PREPULSE INHIBITION

OF THE POST-AURICULAR REFLEX

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In Partial Fulfillment

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Master of Arts

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by

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PREPULSE INHIBITION

OF THE POST-AURICULAR REFLEX

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ABSTRACT

It is well established that a weak lead stimulus will inhibit the startle response to a more intense subsequent stimulus, given an appropriate lead time (i.e. 80-400 ms). This effect is termed prepulse inhibition (PPI), and is a widely used method for studying attention. PPI of the post-auricular reflex (PAR), another startle response, has yet to be directly tested in human participants.

Purpose: The present study tested whether the post-auricular reflex, a weak electrical response of the muscle behind the ear, exhibits prepulse inhibition (PPI).

Methods: Twenty-five healthy young adult participants were prompted to indicate the number of target stimuli (pink checkerboards) they saw amid non-targets (red checkerboards). Most of these visual stimuli were immediately followed by a train of six white noise bursts (105 dB SPL). Amplitudes of the PARs elicited by these noise burst were compared between trials with and without a visual prepulse (i.e., checkerboard). Responses for each of the six acoustic startle probes were quantified separately for the left and right post-auricular muscles.

Results: Planned comparisons indicated the presence of PAR inhibition for trials with a visual prepulse, compared to trials without a visual prepulse (control trials). This effect was observed when the lead time was 100 ms. A sustained facilitation effect was observed at later lead times for trials with a visual prepulse when compared to control. Supplementary analyses indicated an absence of habituation across 3,600 reflexes per subject.

Conclusions: The post-auricular reflex does exhibit prepulse inhibition, albeit brief in duration. Potentiation of amplitude was observed at intermediate and long lead times, likely due to an arousal effect. Similar to other oligosynaptic reflexes, the PAR is resistant to habituation.

INTRODUCTION

Understanding attentional processes and their underlying neural correlates is of fundamental interest within the field cognitive neuroscience. A useful approach to understanding low-level mechanisms is investigating the effects of attention on reflexes. Reflexes are ideal for such investigation, as they possess known neural correlates. Therefore, reflexes can be a pure, straightforward measure of simple attentional mechanisms.

A large body of literature exists linking attention and reflexes. A classic example of this relationship is the startle eye-blink reflex (Fillion, Dawson, & Schell, 1998; Blumenthal, 1999). Research has shown that when participants are asked to attend to the location of an intense acoustic stimulus, the startle eye-blink reflex elicited by the stimulus is faster, relative to when they are attending to a different location (Hackley & Graham, 1987, Experiment 1).

In addition to this effect of attending to the startle stimulus, attention effects on a pre-startle stimulus, or lead stimulus, have also been documented using prepulse inhibition. Prepulse inhibition (PPI) is a startle modification technique wherein a lead stimulus is presented within a certain amount of time (20-700 ms) prior to a startle-eliciting stimulus (Blumenthal, 1999). This paring of a lead stimulus with a startle-eliciting stimulus produces a reliable modulation of the startle reflex, specifically, a reduction of reflex amplitude. When subjects are instructed to attend to the lead stimulus (versus not attending or ignoring the lead stimulus), the amount of reflex inhibition in response to the startle-stimulus is significantly greater. This attentional effect on blink reflex inhibition using a lead stimulus has been replicated multiple times across several

sensory modalities (DelPezzo & Hoffman, 1980; Elden & Flaten, 2003). Using a startle modification paradigm termed prepulse inhibition (PPI), Hackley and Graham (1987, Experiment 2) reported that the amplitude of a tactile blink reflex is more strongly inhibited when participants attended to the expected location of a prepulse compared to when they attended to a different location. As a consequence of these findings, the blink reflex continues to be the gold standard for attentional research involving startle modification paradigms. To date, research utilizing other components of startle to measure attentional modulation is scant in the literature.

The purpose of the present experiment is to explore the attentional modulation of another reflex—the post-auricular reflex (PAR). The PAR is a simple, acoustically activated response observed in the muscle behind the ear (Figure 2). This reflex is the fastest response of which humans are capable (Hackley, 1993). Research shows the PAR is impacted by the allocation of attention, in spite of its simplicity and automaticity. In the same study mentioned in the previous paragrah, Hackley and colleagues (1987) reported decreased PAR amplitudes (as measured by electromyography) when participants were attending to the location of a lead stimulus that preceded the startle-eliciting stimulus (a loud, abrupt tone pip), relative to when they were attending to a different location. This finding is comparable to the results of several startle eye-blink studies (DelPezzo & Hoffman, 1980; Hackley & Graham, 1987; reviewed in Filion et al., 1998), wherein greater suppression of eye-blink reflex amplitude was observed for attended as compared to non-attended lead stimuli.

A parallel between lead stimulus modulation of the PAR and the eye-blink reflex has yet to be examined in human subjects. If the PAR behaves similarly to the startle eye-blink response, it may be advantageous for translational research concerning the neural basis of various types of plasticity. Compared to the startle-blink reflex, the PAR's neural circuitry is simpler and better understood, rendering it potentially more beneficial for studies involving attentional processes (a more extensive literature review regarding the neural circuitry of both PPI of the blink reflex and the PAR is provided in Appendix B). The current study will explore the PAR's susceptibility to passive attentional phenomena using a PPI startle modification paradigm. While PPI of the blink reflex has been investigated for decades, PPI of the PAR remains relatively unexplored in the literature.

Inhibition (decrease in reflex amplitude) of the rodent pinna-startle reflex (comparable to the human PAR) and inhibition of the PAR in humans have both been previously documented (Cassella & Davis, 1986; Li & Frost, 2000; Fox et al., 1989). However, the inhibition itself is ambiguous in nature. It is difficult to ascertain whether the inhibition observed in these studies is due to refraction, acoustic middle ear reflexes, habituation, or PPI. Reflex modulation of the PAR would be considered classic PPI if the same neural pathways mediating PPI of startle are involved in producing the inhibition observed. More specifically, the established mechanism consists of an inhibitory pathway from the pendunculopontine tegmental nucleus to the caudal pontine reticular nucleus (the startle center; see Figure 1; also see Appendix B). This inhibitory pathway is responsible for the manifestation of PPI of rat whole-body startle (Koch & Schnitzler, 1997) and possibly human startle eye-blink reflex (Fendt, Li, & Yeomans, 2001; Swerdlow, Geyer, & Braff 2001). Whether it applies to the PAR (Figure 1) is unknown.

There exist two rodent studies (Cassella & Davis, 1986; Li & Frost, 2000) and one human study (Fox et al., 1989) wherein inhibition of the pinna reflex (in rats) or PAR (in humans) was found. In one rodent study, researchers Cassella and Davis (1986) presented rats with an acoustic lead stimulus (55, 60 or 65 dB depending on condition; 20 ms duration), followed 100 ms later by an acoustic startle stimulus (106 dB, 10 ms duration). Cassella and Davis reported the rodents' pinna-retraction reflex was significantly inhibited by the lead stimulus in each prepulse condition, compared to the startle stimulus-alone control. In addition, a study by Li and Frost (2000) also confirmed PPI for rat pinna-startle. They further reported that the inhibition was not impacted by the location of the acoustic lead stimulus relative to the startle stimulus.

A few concerns limit attempts to extrapolate these findings to human subjects.

(1) Possible differences in biological musculature. It is important to consider that the pinna-retraction musculature in rats may not correspond to the post-auricular musculature in humans, since the pinnae are permanently retracted in humans and apes, unlike other species (Johnson, Valle-Inclan, Geary, & Hackley, 2011).

(2) Possible refractory effects. Due to the fact that the lead stimuli used in both studies (Cassella & Davis, 1986; Li & Frost, 2000) were acoustic, the possibility that the lead stimulus itself may have induced a small pinna reflex before the startle stimulus was delivered must be considered. If two reflexes are triggered in rapid succession, the second reflex would be smaller, due to refractory effects. The resulting suppression could have mimicked inhibitory processes.

(3) Possibility of a stapedius reflex. Consider that one middle ear muscle, the stapedius, naturally contracts when the middle ear cavity encounters loud acoustic stimuli

(Moller, 1974). This is an automatic protective mechanism, as the muscle contraction protects the inner ear from noise-induced injury. If the lead stimulus (e.g. an acoustic tone pip) activated this middle-ear muscle contraction, an inhibited PAR may be observed, which would be a mechanism entirely independent of prepulse inhibition. The prepulse intensities used in these studies would be too low to elicit a protective response in humans (85 dB threshold; Moeller, 1974), but the threshold may be different in rodents. Using a non-auditory lead stimulus is advantageous when studying prepulse inhibition of the PAR, as this alternative interpretation for observed inhibition would be eliminated.

Fox and colleagues (1989) reported suppression of the PAR in human subjects using a cutaneous lead stimulus. They found that medial nerve wrist shock, preceding a pair of auditory probes (each 100 dB), inhibited the PAR at a lead time of 40 ms (Figure 3). This finding is important because it argues that the PAR can be inhibited at short lead intervals when using a cross-modal lead stimulus. Whether or not this inhibition is due to the activation of brainstem PPI circuits or general suppression of muscle activity renders the interpretation of PAR inhibition ambiguous. Consider the possibility that the inhibition observed by Fox et al. was due to suppression of the post-auricular muscle and not detection of the cutaneous prepulse, in which case the inhibition would not be a product of PPI. In their study, Fox and colleagues (1989) reported suppression of spontaneous activity in the post-auricular muscle for approximately 60 ms following the wrist shock. The inhibition of the PAR, in this case, could be due to a reduction in the general responsivity of the post-auricular muscle. Other studies have shown the PAR is sensitive to background muscle activity (O'Beirne & Patuzzi, 1999). It should be noted

that Fox and colleagues (1989) were not assessing for PPI of the PAR, specifically in their study.

Current Study

The intent of this experiment was to establish whether the human PAR exhibits PPI comparable to that of the startle blink reflex. To achieve this in an unambiguous manner, I paired a large, visually salient lead stimulus with a series of acoustic startle stimuli. Employing a lead stimulus that is visual, instead of acoustic, will reduce uncertainty in any observed inhibition effects on the PAR because a visual stimulus should not itself trigger a PAR or middle-ear reflex. If inhibition can be observed under these experimental conditions, this would be evidence that the inhibition is not merely due to middle-ear muscle contraction, refraction, or general sensitivity of the muscle response itself, in other words, that the PAR exhibits classic PPI.

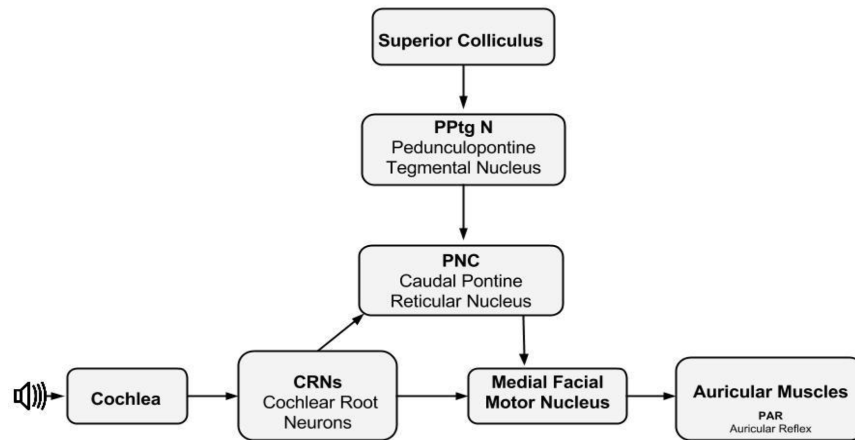

*Figure 1***.** Proposed acoustic pinna reflex pathway (adapted from Horta-Junior et al., 2008). A modulatory pathway has been added (PPtgN).

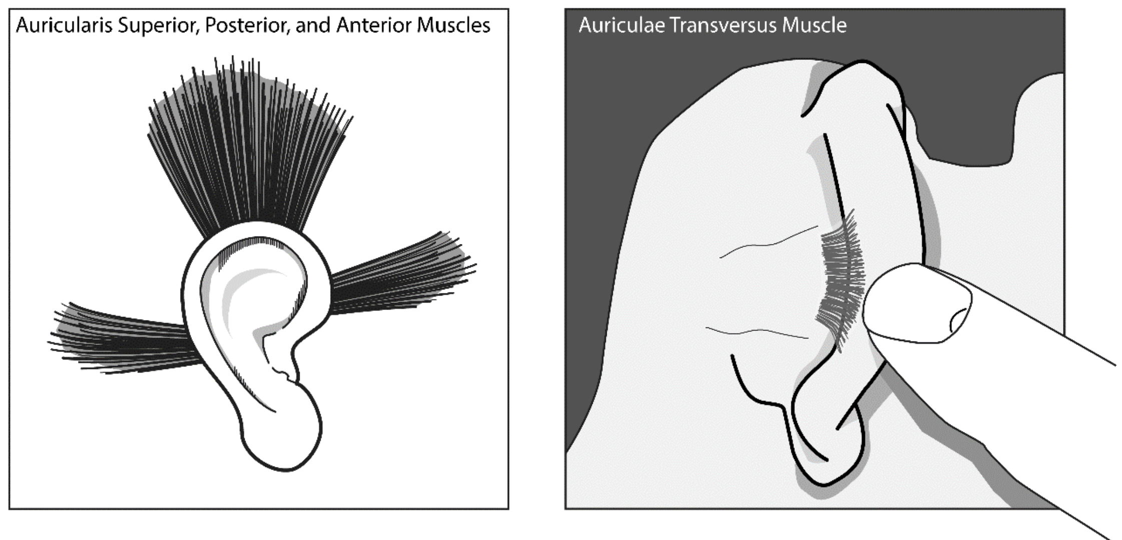

Figure 2. Schematic of the auricular muscles. The second picture (located on the right) depicts the muscles when the ear is pulled forward, which was the method used to locate and place the electrodes over the post-auricular muscles.

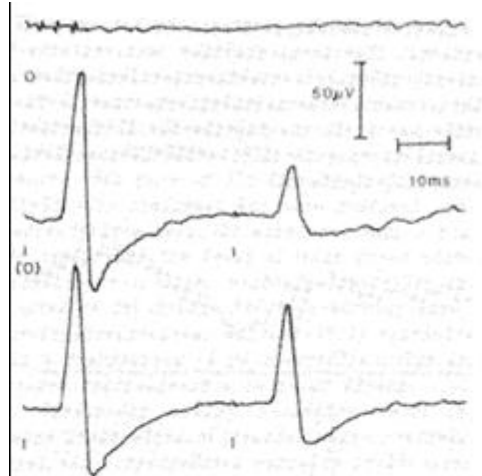

Figure 3. Suppression of activity in the post-auricular muscle (PAM) in human subjects using a shock lead stimulus and train of two acoustic probes (Fox et al., 1989). The top trace shows PAM activity without acoustic or medial nerve stimulation. The middle trace shows medial nerve shock, (o), paired with an acoustic startle probe (100dB), (|) , followed by another identical acoustic startle probe (40 ms lead time interval). The third trace shows the PAM response to a train of two acoustic probes (100dB, 40 ms lead time interval). Note that when pairing an acoustic probe with a wrist shock, the amplitude of the second post-auricular response is especially reduced in size.

METHODS

Participants

Thirty young adults (17 women) who were either graduate or undergraduate students (M= 20 years old) at the University of Missouri participated in this study. Among these, 25 subjects exhibited reflexes with a signal-to-noise ratio adequate for inclusion in the final analysis. Participants were either recruited from an introductory psychology course or paid for their participation at a rate of $8.00 per hour. Ethical approval was granted by the campus Institutional Review Board and written informed consent was obtained from each participant.

Apparatus

Following the consent process, Ag-AgCl surface electrodes (11 mm) were attached using adhesive collars. Participants were then seated in an upright chair positioned with their face 50 cm in front of a 32 cm × 23 cm computer monitor. They were provided with Sony (Model MDR-NC7) headphones. Electrophysiological data were recorded on a desktop computer utilizing NeuroScan acquisition software (NeuroScan, Inc., Herndon, VA). A second computer in the same room was used to run the stimulus presentation program developed for this experiment. This program was created using MatLab software (Math Works, Inc., 2013) in conjunction with Psychophysics Toolbox (Brainard, 1997; Pelli, 1997).

Design and Procedure

Participants were first familiarized with the experimental design via a practice block prior to beginning the experiment. The practice block included 10 of each of the trial types described below, presented in random order. On a typical trial, participants would see a briefly presented pink or red circular checkerboard (similar to a standard dart board) centered around the fixation point (Figures 4 & 5).The checkerboard was about 20˚ in diameter. This visual lead stimulus lasted 80 ms and constituted the beginning of a trial. Six white noise bursts were presented, beginning either simultaneous with the onset of the lead stimulus (Figure 4) or at 100 ms following lead stimulus onset (Figure 5). These two trial types are referred to as “Train Delay: 0 ms” (TD-0) and “Train Delay: 100 ms” (TD-100), respectively. A fixation point was continuously presented throughout each block of trials, even during the inter-trial interval.

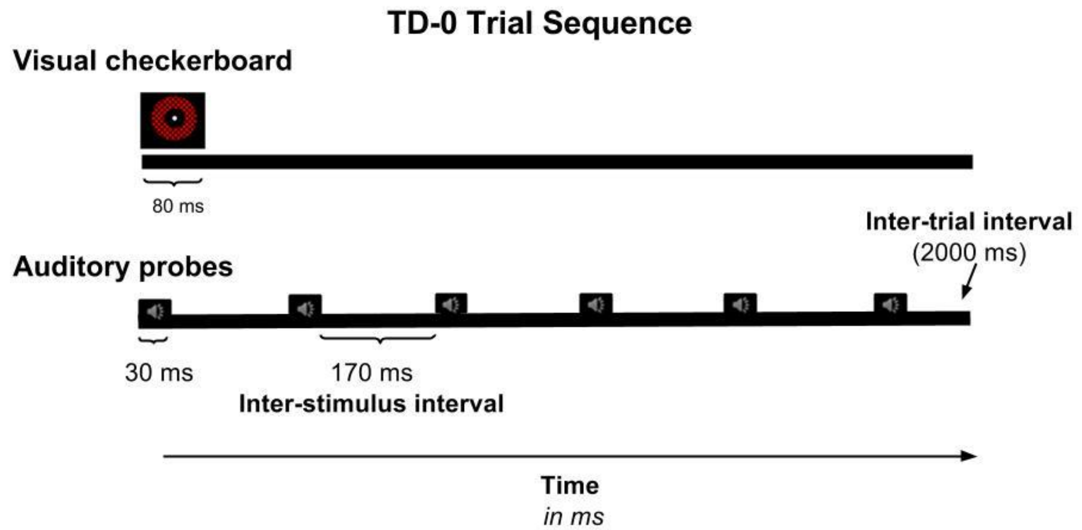

Figure 4. Detailed trial structure for the Train Delay: 0 ms (TD-0) condition. Trials of this type allowed reflex modulation to be tested at lead times of 0, 200, 400, 600, 800, and 1000 ms.

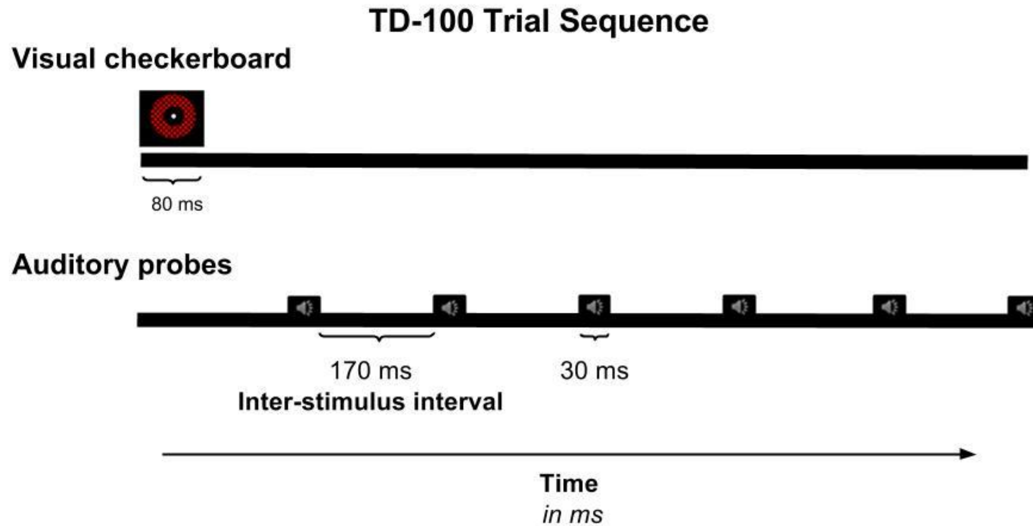

Figure 5. Detailed trial structure for the Train Delay: 100 ms (TD-100) condition. On these trials, modulation of the PAR was assessed at lead times of 100, 300, 500, 700, 900, and 1100 ms.

Auditory control trials consisted of the six white noise bursts only, and will be referred to as the "No Visual Stimulus" (NVS; see Figure 6) trials. The six white noise bursts used in this study were 30 ms in duration, 170 ms apart, and approximately 105 dB SPL-A in amplitude (measured at steady-state). Visual-only trials consisted of the checkerboard with no startle stimuli.

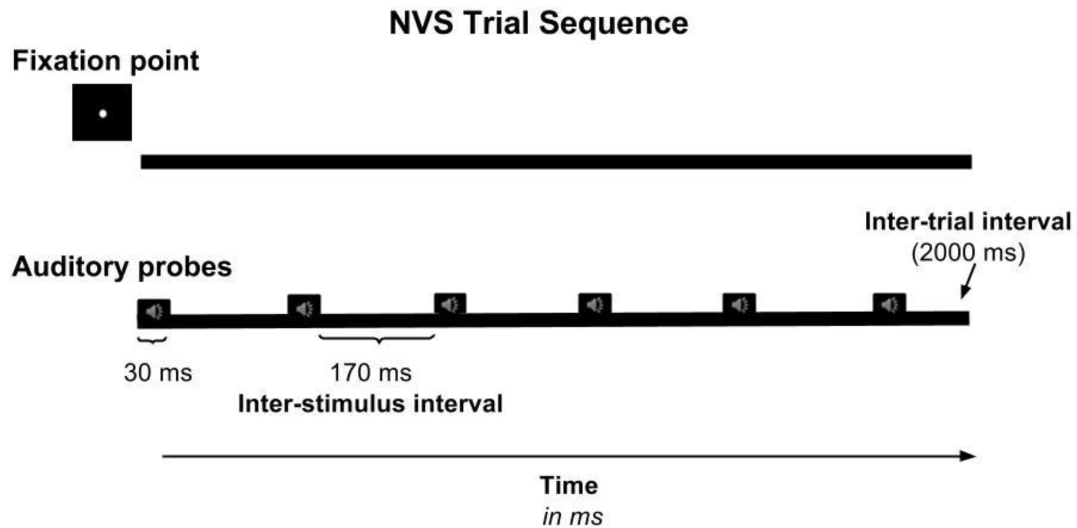

Figure 6. Detailed trial structure for the No Visual Stimulus condition, which was the main control condition.

Trials were randomized within each of 20 blocks, with 40 trials per block. At the end of each block, participants were prompted to indicate the number of pink checkerboards (occurring 7.5% per block) they saw in that block by pressing the corresponding number on the computer keyboard. Accuracy of key responses were not analyzed. This task was designed to encourage participants to pay attention to the visual lead stimuli. Inter-trial intervals (ITIs) lasted 2000 ms. Inter-block intervals were longer, in that participants were given an opportunity to take a short break if they wished. Subjects then pressed a key to indicate they were ready to begin the next block.

Physiological Recordings

Electromyogram (EMG) readings were recorded from the left and right orbicularis oculi and the auricularis posterior muscles (Figure 7). In the latter case, electrode placement was determined by pulling the subject's ears forward to better visualize the muscle location (Figure 1). Vertical and horizontal electrooculograms (EOG) readings were also recorded, utilizing standard periorbital locations (Figure 7). Blinks and eye movements were recorded for the purpose of artifact rejection procedures, which are discussed in the next section. Impedances of 15 kΩ or lower were achieved by rubbing the skin with a small disposable alcohol pad. The Ag/AgCl surface electrodes were attached with double adhesive collars. Raw EMG (filtered online with a bandpass of 3-300 Hz) and EOG (bandpass of .01- 30 Hz) signals were recorded continuously throughout the duration of the experiment, with a sampling rate of 600 Hz.

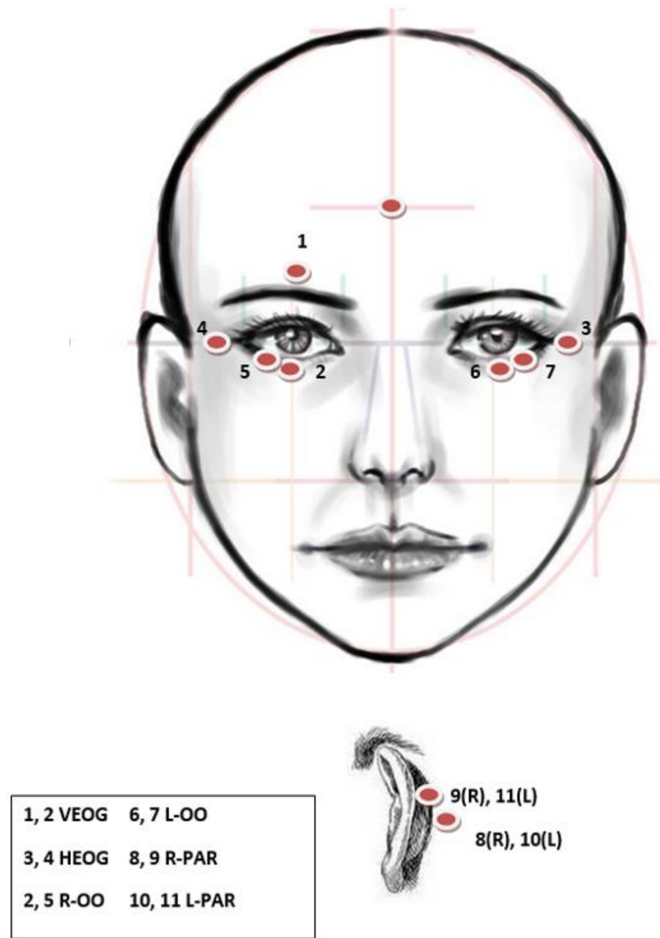

Figure 7. Schematic representation of electrode placement used in this study. VEOG and HEOG (1,2,3,4) indicate vertical and horizontal electrooculogram, L- and R-OO (2,5,6,7) indicate left and right orbicularis oculi, and L- and R-PAR (8,9,10,11) indicate left and right post-auricular muscles.

Data Processing and Analysis

The EMG data were rectified and epoched offline with EEGLab (Delorme & Makeig, 2004) and ERPLab (Lopez-Calderon & Luck 2014) software. Epochs with extensive movement artifacts (e.g. blinks, saccades) were eliminated from data analysis. Left and right post-auricular muscle EMG channels were averaged together. The entire data set was signal averaged, collapsing across conditions. This signal averaged prototypical waveform was then compared to each individual trial. If the correlation

coefficient between the averaged waveform and individual trial was greater than .20, the trial was accepted. Participants were required to have at least 50 good trials in each condition for acceptance. A total of 30 subjects participated in this study, but only 25 met the parameter requirements detailed above. Data from these selected trials and subjects were then re-averaged with separate bins for each condition.

Post-auricular reflex responses to the six probes were quantified separately for each condition by measuring base-to-peak amplitude, with windows beginning at acoustic probe onset and ending 50 ms after onset. Baseline values were the smallest values in the window, peak values were the largest. Six paired t-tests were conducted at each SOA in Train Delay 0 and also in Train Delay 100, comparing the lead stimulus conditions to the No-Visual-Stimulus condition. In addition to this analysis, the data were subjected to two 2x6 repeated measures ANOVAs. These ANOVAs assessed the relationship between ordinal probe position (probe 1 through 6) and trial condition separately for the TD-0 and TD-100 conditions, each compared to the NVS control condition.

Changes in PAR amplitude during the 1100 ms after a target were expected to reveal attention-related effects. However, there is little basis in the literature for clear-cut predictions. The most closely related study, that of Parks, Hilimire, and Corballis (2009) involved monitoring visual task stimuli embedded within a continuous stream of PAR-eliciting clicks. In a condition in which the visual discrimination was especially difficult, smaller PARs were observed. If the pink checkerboards in this study engage more careful visual scrutiny, enhanced PPI may be obtained. Pink (target) and red (standard) lead stimulus trials were analyzed separately.

RESULTS

Red Checkerboard Trials

The trains of stimuli were effective at eliciting reflexes at each ordinal position (probes 1-6) across conditions. Reliable modulation effects were also observed. Paired-sample t-tests comparing PAR peak amplitude at each SOA revealed no effect of the visual prepulse at the shortest lead time in the TD-0 condition (0 ms). There was a significant difference in peak amplitude for the TD-100 condition (M=8.01; SE=1.64) compared to the NVS control condition (M=9.01; SE=1.59) at 100 ms [$t(24) = -2.62$, $p = .015$]. This is the most important finding in this study, in that it is indicative of prepulse inhibition (Figure 8).

Facilitation effects were observed in the TD-100 condition at lead times of 300 ms [$t(24)=3.31$, $p = .003$], 500 ms [$t(24) = 4.64$, $p = .0001$], 700 ms [$t(24) = 5.23$, $p = .00002$], 900 ms [$t(24) = 6.25$, $p = .000002$] and 1100 ms [$t(24) = 5.11$, $p = .00003$]. Similarly, there was a significant enhancement of peak amplitude for the TD-0 condition (M=9.52, SE=1.71) compared to the NVS condition at 400 ms [$t(24)=4.29$, $p = .0003$], 600 ms [$t(24)= 4.12$, $p = .0004$], 800 ms [$t(24)=4.70$, $p = .00009$], and 1000 ms [$t(24)=3.47$, $p = .002$]. These results indicate reflex facilitation at all lead times greater than 200 ms.

Two separate 2x6 repeated-measures ANOVAs revealed main effects for ordinal probe position in the TD-100 vs. NVS condition [$F(5,120) = 14.27$, $p = .00001$] and in the TD-0 vs. NVS condition [$F(5,120) = 12.59$, $p = .00001$]. Amplitude variations by lead time and ordinal probe position are captured in Figure 8 and 9. Main effects of condition type were observed for both the TD-100 [$F(1, 24) = 22.34$, $p = .005$] and TD-0

conditions [$F(1,24) = 16.91$, $p = .001$] compared to control, which mainly reflect prepulse facilitation (PPF). An interaction effect for condition type and ordinal position on PAR peak amplitude for the TD-100 [$F(5,120) = 12.37$, $p = .00001$] and TD-0 conditions [$F(5,120) = 10.57$, $p = .00001$.] indicates that significant differences in amplitude between prepulse and control conditions were dependent on the lead time. The planned comparison described above makes it clear that inhibition occurred at onset asynchronies less than 200 ms and that facilitation occurred at asynchronies greater than 200 ms.

Pink Checkerboard Trials

Similar to the red, non-target checkerboard analyses described above, paired-sample t-tests comparing PAR peak amplitude at each SOA in the pink target checkerboard analysis were conducted. These t-tests revealed sustained facilitation for the TD-100P condition (M=10.05; SE=1.68; "P" stands for "Pink") compared to the NVS control condition (M=8.84; SE=1.59). This was significant across lead times of 500 ms [$t(24)=2.49$, $p = .02$], 700 ms [$t(24)=5.43$, $p = .00001$], 900 ms [$t(24)=5.51$, $p = .00001$], and 1100 ms [$t(24)=4.72$, $p= .00008$]. Facilitation effects were also observed for the TD-0P condition (M=10.39, SE=1.81) compared to the NVS condition at 400 ms [$t(24)=2.79$, $p = .01$], 600ms [$t(24)=4.54$, $p = .0001$], 800 ms [$t(24)=4.85$, $p = .00006$] and 1000ms [$t(24)=4.62$, $p = .0002$]. These results indicate reflex facilitation at all lead times except 200, 300, and 400 ms. There were fewer trials per condition but, nonetheless, the general observed pattern observed on red non-target trials was confirmed. As shown in Figures 8 and 9, the potentiation was even greater than on non-target trials.

Two separate 2x6 repeated-measures ANOVAs revealed main effects for ordinal probe position in the TD-100 vs. NVS condition, [$F(5,120) = 16.21$, $p < .00001$] and in the TD-0 vs. NVS condition, [$F(5,120) = 14.85$, $p < .00001$]. Main effects of condition type (i.e., prepulse vs. control) were observed for the TD-100P condition, [$F(1, 24) = 16.77$, $p < .005$] and TD-0P condition [$F(1,24) = 23.38$, $p < .008$]. This mainly reflects prepulse facilitation (PPF).

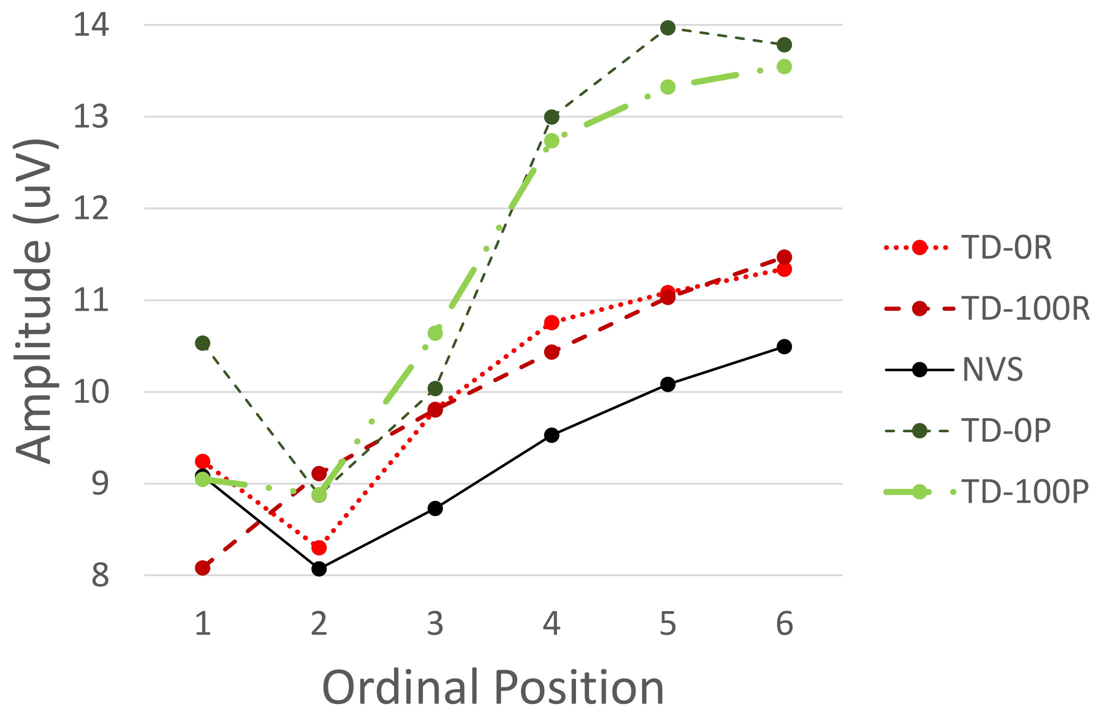

*Figure 8***.** Average PAR peak amplitude as a function of ordinal probe position and lead stimulus condition. TD-0R and TD-0P represent the conditions in which the red (R) or pink (P) checkerboard prepulses onset simultaneously with first acoustic startle probe. TD-100R and TD-100P label the conditions in which the red (R) or pink (P) checkerboard prepulses onset 100 ms before the first startle probe. NVS is the no-prepulse control condition.

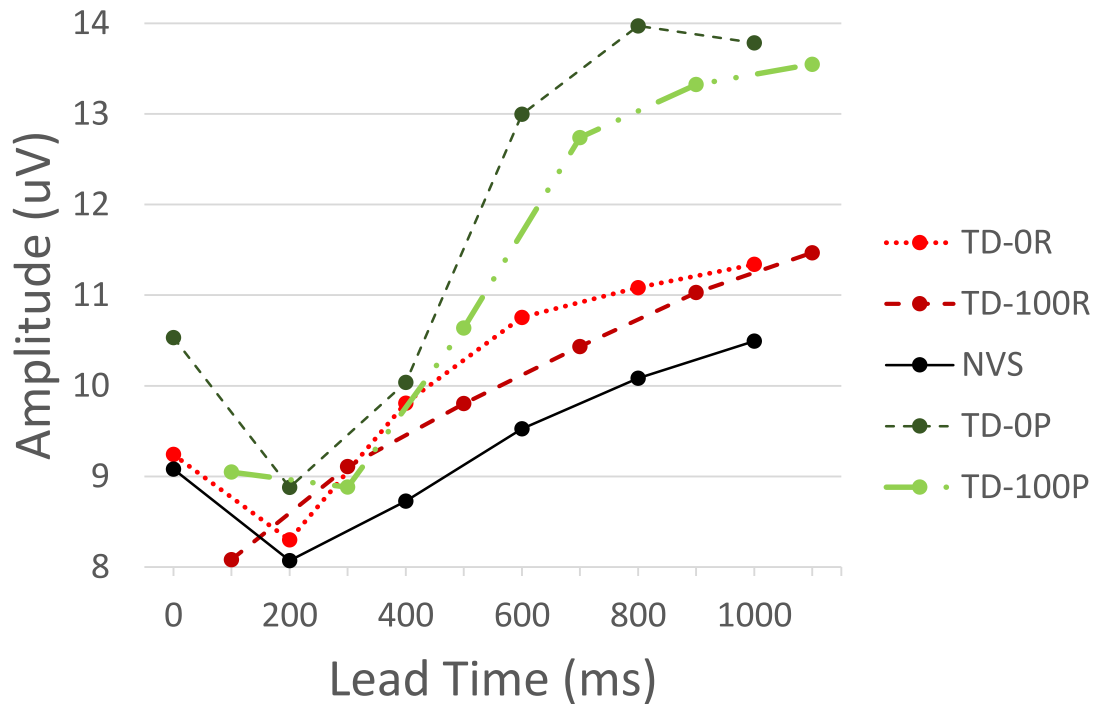

*Figure 9***.** Average PAR peak amplitude as a function of lead time and prepulse condition. Note inhibition at 100 ms lead time followed by a subsequent facilitation that is sustained from about 300 to 1000 ms.

Pink vs. Red Checkerboard Trials

As evidenced by Figure 8, there was a significant enhancement of peak amplitude for the TD-0P and TD-100P (target) conditions compared to the TD-0 and TD-100 (non-target) conditions at ordinal positions 4-6. For the fourth ordinal position, TD-0P was significantly higher than TD-0 (red) *[$t(24)=3.12$, $p = .005$]* and TD-100P was significantly higher than TD-100 (red) *[$t(24)= 4.56$, $p = .0001$]*. For the fifth ordinal position, TD-0P *[$t(24)=4.05$, $p = .0005$]* and TD-100P *[$t(24)= 3.85$, $p = .0007$]* followed a similar trend when compared to the TD-0 and TD-100 (red) conditions,

respectively. For the sixth ordinal position, TD-0P was significantly higher than TD-0 (red) *[t(24)=3.79, p = .001]* and TD-100P was also significantly higher than TD-100 (red) *[t(24)= 3.01, p = .006]*.

Habituation

Comparison of the first and last 10% of trials (deciles) in the NVS condition across ordinal position revealed no significant difference in amplitude [$F(1,24) = 0.31$, $p=.3$] across participants (see Figures 10 & 11). A 2 (decile) x 6 (ordinal position) repeated measures ANOVA was conducted to assess for any significant difference in peak amplitude between the first 10% percent and last 10% of trials in the NVS control condition and whether such differences varied across the six probe positions. The analysis confirmed a main effect for ordinal position [$F(5,120) = 8.06$, $p< .001$] (see Figure 11). Post-hoc comparisons among the six positions clarified this main effect. In the first decile, paired t-tests revealed that the response in the second ordinal position was significantly lower in amplitude compared to the first *[t(24)=2.52, p = .02]* and sixth ordinal positions *[t(24)=4.56, p = .0001]*. In the last decile, mean amplitude in the sixth position was significantly higher compared to the first *[t(24)=3.22, p = .004]* and second *[t(24)=3.71, p = .001]*. Habituation was also assessed across the experiment in its entirety (Figure 12). In this analysis, I collapsed across probe positions 1-6, averaging the PAR response across trials within each tenth of the experiment (averages are indicated in Figure 12). Despite the large number of startle probes per session (3,600), there was no significant decline in average PAR amplitude as a function of decile [$F(9, 216) = 1.49$, *p*

= .21]. Unlike other components of the startle response, such as the startle-blink reflex (Haerich, 1996), the PAR is highly resistant to habituation.

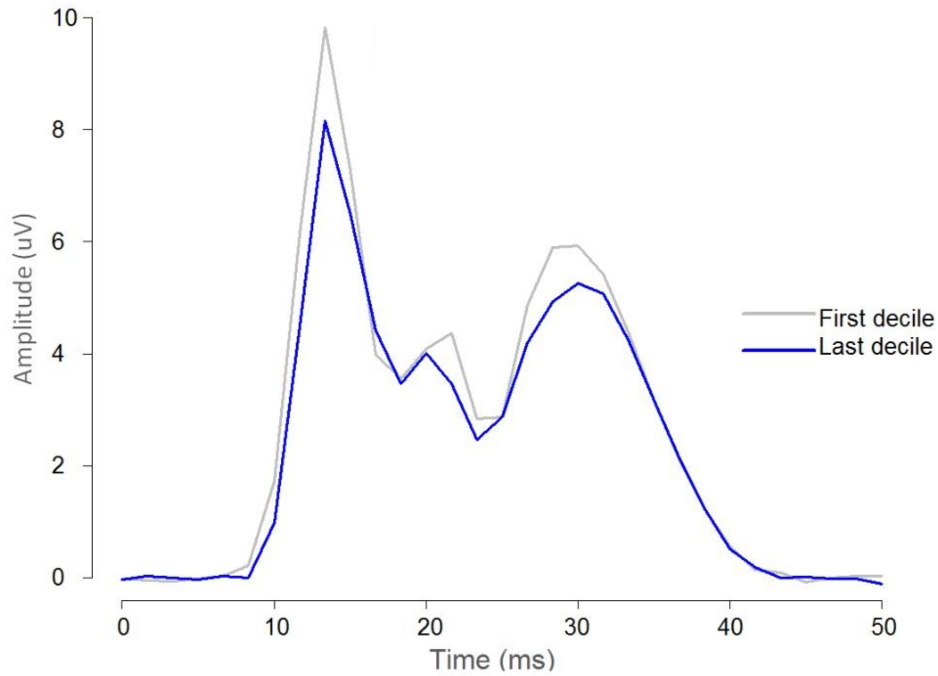

Figure 10. Grand averages for the PAR for the first and last decile of the experiment. This waveform reflects the average post-auricular response at the first acoustic startle probe position. The first decile (grey) is approximately 5% higher in amplitude than the last decile (blue). This difference was non-significant, indicating that the PAR is highly resistant to habituation.

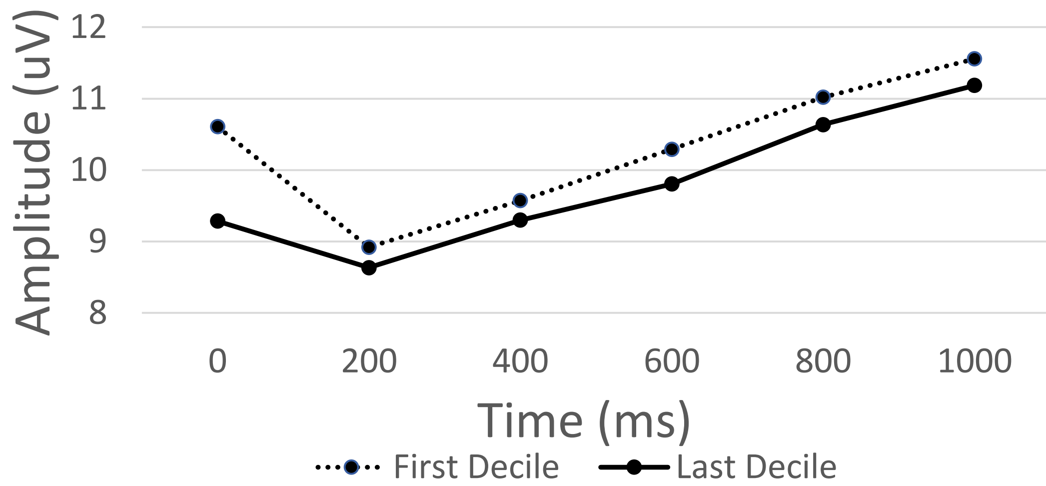

Figure 11. Average PAR amplitudes for the six probe positions recorded during the first and last deciles. An effect of ordinal position was observed, but the interaction with decile was not significant.

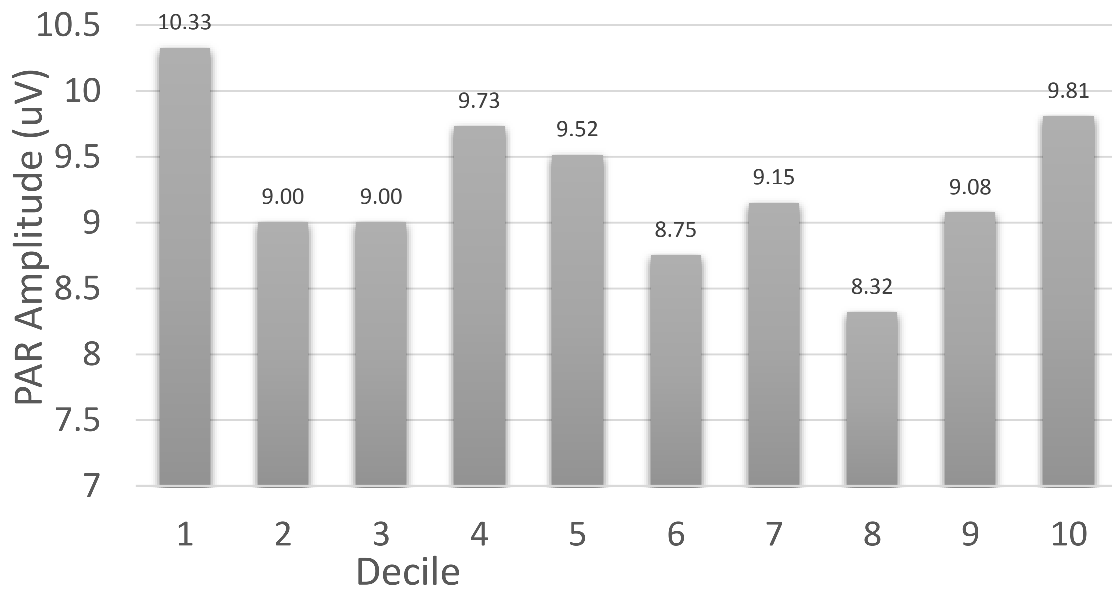

Figure 12. Average PAR amplitude at each decile of the experiment (across-experiment changes).

DISCUSSION

Albeit modest in range and depth, the results of this study provide evidence for PPI of the PAR. Results indicate inhibition only at 100 ms lead time and only on trials in which the lead stimulus was not a target (i.e, only on red checkerboard trials). This finding is comparable to that of Fox and colleagues (1989), in which they report inhibition of the PAR was observed at 40 ms following a shock to the wrist, the only lead time they tested. Rodent studies indicated inhibition of the PAR at 100 ms. Cassella and Davis (1986) reported inhibition of the rodent pinna-startle at 100 ms following the presentation of an acoustic lead stimulus. Li and Frost (2000) showed that the rodent pinna startle response is significantly inhibited following presentation of an acoustic lead stimulus at 100 ms. Again, only one lead time was tested in these studies, Taking the consistency of the four studies into consideration, these findings give credence to the claim that the PAR is modulated by PPI, at least at short lead times.

Facilitation at long lead times

Unexpectedly, the results of this study did not show inhibited PARs at a broad range of lead times. Instead, results showed a sustained facilitation effect at later lead times (Figures 8 & 9). This finding may be explained in a couple of ways.

(1) Facilitation as a function of diminishing PPI and increasing arousal. The results of this study indicate a decay of prepulse inhibition after 100 ms, followed by a facilitation effect sustained from about 300 to at least 1000 ms lead time. This increase in PAR amplitude (Figure 9) may be due to an arousal effect. The mechanism underlying this arousal may be norepinephrine (NE) accumulation at the post-auricular muscle

(PAM) motor neurons, a process which has been shown to facilitate the masseteric (jaw-jerk) reflex in cats (Stafford & Jacobs, 1990 a and b). Much like the PAR, the masseteric reflex is oligosynaptic (neural pathway with only a few synapses), with a short-latency of 15-20 ms (Komiyama et al., 2008). Stafford and Jacobs assessed the masseteric response in cats employing (a) several minutes of 100 dB white noise or (b) 113 dB single clicks at staggered lead times prior to electrically stimulating the proprioceptive nerve coming from the masseter muscle. Stafford and Jacobs reported reflex facilitation (a) throughout the duration of exposure to the acoustic white noise and (b) 100-150 ms after the click was presented. To test their hypothesis that NE augmented the masseter response, the researchers then injected the cats with either a NE (prazosin) or serotonin antagonist (methysergide) under the experimental conditions described above, (a) and (b). In both cases, administration of the NE antagonist eliminated the facilitation effect. The serotonin antagonist had no effect the modulation. It may be that potentiation of the PAR, much like the masseteric reflex, is mediated by NE. Increased NE activity at the PAM motor neurons may be, at least in part, responsible for the facilitation effect I observed (e.g., Figure 9).

(2) Facilitation as a function of muscle tension effects. Previous studies indicate the PAR is sensitive to background muscle activity (Fox et al., 1989; O'Beirne & Patuzzi, 1999). Lundberg and colleagues (1994) reported that mental stress associated with performance of a Stroop task or a mental arithmetic task caused increased EMG activity in a neck muscle. Subjects may be tensing their muscles in response to the loud acoustic stimuli, causing the facilitation effect we observe in Figure 8 and 9. Subjects may tense even more when they encounter the bright pink checkerboard, to which they are

instructed to attend. Detection of a target may have produced a similar effect at later lead times.

Habituation

This study delivered a total of 3,600 acoustic probes per experimental session. Raw EMG data from this experiment shows that this reflex does not disappear over an extended period of time. This presents a question of whether habituation occurs. To my knowledge, no analysis of PAR habituation has been conducted. I assessed habituation effects of the PAR in my experiment in two ways.

(1) Across-experiment changes. PAR amplitudes were assessed at each decile of trials for the NVS condition only. Using the NVS condition avoided confounding across-trial changes in PPI or PPF with reduction of the unmodified PAR. Results indicate the average PAR amplitude in the last decile of the experiment was slightly lower than in the first decile of trials (approximately 5% lower, N.S.; see Figure 10). No significant differences were observed in this analysis across the 10 deciles (see Figure 12). Habituation is defined as an overall decrease in a response following a period of repeated stimulation (Groves & Thompson, 1970). As evidenced in Figure 12, there is no gradual decline in PAR reflex amplitude from the beginning to the end of this experiment. The average response of the PAR, over time was modest in size and erratic in time course. This suggests that the PAR is highly resistant to habituation, even after repeated exposure to many loud, acoustic white noise bursts for a 45 minute experimental session. It would seem that the PAR has minimal long-term plasticity, at least in the sense of habituation.

(2) Within-trial changes. PAR amplitudes on NVS trials were assessed at each ordinal position (startle probes 1-6). A non-monotonic pattern of average amplitude was observed (see Figure 11), wherein the response to the second probe was smaller than that of the first and third responses. This was most likely caused by a refractory effect and a stapedius reflex to the first of six white noise probes. The PAR response decreased in amplitude around 200 ms, which was followed by a gradual recovery (400-1000 ms). The refractory period of the PAR is approximately 90-100 ms (Fox et al., 1989), which is captured in the decreased response to the second white noise probe (Figure 11). Therefore, it seems that the PAR is susceptible to processes of refraction, but only slightly to habituation.

Relation of the PAR to other oligosynaptic reflexes

Much like the PAR, the R1 blink reflex to trigeminal nerve stimulation is oligosynaptic and has a latency of about 10 ms (Ison et al., 1990; Sanes and Ison, 1979). The facilitation effect observed in the current study is similar to the R1 facilitation reported by Sonnenberg and colleagues' (2006). Sonnenberg and colleagues reported facilitated R1 amplitudes from lead times of 80-1000 ms following presentation of a visual lead stimulus, an effect they attributed to arousal. As both the PAR and R1 reflex share similar biological properties (oligosynaptic reflex arc, short onset latency), it may be that the PAR and R1 reflex share a common mechanism in regard to lead stimulus effects.

As mentioned earlier, the masseteric (jaw-jerk) reflex also has a simple neural circuit and short onset latency (Komiyama et al., 2008). In a study on this reflex, Stafford

and Jacobs (1990) demonstrated a facilitation effect on the jaw-jerk response in cats. When cats were exposed to continuous acoustic white noise or loud clicks, Stafford and Jacobs found their masseter response was reliably facilitated. The pattern of facilitation they observed was similar to that of the PAR in this thesis.

The Hoffman (H) reflex is also oligosynaptic and resistant to habituation (Rothwell et al., 1986). This response is a proprioceptive muscle reflex elicited by electrical stimulation, usually in the leg (Akins, 1981). Rothwell and colleagues (1986) reported that the short-latency H-reflex amplitude remained unaffected by repeated stimulation over time, whereas the long-latency response diminished. It may be that simple neural circuitry is conducive to habituation resistance.

Conclusions

In conclusion, prepulse inhibition of the post-auricular reflex was observed at a lead time of 100 ms in human subjects, similar to other findings within the startle blink literature. The inhibition reported here does not last as long as in the other components of human startle, such as the acoustic eye-blink reflex (120-1000 ms.; Sonnenberg et al., 2006). Because the lead stimulus in this experiment was visual and not auditory, we may assume that the PAR modulation reported here is not due to middle-ear reflexes or refraction. This finding is also congruent with previous similar reports in rodent models, wherein the pinna-startle reflex exhibited similar results. Therefore, this could serve as an effective translational paradigm in testing PPI at short lead times. Future research investigating PAR modulation could potentially identify certain neural mechanisms that underlie cognitive processes such as attention in humans.

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APPENDIX A

The purpose of the following Appendix is to provide the reader with further information regarding prepulse inhibition (PPI). To understand PPI, it is important to identify which mechanisms and paradigms allow for its observation. First, I will discuss the startle eye-blink reflex and how it is traditionally studied, via the startle eyeblink modification (SEM) paradigm. I will then outline PPI, a specific type of SEM paradigm, and discuss its attentional modulation and clinical applications.

Startle Blink and the Startle Eyeblink Modification (SEM) Paradigm

The startle eye-blink reflex is a relatively simple brainstem response evoked by tactile, visual or acoustic stimuli (Koch, 1999). It is produced when an organism experiences an intense, abrupt stimulus, causing a rapid (about 30 ms onset latency) and reflexive blink. This pattern of behavior is assumed to function as a protective mechanism, shielding an organism from environmental threat. To better appreciate this simple response, consider this example.

Imagine you're sitting by a campfire. It is night, and very quiet—all you hear is the soft, ambient crackling of firewood at your feet. Unbeknownst to you, someone in your camping party threw an empty aerosol can of sunscreen into the fire pit. BAM! The can explodes, causing the fire to flash violently. In immediate response to this startling event, you jump and close your eyes, automatically. The blink issued to exploding can of Coppertone and the flashing fire was your brainstem hard at work, using the blink reflex to protect your eyes from the fire or the possible shrapnel resulting from the exploding

can. This rapid response occurred before you had an opportunity to decide whether or not the sunscreen was a threat.

Albeit fast and simple, the startle-blink reflex can be modified when certain changes to an organism's sensory environment are made (Filion, Dawson, & Schell, 1998). Modification of the startle-blink reflex is of interest to cognitive psychologists because it may serve as an index of attentional and emotive processes in humans. Before discussing the implications of this research, one must first understand how modification of the startle-blink reflex is achieved.

The startle-blink reflex is modified by pairing a weak, preceding (lead) stimulus with a startle-eliciting stimulus. This lead stimulus, also termed a prepulse, modifies the amplitude of the blink response to the startle-eliciting stimulus. This change in amplitude, whether enhancement or attenuation, mainly depends on the time between the prepulse and the startle stimulus (Blumenthal, 1999). This time period, termed lead time, is also known as stimulus onset asynchrony (SOA) within a startle eye-blink modification paradigm (SEM).

Based on the concept of lead time, there are two main categories of SEM effects: long lead interval (range of approximately 800-7000 ms) and short lead interval effects (range of approximately 70-700 ms). Long lead interval effects are typically employed to examine the emotional modulation of startle blink (Filion et al., 1998). Previous research has shown that the human startle-blink is smaller when viewing pleasant photos and larger when viewing negative photos 2500-5500 ms before the onset of the startle-eliciting stimuli (Bradley et al., 1993a).

Short lead interval effects are categorized in three subclasses: amplitude facilitation effects, latency facilitation effects, and prepulse inhibition. The most thoroughly studied short-lead interval effect of startle modification is the inhibition of startle amplitude, termed prepulse inhibition (PPI). The characteristics of this effect will now be discussed in greater detail.

Prepulse Inhibition (PPI)

Prepulse inhibition is produced by a wide range of stimuli, can occur cross-modally (wherein the lead stimuli and startle stimuli are presented in differing sensory modalities), and occurs within a lead time of approximately 70-700 ms (Filion et al., 1998; Burke & Hackley, 1997; Sonnenberg et al., 2006). In addition to lead time variation, prepulse inhibition may also be influenced by the intensity of the lead stimulus presented. It is important to note that a more salient visual stimulus, or a more intense acoustic stimulus, will typically produce a larger inhibition effect. It may also be noted that the inhibition of startle is not a conditioned response, as PPI is robustly observed at the initial pairing of lead and startle stimuli (Graham, 1980).

PPI and Attention

Prepulse inhibition can be used to study effects of attention (reviewed in Filion et al., 1998). When participants are instructed to attend to the lead stimulus, greater PPI will be observed compared to when subjects are attending away from the lead stimulus (Hackley & Graham, 1987). Consistent with this finding, Filion and colleagues (1993) reported that young adults showed significantly greater PPI at lead times of 120 ms when

instructed to attend to specific acoustic lead stimuli, compared to when they were instructed to ignore acoustic stimuli at the same lead time interval. In other words, attending to the prepulse produces a larger PPI effect on the startle-blink response in humans.

Clinical Applications of PPI

PPI is disrupted in people diagnosed with schizophrenia spectrum disorders (i.e. schizophrenia, schizotypal personality disorder or "psychosis-prone individuals" as distinguished by the Minnesota Multiphasic Personality Inventory, Filion et al., 1998). Using a PPI paradigm, Braff and colleagues (1978; 1992; reviewed in Filion et al., 1998) conducted several studies documenting a deficit of PPI in patients with schizophrenia, a psychiatric disorder associated with an overactivation of dopamine receptors. This finding has been replicated many times using stimuli of the acoustic, tactile and visual modalities (reviewed in Filion et al., 1998). Dawson and colleagues (1993) found that when instructed to attend to an acoustic lead stimulus during a PPI experiment, schizophrenic patients showed no significant differences in reflex amplitude between attending and ignoring the lead stimulus. In this same experiment, healthy controls exhibited the expected pattern of reflex modulation of startle-blink, in that the percentage of blink modification was much higher for the to-be-attended tones (reflecting increased PPI) compared to the ignored tones. PPI deficit in such cases has been attributed to a malfunction of a putative sensory-motor gating system (Braff et al., 1999). This system is conceived of as an internal regulation system responsible for filtering irrelevant stimuli,

allowing for the most salient features of a busy, “stimulus-rich” environment to be processed (Maclaren, Markovic, & Clark, 2014).

APPENDIX B

The purpose of the following material is to provide supplemental information regarding both PPI and the PAR. In the first section of this Appendix, the neural mechanisms underlying PPI are discussed in detail. The second section of this Appendix provides additional information on the PAR not included in the thesis manuscript. A detailed description of the PAR's neural circuitry is also provided in the latter portion of Appendix B.

The Circuitry of PPI

Several midbrain structures, which are sensitive to the modality of the lead stimulus, have been deemed essential in the manifestation of PPI (Fendt, Li, and Yeomans, 2001; Swerdlow, Geyer, & Braff 2001). Studies show if lesioned, the inferior colliculus will totally disrupt PPI for auditory lead stimuli, but not visual stimuli. The central nucleus of the inferior colliculus receives auditory input, which is then sent to the external nucleus. From the external nucleus, information is then relayed to the middle layers of the superior colliculus. The superior colliculus is involved in the expedient processing of visual and tactile lead stimuli within the PPI circuit, as it receives information from medial lemniscus and the retina, in addition to receiving information from the inferior colliculus. The superior colliculus has descending projections to the hindbrain, which allow for an organism to turn away or toward a startling stimulus.

The superior colliculus also projects to the pedunculopontine tegmental nucleus and the laterodorsal tegmental nucleus (Fendt et al., 2001). These structures make up parts of the reticular formation of the midbrain. Previous lesion research has indicated the

pedunculopontine tegmental nucleus and the laterodorsal tegmental nucleus are involved in the mediation of prepulse inhibition. Lesions in both these areas have been shown to dramatically attenuate PPI (as reviewed in Fendt et al., 2001).

The caudal pontine reticular nucleus (PnC) is also critically involved in mediating prepulse inhibition (Fendt et al., 2001). Vestibular and trigeminal neurons project directly to caudal pontine reticular nuclei, otherwise known as the startle center. The PnC is activated by vestibular, acoustic, or tactile lead stimuli. The PnC neurons are shown to be most inhibited by acoustic lead stimuli.

The structures associated with the regulation of PPI have been researched in rat models. Regulation of PPI has been shown to be manipulated by four main limbic cortical regions: the hippocampus, the medial prefrontal cortex, the amygdala and the nucleus accumbens. Admittedly, these regions are structurally distant from the primary startle circuit described in the brainstem. It is not well understood how some of these structures, especially the hippocampus, impact prepulse inhibition. It should be noted that rat models have shown PPI is regulated by dopaminergic receptors, mainly D2 receptors. The involvement of dopamine is considered the common denominator within the limbic structures that are attributed to regulating prepulse inhibition.

When a lead stimulus is of the visual modality (as in this thesis), inhibition of the startle reflex requires participation of the neocortex in stimulus processing, in addition to the brainstem mechanisms that mediate PPI. Sonnenberg and colleagues (2006) investigated the necessity of the striate cortex (V1) in a study of 13 hemianopic patients with unilateral V1 damage. Subjects were presented with a lateralized, grated-light lead stimulus lasting 80 to 1000 ms. This was followed immediately by either an acoustic

startle stimulus (presented binaurally), in the form of a 100 dB (SPL-A) white-noise burst lasting 50 ms, or an air puff to the forehead. Percent-change results for acoustic blink showed that when the visual lead stimulus was within the intact hemi-field, it produced prepulse inhibition at lead times of 120-600 ms. In contrast, when stimulus presentation was in the blind hemi-field, no PPI was observed. Similar results were obtained when the reflexogenic stimulus was an air puff rather than a white noise burst. These findings provides supportive evidence that, for the visual modality, an intact V1 is necessary for PPI to occur.

The Post-auricular Reflex (PAR)

The post-auricular reflex (PAR) is recorded from the post-auricular muscle (PAM) using electromyography. It is triggered by the presentation of abrupt acoustic stimuli (Hackley, 1993). Due to the manner in which this reflex is evoked, the PAR can be studied simultaneously with the blink reflex using a conventional startle eye-blink modification (SEM) paradigm. The PAR is considered a vestigial response in humans, as no movement of the pinnae (external parts of the ears) occurs when the post-auricular muscle is stimulated, even in subjects who possess the ability to voluntarily wiggle their ears (Hackley et al., 1987; Hackley 2015).). It is important to note that the response of the PAR (measured in amplitude) varies between and within subjects. There are several causal factors that modulate response, including muscle tone, background sensory stimulation, head position, and startle stimuli themselves (Fox et al., 1989).

The PAR has a rapid onset latency (8-12 ms), rendering it the fastest known exteroceptive (response evoked from stimuli external to an organism) reflex of which

humans are capable (Hackley 2015). It should also be noted that the post-auricular reflex cannot be elicited by a visual stimulus alone; auditory stimulation is necessary to evoke a reflex. In this study, trials comprised of a visual stimulus only were evaluated for the presence of PARs. There was no reflex in response to the visual stimulus observed in this study. Similarly, Fox and colleagues (1989) found that an electrical stimulus to the wrist did not trigger a PAR.

Benning and colleagues (2004) reported evidence supporting the emotional modulation of the PAR in an experiment utilizing emotion-inducing photographs from the International Affective Picture System (IAPS). They found that the PAR amplitude was most significantly and reliably potentiated while subjects were viewing positively-valenced photographs (pictures inducing feelings of happiness or pleasantness). The PARs elicited by an acoustic startle stimulus, or probe, were larger when subjects were viewing pleasant photos (e.g. erotica), compared to when they were viewing unpleasant photographs (e.g. combat scenes) or photos containing neutral content (e.g. kitchen utensils).

Circuitry of the PAR

Our understanding of PAR circuitry is derived from translational rodent models. Previous research indicates the rodent pinna-startle reflex (in theory translational to a human PAR) is oligosynaptic, involving only a few synapses (Horta-Junior et al. 2007; Gomez-Nieto et al., 2013). The reflex arc of the pinna reflex in rodents begins with the cochlea feeding acoustic information to the cochlear root neurons (CRNs), which then transmit information to the facial motor nucleus as well as the caudal pontine reticular

nucleus (PnC). Cells in the facial nucleus, in turn, activate the post-auricular muscle (Figure 1).

More specifically, projections of the trapezoid body (VNTB) to the CRNs are involved in modulating the acoustic startle reflex (Gomez-Nieto et al., 2013). CRNs receive cholinergic input from VNTB neurons. Researchers have found that the CRNs are the first brainstem neurons to directly initiate (via the PnC) the acoustic startle response in auricular motoneurons. The PnC is a nucleus where sensory and motor information is integrated. It is excited by abrupt acoustic stimuli (Horta-Junior et al., 2008).

APPENDIX C

This Appendix houses the IRB-approved consent and debriefing forms provided to subjects who participated in this experiment.

Consent Form

Prepulse Inhibition of the Post-auricular Reflex

I hereby consent to take part in research conducted by Amy Underwood and Steven Hackley at the Cognitive & Clinical Neuroscience Lab of the University of Missouri.

Purpose: The purpose of this research is intended to investigate perceptual-motor activity that is controlled by the brain stem.

What Is Involved in the Study: My participation will consist of counting the number of pink checkerboard images I see presented on a computer screen. Multiple series of loud clicks will also be presented throughout the experiment. Sensors will be taped to my face to record muscle activity in response to the clicks. My participation in the experiment will consist of a single session, lasting about two hours.

Risks: There is only one aspect of this experiment that poses a greater risk than everyday life. In order to attach the sensors, the skin must be cleansed and exfoliated. If the experimenter rubs too hard, an abrasion may occur. Abrasions require about a week to heal. The experimenter is highly trained and will be very careful. Consequently, the chance of this occurring is minimal, only about 5 percent. I will be told of any significant new information that might affect my willingness to take part in this research.

Benefits: This experiment could lead to advances in knowledge that will benefit humans and animals in general. For example, the reflex methods used in this study are routinely employed to test drugs for schizophrenia. However, this experiment is unlikely to be of any special benefit to me, individually.

Participation: My participation is strictly voluntary; I can quit at any time, even after signing this consent form. I will be given a scheduled break half-way through the video game, and I can take additional breaks whenever I wish.

Course Credit: I am aware that my participation in this study will at least partially fulfill the research requirements for my Psychology 1000 class. I am also aware that there are alternative ways of fulfilling my research requirement (e.g., completing a short paper). These alternatives are described in the syllabus for my Psychology 1000 class. I will be given an amount of credit that is proportional to the amount of time I participated (this is one credit point for each one-half hour of participation, or fraction thereof). I can expect this experiment to last about two hours. If the experiment lasts two hours and one minute, I would receive five credits, not four. If I quit the experiment, my course instructor will not be informed and the researchers will not be upset with me.

Confidentiality: The results of this research may be published, but I will not be identified in any such publication. The data collected for this study will be kept in a locked room, accessible only to laboratory personnel.

Consent: By signing this form, I indicate that any questions I have had up to this point in time have been answered. If I have any further questions, I am to contact Miss Underwood at 1-417-280-5338 or Dr. Hackley at 1-573-882-3277 (112 Psychology Building). I further consent to allow Dr. Hackley, Miss Underwood, and their associates to report their findings to government agencies, funding agencies or scientific bodies and to publish their findings. A copy of this consent form will be provided to me.

If you have questions regarding your rights as a participant in research, please feel free to contact the Campus Institutional Review Board at 1-573-882-9585.

Signature: ______________________________________

Date: ____________

Experimenter ___________________________________

Date: ____________

Debriefing Form

Prepulse Inhibition of the Post-Auricular Reflex

Every animal has a reflex response system. A reflex is an involuntary and almost instantaneous movement in reaction to a stimulus. This experiment is designed to study the human startle reflex. The startle reflex is modulated by the brainstem (in humans) and serves to protect us from harmful or threatening stimuli in our surrounding environment. The startle reflex is seen across the human lifespan and in most all animal species.

In response to a startling stimulus, this armadillo jumps high off of the ground.

The purpose of the experiment you were just in was to study how a weak stimulus can inhibit your startle response to a strong stimulus, or in other words, a neurological phenomenon termed *prepulse inhibition* (PPI). The weak stimuli were the checkerboards that you saw. The strong stimuli were the loud clicks you heard. The absence of PPI in humans can be indicative of some neurological disorders such as schizophrenia and Alzheimer's disease.

In conjunction with PPI, the aim of this experiment was to study the impact of PPI on the *post-auricular reflex*, which is a slight twitch of the muscle behind the ear.

This type of inhibition is useful for testing drugs for schizophrenia. It doesn't last very long, so we are assessing the duration of inhibition by varying the time interval from when you see the checkerboard until you start to hear the clicks.

Every experiment has at least one independent variable and one dependent variable. The *dependent* variable –the one that is measured –was the size of the muscle twitch behind the ear. The *independent* variable –the one that is manipulated—was amount of time between the checkerboard and the first click in each trial.

Thank you for your participation!