Individual Differences in Fear Conditioning: Influence of Compound Presentation of Stimuli and Reinforcement Rate

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Abstract

Trait anxiety has been strongly linked to fear learning and we sought to examine how it can modulate the differences in how people learn fear towards compound stimuli and partially reinforced stimuli. Highly anxious people have been shown to generalize fear stronger than their non-anxious counterparts and to exhibit higher levels of fear towards ambiguous stimuli. A recent study examined the effect of anxiety levels in associating fear towards partially conditioned stimuli by comparing control mice with mice missing a gene responsible for anxiety suppression. After exposing both groups of mice to a light and tone compound that was paired with an aversive stimulus and just the tone itself unreinforced, the researchers noted increased fear levels in the highly anxious mice when exposed to just a tone. This is attributed to anxiety playing a role in causing elevated fear towards partially predictive stimuli. However, studies on the transfer of fear in compound stimuli indicate that they may be processed differently than single-part stimuli and that anxiety may play a role in modulating this. We believe that fear may transfer from the compound stimulus to its individual parts for highly anxious individuals and that this could also explain the results seen in the experiment described above. To our knowledge, no one has yet tested this possibility. In

order to decipher this, we designed five different fear conditioning paradigms. By measuring pupillary responses from participants before and after each type of conditioning in order to measure fear, we were able to measure the effects of conditioning partial predictors of fear and compound stimuli. Additionally, we were able to replicate the described experiment as one of our conditions. Our results from all five conditions revealed no anxiety effect, but we observed prolonged fear retention towards compound stimuli and a configural association of compounds.

1. Introduction

40 million American adults are currently diagnosed to be suffering from some type of anxiety disorder (Kessler et al., 2005). Research over the last few decades has unearthed links between symptoms of anxiety and disruptions in fear expression and inhibition in the context of fear conditioning, creating an interesting framework to better understand underlying mechanisms and develop more effective clinical treatments.

In the next section, I give an overview of fear conditioning, its connection to anxiety, and the research motivating our work. I then describe our experimental setup and the specific measures we sought to observe in our study. Lastly, I give a summary of our results and discuss the potential impact of these results on further research.

2. Background

2.1 *Fear Learning and Fear Inhibition*

2.1.1 *Classical Fear Conditioning*

Ivan Pavlov, a Russian psychologist, was the first to introduce the idea of conditioning in the early 1900's (Pavlov, 1927). In his work with the gastric function of dogs, Pavlov noticed that the animals would start salivating in anticipation of being fed and sought to explain what he called this "psychic secretion". He developed a paradigm, later called classical conditioning, in which he would ring a metronome systematically before presenting food to the dogs. After several repetitions of this procedure, he would observe salivation without even bringing out any food, the ringing of the metronome being enough to cause the dogs to salivate. Pavlov demonstrated that this effect generalized beyond the scope of this experiment by observing the same results when using a touch on the dog's harness, a flash of light, and many other stimuli in place of the metronome.

In any instance of classical conditioning, the presentation of an **unconditioned stimulus (US)** will always cause a specific response. In the case of Pavlov's dogs, the US was the food that was given to the dogs and the resulting response was salivation. Additionally, a neutral stimulus, called the **conditioned stimulus**

(CS), is paired with the US (the metronome in Pavlov's early experiment). After several pairings, an association is created between the two stimuli and the CS will elicit a similar response as the US, even when presented alone. This response, illustrated by the salivation in response to the metronome even in the absence of food, is called the **conditioned response (CR).**

Following Pavlov's experiment, many studies have been conducted in order to examine the pervasiveness of classical conditioning and understand its impact and underlying mechanisms. In particular, classical conditioning has been shown to be a key component of how humans acquire fear to elements in their environment. An influential study conducted by John Watson, known as the "Little Albert experiment", was the first to demonstrate this (Watson, 1920). Watson measured the baseline levels of fear of a young child interacting with animals, mainly a white rat. The 11-month child did not initially exhibit any fear response to any of these animals and spent a large amount of time with the white rat. Watson then began to strike a suspended metal bar with a hammer every time the child touched the rat. This sound would cause the child to cry and the child began to associate the aversive sound (US) with the rat (CS). After some time, the child began to react fearfully towards the rat, even when no sound was present. Additionally, the avoidance behaviors (CR) were shown not just towards the rat, but also towards other furry objects, demonstrating that acquired fear can generalize to objects other than the ones they have been initially conditioned.

2.1.2 *Fear Extinction*

Although appropriate fear learning is important in order to avoid potentially dangerous situations, it is equally important to be able to discern when a once threatening stimulus no longer poses a threat. This form of safety learning is reliant on a process called **extinction**, which allows for inhibition of fear expression in response to a CS that no longer predicts a threat. During extinction, the previously conditioned CS is presented several times without being paired with the US, eventually leading to the elimination of the CR in response to the CS. However, it has been repeatedly shown that the CR can be recovered if the CS is presented again after some time has passed (spontaneous recovery), if the context in which it is presented is changed (renewal), or if a few US are presented beforehand (reinstatement) (Quirk, 2002). Therefore, the previously acquired fear memory is not erased during fear extinction, and the amount of fear expressed in response to the CS depends on the balance between fear expression and fear inhibition.

2.1.3 *Fear Generalization*

As demonstrated by the Little Albert experiment, humans will not only associate a CS with an aversive emotional memory, but they also transfer this fear association to other similar things (Watson, 1920). This phenomenon is known as **fear generalization.** In the case of the experiment mentioned above, the fear of

the white rat generalized to other furry animals. This is an important attribute of fear learning because most stimuli that are important to recognize as threatening are not likely to be absolutely identical every time they are seen. For example, if you have learned that a spider is deadly and should be avoided, it would be important to avoid that type of spider even if you saw a smaller spider in a different location that shared the same defining characteristics. Experimentally, fear generalization has been mainly studied by showing stimuli similar to the original CS on a given dimension after fear conditioning acquisition.

2.2 *Impaired Fear Learning and Anxiety*

While expressing fear and avoiding stimuli previously associated with aversive outcomes is essential for survival, excessive fear expression can lead to maladaptive behaviors. In fact, one of the main symptoms of anxiety disorders is excessive fear (American Psychiatric Association, 2013), and fear learning disruptions have been linked to several anxiety disorders such as PTSD, panic disorders, and phobias (Lissek et al., 2005; Etkin and Wager, 2007; Shin and Liberzon, 2010).

2.2.1 *Panic Disorder*

Panic disorder is characterized by frequent and unpredictable panic attacks. A panic attack is an extremely crippling feeling of fear and danger than can persist for several minutes in the absence of any real danger. Depending on the severity,

there can also be physical effects, causing the person suffering to feel like they are having a heart attack. Panic disorder can also lead to higher levels of general anxiety as the fear of having a panic attack can lead to irrational fear of many of the indicators of their prior panic attacks (Bouton et al., 2001). At extreme levels, this can lead to some people being afraid of vital tasks such as driving because of constant fear of a panic attack. This effect can propagate further and lead to agoraphobia or depression.

The anxiety that results from panic disorder can be understood as inappropriate fear learning. By developing a conditioned fear towards external or internal cues that preceded their prior panic attack(s), people suffering from panic disorder experience anxiety in response to many stimuli that are not indicative of any type of danger or even a panic attack. Experimentally, it has been shown that individuals with panic disorder exhibit higher anticipation of a US when presented with a safe stimulus as opposed to healthy participants (Lissek et al., 2009). They also show greater fear responses to cues that resembled a previously conditioned CS (Lissek et al., 2010), indicating overall excessive generalization in fear learning.

2.2.2 *Phobias*

A phobia is labeled as an inappropriate amount of fear towards something, this fear being typically disproportionate to the threat it actually poses. As they can

occur in the absence of any initial conditioning-like episode - the patient has never necessarily had an aversive outcome paired with the object of their phobia – researchers have thought of phobias as being caused by a deficit in fear extinction rather than in fear acquisition (Field, 2006). In fact, it has been shown that extinction learning is impaired in phobic patients when compared with healthy controls (Hermann et al., 2002), and the main behavior therapy used to treat phobia, exposure therapy, is based on an extinction procedure: patients are exposed to the object of their phobia in a safe environment to try to induce extinction.

2.2.3 *Post Traumatic Stress Disorder*

Post-traumatic stress disorder (PTSD) is one the most widely studied anxiety disorders. After exposure to a traumatic event, patients with PTSD will have nightmares and flashbacks of the traumatic experience as well as general avoidance behaviors and hyperarousal for several months or years. Not every person exposed to a traumatic event will develop those symptoms, indicating that there are individual differences in vulnerability factors for PTSD. In line with this thinking, the persistence of fear responses to reminders of trauma in a safe context as well as the effectiveness of exposure therapy, a treatment designed to focus on extinction impairments, imply that extinction deficits may be a large factor in PTSD occurrence (Rauch et al., 2006; Myers and Davis, 2007; Herry et al., 2010).

2.2.4 *Fear Learning Impairments and Trait Anxiety*

Although there has been extensive work examining the fear learning deficits in clinically anxious populations, there have been significantly fewer studies investigating these same effects for non-clinically anxious individuals. This might be of importance as higher trait anxiety levels in healthy individuals might confer vulnerability to develop anxiety disorders. Understanding the underlying mechanisms could therefore help prevent the occurrence of such disorders. Several studies on subclinical populations have found increased fear responses after conditioning for highly anxious individuals when compared to their low anxious counterparts (Zinbarg and Mohlman, 1998; Baas et al., 2008; Barrett and Armony 2009, Indovina et al., 2011). High anxious individuals also tend to exhibit higher levels of generalization (Dunsmoor et al., 2011b; Dunsmoor et al., 2011c) as well as reduced down-regulation of fear expression both during acquisition (Indovina et al., 2011) and extinction (Baas et al., 2008; Sehlmeyer et al., 2011).

Therefore, examining the role of extinction impairments, excessive overgeneralization, and inappropriate fear learning in maintaining high levels of anxiety in non-clinically anxious individuals could help in understanding the etiology and prevalence of anxiety disorders.

2.3 *Ambiguity and anxiety*

2.3.1 *Anxiety and Ambiguous Cues*

Anxiety levels can also influence people's responses to unpredictable or ambiguous cues. Perceived unpredictability and lack of control have been theorized to play a large role in chronic anxiety (Barlow, 2000). Patients with generalized anxiety disorder tend to process ambiguous stimuli as more threatening than others (Hazlett-Stevens et al., 2004). This effect has also been confirmed in when looking at differences in trait anxiety. In a study using homographs (words with multiple meanings) as ambiguous primes, anxious individuals showed a preference towards a threatening interpretation of an ambiguous stimulus in a lexical decision task (Richards and French, 1992). Further studies have confirmed this effect and shown that high anxious individuals are more likely to view an ambiguous stimulus as a threat in word comprehension (MacLeod and Cohen, 1993; Calvo, Eysenck, and Estevez, 1994). This differential processing of ambiguous stimuli may have ties to impaired fear learning in people with high levels of anxiety.

2.3.2 *Unpredictability in fear conditioning*

In the context of fear conditioning, ambiguity can be manipulated by varying how much the CS predicts the US. When using partial reinforcement rates, not all occurrences of the CS are paired with the US, leading to slower fear conditioning

acquisition but also slower extinction. Hence, more unpredictable cues tend to lead to more long-lasting fear expression.

A recent study has looked at the effects of partial conditioning in serotonin receptor 1A (Htr1aKO) knockout mice, a mice model for anxiety disorders (Tsetsenis et al., 2007). In this experiment, both control and KO mice were trained using a light and a tone as the CS and an electric shock as the US. In their first condition, the experimenters presented light and tone simultaneously three times, always followed by the US. In a second condition, the stimuli from the first condition were shown with the same pattern of reinforcement and the tone was presented alone and unreinforced two additional times. In this case, while the light predicted perfectly the shock, the tone was only a partial predictor. As shown in Figure 1, KO mice showed an increased fear response to the partially conditioned tone when compared to wild-type mice.

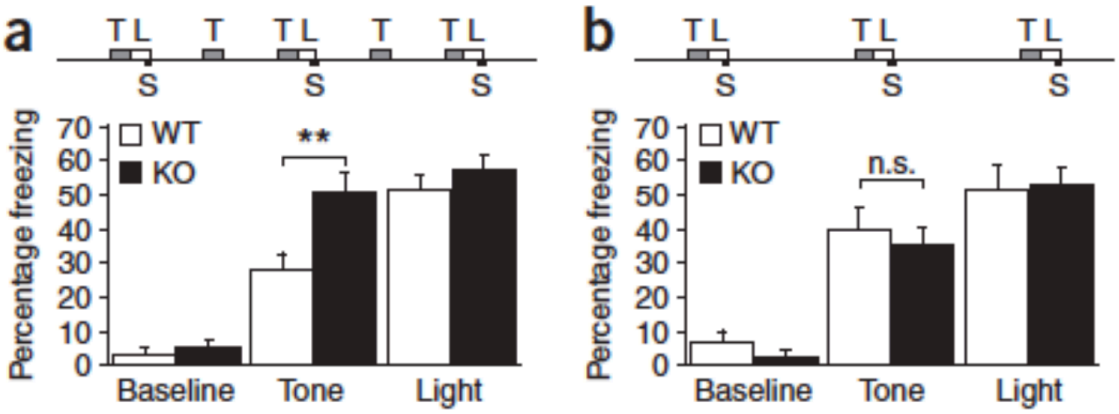


Figure 1: *Freezing responses indicate fear conditioning in mice. The above graphs show the freezing of mice in both of the described conditions. The white bar represents the wild-type mice, which were the control subjects and the black bar represents fear responses for the knockout phenotype.*

The authors interpret this result in the context of ambiguity of the tone cue as it only partially predicts the occurrence of the US. However, high and low trait anxious individuals might also differ in how they treat these different stimuli as the light and tone are presented together, as a **compound stimulus.** Theories of differential conditioning for compound stimuli suggest possible alternatives for the results seen above.

Two primary models exist which explain how compound fear stimuli may be represented. The **configural model** of stimuli association suggests that a stimulus is uniquely associated with a US and is represented as a whole in the mind of an individual (Pearce 1994). Alternatively, the **elemental model** of fear representation (Rescorla and Wagner, 1972) claims that stimuli are encoded as a sum of parts, and each part is individually conditioned to be predictive of fear. One interpretation of the previous results might be that KO mice differ from wild-type mice on how they process and associate fear with each element of compound stimuli.

Drawing upon the results of this study, we developed a paradigm to (i) replicate these findings in a human population and to (ii) disentangle the effects of ambiguity and compound stimulus presentation. We measured pupil diameter as an index of arousal (Reinhard, 2006) while healthy participants with different levels of trait anxiety underwent a fear conditioning paradigm where they were exposed to five different reinforcement schedules. Two of them corresponded to the Tsetsenis conditions and the three additional ones were developed to differentiate between the influence of reinforcement rate and processing of compound stimuli in any differences observed.

3. Materials and Methods

3.1 *Participants*

A total of thirty-two right-handed participants (14 females, mean age ± SD: 22 years ± 4) completed the main task of this experiment after giving informed consent. The study was approved by the UC Berkeley Committee for the Protection of Human Subjects and carried out in compliance with their guidelines. All participants had normal vision acuity and none of them reported any past or present neurological or psychiatric conditions. Two participants were excluded from further analysis, one because of excessive signal dropout in the pupil diameter detection, and the other one because of excessively low pupillary responses. Before the main portion of the experiment, we measured the trait anxiety levels of all participants using the Spielberg State-Trait Anxiety Inventory,

Form Y (STAIt, Spielberger 1983). This provides a quantified measure of trait vulnerability to anxiety, with higher scores corresponding to higher levels of trait anxiety. Researchers validated the effectiveness of this scale by recording higher scores on this assessment for people with anxiety disorders than for control subjects (Bieling et al., 1998; Chambers et al., 2004). We selected participants with a distributed range of anxiety scores in order to measure the effects of anxiety in our experiment. The scores of our participants ranged from **22 to 57**, which falls within the normal range for this age group (Spielberger, 1983). We also administered other questionnaires such as the Eysenck Personality Questionnaire (EPQ) to measure emotional stability, the Mood and Anxiety Symptom Questionnaire (MASQ) to distinguish between dimensions of anxiety and depression, and the CESD-R (Center for Epidemiologic Depression Scale Revised) to measure subscales of depression.

3.2 *Apparatus and Stimuli*

Throughout the course of our experiment, subjects viewed a succession of abstract images generated using a Matlab toolbox that scrambles original pictures while keeping low level visual features, resulting in colorful meaningless textures (Portilla et al., 2000). All stimuli had similar luminance levels in order to minimize differences in light induced pupil constriction in response to the different images. In total, 75 different images were created. These images were used as CS throughout the experiment and were presented alone or in compound (Figure

2, for more details see section 3.3). Several CS presentations co-terminated with an aversive unconditioned stimulus (US) that consisted of simultaneous presentation of an electrical stimulation and a scream. The electrical stimulation was delivered to the wrist of each participant through a WASP electrode using a Digitimer constant current stimulator (Digitimer Ltd.), and the scream was presented through Over-the-Head headphones that participants wore throughout the whole experiment. Both the level of the shock and the scream were set at a level that each participant rated as “uncomfortable but tolerable” and that they were willing to tolerate throughout the whole experiment.

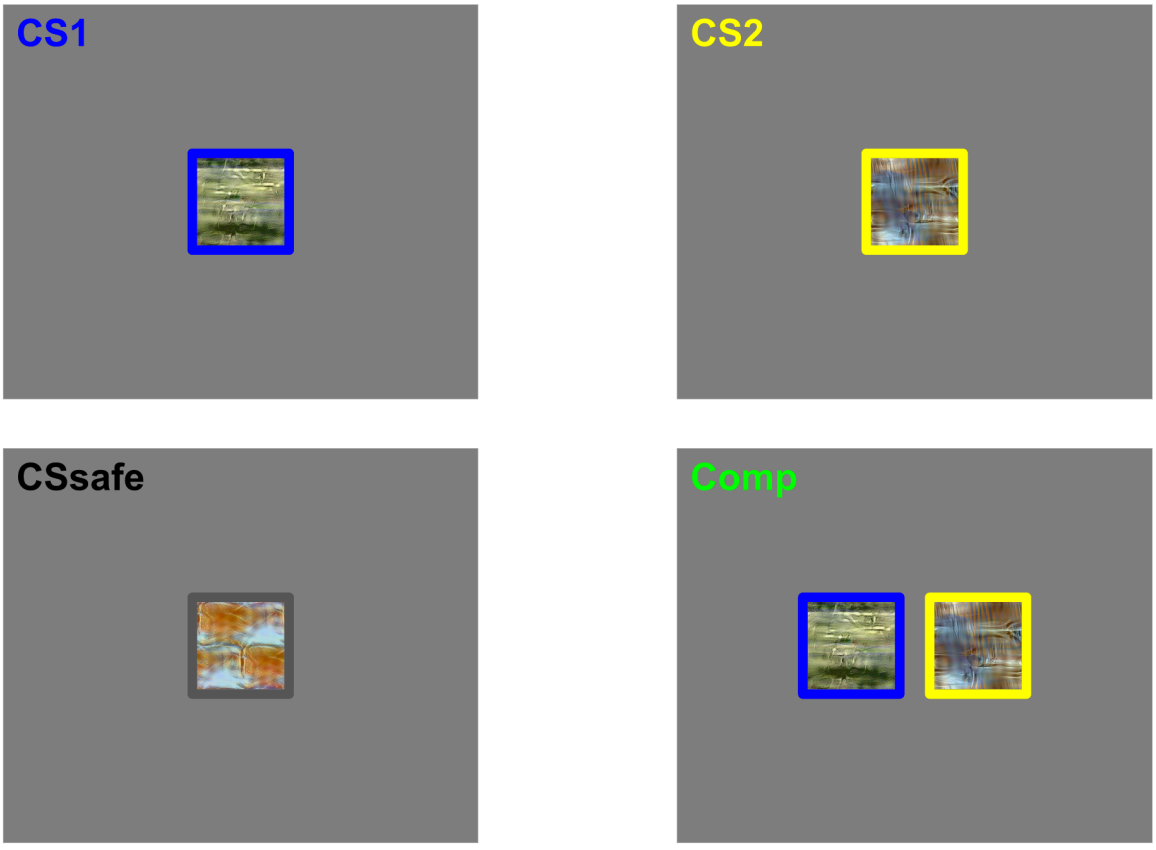


Figure 2: Example Stimuli

These sample images are representative of the stimuli shown throughout the course of the experiment. The colored boxes surrounding them are not present during the actual trials.

3.3 *Experimental Procedure*

Between one and two weeks before the actual experiment, participants came in lab for a short session to fill in questionnaires. If they were eligible for our study, participants were asked to come back for a second two-hour session comprising the main experiment. At the beginning of the second session, participants filled in one additional questionnaire allowing us to measure their level of state anxiety in that particular moment. The stimulation electrode was then placed on the participant's right hand. After calibration procedures to set individual levels for the electric shock and the scream, participants were given the following instructions: "The experiment is divided in five runs of approximately 10 minutes each, with each run divided in five little blocks. At the beginning of each block, a screen indicating "Block n°X" will be presented for a few seconds, followed by repeated successive presentations of three different abstract images that will appear either alone or in compound in the center of the screen. There will be no shock or screams for the first few presentations. Each block has three new images. Your task is to attend to the images and make sure that you are able to differentiate the three different images and to recognize which ones are presented in compound. As much as possible, try not to move or blink to ensure that we can measure your eye movements and pupil diameter." Before each session, an

instruction screen briefly summarized this information as a reminder. Participants had their chin resting on a chin-rest in order to minimize head movement. Stimulus presentation was controlled using PsychoPy, a Python based application (Peirce, 2009). Pupil diameter and eye gaze was measured using an SR Research Eyelink1000 eye tracker (SR Research Ltd.). After the experiment, participants were asked if they noticed anything interesting or strange during the experiment to evaluate their awareness of the experimental manipulations.

3.4 *Design*

Our experiment consisted of five distinct conditions presented in one session. In order to collect a sufficient amount of data and eliminate the effect of condition ordering, we presented all conditions in five sessions, with the order of appearance of counterbalanced across sessions so that each condition would appear at each position in exactly one session. The presentation of one condition during a session is referred to as a condition block. With five total sessions and five condition blocks during each session, we had a total of twenty-five total condition blocks during the experiment. Each condition block used a different set of three images in order to avoid carry on effects from one block to another. Each image appeared alone at the center of the screen. In addition, two of the images were also presented in compound with one on each side of the center of the screen. These images will be referred to as CS1 and CS2. They were set so that the image corresponding to CS1 would appear roughly half of the time on the left

side of the compound for each condition and the other half on the right. The third image will be referred to as CSsafe (see Figure 2). All stimuli were presented for two seconds each, separated by an inter-trial-interval of 4s on average (from 2.95 and 5.05 s).

Each condition block consisted of a habituation, an acquisition and a test phase in that order. The acquisition phase was different for each condition and was the only moment where US could be presented co-terminating with the other stimuli. During both the habituation and test phase, all stimulus types (CS1, CS2, Compound and CSsafe) were presented in a random order twice, never associated with the US. Therefore, the habituation phase allowed us to measure the baseline pupillary responses to all images used in the condition block before any pairing occurred, and the difference in pupil dilation between the test and habituation phases was taken as an index of how much anticipation of the US was associated with each stimulus after conditioning.

Figure 3 illustrates the five different conditions for the acquisition phase. Conditions 1 and 2 correspond to the original Tsetsenis conditions, where the compound is always reinforced, with additional unreinforced presentations of CS1 for condition 2. Conditions 3 and 4 corresponded to control conditions for condition 1 and 2, where CS1 was associated with the US instead of the compound. If individuals treat the compound as a “whole” and not as a sum of

parts, responses to the compound in the test phase of conditions 1 and 2 should be comparable to responses to CS1 during test phase of conditions 3 and 4. In condition 5, both CS1 and CS2 were associated with the US, but CS1 with a 100% reinforcement rate and CS2 with a 50% reinforcement rate. Crucially, this condition was designed to test for differences in contingency encoding without the confound of compound stimulus processing.

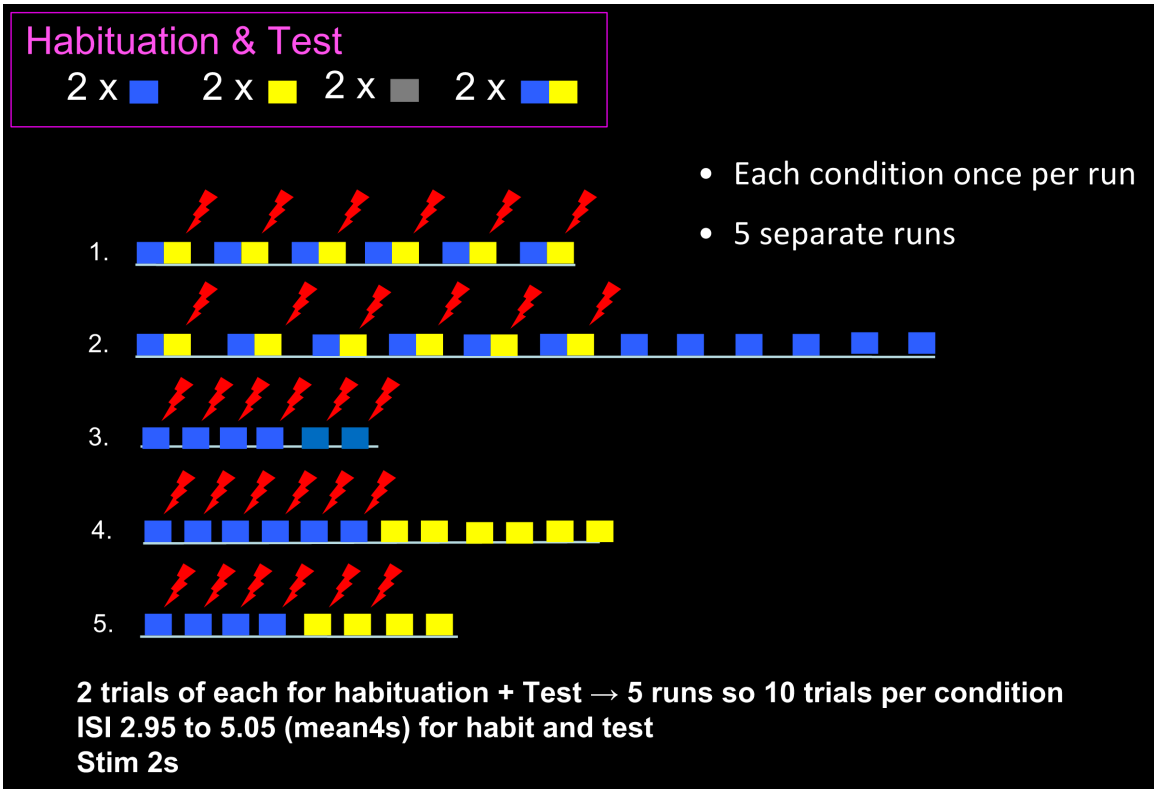


Figure 3: Conditions

Each stimulus is presented twice during the habituation phase at the beginning of each condition block and twice during the test phase at the end of each condition block. A summary of the acquisition phase for each of the conditions can seen above numbered by condition. A

lightning bolt above a block indicates the pairing of that stimulus presentation with a shock and scream. A blue block represents a presentation of the CS1, yellow the CS2, a combination the compound, and a gray block the CSsafe.

3.5 *Pupil Diameter Recording and Analysis*

In order to examine the anticipatory response of subjects throughout our experiment, we measured pupillary diameter for the entire duration of the experiment with a sampling rate of 1000 Hz. Pupil diameter has been shown to be a reliable measure of Pavlovian conditioning to a CS and effective when compound stimuli are used (Reinhard et al., 2006). Furthermore, the pupil response is quick and changes in pupil diameter can be seen less than .5 sec after stimulus onset (Beatty, 1982; Loewenfeld, 1993). Eyeblinks and artifacts were removed and data interpolation was performed using a semi-automatic custom-made Matlab toolbox. After filtering the data with a low pass filter of 3.75Hz cutoff frequency (Nassar, 2012), the signal was cut into 4.2s epochs (form 200 ms to 4s after stimulus onset), the average signal across the 200ms baseline period was subtracted from each data point for each trial, and data was z-scored for each session separately. Trials where more than half of the epoch was missing were rejected, and participants for whom we had to reject more than three repetitions for any given condition were rejected from the overall analysis. We then took the average from 1 to 3 s after the stimulus onset as our index for pupillary response for each trial as this corresponded to when we observed our

peak pupil dilation. We then averaged this index for each stimulus and each condition across all sessions separately for the second repetition of the habituation phase (to avoid contamination of baseline measurements by novelty). We then submitted the data to two-way repeated measures ANOVA's with experimental phase (2 phases: habituation and test first trial) and stimulus (4 stimuli: CS1, CS2, Compound and CSsafe) as factors for each condition separately. Follow up direct comparisons between individual conditions were assessed using two tailed paired t-tests We then examined the impact of anxiety in each one of these effects by looking at Pearson correlations between differential pupillary responses and anxiety scores.

4. Results

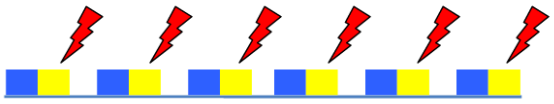
In total, data was available for 30 participants. We will present the pupillary diameter measured in response to each stimulus type (CS1, CS2, Compound ad CSsafe) for habituation and test for each condition separately. Increased pupillary response in test as compared to habituation is an index of successful fear association.

Figure 4.1: Legend of Graph Symbols:

*	*This symbol indicates a significant difference with a p-value of .05 or under*
**	*This symbol indicates a significant difference with a p-value of .01 or under*
***	*This symbol indicates a significant difference with a p-value of .001 or under*
⊙	*This symbol indicates a significant difference between response values for the test1 and habit phases for the stimulus*
△	*This symbol indicates a significant difference between response values for the test2 and test1 phases for the stimulus*

4.2 *Summary of Results for Conditions*

4.2.1 *Condition 1*



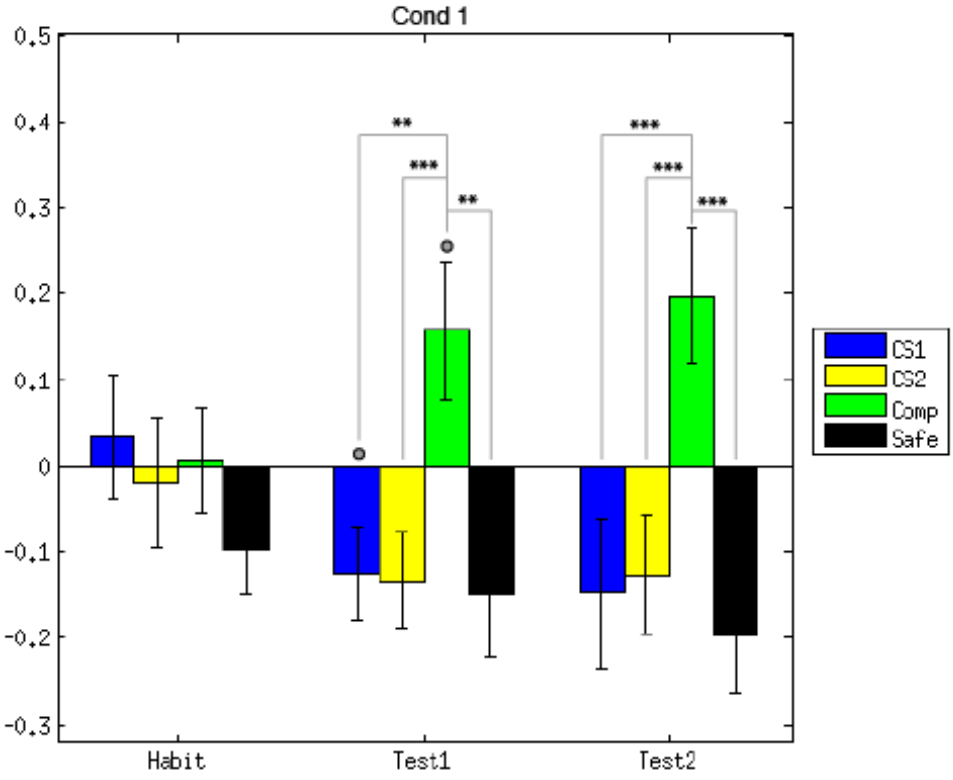


Figure 4.2: Results for Condition 1

An ANOVA with phase (Habit and Test1) and stimulus as factors showed a significant main effect of stimulus ($F(3,87) = 3.717$, $p=0.014$) and a main interaction of experimental phase by stimulus ($F(3,87) = 3.379$, $p=0.019$). Follow up two-tailed t-tests showed that during the first trial of the test phase, pupillary response was significantly higher to the compound stimulus than to all other stimuli (all t-values > 2.782 and all p-values < 0.009). Furthermore, participants' average response to the compound stimulus was higher during the first trial of the test phase than during habituation ($t = -2.168$, $p = 0.039$). Interestingly, the pupillary response was smaller during the first trial of test for CS1 ($t= 2.259$, $p= .032$) and CS2 exhibited the same trend ($t=-2.168$, $p= .039$). Taken together, these results show that participants developed a conditioned response to the compound after fear conditioning acquisition but not to its parts.

Another ANOVA taken between the two trials of the test phase also showed a significant effect of stimulus ($F(3,87) = 12.438$, $p=0.000$). However, there was no interaction of experimental phase by stimulus. T-tests revealed that the pupillary response to the compound stimulus was still higher than to the other stimuli in the second trial of the test phase (all t-values ≥ -4.146 and all p-values ≤ 0.000). These results demonstrate that subjects maintain the conditioned response throughout the entire test phase.

4.2.2 *Condition 2*

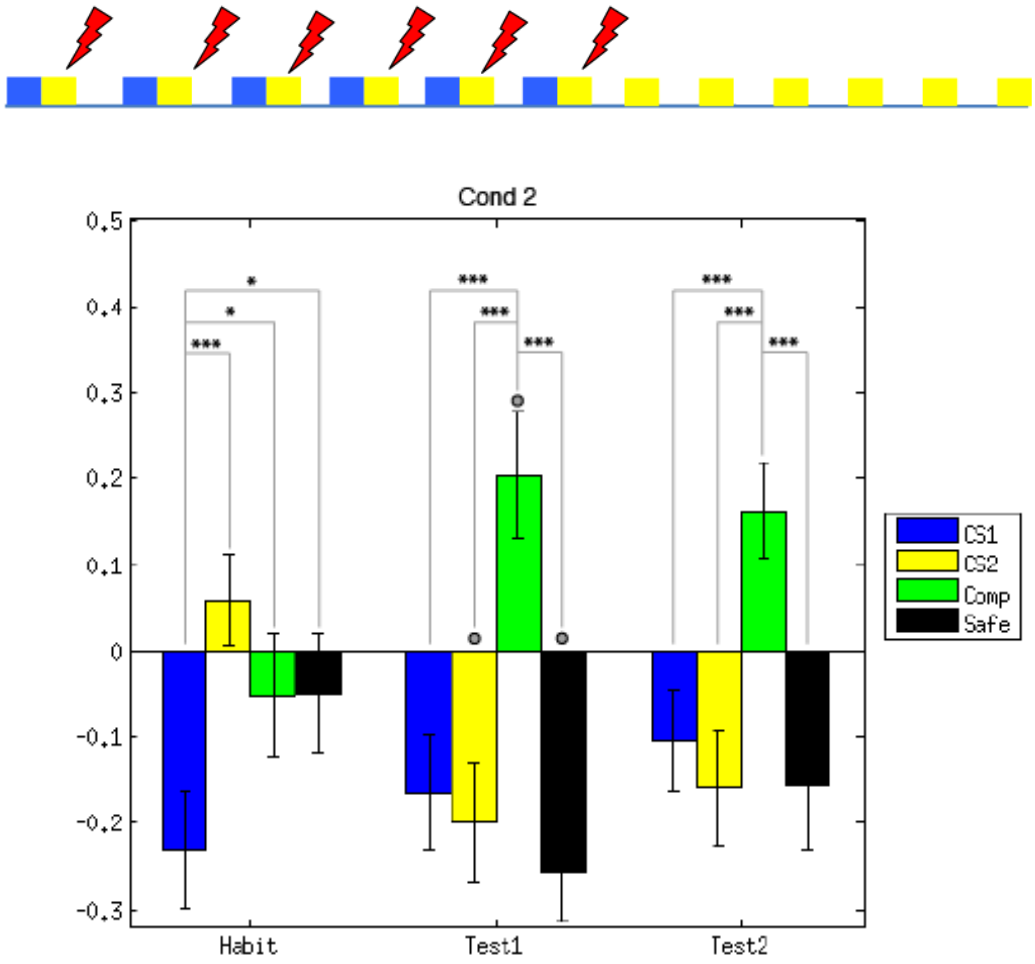


Figure 4.3: Results for Condition 2

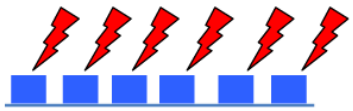
An ANOVA on the second trial of habituation with stimulus as the only factor revealed a significant main effect of stimulus ($F(3,87) = 5.185$, $p = .002$) during this phase. Two-tailed t-tests showed a significant difference between the pupillary response to CS1 and all of the other stimuli presented during the second phase of habituation (all t-values ≥ -3.782, all p-values $\leq .05$). As habituation occurs before any type of conditioning, we would not expect any

significant variance and therefore examined this effect to see why this was the case. We checked the side of the screen on which each stimulus was presented, the order in which condition 2 was seen within sessions, which conditions were shown before condition 2, and the specific images shown during condition 2 to ensure that neither of these factors were randomized in a manner that was heavily weighed in favor of a specific arrangement and could thereby contribute to the low response towards CS1 that was observed during the habituation phase. After testing the significance of each of the above factors, we were not able to find any explanation for this low CS1 value. Other values for this condition appear to exhibit normal patterns.

Just as in the previous condition, an ANOVA with experimental phase (Habit and Test1) and stimulus as factors indicated a main effect of stimulus ($F(3,87) = 7.22$, $p=0.00$) and a significant interaction of phase by stimulus ($F(3,87) = 10.901$, $p=0.000$). Also in line with the results seen in condition 1, t-tests showed a higher pupillary response to the compound stimulus during the first trial of the test phase than to other stimuli in this phase (all t-values ≥ -5.024, all p-values ≤ 0.00) and to the compound stimulus during habituation ($t = -3.249$, $p = 0.003$). A decrease in the response to CS2 ($t = 3.376$, $p = 0.002$) and to CSsafe ($t = 2.282$, $p = 0.03$) was also seen in Test1. These results show successful conditioning of subjects to the compound stimulus but a lack of transfer from this fear to a fear of its parts.

An ANOVA taken with the two trials of the test phase and stimulus as factors showed a significant effect of stimulus ($F(3,87) = 12.304$, $p=0.000$). Pupillary response to the compound stimulus remained higher than responses to other stimuli during the second trial of the test phase (all t-values ≥ -3.922, all p-values ≤ 0.003). Similarly to condition 1, fear expression towards a conditioned compound stimulus was maintained throughout both test trials.

4.2.3 *Condition 3*



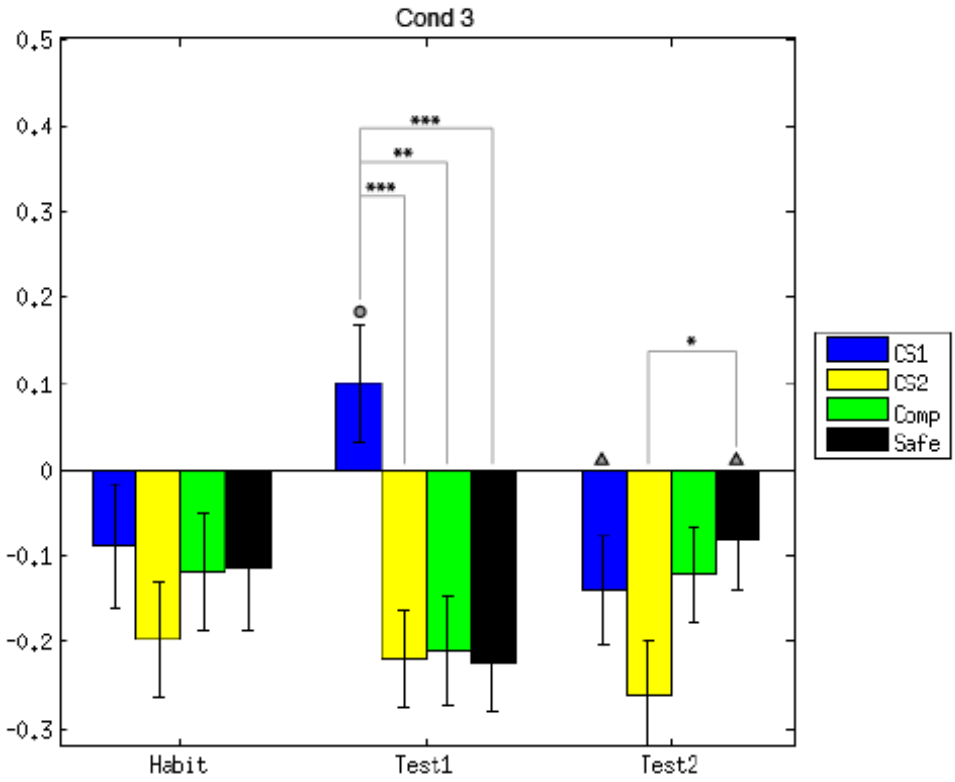


Figure 4.4: Results for Condition 3

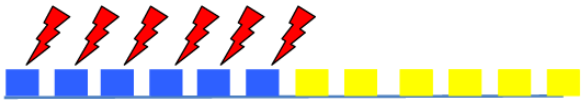
As in the prior two conditions, the ANOVA with phase (Habit and Test1) and stimulus as factors showed a significant main effect of stimulus ($F(3,87) = 5.269$, $p=0.002$) and a main interaction of experimental phase by stimulus ($F(3,87) = 3.963$, $p=0.011$).

Using a two-tailed t-test, we found a significant difference for average pupil diameter in response to CS1 presentation between when it was presented in the habituation phase and when it was presented in the first test trial ($t = -2.721$, $p = 0.011$). This increased fear response in the first test phase was also significantly higher than values for all other stimuli during the first test phase (all t-values ≥ 3.361, all p-values ≤ 0.002).

An ANOVA with phase (Test1 and Test2) and stimulus as factors showed a significant main effect of stimulus ($F(3,87) = 5.269$, $p=0.002$) and a main interaction of experimental phase by stimulus ($F(3,87) = 6.098$, $p=0.001$).
The pupillary response to CS1 decreased between the two test trials ($t = 3.676$, $p = .001$). The response to CSsafe significantly increased between test phases 1 and 2 ($t = 2.570$, $p = .016$), making its elicited pupil response significantly higher than that obtained by CS2 ($t = 2.185$, $p = .037$). These results indicate that subjects developed a fear response towards CS1 after fear conditioning acquisition, but do not retain this fear response until the second test trial of the

test phase. However, these results should be interpreted with caution, as the low pupillary response towards CS1 in the habituation phase is unexplained and could have unknown implications for the rest of the results.

4.2.4 *Condition 4*



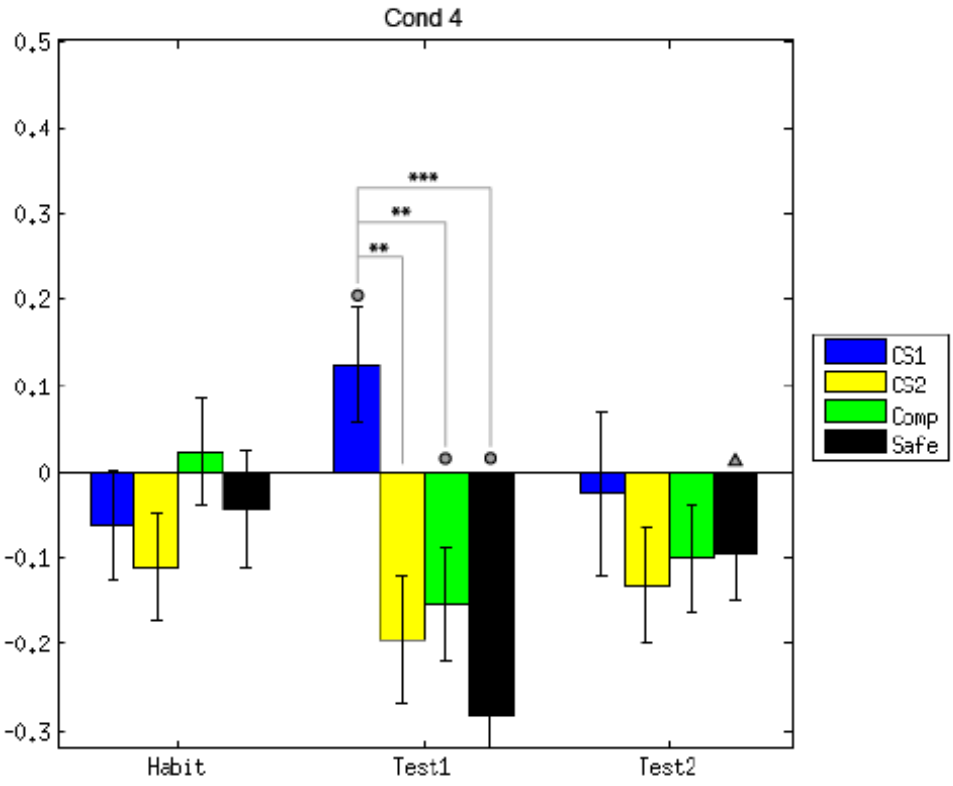


Figure 4.2: Results for Condition 4

The ANOVA revealed an effect of stimulus ($F(3,87) = 4.246$, $p=0.008$), experimental phase ($F(3,87) = 4.389$, $p=0.045$), and an interaction of stimulus

and phase ($F(3,87) = 5.482$, $p=0.002$) when stimulus and phase (Habit Test1) were used as factors.

As in condition 3, further t-tests showed that participants had a higher pupillary response to CS1 than to any of the other stimuli in the first trial of the test phase (all t-values ≥ 2.764, all p-values ≤ 0.01) and had a higher response to CS1 in the first trial of the test phase than in the habituation phase ($t = -2.5$, $p = .018$). The decrease of pupil diameter in response to unconditioned stimuli between habituation and the first trial of the test phase as seen in condition 1 was present during this condition as well. This trend is seen for the compound stimulus ($t = 1.954$, $p = .06$) and is significant for the safe stimulus ($t = 3.117$, $p = .004$).

Another ANOVA with phase (Test1 Test2) and stimulus as factors showed a significant main effect of stimulus ($F(3,87) = 5.193$, $p=0.002$) and an interaction between phase and stimulus ($F(3,87) = 3.218$, $p=0.027$). T-tests revealed no significant differences between any of the stimuli in the second trial of the test phase and no significant differences between any of the stimuli in the second trial of the second phase and their correspondents in the first trial of test. These results show that a conditioned response towards CS1 is learned, but is not retained throughout the entire test phase, as the pupillary response to the CS1 is no longer significantly higher than the response to the other stimuli in the second trial of the test phase.

4.2.5 *Condition 5*

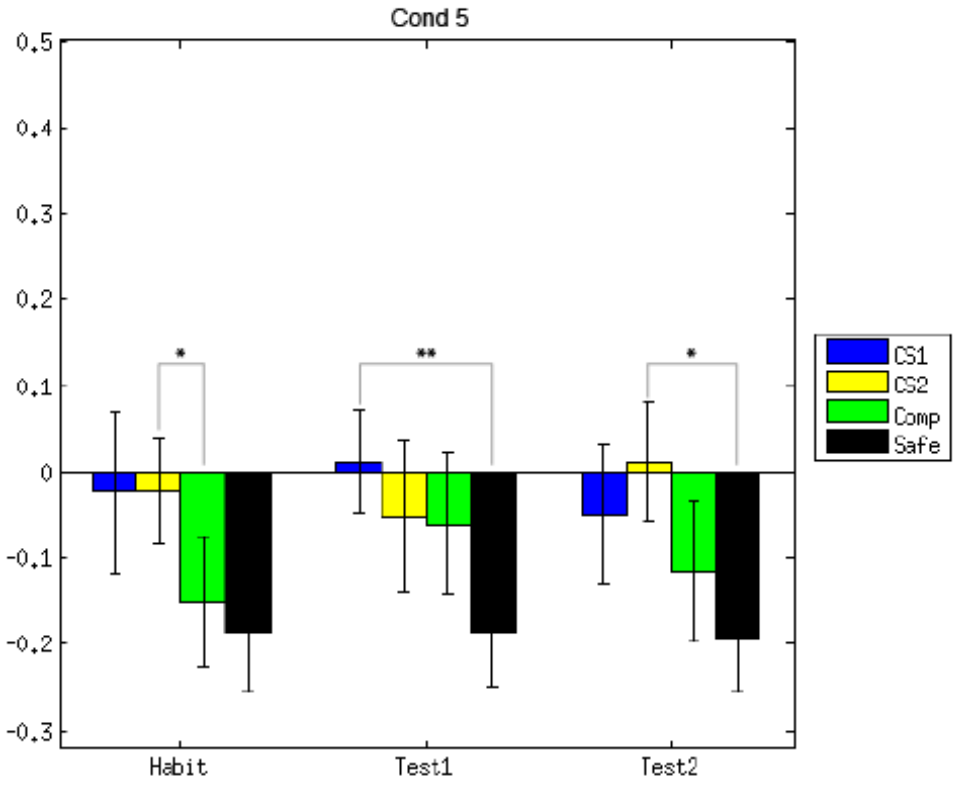


Figure 4.2: Results for Condition 5

ANOVA's conducted with every combination of experimental phase and stimuli as factors and with every experimental phase as a sole factor revealed no significant effects.

As there are only a few viewings of each stimulus during the acquisition phase, it appears that participants are not able to learn any fear associations. In the debriefing that occurs after the experiment, most participants claimed to be confused by this condition and were not sure of what was going to be paired with the US.

4.2.6 *Correlations with Anxiety Scores*

We find no conditioned response to the parts of the compound when a compound stimulus is conditioned. A Pearson correlation between the anxiety levels of participants and the increase in pupillary response to the CS1 and CS2 between habituation and test in conditions 1 and 2 was not significant.

Likewise, no significant correlations with anxiety were measured in the case of partial conditioning. We calculated Pearson correlations between participants' anxiety levels and their pupillary response to CS2 in condition 5, the partially conditioned stimulus. However, given that no main effect was seen in condition 5, this result should be taken with caution.

Our experiment as a whole does not seem to show an effect of anxiety on how fear is associated with each part of a compound, but we observed that our sample population seems to condition to the compound as a whole and not transfer the fear of the compound to its parts. This configural processing is seen for all subjects, without a significant correlation to anxiety scores.

4.2.7 *Further Analyses*

Although our lab was limited by time, there are analyses we would have liked to conduct that may have produced interesting results. Using the data from only the first two sessions of the experiment may be conducive to seeing stronger effects as the participants have been exposed to fewer stimuli and are less tired at the beginning of the experiment. Another possible way to strengthen the effects in the data would be to exclude participants who did not show successful conditioning to the compound stimulus in the first condition or successful conditioning to CS1 in the third condition. Using only participants who conditioned well to the basic conditions may provide better results. Lastly, it may also be interesting to only consider the data from participants whose anxiety scores are on the extreme ends of the spectrum. This would allow us to compare two groups of participants rather than look at correlations and may also show stronger effects.

5. Discussion

5.1 *Main Effects*

5.1.1 *Configural Processing*

In each of the conditions, with the exception of condition 5, there was evidence of configural association of stimuli. The configural model states that there is no transfer of fear from a compound stimulus to its individual parts (Pearce, 1994). This type of fear transfer was never seen and fear transfer in the opposite

direction (parts to compound) was also not seen. Holding the configural model to be true, one would expect the same results from condition 1 and condition 3. If the compound is treated as its own entity and not as a summation of two other stimuli, the two conditions are identical. As the configural model would predict, both resulted in a heightened fear expression of the conditioned stimulus during the first trial of the test phase without any fear transfer. In the same line of thinking, conditions 2 and 4 should also be identical. Just as in the previous comparison, they yielded the same results for the conditioned stimulus. This trend did not show any correlation with anxiety measurements. There were no significant correlations when the differences in pupillary response to CS1 and CS2 between conditions 1 and 2 were calculated during the test phase. Moreover, there were also no significant correlations between the differences in response towards CS1 and CS2 between the habituation and test phases. An elemental model would suggest that participants in condition 1 would show strong fear responses towards the CS1 and CS2 in the first test trial, which was not the case. This seems to indicate that people show a strong configural processing of stimuli with association to fear regardless of their trait anxiety levels.

5.1.2 *Compound Retention*

The first trial of the test phase was used to look at how strongly fear was associated with a stimulus because decay in the conditioned response is seen during the second trial of test. However, this effect does not seem to be present

when the conditioned stimulus is a compound stimulus. In conditions 1 and 2, when a compound stimulus served as the conditioned stimulus, there is no significant difference between the pupillary responses to the compound stimulus in the two trials of test. Furthermore, the response to the compound stimulus during the second trial of test is still significantly higher than the responses towards all other stimuli. However, in conditions 3 and 4, when a single image was presented as the conditioned stimulus, this was not the case. In condition 3, the difference in pupil diameters between the two trials of the test phase was significant and the pupillary response to CS1 (the CS) was not significantly different than the response values for other stimuli shown in the same trial. Though there was not a significant difference between CS1 in the two test trials, there was an observable decrease and the CS1 response value was no longer significantly higher than the other stimuli during the second test trial. This trend seems to imply a lack or a delay of fear extinction for a compound stimulus.

5.1.3 *Safety Learning*

All of the stimuli that are not associated with a US during the acquisition phase show strong decreases in elicited fear responses between the second trial of the habituation phase and the first trial of the test phase. Several of these decreases are significant and show a general trend towards slightly increasing during the second trial of the test phase. Safety learning appears to be occurring for the unconditioned stimuli.

5.2 *Tsetsenis Study*

Although there are many observable differences between the experimental design of Tsetsenis and our current experiment, our primary results seem inconsistent with those produced by Tsetsenis. In condition 2, the condition that we created in order to replicate the main anxiety effect of his study, the compound stimulus is perfectly paired with the US whereas one of its constituent parts is shown unreinforced with the US and the other is never seen during conditioning. If his results were perfectly replicated, we would expect to see an increased response to the unreinforced stimulus (CS2) by high anxious individuals and a high response to the elemental stimulus that was only seen as part of the compound (CS1) by all individuals. However, neither of these effects was seen, implying that people do not encode fear to the individual parts of a compound and that no partial conditioning is occurring in this scenario.

Despite our attempt to replicate the experimental parameters as closely as possible, there still exist many possible reasons for the disparity in our results. Firstly, our use of abstract images instead of using a light and a tone as the stimuli could have had an effect on the stimuli processing. The different modalities that comprised the compound stimulus in the Tsetsenis study may be processed differently and may not be seen as a compound the same way that two stimuli of the same modality are. One could possibly be preferentially processed or they could be processed completely independently. Another factor

that may have impacted our results was the spatial placement of stimuli in our presentation of a compound stimulus. When a non-compound stimulus was shown, it was seen in the middle of the screen. However, during the presentation of a compound stimulus, the two parts of the stimuli were each presented next to each other, with a slight gap in between them. This results in neither part of the compound being presented in the same part of the screen as it is when presented alone. This change may make it more difficult to recognize the individual parts of a compound stimulus. Changes in spacing between the parts of a compound may play a role in the way that compound stimuli are processed in this context and this effect is something worth examining in further studies. Also, the images that we showed participants were not part of a background. The light and tone presentation in the Tsetsenis condition were presented in a naturalistic setting and were administered in the context of a normal background. The isolated presentation of our stimuli may have also impacted how the stimuli were processed. Another possible explanation is the difference of stimuli processing in humans and rats. Although many similarities have been noted in the way that both learn fear through conditioning paradigms, many differences may still exist and play a larger role when compound stimuli are processed. Lastly, the knockout mice that were used in our comparison study all exhibited high anxiety levels comparable to people who are clinically anxious. Our subjects ranged in terms of their anxiety scores and we measured effects in our data and how these differences correlated with anxiety scores as opposed to separating

the subjects into two distinct groups of high and low anxiety levels. Had we instead looked at clinically anxious individuals and compared this to a control group, our results may have varied.

5.3 *Fear Learning Impairments in Anxiety*

Though people with high levels of anxiety have problems with fear learning and show excessive generalization of fear stimuli (Dunsmoor et al., 2009), this does not appear to be due to any differences in processing of compound stimuli. Highly anxious people do not tend to encode fear memories to individual parts of a compound that they have been conditioned to fear. However, shared features to a fear stimulus still do play a role in fear generalization (Lissek et al., 2010). It is possible that fear may generalize to things that have an overall perceived similarity to an object that is already feared.

Although extinction occurs naturally through constant exposure to a conditioned stimulus unreinforced, our findings indicate that this process may be slower in response towards compound stimuli. To facilitate the process of assisting people with fear inhibition deficits, a treatment method known as exposure therapy is often used. This involves showing patients triggers of their fear in a safe context unreinforced. This has been shown to effective in the treatment of phobias and useful in other anxiety related disorders such at PTSD (Pull, 2008). In order to account for the slowed decay for compound stimuli, it may be effective to gear

further research towards examining the root of this effect and account for this difference in the utilization of therapies such as exposure therapy.

5.4 *Further Research*

In order to clarify many of the ambiguities existing after analyzing the results of this experiment, our lab plans on conducting a follow up experiment in order to effectively examine the effect of anxiety on partial conditioned stimuli. We plan to accomplish this through creating more robust conditions consisting of more trials to make the conditions more clear. Additionally, we intend to utilize neutral objects as our stimuli rather than abstract images. This will allow us to see if our results exist in more naturalistic settings. We also plan to look at the effect of conserving the spatial position of the parts when presented alone, and changing the space between the parts in the compound. Moving them far enough away may create a bias towards elemental processing, which may show vastly different results.

Conclusions

Trait anxiety does not appear to play a role in compound stimuli processing and all people appear to process compound stimuli in a configural manner. Compound stimuli also have a delayed extinction period. Further studies are necessary to make any conclusions about partially conditioned stimuli.

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