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Investigating the Physiological and Behavioral Responses to Fear in a Virtual Reality Fear Conditioning Paradigm

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Masters of Science Thesis

Investigating the Physiological and Behavioral Responses to
Fear in a Virtual Reality Fear Conditioning Paradigm

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Abstract

After a traumatic event such as a car accident, personal attack, or combat exposure, some individuals develop posttraumatic stress disorder (PTSD), which is a debilitating and chronic hypersensitivity to stimuli that are associated with the original trauma. PTSD symptoms can be studied using a fear conditioning paradigm. Whereas there are hundreds of scientific studies examining how nonhumans learn and extinguish fear responses, there are significantly fewer studies with humans. Moreover, the measures of fear are often quite different across species. Thus, in order to better understand the mechanisms underlying human fear, the aim of the current study was to examine the physiological and behavioral mechanisms underlying fear conditioning. Specifically, we sought to find whether humans would display conditioned fear in a virtual environment paired with an aversive event and whether humans exhibit freezing behavior in response to the conditioned stimulus. Using a virtual reality version of a classic fear conditioning paradigm, our main findings show that humans have a strong physiological response to the US and the CS; however, humans do not show a freezing response similar to the response seen in rodents. In addition, it was seen through extinction that there was a significant interaction between the trials and the CS+ and CS- presentation, with a stronger stimulus showing trial differences. Finally, it was found that extinction is not context-specific, an idea that has been debated in the literature, as no spontaneous recovery was seen. These findings provide novel understanding of how humans process fear and, perhaps most importantly, give insight into potential targets for clinical PTSD.

Chapter 1: General Introduction

Experiencing stress is a natural biological process that many organisms experience regularly, as fear and anxiety produced from a stressful event are adaptive in nature, helping an organism to protect itself from danger (Mineka & Oehlberg, 2008). As is the case with many other beings, humans have developed systems to regulate the physiological response that the body has to stress in order to remain at homeostatic levels; however, a stressful experience can influence cognition and emotional processing in a detrimental way (Jackson et al., 2006). It has been shown that animals who are exposed to stress in an uncontrollable manner will show alterations in their learning that produce maladaptive fear and anxiety (Maier, 1993). In humans, it is thought that extremely stressful events can impact emotional learning in a pathological way, consistent with the symptoms of anxiety disorders, particularly Post-Traumatic Stress Disorder, or PTSD (Pitman, 1989). Given the encumbering symptoms of disorders like PTSD, it is crucial to develop effective treatment methods to treat this class of disorders.

Fear and Post-Traumatic Stress Disorder

Although there are multiple types of trauma- and stressor related disorders, one of the most studied is Post-Traumatic Stress Disorder (PTSD). Those who are diagnosed with PTSD have experienced or witnessed a traumatic event, causing them to respond with fear, helplessness, horror, or another intense emotion (Keane et al., 2008). When PTSD was first conceptualized by clinicians, it was thought that the likelihood of a human to experience a significant trauma to lead to this pathological response was relatively rare, as this response could only happen from experiencing extreme stressors like war, an assault, or being a part of a natural disaster (Keane et al., 2008). However, it has been realized in the years since PTSD was

conceptualized that it was extremely likely that a human will experience a trauma in their lifetime. In the National Comorbidity Survey, it was found that 60% of men and 51% of women has experienced a traumatic event in their lifetime (Kessler et al., 1995).

Fortunately, not all those who experience a traumatic event go on to develop PTSD. It is thought that a significant trauma triggers PTSD symptoms in those who are already psychological vulnerable; however, the likelihood of developing PTSD increases as the trauma becomes more frequent, gruesome, or more severe (Sutker & Allain, 1996). Lifetime prevalence rates range from approximately 6-9% in the general population (Kessler et al., 2005). Interestingly, PTSD is more prevalent in women than it is in men, with prevalence rates in women ranging from approximately 10-12% while rates range from approximately 5-9% in men (Resnick et al., 1993). As expected, certain groups within the population, particularly veterans, are more susceptible to developing PTSD, with rates being higher partially because of the stress of readjusting to civilian life after being deployed (Keane et al., 2008).

Anxiety disorders like PTSD can be modeled by a classic learning paradigm. Classical conditioning is a learning paradigm where there is an unconditioned stimulus, an unconditioned response, a conditioned stimulus, and a conditioned response that are used. The same four components of a classical conditioning paradigm can be used to model a trauma. One can associate each component with part of a traumatic experience. For instance, in a trauma situation involving a car accident, one could assume that an unconditioned stimulus would be the car accident while an unconditioned response would be the reaction to the car accident (i.e., fear, stress, anxiety, etc.). A conditioned stimulus would be anything that became associated with the car accident, such as a bridge that was seen prior to the car accident. Finally, a conditioned

response would be produced when the conditioned stimulus elicits a response that is similar to the unconditioned response.

Fear Conditioning

Classical conditioning has long been used as a model for pathological forms of fear and anxiety (Baas, et al., 2008). One form of classical conditioning, called fear conditioning, involves learning that certain stimuli in the environment predict an aversive event, meaning that the mechanisms involved in fear conditioning are how humans learn to fear certain people, places, objects, and/or animals (Maren, 2001). This type of learning is seen in animals, as well. Evolutionarily, fear conditioning assists in helping animals survive when faced with a threat presently or in the future, making it a vital part of defensive behavior for many animals (Maren, 2001). Fear conditioning involves the use of both memory and emotional processes, which is why it is thought that disturbances in fear conditioning may underlie disorders of fear and anxiety, such as PTSD (Rosen & Schulkin, 1998).

Fear conditioning has been extensively validated as a model of anxiety disorders through various studies done with both animals and humans. The first recognized study done with fear conditioning, and perhaps the most well-known study of this phenomena, was the Little Albert experiment done by John Watson, where an infant named Albert was conditioned to fear a white rat through its pairing with a loud and aversive noise after the rat had been presented (Watson & Rayner, 1920). Eventually, with just the presentation of the rat, Albert got visibly upset and tried to move away from the animal, thus proving his learning of the association between the rat and the loud, aversive noise that was presented to him (Watson & Raynor, 1920).

Since the famous Little Albert experiment, fear conditioning has been used in various experiments that have told more about the paradigm and the processes involved in fear conditioning. Generally, fear conditioning paradigms involve a learning phase, an extinction phase, and some form of reinstatement or reacquisition depending upon the specific research design. In fear conditioning, the experiment can use a cue to signal an aversive event or an aversive event can occur only in a specific context. Studies that have been done will usually employ just one of these to signal the aversive event to the animals. For example, in a study done by Fanselow (1980), a shock was used as an aversive event. The shock was only given in a particular context and the assessment of fear was made either in the context that the animals were shocked in or it was assessed in a different context. This allowed the researcher to see if the animals learned that the context was predicting the aversive shock; thus, the animals should only fear the context that they were shocked in and should not fear a neutral context if the association was made correctly (Fanselow, 1980). In a cued fear conditioning paradigm, numerous different things can be used as a cue, such as an odor, a tone, or a light, to name a few. As an example, in a study done by Otto, et al. (2000), an odor was used to cue an aversive foot shock. Whether a cue or context is used in the fear conditioning paradigm, it has been shown that animals quickly learn the predictive value of a cue or a particular context presented with an aversive event, which validates the paradigm as a form of fear-induced learning.

Much of the literature supports the notion that extinction of conditioned fear is context-specific (Bouton, 2004; Bouton & King, 1983; Bouton & Ricker, 1994; Neumann & Longbottom, 2008; Polack, et al., 2013; Vervliet, et al., 2013). In a study conducted by Langton and Richardson (2009), they compared the effects of context change on extinction and re-extinction in rats. The researchers used two different chambers that each had different floors,

background noise, and smells (Langton & Richardson, 2009). The rats received a noise CS in only one of the contexts (Langton & Richardson, 2009). They found that both extinction and re-extinction are context-specific, as a renewal effect occurred in both cases (Langton & Richardson, 2009). Numerous studies have been done that show similar results in humans. In a study conducted by Milad, et al. (2005), the experimenters employed a two-day fear conditioning and extinction protocol. Conditioned stimuli were presented with an electric shock in one context but it was extinguished in a different context (Milad, et al., 2005). They then examined extinction recall and renewal 24 hours after training took place (Milad, et al., 2005). It was found that skin conductance responses were smaller when the conditioned stimulus was presented in the extinction context, but the response was renewed when the conditioned stimulus was presented in the original conditioning context (Milad, et al., 2005). Taken together, these studies show the context-specificity of extinction.

Although much fear conditioning work has been done using animal models, fear conditioning paradigms can also be used in experiments with humans. Through the same fear conditioning procedure used with animals, humans can also be conditioned to fear a particular cue or a certain context when they have experienced something aversive paired with it. As was previously mentioned, one of the earliest experiments done with fear conditioning involved exposing a baby to an aversive noise after a rat was presented (Watson & Rayner, 1920). Since then, fear conditioning has continued to be studied by many researchers. For instance, in a study done by Lake, et al. (2017), human participants were conditioned to fear a particular colored tile that predicted an aversive event a shock. However, this paradigm can get more complex when one puts two conditioned stimuli into the experiment with only one serving as the cue, which is referred to as discriminative fear conditioning (Lake, et al., 2017). For instance, in the above-

mentioned study done by Lake, et al., there were two colored tiles presented to participants, but only one was the cue for the shock while the other cued safety. In this case, the colored tile that cued the shock would be referred to as the CS+ while the colored tile that cued safety to the participant would be referred to as the CS-. Humans are also able to condition to a specific context, which Baas et al. demonstrated in their study done in 2004, where one room was associated with a shock while another room was not. When the results from the human fear conditioning experiments are compared to those done with animals, the results are similar in that humans also show that they learn the association well and will eventually learn to fear the conditioned stimulus before the aversive event has even occurred, showing the translational value of this research.

Neurobiological Features of Fear Conditioning

Fear conditioning allows organisms to make neural representations of their world through the learning processes that take place during conditioning. In order to make these representations, there is heavy involvement from multiple brain regions and substances. The neural mechanisms underlying fear conditioning were first discovered when scientists observed deficits in emotional processing due to brain damage in the temporal lobe of monkeys (Kluver & Bucy, 1937). Behavioral changes, including hypersexuality, visual agnosia and, most notably, a loss of fear, were seen with the brain damage to the temporal areas, drawing attention to this cortex for, among other reasons, fear modulation (Kluver & Bucy, 1937). Weiskrantz (1956) made the discovery that the loss of fear came from amygdala damage deep in the temporal lobe. Since Weiskrantz, the involvement of the amygdala in the fear response has been seen many times over.

However, although scientists knew that the amygdala was involved in modulating fear, it was unclear until later that the amygdala was also involved in fear learning processes. Although those that made this discovery used learning and memory tasks, such as instrumental avoidance tasks, a classic fear conditioning paradigm was also used by many to realize the involvement of the amygdala. Using a contextual fear conditioning paradigm, it was seen that lesions to the amygdala reduce a fear response in animals after a shock and also eliminate the fear response that is caused by the presence of a predator, showing that the amygdala is required to make fear-associated memories (Blanchard & Blanchard, 1972).

In addition to the amygdala, the hippocampus also plays a substantial role in fear conditioning, especially with contextual conditioned stimuli (Maren, 2001). It has been realized that the hippocampus is responsible for putting together contextual representations and then sending these representations to the amygdala where they are connected to an unconditioned stimulus (Maren, 2001). This connection was made when experimenters lesioned the dorsal hippocampus and saw that both acquisition and the fear produced by contextual fear conditioning were both impaired (Phillips & LeDuox, 1992). Other studies have shown that the point during training that the dorsal hippocampus is lesioned impacts fear learning and learning strategy; however, the dorsal hippocampus is heavily involved in any strategy employed during fear learning. Interestingly, impairments were originally only seen in these areas when the dorsal hippocampus was lesioned but impairments in auditory fear conditioning were seen when the subiculum was lesioned (Maren, et al., 1997), showing that the hippocampus has wide involvement in fear conditioning. More recent evidence has shown that the fornix and entorhinal cortex are also involved in these processes. In a study done by Ji and Maren (2008), it was seen that rats with lesions to the entorhinal cortex or to the fornix showed an impaired fear response

when placed back in the context that they had previously experienced a shock in. he results of this study and the others done before it generally show that the cortical and subcortical areas of the hippocampus are required for contextual fear memory retrieval.

There is a critical role played by various neurotransmitters and substances in the brain in the processes underlying fear conditioning. One essential neurotransmitter to fear conditioning is glutamate. In the amygdala, the basolateral complex (BLA) is vital to acquisition, expression and extinction of conditioned fear (LeDoux, 2000). Within the BLA, it has been seen that glutamate receptors are important for these processes, as well (Zimmerman & Maren, 2010). It has been seen that when AMPA receptors are antagonized, the expression of conditioned fear is impaired (Walker, et al., 2005). Furthermore, when an NMDA receptor antagonist is administered, the acquisition and extinction of conditioned fear is prevented (Walker, et al., 2005). Clinically, most anti-anxiety medications act on monoamines (Lapidus, et al., 2013); however, preclinical and clinical data have pointed to glutamate as a potential treatment target, as it is believed that targeting a non-monoaminergic system may be more effective and faster acting (Lapidus, et al., 2013).

Cortisol, a glucocorticoid hormone, is widely known to be released in response to stress. Diseases characterized by sustained hypercortisolism, or an excessive amount of cortisol in the blood, have been linked to human learning and memory impairments in disorders like Alzheimer’s disease or Cushing’s syndrome (McEwen & Sapolsky, 1995). Previous animal studies have shown that under prolonged stress induced by exposure to glucocorticoids or to stress over a 21 day period, atrophy of the dendritic branches could be produced in the CA3 region of the rat hippocampus (Woolley et. al., 1990; Watanabe et. al., 1992). Human clinical data has also supported this idea of brain abnormalities; high levels of glucocorticoids can cause

reversible dendritic alterations or even permanent neuronal loss, depending on the amount of exposure to stressful situations (McEwen & Sapolsky, 1995). It has been thought by some that there is enhanced amygdala activity but reduced hippocampal activity in times of high stress when more cortisol is circulating in the system (Sapolsky, 2003).

While both cortisol and glutamate are involved in the processes of fear conditioning, serotonin has also been shown to be specifically involved in the fear and anxiety responses during a fear conditioning paradigm (Hashimoto, et al., 1999). It has been seen clinically that various serotonin-related agents, such as selective serotonin reuptake inhibitors, 5-HT_{1A} receptor agonists, 5-HT_2 receptor antagonists, and monoamine oxidase inhibitors, are effective in treating anxiety disorders (Hashimoto, et al., 1999). However, it was unclear in the research whether increasing serotonin is raising anxiety levels or lowering them (Iversen, 1984). Thus, Hashimoto et al. sought to examine a more specific role for serotonin in terms of fear and anxiety through studying the effect of conditioned fear stress on extracellular serotonin levels in the rat medial prefrontal cortex by using a microdialysis technique while also observing the freezing behavior of the animals (Hashimoto, et al., 1999). Through the use of citalopram, a selective serotonin reuptake inhibitor, they found that conditioned fear stress increased extracellular serotonin levels in the medial prefrontal cortex while they observed a subsequent decrease in freezing behavior (Hashimoto, et al., 1999). These findings helps to settle the debate about the role of serotonin in fear and anxiety in rodents, as it showed that selectively increasing serotonin in the brain decreases anxiety.

Measuring Fear

In fear conditioning, there are numerous ways to measure fear, including physiological mechanisms. In animals, it has been seen that there are physiological changes in the animal as they become more anxious. During tests of anxiety, it has been shown that animals groom, urinate, and defecate as they become more anxious (Pavlova & Rysakova, 2015). In addition, a fear response in animals can also be measured through heart rate (Antoniadis & McDonald, 1999). In a study conducted by Antoniadis and McDonald (1999), the researchers used all of these various physiological measures of fear in a contextual fear conditioning paradigm that used two different chambers to assess fear across four test sessions. The researchers observed a higher heart rate across time within the sessions in the paired chamber (Antoniadis & McDonald, 1999). They found significantly greater amounts of urination and defecation in the paired chamber (Antoniadis & McDonald, 1999). These results help to explain the various physiological mechanisms that can be used to assess fear in rodents.

In humans, different physiological mechanisms are employed. Arguably the most common measure of anxiety in humans is skin conductance response, or SCR, which is used in many studies that employ a fear conditioning paradigm (Merz et al., 2010; Jackson et al., 2006; Stark et al., 2006; Milad et al., 2005; Coelho et al., 2015; Lake et al., 2017). SCR falls under the umbrella of electrodermal activity (EDA) measures (Braithwaite, et al., 2015). Broadly, SCR measures autonomic changes in the electrical properties of the skin; more specifically, SCR measures the flow of current between two points on the skin after an electrical potential has been applied (Braithwaite, et al., 2015). It has been argued that EDA, which SCR is included in, is the most useful way to detect changes in emotional states that can be traced back to sympathetic system arousal because it is the only psychophysiological variable that is not influenced by

parasympathetic activity (Braithwaite, et al., 2015). In the fear conditioning paradigm used by Lake et al. (2017), they saw a large increase in SCR response to the conditioned stimulus. Changes in heart rate can also be used to measure levels of anxiety in humans as well as in animals. Resting state heart rate variability (HRV) is the primary cardiac measure used in several studies (Wendt, et al., 2015; Liu, et al., 2013; Pappens, et al., 2014). HRV is simply a different way of looking at the activity of the vagus nerve (Pappens, et al., 2014). Whereas SCR is an indicator of arousal, HRV is used experimentally to examine inhibition and adaptability (Pappens, et al., 2014). It has been seen that those with cardiac deceleration have impaired fear conditioning while those without cardiac deceleration are able to condition to the paradigm and show an increased heart rate as anxiety increases (Sevenster et al., 2015).

Behaviorally, there are different ways to measure anxiety in animals compared to humans. In both animal and human fear conditioning paradigms, a startle response is used by some researchers as a measure of anxiety and/or fear (Dunsmoor, et al., 2014). In humans, a startle response is measured through EMG that is recorded from the orbicularis oculi muscle, the muscle that controls the eyelid (Dunsmoor, et al., 2014). In animals, it is measured through muscle contraction in the body after an acoustic cue (Zhang & Li, 2016; Russo & Parsons, 2017). Some researchers prefer to use a startle response as a behavioral measure because, compared to SCR, it is not as sensitive to attentional processes (Bocker, et al., 2004). In addition, amygdala-based fear circuits play a central role in startle in both humans and animals (Hitchcock & Davis, 1986), which is different than the basis of SCR. Although it is a different measure, results are similar to SCR and heart rate, in that there is an increased startle response to the conditioned stimulus as anxiety increases. In the previously mentioned study done by Antoniadis and McDonald (1999), they also examined various behavioral mechanisms in rodents, including

ultrasonic vocalizations, locomotion, and a preference test when the experiment involves contextual fear conditioning. The results of this contextual fear conditioning study showed that the rats preferred the chamber that was unpaired with the aversive event (Antoniadis & McDonald, 1999). The researchers also observed more ultrasonic vocalizations across time within the sessions in the paired chamber (Antoniadis & McDonald, 1999). They found significantly lower amounts of locomotion in the paired context (Antoniadis & McDonald, 1999).

More specifically, in animal paradigms, most experimenters measure fear in terms of freezing behavior, as it is a species-specific defense reaction that does not require any training in order to be produced (Bolles, 1970). Freezing behavior can be defined as the animal being in a crouching posture while also being completely immobile for one second or more (Blanchard and Blanchard, 1969). It has been found that the basolateral amygdala (BLA) is very much involved in the performance of this behavior, as pharmacological manipulations and lesions impact the behavior (McDannald & Galarce, 2011). In one such study that examined the connection between the BLA and freezing behavior, rats received 10 tone-shock pairings in one context, which was considered a remote memory as they were expected to remember this pairing for a substantial amount of time (Gale, et al., 2004). Then, the rats received another 10 tone-shock pairings 16 months later with a novel tone and context, which was considered a recent memory (Gale, et al., 2004). One day after the recent training took place, rats received BLA lesions or sham lesions of the BLA (Gale, et al., 2004). It was found that the rats with the sham lesions showed high and comparable freezing with all context-tone pairings while the BLA-lesioned rats showed large freezing deficits with both recent and remote tests (Gale, et al., 2004). These results showed that the BLA plays a substantial role in the permanent storage of fear memories,

which then impacts the expression of this fear in later tests (Gale, et al., 2004). It has been shown in previous studies that freezing behavior is associated with bradycardia (Vianna, et al., 2005; Walker & Carrive, 2003). Although freezing has never been examined in humans in the same way that it is examined in animals, some researchers have used bradycardia as a way to investigate whether humans exhibit this behavior as it is a response that is associated with freezing behavior (Vianna, et al., 2005; Walker & Carrive, 2003). Using this as a measure, it has been shown that humans do exhibit freezing in terms of a slowing of heart rate in response to a fear-inducing event (Lang & Davis, 2006); however, freezing in the classic sense has never been reported in humans in any fear conditioning study that has been done to date.

Using Virtual Reality to Study Fear

In both humans and animals, fear conditioning has been extensively validated as a paradigm (Baas, et al., 2008; Maren, 2001; Rosen & Schulkin, 1998; Watson & Rayner, 1920; Fanselow, 1980; Otto, et al., 2000). As previously discussed, many different researchers employ numerous techniques to study fear and anxiety. One way is to use computerized fear conditioning, which presents stimuli to participants on a computer in a two-dimensional format. In one such study that used a computerized fear conditioning technique in humans, the researchers were interested in examining the differences in acquisition of conditioned fear between men and women that are diagnosed with PTSD (Inslicht, et al., 2013). Participants were shown computer-generated colored circles that were either paired or unpaired with an electrical shock (Inslicht, et al., 2013). Skin conductance levels were assessed throughout the entirety of the experiment (Inslicht, et al., 2013). It was found that women had greater differential

conditioned skin conductance responses compared to men, which suggests women have greater acquisition of conditioned fear (Inslicht, et al., 2013).

However, one more modern technique that has been used by researchers recently is virtual reality. Virtual reality tests were developed and used with fear conditioning because it allowed for generalizations to be made between animal and human work. For instance, with virtual reality, it is possible to use different spatial contexts, like those used in animal work, while the subject is stationary in the laboratory (Pine, et al., 2001). In addition to the contextual benefits of using virtual reality, experimenters also found that virtual reality provided a more stimulating environment for the subjects when compared to standard fear conditioning experiments that had previously been done (Baas, et al., 2004) as well as a way to consistently monitor the movement of subjects during all phases of fear conditioning (Pine, et al., 2001). Allowing participants to move freely in a controlled environment while still being confined to a laboratory is an advantage of using a virtual reality paradigm that many researchers have taken advantage of (Grillon, et al., 2006).

Since virtual reality was adapted to be used as a fear conditioning paradigm, several researchers have proven its validity as a way to assess fear and anxiety. Virtual reality was first established as a valid measure to use to measure cued fear (Pine, et al., 2001). In one such study that validated virtual reality as a way to measure cued fear, two colors lights were shown to participants, with one predicting an air puff while the other predicted nothing (Pine, et al., 2001). The researchers found that participants were able to adequately condition in a virtual reality context, as similar results were found using virtual reality compared to previous studies that did not use this technique (Pine, et al., 2001). In 2004, Baas et al. were interested in using virtual reality to condition participants to a context, as it had only been used to condition subjects to a

cue previously. They found that it was just as adequate in conditioning subjects to a context as it was in conditioning them to a cue (Baas, et al., 2004).

After virtual reality was validated as a way to study both cued and contextual fear conditioning, researchers were then able to use virtual reality as a measure of more anxiety-specific behaviors. One such study was done by Baas et al. in 2007, where they examined whether a deficit in fear conditioning equated to maladaptive fear. They predicted that the failure to learn the associations put forth in the fear conditioning paradigm would result in higher contextual fear and trait anxiety (Baas, et al., 2007). To examine this, they used two different virtual reality contexts and a shock as an aversive stimulus as well as skin conductance response recordings and fear-potentiated startle (Baas, et al., 2007). They found that their hypothesis was supported in that those participants who failed to condition to the predictive cue showed chronic anticipation of danger in the threat context and also showed high levels of fear as measured through self-reported fearfulness and no difference of startle in the presence or absence of the cue in the threat context (Baas, et al., 2007). They also found that virtual reality is a useful tool for studying contextual fear conditioning in relation to anxiety, as all participants showed increases in shock expectancy, subjective reports of fearfulness, and startle reactivity (Baas, et al., 2007). Furthermore, using a virtual reality version of a fear conditioning paradigm gave the researchers similar skin conductance responses compared to a non-virtual paradigm, helping to show that this is a valid way to examine fear and anxiety in an immersive environment that would not be able to be used in non-virtual fear conditioning paradigms (Baas, et al., 2007).

Chapter 2: Present Experiment

Introduction to the current study

Clinically, anxiety disorders like PTSD are treated using exposure treatment, which involves exposing a patient to a feared object and/or situation in a controlled and gradual manner so that the patient can learn that the object of their fear is not threatening to them. Exposure therapy was first developed roughly sixty years ago when Wolpe (1958) put forth his idea of systematic desensitization. In his desensitization treatment for anxiety, Wolpe had his patients develop muscle relaxation skills before he instructed them to imagine a hierarchy of anxiety-provoking scenes that could be counteracted with muscle relaxation (McNally, 2007). Over the years, clinicians concluded that the key element for reducing fear was exposure to a cue (Marks, 1978). Exposure treatment uses the process of extinction, which is the process in which there are repeated pairings of the conditioned stimulus without any pairing to the unconditioned stimulus, resulting in a decreased conditioned response. One of the more difficult components of exposure treatment is when it should be initiated and to whom (Gray & Litz, 2005). Some research indicates that those who do experience distress after a trauma should be given treatment shortly after the trauma. In a study of rape and aggravated assault victims conducted by Foa, et al (1995), treatment was delivered ten days after the initial trauma in an effort to not worsen the maladaptive symptoms that may arise. They found that those who went through this treatment course showed less PTSD and depressive symptoms (Foa, et al., 1995). Bryant, et al. (1998) performed a similar experiment where treatment was given to traumatized individuals who had experienced a life threatening car accident within the month prior to seeking treatment. They also found that those who went through this treatment showed fewer PTSD and depressive symptoms at follow-up appointments (Bryant, et al., 1998).

More recently, there has been disagreement among clinicians about when to initiate exposure treatment. As Foa, et al. and Bryant, et al. demonstrated, it is possible that early exposure and debriefing are the most beneficial to the patient, as their results showed that early exposure treatment may be critical for recovery. On the other hand, it has been shown that early interventions may be ineffective, meaning that an early intervention may not help someone who has experienced a trauma to overcome the trauma symptoms (Bisson, 2003). In fact, there is some evidence showing that intervening too early, particularly when the intense and acute stress of the experience was not waned, might exacerbate the relapse of fear in a patient (Rothbaum & Davis, 2003). Researchers have noted that individuals who develop PTSD may be resistant to extinction, which could heavily impact exposure treatment (Rothbaum & Davis, 2003). In addition, it has been seen that early one-time interventions may be worse for extinction whereas exposure treatment delivered over multiple sessions weeks after the trauma has occurred is effective in treating the developing symptoms associated with PTSD (Rothbaum & Davis, 2003). Some have pointed out that someone who becomes extremely distressed after experiencing a trauma may be more concerned with basic needs, such as safety or shelter, meaning that they may not be in a position to process their trauma or benefit from any treatment (Gray & Litz, 2005).

To address this problem, Maren and Chang (2006) performed an experiment in animals where timing was a key factor. They were interested in whether an early intervention delivered minutes after fear conditioning has taken place would produce superior extinction relative to a standard delayed intervention of 24 hours. During conditioning, an auditory conditioned stimulus was paired with a noxious foot shock in a novel chamber. The early intervention in this study was 15 minutes while the long break was 24 hours. The researchers measured fear in the animals

used in the study in terms of freezing, a behavior where the animal becomes immobile that is most common in animals of prey (Maren & Chang, 2006).

During conditioning, animals showed low levels of freezing during baseline with freezing behavior only emerging after the first trial of the experiment. After conditioning, the animals either received the early break (15 minutes) or the long break (24 hours). Then, extinction was done. During extinction, it was found that all animals exhibit high levels of fear before the onset of extinction during a baseline trial; however, animals that received the early break showed significantly higher levels of fear at baseline compared to the animals that received the long break. But, all animals extinguish by the end of the extinction trials regardless of timing condition. Clear differences were observed during retention, where animals in each group showed differences in terms of retention of the extinction memory. Specifically, only rats that had the long break show a reduction in freezing behavior over time. With their study, Maren and Chang showed that when an animal is in a high fear state, immediate extinction training is worse than delayed extinction training. This was seen when, during retention, spontaneous recovery occurred only after an early intervention while it remained inhibited in rats with a long break.

Although the results above indicate that there is a difference in fear after a short or long break, the results from the extinction sessions are particularly important to the issue of timing in exposure therapy. Maren and Chang (2006) found that there were much higher levels of fear before extinction took place for the rats that had the short break compared to those who had the long break. Therefore, the researchers investigated whether the different levels of fear before extinction training contributed to the differences in long-term extinction that were seen between the groups (Maren & Chang, 2006). Rats went through the same behavioral procedures described above except that they only had one conditioning trial and extinction and retention testing were

conducted outside of the conditioning context in order to reduce the level of fear before extinction took place (Maren & Chang, 2006). They found that freezing behavior was greatly reduced before extinction training in both groups, indicating that early extinction is effective for suppressing fear long-term when the level of fear is low prior to extinction training (Maren & Chang, 2006). Taken together, the results of this study suggest that the timing of extinction is a critical component of effective exposure treatment and that different traumas may require different treatments clinically (Maren & Chang, 2006).

Thus, in the present study, we sought to replicate some of these findings in humans. Specifically, we aimed to determine whether humans would display conditioned fear physiologically and behaviorally in a virtual environment paired with an aversive event. Also, we aimed to determine the strength of the fear response to two different stimuli in an effort to establish different levels of 'trauma'. We hypothesized that participants will show increased skin conductance response when exposed to the cue that was previously paired with aversive stimuli and that participants will show more freezing behavior (a behavior never before reported in humans) to the CS+ compared to the CS- during conditioning. We also hypothesized that the skin conductance response would decrease during extinction and would subsequently increase during reinstatement when the participant was exposed to the same context that conditioning took place in. Furthermore, we hypothesized that a shock would be a stronger aversive stimulus compared to a scream, as indicated by larger skin conductance responses.

Materials and Methods

Experiment 1:

Participants

One hundred and thirteen University of Connecticut undergraduates were recruited from introductory psychology classes. Of these participants, seventy-two participants' data was discarded due to ineligibility. To be eligible, participants must not have had any preexisting heart or neurological conditions; if participants noted having either, they were not allowed to participate in the study in any capacity. Additionally, in order to be included, the participant must have correctly identified the CS+ on a post-test survey with a confidence rating in that choice of 5 or more. Additionally, a visual skin conductance response to the aversive scream must have been present after the first presentation of the CS+ in order to be included in the final analysis. Previous studies have excluded participants due to similar criteria in regards to heart and neurological health as well as the participant needing to respond to the aversive event in order to be included (Levar, et al., 2017; Schiele, et al., 2015; Dunsmoor, et al., 2009); however, using a confidence rating is a novel technique that the current study sought to examine. This resulted in usable data from forty-one undergraduates. Fourteen of the participants were male with a mean age of 19.07. Participants received class credit for their participation. The University of Connecticut Institutional Review Board approved this study.

Apparatus

Stimuli were presented on a standard 17-inch computer monitor. Screams were presented at 90 dB through headphones. Participants were seated at the computer and navigated through the virtual environments using a joystick. Physiological measurements were taken from the participants using a Biopac Systems MP150 data acquisition system. The Biopac MP150 system was connected via an Ethernet cord to a laptop that was running Biopac Acqknowledge software, version 3.8.1. The Biopac MP150 system received digital TTL signals through its isolated digital

interface connecting to the parallel port on the stimulus computer running the VR software E-prime. Electrodermal activity (EDA) was collected continuously from two disposable electrodes on the middle and pointer fingers on the non-dominant hand. Electrocardiogram (ECG) was obtained via two disposable electrodes attached on the upper left pectoral and the lower right abdomen.

Procedure

Participants signed up for a one-hour testing session. After consent was obtained from each participant, they completed a general questionnaire. Questions included information about basic demographics and gaming experience but also asked for a neurological history and heart health history because anyone who had a pre-existing heart condition or seizure disorder was not allowed to participate. After the questionnaire is completed, electrodes were placed on the index finger and the middle finger of the participant's non-dominant hand (EDA) and on their chest (ECG).

After the physiology set-up, participants were instructed to keep their non-dominant hand still while using their dominant hand to navigate the joystick as naturally as possible but to keep moving throughout all of the sessions. Participants go through four virtual testing sessions on the computer. The first two sessions were for conditioning, the third served as an extinction session, and the fourth was for recovery. For conditioning, either a red or green colored floodlight, termed the CS+, was presented for 8 seconds. After 7.5 seconds of the CS+ light, a 90 dB scream sound was presented for .5 seconds. A different colored light was presented for 8 seconds; however, there was no aversive stimulus paired with this stimulus. This light was termed the CS- (Figure 1a and 1b).

Within each session, participants were confined to one of the virtual rooms for up to 240 seconds. During each acquisition session, participants were presented with 12 CS exposures (6 CS+, 6 CS-) separated by ~20 seconds; however, two of the CS exposures were not paired with an aversive scream. During extinction sessions, participants are placed into room B for 240 seconds. Participants were exposed to the CS+ and the CS- 6 times each, but no US was presented. Then, participants took a 10-minute break prior to completing the recovery session. Similar to the extinction session, the recovery session allowed participants to be exposed to the CS+ and the CS- 6 times each, but no US was presented.

Each participant was exposed to room A twice for conditioning, exposed to room B for extinction, and was either placed in room A or room B for recovery. Rooms were unique in terms of colors, furniture, floor and layout, but were the same size (Figure 2). Room pairings with the US (scream) were counterbalanced across participants. Recovery took place either in the room that acquisition took place in or in the room that extinction took place in to test the notion of extinction being context-specific. Participants then completed a questionnaire that asked them to say which colored light was the CS+ (i.e., predicted the scream) and how confident they are in that choice. This served as an assessment of how well the participant learned the task. Finally, they completed a cyber-sickness questionnaire. This addressed numerous potential outcomes of cyber-sickness (i.e. fatigue, boredom, headache, nausea, faintness, confusion, etc.). Participants indicated whether they did or did not experience any of the symptoms. If they indicated yes, they also provided the level of severity (slight, moderate, or severe). If any participant noted a moderate or severe level of severity for any symptom that indicated they were motion sick during the task, they were excluded from the data analysis.

Results

Statistical Data Analysis*:*

The values used for all measures concerning the effect of the unconditioned stimuli was a difference score that describes the difference between baseline and the maximum skin conductance response that occurred in the 8 seconds after the unconditioned stimulus had been presented. More specifically, the average of the baseline response that occurred in the 1 second prior to each conditioned stimulus presentation was subtracted from the maximum response that occurred in the 8 seconds after the unconditioned stimulus presentation. For the analyses associated with the conditioned stimulus, a difference score was used that demonstrated the difference between the average baseline response for the 1 second prior to the conditioned stimulus presentation and the maximum skin conductance response that occurred during the 8 second conditioned stimulus presentation. Any data with artifacts or invalid readings was removed from the final data set; an artifact was defined as an apparent SCR response that was not due to any experimental features or manipulations while invalid readings occurred from technical issues. The baseline recording was considered to be the 1-second interval before the CS+ or CS- onset. The recording during the CS+ or CS- presentation was taken during the 8-second CS presentation. Finally, the recording after the US was taken from the first 8-seconds after the US presentation. Those participants that were included in the final data analyses had to have selected the correct CS+ and had a confidence rating in that choice of a 5 or above on the exit survey while also having passed the screen for neurological and heart problems. 36% of the total participants for Experiment 1 and 27% of total participants from Experiment 2 were included in the final analysis. For the skin conductance data, individual data points that were 3 standard deviations above or below the mean were removed and left blank during the final data

analysis. For the freezing data, the participants from the shock skin conductance data were only included in these analyses if they froze to the unconditioned stimulus more than 1 standard deviation below the mean (13% of participants). Freezing was examined using a Matlab code that calculated freezing based on joystick movement on a XYZ plane. This way of examining freezing allowed the actual movements of the participants to be analyzed as opposed to examining freezing of the avatar within the virtual reality environment. Repeated measures ANOVAs were used to analyze all acquisition data, two-way ANOVAs were used for the extinction data, and independent samples t-tests were used to analyze the recovery data. Only trial 1 in the recovery session was examined, as the goal of this session was to examine spontaneous recovery, which would be seen in the first trial. The general significance level that was used in these analyses was $p=0.05$.

Results

In the first acquisition session, the skin conductance response to the scream was compared to the response after no scream. The response to the scream was significantly larger than the response to no scream ($F(1, 35) = 49.27$; $p < 0.01$; Figure 3). Then, the response to the CS+ presentation was compared to the response to the CS- presentation, and it was seen that the skin conductance response was significantly greater than the response to the CS- ($F(1, 31) = 8.07$; $p = 0.01$; Figure 4). The same two analyses were done for the second acquisition session. The skin conductance response to the scream in this session was significantly larger than the response to no scream ($F(1, 34) = 16.38$; $p < 0.001$; Figure 3) and the response to the CS+ presentation was significantly larger than the response to the CS- ($F(1, 33) = 11.67$; $p = 0.002$; Figure 4).

During the extinction session, a two-way ANOVA was conducted that examined the effect of trial and CS+/CS- on SCR response post-CS presentation. There was a statistically significant interaction between the effect of trial and CS+/CS- on SCR response ($F(5, 480) = 2.39$; $p = 0.04$); however, no significant effect was observed when trial was examined alone ($F(5, 480) = 2.01$; $p = 0.08$) or when CS+ and CS- were examined alone ($F(1, 480) = 3.4$; $p = 0.07$). During the CS presentation, the same analyses were conducted. No significant interaction was found between the effect of trial and CS+/CS- on SCR response ($F(5, 480) = 0.553$; $p = 0.74$). For the recovery session, data of participants who were placed in the same room for acquisition and recovery ('Same') were compared to those who were placed in a different room for acquisition and recovery ('Different') for trial 1 only to examine if spontaneous recovery occurred in the 'same' condition, as this tested the context-specificity of extinction since this condition involved participants being in a different room for extinction than recovery took place in. It was found that there were no significant differences between the same and different rooms in the first trial of the recovery session after the CS+ presentation ($t(38) = -0.76$; $p = 0.45$; Figure 7) and during the CS+ presentation ($t(33) = -0.33$; $p = 0.75$; Figure 8), showing that the location of extinction in this experiment was not context-specific.

Materials and Methods

Experiment 2:

Participants

One hundred and thirty-one University of Connecticut undergraduates were recruited from introductory psychology classes. Of these participants, ninety-five participants' data was discarded due to ineligibility. The same eligibility criteria from Experiment 1 were used for

Experiment 2. Similar to Experiment 1, discarding data is seen in other studies (Levar, et al., 2017; Schiele, et al., 2015; Dunsmoor, et al., 2009); however, using a confidence rating as opposed to only relying on the data to exclude participants is a novel technique. This resulted in usable data from thirty-six undergraduates. Twenty-one of the participants were male with a mean age of 19.06 years. All participants received class credit for their participation. The current study was approved by the University of Connecticut Institutional Review Board.

Apparatus

The apparatus used in Experiment 2 is the same as the apparatus used in Experiment 1, except for the following changes. The headphones were used in order to hear sounds from the virtual environment as opposed to hearing a scream. The same physiological setup and measurements used in Experiment 1 were used in Experiment 2. The aversive event in Experiment 2 was a shock. Shocks were delivered through a Biopac Systems STIMSOC attached to the Biopac Systems MP150 system. The participants received the shocks through two disposable electrodes placed on the non-dominant forearm.

Procedure

As was the case in Experiment 1, participants signed up for a one-hour testing session, where they completed a consent form and the same general questionnaire. After the same physiology set-up as Experiment 1, participants first set their own shock level during a test VR session that a trained experimenter administers. To do this, the experimenter set the STIMSOC box to the 200V setting. Then, a test VR session was started, which allowed the experimenter to shock the participant. Using a knob located on the MP150 box and the ‘Z’ key on the keyboard

of the computer that the test sessions took place on, the knob was slowly moved up while the ‘Z’ key was pressed until the participant stated that the shock was aversive but not painful. The process of setting the shock took approximately 30 seconds per participant, with participants receiving a maximum of 10 shocks before settling on a level. Then, participants completed four virtual testing sessions on the computer where they were instructed not to move their non-dominant hand and to keep moving throughout the sessions. Similar to Experiment 1, one green or red colored light, termed the CS+, was presented to the participant for 8 seconds. After 7.5 seconds of the CS+ light, a shock was presented for .5 seconds. A different light was presented for 8 seconds; however, there was no aversive stimulus paired with this colored light. This light was termed the CS-.

As was the case in Experiment 1, participants were confined to one of the VR rooms for up to 240 seconds with the same amount of exposures to the CS+ and the CS-. The exposure to the VR rooms during each session as well as the colors, furniture, floor and layout of each VR room were the same as in Experiment 1. Extinction and recovery were conducted in the same way as previously described in Experiment 1. Recovery took place either in the room that acquisition took place in or in the room that extinction took place in to test the notion of extinction being context-specific. Participants then completed the same questionnaires as in Experiment 1 to finish the experiment.

Results

For the first session of acquisition, the skin conductance response to the shock was compared to the response after no shock. There was a significant difference between the response after receiving a shock compared to the response after no shock was received ($F(1, 31) = 44.65$; $p < 0.01$; Figure 9). The response to the CS+ was then compared to the response to the

CS-, and the skin conductance response to the CS+ was significantly larger than the response to the CS- ($F(1, 30) = 19.42$; $p < 0.01$; Figure 10). In the second acquisition session, the same two analyses were conducted. It was seen that the skin conductance response to the shock was significantly different than the response after no shock was received ($F(1, 31) = 44.36$; $p < 0.01$; Figure 9) and that the response to the CS+ was significantly different than the response to the CS- ($F(1, 30) = 25.02$; $p < 0.01$; Figure 10).

During the extinction session, a two-way ANOVA was conducted that examined the effect of trial and CS+/CS- on SCR response post-CS presentation. There was a statistically significant interaction between the effect of trial and CS+/CS- on SCR response ($F(5, 420) = 2.99$; $p = 0.01$); however, no significant effect was observed when trial was examined alone ($F(5, 420) = 1.57$; $p = 0.17$) but a significant effect was seen when CS+ and CS- were examined alone ($F(1, 420) = 51.58$; $p < 0.001$). During the CS presentation, the same analyses were conducted. A significant interaction was found between the effect of trial and CS+/CS- on SCR response ($F(5, 420) = 3.57$; $p = 0.004$) with significant results being seen when trial was examined alone ($F(5, 420) = 3.76$; $p = 0.002$) and when CS+ and CS- were examined alone ($F(1. 420) = 42.43$; $p < 0.001$). Post hoc analyses revealed a difference between trial 1 and trial 6 ($p = 0.001$), indicating that extinction took place. For the recovery session, the same method of using data from participants who were placed in the same room for acquisition and recovery (‘Same’) and comparing it to those who were placed in a different room for acquisition and recovery (‘Different’) was used. It was found that there were no significant differences between the same and different rooms in the first trial of the recovery session after the CS+ presentation ($t(34) = -1.45$; $p = 0.16$; Figure 13) and during the CS+ presentation ($t(34) = -1.46$; $p = 0.16$; Figure 14), showing that the location of extinction in this experiment was not context-specific.

Scream Versus Shock

In order to assess which stimuli was stronger so that future experiments could be conducted, analyses comparing the CS+ data from both shock and scream were conducted. For the first session of acquisition for both stimuli, there was a significant difference between the responses to the shock and to the scream ($F(1, 31) = 4.77$; $p = 0.04$; Figure 15). There was also a significant difference between shock and scream when the CS+ was presented ($F(1, 29) = 6.41$; $p = 0.02$; Figure 16), meaning that the response to the CS+ is higher in the shock experiment compared to the scream experiment. Similar results were found in the second acquisition session for both stimuli. It was found that there was a significant difference between the response to the shock and scream ($F(1, 28) = 19.4$; $p < 0.01$; Figure 15) and that there was a significant difference between the skin conductance response during the CS+ presentation for both stimuli ($F(1, 28) = 18.61$; $p < 0.01$; Figure 16).

During the extinction session, a two-way ANOVA was conducted that examined the effect of trial and CS+/CS- on SCR response post-CS presentation. There was a statistically significant interaction between the effect of trial and CS+/CS- on SCR response ($F(5, 450) = 2.91$; $p = 0.01$); however, no significant effect was observed when trial was examined alone ($F(5, 450) = 1.96$; $p = 0.08$) and no significant effect was seen when CS+ and CS- were examined alone ($F(1, 450) = 1.6$; $p = 0.21$). During the CS presentation, the same analyses were conducted. A significant interaction was found between the effect of trial and CS+/CS- on SCR response ($F(5, 450) = 3.29$; $p = 0.01$) with significant results being seen when trial was examined alone ($F(5, 450) = 5.09$; $p < 0.01$) and when CS+ and CS- were examined alone ($F(1. 450) = 54.61$; $p < 0.01$). Post hoc analyses revealed a significant difference between trial 1 and trial 6 ($p = 0.001$), indicating extinction took place.

Freezing

Based on the comparison made between shock and scream, it was determined that shock is a stronger aversive stimulus. Because of that, the freezing data was only taken from shock participants that froze at least one standard deviation above the mean. As was previously mentioned, in the animal literature, freezing is defined as one second or more of no movement Blanchard & Blanchard, 1969); however, this definition does not apply for humans. After testing several methods, the best approach to looking at freezing behavior in humans and the method that was used in the data analysis below was a speed analysis based on joystick movement. This analysis used joystick position as a measure of freezing instead of using the avatar in the virtual reality paradigm by employing a XYZ plane to quantify joystick position. During the first session of acquisition, the amount of freezing in response to the unconditioned stimulus was compared to the freezing when no unconditioned stimulus was presented. There was not a significant difference between the freezing behavior after receiving a shock compared to the behavior after not receiving a shock ($F(1, 15) = 2.77$; $p = 0.12$; Figure 19). The freezing behavior caused by the CS+ was then compared to the behavior caused by the CS-, and the freezing behavior in response to the CS+ was not significantly different than the behavior during the CS- presentation ($F(1, 15) = 1.15$; $p = 0.3$; Figure 20), which was not unexpected as learning was taking place. In the second acquisition session, the same two analyses were conducted. It was seen that the freezing behavior in response to the shock was not significantly different than the behavior after no shock was received ($F(1, 15) = 0.07$; $p = 0.79$; Figure 19) and that the amount of freezing to the CS+ was not significantly different than the amount of freezing to the CS- ($F(1, 15) = 1.13$; $p = 0.3$; Figure 20).

During the extinction session, a two-way ANOVA was conducted that examined the effect of trial and CS+/CS- on SCR response post-CS presentation. There was a statistically significant interaction between the effect of trial and CS+/CS- on SCR response ($F(5, 180) = 2.84$; $p = 0.02$); however, no significant effect was observed when trial was examined alone ($F(5, 180) = 0.27$; $p = 0.93$) and no significant effect was seen when CS+ and CS- were examined alone ($F(1, 180) = 0.003$; $p = 0.96$). During the CS presentation, the same analyses were conducted. No significant interaction was found between the effect of trial and CS+/CS- on SCR response ($F(5, 180) = 1.74$; $p = 0.13$). For the recovery session, the same method of using data from participants who were placed in the same room for acquisition and recovery ('Same') and comparing it to those who were placed in a different room for acquisition and recovery ('Different') was used. It was shown that there were no significant differences between the same and different rooms after the CS+ presentation ($t(14) = .33$; $p = 0.75$; Figure 23) and during the CS+ presentation ($t(14) = -0.76$; $p = 0.46$; Figure 24), showing that the location of extinction was not context-specific and did not impact freezing behavior.

Chapter 3: General Discussion

The current experiments were conducted in order to extend some of the findings from a study done in rodents by Maren and Chang (2006), which suggested that different traumas might require different clinical treatments. In the study conducted by Maren and Chang (2006), they were able to manipulate trauma severity through changing the location of extinction and retention, which showed that freezing behavior was greatly reduced before extinction training in both the short and long break groups. This indicated that early extinction is effective for suppressing fear long-term when the level of fear is low prior to extinction training (Maren &

Chang, 2006). Specifically, the current study was undertaken in order to determine whether humans would display conditioned fear physiologically and behaviorally in a virtual environment paired with an aversive event. This study was also done so that the strength of different stimuli could be defined in an effort to establish different levels of 'trauma' for future studies.

Overall, the results from the present study help to support previous literature indicating that humans do display conditioned fear physiologically in a virtual reality paradigm. This was seen in Experiment 1 and Experiment 2 in both acquisition sessions. In Experiment 1, the physiological response to the scream and the response to the CS+ were significantly greater than the response to the CS-. In Experiment 2, the response to the shock and the response to the CS+ were significantly different than the response to the CS-. It was shown that this conditioned fear continues into extinction, but, through data analysis, it is clear that in Experiment 2 the difference decreases as the session goes on. In recovery, the data showed that extinction is not context-specific. The results also indicated that the shock is a stronger aversive stimulus physiologically compared to the scream as evidenced by the significant differences seen in both acquisition sessions between the two stimuli. In addition, in comparing the two stimuli, it was seen that there was a significant difference in the physiological responding between the shock and the scream in extinction, although data analysis indicates that this difference decreases as the participant proceeds through the session.

Contributions of the Current Work to the Literature

Both Experiment 1 and Experiment 2 demonstrated that humans do display conditioned fear based on their skin conductance response in a virtual reality environment. Specifically, in

both experiments, participants in acquisition sessions 1 showed a significantly larger skin conductance response to the CS+ compared to the CS-. They also showed a larger skin conductance response to the US compared to the CS-. The same results were found for acquisition 2, where there was a significantly larger skin conductance response to the CS+ and in response to the US compared to the response to the CS-. These findings, which suggest that humans do display conditioned fear, are consistent with those found in the literature on human fear conditioning. In a study conducted by Olsson and Phelps (2004), they compared fear learning either through fear conditioning or without a direct experience, such as observing or through verbal instructions. In each group, the authors examined whether the participants had different responses to the CS+ (Olsson & Phelps, 2004). The responses were measured using a skin conductance response, which was significantly larger when the CS+ was presented compared to the CS- in the acquisition phase (Olsson & Phelps, 2004). Similar results were seen in a study involving discriminative fear conditioning, which involved learning that one cue predicted an aversive event while the other predicted safety (Lake, et al., 2016). In this particular study, one cue indicated an aversive shock (CS+) while the other predicted non-aversive tactile stimulation (CS-). The response to the CS+ was significantly larger than the response to the CS- during the conditioning phase of the experiment (Lake, et al., 2016), which is consistent with the results found in the present study. The finding that humans do display conditioned fear physiologically has carried over to the virtual reality studies that have been conducted. In a study done by Baas et al. (2004), subjects were steered through a virtual environment that included two distinct rooms connected by a street scene. In one room, a colored panel on the wall, which served as the CS, indicated that a shock would be received while, in the other room, a different colored panel indicated that the room was ‘safe’ (Baas, et al., 2004). Using eye blink startle as

their physiological measure, it was found that the startle response was significantly higher in response to the CS+ compared to the CS- (Baas, et al., 2004). The results of the present study are highly supported by the literature in the field; however, the present study is one of few to employ both skin conductance response and virtual reality techniques to study fear conditioning. Given these observations, it is important for future work to focus on using these measures together to effectively study their use in the field as well as to make comparisons with other measures, such as cardiac response.

During extinction, the results from Experiment 1 showed that there was a statistically significant interaction between trial and CS+/CS- with no effect seen when trial and CS+/CS- were examined alone. In Experiment 2, the response to the CS+ was effectively extinguished. Although the results from Experiment 1 were not quite as clear as the results from Experiment 2, Experiment 2 clearly suggests that extinction took place. As was the case with the results from the acquisition sessions, a fear response being successfully extinguished has been seen in the literature on the subject in both humans and animals. In the previously mentioned animal model of fear conditioning, Maren and Chang (2006) conducted an extinction session either after a 15-minute delay or after a 24-hour delay. In the extinction session alone, animals showed similar responses to humans, in that the fear response on the first trial was large as measured by freezing behavior, and it gradually decreased as extinction went on, regardless of the timing of the extinction session in this study (Maren & Chang, 2006). This shows that the response that we saw in the current study during extinction is similar to what is seen in the animal literature on the subject.

In the human literature, extinction results are similar to those seen in the current study and in animal models of fear. In a study investigating stressor controllability as a way to examine

the behavioral consequences associated with experiencing a trauma, researchers randomly assigned participants to an escapable stressor condition, a yoked inescapable condition, or a control condition where no stress was involved (Hartley, et al., 2014). Physiological measurements were taken from those in the control condition so that the measurements of those in the stressor conditions could be compared (Hartley, et al., 2014). It was found that those in the escapable stressor condition and in the control condition showed significantly reduced skin conductance responses from acquisition to extinction (Hartley, et al., 2014). A similar reduction in a fear response between acquisition and extinction in humans has been seen in several other studies. In one such study, researchers investigated the effect of electrical brain stimulation on fear extinction as a way to assess novel treatment methods in anxiety disorders (Abend, et al., 2016). Electrical stimulation that targeted the medial prefrontal cortex was applied on day 2 of the 3-day study (Abend, et al., 2016). Using skin conductance response and self-reports, it was seen that the response to the CS+ was no longer significantly different than the response to the CS- by the end of the extinction session for the group that received the electrical brain stimulation (Abend, et al., 2016). The results from the study performed by Abend, et al. (2016) indicate, not only should electrical brain stimulation be considered for novel treatment, but also that there is a decrease in responding between acquisition and extinction. Thus, the results found in the present study regarding extinction are supported by the literature in both animals and humans.

The results from the recovery session indicated that, in the present study, extinction was not context-specific since there were no significant differences between the same and different rooms after the US presentation or during the CS+ presentation in both Experiments 1 and 2. These results were unexpected, as numerous studies have indicated that extinction is, in fact,

context-specific (Bouton, 2004; Bouton & King, 1983; Bouton & Ricker, 1994; Neumann & Longbottom, 2008; Polack, et al., 2013; Vervliet, et al., 2013). By this, it is meant that after a CS has been extinguished, the CR should only be renewed when the CS is presented outside of the extinction context (Goode & Maren, 2014). One example of this was found in a study conducted by Bouton and King (1983). Four experiments were conducted to test the influence of contextual stimuli on rodents' fear response to an already-extinguished CS (Bouton & King, 1983). They found that when the rats received repeated pairings of a CS with a shock in one context and then went through extinction of the CS in another context, fear was renewed only in the original context, showing the context-specificity of extinction (Bouton & King, 1983). However, to complicate the matter further, there is evidence from animal models that extinction may not be context-specific (Westbook, et al., 2002). In a study conducted by Westbrook, et al. (2002), the experimenters extinguished two CSs in two different contexts that were both different than the context where conditioning took place (Westbrook, et al., 2002). After, the rats received a footshock reminder in one of the two extinction contexts and then were tested for retention in a neutral context (Westbrook, et al., 2002). The rats showed more fear to the CS that was extinguished in the same context that the footshock reminder took place in, suggesting that extinction may not be context-specific (Westbrook, et al., 2002). Although it is a common belief that extinction is context-specific, with the current experimental design and with the evidence presented in the literature, it seems as though extinction may not be context-specific for every experimental designs.

The comparison between the shock and scream stimuli is an important component that is necessary to better treatment methods for anxiety disorders, as there are minimal studies done that compare stimuli in this way. In the present study, it was found that, when comparing shock

and scream, the shock was a stronger aversive stimulus, as seen in the response to the US and in response to the CS+ in both acquisition 1 and 2. During extinction, results indicated that there were significant differences between the two stimuli with a decrease in SCR response during the CS presentation, indicating that they extinguish in the same way regardless of the initial start point for the SCR response.

Clinically, there is a large debate in regards to when exposure therapy, the current primary method of treatment after one has experienced a trauma, should occur. Some clinicians believe that, regardless of the type of trauma experienced, one should receive immediate therapy (Foa, et al., 1995) while others believe that, depending upon the severity of the trauma, perhaps therapy should be postponed to a later date (Gray & Litz, 2005). In an animal model designed to address this clinical debate, Maren and Chang (2006) concluded that when an animal has experienced an aversive stimulus, immediate extinction training is worse than delayed extinction training. They were able to determine this after seeing that spontaneous recovery only returned after immediate extinction training had taken place while it remained inhibited in rats that had a longer extinction training period. This study showed that, in animals, extinction practices should be altered depending on the strength of the stimulus. From the comparison between the shock and scream stimuli in the current study, it is clear that one stimulus is stronger than the other based on the significant differences in physiological responding. Future work should further examine the findings from the Maren and Chang (2006) experiments, as they have substantial implications for anxiety treatment research if they can be duplicated in humans. Experiments should be conducted in an effort to understand the impact of the timing of extinction; Maren and Chang (2006) reported that longer extinction training had more long-term effects in reinstatement compared to immediate extinction training. To study this in humans using the

current experimental design, changing the delay between extinction and the recovery session would allow experimenters to examine the impact of timing of extinction on a recovered fear response. Also, experiments could focus on determining whether a stronger stimulus requires more time between extinction and reinstatement compared to a weaker stimulus, as was seen in the Maren and Chang (2006) study. This could be examined in humans using the current experimental design by changing the timing between extinction and recovery for the shock and the scream conditions to see if the timing should be longer for the stronger stimulus (shock) compared to the weaker stimulus (scream).

In animal models of fear conditioning, freezing is the primary method of measuring fear. Freezing can be defined as the animal assuming a crouching posture while also being completely immobile for one second or more (Blanchard and Blanchard, 1969). Although this behavior has never been reported in humans, it is widely used in animal models of fear conditioning. For example, in the previously mentioned Maren and Chang (2006) study, freezing was the measure of fear in the animals. In addition to that study, many other studies employed freezing as a measure of fear (Fanselow, 1980; Ji & Maren, 2008; Hashimoto, et al., 1999). In one such study conducted by Fanselow (1980), rats received a shock in one context and then post-shock freezing was assessed in the same context or in a different one. Freezing was measured either directly after a shock had been received or 24 hours after (Fanselow, 1980). It was found that post-shock freezing was reduced when the animals were tested in a different context than the one that the shocks were originally administered in while the 24-hour delay did not reduce freezing (Fanselow, 1980). Taken together, these studies show that, not only is freezing behavior a validated measure of anxiety, but that it can be used in a wide variety of studies examining anxiety.

In the current study, we sought to examine whether humans also display freezing behavior, as this has never been examined before in the human fear conditioning literature. Based on these results, it would seem that humans do not freeze, as there were no significant differences in the freezing behavior to the CS+ compared to the CS- in any session. As was previously mentioned, the data were adjusted so that anyone who did not fit the freezing criteria would be eliminated. This was done by eliminating anyone who did not freeze one standard deviation or more above the mean. After these adjustments, there were still no significant differences even though it is clear from the raw data values that some individual participants have much higher levels of freezing compared to others (Figure 25). In the current study, the data were adjusted by only including the participants if they froze to the unconditioned stimulus more than 1 standard deviation below the mean. With this in mind, one explanation for why we did not see any results in human freezing could be that the data were not adjusted in an appropriate way. In the present study, we explored multiple different options for examining the freezing data. The data were initially analyzed in the same way that it is in the animal literature, with freezing meaning that the participants had to freeze for one second or more to be included in the data analysis. After this analysis did not yield the appropriate results, we then performed a speed analysis based on the avatar so that freezing then simply meant slowing down. However; this also did not give the results that we needed, so the current approach was then undertaken. Perhaps different criteria or analysis would yield different results.

Additionally, research has recently been published in rodent models of fear conditioning, which has shown that ‘darting’ behavior is another fear response that occurs primarily in females (Gruene, et al., 2015). This research was initially undertaken with the theory that relying on fear expression to automatically mean an absence of movement limits consideration of any other

expression of fear learning (Gruene, et al., 2015). With that idea in mind, the researchers realized that animals that they initially believed were not freezing were actually exhibiting a 'darting' behavior, which simply means that, instead of not moving, they were making quick movements around their environment (Gruene, et al., 2015). As is the case with freezing, it was found that this behavior appeared during conditioning and then disappeared during extinction (Gruene, et al., 2015). With this idea in mind, perhaps humans exhibit a 'darting' response more often than a freezing response, which would mean the data would need to be examined in a new way in future studies as this would indicate that freezing does not capture all motor-related fear behaviors in humans. Additionally, it would appear as though habituation takes place during freezing, which can be seen by examining the graphs. It seems as though the participants initially freeze, as the freezing response to the CS+ is lower than the freezing response to the CS- in the first trial of the experiment. However, this response quickly diminishes, which resembles what one would expect to see during habituation. This is an idea that could be examined more in the future in hopes of seeing a stronger freezing response, as addressing why the participants habituate so quickly would aid in explaining the freezing results further. Perhaps using a stronger stimulus or shorter trials would lengthen the amount of time prior to participants habituating; this idea can be examined in future studies.

Whereas in the animal models, most rodents freeze, perhaps this is a behavioral characteristic that only a small amount of humans show. It is possible that, in humans, there is a factor that determines whether humans are 'freezers' or 'non-freezers', such as if they have higher trait anxiety. If this was the case, it would mean that we should not anticipate seeing freezing in the majority of the participants. Another point to consider is that this behavior has never been looked at in humans before; as such, there is a chance that the way it was quantified

in the current study or that the task itself is not the most effective means of examining this behavior. In rodent literature, some experimenters have scored freezing behavior by hand, meaning that a trained experimenter watches video of a session and tallies the amount of freezing that took place. Perhaps video-taping participants is a more effective way of quantifying freezing in humans, as it would eliminate any artificial recordings taken from the computer. Future experiments should explore this as a potential option for examining this phenomenon in greater detail in humans. In addition, one limitation of our study was that we told participants to keep moving in an effort to counteract some of their natural inclination to not move throughout the experiment. Telling participants to move when one measure of fear that we examined was freezing may have greatly impacted the results that we have. In the future, task instructions will be altered so that participants are encouraged to move as naturally as possible, even if that includes stopping. Even though some of our findings deviate from what was originally anticipated in that extinction was not context-specific and all humans do not show freezing behavior, we are confident that the results indicate that humans do display conditioned fear in a virtual environment paired with an aversive event and that the relative strength of specific stimuli has been defined in an effort to establish different levels of 'trauma' for future research.

It is important to note that the current study excluded the majority of participants for each of the experiments that were performed. Although other studies that use a human fear conditioning paradigm and measure fear with physiological measures also exclude many of their participants (Inslicht, et al., 2013; Levar, et al., 2017), the parameters of the current study caused us to exclude more than is normally seen in other studies. This could have impacted our data analyses in terms of significance and also could have eliminated participants who responded well to the unconditioned and/or conditioned stimuli but did not meet a specific criteria. Future work

may employ different techniques for exclusions, such as excluding only based on responding or technical difficulties instead of on a confidence rating. However, the current results still agree with literature on the topic as was detailed previously so it is clear that the exclusions did aid in a positive way, at least upon initial examination.

Future Directions

The present study has provided novel and informative data in understanding the human physiological response to fear in a virtual reality task, helping to further validate virtual reality as a way to assess these responses and as a way to study anxiety and trauma. This was found through examining the physiological responses to two different aversive stimuli that were presented during a virtual reality fear conditioning paradigm. Additionally, these results provide a basis for future studies to examine these responses more thoroughly so that anxiety and trauma clinical treatment can be improved in the future, in that the results from comparing the shock and scream responses may be beneficial to studying exposure therapy in more detail.

In Maren and Chang's (2006) study, they were interested in whether an early intervention delivered minutes after fear conditioning had taken place would produce superior extinction relative to a standard delayed intervention of 24 hours. They found that the animals in each group showed differences in terms of retention of the extinction memory, where only rats that had the long break show a reduction in freezing behavior, suggesting that extinction timing could be a factor in trauma treatment in humans. In the current study, we were able to establish a model to study this issue in humans, which will be used in the future to experiment with delayed extinction in an effort to examine whether the results discovered by Maren and Chang (2006) will translate to humans. Maren and Chang (2006) also showed that when an animal is in a

fearful state, immediate extinction training is worse than delayed extinction training, which was seen with spontaneous recovery occurring only after an early intervention while it remained inhibited in rats with a long break between conditioning and extinction. This showed that the intensity of a stimulus could be another factor in the treatment of trauma in humans. Although it has already been seen that there are differences in the strength of two USs, it is of interest to examine how the strength of each stimulus impacts the timing of extinction. The paradigm from the current study could be easily adapted to test the timing of extinction by simply changing the time of the break between the extinction and reinstatement sessions. This would be of particular interest because it would significantly help clinicians to provide appropriate care to those who have experienced a trauma.

Having anticipated that we would see that humans do exhibit freezing behavior, it was surprising to see no statistically significant differences between the CS+ and the CS-; however, it is intriguing that certain individuals show this classic rodent behavior. This may indicate that it is a behavior that is uncommon among humans, but a select few show it. It would be of interest to study those who do exhibit this behavior to examine any potential differences that they may have that would categorize them as a 'freezer.' It is also of importance to further examine the task and the quantification methods for this behavior, as one or both should be adjusted in the future to better study this behavior. For instance, it may be possible that it is difficult to examine freezing in a virtual reality paradigm because of the added component of an avatar being controlled by the participant. Experimentally, videotaping participants or examining another motor behavior during each session could fix this. In addition, it could also be possible that the methods used to analyze the freezing data need to be adjusted. As this has never been done before in humans,

quantifying freezing in humans would be an interesting phenomenon to study in the future, specifically in terms of determining how best to examining this behavior.

As previously mentioned, fear conditioning is used as a model of anxiety disorders. As such, future work might focus on examining the differences in terms of physiological responding and behavior between non-anxious and anxious individuals. In addition, individuals who have undergone a trauma may have different behavioral and physiological responses in response to different extinction timings. We believe that those who are anxious or who have undergone a trauma would have higher baseline SCR levels and would have a stronger response to an aversive event compared to those that are not anxious or have not undergone a trauma. It would also be of interest to examine the freezing behavior in anxious and non-anxious individuals. It has been seen in the animal literature that rats with a genetic predisposition for anxiety freeze more (Salchner, et al., 2006), so perhaps the same would be true in humans who are anxious, which could lead to a differentiating factor in the freezing responses we saw in the current study. Anxiety-ridden individuals and those who have experienced a trauma are the populations that will benefit from this research; therefore, it is of interest to know how they respond in this paradigm so that treatment methods can be adapted.

Both the amygdala and the hippocampus play substantial roles in fear conditioning. It was first known that the amygdala was involved in modulating fear; however, it was learned later that the amygdala was involved in fear learning processes. Specifically, it was seen that, in addition to other roles, the amygdala is vital in order to make fear-associated memories (Blanchard & Blanchard, 1972). The hippocampus also plays a crucial role in fear learning, as it sends contextual representations to the amygdala to be connected to the unconditioned stimulus, thus allowing fear learning to take place (Maren, 2001). As was previously mentioned, much

work has already been done in establishing the importance of the amygdala and the hippocampus as key components in the neurocircuitry behind fear conditioning; however, there is minimal information on the neurocircuitry underlying freezing behavior, which could be of interest to study in the future in order to better understand the behavior in general. Future work may begin to use brain-imaging techniques with a similar paradigm in order to add to the already existing literature on these two structures in an effort to better understand their involvement with fear conditioning generally and also with different stimuli and behaviors.

Conclusions

In summary, the current experiment has provided a concrete foundation for future work in that it characterized the physiological characteristics of human fear in response to two different stimuli. Taken together, the results show that (1) humans do display conditioned fear physiologically in a virtual reality paradigm, (2) extinction is not context-dependent, and (3) a shock is a stronger aversive stimulus compared to a scream. These results also validate using a virtual reality paradigm for fear conditioning studies with the hope of better understanding human anxiety and trauma. Most importantly, the current findings, in combination with the research ideas that have been proposed, will provide improved treatment methods using exposure therapy for anxiety disorders such as PTSD and will allow for insight into the mechanisms underlying these disorders so that they can be better understood by psychologists.

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Figures

	CS+	Reinstatement Room
Condition A	**Green**	**Same**
Condition B	**Green**	**Different**
Condition C	**Red**	**Same**
Condition D	**Red**	**Different**

Figure 1A: Diagram depicting the four different conditions with the CS+ and reinstatement room for each, showing how the CS+ and reinstatement rooms are counterbalanced across conditions.

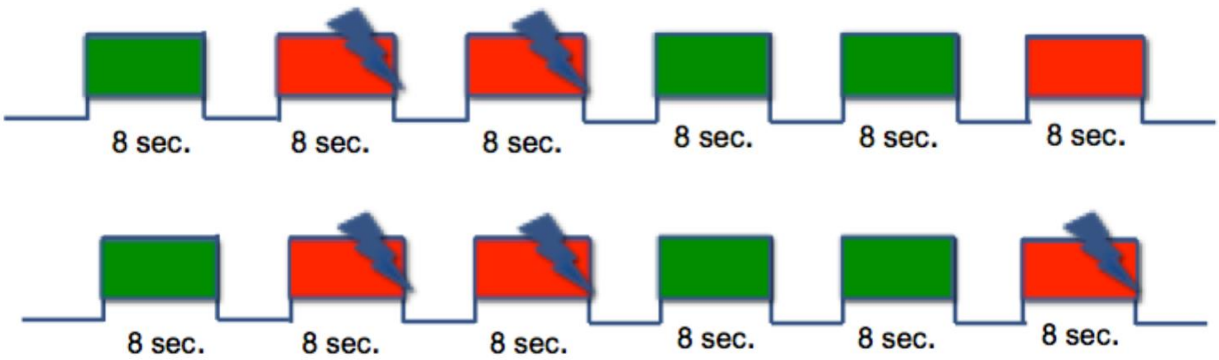


Figure 1B: A sample acquisition session for one participant. Each CS is presented 6 times per session with an inter-trial interval of 20 seconds +/- 4s; however, the US is only presented with the CS+ 5 of the 6 times each session.

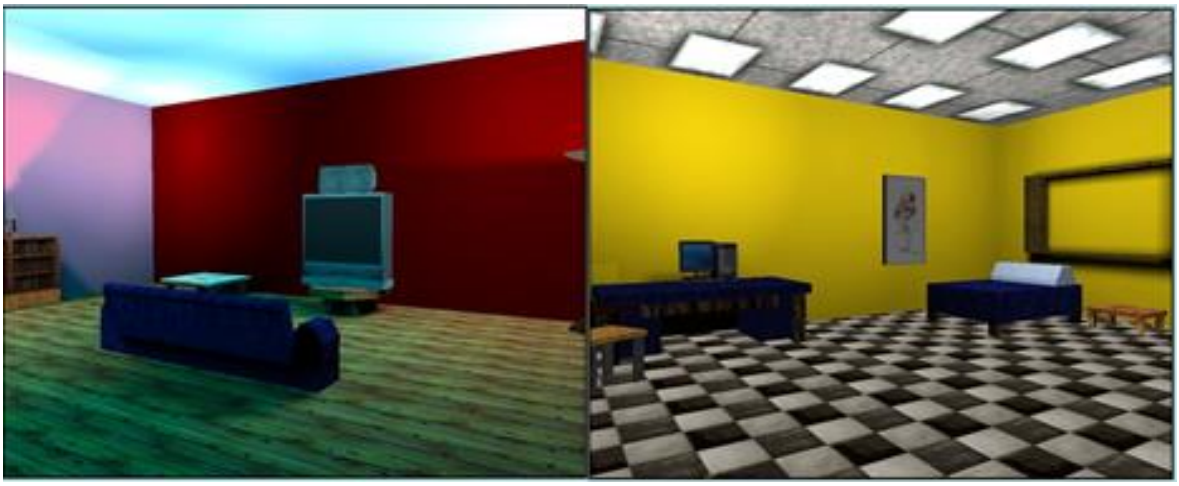


Figure 2: Both rooms were identical in shape and size, but contained different items, colors, and patterns.

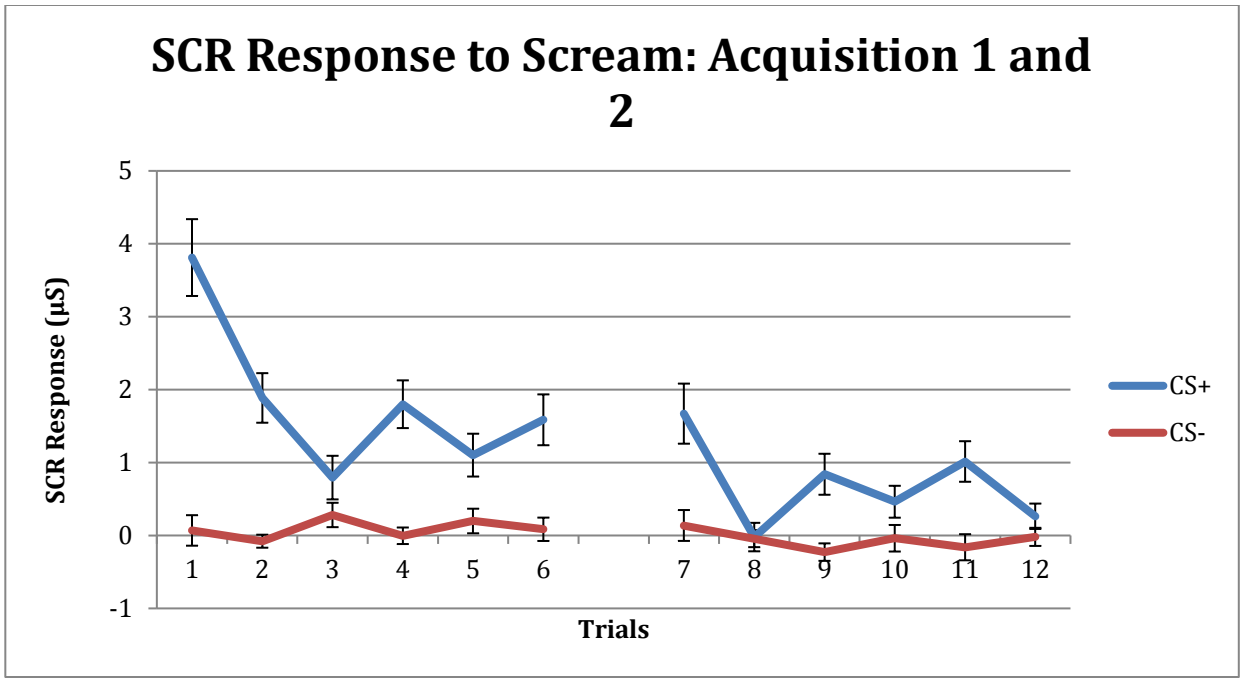


Figure 3: The SCR response to the scream in Acquisition 1 is significantly greater than the response after the CS- presentation ($F(1, 35) = 49.27$; $p < 0.01$). The SCR response to the scream in Acquisition 2 is also significantly greater than the response after the CS- presentation ($F(1, 34) = 16.38$; $p < 0.01$).

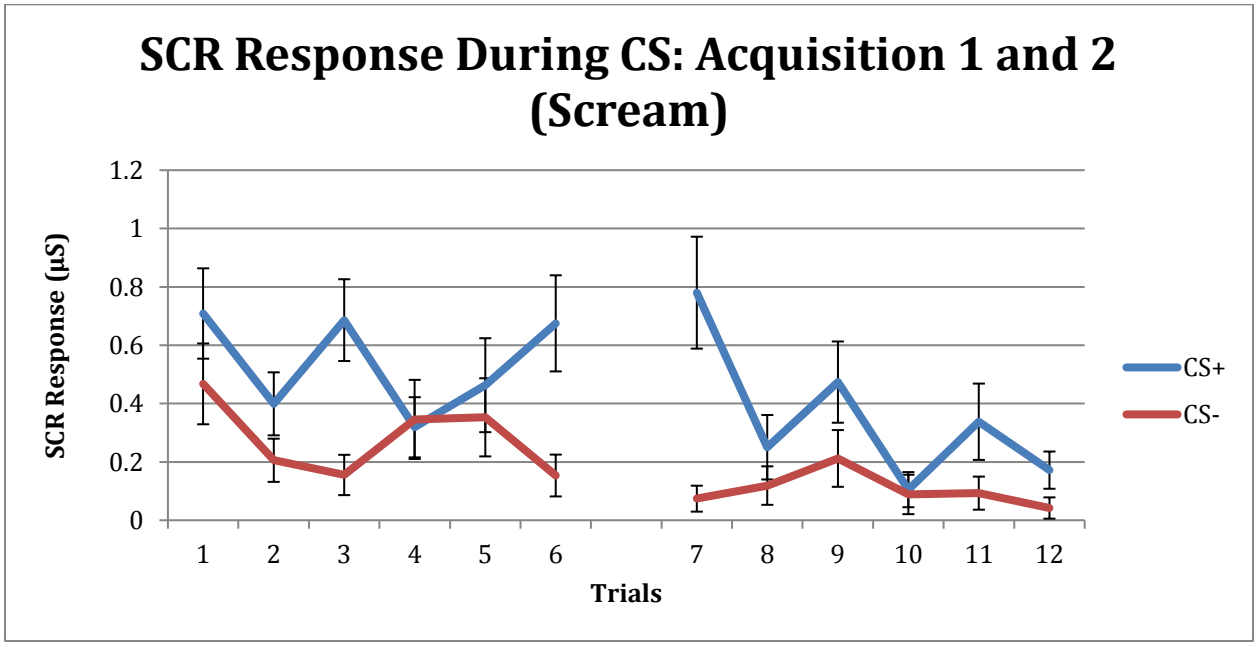


Figure 4: The SCR response in Acquisition 1 to the CS+ presentation was significantly greater than the response to the CS- presentation ($F(1, 31) = 8.07$; $p = 0.01$). The SCR response to the CS+ presentation was significantly greater than the response to the CS- presentation in Acquisition 2 ($F(1, 33) = 11.67$; $p = 0.002$).

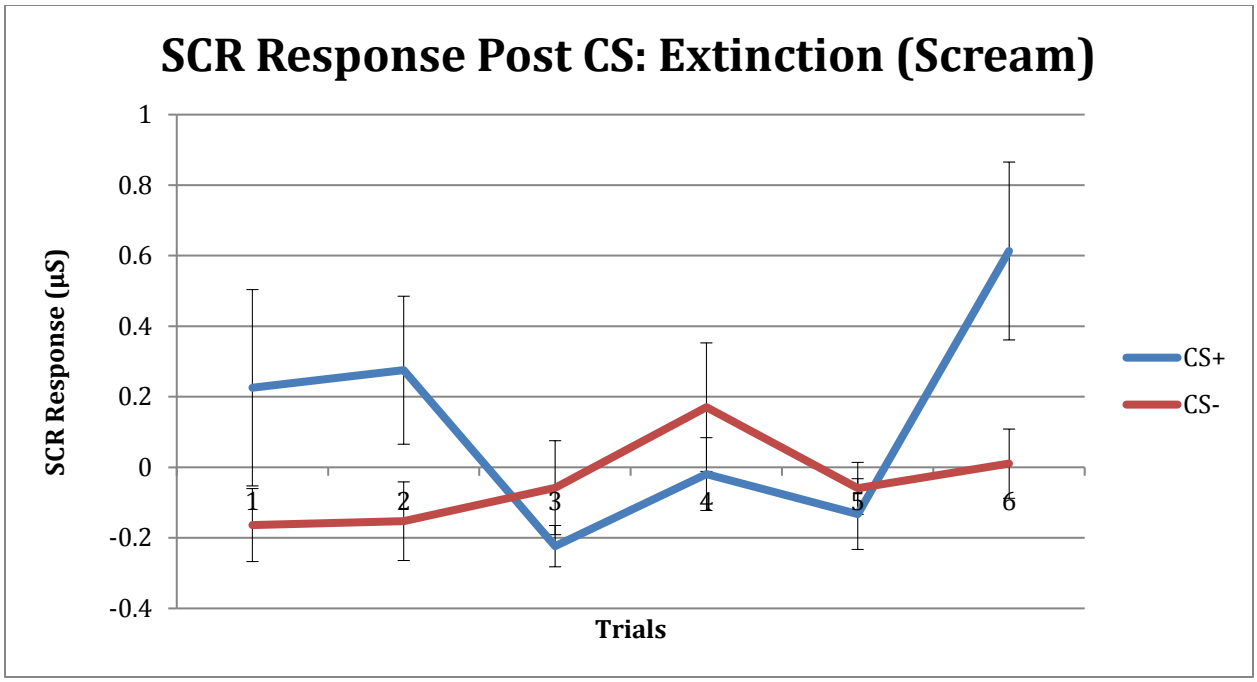


Figure 5: A significant interaction was seen between the effect of trial and CS+/CS- on SCR response (F(5, 480) = 2.39; p = 0.04) with no significant effect seen when trial and CS+/CS- were examined alone.

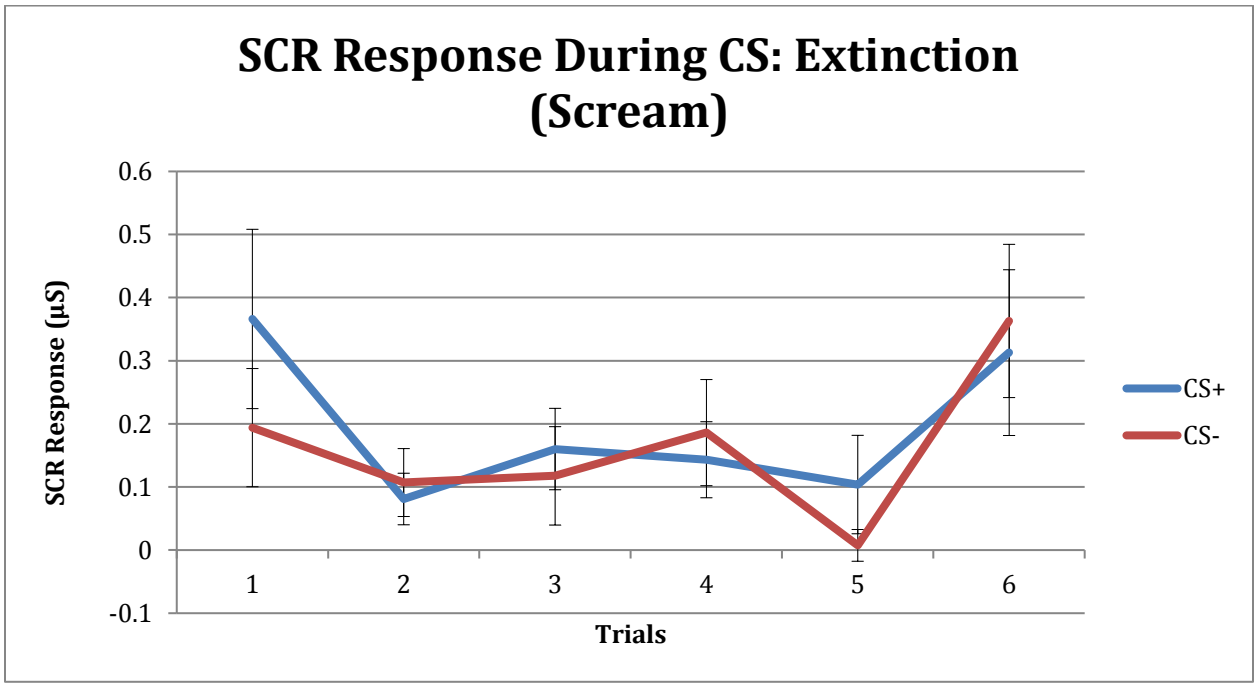


Figure 6: No significant interaction was seen between the effect of trial and CS+/CS- on SCR response (F(5, 480) = 0.553; p = 0.74).

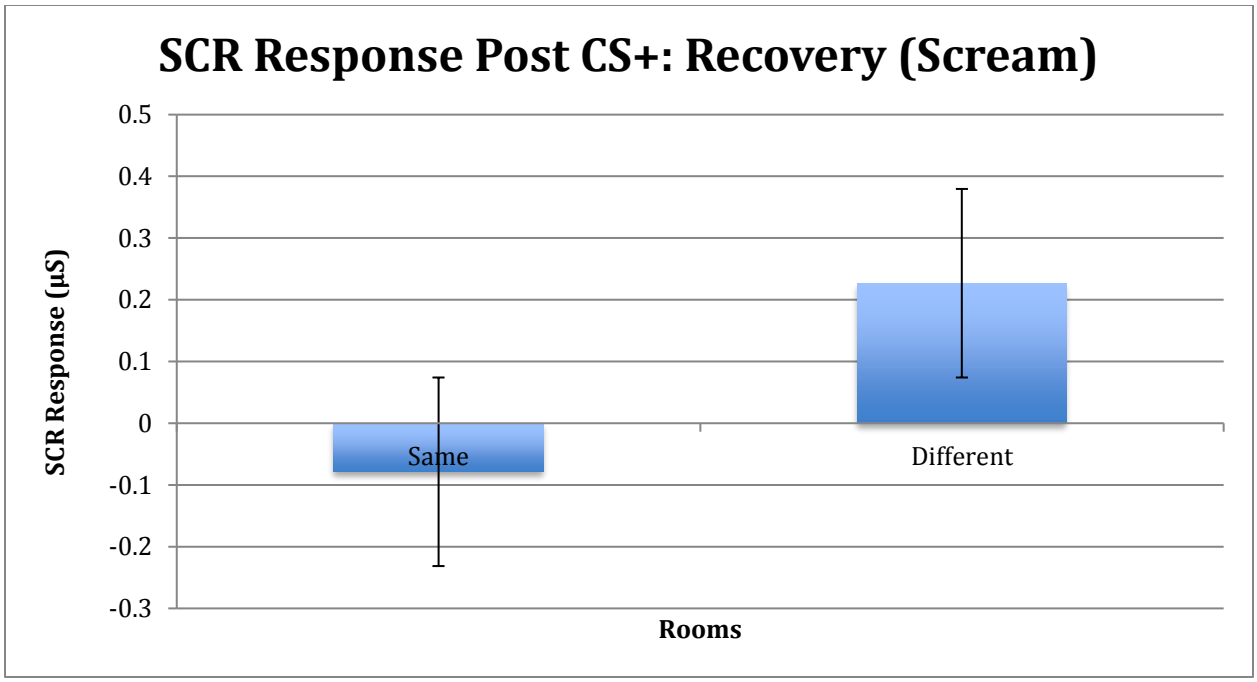


Figure 7: No differences were observed when the acquisition and recovery rooms were the same compared to when they were different after the CS+ and CS- presentations (t(38) = -0.76; p = 0.45).

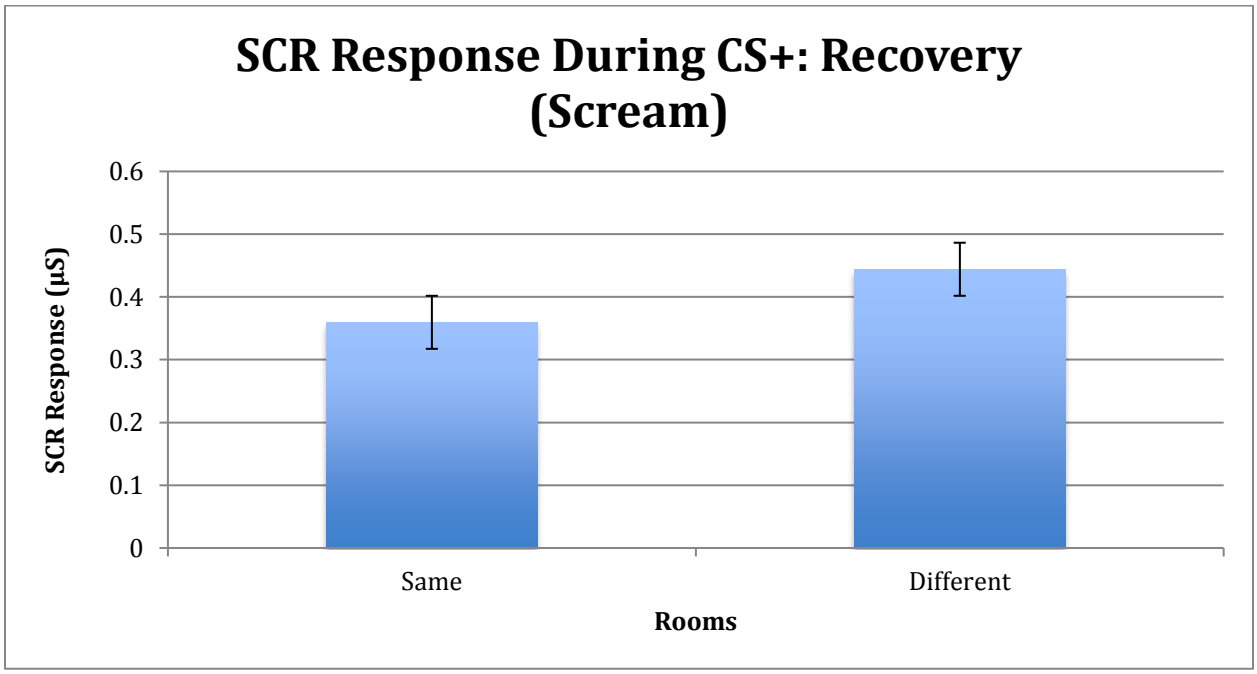


Figure 8: No differences were observed when the acquisition and recovery rooms were the same compared to when they were different during the CS+ and CS- presentations (t(33) = -0.33; p = 0.75).

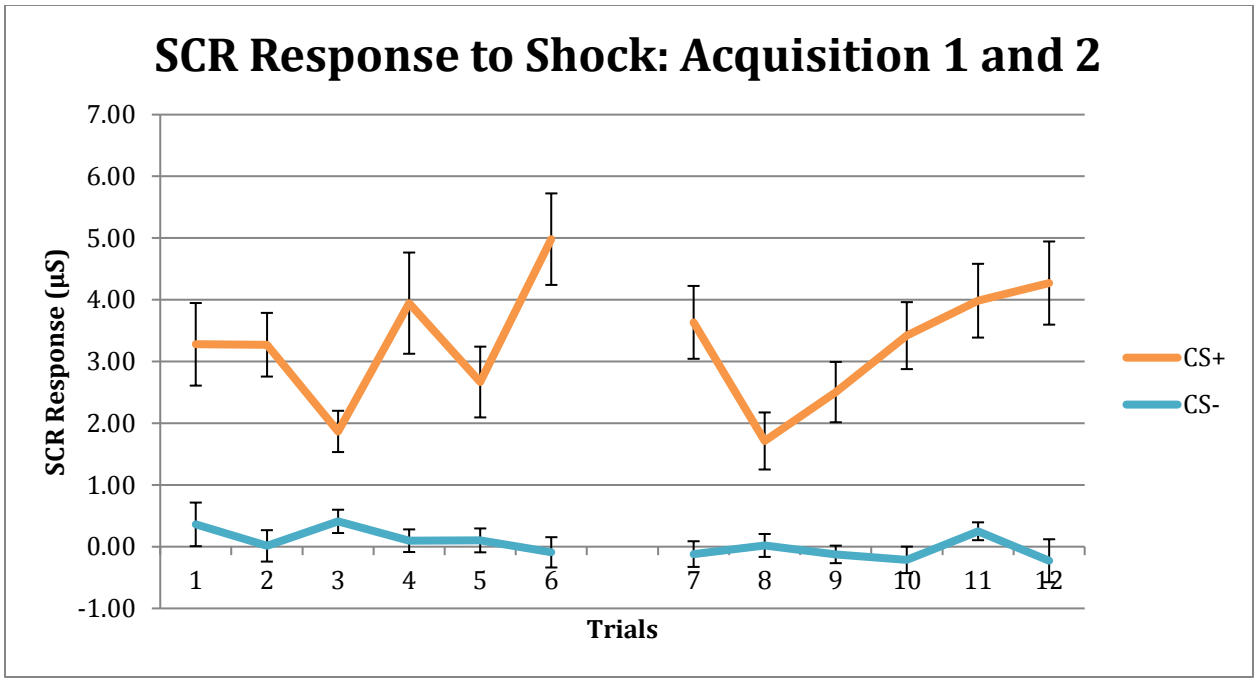


Figure 9: The SCR response to the shock in Acquisition 1 is significantly greater than the response after the CS- presentation ($F(1, 31) = 44.65$; $p < 0.01$). The SCR response to the shock in Acquisition 2 is significantly greater than the response after the CS- presentation ($F(1, 31) = 44.36$; $p < 0.01$).

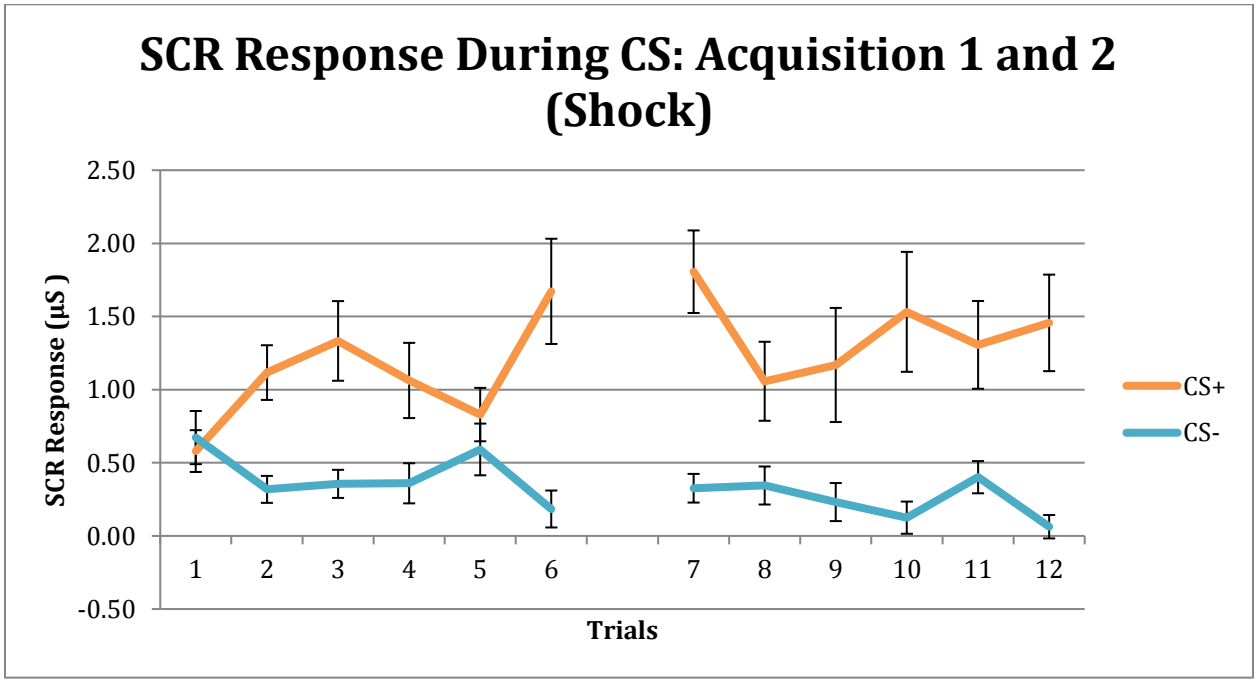


Figure 10: The SCR response to the CS+ presentation in Acquisition 1 was significantly greater than the response to the CS- presentation ($F(1, 30) = 19.42$; $p < 0.01$). The SCR response to the CS+ presentation in Acquisition 2 was significantly greater than the response to the CS- presentation ($F(1, 30) = 25.01$; $p < 0.01$).

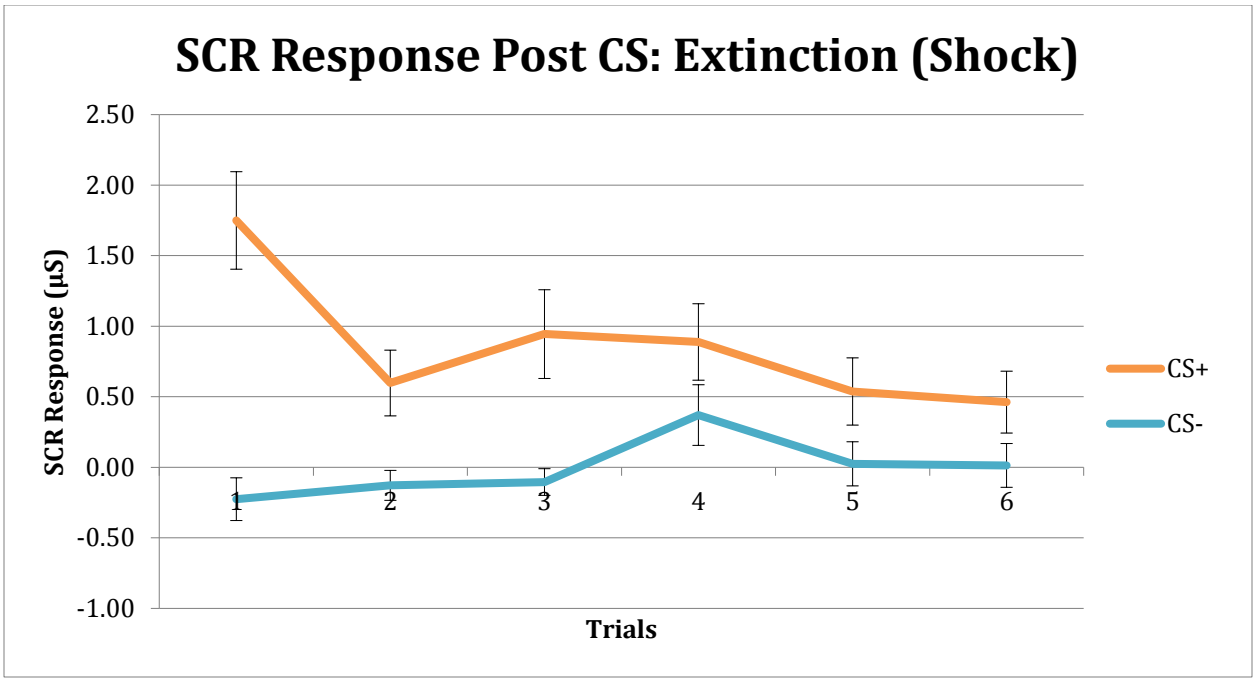


Figure 11: A significant interaction was seen between the effect of trial and CS+/CS- on SCR response ($F(5, 420) = 2.99$; $p = 0.01$) with a significant effect seen when only CS+/CS- was examined alone ($F(1, 420) = 51.58$; $p < 0.001$).

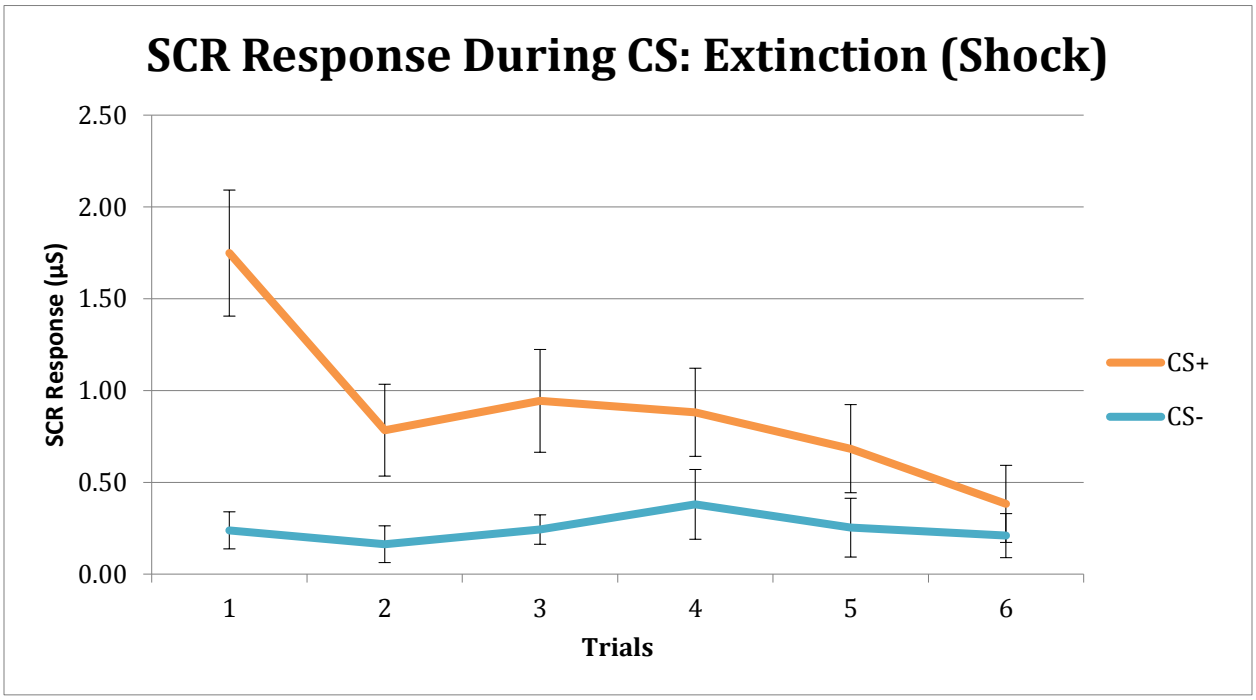


Figure 12: A significant interaction was seen between the effect of trial and CS+/CS- on SCR response ($F(5, 420) = 3.57$; $p = 0.004$) with a significant effect seen when trial was examined alone ($F(5, 420) = 3.76$; $p = 0.002$) and when CS+/CS- were examined alone ($F(1, 420) = 42.43$; $p < 0.001$). Post hoc analyses revealed a significant difference between trials 1 and 6 ($p = 0.001$).

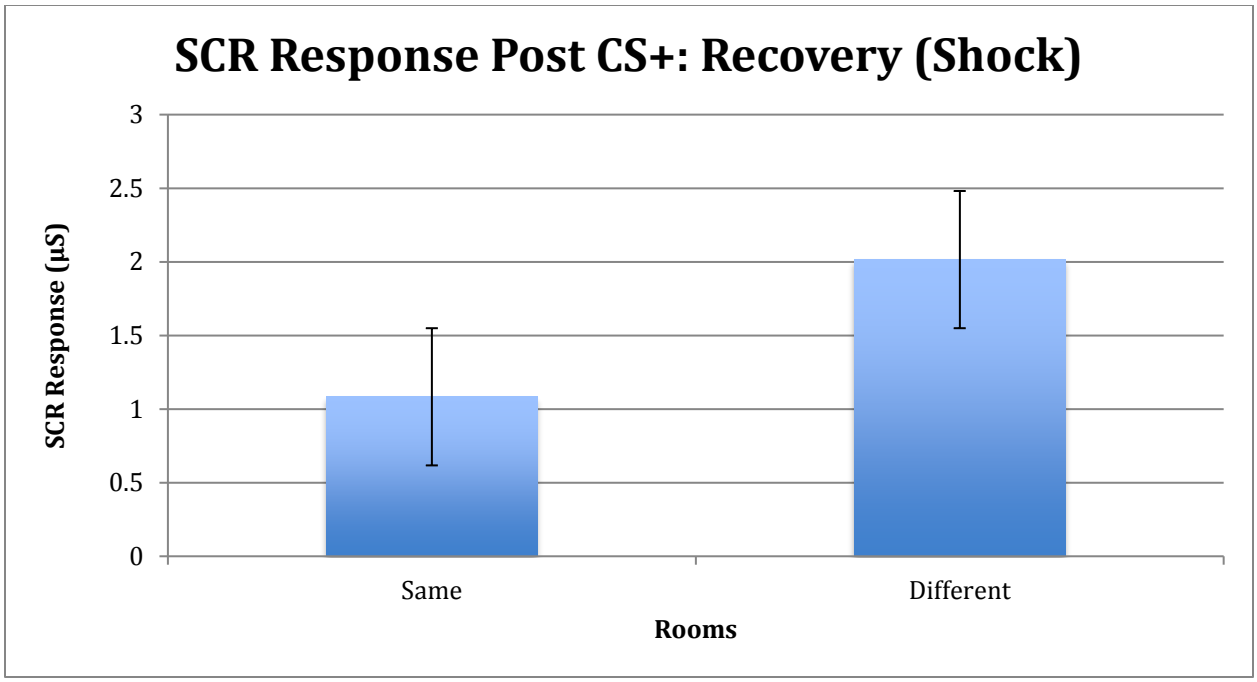


Figure 13: No differences were observed when the acquisition and reinstatement rooms were the same compared to when they were different after the CS+ and CS- presentations (t(34) = -1.45; p = 0.16).

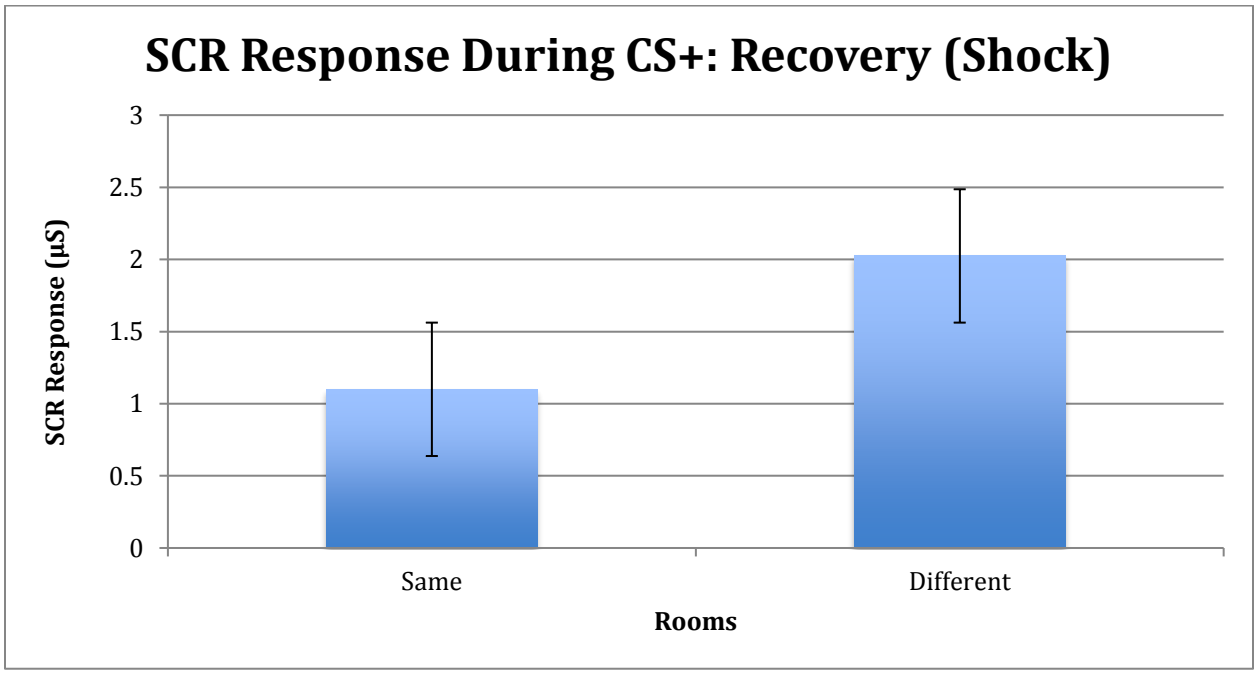


Figure 14: No differences were observed when the acquisition and reinstatement rooms were the same compared to when they were different during the CS+ and CS- presentations (t(34) = -1.46; p = 0.16).

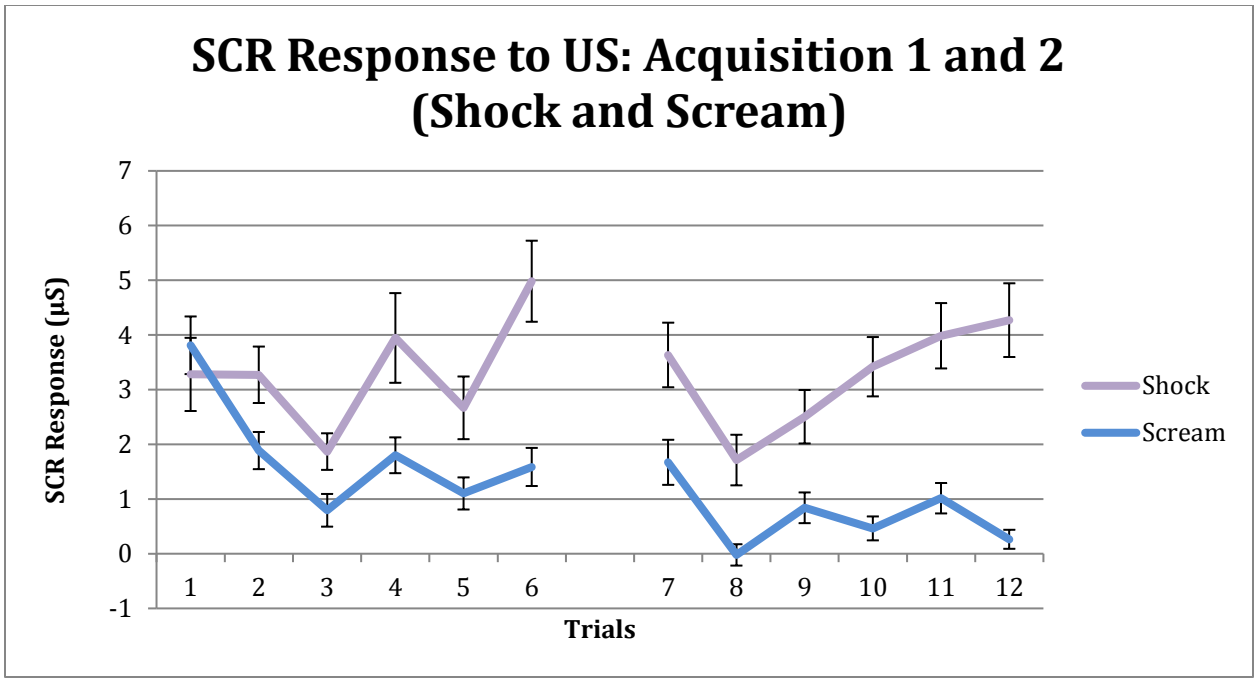


Figure 15: The SCR response to the shock is significantly greater than the response to the scream in Acquisition 1 ($F(1, 31) = 4.77$; $p = 0.04$) and in Acquisition 2 ($F(1, 28) = 19.4$; $p < 0.01$).

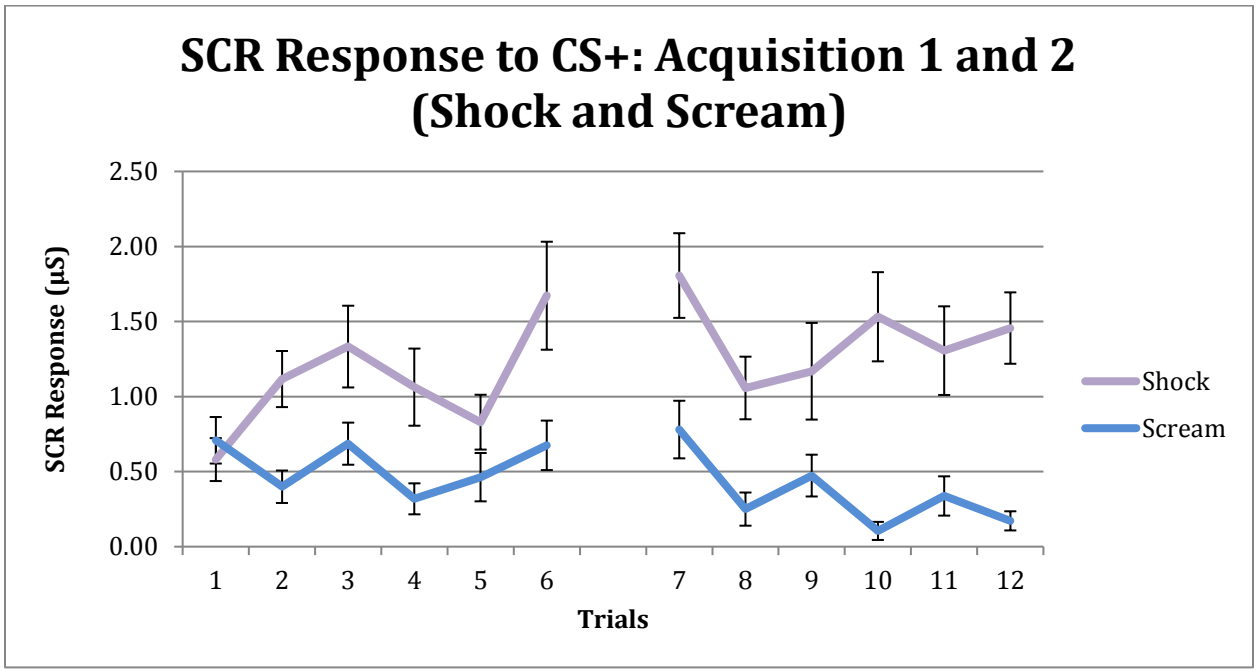


Figure 16: The SCR response to the CS+ presentation shown prior to a shock was significantly larger than the response to the CS+ presentation before the scream in Acquisition 1 ($F(1, 29) = 6.41$; $p = 0.02$) and in Acquisition 2 ($F(1, 28) = 18.61$; $p < 0.01$).

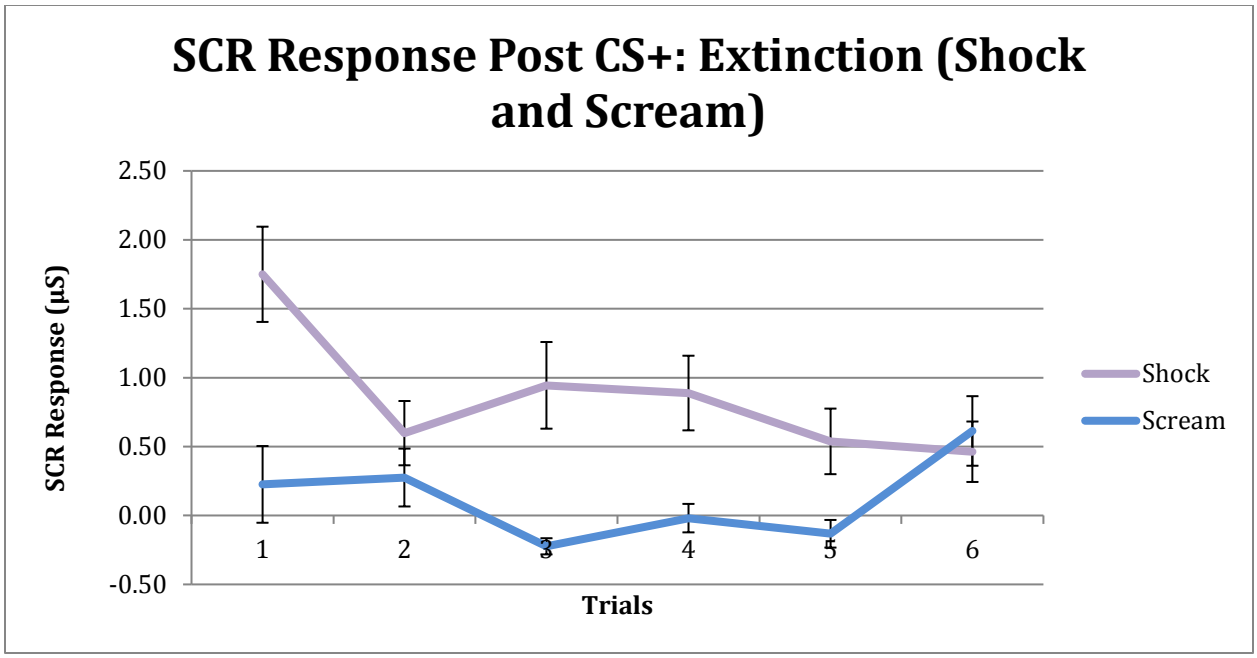


Figure 17: A significant interaction was seen between the effect of trial and CS+/CS- on SCR response ($F(5, 450) = 2.91$; $p = 0.01$) with no significant effect seen when trial and CS+/CS- were examined alone.

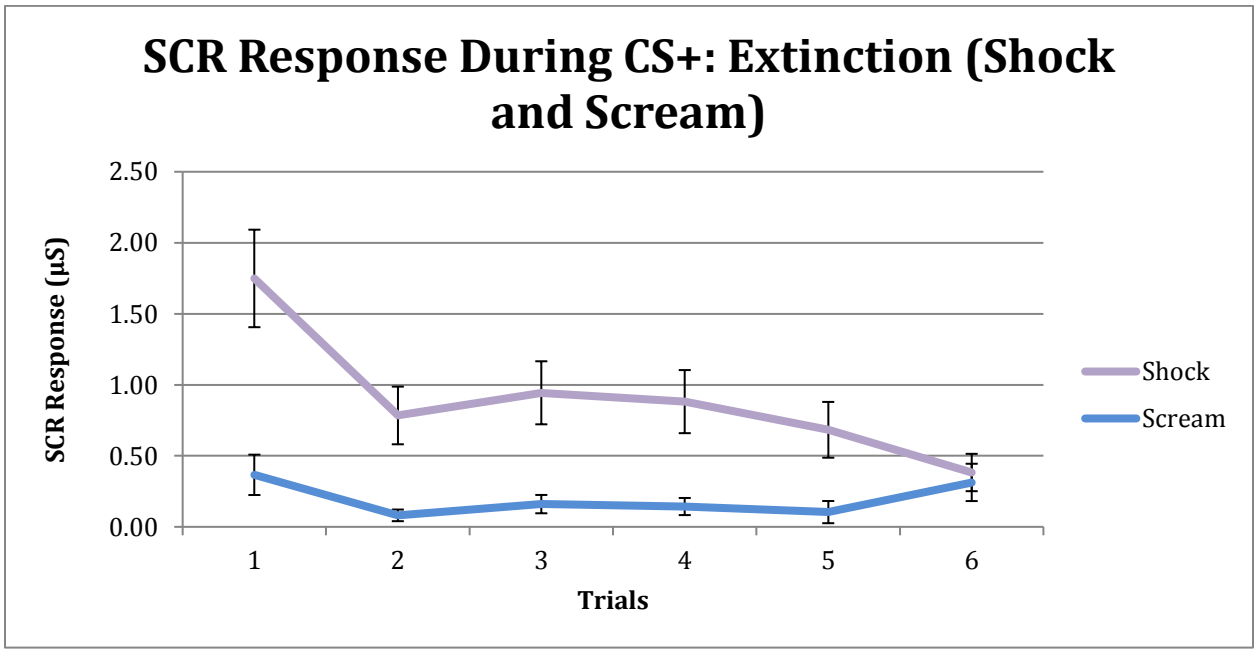


Figure 18: A significant interaction was seen between the effect of trial and CS+/CS- on SCR response ($F(5, 450) = 3.29$; $p = 0.01$) with significant effects being seen when trial was examined alone ($F(5, 450) = 5.09$; $p < 0.01$) and when CS+/CS- was examined alone ($F(1, 450) = 54.61$; $p < 0.01$). Post hoc analyses revealed a significant difference between trials 1 and 6 ($p = 0.001$).

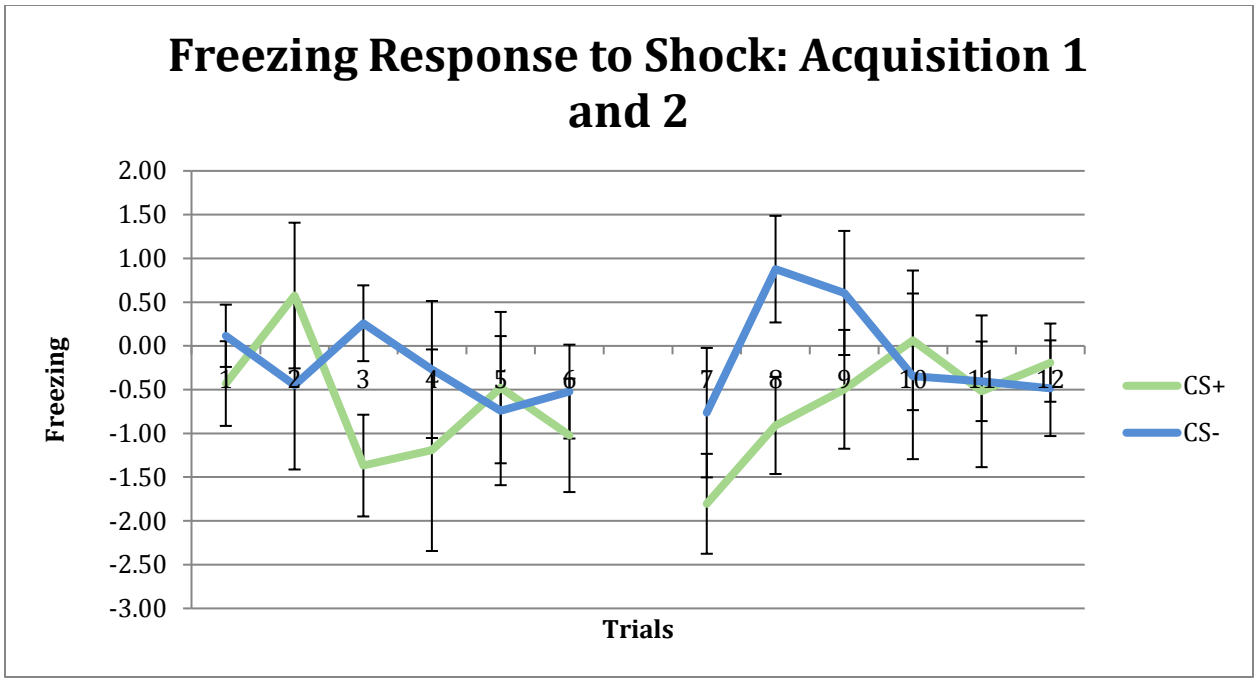


Figure 19: There is no significant difference between the freezing response to the shock and the response after the CS- presentation in Acquisition 1 ($F(1, 15) = 2.77$; $p = 0.12$) or in Acquisition 2 ($F(1, 15) = 0.07$; $p = 0.79$).

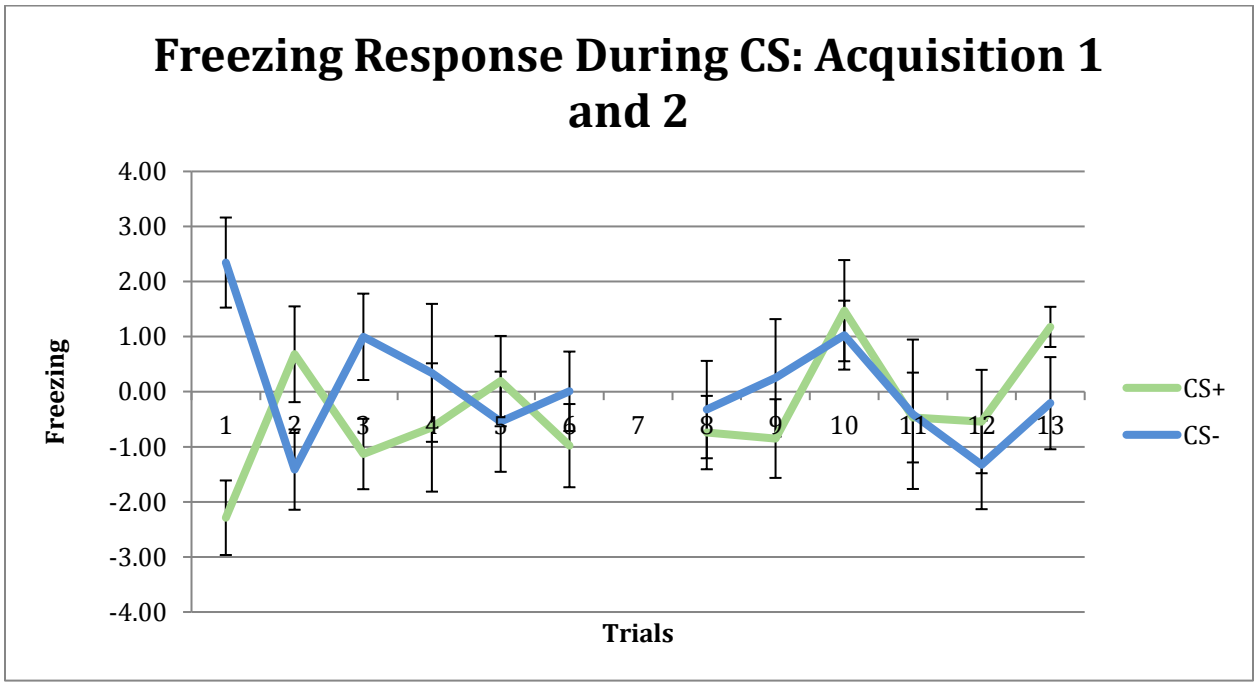


Figure 20: There is no significant difference between the freezing response during the CS+ presentation and the response to the CS- presentation in Acquisition 1 ($F(1, 15) = 1.15$; $p = 0.3$) or in Acquisition 2 ($F(1, 15) = 1.13$; $p = 0.3$).

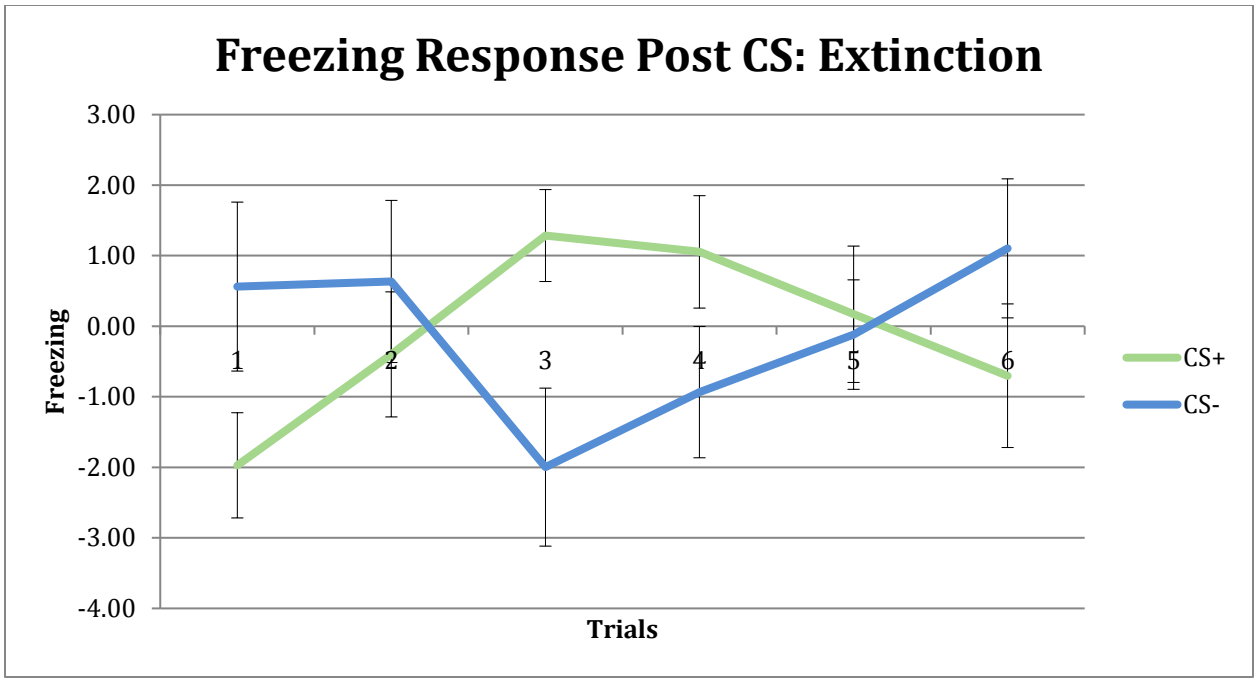


Figure 21: A significant interaction was seen between the effect of trial and CS+/CS- on SCR response (F(5, 180) = 2.84; p = 0.02) with no significant effect seen when trial and CS+/CS- were examined alone.

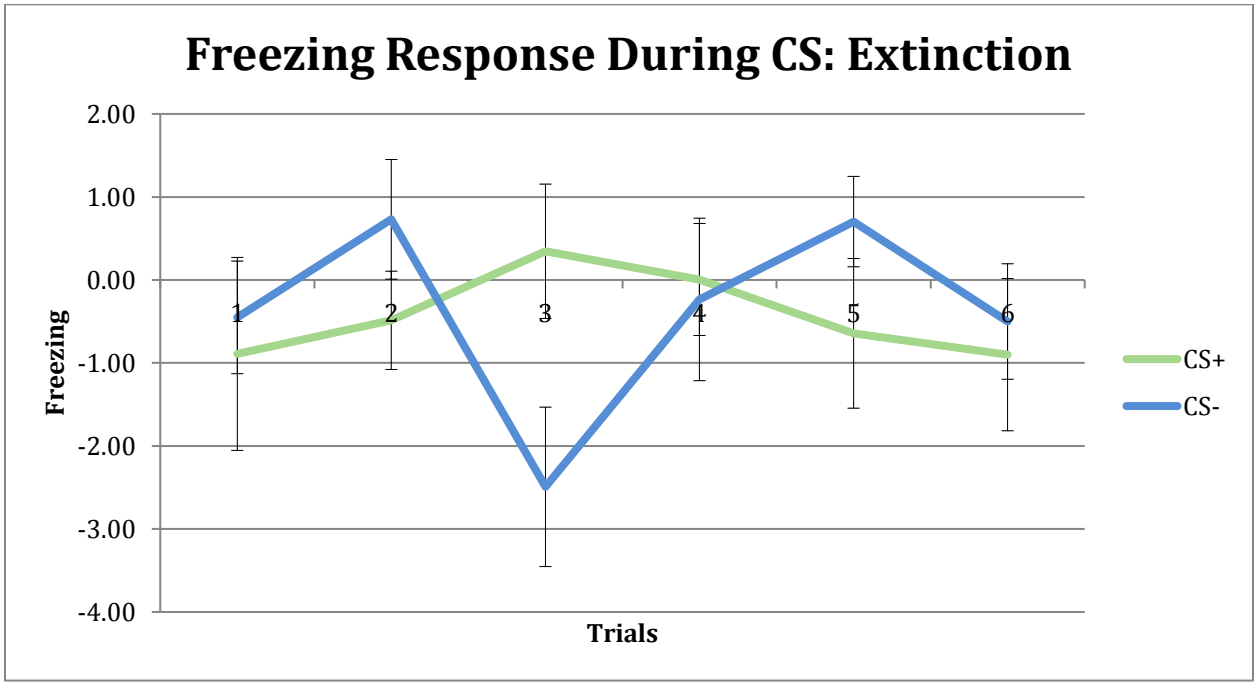


Figure 22: No significant interaction was seen between the effect of trial and CS+/CS- on SCR response (F(1, 180) = 1.74; p = 0.13).

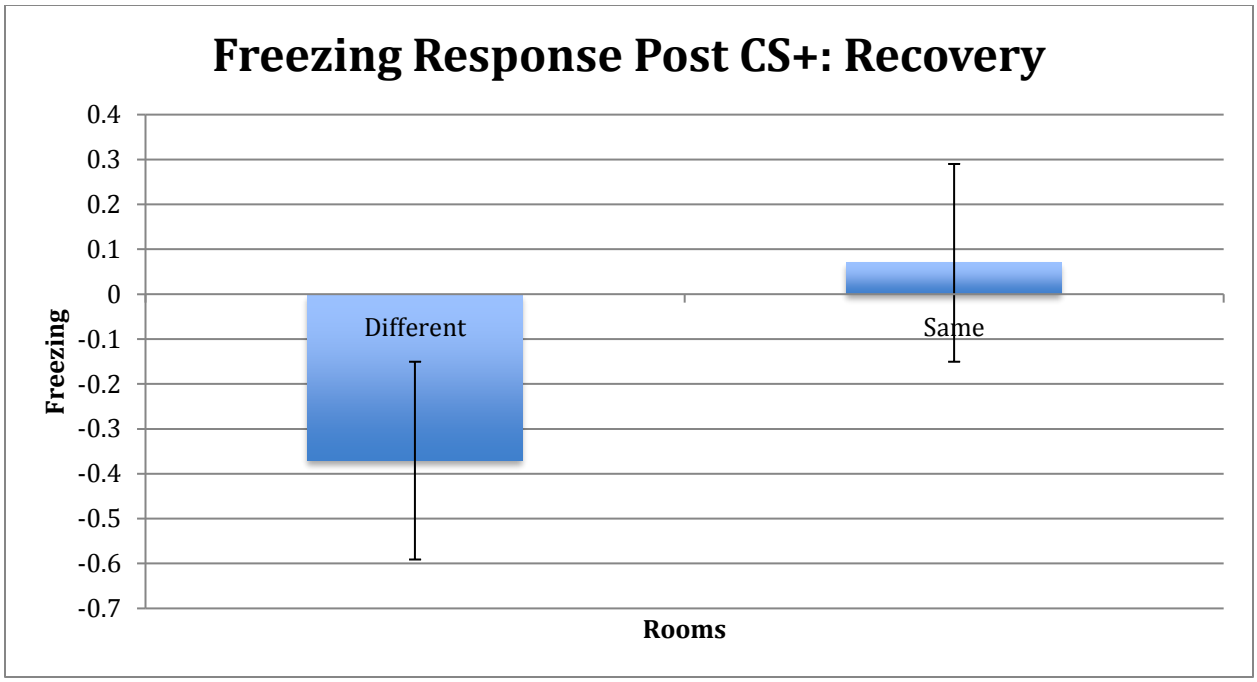


Figure 23: No differences were observed when the acquisition and reinstatement rooms were the same compared to when they were different after the CS+ and CS- presentations (t(14) = .33; p = 0.75).

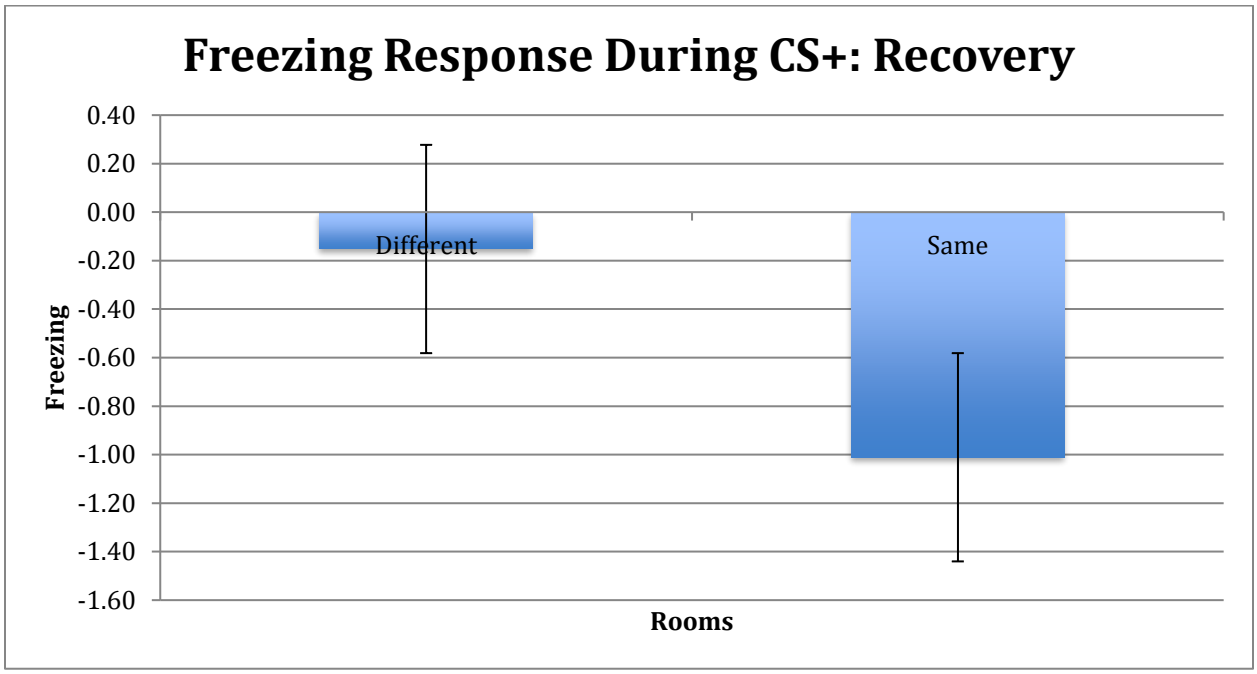
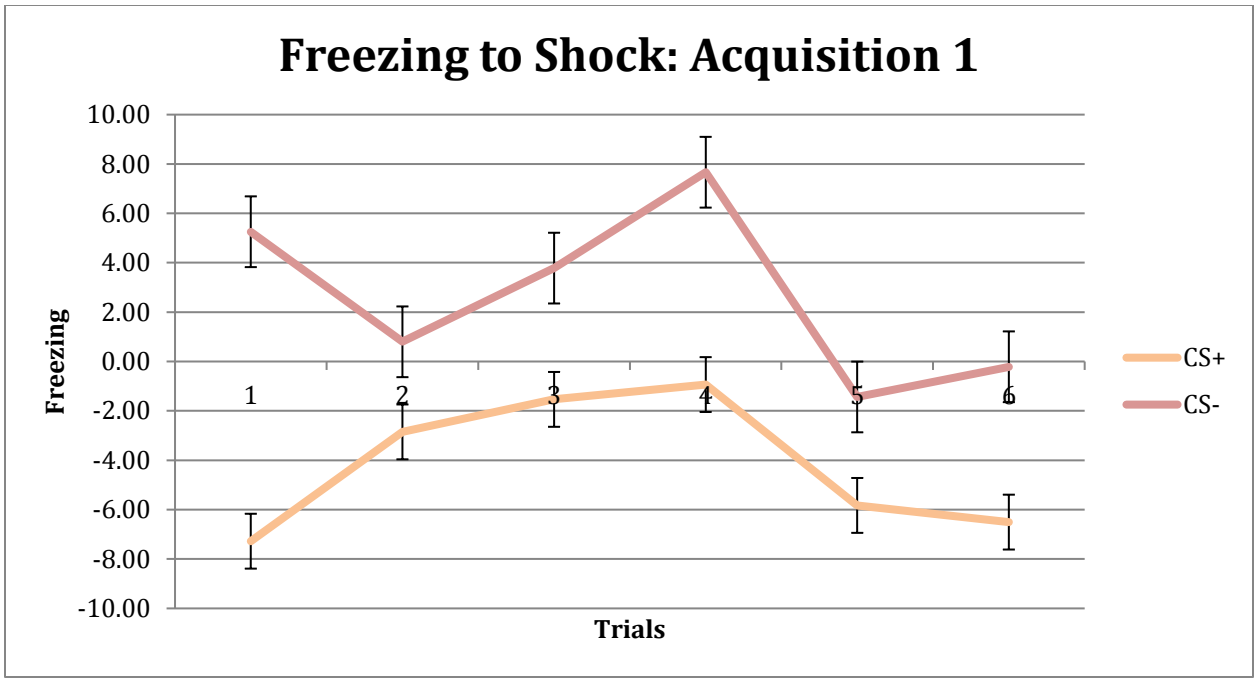


Figure 24: No differences were observed when the acquisition and reinstatement rooms were the same compared to when they were different during the CS+ and CS- presentations (t(14) = -0.76; p = 0.46).



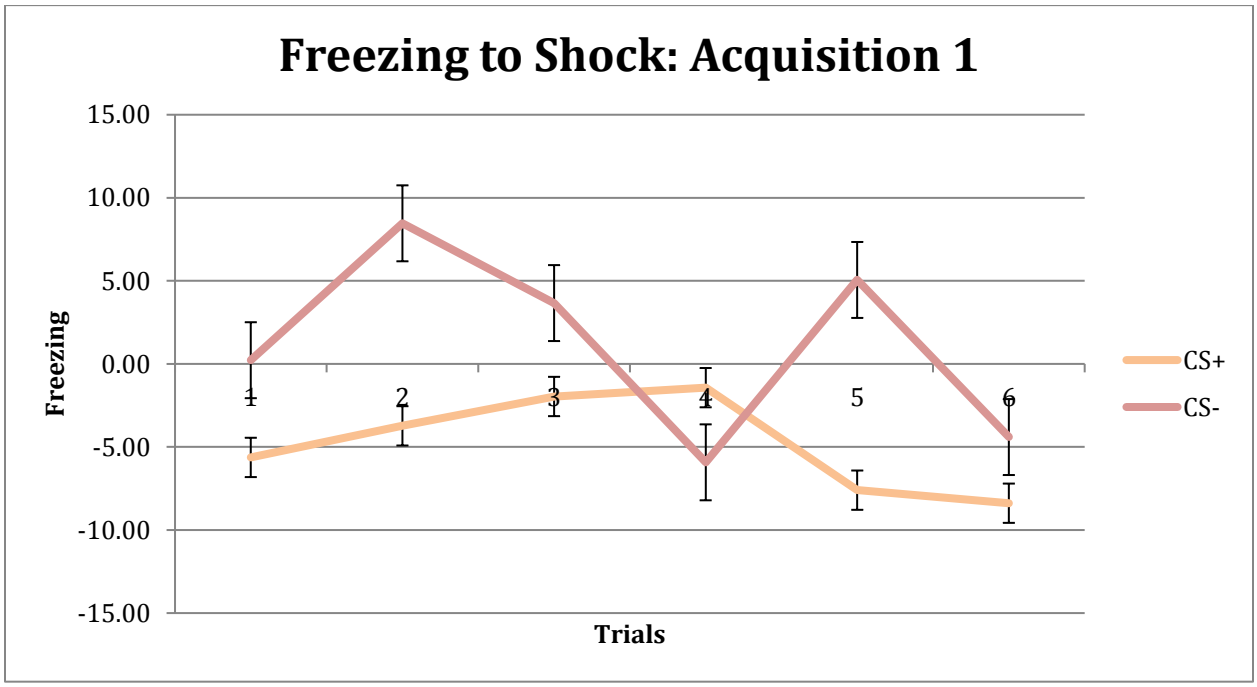


Figure 25: Freezing results for Acquisition 1 for two individual participants. Both participants show a reduction in movement after a shock has occurred (CS+) compared to when the CS- is presented.