# The Role of Spatial Location in Threat Memory: Modulation of Learning and Discrimination

**by**

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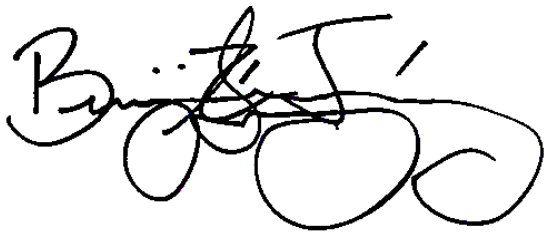
**Declaration:**

I, Benjamin Suarez Jimenez, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

**Signed:** 

**Date:** June 9, 2016

**Abstract:**

Learning about dangers in our environment is a vital adaptive behavior, and many have studied the association of environmental cues with danger or safety. However, the outcome associated with a specific environmental cue can depend on where it is encountered, and relatively little is known about the neural mechanisms behind location-specific threat learning within a single environment.

Through a series of experiments, I developed a novel virtual reality task comprised of safe and dangerous zones within a single environment. Healthy volunteers explored this environment while 'picking flowers', which they were told might contain bees. On contacting a flower, participants were frozen for a short period of time and, if 'stung,' received a mild electric shock at the end of this period. Participants had the opportunity to learn that bees only inhabited flowers in one 'dangerous' half of the environment.

Participants were able to discriminate zones that predict safety and threat within a single environment, with galvanic skin responses and subjective reports increasing as they approached and picked flowers in the dangerous half of the environment. Using functional magnetic resonance imaging, I found posterior medial temporal lobe structures (parahippocampus, posterior hippocampus) to be involved in memory for object locations. In contrast, anterior hippocampus, amygdala, and ventromedial prefrontal cortex showed greater activity when approaching flowers, but this activity did not differentiate between safe and dangerous zones. However, once participants reached a flower in the dangerous zone, increased activity was seen in areas associated with imminent threat, such as the midbrain/periaqueductal gray, dorsal anterior cingulate, and insula cortices.

These results are the first to reveal mechanisms of location-specific threat learning in humans, in the absence of obvious boundaries delineating safety and danger zones. In the future, I hope this new paradigm will be used to understand

the overgeneralization of threat in anxiety disorders and post-traumatic stress disorder.

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# Dedication

This thesis is dedicated to those struggling with Anxiety and PTSD.

*"Never, never, never give up!"* - Winston Churchill

# Acknowledgment

Looking back, I have to acknowledge that I have never been alone in this long and arduous process. I refer not only to this thesis and PhD, but also through the path that got me here (I might need another thesis for my acknowledgements).

First, I want to thank my PhD supervisors, at UCL, Neil Burgess and John King, at the NIMH, Monique Ernst, Christian Grillon, and Daniel Pine. Their support and dedication had no bounds. They are exemplar supervisors and mentors who opened countless doors for me and guided me on the path of becoming an independent researcher. I am a better scientist for working with them and for the opportunities they provided me.

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I would like to thank the members of my groups, past and present, at the NIH and at the ICN for their continuous help and support. Especially I want to thank James Bisby, he is a great mentor and I will be eternally grateful for all the hours he spent teaching me how to create, conduct, and analyze fMRI data and virtual reality tasks. In addition, I am very lucky to have gotten the help and guidance from such talented scientist such as Nicholas Balderstone, Daniel Bush, Adam Gorka, Aidan Horner, Raphael Kaplan, and Salvatore Torrisi.

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Finally, I would have never made it this far without the immeasurable love, advice, and support of my friends and family who always believed in me.

Woot woot!

# Preface

Learning about potential dangers within our environment is essential to survival. During exploration of novel environments, one must learn about the objects we encounter and whether they should be approached with caution. Learning about and reacting to dangerous aspects of an environment can result in increases in both anxiety, an emotional anticipatory response to potential threats, and fear, a response evoked by an imminent acute threat from a discrete stimulus (Blanchard & Blanchard, 2008; Davis et al., 2010; Sylvers et al., 2011; Tovote et al., 2015). However, these responses become maladaptive in anxiety disorders, which are characterized by chronic and excessive fear and anxiety. One theoretical model of pathological anxiety is based on a deficit in learning to distinguish threat from safe cues or contexts (Grillon 2002). The present thesis aims at examining this model and its neural correlates to further understand the neural mechanisms underlying learning about location-specific danger within an environment in healthy adults. Based on these findings, future research can be designed to address how these neural mechanisms are perturbed in individuals with anxiety disorders. More specifically, the question examined here concerns the learning of contextual threat, focusing on spatial information processing paired with aversive learning, functions known to depend heavily on the integrity of hippocampal (HPC), amygdala, and prefrontal cortex (PFC).

Anxiety and fear manifests as worry and uneasiness accompanied by somatic symptoms, including changes in blood flow, blood pressure, heart rate, pupillary diameter, and perspiration. The latter, indexed by electrical skin conductance response (SCR), is one of the most reliable measures of physiological anxiety and fear, and the main physiological response used in this study concurrently with neuroimaging data collection and subjective reports. The paradigm used in this study probes “differential aversive context conditioning”, i.e., learning to distinguish threat from safe contexts. The basic concept of aversive conditioning consists of learning the association of a neutral stimulus (cue conditioning) or context (context conditioning) with an aversive stimulus. When a

neutral stimulus or context (e.g., a tone or a room, respectively) is associated repeatedly with an aversive stimulus (e.g., an electric shock), the neutral stimulus or context begins to be anticipated as aversive, triggering a fear or anxious response similar to the one generated by exposure to the aversive stimulus. Differential conditioning is the learning of the association of two different neutral stimuli (i.e., cues, or contexts), one with an aversive stimulus, and the other with an absence of the aversive stimulus. The conditioned stimulus (CS) that carries the aversive tag is termed CS+, and the CS that carries the safe tag is termed CS-.

Traditionally, the process of discriminating between safety and threat has only been studied using either two separate contexts or two discrete stimuli (Herry et al., 2008; Maren & Holt, 2000; Milad et al., 2007; Orsini, Hyun Kim, Knapska, & Maren, 2011). These paradigms inform the processes of threat learning, but do not appropriately address the process of discrimination. Using two contexts or stimuli creates divergent memories during initial conditioning, creating separate memories for each context or stimulus. In other words, traditional context conditioning creates an emotional memory for the CS+ and an unemotional memory for the CS-. My task addresses this gap by using a single environment, creating a single emotional memory, which requires location-specific information to discriminate between the CS+ and CS-. As a result, it allows for better understanding of neural mechanisms necessary for discrimination within an environment.

Therefore, to investigate the processes involved in distinguishing between threatening and safe conditions determined by spatial locations, my thesis aims to investigate the neural bases of contextual threat learning using functional magnetic resonance imaging (fMRI). In addition, I aim to study the reactions of the brain as aversive stimuli are encountered in a virtual environment I created, to understand

how threat-related responses depend on the surrounding context, and how these responses are reactivated in subsequent visits to the aversive locations.

The overall purpose of this thesis is to develop a paradigm to further asses the neural basis of contextual aversive conditioning. This paradigm will be used to identify the brain areas active during location-specific context conditioning, so that this information can be applied to the understanding, diagnosis, and treatment of anxiety disorders in the future. In the first chapter of this thesis I discuss anxiety, its physiological and cognitive manifestations, and how they are traditionally studied in research. Especially, I focus on context conditioning and the differences between healthy participants and patients suffering from anxiety disorders. I will then discuss the brain areas associated with context conditioning and threat learning. Particularly, I focus on the HPC, amygdala, PFC, and cognitive interactions due to their engagement in traditional conditioning studies. In addition, I briefly introduce some of the fMRI and virtual reality methodology used in my experiments. Then I go over the 11 experiments I carried out in order to create and validate the virtual reality paradigm I used in the 12th experiment, while participants where in the fMRI scanner (Table 1). Lastly, I conclude by discussing the potential implications, the future directions, and follow-up experiments of this paradigm.

***Table 1: Experiment descriptions.***

| Experiment (n) | n (male) | Number of Trials | Reinforcement of CS+ | CS+ Type | % of learners (% male) | Special note |
|---|---|---|---|---|---|---|
| **1.1** | 21 (17) | 40 | 65%, with increments of 15% | Shock | 62% (84%) | Reinforcement of shock increased as participant approached dangerous hive |
| **1.2** | 29 (23) | 80 | 65%, with increments of 15% | Shock | 83% (75%) | Reinforcement of shock increased as participant approached dangerous hive |
| **1.3** | 20 (1) | 40 | 95% | Shock | 85% (05%) | None |
| **1.4** | 25 (9) | 80 | 60% | Shock | 80% (40%) | None |
| **2.1** | 10 (3) | 80 | 35% | Shock | 60% (34%) | None |
| **2.2** | 11 (2) | 80 | 50% | Shock | 73% (25%) | None |
| **2.3** | 27 (11) | 80 | 50% | Shock | 78% (47%) | Participants movement restriction period was jittered from 2-8 sec |

| Experiment (n) | n (male) | Number of Trials | Reinforcement of CS+ | CS+ Type | % of learners (% male) | Special note |
|---|---|---|---|---|---|---|
| **3.1** | 20 (6) | 80 | 65% | Scream | 90% (28%) | None |
| **3.2** | 9 (1) | 40 | 95% | Scream | 89% (13%) | None |
| **3.3** | 18 (6) | 80 | 40% | Scream | 95% (36%) | None |
| **3.4** | 24 (3) | 80 | 50% | Scream | 83% (10%) | Participants movement restriction period was jittered from 2-8 sec |
| **4** | 23 (14) | 80 | 50% | Shock | 78% (62%) | FMRI, Participants movement restriction period was jittered from 2-8 sec |

# Chapter 1

## Introduction: Anxiety and context

## What are anxiety disorders?

Anxiety disorders are highly prevalent and debilitating psychiatric disorders (Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995; Neria, Nandi, & Galea, 2008; Neria, DiGrande, & Adams, 2011). The National Institute of Mental Health (NIMH) reports that anxiety disorders affect about 19 million adults in the USA alone. Mostly these disorders begin between childhood and early adulthood, occurring at a higher frequency in females than males. Symptoms of anxiety include worry and uneasiness, accompanied by somatic symptoms like sweating and elevated heart rate. This cluster of symptoms is similar to fear, but anxiety differs from fear because the symptoms appears in the absence of a clear threat stimulus and are often sustained for extended periods of time. While occasional bouts of anxiety are extremely common, anxiety disorders are generally not diagnosed unless the anxious symptoms are frequent, prolonged, and impairing, as outlined in the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5). Diagnosis of an anxiety disorder includes the presence of symptoms for at least 6 months, and requires that symptoms are so severe that they interfere with a person's ability to lead a normal life. Anxiety disorders include panic disorder, social anxiety disorder, stress disorders, specific phobias, separation anxiety disorder, and generalized anxiety disorder. Other pathological anxiety includes acute stress disorder and post-traumatic stress disorder (PTSD). PTSD was classified as an anxiety disorder in DSM-IV, but has been reclassified as a trauma and stressor related disorder in DSM-5. Still, PTSD shares many symptoms with anxiety disorders. The symptoms of anxiety disorders depend and vary with the type of disorder, still, some of the most general and common symptoms are feelings of panic and uneasiness, uncontrollable worry, sleep perturbations, shortness of breath, and palpitations, among others.

All psychiatric diagnoses are based on self-reported symptoms rather than an underlying mechanism. Based on the multidimensional and broad range of

symptoms presented from patient to patient, research has been trying to find biologically-informed markers of psychiatric disorders. For this purpose, the NIMH developed the Research Domain Criteria (RDoC) to integrate self-reported symptoms with behavioral, neural circuity, genetic, and molecular findings to address psychopathologic research and create biologically-informed psychiatric diagnosis.

## Why are anxiety disorders bad?

The physiological manifestations of anxiety can be both unpleasant and result in detrimental health consequences such as hyper-secretion of cortisol, increased blood pressure, and heart rate. The primary behavioral consequences of anxiety are avoidance and hyper-vigilance for threat, which can lead to a lower quality of life. Anxiety disorders can also disrupt normal personal and social development, especially when they begin early in life. Anxiety can cause adaptive responses when it occurs infrequently, and in response to legitimate threats, but in anxiety disorders, these responses become chronic and exaggerated and, in many cases, without a legitimate stimulus. Such exaggerated responses can result from emotional dysregulations of brain systems involved in defensive responses (Pine, 2007). Most anxiety disorders typically appear in childhood and adolescence, and although for most individuals the disorder remits, for some it does not. The appearance of anxiety in childhood substantially increases the likelihood of chronic psychiatric conditions throughout life, particularly for internalizing disorders like anxiety and depression.

## How anxiety is typically studied in experimental paradigms

One of the most common ways to study anxiety is through aversive conditioning, which is a form of learning where a neutral stimuli or context (e.g., a tone or a room, respectively) is associated with an aversive stimulus (e.g., an electric shock), resulting in the expression of fear and anxiety response to the originally neutral stimuli or context. Many studies of anxiety use electric shock or loud noises to elicit a physiological or psychological response.

Several studies have examined ways to minimize the return of anxious responses. To do this minimization, many studies investigate eliminating the learned fear or anxiety response (called “extinction”) by presenting the original neutral stimulus or context without the aversive stimulus, until the expression of the anxious response vanishes. The return of the extinguished conditioned response initially observed, when the threat stimuli is encountered outside of the extinction context, is known as “renewal”. Spontaneous recovery (SR) is another phenomenon that results in the return of fear or anxiety in the extinction context after a period of time has elapsed since extinction. SR has been observed after a long delay following the extinction training. A couple of studies have examined renewal of fear or anxiety and spontaneous recovery of aversive conditioning by changing the time between learning and extinction (Huff, Alba Hernandez, Blanding, & LaBar, 2009; Vervliet, Baeyens Van den Bergh, & Hermans, 2012). Findings show that delayed extinction training shows a brief form of fear or anxiety renewal that re-extinguished inside the testing session. Furthermore, spontaneous recovery is weaker after delayed extinction than after extinction conducted immediately after aversive conditioning. Extinction conducted after conditioning yields both spontaneous recovery and prolonged fear or anxiety renewal (Huff, Alba Hernandez, Blanding, & LaBar, 2009; Vervliet, Baeyens, Van den Bergh, & Hermans, 2012).

## Physiological manifestation of anxiety

Physiological responses have often been observed and correlated with anxiety disorders and have been used as measures of anxiety. The main physiological response to threats is the release of cortisol, a hormone released in response to stress, fear, or anxiety by the hypothalamic-pituitary-adrenal (HPA) axis. Grillon et al. (2011) found that administering cortisol (hydrocortisone), in humans, selectively increased anxiety, but not fear, supporting the idea that cortisol plays a specific role in anxiety. Thus, in order to prepare for a fight or flight situation, the release of cortisol increases blood flow and pressure, heart rate, perspiration, and causes the pupils to dilate. This response causes several physical effects that include heart palpitations (tachycardia), shortness of breath, sweating, and in extreme cases nausea, stomachaches, and even headaches. Cortisol is a direct measure of anxiety, which can be extracted from blood or saliva, but indirect measures of anxiety, such as perspiration, are most commonly used. One example of a psychophysiological measurement is skin conductance. This method measures the electrical conductance of the skin caused by variation in the skin moisture (sweat) (Figure 1).

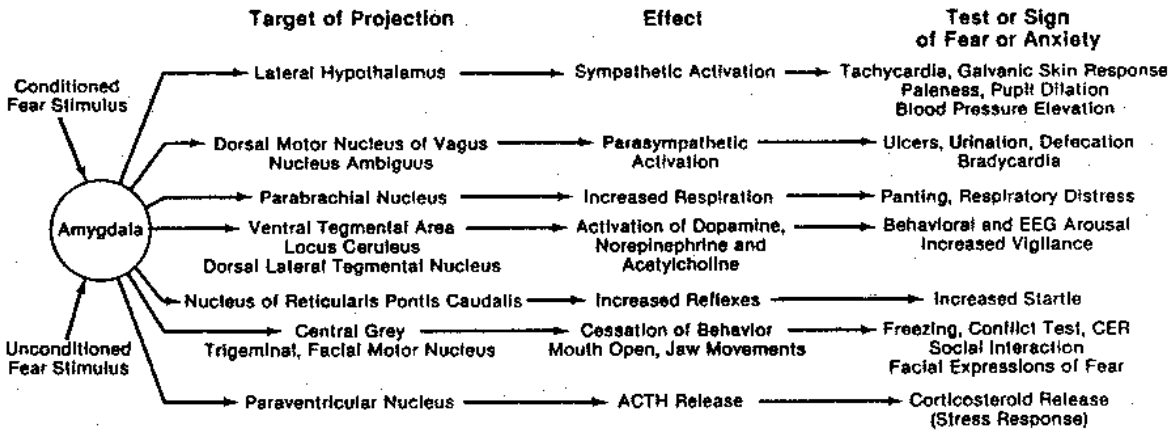


***Figure 1: Physiological and cognitive manifestations of anxiety.***

*Schematic diagram showing direct connections between the amygdala and other brain areas with their physiological and cognitive effects*

*(http://www.benbest.com/science/anatmind/anatmd7.html).*

## Cognitive manifestations of anxiety

Experimental paradigms, such as aversive conditioning, have revealed that maladaptive responses to threat involve cognitive distortions that underlie anxiety in the presence of a threat. Anxiety can enhance or impair cognitive processing (Dolcos, Iordan, & Dolcos, 2011). It is often accompanied by a higher expectation and increasing thoughts of threat, which are associated with increased avoidance, vigilance, and attention to potentially dangerous stimuli (Epistein, 1972; Rosen, Schulkin, 1998). Previous studies have shown that anxiety, in healthy individuals, produced by threat of shock increases attention control, supporting the benefits of anxiety as an adaptive response (Grillon, Robinson, Mathur, & Ernst, 2015; Robinson, Krimsky, & Grillon, 2013). Subtler factors also influence the learning and expression of anxiety. Anxiety can be affected by the closeness and similarity of

events or stimuli (Lissek et al., 2008). Expectation and uncertainty also play a role in fear or anxiety responses. The absence of an expected learned aversive event (a form of prediction error) has been shown to produce a high reactivity to the stimulus. In turn, prediction error created an uncertainty to the stimulus that later elicited generalization to stimuli that never predicted the US (Dunsmoor & LaBar, 2012). The ability to successfully predict and differentiate threatening from safe cues, or events, requires flexible learning to update previously learned information, as we gather more data, or as it changes in the context of environment. Furthermore, this knowledge should be used to make decisions in novel situations.

Inflexibility to adjust to changing conditions has been attributed to anxiety disorders. That is, anxious people show deficits in their ability to flexibly "shift" or update fear and anxious responses (Schiller, Levy, Niv, LeDoux, & Phelps, 2008). It is thought that flexible learning uses more spatial strategies, i.e., using the environment and surrounding landmarks to create a spatial representation during learning that can be transferred to novel situations. That is, flexible strategies require an allocentric approach, which is an approach where the reference frame is based on the external environment, and it is independent of one's current location in the environment. Therefore, the location of one object is defined relative to the location of other objects. On the other hand, stimulus driven strategies, i.e., using a single stimulus or an egocentric perspective of the environment, have been shown to be more structured and inflexible (Mishkin & Petri, 1984; O'Keefe & Nadel, 1978). Inflexible learning strategies (i.e., stimulus driven strategies), engage an egocentric approach, which is an approach where the reference frame is based on one's own location within the environment; the location of one object is defined relative to the body axes of the self. Anxiety disorders and post-traumatic stress disorder (PTSD), have been found to disturb allocentric spatial processing of the environment (Smith, Burgess, Brewin, & King, 2015). Even in healthy adults, stress and anxiety have been shown to stimulate this inflexible stimulus driven

strategy, which is postulated to be less cognitively demanding than a spatial strategy (Kim, Lee, Han, & Packard, 2001; Schwabe, Wolf, & Oitzl, 2010). This inflexibility of learning has been shown to be modulated by stress, and is particularly aggravated by early life and prenatal stress (Schwabe, Wolf, & Oitzl, 2010; Schwabe et al., 2007; Schwabe, Bohbot, & Wolf, 2012).

As presented, a person's context often seems to play an important role in threat learning. Studies have looked at the effect of conditioning in one environment and extinguishing the learned behavior in another environment (Herry et al., 2008; Orsini et al., 2011). Patients with anxiety disorders often overgeneralize threat into inappropriate contexts. That is, inflexible learning strategies often leads to very concrete association of stimulus-cue representation. For example, the previous association of a light with an electric shock in a given context provokes an anxious response, which can also be found in distinct safe environments where the light has never been paired with a shock. Therefore, even if anxious responses can be extinguished in one context, overgeneralization may lead to persistence of anxiety in other contexts. Flexible learning requires a level of awareness of the context, and other signals, in order to differentiate between a safe and a dangerous stimulus. For example, cues and context might predict the averseness of an event or stimulus, therefore attention to these signals are important to elicit appropriate behavioral responses. Without awareness of the cue or context association, even healthy participants have generalized fear and anxious responses in the presence of safety signals (Baas, van Ooijen, Goudriaan, & Kenemans, 2008; Baas, 2013).

## Contextual aversive conditioning

Experimental aversive conditioning paradigms in animals and in humans have revealed that anxiety and fear can be modulated by the context. Context is a

very important aspect of aversive conditioning because context helps manifest appropriate behavioral and anxious responses when there is no danger. For example, a study with rodents reported that, after cue conditioning, rodents still displayed anxious responses in the conditioning chamber in the absence of the CS+ (Blanchard & Blanchard, 1972). Similarly, in humans, the context in which the cue conditioning occurs has also been shown to be encoded during aversive conditioning. This aspect creates a context-dependent anxious response in which the cue triggers a strong conditioned response in the conditioned context. Furthermore, the same cue when presented in a novel context has been shown to have a weaker conditioned response (Alvarez et al., 2008; Ameli, Ip, Grillon, 2001; Grillon, 2002; Huff et al., 2010). An individual who cannot differentiate between a safe and a threatening context manifests exaggerated anxious responses, which underlie anxiety disorders (Grillon, 2002; Pine, 2007).

Accordingly, aversive conditioning paradigms have shown that a fear and anxious response can be triggered not only by the presentation of an aversive conditioned stimulus, but also by the presentation of the context where the association was made. Typically, healthy individuals are able to distinguish between a safe and dangerous context. For example, healthy participants can learn that a light in context A signals threat, but it does not in context B. That is, when healthy individuals associate a cue to an aversive stimulus, they display a fear and anxious response that is dependent on the context in which the association was made (CS+). However, the same cue in a different context (CS-) elicits a weaker fear response. By contrast, individuals with anxiety disorders often exhibit a higher fear response to the CS-, similar to the CS+, which healthy individuals do not show. In addition, they also display a higher reactivity to the CS+ compared to healthy individuals (Britton, Lissek, Grillon, Norcross, & Pine, 2011; Grillon, Pine, Lissek, Rabin, & Vythilingam, 2009; Kheirbek, Klemenhagen, Sahay, Hen, 2012; Pine, 2007). These experimental findings have suggested that the

inability to distinguish threatening from safe conditions is a cardinal characteristic of pathological anxiety. That is, healthy individuals display a pattern of reactivity to the conditioned stimulus paired with a dangerous cue (CS+), but not to the conditioned stimulus unpaired with a dangerous cue (CS-). On the other hand, patients with anxiety disorders react to both CS+ and CS-. This inability to identify danger signals (CS+) leads to a heightened anxious state, where cue discrimination acts as an important regulator of fear and anxious responses (Baas, 2013; Baas & Heitland, 2014). This overgeneralization of fear and anxious responses to emotional stimulus is a common trait of anxiety disorders.

The described experimental findings show that the inability to distinguish threatening versus safe conditions is a prevalent characteristic of anxious behavior. Context and aversive events seem to play an important role in fear and anxiety, with psycho-physiological correlates that have been used to measure fear and anxiety responses. Still, anxiety disorders are mostly defined based on self-report of distress and avoidance. Therefore, finding biological markers of anxiety disorders, such as brain activity, may be essential for improving diagnosis, treatment, and even prevention of these debilitating disorders.

# Chapter 2

## Brain activity during threat learning

Pattern separation is the ability to separate components of memories into distinct experience representations to make them unique and distinguishable (Watson & Rayner, 1920). For example, everyday routines, like parking your car at work, might start to mush together after doing it several days in a row. Pattern separation allows us to take these similar everyday memories and separate them by time, making finding your car in the parking lot easier. On the other hand, generalization is making broad representations of a memory, for example identifying a duck as a bird, only by knowing its characteristics of having feathers, a beak, lying eggs, etc. Both pattern separation and generalization are important components for making decisions about the environment and their dangers. Animal and human research suggests that contextual learning and pattern separation underlie the process of discrimination between safety and threat. When pattern separation or generalization are not properly controlled, it can lead to an overgeneralization of the memory.

The famous case of little Albert is a great example of overgeneralization. After aversive conditioning to a white mouse, little Albert started exhibiting fear responses to other white furry objects, even the experimenter's beard. Overgeneralization involves a response to a neutral stimuli similar to the response provoked by an aversive event, even in the presence of safety cues. Furthermore, overgeneralization is thought to be a trait that underlies pathological anxiety and PTSD (Britton, Lissek, Grillon, Norcross, & Pine, 2011; Grillon, Pine, Lissek, Rabin, & Vythilingam, 2009; Grillon, 2002; Herry et al., 2008; Kheirbek, Klemenhagen, Sahay, & Hen, 2012; Lissek et al., 2008; Orsini, Hyun Kim, Knapska, & Maren, 2011; Pine, 2007), where patients have difficulty regulating their response between threat and safe contexts. Although data suggests that these two processes are compromised in clinical anxiety and PTSD, the underlying neural basis of safety and threat discrimination is poorly understood even in healthy adults. Still, research using functional neuroimaging, neurocognitive assessment, and animal models has implicated several brain regions that are likely to be involved in the process of

pattern separation and context conditioning. These include the amygdala, the hippocampus (HPC), and the prefrontal cortex (PFC), mainly the ventral prefrontal cortex (vPFC).

It is hypothesized that contextual learning depends heavily on the integrity of the PFC and the HPC (Kim, Lee, Han, & Packard, 2001; Maren & Holt, 2000; Milad et al., 2007; Schwabe et al., 2007; Schwabe, Bohot, & Wolf, 2012). Specifically, the anterior HPC (aHPC) is associated with representation of threat and the ventromedial PFC (vmPFC) in the representation of safety signaling after 'extinction' of the CS+ (Bannerman et al., 2004; Burgess, Maguire, & O'Keefe, 2002; Fanselow & Dong, 2010; Gruber & McDonald, 2012; Laird et al., 2011; Linnman et al., 2012; Milad & Quirk, 2002; Wang et al., 2012). In addition, dorsal anterior cingulate cortex (dACC) activity has been implicated in threat signaling and fear renewal (Linnman et al., 2012; Robinson, Charney, Overstreet, Vytal, & Grillon, 2012; Robinson et al., 2014; Shin et al., 2009; St. Jacques, Dolcos, & Cabeza, 2010).

Pattern separation is also associated with the hippocampal formation. The process of pattern separation, which takes similar experiences and cues, such as context, and encodes them as distinct memories, is mainly attributed to dentate gyrus (Bakker, Kirwan, Miller, & Stark, 2008; Leutgeb, Leutgeb, Moser, & Moser, 2007; Marr, 1971). The PFC and HPC are highly interconnected areas implicated in exchange of information to support learning and memory, especially, when the outcome of an event is determined by the context (Roy, Shohamy, & Wager, 2012). It has been proposed that deficits in the medial PFC (mPFC) and HPC integrity lead to overgeneralization through faulty contextual learning and pattern separation (Kheirbek, Klemenhagen, Sahay, & Hen, 2012). To further support this point, it has been found that patients with anxiety disorders and PTSD show reduced grey matter volume and activity in the mPFC and HPC compared to

healthy individuals (Linnman et al., 2012; Robinson, Charney, Overstreet, Vytal, & Grillon, 2012; Robinson et al., 2014; Shin et al., 2009; Sotres-Bayon, Sierra-Mercado, Padilla-Delgado, & Quirk, 2012; St. Jacques, Dolcos, & Cabeza, 2010; Wang et al., 2010). This research supports the importance of an intact PFC, amygdala, and hippocampus, especially the hippocampus due to its ability to separate similar memories into distinct memories, for context conditioning and discrimination.

## Amygdala

One key brain region in processing threat and anxiety is the amygdala. One of its functions is to process memories and emotional reactions. These functions rely on the amygdala's wide connectivity with other brain areas related to the processing of threat. Anticipation of loss activates the amygdala and the hippocampus, strengthening their functional connectivity (Hahn et al., 2010). Other important connections of the amygdala are with the orbital and medial PFC, including the anterior cingulate cortex, as well as with subcortical structures, including hypothalamus, periaqueductal gray, many brainstem nuclei, and peripheral components of the autonomic nervous system (Figure 2). The functional role of these brain structures depends on neurotransmitters, such as serotonin and norepinephrine, and their receptors. Their function has been further explored by the action of specific drugs, such as the anxiolytic effect of selective serotonin re-

uptake inhibitors (SSRI), which act on serotonin reuptake transporters and are widely prescribed for treating mood and anxiety disorders.

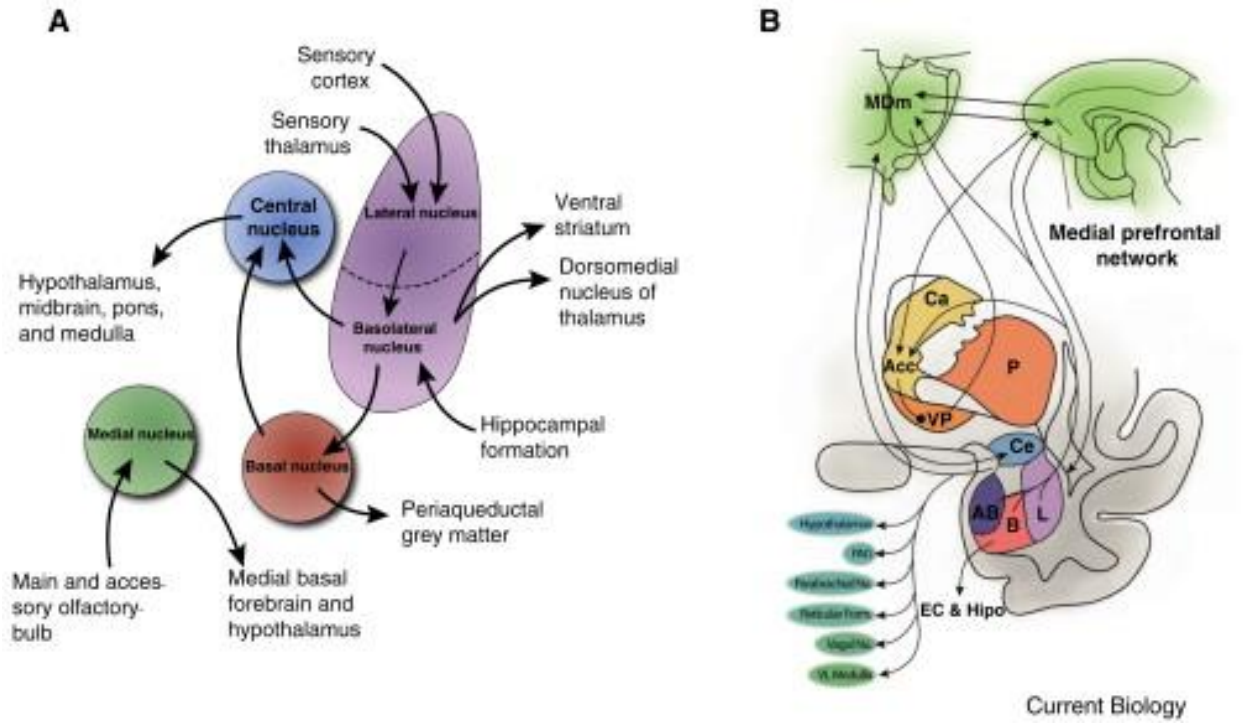


***Figure 2: The Amygdala.***

*A) Main amygdala nuclei and their inputs and outputs. B) Amygdala connectivity with other brain areas.*

*(http://www.sciencedirect.com/science/article/pii/S0960982212014352)*

The amygdala is implicated in the storage of threat memory and the ability to discriminate between conditioned stimulus (CS+ and CS-) (Britton, Lissek, Grillon, Norcross, & Pine, 2011). In classical aversive conditioning experiments, the learning of an association between a simple neutral stimulus (CS), such as a light or tone, with an aversive stimulus (unconditioned stimulus, US), such as a painful electric shock, has been closely identified with the basolateral amygdala in

both rodents and humans (Alvarez et al., 2008; Bechara et al., 1995; Blanchard & Blanchard, 1972; Britton, Lissek, Grillon, Norcross, & Pine, 2011; Chudasama, Izquierdo, & Murray, 2009; Feinstein, Adolphs, Damasio, & Tranel, 2011; Kalin, Shelton, & Davidson, 2004; LaBar, LeDoux, Spencer & Phelps, 1995; Machado, Kazama, & Bachevalier, 2009). Amygdala activity differences, between CS+ and CS-, have been found in participants who showed successful discriminative threat learning, i.e., higher activity in the amygdala during presentation of CS+ for participants with successful threat learning (van Well et al., 2012). This finding is consistent with studies showing impaired aversive conditioning due to amygdala inactivity in both humans and animals (Bechara et al., 1995; Blanchard & Blanchard, 1972; Chudasama, Izquierdo, & Murray, 2009; Feinstein, Adolphs, Damasio, & Tranel, 2011; Feinstein et al., 2013; Kalin, Shelton, & Davidson, 2004; LaBar, LeDoux, Spencer & Phelps, 1995; Machado, Kazama, & Bachavalier, 2009). In addition, amygdala activity has been correlated with changes in skin conductance during aversive conditioning (Petrovic, Kalisch, Pessiglione, Singer, & Dolan, 2008).

More specific activity responses to aversive conditioning are found in different nuclei of the amygdala. Both basolateral and centro-cortical amygdala nuclei are able to discriminate responses to conditioned stimuli (Bach, Weiskopf, & Dolan, 2011). A cluster of neurons in the basal nuclei of the amygdala (BA) shows a selective increase in CS+-evoked spike firing during and after aversive conditioning. After extinction training, another cluster of neurons had the same selective increase in spike firing for CS+ extinction. This effect suggests that two types of neurons represent functionally distinct classes that can discriminate between extinguished and non-extinguished stimuli (Herry et al., 2008). Therefore, it is arguable that BA activity is necessary for the acquisition of extinction and for context-dependent fear renewal. Furthermore, while the dorsal amygdala, which include the central nucleus, medial nucleus, and anterior amygdala area, shows a decline in activity to an extinguish stimulus over time, the ventral amygdala, which

is comprised of the basolateral complex and cortical nucleus, shows a persistent activity for previously threat-related stimuli (Morris & Dolan, 2004). Moreover, basolateral nucleus of the amygdala (BLA) inactivity decreased the activity of projection cells in prelimbic PFC (PL), and reduced PL responses to the conditioned tone. In contrast, vHPC inactivity decreased activity of interneurons in PL and increased PL conditioned tone responses (Sotres-Bayon et al., 2012). This activity pattern supports the idea that amygdala, especially the basolateral amygdala, sustains the initial learning of threat, as well as a more time-enduring threat memory storage.

## Prefrontal cortex

The PFC is implicated in decision-making and executive function, and is one of the last brain regions to reach maturity in the late teenage years or early adulthood. Because of this slower development, there is a growing area of interest in the PFC and differences in overgeneralization of threat between younger and adult populations. When compared to younger adults, older adults experience negatively valence pictures as being less negative (Lau et al., 2011; St. Jacques, Dolcos, & Cabeza, 2010). This difference in experience might be because adults have greater functional connectivity between the right amygdala and ventral anterior cingulate cortex (vACC), possibly reflecting increased emotional regulation, and decreased functional connectivity with posterior brain regions, resulting in decreased perceptual processing, (St. Jacques, Dolcos, & Cabeza, 2010). Moreover, adolescents tend to report less discrimination between threat/safety cues. Adolescents engage early-maturing PFC regions when compared to adults who engage late-maturing PFC regions during threat/safety discrimination (Lau et al., 2011). This suggests there is an age-related difference in threat/safety discrimination due to maturational differences in these brain areas.

Furthermore, older children (11-13) show greater threat learning and a similar pattern of generalization to that of a young adult, when compared to younger children (8-10). In addition, the fact that only children who correctly identify the CS+ display fear-potentiated startle, specifically to the CS+, shows that threat learning requires some degree of contingency awareness (Glenn et al., 2011). These studies support the view that PFC plays an important role in discrimination, and that a fully developed and functional PFC is needed for superior discrimination between stimuli.

The engagement of the ventromedial PFC (vmPFC) is also associated with learning flexibility, particularly when dissociating between a safe stimulus previously predictive of danger ("extinction") and a dangerous stimulus previously predictive of safety (a "reversal learning" paradigm) (Schiller, Levy, Niv, LeDoux, & Phelps, 2008). More specifically, rapid reversal of acquired fear and anxious responses is associated with the orbitofrontal cortex (Morris & Dolan, 2004). This flexibility to reverse the fear and anxious response is also thought to be mediated by a widespread network that includes the amygdala, striatum, and vmPFC (Schiller, Levy, Niv, LeDoux, & Phelps, 2008). On the other hand, neural activity during omission of an expected aversive event has been observed in the dorsolateral PFC (dlPFC), along with the anterior cingulate gyrus, parietal cortex, and striatum (Dunsmoor & LaBar, 2012). In addition, extinction depends on the vmPFC. Moreover, the thickness of the vmPFC has been correlated with the degree of extinction retention (Hartley, Fischl, & Phelps, 2011).

These studies suggest that the PFC, especially the mPFC, plays an important role in the modulation of threat-related processing via its interaction with other brain areas, such as the amygdala.

## Hippocampus

A substantial body of evidence emphasizes the importance of the HPC in the formation of new memories, as well as spatial navigation (Fanselow & Dong, 2010; O'Keefe & Nadel, 1978; Scoville & Milner, 1957; Squire, 1992). Going beyond the crucial mnemonic role of the hippocampus itself, the hippocampal region, along with the dorsal striatum, sensory cortex and amygdala has been identified as part of the multiple memory systems perspective (Ashby & O'Brien, 2005; Berger, 2006; Packard & Cahill, 2001). McDonald, Devan, & Hong (2004), suggest that these brain regions, involved in the organization of memory, also play an important role in emotion, decision-making, and personality. Moreover, it has been proposed that there is an emotional memory network in the brain composed of striatum, amygdala, hippocampus, and prefrontal cortex, involved in context, emotion, and pursuit of goals (Gruber & McDonald, 2012). The context-dependence of aversive conditioning, i.e., expression of the anxious response being dependent upon re-exposure to the environment in which the learning occurred, depends heavily on the hippocampus (Malin & McGaugh, 2006; Roozendaal, Griffith, Buranday, De Quervain, & McGaugh, 2003). Consistent with its role in spatial and episodic memory (Burgess, Maguire, O'Keefe, 2002), the hippocampus is thought to create a stable spatial representation of the location in which emotional (threatening) events occurs (Wang et al., 2012). Furthermore, aversive conditioning to a series of stimuli, such as context and spatial location, is strongly dependent on the HPC (Kjelstrup et al., 2002).

Within the hippocampus there is a population of cells, called place cells, that becomes active in a particular place within an environment (O'Keefe et al., 1998; O'Keefe & Dostrovsky, 1971; O'Keefe & Nadel, 1978). Place cells are active in an environment signaling the entire context, and collectively they are thought to act as a cognitive representation of specific-locations in space, forming a "cognitive

map" of the environment (O'Keefe & Nadel, 1978). Moreover, specific reactivation of hippocampal neurons, active during learning, is sufficient to induce the same behavior. This specific-location place cell representation can support the association of negative (and positive) events to that place within an environment. For example, an optogenetic study in mice reactivated hippocampal neurons, which were active during aversive conditioning learning, and found the same freezing behavior observed during conditioning. Interestingly, this effect was only seen inside the conditioning context and not in a different context, supporting the context-specific role of these cells (Liu et al., 2012). Moreover, it has been shown that the conditioned response of place cells is driven by their location-specific firing, in other words, after conditioning place cells responded only when the rat was within the place cell field (Moita et al., 2003). Additionally, Moita et al. (2004) showed that aversive conditioning causes a place cell remapping of the environment, even if the environment itself remains the same. This place cell remapping might be due to new spatial representation of the environment, which in turn could help the organism discriminate valance within the environment to motivate appropriate behaviors. Although most of this experiments have been done with aversive conditioning, other experiments showed that spatial cells in the hippocampus also support the association to reward and direct spatial behavior (de Lavilleon et al., 2015; Redondo et al., 2014).

More specifically, the hippocampus has been divided into ventral hippocampus (vHPC), which is associated with stress, emotion, and affect, and the dorsal hippocampus (dHPC), which is associated with cognitive functions such as spatial navigation (Fanselow & Dong, 2010; Viard et al., 2011).

In mice, it has been suggested that the mPFC holds the representation of anxiogenic environments carried by inputs from the vHPC (Adhikari, Topiwala, & Gordon, 2011). In support of this idea, there is a high baseline correlation of theta-frequency activity in the mPFC and vHPC, which increases in anxiogenic contexts.

This is even more so in a genetic mouse model (Serotonin 1A receptor knockout mice) that exhibits increased anxiety-like behavior, and which displays higher activity in mPFC theta power that is predictive of avoidance of aversive areas (Adhikari, Topiwala, Gordon, 2010). Lesions to the vHPC impair defensive anxious responses during exposure to potentially threatening environments (Kjelstrup et al., 2002). Higher neuronal activity in the amygdaloid basal nuclei projecting neurons in the vHPC and PL is found outside the extinguished context when compared to the neuronal activity in the extinction context. Furthermore, disconnections of the vHPC from either the BA or PL eliminate renewal (Orsini, Hyun Kim, Knapska, & Maren, 2011). This suggests that convergent inputs from both the vHPC and PL in the BA mediate the contextual control of anxiety after extinction.

It has been suggested that deficient hippocampal modulation from the PFC may underlie some emotional disorders. Rhesus monkey experiments have demonstrated that the central nucleus region of the amygdala and the anterior HPC play an important role in the neural circuit predictive of anxious temperament. This anxious temperament is highly heritable, especially metabolic activity in the hippocampus (Oler et al., 2010). Even though these two brain regions are closely linked, differential influence of genes and environment mediate anxious temperament and the risk of developing anxiety disorders.

The dHPC is theorized to store contextual threat memory (Kim & Fanselow, 1992). Damage to the dHPC results in retrograde amnesia for recently acquired contextual threat memories, but anterograde amnesia can be overcome with sufficient training (Kim & Fanselow, 1992; Zelikowsky et al., 2013). Importantly, it has been shown that the brain can recruit the mPFC, which serves as a long-term storage of contextual threat memories, in the absence of the dHPC (Zelikowsky et al., 2013). Still the memories formed outside dHPC decay with time, suggesting

that the dHPC is needed for permanence of contextual threat memories (Zelikowsky, Bissiere, & Fanselow, 2012). Overall, the HPC is thought to be responsible for episodic and spatial emotional memories by creating a stable and permanent spatial representation of the location in which emotional (threatening) events occurs (Want et al., 2012). Reduced activity in the amygdala and HPC during successful encoding of trauma memories might reflect encoding of general trauma representations, instead of detailed and contextual trauma memories (Pannu Hayes et al., 2011). These findings suggest the HPC and its interactions with other brain areas might serve not only as a memory structure (dHPC), but also as a modulator for neural processing of anxiety (vHPC), especially in relation to the context of a threatening event.

## The anxious brain

One potential characterization of anxious brain states might come from analysis of 'resting-state' fMRI and positron emission tomography (PET). During resting state, the intrinsic connectivity network refers to the activity of brain areas during a "resting state" where the participant is not doing anything, which includes large-scale functionally connected brain networks. Resting state is an approach that can be used to help understand the difference between the brains of anxious participants compared to healthy ones. It has been found that a network group cluster, which included areas such as limbic and orbitofrontal cortex, is involved in emotional tasks, such as discrimination of emotional faces and pictures, or emotional autobiographical memories (Laird et al., 2011). Furthermore, during PET scans, resting amygdala metabolism has been positively correlated with activity in the vmPFC and negatively correlated with activity in the dACC during extinction training (Linnman et al., 2012). The opposite is seen during extinction recall, resting amygdala metabolism negatively predicted activity in the vmPFC

and positively correlated with activity in the dACC. In addition, skin conductance of fear and anxiety expression during extinction recall was predicted by resting metabolism in the dACC (Linnman et al., 2012). This result suggests that resting brain metabolism predicts neuronal activity and skin conductance changes associated with recall of extinction memory. Research has shown that greater early life stress (ELS) predicts increased childhood cortisol levels, which was correlated with decreased amygdala-vmPFC resting-state functional connectivity (rs-FC) (Burghy et al., 2012). This effect was inversely correlated with concurrent anxiety symptoms, but positively correlated with depressive symptoms (Burghy et al., 2012). Thus, there might be opposing amygdala-vmPFC modulation of anxiety and depression from childhood cortisol levels that function through adolescence.

Compared to healthy subjects, individuals with anxiety disorders display higher reactivity to stimuli that signal danger, as well as those that signify safety cues (Britton, Lissek, Grillon, Norcross, & Pine, 2011; Grillon, Pine, Lissek, Rabin, & Vythilingam, 2009; Kheirbek et al., 2012; Pine, 2007). Patients with PTSD or generalized anxiety disorder (GAD) show enhancement of fear to predictable threats, but only patients with PTSD display elevated anxiety during unpredictable conditions (Grillon, Pine, Lissek, Rabin, & Vythilingam, 2009). These studies show that anxiety is characterized by a maladaptive fear response, which is frequently expressed as an overgeneralization of threat, or fear in the presence of both threatening and non-threatening (safe) conditions. In addition, an intolerance of uncertainty is also present. Anxious participants have higher fear and anxiety levels overall, which during a threat appraisal recall task, anxious participants exhibited reduced activity in the subgenual anterior cingulate and in the vmPFC, compared to healthy participants (Britton et al., 2013). In the last decade, several lines of evidence have indicated that PTSD is mediated by dysfunctional processes involving the brain's threat-circuitry network, including impaired discrimination between dangerous and safe stimulus (Shvil, Rusch, Sullivan, & Neria, 2013).

Although much is known about the psychophysiology of anxiety disorders, the neural systems underlying these disorders and their development are still not fully understood. Much work has been done trying to describe and understand anxiety in relation to the brain. Most of this work has focused on amygdala-based tasks, using divergent stimuli or contexts, which might be creating different memory for each of the components presented. In contrast, the task I created in this study and thesis addresses this gap by using a single environment, creating a single emotional memory, which requires location-specific information to discriminate between the CS+ and CS-. As a result, it allows for better understanding of neural mechanisms necessary for discrimination within an environment. This research will aid in the characterization of the neural and behavioral substrates of threat overgeneralization.

# Chapter 3

## Methods

## Methods review

All of the experiments described in this thesis employed a virtual reality task coupled with recordings of skin conductance and subjective measures of anxiety. As explained above, in order to prepare for a fight or flight situation due to exposure to a believed or direct threat, the body releases cortisol, which increases perspiration levels. Therefore, skin conductance, which depends on skin humidity (perspiration), is often used as an indirect measure of anxiety. Skin conductance includes two types of responses. The first, ***tonic response***, is a slow and prolonged response to general changes in autonomic arousal. Tonic response is measured by variations in sweat levels in long periods of time, known as skin conductance level. The second, ***phasic response***, is a rapid autonomic response to specific elements, events, or signals. Phasic response is measured by variations in sweat levels in short periods of time before or after an event or signal, known as skin conductance response. In Chapter 7, while participants performed the virtual reality task, in addition to skin conductance and expectancy ratings, I employed functional magnetic resonance imaging (fMRI) to assess the role of the amygdala, mPFC, and HPC in the learning and discrimination between safe and dangerous context.

In this chapter, I will provide an introduction to the benefits of using virtual reality as opposed to traditional threat learning experimental designs. In addition, I will provide a brief introduction to fMRI data acquisition and pre-processing. Further data analysis will be discussed in Chapter 7. For an in depth overview of the methods and procedures of fMRI not discussed in this chapter, I recommend the books “MRI The Basics” by Hashemi, Bradley, & Lisanti (2010), and “Functional Magnetic Resonance Imaging” by Huettel, Song, & McCarthy (2009).

## Virtual reality

Virtual reality (VR) is a term that applies to computer-generated environments that can simulate the physical presence of a participant in place of the real world. Most of these environments are visually displayed through a computer screen, but sometimes headsets are added. Virtual reality is becoming a great tool to study context-dependent memories, as it can create 3D conditions of context specificity from the first person perspective as opposed to simpler 2D stimuli presented on the screen. The use of VR enhances the sense of presence or immersion in the context, making the illusion of actually being present or immersed in the virtual world. For example, VR has been found to activate the same regions thought to be involved in physical navigations (Doeller et al., 2010; Hartley et al., 2003; Maguire et al., 1998). Pine et al. (2002), used fMRI to study brain areas activated during memory-guided navigation in virtual reality. They found that adolescents and adults have similar memory-guided navigation abilities, which correlated with BOLD signal in areas such as the right frontal and anterior medial temporal lobe. Furthermore, they found that adults have better allocentric memory abilities related to temporoparietal association cortex and the cerebellum. One study used fMRI while participants learned the location of objects by collecting and replacing them in a virtual environment. They found right posterior hippocampal activity reflected learning and remembering of boundary-related locations. In addition, the right dorsal striatal activity reflected learning and remembering landmark-related locations (Doeller, King, & Burgess 2008). These findings strongly support the idea of parallel memory systems centered on the hippocampus and dorsal striatum.

Furthermore, developmental and pathology studies using VR support the sensitivity of this tool reflective of differences between study cohorts. Spatial navigation in children is characterized by impaired performance, which includes

more heading direction errors and worse accuracy (Sneider et al., 2015), an effect that has been also found in anxious children (Mueller et al., 2009). In addition to age, sex also influences heading errors, where males have an overall advantage (Sneider et al., 2015). Likewise, depressed adult patients perform significantly worst at finding location on a virtual reality navigation task when compared to healthy adults (Gould et al., 2007). This difference could be due to underdevelopment or deficits in activity in brain areas related to spatial navigation, such as the PFC and HPC.

In VR, unlike more traditional contexts, participants can interact spatially with threat stimuli. Thus avoidance, the act of avoiding a possible threatening situation or stimuli, is a key behavioral manifestation of anxiety that can be mapped directly on to neural circuitry in VR-based tasks. This avoidance behavior is highly associated with maladaptive anxiety expression during extinction training. A study found that avoidance behaviors reduced extinction learning, likely due to the lack of exposure to the stimulus now predicting safety (Cornwell, Overstreet, Krimsky, & Grillon 2013). Activity in the right anterior hippocampus and bilateral amygdala related to a CS+ has been found in context conditioning. Furthermore, context conditioning has been associated with activity in posterior orbitofrontal cortex, medial dorsal thalamus, anterior insula, subgenual anterior cingulate, and parahippocampal, inferior frontal, and parietal cortices (Alvarez et al., 2008). Huff et al. (2010) described the use of a fully immersive virtual reality 3D context for aversive conditioning. They used skin conductance to compare the fully immersive environment with a laboratory setting, and found that immersion is a reliable and informative way to conduct aversive conditioning studies (Huff, Zielinski, Fecteau, Brady, & LaBar, 2010). In addition, Huff et al. (2011) used full immersion 3-D virtual reality in humans to study context-specificity of cued aversive conditioning with electric shock. They found that context threat learning occurs rapidly in humans. Differential cued aversive conditioning in humans is slower to occur than contextual conditioning. Still, cued threat learning is retained in a context-specific

manner 24h after training (Huff et al., 2011). Furthermore, conditioned response can be modulated by spatial context. In addition, increased fear and anxiety responses to a CS+ were found when CS+ were presented in a dangerous context when compared to a safe context (Degeilh et al., 2012).

Findings with VR tasks support animal and human anxiety models suggesting that the hippocampus, PFC, and amygdala play an important role in contextual threat learning. VR is an optimal approach for studying context conditioning and to help elucidate how more complex environmental situations are modulated by the aforementioned brain areas.

## Basics of functional MRI acquisition, pre-processing and analysis

Magnetic resonance imaging employs a strong magnetic field to record images of biological tissue. By changing magnetic gradients and oscillating electromagnetic fields, the scanner is able to create images as these energy fields are absorbed by molecules in the body. After being absorbed, this energy is later emitted by these molecules, whose number determines the energy level. As such, the scanner is able to detect this energy and differentiate tissue types, i.e., grey and white matter in the brain.

The body is mostly composed of hydrogen molecules (water), and so is the brain. Under normal conditions, these molecules are spinning randomly on an axis. In order to create the images, the MRI creates a strong electro-magnetic field (static field) to align these hydrogen molecules using a static coil. After the molecules aligned, a magnetic pulse is emitted by the radio-frequency coil, causing some of the molecules to absorb this energy and flip their spin axis 90 degrees to the transverse axis. Subsequently, this energy is released by the molecules as

they return to their aligned state. This energy is picked up by the radio-frequency coil to create the images. Furthermore, a gradient field is produced by a gradient coil during this process to distort the static field in a predictable way, in so determining the position of the energy signal being picked up by the coil. Depending on the type of tissue, this signal might take longer, i.e., molecules in water take longer (relaxation time) to return to their aligned state than molecules in fat. The MRI physicists take full advantage of this knowledge to differentiate between tissues in the brain and create high resolution structural images (T1-weighted images).

Functional MRI (fMRI) is a non-invasive tool extensively used in humans to measure brain activity with a relatively high spatial (~mm) and reasonable temporal resolution (~sec). FMRI uses the concept above to generate functional images by measuring changes in the oxygenation of the blood, a correlate of neuronal activity. Neuronal activity increases metabolic requirements, delivered through the vascular system in the form of glucose (ATP) and oxygen. Oxygen is bound to hemoglobin molecules, which has magnetic properties when deoxygenated. In turn, this deoxygenation of the blood creates a blood oxygen level-dependent (BOLD) signal, which changes according to function (task), and which is measured by the scanner to create T2-weighted images.

After being collected, T2-weigthed images must go through a series of “pre-processing” steps before analysis. First, the images must be realigned, as participants’ head movement causes the images to shift position. This step is necessary to align the images to each spatial location. Slice-time correction is another step necessary to account for the variability in time between each image acquired. Afterwards, the T1-weigthed structural images are co-registered to the T2-weighted functional images, so that the functional activity can be viewed over the corresponding anatomy. Finally, to look at group level analysis, individual

brains are normalized to a standard space (e.g., MNI template) and smoothed to reduce any possible anatomical variability between subjects.

After the data is acquired and pre-processed, it is ready for analysis. The first step is to carry a "first level" (*subject level*) analysis, in which the magnitude of beta weights (the regression coefficients for standardized data) corresponds to a condition that it can be compared to. Identical tests are carried out for each voxel in the brain, resulting in 3-dimensional "contrast maps". One common way to analyze these data is by an "event-related design", which is used to detect changes in the BOLD response to neural activity in response to events presented in a random fashion. The idea is to try to model or measure the transient changes in BOLD response, as the events are presented, while image acquisition is on-going. Event-related designs are analyzed by estimating thousands of univariate general linear models (GLMs), one for each voxel in each subject, using ordinary least square regression:

$$\gamma = \beta_0 + \beta_1 X_1 + \ldots + \beta_n \chi_n + \varepsilon$$

The $\gamma$ variable on the left corresponds to the BOLD signal value, i.e., the measured time course of a single voxel, which is explained by the terms on the right side of the equation (the experimental data). On the right, $\beta$ corresponds to the contribution of the regression factor to the overall data (parameter weights or beta values). $\beta_0$ would be the baseline signal intensity on each voxel and any constant activation throughout the experiment. The $\chi$ is the explanatory variables that represent factors that may contribute to the data. Variables that are of interest in the experiment (i.e., conditions) and those that are considered noise (i.e., head movement). Finally, $\varepsilon$ is the error or residual noise in the data (measurements errors).

The contrast maps are then analyzed in a "second level" (*group level*) analysis. During the second level analysis, inferences are drawn across each subject using a random effects analysis, resulting in 3-dimmensional maps of t-statistics that reflects the strength of the statistical difference between conditions. This uses another GLM:

$$\boldsymbol{\gamma = G \times \beta \times \varepsilon}$$

The fMRI data (***γ***) is represented in a data matrix consisting of *n* time points by *V* voxels. The design matrix (***G***), which is the linear model to be evaluated constructed by the experimenter based on the hypothesized effects of the experimental manipulations, consists of *M* regressors, each *n* time points in length. The parameter matrix (***β***) contains *M* parameter each weights (beta values) and *V* voxels, so that each cell indicates the *β*–value for a given voxel. The error matrix (***ε***) is an n-by-V matrix, of the residual error for each voxel. In Statistical Parametric Mapping (SPM; Welcome Trust Centre for Cognitive Neurology), the regression equations are represented as the design matrix.

I will use this GLM analysis to build the design matrix and understand the participants' brain activity, while they are learning to discriminate between the safe and dangerous area, in the virtual reality task.

# Chapter 4

## Experiment 1: Behavioral Study of Context Discrimination: Understanding the Effects of Shock Predictability and Number of Trials

## Precis

I was interested in whether participants could learn to discriminate between the danger and safety of cues when these cues were in the same context, but in different locations in this context (e.g., dangerous vs. safe zones), or whether they would generalize the threat of shock to the whole environment. I expected to detect learning of the contextual threat through differential skin conductance response (SCR), which should increase, in the dangerous zone as subjects learn the threat. In order to test this hypothesis, I created a novel virtual reality game, using Unity software (Unity Technologies, USA), to examine learning and discrimination between danger and safety zones within an environment. The environment consisted of a circular environment, with distal cues (e.g., clouds, mountains, and sun), with two beehives at opposite ends of the environment to serve as landmarks. Participants were asked to collect flowers, while viewing this scene, which could co-terminate with a bee-sting (shock). The participant was unaware that the environment was divided in half, where one beehive was dangerous, and the flowers around that beehive were sometimes paired with a shock, while the other one was safe and never paired with a shock. For the first set of experiments, I manipulated the reinforcement of a flower being paired with a shock (reinforcement rate) and the number of trials (flowers) the participant had to collect to finish the task. During the task, the reinforcement of the CS+ (shock) would increase depending on the distance to the center of the dangerous zone, within a low number of trials (Experiment 1.1), or a high number of trials (Experiment 1.2). Furthermore, I tested the effects of a high and stable reinforcement of CS+ in the dangerous zone with a low number of trials (Experiment 1.3), and the effects of a low and stable reinforcement of CS+ in the dangerous zone with a high number of trials (Experiment 1.4). These manipulations helped me understand how the reinforcement of shock and the number of trials played a role in the predictability of the shock, therefore helping with the participant's discrimination of the safe and dangerous zone.

It was expected that over time, participants would learn to discriminate between the dangerous and the safe zone in the environment. This discrimination would be indexed by differential skin conductance to the safe vs. threat context. Skin conductance would increase in the dangerous zone, during learning (Experiment 1.1). Furthermore, increasing the number of trials, would help participants improve their discrimination between the dangerous and safe zones (Experiment 1.2). That is, healthy participants would be able to discriminate when a flower is dangerous, and when it is not, based on its location within the environment with a better accuracy. Furthermore, I predicted that even with a low number of trials, a high and stable reinforcement of shock would allow sufficient learning (Experiment 1.3), similar to high number of trials, with a low and stable reinforcement of shock (Experiment 1.4).

## Method

***Participants:*** All participants (age range: 20-30 years) were recruited from the University College London student population. Before taking part, all participants provided written informed consent and, after completion, were debriefed and reimbursed for their time. The study was approved by the UCL Research Ethics Committee. All participants were right-handed and free from neurological or psychological impairment, and had not participated in any version of the task previously (all participants were naïve to the task).

***Virtual environments and conditioned stimuli:*** A circular virtual environment was created using Unity software (Unity Technologies, USA). The environment comprised of a circular grassland with a single perimeter wall (boundary), some distal cues (mountains, sun and clouds) for orientation and two local landmarks (beehives) used for localization within the environment (Figure 3

& 4). The environment was presented in a first person perspective and participants could explore using a button box with buttons corresponding move forward and turn left or right. Participants were not allowed to move backwards, to ensure they were aware of their location.

***Skin conductance:*** Skin conductance was measured as an index of arousal via 8mm Ag/AgCl electrodes attached to the medial phalanges of the index and middle fingers of the participant's left hand. Data were acquired using a custom-build constant voltage coupler (2.5V) with output converted into an optical pulse frequency. The optical signal was then converted to voltage pulses and recorded (Micro 1401/Spike 2, Cambridge Electronic Design, Cambridge, UK).

***Procedure:*** During the task, participants were instructed to move around the environment and pick flowers that appeared one at a time in random locations. On contact with a flower, the participant's key (movement) was frozen for 3000ms. This period will be referred to as "freezing" period. There were 80 trials (flowers), with 40 trials situated in each half of the environment. Half of the environment was associated with danger; flowers picked in this zone reinforced with shock (danger). On the other hand, flowers picked in the other half were never paired with shock (safe). The zones were counterbalanced across participants (Figure 5). After the freezing period had ended, participants were free to move and the next flower appeared in the environment for the next trial. Shocks were applied using a Digitimer DS7A electrical stimulator (Digitimer, Welwyn Garden City, UK) and were delivered to the left hand with intensity up to 20mA for 2ms duration through a silver chloride electrode. Shock intensity was individually adjusted prior to starting the experiment. During this workup procedure, participants were given a series of shocks, starting at a low intensity (1.2 mA), and asked to rate "how painful" each shock was on a 1-10 scale. Shock intensity was increased until reaching a level that was aversive but not painful. Subjectively the participants 10 was the aimed

threshold, where the shock was not painful but aversive and undesirable, without excessive movement from the participant.

Participants also performed spatial-memory trials within the same environment in the absence of shocks, interleaved with the flower-search trials. The spatial memory trial occurred after every 4 threat learning trials. On each spatial-memory trial, subjects were required to learn the location of one of 4 objects (wooden box, gas can, book, and clock; Figure 6), which appeared in distinct locations; two objects appeared in each half of the environment (Figure 7). For the first 4 spatial memory trials, the object appeared in a 'home' location, and participants were instructed to collect the object and memorize its location. After the initial 4 spatial memory trials, 16 memory trials were carried out (4 per object) during the experiment. During these trials, the participants' memory for object locations was tested. A static image of the object was presented in the top left corner of the screen, and the participant was required to move their position to the object's home location. Upon arriving to the guessed home position of the object, participants pressed a button to indicate their response. After responding, a feedback phase was presented in which the object appeared in its correct location and the participant had to collect it, strengthening the object location memory for the next time the same object was presented.

At the end of the experiment, participants were asked to name the four objects and their locations used during the spatial memory task and to explain the contingencies of danger and safety during threat learning. Participants who were unable to provide the objects name and position were excluded from the final analysis. Participants were verbally instructed using the following script:

*"The story is that you are trying to build a garden for that house (point at the house in the screen) by collecting those flowers (point at the flower in the screen). As you collect flowers these might have a bee inside of them which will sting you*

*and it will be represented by the electric stimulus (point at the electrode). In this environment there will be 2 beehives, one is safe and the other one is dangerous. As you collect flowers try to see if there is any relationship between the position of the beehives, the flowers, and if you are receiving a sting or not. In addition to this, you will be asked to collect other type of objects that are not flowers, please try to remember the exact location of where you pick the object from because you will be asked to place or return the object to where you found it."*

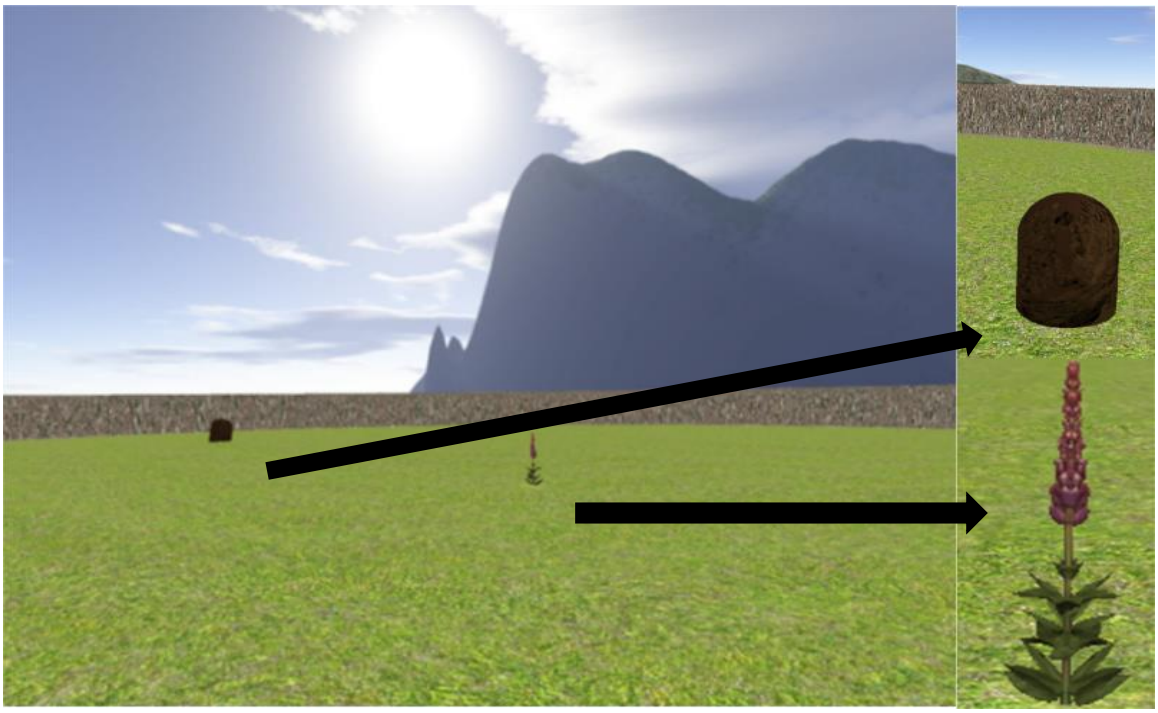

***Figure 3: First person view of the environment, flower, and beehive.***

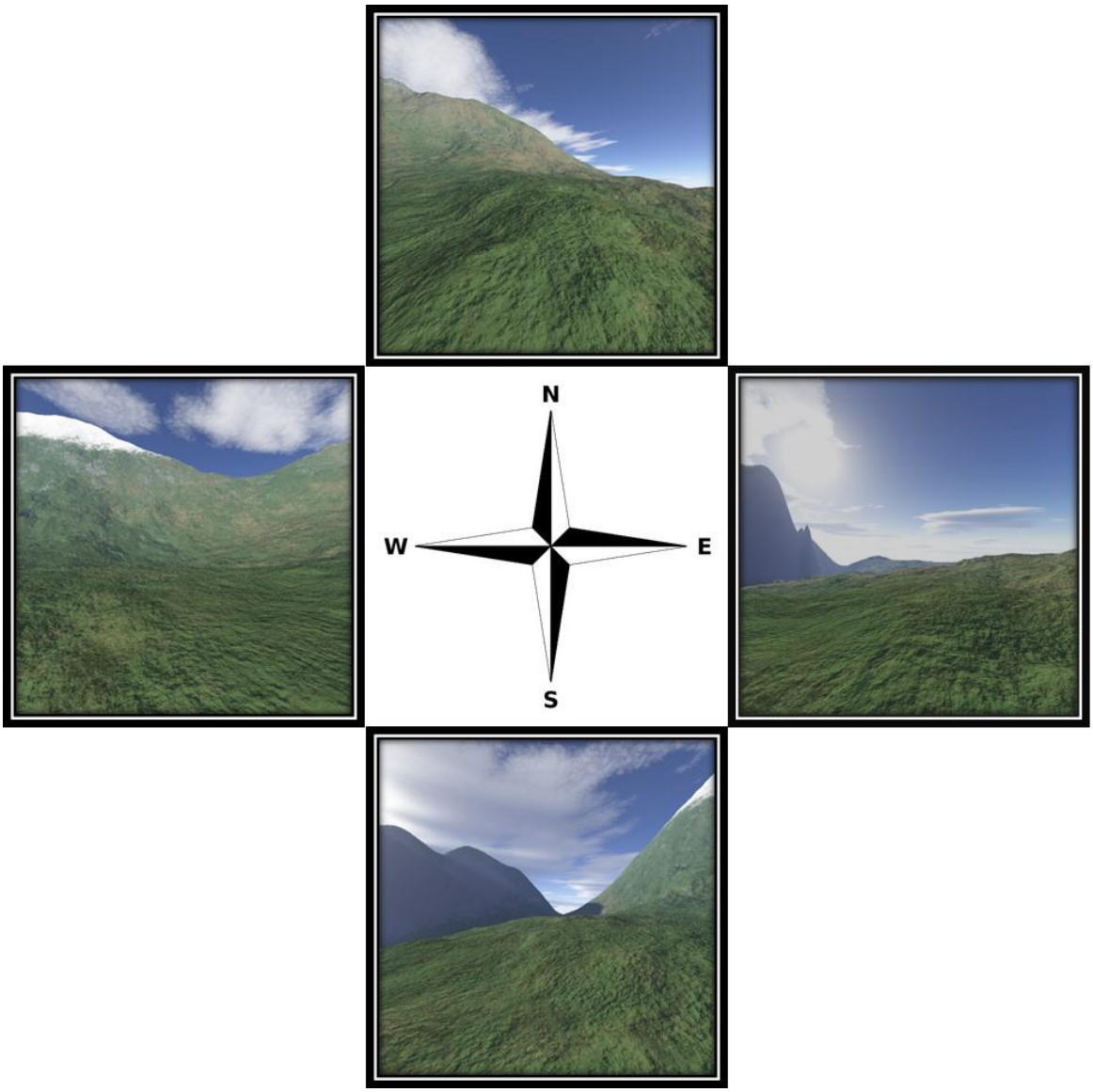


***Figure 4: 360˚ view of the environment surroundings.***

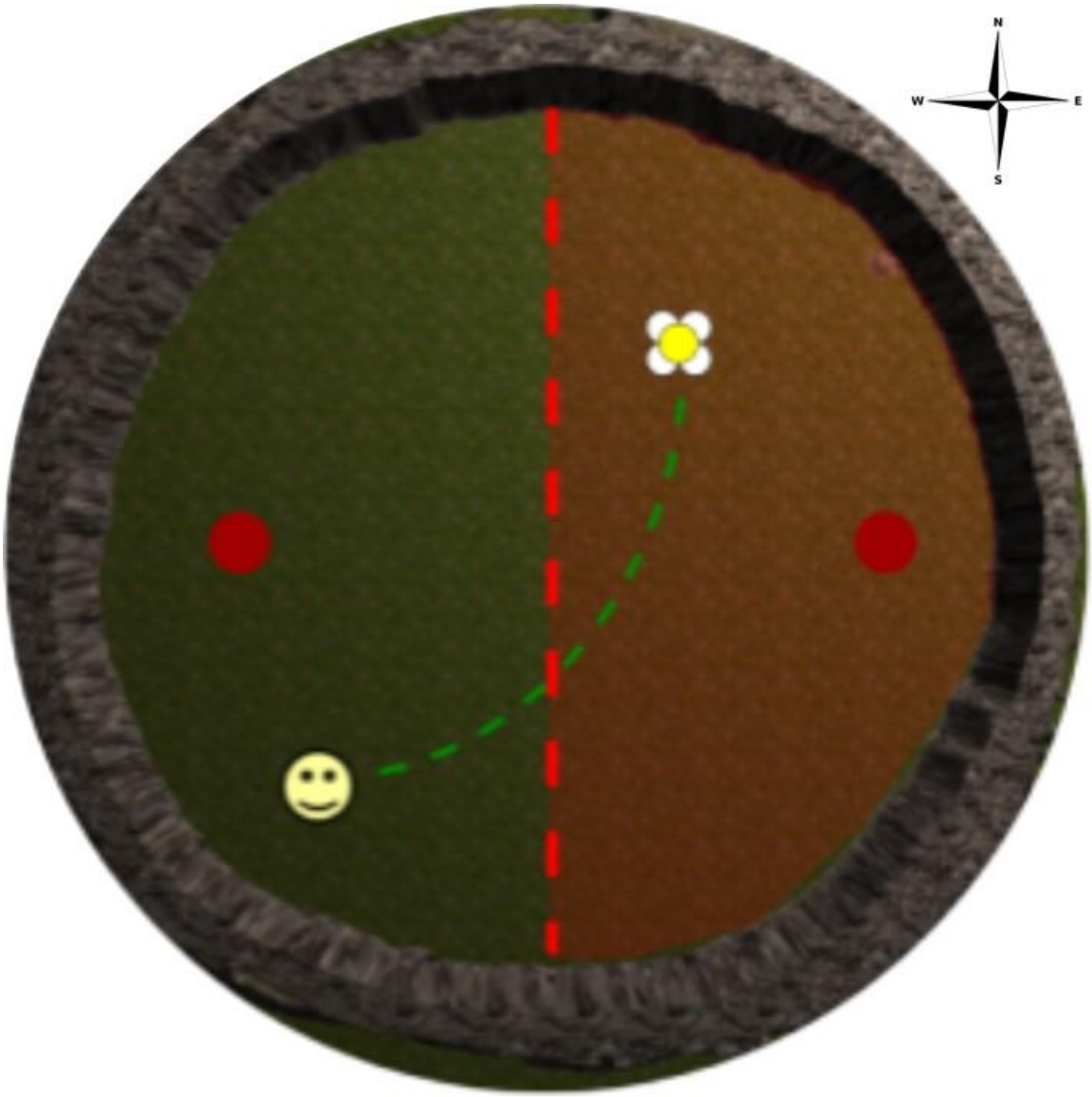


***Figure 5: Environment map, showing the divisions of the stage by zones. Participants do not see any divisions within the environment or in the floor, this image is for display purposes only.***

***Figure 6: Pictures of the objects being collected by participants in the environment.***

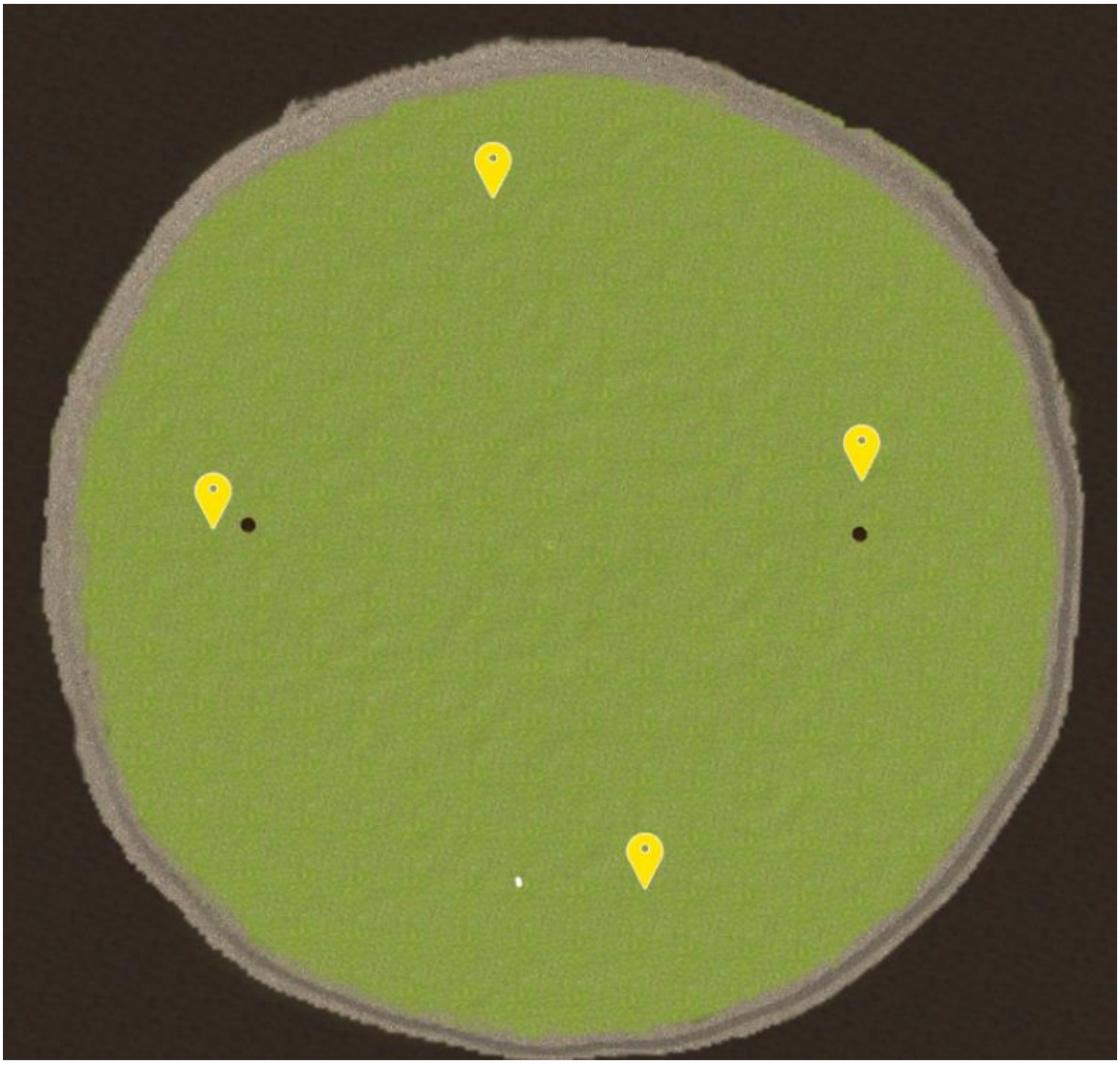

***Figure 7: Overview of the environment. The locations of the objects are marked in yellow. Participants do not see any markings within the environment or on the floor, this image is for display purposes only.***

***Data analysis:*** Skin Conductance Response (SCR) was used to measure reactivity throughout the test and during each trial. Two ring electrodes were placed on the left hand, one on the index finger and one on the middle finger,

recorded SCR. For phasic SCR data, SCR were calculated for every trial by subtracting the minimum skin conductance (baseline) during the freezing period from the maximum response (peak) before the participant is able to move again. Any response difference under 0.03 micro Siemens was scored as zero. SCR were log transformed (log [1+SCR]) to normalize the distribution, and then a range correction ([SCR-SCRmin]/[SCRmax-SCRmin]) was applied to control for individual variation in SCR (Lykken, 1972). The SCRmin and SCRmax used in this correction were the maximum and the minimum response for each participant from all their SCR during the experiment. SCRs were then averaged into four blocks representing the first (t1), second (t2), third (t3), and fourth (t4) quarters of the experiment, with each block including 10 trials per condition (safe and danger). SCRs were analyzed by using a General Linear Model (GLM) for repeated measures. Bonferroni-corrected post-hoc comparisons were conducted. For all analyses, an alpha level of 0.05 was used.

Finally, performance on the spatial memory task was analyzed by assessing distance error on each test trial. This error was calculated by taking the distance in virtual meters between the participant's response location when replacing the object and its correct location within the environment. Distance error was taken from each trial and averaged into 4 blocks (1 trial from each object in each block; 4 trials per block). All results were analyzed using a General Linear Model (GLM) for repeated measures using 2x4 ANOVAs to look for changes between conditions (safe, danger) and block (1 to 4). Bonferroni-corrected post-hoc comparisons were conducted and an alpha level of 0.05 was used.

# Experiment 1

## Experiment 1.1: Increasing reinforcement of shock & 40 trials

### Introduction

With this initial experiment I sought to create a virtual reality paradigm to examine the processes involved in learning and discriminating between a safe and dangerous zone within a single environment, using location-specific cues from an aversive stimulus. In preparation for fMRI studies, with this exploratory experiment I wanted to examine the effect of location-specific context conditioning.

With this paradigm, I predicted that over time participants would be able to discriminate between the dangerous and the safe zones of the environment, which would be indicated by SCR that increased in the dangerous zone during learning. With the object task I verified that participants were engaging in the task. In addition, I expected an increased awareness of spatial location if they were able to discriminate safety vs danger on the basis of location. Furthermore, I hypothesized that the emotional aspect of the zones might enhance or impair the cognitive processing of the objects' location.

### Methods

***Participants:*** twenty-one healthy volunteers (17 males), with an average age of 23 years, were recruited for this experiment.

***Aversive conditioning procedure:*** The participant had to collect 2 flowers in between objects. There were 40 flowers in total, an average of 19 trials in the dangerous zone (11 CS+). For this experiment, the dangerous half of the stage was further divided in 3 dangerous zones. That is, there were three levels of danger within the dangerous zone, which increased the reinforcement of shock as the participant approached the dangerous side (the hive). The reinforcement of shock would increase, in increments of 15%, starting at a 65% reinforcement of shock, once the participant entered the outermost part of the dangerous zone. At the end of the experiment, participants were asked to identify the dangerous side. Only 13 participants (62%) were able to identify the dangerous zone and were considered "learners" (11 males).

## Results

### Object location memory

A 2x4 within-subjects ANOVA (zone x block) measuring error rate of placing the objects showed that error decreased significantly over block for learners and non-learners ($F(3,60)=17.72$, $p<0.01$) (Figure 8a). It was independent of the zone ($F(1,20)=0.16$, $p>0.05$) in which the object was found and there was no significant interaction ($F(3,60)=1.79$, $p>0.05$). The error rate of placing the objects for the non-learners showed a significant effect of block ($F(3,15)=9.18$, $p<0.01$), which shows a reduction of error over time. There was no significant effect of zone ($F(1,5)=0.38$, $p>0.05$) or zone x block interaction ($F(3,15)=1.25$, $p>0.05$) (Figure 8b). The error rate of placing the objects of the learners showed a significant difference of block ($F(3,18)=9.41$, $p<0.01$), which shows a reduction of error over time. There was no significant effect of zone ($F(1,6)=0.95$, $p>0.05$) or zone x block interaction ($F(3,18)=0.62$, $p>0.05$) (Figure 8c).

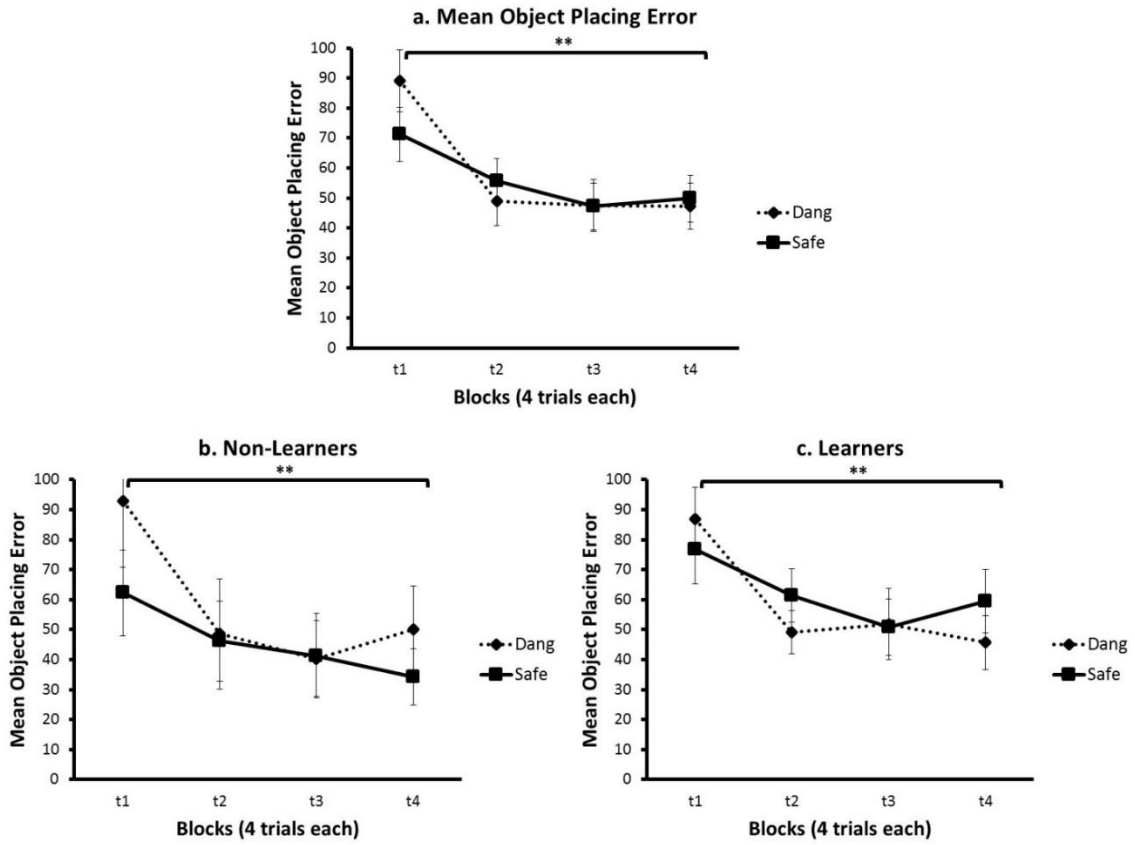


***Figure 8: Experiment 1.1 Mean object placement error.***

*Mean object placement error, for the safe zone (Safe) and the dangerous zone (Dang) over time for a) learners and non-learners together, b) non-learners, and c) learners. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

## Skin conductance response

A 2x4 within-subject ANOVA (zone x block) was performed on the phasic SCR data for the learners and non-learners. There was a trend towards a difference in the zone ($F(1,20)=3.68$, $p=0.069$). There was no significant effect of block ($F(3,60)=0.00$, $p>0.05$), or zone x block interaction ($F(3,60)=0.98$, $p>0.05$)

for the data of the learners and non-learners together (Figure 9a). A t-test between dangerous and safe zone at each block point showed a significant difference only for t2 ($p<0.0125$ Bonferroni corrected). The phasic SCR data for the non-learners showed a significant difference of zone x block interaction ($F(3,21)=3.37$, $p<0.05$), which showed an increased SCR over time blocks in the safe zone, while SCR decreased over time blocks in the dangerous zone. There was no significant effect of block ($F(3,21)=0.62$, $p>0.05$) or zone ($F(1,7)=0.12$, $p>0.05$) (Figure 9b). The phasic SCR data for the learners showed a trend difference of zone ($F(1,12)=3.86$, $p=0.073$), showing higher SCR during the danger zone compared to the safe zone. There was no significant effect of block ($F(3,36)=0.06$, $p>0.05$) or zone x block interaction ($F(3,36)=0.67$, $p>0.05$) (Figure 9c). A t-test between dangerous and safe zone at each block point showed a significant difference for t2 and t4 ($p<0.05$ uncorrected), but they were not significant when Bonferroni corrected.

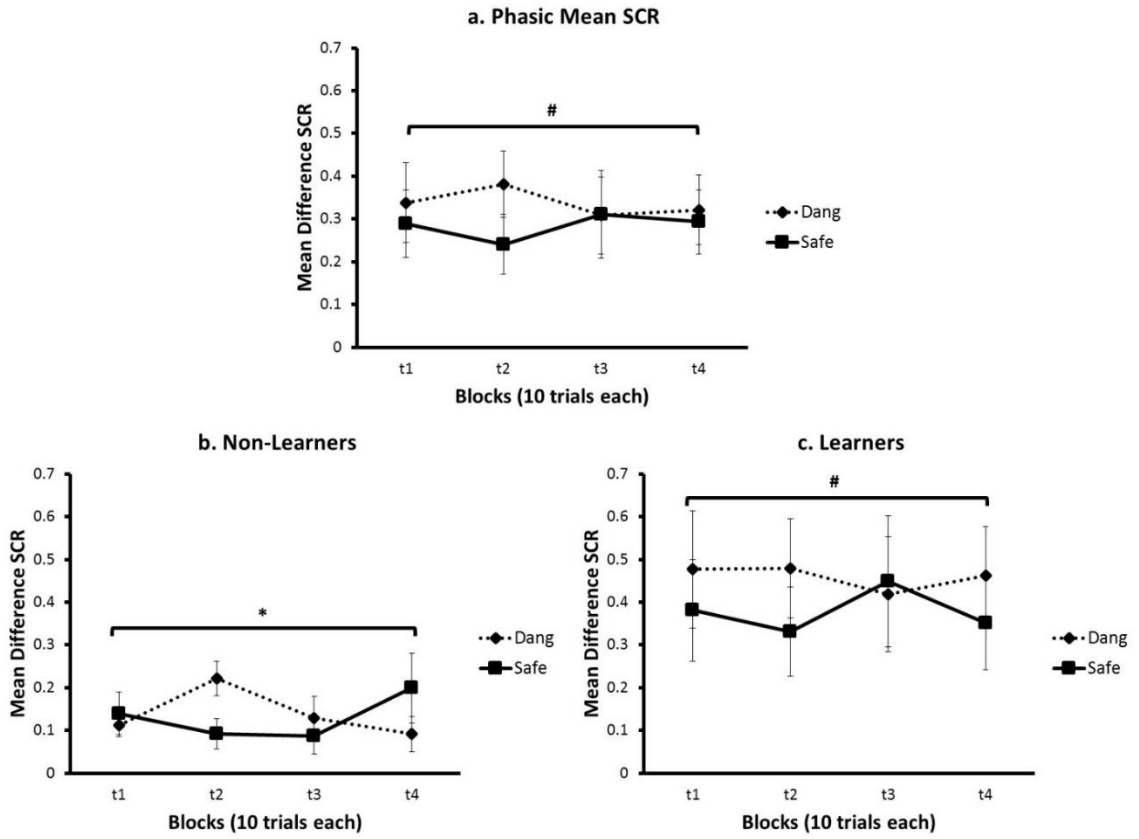


***Figure 9: Experiment 1.1 Mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) zone over time for a) learners and non-learners together, b) non-learners, and c) learners. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

Since I did not observe a zone x block interaction over the four quarters of the experiment, and there were visible differences between SCRs in the safe and dangerous zones in the first quartile, I decided to take a closer look in that first block point. To do this comparison, I divided the first quartile into 4 more quartiles. A 2x4 within-subject ANOVA (zone x block) of phasic SCR data for the learners and non-learners showed no significant effect of block ($F(3,60)=0.71$, $p>0.05$), zone ($F(1,20)=0.43$, $p>0.05$), or zone x block interaction ($F(3,60)=1.44$, $p>0.05$)

(Figure 10a). The phasic SCR data for the non-learners showed no significant effect of block ($F(3,21)=0.32$, $p>0.05$), zone ($F(1,7)=0.39$, $p>0.05$), or zone x block ($F(3,21)=0.29$, $p>0.05$) (Figure 10b). The phasic SCR data, for the learners, showed no significant effect of block ($F(3,36)=0.49$, $p>0.05$), zone ($F(1,12)=0.63$, $p>0.05$), or zone x block interaction ($F(3,36)=1.53$, $p>0.05$) (Figure 10c).

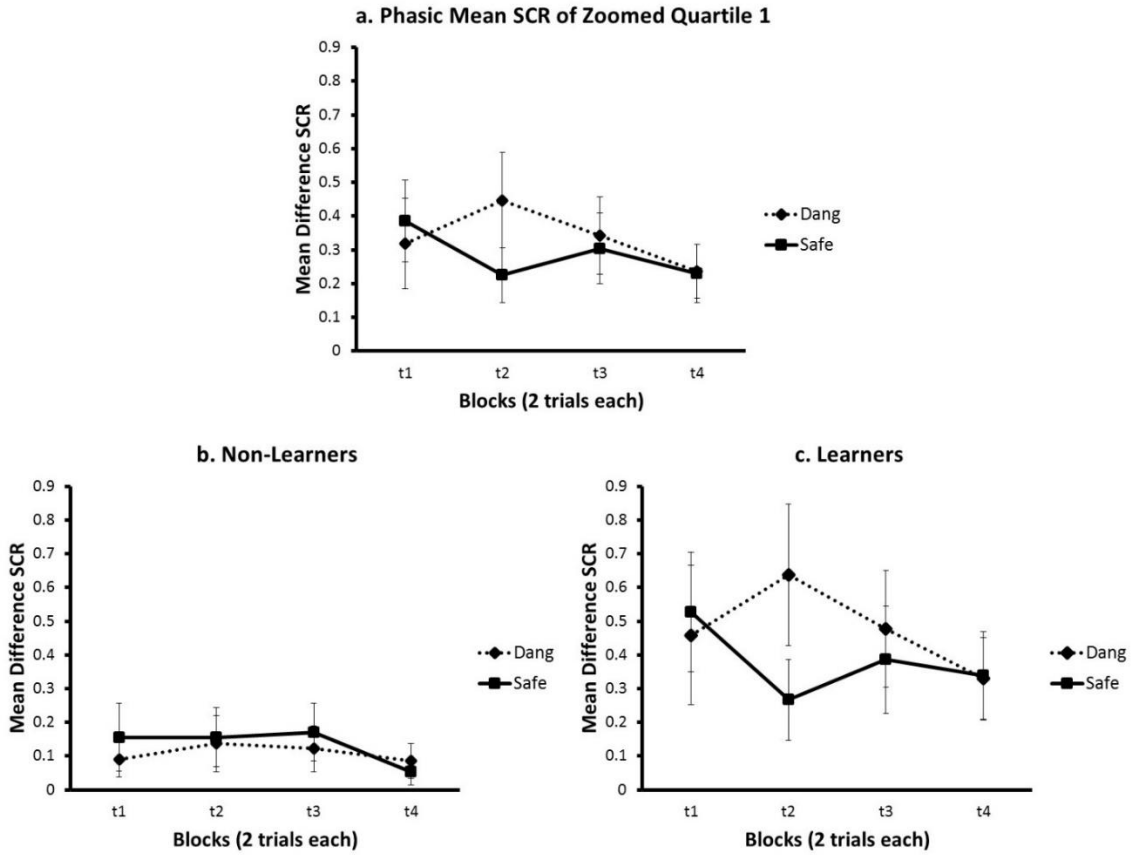


***Figure 10: Experiment 1.1 Zoomed quartile 1 mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) over the first quartile of experiment time for the a) non-learners and learners taken together, b) non-learners, and c) learners. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

### Conclusions

In this experiment, the findings show that participants had no problem learning the positions of the objects in the environment, regardless of the zone in which they were found. In contrast, they failed to show learning of the differential threat of shock within each zone. This failure can be seen by the high number of non-learners, but also by the mixed pattern of SCRs over time in the experiment. Nonetheless, it seems that learners have a higher SCR to the dangerous zone vs. safe zone, while this effect is not present in the non-learners.

I thought that the number of trials would play a role in the ability of the participants to learn the discrimination, so that increasing the number of trials should create bigger differences between zones and also allows more trials to capture the change required to show that learning occurred. To verify this hypothesis, I decided to extend the number of trials within the experiment.

## Experiment 1.2: Increasing reinforcement of shock & 80 trials

### Introduction

From the first experiment, I saw that although those who learned the difference between the zones had a trend for differential SCR to the flowers in danger vs. safety zone compared to those who did not learn the flower-shock contingency, neither group showed a robust significant SCR differences between the zones.

I predicted that more training is required for the participants to learn and distinguish between dangerous and safe zones. Therefore, increasing the number

of trials could allow participants greater learning and discrimination. This effect would be reflected not only as a higher SCR difference between the zones, but also as a decrease in the number of non-learners. Thus, I decided to extend the number of trials within the experiment.

## Methods

***Participants:*** twenty-nine healthy volunteers (23 males), with an average age of 23 years, were recruited for this experiment.

***Aversive conditioning procedure:*** Each participant had to collect 4 flowers in between objects. There were 80 flowers in total, with an average of 33 flowers in the dangerous zone (20 CS+). For this experiment, the dangerous half of the stage was further divided in 3 dangerous zones. That is, there were three levels of danger within the dangerous zone, which increased the reinforcement of shock as the participant approached the dangerous side (the hive). The reinforcement of shock would increase, in increments of 15%, starting at 65% reinforcement of shock, once the participant entered the outermost part of the dangerous zone. At the end of the experiment, participants were asked to identify the dangerous side. 24 participants (83%) were able to identify the dangerous zone and were considered “learners” (18 males). Already, this increase in learner percentage suggested that extending the number of trials within the experiment allowed participants to better discriminate the safety vs. the dangerous zone.

## Results

### Object location memory

A 2x4 within-subjects ANOVA (zone x block) of object placement error rate showed that error decreased significantly over block for learners and non-learners ($F(3,84)=12.47$, $p<0.05$) (Figure 11a). It was independent of the zone ($F(1,28)=0.07$, $p>0.05$) in which the object was found, and there was no significant interaction ($F(3,84)=12.47$, $p>0.05$). The error rate of object placement for the non-learners showed no significant effect of block ($F(3,12)=0.57$, $p>0.05$), zone ($F(1,4)=1.28$, $p>0.05$), or zone x block interaction ($F(3,12)=0.52$, $p>0.05$) (Figure 11b). For the learners, the error rate of placing the objects showed a significant effect of block ($F(3,69)=12.23$, $p<0.01$), which shows a reduction of error over time. There was no significant effect of zone ($F(1,23)=0.00$, $p>0.05$) or zone x block interaction ($F(3,69)=1.69$, $p>0.05$) (Figure 11c).

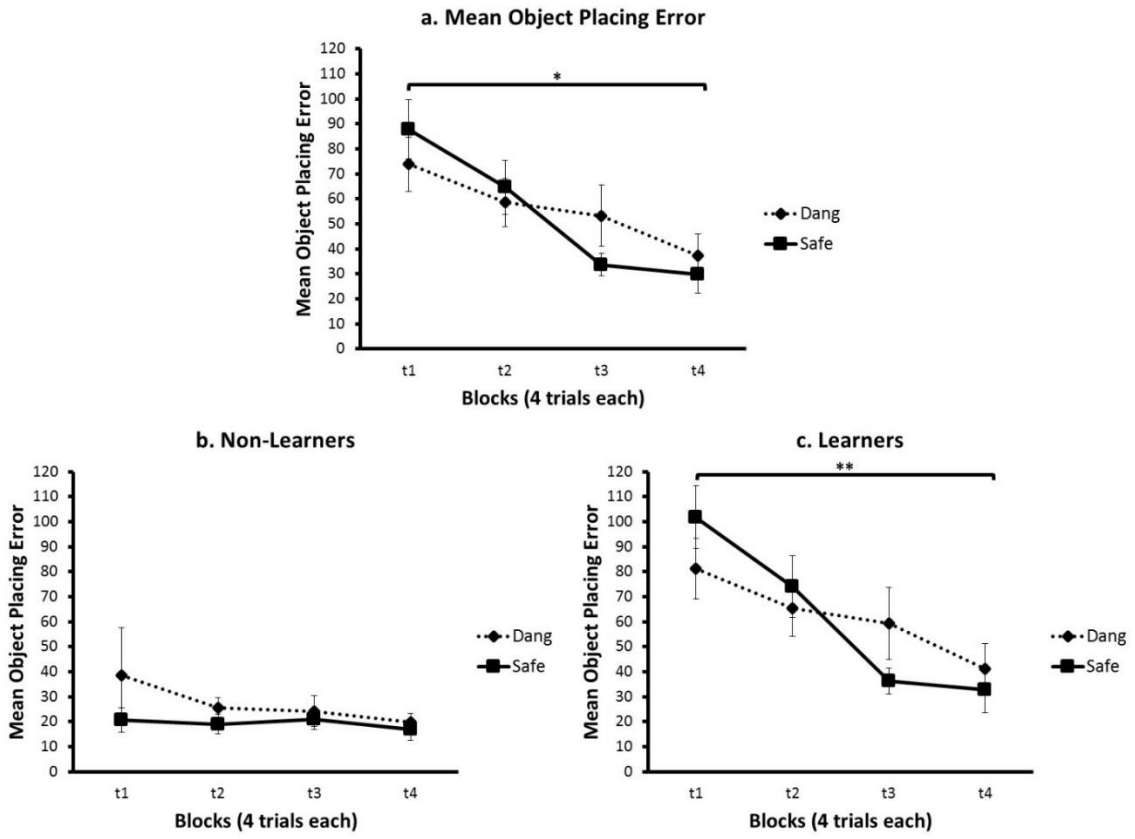


***Figure 11: Experiment 1.2 Mean object placement error.***

*Mean object placement error, for the safe zone (Safe) and the dangerous zone (Dang) over time for a) learners and non-learners together, b) non-learners, and c) learners. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Skin conductance response**

A 2x4 within-subject ANOVA (zone x block) on phasic SCR data for the learners and non-learners showed a significant effect of block ($F(3,84)=7.57$, $p<0.01$) and zone ($F(1,28)=10.43$, $p<0.01$), showing a higher SCR to the dangerous zone compared to the safe zone. There was no significant zone x block interaction ($F(3,84)=1.22$, $p>0.05$) (Figure 12a). In addition, a t-test between

dangerous and safe zone at each block point showed a significant difference only for t3 and t4 ($p<0.05$ uncorrected), but only t3 was significant after correction ($p<0.0125$ Bonferroni corrected). The phasic SCR data for the non-learners only showed a significant effect of block ($F(3,12)=3.73$, $p<0.05$), indicating SCR's decreased over time. There was no significant effect of zone ($F(1,4)=0.99$, $p>0.05$) or zone x block interaction ($F(3,12)=1.50$, $p>0.05$) (Figure 12b). The phasic SCR data, for the learners, showed a significant effect of block ($F(3,69)=4.75$, $p<0.01$) and zone ($F(1,23)= 3.37$, $p<0.01$), where there was an increase of SCR to the dangerous zone compared to the safety zone. The effect of block showed a reduction in SCR over block, in both the dangerous and safe zone, suggesting habituation to the shock over time. There was no significant zone x block interaction ($F(3,69)=0.95$, $p>0.05$) (Figure 12c). A t-test between dangerous and safe zone at each block point showed a significant difference for t1, t3, and t4 ($p<0.05$ uncorrected), but they were not significant after Bonferroni correction.

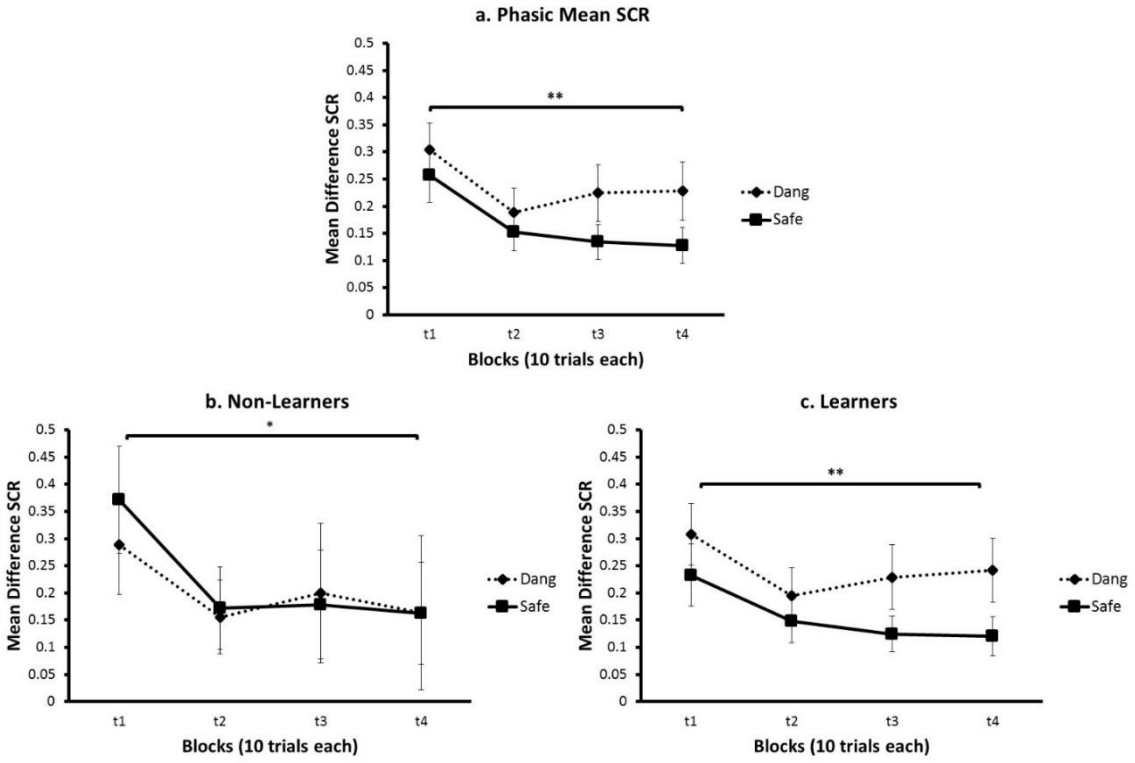


***Figure 12: Experiment 1.2 Mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) zone over time of a) learners and non-learners together, b) non-learners, and c) learners. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

Focusing on the first quartile, a 2x4 within-subject ANOVA (zone x block) on phasic SCR data of the learners and non-learners showed no significant effect of block ($F(3,84)=1.54$, $p>0.05$), zone ($F(1,28)=2.86$, $p>0.05$), or zone x block interaction ($F(3,84)=2.21$, $p>0.05$) (Figure 13a). The phasic SCR data for the non-learners showed a significant effect of block ($F(3,12)=7.57$, $p<0.01$), and a trend for the zone ($F(1,4)=7.22$, $p=0.055$). There was no significant zone x block interaction ($F(3,12)=0.77$, $p>0.05$). However, this data should be considered cautiously since there were only 5 non-learners (Figure 13b). The phasic SCR

data, for the learners, showed a significant effect of zone ($F(1,23)=5.49$, $p<0.05$), where SCR was higher for the danger zone compared to the safe zone. There was no significant effect of block ($F(3,69)=0.99$, $p>0.05$) or zone x block interaction ($F(3,69)=1.90$, $p>0.05$) (Figure 13c). A t-test between dangerous and safe zone at each block point showed a significant difference for t3 ($p<0.05$ uncorrected) and a trend for t4 ($p=0.071$ uncorrected) that were not significant after Bonferroni correction.

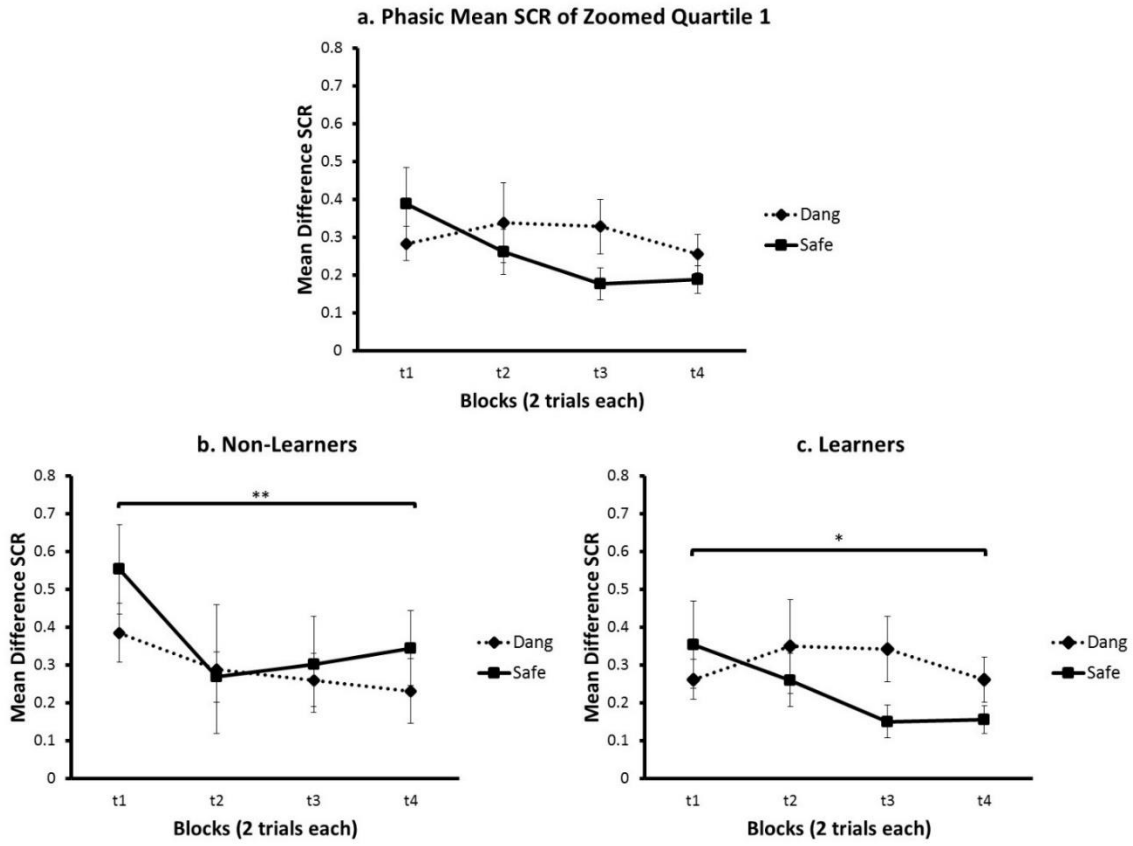


***Figure 13: Experiment 1.2 Zoomed quartile 1 mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) over the first quartile of experiment time for the a) non-learners and learners taken together, b) non-learners, and c) learners. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

### Conclusions

Experiment 1.2 showed that increasing the number of trials within the experiment resulted in a higher number of learners compared to non-learners. I showed that increasing the number of trials resulted in greater learning discrimination of the dangerous vs. safe zone, for those who learned the rule. Furthermore, when I took a closer look into the first quartile of the data, I saw that the learners are able to discriminate between zones early in the experiment. Furthermore, like in experiment 1.1, participants were able to correctly place the object in the environment regardless of the zone it was found in.

Although I saw successful learning in this experiment, I saw a high number of non-learners (17%). I wondered if I could use a smaller number of trials, but still retain good learning by increasing the reinforcement of shock, or predictability (a detail that was not disclosed to the participants), that way reducing the number of non-learners. Because of this hypothesis, I decided to re-create experiment 1.1, but eliminated the change in reinforcement of shock as the participant approached the dangerous side (the beehive), and just had the shock constant at a high level across the dangerous zone.

## Experiment 1.3: 95% reinforcement of shock & 40 trials

### Introduction

From the previous, I saw that the number of trials plays an important role in the learning and discrimination of dangerous vs. safe zones. A higher number of trials increased the window of learning and discrimination, while the reinforcement

of shock increased as the participant got closer to the center of the dangerous zone. This discrimination task resulted in a higher SCR in the dangerous zone for the learners, but not for the non-learners.

Still, I wanted to see if I could reduce the number of non-learners. I thought that the predictability of the CS+ was also playing an important role in learning the different zones. Since the reinforcement of shock in the danger zone varied in the previous experiments, I thought that the predictability of the CS+ might be causing some confusion. This issue would explain the mixed SCR result in experiment 1.1, and why increasing the duration of the experiment yielded better learning and discrimination. I predicted that keeping a stable reinforcement of shock would help with the learning process regardless of the number of trials. Furthermore, having a high predictability for the CS+ could accelerate the learning process, and reduce the number of non-learners. To test if the reinforcement of shock plays a role in learning, I decided to re-create experiment 1.1, keeping a low number of trials, but keeping a high, stable reinforcement of shock throughout the dangerous zone.

## Methods

***Participants:*** 20 healthy volunteers (1 male), with an average age of 23 years, were recruited for this experiment.

***Aversive conditioning procedure:*** Each participant had to collect 2 flowers in between objects. There were 40 flowers in total, an average of 18 flowers in the dangerous zone (17 CS+). For this experiment, the stage was divided in half; one half was dangerous and the other was safe. The reinforcement of shock was 95% and remained stable throughout the dangerous zone. At the end of the experiment, participants were asked to identify the dangerous zone. 17

participants (85%) were able to identify the dangerous zone and were considered “learners” (1 male).

## Results

### Object location memory

A 2x4 within-subjects ANOVA (zone x block) measuring error rate of object placement showed that error decreased significantly over block for learners and non-learners ($F(3,57)=10.48$, $p<0.01$) (Figure 14a). It was not dependent on the zone ($F(1,19)=1.80$, $p>0.05$) the object was found and there was no significant interaction ($F(3,57)=0.71$, $p>0.05$). The error rate of placing the objects for the non-learners showed a trend effect of block ($F(3,6)=4.34$, $p=0.06$) and zone x block interaction ($F(3,6)=4.66$, $p=0.052$), showing a decrease of error over time for the danger zone and an increase in the safe zone. There was no significant effect of zone ($F(1,2)=0.53$, $p>0.05$) (Figure 14b). The error rate of placing the objects of the learners showed a significant difference of block ($F(3,48)=7.25$, $p<0.01$), which shows a reduction of error over time and a trend effect of zone ($F(1,16)=4.05$, $p=0.061$). It also showed a higher error of placing the object in the safe zone compared to the dangerous zone. There was no significant zone x block interaction ($F(3,69)=1.69$, $p>0.05$) (Figure 14c).

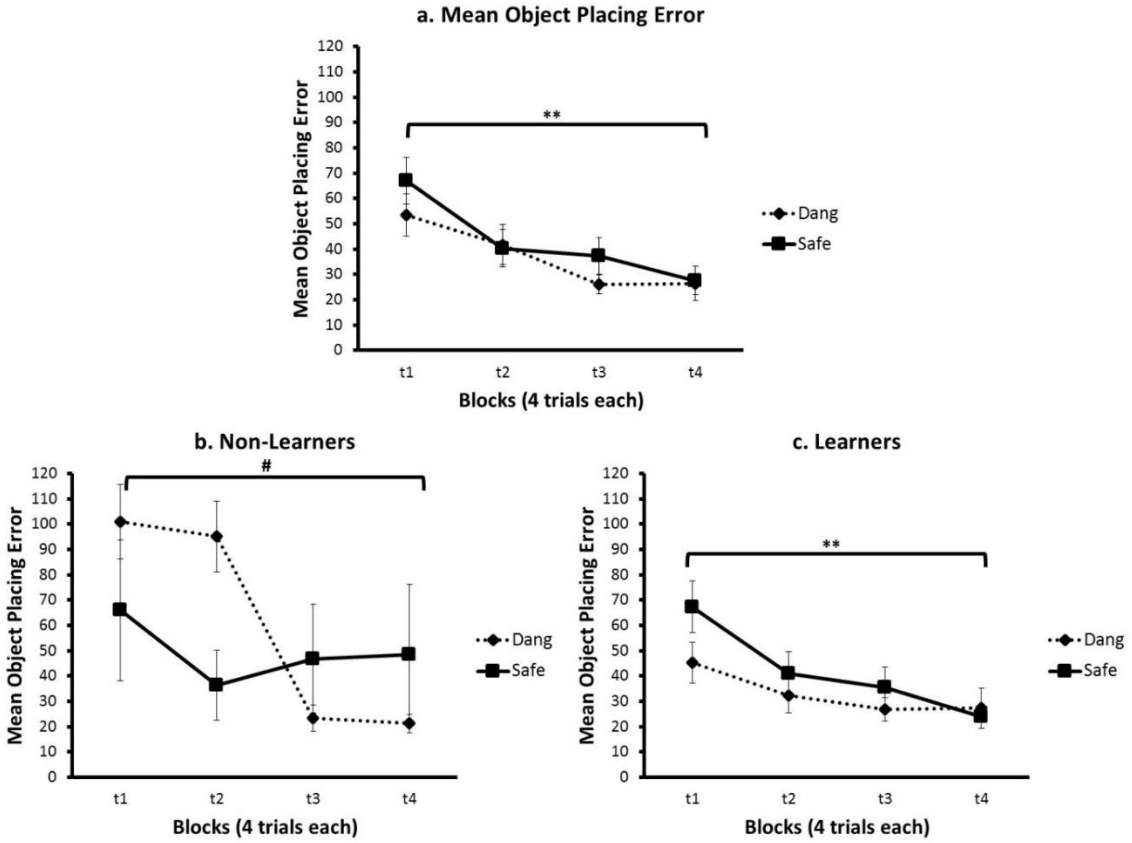


***Figure 14: Experiment 1.3 Mean object placement error.***

*Mean object placement error, for the safe zone (Safe) and the dangerous zone (Dang) over time for a) learners and non-learners together, b) non-learners, and c) learners. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

## Skin conductance response

A 2x4 within-subject ANOVA (zone x block) on phasic SCR data of the learners and non-learners showed a significant effect of zone ($F(1,19)=16.16$, $p<0.01$), with higher SCR to the dangerous zone compared to the safe zone. There was no significant effect of block ($F(3,57)=1.00$, $p>0.05$) or zone x block interaction

(F(3,57)=0.59, p>0.05) (Figure 15a). A t-test between dangerous and safe zone at each block point showed a significant difference at t1, t2, and t4 (p<0.05 uncorrected), but only t1 and t2 remained significant after correction (p<0.0125 Bonferroni corrected). The phasic SCR data for the non-learners showed no significant effect of block (F(3, 6)=0.64, p>0.05), zone (F(1,2)=1.89, p>0.05), or zone x block interaction (F(3,6)=0.82, p>0.05) (Figure 15b). The phasic SCR data, for the learners, showed a significant effect of zone (F(1,16)=18.95, p<0.01), where there was an increase of SCR to the dangerous zone compared to the safety zone. There was no significant effect of block (F(3,48)=1.68, p>0.05) or zone x block interaction (F(3,48)=1.52, p>0.05) (Figure 15c). A t-test between dangerous and safe zone at each block point showed a significant difference for t1, t2, and t4 (p<0.05 uncorrected), but only t2 remained significant after correction (p<0.0125 Bonferroni corrected).

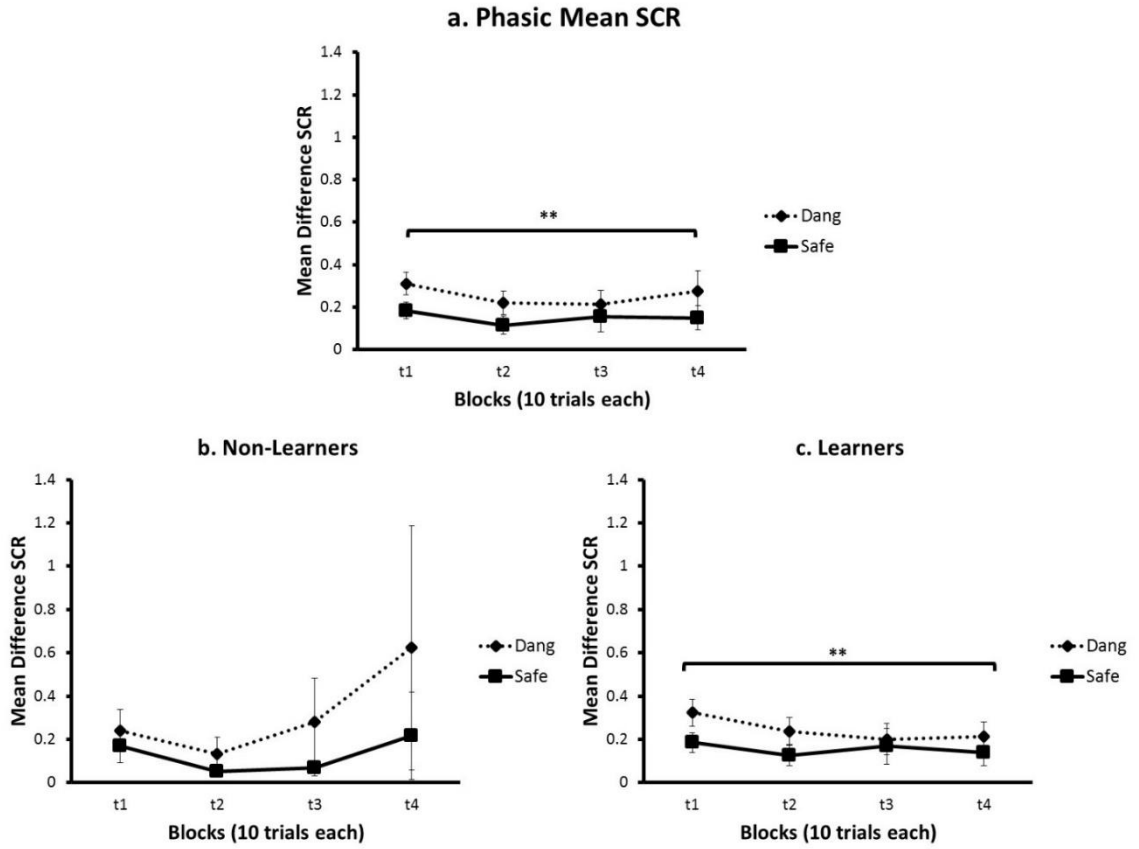


***Figure 15: Experiment 1.3 Mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) zone over time of a) learners and non-learners together, b) non-learners, and c) learners. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

Focusing on the first quartile, a 2x4 within-subject ANOVA (zone x block) on phasic SCR data of the learners and non-learners showed a significant effect of zone ($F(1,19)=10.14$, $p<0.01$), revealing a higher SCR to the dangerous zone compared to the safe zone. There was no significant effect of block ($F(3,57)=0.22$, $p>0.05$) or zone x block interaction ($F(3,57)=0.64$, $p>0.05$) (Figure 16a). A t-test between dangerous and safe zone at each block point showed a significant difference for t1, t3, and t4 ($p<0.05$ uncorrected), but none were significant after

Bonferroni correction. The phasic SCR data for the non-learners showed no significant effect of block ($F(3,6)=1.04$, $p>0.05$), zone ($F(1,2)=4.39$, $p>0.05$), or zone x block interaction ($F(3,6)=1.00$, $p>0.05$). However, this data should be considered carefully since there were only 3 non-learners (Figure 16b). The phasic SCR data, for the learners, showed a significant difference of zone ($F(1,16)=7.88$, $p<0.05$), showing a higher SCR to the dangerous zone compared to the safe zone. There was no significant effect of block ($F(3,48)=0.13$, $p>0.05$) or zone x block interaction ($F(3,48)=0.40$, $p>0.05$) (Figure 16c). A t-test between dangerous and safe zone at each block point showed a significant difference for t1 ($p<0.05$ uncorrected) and a trend for t3 ($p=0.067$ uncorrected), but they were not significant after Bonferroni correction.

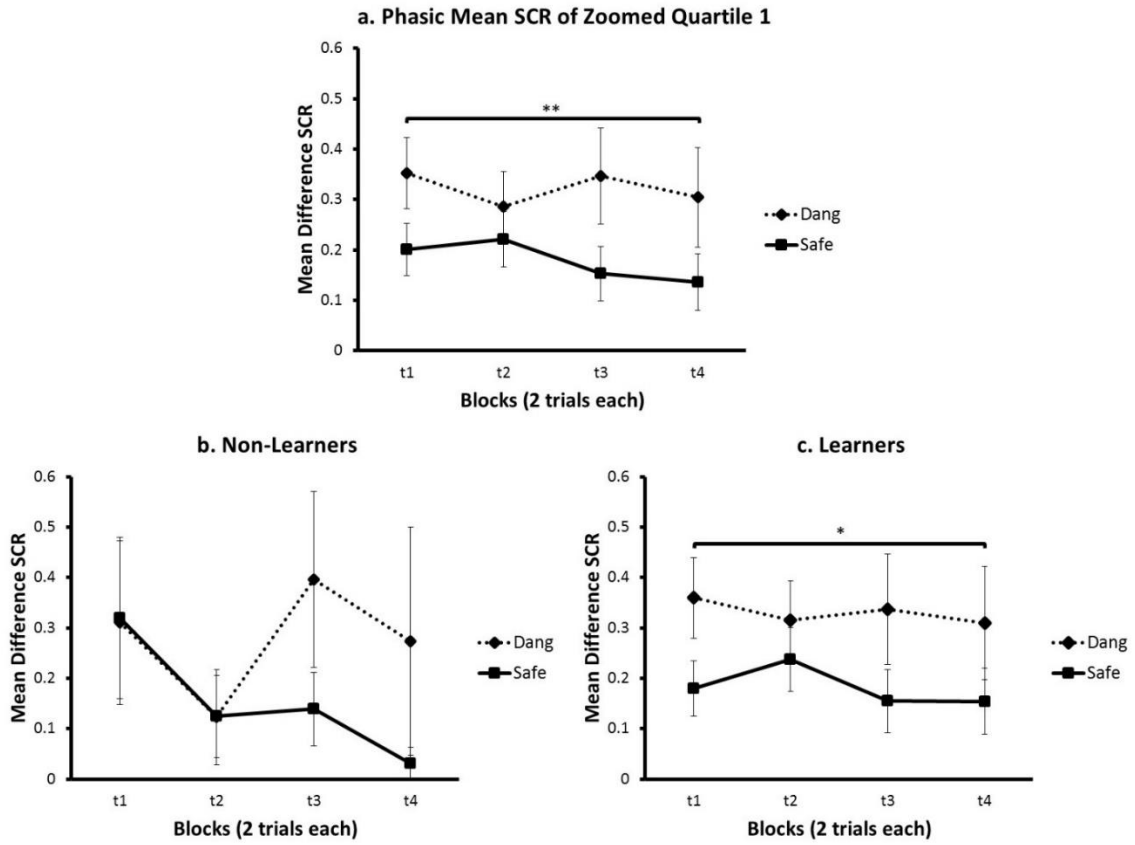


***Figure 16: Experiment 1.3 Zoomed quartile 1 mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) over the first quartile of time of the experiment for the a) non-learners and learners taken together, b) non-learners, and c) learners. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

## Conclusions

Experiment 1.3 showed that increasing the reinforcement of shock within the experiment resulted in a higher number of learners compared to non-learners. Increasing the reinforcement of shock resulted in a higher discrimination as shown by higher SCR in the dangerous vs. safe zone. Furthermore, when I took a closer

look into the first quartile of the data I saw that the learners were still able to discriminate between zones early in the experiment. In this experiment, I was not able to see the learning of contextual threat as I did not see differential SCR that increased during learning, since I saw high levels of SCR to the dangerous zone already in the first trials of the experiment. This response could be an effect of the instructions given at the beginning of the experiment and the high reinforcement rate. Although they were not explicitly told that the stage was divided in half, they were told that there is a safe beehive and a dangerous one. Thus, it might be easy for them to identify the dangerous zone due to the high reinforcement rate (predictability). Consistently with the previous experiments, I saw that participants were able to learn the correct location of the objects in the environment over time. Still, in the learners there was no significant difference in object placement error between safe and dangerous zone. On the other hand, non-learners showed a steeper reduction of object placement error in the dangerous zone compared to the safe zone. However, there were only 3 non-learners, so this interaction of zone x block should be considered cautiously.

# Experiment 1.4: 60% reinforcement of shock & 80 trials

## Introduction

From the previous experiment, unlike experiment 1.1, I saw that learning and discrimination of dangerous and safe zones was possible even with a low number of trials, as long as there was a high and predictable reinforcement of shock. A higher reinforcement made the difference between the two zones extremely noticeable, resulting in higher predictability of shock, and allowing for higher discrimination starting early on in the experiment. This predictability not only

resulted in a relatively higher SCR in the dangerous vs. safe zone, in the learners, it also kept the number of non-learners low.

Since the predictability is important in the learning of the CS+, I wanted to see if I could have successful learning even with a low reinforcement of shock, as long as there is enough time to learn the rule. Unlike the first two experiments, consistent reinforcement of shock, whether high or low, should yield successful discrimination of the two zones. I saw in previous experiments that high predictability produced fast and successful learning. Therefore, I predicted that a low predictability, with a high number of trials, would produce a slow but successful learning of the contextual aversive response. In this experiment, I expected to measure learning of the contextual anxiety response via differential SCR that increase, in the danger zone, during learning. To test this effect, I decided to re-create experiment 1.2. I kept a high number of trials, but with a low stable reinforcement of shock in the dangerous zone. In addition, I decided to add a self-reported expectancy rating as a secondary measure of learning.

## Methods

***Participants:*** 25 healthy volunteers (9 males), with an average of 23 years, were recruited for this experiment.

***Aversive conditioning procedure:*** Each participant had to collect 4 flowers in between objects. There were 80 flowers in total, with an average of 37 flowers in the dangerous zone (23 CS+). For this experiment, the stage was divided in half, one half was dangerous and the other was safe. The reinforcement of shock was 60%, and remained stable as the participant approached the dangerous hive. At the end of the experiment, participants were asked to identify

the dangerous side, 20 participants (80%) were able to identify the dangerous zone and were considered “learners” (8 males).

***Expectancy ratings procedure:*** Each time the participant collected a flower, the question *“How likely is a sting to occur?”* would appear before the freezing period (Figure 17). During this period, they were asked to make a simple expectancy rating from 0 to 9 on how likely they were to receive a shock (0 for no shock, 9 for definite shock). This was performed via button press, using one button to decrease the rating and another button to increase it.

***Expectancy ratings analysis:*** Expectancy ratings taken at the beginning of each freezing period were analyzed in a similar way to skin conductance. Each rating provided (0-9) was averaged across trials to create 4 equal blocks in the safe and danger conditions (10 trials in each block).


***Figure 17: Threat expectancy rating question.***

## Results

### Object location memory

A 2x4 within-subjects ANOVA (zone x block) measuring error rate of object placement showed that error decreased significantly over block for learners and non-learners ($F(3,72)=15.40$, $p<0.01$) (Figure 18a). It was not dependent on the zone ($F(1,24)=0.00$, $p>0.05$) in which the object was found and there was no significant interaction ($F(3,72)=0.81$, $p>0.05$). The error rate of placing the objects for the non-learners showed no significant effect of block ($F(3,12)=3.05$, $p>0.05$),

zone (F(1,4)=0.10, p>0.05), or zone x block interaction (F(3,12)=0.40, p>0.05) (Figure 18b). The error rate of placing the objects of the learners showed a significant difference of block (F(3,57)=14.44, p<0.01), which showed a reduction of error over time. There was no significant effect of zone (F(1,19)=0.08, p>0.05) or zone x block interaction (F(3,57)=0.71, p>0.05) (Figure 18c).

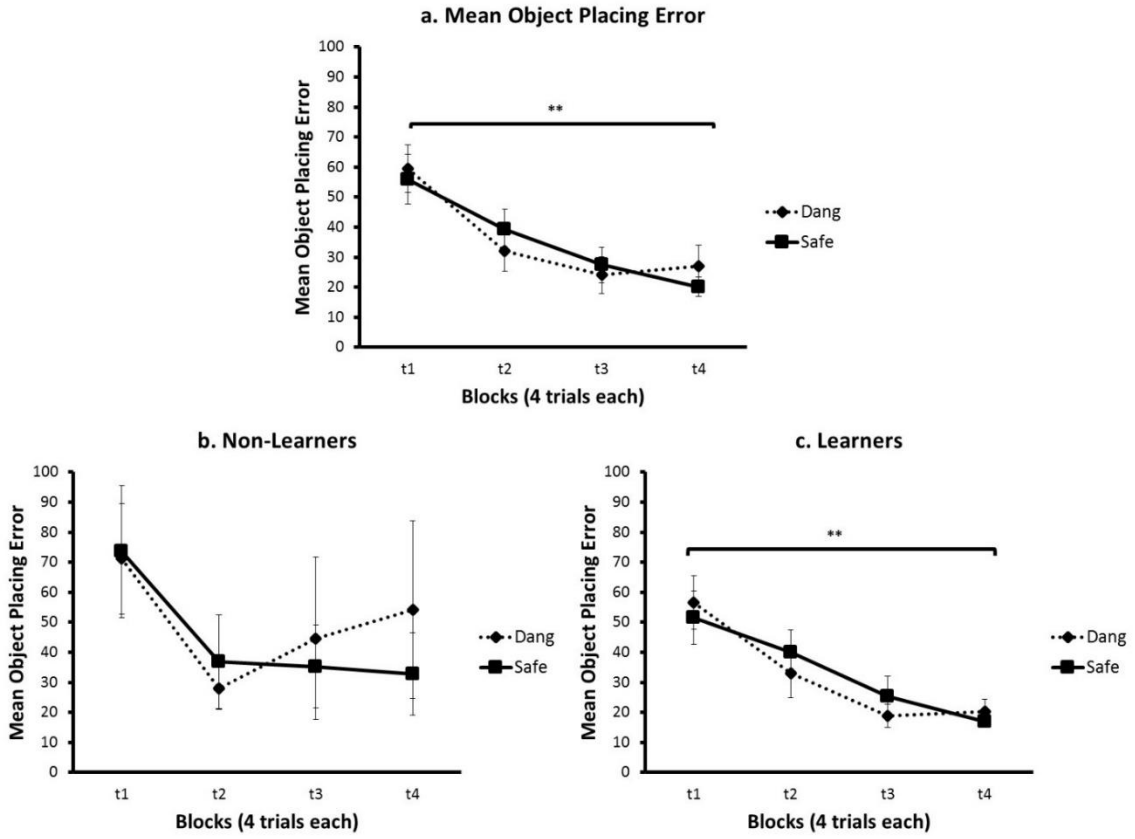


***Figure 18: Experiment 1.4 Mean object placement error.***

*Mean object placement error, for the safe zone (Safe) and the dangerous zone (Dang) over time for a) learners and non-learners together, b) non-learners, and c) learners. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Skin conductance response**

A 2x4 within-subject ANOVA (zone x block) on phasic SCR data of the learners and non-learners showed a significant effect of block ($F(3,72)=25.00$, $p<0.01$) and zone ($F(1,24)=6.93$, $p<0.05$), showing a higher SCR to the dangerous zone compared to the safe zone, in which SCR reactivity seemed to decrease over time. There was no significant zone x block interaction ($F(3,72)=1.76$, $p>0.05$) (Figure 19a). A t-test between dangerous and safe zone at each block point showed a significant difference for t2, t3, and t4 ($p<0.05$ uncorrected), but only t4 was significant when corrected ($p<0.0125$ Bonferroni corrected). The phasic SCR data for the non-learners showed a significant effect of block ($F(3,12)=10.15$, $p<0.01$) where, over time, SCR reactivity seemed to decrease. There was no significant effect of zone ($F(1,4)=0.04$, $p>0.05$) or zone x block interaction ($F(3,12)=1.76$, $p>0.05$) (Figure 19b). The phasic SCR data, for the learners, showed a significant effect of block ($F(3,57)=17.07$, $p<0.01$) and zone ($F(1,19)=7.65$, $p<0.05$), showing a higher SCR to the dangerous zone compared to the safe zone, in which SCR reactivity seemed to decrease over time. There was no significant zone x block interaction ($F(3,57)=1.77$, $p>0.05$) (Figure 19c). A t-test between dangerous and safe zone at each block point showed a significant difference for t2, t3, and t4 ($p<0.05$ uncorrected), but only t4 was significant after correction ($p<0.0125$ Bonferroni corrected).

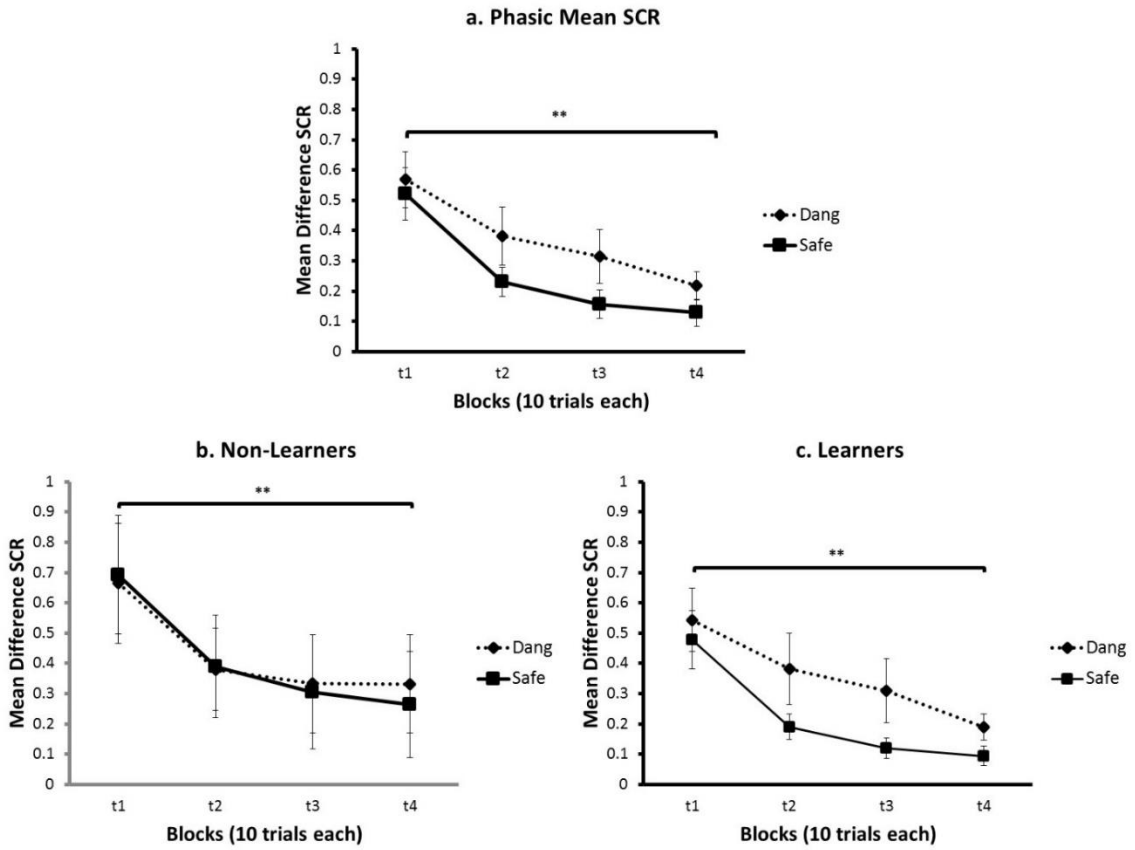


***Figure 19: Experiment 1.4 Mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) over time for a) non-learners and learners taken together, b) non-learners, and c) learners. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

Focusing on the first quartile, a 2x4 within-subject ANOVA (zone x block) on phasic SCR data of the learners and non-learners showed a significant effect of block ($F(3,72)=20.97$, $p<0.01$), indicating a reduction of SCR over time. There was no significant effect of zone ($F(1,24)=1.66$, $p>0.05$) or zone x block interaction ($F(3,72)=0.72$, $p>0.05$) (Figure 20a). The phasic SCR data for the non-learners showed a significant effect of block ($F(3,12)=4.88$, $p<0.05$), where the SCR reactivity decreased over time. There was no significant effect of zone

(F(1,4)=0.04, p>0.05) or zone x block interaction (F(3,12)=2.15, p>0.05). However, this data should be considered with caution, since there were only 5 non-learners (Figure 20b). The phasic SCR data, for the learners, showed a significant effect of block (F(3,57)=16.42, p<0.01), showing a reduction of SCR over time. There was no significant effect of zone (F(1,19)=2.51, p>0.05) or zone x block interaction (F(3,57)=1.54, p>0.05) (Figure 20c).

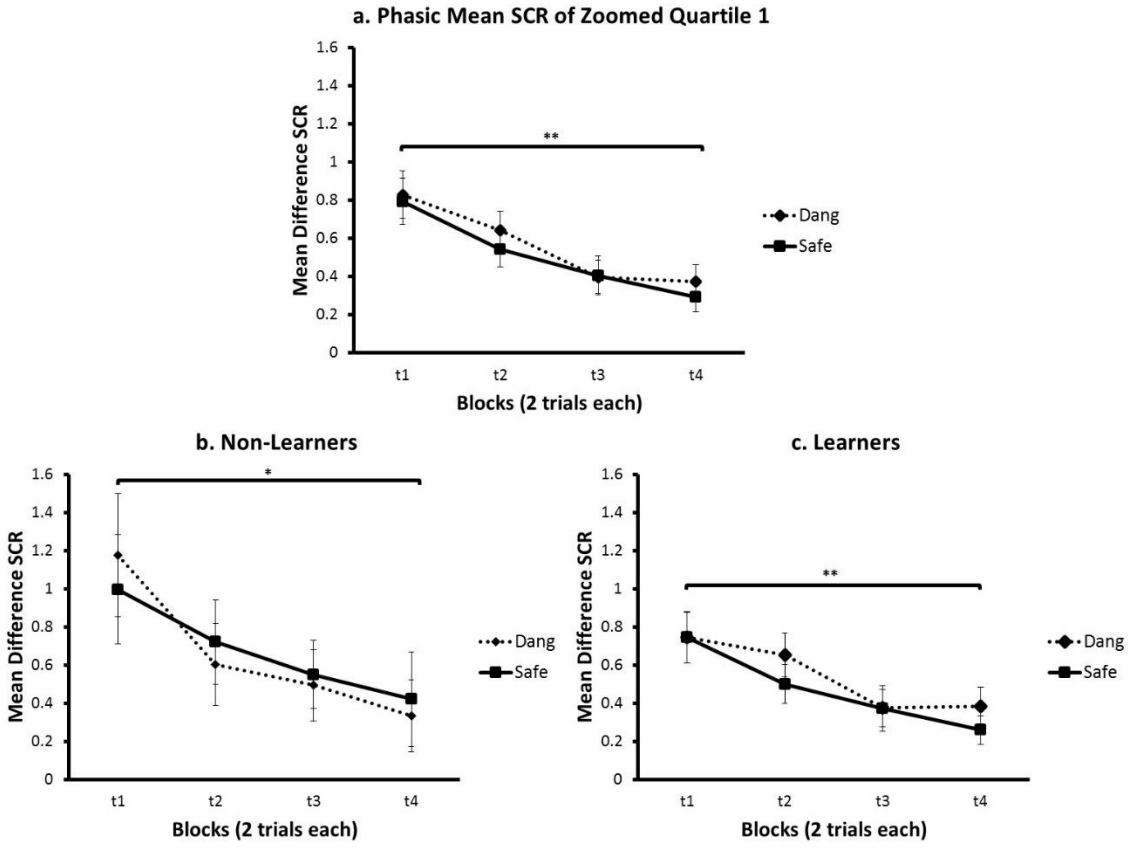


***Figure 20: Experiment 1.4 Zoomed quartile 1 mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) over the first quartile of experiment time for the a) non-learners and learners taken together, b) non-learners, and c) learners. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Threat expectancy rating**

A 2x4 within-subject ANOVA (zone x block) of threat expectancy rating of the learners and non-learners showed a significant effect of block ($F(3,57)=3.09$, $p<0.05$), zone ($F(1,19)=50.31$, $p<0.01$), and zone x block interaction ($F(3,57)=11.68$, $p<0.01$), showing a higher expectancy of shock in the dangerous zone compared to the safe zone over block (Figure 21a). In addition, a t-test between dangerous and safe zones at each block point showed a significant difference for t1, t2, t3, and t4 ($p<0.0125$ Bonferroni corrected). The threat expectancy rating for the non-learners showed a significant effect of zone ($F(1,3)=36.75$, $p<0.01$), and zone x block interaction ($F(3,9)=4.90$, $p<0.05$), showing a higher expectancy of shock in the dangerous zone compared to the safe zone over time. There was no main effect of block ($F(3,9)=0.84$, $p>0.05$) (Figure 21b). A t-test between dangerous and safe zone at each block point showed a significant difference only for t3 ($p<0.0125$ Bonferroni corrected). The threat expectancy rating, for the learners, showed a significant difference of block ($F(3,45)=4.56$, $p<0.01$), zone ($F(1,15)=103.16$, $p<0.01$), and zone x block interaction ($F(3,45)=12.00$, $p<0.01$), showing a higher expectancy of shock to the dangerous zone compared to the safe zone over block (Figure 21c). In addition, a t-test between the dangerous and safe zones, at each block point, showed a significant difference for t1, t2, t3, and t4 ($p<0.0125$ Bonferroni corrected).

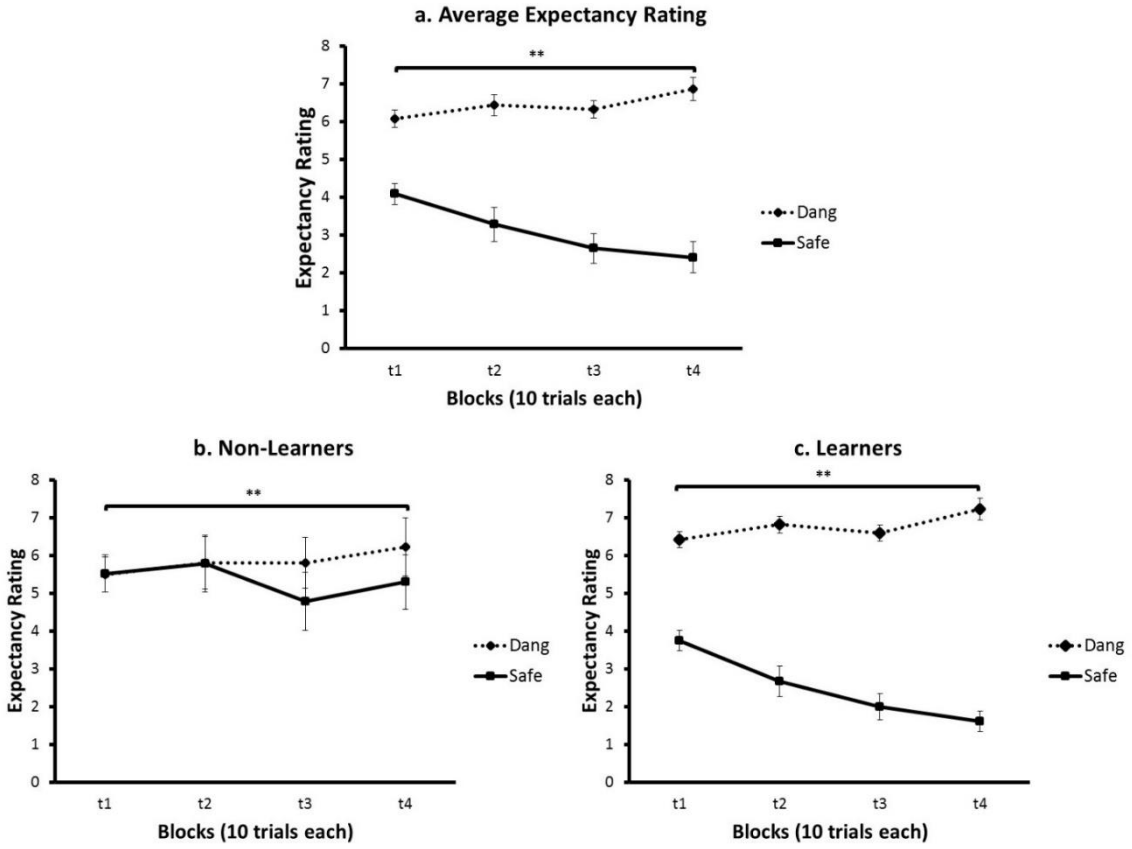


***Figure 21: Experiment 1.4 Average expectancy ratings.***

*Average expectancy ratings of safe (Safe) and dangerous (Dang) zone over time of a) learners and non-learners together, b) non-learners, and c) learners. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

## Conclusions

Here, I showed successful contextual threat learning with a low reinforcement of shock within the experiment, which resulted in a higher number of learners compared to non-learners. When I took a closer look the first quartile of the data, I see that the learning was slower in this experiment. These two last

experiments suggest that having a consistent reinforcement of shock results in accurate contextual threat learning, whose speed depends upon the predictability of shock. Having a low reinforcement of shock results in differential SCR that increases during learning in the dangerous zone. Furthermore, consistent with SCR, expectancy ratings show that learning does occur over time for the learners. I see differential expectancy ratings that increase during learning in the dangerous zone. Finally, consistent with the previous experiments I see that participants are able to learn the correct location of the objects in the environment over time.

## Discussion

In summary, the current series of experiments were designed to create a paradigm that could be used to study learning and discrimination of dangerous and safe stimuli. In general, the results indicate that (a) during unpredictable conditions a low number of trials yields a discrimination of the CS+ which might be confusing for those who are unable to identify the dangerous zone (Experiments 1.1); (b) increasing the duration of the experiment, by increasing the number of trials, increases the discrimination of the CS+, allowing better learning discrimination of the dangerous zone (Experiment 1.2); (c) during a predictable situation, where there is a high predictability of the CS+, even a low number of trials can create a fast and accurate learning and discrimination of the CS+ (Experiment 1.3); (d) a stable low reinforcement of shock with a high number of trials results in slow but accurate learning and discrimination of the CS+; (e) regardless of the conditions of the experiment, participants had no trouble learning the correct location of the objects in the environment over time. Since my main interest is elucidating how safety and danger boundaries are normally formed, I decided to concentrate the rest of the thesis only on the learners group.

# Chapter 5

## Experiment 2: Behavioral Study of Context Discrimination: Creating the Virtual Reality Task Settings for an fMRI Experiment

## Precis

Experiment 1 showed a) participants were able to discriminate between safety and danger, b) stable and predictable shock reinforcement increased the number of learners, and c) the reinforcement of shock modulates the speed in which participants learned the contingencies. Based on these results, I decided to focus on the learners in order to continue investigating how safety and danger boundaries are formed using location-specific information.

With the information from experiment 1, I set out to find the perfect settings to test location-specific threat learning in the fMRI scanner. I wanted to use a stable reinforcement of shock, to avoid confusion amongst the participants, but use a low enough reinforcement that would enable a slow learning of the contingencies, in order to compare the brain areas needed to discriminate between safety and danger within one environment. In other words, I wanted learning to occur reliably over a large number of trials and observe learning over time. In addition, I wanted to reduce the number of shocks to avoid any artifact confounds that these might create during the fMRI data acquisition and analysis. In order to do this, I would have to either remove the trials that included the electrical stimulus or mitigate the artifacts. Therefore, a lower number of CS+ trials was necessary to keep the majority of the data.

The present study examined learning and discrimination between danger and safety, with a stable and low (lower than Experiment 1.4) reinforcement of shock in the dangerous zone, with a high number of trials to reduce the number of shocks given in the experiment (Experiment 2.1 & 2.2). In addition, I randomized (jittered) the virtual freezing time as an alternative to keep the electric shock trials in the fMRI data analysis (Experiment 2.3).

It was expected that over time, participants would learn to discriminate between the dangerous and safe stimuli in the environment through differential

SCR and expectancy ratings that would increase, in the dangerous zone, during learning. I expected that learning the discrimination in experiment 2.1 would take longer than in experiment 2.2 or experiment 1.4 since it was the lowest reinforcement of shock I used. Regardless, while using a stable and predictable CS+, I expected learning in all of the experiments in this chapter, and that jittering (random variability in time of freezing after collecting a flower) would not cause any difference in learning (Experiment 2.3).

## Method

The methods used in this experiment were roughly the same as the ones described in Chapter 4, alterations to the methods are described in each experiment. All experiments in this chapter, and beyond, include the expectancy ratings.

***Expectancy ratings procedure:*** During the task, participants were instructed to move around the environment and pick flowers that appeared one at a time in random locations. On contact with a flower, the participant's movement was frozen for a short duration. During this period, they were asked to make a simple expectancy rating from 0 to 9 on how likely they were to receive a shock (0 for no shock, 9 for definite shock; Figure 22). This response was performed via button press, using one button to decrease the rating and another button to increase it.

***Expectancy ratings data analysis:*** Expectancy ratings taken at the beginning of each freezing period were analyzed in a similar way to skin conductance. Each rating provided (0-9) was averaged across trials to create 4 equal blocks in the safe and danger conditions (10 trials in each block).


***Figure 22: Threat expectancy rating question.***

# Experiment 2

## Experiment 2.1: 35% reinforcement of shock & high number of trials

### Introduction

From Chapter 4, I saw that learning and discrimination of dangerous and safe zones is possible even with a low reinforcement of shock, as long as there are a high number of trials, which allows more time for successful learning. A higher number of trials increased the time window for participants to learn the difference between each zone and, in doing so, allowed for higher discrimination. This adjustment not only resulted in a higher SCR in the dangerous zone, in the learners, it also kept the number of non-learners low.

Since I have seen that predictability is important in learning the CS+, I wanted to see if I could have successful learning even with a minimal reinforcement of shock, as long as there is enough time to learn the rule. Unlike in the first experiments, with consistent reinforcement of shock (either high or low) yielding successful discrimination of the two zones, and where the high predictability produced fast and successful learning, I expect that a lower predictability, with a high number of trials, will produce slow, but successful, learning. To test if the number of trials plays a role in the learning of the rule, I decided to re-create experiment 1.4. I kept a high number of trials, but lowered the reinforcement of shock in the dangerous zone.

## Methods

***Participants:*** 10 healthy volunteers (3 males), with an average of 22 years, were recruited for this experiment.

***Aversive conditioning procedure:*** Each participant had to collect 4 flowers in between objects. There were 80 flowers in total, an average of 40 flowers in the dangerous zone (14 CS+). Each time the participant collected a flower, the participants would be frozen in place for 3000ms. The reinforcement of shock was 35%, and remained stable as the participant approached the dangerous hive. At the end of the experiment, participants were asked to identify the dangerous side. 6 participants (60%) were able to identify the dangerous zone and were considered "learners" (2 males).

## Results

### Object location memory

A 2x4 within-subjects ANOVA (zone x block) measuring error rate of object placement for learners, showed no significant effect of block ($F(3,15)=2.52$, $p>0.05$), zone ($F(1,5)=3.36$, $p>0.05$) or interaction between zone x block ($F(3,15)=2.86$, $p>0.05$) (Figure 23).

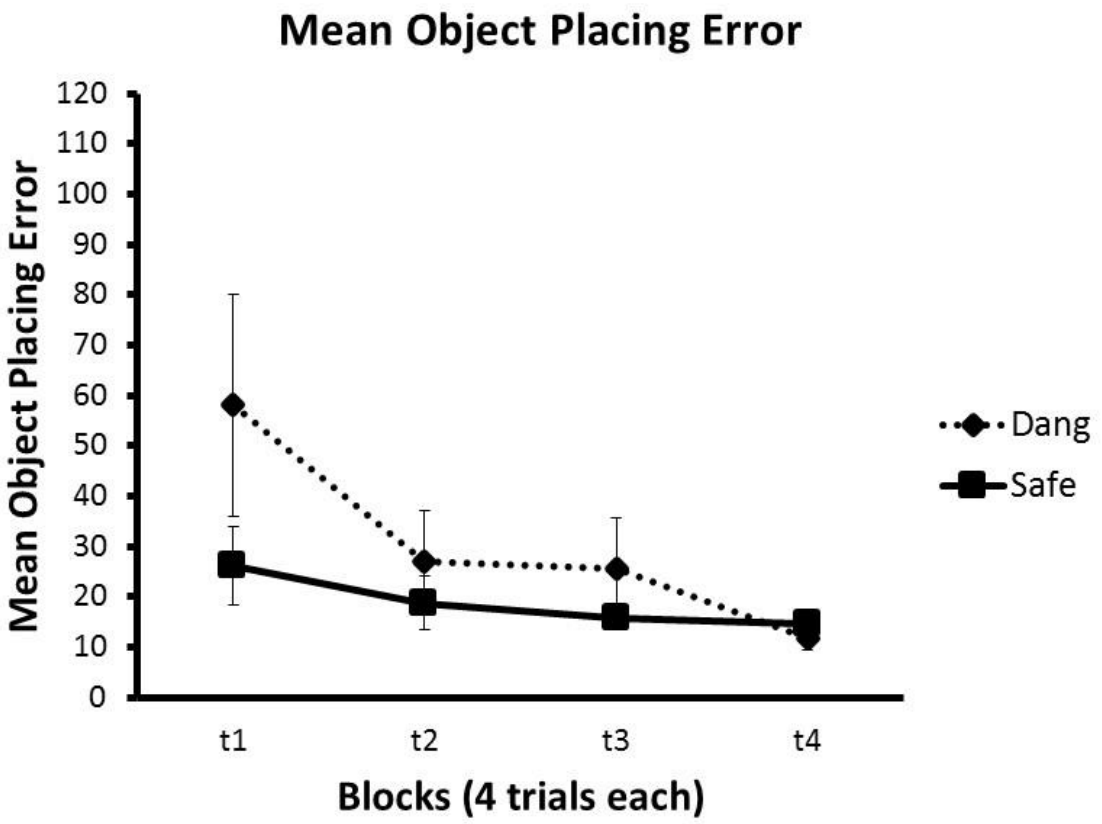


***Figure 23: Experiment 2.1 Mean object placement error.***

*Mean object placement error, for the safe zone (Safe) and the dangerous zone (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Skin conductance response**

A 2x4 within-subject ANOVA (zone x block) phasic data of the learners showed no significant difference of block ($F(3,15)=0.64$, $p>0.05$), zone ($F(1,5)=3.79$, $p>0.05$), or zone x block ($F(3,15)=0.40$, $p>0.05$) (Figure 24).

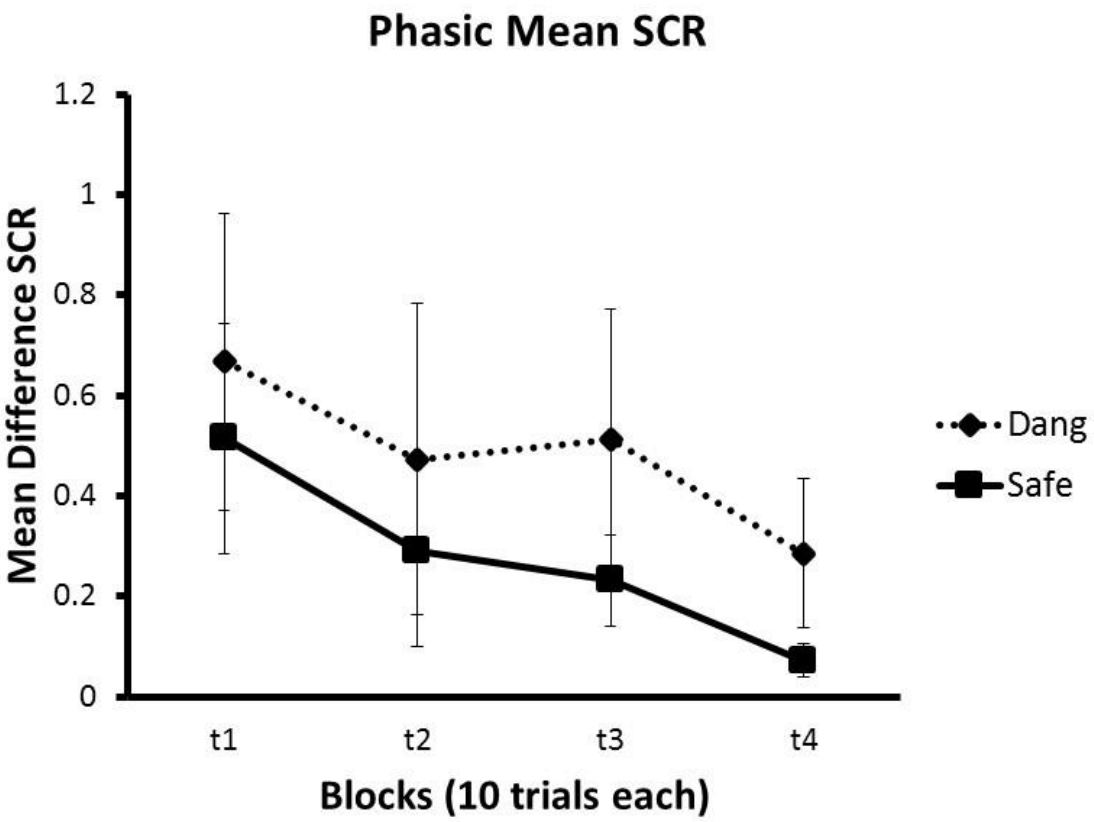


***Figure 24: Experiment 2.1 Mean phasic SCR.***

*Mean phasic SCR of safe (square) and dangerous (diamond) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

Since I did not observe a zone x block learning interaction, and there were visible differences starting in the first quartile in time, I decided to take a closer look at that 1st block point. To examine this block point, I divided the first quartile into 4 more quartiles. A 2x4 within-subject ANOVA (zone x block) phasic data of the learners showed a significant difference over block ($F(3,15)=4.22$, $p<0.05$), showing a reduction of SCR over time. There was no significant effect of zone ($F(1,5)=4.78$, $p>0.05$) or zone x block ($F(3,15)=1.05$, $p>0.05$) (Figure 25).

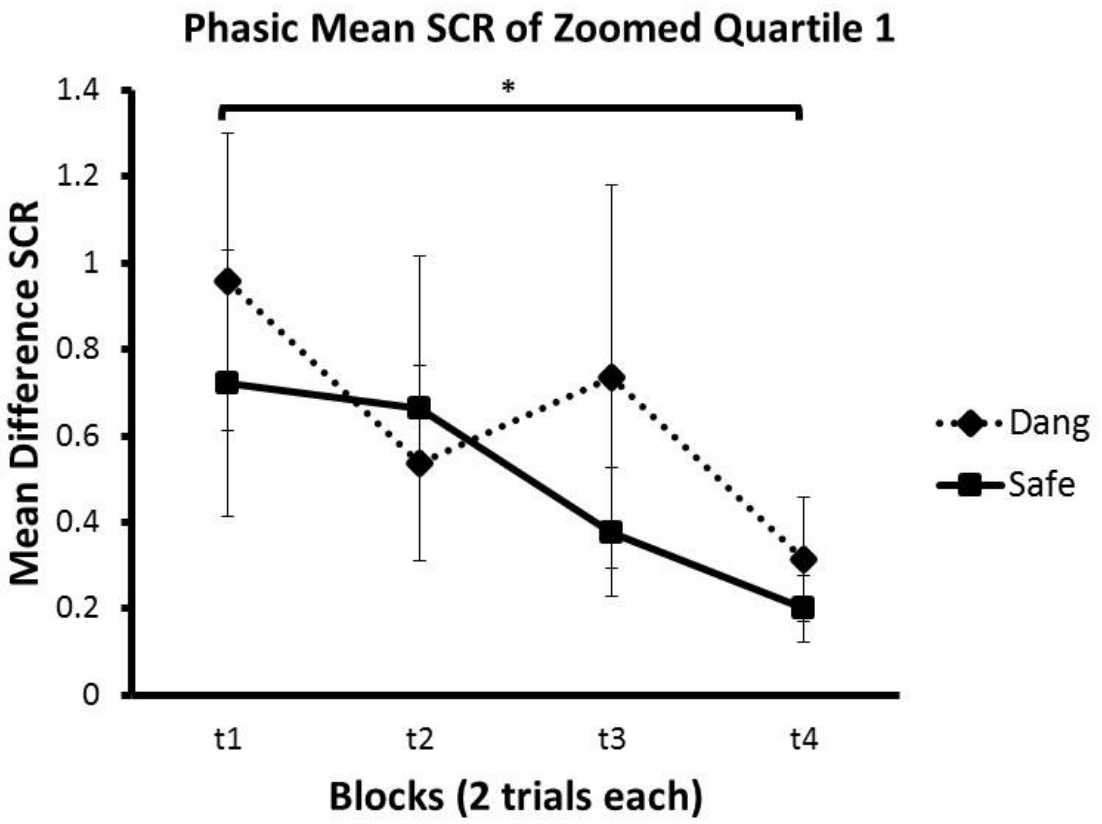


***Figure 25: Experiment 2.1 Zoomed quartile 1 mean phasic SCR.***

*Mean phasic SCR of safe (square) and dangerous (diamond) over the first quartile of time of the experiment. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Threat expectancy rating**

A 2x4 within-subject ANOVA (zone x block) of the threat expectancy rating of the learners showed a significant difference of block ($F(3,15)=14.32$, $p<0.01$), zone ($F(1,5)=98.08$, $p<0.01$), and zone x block ($F(3,15)=11.61$, $p<0.01$), showing a higher expectancy of shock in the dangerous zone compared to the safe

zone over block (Figure 26). A t-test between dangerous and safe zone at each block point showed a significant difference for t1, t2, t3, and t4 ($p<0.05$ uncorrected), only t2, t3, t4 were significant when corrected ($p<0.0125$ Bonferroni corrected).

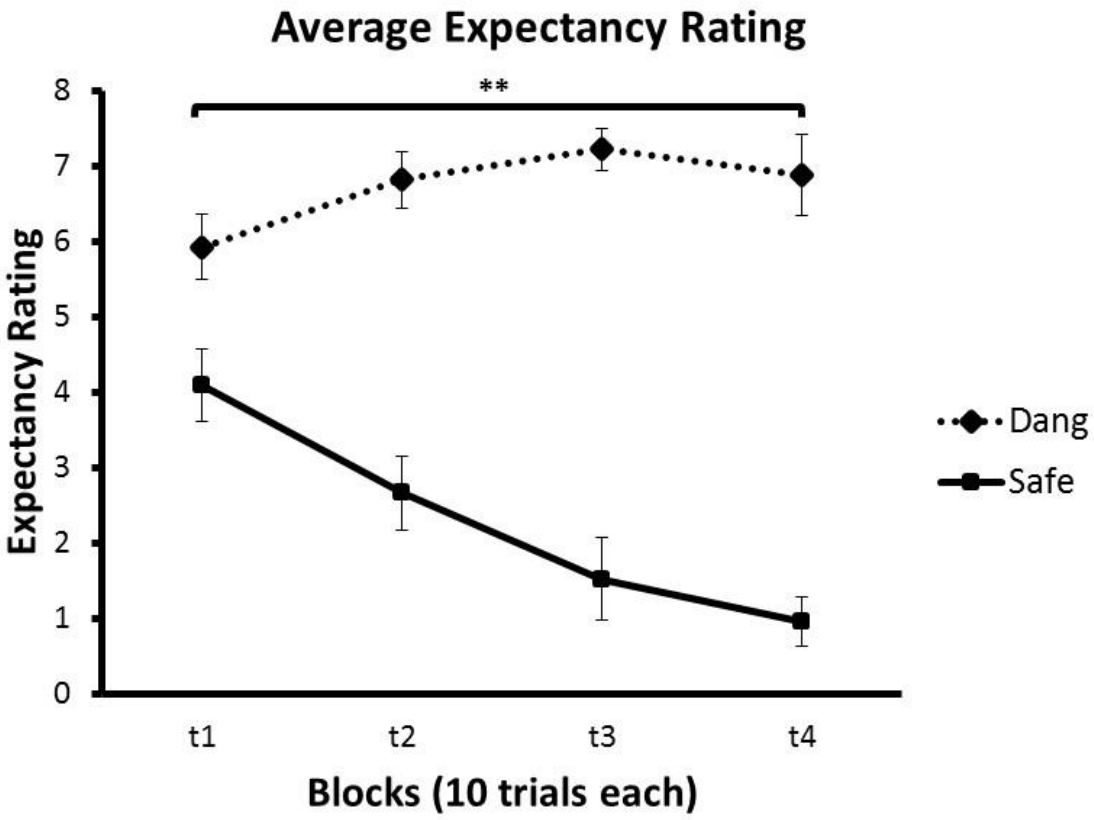


***Figure 26: Experiment 2.1 Average expectancy ratings.***

*Average expectancy ratings of safe (squares) and dangerous (diamond) zone over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

### Conclusions

Here I showed that, unlike in experiment 1.4, a low reinforcement of shock resulted in successful learning only in the expectancy rating data when the participant was given enough time to learn the differences between the zones, but not in the SCR. Furthermore, in this experiment I had a higher number of non-learners. These results suggest that having a low reinforcement of shock, lower than in experiment 1.4, resulted in inaccurate somatic aversive response (SCR). On the other hand, explicit aversive responses (expectancy ratings), showed that the participants were able to accurately predict where they might expect a shock to occur. However, this data should be considered carefully since there are only 6 learners. Still, this finding supports that there are some visual SCR differences in danger vs. safe zones (Figure 24). Consistent with the previous experiments, I saw that participants were able to learn the correct location of the objects in the environment over time.

## Experiment 2.2: 50% reinforcement of shock & high number of trials

### Introduction

From experiment 2.1, I saw that learning and discrimination of dangerous and safe zones only happened explicitly (expectancy ratings) compared to somatic aversive responses (SCR) with a low reinforcement of shock. I saw that with a reinforcement of 35% of shock there was no significant difference of somatic aversive response (SCR) to each zone, but I did see significant differences of explicit aversive responses (expectancy ratings) to each zone over time. However,

visually there were some SCR differences between safe and dangerous zone (Figure 24), but the experiment yielded too many non-learners.

Since I previously saw that predictability is important in learning and accurate fear response of the CS+, I wanted to see if I could have successful implicit and explicit aversive response learning with a 50% of shock, while maintaining a low number of non-learners. My aim was to take this experiment to the fMRI scanner, therefore I thought that having 50% of the trials without an electric shock would yield enough data points, in case of carryover from the shock in the imaging data. I saw that high predictability produced fast and successful implicit and explicit aversive response learning (Experiment 1.3 & 1.4), while a lower predictability only produced successful explicit aversive response learning and reduced the numbers of learners (Experiment 2.1). I hypothesized that a 50% reinforcement of CS+ should yield results similar to experiment 1.4, where there was successful explicit and somatic aversive response learning, while maintaining a high number of learners.

## Methods

***Participants:*** 11 healthy volunteers (2 males), with an average of 23 years, were recruited for this experiment.

***Aversive conditioning procedure:*** Each participant had to collect 4 flowers in between objects. There were 80 flowers in total, an average of 40 flowers in the dangerous zone (20 CS+). Each time the participant collected a flower, the participants would be frozen in place for 3000ms. The reinforcement of shock was 50%, and remained stable as the participant approached the dangerous hive. At the end of the experiment, participants were asked to identify the dangerous side.

8 participants (73%) were able to identify the dangerous zone and were considered “learners” (2 males).

## Results

### Object location memory

A 2x4 within-subjects ANOVA (zone x block) measuring error rate of object placement showed that error decreased significantly over block for learners ($F(3,21)=9.16$, $p<0.01$). It was not dependent on the zone ($F(1,7)=0.00$, $p>0.05$) or interaction between zone x block ($F(3,21)=0.55$, $p>0.05$) (Figure 27).

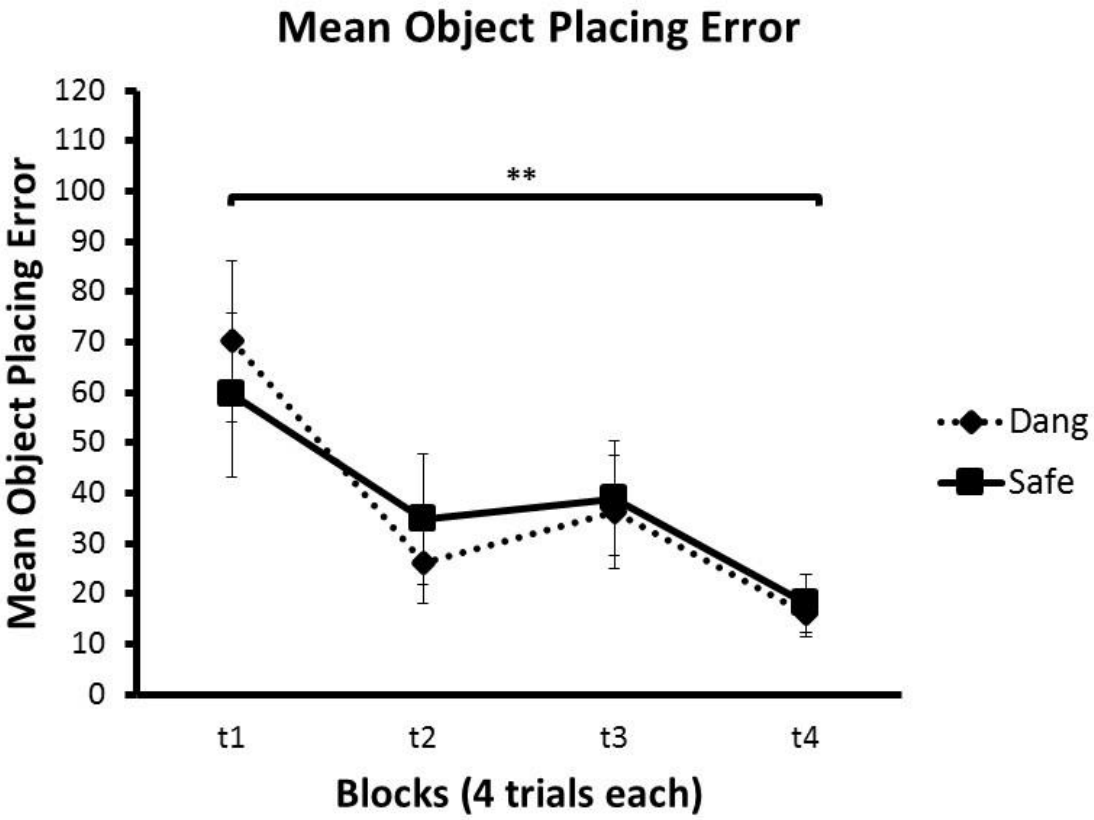


***Figure 27: Experiment 2.2 Mean object placement error.***

*Mean object placement error, for the safe zone (Safe) and the dangerous zone (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Skin conductance response**

A 2x4 within-subject ANOVA (zone x block) on phasic SCR data of the learners showed a significant effect of block ($F(3,21)=4.35$, $p<0.05$), where over time SCR reactivity seemed to decrease. There was no significant effect of zone

(F(1,7)=3.72, p>0.05) or zone x block interaction (F(3,21)=0.10, p>0.05) (Figure 28).

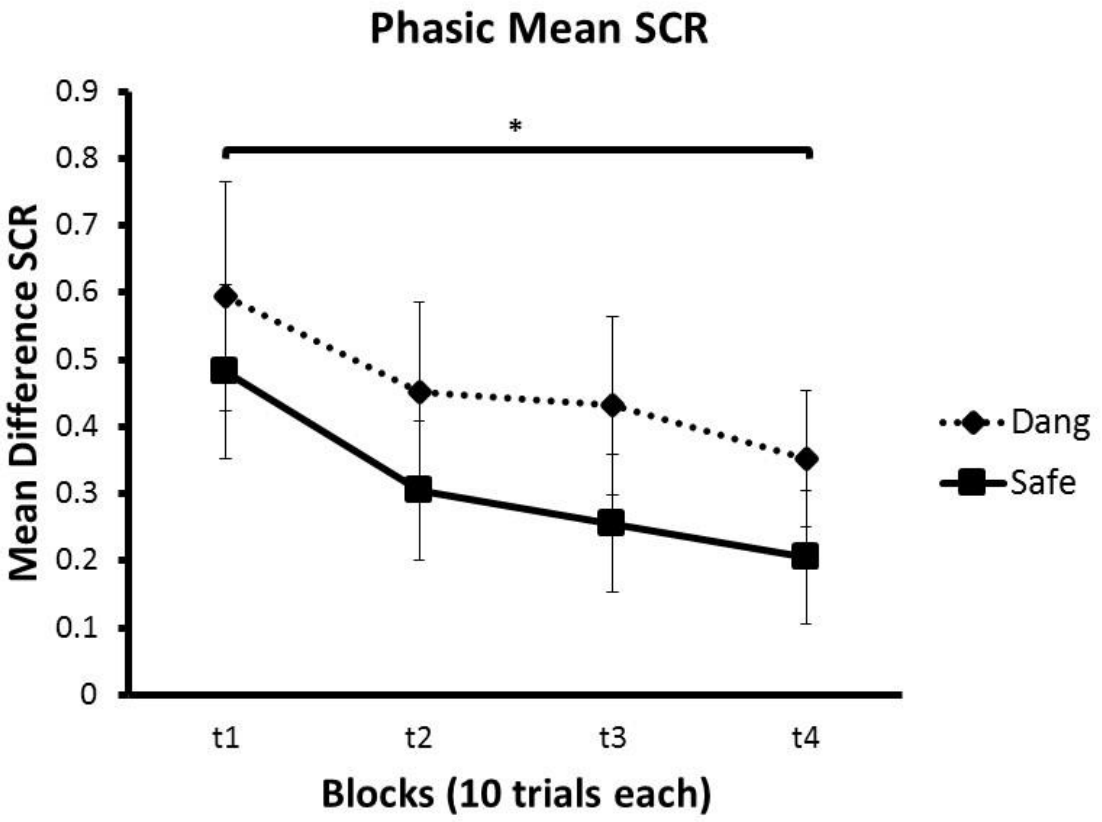


***Figure 28: Experiment 2.2 Mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

Focusing on the first quartile, a 2x4 within-subject ANOVA (zone x block) on phasic SCR data of the learners showed a significant effect of block (F(3,21)=3.51, p<0.05) and a trend for zone (F(1,7)=4.32, p=0.07), showing a trend of higher SCR to the dangerous zone compared to the safe zone, which SCR

reactivity seemed to decrease over time. There was no significant zone x block interaction (F(3,21)=0.66, p>0.05) (Figure 29).

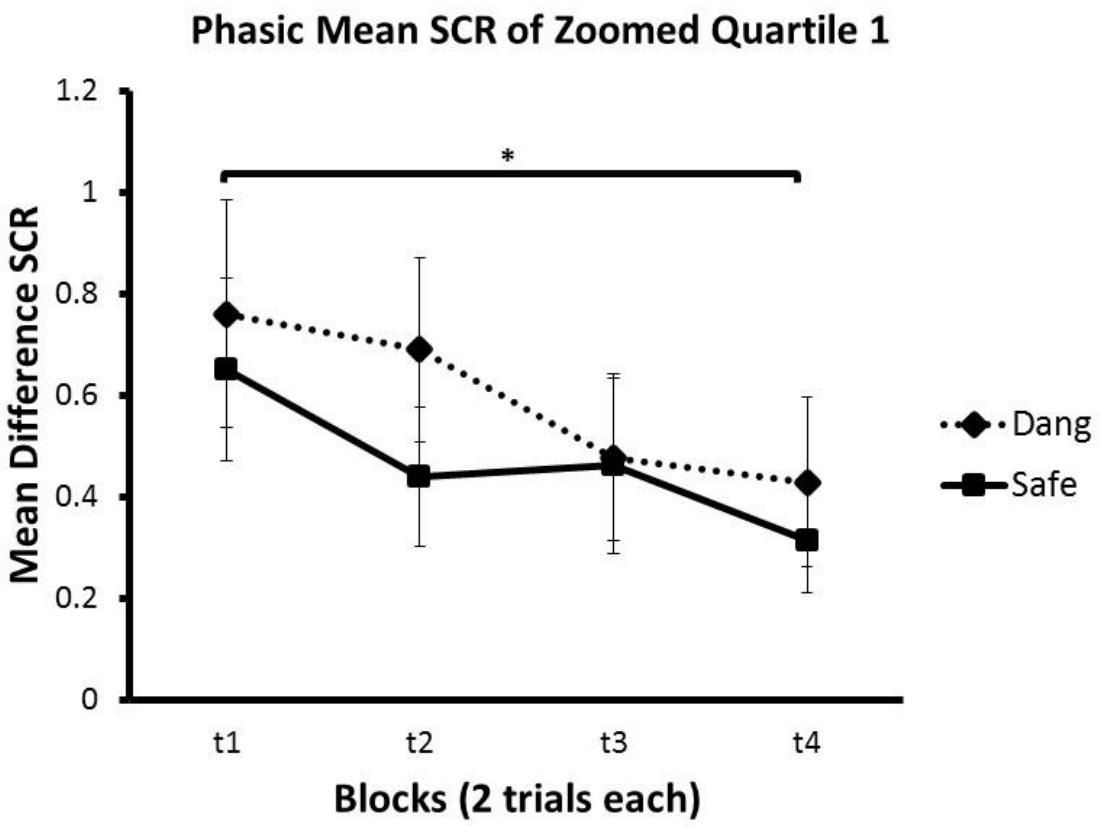


***Figure 29: Experiment 2.2 Zoomed quartile 1 mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) over the first quartile of time of the experiment. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Threat expectancy rating**

A 2x4 within-subject ANOVA (zone x block) of threat expectancy rating of the learners showed a significant effect of zone (F(1,7)=80.39, p<0.01),

and zone x block interaction ($F(3,21)=6.33$, $p<0.01$), showing a higher expectancy of shock in the dangerous zone compared to the safe zone, over time. There was no significant effect of block ($F(3,21)=2.40$, $p>0.05$) (Figure 30). A t-test between dangerous and safe zone at each block point showed a significant difference for t1, t2, t3, and t4 ($p<0.05$ uncorrected) but only t2, t3, and t4 were significant when corrected ($p<0.0125$ Bonferroni corrected).

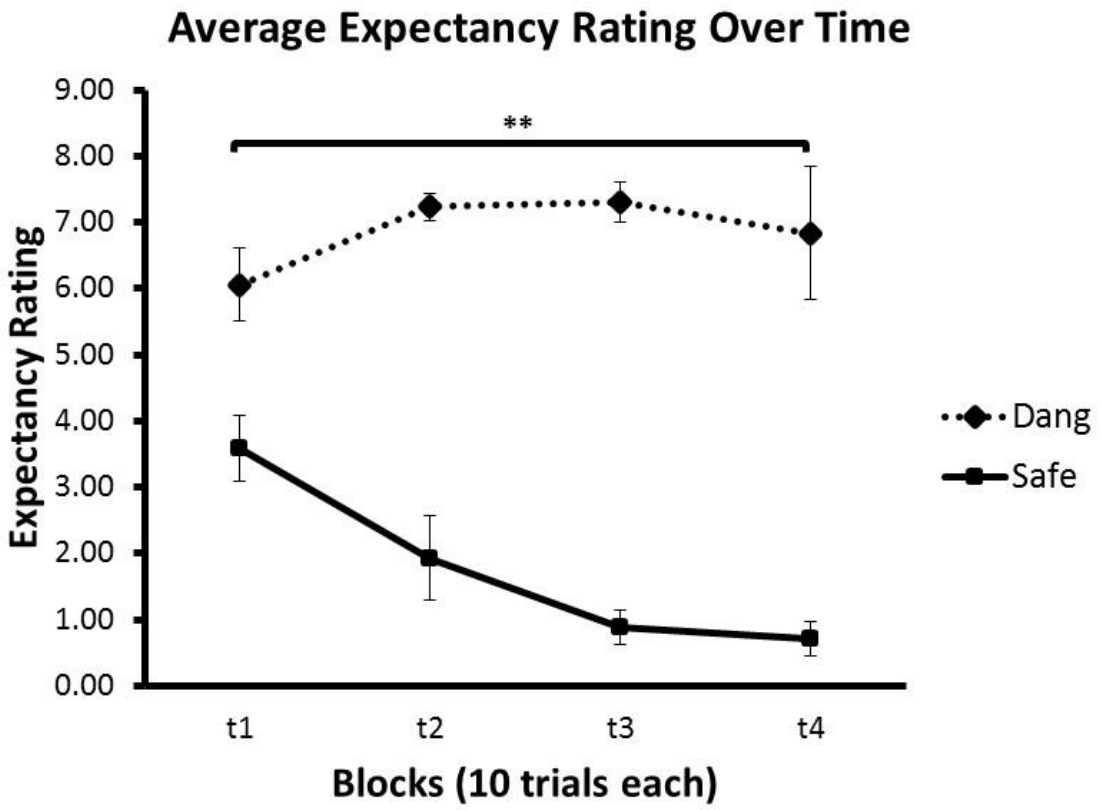


***Figure 30: Experiment 2.2 Average expectancy ratings.***

*Average expectancy ratings of safe (Safe) and dangerous (Dang) zone over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

### Conclusions

Like experiment 2.1, I showed successful explicit aversive response (expectancy rating) learning with a 50% reinforcement of shock within the experiment, but unsuccessful somatic aversive response (SCR) learning to each of the zones. Still, I saw the same pattern of learning in those who learn to differentiate between the safe and dangerous zone. Since this was a pilot study, I only had 8 learners, which could explain the lack of significance in SCR. Likewise, this could suggest that explicit threat learning occurs initially faster than somatic aversive response learning. In other words, before internalizing a fear and anxious response the participant must not only be conscious of the aversive event, but also confident they will be sheltered in the safe zone. Finally, consistent with previous experiments, I saw that participants were able to learn the correct location of the objects in the environment over time.

## Experiment 2.3: 50% reinforcement of shock & high number of trials: *With jittered freezing period time*

### Introduction

From the previous experiments (Experiment 2.1 & 2.2), I saw that learning and discrimination of dangerous and safe zones only happened explicitly (expectancy ratings) compared to somatic aversive responses (SCR). However, I still saw the same pattern of learning in those pilot studies, one with a high number of non-learners (Experiment 2.1) and the other with a low number of non-learners (Experiment 2.2). Therefore, I decided to continue with the 50% reinforcement of shock.

Since my next step was to take the paradigm to the scanner, I decided to recreate experiment 2.2, with a jittered (random variability) time of freezing after collecting a flower, in order to avoid confounds of the shock in the BOLD signal. I expected that jittering the time of freezing would not affect the participant's ability to learn the difference between the safe and dangerous zone and still retain a high number of learners.

## Methods

***Participants:*** 27 healthy volunteers (11 males), with an average of 23 years, were recruited for this experiment.

***Aversive conditioning procedure:*** Each participant had to collect 4 flowers in between objects. There were 80 flowers in total, an average of 40 flowers in the dangerous zone (20 CS+). Each time the participant collected a flower, the participants would be frozen in place for 2000-8000ms. The reinforcement of shock was 50%, and remained stable as the participant approached the dangerous hive. At the end of the experiment, participants were asked to identify the dangerous side. 21 participants (78%) were able to identify the dangerous zone and were considered "learners" (10 males).

## Results

### Object location memory

A 2x4 within-subjects ANOVA (zone x block) measuring error rate of object placement showed that error decreased significantly over block for learners

(F(3,60)=9.09, p<0.01). It was not dependent on the zone (F(1,20)=0.00, p>0.05) or interaction between zone x block (F(3,60)=0.49, p>0.05) (Figure 31).

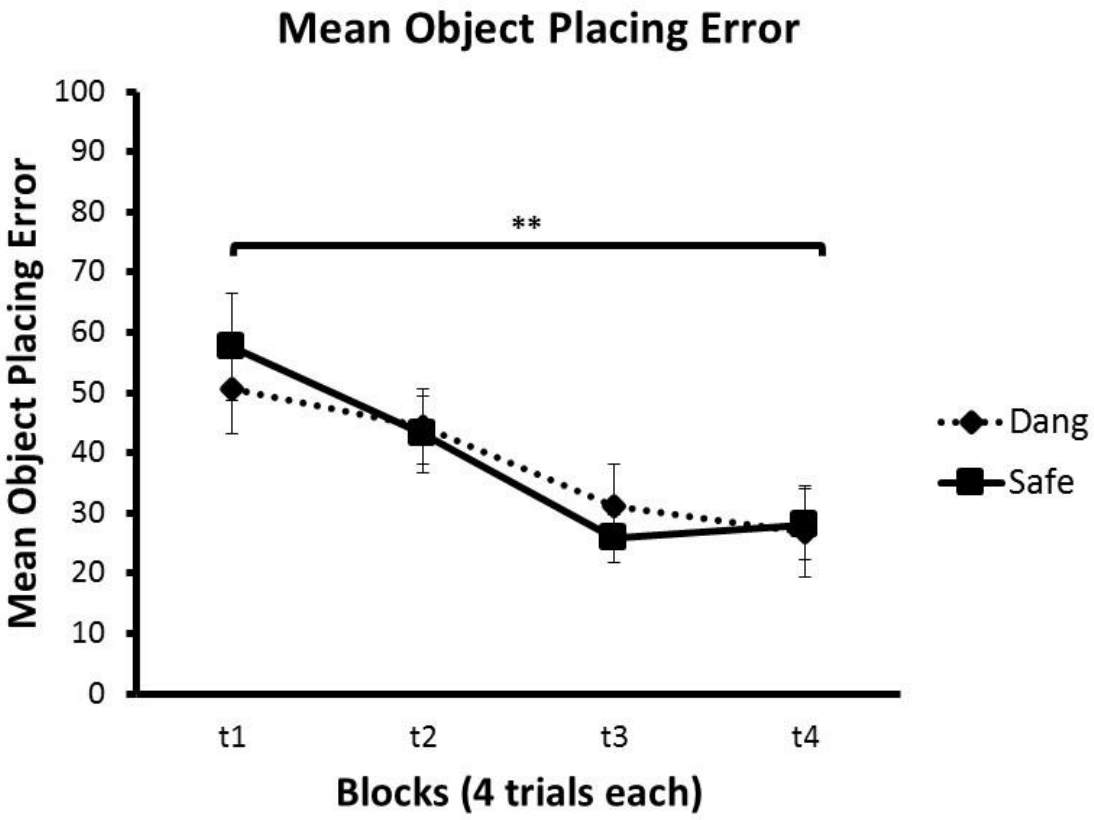


***Figure 31: Experiment 2.3 Mean object placement error.***

*Mean object placement error, for the safe zone (Safe) and the dangerous zone (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Skin conductance response**

A 2x4 within-subject ANOVA (zone x block) on phasic SCR data of the learners showed a significant effect of block (F(3,60)=4.42, p<0.01), and zone

(F(1,20)=15.98, p<0.01), where there was higher SCR reactivity in the dangerous zone compared to the safe zone. There was no significant zone x block interaction (F(3,60)=2.07, p>0.05) (Figure 32). A t-test between dangerous and safe zone at each block point showed a significant difference for t2, t3 (p<0.0125 Bonferroni corrected), and an uncorrected trend at t4 (p=0.063 uncorrected).

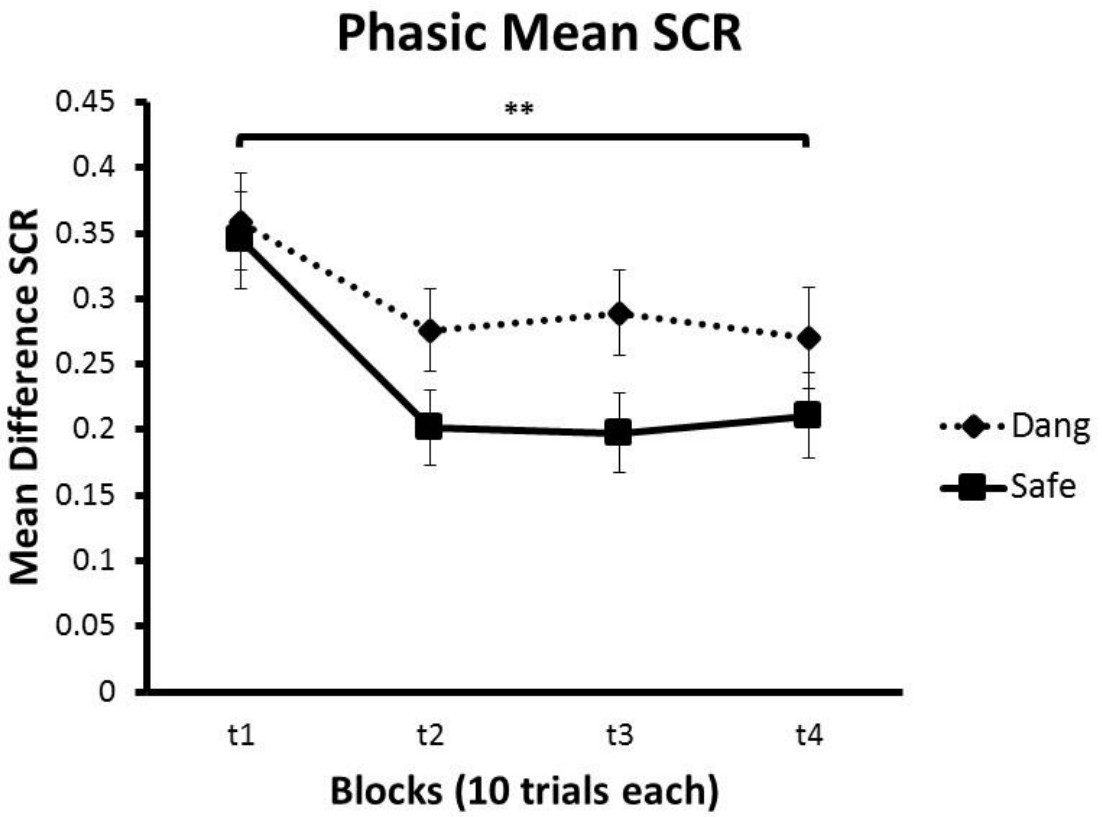


***Figure 32: Experiment 2.3 Mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

Focusing on the first quartile, a 2x4 within-subject ANOVA (zone x block) on phasic SCR data of the learners showed a significant effect of block

(F(3,60)=2.85, p<0.05), showing SCR reactivity decreased over time. There was no significant effect of zone (F(1,20)=0.53, p>0.05) or zone x block interaction (F(3,60)=2.17, p>0.05) (Figure 33).

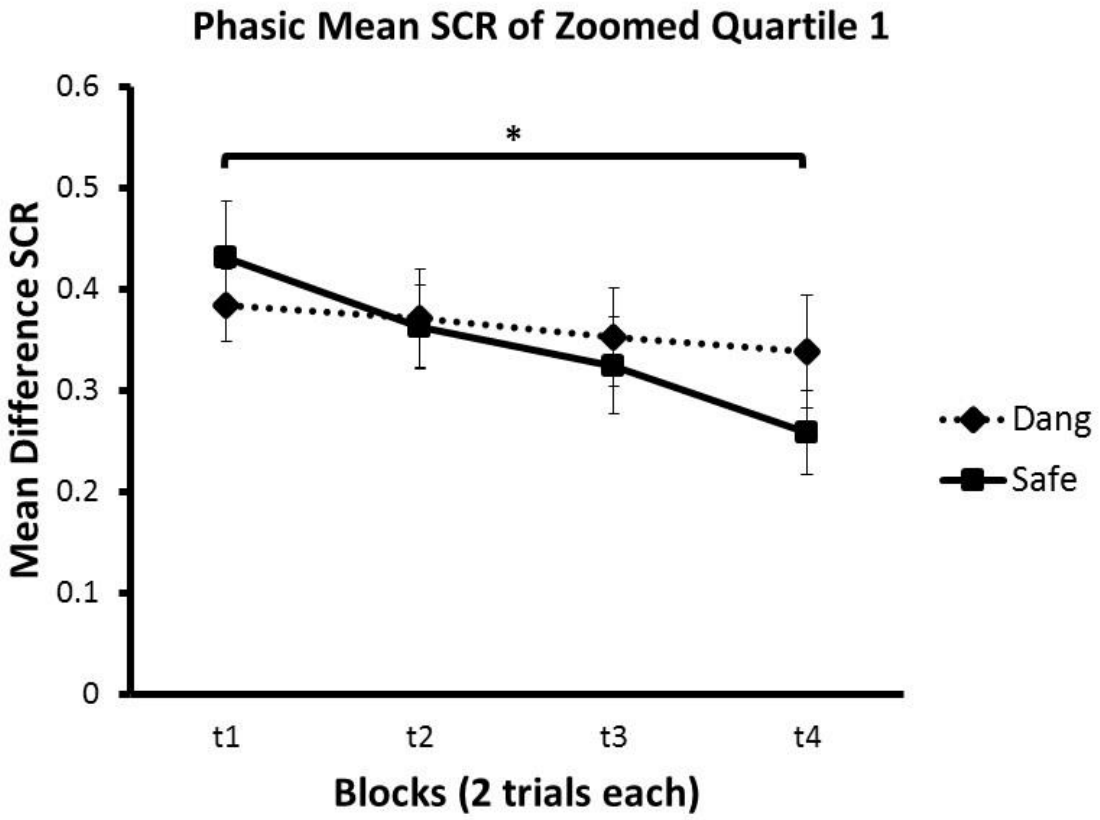


***Figure 33: Experiment 2.3 Mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) over the first quartile of time of the experiment. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Threat expectancy rating**

A 2x4 within-subject ANOVA (zone x block) of threat expectancy rating of the learners showed a significant effect of block ($F(3,60)=14.51$, $p<0.01$), zone ($F(1,20)=210.47$, $p<0.01$), and zone x block interaction ($F(3,60)=39.47$, $p<0.01$), showing a higher expectancy of shock in the dangerous zone compared to the safe zone over block (Figure 34).

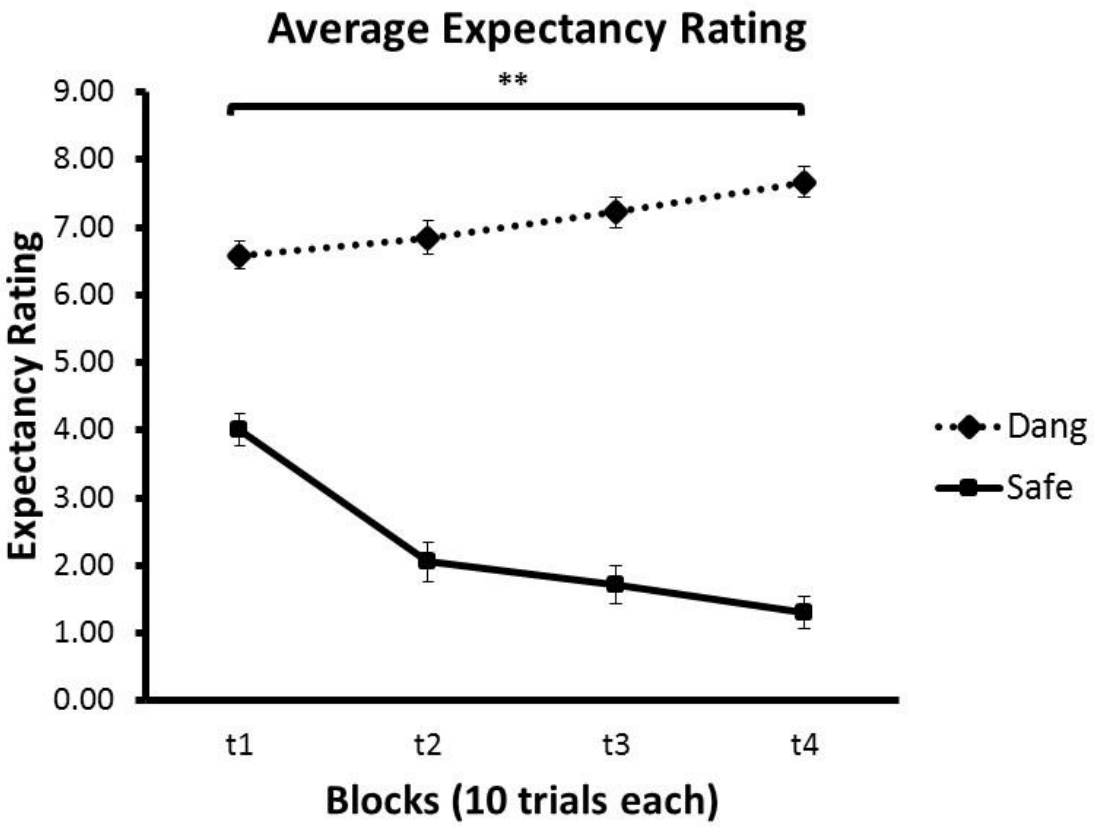


***Figure 34: Experiment 2.3 Average expectancy ratings.***

*Average expectancy ratings of safe (Safe) and dangerous (Dang) zone over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

## Conclusions

Like experiment 1.4, I showed successful explicit aversive response (expectancy rating) learning and somatic aversive response (SCR) learning in each of the zones with the jittered freezing time. Based on these results, I will take this paradigm to the fMRI scanner for brain analysis. Finally, consistent with the previous experiments, I saw that participants were able to learn the correct location of the objects in the environment over time.

## Discussion

The previous series of experiments were designed to find the best settings for the paradigm where (1) participants could slowly learn to discriminate between the safe and dangerous zone of the environment over time; (2) yield a high number of learners; and (3) have a reduced number of shocks to avoid any confounds in the BOLD response. Overall, the results suggested that (a) participants were able to discriminate between the safe and dangerous zone using a low number of CS+ (Experiment 2.1 & 2.2); (b) even through a stable reinforcement of shock and long duration of the experiment, a low reinforcement of shock (35%) caused a high number of non-learners (Experiment 2.1), which was reduced by increasing the reinforcement of shock to 50% (Experiment 2.2). Since I couldn't reduce the number of shocks to 35% without affecting the number of learners, I found jittering the time of freezing was an effective measure to (1) maintain participants' ability to discriminate between safety and danger zones along a slow but effective trajectory, (2) maintain a high number of learners, and (3) by jittering the time before the delivery of the CS+, I will be able to keep all the trials for the fMRI Analysis (Experiment 2.3). Based on these experiments, I decided to maintain a

50% reinforcement of shock with a jittered freezing period as the optimal settings for the experiment, which will be repeated inside the fMRI scanner to assess the neural correlates of location-specific threat learning and discrimination of safety and danger within an environment.

# Chapter 6

## Experiment 3: Behavioral Study of Context Discrimination and Cognitive Map Formation: Using aversive screams

## Precis

Since some of my future aims included the use of this paradigm to study overgeneralization in patients with anxiety disorders, children and adults, I wanted to validate the task using an aversive alternative to shocks that would be more feasible with these vulnerable populations. In particular, ethical concerns prevent the use of electrical shocks in children. In addition, anxious patients, adults and children, might be discouraged from participating in the study due to the highly aversive nature of electrical shocks, in addition to being inside the enclosed space of the fMRI. As an alternative to shock, other studies have used auditory threats (e.g., loud white noise) as an unconditioned stimulus. Recently, studies have also been using the sound of a screaming lady as an unconditioned stimulus, as a reasonable alternative to an aversive shock (Britton, Lissek, Grillon, Norcross, & Pine, 2011). This novel approach has been examined in a study of anxiety disorders in youth, which found greater fear response to the CS+ compared to the CS-, for anxious participants relative to healthy ones (Lau et al., 2008). Therefore, I decided to try using an aversive sound, the screaming lady paradigm, which has been shown to be highly aversive, but less so than electrical shocks.

The following set of experiments tested the same paradigm, examining learning and discrimination between danger and safety within a single environment, using an aversive scream instead of electrical shocks. With the knowledge acquired from the previous experiments, and knowing that the screaming lady paradigm is, although aversive, less aversive than an electrical shocks, I decided to (a) replicate experiment 1.4, with a stable reinforcement of shock increased to 65% (Experiment 3.1); (b) replicate experiment 1.3, having a high and stable reinforcement of screams with a low number of trials (Experiment 3.2); (c) replicate experiment 2.1, with a low and stable reinforcement of screams with a high number of trials, again with a slightly raised reinforcement of scream to account for the slightly less averseness of the scream (Experiment 3.3); and (d)

replicate experiment 2.3, with a stable reinforcement of scream using a high number of trials and adding the jittered freezing block (Experiment 3.4).

It was expected that, over time, participants would learn to discriminate between the safe and dangerous zone indexed by differential SCR and expectancy ratings that increased, in the dangerous zone, during learning. Like experiment 1.4, I expected, that a 65% reinforcement of scream would yield a slow yet accurate learning to discriminate between the safe and dangerous zone (Experiment 3.1), while increasing the reinforcement of scream and reducing the number of trials would produce fast and accurate learning, like experiment 1.3 (Experiment 3.2). Furthermore, I predicted that a low reinforcement of screams with a high number of trials would produce a slower ability to discriminate between the safe and the dangerous zone (Experiment 3.3). Finally, I sought to replicate the optimal experiment for the scanner, experiment 2.3, using screams (Experiment 3.4) to test if this could be a viable alternative instead of using electrical shocks inside the scanner.

## Method

The methods used in this experiment were the same as the ones described above, alterations to the methods are described in each experiment. All experiments in this chapter use an aversive scream instead of electrical shocks.

***Scream procedure:*** Half of the environment was associated with danger, flowers picked in this zone were reinforced with an aversive scream (danger), while flowers picked in the other half never paired with an aversive scream (safe). The scream was a 95dB screaming lady lasting 1181ms, delivered through

headphones. The scream was the same used by Britton et al. (2011) and Lau et al. (2008).

# Experiment 3

## Experiment 3.1: 65% reinforcement of scream & high number of trials

### Introduction

From the previous experiments, I saw that with a shock reinforcement of 50% or more, implicit and explicit learning was successful in maintaining a high number of learners, and that the higher the reinforcement, the faster the learning.

Likewise, I wanted to see if I could repeat the findings, having successful implicit and explicit aversive response learning, with an auditory stimulus instead of a shock. Therefore, I decided to use the screaming lady paradigm instead of an electrical shock. I hypothesized that a 65% reinforcement of CS+ should yield results similar to experiment 1.4 (60% reinforcement of shock), where there was successful explicit and somatic aversive response learning using an electric stimulus.

### Methods

***Participants:*** 20 healthy volunteers (6 males), with an average of 23 years, were recruited for this experiment.

***Aversive conditioning procedure:*** Each participant had to collect 4 flowers in between objects. There were 80 flowers in total, an average of 40 flowers in the dangerous zone (26 CS+). Each time the participant collected a flower, the participants would be frozen in place for 3000ms. The reinforcement of scream

was 65%, and remained stable as the participant approached the dangerous hive. At the end of the experiment, participants were asked to identify the dangerous side. 18 participants (90%) were able to identify the dangerous zone and were considered “learners” (5 males).

## Results

### Object location memory

A 2x4 within-subjects ANOVA (zone x block) measuring error rate of object placement showed that error decreased significantly over block for learners ($F(3,51)=9.26$, $p<0.01$). It was not dependent on the zone ($F(1,17)=0.60$, $p>0.05$) or interaction between zone x block ($F(3,51)=1.55$, $p>0.05$) (Figure 35).

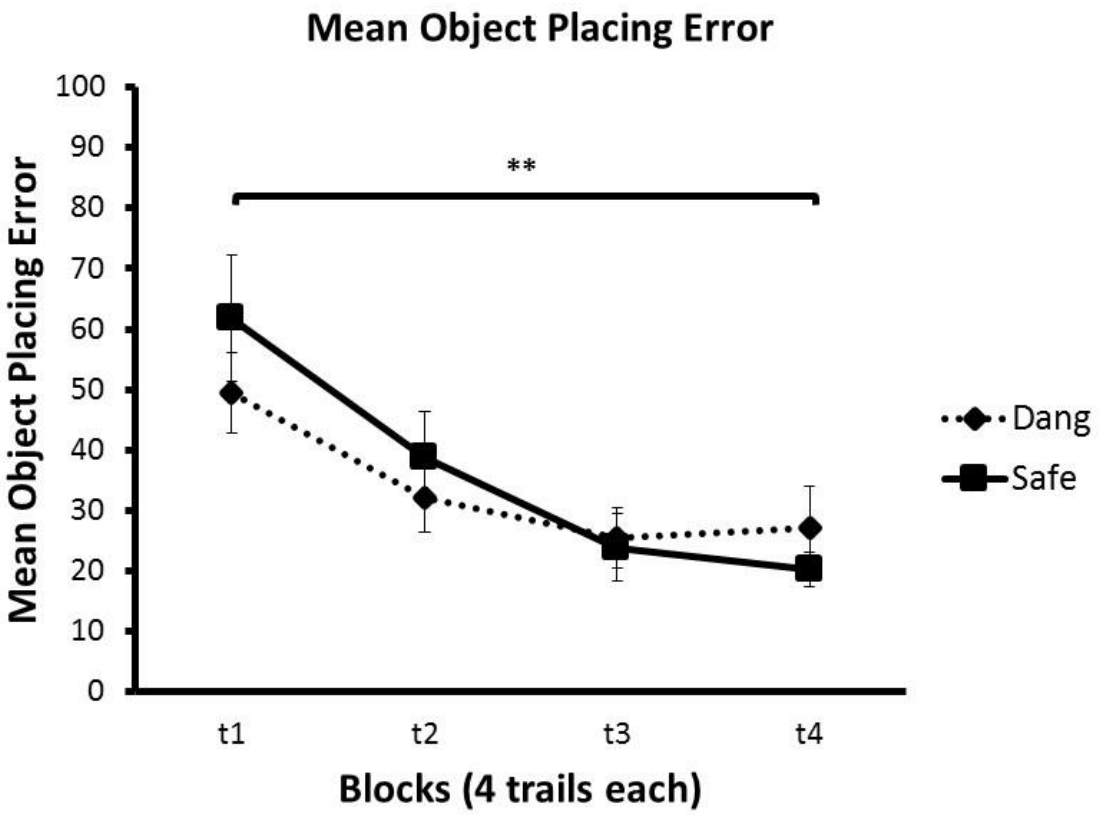


***Figure 35: Experiment 3.1 Mean object placement error.***

*Mean object placement error, for the safe zone (Safe) and the dangerous zone (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Skin conductance response**

A 2x4 within-subject ANOVA (zone x block) on phasic SCR data of the learners showed a significant effect of block ($F(3,51)=3.07$, $p<0.05$) where SCR decreased over block time, suggesting habituation to the scream. There was no

significant effect of zone ($F(1,17)=0.004$, $p>0.05$) or zone x block interaction ($F(3,51)=0.02$, $p>0.05$) (Figure 36).

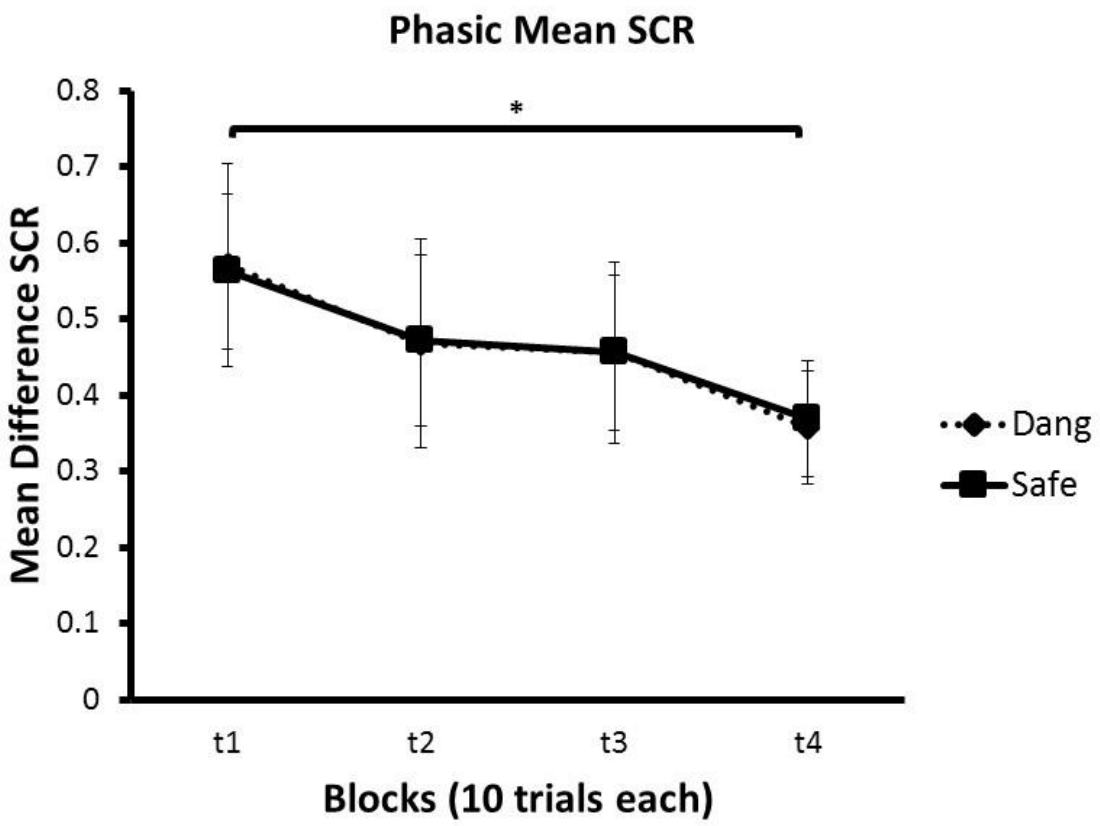


***Figure 36: Experiment 3.1 Mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Threat expectancy rating**

A 2x4 within-subject ANOVA (zone x block) of threat expectancy rating of the learners showed a significant effect of block ($F(3,51)=3.77$, $p<0.05$), zone

(F(1,17)=120.38, p<0.01), and zone x block interaction (F(3,51)=25.74, p<0.01), showing a higher expectancy of scream in the dangerous zone compared to the safe zone over block (Figure 37).

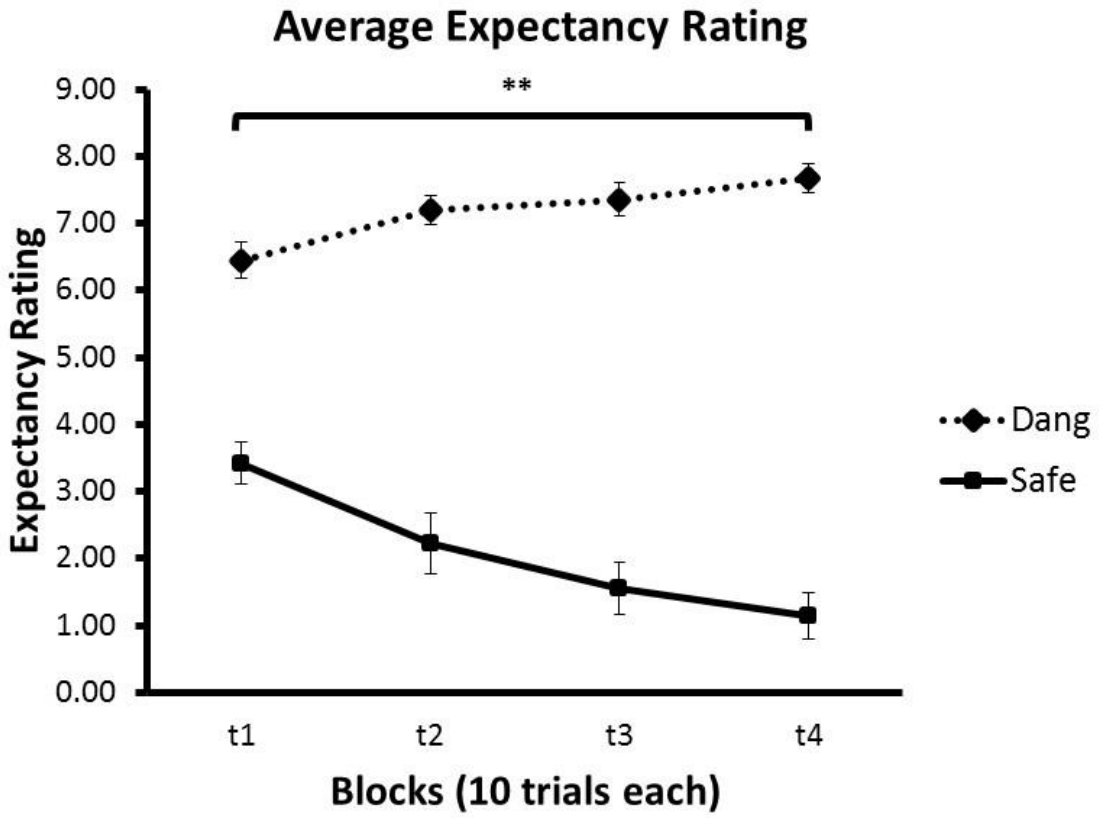


***Figure 37: Experiment 3.1 Average expectancy ratings.***

*Average expectancy ratings of safe (Safe) and dangerous (Dang) zone over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

To make sure the scream was eliciting the expected fear response, I did a 2x4 within-subject ANOVA (zone x block) on phasic SCR, after the scream onset data of the learners. The data showed a significant effect of zone (F(1,17)=19.61, p <.01), where the SCR reactivity was higher for the dangerous side compared to

the safe after a scream was delivered. There was no significant effect of block ($F(3,51)=0.15$, $p>0.05$) or zone x block interaction ($F(3,51)=1.89$, $p> .05$) (Figure 38).

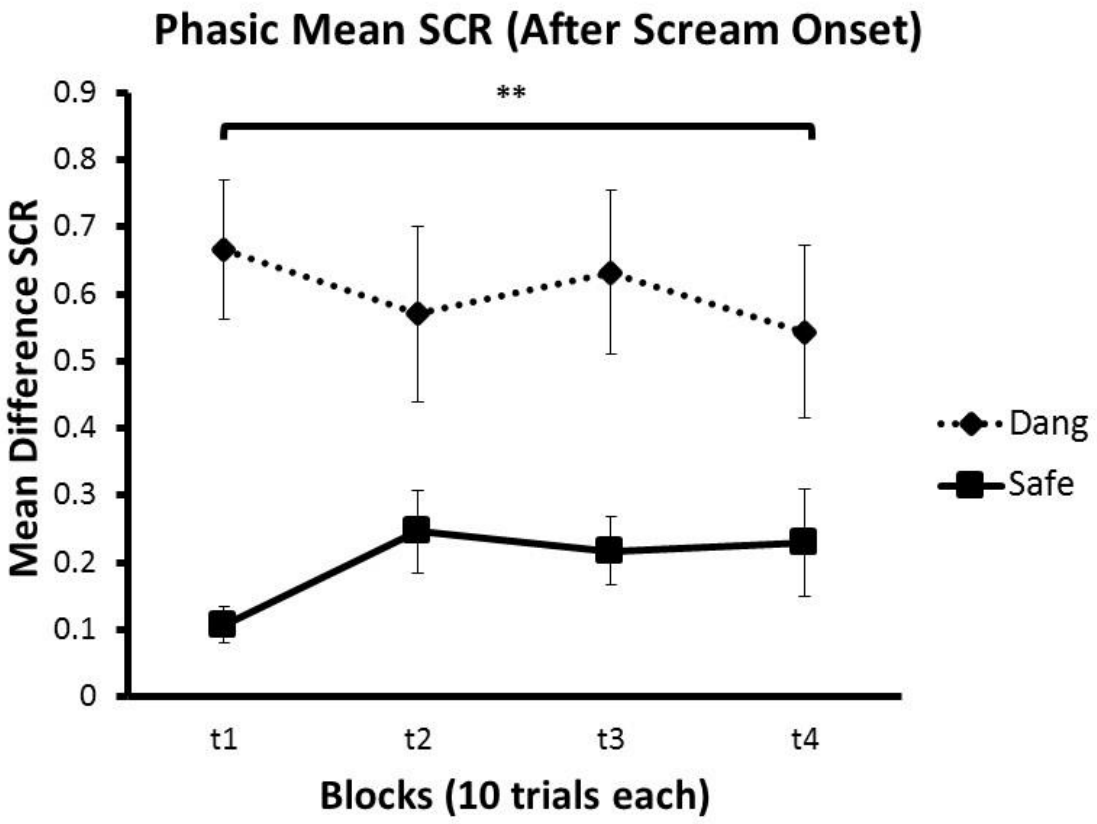


***Figure 38: Experiment 3.1 Mean phasic SCR after scream onset.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) after scream onset over time for the non-learners and learners taken together. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

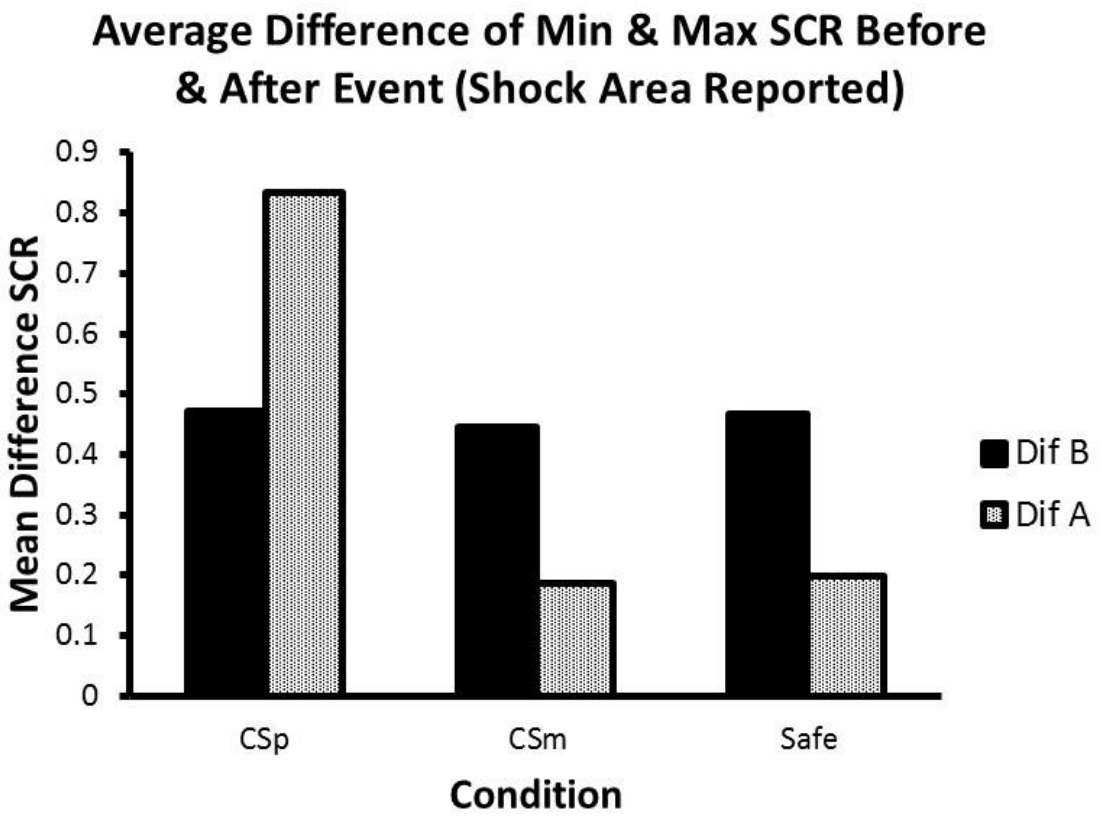


***Figure 39: Experiment 3.1 Mean phasic SCR difference before and after scream onset.***

*Mean Phasic SCR of before scream onset (Dif B) and after scream onset (Dif A) for flowers picked in the safe zone (safe) and in the dangerous zone; with shock (CSp) and without shock (CSm) after flower pickup.*

## Conclusions

Like the previous experiments, I showed successful explicit learning response (expectancy rating) learning here, but unlike previous experiments there

seemed to be unsuccessful somatic aversive response (SCR) learning in each of the zones. The data showed that the scream evoked a large SCR response (Figure 39), suggesting that the scream was generating a fear response. Still, there seemed to be no SCR difference before the scream onset between the dangerous zone and safe zone over time. This change could mean that either the scream was not powerful enough for threat learning, or that the participants were not able to successfully internalize the difference between zones (SCR), even though they were able to explicitly determine which was the dangerous zone (expectancy data). These results were similar to the results of experiment 2.1, where there was successful explicit aversive response learning, but not somatic aversive response learning using an electric stimulus. To determine if the scream was powerful enough to elicit a successful SCR threat learning response, I re-ran this experiment using a 95% reinforcement of scream (described below in Experiment 3.2), similar to experiment 1.3. Finally, consistent with the previous experiments, I saw that participants were able to learn the correct location of the objects in the environment over time.

## Experiment 3.2: 95% reinforcement of scream & low number of trials

### Introduction

From experiment 3.1, I saw that learning and discrimination of dangerous and safe zones only happened explicitly (expectancy ratings) compared to somatic aversive responses (SCR) with a 65% reinforcement of scream. I concluded that either the scream was not powerful enough for threat learning or that the participants were not able to successfully internalize the difference between zones (SCR), even though they are able to explicitly determine which was the dangerous

zone (expectancy data). Still, the data showed that the scream was eliciting a higher SCR response after onset of the scream, compared to when it was not present.

I wanted to see if by increasing the reinforcement of scream to 95%, I could find successful implicit and explicit aversive response learning. Therefore, I decided to repeat the screaming lady with a 95% reinforcement of CS+.

## Methods

***Participants:*** 9 healthy volunteers (1 male), with an average of 23 years, were recruited for this experiment.

***Aversive conditioning procedure:*** The participant had to collect 2 flowers in between objects. There were 40 flowers in total, an average of 20 flowers in the dangerous zone (19 CS+). Each time the participant collected a flower, the participants would be frozen in place for 3000ms. The reinforcement of scream was 95%, and remained stable as the participant approached the dangerous hive. At the end of the experiment, I asked participants to identify the dangerous side. 8 participants (89%) were able to identify the dangerous zone and were considered “learners” (1 male).

## Results

### Object location memory

A 2x4 within-subjects ANOVA (zone x block) measuring error rate of object placement showed that error decreased significantly over block for the learners

(F(3,21)=3.36, p<0.05). It was not dependent on the zone (F(1,7)=0.64, p>0.05) or interaction between zone x block (F(3,21)=0.48, p>0.05) (Figure 40).

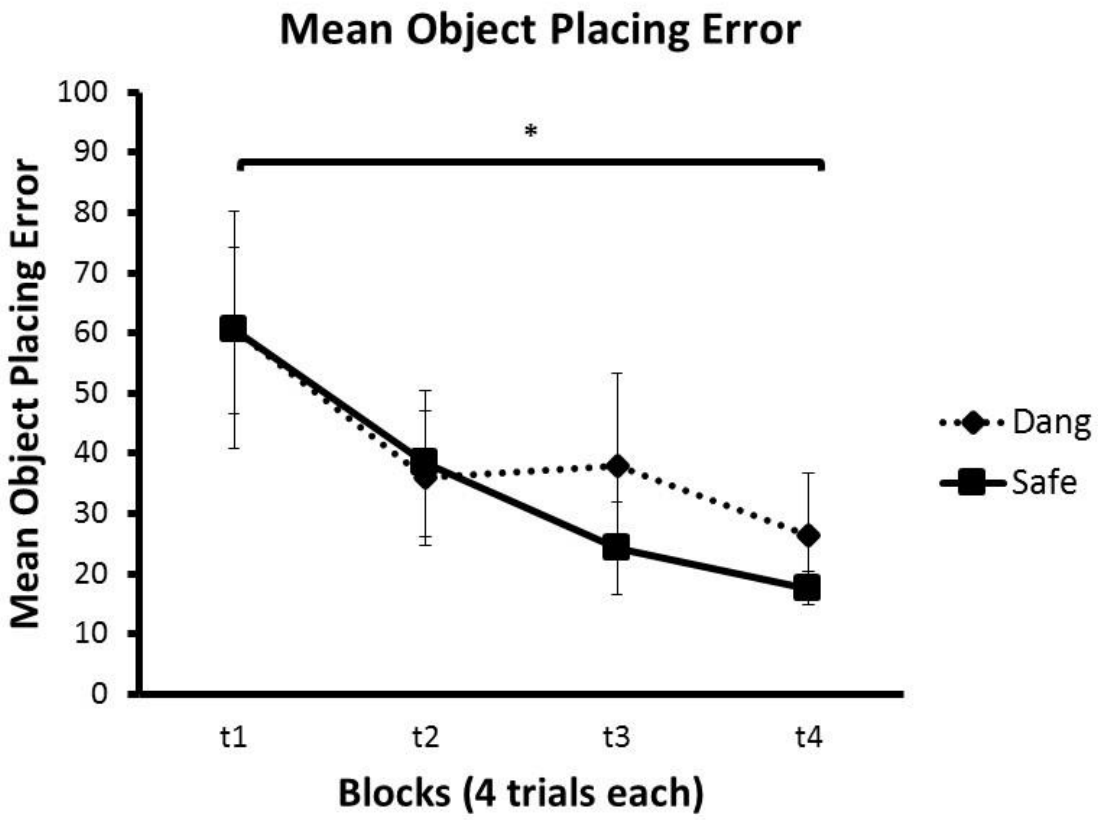


***Figure 40: Experiment 3.2 Mean object placement error.***

*Mean object placement error, for the safe zone (Safe) and the dangerous zone (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Skin conductance response**

A 2x4 within-subject ANOVA (zone x block) on phasic SCR data of the learners showed a trend effect of block (F(3,21)=2.59, p=0.079), where over time

the SCR decreased slightly. There was no significant effect of zone ($F(1,7)=0.09$, $p>0.05$) or zone x block interaction ($F(3,21)=0.29$, $p>0.05$) (Figure 41).

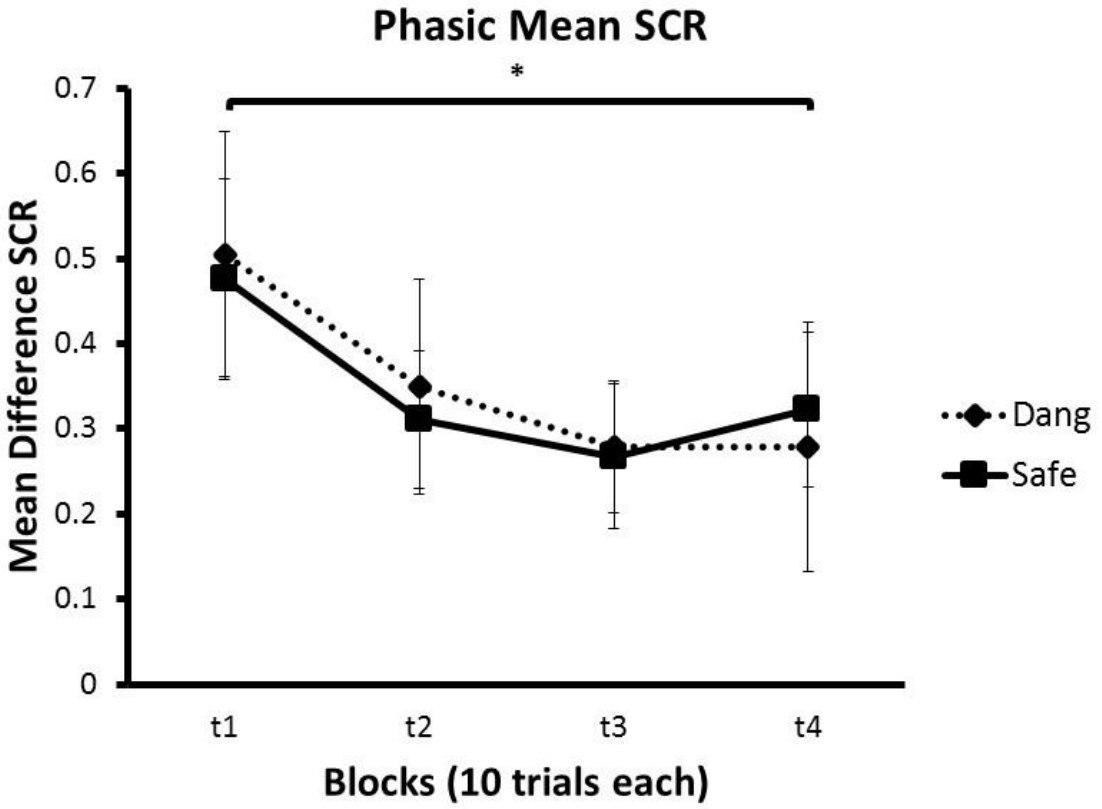


***Figure 41: Experiment 3.2 Mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Threat expectancy rating**

A 2x4 within-subject ANOVA (zone x block) of threat expectancy rating of the learners showed a significant effect of zone ($F(1,7)=54.92$, $p<0.01$), and zone

x block interaction (F(3,21)=8.61, p<0.01), showing a higher expectancy of scream to the dangerous zone compared to the safe zone over time. There was no main effect of block (F(3,21)=0.06, p>0.05) (Figure 42).

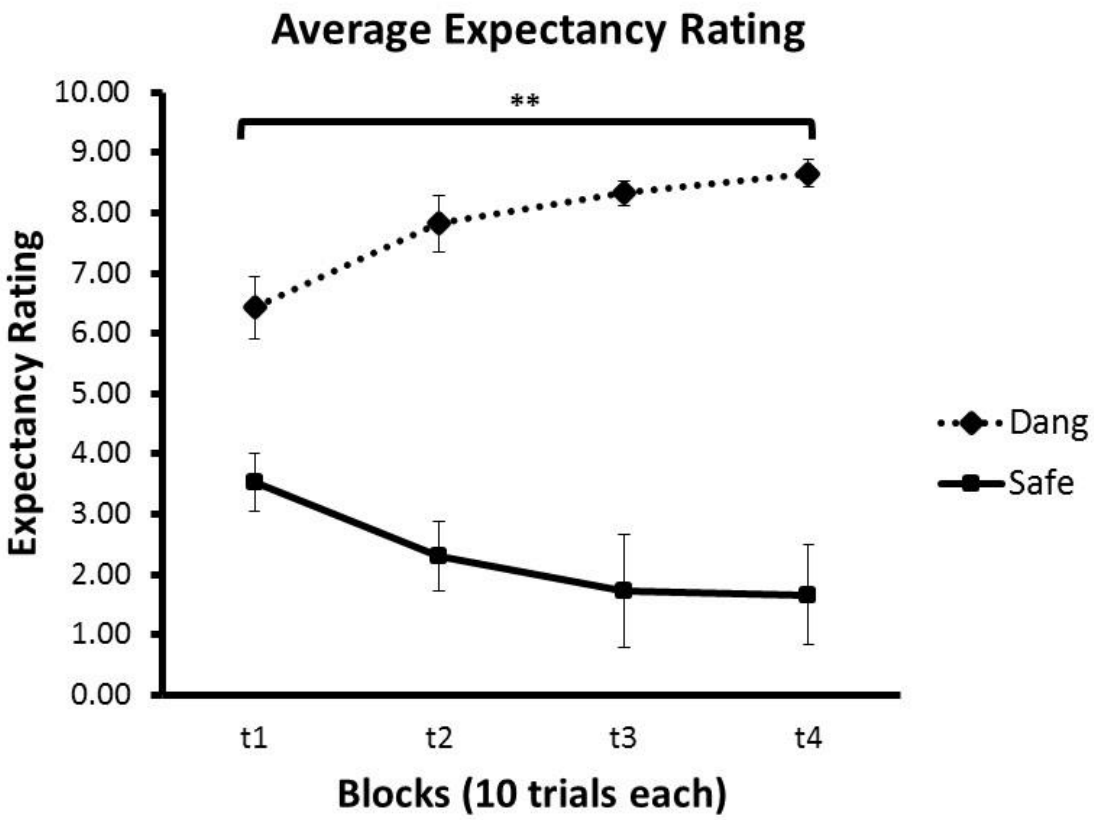


***Figure 42: Experiment 3.2 Average expectancy ratings.***

*Average expectancy ratings safe (Safe) and dangerous (Dang) zone over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

### Conclusions

Like experiment 3.1, I showed successful explicit aversive response (expectancy rating) learning here, and again I found unsuccessful somatic aversive response (SCR) learning to each of the zones. The data showed that the scream was eliciting a higher SCR response after onset of the scream, compared to when it was not present, suggesting that the scream was generating a fear response. Still, there seemed to be no SCR difference before the scream onset between the dangerous zone and safe zone over time. I posited that the scream was creating an anxiogenic environment. Although participants were able to distinguish where the safe and dangerous zones were, they still felt anxious in the safe zone. To determine if this was the case, I re-ran this experiment using a 40% reinforcement of scream (as described below in Experiment 3.3). Having a lower reinforcement of scream should give participants enough information to distinguish the two zones and elicit a fear and anxious response. Meanwhile, a lower amount of scream might help them feel safer in the safe zone. Finally, consistent with the previous experiments, I saw that participants were able to learn the correct location of the objects in the environment over time.

## Experiment 3.3: 40% reinforcement of scream & high number of trials

### Introduction

From the previous experiment 3.2, I still saw that learning and discrimination of dangerous and safe zones only happened explicitly (expectancy ratings), but not implicitly (SCR), with a 95% reinforcement of scream. I started thinking that the paradigm might be too emotionally charged and so the participants were

overgeneralizing the fear and anxious response throughout the whole environment, even though they were able to explicitly determine the dangerous from the safe zone (expectancy data).

To test if the participants are indeed overgeneralizing the fear and anxious response because of the high reinforcement of scream, I lowered the reinforcement of scream to 40%, to see if I could find successful implicit and explicit aversive response learning. Therefore, I decided to repeat the screaming lady trial with a 40% reinforcement of CS+.

## Methods

***Participants:*** 18 healthy volunteers (6 males), with an average of 23 years, were recruited for this experiment.

***Aversive conditioning procedure:*** Each participant had to collect 2 flowers in between objects. There were 80 flowers in total, an average of 40 flowers in the dangerous zone (16 CS+). Each time the participant collected a flower, the participants would be frozen in place for 3000ms. The reinforcement of scream was 40%, and remained stable as the participant approached the dangerous hive. At the end of the experiment, participants were asked to identify the dangerous side. 17 participants (95%) were able to identify the dangerous zone and were considered “learners” (6 males).

## Results

### Object location memory

A 2x4 within-subjects ANOVA (zone x block) measuring error rate of object placement showed that error decreased significantly over block for the learners ($F(3,48)=2.75$, $p=0.05$). It was not dependent on the zone ($F(1,16)=0.18$, $p>0.05$) or interaction between zone x block ($F(3,48)=0.23$, $p>0.05$) (Figure 43).

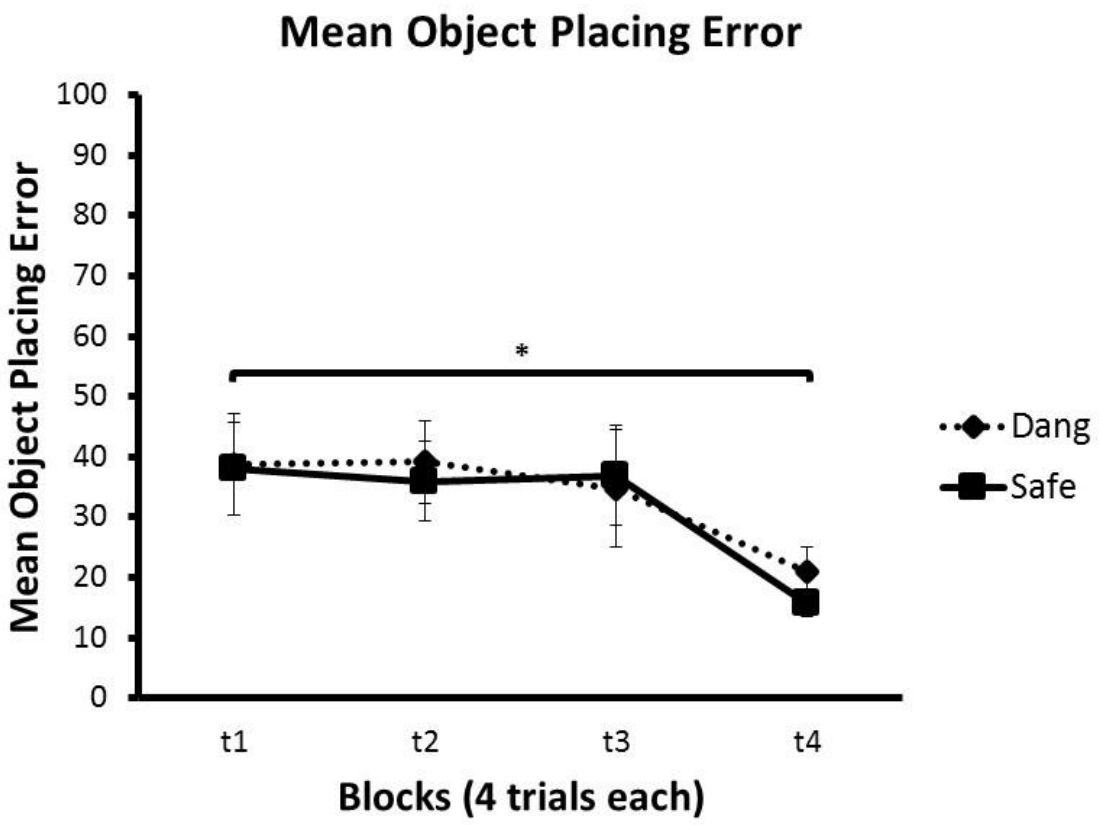


***Figure 43: Experiment 3.3 Mean object placement error.***

*Mean object placement error, for the safe zone (Safe) and the dangerous zone (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

### Skin conductance response

A 2x4 within-subject ANOVA (zone x block) on phasic SCR data of the learners showed no significant effect of block ($F(3,51)=0.80$, $p>0.05$), zone ($F(1,17)=1.35$, $p>0.05$), or zone x block interaction ($F(3,51)=0.89$, $p>0.05$) (Figure 44). A t-test between dangerous and safe zone at each block point showed no significant difference for t1, t2, t3, or t4 ($p>0.05$ uncorrected), although there was a trend difference at t4 ($p=0.077$ uncorrected).

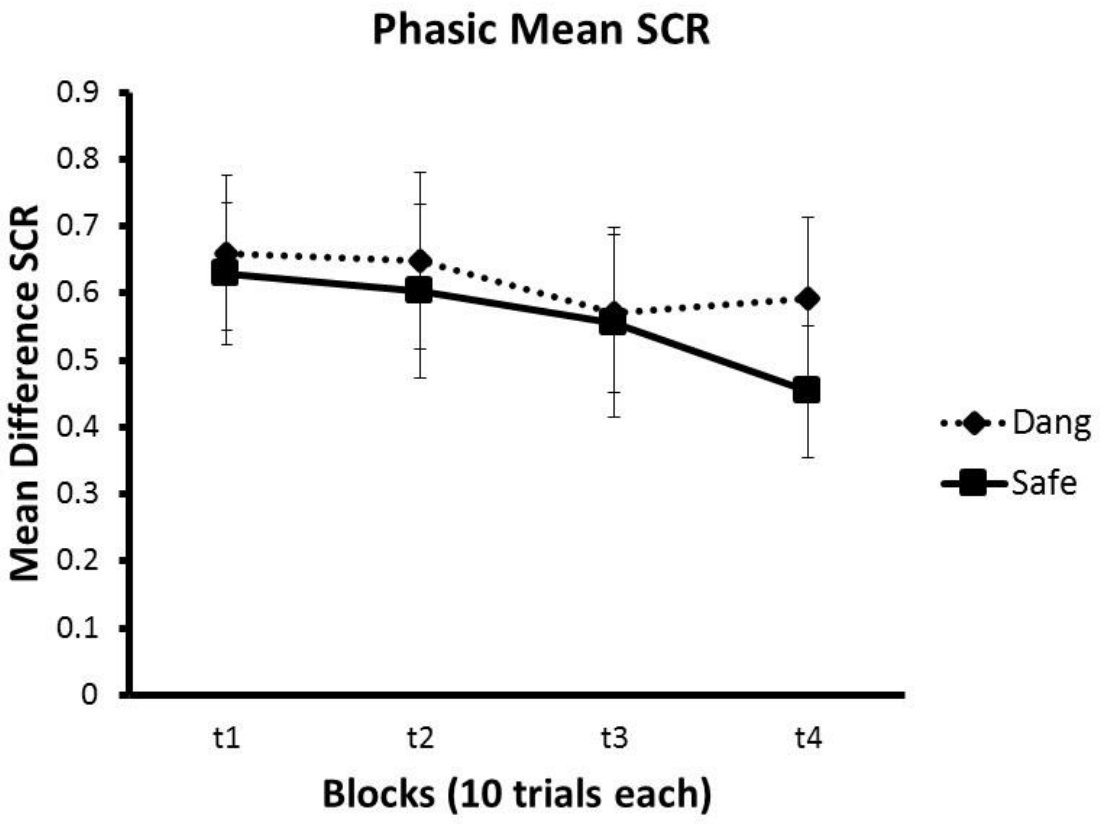


***Figure 44: Experiment 3.3 Mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Threat expectancy rating**

A 2x4 within-subject ANOVA (zone x block) of threat expectancy rating of the learners showed a significant effect of block ($F(3,48)=4.86$, $p<0.01$), zone ($F(1,16)=91.26$, $p<0.01$), and zone x block interaction ($F(3,48)=21.84$, $p<0.01$), showing a higher expectancy of scream in the dangerous zone compared to the safe zone over block (Figure 45).

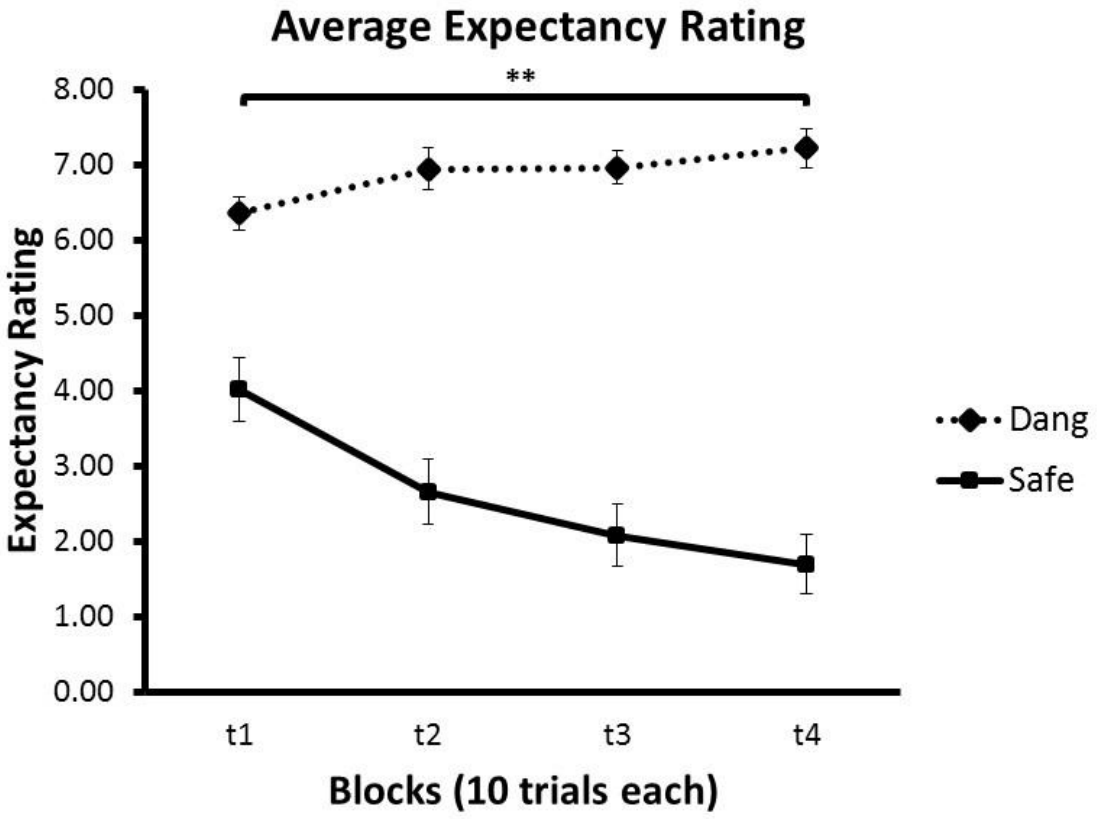


***Figure 45: Experiment 3.3 Average expectancy ratings.***

*Average expectancy ratings safe (Safe) and dangerous (Dang) zone over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

## Conclusions

Like the previous experiments, I showed here successful explicit aversive response (expectancy rating) learning, and again I found unsuccessful somatic aversive response (SCR) learning to each of the zones. The data from experiment 3.1 showed that the onset of the scream was eliciting a higher SCR response compared to when it was not present, suggesting that the scream was generating

a fear response. Still, there seemed to be no significant SCR difference before the scream onset between the dangerous zone and safe zone over time. In addition, SCR data showed an uncorrected t-test trend difference at SCR t4, suggesting that, by the end of the experiment, the participants' SCR was starting to reflect the safe and dangerous zone discrimination. These results suggested that either the paradigm was creating an overly anxiogenic environment, or the participants were receiving too few screams that would allow them to properly predict the scream, and therefore feel comfortable in the safe zone. To determine if this is the case, increasing the reinforcement of scream could give participants enough information to distinguish between the two zones and still elicit a fear and anxious response. Finally, consistent with the previous experiments, I saw that participants were able to learn the correct location of the objects in the environment over time.

## Experiment 3.4: 50% reinforcement of scream & high number of trials: *With jittered freezing period time*

### Introduction

From the previous scream experiment, I saw that learning and discrimination of dangerous and safe zones only happened explicitly (expectancy ratings) compared to somatic aversive responses (SCR) with a 40% reinforcement of scream. Although, in the last experiment I saw a trend difference in t4 for the SCR phasic data, suggesting that participants were beginning to learn the difference between zones. I thought that this could either be that participants were slower to feel comfortable in the safe zone, or that, because of the low reinforcement of scream, they were unsure as to whether they would get a scream or not.

Since the 50% reinforcement rate with jitter time during the freezing period worked so well, I decided to test if the participants were indeed overgeneralizing the fear and anxious response because of the low reinforcement of scream. I increased the reinforcement of scream to 50% and jittered the freezing time to see if I could find successful implicit and explicit aversive response learning. Therefore, I decided to repeat the screaming lady trial with a 50% reinforcement of CS+.

## Methods

***Participants:*** 24 healthy volunteers (3 males), with an average of 23 years, were recruited for this experiment.

***Aversive conditioning procedure:*** Each participant had to collect 4 flowers in between objects. There were 80 flowers in total, an average of 40 flowers in the dangerous zone (20 CS+). Each time the participant collected a flower, the participants would be frozen in place for 2000-8000ms. The reinforcement of scream was 50%, and remained stable as the participant approached the dangerous hive. At the end of the experiment, participants were asked to identify the dangerous side. 20 participants (83%) were able to identify the dangerous zone and were considered “learners” (2 males).

## Results

### Object location memory

A 2x4 within-subjects ANOVA (zone x block) measuring error rate of object placement showed significantly effect of block ($F(3,57)=20.79$, $p<0.01$), and zone ($F(1,19)=5.86$, $p<0.05$), showing greater error of placing the object in the safe

zone. Still, error decreased over time regardless of the zone. There was no significant interaction between zone x block (F(3,57)=1.51, p>0.05) (Figure 46).

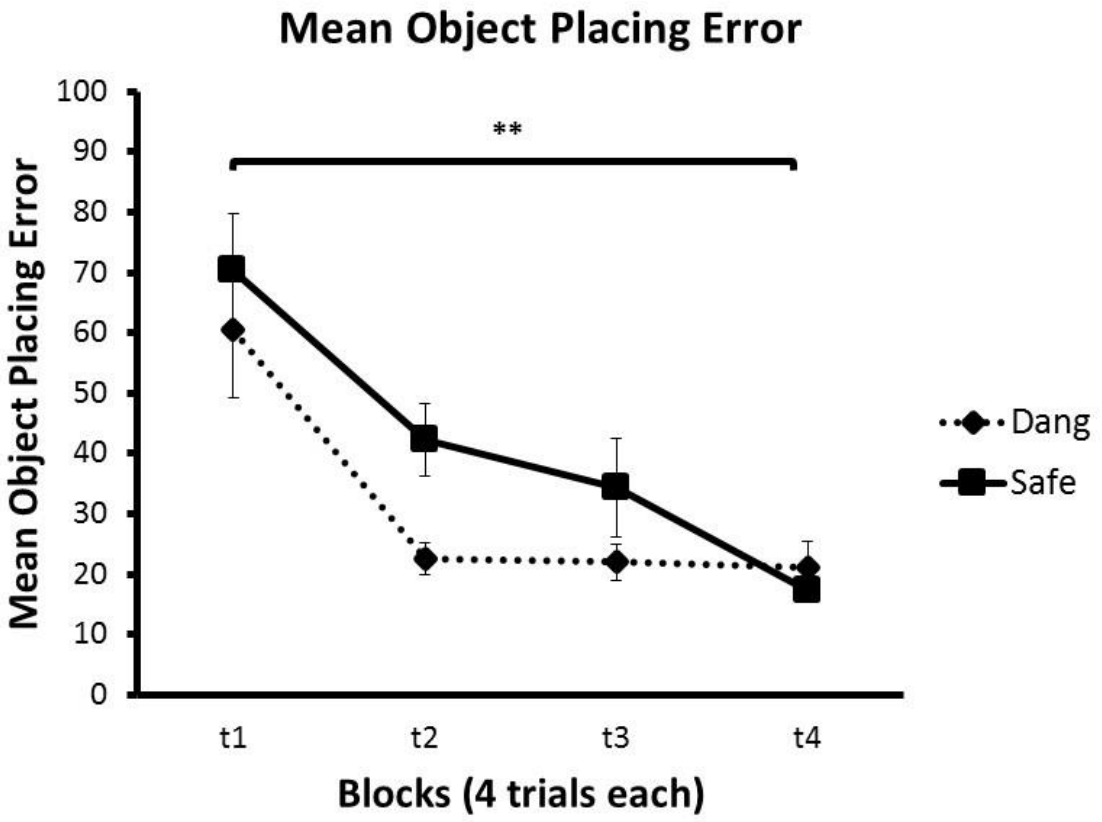


***Figure 46: Experiment 3.4 Mean object placement error.***

*Mean object placement error, for the safe zone (Safe) and the dangerous zone (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Skin conductance response**

A 2x4 within-subject ANOVA (zone x block) on phasic SCR data of the learners showed a trend effect in zone (F(1,5)=5.67, p=0.063), where the SCR in

the dangerous zone was higher than in the safe zone. There was no effect of block ($F(3,15)=0.40$, $p>0.05$) or zone x block interaction ($F(3,15)=0.69$, $p>0.05$) (Figure 47). A t-test between dangerous and safe zone at each block point showed no significant difference for t1, t2, t3, or t4 ($p>0.05$ uncorrected), although there was a trend difference at t2 ($p=0.070$ uncorrected).

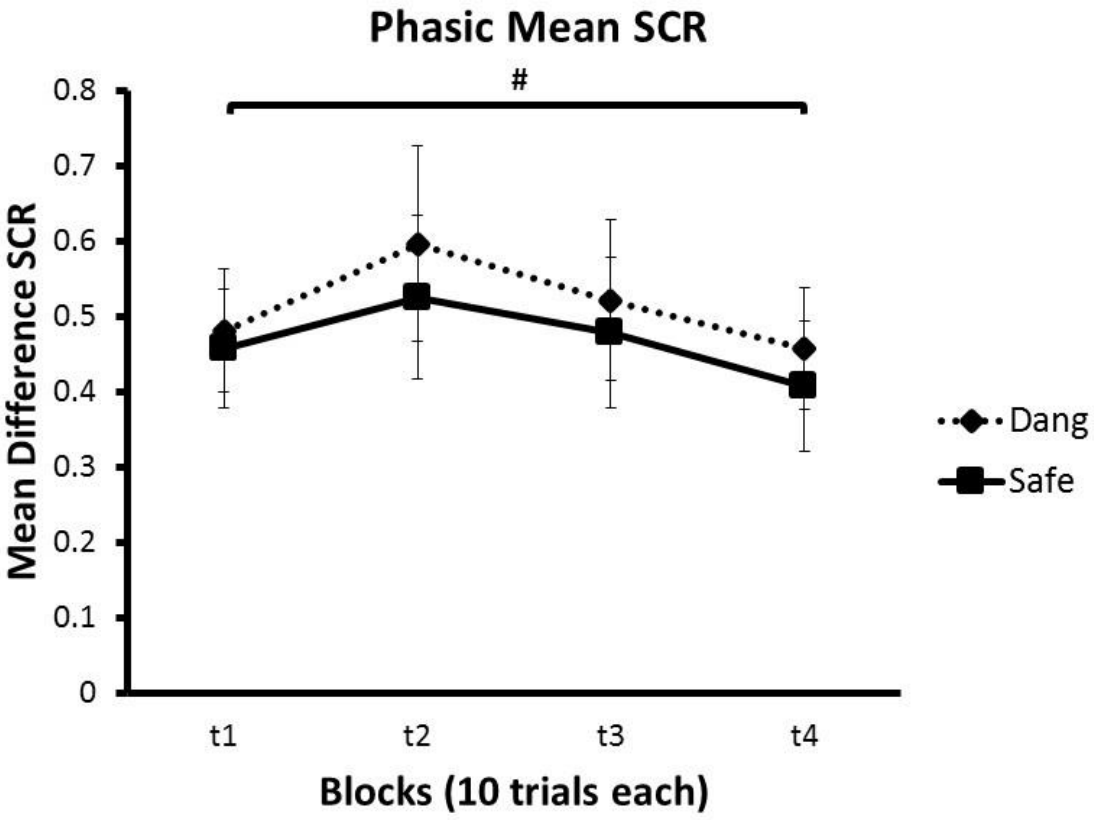


***Figure 47: Experiment 3.4 Mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Threat expectancy rating**

A 2x4 within-subject ANOVA (zone x block) of threat expectancy rating of the learners showed a significant effect of block ($F(3,15)=4.61$, $p<0.05$), zone ($F(1,5)=85.64$, $p<0.01$), and zone x block interaction ($F(3,15)=7.20$, $p<0.01$), showing a higher expectancy of scream in the dangerous zone compared to the safe zone over block (Figure 48). A t-test between dangerous and safe zone at each block point showed a significant difference for t1, t2, t3, or t4 ($p>0.0125$ Bonferroni corrected).

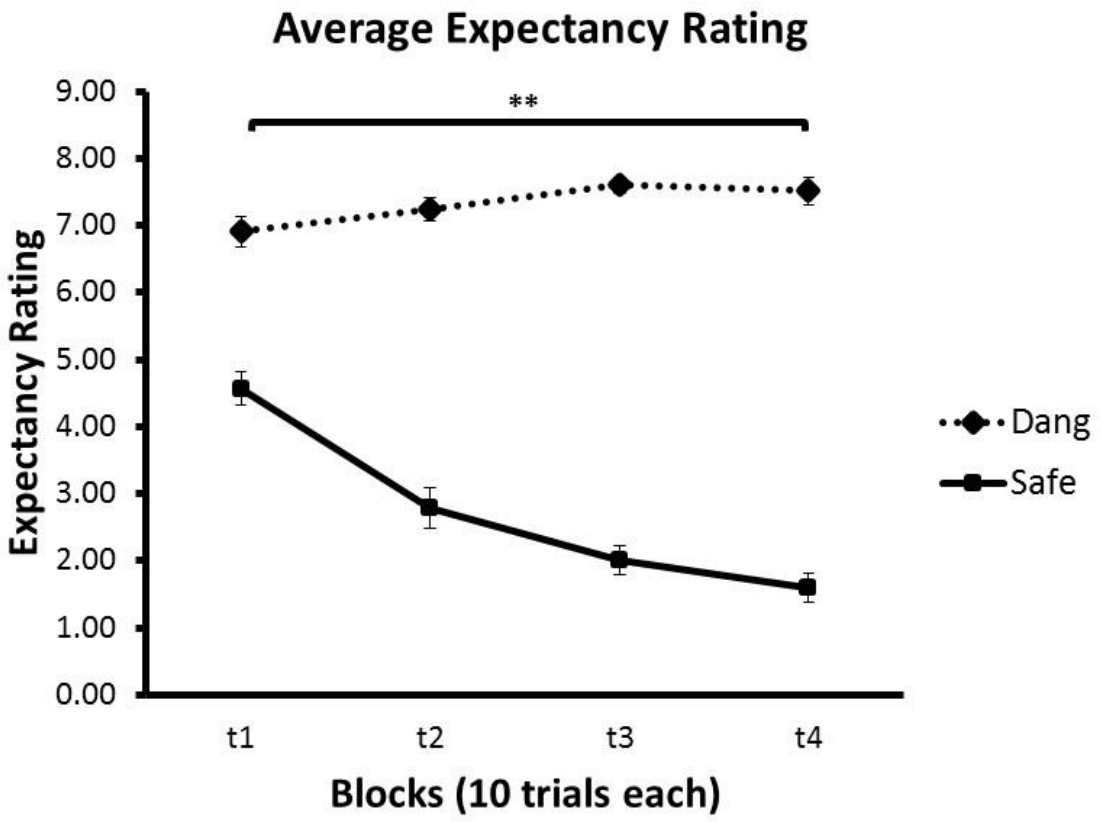


***Figure 48: Experiment 3.4 Average expectancy ratings.***

*Average expectancy ratings safe (Safe) and dangerous (Dang) zone over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

## Conclusions

Like the previous experiments, I showed successful explicit aversive response (expectancy rating) learning, and again I failed to find significant somatic aversive response (SCR) learning to each of the zones. Although, the data showed a trend where there was a higher SCR in the dangerous zone, compared to the safe zone. These results suggest that even though the participants were learning to distinguish between the safe and dangerous zone (expectancy results), there was no SCR difference between zones. Finally, consistent with the previous experiments, I saw that participants were able to learn the correct location of the objects in the environment over time.

## Discussion

These series of experiments were designed to substitute electrical shocks with an aversive scream. I thought that validating an aversive scream paradigm would facilitate the future recruitment of vulnerable populations that might find the combination of electric stimulus and fMRI (due to its' enclosed space) too unpleasant to participate. Furthermore, this scream paradigm could have been used to study other population where electrical shocks might be unethical (e.g., children). In general, the results showed that participants could explicitly identify the dangerous zone, as reflected by the expectancy ratings and the end of test questionnaires. Regardless, I did not find any significant difference in the SCR data. I propose two possible scenarios, either the scream was extremely aversive,

creating an overly anxious environment, or the opposite, where the scream was not aversive at all, therefore participants were very comfortable with it. Further studies are necessary to provide a definite conclusion, but I propose several thoughts. An overly anxiogenic environment could be supported by 1) high levels of SCR, which were very similar to those found in the shock paradigm, ranging from 0.4-0.8. Still, this could be due to high levels of baseline SCR in the participants from these experiments. 2) 95% reinforcement of scream (Experiment 3.2) yielded no significant difference in SCR, though in the 95% reinforcement of shock (Experiment 1.3), the high predictability of shock produced fast and accurate discrimination between the two zones. 3) A low reinforcement of scream started producing some SCR differences (albeit a trend) by the end of the experiment, suggesting that reducing the number of screams was having a less anxiogenic effect, though the low number of screams might have caused a lower predictability of screams that slowed the discrimination learning (Experiment 3.3). This last interpretation was further supported by experiment 3.4, 50% reinforcement of scream, where there was a trend for zone discrimination. This last experiment suggested that participants had enough information to discriminate the zones, at a 50% reinforcement rate, although the averseness of the scream might also be occluding the effects from significance. Based on the difficulty of finding the most effective settings that produce clear and significant results, I decided to abandon the scream paradigm and focus on the shock paradigm to continue with experiment 2.3 in the fMRI scanner. Still, it is worth noting that previous experiments using the screaming lady paradigm has focused on teenagers and children. I used the screaming lady in adults, which might reflect a higher emotional arousal by screaming due to adults lack of exposure to screams. On the other hand, children and teenager might be more accustomed to loud screams either at play or arguments with other children or used as discipline by adults like caretakers and/or teachers.

# Chapter 7

## Experiment 4: Neural Correlates of Location-Specific Context Discrimination and Cognitive Map Formation

# Experiment 4

## Experiment 4: 50% reinforcement of shock & high number of trials: With jittered time inside fMRI scanner

### Precis

From experiment 2.3, I saw that learning and discrimination of dangerous and safe zones was possible even with 50% reinforcement rate with a jittered time before the stimulus onset (electric shock). This reinforcement rate resulted in a higher SCR in the dangerous zone, in the learners, while also keeping the number of non-learners and shocks low.

It remains unclear how neural networks allow specific cues encountered in particular locations within a context to become associated with an aversive outcome. Therefore, the current study used virtual reality and functional magnetic imaging (fMRI) to map this network. Participants had to use spatial-information to learn to discriminate between a safe and a dangerous zone while collecting flowers. Flowers in the dangerous zone were paired with an electrical stimulus reinforced 50% of the time, like in experiment 2.3. The task was designed to reveal brain areas involved in learning locations in the environment predictive of safety and danger. In particular, to assess the role of the amygdala, mPFC, and HPC in the learning and discrimination between safe and dangerous contexts.

It was expected that, over time, participants would learn to discriminate between the dangerous and safe flower in the environment through differential SCR and expectancy ratings that would increase in the dangerous zone, during learning. In addition, during learning, it was expected that amygdala and hippocampal activity would increase in the dangerous zone, based on their role in

context aversive conditioning. On the other hand, vmPFC activity was expected to increase in the safe zone due to its role in safety signaling (Bannerman et al., 2004; Burgess, Maguire, & O'Keefe, 2002; Fanselow & Dong, 2010; Gruber & McDonald, 2012; Laird et al., 2011; Linnman et al., 2012; Milad & Quirk, 2002; Wang et al., 2012).

## Method

The methods used in this experiment were the same as the ones described above in experiment 2.3. The major difference was that this experiment was conducted while the participant was inside an fMRI scanner.

***Participants:*** 27 healthy volunteers (14 males) were recruited for this experiment. Four participants were excluded from the analysis due to technical issues during scanning, and two further participants were omitted since they were unable to explain the shock contingencies between the locations at the end of the task. Therefore, the data was analyzed from the remaining 21 participants (13 males), with an average of 24 years.

***Aversive conditioning procedure:*** Each participant had to collect 4 flowers in between objects. There were 80 flowers in total, an average of 40 flowers in the dangerous zone (20 CS+). Each time the participant collected a flower, the participants would be frozen in place for 2000-8000ms. The reinforcement of shock was 50%, and remained stable as the participant approached the dangerous hive. At the end of the experiment, participants were asked to identify the dangerous side. 21 participants (78%) were able to identify the dangerous zone and were considered "learners".

**Skin conductance level:** In addition to SCR, skin conductance levels (SCL) was measured. Mean skin conductance level during each approach quantified tonic skin conductance levels as participants navigated towards the flower. SCL was quantified for the period from flower appearance until trial completion. Skin conductance level was calculated by measuring the mean skin conductance from the beginning of active approach until before the flower was picked for each trial. Any response difference under 0.03 micro Siemens was scored as zero. SCL were log transformed (log [1+SCL]) to normalize the distribution and then range correction ([SCL-SCLmin]/[SCLmax-SCLmin]) was applied to control for individual variation in responding (Lykken, 1972). SCL were averaged into four equal blocks across the duration of the experiment, with each block including 10 trials per condition (safe and danger).

**FMRI acquisition:** Blood oxygen level-dependent T2*-weighted functional images were acquired on a 3T Trio system (Siemens, Germany) using echo-planar imaging (EPI) with a 32 channel head coil. Images were acquired obliquely at 45° with the following parameters: repetition time, 3,360ms; echo time, 30ms; slice thickness, 2mm; inter-slice gap, 1mm; in-plane resolution, 3×3mm; field of view, 64×72mm$^2$; 48 slices per volume. A field-map using a double echo FLASH sequence was recorded for distortion correction of the acquired echo planar imaging (EPI) (Weiskopf et al. 2006). After the functional scans, a T1-weighted 3-D MDEFT structural image (1mm$^3$) was acquired to co-register and display the functional data.

**FMRI analysis:** Data processing and analysis were performed using the statistical parametric mapping software (SPM8) (http://www.fil.ion.ucl.ac.uk/spm). EPI images were first preprocessed using a bias correction to control for within volume signal intensity difference, unwarping, and realignment to correct for movement and slice-time correction. Images were then spatially normalized to the MNI template using parameter estimates from warping each participant's structural

image to a T1-weighted average template image. All images were finally smoothed using an 8mm FWHM Gaussian kernel.

Statistical analyses occurred in two stages. The first-level model included 13 regressors of interest. Four separate regressors were created for approach periods, starting from the end of the first quarter of each approach period to the point in which that flower was reached. Using a boxcar function, the regressors consisted of a 2x2 design (zone x block), divided by zone (safe or danger) and by block (early or late within the experiment, split into halves). A further four regressors were created for the freezing period of each trial, starting after the participant had rated their shock expectancy, for the duration of the freezing period. These regressors were separated in the same way as approach periods (i.e., a 2x2 design which factors zone and block). The end of each trial was also modeled using a stick function to account for whether participants received a shock or not, across danger and safe conditions (3 regressors: danger-shock; danger-no-shock; safe-no-shock). Finally, trials when participants were replacing objects on the spatial memory task were modeled by using a boxcar function during the approach period to the location where a response was made (2 regressors, first and second half of the experiment). Six regressors of no interest were also added to the model representing movement parameters estimated during realignment. Parameter estimates for conditions of interest were then entered into second level GLMs.

All analyses report family-wise error ($p<0.05$ FWE) corrected effects across the whole brain. Given the *a priori* hypotheses, effects in bilateral hippocampus, amygdala, and mPFC that survive small-volume correction (SVC; $p<0.05$ FWE) were reported. A bilateral mask comprising the hippocampus, a bilateral mask for the amygdala, and a bilateral mask for the mPFC that included the orbito-frontal gyrus, medial frontal gyrus, and anterior cingulate and medial cingulate gyrus was created, defined using the Automated Anatomical Labeling atlas (AAL; Tzourio-

Mazoyer et al., 2002), and implemented using the WFU Pickatlas toolbox in SPM8 (Maldjian et al., 2003).

To examine approach periods during threat learning, a second-level model was created to contrast approach to flowers associated with safety or danger and whether they were collected during the first or second half of the experiment. Therefore, approach periods were analyzed using a 2x2 ANOVA (zone, block). Periods when the flower was picked and participants were held stationary were analyzed in a similar second level model using a 2x2 ANOVA (zone, block). Finally, approach periods during threat learning (approaching flowers) was compared with approach periods during the spatial memory task (approaching location to replace the object). A second-level model was created contrasting approach periods for threat learning (collapsing across safety and danger) with approach during spatial memory across the first and second half of the experiment using a 2x2 ANOVA (task, block).

For any significant interaction, the eigenvariate were extracted through SPM8 MarsBaR (http://marsbar.sourceforge.net) toolbox using a 6mm sphere at the peak of the activity in the regions of interest. The extracted values were analyzed in SPSS 22 on a 2x2 ANOVA (task x block), and further analyzed through a 2-sample t-test and Bonferroni corrected.

**Functional connectivity analyses:** Functional connectivity was assessed at group level using psychophysiological interactions (PPI) analysis using the SPM8 generalized psychophysiological interaction toolbox (gPPI; McLaren, Ries, Xu, & Johnson, 2012; https://www.nitrc.org/projects/gppi). The gPPI toolbox compares functional connectivity (i.e., between-region correlations in activity across trials) to a single seed region across tasks, while accommodating for multiple task conditions in the same PPI model. The seed regions were selected based on *a priori* hypothesis of the connectivity of the vmPFC, dACC, PAG, and

hippocampus to other areas during the task. Peak activation from these areas in the group level analysis, for approach and freezing periods, were used to create volumes of interest for each subject. The seed time series activity was extracted using a 6mm sphere at the center of the activation peak. Each seed region was assessed for task connectivity during active approach and freezing period. The individual t-contrast images of the interaction from the gPPI were examined using a group level one-sample t-test. The group PPI were detected using t-tests with threshold of $p<0.001$ uncorrected.

## Results

### Object location memory

A 2x4 within-subjects ANOVA (zone x block) measuring error rate of object placement showed significantly effect of block ($F(3,63)=14.98$, $p<0.01$), showing error decreased over time regardless of the zone. There was no significant effect of zone ($F(1,21)=0.94$, $p>0.05$) or interaction between zone x block ($F(3,63)=0.96$, $p>0.05$) (Figure 49).

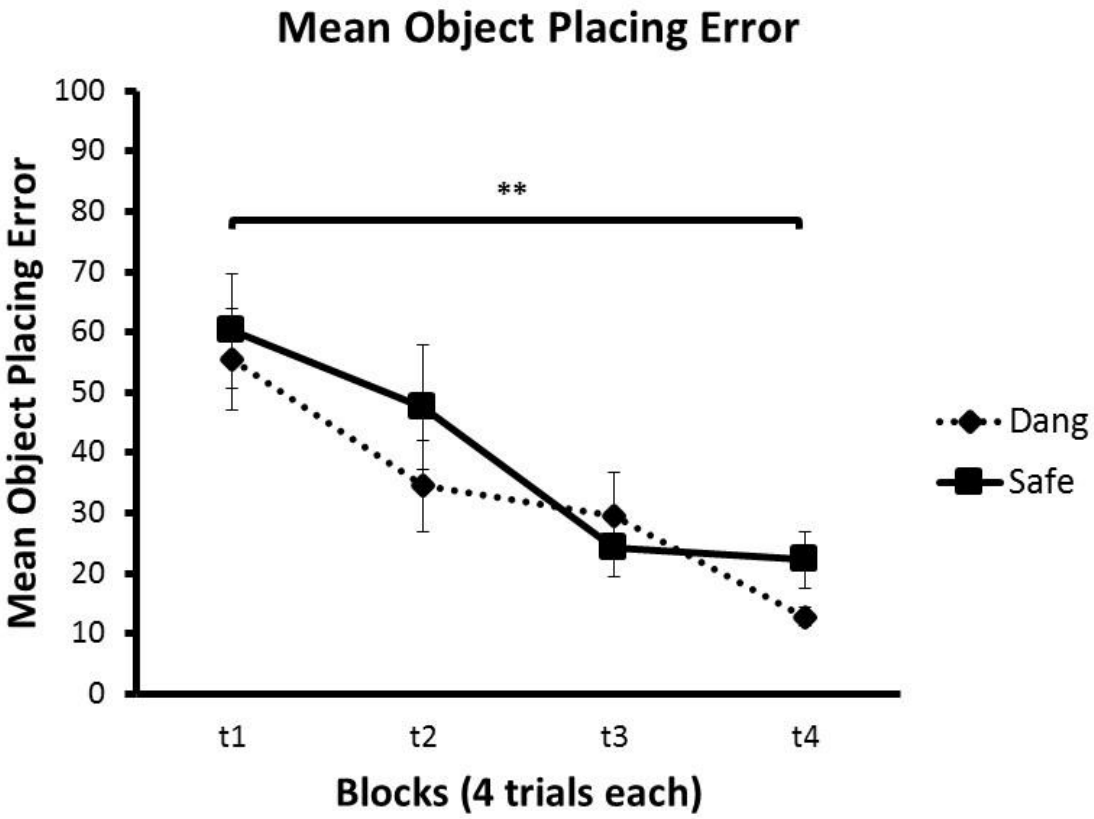


***Figure 49: Experiment 4 Mean object placement error.***

*Mean object placement error, for the safe zone (Safe) and the dangerous zone (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Skin conductance level**

I first compared SCL (tonic changes in skin conductance) during periods when participants approached a flower in dangerous relative to safe contexts. A 2x4 within-subject ANOVA (zone x block) on phasic SCL data of the learners show a significant effect of zone ($F(1,21)=8.92$, $p<0.01$), where the SCL in the dangerous

zone is higher than in the safe zone. There was no effect of block ($F(3,63)=1.01$, $p>0.05$) or zone x block interaction ($F(3,63)=1.37$, $p>0.05$) (Figure 50).

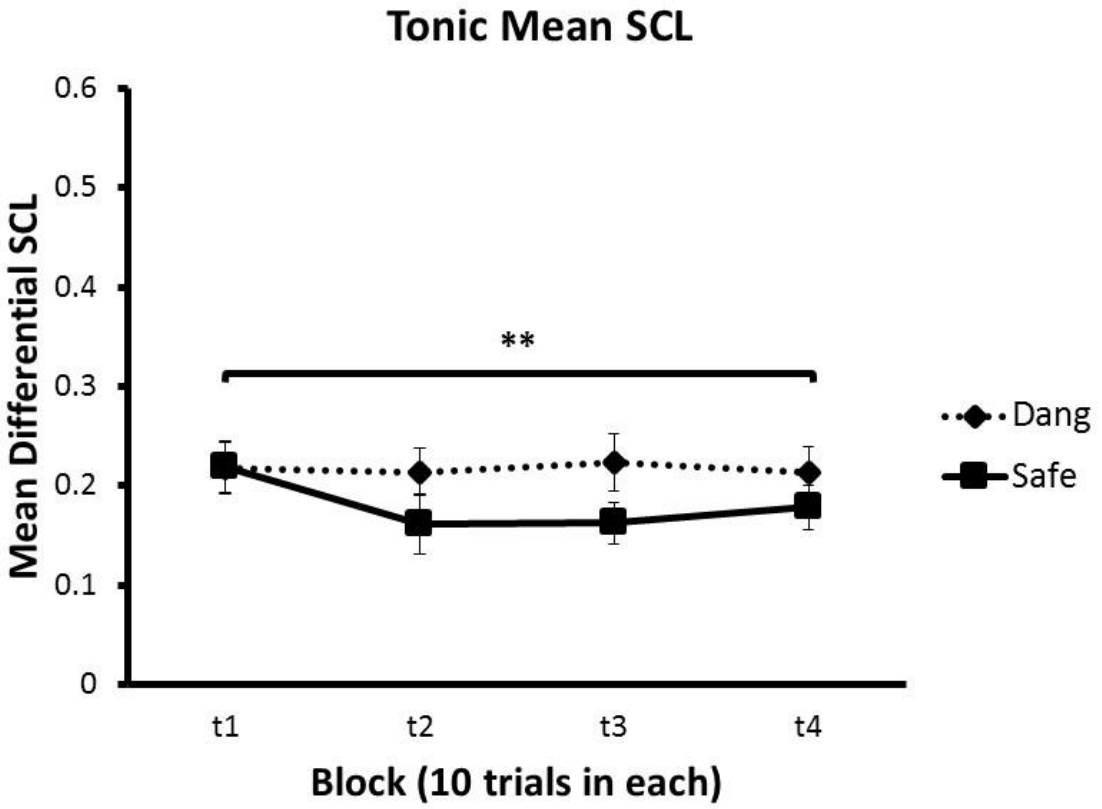


***Figure 50: Experiment 4 Mean phasic SCL.***

*Mean phasic SCL of safe (Safe) and dangerous (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Skin conductance response**

Next, I examined SCR immediately after participants contacted a virtual flower, leading their movement to be restricted (freezing). A 2x4 within-subject

ANOVA (zone x block) on phasic SCR data of the learners showed a significant effect of zone ($F(1,21)=7.76$, $p<0.01$), where the SCR in the dangerous zone was higher than in the safe zone. I also saw a significant effect of block ($F(3,63)=16.06$, $p<0.01$) reflecting a general decrease in SCRs as the experiment progressed (possibly due to habituation to shock intensity). There was no zone x block interaction ($F(3,63)=1.69$, $p>0.05$) (Figure 51).

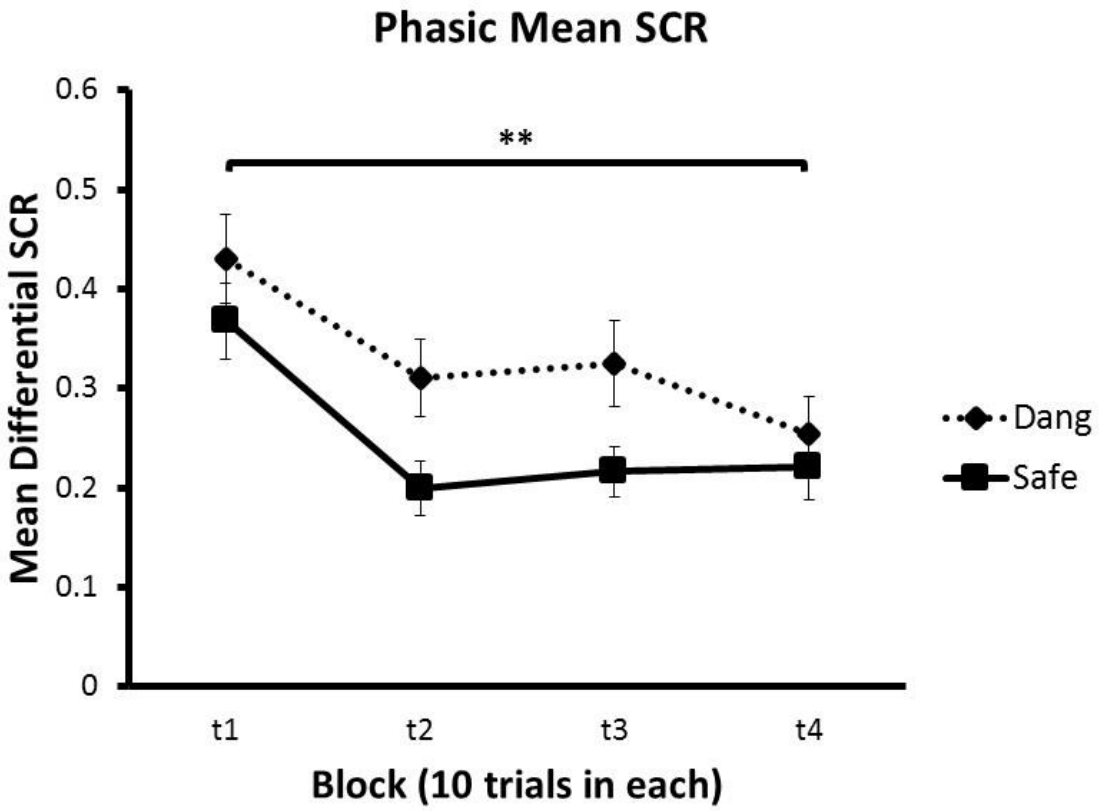


***Figure 51: Experiment 4 Mean phasic SCR.***

*Mean phasic SCR of safe (Safe) and dangerous (Dang) over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Threat expectancy rating**

A 2x4 within-subject ANOVA (zone x block) of threat expectancy rating of the learners showed a significant effect of block ($F(3,63)=9.98$, $p<0.01$), zone ($F(1,21)=135.55$, $p<0.01$), and zone x block interaction ($F(3,63)=20.76$, $p<0.01$), showing a higher expectancy of scream in the dangerous zone compared to the safe zone over block (Figure 52).

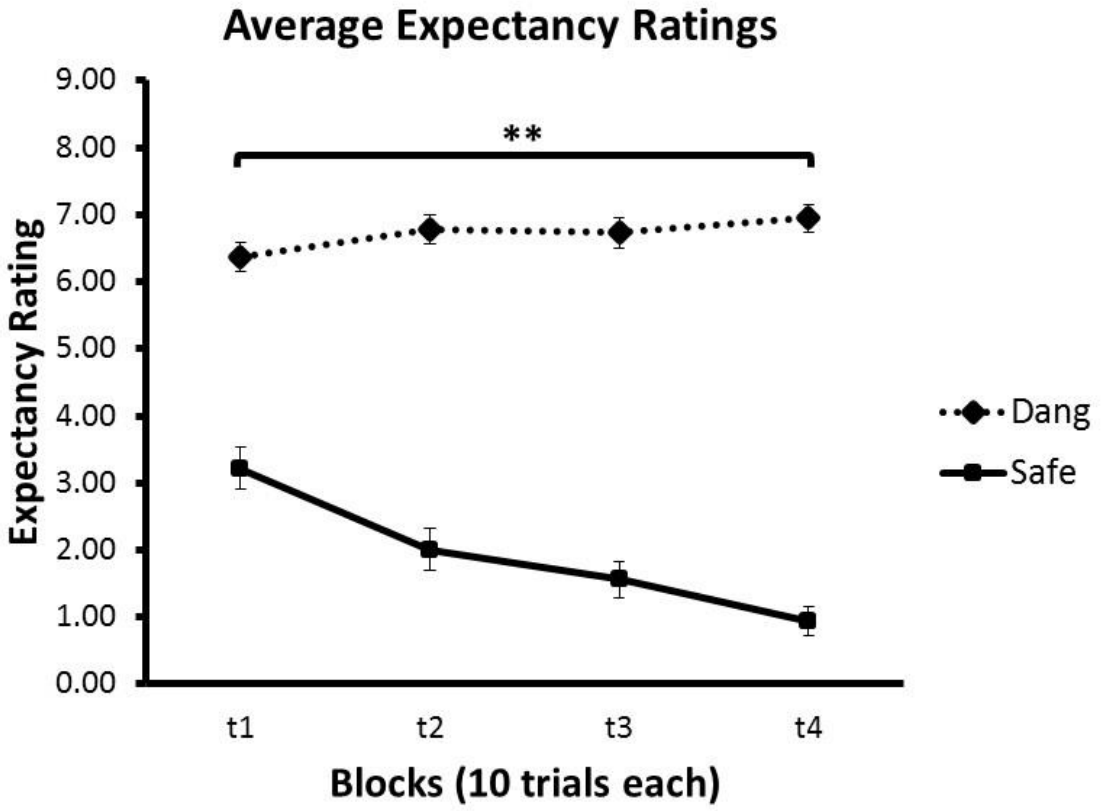


***Figure 52: Experiment 4 Average expectancy ratings.***

*Average expectancy ratings safe (Safe) and dangerous (Dang) zone over time. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

**Approaching a threat within an environment**

I next compared brain regions engaged when individuals navigated towards flowers approached in dangerous relative to safe environmental zones. Trials began when a flower appeared and ended when the flower was contacted. I compared brain activity in the last three quarters of dangerous and safe trials. I also examined a time factor by collapsing trials into two blocks comprising the first and second half of learning, resulting in a 2x2 ANOVA (block, zone).

When approaching flowers associated with danger as opposed to safety (danger > safe), greater activity manifested in dorsal anterior cingulate cortex (dACC; $p<0.05$ FWE; see Figure 53A), with no areas of activity in the reverse contrast (safe > danger). I next examined time-related variation in response to these cues. First, I compared time-related changes in activity between the first and last half of the experiment, irrespective of whether or not the flower was positioned in a dangerous or safe zone of the environment. For the late relative to early period, I saw greater activity in posterior cingulate cortex, vmPFC, and right hippocampus ($p<0.05$ FWE; greater activity was also seen in the left hippocampus using small volume correction, $p<0.05$ FWE SVC; see Figure 53B and C). The reverse contrast, identifying areas more involved during early trials (first half > last half), showed greater activity in the right insula ($p<0.05$ FWE).

In summary, while vmPFC and hippocampus showed non-specific increases in activity during approach periods as learning progressed, dACC demonstrated a more selective increase for flowers approached in the danger zone, compared to safe zone, on the environment throughout the whole task.

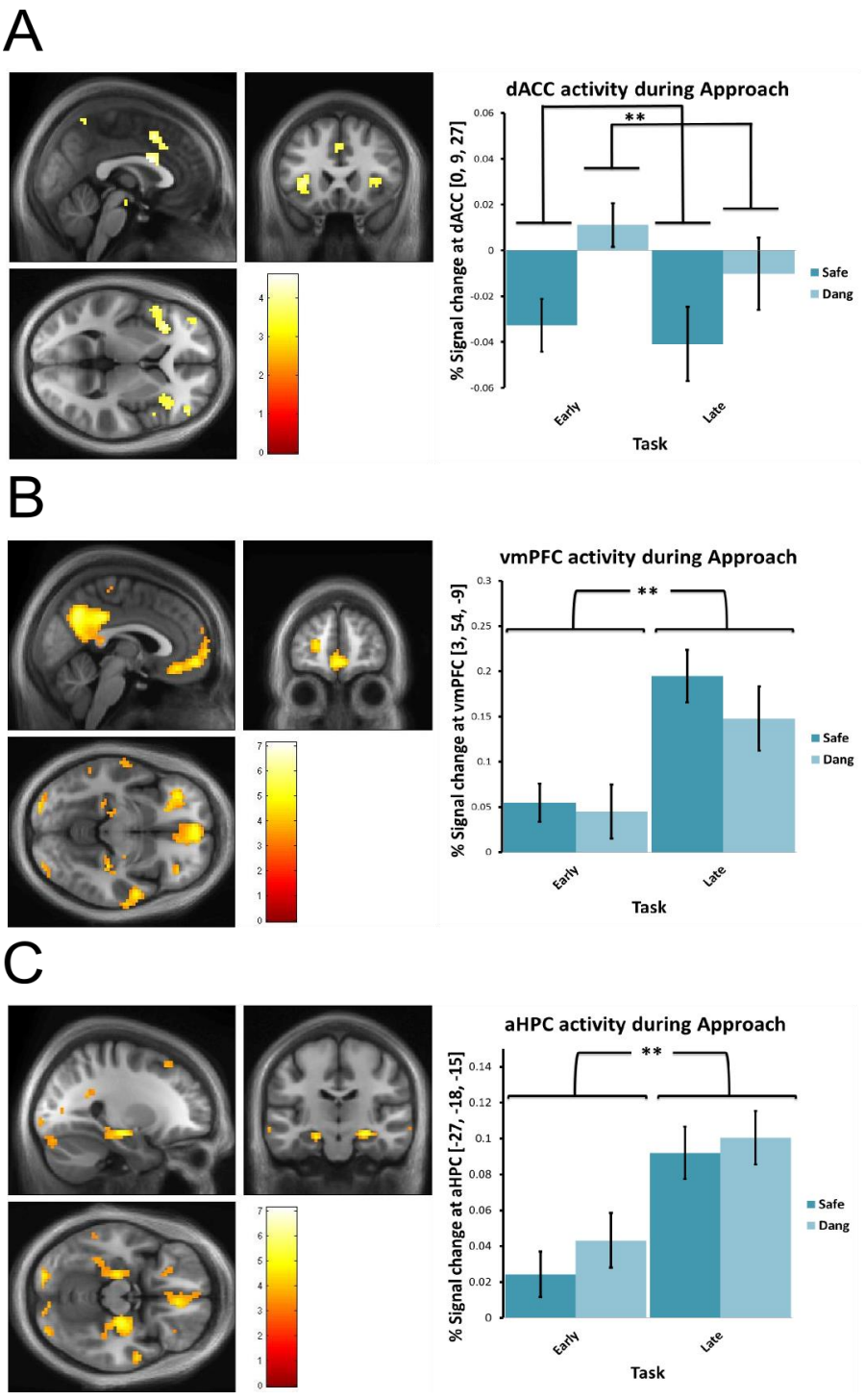


***Figure 53: Brain activity of approaching a flower within the environment.***

*(A) dACC shows greater activity for flowers approached in the dangerous compared to safe zone of the environment across the whole test session. Irrespective of the location of flowers (B) vmPFC and PCC and (C) hippocampus show greater activity during the last half of the learning session (second > first half of active approach). All images are presented at p<0.001 uncorrected for display purposes. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

***Table 2: Brain regions active during active approach contrasts at 0.05 FWE.***

| Contrast | Brain Area | X | Y | Z | Z scores |
|---|---|---|---|---|---|
| **Approach Danger > Safe** | | | | | |
| | **Dorsal Anterior Cingulate Cortex** | 0 | 9 | 27 | 4.24 |
| **Approach Late > Early** | | | | | |
| | **Left Angular Gyrus** | -45 | -72 | 30 | 6.04 |
| | **Left Posterior Medial Cingulate Cortex** | -3 | -39 | 36 | 5.53 |
| | **Left Middle Frontal Gyrus** | -24 | 24 | 51 | 5.27 |
| | **Right Precuneus** | 6 | -57 | 39 | 5.21 |
| | **Right Posterior Cingulate Cortex** | 9 | -48 | 15 | 4.76 |
| | **Right Anterior Hippocampus** | 27 | -18 | -15 | 4.75 |
| | **Left Anterior Hippocampus** | -21 | -21 | -18 | 4.49 |
| | **Ventromedial Prefrontal Cortex** | 3 | 54 | -9 | 4.68 |
| **Approach Early > Late** | | | | | |
| | **Right Inferior Frontal Gyrus** | 51 | 12 | 15 | 5.29 |
| | **Right Postcentral Gyrus** | 63 | -18 | 33 | 5.08 |
| | **Left SupraMarginal Gyrus** | -63 | -24 | 30 | 4.88 |
| | **Right Insula** | 33 | 27 | 3 | 4.8 |
| | **Right SupraMarginal Gyrus** | 57 | -36 | 36 | 4.79 |

## Anticipating an aversive outcome

I next examined brain regions engaged during participants' freezing in dangerous relative to safe environmental zones. As above, a time factor was

assessed by collapsing trials into two blocks comprising the first and second half of learning.

When frozen in zones predictive of danger (danger > safe), greater activity was seen in an area of the midbrain including the periaqueductal gray, and in the caudate, dACC, and bilateral insula ($p<0.05$ FWE; see Figure 54A and B). Freezing in areas predicting safety (safe > danger), revealed a tendency, in line with the *a priori* hypothesis, of activity in vmPFC that did not survive FWE correction ($p<0.001$ uncorrected; see Figure 54C). Activity during the late, relative to the early, periods (last half > first half), showed increased activity in bilateral posterior hippocampus ($p<0.05$ FWE SVC), with no areas of activity in the reverse contrast (first half > last half).

Therefore, a number of areas including dACC, insula, midbrain, and caudate were found to be involved during freezing to a flower located in a zone of the environment predicting danger, while vmPFC was more active to flowers in the safe area.

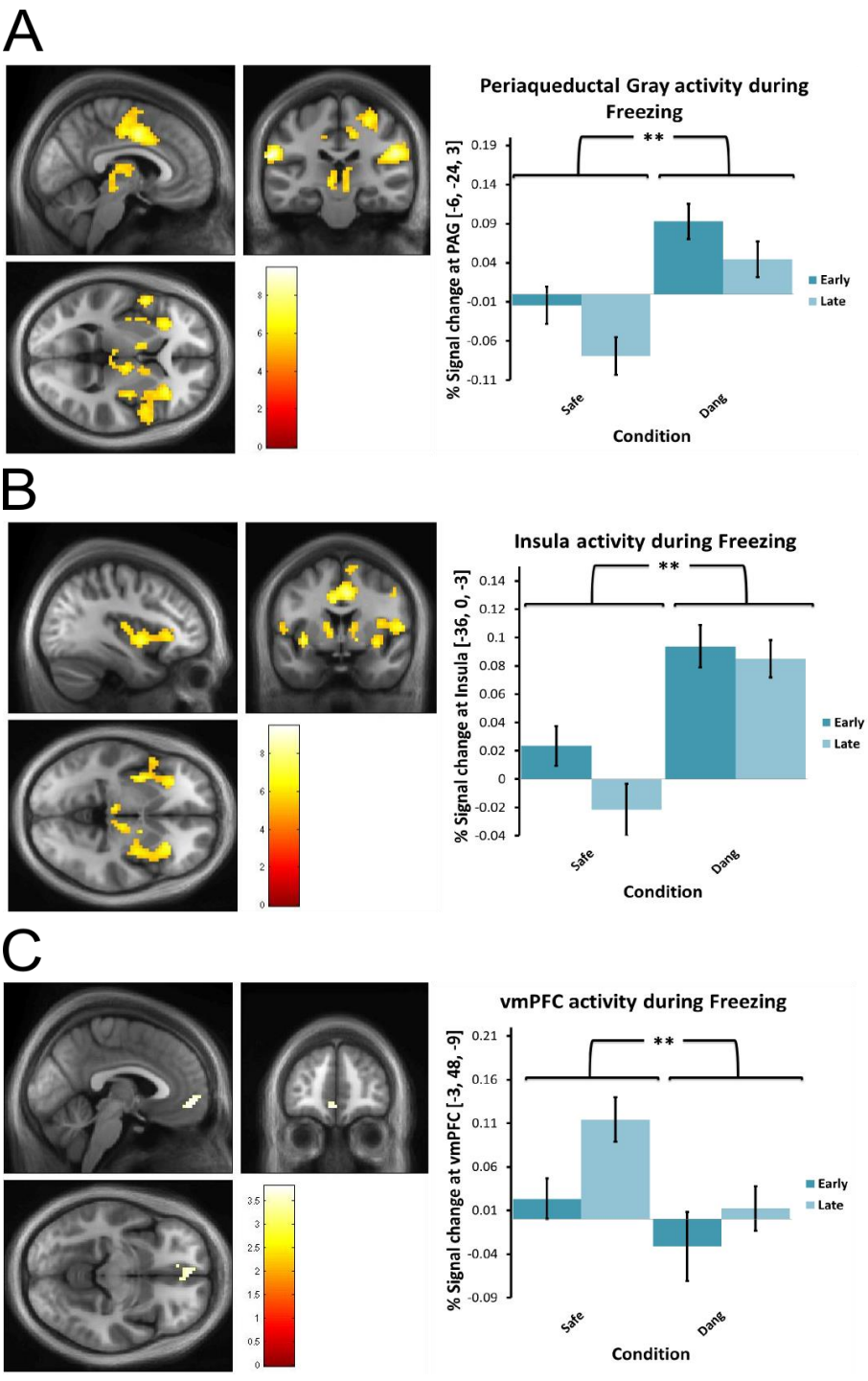


***Figure 54: Brain activity of anticipating the outcome of picking a flower within the environment.***

*Contrasting periods when participants were frozen (picking the flower) showing greater activity in (A) periaqueductal gray, dACC and (B) bilateral insula (p<0.05 FWE) when flowers were picked in dangerous versus safe locations of the environment. The reverse contrast showed (C) greater activity in the vmPFC (p<0.001 uncorrected) when flowers were picked in safe versus dangerous location of the environment. All images are presented at p<0.001 uncorrected for display purposes. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

***Table 3: Brain regions active during freezing period contrasts at 0.05 FWE.***

| Contrast | Brain Area | X | Y | Z | Z scores |
|---|---|---|---|---|---|
| **Freezing Danger > Safe** | | | | | |
| | **Left SupraMarginal Gyrus** | -66 | -24 | 21 | 7.38 |
| | **Right SupraMarginal Gyrus** | 54 | -21 | 24 | 7.1 |
| | **Dorsal Anterior Cingulate Cortex** | 6 | 0 | 39 | 6.66 |
| | **Right Postcentral Gyrus** | 21 | -42 | 63 | 6.53 |
| | **Left Insula** | -36 | 0 | -3 | 6.24 |
| | **Right Thalamus** | 12 | -18 | 9 | 6.02 |
| | **Right Insula** | 35 | 3 | -5 | 5.77 |
| | **Periaqueductal Gray** | 6 | -24 | 3 | 5.11 |
| | **Posterior Medial Cingulate Cortex** | -12 | -27 | 39 | 5.04 |
| | **Left Middle Temporal gyrus** | -51 | -63 | 9 | 4.98 |
| | **Right Superior Frontal Gyrus** | 18 | -12 | 72 | 4.79 |
| | **Left Cerebellum** | -21 | -57 | -51 | 4.77 |
| | **Left Dorsomedial Prefrontal Cortex** | -6 | -9 | 66 | 4.75 |
| **Freezing Safe > Danger** | | | | | |
| | **Ventromedial Prefrontal Cortex** | -3 | 48 | -9 | 3.59 |
| **Freezing Late > Early** | | | | | |
| | **Left Inferior Parietal Lobule** | -30 | -78 | 48 | 5.37 |
| | **Left Angular gyrus** | -45 | -72 | 42 | 4.84 |
| | **Right Posterior Hippocampus** | 33 | -33 | -3 | 4.25 |
| | **Left Posterior Hippocampus** | -30 | -33 | -6 | 4.22 |

**Differences between approaching objects and flowers**

During learning, participants were required to perform two different types of task within the same environment (see methods for further description of tasks): 1) approaching flowers whose locations need not be remembered, but which might be related to a threat of shock; or 2) approaching objects whose locations needed to be remembered, but which were never related to a threat of shock. I compared brain regions engaged when individuals navigated towards a flower, collapsed across dangerous and safe conditions, relative to navigating towards an object (mean duration = 14.91 sec, SD=6.89 sec) in a 2x2 ANOVA (block, task).

When approaching flowers as opposed to objects (threat learning > object memory), greater activity manifested in a range of structures, including a large area of mPFC, bilateral hippocampus, the amygdala, middle and posterior cingulate cortex, insula, middle temporal gyrus and lateral parietal regions ($p<0.05$ FWE). A task x block interaction revealed significant difference of activity in vmPFC ($p<0.05$ FWE; see Figure 55A).

To further understand the significant task x block interaction, a 2x2 ANOVA (task x block) follow-up analysis was done using a 6mm sphere in the vmPFC peak activity. The results showed a significant task x block interaction ($F(1,20)=17.60$, $p<0.01$) and significant main effects of block ($F(1,20)=9.51$, $p<0.01$) and task ($F(1,20)=59.68$, $p<0.01$). A paired—sample t-test revealed no significant difference in vmPFC activity in early relative to late blocks of the flower task ($p>0.05$), while the object task revealed a significant difference in early relative to late blocks of the object task ($p<0.0125$ Bonferroni corrected), showing a reduction in vmPFC activity over time.

A similar pattern of activity manifested in left hippocampus ($p<0.05$ FWE SVC; see Figure 55B), where a follow-up 2x2 ANOVA (task x block) showed a significant task x block interaction ($F(1,20)=16.59$, $p<0.01$) and significant main

effect of task ($F(1,20)=43.27$, $p<0.01$). There was no significant main effect of block in the left hippocampus ($F(1,20)=1.06$, $p>0.05$). A paired sample t-test revealed a significant difference in left hippocampal activity between the early and late blocks of the flower task ($p<0.0125$ Bonferroni corrected), the object task also revealed a significant difference between the early and late part of the task ($p<0.01$ Bonferroni corrected), showing an increase over time in the left hippocampus during the flower task and a reduction in activity over time in the object task.

When approaching objects as opposed to flowers (object memory > threat learning), greater activity was seen in bilateral parahippocampal gyrus, intraparietal sulcus, precuneus, and cuneus ($p<0.05$ FWE; see Figure 55C).

In summary, while vmPFC, hippocampus, and amygdala were involved in threat learning, with greater activity observed as threat learning progressed, the parahippocampal gyrus showed more involvement in the learning of object locations within the environment.

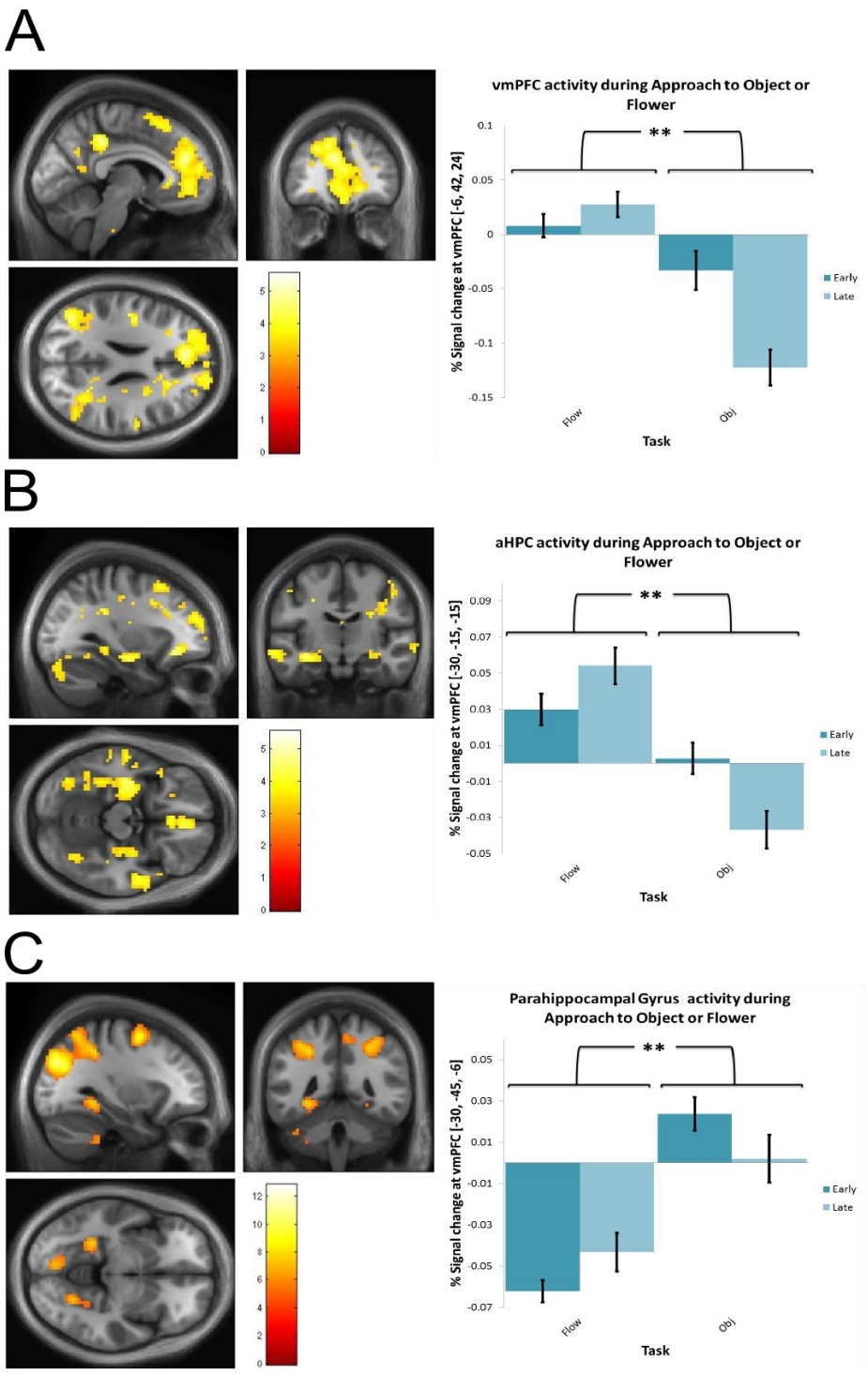


***Figure 55: Brain activity differences between approaching objects during threat learning and spatial memory.***

*Activity in (A) vmPFC (p<0.05 FWE; -6, 42, 24) and (B) hippocampus (p<0.05 FWE SVC; Left hippocampus -30, -15, -15) increased from the first half to the second half of threat learning compared to the object location task (images shown at p<0.001 uncorrected). Activity in (C) parahippocampal gyrus (p<0.05 FWE), was greater during object-location learning compared to threat learning. Error bars show SEM. *p<0.05, **p<0.01, #p<0.08.*

***Table 4: Brain regions active between approaching objects contrasts at 0.05 FWE.***

| Contrast | Brain Area | X | Y | Z | Z scores |
|---|---|---|---|---|---|
| **Flower > Object Task** | | | | | |
| | **Ventromedial Prefrontal Cortex** | 12 | 51 | 36 | >8 |
| | **Left Middle Temporal Gyrus** | -48 | 0 | -27 | >8 |
| | **Right Angular Gyrus** | 54 | -60 | 33 | >8 |
| | **Left Cerebellum** | -27 | -78 | -33 | >8 |
| | **Right Inferior Occipital Gyrus** | 33 | -90 | -6 | >8 |
| | **Right Cerebellum** | 30 | -81 | -30 | 7.73 |
| | **Left Middle Occipital Gyrus** | -30 | -93 | -6 | 7.52 |
| | **Right Anterior Hippocampus/Amygdala** | 27 | -9 | -15 | 7.45 |
| | **Right Posterior Cingulate Cortex** | 6 | -51 | 30 | 7.12 |
| | **Left Postcentral Gyrus** | -42 | -21 | 45 | 6.68 |
| | **Left Anterior Hippocampus/Amygdala** | -24 | -12 | -15 | 6.67 |
| | **Right Postcentral Gyrus** | 27 | -27 | 60 | 6.67 |
| | **Left Middle Frontal Gyrus** | -36 | 18 | 48 | 6.62 |
| | **Right Cuneus** | 9 | -81 | 27 | 5.78 |
| | **Left Postcentral Gyrus** | -18 | -42 | 69 | 5.26 |
| | **Right Calcarine Gyrus** | 15 | -81 | 6 | 4.94 |
| **Task x Block Interaction** | | | | | |
| | **Left Middle Frontal Gyrus** | -21 | 24 | 48 | 4.93 |
| | **Left Posterior Middle Cingulate Cortex** | -3 | -39 | 42 | 4.77 |
| | **Left Angular gyrus** | -45 | -72 | 27 | 4.77 |
| | **Ventromedial Prefrontal Cortex** | -6 | 42 | 24 | 4.67 |
| | **Left Hippocampus** | -33 | -15 | -15 | 4.36 |
| | **Right Hippocampus** | 33 | -27 | -12 | 3.57 |
| **Object > Flower Task** | | | | | |
| | **Right Superior Parietal Lobule** | 18 | -66 | 51 | >8 |
| | **Left Middle Frontal Gyrus** | -24 | 0 | 54 | >8 |
| | **Right Middle Frontal Gyrus** | 24 | 0 | 54 | >8 |
| | **Right Linual Gyrus** | 24 | -63 | -3 | 6.75 |
| | **Left Parahippocampal Gyrus** | -30 | -45 | -6 | 6.71 |
| | **Right Cerebellum** | 12 | -51 | -51 | 6.7 |
| | **Left Cerebellum** | -12 | -54 | -48 | 6.51 |
| | **Right Parahippocampal Gyrus** | 27 | -45 | -9 | 4.86 |
| | **Left Inferior Frontal Gyrus** | -42 | 27 | 27 | 4.84 |

**Coordinating spatial information with anxiety during approach**

Given the particular importance of the hippocampus in spatial coding, I was interested in whether I could see evidence of connectivity increases with areas involved in influencing threat. Therefore, I performed a psychophysiological interaction (PPI), using the hippocampus (L: -21, -21, -18; R: 27, -18, -15) as a seed region (defined from the flower approach contrast) and looked for areas showing greater connectivity for flowers associated with danger compared to safety. When approaching flowers associated with danger, I saw increases in functional connectivity between the right hippocampus and bilateral insula ($p<0.001$ uncorrected; see Figure 56A). In addition, during active approach (late > early) I saw increases in functional connectivity between the hippocampus and vmPFC ($p<0.001$ uncorrected; see Figure 56B).

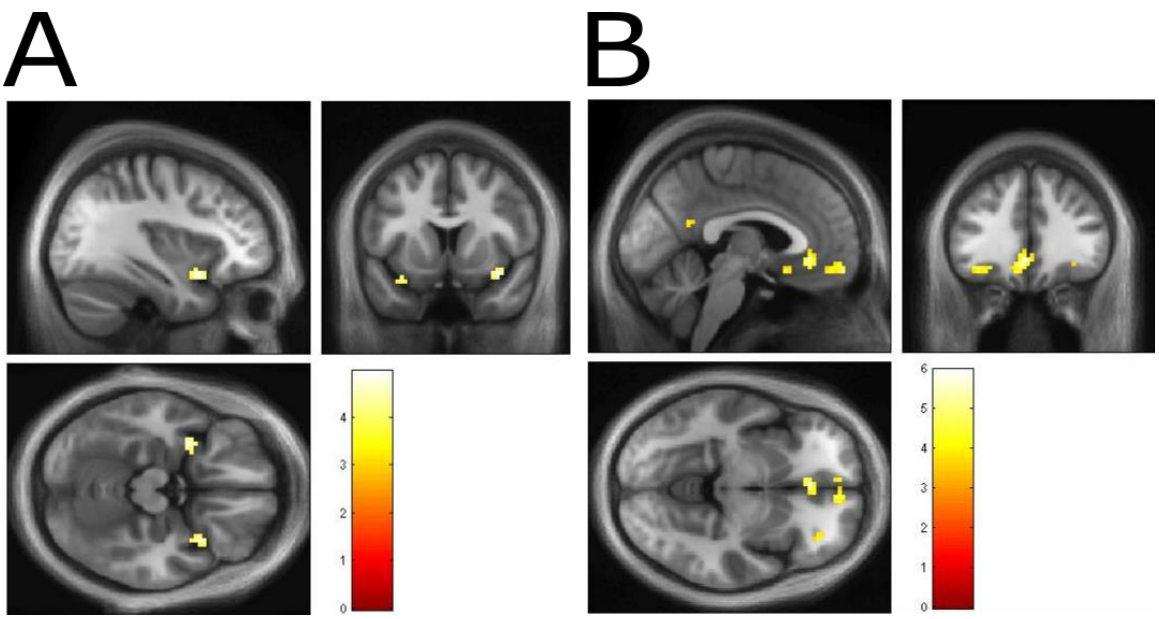


***Figure 56: Brain activity connectivity of approaching a flower within the environment.***

*Hippocampus showed functional connectivity with (A) bilateral insula (p<0.001 uncorrected) while approaching flowers associated with danger compared to safety, and (B) vmPFC (p<0.001 uncorrected) during the late part of active approach.*

**Anticipatory threat network during freezing**

Previous work has suggested that midbrain areas, particularly the periaqueductal gray, play an essential role in controlling defense reactions during imminent threat (LeDoux, Iwata, Cicchetti, & Reis, 1988). Therefore, I performed another PPI, using the PAG (6, -24, 3) and dACC (6, 0, 39) as seed regions (defined from the freezing contrast) and looked for areas showing greater connectivity for flowers associated with danger compared to safety. Periaqueductal gray activity during freezing (danger > safety) showed connectivity with left insula and caudate, and mPFC during the late part of active approach (p<0.001 uncorrected; see Figure 57A). The dACC activity during freezing (danger > safety) showed connectivity with bilateral insula activity during freezing in the danger zone (p<0.001 uncorrected; see Figure 57B).

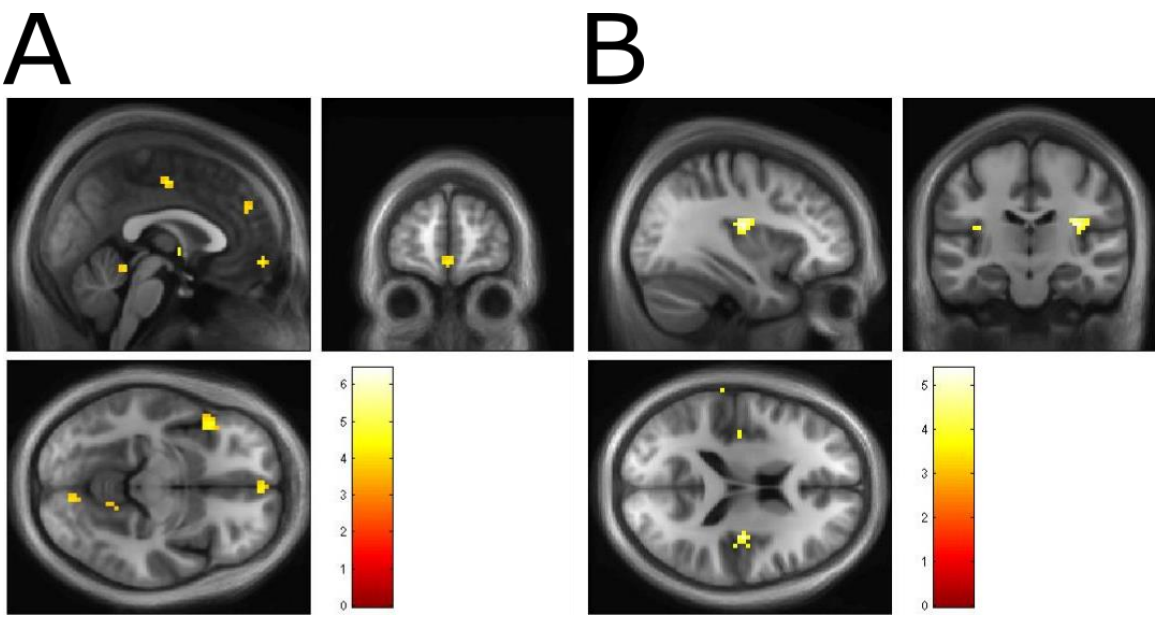


***Figure 57: Brain activity connectivity of anticipating the outcome of picking a flower within the environment.***

*Periaqueductal grey showed functional connectivity with (A) insula, caudate, and mPFC (p<0.001 uncorrected) during the late part of active approach. Dorsal ACC activity showed functional connectivity with (B) insula (p<0.001 uncorrected) during freezing in the danger zone.*

**Summary**

Like previous experiments, participants demonstrated location-specific threat, indexed by greater skin conductance when approaching flowers and during movement restriction (freezing) in dangerous relative to safe virtual zones. In addition, they demonstrated threat learning over time through differential shock expectancy ratings, which were higher for flowers in the danger vs. safe zone. During the approach to all flowers, irrespective of danger, activity increased over

learning trials in the hippocampus and vmPFC. When approaching flowers in threat relative to safety zones, activity increased in the dorsal ACC. The dACC, along with the insula, midbrain areas, and caudate were also active during the freezing period within the dangerous zone. On the other hand, during the freezing period within the safe zone, the vmPFC showed a tendency of activity that is consistent with many studies showing the vmPFC activity correlated with reward, safety, and subjective value (Adhikari, Topiwala, & Gordon, 2010; Dunsmoor & LaBar, 2012; Morris & Dolan, 2004; Schiller, Levy, Niv, LeDoux, & Phelps, 2008).

Finally, when looking at the object task, participants showed reduced object placement error over time, regardless of the zone where the object was found. The object placement task showed greater activity in the parahippocampal gyrus, precuneus, and cuneus when compared to the flower task. Conversely, the flower task showed greater activity in a wide range of areas that included the hippocampus, amygdala, and mPFC.

These findings provide the first evidence of location-specific threat learning in humans, highlighting a role for the hippocampus and vmPFC in processing cognitive maps within a single spatial context.

## Discussion

The main objective of this study was to examine the neuronal basis of contextual aversive conditioning using a VR task paired with fMRI, with the goal of understanding the neural mechanisms underlying spatial contextual discrimination within a single environment. Here, I show that healthy individuals were able to discriminate between two similar zones within one environment by using location-specific information from previous experiences within the environment. While participants collected flowers within the environment, they learned to discriminate

between the safe and the dangerous zone by identifying the areas where they were more likely to get an electrical shock (CS+). Behavioral data showed successful contextual threat learning through higher SCR and expectancy ratings to the dangerous zone than the safe zone.

The fMRI results revealed several unexpected results. First, vmPFC, hippocampus, and PCC showed increased activity when approaching a flower during the second half of the experiment compared to first half, irrespective of the zone in which the flower was found. Furthermore, aHPC showed changes in functional connectivity with the vmPFC during active approach in the second half of the experiment. These results suggest these areas are key in learning about the environment and the associations between threat and safety – they might inform other areas involved more in the behavioral response. This theory is further supported by the tasks comparison interaction, where I saw activity in the hippocampus and vmPFC decreased for the object task (a non-emotional task) over time, while increasing in the flower task (emotional task). These results further support the interpretation that these areas are active during learning about the environment and discriminating between threat and safety – emotional valence of the environment. This effect might reflect that hippocampal place cells become associated with the presence of salient stimuli (flowers) and whether or not they might contain bees. Furthermore, during ecological behavior, the hippocampus and vmPFC activity might reflect their role in evaluating what will happen next, for example, appraising value (positive or negative) of upcoming states (King et al., 2005; Viard et al., 2011).

On the other hand, I find a greater activity of the parahippocampal gyrus during the object task, when contrasted to the flower task. The parahippocampal gyrus is a well-known area that supports memory encoding and retrieval (Hayes, Nadel, & Ryan, 2007). These results support the idea that the emotional memory

of the environment is maintained by the hippocampus, while the non-emotional memory of the environment is maintained by the parahippocampal gyrus.

The mPFC has been suggested to have a central role in threat learning and extinction regulation and expression. The function of the mPFC in threat-related discrimination has been divided into dACC and dmPFC associated with the expression of threat (Mobbs et al., 2007; Mobbs et al., 2009), and over activity of these areas have been linked to anxiety disorders and stress (Robinson et al., 2012; Robinson et al., 2014). In addition, vmPFC has been associated with threat suppression (Milad et al., 2007; Phelps et al., 2004; Schiller et al., 2008), avoidance (Adhikari, Topiwala, & Gordon, 2010; Machado, Kazama, & Bachevalier, 2009), and extinction of fear and anxious responses (Milad & Quirk, 2002; Morgan & LeDoux, 1995). The vmPFC has been implicated in the 'extinction' of fear responses to the CS+ during repeated presentations without aversive stimulus (Milad & Quirk, 2002; Morgan & LeDoux, 1995). In addition, the vmPFC has been implicated not only in fear and anxiety modulation, but also in tracking positive rewards (Morrison, Saez, Lau, & Salzman, 2011; Saez, Rigotti, Ostojic, Fusi, & Salzman, 2015). Activity in the vmPFC has been generally attributed to safety signaling, particularly after extinction (Milad & Quirk, 2002; Morgan & LeDoux, 1995).

Neuroimaging studies in humans have shown that activity in the vmPFC increases during the presentation of CS-, while decreasing during presentations of the CS+ (Schiller, Levy, Niv, LeDoux, & Phelps, 2008; Schiller & Delgado, 2010). This contingent might argue that the vmPFC activity I found is due to the nature of the paradigm; where participants have to approach every flower, therefore reflecting avoidance inhibition or safety signaling to increase approach. Several of my findings suggest that this assumption is unlikely. First, I see this vmPFC-aHPC activity by the end of the experiment. If vmPFC was acting as a behavioral modulator for safety or approach, I would see no difference in activity within the

experiment or greater vmPFC activity at the beginning of the experiment when the shocks are less predictable. Second, my object task findings support that indeed this activity is related to anxious representation of the environment-cue association, as I find greater vmPFC-aHPC activity during the flower task when compared to the object task. Interestingly, in many studies, patients with anxiety disorders display an inability to discriminate between dangerous and safe context, overgeneralizing the anxious response (Britton, Lissek, Grillon, Norcross, & Pine, 2011; Pine, 2007; Grillon, Pine, Lissek, Rabin, & Vythilingam, 2009; Kheirbek et al., 2012). In general, contextual threat discrimination has been attributed to neuronal systems that regulate emotion and memory, especially the hippocampus (Fanselow & Dong, 2010; Kheirbek et al., 2012). Accordingly, decreased hippocampal volume and hippocampal dysfunctions have been associated with anxiety disorders and PTSD (Fanselow & Dong, 2010; Kheirbek, Klemenhagen, Sahay, & Han, 2012; Sotres-Bayon et al., 2012).

Once the flower is reached, areas often involved during immediate threat (PAG, insula, dACC) are seen when a shock is imminent (danger > safety). Conversely, vmPFC activity (safety > danger) shows a preference for flowers reached in the safe zone of the environment, which might be reflective of positive value or safety signaling. These areas showed a pattern of connectivity with other areas I saw involved in the discrimination of the two zones within the environment. Activity in the periaqueductal gray shows functional connectivity with the insula, caudate, and mPFC during the late part of active approach. Similarly, dACC showed connectivity with bilateral insula activity during freezing in the danger zone. These results suggest a signaling process for threat expression or inhibition of threat suppression.

The activity in the dACC, PAG, and Insula support the existing literature about their role in aversive learning. Activity in the dACC, and connectivity with the

amygdala, has been shown to increase during processing of threat, especially under anxious conditions (Robinson et al., 2012). Furthermore, over-activity of the dACC has been found in patients with anxiety disorders (Robinson et al., 2014). The periaqueductal gray has been implicated in aversive conditioning, especially in the modulation of autonomic conditioned responses (LeDoux, 1988). The insula has been proposed to have a similar role in autonomic responses to emotional stimuli. Specifically, studies have found increases in activity during expectation of aversive stimulus (Berns et al., 2006; Dunsmoor, Bandettini, & Knight, 2007; Phan, Wager, Taylor, Loberzon, 2002). Still, areas activated during freezing, such as dACC, insula, and midbrain areas, might be reflective of valuation of valence and not specific to threat signaling. Further studies are required to pick apart if the activity in these areas are specific to threat or general valence evaluation. However, there is a huge body of literature that points to the general recruitment of these areas during threat and pain valuation and expectation.

The amygdala has been consistently associated with cue aversive conditioning, and the acquisitions and expression of threat (Buchel & Dolan, 2000; Cheng, Knight, Smith, & Helmstetter, 2006; Knight, Nguyen, Bandettini, 2005; LeDoux, 2000). In the study, I did not see any particular variation of activity in the amygdala throughout the flower task. One reason might be the quick adaptability found in the amygdala (LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; Buchel, Morris, Dolan, & Friston, 1998). Nonetheless, when I compared the object task to the flower task, I find higher activity in the amygdala during the flower task. In recent years, several studies have found that the amygdala is active in negative, as well as positive, reinforcement, implying a more complex structure than just signaling CS-US associations (Dolan, 2007; Peck, Lau, & Salzman, 2012; Peck & Salzman, 2014; Saez, Rigotti, Ostojic, Fusi, & Salzman, 2015). This finding might suggest that the lack of amygdala activity differentiation might be due to participants seeing the flowers in the safe zone as positive, since it is a relief not to get a shock.

Other studies have failed to find any significant activity of the amygdala (Onat & Buchel, 2015), and yet others suggest that the amygdala might be active throughout the whole experiment, tracking not only the value, but also other events in the task, like a fixation cross (Belova, Paton, & Salzman, 2008; Paton, Belova, Morrison, & Salzman, 2006). The authors of these studies posited that the amygdala might be tracking the overall value of an organism's situations, as they appear in the environment. I postulate, with the results found in this thesis, that the amygdala has a passive role during context valence representation, whereby with the presence of a threat or safety the amygdala activity is modulated by areas such as the vmPFC, aHPC, and dACC to calculate its value. Therefore, I see an overall activity of the amygdala, during the flower task, as each flower needs to be re-valued depending on the zone where it is encountered. Regardless, due to the divided and mixed literature of the amygdala, more research is needed to tease apart the role of this area.

In conclusion, these results suggest that areas typically associated with specific safety signaling might be involved in a process of discrimination, rather than specific valence signaling. This process of discrimination was characterized by activity in the vmPFC and hippocampus after learning the environment's contingencies. Furthermore, areas involved in threat signaling, such as the dACC and PAG, might be recruited in order to warn the individual of an impending or approaching danger. Most importantly, these areas seem to be highly activated while doing an emotional task, as opposed to an unemotional task. These results illustrate a novel task to study the neural basis of threat learning and discrimination. Moreover, studies of patients with anxiety disorders and PTSD could benefit from this task as a way to investigate the malfunctions in neural mechanisms of threat discrimination underpinning context generalization in these clinical populations.

# Chapter 8

## General Discussion

## Overview

Learning about potential dangers in our environment is a vital adaptive behavior. Research has identified a key network of brain areas involved in forming associations between environmental cues or contexts, and whether they predict danger or safety. However, in some situations, an aversive outcome associated with an environmental cue might be determined by the specific location it is encountered within the world. Little is known about the neural mechanisms behind location-specific threat learning within a single environment in humans. I thought that through involvements of the mPFC, amygdala, and hippocampus there would a modulation in valence signaling that would guide specific-location behavior. That is, locations in space, that participants associated with safety or danger, would be represented by these brain areas, in particular the hippocampus due to place cells cognitive mapping. Through a series of experiments, I attempted to elucidate into the neurobiology of location-specific threat learning by measuring physiological responses to anxiety in conjunction with functional magnetic resonance imaging (fMRI).

Through the first series of experiments (Chapter 4), I developed and validated a novel virtual reality task aimed to examine learning valence discrimination within an environment using location-specific information. The task consisted of safe (CS-) and dangerous (CS+) zones, within a single environment. Healthy volunteers explored a single virtual environment and were instructed to collect flowers in the environment. On collecting a flower, participants were frozen for a short period and informed that a bee might be inside the flower and could sting them (shock co-terminated with trial offset). Only flowers appearing in one half of the environment were paired with a shock. Participants had to use spatial-information to learn to discriminate between both zones. Here I showed that participants were able to discriminate, within a single environment, zones that predict safety and threat. Participants demonstrated location-specific anxiety and

fear indexed by greater skin conductance when approaching flowers and during freezing in the dangerous, compared to safe, zone of the environment.

Through the next series of experiments (Chapter 5), I used this VR reality task to further determine the optimal settings in the paradigm to study location-specific threat learning. During these experiments, I tested several avenues to diminish any artifact that the electrical shocks might cause in the fMRI data analysis, by either reducing the number of shocks, or jittering the time the expectation period before that shock lasted. I showed that jittering the time of freezing was the optimal solution, while maintaining learning and discrimination of the safe and dangerous zone of the environment.

Subsequently, I developed another series of experiments (Chapter 6), where I tried to validate the same task using an aversive scream. The purpose of these experiments was to find an alternative to the electrical shocks, in order to use this task in vulnerable populations, such as children or patients with anxiety disorders, that ethically the use of shock was not permitted or might be too aversive. Although I did not find differential skin conductance response to threat compared to safety, I did find higher expectancy ratings to scream in the threatening zone of the environment compared to the safe zone. These results suggest that predicting the scream is not enough to regulate contextual anxiety.

Finally, the last experiment (Chapter 7) discusses the use of the task during fMRI scanning. The task was designed to reveal brain areas involved in learning locations in the environment predictive of safety and danger. The fMRI was coupled with physical measures of anxiety, such as skin conductance response (SCR; measuring sweat as an index of anxiety) and subjective ratings of anxiety. I identified several neural circuits recruited in the formation of spatial safe and threat cognitive maps within an environment. During the approach to a flower, there was activity that increased over learning trials in the hippocampus and vmPFC,

irrespective of the location of the flower. Specific to threat zones, there was increased activity in the dorsal ACC that was specific to approaching flowers in locations predicting danger compared to safety. Upon reaching the flower, activity increased in areas involved during immediate threat (PAG, insula, dACC) when a shock was imminent. With this experiment, I provide the first evidence of location-specific threat learning in humans. My findings highlight a role for the hippocampus and vmPFC in processing cognitive maps within a single spatial context. In particular, if the association of threat/safety to location is via association to place cells in the hippocampus, then it would be expected that place cells with a range of spatial scales of firing fields to be involved, i.e., a range of posterior-anterior regions (Kjelstrup et al., 2008)

These findings have important implications for the way in which we learn about threatening situations, and how these neural mechanisms might break down in anxiety disorders. Taken together, this is the first time threat learning discrimination has been demonstrated within one environment without boundaries delineating safety and danger. These results are important since we experience the world in a continuous manner, where boundaries are not always as clear-cut as they tend to be under laboratory constraints. Importantly, elucidating how these boundaries are normally formed is vital to clarify the process of overgeneralization of threat in those afflicted by anxiety disorders and PTSD.

## Future directions

From my acquired knowledge of spatial navigation in virtual reality and anxiety disorders, my goal is to further understand brain areas involved in threat learning and discrimination, especially safety learning. Using fMRI in humans, I aim to further investigate the neural circuits involved in safety learning and threat discrimination within an environment. A hallmark of clinical anxiety and PTSD is

the inability to discriminate between safety and threat. Using the virtual reality paradigm that I developed, I have identified several brain areas engaged in threat learning discrimination within an environment in healthy volunteers. I am interested in understanding why patients with anxiety disorders and PTSD are not able to make this safe/threat discrimination, and the underlying dysfunction in the brain areas associated with threat learning and discrimination.

My first aim is to use the virtual reality paradigm that I have developed to further explore the neural mechanisms underlying threat discrimination in anxiety disorders (i.e., generalized anxiety disorder, social anxiety disorder, panic disorder, etc.) and PTSD. Utilizing the results from healthy adults, I want to further understand the brain areas that differ in anxiety disorders, PTSD, and healthy volunteers. Once I gather the results of patients with anxiety disorder, I will be better equipped to further understand necessary brain areas for safe/threat discrimination. Generally, greater dACC activity is found in patients with anxiety disorders and PTSD (Robinson, Charney, Overstreet, Vytal, & Grillon, 2012; Robinson et al., 2014). Still, little is known if this dACC effect is due to an over activity of the area or an under activity of other modulatory brain areas, such as the vmPFC and aHPC. Differences in dACC modulation deficits between anxiety disorders and PTSD is also possible. The aHPC and mPFC are necessary to discriminate between safety and threat by creating a cognitive representation of the environment. Thus, HPC and mPFC dysfunctions associated with anxiety disorders and PTSD might prevent patients from accurately discriminating between threat and safety within the environment. I predict that patients will demonstrate a weaker ability to distinguish safe and dangerous areas of the environment compared to healthy controls. This should be evidenced by lower accuracy at predicting the likelihood of experiencing an aversive shock in the correct area of the environment. Furthermore, patients will show lower vmPFC and aHPC activation compared to controls. This difference in activation should be

reflective of patients' inability to discriminate between safe and dangerous areas within the environment. Finally, patients would reflect greater dACC activity compared to controls, while navigating the safe and dangerous area of the environment. Alternatively, higher emotional reactivity decreases cognitive functions, preventing patients from accurately discrimination between threat and safety within an environment. Over activation of the dACC found in pathological anxiety and PTSD is associated with maladaptive fear and anxiety expression. Consequently, valence discrimination is dependent on appropriate activation of the dACC. In such a case, I predict that patients should reflect greater dACC activity, but no difference in aHPC or vmPFC activity, compared to controls, while navigating the safe and dangerous area of the environment.

My second aim is to further understand the role of each brain area previously identified (e.g., mPFC and midbrain areas) in healthy participants. The vmPFC is theorized to be involved in safety signaling in the brain (Schiller, Levy, Niv, LeDoux, & Phelps, 2008). Still, I find vmPFC involvement throughout both safe and threatening zones of the environment, suggesting a role in spatial mapping discrimination and value, rather than a specific role to safety signaling. Similarly, in the literature, greater aHPC activity is associated with threatening environments (Adhikari, Topiwala, Gordon, 2010). Again, I find aHPC involvement mainly as a signal of context discrimination, rather than specific to threat zones. Consistently with the literature, I find greater dACC activity associated to danger. Still, it is necessary to tease apart these findings before any conclusions can be made. Teasing apart these signals can help us understand if the role of these brain areas is indeed related to discrimination, valence signaling, or both; and if they differ in pathological anxiety and PTSD.

## Follow-up Experiments

There are several follow-up experiments that this paradigm could be useful for. First, my initial paradigm could be used to study neural circuits underlying discrimination learning in patients with anxiety disorders and PTSD. In addition, the paradigm could study learning differences within healthy volunteers. Throughout my experiments I have seen healthy participants who are unable to identify the safe zone within the environment. The number of non-learners can be increased or decreased by variations in the number of trials and the reinforcement rate of the aversive stimuli within the environment. That is, an increase in shocks within the dangerous part of the environment increases participant's accuracy to discriminate between the safe/threat zones of the environment. This result suggests that predictability within the environment contributes to successful discrimination. This paradigm can be a useful application to study the areas of the brain that are engaged in successful discrimination and differences within healthy volunteers will give a better insight of brain areas necessary for safe/threat discrimination. Furthermore, differences in brain activity within patients, with pathological anxiety or PTSD, and healthy volunteers who overgeneralize threat into safety zones will help further understand discrimination inabilities in these disorders. Overall, further experimentation will give a better understanding of mechanisms that go awry in patients and help further elucidate healthy from aberrant brain activity.

### Task modification

These populations of healthy volunteers (learners and non-learners) and patients can be used to further understand the role of each brain area identified in my initial experiments in safety and threat signaling (e.g., mPFC and midbrain). In

order to do this, there are potential modifications that can be made to my existing virtual reality task that could be made to assess the role of each brain area during threat learning within an environment.

One way to further specify the roles of the brain areas that I identified in the discrimination of safe and threat zones within an environment (vmPFC & aHPC) would be to make both zones perceptually distinct. Currently, to study learning discrimination over time, participants must create and maintain boundaries ("internal mapping") within the environment; the environment itself is uniform and does not have marked boundaries. Creating marked boundaries will reduce the activity of brain areas involved in the discrimination process, revealing activity of brain areas more specifically related to emotional valence within the environment.

Another potential modification would be to give participants a stressor before the experiment. Although the experiment on its own has a layer of stress, based on the nature of the task, adding another layer of stress prior to the experiment might allow for the effects of stress to be examined independently to learning the context discrimination itself. Thus, it is possible that the function of brain areas like the aHPC will be impaired by the additional stress (see e.g. Bisby et al., 2016), increasing the number of people who are unable to discriminate between safe and threat, and allowing for further investigations of symptoms of overgeneralization.

In order to look at the involvement of the dACC in threat signaling, another modification could be to change the aversive component of my original task into a reward component. This modification would diminish any threat signaling activity within the experiment, and increase activity in brain areas that signal safety and reward; maintaining activity of brain areas related to internal valence mapping of the environment. Another approach to explore is the use of reversal learning, a switch between safety and threat in the environment. In doing so, switching the

activity of brain areas related to threat signaling to areas previously predicting of safety. This approach is useful to study brain areas related to safety signaling, reflected in areas previously predictive of threat, and vice-versa. Another approach to study valence signaling is extinction. By training participants to re-learn or “extinguish” the association of the stimuli and the aversion that accompanied it, I expect a decrease of activity in brain areas reflective of threat (dACC), and an activity increase in areas related to safety. This test would help assess if the vmPFC really plays a role in extinction. Finally, pairing the shock to random times, while navigating the dangerous environment, instead of paired to a stimulus allows for a deeper understanding of attention to threating zones, rather than threatening cues within the environment. I hypothesize that adding a higher level of unpredictability will increase activity in brain areas related to threat signaling.

In addition to fMRI, these studies could be carried out using Magneto encephalogram (MEG). MEG is another brain imaging technique that surpasses fMRI temporal resolution, at the cost of spatial resolution. In other words, MEG can assess events in time with a higher accuracy than fMRI, although without the precision in space that fMRI offers. Still, MEG results can further elucidate the activity in the mPFC, and its theta phase coherence in spatial learning with the medial temporal lobe, as shown in other threat inducing experiments (Jones & Wilson, 2005 in rodents; Kaplan, et al., 2014; Watrous et al., 2013 in MEG) and in anxiety testing (Adhikari, Topiwala, & Gordon, 2010 in rodents; Cornwell et al., 2012 in MEG).

In summary, future uses of the paradigm I developed could shed further light on the neural circuits modulating safety and threat signaling that support appropriate threat learning and discrimination within an environment.

## Conclusion

This thesis demonstrated that participants are able to use location-specific information to determine threat and safety zones within one environment. Through the use of the threat learning virtual reality paradigm, I found evidence that participants are able to discriminate safe/danger zones within an environment, by differential SCR and expectancy ratings. Further, the hippocampus and vmPFC show involvement in discriminating threat and safe zones within one environment. Additionally, I found that the dACC is involved in the process of threat signaling during approach and freezing. Eventually, I aim to use this knowledge in valence signaling and discrimination to 1) study the effects of psychopharmacological agents in discrimination learning and 2) to create new virtual reality environments to study the activity of these brain areas in tasks related to approach, avoidance, decision making, and attention biases. In this way I hope to further understand the role of mPFC and aHPC in discrimination and safety learning, and their performance in pathological anxiety and PTSD. In the long term, I am confident that this research will shed light on the specific role of brain areas needed for threat learning and discrimination within an environment, which will help advance the development of effective diagnostics and treatments for clinical anxiety and PTSD.

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