

POSTAURICULAR AND BLINK REFLEXES TO STARTLE PROBES
AND AUDITORY CLICKS

By

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CHAPTER I

INTRODUCTION

General Introduction

An abundance of data demonstrates that the startle eyeblink reflex (SBR) is modulated by emotional contexts; that is, the more negative affect an individual is experiencing, the greater his or her relative startle eyeblink will be (e.g., Lang, Bradley, & Cuthbert, 1990). This measure has been applied extensively and in myriad ways to investigate normal and abnormal negative emotional processing (see Grillon & Baas, 2003; Vaidyanathan, Patrick, & Cuthbert, 2009; Vaidyanathan, Patrick, & Bernat, 2009). Despite its ability to index negative emotionality, this reflex is not a reliable indicator of positive emotionality (see Jackson, Malmstadt, Larson, & Davidson, 2000).

The postauricular reflex (PAR), a vestigial muscle response in humans (Gray, 1901/1995), shows the opposite pattern of modulation; this reflex's magnitude is greatest during the experience of positive emotion (Benning, Patrick, & Lang, 2004); thus, PAR can be used as an index of positive emotionality. Because this measure is relatively new to the field of psychology, little is known about the time course of its modulation and how to collect it. Understanding more about these parameters is a critical step in utilizing PAR as a psychophysiological measure of positive emotion.

Elicitation of the Reflex

Startle Blink Reflex

Postauricular (PA) and startle blink reflexes both occur in response to a sudden and intense stimulus. In the case of SBR, the startling stimulus represents a threat to which the body must orient (Blumenthal & Berg, 1986). In recent applications of SBR methodology, the stimulus typically used to elicit SBR is a loud noise, referred to as a startle probe (Blumenthal, Cuthbert, Fillion, Hackley, Lipp, & van Boxtel, 2005). The startle probe results in the contraction of the orbicularis oculi muscles, or an eyeblink, within 20-120 ms after the onset of the probe (i.e., the latency period). Activity during this period is measured by placing EMG electrodes over the orbicularis oculi muscles, and SBR is quantified as the peak activity during that window (Blumenthal et al., 2005).

SBR is thought to tap into the defensive system; the eyeblink response represents the most persistent and consistent component of the overall startle response, which generally occurs to threat in the environment (Landis & Hunt, 1939). This theory helps explain why SBR is largest during images of threat (such as images of guns pointed at the viewer; Gard, Gard, Mehta, Kring, & Patrick, 2007), which directly activate the defensive system more so than other aversive visual stimuli (such as images of mutilation).

Postauricular Reflex

PAR is also elicited by a startle probe. The probe results in contraction of the postauricular muscles, a small band of muscles behind the ear (Benning et al., 2004). The reflex has a latency period of 8-30 ms after probe onset (see Benning et al., 2004); as such, PAR is quantified as the peak response in that window. Unlike SBR, PAR is a microreflex: to extract a meaningful peak, several trials must be averaged together (Hackley, Woldorff & Hillyard, 1987).

It is logical that a startle probe would result in increased SBR – the loud probe represents a potential threat to which the body must orient. Why PAR is also elicited to the startle probe is less clear. This is particularly counterintuitive in light of findings that PAR likely taps into the appetitive system. Such research reveals that although postauricular muscles are vestigial in humans, they are used by other mammals to pull the ears back. In juveniles, this action signals to mothers the desire to nurse, and subsequently, facilitates the process of nursing. Therefore, the postauricular muscles may have evolved to activate during appetizing stimuli; as a result, PAR is largest during when elicited in an appetitive state (Johnson, Valle-Inclán, Geary & Hackley 2012). This is consistent with research that shows PAR is the greatest during images of food and erotic scenes (Sandt, Sloan & Johnson, 2009), which should directly activate the appetitive system more so than other pleasurable stimuli (such as images of cute animals; Gard et al., 2007). Currently, it is unclear whether PAR and SBR are opposing indexes of a broader startle reflex or are instead independent reflexive measures of two separate processes.

Emotional Modulation of the Reflexes

Startle Blink Reflex

As SBR is a component of defensive responding, it is not surprising that a large body of literature shows SBR is largest during the experience of negative emotions (i.e., greater reflexes to negative emotions relative to neutral or pleasant emotions): the reflex is emotionally modulated. As a result, SBR can be used in the lab to assess negative emotion at different time points. Because the equipment and expertise required to collect SBR data is minimal, SBR provides a relatively simple and inexpensive methodology. Furthermore, as its latency period is under 120 ms, it also provides a temporally-specific method of assessing negative emotionality.

Many research programs have taken advantage of this valuable methodology. Many paradigms have utilized SBR to reveal information about normal and abnormal emotional processing. A relatively basic and common experimental paradigm that has been utilized to examine negative emotionality is the picture-viewing paradigm (e.g., Bradley, Codispoti, & Lang, 2006; Bradley, Cuthbert & Lang, 1993; Vrana, Spence, & Lang, 1988). In this paradigm, participants view a standardized set of pleasant, neutral, and aversive images from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 2005). A loud noise probe is played during each image on a computer screen. The intensity of SBR to this probe, relative to each individual's baseline SBR reactivity, provides an index of negative emotionality. This paradigm consistently yields a pattern of greatest SBR during aversive images, and lowest SBR during pleasant images in healthy

participants (Bradley, Lang & Cuthbert, 1999; Bradley et al., 2006; Gard et al., 2007; Vrana et al., 1988). Additionally, this pattern of modulation varies across psychopathologies; for example, individuals with depression show blunted emotional modulation of SBR (e.g., Dichter, Tomarken, Shelton, & Sutton, 2004). Therefore, SBR can be used to assess individual differences in negative emotional processing across psychopathologies, and in normal-range emotional functioning.

Postauricular Reflex

Unfortunately, SBR is not a reliable indicator of positive emotion. Although pleasant images can result in decreased SBR, even relative to neutral pictures, this pattern is not consistent (see Johnson, Jackson, Malmstadt, Larson, & Davidson, 2000) and is more likely measuring a lack of negative emotion, rather than the presence of positive emotion. Recently, PAR was investigated as a potential measure of the reverse of SBR: a reflex emotionally modulated during pleasant emotions. Benning and colleagues (2004) employed a standard picture-viewing paradigm, and measured PAR in response to a standard startle probe. They found that PAR was significantly potentiated during pleasant images vs. aversive images, and that this effect was specific to highly arousing pictures.

These findings led Benning et al. (2004) to suggest that potentiated PAR might be a measure of positive emotionality. Supporting this assertion, many investigators have found potentiated PAR during pleasant images since this original study (Dichter, Benning, Holtzclaw, & Bodfish, 2010; Hess, Sabourin, & Kleck, 2007; Quevedo, Benning, Gunnar, & Dahl, 2010; Sandt et al., 2009). Potentiation of PAR

has also been found during pleasant vs. neutral or aversive emotional sounds (Benning, 2011), suggesting the reflex measures positive emotion across sensory modalities. PAR has also been measured before a pleasant event occurs; specifically, consuming chocolate (Hackley, Muñoz, Hebert, Valle-Inclan, & Vila, 2009). This work suggests that PAR can be used to measure anticipation of pleasant stimuli in addition to consummation of pleasant stimuli, enhancing its applicability. Thus, the limited literature that exists at this point supports the role of PAR as an index of positive emotion, and a useful compliment to SBR methodology.

PAR has quickly proven valuable in elucidating individual differences in positive emotionality in several different populations. Dichter and colleagues (Dichter, Benning, Holtzclaw, & Bodfish, 2010) found increased PAR to aversive images in children with Autism, suggesting general abnormalities in emotional processing. Lubman and colleagues (Lubman, Yucel, Kettle, Scaffidi, MacKenzie, Simmons, & Allen, 2009) found increased PAR to drug images and decreased PAR to natural reinforcers in opiate-dependent individuals. In addition to providing valuable information about positive emotional processing in these populations, this sensitivity to individual differences has the potential to inform intervention, treatment, and possibly to identify endophenotypes.

Time Course of Emotional Modulation

Understanding the time course of emotional modulation is critical knowledge for developing paradigms that utilize SBR and PAR. Pictures are complex stimuli, and studies of SBR magnitude and modulation during picture processing have

revealed a dynamic interplay between attention and emotion (see Bradley et al., 2006; Bradley et al., 1993). Studies have examined time course of emotional modulation of SBR using the classic picture-viewing paradigm. During the 6 s presentation of an IAPS image, researchers have varied the onset of the probe to identify the point at which SBR is consistently emotionally modulated.

Such time course analyses reveal that SBR shows a facilitatory effect lasting around 50 ms, in which SBR is significantly greater than other all other time points. This period likely reflects an initial period of orienting to the stimulus. This facilitation is followed by a 100-250 ms period of inhibition, during which participants demonstrate the lowest SBR. This effect is particularly strong during valenced images (i.e., pleasant and aversive, but not neutral). This is likely the result of attentional resources allocated to processing the more affectively complex images. Finally, a consistent pattern of emotional modulation emerges around 3 s, in which SBR during aversive pictures is greater than that during pleasant pictures (see Bradley et al, 1993; Bradley et al., 2006).

These findings have been critical to developing an understanding of the emotional and attentional processes involved in the emotional modulation of SBR. These findings have greatly improved study designs utilizing SBR as well; for example, we now know the startle probe should be placed no earlier than 3 s after picture onset in a standard picture-viewing paradigm (Bradley et al., 2005). However, despite providing such valuable information about SBR, this work is limited by the necessity of using a loud startle probe to elicit SBR. Because the probes are aversive, they must be spaced out over time. In picture-viewing

paradigms, only one probe is typically presented during each image. Thus, to ascertain the time course of emotional modulation, researchers must not only vary probe presentation within-subjects, but between-subjects as well. This process yields an analysis that is the result of SBR data averaged across trials, and across participants.

Currently, there has been no analysis of the time course of emotional PAR modulation. This is in large part due to the limitations of using the startle probe to elicit PAR: in addition to the limitation of using the probe to collect SBR, PAR is further complicated as a microreflex. Because many trials must be averaged together to extract a meaningful PAR response, many more trials would have to be collected than have been used to study emotional SBR modulation's time course. Thus, a similar analysis of the time course of PAR would be unnecessarily time-consuming, cumbersome, and without a reliable and straightforward capacity for statistically analysis.

Using Clicks to Elicit Postauricular Reflexes

PAR is currently limited in its collection parameters: little work has been done to understand how to best collect PA reflexes. Instead, PA reflexes are typically collected using the same parameters used to collect startle blink reflex, which is aversive and methodologically limiting. However, the startle probe has remained the standard for collecting PAR for several reasons: 1) startle parameters are effective at eliciting modulation of the PA reflex; 2) using the same elicitation stimulus allows the simultaneous collection of levels of positive and negative

emotionality; 3) the ideal parameters for the startle eliciting acoustic probe have been well established, whereas the same has not been established for the PAR. Despite the conceptual and methodological convenience of collecting PAR and SBR concomitantly, PAR differs fundamentally from SBR as an index of positive emotionality. Thus, it is critical to establish how to best collect the PAR in its own right.

Because of the aversive and methodologically limiting nature of the standard startle probe, elucidating the ideal parameters of the probe is a priority in advancing PAR methodology. The loud probe was designed to tap into the defensive system: a loud probe is thought to be most effective at eliciting SBR because of its threatening and aversive nature (Lang, Bradley, & Cuthbert, 1997): it causes the listener to orient to the probe, and prepare a defensive response (Blumenthal & Berg, 1986). As PAR is thought to be part of the appetitive system, and not the defensive system, this probe is likely not ideal for eliciting the reflex.

SBR literature reveals many essential parameters to consider when developing a reflex-eliciting stimulus. Decades of research have resulted in the elucidation of several key features that influence the intensity and modulation of SBR: decibel (dB) level (i.e., volume of the probe), duration (i.e., length of the probe), bandwidth (i.e., frequency), and rise time (i.e., how quickly the stimulus reaches its full amplitude; see Blumenthal et al., 2005). The ideal probe for collecting startle blink reflex is around 100 dB, 50 ms, broadband bandwidth (i.e., white noise), and has a nearly instantaneous rise time (see Blumenthal et al., 2005, for a committee report of collection guidelines).

A starting point for elucidating more versatile collection parameters comes from the audiology literature. Although PAR is new to psychology, it has been used by audiology as a screening tool for deafness in infants for decades (Agung, Purdy, Patuzzi, O'Beirne, & Newall, 2005). This literature does not use a standard startle probe to elicit PAR, instead it uses a variety of noises to elicit the reflex. These noises differ from the startle probe in many ways: they are shorter (as short as 100 microseconds; Agung et al., 2005), softer (e.g., 50 dB; see O'Beirne & Patuzzi, 1999), and have varying levels of rise time (see Agung et al., 2005) compared to the standard startle probe. Although this literature demonstrates that PAR can be obtained without the standard startle probe, it is unclear whether PAR elicited by a softer noise with these parameters is emotionally modulated. However, this literature serves as a good starting point for attempting to identify a noise less aversive than the startle probe that can elicit emotionally modulated PAR.

Identifying a softer stimulus that is successful at producing emotionally modulated PAR has many implications for its use: Using a softer stimulus that does not engage the defensive response system will likely increase the effectiveness of eliciting the response. Indeed, it may provide a test of the separability of the PAR and SBR circuits. Furthermore, a softer noise could be presented more frequently than a loud, aversive stimulus. This would allow the measurement of positive emotionality during the course of an experimental paradigm, and a more reliable analysis of the time course of PAR.

Current Study

The goal of the current study was to investigate the possibility that softer noises can result in emotionally modulated PAR, and use these versatile stimuli to examine the time course of the emotional modulation of the reflex. To do so, the current study employed a standard picture-viewing paradigm, in which pictures were presented for 6 seconds each. A single startle probe was presented during the presentation of each image. This served as a manipulation check to ensure we were successfully inducing emotion in participants. We expected to see potentiated PAR to pleasant images and SBR to aversive images, consistent with existing literature. In addition, “clicks,” or noises similar to those used in the audiology literature (Agung et al., 2005) were played every 100 ms. These stimuli had the following properties: 65 dB, 100 microseconds, with an instantaneous rise time. We predicted that these clicks would yield a similar pattern of emotional modulation as startle probes, with potentiation to pleasant images compared to aversive and neutral images.

Using the clicks to elicit PAR allowed a nuanced depiction of the time course of its modulation. We aggregated responses to clicks over 500 ms time bins, for a total of 12 time bins during the 6 s picture presentation. We excluded all time bins that contained a probe from this analysis. We expected to see a pattern similar to that of SBR, with an initial facilitation, followed by inhibition, and finally a consistent pattern of modulation. However, to the extent that SBR and PAR have different neural circuitries, the shorter and softer clicks may give rise only to PAR, not SBR. Nevertheless, because PAR has a much faster latency than SBR (8-35 ms v. 20-120 ms), we expected consistent modulation to occur more quickly than SBR.

Furthermore, we assessed the clicks' potential to elicit PAR that is sensitive to individual differences. Thus far, probe-elicited PAR has shown sensitivity to individual differences: as such, it has the potential to reveal clinically meaningful distinctions in positive emotional processing. The current study examined the clicks' sensitivity to individual differences in depression, a psychopathology tied to low positive emotionality (Tellegen, 1985), to ensure sufficient potentiation of PAR.

CHAPTER II

METHODS

Participants

Participants were 85 undergraduates recruited from Psychology classes at Vanderbilt University. Students received course credit for their participation.

Stimuli

The startle probe was a bilateral 50 ms, 105dB white noise probe with nearly instantaneous rise time. These probes were presented at 3, 4, or 5 seconds after image onset. A total of 52 images from IAPS (Lang et al., 2005) were selected for use in this experiment. Four images were presented without startle probes to reduce predictability. The remaining 48 consisted of 16 pleasant, 16 neutral, and 16 aversive images. There was an equal distribution of specific image categories.¹ All image contents were gender balanced on dimensions of normatively rated valence and arousal.

¹ Content categories consisted of the following IAPS pictures: adventure: 5623, 8034, 8180, 8210; nurturant: (1811, 2071, 2160, 2340/ 1463, 1722, 2341, 2655); erotic: 4640, 4660, 4680, (4255/4572); food: 7200, 7230, 7260, 7460; buildings: 5731, 7180, 7490, 7491; humans: 2190, 2393, 2870, 2890; landscapes: 5120, 5390, 5740, 9210; objects: 7002, 7004, 7034, (7031/7038); disgust: 9342, 9520, 9560, 9830; mutilation: (3051, 3061, 9253, 9420/9042, 9265, 9440, 9490); threat: 6250, 6260, 9630, (6243/6190); victim: 6570, 9920, (6312, 6540/6530, 6561). Pictures not in parentheses were presented to participants of either gender; pictures within parentheses to the left of the slash were presented only to men, and those within parentheses to the right of the slash were presented only to women.

The PA-eliciting “clicks” in this study were bilateral 100 μ s, 65 dB white noise clicks with nearly instantaneous rise time. Following image onset, these clicks were presented every 100 ms, except when the standard startle probe was played.

Images and probe placement were counterbalanced in this study: Two sets of four different serial positions of stimuli were used, one set designed for women and the other for men. In all of the stimulus orders, no more than two stimuli of the same valence were presented next to each other, and the same image content were never placed next to each other.

Physiological Measures

PAR was collected using the parameters described by O’Beirne & Patuzzi (1999). A pair of 4 mm Ag-AgCl electrodes were placed over the postauricular muscle of each ear, identified by locating the fibrous strip connecting the pinna (outer ear) and scalp. One electrode was placed over the muscle on the scalp, and the other adjacent to the first on the pinna. Prior to placement, the sites were scrubbed with an abrasive gel to reduce impedances below 10 kHz. Raw electromyographic (EMG) signals for each ear were recorded for 50 ms before noise probe onset to obtain baseline, and 8-35 ms after the probe to locate PAR.

Because PAR is a microreflex, the activity was aggregated over several trials using rectified waveforms (Hackley, Woldorff & Hillyard, 1987). In this study, clicks were aggregated by valence and 500 ms time bins (yielding a total of 36 average waveforms; 12 time bins for each valence). PAR to clicks were then aggregated by valence, yielding a total of 3 average waveforms. Because of the large variation in

individuals' PA reflex responses, the magnitudes of the average waveforms were then z-scored within each participant for valence and time analysis.

SBR was also collected using a pair of 4 mm Ag-AgCl electrodes according to the collection parameters specified in Blumenthal et al. (2005); one electrode was placed 5 mm below the lid of the right eye, directly under the pupil. The second was placed adjacent to first, toward the outer canthus. The site was also scrubbed to reduce impedances below 10 k Ω . Raw EMG signals were recorded 50 ms prior to each probe to establish a baseline, and 30-120 ms following the onset of the probe to obtain the peak of the reflex.

Both postauricular and startle blink channels were sampled at 2000 Hz with a NeuroScan SynAmps² bioamplifier (Compumedics, Charlotte, NC) at DC with a 500 Hz low-pass filter.

Procedure

Participants arrived and completed a consent form. Next, they were given a verbal description of the psychophysiology set up. They then completed study questionnaires, including the Self-Rating Depression Scale (SDS; Zung, 1965), while electrodes were attached. Once setup was complete, they were instructed to watch the images on the screen and to focus on a fixation cross in the absence of an image. They were also told they would hear brief noises through headphones, which they could ignore. They were asked to remain as still as possible throughout the data collection portion of the experiment.

They were then presented with three images and noise probes which served as habituation trials, and given demonstrations of Self-Assessment Manikin ratings (SAM; Bradley & Lang, 1994), which were used to assess valence and arousal after each image during the experiment. These habituation trials were not included in analyses. They were then given the opportunity to ask any questions they had, after which the experimenter left the room and the experiment began.

Each image was presented for 6 seconds. As in the habituation trials, participants completed SAM ratings, which asked them to self-report on valence and arousal of their current emotional state after each image. Following completion of the SAM ratings, participants were instructed to focus on a fixation cross until the next image appeared.

CHAPTER III

RESULTS

Analyses

To examine emotional modulation of SBR to the standard startle probe, we performed within-subject repeated-measures analysis of variance (ANOVA) to assess the main effect of valence. We also performed an ANOVA to examine the main effect of valence on PAR to standard startle probes. We conducted follow-up pairwise comparisons of magnitude at each valence. We also conducted an ANOVA to examine the emotional modulation of clicks for PAR, excluding all responses elicited by a startle probe. We conducted follow-up pairwise comparisons of PAR magnitude at each valence.

To examine the time course of emotional modulation of PAR, we looked at PA activity in 500 ms time bins. We aggregated the responses to clicks over each 500 ms time bin, such that each bin had 5 clicks. Bins that contained a response to the startle probe were excluded from analysis. To assess the time course of potentiation, we conducted pairwise comparisons between the three valence categories at each time bin.

To examine sensitivity to individual differences of depression to probes, we conducted correlations between SDS scores and PAR modulation. For probes, we conducted a single correlation. For clicks, we performed correlations between SDS scores and PAR magnitude at each time bin.

Emotional Modulation to 105 dB Probes

Figure 1 depicts SBR to the startle dB probes. There was a significant main effect of valence on eyeblink magnitudes, $F(2,164) = 6.473, p = .002$. Follow-up pairwise comparisons revealed that the three valence groups were significantly different from each other: Aversive images resulted significantly greater SBR magnitude than neutral and pleasant, $t(84)s < -1.122, ps < .05$. SBR magnitude during neutral and pleasant pictures were not significantly difference, $t(84) = .056, p = .281$.

Figure 2 displays PAR to the startle probes. There was a significant main effect of valence on postauricular reflex magnitude, $F(2,168) = 3.388, p = .037$. Follow up pairwise comparisons revealed that pleasant images revealed significantly greater PAR than neutral and aversive images, $t(84)s > 2.07, ps < .05$, but PAR during neutral and aversive pictures were not significantly different from each other, $t(84) = .069, p = .941$.

Emotional Modulation to 65 dB Clicks

Figure 3 displays the grand average waveforms for postauricular and orbicularis oculi muscle activity elicited by the 65 dB clicks. Whereas click-related activity was apparent for both the left and right postauricular muscles, there was no discernable activity across participants for the orbicularis oculi. Visual inspection of individual participants' waveforms confirmed that there was discernible

waveforms of valence-level aggregates. Thus, only click-elicited PARs were analyzed further.

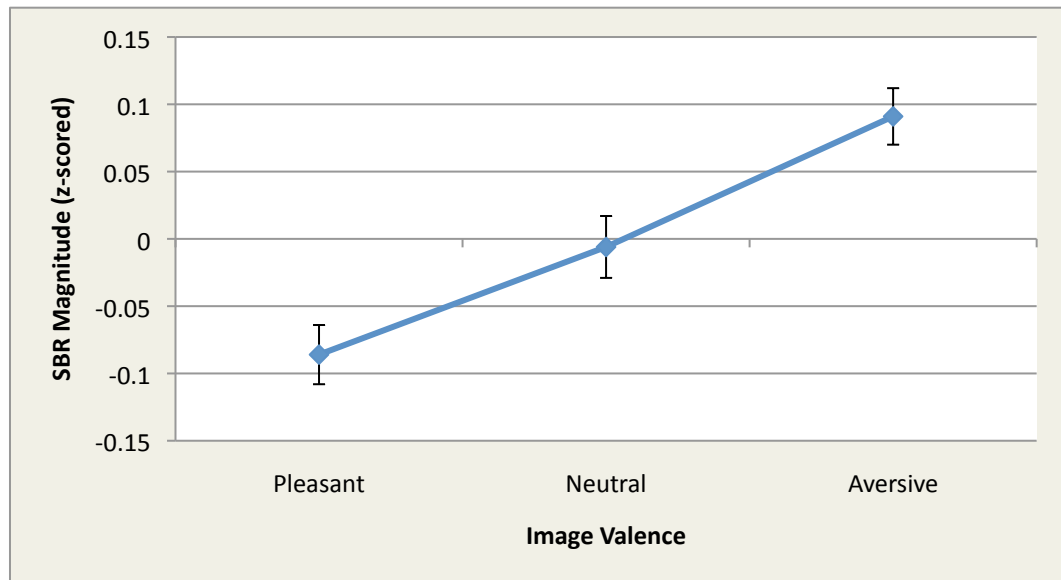


Figure 1. Emotional modulation of startle blink reflexes elicited by 105 dB probes. Error bars represent the standard error of the mean.

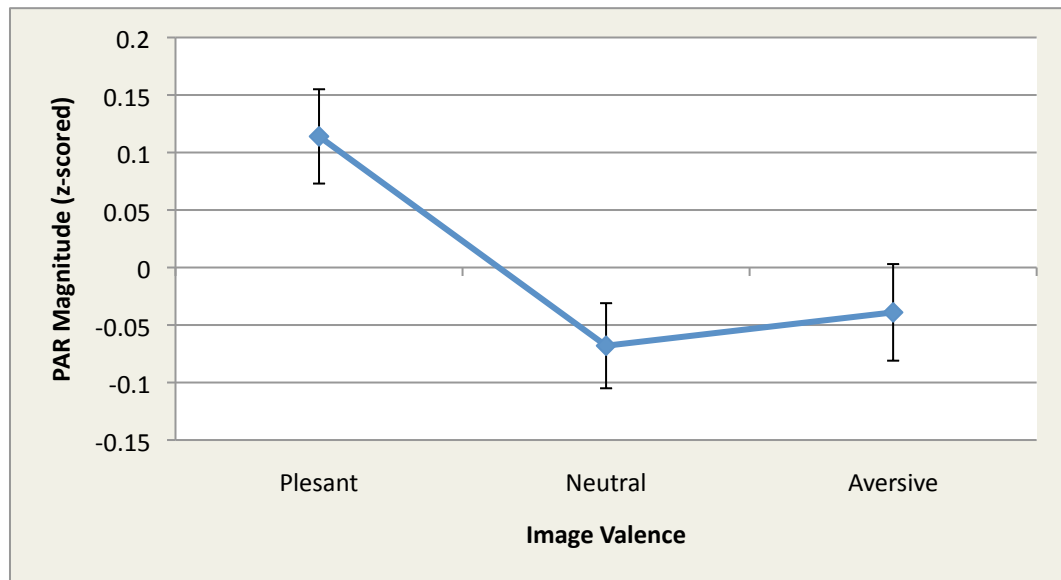


Figure 2. Emotional modulation of postauricular reflexes elicited by 105 dB probes. Error bars represent the standard error of the mean.

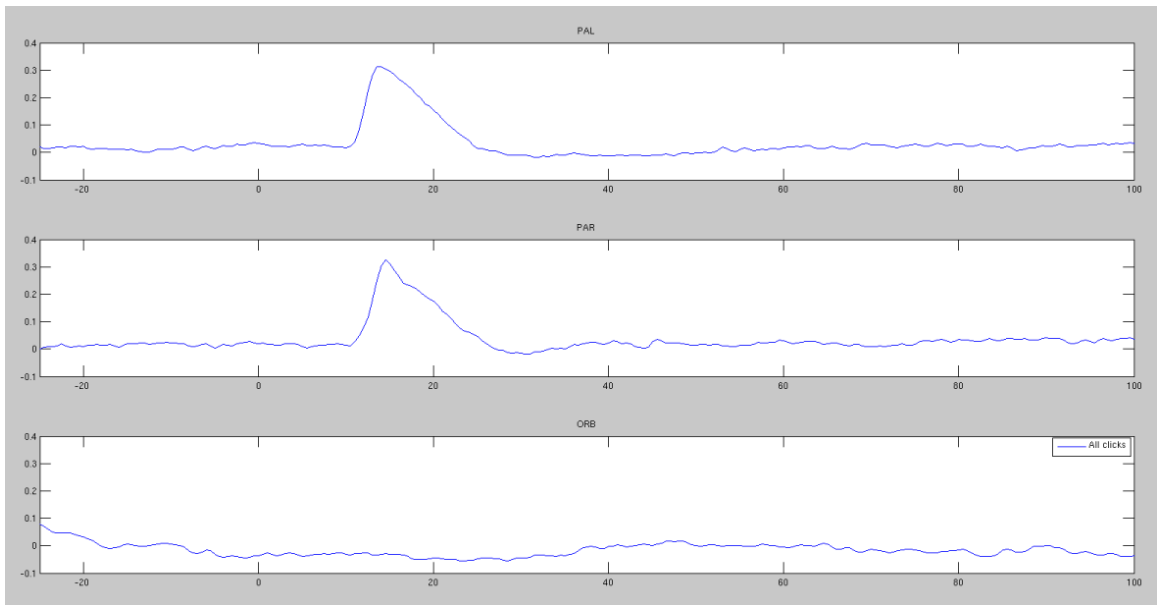


Figure 3. Mean postauricular activity in the left ear(PAL), postauricular activity in the right ear (PAR), and orbicularis activity (ORB) to clicks. Activity is presented from 25 s prior to click onset, and 100 ms post-click onset.

As evident in Figure 4, there was a significant main effect of valence on PAR magnitude to the clicks, $F(1.86,136) = 3.46, p = .037$. Pairwise comparisons revealed that PAR was greater during pleasant vs. aversive images, and during pleasant vs. neutral images ($p < .05$).

Time Course of PAR Modulation

There was a significant main effect of time on PAR magnitude, $F(9.94,726) = 2.32, p = .011$. Figure 5 depicts the time course of PAR potentiation over the course of the image. The first time bin shows an elevation of PAR during pleasant, neutral, and aversive images. There were no significant differences between the three valences. Emotional PAR modulation first appears during the 2000 ms time bin, but it is not consistent throughout the duration of the image onset. Pairwise comparisons revealed that PAR was larger during pleasant than aversive images at 2000, 4000, and 5500 ms, $t(84)s > 2.11, ps < .05$.

PAR Relationships with Individual Differences

To analyze sensitivity to individual differences in depression in response to probes, a correlation between PAR modulation and SDS scores was conducted. There was a significant negative correlation between modulation and SDS scores, $r(83) = -.282, p = .014$.

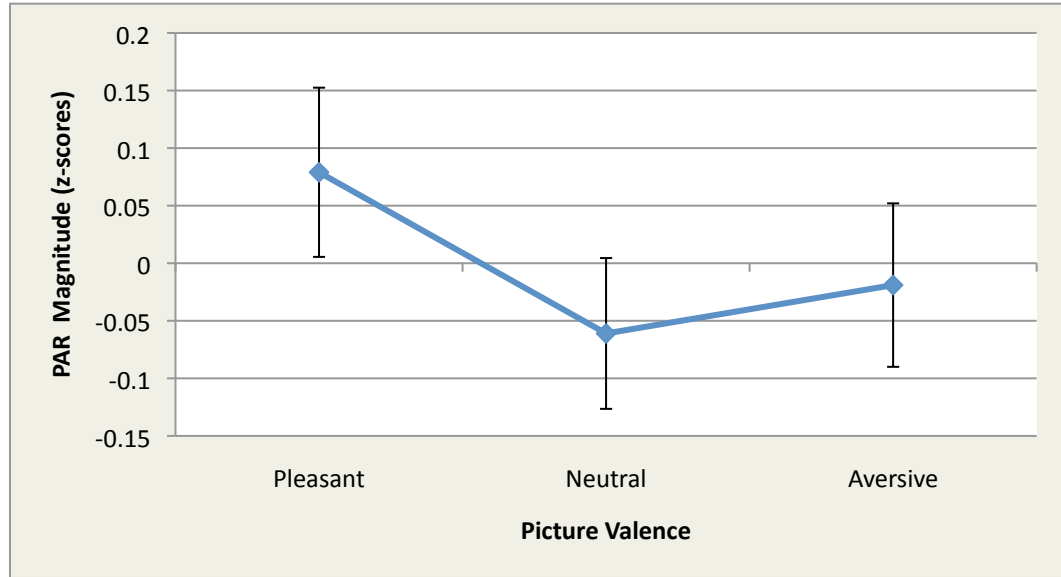


Figure 4. Emotional modulation of postauricular reflexes elicited by 65 dB clicks. Error bars represent the standard error of the mean.

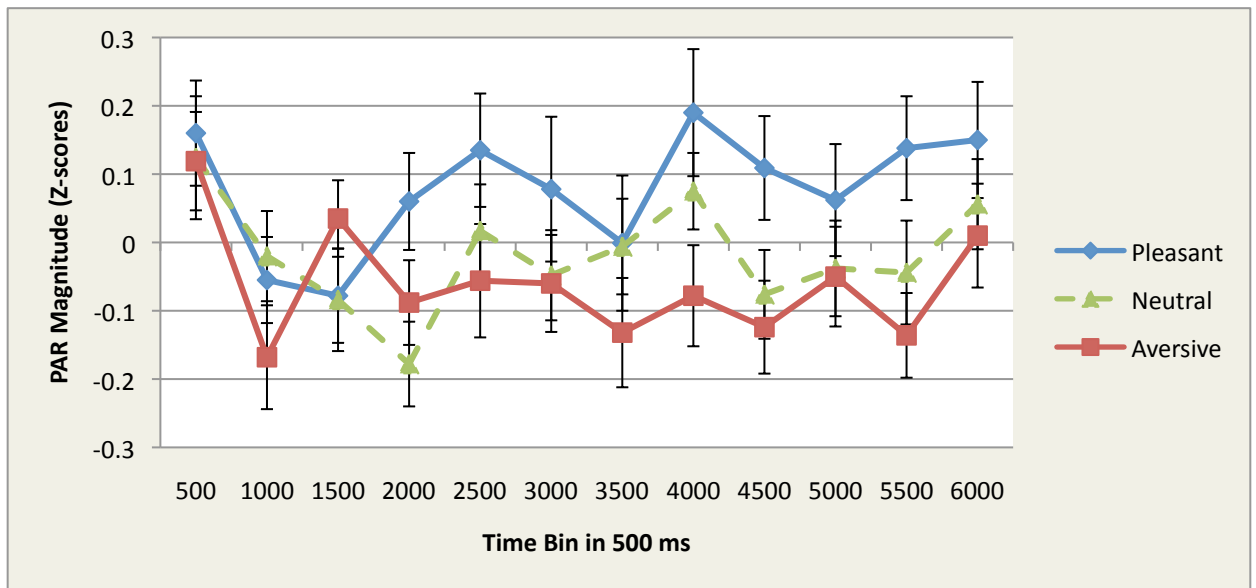


Figure 5. Time course of postauricular reflex modulation during picture presentation.

The time course of PA is currently; thus, the optimal window for the placement of elicitation stimuli for PA is unknown. Because of this limitation, correlations between SDS scores and PAR modulation were conducted at each time point. There were no significant correlations, $|r(83)|s < .2, ps \geq .07$.

CHAPTER IV

DISCUSSION

General Discussion

In addition to standard startle probes, the current study used clicks – a more versatile, less aversive, and perhaps more appropriate stimulus – to elicit PAR. Using clicks allowed the separation of SBR and PAR, and it also facilitated an examination of the time course of emotional PAR modulation. PAR and SBR to standard startle probes both showed the expected pattern of emotional modulation. However, only PAR was obtained to the clicks, with greater PAR during pleasant images, relative to neutral and unpleasant. After what appears to be a 1500 ms orienting period, the PA reflex was only potentiated during pleasant images at 3 of 9 expected time bins. Despite this lack of consistent modulation to the clicks, the overall effect of valence on PAR potentiation to clicks leads us to consider that the standard startle probe may not be necessary to elicit emotionally modulated PAR. A click with different parameters may yield the desired pattern of emotional modulation of PAR.

In addition to replicating the basic pattern of emotional modulation of PAR to standard startle probes, the current study also revealed sensitivity to individual differences in depression with PAR. Specifically, depression was associated with decreased PAR during emotionally valenced images, and increased PAR during neutral images. This abnormality is interesting in light of SBR literature that shows

a lack of SBR modulation in depressed patients (Dichter et al., 2004). Unfortunately, this sensitivity of PAR to depression was not present in response to clicks. Because sensitivity to individual differences is a critical characteristic for the clinical utility of PAR, and because it is present in response to startle probes, the parameters of the click used in this study are not acceptable for clinical investigations of positive emotionality and thus are not ideal.

Nevertheless, the use of clicks instead of probes allowed the analysis of the time course of PAR, which revealed a similar pattern of modulation as SBR: an initial orienting period was followed by emotional modulation between 1500 – 2000 seconds. Although we hypothesized that PAR would show a pattern of emotional modulation earlier than SBR, the time course of modulation actually lagged that of the SBR. Whereas the SBR is facilitated only at 50 ms post-picture onset and is inhibited from 300-800 ms (Bradley et al., 2006), PAR was facilitated for 500 ms in our study, with inhibitions lasting at least 1500 ms. Furthermore, SBR is greater during aversive than pleasant pictures as early as 500 ms after picture onset (Bradley et al., 2006), whereas PAR during pleasant pictures was not greater than that for aversive pictures until 2000 ms. Modulation was not consistent throughout the image presentation, but it did occur as late as 5500 ms, suggesting PAR modulation is maintained throughout the duration of the image presentation, just as SBR shows consistent modulation throughout image presentation in healthy controls (Bradley et al., 2006; but see Dichter & Tomarken, 2008, for individual differences in this pattern). This pattern should continue to be investigated, as the pattern of modulation likely varies with different elicitation parameters.

Overall, the data support the notion that the standard probe may not be necessary to elicit emotionally modulated PAR. However, the click used in this study was not sufficient to elicit this emotional modulation consistently, nor did it show sensitivity to depression. Future work should be conducted to further investigate ideal click parameters.

Limitations

Because two properties of the standard startle probe were altered, it is unclear how each affected PA responses. It is possible that changing one property would have resulted in the desired effect, or that altering both was more effective than isolating only one. For example, it is possible that a 65 dB click with a duration of 50 ms would be more successful at eliciting emotionally modulated PA reflexes than a 65 dB click with a duration of 100 μ s. Alternatively, louder clicks elicit larger PAR (O'Beirne & Patuzzi, 1999), suggesting that a 100 μ s click at a higher volume would yield more robust PAR.

It is also possible that varying startle probe characteristics left unaltered, in isolation or combination, could have produced more robust PAR. Specifically, the rise time of the PAR-eliciting stimulus may be different than the startle probe. Startle probes have near instantaneous rise time (Blumenthal et al., 2005), as quicker rise times result in larger and more consistent SBR (Blumenthal, 1988). This effect is thought to be the result of creating a more startling stimulus, which results in greater SBR because the reflex is part of the defensive system, and thus designed to attend to sudden changes in the environment (Blumenthal & Berg,

1986). Because the PA reflex is thought to tap into the appetitive system (Johnson et al., 2012), it may not be necessary to use a sudden probe. In fact, the startling nature of the probe might have a negative impact on the participant. Agung et al. (2005) found that 10 ms clicks with rising frequency, referred to as “chirps,” yielded larger PAR than a 100 μ s click like that used in the current study. Although it is unclear whether the duration or rising frequency of the chirp drove this effect, it is possible that the using an elicitation stimulus with longer rise time might result in increased modulation and sensitivity to individual differences. Future studies should examine the necessity of an instantaneous rise time for the ideal PAR-eliciting noise.

The current study is not a traditional picture-viewing paradigm in which only one reflex eliciting noise is presented during the presentation of the image; instead, many stimuli were presented consecutively. It is possible that the presentation of stimuli in this way had an attentional effect on the participant. Prepulse inhibition (PPI), for example, refers to the phenomenon that placing a probe 30 – 500 ms before another standard startle probe will reduce startle reflex activity to the second probe (Blumenthal, 1999). It is possible that placing the clicks only 100 ms apart resulted in a similar effect, though it is currently unknown if PA reflexes are also affected by PPI in the same way as SBR. Exploratory analyses of the current data do not support the existence of a PPI effect, but the possibility cannot be ruled out.

Finally, as participants were undergraduate volunteers, individual differences in depression were limited. Sensitivity to individual differences may be

present in more diverse populations, or populations with more extreme psychopathology. Notably, however, the standard startle probe elicited emotionally modulated PAR that was sensitive to individual differences; thus, the click employed in this study may not be sufficiently sensitive to individual differences, even if the sample were more diverse.

Future Directions

Existing literature demonstrates that like SBR, PAR is a flexible measure that can be used in myriad research paradigms. It is relatively simple, unambiguous, and inexpensive to collect relative to other indexes of positive emotion. For example, fMRI is expensive and has poor temporal resolution; zygomaticus EMG recordings are not always specific to positive emotions (Johnson et al., 2012). Self-report of emotions are subjective and have been demonstrated as inconsistent with SBR data in clinical populations (e.g., Dichter & Tomarken, 2008, Kring & Moran, 2008). PAR is a useful stand-alone measure of positive emotion. It can also be used as a compliment to other measurements of positive emotionality.

Future work should continue to examine the parameters of the PAR-eliciting noise. The dB level of the noise should be assessed, to achieve a level that is sufficient to obtain emotionally modulated PA reflex activity that is sensitive to individual differences, yet minimizes its aversive impact. Future studies should also consider altering the rise time of the noise, to determine whether or not an instantaneous rise time is ideal for collecting PA.

Work should continue to consider the time course of modulation, and attempt to better understand attentional effects on PAR. Understanding the effect of PPI on PAR is a critical next step to PAR study designs. Some research suggests that PAR is smaller when attention is directed away from an elicitation stimulus, consistent with a pattern of PPI (Hackley et al., 1987). If PPI effects do exist for the PAR, it likely has a different time course that deserves its own research attention. Understanding the time course and attentional effects of PAR will improve study design and allow the examination of the development of positive emotions, which may have clinical significance in its own right.

SBR has been invaluable in our understanding of emotional processing across psychopathologies. It has revealed valuable information about specific psychopathologies related to anxiety and fear (see Grillon & Baas, 2003, for a review), and recently has been used to conceptualize dimensional models of these forms of psychopathology. For example, Vaidyanathan and colleagues (2009) administered a standard picture-viewing paradigm in addition to a diverse battery of questionnaires that assessed fearfulness and fearlessness. They found that in general, fearfulness (e.g., specific phobias) was positively correlated with startle reactivity, and fearlessness (e.g., psychopathy) negatively correlated with startle activity. The group suggests that SBR may serve as an index of a bipolar fear dimension in dimensional models of psychopathology.

PAR has a similar potential to enhance our understanding of how positive emotionality manifests in different disorders. For example, melancholic depression is characterized by anhedonia, or a lack of emotion (APA, 2000). PAR offers the

unique potential to measure levels of positive emotionality in this population: to measure baseline differences in positive emotionality using a picture-viewing paradigm, to differentiate melancholic depression from other subtypes using psychophysiology, to test specific parameters under which anhedonia is (or is not) present, and potentially identify deficits in positive emotion as an endophenotypes for identifying melancholic depression before it manifests..

PAR is a new and promising index of positive emotionality. Although using parameters established for a standard startle probe has proven to be effective, the reflex likely has its own set of ideal parameters that will enhance the validity of the measure, and create a more flexible and diverse index of positive emotionality.

REFERENCES

- Agung, K., Purdy, S.C., Patuzzi, R.B., O'Beirne, G.A. & Newall, P. (2005). Rising-frequency chirps and earphones with an extended high-frequency response enhance the post-auricular muscle response. *International Journal of Audiology*, 44 (631-636). doi:10.1080/14992020500266613
- American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., text rev.). Washington, DC: Author.
- Benning, S. D., Patrick, C. J., & Lang, A. R. (2004). Emotional modulation of the post-auricular reflex. *Psychophysiology*, 41(3), 426–432. doi: 10.1111/j.1469-8986.2010.01071
- Benning, S.D. (2009, October). *Reduced appetitive postauricular reflex potentiation in depressed undergraduates*. Poster session presented at the annual meeting of the Society for Psychophysiological Research, Berlin, Germany.
- Benning, S.D. (2011). Postauricular and superior auricular reflex modulation during emotional pictures and sounds. *Psychophysiology*, 48, 410-414. doi: 10.1111/j.1469-8986.2010.01071
- Blumenthal, T. D. & Berg, W. K. (1986). Stimulus rise time, intensity, and bandwidth effects on acoustic startle amplitude and probability. *Psychophysiology*, 23, 635–641. doi: 10.1111/j.1469-8986.1986.tb00682
- Blumenthal, T. D. (1988). The startle response to acoustic stimuli near startle threshold: Effects of stimulus rise and fall time, duration, and intensity. *Psychophysiology*, 25, 607–611. doi: 10.1111/j.1469-8986.1988.tb01897
- Blumenthal, T.D. (1999). Short lead interval modification. In M.E. Dawson, A.M. Schell, A.H. Bohmelt (Eds.), *Startle modification: Implications for neuroscience, cognitive science, and clinical science* (pp. 51-71). Cambridge, UK: Cambridge University Press.
- Blumenthal, T.D., Cuthbert, B.N., Filion, D.L., Hackley, S., Lipp, O.V., & van Boxtel, A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology*, 42, 1-15. doi: 10.1111/j.1469-8986.2005.00271
- Bradley, M. M., Cuthbert, B. N. & Lang, P. J. (1993). Emotion, novelty, and the startle reflex: Habituation in humans. *Behavioral Neuroscience*, 107, 970–980. doi: 10.1037/0735-7044.107.6.970

- Bradley, M. M., & Lang, P. J. (1994). Measuring emotion: The Self- Assessment Manikin and the semantic differential. *Journal of Behavior Therapy and Experimental Psychiatry, 25*, 49–59. doi: 10.1016/0005-7916(94)90063-9
- Dichter, G.S., Benning, S.D., Holtzclaw, T.N., & Bodfish, J.W. (2010). Affective modulation of the startle eyeblink and postauricular reflexes in autism spectrum disorder. *Journal of Autism and Developmental Disorders, 40* (858-869). doi: 10.1007/s10803-009-0925-y
- Dichter, G. S., Tomarken, A. J., Shelton, R. C. & Sutton, S. K. (2004). Early- and late-onset startle modulation in unipolar depression. *Psychophysiology, 41*, 433–440. doi: 10.1111/j.1469-8986.00162.x
- Dichter, G. S., & Tomarken, A. J. (2008). The chronometry of affective startle modulation in unipolar depression. *Journal of Abnormal Psychology, 117*, 1–15. doi: 10.1037/0021-843X.117.1.1
- Gard, D.E., Gard, M.G., Mehta, N., Kring, A.M & Patrick, C.J. (2007). Impact of motivational salience on affect modulated startle at early and late probe times. *International Journal of Psychophysiology, 66*, 266-270. doi: 10.1016/j.ijpsycho.2007.05.005
- Gray, H. (1901/1995). *Anatomy: Descriptive and surgical* (15th edn). New York: Barnes and Noble Books, Inc.
- Grillon, C., & Baas, J. (2003). A review of the modulation of the startle reflex by affective states and its application in psychiatry. *Clinical Neurophysiology, 114*, 1557–1579. doi: 10.1016/S1388-2457(03)00202-5
- Grillon, C. & Davis, M. (1997). Fear-potentiated startle conditioning in humans: explicit and contextual cue conditioning following paired vs. unpaired training. *Psychophysiology, 34*, 451–458. doi: 10.1111/j.1469-8986.1997.tb02389
- Hackley, S. A., Muñoz, M. A., Hebert, K., Valle-Inclan, F., & Vila, J. (2009). Reciprocal modulation of eye-blink and pinna-flexion components of startle during reward anticipation. *Psychophysiology, 46*, 1154– 1159. doi: 10.1111/j.1469-8986.2009.00867
- Hackley, S. A., Woldorff, M., & Hillyard, S. A. (1987). Combined use of microreflexes and event-related brain potentials as measures of auditory selective attention. *Psychophysiology, 24*, 632–647. doi: 10.1111/j.1469-8986.1987.tb00343
- Hamm, A.O. & Stark, R. (1993). Sensitization and aversive conditioning: effects on the startle reflex and electrodermal responding. *Integrative physiological and behavioral science, 28*, 171-176. doi: 10.1007/BF02691223

- Hess, U., Sabourin, G., & Kleck, R. E. (2007). Postauricular and eyeblink startle responses to facial expressions. *Psychophysiology*, *44*, 431–435. doi: 10.1111/j.1469-8986.2007.00516.x
- Jackson, D.C., Malmstadt, J.R., Larson, C.L. & Davidson, R.J. (2000). Suppression and enhancement of emotional responses to unpleasant pictures. *Psychophysiology* *37*, 515–522. doi: 10.1017/S0048577200990401
- Johnson, G.M., Valle-Inclán, F., Geary, D.C. & Hackley, S.A. (2012). The nursing hypothesis: An evolutionary account of emotional modulation of the postauricular reflex. *Psychophysiology*, *49* (178-185). doi: 10.1111/j.1469-8986.2011.01297
- Kring, A.M. & Moran, E.K. (2008). Emotional response deficits in schizophrenia: Insights from affective science. *Schizophrenia Bulletin*, *34*, 819-834. doi: 10.1093/schbul/sbn071
- Landis C, Hunt WA. The startle pattern. New York: Farrar and Rinehart Inc; 1939.
- Lang, P.J., Bradley, M.M. & Cuthbert, B.N. (1990). Emotion, attention, and the startle reflex. *Psychological Review*, *97*, 377-395. doi: 10.1037/0033-295X.97.3.377
- Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2005). International affective picture system (IAPS): Digitized photographs, instruction manual and affective ratings. Technical Report A-6. Gainesville, FL: University of Florida. doi: 10.1016/j.epr.2006.03.016
- Lubman, D.I., Yucel, M., Kettle, J.W.L., Scaffidi, A., MacKenzie, T., Simmons, J.G. & Allen, N.B. (2009). Responsiveness to drug cues and natural rewards in opiate addiction. *Archives of General Psychiatry*, *66* (205-213). doi: 10.1001/archgenpsychiatry.2008.522
- National Institution of Deafness and Other Communication Disorders (2011). *How loud is too loud?* Retrieved from <http://www.nidcd.nih.gov/health/hearing/pages/ruler.aspx>
- O'Beirne & Patuzzi, R.B. (1999). Properties of the sound-evoked post-auricular muscle response (PAMR). *Hearing Research* *138*, (115-132). doi: 10.1016/S0378-5955(99)00159-8
- Quevedo, K., Benning, S.D., Gunnar, M.R. & Dahl, R.E. (2010). The onset of puberty: Effects on the psychophysiology of defensive and appetitive motivation. *Developmental Psychopathology*, *21*, 27-45. doi: 10.1017/S0954579409000030
- Sandt, A. R., Sloan, D. M., & Johnson, K. J. (2009). Measuring appetitive processing with the postauricular reflex. *Psychophysiology*, *46*, 491–497.

- Sollers, J. J. & Hackley, S. A. (1997). Effects of foreperiod duration on reflexive and voluntary responses to intense noise bursts. *Psychophysiology*, *34*, 518–526. DOI: 10.1111/j.1469-8986.1997.tb01738.
- Vaidyanathan, U., Patrick, C.J., & Bernat, E.M. (2009). Startle reflex potentiation during aversive picture viewing as an indicator of trait fear. *Psychophysiology*, *46* (75-85). doi:10.1111/j.1469-8986.2008.00751.
- Vaidyanathan, U., Patrick, C.J., & Cuthbert, B.N. (2009). Linking dimensional models of internalizing psychopathology to neurobiological systems: Affect-modulated startle as an indicator of fear and distress disorders and affiliated traits. *Psychological Bulletin*, *135* (909-942). doi: 10.1037/a0017222.
- Vrana, S. R., Spence, E. L., & Lang, P. J. (1988). The startle probe response: A new measure of emotion? *Journal of Abnormal Psychology*, *97*, 487–491. doi: 10.1037/0021-843X.97.4.487
- Zung, W.W. (1965). A self-rating depression scale. *Archives of General Psychiatry*, *12*, 63-70.