Prepulse inhibition of the acoustic startle reflex: Comparing low and high trait anxious individuals

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PREPULSE INHIBITION OF THE ACOUSTIC STARTLE REFLEX: COMPARING LOW AND HIGH TRAIT ANXIOUS INDIVIDUALS

by
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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College

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ABSTRACT

This investigation examined whether prepulse inhibition of the acoustic startle reflex was impaired among individuals with high trait anxiety compared to controls. PPI has traditionally been theorized to be a psychophysiological index of information processing and sensorimotor gating, and is now being used to help identify various psychological disorders. Although results of existing research on reduced PPI across the anxiety spectrum are equivocal, previous findings indicate that highly anxious (HA) participants exhibit significantly reduced PPI at lead intervals of 60 ms relative to low anxious (LA) controls. This study paired a 70 dB (A) white noise prepulse stimulus with a 100 dB (A) white noise startling stimulus at a 60 msec discrete lead interval. The results showed a higher baseline startle response and a trend toward increased response probability among the HA subgroup compared to controls. Due to a large number of nonresponse trials, analysis of PPI between groups could not be performed, but the data that was obtained revealed a 2.2% reduction in PPI among highly anxious participants. The current results highlight the potential for PPI to index sensorimotor gating deficits among anxious populations.
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CHAPTER I
Introduction

The acoustic startle reflex (ASR) is a defensive response to an intense, abrupt auditory stimulus that results in contraction of facial and skeletal muscles (Romirez-Moreno & Sejnowski, 2012). This “descending flexor wave reaction” (Grillon, 2002) creates a crouch-like posture that probably protects the organism from harm (Hoffman & Ison, 1980). According to Koch, the response also involves an arrest of ongoing behavior and an increased heart rate, which may have a protective function and prepare the individual for a flight or fight response (1999). Although the startle reflex occurs across multiple modalities (Braff, Geyer, & Swerdlow, 2001), this review focuses on the response elicited by auditory stimuli. The magnitude of the startle reflex can be modified (i.e. enhanced or inhibited) by various methods. Startle magnitude is increased via sensitization, fear-potentiation, and drug-induced enhancement; it is decreased by habituation, prepulse inhibition, drug-induced inhibition, and the attenuation by positive effect (Koch, 1999). Inhibition of the ASR was first demonstrated by Peak in 1939, and in 1965 Hoffman and Searle exhibited startle attenuation by presenting a weak noise pulse 20-500 ms before a startle-eliciting stimulus. The term prepulse inhibition (PPI), however, was not suggested until 1971 by Ison and Hammond (Fendt, Li, & Yeomans, 2001). PPI has been defined as “the normal unlearned suppression of the startle reflex when the intense startling stimulus is preceded by a weak sound pulse.”
prepulse stimulus” (S. Ludewig, K. Ludewig, Geyer, Hell, & Vollenweider, 2002). It gained attention as an operational measure of sensorimotor gating, which refers to the ability to filter (i.e. gate) irrelevant information. Many researchers lost interest in the topic as Pavlovian conditioning came about (Hoffman & Ison, 1980), but a reemergence of startle modification studies occurred in the 1960’s. The number of cross-species translational studies have increased tremendously in the last 30 years (Braff et al. 2001), as prepulse inhibition has proven to be a useful tool in identifying neural mechanisms underlying cognition and behavior.

Additionally, PPI is now being used to study various psychological disorders. For instance, decreased startle inhibition has been established across schizophrenia spectrum disorders. More recently, researchers have directed their attention to the anxiety spectrum, but results have been mixed. Some studies indicate reduced PPI in anxiety disorders such as post-traumatic stress disorder (Grillon, Morgan, Southwick, Davis, & Charney, 1996), obsessive-compulsive disorder (Hoenig, Hochrein, Quednow, Maier, & Wagner, 2005), panic disorder (Ludewig et al., 2002), and high trait anxiety (Duley, Hillman, Coombes, & Janelle, 2007). However, Franklin and colleagues cited 5 studies that found no correlation between anxiety and reduced PPI (2009). They hypothesize that an inappropriate signal-to-noise ratio (SnR) used in the methodologies was likely the cause of null findings, and that an SnR of +15 dB is ideal to elicit PPI variations in individuals with mental health conditions (Franklin, Bowker, & Blumenthal, 2009). Determining the relationship between psychiatric disorders and startle modification will help us gain a more complete understanding of the neural mechanisms underlying these conditions.
Purpose of the Study

The purpose of this study was to determine if prepulse inhibition of the acoustic startle reflex is reduced in individuals with high trait anxiety compared to controls. Specifically, we wanted to know if using the recommended +15 dB signal to noise ratio protocol would allow us to replicate the results of the Duley (2007) study.

Hypotheses

The following hypotheses were tested in this study:

Ho1: Preceding an intense startle-eliciting stimulus (100 dB, 40 ms) with a weak non-startling stimulus (70 dB, 40 ms) would result in decreased startle amplitude.

Ho2: Highly trait anxious (HA) participants would exhibit reduced PPI compared to the low trait anxious (LA) group.

Ho3: A 15+ dB signal-to-noise ratio would produce noticeable results between the HA and LA groups.

Operational Definitions

1. Acoustic startle reflex (ASR) is a defensive response to an intense and abrupt sound that results in contraction of facial and skeletal muscles.

2. Pulse is the intense startle-eliciting stimulus.

3. Prepulse is the weak non-startling stimulus that precedes the pulse.

4. Intertrial interval (ITI) is the time between successive stimulus trials.
5. Interstimulus interval (ISI) is the time between the prepulse and the pulse.

6. Prepulse Inhibition (PPI) is a reduction in the startle response when the intense startling stimulus is preceded by a weak prepulse stimulus.

**Delimitations**

The study was limited to the following:

1. Male or female subjects between the ages of 18 and 28.
2. Subjects were considered high anxious (upper quartile) or low anxious (lower quartile) according to the SONA stored Anxiety Assessment Scale.
3. Subjects were not on antidepressants or anxiolytic medications.

**Assumptions**

The following assumptions applied to this study:

1. Subjects had no hearing impairments.
Startle modification studies are necessary to improve our understanding of information processing, and the deficits associated with certain neuropsychiatric disorders. Identifying the neural circuit that regulates PPI will further our knowledge of the neurobiology underlying various brain functions and the pathophysiology of disorders (Swerdlow, Geyer, & Braff, 2001). Several studies have demonstrated dysfunctional information processing and impaired sensorimotor gating in patients with mental health disorders (see Braff et al., 1978; Gillon et al., 1996; Ludewig et al., 2002; Hoenig et al., 2005; Duley et al., 2007; Franklin et al., 2009). The ASR is a useful tool to examine neuropathological impairments of sensory information processing and behavioral plasticity (Koch, 1999). Plasticity is the ability to adapt and change, specifically, the capacity of the CNS to find alternative pathways for sensory perception and motor skills. The ASR exhibits plasticity through prepulse inhibition and habituation. Habituation refers to “the decrement in responding when the same initially novel stimulus is presented repeatedly in the absence of any contingencies” (Ludewig et al., 2002). Reduced amounts of plasticity implicated in schizophrenia and anxiety spectrum disorders may be traced to abnormalities in the neural structures involved in the ASR and PPI pathways. Therefore, evaluating how the different levels of the central nervous system function in processing important sensory information
and filtering out irrelevant stimuli has functional significance in the life of the organism (Blumenthal, 1999). Experimental manipulations such as brain tissue lesions, infusion of pharmacological agents, electrical stimulation, or administration of neurotoxins have been used to study the brain regions involved in PPI (Swerdlow et al., 2001). Swerdlow and Geyer suggest further research to identify where disruptions occur in the neural circuitry of individuals with neuropsychiatric disorders, which would allow for interventions at specific levels of the circuitry to improve sensorimotor gating (1999). Verifying the involvement of proposed structures and neurotransmitter systems would provide an “anatomical framework” to figure out how this behavior could be modified by various treatments (Davis, Walker, & Lee, 1999). Patients with mental health disorders often receive inadequate and inconsistent treatment. For example, of the 18 million people dealing with an anxiety disorder only 23% receive treatment, and treatment options are usually limited to drugs and psychotherapy (Wipfli, Rethorst, & Landers, 2008). The antidepressants prescribed have unpleasant side effects such as drowsiness, light-headedness, headache, dry mouth, gastrointestinal upset, irregular heartbeat, and weight gain. Duley and colleagues claim that “short lead interval startle modification research appears to be a promising tool to evaluate the psychological modulation of anxiety in response to different clinical and pharmacological treatments” (2007). The study they conducted examined the effects of a bout of exercise on the amount of PPI observed in anxious individuals. They found exercise may be an alternative to pharmacological treatment for regulation of stress and anxiety (Duley et al., 2007), and Paluska and Schwenk agree that physical activity could be an important adjunct to pharmacological treatment (2000). Comprehending the relationship between abnormal startle response, anxiety, and exercise could support the use of exercise as a prescription. Overall,
Startle provides a reliable dependent variable and has proven to be “an exceptional tool for the study of emotion and psychopathology” (Cook, 1999). Further studies may yield a more complete understanding of cognitive and behavioral disorders. For example, Grillon et al. believe researching the underlying cause of exaggerated startle in PTSD patients could increase our understanding of CNS regulation of this disorder (1996). Obviously, there is a demand for studies that confirm the results of existing research or provide new information surrounding startle modification.

*Startle Elicitation*

A popped balloon, a crash of thunder, and a dog’s bark are everyday acoustic stimuli that may cause a person to jump. Although startle appears to be relatively simple to evoke, startling stimuli in the experimental setting must have certain characteristics in order to produce the ASR. According to Berg and Balaban, the most important parameters for startle elicitation are stimulus rise time, intensity, duration, and bandwidth (1999). The startle response is enhanced by a short rise time, which is the amount of time it takes the stimulus to reach its full, steady-state amplitude. Ideally, the rise time is instantaneous (Graham, 1975). Hoffman and Ison found that the response only occurred if the startle-eliciting stimulus reached a minimum intensity within a certain amount of time (1980). Additionally, louder pulses elicit the ASR more effectively. Increasing the intensity of the startling stimulus not only increases response amplitude and probability, it also promotes latency facilitation (Blumenthal et al., 2005). The startle threshold is around 80 dB (Koch, 1999), and there is a 50% response probability at 85 dB, so many researchers have employed a 100 dB startling-eliciting stimulus (Blumenthal et al., 2005). Furthermore, stimuli that are longer in duration
generally enhance the startle response up to a certain point. Blumenthal et al. found that the duration should not exceed the response latency period of 30-50 msec (2005), which is the amount of time after stimulus onset that it takes for measurable EMG activity to be observed. Gillon and colleagues stated that, “A typical acoustic startle is a brief (e.g. 40 millisecond) burst of white noise with an abrupt onset and intensity ranging from 90 to 115 A-weighted decibels dBA” (2002). White noise is random and includes all frequencies within the range of human hearing in equal amounts. Wide bandwidth white noise is preferred over narrow bandwidth pure tone, as it has proven to be more effective in producing startle (Graham, 1975). At levels above startle threshold it produces 2.5 times larger amplitudes and increases probability by 50 percent (Berg & Balaban, 1999). In regards to startle modification, recent evidence has confirmed that PPI is elicited best with discrete white noise prepulses (Braff et al., 2001). Because white noise contains all frequencies, it is better able to mask ambient noise. Many studies use background noise of 65-75 dB to mask less intense environmental noise, but both animal and human research suggests that it may be more affective to decrease uncontrolled noise sources or isolate the participant from that noise (Blumenthal et al., 2005). With noise-canceling headphones and background white noise that provides the ideal signal to noise ratio, environmental sound should not be a significant concern.

Startle research lacks standardization in the equipment and protocols used to test subjects. For example, researchers have reported both monaural and binaural use of speakers, over-the-ear headphones, and earphones inserted into the auditory canal. Using headphones is acceptable as long as the shape of the earphone allows for proper calibration of stimulus intensity with a sound meter and as long as the earphones can be properly aligned with the auditory canal (Blumenthal et al., 2005). Before administering the test to a subject, it is
suggested that his or her hearing level is assessed (Grillon et al., 1996). Hoenig et al. 
administered brief hearing screenings to ensure participants’ hearing was within normal 
limits (2005). The choice of which ear to deliver the sound pulses may be important, as 
evidence indicates lateralization in the degree of reactivity to startling stimuli. In a study by 
Grillon et al., greater startle potentiation was reported when startling stimuli were delivered 
to the right ear compared to the left ear, implicating left hemisphere lateralization (2002). 
When creating the testing procedure, one must also consider lateralization effects exhibited 
in the motor response (i.e. eyeblink). According to Braff and colleagues, “the choice of right 
versus left eyeblink measures might be an important factor in identifying abnormalities in 
specific psychiatrically disordered populations, in which dysfunction in the startle regulatory 
circuitry might be lateralized” (2001). Though, other researchers claim the eye used to 
measure EMG activity does not matter as long as it is kept consistent within the study (Berg 
& Balaban, 1999). Lateralization will be discussed further in The Neural Basis of PPI.

**Measurements**

Electromyographic (EMG) activity of the orbicularis oculi muscle appears to be the 
method of choice when measuring the motor response to startling stimuli. Although a full 
body response can occur, the eyeblink component is the most sensitive measure of startle and 
most resistant to habituation (Franklin et al., 2009). Several techniques have been tested to 
determine the easiest and most reliable way to measure eyeblink amplitude. Some measure 
eyelid movement, while others measure action potentials generated within the orbicularis 
oculi muscle. Graham agreed that the eyeblink component of startle is typically measured 
using EMG of the orbicularis oculi muscle, but noted that an accelerometer capable of
measuring small rapid movements can be used (1975). Measuring the eyeblink component of the human startle reflex is an easier and more sensitive method than optic indicator, photocell, or lid potentiometer recordings (Blumenthal, 1996). It is also less obtrusive than other methods, which improves the participants’ comfort during testing. Maximum sensitivity occurs when recording palpebral portions of orbicularis oculi, but it is easier to measure over the orbital area (Berg & Balaban, 1999). It is important to place the electrodes in the same location across participants. First, the skin must be prepared before attaching the electrodes in order to maximize conductivity (Blumenthal et al., 2005). The skin surface should be cleansed of makeup, oil, and dead skin to reduce resistance and recording noise; recording noise should be 2000 ohms or less when measured by an impedance meter (Berg & Balaban, 1999). Once the skin has been prepared properly, the electrodes can be placed according to Blumenthal’s instructions: one placed below the lower eyelid in line with the pupil in forward gaze, a second placed approximately 1-2 cm lateral to the first, and a ground electrode attached on a bony prominence such as the forehead, mastoid, or temple (2005). It is recommended that electrodes be 5 mm or less (Berg & Balaban, 1999), and the electrode collars should not overlap, as this could cause mechanical artifacts (Blumenthal et al., 2005). The EMG signal should also be checked for other artifacts from sources such as power lines. After the electrodes have been secured, the participant is usually instructed to look at a fixation cross and ignore any sounds they hear (Duley et al., 2007).

**Figure 1. Diagram of electrode placement.**
Prepulse Inhibition

The startle response is effectively modified by pairing a subthreshold stimulus with a startle-eliciting stimulus. Prepulse inhibition (PPI) of the startle reflex is observed across species when a weak stimulus of any modality (acoustic, visual or tactile) is presented 30-500 msec before an intense startling stimulus (Swerdlow & Geyer, 1999; Fendt et al., 2001; Koch, 1999). Short lead interval startle modification studies report amplitude inhibition and latency facilitation when the prepulse and pulse are paired at this time interval (see Graham, 1975; Hoffman & Ison, 1980; Filion, Dawson, & Schell, 1998). Latency facilitation is the reduction in the amount of time between stimulus onset and a measurable EMG response. A basic startle eyeblink modification paradigm includes a series of randomized trials of the startle-eliciting stimulus alone, the startle-eliciting stimulus preceded by a weaker lead stimulus, and the lead stimulus alone (Filion et al., 1998). For example, Duley et al. used four blocks of twelve trials, and each block contained 3 pulse-only trials, 6 prepulse-pulse trials at various discrete lead intervals, and 3 prepulse-only trials (2007). The pulse is presented by itself in control trials to provide a baseline for comparison to prepulse-pulse trials and quantification of PPI. Control startle magnitude can vary greatly from person to person, so baseline startle response must be considered across participants (Blumenthal, Elden, & Flaten, 2004). The lead stimulus is presented alone to demonstrate that it does not elicit the startle reflex. As long as the startle response magnitude is significantly less in prepulse-pulse trials relative to control trials, then PPI is present (Blumenthal et al., 2004; Franklin et al., 2009). Research indicates the startle response amplitude can be inhibited by at least 40% (Hoffman
& Ison, 1980), and up to 50-80% in some individuals (Filion et al., 1998). Although it is clear that startle can be modified by the lead stimulus, less is known about the functional role of PPI.

Different hypotheses exist as to why prepulse inhibition occurs, but most agree it has some “important adaptive role” (Graham, 1975). We know the lead stimulus activates neural mechanisms that decrease responsivity to subsequent sensory events for a brief period of time (Braff et al., 2001). As previously stated, PPI is used as a measure of both preattentive information processing and sensorimotor gating (Ludewig et al., 2002). Since humans are exposed to profuse amounts of environmental stimuli, a mechanism to filter irrelevant or repetitive stimuli is necessary. Sensorimotor gating is thought to help focus attention on the most important stimuli in the environment, thus providing selective attention in a stimulus-laden world (Braff et al., 2001; Franklin et al., 2009). Gating deficits may create information overload and contribute to symptoms such as distractibility and intrusive thoughts that characterize certain neuropsychiatric disorders (Filion et al., 1998). Attenuation of the eyeblink component of ASR in response to antecedent stimulation makes the response less distracting, but failure to inhibit startle amplitude correlates with a loss in preattentive processing and increased distraction of motor (blinking) activity (Braff et al., 1978). In other words, important sensory information can be processed more effectively if the flexor reaction of startle is suppressed. In 1975, Graham proposed her protection of processing theory, which states that PPI functions to protect the processing of the lead stimulus from interruption by the startle stimulus. Since the 1970’s research has focused on producing evidence in support this premise. Several authors have confirmed the role of PPI in protecting relevant information presented in the weak lead stimulus so it can be adequately processed without
interference from the startling stimulus (see Braff et al., 2001; Hoenig et al., 2005; Franklin et al., 2009, Ramirez-Moreno & Sejnowski, 2012). Blumenthal conducted a study in which he instructed participants to identify the pitch of the lead stimulus, and found that “lead stimulus identification accuracy was higher on trials on which startle was inhibited” (1996). Therefore, information in the lead stimulus is protected more effectively when the startle response is mitigated. The degree of activation of this protective mechanism determines the amount of PPI observed. Activation is dependent upon stimulus onset asynchrony and aspects of the prepulse; a lead stimulus that is longer in duration and higher in intensity results in greater activation of protective mechanism (Graham, 1975). Characteristics of an effective prepulse are discussed further in the section below. Examining how experimental and clinical manipulations affect PPI may provide further insight into its purpose.

Although there is debate regarding the involvement of attention in startle inhibition, it has been established that PPI is not a learned response. Graham suggested that startle inhibition reflects the protection of preattentive processing (1975), but subsequent studies have shown that directing attention toward a lead stimulus increases the amount of startle inhibition produced (Filion et al., 1998). Blumenthal proposed that short lead interval startle modification can be affected by both automatic and controlled attentional processing since they follow different time courses (1999). At short lead intervals (<60 ms) modulation depends on automatic processes, whereas at longer lead intervals (>120 ms), attention is involved (Filion et al., 1998; Koch, 1999; Braff et al., 2001). The experimental design and instructions given to participants can also affect the degree to which automatic or attentional processes regulate PPI. Whether deficits in PPI indicate impaired sensorimotor gating or impaired attention, they have important implications in neuropsychiatric disorders. Koch
claims, “attentional (‘top-down’) mechanisms obviously affect PPI at the perceptual level, whereas higher levels of stimulus processing are protected by gating mechanisms underlying PPI” (1999). More research is necessary to determine the role of attention in modulating prepulse inhibition, but we are certain that this form of neural plasticity is independent of learning. In fact, the profound inhibitory effect is not a product of learning, sensory masking, or middle ear protective reflexes (Graham, 1975). Given that PPI occurs on the first presentation of a prepulse-pulse pair, there is no possibility of a learning effect (Fendt et al., 2001; Blumenthal, 1999). Additionally, startle inhibition is present in infants, though brainstem structures that mediate PPI may not be fully developed until ages 8-10 (Braff et al., 2001). For these reasons PPI is not associated with conditioning. However, habituation is a form of learning that can occur as the testing session progresses since the stimulus does not predict a biologically significant event. Habituation is not a result of muscle fatigue or blunted sensory receptor responsiveness (Koch, 1999). The amount of startle is reduced after repeated presentation of the startling stimulus, and according to the Law of Dynamic Range, the amount of PPI decreases as the startle response habituates (Blumenthal, 1996; Blumenthal, 1999). Thus, when formulating PPI studies randomization is essential to prevent habituation and to obtain an accurate representation of the participant’s sensorimotor gating abilities.

**Characteristics of the prepulse**

Certain qualities of the lead stimulus such as intensity, duration, signal-to-noise ratio, and interstimulus interval are important to achieve maximal PPI. Bloch’s law states that the influence of the stimulus reflects an interaction of intensity and duration (Braff et al., 2001). Generally PPI follows this principle; as prepulse intensity increases so does the percent
reduction in response amplitude (Blumenthal, 1996). While the prepulse only has to reach detection threshold for measurable effects to occur (Hoffman & Ison, 1980), a lead stimulus of sufficient intensity can activate the startle response itself (Blumenthal, 1999). Acoustic stimuli above 80-85 dB will trigger the ASR in most individuals (Romirez-Moreno & Sejnowski, 2012). Several studies have reported significant PPI with the use of a 70 dB lead stimulus lasting 40-50 ms (see Blumenthal, 1996; Blumenthal et al., 2004; Braff et al., 1978; Duley et al., 2007; Graham, 1975; Grillon et al., 1996). In addition to prepulse intensity, Franklin et al. argue that the signal-to-noise ratio is crucial to obtain reliable results, especially in patients with psychiatric disorders (2009). The signal-to-noise ratio (SnR) refers to the difference between background noise intensity and stimulus intensity. Recent research has indicated that an optimal SnR is +15 dB above background, but success has also occurred with SnRs between +10 dB and +16 dB (Duley et al., 2007; Franklin et al., 2009). Braff et al. emphasize the importance of standardizing the signal-to-noise ratio, and argue that the brain processes involved in regulating PPI cannot be the same when SnR varies so much across studies (2001). Another variable that critically affects startle modification is the time period between the prepulse and pulse, or the interstimulus interval (ISI). Reflex latencies are reduced when lead intervals are less than 100 msec (Swerdlow & Geyer, 1999), whereas a 120 ms ISI produces maximal amplitude inhibition and latency facilitation of the eyeblink response (Braff et al., 1978; Graham, 1975). This indicates that reflex magnitude and latency are independent processes. Blumenthal et al. found that a main effect occurs between prepulse intensity and response latency. Results from their study showed effective startle inhibition with 70 dB prepulses at a 60-ms lead interval but not a 120-ms lead interval (2004). The effects of the lead interval reflect transmission time difference between the PPI.
pathway and the ASR pathway (Ramirez-Moreno & Sejnowski, 2012). Neural circuitry will be discussed further on in this review. Additionally, the intertrial interval, the interval between successive stimulus trials, is another time-sensitive aspect of PPI experimentation. The ITI is typically around 15-20 s, but can range from 8-30+ s (Braff et al. 2001). Randomization of ITIs is crucial to prevent habituation. Clearly, PPI is a time-sensitive measure and careful consideration should be given to the ISI and ITI.

**Quantification of startle and PPI**

When examining the effects of clinical and experimental manipulations on the ASR, quantifying variables such as probability, amplitude, magnitude, latency, and habituation provides valuable information. Response probability is calculated by taking the number of trials with a slope change of 2 analog-to-digital units over 5 ms in the 20-100 ms time window after startle stimulus onset, and dividing that number by the total number of trials presented (Blumenthal, 1996). Probability can vary depending on characteristics of the stimulus and the individual being tested. Response amplitude and response magnitude differ in meaning, but are both measures of the intensity of muscular contractions elicited by the startling stimulus. The term ‘peak amplitude’ is used if the average of the responses excludes nonresponse trials, whereas ‘peak magnitude’ refers to the mean calculated with nonresponse trials that are assigned a value of zero (Berg & Balaban, 1999). Although these terms are often used interchangeably, it is important to note the distinction when conducting startle research in order to compare results to other studies or replicate their findings. Response amplitude is calculated as the difference between the EMG value at response peak and response onset (Blumenthal et al., 2005). This variable is reflective of an individual’s
reactivity to startling stimuli. Response amplitude or magnitude should be measured at baseline, before attempting to modify the ASR. Individual differences in baseline startle are large and experiments should have a within-subject design when studying modification effect (Cook, 1999). Additionally, PPI is quantified with either difference scores or percentage change scores. The difference score takes the difference in amplitude between prepulse and control conditions (Berg & Balaban, 1999; Swerdlow et al., 2001). Blumenthal calculated PPI by subtracting response amplitude in the control condition from the value in the prepulse condition, but they also used a proportion of difference score, as recommended by Hoffman and Ison (Blumenthal, 1996; Hoffman & Ison, 1980). Researchers have compared methods of quantifying PPI and found that proportion of difference (the difference between reactivity on prepulse and control trials, divided by that on control trials) was the most reliable and constant across a variety of conditions (Blumenthal et al., 2004; Filion et al., 1998; Koch, 1999; Ludewig et al., 2002). Percentage change scores are affected less by individual differences in response to control startle stimulus (Berg & Balaban, 1999). Unlike other methods, proportion of difference describes the extent to which the prepulse inhibits the startle response and is considered a direct measurement. However, Braff et al. stated that both percent scores and difference scores are appropriate measures depending on the research design, and can be used together for a more comprehensive examination of PPI (2001). A large degree of variation exists in the methods used to measure inhibition, and standardization of quantification is necessary to help compare PPI research. Although PPI is the primary outcome parameter, response latency and habituation are other commonly used measures (Kohl, Heekeren, Klosterkotter, & Kuhn, 2005). These measures may provide information about the nature of certain psychiatric conditions. For example, Braff et al.
noticed less latency facilitation in schizophrenics (1978), and Ludeweig et al. found that individuals with panic disorder show less habituation compared to controls (2002). Overall, the parameter used to quantify startle and PPI depend on the purpose of the study.

**Evaluating the Data: Filtering, Smoothing and Rectification**

Electromyographic data must be analyzed within a specific time frame after the onset of the startling stimulus for motor activity to be considered a startle response. A voluntary eyeblink can be differentiated from a reflexive eyeblink by the unique latency and form each exhibits (Graham, 1975). The blink reflex is distinguished relatively easily from non-reflexive blinks by examination of blink onset and duration, especially when specific waveform acquisition criteria are used (Braff et al., 2001). If the blink is a reflex, eyelid closure begins within 120 ms of stimulus onset and reaches peak closure within 150 ms (Graham 1975). For this reason, it is recommended to evaluate acoustic eyeblink EMG from 21-150 ms after startling stimulus onset (Berg & Balaban, 1999; Blumenthal et al., 2005; Braff et al., 1978). It is essential to define the response window before taking data, and limit acceptable reflex responses to blinks within a narrow latency window (Blumenthal et al., 2005). One must allow enough time for the response to develop, but longer windows increase the chance of scoring voluntary blinks. Additionally, the EMG waveform has a different appearance depending on whether the blink resulted from startle or volition (See Fig. 2).
After data collection, the raw EMG waveforms must be processed through filtering, rectification and smoothing in order to condition the data for analysis. First, the data must be filtered to eliminate electrical potentials above or below the frequency of interest. A high-pass filter may be applied to remove frequencies below a certain cutoff, whereas a low-pass filter may be applied to remove frequencies above a certain cutoff frequency. Recordings taken from the orbicularis oculi muscle range in frequency from 28 to 512 Hz, but many studies use 90 Hz and 250 Hz as their high-pass and low-pass cutoff frequencies, respectively, because it eliminates 60 Hz line noise and "reduces cross-talk from other muscle sites (Berg & Balaban, 1999). Applying the appropriate filters can also reduce noise from movement and instrumentation (Blumenthal et al., 2005). In addition, the EMG signal oscillates in the positive and negative direction around zero. Rectification involves inverting
any negative (below zero) signals and combining with the positive signals (Berg & Balaban, 1999). This converts all EMG data into absolute values to prevent negative and positive components of the waveform from canceling each other out when processing the data (Blumenthal et al., 2005). Finally, the data is processed through integration or smoothing. Integration entails calculating the area under the curve of the EMG signal. Smoothing involves passing the rectified data through a low pass filter, for which the setting is a time constant (Berg & Balaban, 1999). Blumenthal et al. recommend the time constant be no greater than 10 ms because higher time constants make it more difficult to detect small responses and may cause an overestimation of the number of “nonresponders”, even though they reduce the impact of high frequency fluctuations in the rectified EMG signal (2005). Duley et al. used a 20-200 ms time window for response latency, and processed the raw EMG data with a FIR filter using a low-pass cutoff frequency of 40 Hz (2007).

Prior to data analysis, it is crucial to determine rejection, non-response, and exclusion criteria. Studies have rejected trials with unstable baseline EMG, as this indicates movement artifact. To replace a rejected file, he interpolated the average values for that condition (Blumenthal et al., 1996; Duley et al., 2007). A trial is also rejected if a voluntary blink occurs in the time period immediately preceding stimulus onset or within the interstimulus interval (Berg & Balaban, 1999; Blumenthal et al., 2005). Rejected trials differ from nonresponse trials, which indicate the participant fails to respond to a minimum number of startle-eliciting stimuli (Blumenthal et al., 2005). As previously mentioned, amplitude is calculated if nonresponse trials are to be excluded, whereas magnitude is used if nonresponse trials are included and assigned a value of zero. Determining exclusion criteria before data collection reduces bias and improves the validity of the results.
The Neural Basis of Startle and PPI

Certain aspects of the brain mechanisms underlying PPI remain unclear, but existing research supports the involvement of a few key structures and neurotransmitters. While the neural basis for the whole body startle reaction is understood, knowledge specific to the human blink reflex pathway is incomplete (Berg & Balaban, 1999). Experimental manipulations such as brain tissue lesions, infusion of pharmacological agents, electrical stimulation, or administration of neurotoxins have been used to study the brain regions involved in PPI (Koch, 1999; Swerdlow et al., 2001). These methods provide information related to the anatomical pathway regulating startle modification, but they also reveal how pathology in certain brain regions manifests in some clinical disorders. Separate brain-stem circuits mediate startle and PPI, but it is believed that they converge at a common neural structure. In addition, there may be different modification circuits for various affective influences on startle (Cook, 1999). Sedative and anxiolytic drugs such as clonidine and diazepam reduce the magnitude of startle response, but do not significantly affect the percent score of PPI (Braff et al., 2001). This indicates that the regulation of startle magnitude and PPI may be separable. Below, the primary acoustic startle pathway and proposed PPI pathway are discussed, along with the neurotransmitter systems affecting these circuits.

Primary Acoustic Startle Pathway

The primary startle circuit is located in the ponto-medullary brainstem (Braff et al., 2001; Koch, 1999). Pontine nuclei communicate with the forebrain and the cerebellum, and are involved in motor activity. Evidence indicates that the startle center is in the nucleus
reticularis pontis caudalis (Hoffman & Ison, 1980). The human startle reflex has a short latency (10 msec), which suggests that the primary pathway involved in the ASR is relatively simple and only has 3 synapses: cochlear root neurons, the nucleus reticularis pontis caudalis (PnC), and spinal motor neurons (Davis et al., 1999; Grillon, 2002; Koch, 1999).

Additionally, Hoffman and Ison studied rats and found their response latency to be just 6-8 ms, which led them to believe there was only enough time for one or two synapses between the cochlear nucleus and spinal motor neurons (1980). Animal studies are necessary to increase our understanding of sensorimotor gating. There is a high degree of homology between humans and rodents, and the same parameters can be used to generate the ASR across species (Braff et al., 2001; Koch, 1999). In both species, as sound enters the ear, acoustic information is transmitted along the cochlear nerve to nuclei in the brainstem and midbrain. Cochlear root neurons receive input directly from the cochlea and send axons near the lateral lemniscus (brainstem) and on up to the superior colliculus (midbrain), but collaterals terminate on PnC cells (Berg & Balaban, 1999; Davis et al., 1999). The PnC is labeled the startle center because it communicates with the spinal cord to create the acoustic startle reflex. These synapses are confirmed by lesion studies. Lesions to cochlear root neurons result in the elimination of the startle reflex, and lesions to the nucleus reticularis pontis caudalis eliminated the pinna reflex in rats, which is analogous to the eyeblink component of the human startle response (Davis et al., 1999). While the circuitry of ASR is clear, knowledge of the pathway involved in PPI is incomplete. However, evidence has shown these two circuits share a common structure, the PnC.

As stated by Blumenthal, “The fact that a lead stimulus can modify the response to a startle stimulus proves that the neural signals activated by the two stimuli converge at some
A stimulus intensity threshold around 80 dB must be reached for the information to get passed on to the caudal pontine reticular nucleus, a crucial element in the startle pathway (Kohl et al., 2013). But this response can be modified if a weak lead stimulus is presented just prior to the startle stimulus. The prepulse is thought to activate an inhibitory projection to the startle center (Blumenthal et al., 2004; Franklin et al., 2009; Koch, 1999). The proposed PPI pathway involves key structures including the cortex, striatum, pallidum, and pons, which will be referred to as CSPP circuitry from now on.

**PPI Pathway**

Based on timing approximations of impulse conductance and synaptic transmission, there can only be five neuronal connections at most in the PPI circuit (Swerdlow et al., 2001). Excitatory, fast sensory input is modified by a parallel inhibitory system via a reflex pathway located in the brainstem core (Hoffman & Ison, 1980). Efficient communication between the brainstem and forebrain is responsible for the fast relay of PPI. Sequential and parallel neural connections between the limbic cortex, ventral striatum, ventral pallidum, and the pontine tegmentum are thought to regulate startle modification (Swerdlow et al., 2001), and are involved in the pathophysiology of several neuropsychiatric disorders (Swerdlow &
Geyer, 1999). Understanding the PPI pathway can help us understand the nature of some of these clinical disorders. Currently, research supports the involvement of the inferior colliculus (IC), the superior colliculus (SC), the pedunculopontine tegmental nucleus (PPTg), and the PnC (Koch, 1999). Auditory input from the prepulse excites the IC, SC and PPTg, and the PPTg inhibits the PnC (Kohl et al., 2013). Figure 3 provides a summary of the sequence of synapses involved in this response. Like the ASR pathway, lesion and electrical stimulation methods have proven the role of these structures in mediating PPI. For example, lesions to the IC increase baseline startle and disrupt PPI, whereas electrical stimulation of the IC before startle stimulus creates an inhibitory effect (Fendt et al., 2001). In addition, activation of the SC and PPTg improve perceptual processing, which may have a protective effect.

Several authors have suggested that the PPTg sends an inhibitory cholinergic projection to the PnC to attenuate startle (see Blumenthal, 1996; Fendt et al., 2001; Franklin et al., 2009; Koch, 1999; Romirez-Moreno & Sejnowski, 2012; Swerdlow & Geyer, 1999). The inhibitory input from the PPTg must exceed the excitatory input generated by the startling stimulus in order for a response to be completely prevented, but as long as inhibitory input is present there will be some response attenuation (Blumenthal, 1996). Latent inhibition denotes activation of the startle center by the prepulse occurs whether or not the startling stimulus is presented, but inhibition is actualized when the eliciting stimulus is presented (Blumenthal et al., 2004). The startle-inhibiting role of PPTg neurons may be a secondary effect of their role in alerting the cortex and facilitating survival behaviors. The cholinergic neurons of the PPTg also have excitatory connections with the thalamus, which activates the cortex and excites dopamine neurons (Kohl et al., 2013). Clearly the PPTg has several
important roles, but for our purposes the startle mediating function is of particular interest. Overall, the regulation of PPI is thought to involve limbic cortical inputs to the striatum, striatal connections with the pallidum, and pallidal inputs the pontine tegmentum. Activation of the pontine tegmentum at the PPTg inhibits the PnC so that the response to subsequent stimuli is reduced (Swerdlow & Geyer, 1999). Knowledge of this pathway is helpful when studying the association between neuropsychiatric disorders and impaired PPI.

![Diagram of neural pathway involved in prepulse inhibition]

Figure 3. A basic summary of a proposed neural pathway involved in prepulse inhibition.

**Abnormalities in PPI Circuitry**

Differences in the startle response between individuals with neuropsychiatric disorders and controls are significant. Researching the structural abnormalities present when startle modification is deficient may provide information about the etiology of various disorders. For example, the amygdala is critically involved in fear-potentiated startle,
whereas the bed nucleus of the stria terminalis (BNST) plays a role in the enhancement of startle associated with generalized anxiety (Grillon et al., 2002; Koch, 1999). Fear-potentiated startle is the increase in startle amplitude to startle stimuli delivered in the presence of a conditioned stimulus. An overactive amygdala may be responsible for some of the fearful symptoms and reduced ppi seen in panic disorder (Ludewig et al., 2002). In this way, stress and anxiogenic factors decrease the amount of prepulse inhibition. In addition, the BNST plays an essential role in corticotropin-releasing hormone (CRH) enhanced startle (Davis et al., 1999). Koch agrees that stress hormones and neurotransmitters released in response to aversive events may facilitate neuronal transmission in the ASR pathway. She found that infusions of CRH into the PnC enhanced the startle response (1999). CRH triggers the release of hormones that mediate the stress response, so it is reasonable that administration of the hormone increases the startle response.

Lateralization is another example of how biological abnormalities are associated with impaired PPI. Previous studies have indicated lateralized dysfunction or abnormal asymmetry in several neuropsychiatric disorders (Swerdlow & Geyer, 1999). For instance, right and left asymmetry has been reported in OCD and schizophrenia patients (Braff et al., 2001). Most studies have recorded EMG of the right eye only, and since the information is processed contralaterally, impaired startle inhibition would be associated with abnormalities in left hemisphere forebrain activity (Braff et al., 2001; Swerdlow & Geyer, 1999). However, Duley et al. found that an increase in PPI among high trait anxiety participants was only significant for the left eye following exercise, which implicates greater right hemisphere lateralization (2007). Recent studies have used bilateral blink recording, which may allow for
further examination of hemispheric differences in PPI in psychiatric disorders. More research is necessary to determine lateral differences in PPI regulation among these populations.

Furthermore, researchers speculate the involvement of several neurotransmitters in the mediation and modification of startle. Many of these chemical messengers alter cognition and behavior and are implicated in certain mental health conditions. Glutamate, dopamine, serotonin, acetylcholine (ACh), and GABA converge in the nucleus accumbens of the ventral striatum and are thought to and affect PPI (Koch, 1999; Swerdlow & Geyer, 1999). The nucleus accumbens is an important connection between the forebrain and limbic structures that control cognition and behavior. Cholinergic activation of the hippocampus may impair PPI by stimulating glutamate release in the nucleus accumbens (Swerdlow & Geyer, 1999). The hippocampus is a subregion of the limbic cortex and evidence supports its function in sensorimotor gating via glutamatergic activity (Swerdlow et al., 2001). Glutamate acts on NMDA or non-NMDA receptors in the PnC (Davis et al., 1999; Koch, 1999), which plays a critical role in ASR and PPI, as previously discussed. Blocking glutamate receptors with NMDA antagonists reduces PPI and produces symptoms of schizophrenia (Swerdlow & Geyer, 1999). Although several questions remain about the effects of glutamate, research of this type benefits our comprehension of the interaction between decreased startle inhibition and psychiatric disorders. Additionally, the nucleus accumbens may serve a major role in mediating dopamine activity (Swerdlow & Geyer, 1999). Tricyclic substances (antidepressants) have a disruptive effect on PPI, which implies that dopaminergic systems are involved in the regulation of startle modification (Hoenig et al., 2005). Injecting dopamine (DA) agonists and activating D1 and D2 dopamine receptors facilitates the startle response (Braff et al., 2001; Fendt et al., 2001; Swerdlow & Geyer, 1999). Psychiatric
disorders that involve irregular dopamine production often exhibit abnormal sensorimotor gating. For instance, dopamine and serotonin systems are involved in the regulation of cortico-striato-pallido-pontine circuits, but are also implicated in the pathophysiology of OCD (Hoenig et al., 2005). However, more research is necessary to confirm the mechanism by which the neurotransmitter affects PPI. Furthermore, antipsychotics work by altering the effects of dopamine, serotonin, ACh and other chemicals in the brain that change mood and behavior. The clinical efficacy of antipsychotic medications correlates with their ability to normalize sensorimotor gating (Swerdlow & Geyer, 1999). Clearly, the nucleus accumbens and associated neurotransmitters affect startle modification by means that are not completely understood, but research in this field holds the possibility to help treat a variety of neuropsychiatric disorders.

**Neuropsychiatric disorders**

Neuropsychiatric disorders that exhibit reduced PPI are usually characterized by high distractibility and an inability to filter out irrelevant stimuli due to deficits in sensorimotor gating and abnormalities in CSPP circuitry (Blumenthal, 1999; Duley et al., 2007; Ludewig et al., 2002; Swerdlow & Geyer, 1999). Clinical evidence shows impaired startle modification in schizophrenia, schizotypal personality disorder, obsessive-compulsive disorder (OCD), Huntington’s disease, Tourette syndrome, attention-deficit/hyperactivity disorder (ADHD), temporal lobe epilepsy with psychosis, enuresis, and post traumatic stress disorder (PTSD) (Blumenthal, 1999; Braff et al., 2001; Hoenig et al., 2005; Koch, 1999; Swerdlow & Geyer, 1999). Extensive research demonstrates impaired PPI in schizophrenics, however anxiety spectrum disorders have received less attention. Deficits in information...
processing are a defining feature of schizophrenia and may result from sensory overload (Braff et al., 1978; Filion et al., 1998). In other words, a loss of preattentive filtering causes a flood of sensory stimuli and manifests as symptoms of schizophrenia. Braff et al. found that patients exhibit significantly less startle inhibition than controls at a 60 ms lead interval (1978). Additionally, relatives of schizophrenia patients show decreased PPI compared to controls, which indicates PPI is an important tool for genetic studies (Braff et al., 2001). Determining the neural basis of PPI abnormalities in schizophrenics could provide more effective treatment options for the disorder.

Unlike schizophrenia research, examinations of PPI deficits in anxiety spectrum disorders have been inconclusive so far. Evidence suggests reduced startle inhibition in PTSD, OCD, panic disorder (PD), and high trait anxiety (Franklin et al., 2009). For example, Grillon et al. demonstrated reduced PPI in veterans with PTSD compared to civilian controls, although baseline startle was not amplified (1996). According to the DSM-IV-TR, an exaggerated startle response is a diagnostic criterion for PTSD (American Psychiatric Association, 2000). Patients exhibit a lower threshold for startle elicitation and decreased PPI compared to controls (Braff et al., 2001). Sensorimotor gating deficits are thought to contribute to PTSD symptoms such as intrusive thoughts and flashbacks, but this may result from “an acute state of conditioned fear or anxiety, rather than a persistent trait variable” (Grillon et al., 1996). Further assessment of baseline startle levels and startle modification in PTSD patients is necessary to support these findings. In addition, Hoenig et al. studied PPI in individuals with OCD and found that startle inhibition was 19.3% less in unmedicated OCD patients compared to controls, but significant impairments were only present at the most intense prepulse intensity (2005). They believe the inability to inhibit intrusive thoughts and
compulsions is caused by abnormalities in fronto-striatal brain regions involved in sensorimotor gating (Hoenig et al., 2005). Furthermore, PD is another anxiety disorder in which individuals are unable to inhibit their response to internal and external stimuli. Ludewig et al. found that PD patients show less habituation and reduced PPI compared to controls, which may indicate a problem in early information processing (2002). Habituation is necessary to discriminate relevant from irrelevant stimuli. Decreased levels could be responsible for the symptoms of intense fear and hypervigilance to bodily sensations experienced by individuals with this anxiety disorder. Research evaluating anxiety states such as fearfulness, phobias, and stress disorders have shown that high-fear groups (Fear Survey Schedule) have a larger startle response during negative imagery than low-fear groups (Filion et al., 1998). Additionally, data shows high trait anxiety correlates with reduced PPI (see Duley et al., 2007; Grillon et al., 1996; Ludewig et al., 2002). There is a difference between fear and anxiety, and it is important to take this into consideration when studying various disorders in the anxiety spectrum.

**Anxiety and PPI**

Anxiety is a general state of discomfort and apprehension in anticipation of a potential threat (Grillon, 2002; Koch, 1999). It is a more chronic, future-oriented response, whereas fear is an acute response to an explicit threat (Barlow, 2002; Grillon, 2002). While both arouse the central nervous system, symptoms of anxiety present in a different manner than fear. Symptoms include avoidance, escape, hypervigilance, exaggerated startle, and physiologic signs of arousal such as rapid heart rate, sweating, nausea, chills, trembling, and hyperventilation (Wipfli et al., 2008). These signs of anxious apprehension are caused by a
perceived inability to control upcoming events. A sense of uncontrollability is central to anxiety, and hypervigilance indicates that the individual is ready to deal with potentially negative events (Barlow, 2002). If danger is unpredictable, the person cannot distinguish between periods of danger and safety, and remains in a continuous state of anxiety (Grillon, 2002). That is, defense mechanisms are continuously activated in case a threat is realized. According to Barlow, the function of anxiety or stress is “to prepare the organism both biologically and psychologically to meet the challenges and conflicts of day-to-day life” (2002). A moderate amount of anxiety increases performance, but beyond a certain level it can hinder it. Studies often report using state anxiety and trait anxiety as variables in the research design. State anxiety is an acute psychological response that is more situational, whereas trait anxiety is a chronic tendency to become anxious (Paluska & Schwenk, 2000).

Studies show heightened baseline startle and reduced PPI in anxious patients. For example, Gillon found that baseline ASR is elevated in individuals who have been exposed to chronic stress, as CRH has anxiogenic effects that may contribute to the potentiation of startle (2002). As previously discussed, the effects of CRH on the startle response have been evidenced by Davis et al. and Koch. Alternatively, in support of the sensory overload theory, Ludewig et al. believe impaired information processing causes an overwhelming inundation of information that is a source of anxiety (2002). They found a significant correlation between high trait anxiety, as measured by the State Trait Anxiety Indicator (STAI), and decreased PPI (Ludewig et al., 2002). While both of these theories are legitimate, more studies are necessary to provide supporting evidence and replicate the results of existing research. Anxiety spectrum disorders are often associated with reduced PPI, but there have been very few studies that have explored how anxiety spectrum constructs are associated
with PPI in normative samples (Franklin et al., 2009). Franklin et al. conducted a study on Cluster C personality disorders, and found that all anxiety spectrum disorders were significantly negatively correlated with PPI, with the exception of obsessive-compulsive personality disorder (2009). Cluster C consists of fearful-anxious personality disorders of the avoidant, dependent, and obsessive-compulsive types (DSM-IV-TR, American Psychiatric Association, 2000). While most research has focused on establishing that PPI is reduced in certain neuropsychiatric conditions, Duley et al. went one step further. They examined how the anxiolytic effects of exercise affect sensorimotor gating deficits in patients with high trait anxiety (2007). PPI was impaired in high trait anxiety individuals following a quiet rest session, but no group differences were seen following the exercise session (Duley et al., 2007). For years exercise has been known to reduce stress, but the exact mechanism by which this occurs is still uncertain. Duley’s study shows that PPI may be a necessary tool to evaluate how exercise mitigates anxiety.

Extensive research has proven exercise has the ability to improve symptoms of anxiety and other mental health disorders. In fact, exercise alone is as effective at reducing anxiety as psychotherapy, meditation, and relaxation techniques, and nearly as effective as pharmacotherapy (Paluska & Schwenk, 2000; Wipfli et al., 2008). Pharmaceuticals provide the most rapid benefits for patients with depression and anxiety, but combination therapy using exercise interventions as adjunctive treatment may be more beneficial long-term (Stathopoulou, Powers, Berry, Smits, & Otto, 2006). Many adults do not meet the physical activity recommendation set by the American College of Sports Medicine, and this may be especially detrimental for mental health disorders. Physical inactivity puts individuals with neuropsychiatric disorders at increased risk of morbidity (Paluska & Schwenk, 2000),
whereas higher levels of physical activity are associated with higher quality of life (Stathopoulou et al., 2006). Guszkowska claims that exercise primarily effects the somatic aspects of anxiety (i.e. muscle tension, pain, rapid heart rate), but a distractive mechanism may serve to direct attention away from daily worries and reduce anxious thoughts (2009). In support of the distraction method, Stathopoulou et al. suggest exercise promotes increased coping abilities among anxiety patients and may alter the accessibility or intensity of worries. However, there are various theories that attempt to rationalize the anxiolytic effects of exercise. For example, the monoamine hypothesis proposes that exercise increases transmission of monoamines (norepinephrine, dopamine and serotonin), and the endorphin hypothesis proposes that exercise increases endorphin secretion, which reduces pain and causes euphoric feelings (Paluska & Schwenk, 2000). Furthermore, different results have been reported with state versus trait anxiety groups. In Guszkowska’s study, participants with high pre-exercise state anxiety had the largest reduction in anxiety post-exercise, and results were largely independent of trait anxiety (2009). Paluska and Schwenk also found that acute anxiety responds better than chronic anxiety. However, programs exceeding 21 minutes per session for more than 10 weeks produced significant reductions in trait anxiety, with maximum benefits with 40 minute sessions (2000). There is a dose-response relationship between exercise and mental health that shows as physical activity levels approaches 12.5 kcal · kg-1 · week-1, greater reductions in anxiety result (Wipfli et al., 2008). However, benefits can be obtained from even a single bout of exercise. To evaluate how an exercise session affected anxiety and PPI, Duley et al. had participants participate in 30 minutes of exercise at 70% heart rate max before administering the STAI and PPI procedure. Exercise was found to have a normalizing effect, as it moderated the sensorimotor gating deficits
exhibited by anxious patients (Duley et al., 2007). Therefore, aerobic exercise may be a treatment option for anxiety disorders that is cost-effective and has fewer side effects than traditional drug therapy. In addition, it is possible that a minimum amount of exercise might be necessary to keep anxiety at healthy levels (Broman-Fulks et al., 2004).
CHAPTER III
Materials and Methods

The purpose of this study was to determine if prepulse inhibition of the acoustic startle reflex is reduced in individuals with high trait anxiety compared to controls. Five male and seventeen female students (age 18-28) were recruited by email from the University of Mississippi. Participants were recruited from a larger screening sample of 176 students who had previously completed the Depression Anxiety Sensitivity Scales (DASS-21) assessment. Individuals from ascending lower quartile scores and descending upper quartile scores were contacted, and subjects were randomly selected from those who replied with the intention of obtaining an equal ratio of high and low anxious participants. The high trait anxious (HA) group \((n = 12)\) included two males and ten females, with a mean age of 20.00 (SD=1.13) and mean DASS score of 17.8 (SD = 5.9). The low trait anxious group (LA) included three males and seven females, with a mean age of 19.90 (SD =1.29) and mean DASS score of 3.24 (SD = 2.8). Subjects completed a University approved informed consent prior to participation, and all procedures were approved by the Institutional Review Board of the University of Mississippi. None of the participants reported any hearing impairments or use of anxiety medication. Subjects received research credit and a fifteen dollar gift card in exchange for their participation.
**Questionnaires—DASS 21**

The Depression Anxiety Sensitivity Scales (DASS) were used to index the severity and frequency of anxious symptoms. The questionnaire includes 42 negative symptoms; 14 each cover depression, anxiety, and stress. The DASS-21 is a shortened version that includes 21 items and takes less time to administer. To convert to full scale scores, the DASS-21 scores are multiplied by two. The anxiety scale mainly includes symptoms of autonomic arousal, whereas the stress scale covers symptoms of tension, irritability, and overreaction (Lovibond, 2006). The scales yielded a spectrum of psychological distress, and participants were randomly selected from the upper and lower quartiles.

**Prepulse and pulse stimuli**

Sound files were created using a waveform generator in Biopac software. All sound stimuli profiles were created and subsequently presented at 20 KHz. A background of 55 dB (A) white noise was created to obscure ambient noise (~53 dB) and underscore the stimulus presentation. Acoustic stimuli were delivered binaurally using Sennheiser headphones (HD 518). The prepulse stimulus was a 40 ms broadband white noise presented at 70 dB (A). The pulse was a 40 ms broadband white noise presented at 100 dB (A). Both the prepulse and pulse stimuli had near instantaneous rise times. Sound calibration was completed using a RadioShack (33-2055, Fort Worth, TX) digital sound meter.

**Procedure**

After the completion of a University approved informed consent, participants sat in a chair in a quiet room (ambient noise level ~53 dB). The subjects’ skin under the eye and
behind the ear was cleansed with rubbing alcohol to prevent interference with the electrodes. A ground electrode was placed on the bony process behind each ear. Two lead electrodes were affixed on each side of the face to pick up activity in the orbicularis muscles surrounding the eye. One electrode was placed below the lower eyelid in line with the pupil in forward gaze, and a second was placed approximately 1-2 cm lateral to the first. The subjects received instruction to maintain their gaze on a specified area on a wall located approximately 2 meters in front of them before putting on the headphones. Participants were informed that they would hear sounds coming from the headphones that they should try to ignore. The subjects were alone during testing, aside from the individual collecting data that was out of sight. The entire testing session lasted about 20 minutes, and was comprised of 3 blocks of 15 trials. Each block contained 5 trials each of prepulse–pulse pairs at a 60 ms discrete lead interval, 5 pulse only trials, and 5 prepulse only trials (i.e., 45 total). The stimulus order was randomized for each participant, as was the intertrial interval which included 15, 21, and 28 second ITI times. Following the testing session, subjects were thanked for their participation and awarded a gift card and psychology research participation hour.

Data analysis

Data were originally sampled at 20 KHz using an MP150 control module and Acknowledge software (BIOPAC Systems, Inc.). Recorded data included the stimulus tone and the EMG for the left and right eye. This is a high sampling rate for EMG, but it was found that the presented tone was distorted through the headphones if it was not sampled at the same sampling rate as it was originally created with the waveform generator. Data were
exported to Matlab (The Mathworks Inc.) for subsequent analysis. In Matlab, data were ‘resampled’ or down sampled to reduce the effective sampling rate to 1000Hz. EMG channels were filtered with a notch and band pass filter (IIR notch = 60 Hz, 4\textsuperscript{th} order Butterworth band pass = 10-250 Hz). The EMG were then rectified and smoothed using a 5 point averaging window. Peak EMG values between 20 and 180 msec post tone were stored for both startle and prepulse startles. Repeated Measures ANOVA was used to test for significant differences for startle type and anxiety status between cohorts.
CHAPTER IV

Results

Nonresponse and rejection criteria were established prior to analyzing the data. A nonresponder is defined as a participant who fails to respond on a predetermined number of stimulus presentations. Subjects had to respond to at least five of the startle stimulus trials, and to at least four of the prepulse-startle paired stimulus trials. Trials were excluded if unstable baseline EMG activity was observed, or if a voluntary blink occurred near the stimulus onset. Unstable baseline activity often indicates noise or movement artifact. There were a total of 15 nonresponders, and of these, the majority were LA subjects. While only five participants completely failed to respond to either stimulus condition, 15 participants had less than four responses on prepulse-startle trials.

Response Probability

A general linear model with repeated measures was performed for the analysis of response probability. A 2 (Group: LA, HA) x 2 (Stimulus: startle, prepulse-startle) multivariate ANOVA was conducted to compare response probability for the startle and prepulse-startle conditions between the LA and HA cohorts. The response probability within subjects did not reach significance, $F (1, 20) = 0.848, p = 0.368, df = 1$, which indicates that the probability of responding to the pulse was similar to the probability of responding to the prepulse-pulse paired stimuli. When comparing response probability measures between
subjects, results approached but did not reach significance, \( F(1, 20) = 3.834, p = 0.064, \text{df} = 1 \). The mean response probability for LA subjects on pulse only trials was 45%, versus 72% for the HA group. The mean response probability for LA subjects on prepulse-startle trials was 12%, compared to 27% among HA participants. Clearly, high anxious individuals have an increased sensitivity to startle stimuli and respond more frequently. This was expected, as the literature supports an elevated baseline startle response for people with anxiety. With more participants, the between subjects comparison of response probability would likely reach statistical significance.

Figure 4. Response probability between LA and HA cohorts on startle-only trials (1) and prepulse-startle trials (2).
Prepulse Inhibition

Since only two LA participants responded to four or more prepulse-pulse trials, an ANOVA analysis could not be performed for this variable. Response amplitude was compared between groups for startle stimuli with a paired-samples T-Test. Analysis revealed a significant difference in response amplitude between and the LA and HA groups ($t = -2.494$, $p = 0.032$, $df = 10$). The mean response amplitude for startle stimuli among the LA participants was 0.041 V, whereas HA participants averaged 0.075 V. Although a complete analysis of prepulse-pulse data could not be performed, our calculations suggest that HA individuals exhibit impaired PPI compared to controls. The proportion of difference for the LA group was 40.1%, compared to 37.9% for the HA group.
CHAPTER V
Discussion

Previous researchers have called for further examination of the relationship between anxiety and sensorimotor gating (Franklin et al., 2009). The purpose of this study was to determine if prepulse inhibition of the acoustic startle reflex is reduced in individuals with high trait anxiety compared to controls when using a SnR of +15 dB. We examined startle modification in anxious participants using a short lead interval protocol. In support of previous research indicating higher baseline startle in individuals experiencing stress or anxiety (Grillon, 2002), our results revealed a significant difference in startle amplitude between high and low anxious subgroups. However, an insufficient number of responders for the prepulse-pulse condition prevented a legitimate comparison of PPI between cohorts. For this reason, our first and second hypotheses could not be supported. Ho1 stated that “preceding an intense startle-eliciting stimulus (100 dB, 40 ms) with a weak non-startling stimulus (70 dB, 40 ms) would result in decreased startle amplitude”. Although there was inadequate data to draw concrete conclusions, calculations on the figures obtained showed decreased response amplitude for the prepulse-pulse condition compared to the startle only condition. Ho2 stated that “highly trait anxious (HA) participants would exhibit reduced PPI compared to the low trait anxious (LA) group”. Again, there were too many nonresponders for conclusions to be made for this assertion, but calculations on the data obtained.
demonstrate a 2.2% difference between the LA and HA groups, indicating impaired sensorimotor gating among highly anxious individuals. For the third hypothesis, it is possible that the 15+ dB SnR was not ideal for this experiment. As expected, none of the participants responded to the 70 dB (A) prepulse only stimulus. This stimulus is below the startle threshold of 85 dB (A) and should not elicit a response. All but five of the 22 participants responded to the 100 dB (A) startle only stimulus. However, several participants responded to the prepulse-pulse stimuli during the interstimulus interval, and could not be counted since the response did not fall in the 20-200 ms interval after the pulse onset. The Duley (2007) study reported a 60 dB ambient noise level, but did not state whether background noise was created to mask the ambient noise. Since the SnR used was unknown, it was difficult to replicate the results. Overall, this study supported a general trend toward HA subjects responding more intensely (i.e. increased response amplitude) and more frequently (i.e. increased response probability) than controls.

*Right Hemisphere Lateralization*

Since Duley et al. found that an increase in PPI post-exercise was only significant for the left eye in HA participants (2007), this study analyzed EMG activity from the left eye only. While the goal of the current study was to establish whether PPI is diminished among anxious individuals, our research will continue with the intention of replicating the results of Duley’s study (2007). That is, the amount of prepulse inhibition among high and low anxious individuals will be examined after a bout of acute exercise compared to a quiet rest session.

*Environmental Noise*
A quiet testing environment is essential to the reliability of the results obtained. The ambient noise in the lab was found to be ~53 dB during ideal conditions. However, the lab is located in a building shared by other professions, and we found it difficult to completely control external noise throughout the testing procedure. The headphones block a certain degree of external noise, but this may have distracted participants or affected the impact of startling stimuli to a minimal extent.

_Habituation and Attention_

A reduction in the amount of prepulse inhibition can occur as the testing session proceeds due to a change in startle reactivity. Blumenthal showed that the lead stimulus continues to have the same inhibitory effect throughout the test, but that a subject’s sensitivity to startling stimuli decreases with repeated presentation (1999). While measures were taken to prevent habituation (i.e. randomization of trial order and ITI duration), reduced startle reactivity may have occurred due to the length of the test and the number of trials presented. Each subject was presented with 3 blocks of 15 trials (i.e. 45 total trials), and each block lasted approximately 5 minutes. The ITI durations of 15s, 21s, and 28s were chosen based on previous research in order to maximize response probability. For example, Franklin et al. (2009) used 14 to 23 second intertrial intervals, and Blumenthal et al. (2004) used 25 to 35 s intervals. A total testing time of 15 minutes seemed to produce fatigue among participants. Therefore, future studies should consider the length of the time the subject is continually being presented with sensory information. While the subject’s alertness may affect startle reactivity, PPI reflects preattentive processing at lead intervals <120 ms and is
not influenced by attentional factors (Braff 2001). Since the interstimulus interval in our study is 60 ms, attention is not a factor in the results of this experiment.

**Recommendations**

In order to replicate the results of existing research, it is necessary to establish a standard method of measuring stimulus intensity. While most researchers report the device (i.e. headphones, speakers) used to administer stimuli, none have described detailed methodology for measuring stimulus intensity. For example, Duley et al. (2007) reported using a Radioshack sound meter, but did not say whether there was direct contact between the headphone and sound meter. Future research should consider whether the distance of the external ear canal should be taken into account when configuring the intensity of the prepulse and pulse stimuli.
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Effects on Human Startle Reflex in Normals and Schizophrenics. *Psychophysiology,

of startle: normal subjects, patient groups, and pharmacological studies.
*Psychopharmacology, 156*(2/3), 234.


http://dx.doi.org/10.1016/j.paid.2008.11.004


Good afternoon,

Your email came up because a survey you took indicates that you may be experiencing some stress.

I am an exercise science student at Ole Miss and I’m working on a study that is testing people who regularly feel worried or anxious. The goal is to determine whether the brain processes auditory information differently in people who are under stress.

People show a startle response to a loud, sudden noise. But if a weaker noise occurs shortly before the loud noise, most individuals are less startled. There is some research that shows people who exhibit anxious symptoms startle just as much to either stimulus. This implies that there is a difference in how information is processed by the brain. This study will provide insight into the nature of anxiety.

This study has been reviewed by The University of Mississippi’s Institutional Review Board. If you participate you can receive up to 1 hour of research credit and a $15 Walmart gift card. All you will have to do is come for a 30 minute testing session, where you will sit in a chair and listen to some sounds.

If you wish to participate, please e-mail me back so I can set up a time for you to come to the lab. Your participation will be confidential and there is no risk to you.

If you have any questions feel free to contact me or Dr. Waddell. We would greatly appreciate your participation.

Thank you,
Krista Sturm

Krista Sturm
B.S. Exercise Science
University of Mississippi
klsturm@go.olemiss.edu
314-283-6390

Dwight Waddell, M.S., Ph.D.
Biomedical Engineering
University of Mississippi
waddell@olemiss.edu
662-202-4356
APPENDIX B:

IRB Approval & Consent Form

Office of Research and Sponsored Programs
The University of Mississippi
109 Berry Hall
P.O. Box 907
University, MS 38677
Office (662) 915-7485
Fax (662) 915-7315

Ms. Krista Sturm
Exercise Science
University, MS 38677

IRB Protocol #: 14-032
Title of Study: Pre-Pulse Inhibition Measures and Trait Anxiety
Approval Date: December 6, 2013
Expiration Date: December 5, 2014

12/6/2013

Dr. Dwight Waddell
Electrical Engineering
University, MS 38677

Dear Ms. Sturm and Dr. Waddell:

This is to inform you that your application to conduct research with human participants has been reviewed by the Institutional Review Board (IRB) at The University of Mississippi and approved as Expedited under 45 CFR 46.116, 4 and 7.

Research investigators must protect the rights and welfare of human research participants and comply with all applicable provisions of The University of Mississippi’s Federal wide Assurance 00000802. Your obligations, by law and by University policy, include:

- Research must be conducted exactly as specified in the protocol that was approved by the IRB.
- Changes to the protocol or its related consent document must be approved by the IRB prior to implementation except where necessary to eliminate apparent immediate hazards to participants.
- Only the approved, stamped consent form may be used throughout the duration of this research unless otherwise approved by the IRB.
- A copy of the IRB-approved informed consent document must be provided to each participant at the time of consent, unless the IRB has specifically waived this requirement.
- Adverse events and/or any other unanticipated problems involving risks to participants or others must be reported promptly to the IRB.
- Signed consent documents and other records related to the research must be retained in a secure location for at least three years after completion of the research.
- Submission and approval of the Progress Report must occur before continuing your study beyond the expiration date above.
- The IRB protocol number and the study title should be included in any electronic or written correspondence.

If you have any questions, please feel free to contact the IRB at (662) 915-7482 or irb@olemiss.edu.

Sincerely,

Dr. Thomas W. Lombardo, Ph.D.
Director, Division of Research Integrity & Compliance
CONSENT FORM

Consent to Participate in an Experimental Study

Title: Pre-Pulse Inhibition And Anxiety In A College-Aged Population

Investigator
Krista Sturm
Division of Exercise Science
Kinnard, Room 275-F
The University of Mississippi
ksturn@gateway.olemiss.edu
1.314.283.5390

Advisor
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1.662.242.4356

☐ By checking this box I certify that I am above 18 years of age but not older than 28.

Description
We are investigating the effect of loud noise on the acoustic startle reflex. In psychology, ‘acoustic startle’ is defined as a “defensive reaction that occurs in response to a sudden and intense sound.” The magnitude of the startle reaction is quantified by how much or how hard you blink your eyes in response to a loud noise. It has been determined that the level of anxiety a person is feeling may be correlated in some way with their startle response.

You will be asked to sit in a comfortable chair for approximately 20 minutes while wearing a pair of headphones. In addition to the headphones, a pair of electrodes (about the size of a small round Band-Aid) will be placed below your eyes to record the electrical activity of your “eye-blink” which is your body’s response to startle. You will listen to 3 trials of pre-recorded white noise (each lasting about 5 minutes) consisting of ambient or background noise, and loud infrequent clicks (about .1 second in duration) which will be much louder than the background noise.

Risks and Benefits
The loud clicks may cause you to jump, or blink a little bit, there is no danger whatsoever to your hearing.

Cost and Payments
The tests will take about 20-25 minutes to complete. For completion of the study you will receive a 15 dollar gift certificate to Walmart. You will also receive 30 minutes of experimental course credit for being part of this project whether you actually complete the entire protocol or decide to stop.
Confidentiality:
We will not put your name on any of your tests. The only information that will be on your test materials will be your gender (whether you are male or female) and your age. Therefore, we do not believe that you can be identified from any of your tests.

Right to Withdraw:
You do not have to take part in this study. If you start the study and decide that you do not want to finish, all you have to do is tell Krista Sturm or Dr. Dwight Waddell in person, by letter, or by telephone at the Department of Electrical Engineering, 915-2623. Whether or not you choose to participate or to withdraw will not affect your standing with the University, nor will it cause you to lose any benefits to which you are entitled.

The researchers may terminate your participation in the study without regard to your consent and for any reason, such as protecting your safety and protecting the integrity of the research data.

IRB Approval
This study has been reviewed by The University of Mississippi's Institutional Review Board (IRB). The IRB has determined that this study fulfills the human research subject protections obligations required by state and federal law and University policies. If you have any questions, concerns, or reports regarding your rights as a participant of research, please contact the IRB at (662) 915-7482.

Statement of Consent
I have read the above information. I have been given a copy of this form. I have had an opportunity to ask questions, and I have received answers. I consent to participate in the study.

_________________________  ________________
Signature of Participant     Date

_________________________  ________________
Signature of Investigator    Date

NOTE TO PARTICIPANTS: DO NOT SIGN THIS FORM
IF THE IRB APPROVAL STAMP ON THE FIRST PAGE HAS EXPIRED.