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PREFRONTAL CORTEX ACTIVITY DURING ATTENTIONAL BIAS CONDITIONING WITH FEARFUL FACES: A NEAR-INFRARED SPECTROSCOPY ANALYSIS

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By

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ABSTRACT

PREFRONTAL CORTEX ACTIVITY DURING ATTENTIONAL BIAS CONDITIONING WITH FEARFUL FACES: A NEAR-INFRARED SPECTROSCOPY ANALYSIS

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Observing a fearful facial expression elicits an automatic orienting of attention. Past research focusing on fear conditioning has used an unconditioned stimulus that the participant directly experiences (e.g. shock). Other research has focused on observational fear learning where the participant watches another individual receive the stimulus. The aim of this study was to condition a colored square with a fearful face in the dot-probe task. Orienting toward and disengagement from fearful faces was also examined using the dot-probe task. In addition, hemodynamic responses in the prefrontal cortex (PFC) were measured using near-infrared spectroscopy (NIRS). It was hypothesized that after pairing a neutral stimulus (a colored square) with a fearful face, attentional bias toward the fearful face would transfer to the neutral stimulus. It was further hypothesized that the medial PFC (mPFC) would be more active during learning and extinction and that the mPFC would be involved with early and late phases of attention processing. The results suggest that attentional bias toward fearful faces did not transfer to a colored square. However, this study did find an orientating of attention toward fearful faces and a delayed disengagement of attention from fearful faces. Analysis of the NIRS data was purely exploratory and indicated that the mPFC was involved in orienting attention toward the fearful face while the lateral PFC was involved in delayed disengagement of attention.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Recognizing faces is an important behavior that aids in communication with other individuals. As Bruce and Young (1986) stated, a face can communicate different characteristics such as identity, age, gender, and emotion. Even though the identity of a person can be determined through other physical features—body shape and voice—the face is the most reliable way to correctly identify an individual. Being able to recognize a loved one, a colleague, and even an enemy is important for survival because one must know with whom to share resources and whom to avoid. Neurological studies have examined how faces are processed in the brain. In human and nonhuman primates, researchers found, through lesion studies, a double dissociation between processing identity and emotional expression (Haxby, Hoffman, & Gobbini, 2002; Humpherys, Donnelly, & Riddoch, 1993). That is, certain areas of the brain respond to the identity of the face while other areas respond to the expression of the face. Ekman (1999) hypothesized that a vital aspect of social functioning is being able to accurately interpret another's emotional facial expression. For example, it would not be socially acceptable to react to someone's sad facial expression with a smile. Recognizing that the individual's facial expression is sad would assist in making actions during the social interaction more appropriate.

Perceiving emotional faces causes neural and behavioral responses. When participants were presented with faces expressing pain, their cortical areas of the brain that respond to direct experience of pain were responsive (Botvinick et al., 2005). Research has shown that fearful and angry facial expressions capture visuospatial

attention more so than neutral facial expressions when participants are consciously aware (Mogg & Bradley, 2002; Pourtois, Grandjean, Sander, & Vuillemier, 2004) and when conscious awareness is restricted (Carlson & Reinke, 2008; Mogg & Bradley, 2002). Restricting conscious awareness is accomplished by backward masking. Backward masking refers to presenting a stimulus, a fearful face, for a short time, 33 ms, and replacing it with a neutral stimulus, a neutral face, making it difficult to identify the initial stimulus.

Two different ways we learn about fear are through direct and indirect experiences. Fear can be learned through personally experiencing an aversive stimulus that caused pain or sadness (i.e. Pavlovian conditioning). Fear can also be learned through witnessing another individual experience an aversive stimuli (i.e. observational learning). Past research suggests that the PFC is involved in fear learning (Olsson, Nearing, & Phelps, 2006). Olsson and Phelps (2007) stated that a fearful faces would be salient enough to be as efficient as direct experience. However, this claim has not yet been empirically tested. The aim of this study was to examine whether pairing a fearful face with a neutral stimulus (i.e. a colored square) would cause an attentional bias toward the neutral stimulus at a later time when it was presented alone without the fearful face. This study also examined the neural correlates in the PFC associated with fear conditioning.

Emotional Faces and Attentional Bias

Darwin's *The Expressions of the Emotions in Man and Animals* (1872) discussed the biological origins of emotional facial expressions. Darwin described how in order for facial expressions to be innate, they must be produced by all healthy humans and used in the same context. Darwin hypothesized that if humans living in different regions, cut off from European influences, displayed the same emotional facial expressions as Europeans, then the facial expressions must have a biological basis. Darwin collected evidence that supported his hypothesis by asking Englishmen living in remote areas to document the facial expressions of the local people and the meanings behind the facial expressions. Even though his findings were criticized because he was relying on the testimonies of untrained observers, his theory was later supported by the research of Ekman and Friesen (1971) who assessed the Fore: a people that lived in New Guinea in isolation for 12 years prior to their study. The researchers found that after reading emotional stories, the Fore people picked a facial expression that expressed the emotion portrayed in the stories. These results suggested that emotional faces have a biological basis because even though the Fore people lived in isolation, they displayed the same facial expressions as the Europeans.

Even though facial expressions are an important aspect of social communication, facial expressions might have evolved primarily to benefit the expresser. Susskind et al. (2008) examined the functionality of fearful and disgusted facial expressions. They asked participants to make one of the emotional facial expressions and then tested the physiological effects. The participants that expressed a fearful face—eyebrows raised,

upper eyelids raised, nostrils flared, and jaw dropped—had a greater visual field and greater air volume intake despite using the same number of respirations than individuals that expressed a neutral face. Participants that displayed a disgusted face—raised upper lip, winkled nose, and raised cheeks—had a smaller visual field and less air volume intake than individuals that expressed a neutral face. Their results suggested that fearful facial expressions increased sensory acquisition, whereas disgust expressions restricted sensory acquisition. When an individual sees an object that causes a fear response (e.g. a bear), it would be advantageous for that individual to have increased air intake and visual field, whereas an individual that experiencing disgust (e.g. eating something rotten) would need to limit their senses as to prevent further intake of the object that was disgusting. Neuroimaging research has indicated that recognition of emotional faces relies upon mirroring the motor action to better understand the expresser's mental state (Adolphs, 2002). When individuals observes another expressing fear, they themselves might express fear to increase sensory acquisition to the environment.

Perceiving another's emotional face serves as an evolutionary benefit to understanding the world. Observers who perceives an angry face directed toward them must understand what it means—perhaps the person is angry with them—and the observer might need to react quickly to avoid an attack. The same may be true with happiness. The perception of a happy facial expression directed toward an observer is pleasing and may serve to reinforce the individual to continue the behavior that warranted the smile. Emotional faces are salient enough that they do not need to be fully processed in the visual cortex before individuals react to them (Carlson & Reinke, 2008). A particularly important emotional face needing rapid processing in the brain is a fearful

face. A fearful face is biologically important for humans because it indicates a potential threat in the environment and causes the amygdala, an important structure in the fear response network, to activate the fight or flight response (Adolphs, Tranel, Damasio, & Damasio, 1994).

Research on humans indicates that some visual information gets crudely processed in cortically blind patients via a direct route to the amygdala through the superior colliculus and pulvinar nucleus of the thalamus (Morris, DeGelder, Weiskrantz, & Dolan, 2001). The human brain has two different routes that sensory information takes for processing: the "low road" and the "high road." LeDoux (1996) described the low road as a quick and dirty analysis of the world; meaning, it processes sensory information quickly, but with little detail. This road travels through subcortical structures: specifically the thalamus to the amygdala. The high road is a relatively slow, but more detailed, path traveling through the sensory cortex which allows the amygdala to reevaluate whether or not the initial reaction was warranted (*see Figure 1*). Consider a hiker who is walking through the woods. In the distance, through his peripheral vision, he spots a large, round, hairy object. His attention is allocated to the object and he feels his heart rate increase only to realize that the object is a mossy tree stump. The hiker's heart rate slows back to the previous rate and he continues the hike. The visual information of the large object went through the thalamus and then divided into two separate pathways: one straight to the amygdala and one traveling through the cortex. Through prior experience, the amygdala learned that a large, round, hairy object in the woods could have been a bear, so the amygdala activated the fight or flight response. As the cortex processed the information, a more clear perception of the object appeared and it inhibited that response.

These two different paths help explain why conscious awareness is not needed for orienting of attention (Carlson & Reinke, 2008).

Experimenters have measured the allocation of attention toward fearful stimuli in various attention tasks. Attention has multiple facets, and different tasks could be used to measure the different facets. The emotional Stroop task is one of the most widely used attention tasks typically using emotional words in different colored font (Williams, Mathews, & MacLeod, 1996). When emotional words are presented, participants typically take longer to determine the color of the word because their attention is focused on the meaning of the word. Attentional blink is another attention task using a list of words with two targets in each list. The first target is neutral and the second is either neutral or negative. Participants are typically better at identifying the negative words suggesting that the negative words overcame the typical "attentional blink" caused by the first word, whereas neutral words do not (Anderson, 2005). Another method is the visual search task. During this task, the participant is presented with neutral targets and negative targets (e.g. fearful or angry faces). Participants are asked to identify whether the targets being presented are negative or neutral. Participants are typically better at detecting negative than neutral targets (Fox et al., 2000). These methods of measuring attention are effective, but they have limitations in accurately measuring both the initial orientating, where attention is captured by a stimulus, and later disengagement, where attention is held and then disengages from the location it was oriented toward.

The dot-probe task can be used to measure the orientating and disengagement of attention. This task could use images of animals or objects (e.g. snakes or plants; Carlson, Fee, & Reinke, 2009), or images of facial expressions (Carlson & Reinke, 2008). The

dot-probe task effectively measures covert spatial attention. Covert spatial attention does not require eye movement, whereas overt spatial attention does (Bradley, Mogg, & Millar, 2000). The task is computer based and typically begins with a fixation cue on the center of the screen. After a set amount of time, two stimuli appear simultaneously on both sides of the fixation cue: typically one is affective and the other is neutral. The two stimuli disappear and a target (i.e. dot) appears on either side of the screen. The participant's task is to focus on the fixation cue and indicate on which side of the screen the dot appeared. When the dot appears on the same side of the screen as the affective stimulus, it is called a congruent trial, and when the dot appears on the same side as the neutral stimulus, it is called an incongruent trial. The capture of attention is measured by the difference in response time between congruent and incongruent trials. Participants' response time is typically faster in congruent trials (i.e. dot appears behind a fearful face) because attention is already oriented to that location. Response times for incongruent (i.e. dot appears behind a neutral face) trials are typically longer because attention is focused toward the fearful face on the other side of the screen. Research has found that participants respond faster to congruent than incongruent trials when using negative facial expressions (Carlson & Reinke, 2010; Fox, 2002; Mogg & Bradley, 2002) and emotional images such as snakes and guns (Carlson, et al., 2009).

Attentional bias consists of three facets: (a) orienting toward a stimulus, (b) focusing or engaging to the attended stimulus, and (c) disengaging attention from said stimulus (Posner, 1980). A benefit to using the dot-probe task is that it measures attention in two different ways: orientating and disengagement. In addition to congruent and incongruent trial types, for a baseline measure, two neutral or two affective stimuli might

be used. By adding this baseline (e.g. two neutral faces or two fearful faces), orienting and disengagement can also be determined. Typically, reaction times (RT) for baseline trials are faster than incongruent, but slower than congruent trials (Carlson & Reinke, 2008).To measure orienting of visuospatial attention, congruent trials are compared to baseline trials. If RT during congruent trials are faster than baseline, there was an orienting effect. Attention is not typically oriented toward either side of the screen during baseline trials because one stimulus is not more salient than the other. By subtracting the difference between incongruent trials from baseline trials, the amount of time it takes to disengage from the affective stimuli can be calculated. As mentioned earlier, incoming visual information, as theorized by LeDoux (1996), takes two different pathways: the low road and the high road. If the stimuli were neutral, then the attention would disengage, but if the stimuli were salient, then attention would be held at that location for a longer period of time (i.e. delayed disengagement). This study aimed to measure disengagement and orienting of attention by using baseline trials.

Neural Correlates of Attention to Fearful Facial Expressions

As stated previously, sensory information is projected to the amygdala and the sensory cortex from thalamus. Adolphs et al., (1994) studied fear recognition in patient S.M., a woman with Urbach-Wiethe disease, which caused almost complete bilateral amygdala damage while sparing the hippocampus and other surrounding neocortical structures. S.M. showed an impairment in recognizing fearful facial expression and recognizing multiple emotions within one facial expression. She rated anger, fear, and surprise facial expressions as less intense than a brain damaged control group. She did not however, have any difficulty with recognizing facial identity. S. M. also had difficulty drawing a fearful facial expression. She was, however, able to accurately draw other emotional expressions (i.e. happy, sad, angry, disgusted, and surprised).

Patients with cortical blindness showed amygdala activation when presented with fearful faces (Morris et al., 2001). The amygdala is involved in orienting rapidly to threating stimuli such as a fearful face. The anterior cingulate cortex (ACC), the medial prefrontal cortex (mPFC; Liddell et al., 2005), and the anterior insula (Morris et al. 1998) are involved in the modulation of attention to fearful facial expressions. The visual cortex (Morris et al., 1998) and fusiform face area (Pessoa, McKenna, Gutierrez, & Ungerleider, 2002) are active when attending to fearful facial expressions, more so than when viewing neutral facial expressions, and correlate with amygdala activation, but the visual cortex is not necessary for processing fearful facial expressions (Morris et al., 2001). Incoming visual information from the retina is sent to the superior colliculus, then to the pulvinar nucleus of the thalamus (Liddle et al., 2005). Then the pulvinar nucleus sends

information to the amygdala (the low road; LeDoux, 1996; Liddle et al., 2005; Morris et al., 2001). The amygdala and the mPFC are responsible for orienting visuospatial attention toward a threat related stimulus (Liddle et al., 2005) and the lateral PFC (lPFC) is responsible for attention control (Bishop, 2008; *see Figure 1*).

Fear Conditioning

Fear conditioning in non-humans and humans has frequently been studied using Pavlovian conditioning. Pavlovian conditioning research began with Russian physiologist Ivan Pavlov (1927/2010), when he demonstrated that a dog will naturally salivate when presented with food and typically will not for a sound, but if the food is paired with a sound enough times, the sound alone would produce salivation in the dog. Since Pavlov, there has been a wealth of literature describing Pavlovian conditioning and its various aspects. A basic definition of Pavlovian conditioning, that Rescorla (1988) critiqued, is an unconditioned stimulus (US) that causes an unconditioned response (UR) is paired with a conditioned stimulus (CS) enough times to cause a conditioned response (CR) when the CS is presented alone. The CR is typically less intense than the UR. The given definition is a basic understanding of Pavlovian conditioning and does not fully describe the complexities of the field.

Rescorla (1988) mentioned three issues that are commonly underrepresented in the common Pavlovian conditioning definition: circumstances, content, and the effects on behavior. The common understanding of Pavlovian conditioning is that the presentation of the US and CS needs to be contiguous for conditioning to happen. However, as Rescorla (1988) stated, there are circumstances involved in the effectiveness of conditioning. Forming associations between the CS and US is not dependent upon contiguity but rather on the information about the US provided by the CS. One way this is demonstrated is through the blocking effect. The blocking effect describes a circumstance in which two stimuli are presented simultaneously but one CS-US

association blocks the other stimuli from forming an association with the US. For example, a rat that had previous exposure to light and shock pairing and then light and sound paired simultaneously with shock might have difficulty in forming an association with the sound-shock pairing. In effect, the light stimulus prevented, or blocked, the sound-shock pairing.

Another underrepresented attribute of Pavlovian conditioning is the content of what was learned. Rescorla (1988) described how Pavlovian conditioning is not simply learning an association between a CS and US but many different associations are formed. Overshadowing is a similar effect which states that in a compound stimulus, the most salient attribute of the stimulus will form an association with the US. After a pigeon is conditioned to respond to a red triangle to receive a food pellet, the pigeon might respond to a different colored triangle. In this example the shape was more salient than the color. The third issue is how behavior is affected by conditioning. Many believe that the CS causes the same response that was initially caused by the US. Rescorla (1988) provided an example of why this is not true. After pairing a tone with a shock the tone would cause a rat to freeze, whereas after pairing a prod alone with a shock the prod would cause the rat to run and hide. Both have very different responses but both provide evidence that learning took place.

A famous fear conditioning study was demonstrated in Watson's Little Albert experiment (Watson & Rayner, 1920). Even though there were many ethical issues with the experiment, it contributed significantly to our understanding of fear conditioning. Watson's subject, Little Albert, was an 11-month-old infant who displayed no fear, only curiosity, toward a white rat. When Little Albert reached out to pet the white rat, the

experimenter would hit a steel bar with a hammer close to Little Albert's head causing a loud, startling noise: the US. Little Albert fell forward crying, the UR, and when the white rat was placed near his hand, he retracted his arm and start crying: the CR. The response generalized to other white furry items such as a white rabbit and even a man's beard.

Pavlovian conditioning of fear has also been effective in causing an attentional bias as the CR. Research using Pavlovian conditioning to measure spatial attentional bias during the dot-probe task found that by pairing threatening images (CS+) with loud (~100 dB) white noise (US) reaction times (RT) for the CS+ were faster than threatening images not paired (CS-) (Armony & Dolan, 2002; Beaver, Mogg, & Bradley, 2005). These studies are examples of first-order conditioning; that is, the US is paired with a CS. In higher-order conditioning, or second-order conditioning, the US is paired with the CS (i.e. S1), and then the S1 is paired with another CS (i.e. S2; Gewirtz & Davis, 2000). In the present study, the fearful face may be conceptualized as an S1 since it would have presumably been paired with threat related, or unconditioned, stimuli throughout life. Hence, pairing the fearful face with a square may be described as an examole of higher order conditioning.

Fear can also be learned by observing other individuals receiving the US through observational learning. Observational learning involves a participant who observes another participant receiving the US. Hygge and Öhman (1978) had participants observe the confederate's fearful expressions, the confederate's whole body was in view, while viewing fear-relevant stimuli (e.g. spiders) or fear-irrelevant stimuli (e.g berries). They found that the participants' skin conductance (CR) increased when viewing the images

that were paired with the confederate's fearful expressions. Olsson and Phelps (2004) used three different conditioning methods: Pavlovian conditioning, observational learning, and instructed learning. Each group went through the same basic design, consisting of three phases; habituation for 8 trials, acquisition for 24 trials, and extinction for 20 trials. In the Pavlovian conditioning group, they paired an angry facial expression with a shock. In the observational learning group, the participants watched a confederate receive a shock when a certain angry facial expression was displayed. The instructional group was told that when presented with the angry facial expression, they would receive one to three shocks during the acquisition phase. Each group was presented with stimuli under two conditions: masked and unmasked. They found that learning occurred in all three groups by measuring skin conductance, the CR, when the stimuli were not masked. However, when they masked the stimuli, only the Pavlovian conditioning and observational learning groups displayed the CR. Similarly, Ma, Huang, and Wang (2013) examined observational learning using colored squares (i.e. red and green squares). The participant watched a video of a confederate being shocked when a certain colored square appeared. They found that the participant had increased skin conductance when they were shown the colored square that was paired with the shock.

Neural Correlates of Fear Conditioning

Scientists have focused on the amygdala, a structure found in the medial temporal lobe, in fear conditioning. As stated previously, the amygdala is involved in the attentional response to fearful faces (Anderson & Phelps, 2001; Morris et al., 2001) and the preprocessing of potentially threatening stimuli (LeDoux, 1996). Lesion studies have shown that when the amygdalae are removed, the CR from fear conditioning is impaired in several different animal species. In rats, acquisition and expression of the CR is impaired when the amygdalae is removed (Iwata, LeDoux, & Reis, 1986). Humans that had a unilateral temporal lobectomy showed an impairment in fear conditioning, with no left and right differences (LaBar, LeDoux, Spencer, & Phelps, 1995), consistent with the animal models (Iwata, et al., 1986). Neuroimaging studies have also found that there is an increase of blood flow to the amygdalae in fear conditioning studies (Morris, et al., 1996); however, the amygdala is only a part of the neural network involved in fear conditioning and processing.

In terms of fear conditioning, the function of the amygdala is better understood when it is examined together with other regions in a network within the brain. Patients with bilateral or unilateral amygdala damage could verbally state the US was being paired with the CS, but they showed difficulty in conditioning to the CS (LaBar et al. 1995). However, the hippocampus is involved in declarative and explicit memory and Bechara et al. (1995) showed that bilateral damage to the hippocampus impaired the ability to form a declarative memory about which stimuli were paired with the US, but hippocampal damage did not affect the CR. Thus, the amygdala is necessary for fear

conditioning, but the individual does not need to be aware of the conditioning to form a CR. Neuroimaging studies have suggested that the amygdala may receive secondary representations of the CS and US, and contextual information from the hippocampus, ACC, and the anterior insula (Critchley, 2005; Olsson & Phelps, 2007).

The medial prefrontal cortex (mPFC) is also involved in fear conditioning and observational fear learning. Research has suggested that the mPFC is a critical component of extinction in rats (Quirk, Garcia, & González-Lima, 2006) and in humans (Phelps, Delgado, Nearing, & LeDoux, 2004). Extinction occurs when the CS alone is presented enough times that it no longer causes the CR. The mPFC is of more particular interest in fear conditioning. There is a neural network linking the mPFC and the amygdala in fear acquisition (Burgos-Robles, Vidal-Gonzalez, & Quirk, 2009) and in extinction (Vouimba & Maroun, 2011). In observational learning, the mPFC is associated with individuals being cognizant of others', and their own, internal state and/or emotions (Amodio & Frith, 2006) and may be directly involved in the social learning of fear (Olsson, Nearing, & Phelps, 2006). A recent meta-analysis indicated that the rostral PFC (Brodmann's area 10) has at least three subdivisions: the lateral is for episodic memory, the dorsal is for multitasking, and the anterior/medial is for mentalizing (i.e. empathizing with another's emotional state; Gilbert et al., 2006). They also noted that this area is sensitive to social and emotional experiments. These data suggested that the mPFC is an important structure in Pavlovian conditioning and in observational learning by understanding another's internal emotion. The research on attention and fear conditioning suggests that there is a reciprocal relationship between the mPFC and the amygdala (Liddell et al. 2005); however, the methods used in most of the previously

stated articles did not collect real time data to examine what the mPFC does when initially confronted with a fearful face and then disengages to locate another stimulus (i.e. incongruent trial in the dot-probe task).

Current Methods for Imaging Brain Hemodynamics

Most neuroimaging studies that examine fear conditioning use functional magnetic resonance imaging (fMRI), which measures the changes in blood oxygen levels in the brain. Neurons' main metabolites are glucose and oxygen; therefore, when neurons in a certain area of the brain are active, they consume glucose and oxygen to synthesize adosine triphosphate (ATP), a molecule that neurons use for energy, which then requires the vascular system to resupply the neurons with more glucose and oxygen. The metabolic process causes a drop in the blood oxygen level: an increase in deoxygenated hemoglobin, or HbR, in the area (Huettel, Song, & McCarthy, 2009). HbR makes up roughly 3% of the total hemoglobin (Hb) in the blood (Wyka, Mathews, & Rutkowski, 2011). It is worth noting that fMRI does not measure neural activity directly, but rather, blood oxygen levels which have been shown to strongly correlate with neural activity (Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001). A benefit of fMRI is that a researcher could examine any area of the brain with good spatial resolution. As stated earlier, the amygdala is an important structure in fear conditioning. Functional MRI is capable of measuring the hemodynamic activity in subcortical regions such as the amygdala, while measuring whole brain activity. Disadvantages associated with fMRI include poor temporal resolution, typically a sampling rate of 2 seconds (plus or minus 1 second; Huettel, Song, & McCarthy, 2009), and the required apparatus restricts the participants' movement which might be uncomfortable. Thus, our understanding of human fear conditioning has relied on fMRI which has provided valuable knowledge

about the neural correlates involved; however, the poor temporal resolution of fMRI limits our understanding of temporal aspects of fear learning.

The present study used near-infrared spectroscopy (NIRS) to measure frontal lobe activity while performing a dot-probe task. Like fMRI, NIRS measures the change in HbR. But in contrast to fMRI, NIRS also measures the change in oxygenated hemoglobin (HbO) on the cortical surface of the brain using near-infrared light (Scholkmann et al., 2014). As Boas and Franceschini (2009) discussed, NIRS uses two different wave lengths to measure HbR and HbO to obtain the best estimation of hemoglobin concentration. Visible light (wave lengths of near-infrared light lower than 650 nm) is too easily absorbed by hemoglobin, and water absorption increases significantly at wave lengths over 950 nm. The sources emit the light and the detectors detect the light. The sources and detectors should be spaced about 3 cm apart for an adult participant. Not enough light reaches a detector that is more than 5 cm away, and if a separation occurs less than 3 cm away, the light being detected does not represent the cortical surface. There are a few benefits to using NIRS as a method of neuroimaging. First, the temporal resolution of NIRS is much greater than fMRI; NIRS measures hemodynamic signals at a rate of 50-500 Hz, this study used 50 Hz (Boas & Franceschini, 2009). Second, the participant is relatively more comfortable and has more freedom of movement in comparison to fMRI participants. Third, previous NIRS research has indicated that the HbO is more sensitive to neural activity than HbR (Ma, et al., 2014; Hatakenaka, Miyai, Mihara, Sakoda, & Kubota, 2007; Vermeij, van Beek, Olde Rikkert, Claassen, & Kessels, 2012). Thus, using NIRS in this study has the following advantages: there is greater temporal resolution, it is more comfortable for the participant, and it has the ability to measure HbO in addition to

HbR. However, a 3-D image of the participants' cortical surface and scalp is needed to be able to conduct statistical analysis (Huppert, et al., 2009). The values of hemodynamic response function (HRF) for each participant were extracted, however, the extracted HRF values were not the same as the values on the graph given by HomER2. Because statistical analysis of NIRS typically involves using software that requires a 3-D image, the MatLab code for extracting the HRF has not been completely developed. This study did not have access to a 3-D digitizer or MRI to obtain the 3-D image, therefore, the NIRS data collected was not statistically analyzed.

Hypotheses

Past research has used Pavlovian conditioning methods, using shock or a loud noise as the US, and has caused an attentional bias toward a CS (Armony & Dolan, 2002; Beaver, Mogg, & Bradley, 2005). Research using observational methods were also been successful at creating an increase in skin conductance response toward a CS after the participant observed a confederate's fearful reaction toward the CS (Olsson & Phelps, 2004). The purpose of this study was to identify the importance of the fearful facial expression in fear learning. Most individuals have an attentional bias toward fearful faces even when they are not aware of the fearful face (Carlson & Reinke, 2008). It is unknown whether or not a fearful facial expression alone is salient enough to cause an attentional bias toward a CS. The present experiment examined the attentional responses to a CS within the dot-probe task after conditioning with fearful faces. There were four different blocks of the dot-probe-task. The first block of the dot-probe task was baseline one, where two different colored squares were presented alone. The second block was baseline two, a standard dot-probe task with fearful and neutral faces. Block three was acquisition, where the colored squares were paired with either the neutral face or the fearful face. The fourth block was testing, where the colored squares were presented alone. This experiment used NIRS to measure frontal lobe activity during the four blocks. The hypotheses were: (a) there would be no difference in RT between the two neutral colored squares during the baseline phase, (b) during baseline two, the RTs for congruent trials would be faster than baseline trials and RT for baseline trials would be faster than incongruent trials, (c) RT for congruent trials would be faster than incongruent trials

during acquisition, and (d) the RT would be faster during the CS+ congruent trials during block four (testing). We hypothesized the NIRS data would indicate that the mPFC would have two peaks of HbO concentration during incongruent trials, one indicating orienting, the other indicating attentional control to relocate attention; in addition, we expected the mPFC to be more active during learning.

METHODS

Participants

This study had 29 participants (9 males and 20 females; age $M = 21$, $SD = 2.45$; 5 participants identified as left handed). A power analysis was conducted with block two's data from the first 12 participants. We chose to use the data from block two because it is essential to have an attentional bias toward fearful facial expressions to have a chance of using fearful faces for conditioning. The analysis indicated that we would need at least 20 participants with $\alpha = .01$ and power = .9. The participants were Northern Michigan University students who received extra credit in their psychology course for their time. The restrictions for this experiment included ages 18 through 38 years, and normal or corrected to normal vision. They were informed that their information would remain confidential. The participants reported their handedness, age, gender, and they read and signed the informed consent before participating in the experiment. They were asked if they had any questions and were told that they could withdraw at any time without losing course extra credit. The Institutional Review Board at Northern Michigan University approved this experiment.

Materials

Stimuli

Fearful and neutral facial expressions were used from a standard facial database (Gur et al., 2002). There were four fearful and four neutral faces: two males and two females for each expression. There were two colored squares made in Microsoft Power Point. The two squares were either blue or green. For half of the participants, the blue square was the CS- (paired with the neutral face) and the green square was the CS+ (paired with the fearful face). The other half had the blue square as the CS+ and the green square as the CS-.

Task

The dot-probe task using these images was programed using E-Prime 2 software. The task was presented on a 60 Hz 16" PC computer monitor. Each trial of the experiment began with a white fixation cue (+) in the center of a black screen for 1000 ms. Two stimuli were presented simultaneously on both sides of the fixation cue. After the stimuli were presented, a small dot appeared on either side of the screen. Afterwards, the participant indicated on which side of the screen the dot appeared using a response box. Then the word "Rest" appeared for 7 seconds. The participant used the right index-finger to indicate left-sided target dots by pressing the "1" key on the number pad of a keyboard and their right middle-finger to indicate right-sided target dots by pressing the "2" key. It was considered a directed attention trial when a fearful face and neutral face appeared simultaneously. When the dot appeared behind the CS+ and the fearful face, it was considered a congruent trial. When the dot appeared behind the CS- and the neutral

face, it was considered an incongruent trial. Baseline trials were assessed by presenting a neutral face with a neutral face during the second block.

There were four blocks in the experiment. In each trial of every block, the dot appeared 50 ms post-stimulus and disappeared when the participant indicated the location of the dot. The first block had 40 trials of only the two squares not paired with faces. The squares were presented for 100 ms (*Figure 2*). The second block was the second baseline in which just faces were presented for 133 ms and had 90 trials equal with amounts of congruent, incongruent, and baseline trials. The third block was the acquisition phase: there were 84 trials with an equal number of congruent and incongruent trials. During these trials, the squares were paired with the faces. The squares appeared for 100 ms, and the faces replaced the squares for 100 ms (*Figure 3*). The fourth block was the testing phase: 60 trials had an equal number of congruent and incongruent trials where the squares were presented for 100 ms without the faces.

Imaging Equipment: NIRS

This study measured relative HbO and HbR differences between trial types in the PFC using TechEN CW6 NIRS, a continuous wave-type system. The CW6 emits two wave-lengths of light, 690 nm and 830 nm, optimal for measuring HbO and HbR. The sources were recalibrated to the proper mill-watts (mW), 690 nm were set to 12 mW and 830 nm were set to 6 mW. The optode array used eight sources of light and nine detectors covering the lateral to medial anterior PFC (*Figure 4*). The array was custom-made for this study using plastic and foam padding provided by TechEN. The nine detectors were 3 cm away from the sources, which is the optimal distance for measuring the cortical surface of the brain (Boas & Franceschini, 2009). The array utilized the 10-20 clinical

EEG system to standardize the placement of the cap across participants. To accomplish this, the middle detector (number 5) was placed on the Fpz coordinate. Ten percent of the distance from the nasion to the inion is the Fpz coordinate. The cap was secured with an elastic bandage to have even pressure on the array and was affixed with a strip of hook and loop fastening fabric. The sampling rate was set at 50 Hz. A TechEN 8 BNC connector was used for E-Prime to send stimulus markers to the CW6 system to indicate the timing and type of trial. The medial channels were of particular interest because they reflected activation around the mPFC.

Questionnaire

This study used the Speilberger State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, & Lushene, 1970) to identify individual differences in anxiety. Past research has shown that trait anxiety correlates with greater attentional bias toward threat and reduced PFC activity during attention tasks (Bishop, 2008). The questionnaire data were compared with the dot-probe data using a correlation test.

Procedure

The participants read over the consent form and were asked if they had any questions. After they acknowledged that they fully understood their rights as a participant, they were asked to sign the consent form. The participant was seated 59 cm away from the computer screen to ensure the presentation of the two faces, to the left and right of the fixation cue, were separated by 14° and subtended 5 × 7° of the visual angle (Carlson et al., 2012). Then the participant was told that the experimenter would measure their head to obtain the 10 – 20 coordinates. The middle detector (number 5) was placed on the Fpz location. When the NIRS array was secured on the participant's forehead, the experimenter checked the connection of the sources and detectors using the CW/6 program to ensure there was a reliable signal from source to detector. Instructions for the dot-probe task were then read from a script (*Appendix A*), and the participant was asked if he or she had any questions. Before the dot-probe task began, they read a set of instructions that appeared on the computer screen reiterating what was already read to them. Upon completion of the dot-probe task, the NIRS array was removed from the forehead and they were asked to complete the STAI. STAI was given after the dot-probe task to prevent emotional priming. When they finished the STAI, they were given a debriefing, spoken and written, and a research participation card to give to their instructor for course credit. The experiment lasted approximately 1 hour from start to finish.

Data Analysis

Behavioral data

Correct trials consisted of accurate indication of whether the dot was on the right or left side of the computer screen. Only correct trials that had RT within 100 ms through 750 ms were used in the analysis. This time frame was used to filter out premature and delayed responses that were not related to allocation of attention (Carlson & Reinke, 2008; see Behavioral Results below for how much data was removed). A mixed factor 4 × 2 × 2 (block × trial type × color) analysis of variance (ANOVA) was conducted using the Statistical Program for Social Sciences (SPSS) version 21.0 (IBM Crop., 2012), followed by post-hoc pairwise comparisons when appropriate. Attentional bias was calculated by subtracting the congruent trials from the incongruent trials. In a separate follow up analysis of block two, a repeated measures ANOVA was conducted comparing congruent, incongruent, and baseline trials. The disengagement effect was calculated by subtracting incongruent trials from baseline trials. Orienting was calculated by subtracting congruent trials from baseline trials. This study expected to see no significant difference in RT between trials in the first block; a significant difference between all trial types during second block (i.e. RT for congruent would be faster than neutral and incongruent, RT for incongruent would be slower than neutral and congruent); a significant difference in RT between congruent and incongruent in the third block; and a significant difference between trials during the fourth block (i.e. RT for congruent trials would be faster than incongruent).

Imaging data

HomER 2, a MatLab based program, was used to process the NIRS data (Huppert, Diamond, Franceschini, & Boas, 2009). First optical intensity was converted into optical density (OD). Then a principal component analysis was used for motion correction. A value of .8 was used to conservatively remove variance accounting for the motion artifacts (Brigadoi, et al., 2014). HmrMotionArtifact, a motion detection algorithm, was applied to the OD to identify motion artifacts. The algorithm identifies data-points that exceed a threshold in change of amplitude (AMPThresh) and standard deviation (SDThresh) within a period of time (tMotion) and marks the data-points within a time frame (tMask). For this study, AMPThresh = 0.1, SDThresh = 50, tMotion = 0.5, and tMask = 1 (Huppert, et. al., 2009). Data 1 second before, and 6 seconds after were omitted if the stimulus marker was excluded for motion artifacts. A low bandpass filter was used to smooth out cardiac responses in the data. The OD was then converted into hemoglobin concentration using the modified Beer-Lambert law. The modified Beer-Lambert law is an empirical equation that describes the absorption of OD in a concentration of scattering tissue (Boas et al., 2001). To obtain an average HRF the block average time range was set to one second before and 6 seconds after the stimulus mark. Statistics were not conducted on the HRF due to difficulty in reliably quantifying absolute hemoglobin concentration changes without a three dimensional image (e.g. MRI) representing each individual participants' brain structure (Huppert, et. al., 2009). A visual inspection was done to analyze the NIRS data.

RESULTS

Behavioral results

Four participants (two males and two females; age M = 20.25, SD = 1.5; one participant identified as left handed) were excluded from the analysis. One had very poor accuracy (M = .67, SD = .47, see *Figure 5*) and three for delayed RT during the entire experiment (M = 1226.78, SD = 1668.84; M = 720.36, SD = 429.08; M = 426.81, SD = 326.42; see *Figure 6*). A 4 × 2 × 2 ANOVA (block × trial type) was conducted with the 25 participants' data that were included, (four identified as left handed; seven identified as males; and overall age M = 21.04, SD = 2.55). Only correct trials that were between 150 ms and 750 ms were used in the analysis, this removed 5% of the trials. There were significant main effects for block, $F(3, 72) = 3.11$, $p = .032$, $\eta^2_p = .12$ and for trial type, $F(1, 24) = 16.89$, $p < .0001$, $\eta^2_p = .39$. However, these effects were subsumed and better explained by a significant two way interaction between block and trial type, $F(3, 72) = 6.71$, $p < .0001$, $\eta^2_p = .23$. Least Significant Difference (LSD) post-hoc analysis was conducted to examine the possible differences in RT for congruent trials and incongruent trials within each block. In block one, there was no difference between congruent (M = 334.22, SE = 6.85) and incongruent (M = 331.61, SE = 6.01; p = .55). In block two, congruent (M = 330.39, SE =3.80) was significantly faster than incongruent (M = 350.19, SE = 4.89; $p < .0001$). In block three, congruent (M = 332.76, SE = 3.81) was significantly faster than incongruent (M = 343.42, SE = 3.59; p = .006). In block four,

there was no significant difference between congruent (M = 324.73, SE = 4.69) and incongruent (M = 327.79, SE = 4.49; p = .328; *Figure 7*). There was no interaction between block, trial type, and color, $F(3, 72) = 1.15$, $p = .337$, $\eta^2_p = .04$.

To examine the orienting and disengagement of visuospatial attention, a one way repeated measures ANOVA was conducted for block two. There was a significant difference between the three trial types, $F(2, 50) = 15.43$, $p < .0001$, $\eta^2_p = .39$. A LSD post-hoc analysis revealed that congruent (M = 330.60, SE = 3.87) was significantly faster than baseline (M = 342.63, SE = 4.67) p = .003 and incongruent (M = 350.40, SE = 4.90; $p < .0001$). Baseline was also significantly faster than incongruent (p = .02; *Figures 8 and 9*).

Correlation analyses were conducted with attention effect scores (congruent minus incongruent) for each block, STAI scores (M = 41.21, SD = 10.87), and age. STAI scores did not correlate with attention effect from block one (r = -.186, p = .373), block two (r = -.122, p = .562), block three (r = -.377, p = .063), or block four (r = -.061, p = .939). There was no correlation between STAI and orienting toward the fearful face (r = .141, p = .121) or disengagement from fearful faces (r = .318, p = .121). There was also no correlation between STAI and age (r = .169, p = .419). There was no correlation between age and attention effect in block one (r = .237, p = .254), block two (r = -.305, p = .139), block three (r = -.133, p = .528), or block four (r = -.005, p = .983). Also, there was no correlation between age and orienting toward fearful faces (r = -.355, p = .082) or disengagement from fearful faces (r= -.032, p = .881).

NIRS results

This study was interested in the relative changes in hemoglobin that correlate with attentional bias. For this reason, only the NIRS data from the participants that displayed an attention effect was used to make the graphs of the average changes in hemoglobin for each trial type in each block. In addition to the four participants removed from the behavioral data, one participant was not included in analyzing the NIRS data due to no stimulus markers. There did not seem to be a difference during the first block (N = 23, 15 participants remained after motion correction), suggesting that there was no difference in PFC activity when the dot appeared spatially congruent with either colored square (*Figure 10a and 10b*). During block two (N = 21, 12 participants remained after motion correction), however, there did seem to be a difference in hemoglobin changes between trials. The channels located on the mPFC had greater changes in HbO (peak close to -0.005) than the more lateral channels (peak close to -0.0025) during congruent trials. Incongruent trials appeared to require the medial and bilateral areas of the PFC for reorienting visuospatial attention (peak close to -0.0075, supporting the hypothesis). Baseline trials appeared to require the PFC more than the other two trial types (peak close to -0.01; *Figure 11a, 11b, and 11c*). The congruent trials in block three (N = 20, 11 participants remained after motion correction) were similar to the congruent trials in block two; however, the incongruent trials in block three appeared to have less activity than incongruent trials in block two (*Figures 12a and 12b*). In block four, data from only five participants was usable due to motion and thus, not enough data was available for

NIRS analysis. The increased motion artifacts throughout the experiment could indicate that the array was not properly secured.

DISCUSSION

The present study examined if attentional bias toward fearful facial expressions could be transferred to a neutral stimulus after pairing the neutral stimulus with the fearful face in a dot-probe task. This study also measured the neural mechanisms that correlated with attentional bias to fearful faces and fear learning using NIRS. Four different blocks were used: block one was to determine if there was a preexisting attentional bias toward either a blue or green square; block two was used to examine attentional bias toward fearful faces, or more specifically, orienting attention toward and disengaging attention from fearful faces; block three was the conditioning or acquisition block in which the squares appeared before the faces; and block four was to test whether or not the attentional bias toward fearful faces transferred to the CS+. The behavioral results indicated that there was no initial attentional bias toward either the blue or the green squares before pairing, supporting the hypothesis. The hypotheses for the second block stated that RT for congruent trials would be faster than incongruent trials, congruent trials would be faster than baseline trials, and incongruent trails would be slower than baseline trials. These hypotheses were supported with RT for congruent trials being faster than incongruent trials. These effects mean that there was an attentional bias toward the fearful facial expression. There also appeared to be orienting and disengagement effects; meaning that attention was quickly oriented toward the fearful face and disengagement from the fearful face was delayed. There was also an attentional bias toward the fearful facial expression during the acquisition block when the squares preceded the faces; however, there was no attentional bias toward the CS+ during the

testing block. This indicated that paring a neutral colored square with a fearful face did not elicit a transfer of attentional bias to the colored square, and the main hypothesis of the study was not supported. Trait anxiety did not correlate with attentional bias. However, the NIRS data from block two provided some interesting results about PFC activity associated with the orienting and disengagement of attention. Statistical analysis was not conducted with the NIRS data because this study did not have a 3-D digitizer (see Data Analysis section). Nevertheless, the mPFC seemed to be correlated with orienting attention toward the fearful face, whereas the lateral PFC (lPFC) seemed to be correlated with disengaging and/or relocating attention away from the fearful face.

Behavioral Discussion

In the first block we examined if a blue square or a green square would capture visuospatial attention. This comparison could only state whether blue is more salient than green, or vice versa. We did not expect to see a difference in RT to either trial type. Trial types were called congruent (dot was spatially congruent to the soon to be CS+) and incongruent (dot was spatially incongruent to the soon to be CS+). The results indicated that RT to targets preceded by blue or green squares were equal, and thus, there was no attentional bias toward either colored square. Neither stimulus was more salient than the other, thereby supporting the hypothesis.

Attentional bias toward fearful faces was examined in block two. Prior research has examined attentional bias toward fearful faces using the dot-probe task (Carlson & Reinke, 2010; Fox, 2002) and found that RT for congruent trials was faster than incongruent trials. This study found similar results. The difference in RT is thought to mean that visuospatial attention was captured by the fearful facial expression; therefore, reacting to the dot during a congruent trial would cause a faster response when compared to an incongruent trial. During an incongruent trial, attention would need to be redirected to the other (opposite) side of the computer screen to locate the target dot. This study also used a baseline condition (two neutral facial expressions) to examine orienting and disengagement.

To examine the three-step process of attention that Posner (1980) proposed (i.e. orienting, engaging, and disengaging), congruent and incongruent trials were compared to baseline trials. Consistent with prior research, RT was faster for congruent trials than

RT for baseline trials and RT for baseline trials were faster than RT for incongruent trials (Carlson & Reinke, 2010). This indicated that attention was oriented toward the fearful facial expression, was engaged in that location, and then disengaged from that location. Because the stimuli were equally salient during baseline trials, attention was not oriented toward either side of the screen during those trials. This provided a baseline to identify how long it took to indicate where the target dot was located without attention being captured by previous stimuli. Because we found that congruent was faster than baseline, this study can conclude that attention was oriented toward the fearful facial expression. In addition, since RT during incongruent trials was slower than baseline trials, this study can conclude that attention was held, or engaged, to the location of the fearful facial expression, and then disengaged and reoriented toward the target dot. This study can conclude that the participants reoriented attention toward the target dot and did not respond to the absence of the dot. If the participants were responding to the absence of the dot, then the RTs for congruent and incongruent trials would not be different. Thus, these results support the three step process of attention: (a) orienting, (b) engaging, and (c) disengaging (Posner, 1980).

As stated earlier, facial expressions can communicate to an individual information about his or her environment. Orienting toward a fearful facial expression would be advantageous because it would allow the opportunity to identify a potential threat within the environment. Past research has indicated that when individuals observe a fearful facial expression, they naturally attend to the eyes, with the exception of patient S. M. (Adolphs et al., 1994). Carlson and Reinke (2014) have also demonstrated similar orienting and disengagement effects to fearful eyes. These researchers used fearful and

neutral eyes without other facial features during the dot-probe task. They found that participants rapidly oriented toward the fearful eyes, and had a delayed disengagement from the fearful eyes. If fearful facial expressions indicate that there is a threat in the environment, then the eyes might be the signal that points to the location of the threat. The gaze direction of the fearful eyes would indicate the source of potential threat. Research has shown that attention reorients toward the direction of the gaze (Fox et al. 2007; Mathews et al. 2003). This present study used facial expressions where the gaze was directed forward, toward the participant. This could help explain why there was a delayed disengagement effect. If fearful eyes capture attention and the direction of the eyes signal the location of the threat, then attention might be held at the location of forward gaze eyes until further information, such as gaze direction, is obtained.

Block three attempted to pair the neutral stimuli with the facial expressions. For about half the participants the blue square was the CS+, which was paired with the fearful face and the green square the CS-, which was paired with the neutral face. The other half of the participants had the green square as the CS+ and the blue square as the CS-. The results indicated that even with presenting the squares before the faces, there was still an attentional bias toward the fearful facial expression because RT was faster for congruent trials than incongruent trials. This block did not utilize baseline trials because we wanted to present the squares an equal number of times. The CS- would have been presented more frequently if baseline trials were included.

To test the effects of the conditioning phase, we only showed the squares in block four. This study hypothesized that after pairing colored squares with faces, there would be an attentional bias toward the CS+. There was no difference in RT to either trial type,

congruent or incongruent, suggesting that attentional bias toward fearful facial expressions did not transfer to the colored squares under these experimental conditions.

There are some Pavlovian conditioning concepts that may have had a role in why learning did not take place. As stated earlier (see Fear Conditioning section), learning is dependent on the history of the stimuli. Participants were pre-exposed to the neutral stimuli (i.e. colored squares) in block one. Björkstrand (1990) found that by pre-exposing the CS, participants were less likely to learn from acquisition. However, this study needed to determine if there was an attentional bias toward either colored square because it was not previously tested. Future research may choose to exclude block one to avoid stimulus pre-exposure. Overshadowing could also have affected the lack of learning. Again, overshadowing is when one attribute of a stimulus is more salient than another. The neutral stimuli used in this study had two attributes: square shape and color. It is possible that the square shape was more salient than color, which would make it difficult to discriminate between the two stimuli during the testing phase. To avoid overshadowing, future research could use two different shapes that are also different colors (e.g. a blue square and a green circle).

Past research has been able to transfer attentional bias toward a CS after pairing the CS with an aversive stimulus (e.g. white noise; Armony & Dolan, 2002; Beaver, Mogg, & Bradley, 2005). However, these studies paired stimuli with a direct threat and not an indication of potential threat (e.g. a fearful face). A direct threat (i.e. shock) is more salient then an indication of potential threat (i.e. fearful face). Typically in higher-order conditioning studies (see Fear Conditioning section), the response to the CS becomes weaker than the response in first-order conditioning (Gewirtz & Davis, 2000).

The response to the colored square may be too weak for a significant difference in the dot-probe task. Previous research was successful in detecting differences in skin conductance responses after conditioning (Olsson & Phelps, 2004), which may be more sensitive than RT in the dot-probe task. In addition, Armony and Dolan (2002) used angry facial expressions as the CS. Using an angry facial expression, or another threat-related stimulus, as the CS, rather than a colored square, may be more effective in conditioning attentional bias. Typically in Pavlovian conditioning paradigms, the CS is presented before the US (Armony & Dolan, 2002; Beaver, Mogg, & Bradley, 2005; Watson & Rayner, 1920; Rescorla, 1988). But since a fearful face is an indication of threat and not the source of the threat (i.e. a shock), it may be beneficial to display the neutral stimulus after the facial expression.

Research in observational fear learning found that after observing another individual receive a shock only when the CS was present, the observer had increased skin conductance when they viewed the stimuli that was paired with the shock regardless of the CS being fear relevant or not (Hygge & Öhman, 1978; Ma et al., 2013). Increased skin conductance is thought to be an aspect of the initial fear response, as is orienting attention toward a threat related stimulus (Carlson & Reinke, 2010; Fox, 2002; LeDoux, 1996; Mogg & Bradley, 2002; Posner, 1980). One of the differences between previous research and the current study is that we presented the CS+ and the CS- simultaneously. This is important because the attention is not oriented toward the location of the CS+ until the fearful face was present and the CS+ was gone. Future studies could display the stimuli individually and then test for attentional bias.

There is also the possibility that because the response to a static fearful face may not be equivalent to directly experiencing or watching another experience an aversive stimulus, more pairing might be necessary to transfer attentional bias from fearful faces to colored squares. Previous observation fear conditioning research has had the participant view a confederate's whole or partial body (Ma et al., 2013). Viewing an actual fear response may be different than viewing a static image representing a fear response. Seeing another individual receive a shock might indicate to the observer that they might also receive a shock. Also, in the previously mentioned observational fear conditioning studies, the confederate received a shock, which for some would cause a mild sensation of pain. That being said, the participants are observing a pain response in addition to, or instead of, a fear response.

As discussed earlier, attention is oriented toward the eyes of a fearful facial expression and is held in said location until further information is given (i.e. gaze direction; Fox et al. 2007; Mathews et al. 2003). Because the faces we used were static, the observer did not receive further information. Hooker, Germine, Knight, and D'Esposito (2006) paired gaze direction of happy and fearful facial expressions with geometric shapes. One shape was always paired with the happy face, and the other was paired with the fearful face. They also had the participants view facial expressions without any shapes. The researchers found that the amygdala was more responsive when the shapes were paired with the facial expression than when only the facial expressions were shown. In a gaze direction study, it is clear what the expresser is fearing. In a forward gaze conditioning study, this might not be clear. Pairing a fearful gaze direction with a neutral stimulus could condition an attentional bias toward the CS.

Nonetheless, the data from this study suggested that fearful facial expressions which do not gaze toward a stimulus are not salient enough to use in a Pavlovian conditioning/observational learning paradigm. This could mean that more than a facial expression would be needed for fear conditioning to take place. In observational learning research, the participant typically viewed the individuals reacting to an adverse stimulus and not just a static facial expression (Ma et al., 2013), and it may be necessary for the fearful eyes to gaze toward the stimulus (Hooker et al., 2006). Observing a stimulus and then a fearful face, with the gaze directed at the observer, is not a situation that would likely happen in nature and may not be relevant in fear learning. Rather, observing a fearful face gazing toward a stimulus of potential threat may be more relevant and more likely to happen in nature.

Trait anxiety was also examined in this study. A correlation was run with the behavioral data to determine if trait anxiety was related to attentional bias toward fearful facial expression. This study did not find a correlation between trait anxiety and attention effect, orientating, or disengagement scores. Past research suggests that trait anxiety may be correlated with delayed disengagement from threat-related stimuli (Carlson & Mujica-Parodi, 2015; Fox, Russo, & Dutton, 2002; Koster et al.2006), whereas the data from this study did not statistically support those findings, but was trending in that direction. The difference in findings may have been due to the small sample size and not seeking out individuals with high levels of trait anxiety: Koster and colleagues (2006) selected participants that had high trait anxiety (M = 55.7) and low trait anxiety (M = 28.9). This study did not select individuals based upon trait anxiety; therefore, the anxiety data from

this study did not have as much variability as previous research (*Minimum* = 26, *Maximum* = 67, *M* = 41.21, *SD* = 10.87).

NIRS Discussion

Statistical analysis of the NIRS data was not applicable due to not having anatomical information. As Huppert and colleagues (2009) discussed, obtaining an absolute quantification of hemoglobin changes is contingent on the knowledge of path-length and partial volume of tissue. The head and cortical tissues vary within and between individuals and the light scatters differently through different tissues. Without knowledge of the tissues that the light scatters through, accurate hemoglobin concentration changes are difficult to assess. In addition to not having structural information, much of the data collected was rejected due to motion artifacts. Even with the limited analysis of the NIRS data, the usable data suggested that the PFC is differentially active during different trial types.

In block one, we hypothesized that there would be no evidence of attentional bias toward either colored square. We found in the behavioral data that there was no difference in RT for each trial type and the NIRS data would also suggest that there was no difference between the trials. This would be consistent with the idea that there is no attentional or neurological preference for either a blue square or a green square. Future research would be able to use these stimuli in further attentional bias or conditioning research requiring equally salient neutral stimuli.

As previously mentioned, the behavioral data from block two suggested that fearful facial expressions capture and hold visuospatial attention. During congruent trials, the channels located near the mPFC indicated greater changes in HbO concentrations compared to the channels located near the lPFC. This finding is consistent with previous

research that stated that the mPFC was correlated with orienting attention toward fearful facial expressions (Liddle et al., 2005). Past research has identified a network between the anterior cingulate cortex (ACC)—a structure near the mPFC—and the amygdala (Bishop, 2008; Carlson et al., 2014 Carlson, Reinke, & Habib, 2009). This network is involved in detecting and orienting attention toward its location. The results of this study cannot accurately state that the activation that was detected is correlated with activity in the ACC because the ACC is below the cortical surface. However, the results do suggest that the mPFC is involved in detecting and orienting toward fearful facial expressions.

The difference between the congruent and incongruent trials is the location of the dot. It is logical that the same mPFC activity would be seen in both trials because a fearful face is present in both; however, during the incongruent trials, attention needs to be relocated toward the target dot. There was a delayed disengagement of attention from the fearful facial expression during incongruent trials as depicted by the delay in RT in comparison to baseline trials. The possible increase in lPFC activity during incongruent trials showed that the lPFC is correlated with the control and relocation of visuospatial attention. This is consistent with previous research that indicates that damage to the lPFC can cause an inhibited response in attention control (Bishop, 2008; Dias, Robbins, & Roberts, 1996). However, the hypothesized two peaks in the mPFC were not supported. The peak for incongruent did, however, peak earlier (around 3-4 seconds) than congruent trials (around 4-5 seconds; see *Figure 10*). As Bishop (2008) stated, the ACC and the lPFC are both involved in detecting and attending toward a stimulus. More accurately, the ACC is involved in detecting attention toward a threat (i.e. a fearful face),

whereas the lPFC is involved in orienting attention toward a cognitively "desired" or task relevant stimulus (i.e. a dot).

Prior research has identified a neural network for attentional bias toward threat. Visual information gets sent from the retina to the superior colliculus, then to the pulvinar nucleus of the thalamus, the pulvinar projects to the amygdala (LeDoux, 1996; Liddle et al., 2005; Morris et al., 2001) and visual information is also sent from the thalamus (lateral geniculate nucleus) to the visual cortex (LeDoux, 1996). There is also an amygdala-prefrontal network that allows for reciprocal communication to travel back and forth between the two structures. In terms of this experiment, the mPFC is involved in the initial detection and orienting of visuospatial attention (Liddle et al., 2005) and the lPFC is involved in attention control (Bishop, 2008; Dias, Robbins, & Roberts, 1996). This is, to the best of our knowledge, the first NIRS experiment to measure attentional orienting and disengagement to fearful facial expression. Interestingly, baseline trials appeared to have the most activity in the entire PFC when compared with congruent and incongruent. This was interesting because this was the opposite of what was expected. During the baseline trials there were no orienting or disengagement effects, which correlate with PFC activity, and yet there was more activity in the PFC during these trials. These results might indicate that directing attention toward two locations is more cognitively demanding than directing attention toward one location. Similarly, the automatic attentional bias toward fearful faces may be less of a cognitive load and require less PFC involvement than when there is no automatic attentional response.

The NIRS data from block three was consistent with the data from block two in terms of the mPFC having greater HbO concentration changes than the more lateral

channels during congruent trials. But, the NIRS data for the incongruent trials were different between blocks two and three. This could indicate that there was a habituation effect. The overall activation was not as extreme in block three but the right lPFC channels were more active than the mPFC and left lPFC channels.

The neural correlates of learning cannot be concluded from this experiment because there was no evidence that learning took place. The NIRS data from block four displayed too much noise due to motion artifacts to be able to discuss what the data would suggest.

Limitations

This study had several limitations. One could be that the pairing of stimuli was done via dot-probe task. Ma and colleagues (2013) had a participant passively view another participant receive a shock when the CS+—either a red or green square—appeared on the computer screen. Pairing the squares with the faces individually may be more effective in learning to respond to the CS+. Another limitation was the length of time it took to complete the study: the task took about 50 minutes. On an anecdotal note, many participants complained that they were losing focus about halfway through the experiment. The fact that much of the data was removed because of motion artifacts, especially in block four, might have been caused by the long duration of the experiment. The lengthy duration was difficult to avoid due to the necessity of a rest period after each trial for NIRS data acquisition. Future studies could examine the behavioral data without using NIRS, which would not require the rest period. This could shorten the duration of the study to about 15 minutes. The method of applying the NIRS array to the forehead might have been another contributor to the motion artifacts. In this study, we used an elastic bandage and a strip of hook and loop fastening fabric to secure the array to the head, but it might not have been secure enough to prevent movement. A cap that uses the 10-20 coordinates might be a more effective way of preventing movement of the array. Skin conductance responses could have also been measured to more closely align with previous fear conditioning studies.

Conclusion

Even though the main hypothesis was not supported, this study provided data that further supported some theories and may add to others. There is no evidence of attentional bias toward either a green or a blue square indicating that these colors can be used as neutral stimuli in future research of attentional bias. The behavioral data from block two suggested that participants rapidly oriented toward the fearful facial expression, held attention in that location, and then disengaged attention. Although only exploratory, the NIRS data suggest that the mPFC is involved in automatically orienting attention toward the fearful facial expression and the lPFC is involved in relocating attention.

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APPENDIX A

Instruction Script

Protocol for "Attentional bias conditioning"

Greet & Welcome the Participant – Get their information onto the sheet of paper

1 Seat and Give the participant the consent form and allow them time to read it over.

a. While they read it over, enter their data into the computer program and start up the NIRS program.

b. Once they are finished with the consent form, ask them if they have any questions and would like a copy of the consent form.

c. Sign their consent form and keep the signed copy, File it away.

d. Remind the participant that they are volunteering to par take in the study and they can leave any time without penalty. However to receive extra credit they must participate in the full experiment.

e. ASK them to **TURN OFF** or **SILENCE** their CELL PHONES and leave it in the experimenter's room.

2 Seat the participant 59cm from the screen. Apply the NIRS equipment to the participant, turn off the lights, and test to make sure it is recording. – Tell them NOT to make too many facial movements.

a. Ask if it is comfortable, and Give them the following instructions:

Dot-Probe Task: *Each trial of the experiment will start with a small '+' (plus sign) in the center of the screen. At all times keep your eyes fixated on the plus sign. After an initial*

period of fixation two stimuli will be briefly presented: one on each side of the screen. After these stimuli disappear, a small dot will appear either on the left or on the right side of the screen. Your task is to locate this dot: left or right. To do this, use your right hand. Use your right index finger on the "1" button on the key board to indicate left-sided target dots. Use your right middle finger on the "2" button on the key board to indicate right-sided target dots. IT IS IMPORTANT THAT YOU RESPOND AS QUICKLY AS POSSIBLE. AS SOON AS YOU LOCATE THE DOT MAKE A RESPONSE. The experiment will be divided into several blocks. Between block you can take a small break, if you like. When you are ready to begin the next block press the "1" button. DO YOU HAVE ANY QUESTIONS?

3 After the experiment administer the STAI-T Questionnaire, and ask again if they have any questions.

4 Give the Participant the debriefing statement and participation card. Ask one more time if they have any questions about the study

Figure 1

Model for Attentional Bias toward Threat

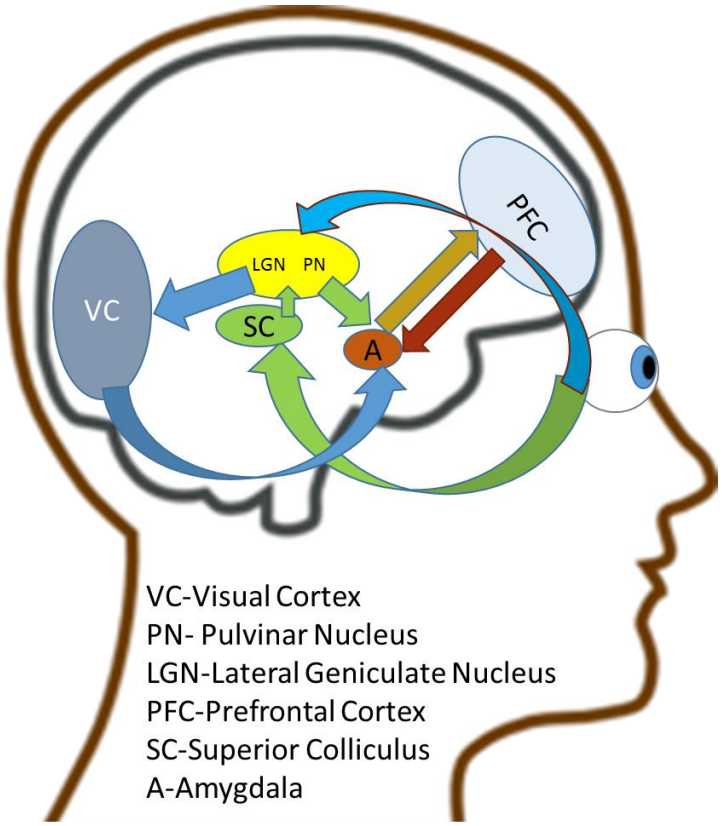


Figure 1: Model for attentional bias toward threat. Green arrows represent the "low road" and the blue arrows represent the "high road." The red and orange arrows represent the feedback loop to and from the amygdala and the prefrontal cortex.

Figure 2

Dot-Probe Task Baseline One and Testing Block Example

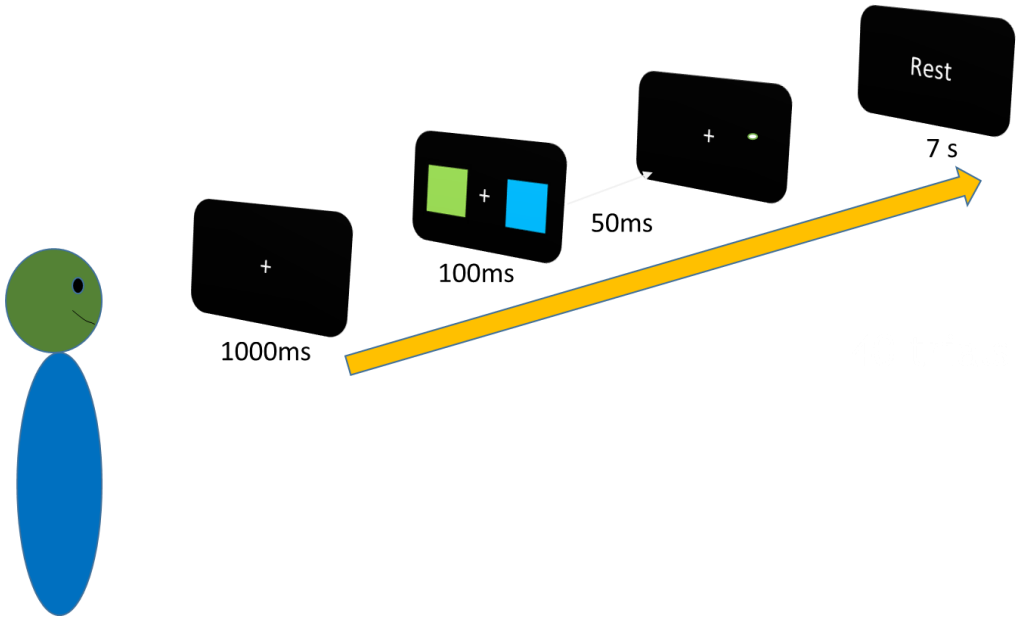


Figure 2: Example of a trial only consisting of squares in the dot-probe task. Used in blocks 1 and 4.

Figure 3

Dot-Probe Task Conditioning Block Example

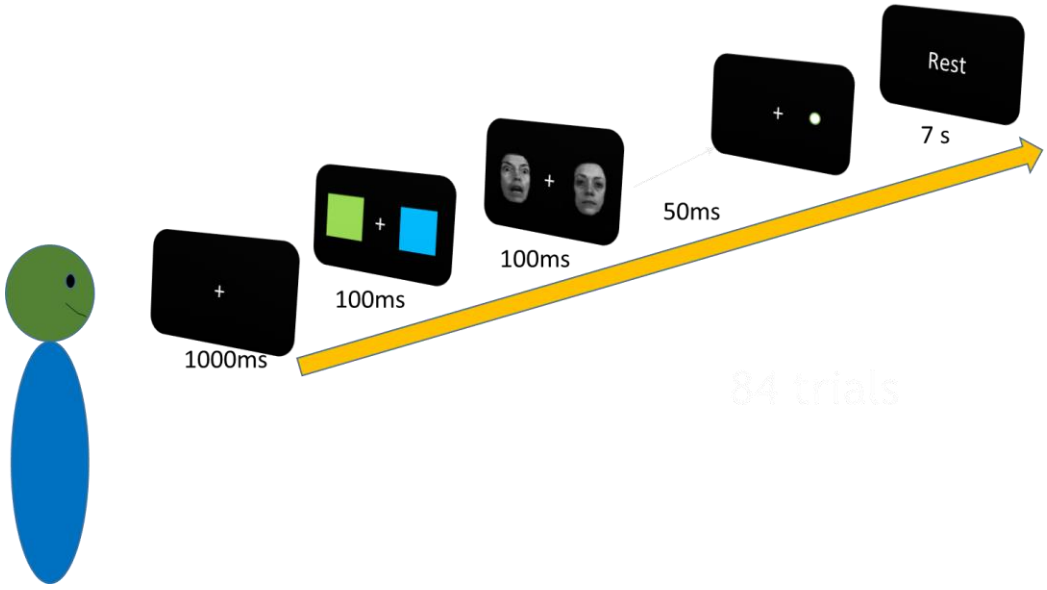


Figure 3: Example of conditioning trial with squares and faces in the dot-probe task. This is an example of an incongruent trial.

Figure 4

NIRS Array Placement

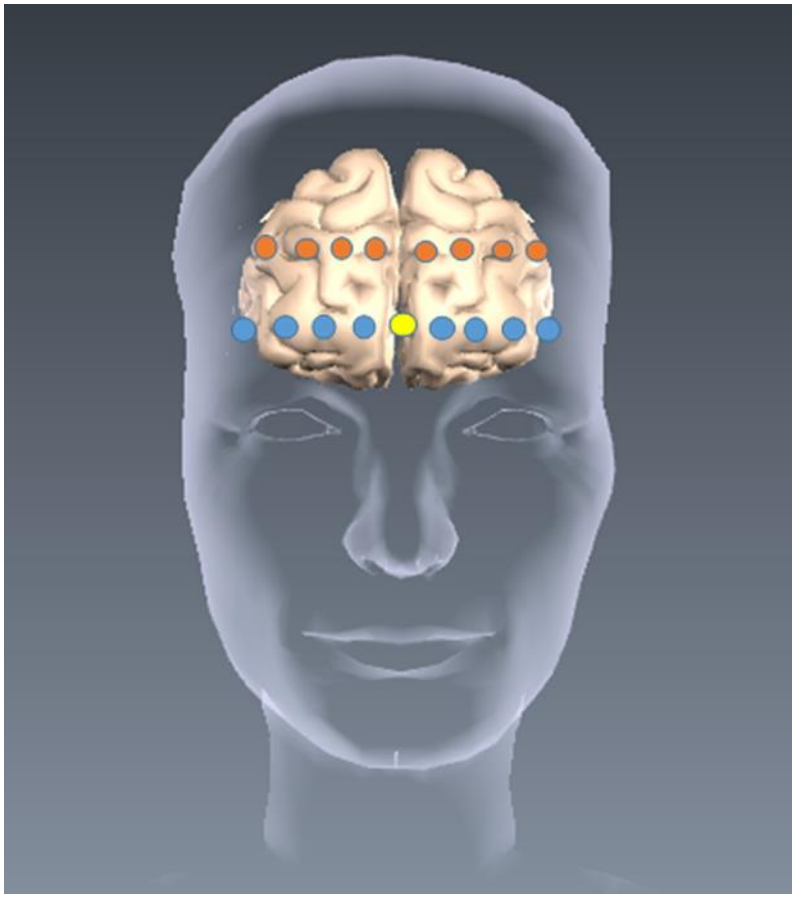


Figure 4: The red dots are the sources of near-infrared light. The blue are the detectors. The yellow dot is the 5th detector that was located on the Fpz.

Figure 5

Dot-Probe Task Accuracy Plot

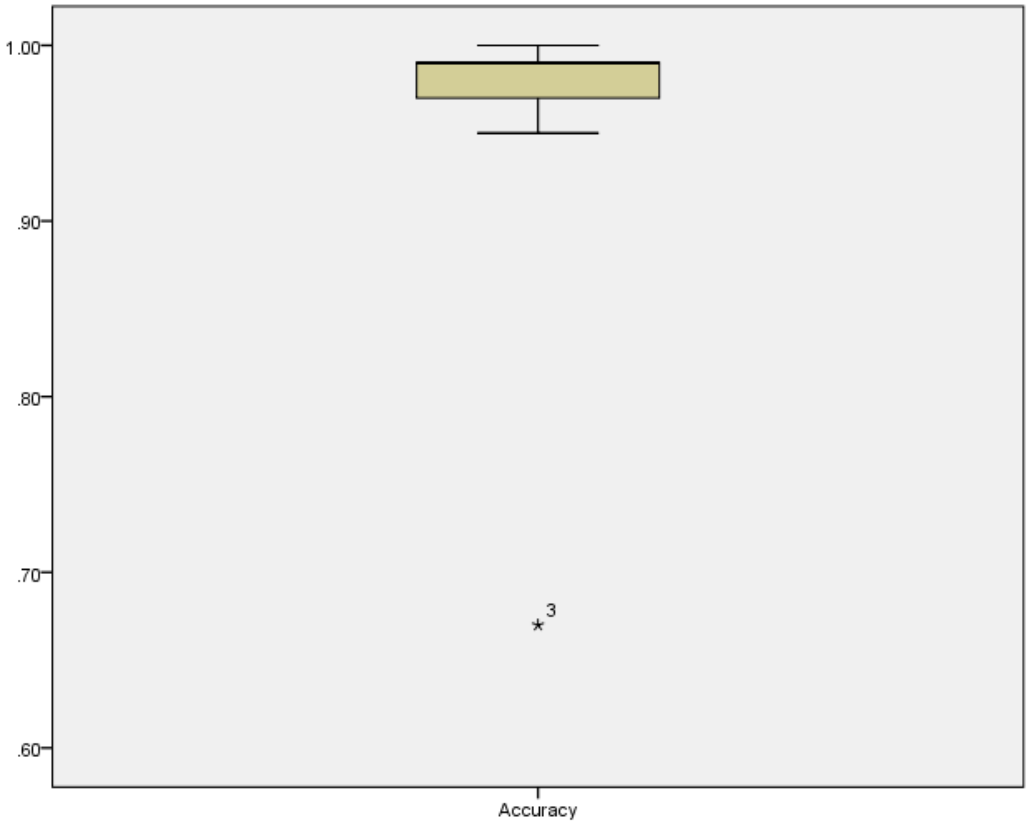


Figure 5: This box plot shows the accuracy for all the participants. The star indicates an outlier (M = .67, SD = .47).

Figure 6

Dot-Probe Task Reaction Time Averages Plot

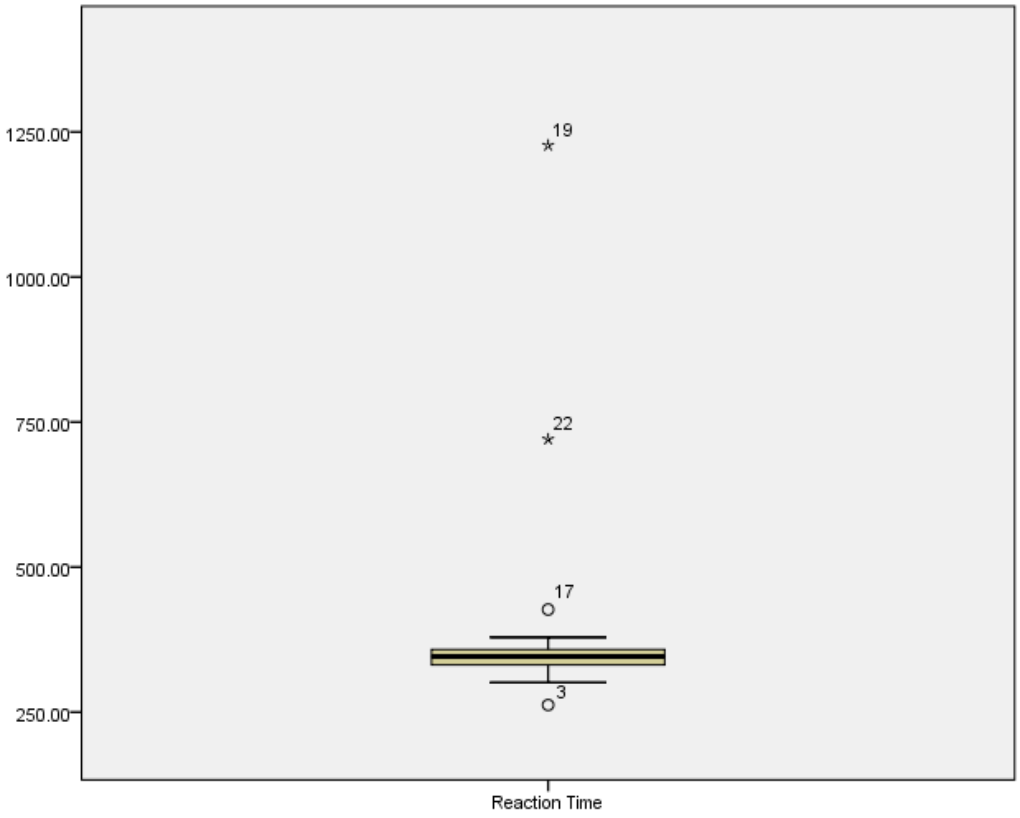


Figure 6: This box plot shows the RTs in ms for all the participants. The two stars (19 and 22) and the circles (3 and 17) are outliers in terms of overall RT in ms. The data from 19 (*M* = 1226.78, *SD* = 1668.84), 22 (*M* = 720.36, *SD* = 429.08), and 17 (*M* = 426.81, *SD* = 326.42) were excluded from the analysis (3 was already excluded for accuracy)

Figure 7

Block by Trial Type Bar Graph

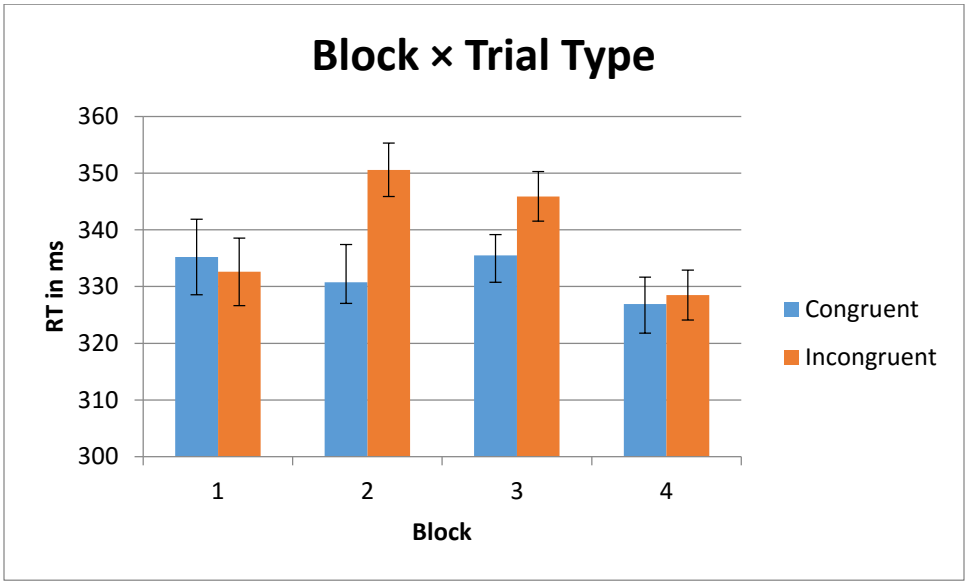


Figure 7: RTs for congruent and incongruent trials in each block. Blocks 1 and 4 are not significantly different, but blocks 2 and 3 are significantly different, *p*s < .01. There was no attentional bias toward either colored square before or after conditioning, but there was an attentional bias toward the fearful faces in blocks 2 and 3.

Figure 8

Block Two's Reaction Time Graph

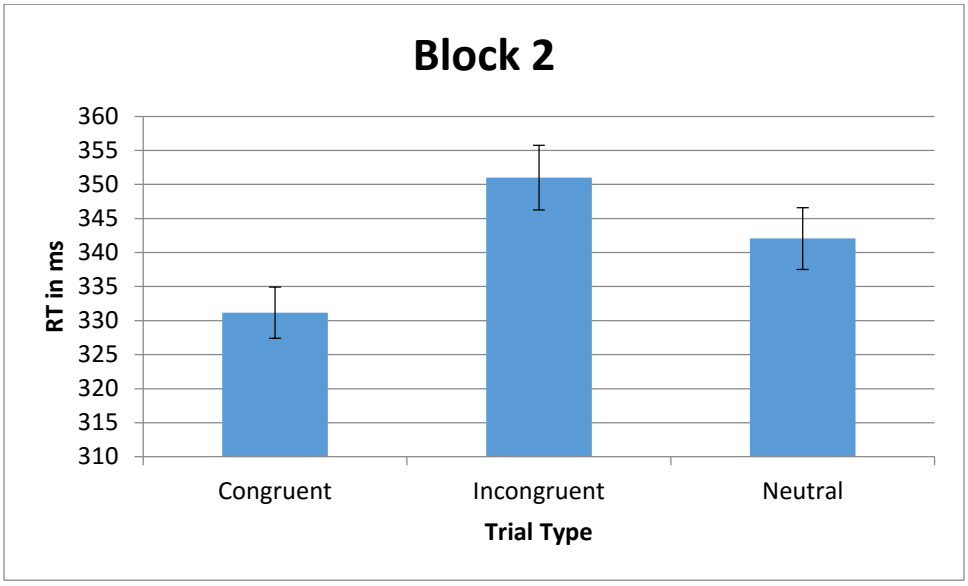


Figure 8: RT for congruent, incongruent, and baseline conditions for block 2. All conditions were significantly different from each other, *p*s < .05.

Figure 9

Orienting and Disengagement Effects

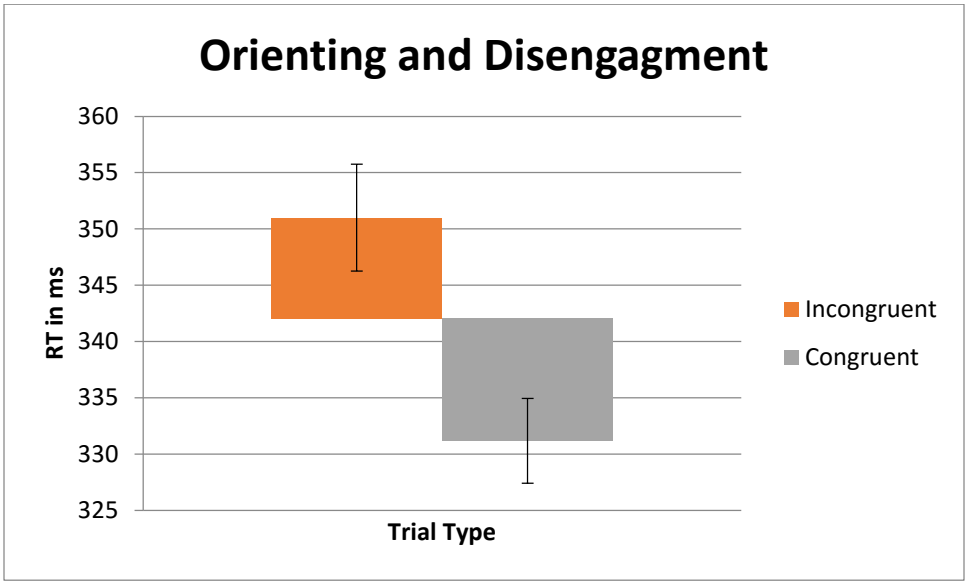


Figure 9: RT for congruent and incongruent trials compared to baseline. Congruent was significantly faster than baseline, $p < .05$, incongruent was significantly slower than baseline, $p < .05$. This graph shows that there was an initial orienting effect (congruent < baseline) toward the fearful faces and there was a delayed disengagement effect (incongruent > baseline) from fearful faces.

Figure 10a

NIRS Data for Congruent Trials from Block One

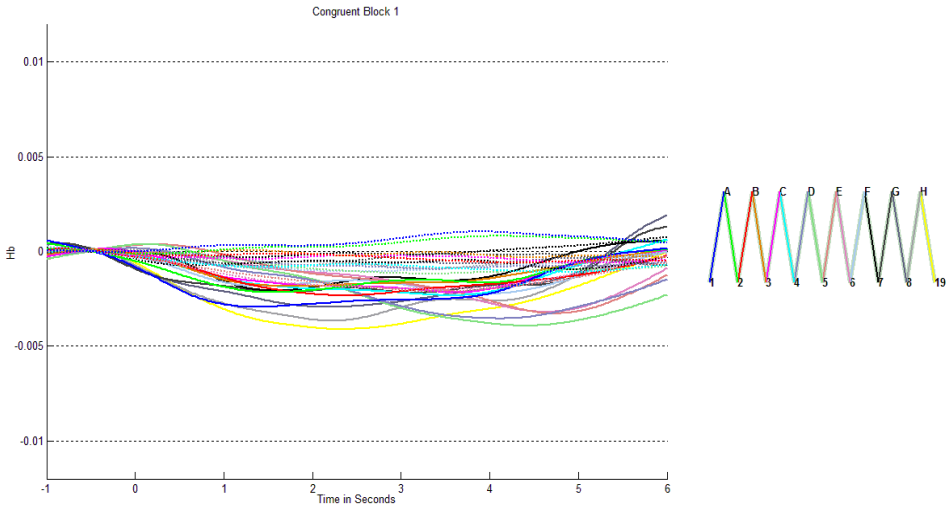
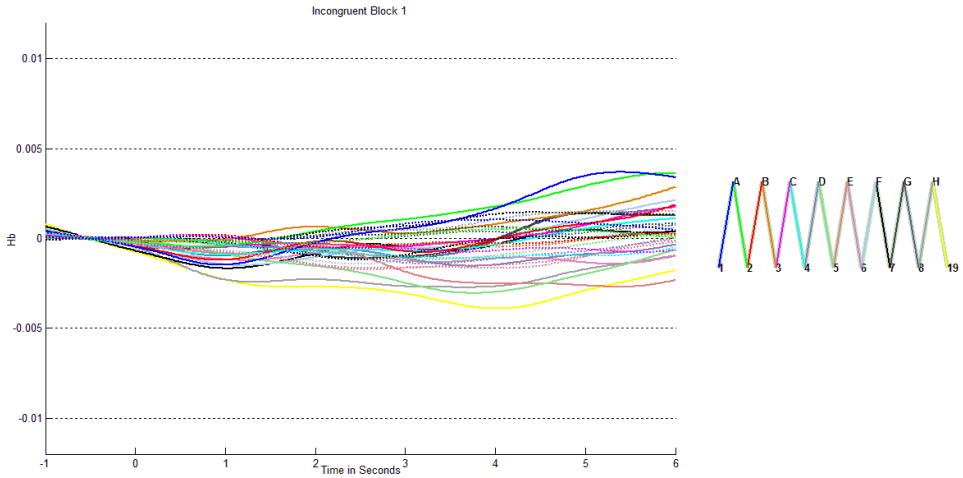


Figure 10b

NIRS Data for Incongruent Trials from Block One



Figures 10a and b: Figure 10a shows the HbR and HbO changes during congruent trials in Block 1 (*N* = 15). The pairing has not happened yet. In this block congruent means the dot appeared behind the square that will be paired with the fearful face. Figure 10b shows the changes during the incongruent trials in Block 1. The y axis indicates the relative change in oxygenated hemoglobin concentration in the prefrontal cortex. The trial started on the "0" marker. The dotted lines show the HbR and the solid line shows the HbO. There seem to be no major differences between congruency type.

Figure 11a

NIRS Data for Congruent Trials from Block Two

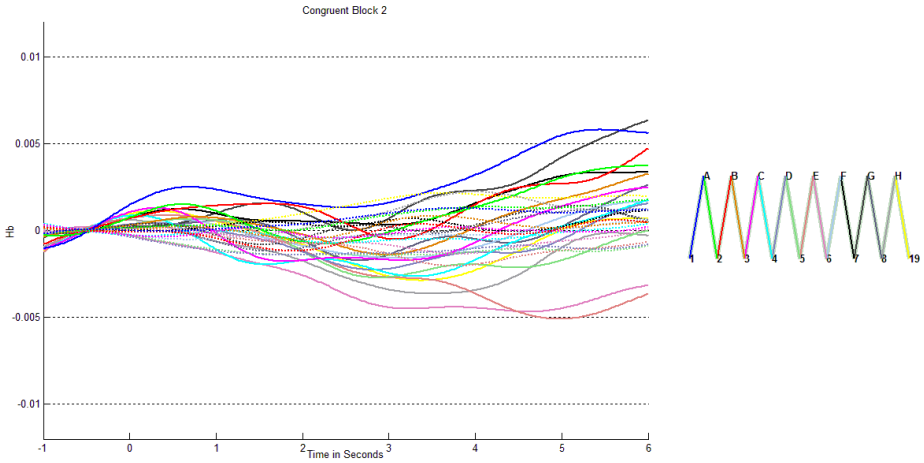


Figure 11b

NIRS Data for Incongruent Trials from Block Tow

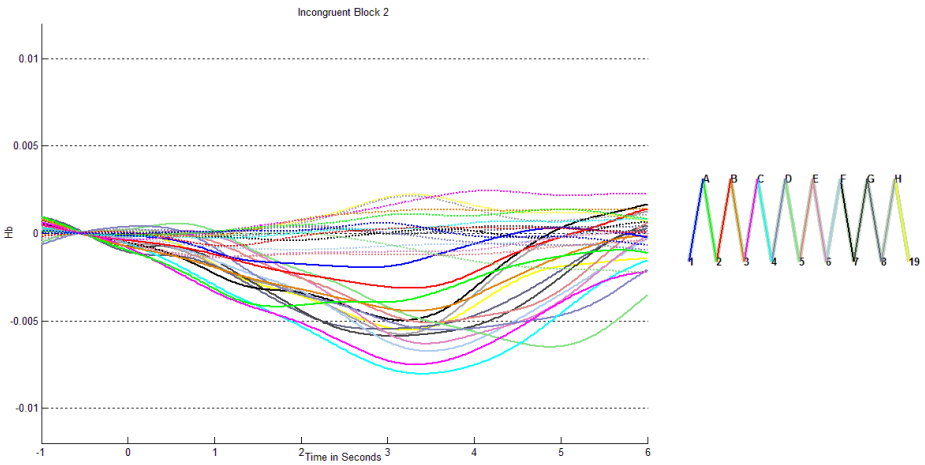
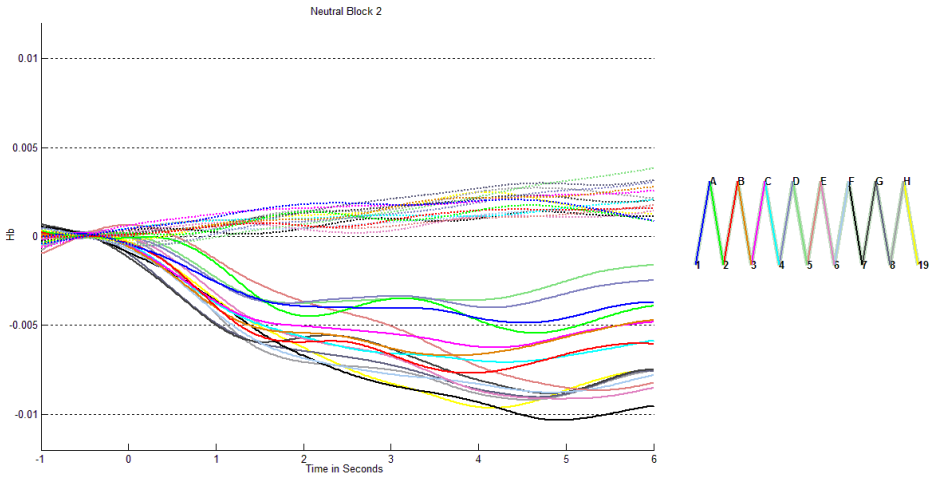


Figure 11c

NIRS Data for Baseline Trials from Block Two



Figures 11a, b, and c: Figure 11a is congruent trials, 11b is incongruent trials, and 11c is baseline trials during Block 2 for the participants that had an attention effect (N = 12). The y axis indicates the relative change in oxygenated hemoglobin concentration in the prefrontal cortex. The trial started on the "0" marker. The dotted line shows the HbR and the solid line shows the HbO. Incongruent and baseline trials seem to have greater HbO changes than congruent trials. This indicates that activity in the mPFC is correlated with orienting attention toward the fearful face (congruent trials), activity in the lPFC is correlated with relocating attention (incongruent trials), and the PFC is active during baseline trials.

Figures 12a

NIRS Data for Congruent Trials from Block Three

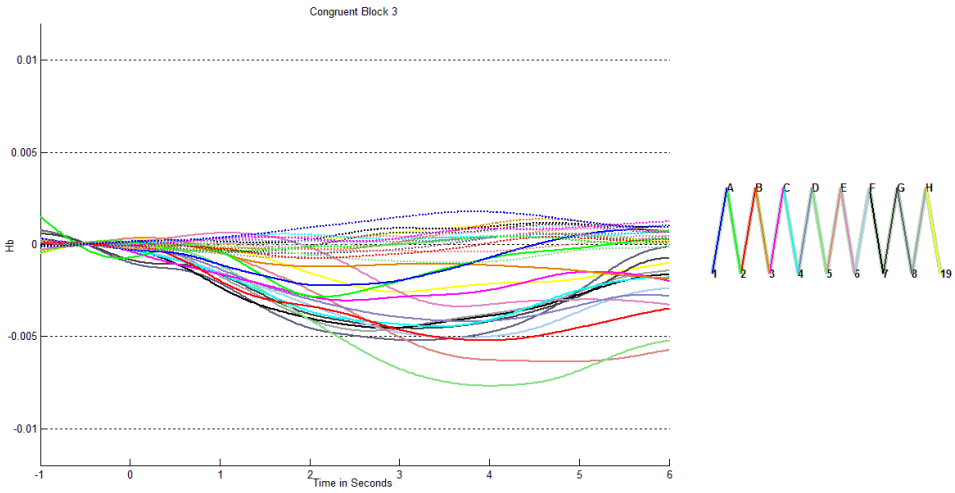
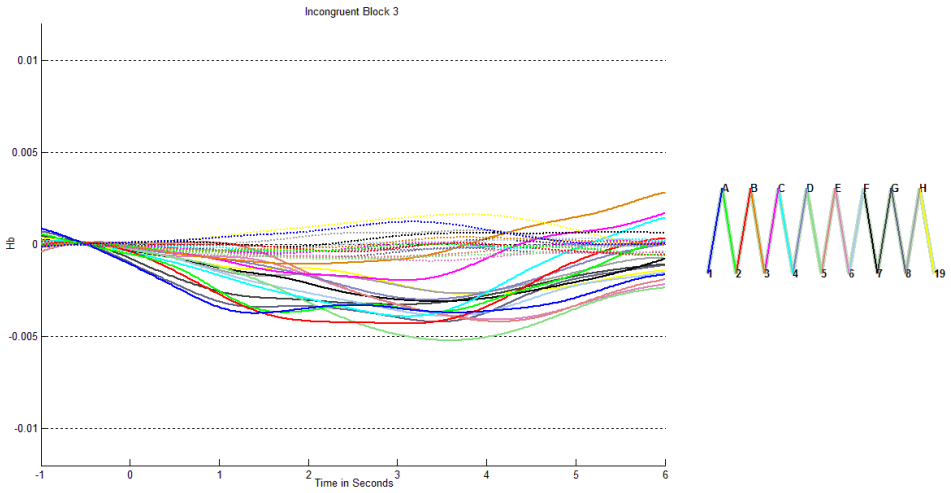


Figure 12b

NIRS Data for Incongruent Trials from Block Three



Figures 12a and b: Figure 12a shows congruent trials and 12b shows incongruent trials during Block 3 for the participants that had an attention effect (N = 11). The y axis indicates the relative change in oxygenated hemoglobin concentration in the prefrontal cortex. The trial started on the "0" marker. The dotted lines show the HbR and the solid line shows the HbO.