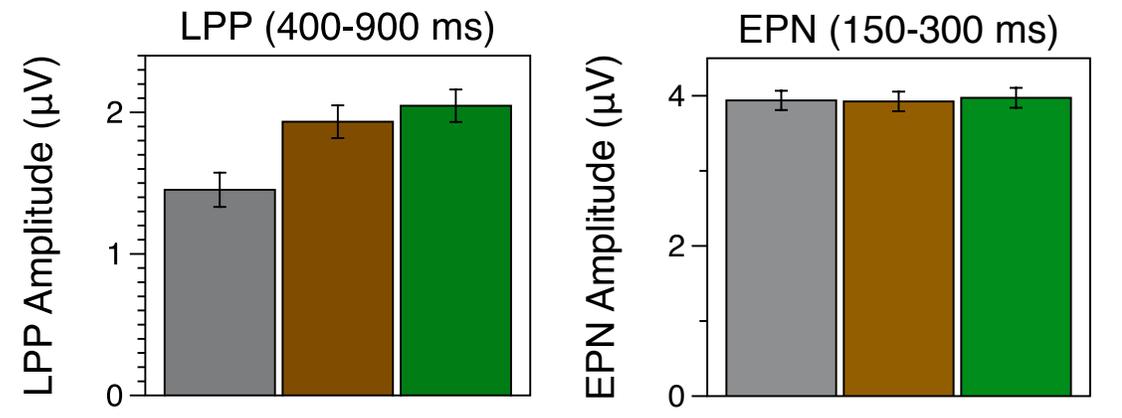


Figure 4. Experiment-wide LPP and EPN amplitudes by block. Post-hoc Tukey tests show that the LPP amplitude for block one is lower than blocks two and three ( $p < .01$ ).

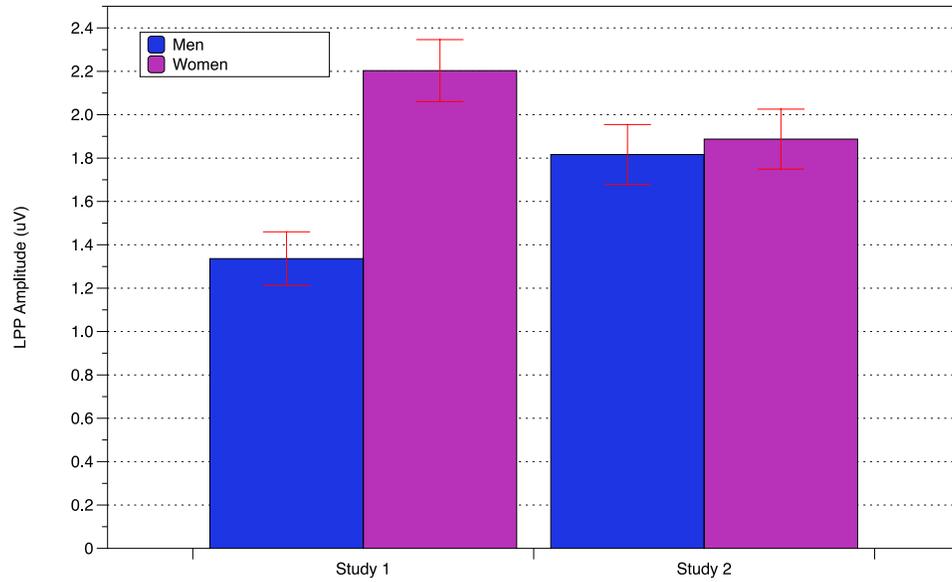


Figure 5. LPP amplitude for Study x Sex interaction. A Tukey test of mean amplitudes ( $\mu\text{V}$ ) indicates that women had a elevated LPP amplitude in Study 1 when compared to men ( $p < .001$ ). Men's LPP amplitude was lower in Study 1 compared to both sexes in Study 2 ( $p < .05$ ).

A significant main effect of sex was found on EPN amplitude,  $F(1,2515) = 97.85$ ,  $p < .001$ , indicating that women showed greater amplitude in response to all categories of images (Figure 6). There was no effect of block on EPN amplitude.

We did not find a main effect of Study, or a Study x Category interaction on EPN amplitude (Figure 7).

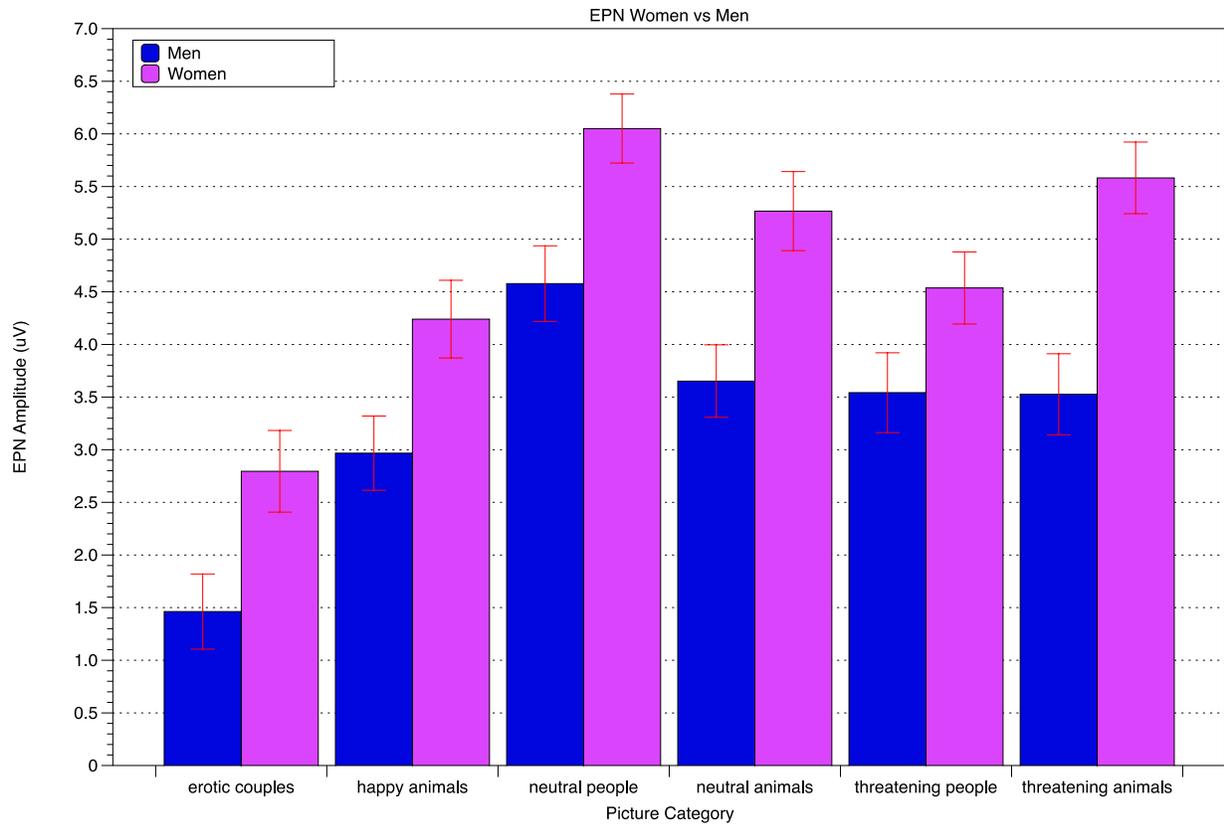


Figure 6. EPN amplitude by sex. Average event-related potential ( $\mu\text{V}$ ) for each image category, separated by sex.

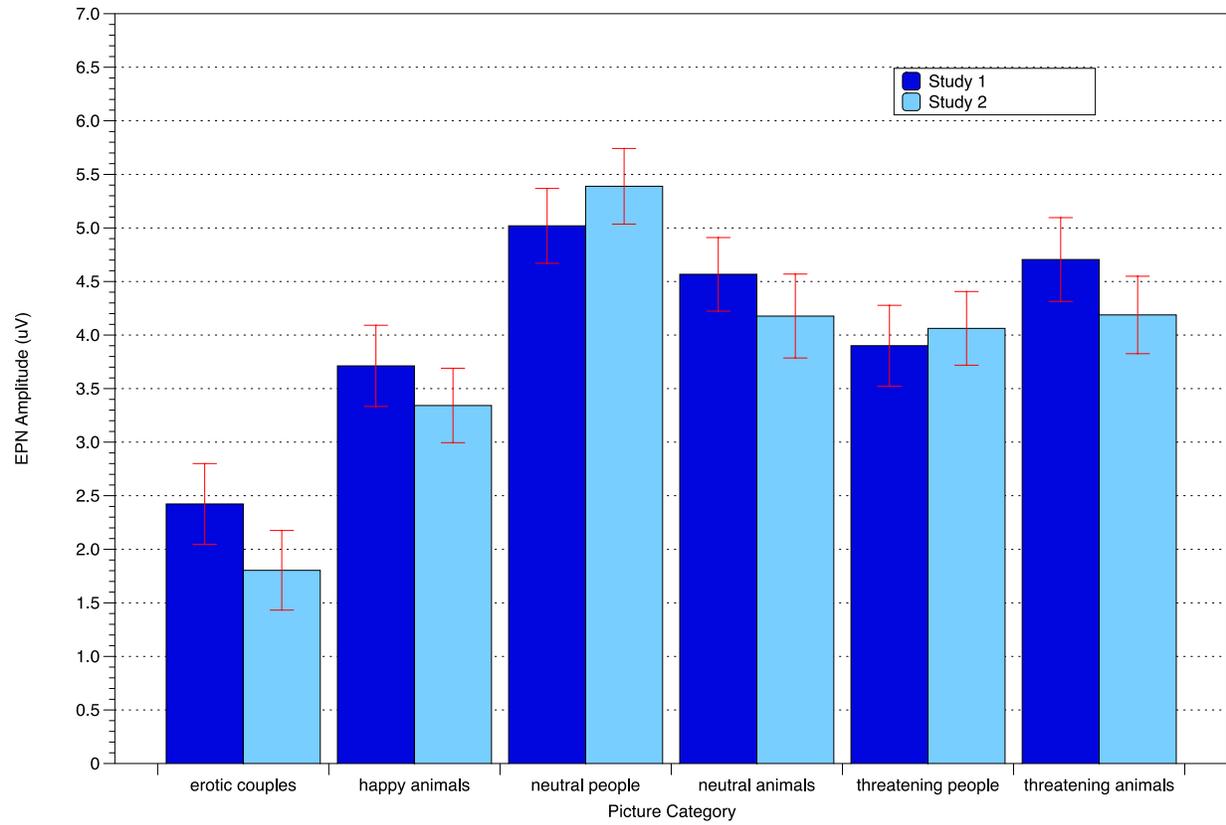


Figure 7. EPN amplitude by study. Average event-related potential ( $\mu\text{V}$ ) for each image category, separated by study.

### Supplementary ERP analyses

LPP amplitude for each category across blocks is displayed in Figure 8. We examined within-block trial data for Study 1 (Figure 9) and Study 2 (Figure 10) to determine if there were linear trends contributing to the main effect of block on LPP amplitude. In these figures, pictures from each category are arranged by picture presentation order, and fit with a linear line for each block. For some categories, weak to moderate correlations ( $0.3 < r < 0.5$ ) can be seen between LPP amplitude and presentation order. Furthermore, these linear trends appear to be fairly consistent across study, as can be seen in Figures 9 and 10.

We also compared the EPN and LPP amplitudes for each picture stimulus, and found that the two measures are correlated ( $r = -0.68$ ; Figure 11). These results are similar to previous work [Sabatinelli 2013].

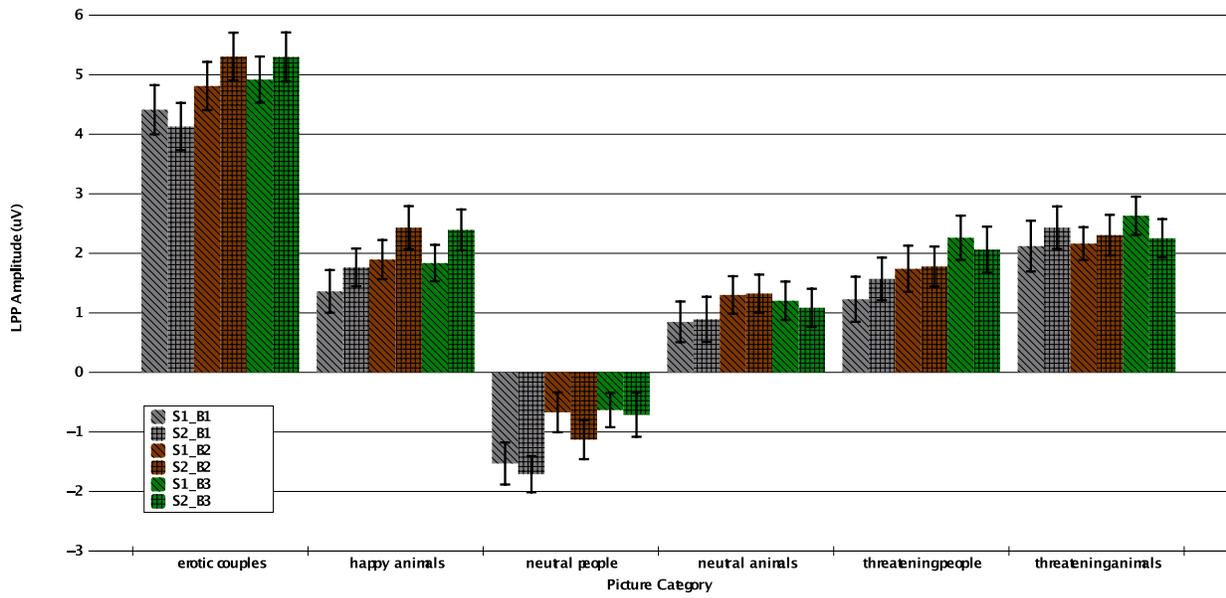


Figure 8. LPP amplitude by study and block. Average event-related potential ( $\mu\text{V}$ ) for each image category, separated by study and block.

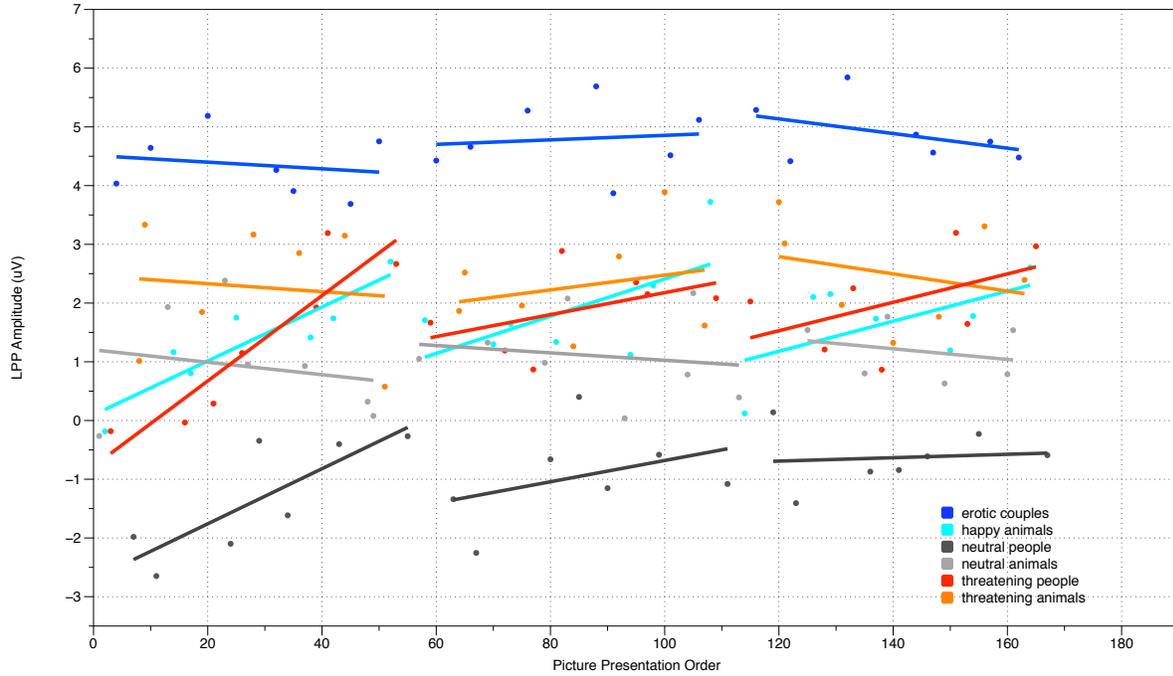


Figure 9. LPP amplitude by picture presentation order for Study 1. Average LPP amplitude ( $\mu\text{V}$ ) for each ordered trial, with a linear fit for trials for each category, within blocks.

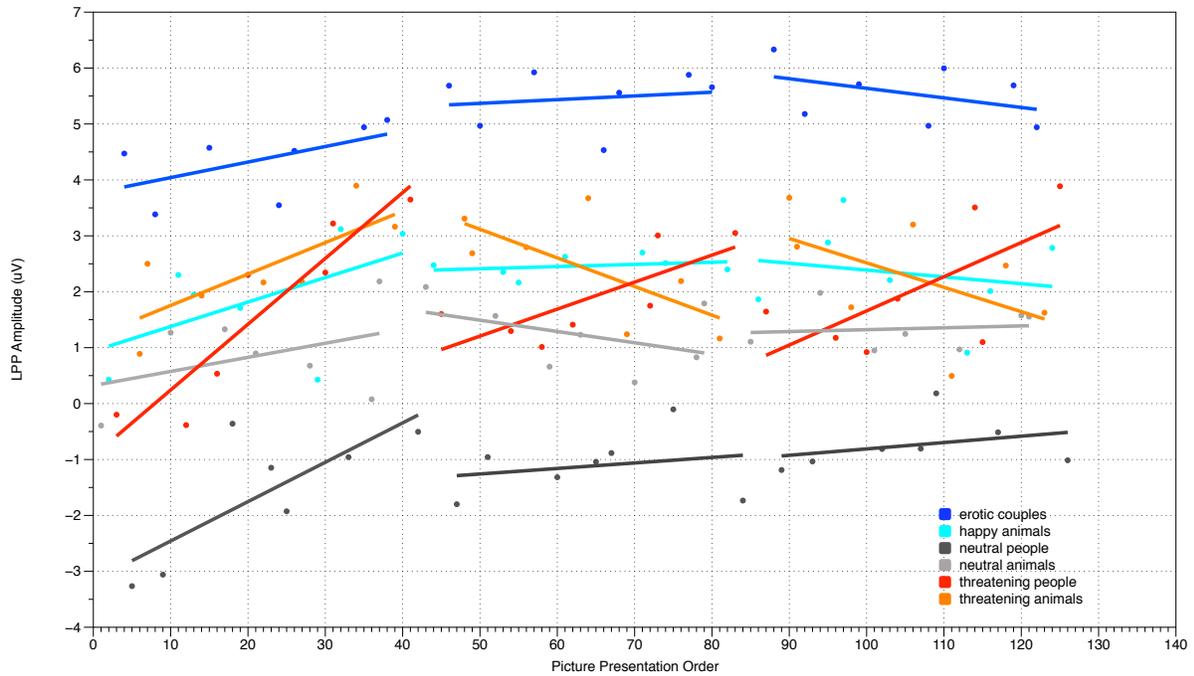


Figure 10. LPP amplitude by picture presentation order for Study 2. Average LPP amplitude ( $\mu\text{V}$ ) for each ordered trial, with a linear fit for trials for each category, within blocks.

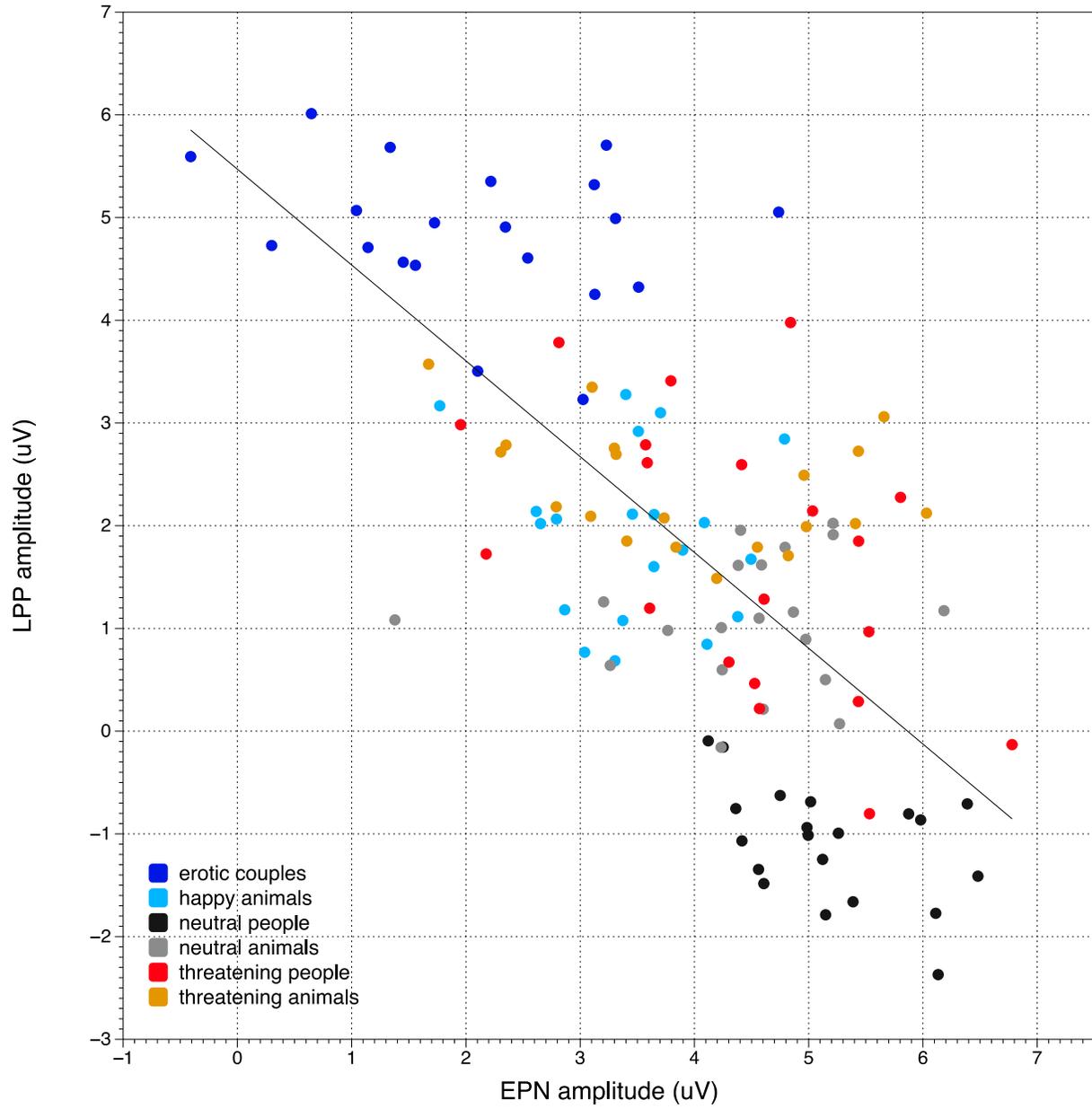


Figure 11. Correlation of EPN and LPP amplitude. Each colored dot represents an image. A linear fit indicates a strong correlation ( $r = -0.68$ , R-squared = 0.46).

### Scene ratings

A main effect of category on pleasantness rating [ $F(5,834)=383.641, p<0.05$ ] indicates that some scene categories were more pleasant than others, as expected. A main effect of Study on pleasantness rating [ $F(1,834)=4.234, p<0.05$ ] was found. Further, a Study x Category interaction indicates that erotica images were rated as more pleasant in Study 1 compared to Study 2 [Tukey test;  $p<0.01$ ]. A Sex x Category interaction [ $F(5,834)=31.34, p<0.05$ ] was also found. Post-hoc Tukey tests reveal this as a higher pleasantness rating for erotica by men, compared to women (Figure 12).

A main effect of category on arousal rating [ $F(5,834)=142.027, p<0.05$ ] indicates that some scene categories were more arousing than others, as expected. A Sex x Category interaction [ $F(5,834)=8.561, p<0.05$ ] was also found. Post-hoc Tukey tests ( $p<0.01$ ) indicate that men rated the erotica category higher than women, and women rated the happy animal category higher than men (Figure 13).

The ratings data indicate that women find erotic couples less pleasant than men. However, the variance in ratings for the erotic couples category is greater than the other categories for both genders. By examining this more closely, we found that the average rating for erotic images spanned the full range of the pleasantness scale, depending on the participant. This raises the question of whether the brain response to erotic couples is consistent within each sex, or whether it varies by rated pleasantness.

To assess this, we separated the participants into quartiles based on their average pleasantness ratings for each category. Then, we examined whether the LPP amplitude exhibited a decreasing or increasing trend based on pleasantness rating for each category. Figure 14 shows

the relationship between LPP amplitude and rated pleasantness of all participants. This analysis did not yield any clear trends.

Since men and women in this experiment rated scenes different according to category, we also examined these data stratified by sex. Figure 15 shows the relationship between LPP amplitude and rated pleasantness of women, in quartiles. For erotica and threatening people, there is a decrease in LPP amplitude as the pleasantness rating increases. Figure 16 shows the relationship between LPP amplitude and rated pleasantness of men, in quartiles.

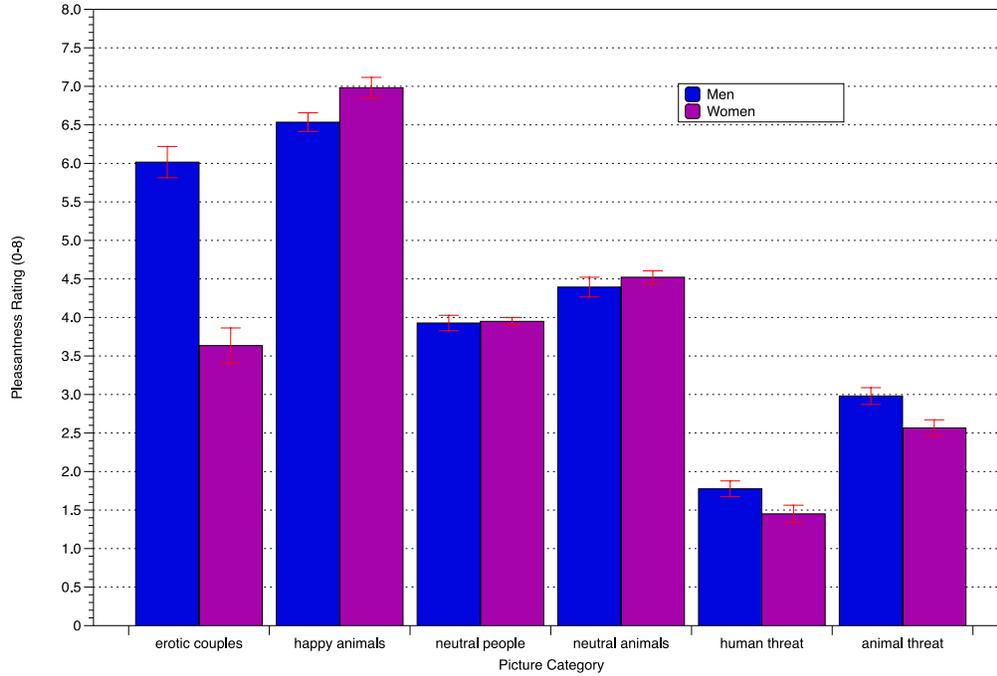


Figure 12. Scene ratings for pleasantness by sex. Average experiment-wide rated pleasantness for images from each category, separated by sex.

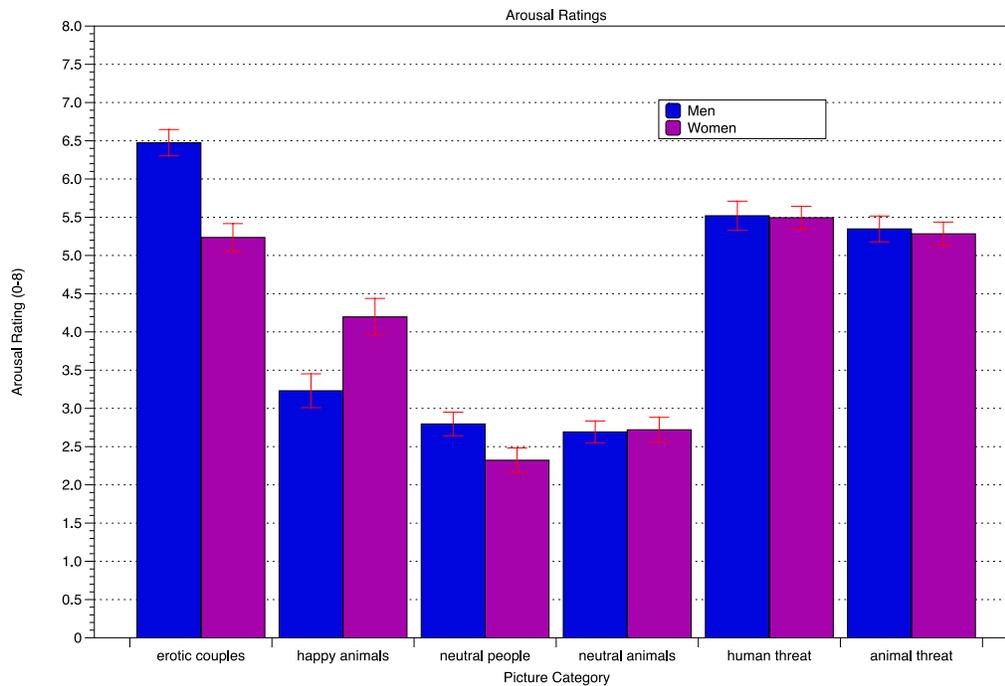


Figure 13. Scene ratings for arousal by sex. Average experiment-wide rated pleasantness for images from each category, separated by sex.

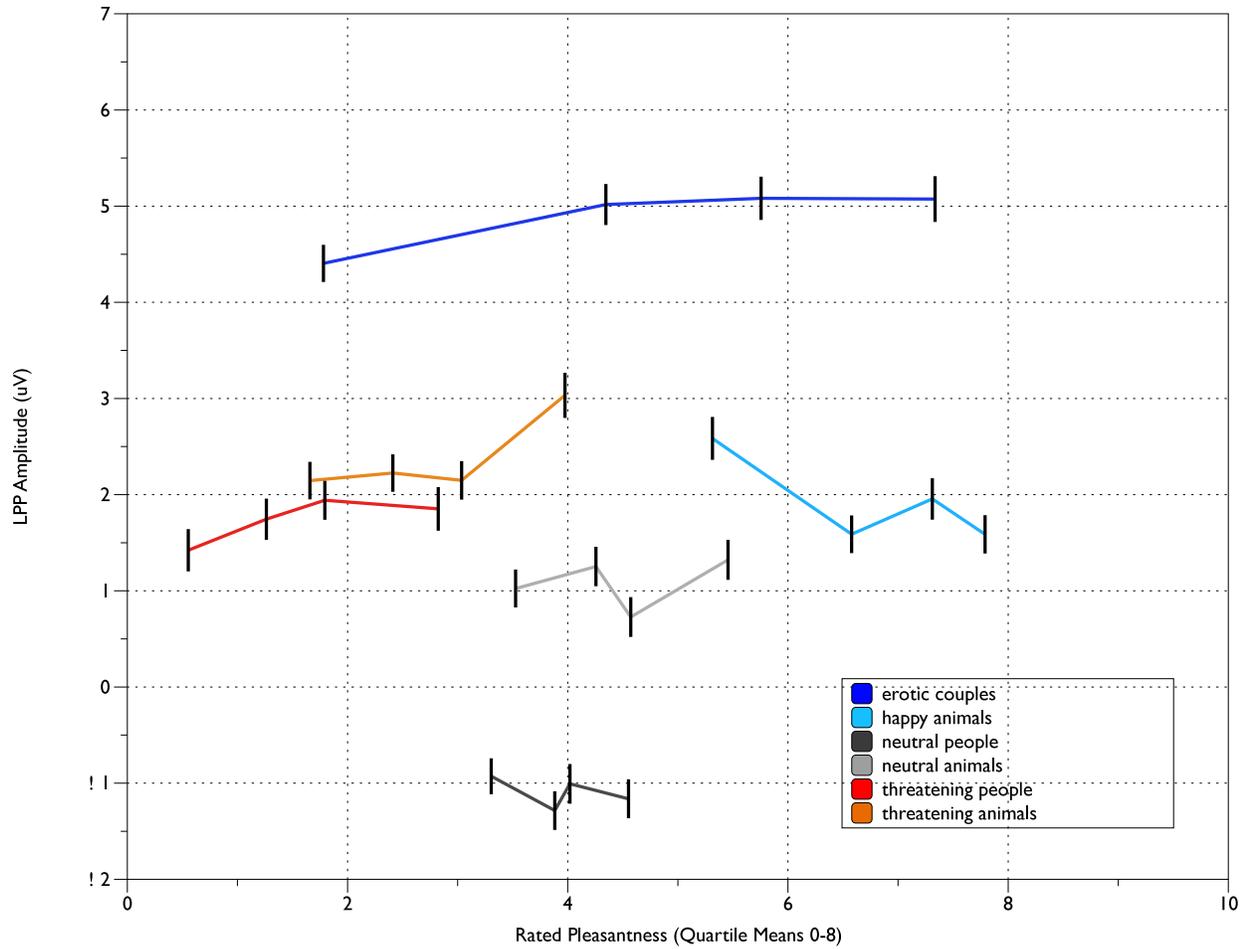


Figure 14. LPP amplitude by rated pleasantness quartile. Average experiment-wide LPP amplitude ( $\mu\text{V}$ ) for all participants, separated into quartiles according to each participant's average rated pleasantness for image categories. Error bars represent standard error of the mean.

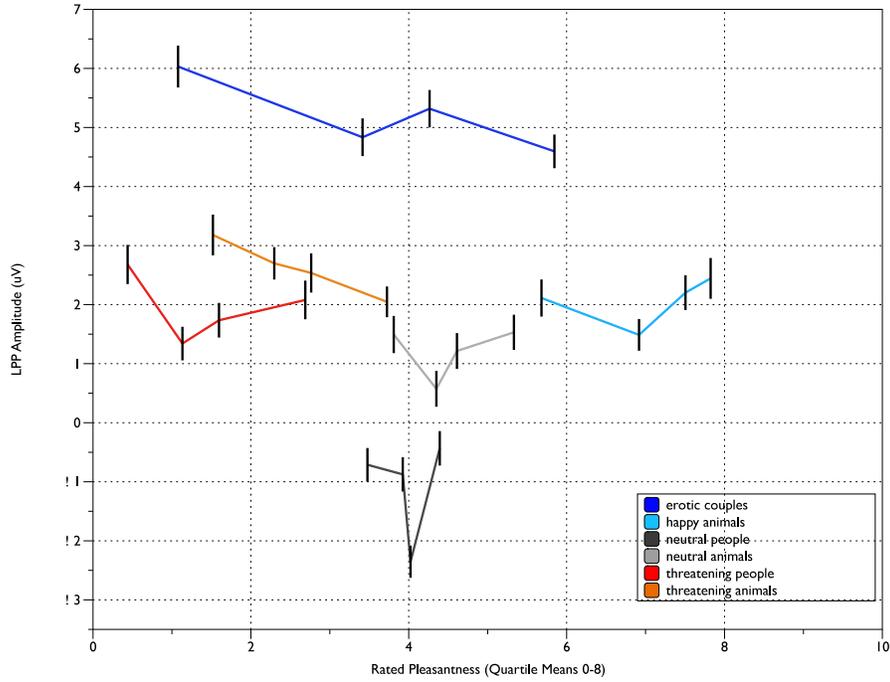


Figure 15. LPP amplitude by rated pleasantness quartile for women.

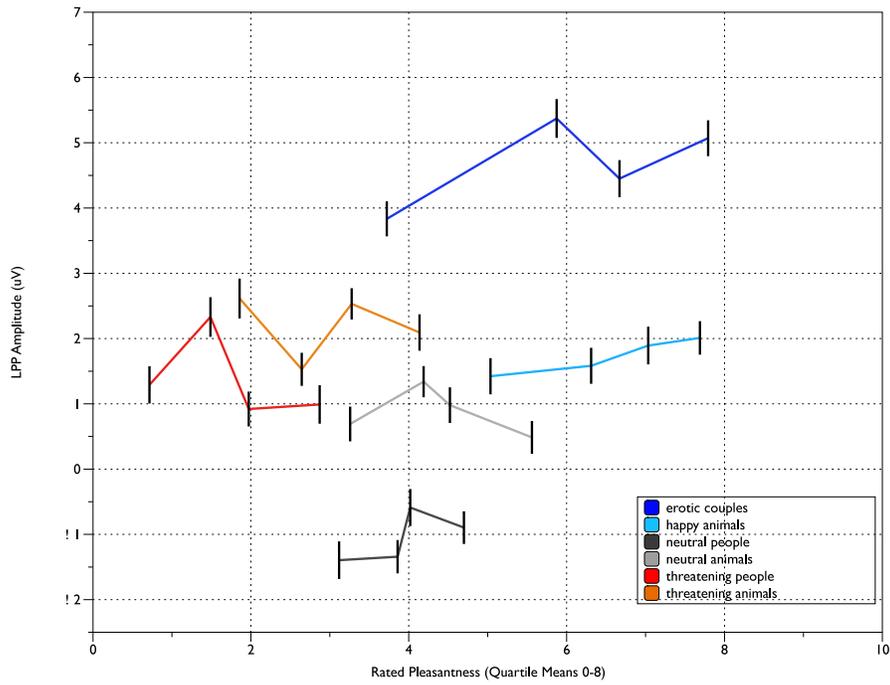


Figure 16. LPP amplitude by rated pleasantness quartile for men.

### Skin conductance and heart rate

A main effect of category on skin conductance [ $F(5,744)=8.113, p<0.001$ ], followed up by a post-hoc Tukey test indicates that the erotic couples elicited stronger SCRs than all other categories. A main effect of Study on skin conductance [ $F(5,744)=5.909, p<0.05$ ] was found, indicating that higher amplitude SCRs were generated in Study 1.

A main effect of category on heart rate [ $F(5,828)=7.409, p<0.001$ ], followed up by post-hoc Tukey tests indicate that the erotic couples, and threatening scenes induce stronger heart rate deceleration compared to less arousing categories. A main effect of sex on heart rate [ $F(1,828)=8.326, p<0.005$ ] was found, indicating that women had stronger heart rate deceleration. Furthermore, a Study x Sex interaction was found [ $F(1,828)=8.345, p<0.001$ ], indicating that women had stronger heart rate deceleration in Study 2 compared to Study 1.

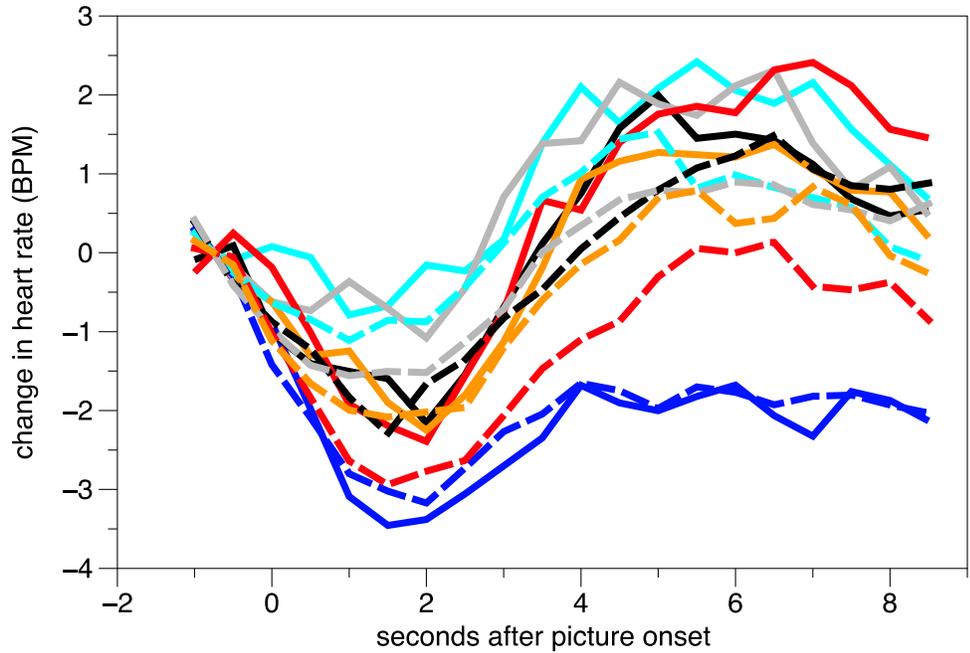


Figure 17. Heart rate response by study. Change in heart rate (beats per minute) after image onset for Study 1 (solid lines) and Study 2 (dashed lines).

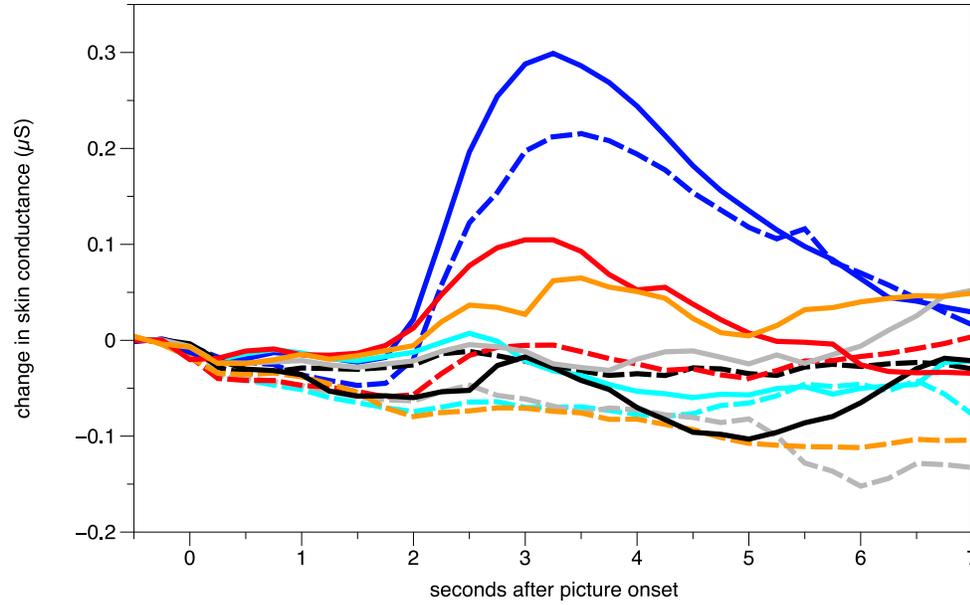


Figure 18. Skin conductance response by study. Change in skin conductance (microsiemens) after image onset for Study 1 (solid lines) and Study 2 (dashed lines).

## CHAPTER 5

### DISCUSSION

#### Emotional perception overview

Emotional perception allows us to discriminate between evolutionarily significant stimuli in the environment, and objects that do not have a strong bearing on our survival. Viewing an emotional scene leads to enhanced cortical and peripheral activation, mobilizing attention and physiological resources that prepare us to act. By measuring emotion-modulated activation in EEG, heart rate, and skin conductance, we can better understand factors that can impact emotional perception. One potential factor that may impact emotional perception is anticipation. If a person anticipates a forthcoming event, it is possible that top-down brain activity may alter regions involved in emotional perception. Previous research has demonstrated that participants can implicitly learn about the composition of the stimulus set, and that this may cause preparatory activity in cortex [Altamura 2014]. In this work, we examined whether stimulus set proportions led to variability in cortical and peripheral measures that are reliably modulated by the perception of emotional stimuli.

#### Development of a new image set

For this study we utilized a newly gathered set of images that were standardized in terms of luminance, complexity, and other criteria. These scenes yielded valence and arousal ratings comparable to the normative ratings for IAPS images [Lang 1997] for most categories. However, we found sex differences in the arousal and valence ratings for erotic couples and happy animals. To summarize, men rated erotic couples higher in arousal and pleasantness,

while women rated happy animals higher in arousal (Figures 12 & 13). Previous studies have demonstrated sex differences using IAPS images, indicating that men have a stronger coupling of pleasure and arousal, while women have stronger coupling of unpleasantness and arousal [Bradley 2001b]. Additionally, neuroimaging research suggests that women may be more sensitive to threat stimuli compared to men [Killgore 2001; Lee 2002; Sabatinelli 2004; Stevens & Hamann 2012].

The disparity in ratings between men and women, as well as within-sex variability, could potentially reflect individual differences in brain activity. To investigate how subjective ratings might relate to ERP measures, we examined the amplitude of the LPP with respect to how highly participants rated each category in terms of pleasantness (Figures 14-16). Similar to past work, subjective ratings of scenes do not appear to have a consistent influence on LPP amplitude within a category [Polich 2008].

#### Emotion modulation of cortical and physiological indices

The emotional modulation of the EPN is thought to reflect selection of significant stimuli for prioritized processing [Schupp 2003a; Schupp 2004a]. We observed enhanced EPN amplitudes for categories that are rated highly in arousal, replicating prior work [Schupp 2003a; Sabatinelli 2013]. The scalp topography of this ERP was also in agreement with previous work, showing activation bilaterally in the occipitotemporal cortices [Junghofer 2001; Schupp 2003a]. Additionally, we observed a stronger LPP in response to emotional stimulus categories. This enhanced LPP activity reflects an elevated level of cortical processing of emotional stimuli. It may reflect an increase in attentional resources devoted toward the stimuli [Schupp 2003a], evaluation of the stimulus [Schupp 2007b], or activation of the brain's defensive or appetitive motivational systems [Bradley 2000]. The scalp topography of the LPP was similar to prior

work, located over centroparietal sensors [Codispoti 2007; Sabatinelli 2013]. These findings indicate that the images used in these study reliably reproduced the expected emotion-modulated response in the EPN and LPP.

Emotional stimuli also produce emotional responses in heart rate and skin conductance [Codispoti 2001]. The initial deceleration of heart rate is thought to occur when we attend to visual stimuli, and visually process them [Lacey & Lacey 1970]. We observed a significant deceleration of heart rate after viewing images of erotic couples, compared to neutral images (Figure 17). These data are not fully reflective of the expected changes in heart rate, since threatening animals and threatening humans did not cause statistically significant deceleration in heart rate. However, these results may be due the use of stimulus set containing new images, or images that are not from the highest arousing negative categories (i.e. mutilation or contamination).

Skin conductance responses are more responsive to both pleasant and unpleasant images compared to neutral images [Codispoti 2001; Bradley 2001a; Amrhein 2004]. Additionally, there is some evidence that highly arousing categories generate a strong sympathetic reaction, causing skin conductance to be exceedingly high [Bradley 2001a]. This effect was seen in our data, where the skin conductance level of the highest arousal category (erotic couples) generated significantly larger SCRs than all other categories. Furthermore, the threatening animal and threatening human categories produced larger SCRs than many of the other categories across experiments (Figure 18).

#### Stimulus set probability does not influence emotional modulation

The aim of the present work was to determine whether stimulus set proportions would bias the activity of the well-established emotional modulation of the EPN, LPP, heart rate, or

skin conductance. We found that the proportion of emotionally arousing images that differed between studies did not influence the level of emotional modulation of these indices. We did not find any significant or marginal Category x Study interactions for cortical or peripheral measures, indicating that the stimulus set proportionality did not affect the extent to which participants were engaged by emotionally arousing images relative to neutral.

We found an overall higher skin conductance response in Study 1, compared to Study 2 (Figure 18). This may be a result of carry-over effects in SCR from a higher incidence of preceding pleasant and unpleasant images, which has been shown previously [Fujimura 2013]. Alternatively, this could be a result of showing fewer images per block in Study 2. There was no Category x Study interaction however, suggesting that image proportions do not affect the emotional modulation of skin conductance.

In addition, we found sex differences for the overall amplitude of the LPP, EPN, and heart rate. This adds to existing literature demonstrating sex differences during emotional scene viewing in early centroparietal N100 and N200 ERP components [Lithari 2009], heart rate and skin conductance [Bradley 2001b]. In Lithari (2009), women displayed greater negativity in the N100 and N200 after viewing emotional stimuli. However, their study may have been unable to detect EPN modulation due to the use of a spatially limited electrode derivation (10-20 international). The existence of sex differences in the visual cortices is supported by neuroimaging work [Lang 1998; Sabatinelli 2004]. In these studies, men displayed enhanced BOLD activity in response to pleasant images compared to women, and women showed enhanced bold activity in response to aversive images compared to men. Our study differed from the literature by observing that women had an enhanced EPN in response to all categories of stimuli, rather than solely emotional stimuli. With regard to peripheral measures, Bradley

(2001b) reported stronger deceleration of heart rate, and more pronounced skin conductance in women when viewing unpleasant scenes. We observed a main effect of sex on heart rate deceleration, indicating that women had stronger heart rate deceleration regardless of scene category. Further, we did not find an effect of sex on skin conductance.

An unpredicted finding of this work was the main effect of experimental block on LPP amplitude. Our results show that the overall amplitude of the LPP increased within the first block and plateaued during blocks 2 and 3, a finding that is not seen in the EPN (Figure 3). The plateauing effect appears to occur in most categories (Figures 9 & 10), suggesting that there may be an upper bound on the LPP's amplitude that is different for each category. Previous work has described habituation of the LPP in response to repetitive presentation of an image within and across experimental blocks [Sambeth 2003]. However, presenting a novel stimuli after a set of repeating images elicits a full amplitude LPP [Codispoti 2006]. A plateauing of the response to serially presented novel images has not been observed in prior work. The LPP is generally thought of as an index of enhanced attentional processing of the emotional content of stimuli, and is insensitive to color, stimulus size, and blurring [De Cesarei & Codispoti 2006; Codispoti 2012]. The emotional modulation of scene categories is distinct across blocks in both studies, suggesting that this effect may be a result of altered attention, fatigue, or mood.

We also observed a correlation between EPN and LPP amplitude, shown in Figure 11. The clustering of images from each category in this figure is similar to that found in previous work (Sabatinelli 2013). Both the EPN and LPP are modulated by emotional arousal, therefore this correlation is not surprising. Interestingly, the animal categories overlap to a greater degree than the categories involving humans, and have less extreme EPN and LPP amplitudes overall.

### Conclusions and future directions

This study demonstrates that several measures of cortical and peripheral activity reliably distinguish emotional stimuli from neutral stimuli. However, we found that the relative proportion of emotionally arousing to neutral stimuli, at least across the proportions investigated here, does not have an appreciable affect on the emotional modulation of event-related potentials or peripheral autonomic activity. This suggests that the proportion of arousing to non-arousing stimuli in visual perception has a negligible impact on the cortical and physiological responses to individual images. It is possible that the proportions of images used (50/50 versus 66/33) were not sufficiently distinct to yield statistically significant results. Future work could include a more broad set of proportions (20/80 & 80/20), which might result in a more salient expectation effect in the participant. If participants are sensitive to the probability of arousing stimuli that they are viewing and this causes anticipation of emotionally engaging stimuli, this anticipation may be stronger when using stimulus sets where the evocative stimuli are more easily predicted. If evidence for this expectation effect were observed, it would support the perspective that top-down brain anticipatory mechanisms mediate the impact of emotional cue processing. Further investigations could identify parametric factors that need to be taken into account when studying emotional perception, and extend our understanding of the preparatory factors that impact emotional perception.

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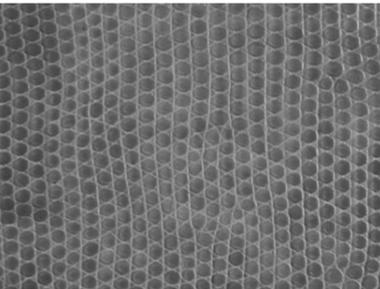
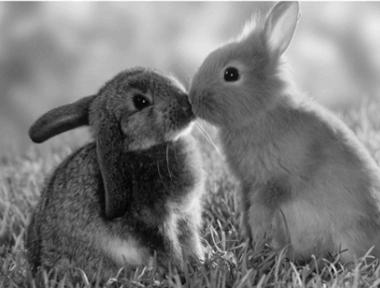
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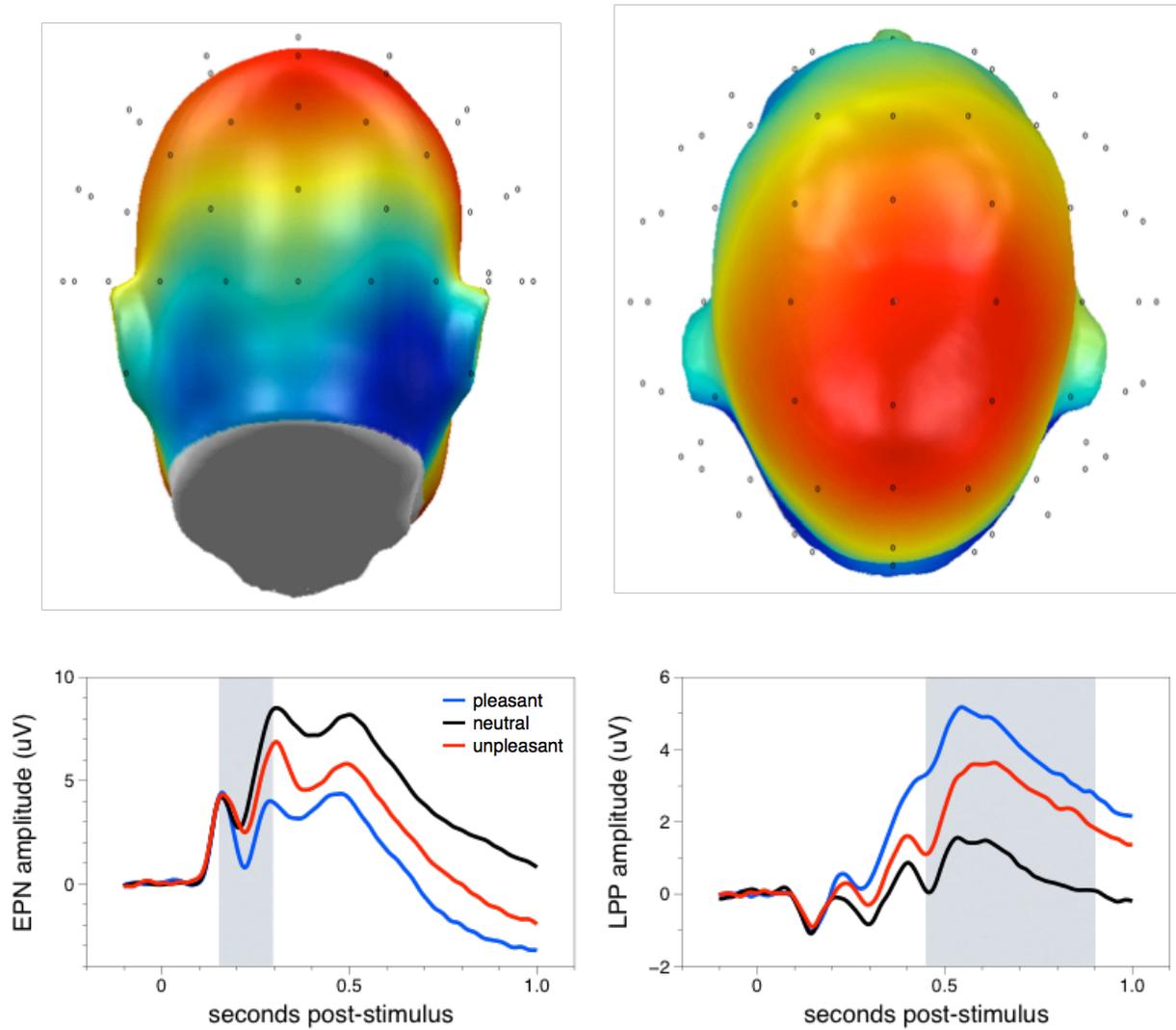
## APPENDICES

### Appendix A



Example images for each picture category in order: erotic couples, neutral people, threatening people, happy animals, neutral animals, threatening animals, neutral textures, neutral faces.

## Appendix B



Topographic plots for the EPN (top-left), and LPP (top-right) obtained by contrasting arousing and non-arousing image categories. The lower figures represent example EPN and LPP timeseries data obtained by averaging the signal from six channels, by valence (pleasant, neutral, unpleasant) using data from both studies. Shaded gray regions represent temporal windows used to obtain EPN and LPP scores from timeseries data.