Neurocircuitry of Generalization of Avoidance Behavior following Pavlovian Conditioning in Adults with High and Low Trait Anxiety

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Abstract

One of the cardinal features of many anxiety disorders is maladaptive avoidance. While behavioral avoidance is important for survival and adaptive when danger is present, in the absence of a threat it is maladaptive. Signaled avoidance depends on Pavlovian learning that a neutral conditioned stimulus signals an ensuing aversive unconditioned stimulus. Maladaptive signaled avoidance could therefore result from abnormalities in Pavlovian conditioning. Overgeneralization of conditioned fear is one such abnormality that has been demonstrated in several anxiety disorders. To assess the relationship between anxiety and generalization of signaled avoidance behavior, 22 participants, with a range of trait anxiety scores split into two group of high and low anxiety, completed a generalization gradient, approach-avoidance fMRI task following Pavlovian discrimination conditioning. Results indicated the expected curvilinear generalization gradient in avoidance responses and ratings of risk, with group differences in avoidance responses. There were several functional regions of interest which also demonstrated the expected curvilinear gradient as well as group differences in percent BOLD signal change across the gradient. This was true for both Pavlovian trials, as well as during the decision making stage of the Instrumental trials. There were also several regions in which activations were significantly related to avoidance behavior. These results indicate that individuals with higher levels of trait anxiety are at increased risk of 'maladaptive' avoidance of safe stimuli that resemble danger-cues. Moreover brain areas such as the anterior insula and subgenual anterior cingulate cortex, and primary visual cortex, which are involved in the Pavlovian generalization of fear, are also involved in the overgeneralization of the avoidance response. Additional unexpected findings highlight the role of the cerebellum, somatosensory cortex, and gender in production and maintenance of an avoidance response.

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Introduction

When a painful or otherwise aversive condition is encountered, escape from that situation is often desirable or necessary. If there are cues that are associated with the occurrence of a painful or aversive event, avoidance of the cues, and therefore the expected aversive event, could be highly adaptive if the cue is a reliable predictor of the aversive event. This last statement is loaded with complexities that have been the topic of much research over the last century. First there is the dissociation of the Pavlovian learning processes that allow an organism to associate cues with events, and second the instrumental learning process, in which an organisms action and the consequences of that action are learned. Also there has been much debate over what is actually avoided, the cues themselves or the expected outcomes. Lastly, if the cue is not reliable, or if associative or instrumental relationships are not reliable or learned appropriately, there could be consequences that result in maladaptive behavior.

While avoidance behavior is important for survival and adaptive when danger is actually present, in the absence of a real threat, avoidance behavior is maladaptive and this type of maladaptive behavioral avoidance is a cardinal feature of anxiety disorders (American Psychiatric Association, 2013). Agoraphobia resulting from panic disorder and specific phobias are characterized by avoidance, Post-Traumatic-Stress Disorder (PTSD) has avoidance as a criterion for the disorder, and social anxiety disorder includes avoidance as a symptom of the disorder.

Research on the mechanisms of avoidance behavior is necessary given its importance both its role as a crucial survival mechanism and it's relevance in clinical disorders. Studies in animals have attempted to identify brain regions and processes that are critical for

avoidance learning and behavior. More recently, these investigations into brain-behavior relationships have begun in humans utilizing technologies such as functional magnetic resonance imaging (fMRI). Studies have also begun to delineate the relationship between avoidance and individual differences such as neuroticism or anxiety. This venture will hopefully lead to the creation of treatments which can reduce the personal, social and societal impact of anxiety and associated avoidance behavior. This dissertation will hopefully contribute to this endeavor as one of the multitude of steps on the path towards understanding the relationships between anxiety and avoidance behavior.

Measuring and Studying Avoidance

Signaled Avoidance

In order to study avoidance, it first needs to be operationalized and measured. There have been a number of different measures that have aimed to do so and these methods a are briefly reviewed here.

The earliest avoidance-learning experiments were conducted at the beginning of the twentieth century using the signaled avoidance procedure which expands on Pavlovian conditioning (Bekhertev, 1913). In Pavlovian fear-conditioning, a previously neutral conditioned stimulus (CS) is paired with an aversive unconditioned stimulus (US) and after repeated pairings, the CS comes to elicit fear by itself and reflexive responses to the CS are measured (Pavlov, 1928). For example, a rat sees a blue light (CS) in the cage turn on, and then receives a shock (US). At first, the rat does not have a fear response to the blue light, but after receiving the shock numerous times after the blue light comes on, the blue light starts to the fear response. In Pavlovian fear-conditioning, the experiment is

fully controlled by the experimenter and the behavior of the subject in no way affects the outcome, that is nothing the rat can do after the presentation of the blue light will stop the shock from occurring. The signaled avoidance procedure first involves classical or Pavlovian conditioning, but the subject is then allowed to perform a response, after the onset of the CS to avoid the US (Hull, 1934). For example the rat in the previous example may be able to run to another chamber, press a lever, etc. as soon as the blue light comes on in order to avoid being shocked. Subjects – both humans and nonhuman animals – typically learn to perform the avoidance response, thereby avoiding the US. This is an example of *active avoidance* behavior, which needs to be differentiated from passive avoidance behavior.

In active avoidance, the subject needs to perform a response in order for the US presentation not to occur. This is contrasted with *passive avoidance* which simply requires not performing a behavior which had previously been reinforced with an unpleasant US in order to avoid further presentations of the US (Lykken, 1957). Both passive and active avoidance are subtypes of instrumental (operant) conditioning, which is a type of learning where behavior is modified by consequences of an action (Skinner, 1937).

Another type of avoidance, termed Sidman avoidance, or free-operant avoidance, differs from signaled avoidance in that there is typically no warning signal that a shock is about to occur and therefore the Pavlovian association does not need to be learned. Instead shocks will continue indefinitely until a response is made which will delay the next shock for a period of time. Continued lever-pressing will continue to delay the

shocks, and the lever press is therefore considered an active avoidance response (Sidman 1955 *A).

Decision Making During Conflict

In many traditional avoidance studies, either signaled or free-operant, there is no drive competing with the drive to avoid. While not having a competing conflict is useful for studying learning, this is not congruent with real life experiences where avoidance necessarily limits approach behaviors that are important for exploring, gathering resources, being social, and the like. Therefore some investigations of avoidance turned to decision making tasks in which rewards and risks are pitted against each other and subjects need to choose between competing options. There are a wide variety of studies that employ this basic structure.

Some decision making tasks employ conditioning, either Pavlovian or instrumental, or some combination of the two. Conditioned suppression studies may for example measure the ability for a Pavlovian conditioned stimulus to modify instrumental responses such as avoidance when the animal is hungry and food is present (Kamin et al., 1963). Many studies using passive avoidance procedures will also rely on conflict to increase motivation to perform the behavior. For instance, rats prefer the dark, but are kept in the light side of a chamber, and are shocked if they move to the dark side (Bourin and Hascoët, 2003). Although termed passive avoidance, they are perhaps better categorized as conditioned suppression. Another similar paradigm is Pavlovian-to-instrumental transfer (PIT) which is used to investigate motivational aspects of the interaction between Pavlovian and instrumental conditioning. Essentially, it involves

training an instrumental response, learning a separate Pavlovian relationship, and testing for the influence of the Pavlovian relationship on the instrumental response without any trained formal association between Pavlovian and instrumental contingencies. In this way the instrumental response can be considered a decision because the subject is able to choose to perform the response or not, based on the anticipated risks and rewards associated with both forms of conditioning.

PIT has been studied extensively in animals (Estes, 1943; Rescorla and Solomon, 1967, Holland et al., 2002), and when studied in humans PIT has mainly been assessed in the appetitive domain (Bray et al. 2008, Talmi et al. 2008). One of the reasons for extensive study in the appetitive domain is the relevance of PIT to drug seeking behavior in response to drug cues in the environment. PIT paradigms offer a controlled experiment that captures this interaction with the CSs that have been paired with drugs motivating a behavioral response to engage in drug seeking behavior (Everitt and Robbins, 2005). More recently, PIT has been adopted by aversive conditioning researchers as it offers promise for investigating fear and anxiety based behaviors such as avoidance (Geurts et al., 2013).

Other studies examine approach-avoidance tendencies through decision making without Pavlovian or instrumental conditioning but still have a balance of potential positive and negative outcomes. This include studies of risk aversion, where decisions that that are taken to minimize negative outcomes, or risk-averse behaviors, are characterized as avoidance tendencies as opposed to approach behavior which is characterized by subjects accepting some level of risk in order to increase the chance of

getting rewards. Many of these studies simply use money as both the reward and losing money as the aversive stimulus (Mohr et al., 2010; Sailer et al., 2008; Paulus et al., 2003), although some studies use a monetary reward and a painful physical stimulus as the aversive stimulus (Talmi et al. 2008).

A**voidance Learning Theories**

A number of theories have been developed to explain how avoidance behaviors are learned and the types of conditions which increase or decrease avoidance behavior. Just as methods are necessary for measuring avoidance, theories are critically important for creating testable predictions and potential treatments for disorders characterized by avoidance. The most influential theories are reviewed here.

Generally, theories of avoidance fall into one of two camps. One camp of these theories are two process theories that reflect the contributions of both Pavlovian and instrumental conditioning on the development and maintenance of avoidance responses, while the other camp of theories highlights cognitive expectancy in avoidance behavior.

At first, it was thought that avoidance learning could be explained completely by Pavlovian conditioning. In early experiments on avoidance, the avoidance response was typically a fleeing behavior. This was the same behavior that was spontaneously elicited by the shock. Therefore it was theorized that the pairings of the CS with the US early in avoidance training (before the avoidance response is learned); lead the CS to elicit a CR that was the same as the UR (fleeing) (Pavlov, 1928). However, experiments in the late

1930s, contradicted this Pavlovian account, suggesting that avoidance learning depends instead on instrumental learning (Skinner, 1937; Thorndike, 1933).

Avoidance Understood Through Thorndike's *Law of Effect*

The main account of instrumental learning was Thorndike's *law of effect* (Thorndike, 1933). Thorndike proposed that stimulus-response (S-R) associations were responsible for learning of instrumental behavior. Specifically, the law of effect states that given a pre-existing situation or stimulus, a response that is accompanied or closely followed by pleasure will reinforce the association between the situation or stimulus (S) and that response (R). Instrumental conditioning theories built on this theory of reinforcement to include the inverse, punishment, which involves the weakening of the relationship between the stimulus and response when the response is followed by displeasure. However, explaining avoidance learning in these now antiquated instrumental terms is difficult because the avoidance itself precludes the organism from experiencing the stimulus.

Mowrer's *Two-Stage Learning Theory*

One prominent account that attempted to overcome the shortcomings of strictly instrumental learning based accounts of avoidance behavior is Mowrer's *two-stage learning theory* (Mowrer, 1947). Mowrer questioned how not experiencing an aversive event could lead to a state of satisfaction. To answer this question Mowrer relied on combining Pavlovian and instrumental learning, hence the name two-factor theory. Additionally, this theory utilizes Clark Hull's drive reduction theory (Hull, 1935).

According to Hull, drive states such as hunger or fear create tension or discomfort which needs to be reduced and this reduction acts as a reward. The first stage of learning in Mowrer's theory includes acquisition of fear to a CS+, paired with an aversive US, through Pavlovian fear-conditioning. In the second stage, this Pavlovian fear motivates instrumental avoidance of the CS+, which leads to anxiety reduction (i.e., relief), and thereby reinforces and strengthens the avoidance response. Importantly for Mowrer's theory, the avoidance response is therefore reinforced by CS termination, not by the actual shock avoidance. This provides an answer to the question of how an event which is not experienced could lead to a state of pleasure or relief as avoidance of the US does not provide relief, it is just a fortunate byproduct. Avoidance behavior is made in response to the CS and essentially is not even avoidance, rather an escape from the CS which does provide relief.

Mowrer's two-stage learning theory does have difficulties explaining some findings in both laboratory and clinical research however. Most importantly, avoidance responses that only serve to prevent the US from occurring and do not have any effect on the presentation of the CS are not predicted by the theory, but are observed (Bolles, Stokes, & Younger, 1966; Rachman and Hodgson, 1974, Rachman, Craske, Tallman, & Solyom, 1986). Generally, both termination of the US and the CS are effective in reinforcing the response, with the highest level of avoidance learning occurring when the response produces both effects. Also, according to the theory, continued anxious reactivity to the CS must be present to maintain the avoidance response, however once the avoidance response is learned and is performed, anxiety in the presence of the CS

decreases, yet the avoidance response persists (Seligman & Campbell 1965, Solomon et al.,1953, Herrnstein, 1969; Rachman, 1977).

Expectancy-Based Account of Avoidance

In order to address the limitations of Mowrer's theory, an expectancy-based account of avoidance learning was presented by Seligman and Johnson (1973). In this account, subjects learn the outcome of making an avoidance response and not making an avoidance response, and then make a decision whether or not to avoid based on a comparison between the expected outcomes. The main principle of Seligman and Johnston's theory is that the avoidance response is driven not by S-R associations, but by expectancies about response-outcome (R-O) contingencies. The theory suggests that during avoidance learning subjects will develop two R-O expectancies: if the avoidance response is performed, no shock will occur; and if it is not the shock will occur. Because subjects prefer no shock to shock, they choose to avoid. This theory explains why subjects make responses to avoid a US when it has no effect on the presence of the CS. This theory assumes that when responses are made to avoid the CS, this is because the subjects evaluating the possible outcomes and only avoiding the CS in order to avoid the shock. This is markedly different from Mowrer's two factor theory. It also explains the persistence of avoidance after fear is extinguished, as one can still hold the cognitive expectancy of safety that is offered by the avoidance after fear reduction. Lovibond et al. (2008) followed up Seligman and Johnson's theory with an expectancy based account of both instrumental, and Pavlovian conditioning. In this model, Pavlovian stimuli associated with shock generate expectancy or anticipation of shock. This expectancy,

once learned cognitively, then triggers autonomic arousal. Instrumental responses are then selected and performed based on a cost-benefit analysis (Lovibond et al., 2008). One strength of this theory is that it relies on a unitary learning system and is therefore a more parsimonious model compared to thetwo-process learning theories.

There is evidence that there is a strong correlation between self-reported shock expectancy and skin conductance in the experiments presented by Lovibond et al. and they argue that expectancy of shock, or threat appraisal, regulates anxiety and that performance of avoidance behavior is selected to reduce the expectancy of a negative outcome. Additional support for a cognitive theory of avoidance comes from a recent study that assessed avoidance to a learned CS+, an instructed CS+ and a derived CS+ which shows that expectancies can be acquired without Pavlovian conditioning and these expectancies influence avoidance behavior (Dymond et al., 2012). In the experiment, the derived CS+ was never associated with shock, but prior to fear conditioning of the learned CS+, the derived CS+ was associatively paired with a CS+ stimulus using corrective feedback. Participants were shown the CS+ that would later be paired with shock and then both the eventual derived CS+, or a CS-, and asked to choose one and were given positive feedback if they chose the former and negative feedback if they chose the latter and only participants who reached a threshold of learning the association between the CS+ and the derived CS+ participated in the fear conditioning phase of the study. Participants made similar avoidance responses to all three different types of conditioned danger stimuli, the learned CS+, the instructed CS+ and the derived CS+., This suggests that subjects made similar cognitive expectancies through direct

experience, by verbal information, and based on a previously acquired relationship between stimuli that influenced their avoidance behavior. Therefore cognitive expectancies alone are capable of eliciting the same avoidance responses that are demonstrated after Pavlovian conditioning.

Cognitive theories of avoidance do have some difficulties explaining avoidance behavior observed in some experiments. For example, animals do still make instrumental responses when the response terminates the CS but is followed by the US (Kamin, 1956), which supports habitual learning more than expectancy learning. Another finding that the expectancy theory does not account for is that across species, unpredictable stimuli elicit greater anxiety than predictable ones (Grillon et al, 2004; Herry et al. 2007). Some of the best evidence for how this contributes to avoidance behavior is demonstrated by measuring rates of return to the experimental context for a second session following conditioning. Subjects who report they are not-aware of the contingency between the CS+ and the US are less likely to return for a second session than those that are aware of the contingency, indicating that unpredictability, which can also be thought of as a lack of cognitive expectancies may actually motivate avoidance behavior (Grillon, 2002). Similarly, after subjects were given either predictable or unpredictable shocks in different virtual reality contexts, they avoided the unpredictable context in a later behavioral test (Grillon et al., 2006). Importantly, shock electrodes had been removed at the time of the behavioral test and therefore the persistence of the conditioned responses, rather than expectancy of shock, seems to account for avoidance. This may be related to the inability of anxious individuals to reduce anxiety despite cognitive awareness that there is no

reason to be anxious (Marks 1969, Mayer et al 2000). Alternatively, unpredictability could result in an expectation that a negative event could happen at any time, which would then lead to avoidance responses to anything that is unpredictable as the expectations are for random negative events. Therefore unpredictability may be just a special type of expectation when an animal has no discrete information to learn from.

Additionally, the expectancy account also requires that an explicit representation of these contingencies (e.g., "blue predicts shock"…") is made by participants to learn the Pavlovian contingencies. However there is evidence that overt reasoning, which is a prerequisite for eliciting emotional responses in the expectancy framework, is in fact preceded by non-conscious emotional biasing processes that aid in developing the cognitive expectancies (Bechara et. al, 1997). Additional evidence against a cognitive expectancy based account is that that Pavlovian conditioning can occur in the absence of contingency awareness (Critchley et al., 2002, Morris et al. 1999).

Decision making models

Two stage avoidance learning theories based on habitual learning, and cognitive expectancy theories based on outcome learning, have been further developed by decision making models. In decision making theory, the goal for an organism is to make optimal decisions, and to do this requires knowing the value of objects and actions in specific situations. Value is multifaceted and subjective. What is valued highly in one situation, i.e. food when hungry, may not be valued as highly the same in another situation when the animal is sated (Redish, 2015). However it is generally thought to be a common currency in which all of the components of multifaceted decisions can be translated into to allow an organism to make an optimal choice (Levy and Glimcher, 2012).

Currently there are three accepted processes/systems that contribute to valuation and decision making: 1) procedural action chains (which are analogous to and extensions of habitual learning and stimulus-response relationships as discussed previously), 2) deliberation (which involves imagining possible outcomes and then choosing an action to get to the best outcome and is an extension of expectancy or response-outcome), and 3) Pavlovian action-selection systems (which differ from the Pavlovian concepts previously discussed and will henceforth be referred to as new Pavlovian) (Rangel et al., 2008, Redish, 2015). Importantly, within the decision making framework it is thought that all of these systems are working simultaneously and there can be conflict between the systems.

Within the habit system, values are estimated though trial and error based on previous experiences (Rangel et al., 2008). One theorized way this is accomplished is through temporal difference learning, where the expected value of actions is calculated by how much better or worse off than expected the organism is after those actions are taken, creating a prediction error that helps the organism learn from experience which actions are best in certain situations (Sutton, 1988). Within this framework, the organism also does not need to create a model of the world in order to imagine outcomes and it has been termed model-free for this reason.

The *actor-critic* model (Barto, Sutton, & Anderson, 1983) is a model-free temporal difference theory of reinforcement learning that has recently been advanced to explain avoidance behavior. In its essence, it is similar to Mowrer's two-factor theory, but differences in the learning mechanisms account for some of the limitations of the two-factor theory (Maia, 2010). The main difference from the original two factor theory is that instead of strengthening or weakening responses based on primary reward or punishment, the responses are strengthened or weakened based on the prediction error that immediately follows the

response. This essentially builds on the Rescorla-Wagner model of Pavlovian fear conditioning in which learning is the result of the discrepancy between what is expected to happen and what actually happens (Rescorla and Wagner, 1972) which was useful in explaining phenomenon such as blocking (Kamin, 1969).

In this model, the critic is responsible for determining values from Pavlovian conditioning, and fear (to the CS+) is the result of a prediction of an aversive outcome (the US). The actor is able to change this prediction through responses and the resulting prediction error. After the critic has learned to fear the CS during fear acquisition, an avoidance response resulting in the lack of shock would lead to a positive prediction error, which would reinforce the avoidance response. Although very similar to the two-factor theory, this important distinction allows the actor-critic model to explain how avoidance responding persists even after fear is extinguished, which is the major criticism of the two-factor theory. After fear is extinguished to the CS, there is no change in prediction error after an avoidance response and therefore the S-R strength remains unchanged.

The model has been successfully applied to account for behavioral and neural findings in conditioning (O'Doherty et al.2004, Maia, 2009). Additionally, the brain regions associated with the 'actor' and 'critic' in this model are regions that are thought to play a role in avoidance learning and behavior (Maia, 2010). Additional evidence for prediction error in reinforcement learning comes from increases in firing rates of midbrain dopamine neurons timelocked to unexpected but not expected rewards (Ljungberg et al. 1992), and the reward prediction error hypothesis of dopamine function has been supported by many scenarios investigating appetitive processing (Bayer and Glimcher, 2005, Cohen et al. 2012).

In contrast to the habit system, within the deliberation system a cognitive model of the environment is made, which allows the organism to predict outcomes of specific actions (i.e. generate expectancies) and compare possible outcome alternatives against each other before a decision is made (Smith et al. 2005, van der Meer and Redish, 2010, Redish, 2015). The first step in this process, imagining a possible future requires episodic future thinking and is thought to involve the hippocampus and the prefrontal cortex (Hassabis and Maguire, 2011). Because it relies on building a model of the world, it is often called model-based. While in model-free learning the organism has to learn from experience based on prediction errors to estimate the value of different states and actions, the values in the model-based learning are because outcomes can be predicted. Support for a model-based approach comes from evidence that animals can learn action-outcome associations that are not taken into account by the model-free approach which only requires stimulus-response relationships (Balleine and Dickinson 1998, Dickinson, 1987). Essentially, the model-based approach is a cognitive planning strategy and the consequences of taking an action given the situation can be calculated by hypothetically completing all possible actions in an internal representation of the environment. This situation-outcome learning model has been used to explain the effects of antipsychotics on avoidance behavior when dopamine availability is included in the model (Smith et al. 2004) and statistically provides a better fit of existing behavioral data than model-free temporal learning does (Smith et al. 2005).

There is evidence that both of these systems are being used for instrumental responses generally (Balleine and Dickinson, 1998) and we can hypothesize that they are both used for avoidance as well. First the responses are made and learned by the cognitive, model-based systems which reflect goal directed behavior but after several repetitions, the behavior becomes

less dependent on this system and switches to the habit based, stimulus-response system (Daw et al., 2005). When this happens, and the brain structures involved with each system can be tested by changing the value of the stimulus in a different context. Only in the deliberation system will the animal take into account current goals and update the value of the stimulus in the current context, which is one of the benefits of this system. The drawback of this system is that it is requires more computation and processing so if values are stable over time, the habit system will be preferred.

The final system which is particularly important to the study of avoidance is the New Pavlovian system. This system is thought to release "hardwired" reactions such as approach behaviors and species-specific defensive responses such as fighting, freezing, and fleeing (Rangel et. al; 2008; van der Meer et al; 2012). As reviewed earlier, learning through Pavlovian associative learning results in a representation that includes an expectancy about what will happen next given what the organism learned has happened previously. This expectancy is thought to contribute to action selection based on that outcome expectancy. It has been shown that Pavlovian action selection will persist despite a change that makes this response detrimental to performance in a task (Breland and Breland 1961; Dayan and others 2006). It can also interact with the other action-selection systems and modulate those responses through Pavlovian-instrumental transfer. This has been demonstrated for both appetitive (Talmi et al., 2008) as well as aversive stimuli (Geurts et al., 2013). Overgeneralization of an avoidance response may therefore result from differences in Pavlovian valuation systems.

Avoidance Behavior in Disorders of Clinical Anxiety

Both the behavioral two factor theories and the cognitive expectancy theories illustrate the pathogenic power of avoidance behavior. Regardless of the learning mechanism which started avoidance behavior, an individual who engages in avoidance is unlikely to extinguish fear to the CS+, or to form new expectancies about the possibility of an aversive outcome given the CS, because avoidance denies the opportunity to experience the CS+ in the absence of the US. This is unique to avoidance behavior and aversive conditioning as in appetitive conditioning the approach response necessitates further interaction with the CS and reinforcer and therefore omission of the reinforcing stimulus can be learned. This conceptualization of avoidance has influenced current etiological models of clinical anxiety implicating avoidance in the maintenance of anxious symptomatology (Barlow, 2002). While different anxiety disorders have different courses, the most significant factors associated with time until remittance across anxiety disorders are duration and severity of avoidance symptoms while anxious arousal symptoms do not offer any additional information in multivariate models (Hendriks et al., 2012). Most treatments for clinical anxiety counter avoidance by utilizing exposure treatments in which patients are directed to confront the feared situation (e.g., Foa & Kozak, 1986; Wolpe, 1982). For these reasons avoidance behavior is maladaptive and considered a critical feature of anxiety disorders and PTSD (American Psychiatric Association, 2013). The following section will review the role of avoidance in these disorders.

PTSD

Posttraumatic stress disorder (PTSD) develops in a subset of individuals following exposure to a real or perceived life-threatening event or an event that threatens the safety or physical integrity of one's self or others. Symptoms in the current Diagnostic and statistical manual of mental disorders (5th ed., [APA, 2013]) are grouped into four clusters, intrusion symptoms, negative alterations in cognition and mood, alterations in arousal and reactivity, and avoidance. Avoidance had been previously included in a cluster with numbing symptoms in the previous edition of the DSM (DSM-IV-TR [APA, 2000]), but research suggested that avoidance was a separate component from numbing (Asmundsun et al., 2004) from both treatment outcome studies (Taylor et al., 2003), and factor analytic studies (King et al., 1998; Asmundson et al., 2000; Simms et al., 2002). Interestingly, one study that still held a three factor theory had active avoidance symptoms grouped with re-experiencing symptoms and passive avoidance grouped with numbing symptoms (Anthony et al., 1999). In the current conceptualization however, passive avoidance does not play a large role and avoidance must be effortful and can take the form of either avoiding thoughts or feelings associated with the trauma, or avoiding external stimuli such as people or places associated with the trauma (APA, 2013).

In theoretical models of PTSD, it has been posited that individuals process trauma-related information by alternating between experience of intrusive symptoms and avoidance and therefore avoidance is a mechanism by which one regulates intrusive symptoms (van der Kolk, 1987). Other theorists have suggested similar mechanisms for avoidance as instrumental attempts to actively curtail intrusive symptoms (Foa et al., 1992), or that avoidance arises as a result of inescapable physiological arousal (Kolb,

1987). Fitting with the evidence for two separate learning mechanisms for avoidance behavior, a recent scale was developed for avoidance behavior following traumatic stress and it identified two factors, one of which was an automatic, more implicit avoidance and the other a more effortful, controlled avoidance (Andrews et al, 2013).

Agoraphobia and Panic Disorder

Agoraphobia resulting from panic disorder is characterized by avoidance (Hara et al. 2012). Agoraphobia is a separate but common complication of panic disorder, and although can be diagnosed with endurance with distress of situations that are difficult to escape in the event of a panic attack, is generally diagnosed with avoidance of those situations (American Psychiatric Association, 2000). Therefore, agoraphobia can be thought of as the avoidance aspect of panic disorder. However, consistent with the theories that fear/anxiety are not perfectly correlated with avoidance, not all individuals who have panic disorder display agoraphobic avoidance, and those who do display avoidance behaviors do not necessarily have panic attacks, leading to the full separation of these disorders in the new DSM (APA, 2013). In patients with panic disorder, having co-morbid agoraphobia is associated with anxiety sensitivity and perceptions of control of threat and emotion. Patients who are fearful of the physical symptoms of anxiety and perceived themselves to have little control over threatening situations and emotions display more avoidance (White et al., 2006).

Avoidance is thought to arise in panic disorder because individuals believe avoidance behaviors will protect them in the event of a panic attack (White & Barlow,

2002). Generally, severity of panic disorder is related to the degree of behavioral avoidance with more severe panic disorder associated with more avoidance (Buller, Maier, & Benkert, 1986). Prospective studies have demonstrated reduced remission rates (Keller et al., 1994), higher impairment (Magee et al., 1996), higher disability (Buller, Maier, Goldenberg, & Lavori, 1991), and more long-term impairment in general (Faravelli & Albanesi, 1987) in panic disorder patients with agoraphobia compared to panic disorder patients without agoraphobia. Additionally, agoraphobia is also associated with less favorable treatment responses (Ehlers, 1995) and higher relapse rates following treatment (Katschnig & Amering, 1998). Likewise, agoraphobia is related to maintenance of panic disorder symptoms in treated samples and recurrence of panic attacks in remitted patients (Ehlers, 1995). This highlights the broad negative impact of avoidance behavior in panic disorder.

Panic disorder with agoraphobia also highlights the role of cognitive expectancy in avoidance behaviors. The occurrence of panic attacks in a specific situation is not significantly associated with avoidance of similar situations. Rather, the anticipation of panic attacks in a situation was correlated with self-reports of avoidance of those situations (Cox et al, 1991). If S-R learning was driving avoidance behavior then individuals with panic disorder would avoid similar situations; however they do not and instead avoid situations when they think they might have a panic attack. While these thoughts may be driven by stimulus relationships that they do not have conscious access to, their behavior is clearly being modified by their expectations. Additional research has shown that expectancy of panic attacks is associated with agoraphobia (Craske, Rapee, &

Barlow, 1988). These findings support Craske and Barlow's (1988) hypothesis that avoidance behavior is more related to the anticipation, rather than the occurrence of the actual attack, or in other words an expectancy of an attack.

Social Anxiety Disorder

Social anxiety disorder (SAD) is characterized by persistent fear and avoidance of social situations (APA, 2013). As with agoraphobia, overt avoidance behavior is not necessary for the diagnosis if the situation is endured with intense anxiety or distress, however it is common. In fact, there is much overlap diagnostically between SAD and avoidant personality disorder which is characterized by a pattern of broad avoidance behaviors especially in the social domain (Taylor et al, 2004) and comorbidity between these two disorders is estimated to be at around 42% (Alden et al. 2002). As with the other anxiety disorders, avoidance is thought to play a key role in maintaining the disorder as it hinders the extinction of fear in social situations and does not allow for social skill development (Clark and Wells, 1995; Rapee and Heimberg, 1997; Stangier et al., 2006). In addition to overt avoidance behaviors such not attending feared social situations all-together, there may be more subtle avoidance or so called safety behaviors in social situations such as averting eye gaze or avoiding conversation in social anxiety disorder (Wells et al., 1996; Horley et al., 2003; Clark and Wells, 1995).

Individuals with SAD do not learn an avoidance response differently than healthy controls in an active avoidance task following Pavlovian conditioning, however they do start with an expectancy bias with higher expectancy of the US on the very first trial of

the task (Ly and Roelofs, 2009). In a probabilistic learning task, individuals with SAD maintain an avoidance response longer than healthy controls (Stevens et al., 2014). Individuals with high social anxiety are quicker to make an avoidance response to both angry as well as happy faces compared to those with low social anxiety(Heuer et al., 2007). Some research has pointed increased activation of the hypothalamus-pituitary-adrenal (HPA) axis corresponding to increased social avoidance in healthy controls (Roelofs et al., 2005) as well as in SAD (Roelofs et al., 2009) during a speeded approach-avoidance task.

Specific Phobia

Specific phobia is a fear of a specific stimulus or situation (blood, heights, closed places, animals, insects, etc.) that invariably produces an anxious response and results in avoidance of the stimulus or situation (APA, 2013). Phobic avoidance is considered the hallmark of the disorder, and generally separates those who have a fear and those who are phobic (Antony and Swinson, 2000). However, despite the role of avoidance in the theory of the disorder, there is relatively little research on the behavioral avoidance in specific phobia. One method that is commonly used is viewing time and visual gaze and phobic subjects do avoid looking at pictures of their phobia (Buodo et al., 2006, Lange et al., 2004, Tolin et al., 1999).

Some theories pose fear-conditioning and negative experiences after encountering a stimulus as the source of the phobia (Kuch et al., 1994; Moore, Brodsgaard, & Birn,

1991), yet others rely on fear learned through observation or just learning information about the stimuli (Rachman, 1977). Some studies of learned fear use measures of avoidance to assess fear. In one study children were read stories about unfamiliar animals, and if the information was negative they reported more fear than if the story was positive. Importantly, the children were slower in approaching a fake cage holding the animal in the negative story (Field and Lawson, 2003). Similarly, children avoid stimuli if it is associated with a negative facial expression made by their mother whereas they approach stimuli after a positive facial expression (Gerull and Rapee, 2002).

GAD

Excessive worry that is difficulty to control has been accepted as the core defining feature of generalized anxiety disorder (GAD). Interestingly GAD is the only anxiety disorder that does not include a behavioral symptom, such as overt avoidance behavior, as a criterion for the diagnosis. This may be because the nature of the worry is usually future oriented and not as specific and therefore behavioral avoidance is not as prominent in the disorder. However, avoidance of thoughts or internal experiences otherwise termed experiential avoidance is quite prominent in GAD (Greenberg and Safran, 1989; Salters et al., 2005). Quite a bit of research has focused on the cognitive avoidance strategies such as thought suppression and distraction which are more pronounced in individuals with GAD (Wells, 2005). One theory posits that worry itself is an avoidance strategy that limits deeper emotional processing and suppresses somatic responses (Borkovec et al., 2004).

Very few studies have examined behavioral avoidance in GAD. One study of behavior therapy for patients with GAD showed that patients with GAD do engage in significant behavioral avoidance, such as not watching the news or avoiding social situations. Success in treatment was related to the degree to which the interventions minimize avoidance behavior and that only patients remitting from GAD reach the low avoidance level of healthy participants. Moreover, initial severity of behavioral avoidance was not correlated with initial treatment success, but higher degrees of behavioral avoidance at the end of treatment predicted worse long-term outcomes (Beesdo-Baum et al, 2012).

Neurocircuitry of Avoidance

The first studies on the neural basis of fear often employed instrumental conditioning procedures measuring avoidance. However, these studies failed to give a comprehensive understanding of the brain mechanisms of fear (Weiskrantz, 1956). During some of the earlier studies, there was less understanding of the complex organization of nuclei and circuits in areas such as the amygdala. Therefore the entire amygdala was removed for some of these studies and in others only some sub-regions were removed, and there was inconsistency in results that led to confusion as to what the role of the amygdala was in avoidance learning (for review see Sarter and Markowitsch, 1985). Additionally, it was recognized that signaled avoidance was a two-process task that first involved learning the Pavlovian association. Therefore research turned to Pavlovian conditioning to study the brain mechanisms of fear because it was a more

simple process and offered more control to the researchers (LeDoux et al. 1984, Davis 1992). However, armed with the knowledge from years of research with Pavlovian conditioning, researchers are now returning to studying the neurocircuitry of avoidance mechanisms and their relationship with Pavlovian brain mechanisms (Choi et al., 2010).

The amygdala

The amygdala is thought to be crucially important for fear learning. The amygdala is part of the limbic system, located in the medial temporal lobe. Fear-potentiated startle ([FPS], the reliable enhancement of the startle reflex when an organism is in a state of fear), in humans is eliminated after temporal lobectomy (Funayama et al., 2001) and amygdala activation to the presentation of fear cues during fear conditioning is shown in fMRI studies (Bremner et al., 2005; Knight, 2005; LaBar et al., 1998; Phelps et al., 2004). PIT has also been shown to involve the amygdala for both appetitive and aversive stimuli in humans (Geurts et al., 2013; Talmi et al., 2008). The amygdala is comprised of several nuclei including the lateral, central, and basal nuclei. The lateral nucleus is thought to be important for forming CS-US associations during fear acquisition and for projecting to the central nucleus and basal nucleus. The basal nucleus is not required for the acquisition and expression of fear conditioning, however it is required for the CS to motivate behavior and thus reinforce responses in aversive instrumental tasks as rats express freezing behavior to CSs after lesions to the basal nucleus do not perform avoidance responses in a two way active avoidance task (Amorapanth et al., 2000, Choi et al., 2010). Lesions of the central nucleus of the amygdala inhibit fear-potentiated startle and freezing in rodents (Davis et al., 1992; LeDoux, 1992) and the central nucleus

is therefore thought to be required for the expression of Pavlovian fear responses. However, the central nucleus is not necessary for the reinforcement of aversive instrumental responses as lesions to the central nucleus do not impair avoidance learning (LeDoux et al. 2009, Choi et al., 2010). In fact, the expression of species specific defensive responses such as freezing which relies on the central nucleus may impair acquisition of avoidance responses. In a group of rats that were initially unable to learn an avoidance response, a lesion of the central nucleus led to improved performance in the avoidance task (Choi et al., 2010). The authors therefore suggest a model in which the lateral nucleus acquires and stores Pavlovian fear associations between the conditioned and unconditioned stimuli and relays this information to the basal and central nuclei. The central nucleus uses this information to perform Pavlovian conditioned responses such as freezing or startle, and the basal nucleus uses the association to motivate active instrumental responses.

There is some evidence however that the central nucleus is also important for conditioned suppression. In a conditioned suppression study food delivery was paired with electric footshocks which suppressed the response to get food in food deprived rats. Lesions of the central nucleus abolished the conditioned suppression effect while rats with lesions of the basolateral complex did not differ from rats with sham lesions (Petrovich et al. 2009). If the conditioned suppression was thought of as a type of instrumental response, the abolition of the suppression in this study would suggest the central nucleus and not the basolateral nucleus is important for action. Alternatively, viewed from the decision making standpoint, this would suggest that conditioned

suppression of this kind may rely on a New Pavlovian system and the central nucleus may be a part of this system. The authors argue that there is evidence that the central nucleus can support aversive learning independent of the basolateral complex (Swanson and Petrovich, 1998; Rosenkranz et al., 2006, Wilensky et al., 2006), and therefore multiple CS–US associations might be generated concurrently within different amygdalar subsystems to support somewhat distinct CS functions. Additional animal studies show that while learning avoidance behavior is dependent on the amygdala, overtraining can render avoidance amygdala-independent (Poremba and Gabriel 1999), and this may be due to the fact that the habit system can take over from the New Pavlovian system which initially instantiated the avoidance response. ().

The amygdala has also shown to be involved in human avoidance (Schlund et al., 2010; Delgado et al., 2009). In a study using fMRI, bilateral amygdala activation was observed to threatening avoidance and escape cues which signaled impending monetary loss (Schlund et al., 2010). Whereas in the avoid condition a response within 8 seconds allowed the subject to avoid monetary loss, when the escape cue appeared money loss started in one second and repeated button presses were needed to prevent further loss although enough button presses did prevent any money loss. Amygdala activation was also seen to the reward cue compared to baseline. Interestingly, the magnitude of amygdala responses within subjects was relatively similar to avoidance, escape and approach cues, but considerable between-subject differences were found. Individual differences in amygdala response to avoidance and escape cues suggests that subjects with hyperactive amygdala may maintain threat-related responses even when aversive

events are consistently avoided, which is consistent with the presentation of clinical anxiety disorders (Schlund and Cataldo, 2010). In a study of healthy participants, amygdala and striatal interactions were shown to underlie acquisition of avoidance following Pavlovian conditioning (Delgado et al., 2009).

Nucleus Accumbens

The nucleus accumbens (NAc) is a brain region located in the ventral striatum which is part of the basal ganglia. The NAc is part of the mesolimbic dopamine system which originates in the ventral tegmental area (VTA) and projects through the NAc to the limbic system (Chronister et al., 1981). The mesolimbic dopamine system has been implicated in processing rewarding information (Wise et al., 1982, Schultz et al., 1997), but the contribution of dopamine and the NAc to punishment and avoidance is less clear.

Experiments in lever-press, free-operant avoidance responding where the lever press cancels an impending shock and is therefore the avoidance response, indicate that dopamine in the NAc is necessary for maintaining the avoidance response (McCullough et al., 1993). Interestingly, when a cue signaling impending shock is added to the free operant responding paradigm, the percentage of shocks avoided increases, but the rate of lever pressing decreases (Sidman 1955, *B). The cue helps the animals to learn when the response is necessary so instead of hitting the lever unnecessarily, they wait until the signal is presented and then perform the avoidance response. Although not explicitly trained as a CS, as lever press responses could be made even before the warning signal comes on which postpones both the next warning cue and the shock and reinforcement was never assured because the avoidance response was allowed and therefore

theoretically the warning signal could never have been associated with US, the warning signal was clearly learned to be associated with the shock.

A recent study found that a warning cue signaling impending shock produced surges in dopamine release that predicted avoidance behavior (Oleson et al., 2012). That is if there was an increase in dopamine at the onset of the warning signal, an avoidance response was likely to follow. Conversely, if avoidance behavior was not performed and therefore the animal was shocked, the onset of the warning signal corresponded to a decrease in dopamine release. Expanding on this they discovered a warning signal decreases NAc dopamine release initially, but as the avoidance response is learned, the warning cue increases dopamine release. The authors conclude that initially, the warning signal is equated with the US (essential has become a CS+), but through training, the cue comes to represent the avoidance response. This fits nicely with a two-factor theory of avoidance. However instead of avoidance providing relief by termination of the CS, the CS has actually become rewarding at its onset due to the animal learning that a response can then be performed which will allow for avoidance of the shock.

Similarly, a human fMRI study showed greater activation of the nucleus accumbens during active avoidance, whereas the nucleus accumbens was deactivated during passive avoidance (Levita et al., 2012). However, another human study demonstrated BOLD signal increases in the ventral striatum during anticipation of an aversive event whether there was an opportunity to avoid the stimulus or not, which argues against ventral striatum activation due to relief of avoiding the aversive event (Jensen et al. 2003). Other human studies have shown that the NAc is involved in both

appetitive and aversive PIT (Geurts et al., 2013; Talmi et al., 2008), and amygdala and striatal interactions were important for acquiring an avoidance response following Pavlovian conditioning (Delgado et al., 2009).

Insula

The insula is also thought to contribute to avoidance learning and maintenance. The insula, an association cortex region of the brain located within the lateral sulcus between the lateral prefrontal cortex and striatum, is thought to play an important role in monitoring internal bodily states and predicting future internal states in response to environmental changes (Craig, 2009). In the appetitive domain, the insula has been implicated in drug craving and urges (Naqvi and Bechara, 2009). During instrumental conditioning, rats with lesions to the insular cortex fail to adjust their choice behavior to variations in motivational manipulations that normally change values of a food reward during learning (Balleine & Dickenson, 1998). The insula has also been implicated in aversive conditioning and whereas fear conditioning can occur without explicit knowledge, awareness of the association and peripheral autonomic arousal are mediated by insular activity (Critchley et al., 2002).

The anterior insula has consistently been associated with anticipation of threat (e.g., Seymour, Singer, & Dolan, 2007; Lissek et al., 2013). Studies of insula lesions in humans have reported alterations in avoidance behavior (Clark et al 2008, Jones et al. 2010). Similarly, human neuroimaging studies and a recent meta-analysis highlight the role of the anterior insula for paradigms involving risk and uncertainty (Mohr et al, 2010,

Sarinopoulos et al., 2010). One study in particular showed that right insula activation was stronger during selection of a risky response versus selecting a safe response in a decision making task, and insula activation was related to avoidance of risky decision making following punishment. Additionally, insula activation was positively correlated with the subjects' degree of harm avoidance and neuroticism as measured personality questionnaires (Paulus et al., 2003).

Insula activation also predicts risk avoidance during financial risk taking tasks (Kuhnen & Knutson, 2005), and insular sensitivity assessed as participants anticipate monetary losses predicts participants' ability to learn to avoid losses several months later, suggesting heightened insular sensitivity promotes learning to avoid loss (Samanez-Larkin et al., 2008). During a fear conditioning task using shock as the US where subjects were allowed to perform an avoidance response, increased BOLD signal in bilateral insula was observed when a response avoiding the US was made, although this was also observed during anticipation of a negative outcome when an avoidance response could not be performed (Jensen et al. 2003). Insula activity was also negatively correlated with both avoidance behavior and amygdala activity in a novel task with healthy 9-13 year olds (Schlund et al., 2010). This study also demonstrated that avoidance behavior did not attenuate basic threat response brain mechanisms which suggests that fear is maintained even when an avoidance response is performed.

Prefrontal Cortex

The prefrontal cortex (PFC) consists of many distinct regions that have dissociable functions and connections to other brain regions, and there is evidence that these regions are important for fear learning and extinction (Sehlmeyer et al. 2009, Phelps et al. 2004). Both animal and human studies provide some evidence of an inhibitory relationship between prefrontal regions and the amygdala during fear extinction or emotional regulation (for review see, Davidson, 2002).

In rats, the medial prefrontal cortex (mPFC) has been shown to be important in inhibitory learning during extinction and to have inhibitory effects on the amygdala (Morgan, Romanski, & LeDoux, 1993; Grace & Rosencrantz, 2002).

Areas of the medial prefrontal cortex in rats have identified as important for instrumental actions. The prelimbic cortex in rats has been identified as critical for functions such as planning, response selection and the control of willful action, which are required for the deployment of action-outcome knowledge in rats (Killcross and Coutureau, 2003, Balleine and Dickenson, 1998). In contrast the infralimbic cortex has been identified as important for stimulus-response relationships (Killcross and Coutureau, 2003).

Even though the PFC has been implicated in instrumental avoidance behavior, it likely codes for higher-order information about the context and internal states of the organism that are not necessary, nor sufficient for the actual learning of avoidance responses and rather serve to mediate the avoidance response. Evidence for this comes from a study where dopamine signaling was restored in dopamine deficient mice and only restoration of dopamine to the striatum and amygdala was necessary for the mice to

learn the avoidance response in a two-way active avoidance task (Darvas et al. 2011). Dopamine was restored using viral gene therapy to reactivate dopamine signaling in select brain regions. Restoring dopamine signaling to only the amygdala and PFC, or PFC and striatum was not sufficient for learning the avoidance response. Also, in overtrained rats, only reactivation of the striatum was required to maintain the avoidance response and the amygdala was no longer necessary to support the avoidance response if it had been overlearned as procedural habit-based learning takes over for the value based learning.

The medial prefrontal cortex (mPFC) also plays a role in human fear and avoidance. One review of human imaging studies posits that the more dorsal regions the mPFC are involved in emotional appraisal and emotional expression, including fear expression, whereas more ventral regions are involved in regulation of fear (Etkin et al., 2011). The ventral medial prefrontal cortex (vmPFC) is thought to play a central role in the inhibition of fear in humans (Phelps et al., 2004).

In a human avoidancestudy, ventral striatum, amygdala and anterior insula activations were significantly stronger during presentation of avoidable aversive cues than for avoidable neutral tones and an interaction analysis in this study showed stronger connectivity between the ventral striatum and the orbitofrontal cortex during aversive than neutral conditions (Bolstad et al., 2013). Another recent human study examined various levels of monetary reward associated with differing probabilities of shock and found that connectivity between insula and orbitofrontal cortex (OFC) was related to individual variability in decision making during trials involving both reward and

punishment (Talmi et al, 2009). Both of these studies highlight the role of orbitofrontal cortex connectivity to other regions mediating the avoidance response.

In summary, mPFC and OFC are considered important for processing both approach- and avoidance-related stimuli. Rather than having a direct role in the acquisition of avoidance behavior, these regions may be responsible for calculating net values and determining choices based on information from other brain regions during decision making (Rangel and Hare, 2010) and regulating limbic and behavioral responses to fear-provoking stimuli (Quirk and Beer, 2006).

Hypothesized Brain Abnormalities Facilitating Heightened Avoidance in Anxiety Patients

Experiments in avoidance behavior in clinically anxious subjects, or subjects with different scores on trait dimensions related to anxiety, highlight the aforementioned brain regions in abnormal avoidance behavior. In subjects with PTSD, avoidance responses to script driven imagery were negatively correlated with left rostral and subgenual ACC and bilateral dorsal ACC activation. Self-reported avoidance was also negatively correlated with activation in right ventral PFC (Hopper et al., 2007).

Intrinsic, not task related, functional connectivity between the anterior insula and the anterior cingulate as well as between the anterior insula and the dorsolateral prefrontal cortex are positively correlated with harm avoidance scores from the Temperament and Character Inventory (Markett et al., 2013). The degree of activation and deactivation of the NAc during passive and active avoidance was associated with individual levels of anxiety

(Levita et al., 2012). Another study tracked trial-by-trial changes in brain activation while adult subjects learned an avoidance response that prevented money loss, and an approach response that produced money gain through trial and error in an fMRI task (Schlund et al, 2011). Both avoidance and approach cues elicited similar increases in activation in a fronto–limbic–striatal network while the outcomes themselves elicited similar activation in frontal and striatal regions. Questionnaire measures of experiential avoidance and self-punishment coping were assessed and higher scores on these measures were associated with decreased activation in frontal regions, anterior cingulate, amygdala and hippocampus.

Apart from the role of these brain areas in avoidance behavior, subjects with clinical anxiety have shown dysfunction in these brain regions more generally. Abnormal amygdala activation has been observed in children with GAD when viewing angry or fearful faces (McClure et al., 2007; Monk et al., 2008) and in adults with GAD when anticipating both aversive and neutral pictures (Nitschke et al., 2009).Children, adolescents and adults with GAD also have larger amygdalae (De Bellis et al., 2000, Schienle, Ebner , and Schäfer, 2011). Greater amygdala activations are also observed in PTSD patients compared to controls when confronted with combat related stimuli (Shin & Handweger, 2009; Rauch et al., 1996; Liberzon et al., 1999, Bremner, et al., 1999; Shin, et al., 1999, Protopopescu et al., 2005), and for non-combat related fearful faces (Rauch et al., 2000; Shin et al., 2005). Increased activation of the amygdala has been also been observed in patients with specific phobia (Dilger et al., 2003; Schienle et al., 2005; Straube, Mentzel, and Miltner, 2006), social anxiety disorder (Lorberbaum et al., 2004; Phan et al., 2006; Stein et al., 2002; Straube et al., 2004; Straube, Mentzel, and Miltner,

2005; Tillfors et al., 2002) and panic disorder patients (van den Heuvel et al. 2005; Maddock et al., 2003, Pfleiderer et al., 2007). Amygdala hyperactivation to public speaking in socially anxious patients (Tillfors et al., 2002) and to trauma related words in PTSD (Protopopescu et al., 2005) also correlates with anxiety symptom severity and amygdala activation decreases after cognitive behavioral treatment (Goosens et al., 2007; Fellingham et al., 2007).

Insula hyperactivation in anxiety disorders during emotional processing has been highlighted in a recent meta-analysis (Etkin and Wagner, 2007). Specifically increased insula activation has been observed in social anxiety disorder (Straub et al. 2004) specific phobia (Dilger et al., 2003; Straube, Mentel and Miltner, 2007) and PTSD (Hopper et al., 2007, Vermetten et al. 2007). As with amygdala activity, insula activation also has been shown to correlate with symptom severity (Shah et al., 2009; Hopper et al., 2007).

Few neuroimaging studies have investigated nucleus accumbens activation in anxiety disorders. A PET study did find increased nucleus accumbens activation in PTSD subjects following exposure to combat sounds (Liberzon et al., 1999) One study in PTSD found lower than normal levels of nucleus accumbens activation in PTSD subjects during reward processing in a risk-related decision making task (Sailer et al, 2008). Decreased striatal activation to reward was also found to correlate with numbing symptoms in individuals with PTSD (Elman et al., 2009).

Subjects with anxiety disorders exhibit OFC, dmPFC, and lateral PFC dysfunction during processing of negative emotional stimuli (Etkin and Wagner, 2007). PTSD subjects also show altered mPFC responses during reward processing in a risk-related

decision making task (Sailer et al, 2008). An fMRI study using discrimination fear-conditioning found decreased ventromedial prefrontal cortex activation during extinction recall the day after conditioning in subjects with PTSD (Milad et al., 2009). Another study using a discrimination fear conditioning experiment with threat of shock found weaker vmPFC activity during threat of shock in PTSD patients compared to controls (Tuescher et al., 2011). Weaker PFC activity is thought to represent a failure in inhibitory processes that can regulate the limbic response to threat. However, in a fear-conditioning study in patients with specific phobia, increases in medial PFC responses were observed (Schweckendiek et al., 2011). The authors suggest this hyperactivation may be an attempt to inhibit strong limbic responses, but also point out evidence contradicting this claim in that the amygdala and mPFC become decoupled during processing of phobic fear (Ahs et al., 2009).

Moving Beyond Pavlovian Fear-Conditioning: Integrating Behavioral Avoidance Measures

In signaled avoidance procedures, whether avoidance behavior is mediated by stimulus-response relationships or response-outcome relationships, the first component of each of these systems involves learning what the dangerous stimulus/situation is. This first step in therefore recognizing what the situation is. When a stimulus is paired with a shock, the amygdala is thought to be involved in associating the representation of that stimulus with the aversive event resulting in a fear response through a process called Pavlovian fear conditioning (Phillips & LeDoux, 1992). Eventually, the stimulus will activate a fear

response even when presented without a shock as the representation of that stimulus has been associated with the aversive event. Therefore, impairments in situation-recognition or Pavlovian conditioning associated with anxiety pathology could have downstream impacts that result in maladaptive avoidance.

One proposed Pavlovian conditioning abnormality in anxiety disorders is overgeneralization of fear. Fear has long been known to transfer, or generalize, to stimuli resembling the original CS (Pavlov, 1927). Because all representations are distributed in the brain and overlap with each other, stimuli will partially activate the representation of other stimuli, and the degree of similarity is proportional to the overlap (McLaren & Mackintosh, 2002). This can be a very useful mechanism as it allows an individual to formulate a fear response to a potentially dangerous event that has never been directly encountered, but resembles a previously encountered situation which resulted in an aversive event. While some degree of generalization is therefore always expected and useful, anxiety patients are thought to overgeneralize. Thus their fear responses may be too broad and extend to stimuli that are not threatening.

The degree of generalization can be measured by measuring *generalization gradients,* or continuous slopes in conditioned responding as the presented stimulus gradually becomes less perceptually similar to the CS (Pavlov, 1927). Previous studies demonstrate overgeneralization of Pavlovian conditioned fear in panic disorder (Lissek et al., 2010), generalized anxiety disorder (Lissek, in press), and preliminarily in PTSD (Lissek & Grillon 2012), as indicated by less steep generalization gradients among those with versus without an anxiety disorder. Evidence linking pathologic anxiety to conditioned

generalization dates back to Watson and Rayner's experiment with 'Little Albert' (1920) and fear generalization has since been adopted as a core feature of anxiety pathology by clinical practitioners and theorists (e.g., Foa, Steketee, & Rothbaum, 1989; Mineka & Zinbarg, 1996).

Gluck and Myers (1993) proposed that the hippocampus mediates stimulus representation. In their theory, the hippocampus works to differentiate cues that are differentially predictive, while at the same time compressing the representation of redundant cues. They propose that other brain regions including sensory cortices then use the hippocampal coding to recode their representations. The hippocampus, working with sensory cortex, is thought to play an important role in fear generalization. Lesions studies in animals demonstrate greater generalization with either hippocampal or sensory cortical lesions (Bucci et al. 2002; Solomon and Moore, 1975). For this reason, the hippocampus plays a central role in a model of fear generalization proposed by Lissek and colleagues (Lissek et al., 2013). In this model, sensory cortex and the hippocampus work together to compare the current stimulus to previously conditioned cues.

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Overgeneralization of fear in anxiety disorders reflects a maladaptive associative learning process where stimulus relationships are altered and (safe) cues that have never been associated with threat become anxiety provoking due to their similarity to threatening cues. Both two-factor theories and cognitive theories would support that fear-generalization will serve to increase behavioral avoidance of these cues as the behavioral response is being selected based on incorrect information about the nature of

the stimulus. However, conditioning experiments in clinical anxiety usually measure physiological indices of anxious motivation, such as increases in heart rate, skin conductance or startle (i.e., fear-potentiated startle) and have mainly stopped short of measuring behavioral avoidance even though much of the pathogenic power of conditioning abnormalities in anxiety disorders may lie in the maladaptive behavior it motivates.

Recent studies from our lab have demonstrated that avoidance decisions following Pavlovian conditioning also form a generalization gradient (van Meurs et al., 2014) and that the strength of acquisition of conditioning predicts future avoidance decisions even though subjects are not aware at the time of acquisition that there will be a decision making task (Lissek and van Meurs, in preparation). Additionally, increases in Pavlovian generalization are associated with increased generalized avoidance. While this relationship was observed in healthy controls, we hypothesize that Pavlovian overgeneralization of the kind found in anxiety disorders will result in heightened, maladaptive, generalized avoidance.

Additional studies provide evidence that Pavlovian conditioning by itself may bias action tendencies. This has been shown in the appetitive domain where approach behavior towards a previously neutral stimulus was quicker after being paired with an appetitive US although there was no cognitive expectation of reward during the approach task and the response was forced and not instrumental in nature (Van Gucht et al., 2008). This avoidance-approach behavior tendency was tested after aversive conditioning with shock and subject's reaction times moving away or avoiding were quicker, and approach was

slower to a previously trained CS+ than to a safety cue (Krypotos et al., 2013). Importantly this was not an instrumental decision making task, rather a speeded reaction time task where behavior explicitly had no effect on CS or US presentation and shock electrodes had been removed before testing of the behavioral tendency. Additionally, this effect disappeared after extinction of fear, and returned after reinstatement of the CS+/US contingency.

Other studies have also demonstrated a relationship between physiological measures of fear and action tendencies. In these studies, the behavior in question was viewing time of aversive stimuli when subjects are allowed to end the viewing with a button press. This measure has been shown to be related to physiological indices of fear in that phobic patients show both greater fear-potentiated startle during viewing of fear-related pictures and shorter subsequent viewing time of fear-related pictures versus controls (Hamm et al., 1997). Similarly, there is a moderately negative relationship between initial startle-potentiation to viewing a trauma related picture and time spent viewing the picture in a later task in recent trauma victims (Elesser et al. 2004). These results indicate that as fear increases, physiological indices such as FPS increase as well, and this is related to an increase in avoidance behaviors resulting in less time viewing the pictures. Slightly conflicting evidence comes from a behavioral avoidance paradigm with patients with panic disorder and startle responses were inhibited while other physiological measures such as heart rate and skin conductance increased in the period directly before an escape response was made (Richter et al., 2012). However the reason startle may have been inhibited in this instance was that this measurement was made in the time course of a single decision, and not relating Pavlovian measured fear at time one

to an avoidance measure at a later time. It also highlights how behaviors stemming from fear may be conflicting and one response would preclude the other.

Alternatively, behavioral avoidance or avoidance tendencies may be impaired with intact Pavlovian conditioning. Many emotional theories include behavior or action tendencies as a distinct response system from the subjective and physiological response systems (Frijda, 1986; Lang et al., 1998). Avoidance behavior then could be dissociated from the physiological indices that are measured during Pavlovian fear conditioning and one of the critiques of simply measuring physiological indices as is routinely done in fear-conditioning experiments is that this ignores the behavioral aspect of anxiety which is not necessary perfectly correlated with physiological measures (Beckers et al., 2013). This is especially important when considering that there are not strong results for Pavlovian fear conditioning abnormalities in non-clinical groups that are at high risk for developing anxiety disorders such as in individuals with high neuroticism or introversion (Davidson et al., 1964, Pineles et al., 2009) and high trait anxiety was found to be associated with superior discrimination learning in a recent study (Indovina et al., 2011).

So, even though Pavlovian fear conditioning abnormalities are assumed to play a role in the etiology of anxiety disorders people who are at risk for the development of some form of anxiety disorder don't always exhibit these abnormalities. Therefore testing all of these systems concurrently may reveal distinct subtypes of clinically anxious patients. This idea was first suggested in the early 1970's in that fear, which could be measured either physiologically or through self-report, could be either synchronous or desynchronous with avoidance behavior (Rachman and Hodgson, 1974). This could lead to targeted

interventions for the specific subtypes of anxious individuals that display distinct patters of abnormal fear/avoidance responding. Additionally, the wealth of information on the neurocircuitry of normal and abnormal Pavlovian fear conditioning in healthy controls and anxiety patients will allow for a more thorough investigation as to the brain mechanisms responsible for maladaptive avoidance, if an avoidance task is placed in the framework of an already established Pavlovian fear-conditioning task such as generalization.

Conclusions

Avoidance behavior in the anxiety disorders is complex and can take many forms from overt evasion to more subtle avoidance of gaze. Avoidance also is not limited to behaviors, and experiential avoidance of thoughts or feelings associated with negative stimuli is a large component of many anxiety disorders. Theoretical accounts of avoidance learning generally posit two mechanisms, one behavioral and one cognitive. The behavioral theories stem from Thorndike's law of effect and S-R learning theory but the limitations in these theories necessitated cognitive theories that incorporate understanding of the response-outcome relationship. Avoidance is a critical component of many anxiety disorders, and has implications for prognosis and response to treatment. Theoretical accounts of avoidance behavior in the anxiety disorders maintain that avoidance behavior precludes the individual from gaining mastery experience with the stimuli that is avoided, which perpetuates fear and anxiety to those stimuli. Exposure therapy in the anxiety disorders is successful because it prevents avoidance and creates positive learning experiences with the feared stimuli.

Avoidance behavior is so central to the concept of fear and anxiety that avoidance behavior was thought to be a direct measurement of fear (Weiskrantz, 1956; Kamin et al., 1963) however the dissociation of fear from performance of an instrumental response and the control offered by Pavlovian conditioning led to dominance of Pavlovian conditioning in the neural mapping of fear. Avoidance behavior still plays a large role throughout the anxiety disorders, yet it remains a vastly understudied area and most research currently employs self-report measures of avoidance rather than experimental manipulations that evoke an avoidance response (Meichenbaum, 1971; Asmundsun et al., 2004; Andrews et al, 2013; Beesdo-Baum et al, 2012). In the last few years there have been a few new paradigms designed to measure avoidance learning and behavior applied to healthy controls and to individuals with anxiety disorders, but this type of research is sparse. Considering the brain regions involved in avoidance behavior and the brain regions typically implicated as functioning aberrantly in anxiety disorders are highly overlapping, the prominence of avoidance behavior in anxiety disorders is not surprising.

Several theorists and researchers have pointed out the lack of research on avoidance behavior and called for a renewed push investigating it (Choi et al., 2010; Beckers et al., 2013). Given the vast literature on Pavlovian conditioning abnormalities in anxiety disorders, a strategy for investigating avoidance behavior after in relationship to Pavlovian conditioning would be useful. For example, generalization of fear has been studied with Pavlovian conditioning, but including a measure of active avoidance will help establish both how maladaptive behavior stems from maladaptive Pavlovian processes, and potentially how maladaptive behavior develops despite normal Pavlovian

processes, which may help identify dissociations in behavior and brain processes in subtypes of anxious individuals. Given the lack of information, additional studies of the neurocircuitry of avoidance behavior are necessary to enhance our understanding of the brain-basis of avoidance behavior which will lead to better diagnostics and treatments for those suffering from anxiety disorders.

Aims

The current study was designed to fill the gap in the literature and provide information on the neurocircuitry of generalization of avoidance behavior after Pavlovian conditioning. This paradigm applies a psychophysiological validated approach avoidance conflict in the context of a 'virtual farmer' computer game (van Meurs et al., 2014). In the game, the participant is a farmer, tasked with harvesting crops. Throughout the game, different shapes are superimposed on the screen with one such shape, paired with electric shock, serving as the conditioned danger-cue (CS+). Other presented shapes, referred to as generalization stimuli (GS), parametrically vary in similarity to the CS+, but are never paired with shock. While playing the game, participants are given the opportunity to avoid shock at the cost of poorer performance (i.e., reduced likelihood of a successful harvest). Consistent with the paradigm from van Meurs et al. (2014), there was no actual reward given to participants. Avoidance responses during CS+ presentations are considered adaptive, even though performance is compromised, because shock is a real possibility. By contrast, avoiding during GS presentations is considered maladaptive because shock is not a realistic possibility and avoiding thus unnecessarily compromises performance on the task.

The aim of this study is to identify differences in the brain mechanisms associated with maladaptive avoidance after conditioned fear generalization between groups with high and low trait anxiety. More specifically, the study aims to: (1) identify brain regions that support avoidance behavior following Pavlovian conditioning and generalization; (*2)* measure the degree to which subjective ratings and brain processes of Pavlovian generalization are associated with maladaptive avoidance responses; and (*3)* To identify brain regions that support anxiety-related aberrancies in these brain substrates of both Pavlovian generalization and generalization of avoidance behavior.

Hypotheses:

This study hypothesizes that individuals with high trait anxiety will demonstrate more maladaptive behavioral avoidance to stimuli that resemble the danger cue. We hypothesize that they will show stronger conditioned generalization than the low trait anxiety control group as evidenced by less steep, downward generalization slopes in fear-related brain areas (amygdala, caudal anterior cingulate, and insula), as well as less steep upward slopes in brain areas associated with fear inhibition (putamen and medial prefrontal and orbitofrontal cortices). Additionally, individuals with high trait anxiety will show less steep slopes of nucleus accumbens activation when an avoidance response is available. We hypothesize that there will be a similar pattern of generalization between brain responses, cognitive expectancy of risk, and avoidance behavior across groups. Additionally we expect that exaggerated Pavlovian conditioning and expectancy of shock in the high trait anxiety group will result in more avoidance. We also hypothesize that if there is a discrepancy between expectancy of risk and avoidance

behavior, there will be increased activation in the nucleus accumbens, medial prefrontal cortex, dorsolateral prefrontal cortex, and orbitofrontal cortex despite increased amygdala and insula activation observed in individuals who do not avoid even though expectancy is high.

METHODS

Participants

This study was approved by the University of Minnesota Institutional Review Board, and the Center for Magnetic Resonance Research. Participants were recruited from the University of Minnesota's Research Experience Program (REP) and were assessed using a brief self-report questionnaire assessing psychological history. Fliers with a link to the questionnaire were also placed in the community. Of the 365 participants who completed the online screening, 35 completed the MRI portion of the study. Participants were asked to participate in the study if they met exclusion and inclusion criteria for the study including magnetic resonance imaging safety criteria (see Appendix A), and were also screened based on the Harm Avoidance (HA1) questions from Cloninger's tri-dimensionality questionnaire (TPQ) (Cloninger et al., 1994). There was not a specific criterion for inclusion in the study based on the HA score. Participants across the entire range including median scores were asked to participate in order to select patients across the entire dimension, but the number of subjects in the high, middle, and low harms avoidance groups were balanced. High harm avoidance was described as scores greater or equal to 1SD above the mean, and the low HA group having scores less than or equal to 1SD below the mean. Participants in the high anxiety group were

allowed to meet criteria for an anxiety disorder, but not any other Axis I disorders. Participants in the low or moderate harm avoidance group with any Axis I diagnoses were excluded. Subjects on psychotropic medications for anxiety were included, however only one subject on medication was enrolled and completed the study. This subject also had greater than 50% of data lost due to movement, and was therefore excluded from analysis. This was the only subject removed due to movement in the scanner.

The eleventh subject in the study had perfect discrimination in the task, and reported that a visual cue in the background of the task was allowing her to do so. As several other participants also had very good discrimination, all 11 subjects were excluded and the background of the task was modified to remove the visual cue. One subject was removed because she noted on her questionnaire that she avoided out of principle, not fear, and did demonstrate adequate learning in the task as she avoided nearly every stimulus. This resulted in a total of 22 participants with a valid dataset.

Although originally intending to group subjects in three groups, due to excluding so many subjects, only two groups could be formed. An additional complication was that subjects all completed the TPQ in full on the day of scanning. 5 of 22 subjects scores so significantly different their groups would have changed based on the Harm Avoidance score. Subjects were therefore grouped into high and low trait anxiety groups on the STAI-T, which was only completed on the day of scanning. Groups were formed based on a cut-off score of 40 (Spielberger, 1983).

Avoidance Task

The avoidance task was presented using the Presentation software package (Neurobehavioral Systems). All instructions and stimuli were presented on a computer monitor in the control room at the CMRR which was then projected onto a mirror that participants can see while they are in the bore of the magnet. The laptop running Presentation software interfaced with a second laptop that delivered mild electric shocks to the ankle of subjects using the PsychLab psychophysiological recording system (Precision Instruments).

Elements of the Task: Conditioned, generalization, and unconditioned stimuli.

While playing the farming game, shapes are superimposed on the screen. These shapes constitute the conditioned and generalization stimuli to which neural responses and self-reported risk of shock are recorded. Additionally, risk for shock during the task depends in part on the size and form of the shape presented in the center of the screen. Specifically, such stimuli consist of circles and triangles of different sizes (see Figure 1). Circular stimuli include eight rings of gradually increasing size with extremes serving as conditioned-danger (CS+) and conditioned-safety cues (CS-). The six rings of intermediary size are generalization stimuli (GSs), and create a continuum-of-similarity between CS+ and CS-. As was done by Lissek and colleagues (2008), responses to every

two GS sizes are averaged yielding 3 classes of GCs (GC_1, GC_2, GC_3).

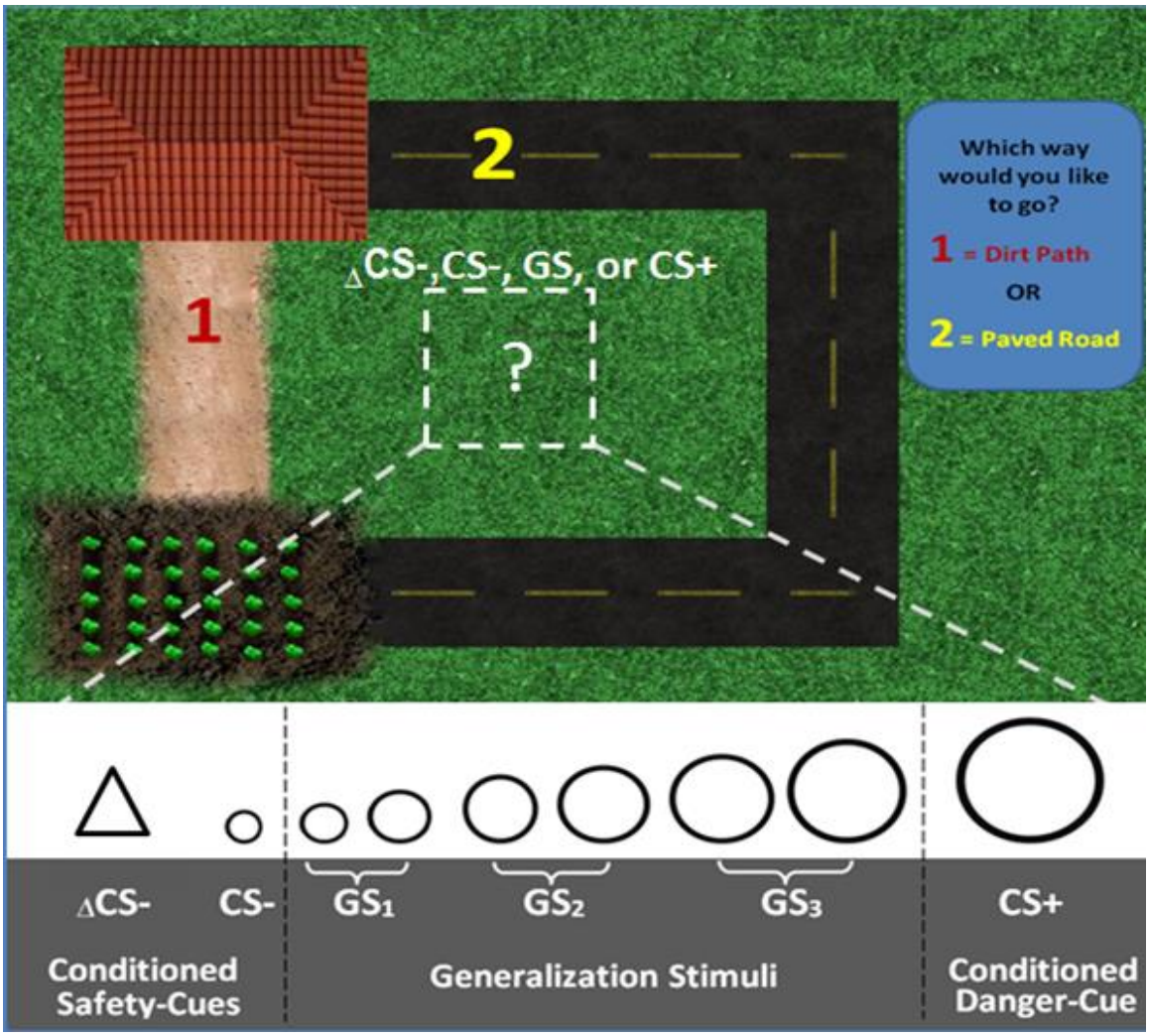

Figure 1. Virtual farmer paradigm and conditioning and generalization stimuli. Replication of image presented to subjects showing shed, garden, the short dangerous road, the long safe road, and a picture of the avatar travelling on the long safe road after a decision to avoid the short dangerous road has been made. Also showing where the stimuli were presented in relation to the other elements. The shape and size of the stimuli are pictured below. The diameter of the small ring is .8" and each ring is 12.5% larger than the smallest ring (i.e., .9", 1.0", 1.1", 1.2", 1.3", 1.4", 1.5"). Width and height are 1.0" for the triangle. CS- = conditioned safety cue; ΔCS-= triangular conditioned safety cue, GS= generalization stimuli, CS+= conditioned danger cue.

This was done to prevent an unrealistically long experiment while still maintaining a gradual continuum of size across circles. Triangles serve as "non-circular" conditioned

safety cues ($_{CS-\Delta}$) and are included to assess the degree to which fear generalizes to all things circular (but not triangular). If anxious participants have a tendency to show greater sensitization, then they might show a fear response to all ring sizes simply because they are similar in shape to the danger cue. Including a control safety cue that is dissimilar in shape to the conditioned and intermediate stimuli allows us to disentangle the contribution of sensitization to our results. Sensitization would be characterized by equivalent reactions to the CS+, CS- and CS-Δ while generalization would show elevations in CS+ and CS- greater than the CS-Δ. Dissociating generalization from sensitization is important for demonstrating that differences between groups are not simply due to greater sensitization in one group. For half of participants, ring sizes from smallest to largest were: CS-, GS_1, GS_2, GS_3, and CS+. For the second half of participants this was reversed (i.e., CS+ is smallest, GS_3 is second smallest, etc.). Thus, regardless of such counterbalancing, GS_3 is most similar to CS+, GS_2 is next most similar, and GS_1 is least similar to CS+ for all subjects. The unconditioned stimulus (US) is an electric shock delivered to the right ankle (3-5 mA, 100-255 ms).

Routes to the Field: Short Path and Long Road

While playing the game, the participants are told their main goal is to harvest crops. They do this by traveling from the shed to their field. There are two ways to get to the field, the short dirt path and the long paved road. Participants are explicitly told the outcome contingencies of the two routes. The short dirt path will always result in a successful harvest, yet also will occasionally cause the farmer to fall of the bike, resulting in a shock for the participant. The long paved road will never be associated with shock,

but because it is a longer route on most trials birds will make it to the field before them and eat their crops. During the task there are two types of trials Pavlovian trials, and Avoidance trials. During the Pavlovian trials, participants are not given a choice and are simply sent down the short dirt path. On Avoidance trials, participants are given the opportunity to choose which route they would like to take to get to the crops. Taking the long paved road is considered an avoidance response as it eliminates the possibility of shock. Avoidance responses during CS+ presentations are considered adaptive, even though performance is compromised, because shock is a real possibility. By contrast, avoiding during GS presentations is considered maladaptive because shock is not a realistic possibility and avoiding thus unnecessarily compromises performance on the task.

Trial Structure

This study consisted of three phases: pre-acquisition, acquisition, and generalization test. The pre-acquisition and acquisition phase of the study only have one trial type, Pavlovian trials. The generalization test has two trials types, Pavlovian trials, and Avoidance trials. During pre-acquisition and acquisition, each trial starts with the onset of the stimuli shape in the center of the screen. This shape remained on the screen for a period of time (jittered [1s,1.5s,2s and 3s] until the farmers started on the dirt path leaving his house to travel to the garden to harvest crops. The presentation of the stimuli shape continued throughout the trial. The avatars trip took a total of 3,4, or 4.5 seconds due to jitter and were a total of either 5s, 5.5s, 6s, or 6.5s. During the pre-acquisition phase, the CS+, the CS-, the CS-Δ and were each presented 4 times with no shock

reinforcement (12 trials total). Pre-acquisition is designed to measure baseline behavioral responses for each stimulus type prior to conditioning. During the acquisition phase, the CS+, the CS-, and the CS-Δ , were each presented 16 times each (48 trials total) with 50% of the CS+ stimuli paired with an electric shock. All CS+ trials were paired with a "virtual shock" to the avatar which reinforced the association without leading to habituation of the shock. On 50% of trials, a message asking the participant to rate their perceived risk of shock came on the screen 1 second after farmer started down the path. Participants were explicitly informed that risk ratings have no effect on shock presentation. At the end of each trial, the participant was rewarded with a congratulatory graphic (the farmer in an ecstatic pose with sparkles and vegetables).

During the generalization test, each trial type (CS+, CS-, 3 classes of intermediate ring sizes, CS-Δ) was presented 16 times for both Pavlovian and Instrumental trials (192 trials total). Each trial started the same way, with a geometric shape being presented superimposed on the game background for 1,1.5,2 or 3 seconds. Then it either proceeded in one of two ways: exactly as previous Pavlovian trials with the farmer leaving the shed down the dangerous path without being given a choice (Pavlovian trial), or an avoidance path-choosing decision (Avoidance trial). On the avoidance trials, 3.5 seconds were allowed for the avoidance decision. Participants were informed if they did not make a choice in time, it would result in the participant taking the dangerous path associated with shock as well as a loss and a message to choose faster. The avatars trip took a total of 1.5, 2.5 or 3 seconds during the avoidance trials for a total trial time of either 7s,7.5s, 8s, or 8.5s. During avoidance trials on successful trials was rewarded with a congratulatory

graphic (the farmer in an estatic pose with sparkles and vegetables), and on unsuccessful trials a disappointing graphic (the farmer with a tear planting more seeds). 100% of the CS+ stimuli were paired with shock when the dangerous path was traveled on avoidance trials. 0% of the CS+ stimuli were paired with shock when the safe road was traveled on avoidance trials.

To control the effects of scanner drift and habituation over time, trial-type order was balanced within each phase. Inter-trial intervals (ITI) of 2, 3, or 4 seconds followed each trial an equal number of times. The duration between the onset of CS+ and the electric shocks was jittered (CS+ trials will be followed by shock 2.5, 4, 5 or 5.5 seconds after the onset of the CS+). The time series of shock delivery was entered into the statistical model as a covariate to control the effects of shock on the BOLD response.

Behavioral ratings

Online risk-ratings. During half of all Pavlovian trials, the question "Level of risk?" appeared at the top of the screen at 2 or 3 seconds post-stimulus onset (1 second after the farmer starts the trip). Participants were cued to use a response box to rate their perceived level of risk for shock on a 3-point Likert scale, where 1= "no risk", 2= "moderate risk", and 3="high risk". Behavioral ratings of risk were assessed quasi-randomly with no more than three consecutive trials prompting risk ratings. Participants were instructed to answer as quickly as possible, and to keep their index, middle, and ring finger on the 1, 2 and 3 buttons respectively. Risk ratings and corresponding response latencies were recorded with Presentation software (Neurobehavioral Systems), and reaction times

exceeding 2.5 standard deviations above the average were considered outliers and discarded.

Retrospective ratings. After acquisition and generalization phases, participants rated the level of anxiety they experienced during CS+, CS-Δ , CS-, and trials using an 11 point Likert scale (0=no anxiety, 10=extreme anxiety). Additionally, participants answered questions about their emotions and decision making during the task. These ratings were completed over the intercom while participants remained in the scanner.

Standardized Questionnaires

Participants completed the Spielberger State and Trait Anxiety Inventory (STAI: Spielberger et al., 1983), the Beck Depression Inventory (BDI: Beck, et al., 1996), the Tridimensional Personality Questionnaire (TPQ: Cloninger et al., 1994), and the Multidimensional Experiential Avoidance Questionnaire (MEAQ: Gámez et al., 2011). The STAI is a 40 question, 4 point Likert scale instrument with has two main scales, one for state and another for trait anxiety. The BDI is a commonly used 20 question measure to assess current depressive symptoms. The TPQ is a 100 question true false inventory with three main scales, each with four subscales. Harm Avoidance measures worry (HA1), fear of uncertainty (HA2), shyness (HA3), and fatigueability (HA4). Novelty Seeking measures exploratory excitability (NS1), Impulsiveness (NS2), Extravagance (NS3), and Disorderliness (NS4). Reward Dependence which measures Sentimentality (RD1), Persistence (RD2), Attachment (RD3) and Dependence (RD4). The MEAQ is a 6 point Likert scale with 56 questions and has 6 subscales; behavioral avoidance which measures active avoidance behaviors, distress aversion which measures how aversive

individuals find distressing states, procrastination which measures putting things off, distraction & suppression which measures doing something else to avoid negative feelings, repression & denial which measures "turning off" emotions or not realizing emotional responses cognitively, and distress endurance which is persevering in the face of adversity.

Other Measures

Patients also completed a sleep and recent use questionnaire. This was done to ensure they had not had caffeine or nicotine within 6 hours of the study and no alcohol for 24 hours prior. Additional weekly use averages were reported as well.

Procedure

Participants were greeted at the CMRR and brought into a preparation room adjoining the scanner. Following informed consent, participants were given a second magnetic resonance imaging safety screening, the sleep and recent use questionnaire, and standardized questionnaires. The participant then read task instructions and completed 6 practice trials of the task. During the instructions participants were told they might learn to predict the shock if they attend to the shapes in the center of the screen, but were not informed of the CS+/US contingency.

After the consent was signed and questionnaires completed, the researcher asked the participant to remove any metal (e.g. piercings, hair clips, belts, etc.) before entering the MRI scanning room. The participants were then led into the room with the scanner and shock electrodes were attached, and a shock workup procedure was completed. . Participants received up to 4 sample shocks and rated the shocks on a 5 point scale (1 =

not painful to 5 = extremely painful). The level and placement of the shocks was adjusted until the participant reported at least a 2, but preferably a 3 or 3.5 on this scale. Participants were reminded that they are free to withdraw from the study at any time prior to the shock workup.

The participants were then situated in the scanner and a T2 scout, localization, and MPRAGE were performed. The three phases of the experiment were then completed over the course of 7 EPI runs with a 5 minute break separating acquisition and generalization, during which participants completed retrospective ratings and another 5 minute break in the middle of the 6 generalization runs. Prior to the start of the generalization phase, subjects were given additional instructions concerning the avoidance portion of the task. Specifically, subjects were told they would now be able to choose the road traveled by the farmer on some trials, and were reminded of the costs and benefits associated with each road. Additionally, subjects practiced using the button box to send the farmer down the long and short road. Retrospective ratings were again completed at the end of the generalization test. All retrospective ratings were performed over intercom while the participant was in the scanner.

MRI procedure

MRI data was collected using a 3-Tesla Siemens scanner with a 20-channel, receive only, headcoil. This scanner is installed at the Center for Magnetic Resonance Research at the University of Minnesota. Participant were taken into the MRI scanner room and asked lie down (head-first, supine) on the table of the MRI-scanner. The

researcher then attached electrodes for providing the electric stimulation to the participant's right ankle.

The researcher showed the participant how to use a handheld MRI-compatible response pad (Lumina LP-404 by Cedrus), provided hearing protection (ear plugs and headphones) and an emergency "squeeze ball" (alerts researcher in control room). A laser-pointing device was used to align the center of the head-coil with the appropriate location on the MRI scanner. When the participant was ready, the researcher moved the table slowly into the bore of the magnet. The researcher then tested the intercom and verified that the participant could see the visual stimuli – which were projected into the participants‟ visual field via a system of mirrors.

The following scans were used: a localizer, T2 scout, a Magnetization Prepared Rapid Gradient Echo sequence (MPRAGE), and seven functional scans of various durations. The localizer and T2 scout scans were used to determine the location of the head within the scanner so that the subsequent structural and functional images could be placed in the correct location. A magnetic field map was also acquired and used in data preprocessing to correct for inhomogeneities and imperfections in the magnetic field during data analysis. Structural MRI scans were collected to provide a detailed representation of the participant's brain on which the functional data can be mapped. The MPRAGE sequence is a high-resolution T1-weighted anatomical scan of the whole brain [224 1.0 mm sagittal slices; FOV=256 mm, T*R*=2530 ms, TE=3.65 ms]. This yields isotropic, 1 mm*3mm voxels that have optimal gray and white matter contrasts. Signal dropout due to air/tissue boundaries were reduced using a higher order shim.

Functional scans were acquired with echoplanar single shot gradient echo T2* weighting (35 3.5 mm sagittal slices; T*R*=2300 ms; TE=28 ms; FOV=224 mm; 1.75*1.75*3.5 mm voxels), resulting in a series of 35 contiguous, 3.5 mm slices covering the entire brain. Four functional volumes were obtained before task onset to allow for signal stabilization. 7 functional scans were collected, one with pre-acquisition and acquisition, and six generalization. 1) pre-acquisition and acquisition (10.63 minutes), 2-7) generalization test (6 runs of 6.47 minutes. Average time for sample shock procedure, subject positioning, scan setup time, and scan collection, was approximately 105 minutes. Data was stored on a secure server hosted by the College of Liberal Arts.

Data Analysis Plan

Image analysis was completed using Analysis of Functional Neural Images (AFNI) software (Cox, 1996). Data preprocessing consisted of the following steps: (1) Slice-timing correction to adjust the amplitude of the signal acquired from each slice to account for the order of slice acquisition within each volume, (2) motion correction (registration to the seventh volume of the first functional imaging scan), (3) spatial smoothing to minimize the effects of anatomical variability (FWHM= 2.5 mm), and (4) normalization to percent signal change using as a baseline each subject's voxel-wise time-series mean. Data from the six separate runs during generalization were concatenated. Any EPI brain volumes with more than 3.0 mm of head motion in any dimension from volume to the next were removed. For a full description of preprocessing steps see Appendix B. If over 35% of the data in an individual's record

was removed, that subject was not included in the analysis. One individual was excluded due to movement.

Individual analyses involved computing functional maps by fitting a general linear model to the data based on the predicted hemodynamic response to the experimental task, and estimating the strength of the BOLD response from the parameters of the general linear model. The general linear model is essentially a multiple regression procedure (Neter et al., 1996). The model includes one time point per volume, one response variable per voxel or region of interest, and a design matrix of predictor variables (explanatory variables) determined by the researchers. Parameter estimates are obtained using the method of least-squares.

During acquisition, the predictor variables were the onset of the stimulus for all 3 stimulus types: ΔCS-, CS-, CS+ (only CS+ trials that did not have an actual shock), through the period where the farmer leaving the shed until either a risk rating or shock could occur, as well as a regressor for reaching the garden. Baseline drift, motion parameters, response time course (button presses), and time course of CS+ paired with shock were modeled as covariates of no interest.

For generalization it was first attempted to model all variables of interest in one regression. However, given the onset of each stimulus type in two conditions and the onset of the farmer in for each stimulus type, the onset of the reaching the garden as a separate variable of interest, as well as the covariates of no interest, there was a lack of power to detect differences across conditions (20 conditions + motion parameters). In order to address this, two separate analyses were completed (See Figures 2.1 and 2.2). In

the first, all Pavlovian stimuli were modeled using a function that consisted of the onset of the stimulus as well as the farmer leaving the shed, up until either a risk rating or shock could have been presented each trial type (ΔCS-, CS-, GC1, GC2, GC3, and CS+). The farmer reaching the garden was kept as a separate variable of interest. Baseline drift, motion parameters, response time course (button presses), and time course of CS+ paired with shock were modeled as covariates of no interest. In the Pavlovian analysis, all avoidance trials were also modeled as covariates of no interest (8 conditions + motion parameters).

In the Instrumental analysis, the onset of the stimulus and the onset of the display asking participants to make their choice were modeled for each stimulus type (ΔCS-, CS-, GC1, GC2, GC3, and CS+). In this analysis baseline drift, motion parameters, response time course (button presses), time course of CS+ paired with shock, as well as reaching the garden were modeled as covariates of no interest (13 conditions + motion parameters).

A final analysis (see Figure 2.3) was completed to address the fact that for the previous analysis, all stimuli of each class were grouped together, regardless of the outcome of the trial, avoid or not-avoid. For this analysis, avoid button presses and non-avoid button presses were modeled as separate regressors. In addition, the time from the onset of the stimulus until the button press was modeled separately for avoided vs. non-avoided trials. Baseline drift, motion parameters, response time course (button presses), time course of CS+ paired with shock, as well as reaching the garden were modeled as covariates of no interest.

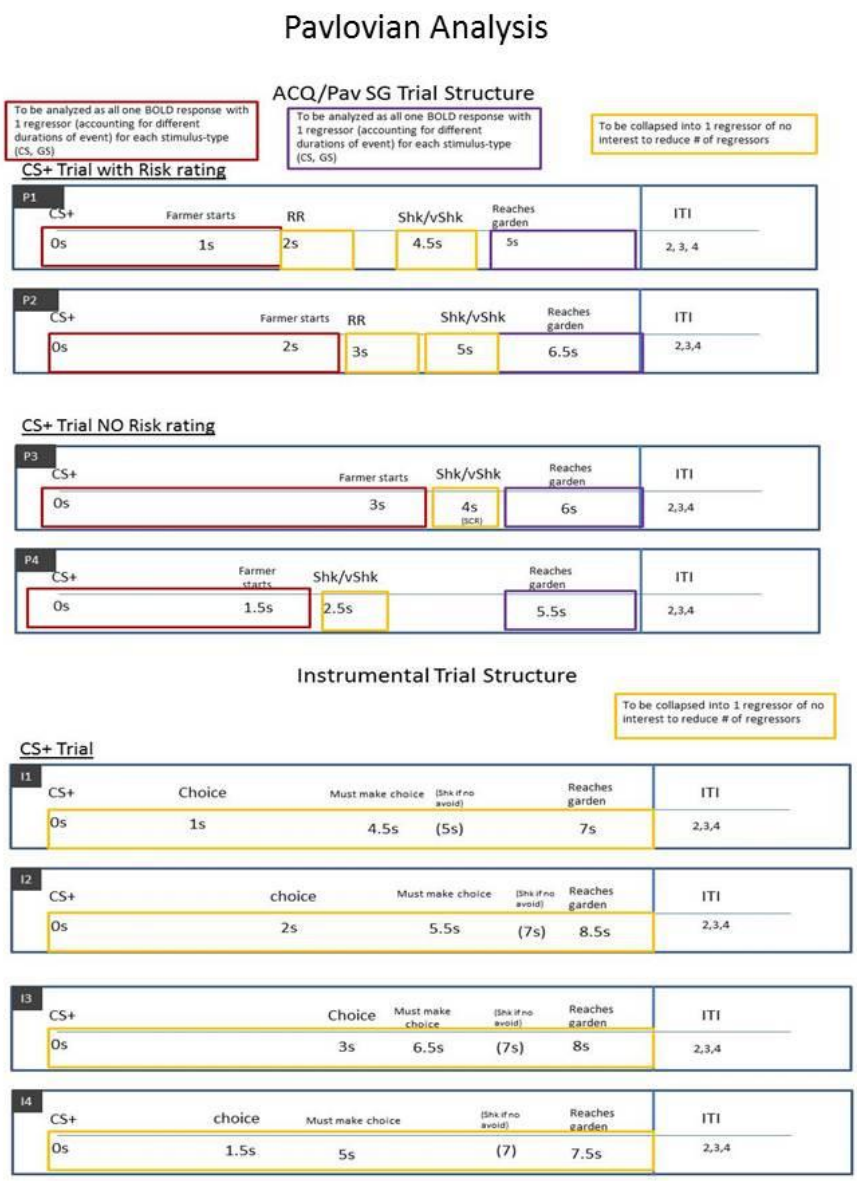

FIGURE 2.1: Visual depiction of the fMRI data analytic plan for Pavlovian Analysis depicted for conditioned danger cue (CS+ type) trials. Timing for conditioned and generalization stimuli depicted in red, timing for reaching the garden depicted in blue, and variables collapsed into a single regressor of no interest in yellow. R*R*=risk rating, Shk/vShk=Shock or Virtual Shock, ITI=Inter-trial-interval; P1, P2, P3 and P4= Jittered Pavlovian trial timings; and I1, I2, I3 and I4=Jittered Instrumental trial timings.

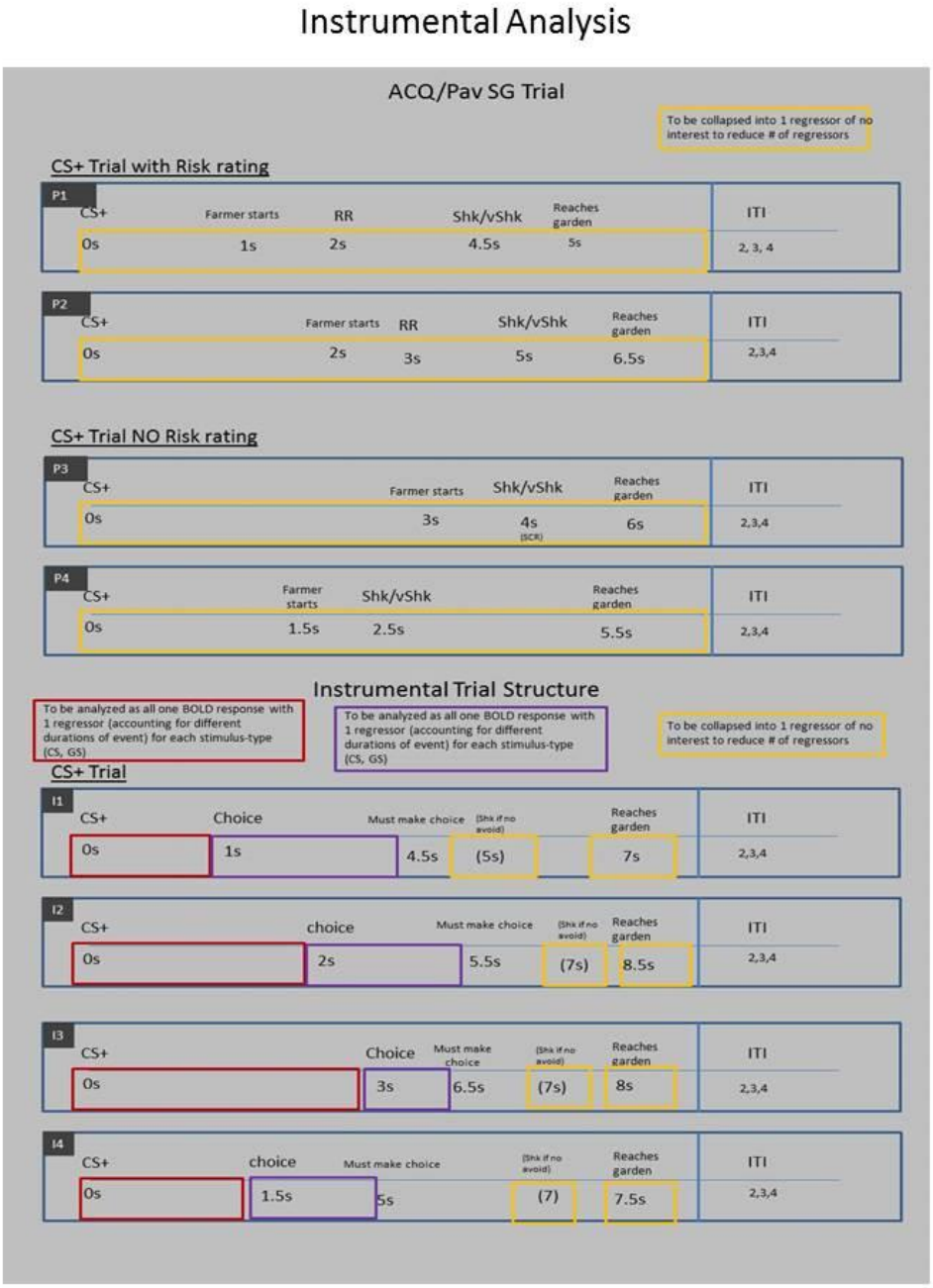

FIGURE 2.2: Visual depiction of the fMRI data analytic plan for Instrumental Analysis depicted for conditioned danger cue (CS+ type) trials. Timing for onset of conditioned and generalization stimuli depicted in red, timing for the duration of the choice making period in blue, and variables collapsed into a single regressor of no interest in yellow. R*R*=risk rating, Shk/vShk=Shock or Virtual Shock, ITI=Inter-trial-interval; P1, P2, P3 and P4= Jittered Pavlovian trial timings; and I1, I2, I3 and I4=Jittered Instrumental trial timings.

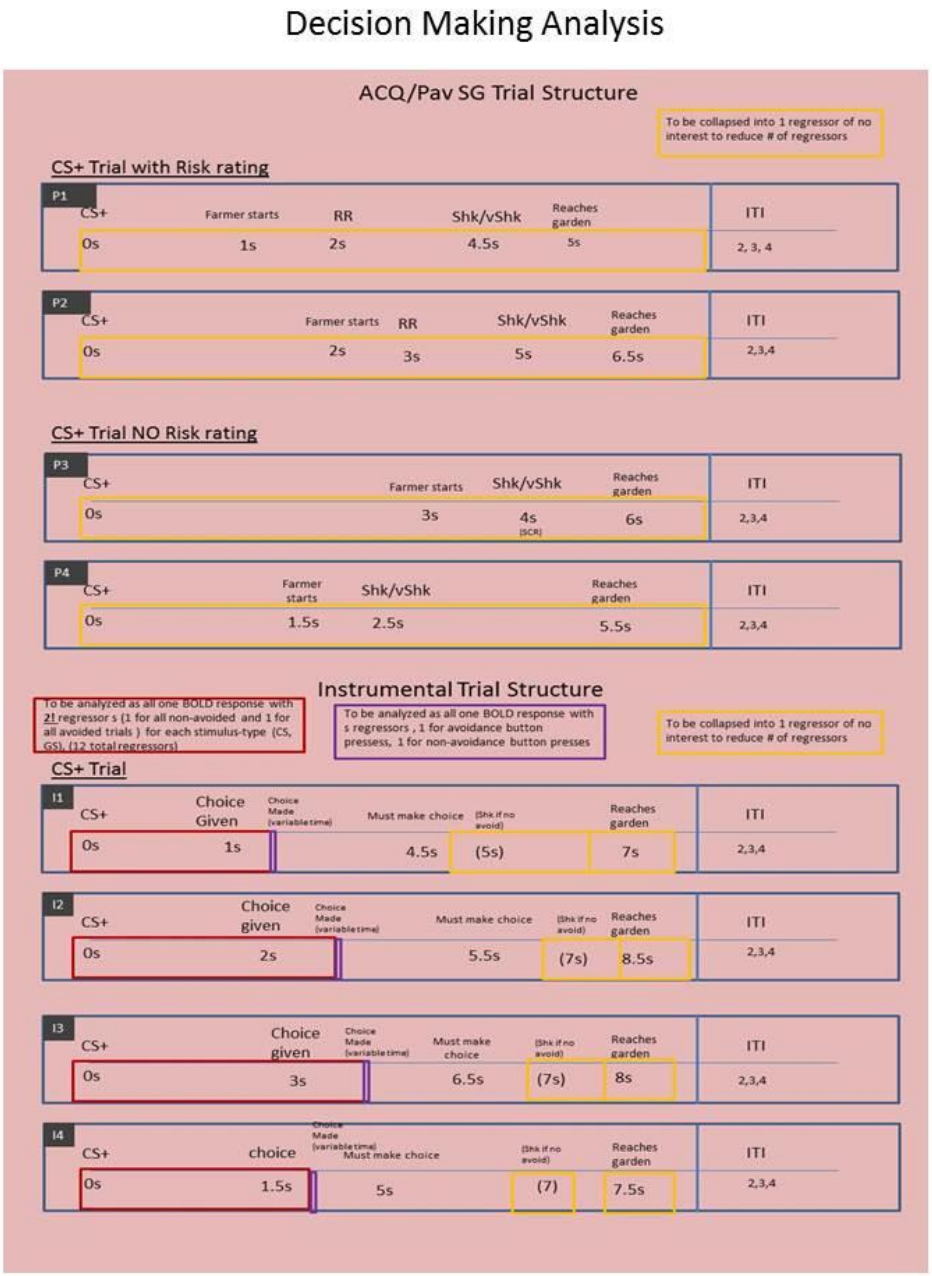

FIGURE 2.3: Visual depiction of the fMRI data analytic plan for Decision Making Analysis depicted for conditioned danger cue (CS+ type) trials. Timing for period from onset of conditioned and generalization stimuli until the choice is made depicted in red (NOTE: Two variables for each stimuli depending on decision outcome), two gamma functions one for avoided choices and one for not avoided choices in blue, and variables collapsed into a single regressor of no interest in yellow. R*R*=risk rating, Shk/vShk=Shock or Virtual Shock, ITI=Inter-trial-interval; P1, P2, P3 and P4= Jittered Pavlovian trial timings; and I1, I2, I3 and I4=Jittered Instrumental trial timings.

The preprocessed time series data was used for group level statistical analysis. The group level analysis involved several steps. First a voxelwise whole brain analyses was conducted for the CS+ (unreinforced) vs. ΔCS- contrast using a paired samples t-test to test the significance of the differences in the parameters (β) between CS+ and CS- using a voxelwise probability of $p \leq .001$. A more stringent voxelwise probability of $p \leq 0005$ was necessary to achieve adequate demarcation between clusters in the Pavlovian analysis. To correct for multiple comparisons and prevent inflation of α (Type I Error), a cluster-size thresholding procedure will be used (Huettel et al., 2004) with a cluster probability of $p \leq .05$. The AFNI program, 3dClustSim, was used to determine the minimum number of voxels necessary to meet the cluster probability which was 26 voxels for the acquisition, instrumental and decision making regressions and 20 voxels for the Pavlovian regression. The CS+ (unreinforced) vs. ΔCS- contrast was chosen over the CS+ (unreinforced) vs. CS- contrast because the latter to control for possible generalization to all circles. However, the pattern of generalization results does not change significantly when analyzing the CS+ (unreinforced) vs. CS- contrast.

In the second step, the average hemodynamic response in each of the voxels that survive the cluster analysis was then calculated for all predictor variables in each analysis listed above. For Acquisition analyses, a 2x3 repeated measures ANOVA (two groups: high anxiety, low anxiety or high HA, low HA; three stimulus types; ΔCS-, CS-, and CS+) was then used to test the significance of these parameters and group differences. For Generalization analyses, a 2x6 repeated measures ANOVA (two groups: high anxiety, low anxiety or high HA, low HA; six stimulus types; ΔCS-, CS-, GC1, GC2,

GC3, and CS+) was used to test the significance of these parameters and differences across groups, as well as to test for linear and quadratic trends across stimulus types as the test stimulus becomes more similar to the CS+. ANOVAs were computed using Wilks's lambda and were followed, when necessary, by quadratic and linear trend analyses and/or paired-samples t tests. Criterion alpha for ANOVAs and follow up statistics was set at $p = .05$. Geisser-Greenhouse corrections were used when there were violations of the sphericity assumption. Rates of avoidance behavior as well as risk ratings during acquisition and generalization were each analyzed with methods mirroring those for the neural indices. .

Several other calculations were completed on the neural and behavioral indices. This included difference scores for activations to each of the stimuli compared to the ΔCS-, as well as contrasts to test for generalization effects such as deviation from linearity and all GCs vs ΔCS-. This was done to in order to correlate these with rates of avoidance for each stimuli compared to a baseline. All correlations were Pearson correlations. Additionally these scores were used to measure relationships with several self-report variables, including questionnaire measures of anxiety, online behavioral responses regarding perceptions of shock risk to the conditioned danger cue, perceptions of risk associated with each path choice, and subjective ratings of the perceived risk of receiving a shock measured after the task is completed. Additionally, a regression analysis using avoidance behavior as the dependent variable was conducted for each of these single scores using forward selection.

Functional Connectivity Analysis

Generalized Psychophysiological interaction (gPPI) was used to estimate the connectivity between brain regions in relation to the generalization task (McLaren et al., 2012). Structurally defined seed regions included the left and right amygdala and the left and right hippocampus based on a priori predictions regarding the fear-conditioning neural network (see Lissek et al., 2013). We predicted increased connectivity between the hippocampus and brain areas associated with fear excitation (e.g., insula) for stimuli with more threat information (closer to CS+), and increased neural connectivity between the hippocampus and brain areas associated with fear inhibition (e.g., vmPFC) for stimuli with more safety information (closer to CS-). We had similar predictions for the amygdala related connectivity. We also predicted that connectivity would differ between groups. Based on previous research using PPI, the criterion alpha was set at $p \leq 0.001$ with no cluster threshold (Passamonti et al., 2009).

RESULTS

Group Characteristics

Participants in the high and low trait anxiety groups who were included in the analysis did not differ in gender, reward dependence, novelty seeking, harm avoidance, depression symptomatology, behavioral avoidance, or perceived pain from shock (see Table 1). Group differences were found in that the low anxiety group had higher distress endurance and used distraction more frequently than the high anxiety group, the high anxiety group used repression more than the low anxiety group, and the low anxiety

group needed significantly higher shock levels to achieve the same level of perceived pain (see Table 1).

Table 1: Means and significance testing statistics for the low anxiety and high anxiety groups for personality measures and shock workup.

Measure	Low Anxiety	High Anxiety		
n	9	13		
			X^2	*p*
% Female	0.56	0.62	0.079	0.561
			t	*p*
Reward Dependence (TPQ)	18.2222	16.3846	.866	.397
Novelty Seeking (TPQ)	16.3333	17.7692	-.709	.487
Harm Avoidance (TPQ)	11.1111	15.0769	-1.470	.157
Beck Depression Inventory	5.3333	7.0000	-1.229	.233
Behavioral Avoidance (MEAQ)	33.5556	29.8462	.974	.342
Distraction (MEAQ)	31.2222	23.5385	3.364	.003
Repression (MEAQ)	28.0000	38.4615	-2.542	.019
Distress Endurance (MEAQ)	56.1111	47.0769	3.444	.003
Shock Perceived Pain	2.9444	3.0385	-.287	.777
Shock Level	221.6667	177.3077	2.424	.025

Note: *X^2= Chi squared statistics, t= t statistic, p= calculated probability, TPQ= Tridimensional Personality Questionnaire, MEAQ= Multidimensional Experiential Avoidance Questionnaire, Shock Perceived Pain level range from 0-5, Shock Level range from 0-255).*

Acquisition

Risk Ratings

There was a main effect of trail-type $F(2,18)=34.629$, $p<.001$, found for levels of perceived risk. This was driven by increased risk to the CS+ compared to both CS- ($t(20)=6.391$, $p<.001$) and CS-Δ ($t(20)=8.496$, $p<.001$). There was a trend towards

higher risk for the circular than triangular safety cue (t(20)2.007, p=.058). There were no differences across groups (p>.1) for either trait anxiety or harm avoidance.

Neurobiological regions

43 fROIs were identified. For each of the fROIs a RM-ANOVA was performed for the % BOLD signal change across the CS+, CS- and CS-Δ . A trend towards significant group*stimulus multivariate effects were found in the right precuneus (F(5,16)=3.353, p=.057) and right cuneus/BA30 (F(5,16)=3.383, p=.055). The right precuneus also had significant within subjects effects (F(5,100)=4.911, p=.012. Significant between subject effects were found in the right inferior parietal lobule (F(1,20)=6.129, p=.022) and a trending effect was found in the left precuneus (F(1,20)=3.035, p=.097). For the responses in the right precuneus, left precuneus, and right cuneus, responses were greatest to the CS+ with deactivations to the CS-Δ and CS-. Additionally for each of these activations the group differences were mainly manifest by less deactivation to the safety cues in the low anxiety group. For the right inferior parietal activation, responses were also greater to the CS+ and driven by activation to the CS+. In this region, the low anxiety group showed greater activations to all stimuli.

Correlation with acquisition risk ratings

Conditioning in each of the fROIs was measured as the difference in percent BOLD signal change to the CS+ vs the CS-Δ as well as the CS+ vs CS-. This was then correlated with the respective difference score for risk ratings. For the CS+ vs CS-Δ contrast there were three significant correlations, the left superior parietal lobule (r=.473, p=.03),the left medial frontal (r=.476, p=.029) and the right insula (r=.645, p=.002).

However, there was one outlier that had rated more risk to the CS-Δ than the CS+, when removed the strength of the correlations fell to left superior parietal (r=.323, p=.165), left medial frontal (r=.255, p=.278) and right insula (r=.432, p=.057). For the CS+ vs CS- contrast there were two significant correlations, in the right anterior insula (r=.462, p=.035), and the right middle frontal (r=.456, p=.038). When the same individual who was an outlier was removed, the strength of the relationships fell to right anterior insula (r=.401, p=.080) and right middle frontal (r=.278, p=.220).

Correlation with later avoidance

There were no significant relationship between conditioning measured during acquisition and later avoidance behavior. This was assessed with the difference score between both CS+ and CS-Δ and CS+ and CS-.

Behavioral Generalization

Risk ratings. A main effect of trial-type, $F(5,100)=62.04$, $p<.001$, was found for levels of perceived risk. Risk ratings evidenced gradients of generalization consisting of both linear, $F(1,20)=95.95$, $p<.001$, and quadratic, $F(1,20)=98.82$, $p<.001$, decreases in perceived risk from CS+ down the continuum-of-size to CS-Δ . Within the high anxiety group, both linear, $F(1,12)=71.408$, $p<.001$, and quadratic, $F(1,12)=38.409$ $p<.001$decreases were observed. This was also true of the low anxiety group with both linear, $F(1,8)=34.164$, $p<.001$, and quadratic, $F(1,8)=69.853$, $p<.001$; however the ratio of linear to quadratic is nearly reversed in each group with the high anxiety group more linear than quadratic and the low anxiety group more quadratic than linear. There was a significant multivariate Group*Stimulus interaction $F(5,16)=2.506$, p=.035. When

compared to CS-Δ as a baseline, there was a large effect of increased risk to the CS+ (d=2.196) GS_3, (d=.92) a moderate increase to GS_2, *(d=.464)* and no effect for GS_1 or CS- (d's <.2). Within the low anxiety group there was a large effect of increased risk to the CS+ (t(8)=4.357,p=.002, d=1.646) but only a moderate effect towards GS_3 (t(12)=1.994, p=.081, d=.697) and no effect for GS_2, GS_1 or CS- (p>.1, d's <.2). Within the high anxiety group there was a large effect of increased risk to the CS+ (t(12)=8.719,p<.001, d=3.392), GS_3 (GS_3, t(12)=5.686, p<.001, d=2.068), GS_2 (t(21)=2.966, p=.012, d=1.267), but not GS_1 or CS- (p>.1, d's <.2). Generalization of risk perception in the high anxiety group can thus be said to extend to GS_3 and GS_2, but not GS_1 whereas the low anxiety group does not generalize risk to any generalization stimuli.

Reaction time for the avoidance decision varied as a function of stimulus only (F(5,15)=.022) and there were no effects of group or interactions with group.

There were no significant main effects of group or interactions when Harm Avoidance was used as the grouping variable (all p's >.2)

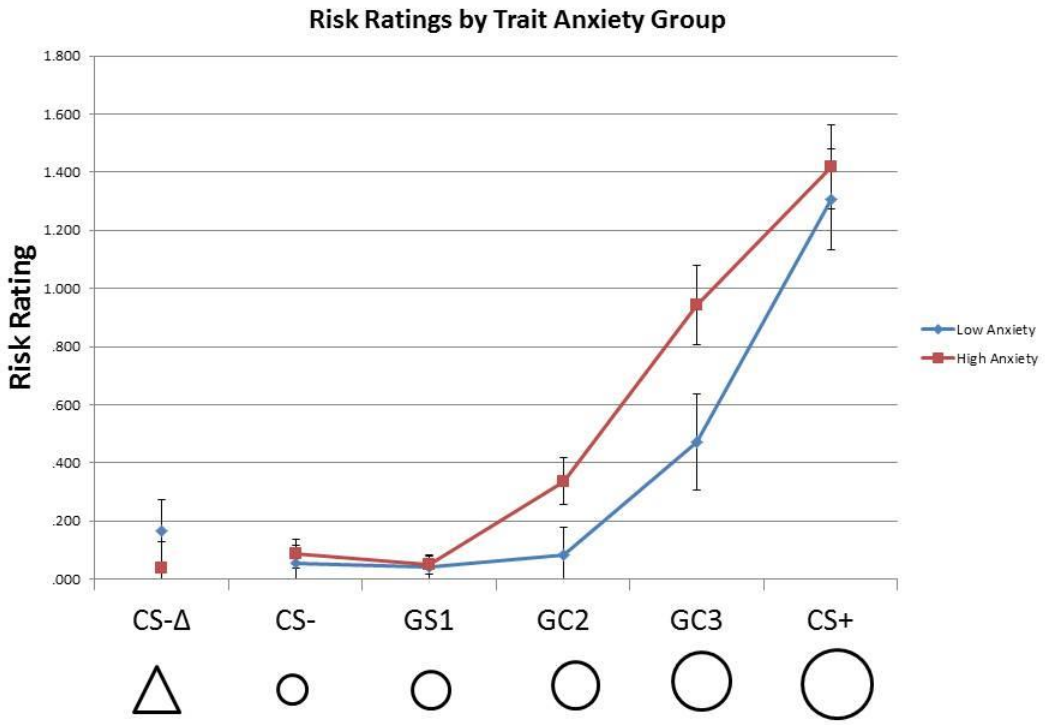

Figure 3: Risk ratings (0 = no risk, 1 = some risk, 2 = a lot of risk) for each group during the generalization phase as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory(blue = low anxiety, red=high anxiety).

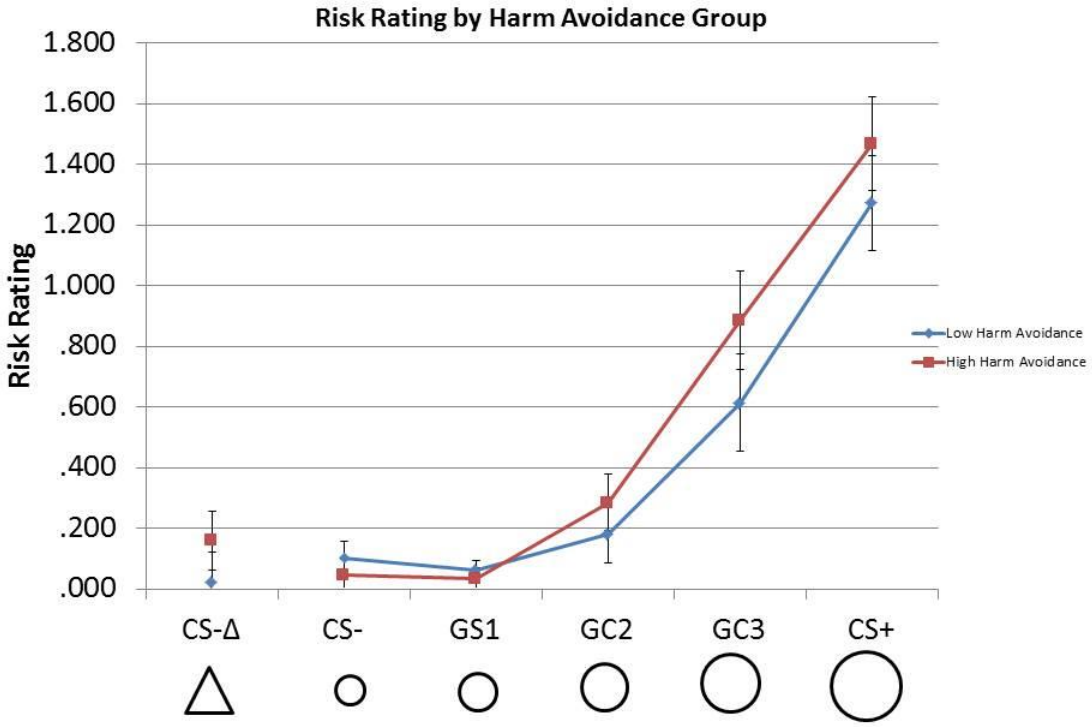

Figure 4: Risk ratings (0 = no risk, 1 = some risk, 2 = a lot of risk) for each group during the generalization phase as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on Harm Avoidance scale on the Cloninger Tridimensional Personality Questionnaire (blue = low harm avoidance, red= high harm avoidance).

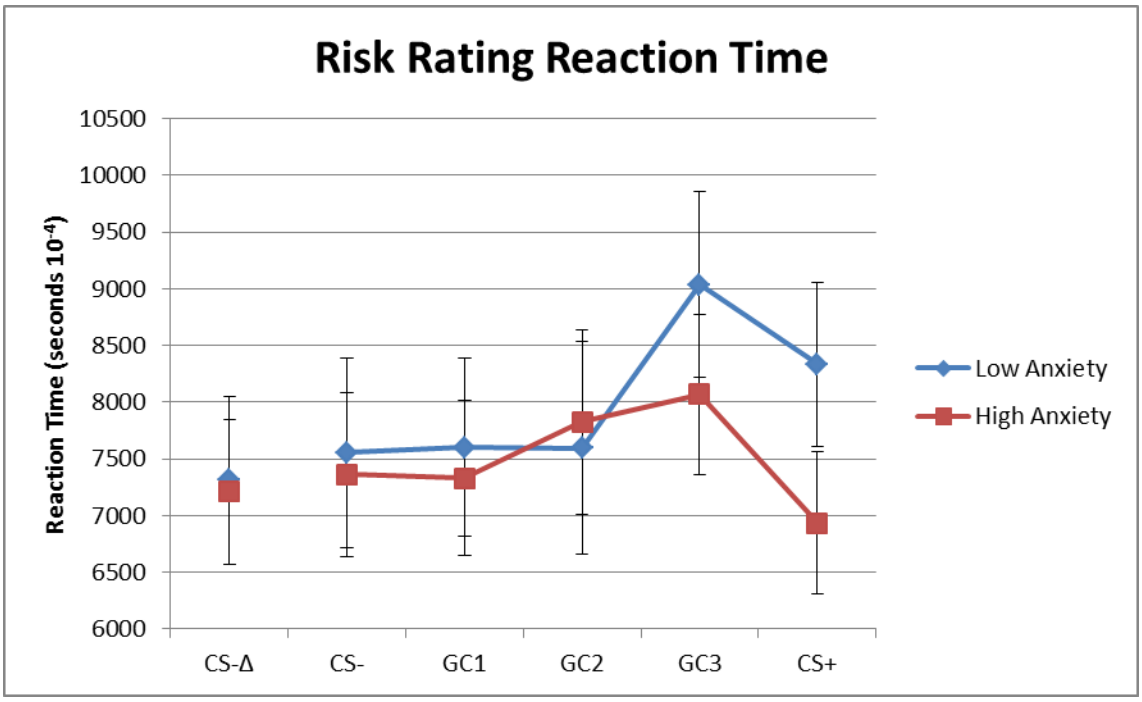

Figure 5: Reaction time in 1/10,000 of a second units for the risk ratings for each group during the generalization phase as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red=high anxiety).

Behavioral Avoidance

Generalization gradients were also present in instrumental avoidance decisions. The main effect of trial-type, $F(5,100)=34.251$, $p<.001$, consisted of linear, $F(1,20)=44.356$, $p<.001$, and quadratic, $F(1,20)=32.766$, $p<.001$, decreases in avoidance from CS+ to GS_3 to GS_2 to GS_1 to CS-. There was no overall group*stimulus type interaction $F(5,100)=1.705$, $p=.14$. Within the high anxiety group, both linear, $F(1,12)=28.991$, $p<.001$, and quadratic, $F(1,12)=14.363$ $p=.003$ decreases were observed. This was also true of the low anxiety group with both linear, $F(1,8)=18.509$, $p=.003$, and quadratic, $F(1,8)=15.788$, $p=.004$. While not as striking as for risk ratings, again the ratio of linear to quadratic is shows the high anxiety group to be more linear.

There was a trend towards a group*stimulus type quadratic interaction $F(1,20)=3.235$, $p=.087$ and a significant group*stimulus cubic trend $F(1,20)=.4.766$, $p=.041$. Relative to CS-Δ , avoidance behavior was increased to CS+, $t(21)=6.887$, $p<.001$, $d=1.822$ and GS_3, $t(21)=5.15$, $p<.001$, $d=1.48$, and GS_2 $t(21)=2.613$, $p=.016$, $d=.748$ but not GS_1, **(**$p=.344$, $d<.2$**)**. Within the low anxiety group there was a large effect of increased risk to the CS+ ($d=1.555$, $t=-3.992$, $p=.004$) and GS_3 ($d=1.298$, $t=-3.091$, $p=.015$) but only a small effect for GS_2 ($d=.289$, $t=-1.456$, $p=.184$), GS_1 or CS- (d's <.2). In contrast within the high anxiety group there was a large effect of increased risk to the CS+ ($d=1.845$, $t=-5.467$, $p<.001$), GS_3 ($d=1.696$, $t=-4.666$, $p=.001$), GS_2 ($d=.853-$, $t=-2.452$, $p=.03$),a moderate effect for GS_1($d=.530$, $t=-1.453$, $p=.17$) and CS- ($d=.463$, $t=-1.258$, $p=.23$) demonstrating more generalization in the high anxiety group.

The reaction time for the avoidance decision had a significant effect of stimulus type ($F(5,15)=5.402$, p=.007) and there was a trending between subjects effect for group ($F(1,19)=3.441$, p=.079). Follow up analysis after stratifying based on the decision made revealed that the low anxiety group took significantly longer to make decisions during the presentation of the CS+ ($t=5.171$, $p<.001$) with a trend towards taking longer for the GC3 ($t=1.991$, $p=.064$).

There were no significant main effects of group or interactions when Harm Avoidance was used as the grouping variable (all p's >.2)

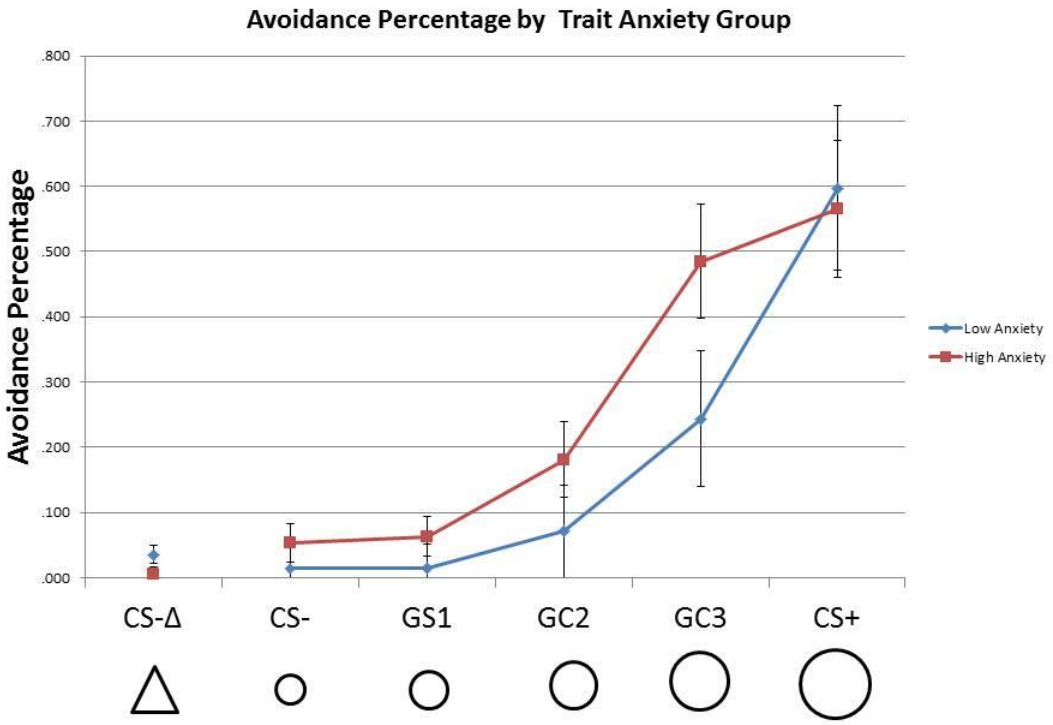

Figure 6: Avoidance as a percentage of trials the long dirt path was chosen for each group during the generalization phase as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red=high anxiety).

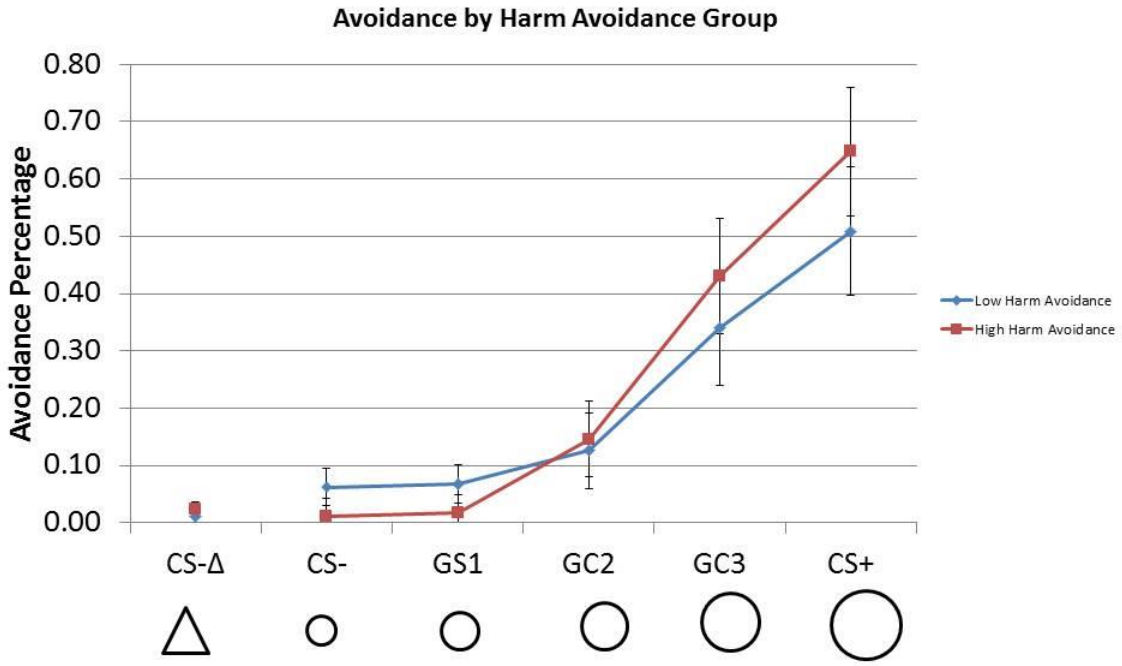

Figure 7: Avoidance as a percentage of trials the long dirt path was chosen for each group during the generalization phase as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on Harm Avoidance scale on the Cloninger Tridimensional Personality Questionnaire (blue = low harm avoidance, red= high harm avoidance).

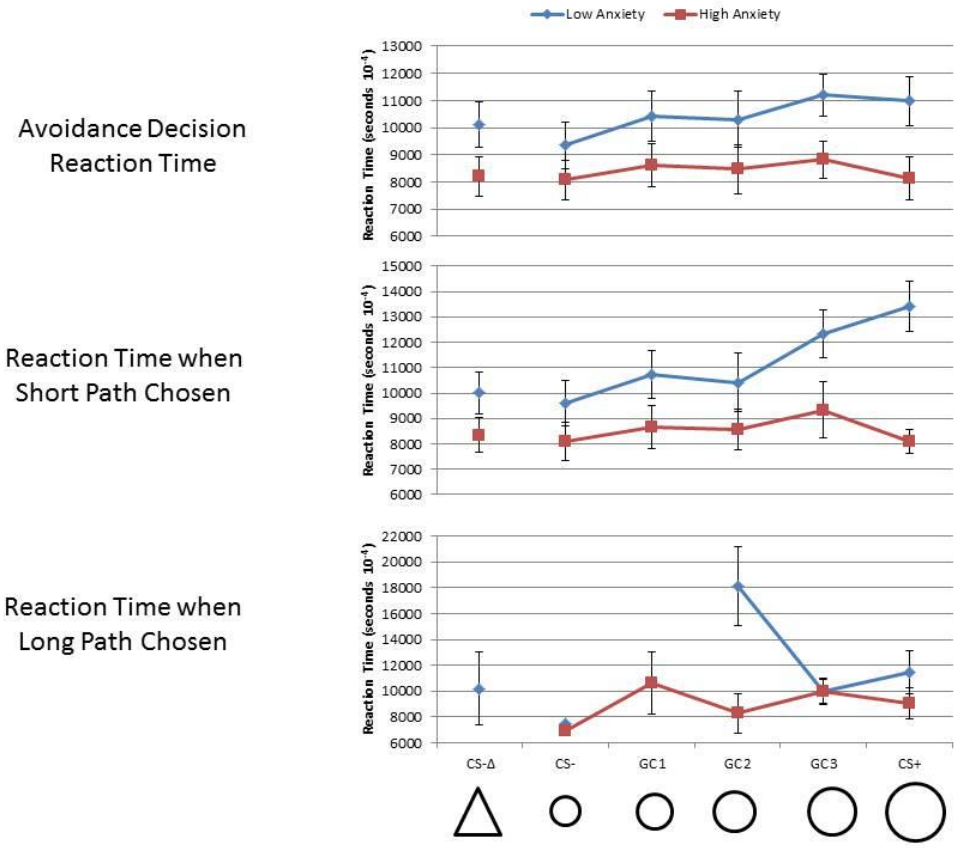

Figure 8: Reaction time in 1/10,000 of a second units for the avoidance decision for each group during the generalization phase as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red=high anxiety). *Top Row:* Reaction Time for all avoidance decisions. *Middle Row:* Reaction time when choosing the short dangerous path. *Bottom Row:* Reaction time when the long safe road avoidance decision was made.

Pavlovian Neurobiological Regions

38 fROIs were identified overall (see table 2). Of those 38, 32 displayed typical generalization gradients with linear or quadratic trends across the gradient.

Table 2: Brain Areas responding differentially to CS+ and ΔCS- during Pavlovian analysis that served as functional regions of interest (fROIs).

Brain Region	Direction	Volume	Peak Coordinates		
			X	Y	Z
Left Insula	CS+ > ΔCS-	4834.156	30	-27	2.5
Right Insula	CS+ > ΔCS-	3569.344	-30	-22.5	-0.5
Left Thalamus	CS+ > ΔCS-	3140.594	1.5	24	1
Right Medial Frontal	CS+ > ΔCS-	2486.75	-1.5	-9	43
Right Thalamus	CS+ > ΔCS-	2390.281	-4.5	18	11.5
Right Precentral Sulcus	CS+ > ΔCS-	2036.563	-36	-1.5	29.5
Right Caudate	CS+ > ΔCS-	1929.375	-9	-6	7
Left Caudate	CS+ > ΔCS-	1865.063	7.5	-3	10
Right Postcentral Gyrus	ΔCS- > CS+	1372	-45	28.5	56.5
Right Precentral Gyrus	ΔCS- > CS+	1146.906	-61.5	4.5	34
Right Primary Visual (BA17)	ΔCS- > CS+	1136.188	-6	93	14.5
Left Middle Occipital (BA18)	ΔCS- > CS+	836.0625	36	87	-2
Left Precentral	ΔCS- > CS+	696.7188	51	10.5	31
Right Postcentral	ΔCS- > CS+	696.7188	-31.5	37.5	59.5
Left Cuneus (BA19)	ΔCS- > CS+	643.125	4.5	90	28
Left Substantia Nigra	CS+ > ΔCS-	610.9688	6	10.5	-11
Precuneus/ Posterior Cingulate	ΔCS- > CS+	610.9688	0	57	23.5
Right Anterior Cingulate	Not Gradient	578.8125	-21	-30	8.5
Right Substantia Nigra	CS+ > ΔCS-	546.6563	-7.5	12	-9.5
Left Postcentral	ΔCS- > CS+	535.9375	48	21	53.5
Midbrain	Not Gradient	503.7813	-1.5	27	-29
Right Superior Temporal	ΔCS- > CS+	460.9063	-63	19.5	4
Right Fusiform	Not Gradient	439.4688	-21	64.5	-9.5
Right Precentral	ΔCS- > CS+	396.5938	-40.5	27	61
Right Thalamus/Red Nucleus	CS+ > ΔCS-	343	-3	16.5	-0.5
Right Anterior Cingulate	Negative	343	-19.5	-22.5	16
Right Inferior Occipital	CS+ > ΔCS-	310.8438	-36	87	-5
Right Superior Frontal	CS+ > ΔCS-	310.8438	-3	-31.5	47.5
Right Red Nucleus	CS+ > ΔCS-	289.4063	-4.5	22.5	-9.5
Right Parahippocampal	Not Gradient	289.4063	-24	46.5	-5
Right Postcentral	ΔCS- > CS+	257.25	-43.5	33	58
Left Subgenual Cingulate	ΔCS- > CS+	235.8125	3	-10.5	-8
BNST	CS+ > ΔCS-	225.0938	10.5	-1.5	-5
Right Posterior Insula	ΔCS- > CS+	225.0938	-34.5	12	19
Right Cingulate	CS+ > ΔCS-	225.0938	-9	-18	32.5
Left Cingulate (BA24)	Not Gradient	214.375	1.5	9	43

Notes: Peak Coordinates are in RAI format. If a direction is indicated, responses in that region followed a gradient, whereas regions where a gradient was not formed were labeled with "Not Gradient". BNST=Bed Nucleus of the Stria Termanalis.

Only two regions showed significant multivariate Group*Stimulus Interactions. The largest region, the left anterior insula demonstrated greater activations to the CS+ in both groups, but larger activations to stimuli on the safety end of the gradient in the high anxiety group compared to the low anxiety group ($F(5,16)=2.9722$, $p=.044$). In the right precentral gyrus there were greater deactivations to the CS+ resulting in a negative gradient, and the high anxiety group showed greater generalization of this deactivation ($F(5,16)=2.927$, $p=.046$).

In the left anterior insula within the low anxiety group the only stimulus significantly different from CS-Δ was CS+ ($t(8)=-3.298$,$p=.011$), but not GS3 ($t(8)=-2.176$,$p=.061$) or GS2, GS1, or CS- (all p's>.2). In the high anxiety group there were significant differences from the CS-Δ in the CS+ ($t(12)=-7.535$, $p<.001$), GC3 ($t(12)=-4.969$, $p<.001$), GC2 ($t(12)=-2.846$, $p=.015$) and the GC1 ($t(12)=-4.391$, $p=.001$) but not to the CS- ($t(12)=-1.761$, $p=.104$). In the right precentral gyrus, within the low anxiety group the only stimulus significantly different from CS-Δ was CS+ ($t(8)=6.623$, $p<.001$), but not GC3 ($t(8)=1.741$, $p=.12$) or GC2, GC1 or CS- (p's>.5). Within the high anxiety group there were significant differences from CS-Δ for CS+ ($t(12)=4.218$, $p=.001$) and GC3 ($t(12)=3.396$, $p=.005$), but not any other stimulus (p's >.5).

There was also a trend for an interaction in the right anterior insula ($F(5,16)=2.837$, $p=.051$). Follow up analysis demonstrated similar differences to the left insula with only the CS+ ($t(8)=-2.931$, $p=.019$) and GC3 ($t(8)=-2.316$, $p=.049$) differing

from CS-Δ in the low anxiety group, but CS+($t(12)=-5.595, p<.001$), GC3($t(12)=-4.252$, $p=.001$), GC2($t(12)=3.221$, $p=.007$), and GC1 ($t(12)=-3.795$, $p=.003$) all differing from CS-Δ in the high anxiety group, again demonstrating overgeneralization.

There were additional significant and/or trending group*stimulus quadratic trends in primary visual cortex, as well as a superior occipital area. Follow-up analysis revealed significant linear and quadratic trends in the low anxiety group, but only significant linear trends in the high anxiety groups in these regions. This pattern (but no significant group*stimulus trend interaction) was also found in many other Pavlovian fROIs, specifically in the left anterior insula, right anterior insula, right precentral gyrus, right anterior cingulate, right substantia nigra , left post central gyrus, right superior temporal gyrus, right post-central gyrus, left bed nucleus of the stria termanalis, right posterior insula, and left cingulate gyrus. The opposite effect with significant quadratic trends in the high anxiety group and not the low anxiety group was found in two midbrain regions, the left substantia nigra and the right substantia nigra.

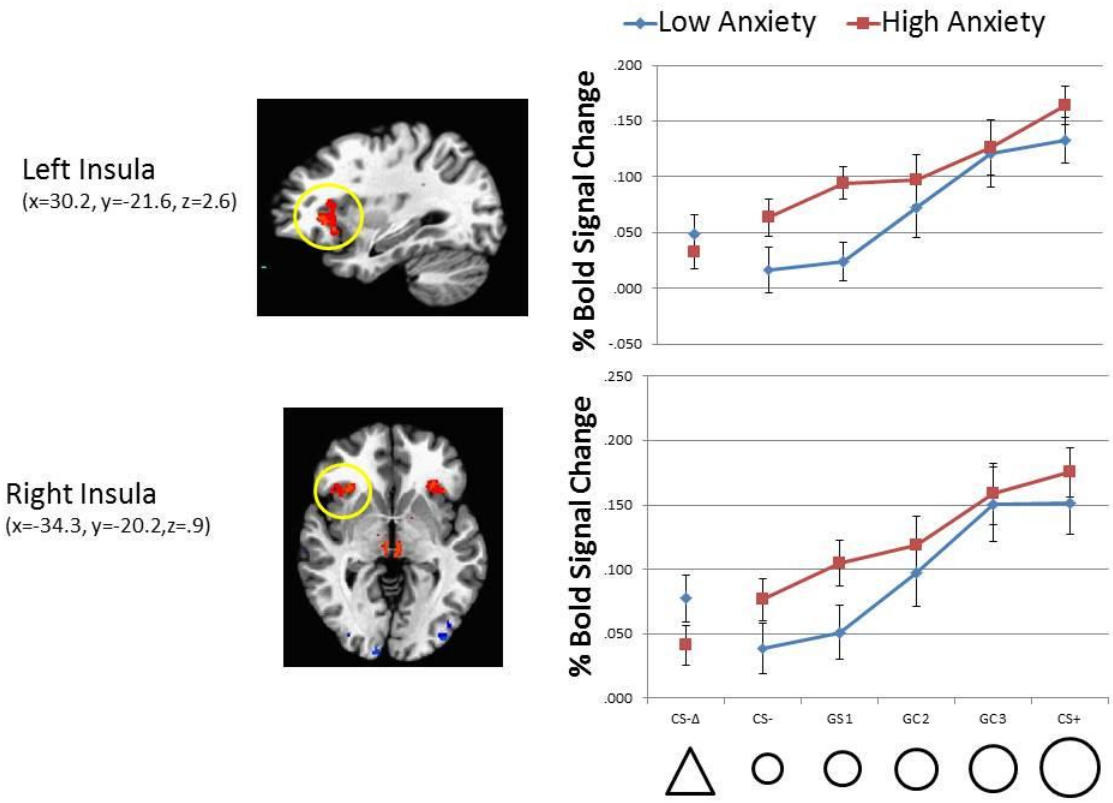

Figure 9: *Left Column:* Description and location of activation during Pavlovian analysis with coordinates in RAI format. *Middle Column:* image of activation for the fROI during Pavlovian analysis. *Right Column:* Percent BOLD signal change in the fROI during Pavlovian analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety).

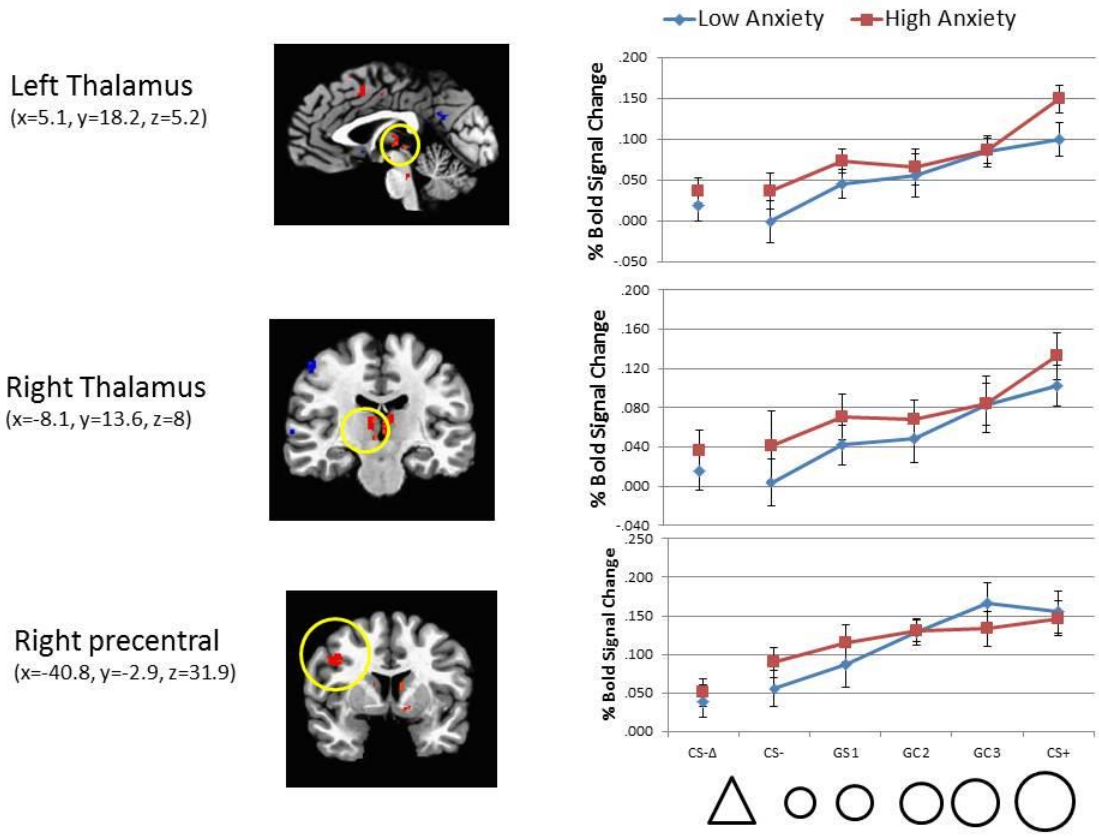

Figure 10: *Left Column:* Description and location of activation during Pavlovian analysis with coordinates in RAI format. *Middle Column:* image of activation for the fROI during Pavlovian analysis. *Right Column:* Percent BOLD signal change in the fROI during Pavlovian analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety).

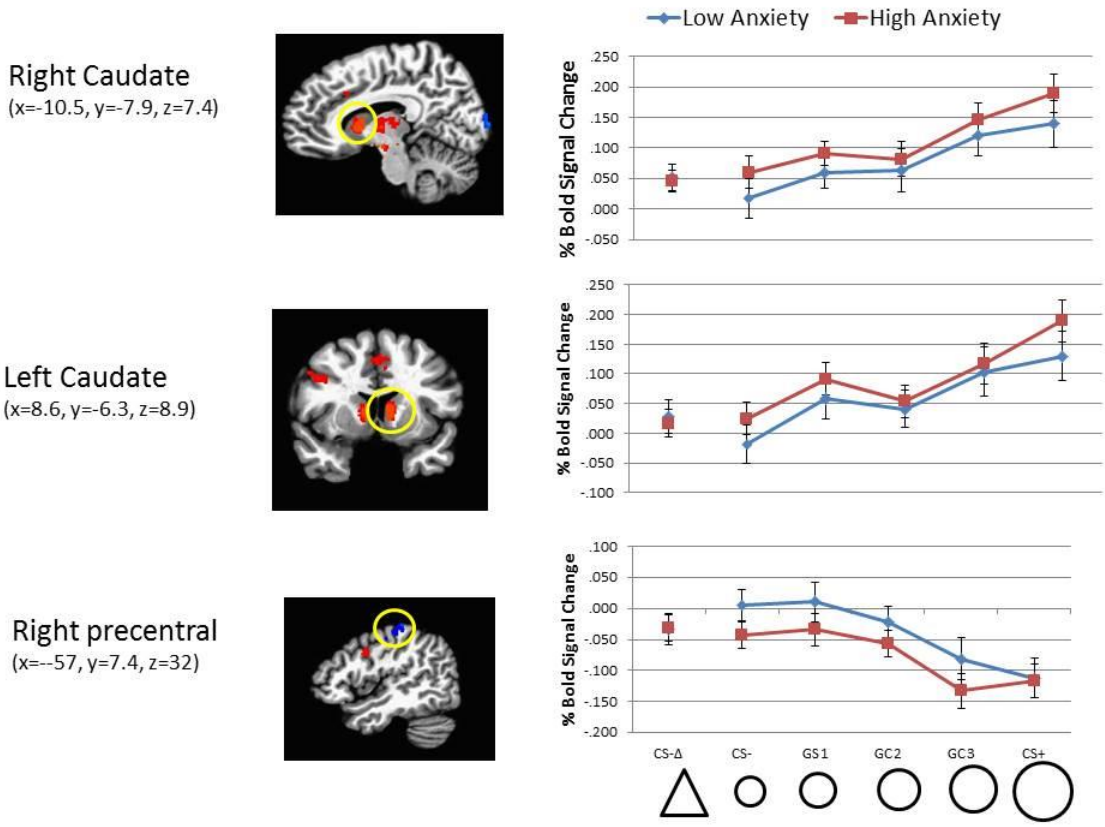

Figure 11: *Left Column:* Description and location of activation during Pavlovian analysis with coordinates in RAI format. *Middle Column:* image of activation for the fROI during Pavlovian analysis. *Right Column:* Percent BOLD signal change in the fROI during Pavlovian analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety).

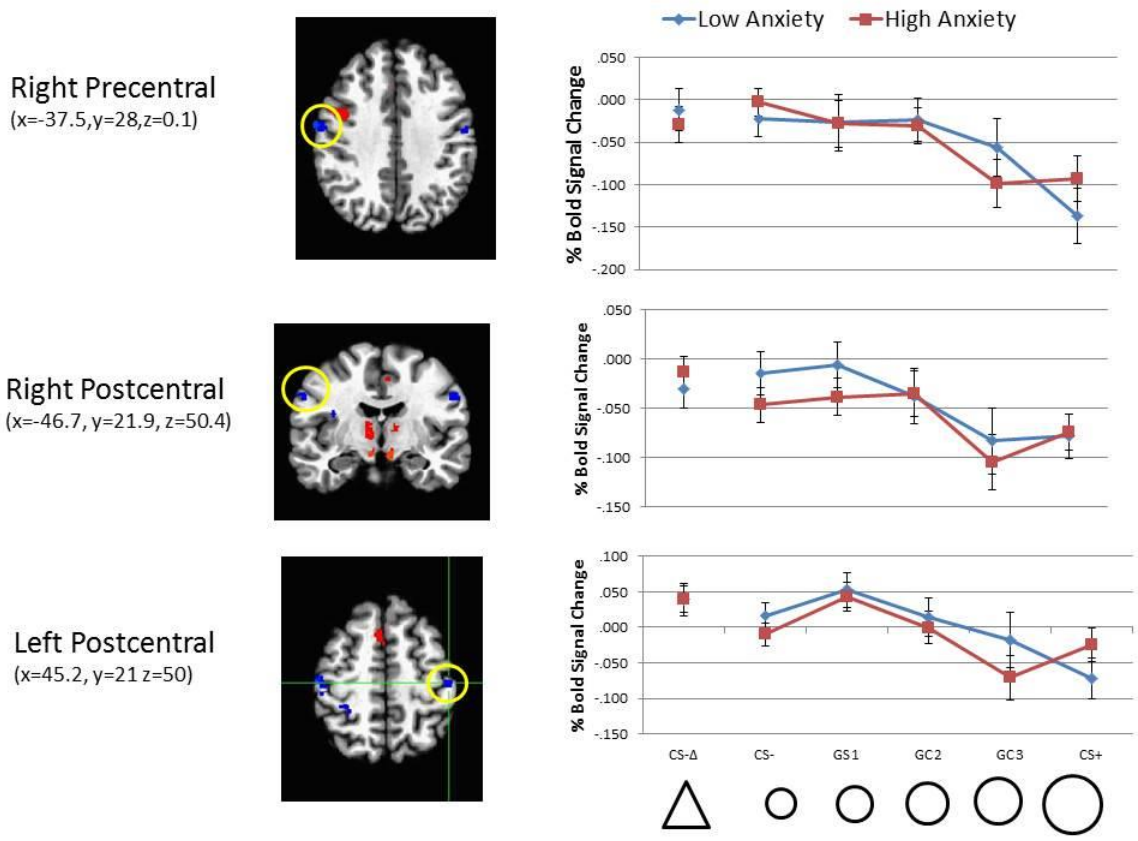

Figure 12: *Left Column:* Description and location of activation during Pavlovian analysis with coordinates in RAI format. *Middle Column:* image of activation for the fROI during Pavlovian analysis. *Right Column:* Percent BOLD signal change in the fROI during Pavlovian analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety).

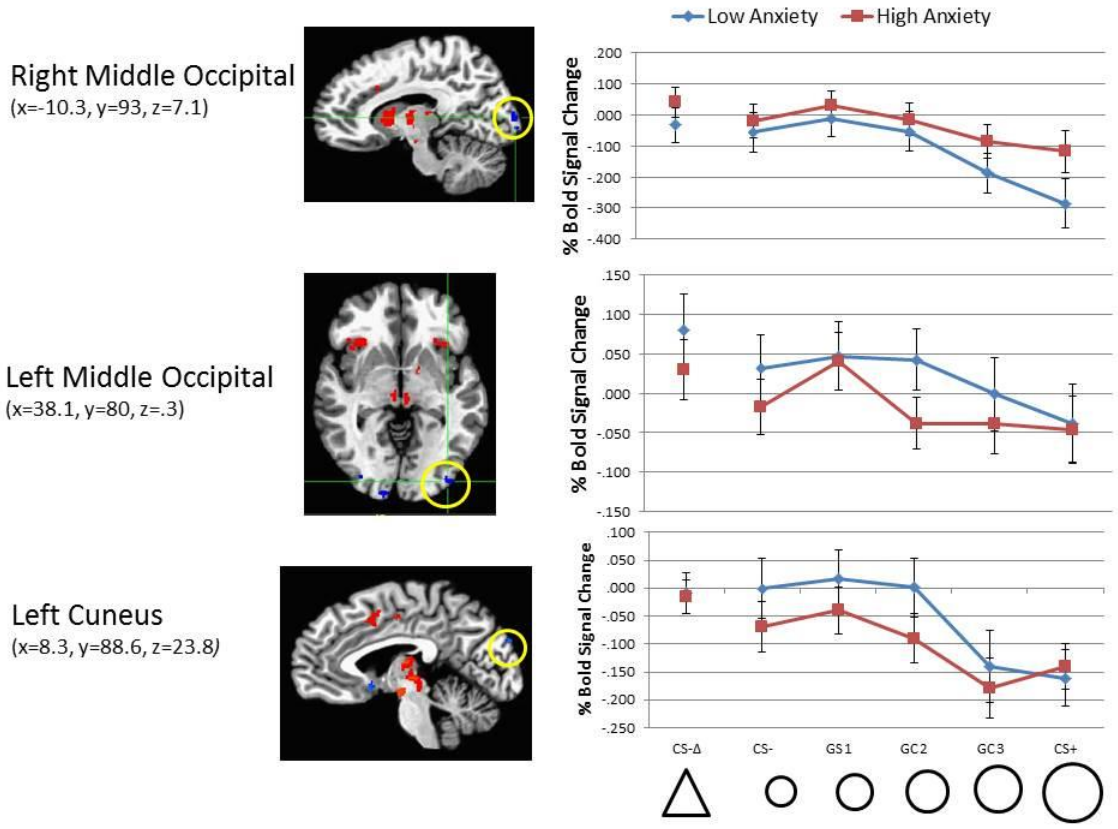

Figure 13: *Left Column:* Description and location of activation during Pavlovian analysis with coordinates in RAI format. *Middle Column:* image of activation for the fROI during Pavlovian analysis. *Right Column:* Percent BOLD signal change in the fROI during Pavlovian analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety).

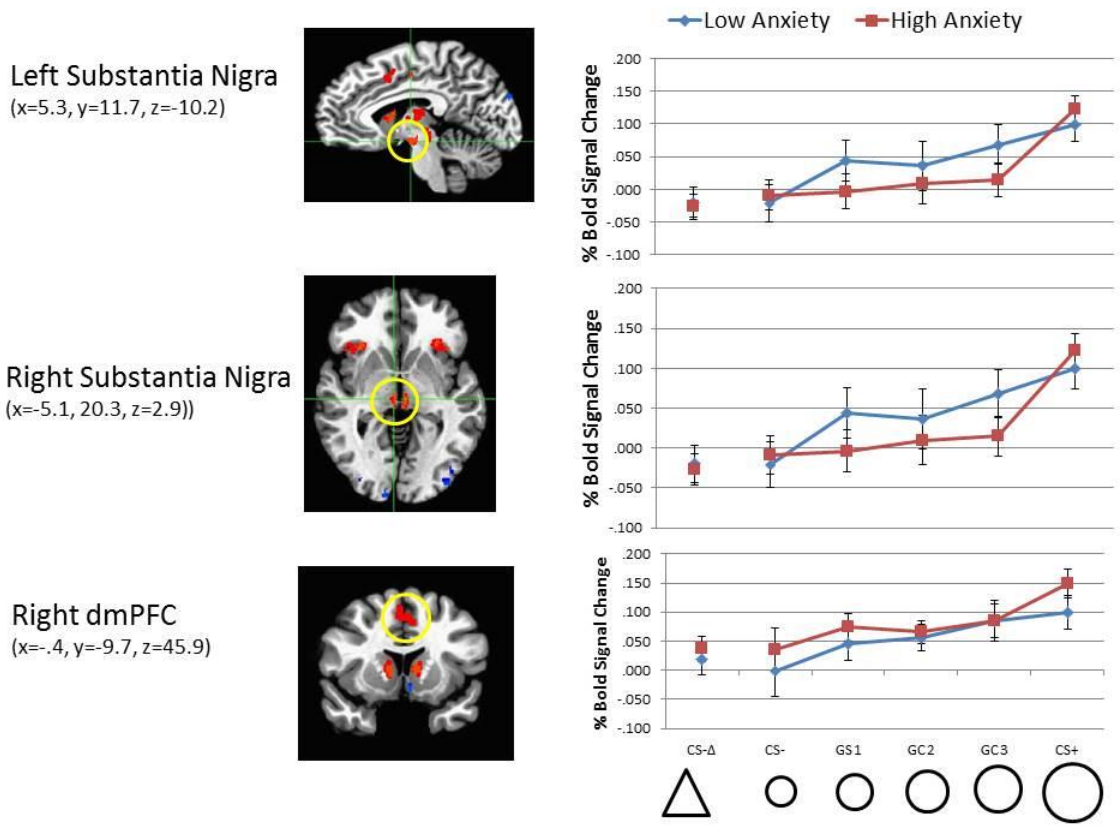

Figure 14: *Left Column:* Description and location of activation during Pavlovian analysis with coordinates in RAI format. *Middle Column:* image of activation for the fROI during Pavlovian analysis. *Right Column:* Percent BOLD signal change in the fROI during Pavlovian analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety).

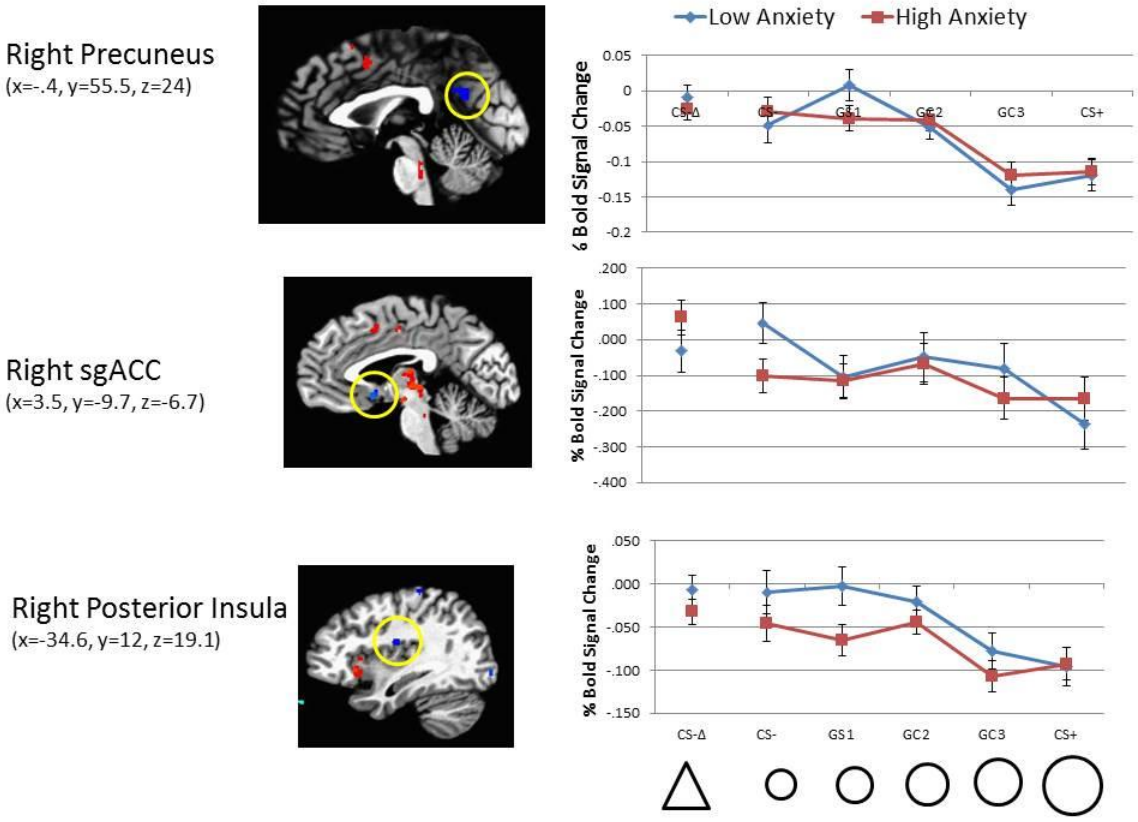

Figure 15: *Left Column:* Description and location of activation during Pavlovian analysis with coordinates in RAI format. *Middle Column:* image of activation for the fROI during Pavlovian analysis. *Right Column:* Percent BOLD signal change in the fROI during Pavlovian analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety).

Pavlovian Neural Correlates of Overall Avoidance

Overall avoidance was measured as total number of times the long path was chosen. This was then correlated with the sum of activation to all stimuli in each fROI. There were significant correlations in many regions including the left thalamus (r=.501, p=.018), the left cingulate gyrus (r=.428, p=.047), the right thalamus (r=.434, p=.049), the right inferior frontal gyrus (r=.465, p=.029), the primary visual cortex (r=.431, p=.045), the right substantia nigra (r=.533, p=.011), and another region in the right substantia nigra (r=.455, p=.033).

However, visual analysis of these correlations revealed an outlier contributing to these correlations. When removed, only a single correlation remained significant; the primary visual cortex (r=.434, p=.049). Overall avoidance was also related to harm avoidance (r=.469, p=.032) but not trait anxiety (r=.23, p>.3)

Pavlovian Neural Correlates of Adaptive Avoidance

Adaptive avoidance was assessed as a difference score between avoidance to the CS+ and avoidance to the CS-Δ . There were only 3 fROIs that were correlated with avoidance, all from negative gradients and therefore inverse correlations. The significant correlations were between avoidance and the right precentral gyrus(r=-.496, p=.019), the right anterior cingulate (r=-.429, p=.046) (ROI 27) and the left subgenual ACC (ROI 34) (r=-.425, p=.048). There were also trends in other inverse gradient fROIs: the left precentral gyrus (r=-.377, p=.083), the right postcentral gyrus (r=-.377, p=.083) another right anterior cingulate region (ROI19) (r=-.375, p=.086). There were also trends with positive gradients in the right substantia nigra (r=.384, p=.078) and the right para-

hippocampal gyrus (r=-.377, p=.083) although the latter activation did not follow a typical generalization gradient.

Using a forward regression model, two regions; the right precentral gyrus and left substantia nigra accounted for 44% of the variance (r=.663, p=.004).

The only region with a difference score that was also related to harm avoidance was the right substantia nigra (r=.552, p=.008). No regions were associated with trait anxiety.

Adaptive avoidance was also assessed with difference scores from the CS-. Again there were significant correlations between avoidance and the right precentral gyrus (r=-.590, p=.004), and the right para-hippocampal gyrus (r=.436, p=.042). Again the latter ROI is not a typical gradient. There were also trends in other inverse gradient fROIs: the left precentral gyrus (r=-.377, p=.083), and a region in the right anterior cingulate cortex (r=-.406, p=.061). There were trends with positive gradients in the left substantia nigra,(r=.369, p=.098) and another right parahippocampal region (r=.413, p=.016). None of these were also associated with either harm avoidance or trait anxiety.

Using a forward regression model, the right precentral gyrus, the two right parahippocampal regions, and the right caudate account for 84% of the variance. (r=.917, p=<.001).

Neural Correlates of Overgeneralized Pavlovian Conditioning, Risk Ratings and Maladaptive Avoidance

The relationships between overgeneralized Pavlovian conditioning in each ROI, risk ratings and maladaptive avoidance were assessed using four methods each discussed separately.

The first method used a difference score between all generalization stimuli and the triangular safety cue ((GC1+GC2+GC3))-CS-Δ). Using this measure of overgeneralization risk ratings and avoidance are highly correlated (r=.813, p<.001). There were no significant correlations between either of these measures of overgeneralization and harm avoidance or trait anxiety. Overgeneralized risk ratings were associated with Pavlovian overgeneralization in the left anterior insula (r=.423, p=.05), the left cuneus (-.616, p=.002), the left postcentral gyrus (r=-.657, p=.001) and the subgenual anterior cingulate cortex (sgACC) (r=-.455, p=.033). Maladaptive avoidance was significantly correlated with overgeneralized activations in these four regions as well; the left anterior insula (r=.432, p=.045), the left cuneus (r=-.504, p=.017), the left postcentral gyrus (r=.-.589, p=.004), and the subgenual anterior cingulate (r=-.549, p=.009). Maladaptive avoidance was also correlated with one region that was not also associated with perceived risk; the right postcentral gyrus (r=-.564, p=.006).

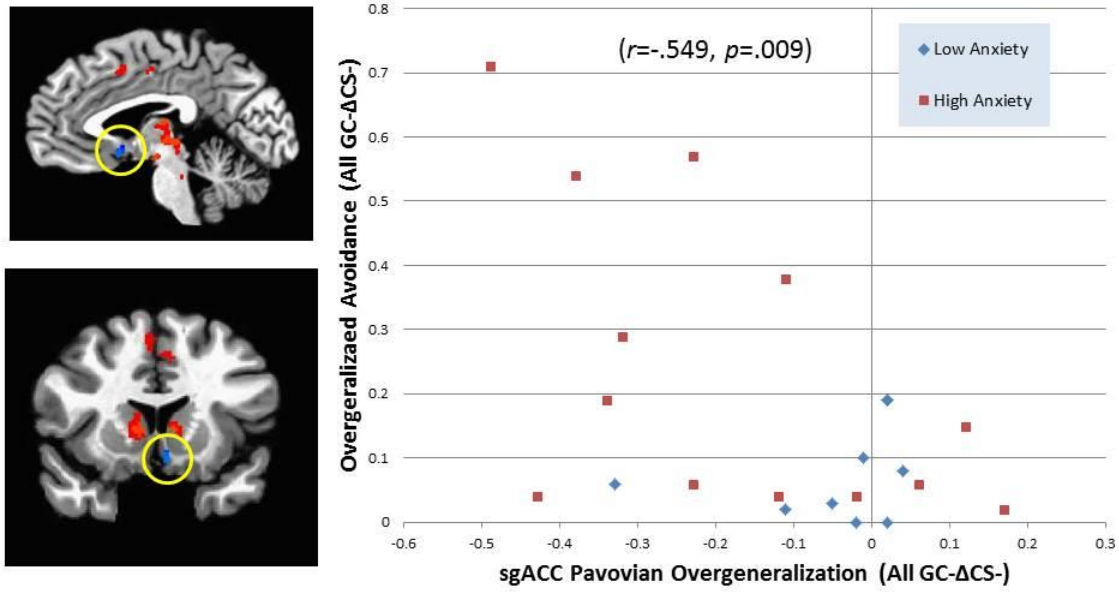

Figure 16: Left: Images showing subgenual anterior cingulate cortex activation (sgACC). Right: Scatterplot showing the relationship between overgeneralization in the subgenual anterior cingulate cortex during Pavlovian trials and overgeneralized avoidance (as measured by a difference score between the average of the generalization stimuli and the triangular conditioned safety cue). Units on the x-axis are in Percent Bold Signal Change and on the y-axis are percentages. Each individual is also color/shape coded for group membership based on trait anxiety on the Spielberger State Trait Anxiety Inventory (red squares are high trait anxiety group, blue diamond are low anxiety group).

When only significant variables were entered together in a regression model, only the left postcentral gyrus region was retained in the model. When not restrained to original predictors, the overall model included the left post-central gyrus, a region in the right anterior cingulate, and the left bed nucleus of the stria termanalis ($F(3,18)=9.307$, $p=.001$, $R=.78$, $R^2=.608$).

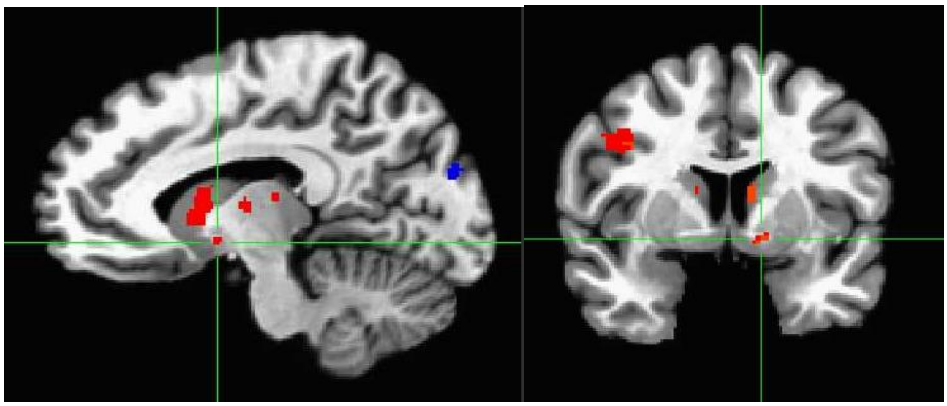

Figure 17: Sagittal and Coronal view of the Bed Nucleus of the Stria Termanalis activation.

The second method of assessing overgeneralization was a deviation from linearity score used in previous research by our group. This score averages the endpoints of the gradient (CS+ and CS-Δ) and then subtracts this from the average of the generalization stimuli ((GC1+GC2+GC3)/3))-((CS+ + CS-Δ)/2). Using this measure, risk ratings and avoidance were only moderately correlated (r=.564, p=.006). There was a trend towards a relationship between overgeneralized risk ratings and higher anxiety (r=.409, p=.059). There were significant correlations between overgeneralized risk ratings and overgeneralization in Pavlovian ROI in one region, the right substantia nigra (r=-.515, p=.014). However, there were several correlations between overgeneralized avoidance and overgeneralization in Pavlovian fROIs: in the left thalamus (r=-.571, p=.006), the right precentral gyrus (r=-.461, p=.031), the right inferior occipital gyrus (r=-.428, p=.047), the left post central gyrus (r=-.651, p=.001), the superior frontal gyrus (r=-.499, p=.018), the right substantia nigra (r=-.453, p=.034) and the right posterior insula (r=-.-557, p=.007).

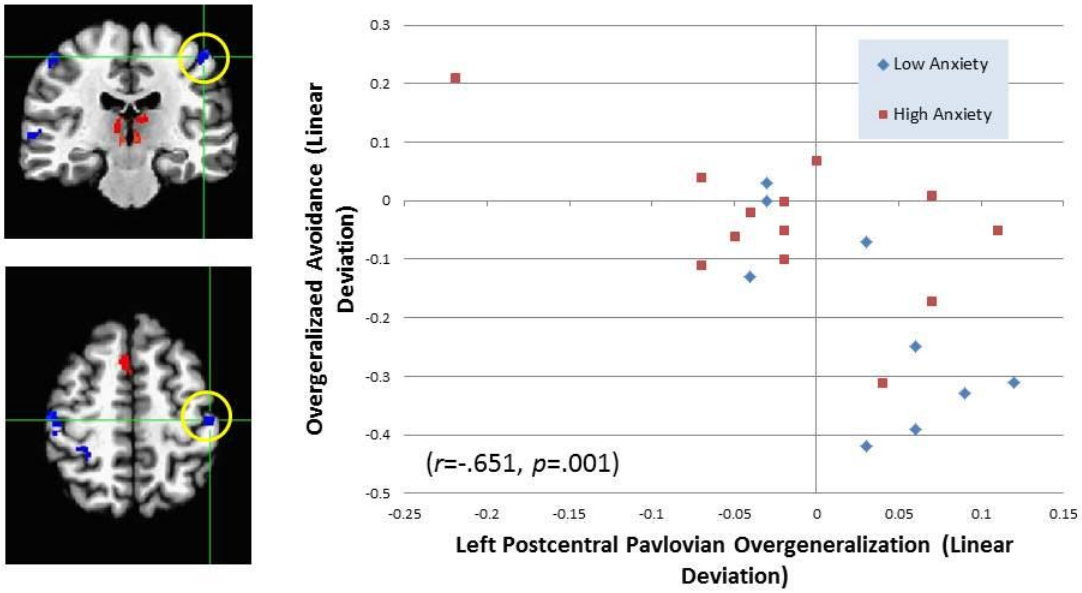

Figure 18: Left: Images showing Left Postcentral gyrus activation. Right: Scatterplot showing the relationship between overgeneralization in the left postcentral gyrus during Pavlovian trials and overgeneralized avoidance (as measured by a deviation from linearity score). Units on the x-axis are in Percent Bold Signal Change and on the y-axis are percentages. Each individual is also color/shape coded for group membership based on trait anxiety on the Spielberger State Trait Anxiety Inventory (red squares are high trait anxiety group, blue diamond are low anxiety group). NOTE: With outlier removed, correlation still significant (r=-.539, p=.012).

When entered into a forward regression model together, no variables other than the left post-central gyrus activation were included in the model. When not constrained to original predictors, a model with the left post-central gyrus, right post central gyrus and the right posterior insula emerged as accounting for most of the variation in avoidance (F=11.964, p<.001, R=.816, R^2=.666).

The third measure simply assessed avoidance to the GC3 vs the triangular safety cue ΔCS-. This measure showed moderately strong correlation between risk ratings and avoidance generalization (r=.606, p=.003). Maladaptive avoidance measured this was also significantly correlated with trait anxiety (r=.424, p=.049), but not harm avoidance (r=.23, p>.1). Maladaptive avoidance to the GC3 was associated with increased generalization in the inferior occipital gyrus (r=.500, p=.018), the right post-central gyrus (p=-.525, r=.012), a region in the right brainstem (r=-.425, p=.048) and the subgenual ACC (p=-.436, r=.042). None of these regions were also associated with overgeneralized risk ratings although there was a trend in the right post-central gyrus (r=-.373, p=.088). There was only one region that was associated with increased risk ratings, the left post-central gyrus (r=-.522, p=.008).

When constrained to significant predictors, only the right post-central gyrus was included in the regression model. When unconstrained, both the right post-central gyrus and the bed nucleus of the stria termanalis were included (F(2,19)=7.328, p=.005, R=.658, R^2=.432).

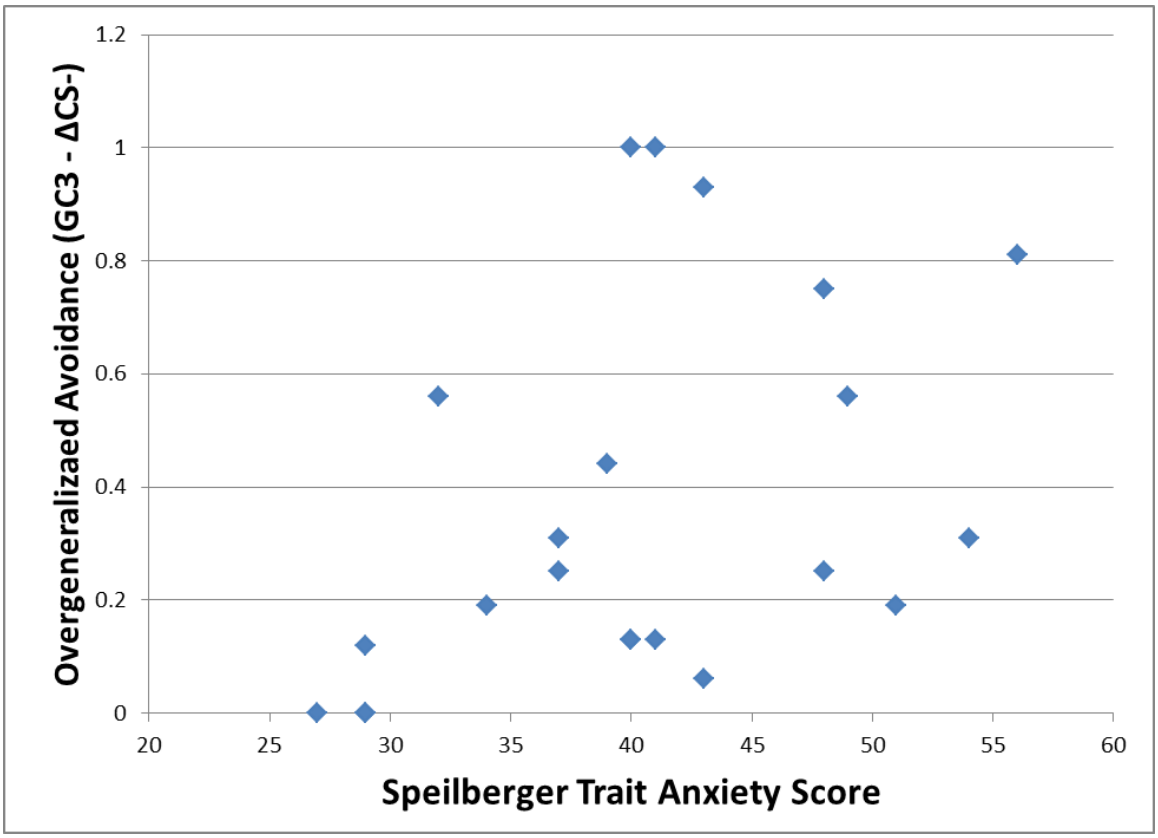

Figure 19: Scatterplot showing the relationship between Spielberger Trait Anxiety and overgeneralized avoidance (as measured by a difference score between the generalization stimulus that was most perceptually similar to the conditioned danger cue).

The fourth measure assessed the difference between the CS+ and the GC3. There was a moderate correlation between avoidance and risk ratings using this measure (r=.574, p=.005). Maladaptive avoidance using this measures demonstrated a moderately strong relationship with %BOLD signal change also using this difference score in the posterior insula (r=-.487, p=.021). This was not at all correlated with risk ratings. However three regions; one in the right primary visual cortex (r=-.510, p=.015), the right thalamus (r=-.488, p=.037), and the right parahippocampal gyrus (r=-.501, p=.018) were all correlated with risk using this measure, but not avoidance.

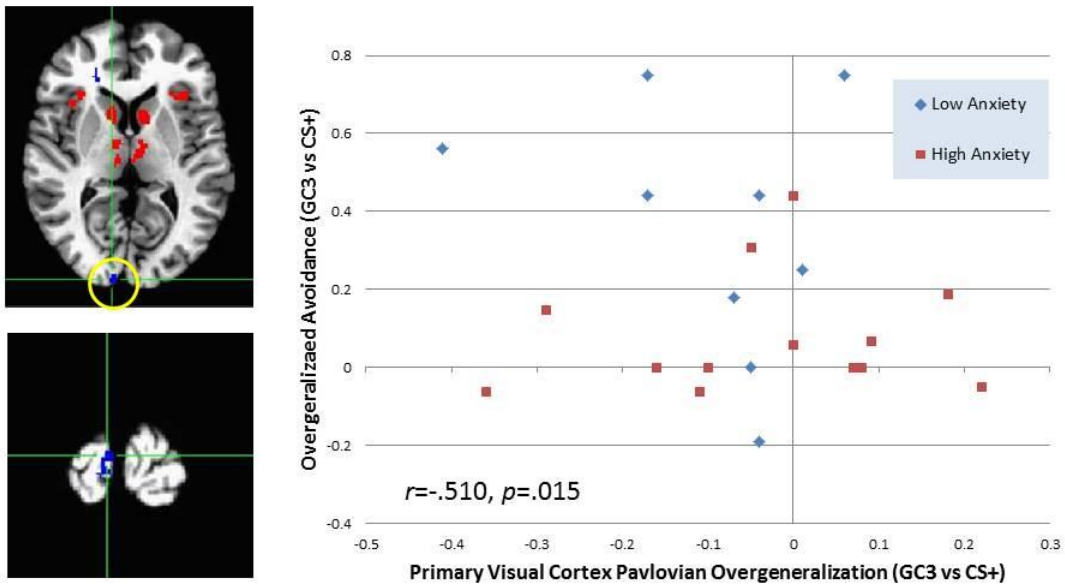

Figure 20: Left: Images showing activation in the right primary visual cortex. Right: Scatterplot showing the relationship between overgeneralization in the right primary visual cortex during Pavlovian trials and overgeneralized avoidance (as measured by a deviation from linearity score). Units on the x-axis are in Percent Bold Signal Change and on the y-axis are percentages. Each individual is also color/shape coded for group membership based on trait anxiety on the Spielberger State Trait Anxiety Inventory. (red squares are high trait anxiety group, blue diamond are low anxiety group).

Instrumental Neurobiological Regions

25 fROIs were identified. All of these demonstrated a gradient, although the gradient was less steep in most regions than in previous studies. Some gradients even demonstrated more of an inverted U shape than a typical quadratic/linear decline (see figures 19-26).

Table 3: *Brain Areas responding differentially to CS+ and ΔCS- during Instrumental analysis that served as functional regions of interest (fROIs).*

			Peak Coordinates		
Brain Region	**Direction**	**Volume**	**X**	**Y**	**Z**
Right Medial Frontal	CS+ > ΔCS-	5916.75	-1.5	-25.5	41.5
Right Anterior Insula	CS+ > ΔCS-	4942.875	-49.5	-19.5	-5
Right dlPFC	CS+ > ΔCS-	3335.063	-46.5	-12	46
Left Anterior Insula	CS+ > ΔCS-	2131.5	28.5	-19.5	-6.5
Right Supramarginal	CS+ > ΔCS-	1525.125	-55.5	45	19
Right Middle Frontal	CS+ > ΔCS-	1451.625	-42	-55.5	8.5
Right Precuneus	CS+ > ΔCS-	1249.5	-15	75	44.5
Left Pyramis	CS+ > ΔCS-	1185.188	7.5	75	-21.5
Right Superior Parietal	CS+ > ΔCS-	1185.188	-28.5	55.5	43
Left Superior Temporal	ΔCS- > CS+	624.75	66	19.5	7
Precentral Sulcus	CS+ > ΔCS-	560.4375	-33	-1.5	32.5
Right Lingual/Fusiform	CS+ > ΔCS-	551.25	-21	69	-11
Right Post Central	ΔCS- > CS+	551.25	-27	30	53.5
Right Superior Temporal	CS+ > ΔCS-	542.0625	-46.5	46.5	13
Right Posterior Cingulate	CS+ > ΔCS-	486.9375	-1.5	24	26.5
Right Middle Frontal	CS+ > ΔCS-	395.0625	-31.5	-51	1
Right Precuneus	CS+ > ΔCS-	376.6875	-7.5	70.5	52
Right Lingual	CS+ > ΔCS-	367.5	-7.5	73.5	4
Right Declive	CS+ > ΔCS-	339.9375	-36	55.5	-20
Right Postcentral	ΔCS- > CS+	339.9375	-16.5	42	62.5
Left Postcentral	ΔCS- > CS+	330.75	24	33	55
Left Culmen	CS+ > ΔCS-	248.0625	33	49.5	-26
Right Precuneus	CS+ > ΔCS-	248.0625	-10.5	64.5	31
Right dlPFC	CS+ > ΔCS-	238.875	-46.5	-9	26.5
Right Precuneus	CS+ > ΔCS-	238.875	-22.5	63	38.5

NOTE: Peak Coordinates are presented in RAI format

During the choice period, there were significant multivariate group*stimulus effects in the right precentral sulcus ($F(5,16)=3.295$, $p=.031$; within subjects $F(5,100)=2.676$, $p=.026$), right postcentral gyrus ($F(5,16)=2.858$ $p=.05$; within subjects $F(5,100)=2.461$, $p=.038$) and the left culmen ($F(5,16)=3.556$, $p=.024$).

In the right precentral sulcus the low anxiety group demonstrated increased responding compared to the CS-Δ for CS+ ($t(8)=-7.287$, $p<.001$), GC3($t(8)=-3.425$, $p=.009$) and GC2 ($t(8)=-2.303$, $p=.05$), but not the GC1 or GC2. In the high anxiety group there was increased BOLD% signal change compared to the CS-Δ in the CS+ ($t(12)=-4.137$, $p=.001$), GC3 ($t(12)=-3.12$, $p=.009$), GC2 ($t(12)=-4.869$, $p<.001$) and GC1 ($t(12)=-5.530$, $p<.001$). In the right postcental gyrus, a negative gradient, the low anxiety group had decreased BOLD% signal change responding to the CS+ only ($t(8)=2.595$, $p=.032$) when compared to CS-Δ . The high anxiety group had decreased BOLD% signal change to the CS+ ($t(12)=4.023$, $p=.002$), GC3 ($t(12)=4.086$, $p=.002$), GC2 ($t(12)=3.483$, $p=.005$), and GC1 ($t(12)=2.612$, $p=.023$).

This effect was reversed in the left culmen as the low anxiety group had increased % BOLD signal change to the CS+ ($t(8)=-6.602$, $p<.001$), and GC3 ($t(8)=-4.398$, $p=.002$), and the high anxiety group only had increased % BOLD signal change to the CS+ ($t(12)=-2.962$, $p=.012$) and not the GC3 ($t(12)=-1.333$, $p=.207$) or any other stimulus.

Additional within-subject group*stimulus differences were found in three separate regions within the right precuneus: (Region 9) ($F(5,100)=2.305$ $p=.05$; group*linear difference $F(1,20)=3.818$, $p=.068$); (Region 17) ($F(5,100)=2.519$, $p=.034$) and (Region 25) ($F(5,100)=2.308$, $p=.035$). In the first precuneus region (9), the low anxiety group had differences from CS-Δ baseline for CS+ ($t(8)=-4.507$, $p=.002$), GC3 ($t(8)=-3.575$, $p=.007$), GC2 ($t(8)=-4.471$, $p=.002$) and even CS- ($t(8)=-2.512$, $p=.036$), but not GC1 ($t(8)=-2.264$, $p=.053$). The high anxiety group had differences from CS-Δ baseline in

CS+ (t(12)=-3.141 p=.009), GC3 (t(12)=-3.387, p=.005), GC2 (t(12)=-3.074, p=.010) and GC1 (t(12)=-2.407, p=.033), but not CS- (t(12)=.085, p=.934). In precuneus region 17, the low anxiety group had differences from CS-Δ baseline for CS+ (t(8)=-5.692, p<.001), GC3 (t(8)=-3.725, p=.006), and GC2 (t(8)=-3.236, p=.012), but not GC1 or CS-. The high anxiety group had differences from CS-Δ baseline in CS+ (t(12)=-3.413 p=.005) and GC3 (t(12)=-2.351, p=.037), but not GC2, GC1, or CS-. In precuneus region 25, the low anxiety group had differences from CS-Δ baseline for CS+ (t(8)=-4.876, p=.001), GC3 (t(8)=-2.337, p=.048), GC2 (t(8)=-3.691, p=.006), and CS- (t(8)=-2.653, p=.029), but not GC1. The high anxiety group had differences from CS-Δ baseline in CS+ (t(12)=-2.432 p=.032) and GC3 (t(12)=-2.935, p=.012), but not GC2, GC1, or CS-.

Additional between subject effects were found in the orbitofrontal cortex (F(1,20)=6.019 p=.023) and a trend toward between group differences in yet another region, and the largest region, in the right precuneus ((1,20)F=3.586, p=.073).

When comparing the high and low anxiety groups within each stimulus class for avoided and non-avoided decisions separately there were several differences. There were differences in the right dlPFC during presentation of the GC3 when the avoidance response was chosen (t(18)=2.104, p=.05) with greater activation in the low anxiety group. In bi-lateral anterior insula the high anxiety group had greater activation during presenation of the GC1 when the short path was taken (right t(20)=2.355, p=.029; left t(20)=-2.439, p=.024). In the left superior temporal gyrus there was greater activation in the low anxiety group during presentation of the CS+ when the short path was taken

(t(16)=2.536, p=.022). In the right precentral sulcus the low anxiety group showed greater activation when the avoidance response was chosen for both GC3 (t(18)=3.154, p=.005) and CS+ (t(19)=2.791, p=.012). The low anxiety group had higher responding to the GC3 when the avoidance response was chosen in one post central region and it was a trending effect in the other postcentral region (fROI13; t(18)=3.417, p=.003; fROI20 t(18)=2.000, p=.061). In one precuneus region (fROI23) the high anxiety group deomonstrated greater activation to the CS+ when the short path was chosen (t(16)=2.331, p=.033).

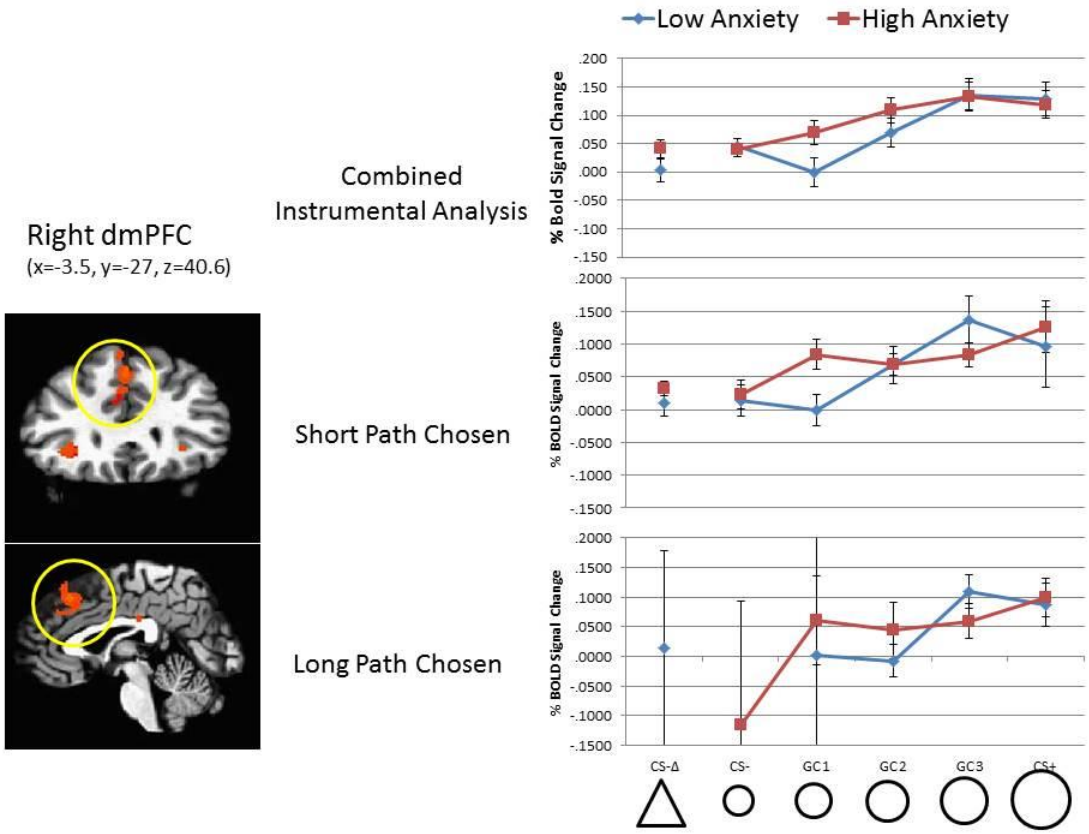

Figure 21: *Left Column:* Description, location and image of activation for the fROI during Instrumental analysis with coordinates in RAI format. *Right Column:* Percent BOLD signal change in the fROI during Instrumental analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety). *Top Right:* Percent BOLD signal change for all trials during the instrumental analysis combined. Middle Right: Percent BOLD signal change only for trials in which the short path was chosen by the participant. Bottom Right: Percent BOLD signal change for only trials in which the long path was chosen. Data was only plotted if there were 2 or more individuals in the group with at least two responses for that stimulus.

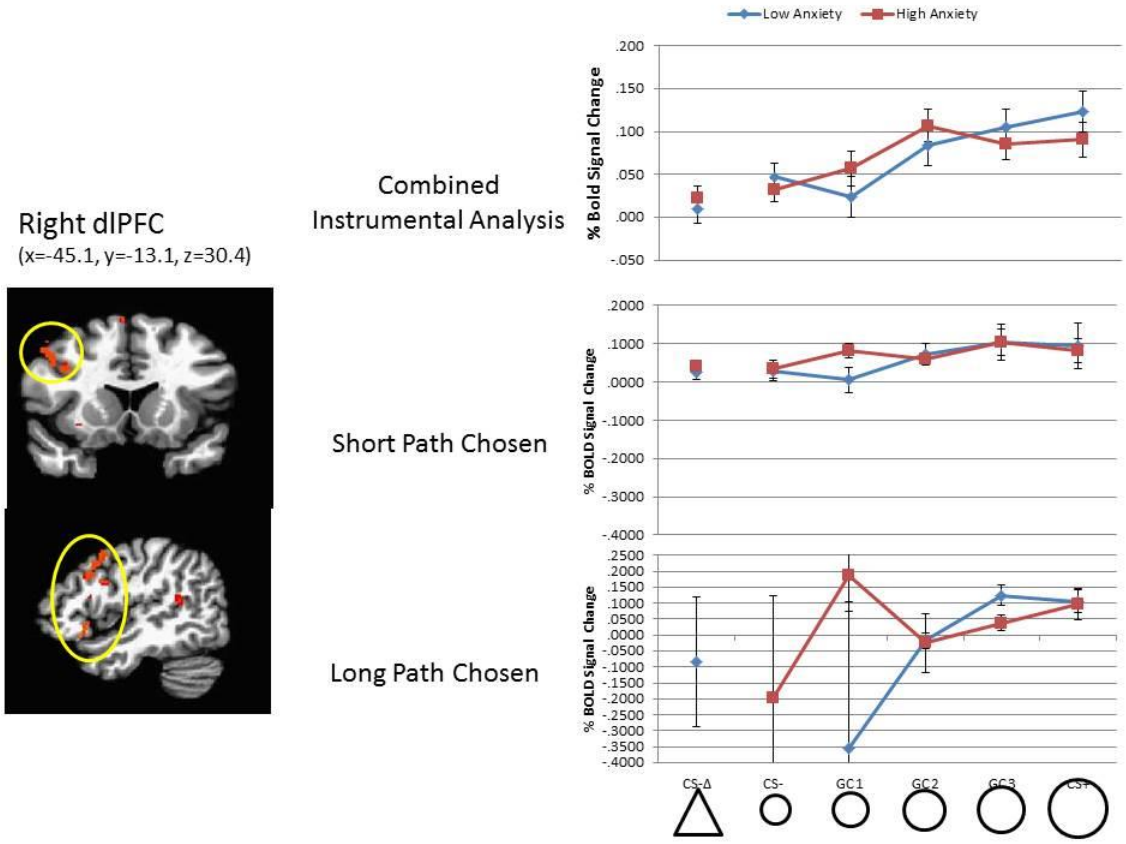

Figure 22: *Left Column:* Description, location and image of activation for the fROI during Instrumental analysis with coordinates in RAI format. *Right Column:* Percent BOLD signal change in the fROI during Instrumental analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety). *Top Right:* Percent BOLD signal change for all trials during the instrumental analysis combined. Middle Right: Percent BOLD signal change only for trials in which the short path was chosen by the participant. Bottom Right: Percent BOLD signal change for only trials in which the long path was chosen. Data was only plotted if there were 2 or more individuals in the group with at least two responses for that stimulus.

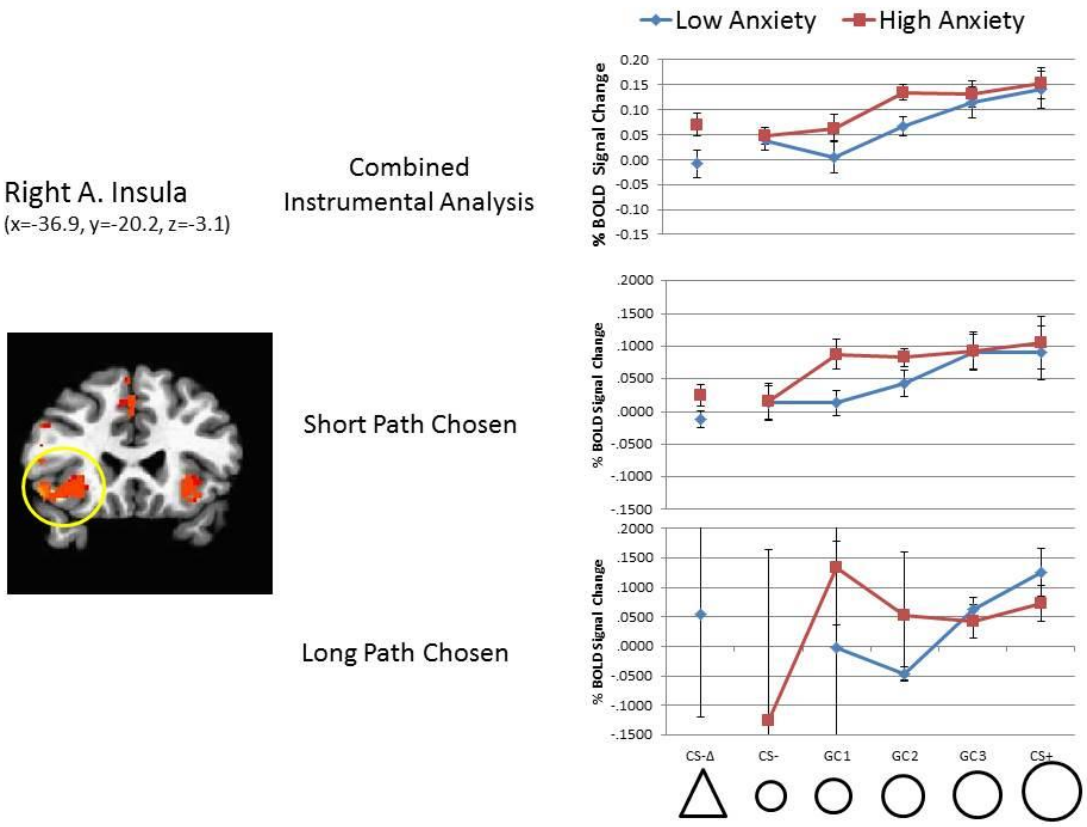

Figure 23: *Left Column:* Description, location and image of activation for the fROI during Instrumental analysis with coordinates in RAI format. *Right Column:* Percent BOLD signal change in the fROI during Instrumental analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety). *Top Right:* Percent BOLD signal change for all trials during the instrumental analysis combined. Middle Right: Percent BOLD signal change only for trials in which the short path was chosen by the participant. Bottom Right: Percent BOLD signal change for only trials in which the long path was chosen. Data was only plotted if there were 2 or more individuals in the group with at least two responses for that stimulus.

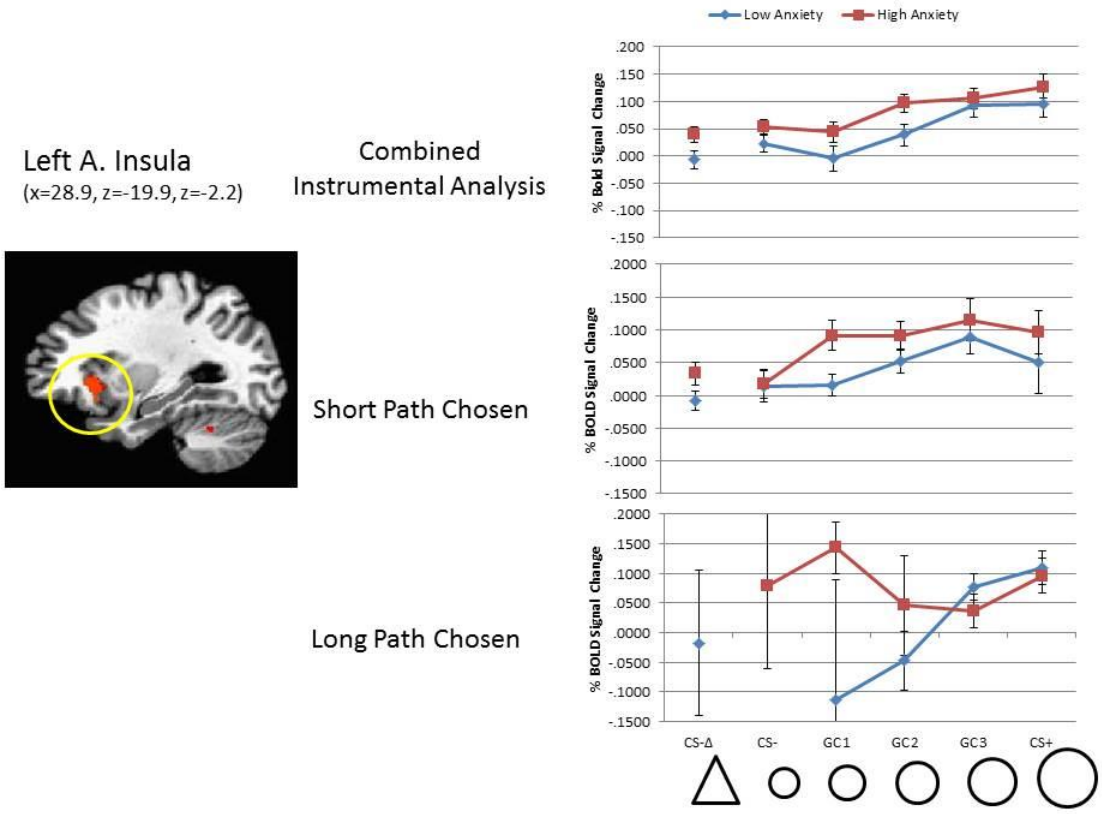

Figure 24: *Left Column:* Description, location and image of activation for the fROI during Instrumental analysis with coordinates in RAI format. *Right Column:* Percent BOLD signal change in the fROI during Instrumental analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety). *Top Right:* Percent BOLD signal change for all trials during the instrumental analysis combined. Middle Right: Percent BOLD signal change only for trials in which the short path was chosen by the participant. Bottom Right: Percent BOLD signal change for only trials in which the long path was chosen. Data was only plotted if there were 2 or more individuals in the group with at least two responses for that stimulus.

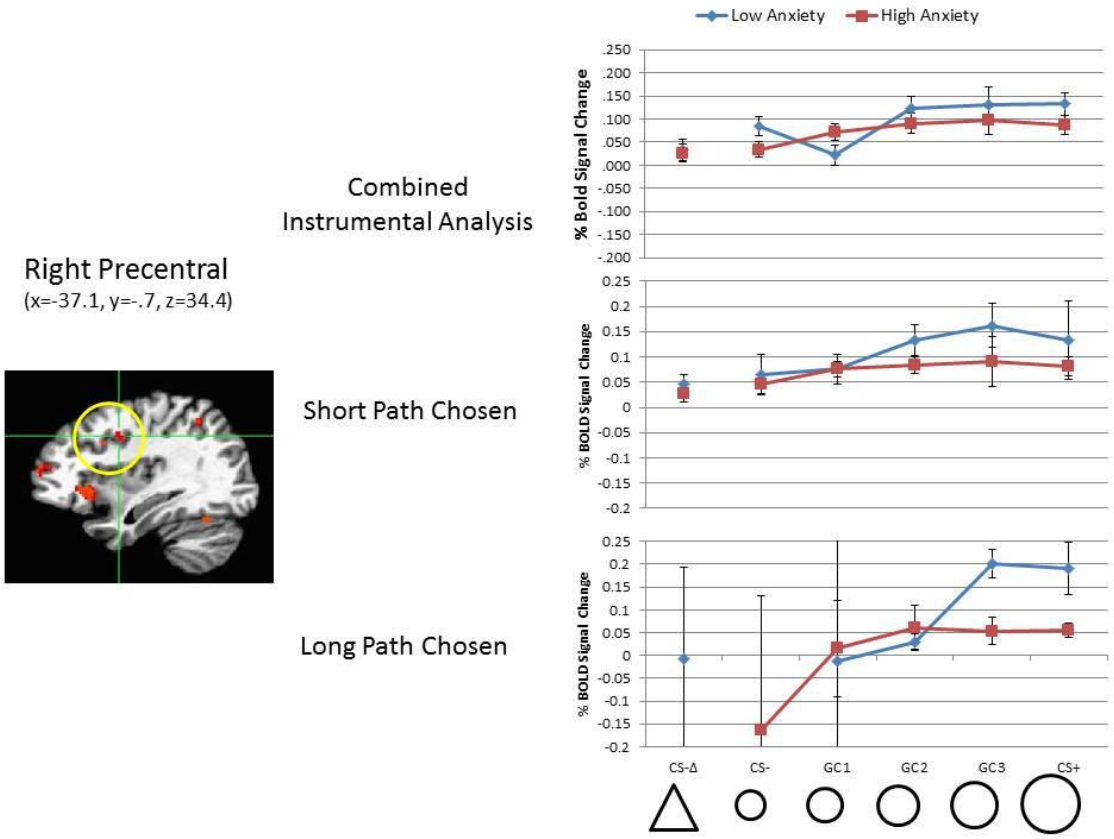

Figure 25: *Left Column:* Description, location and image of activation for the fROI during Instrumental analysis with coordinates in RAI format. *Right Column:* Percent BOLD signal change in the fROI during Instrumental analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety). *Top Right:* Percent BOLD signal change for all trials during the instrumental analysis combined. Middle Right: Percent BOLD signal change only for trials in which the short path was chosen by the participant. Bottom Right: Percent BOLD signal change for only trials in which the long path was chosen. Data was only plotted if there were 2 or more individuals in the group with at least two responses for that stimulus.

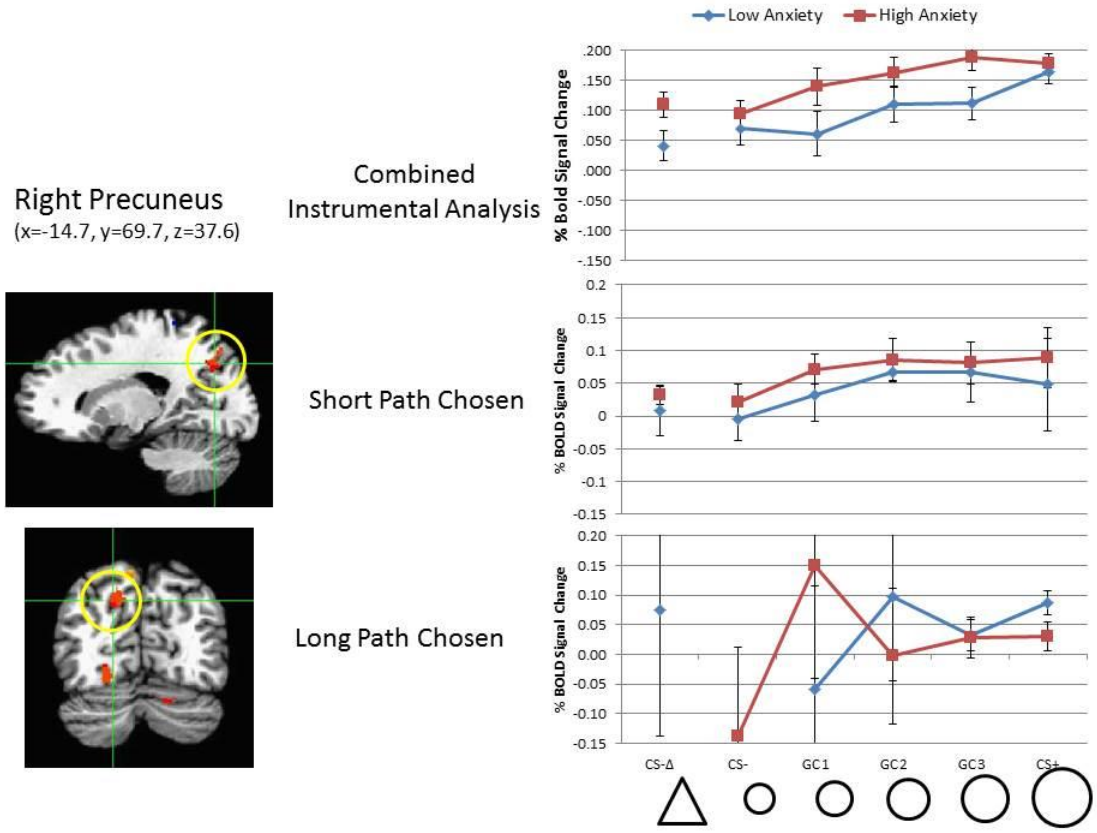

Figure 26: *Left Column:* Description, location and image of activation for the fROI during Instrumental analysis with coordinates in RAI format. *Right Column:* Percent BOLD signal change in the fROI during Instrumental analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety). *Top Right:* Percent BOLD signal change for all trials during the instrumental analysis combined. Middle Right: Percent BOLD signal change only for trials in which the short path was chosen by the participant. Bottom Right: Percent BOLD signal change for only trials in which the long path was chosen. Data was only plotted if there were 2 or more individuals in the group with at least two responses for that stimulus.

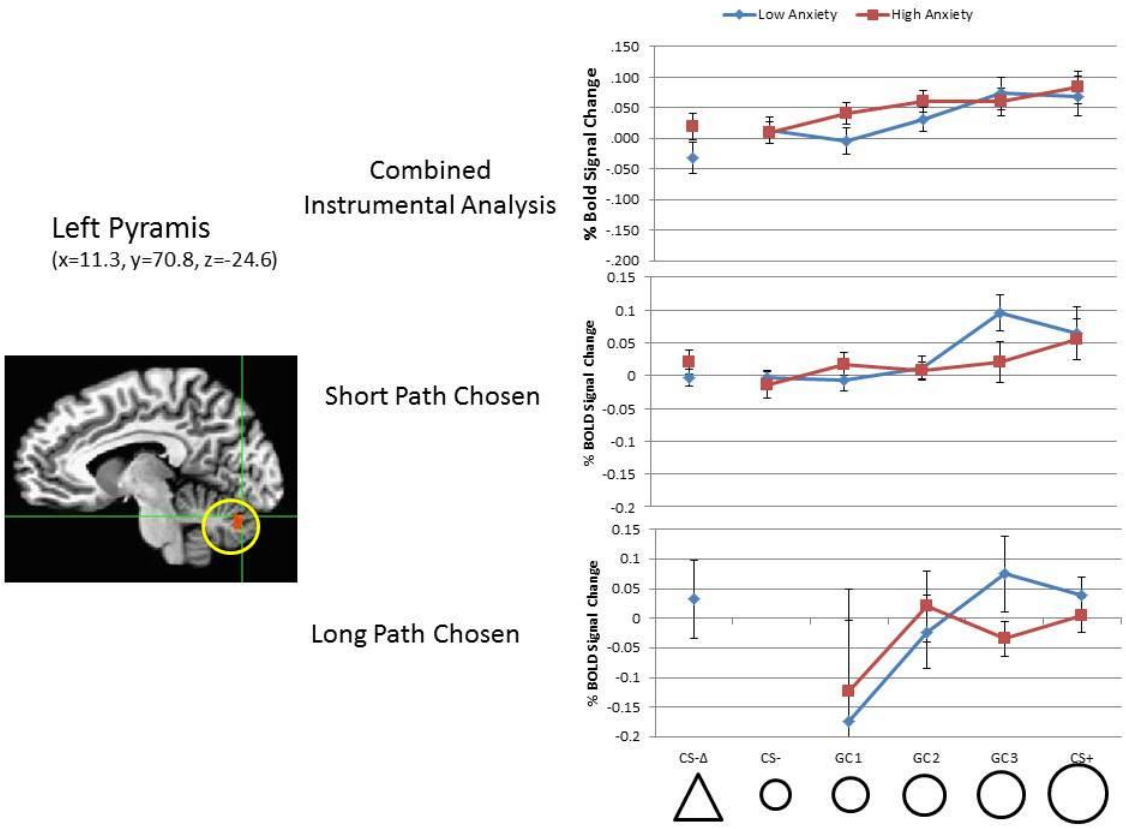

Figure 27: *Left Column:* Description, location and image of activation for the fROI during Instrumental analysis with coordinates in RAI format. *Right Column:* Percent BOLD signal change in the fROI during Instrumental analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety). *Top Right:* Percent BOLD signal change for all trials during the instrumental analysis combined. Middle Right: Percent BOLD signal change only for trials in which the short path was chosen by the participant. Bottom Right: Percent BOLD signal change for only trials in which the long path was chosen. Data was only plotted if there were 2 or more individuals in the group with at least two responses for that stimulus.

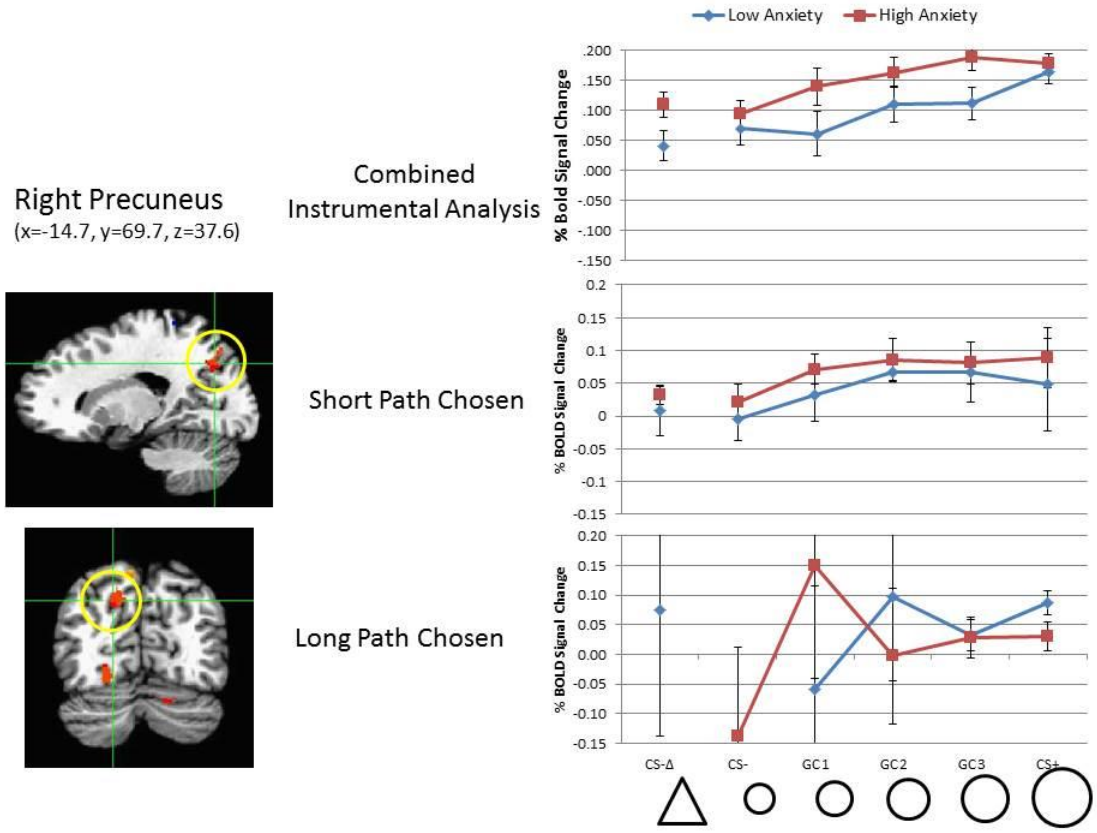

Figure 28: *Left Column:* Description, location and image of activation for the fROI during Instrumental analysis with coordinates in RAI format. *Right Column:* Percent BOLD signal change in the fROI during Instrumental analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety). *Top Right:* Percent BOLD signal change for all trials during the instrumental analysis combined. Middle Right: Percent BOLD signal change only for trials in which the short path was chosen by the participant. Bottom Right: Percent BOLD signal change for only trials in which the long path was chosen. Data was only plotted if there were 2 or more individuals in the group with at least two responses for that stimulus.

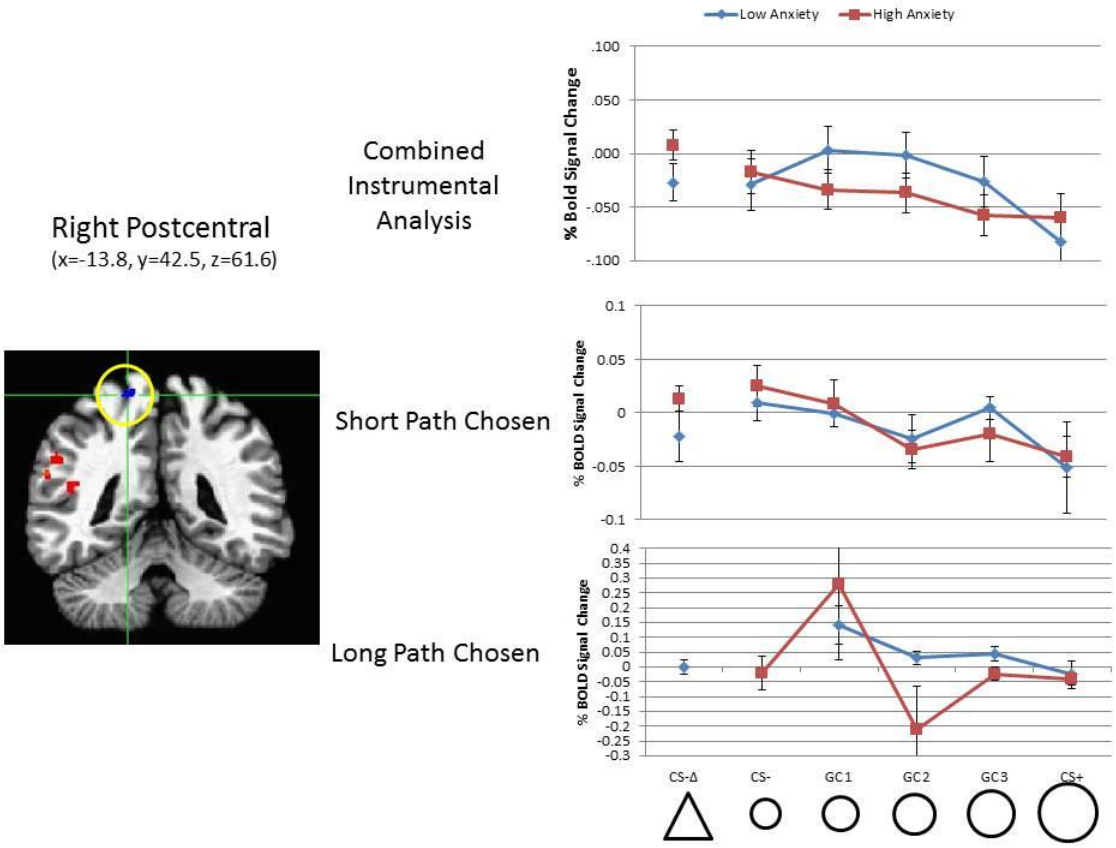

Figure 29: *Left Column:* Description, location and image of activation for the fROI during Instrumental analysis with coordinates in RAI format. *Right Column:* Percent BOLD signal change in the fROI during Instrumental analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety). *Top Right:* Percent BOLD signal change for all trials during the instrumental analysis combined. Middle Right: Percent BOLD signal change only for trials in which the short path was chosen by the participant. Bottom Right: Percent BOLD signal change for only trials in which the long path was chosen. Data was only plotted if there were 2 or more individuals in the group with at least two responses for that stimulus.

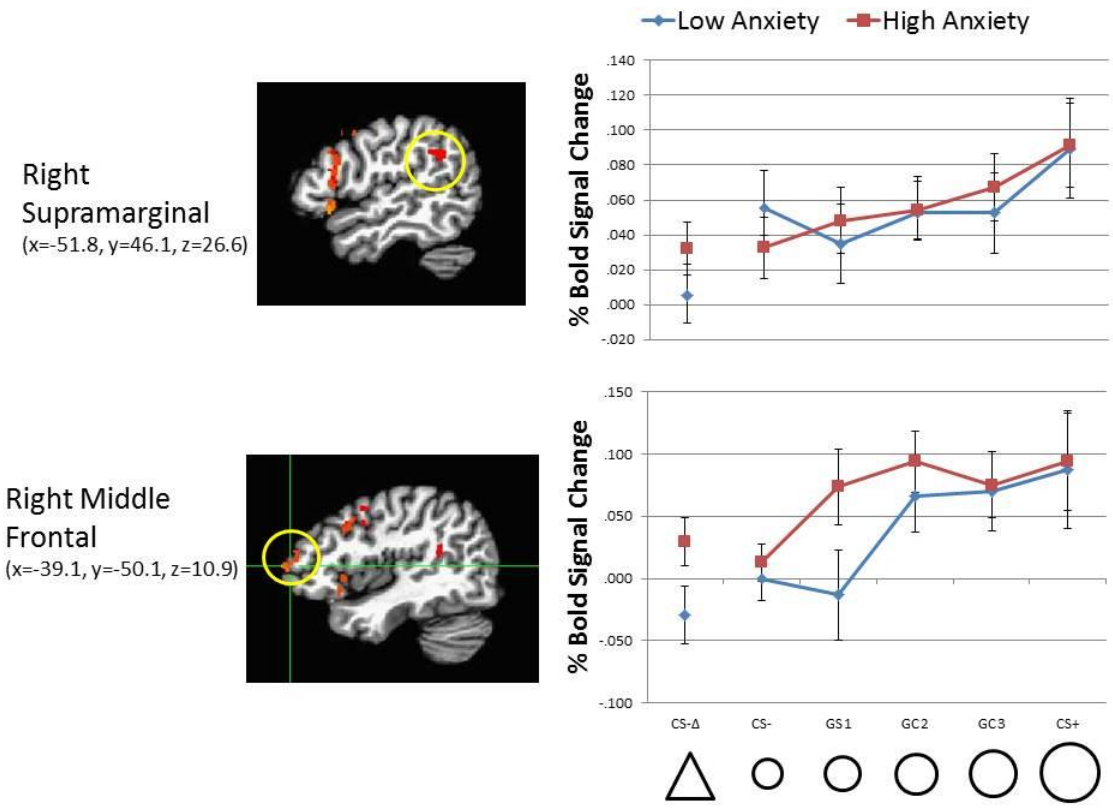

Figure 30: *Left Column:* Description and location of activation during Instrumental analysis with coordinates in RAI format. *Middle Column:* image of activation for the fROI during Instrumental analysis. *Right Column:* Percent BOLD signal change in the fROI during Instrumental analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety).

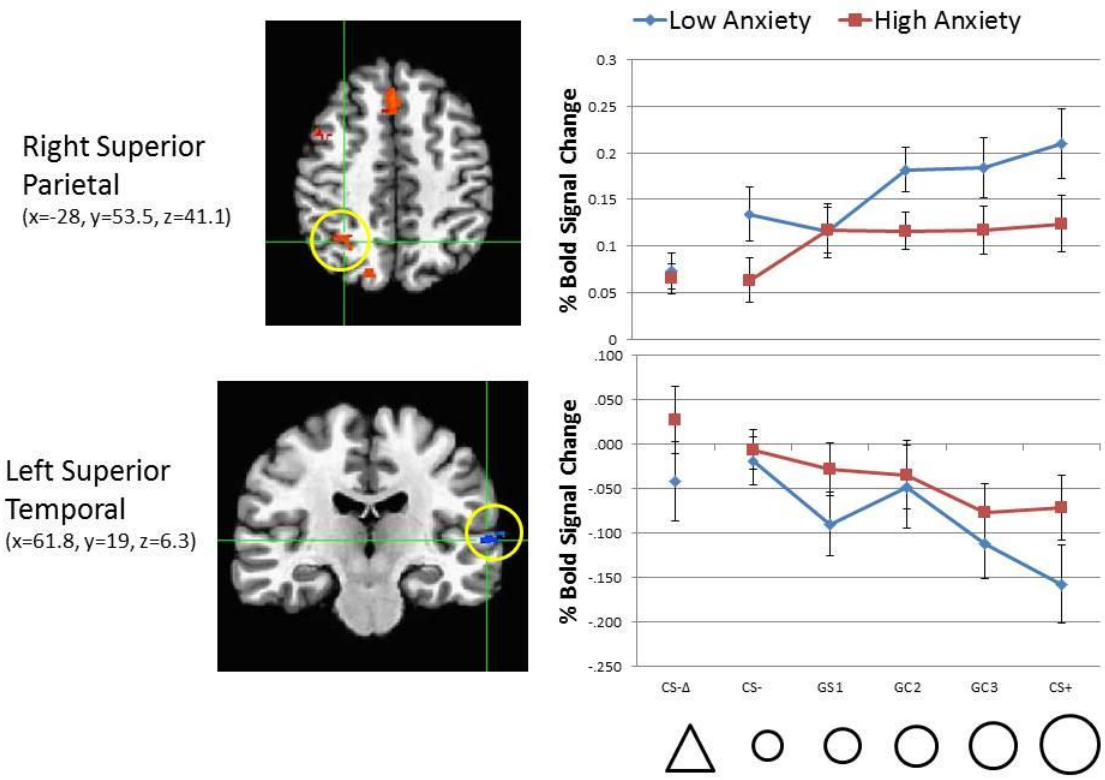

Figure 31: *Left Column:* Description and location of activation during Instrumental analysis with coordinates in RAI format. *Middle Column:* image of activation for the fROI during Instrumental analysis. *Right Column:* Percent BOLD signal change in the fROI during Instrumental analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety).

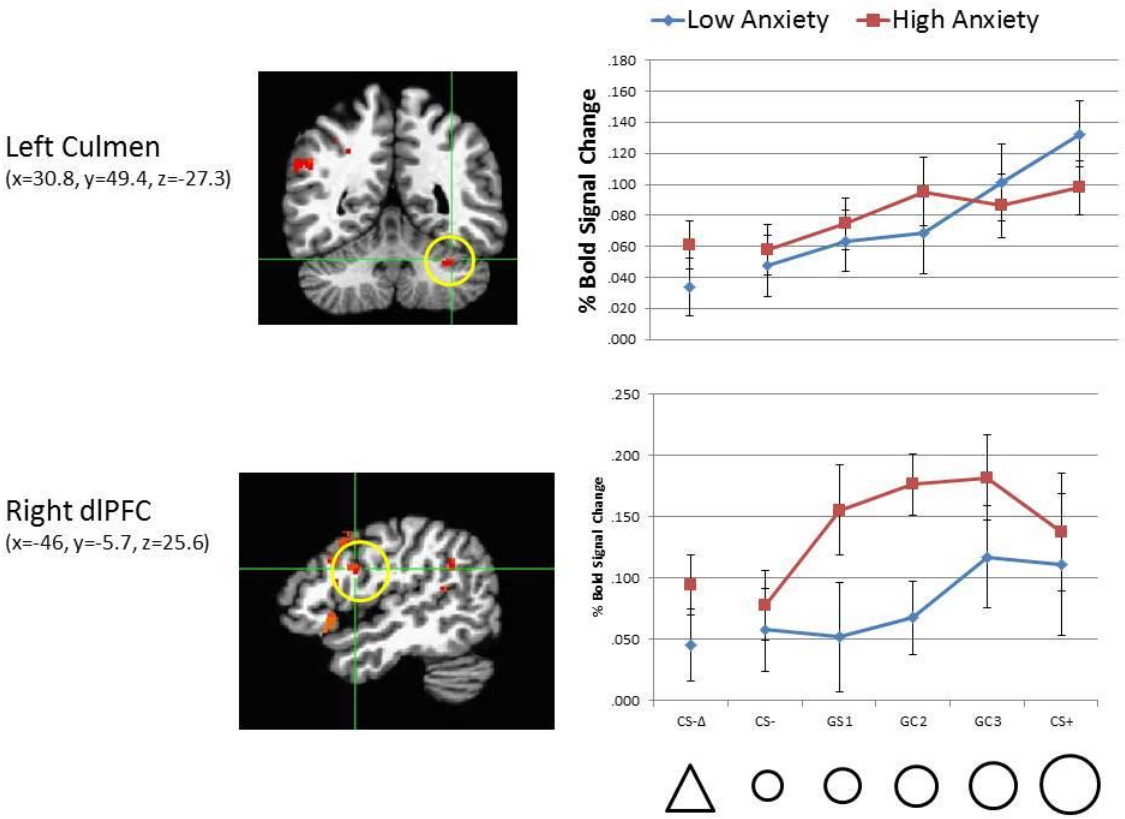

Figure 32: *Left Column:* Description and location of activation during Instrumental analysis with coordinates in RAI format. *Middle Column:* image of activation for the fROI during Instrumental analysis. *Right Column:* Percent BOLD signal change in the fROI during Instrumental analysis as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue) and group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety).

Instrumental fROIs and Overall Avoidance

Overall avoidance was measured as total number of times the long path was chosen. This was then correlated with the sum of activation to all stimuli in each fROI. As with the Pavlovian fROIs and overall avoidance, a single outlier was driving many significant effects and was therefore removed. The effects which remained significant after this outlier removal included correlations were found in the right precuneus (fROI17) (r=.531, p=.013), the left culmen (r=.495, p=.023).

Instrumental fROIs and Adaptive Avoidance

Adaptive avoidance was assessed as a difference score between avoidance to the CS+ and avoidance to the CS-Δ . Again an outlier was driving many significant positive results and therefore only results that were also significant with that outlier removed were included. These included activations in the right precuneus (fROI9) (r=.650, p=.001), right lingual gyrus (r=.561, p=. 008), and another region in the right pre-cuneus (fROI25) (r=.462, p=.035).

Instrumental Overgeneralization and Maladaptive Avoidance

The relationship between Instrumental ROIs and maladaptive avoidance was also assessed using the four methods. For the difference score between all generalization stimuli and the triangular safety cue ((GC1+GC2+GC3))-CS-Δ), there were significant correlations between maladaptive avoidance and overgeneralization in the right dorsal medial prefrontal cortex (dmPFC) (r=.509, p=.016), two regions in the right dorsal lateral prefrontal cortex (dlPFC) (fROI3) (p=.534, r=.01) (fROI24) (r=.477, p=.025), the right precuneus (fROI7) (r=.601, p=.003), the right superior parietal lobule (r=.547,

p=.008), the right postcentral gyrus (*r*=-.483, *p*=.023), and the right fusiform gyrus (*r*=.614, *p*=.002).

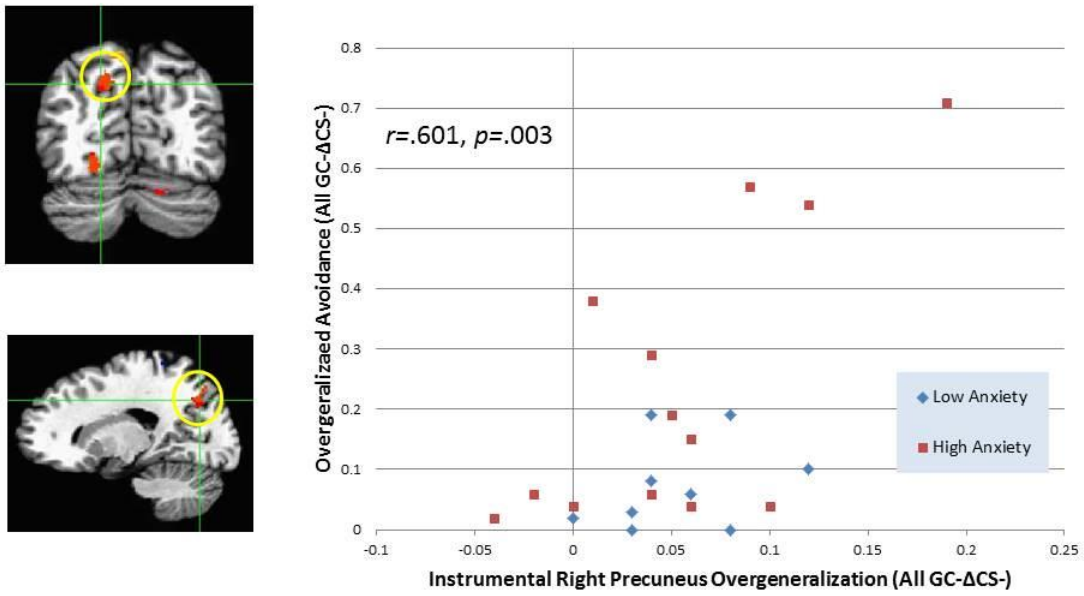

Figure 33: *Left:* Images showing activation in the right precuneus during instrumental generalization. *Right:* Scatterplot showing the relationship between overgeneralization in the right Precuneus during Instrumental trials and overgeneralized avoidance (as measured by a difference score between the average of the generalization stimuli and the triangular conditioned safety cue). Units on the x-axis are in Percent Bold Signal Change and on the y-axis are percentages. Each individual is also color/shape coded for group membership based on trait anxiety on the Spielberger State Trait Anxiety Inventory (red squares are high trait anxiety group, blue diamond are low anxiety group).

For the deviation from linear calculation, most instrumental regions were significantly correlated with avoidance. This included the right dorsal medial prefrontal cortex (dmPFC) (*r*=.679, *p*=.001), the right inferior frontal/anterior insula (*r*=.553, *p*=.008), right dlPFC (ROI3) (*r*=.671, *p*=.001), left inferior frontal/left insula (*r*=.595,

p=.003), right superior temporal (*r*=.533, *p*=.008), right middle frontal (*r*=.597, *p*=.003), right precuneus (fROI7) (*r*=.561, *p*=.007), right brainstem (*r*=.462, *p*=.031), right pre-cuneus (fROI9) (*r*=.575, *p*=.005), right fusiform gyrus (*r*=.575, *p*=.005), right posterior cingulate (*r*=.757, *p*<.001), right middle frontal (.605, *p*=.003), right pre-cuneus (fROI17)(*r*=.441, *p*=.04), right lingual gyrus (*r*=.505, *p*=.015), right pre-cuneus (fROI23) (*r*=.523, *p*=.012), and right dlPFC (fROI24) (*r*=.598, *p*=.003).

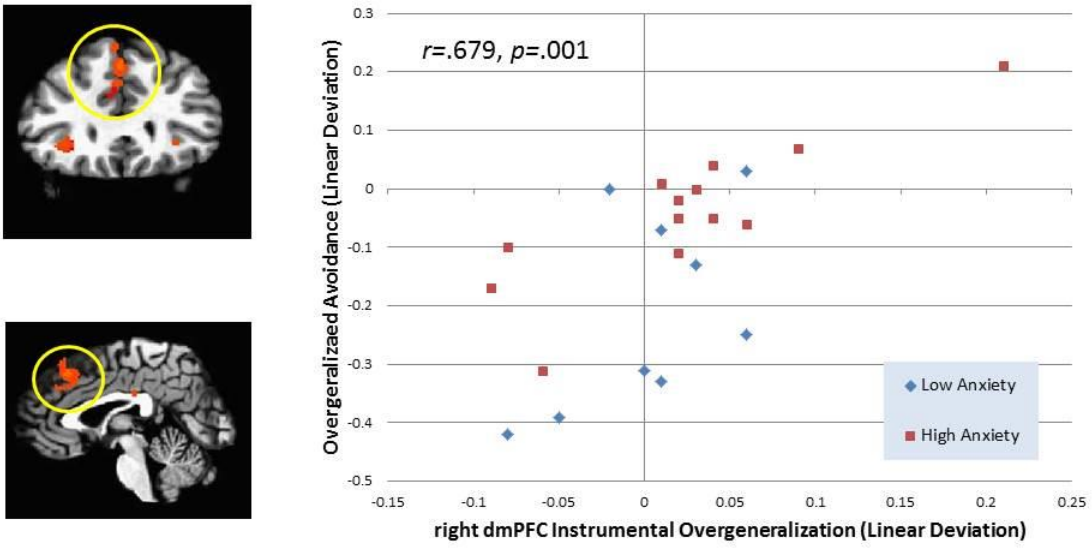

Figure 34: *Left:* Images showing activation in the right doral medial Prefrontal cortex during Instrumental trials: *Right:* Scatterplot showing the relationship between overgeneralization in the right dorsal medial Prefrontal Cortex (dmPFC) during Instrumental trials and overgeneralized avoidance (as measured by a deviation from linearity score). Units on the x-axis are in Percent Bold Signal Change and on the y-axis are percentages. Each individual is also color/shape coded for group membership based on trait anxiety on the Spielberger State Trait Anxiety Inventory (red squares are high trait anxiety group, blue diamond are low anxiety group).

For the GC3 vs CS-Δ comparison, only three instrumental regions were significantly correlated with avoidance: the right fusiform gyrus (r=.620, p=.002) and a region in the right lingual gyrus (r=.458, p=.032) and the right precuneus (fROI25) (r=.534, p=.011).

For the GC3 vs CS+ comparison, there were also three regions that were significantly correlated with avoidance: the right superior temporal gyrus (r=-.448, p=.037), the right middle frontal gyrus (r=.456, p=.033), and the right lingual gyrus (r=.573, p=.005).

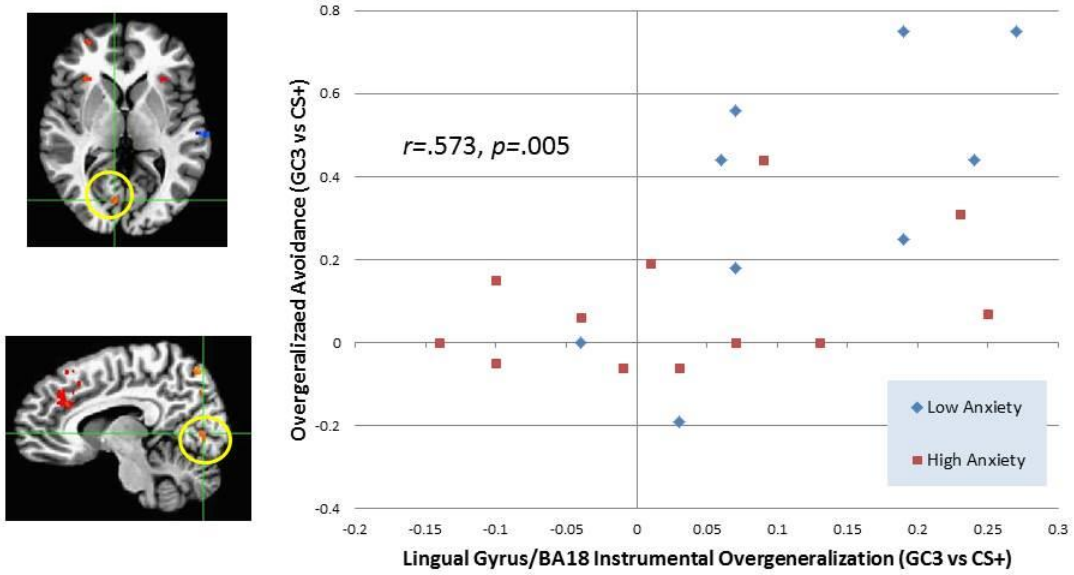

Figure 35: *Left:* Images showing activation in the right lingual gyrus/Brodman Area 18 (BA18) during instrumental trials. *Right:* Scatterplot showing the relationship

between overgeneralization in the right lingual gyrus/Brodmann Area 18 (BA18) during instrumental trials and overgeneralized avoidance (as measured by a deviation from linearity score). Units on the x-axis are in Percent Bold Signal Change and on the y-axis are percentages. Each individual is also color/shape coded for group membership (red squares are high trait anxiety group, blue diamond are low anxiety group) based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, red= high anxiety).

For each of these four comparisons multiple regressions with the significant predictors and then a second regression with all variables was attempted. For each of the regression models only the single largest original predictor was included in the final model.

Relationship between Pavlovian to Instrumental Analysis

All regions, for both the both Pavlovian and Instrumental analysis, that were related to avoidance behavior were correlated with each other in a separate analysis to assess the relationship between regions during different tasks. This was done with several different contrasts.

To test the relationship between adaptive avoidance, the CS+ vs. ΔCS- contrast was used. Activation in the right substantia nigra during Pavlovian conditioning was related to the right dmPFC during Instrumental (r=-.468, p=-.028) and also the right precuneus (fROI7) (r=-.522, p=.013). The left inferior occipital gyrus region during Pavlovian was related to the superior temporal gyrus during instrumental (r=-.474, p=.026). The subgenual ACC activation during Pavlovian was related to the left Uvula/Left Pyramis during Instrumental (r=.448, p=.036).

For the GC vs ΔCS- contrast, there were several significant results. The only Pavlovian region that was significantly correlated with the largest region in the Precuneus

(fROI7), was the subgenual ACC (r=-.588, p=.004). This negative relationship likely reflects the negative gradient in the subgenual and the positive gradient in the precuneus.

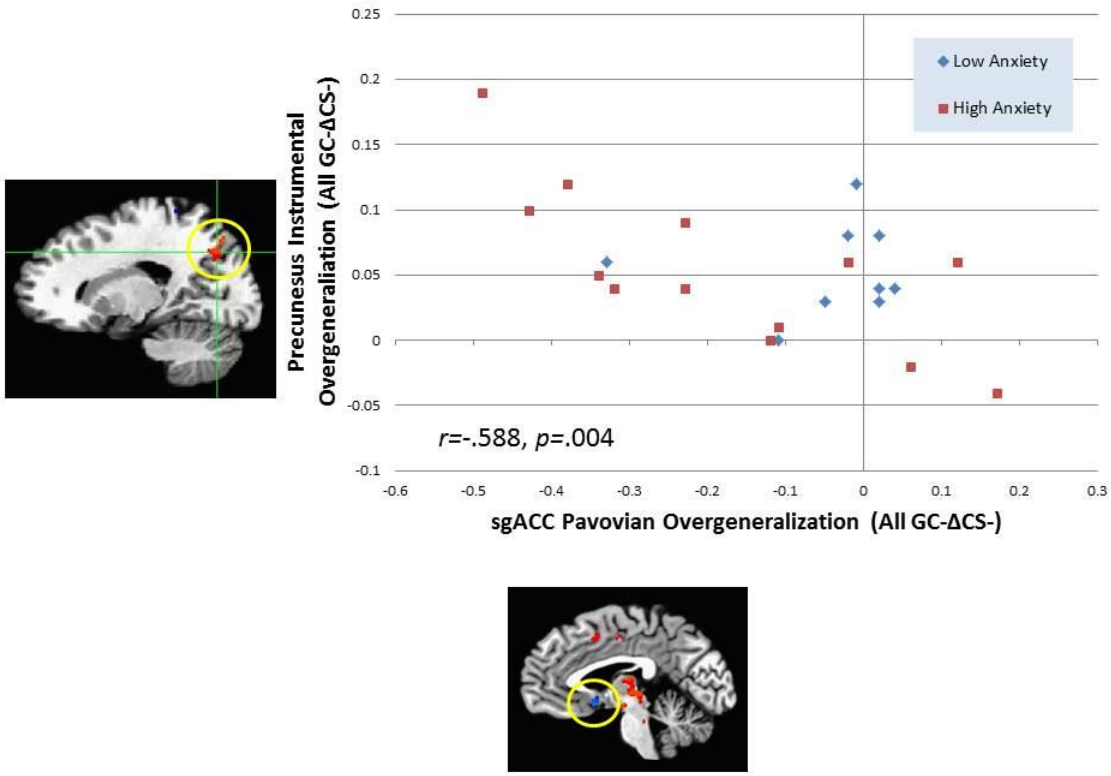

Figure 36: *Top Left:* Image of activation in the precuneus during instrumental trials. *Bottom:* Image of activation in the left subgenual anterior cingulate cortex (sgAGG) during Pavlovian trials. *Top Right:* Scatterplot showing the relationship between overgeneralization in the left subgenual anterior cingulate cortex (sgACC) during Pavlovian trials and overgeneralization in the right Precuneus during Instrumental trials (as measured by a difference score between the average of the generalization stimuli and the triangular conditioned safety cue). Units on both axes are in Percent Bold Signal Change. Each individual is also color/shape coded for group membership based on trait anxiety on the Spielberger State Trait Anxiety Inventory (red squares are high trait anxiety group, blue diamond are low anxiety group).

Additionally there was a relationship between the left substantia nigra during Pavlovian and a smaller region in the precuneus during Instrumental (fROI 25) (r=-.580,

p=.004). This inverse relationship is less clear as both gradients were positive. When expanding beyond the constraint of both regions being related to avoidance, but requiring at least one to be related to avoidance, other relationships emerged. First, bi-lateral anterior insula were related to the declive (left insula r=-.541, p=.009, right insula, r=-.715, p<.001). Second, the right caudate was related to several instrumental regions; the right superior parietal, (r=-.434, p=.044), right post-central gyrus (r=-.503, p=.017), right precuneus (fROI17) (r=-.435, p=.043), and right lingual gyrus (r=-.487, p=.021). All of these are also negative correlations between two positive gradients.

Using the deviation from linear calculation, the left postcentral gyrus during Pavlovian conditioning, was significantly correlated with many Instrumental ROIs including the right dmPFC (r=-.602, p=.003), right anterior insula (r=-.670, p=.001), right dlPFC (fROI3) (r=-.619, p=.002), right precuneus (fROI7) (r=-.568, p=.006), right superior parietal (r=-.552, p=.008), right posterior cingulate (r=-.768, p<.001), and the right dlPFC (fROI24) (r=-.660, p=.001). The left inferior occipital gyrus during Pavlovian conditioning was related to the right precuneus (fROI7) (r=-.486, p=.022), right superior parietal (r=-.625, p=.002), right posterior cingulate (r=-.593, p=.004), right precuneus (fROI17) (r=-.570, p=.006), and another region in the right precuneus (fROI 23) (r=-.524, p=.021). Another inferior occipital region during Pavlovian showed a similar pattern of relationships with instrumental regions in the right dmPFC (-.540, p=.01) right anterior insula (r=-.578, p=.005), right dlPFC (fROI 3) (-.531, p=.011), left anterior insula (r=-.555, p=.007), right supramarginal/inferior parietal/superior temporal (r=-.563, p=.006), right middle frontal (fROI6) (r=-.651, p=.001), right precuneus

(fROI7) (-.548, p=.008), right superior parietal (r=-.583, p=.004), right posterior cingulate (r=-.581, p=.005), right precuneus (fROI 17) (r=-.591, p=.004), another region in the right precuneus (fROI 23) (r=-.641, p=.001) and right dlPFC (fROI 24) (r=-.544, p=.009). The subgenual ACC during Pavlovian showed a relationship with the right dmPFC during Instrumental (r=-.449, p=.036). For all of these, the Pavlovian gradient was negative and the Instrumental gradient was positive.

The left substantia nigra, which was a positive Pavlovian relationship was also inversely related to Instrumental regions in the right dmPFC (r=-.457, p=.033), right anterior insula (r=-.448, p=.037), right dlPFC (r=-.461, p=.031), left anterior insula (r=-.477, p=.025), right supramarginal/inferior parietal/superior temporal r=-.451, p=.035), right middle frontal (r=-.495, p=.019) right precuneus (fROI 7) (r=-.485, p=.022), right superior parietal (r=-.642, p=.001) right posterior cingulate (r=-.641, p=.001), another region in the right precuneus (fROI23) (r=-.468, p=.028) and the right dlPFC (fROI24) (r=-.465, p=.029).

Reward Motivation

Within each identified ROI during the Pavlovian trial, the %BOLD signal change to reaching the garden was also calculated. There were group differences in four regions, the left cuneus (t(20)=2.105, p=.048), the right substantia nigra (t(20)=2.60, p=.017), the right posterior insula (t(20)=2.729, p=.013) and the left cingulate gyrus (t(20)=2.398, p=.026). There was a significant correlation between response to reaching the garden and overgeneralized avoidance using the GS vs CS-Δ contrast as well as one of the generalization deviation from linear measures in the left thalamus/left substantia nigra

(r=.543, p=.009; r=.426, p=.048), as well as in the right inferior occipital (r=.423, p=.05;r=.441, p=.04).

The avoidance decision

None of the several methods of attempting to separate out the avoided trials versus the non-avoided trials across stimulus types yielded fROIs. When all avoided trials and all non-avoided trials across stimulus types were pooled together, 11 fROIs were identified in a contrast during the time of the decision being made between those that were avoided and those that were not avoided. These were analyzed using a Group*Outcome*%Bold Signal change repeated measures ANOVA. There was only an effect of anxiety in one region in the middle occipital gyrus (Within Subjects; F(5,100)=4.283, p=.052; Between Subjects; F(1,20)=3.753, p=.067). This was driven by higher %BOLD signal change in the low anxiety group across both conditions (Avoided mean %Bold Signal Change=.053, mean Non-Avoided =.050) and lower %Bold signal change in the Avoided condition but higher in the non-avoided condition in the high anxiety group (Mean Avoided=-.037, Mean Non-Avoided=.024). There were no correlations between any of these regions and trait anxiety or harm avoidance, however there was a fairly strong relationship between Novelty-Seeking and % Bold signal change in the Approach condition in the left superior parietal lobule/precuneus (r=.633, p=.002).

Connectivity Results

Connectivity was only assessed during the Pavlovian trials during generalization. For Seed 1 from the right amygdala, 61 fROIs (minimum 3 voxels) were identified and

22 fROIs in a priori regions of interest were identified and further analyzed. In connectivity with the right thalamus there was significant group*quadratic interaction ($F(1,20)=8.056$, $p=.01$) as well as a trend to significant multivariate effects ($F(5,16)=2.64$, $p=.063$) and between subjects effects ($F(1,20)=2.254$, $p=.055$). There was also a trend to a significant group*stimulus quadratic interaction in the connectivity with the left middle temporal gyrus ($F(1,20)=3.434$, $p=.079$). There was a between subjects effect in the connectivity with the right middle temporal gyrus ($F(1,20)=4.391$, $p=.049$).

Seed 2, the left amygdala had 85 fROIs (minimum 3 voxels, 18 in a priori regions further analyzed). There were significant group*stimulus multivariate effects in the right anterior cingulate ($F(5,16)=4.998$, $p=.006$; group*stimulus quadratic $F(1,20)=5.249$, $p=.033$), and significant within subject group*stimulus effects in the left posterior insula ($F(5,100)=2.663$, $p=.027$; group*stimulus quadratic $F(1,20)=5.675$, $p=.027$), right lingual gyrus ($F(1,20)=2.59$, $p=.03$; multivariate $F(5,16)=2.4$, $p=.083$), left middle frontal gyrus ($F(1,20)=4.364$, $p=.001$, multivariate $F(5,16)=2.531$, $p=.072$), right posterior cingulate ($F(1,20)=2.619$, $p=.019$), the right amygdala ($F(1,20)=2.36$, $p=.045$) and left inferior frontal gryus ($F(1,20)=3.052$, $p=.013$; multivariate $F(5,16)=2.456$, $p=.078$; group*stimulus quadratic $F(1,20)=5.949$, $p=.024$). There were additional trending effects in the left precuneus (multivariate $F(5,16)=2.33$, $p=.09$, within subjects $F(1,20)=2.239$, $p=.056$).

For the third seed, the left hippocampus, there was a significant group*stimulus quadratic interaction in the left parahippocampal gyrus ($F(1,20)=4.644$, $p=.044$). There

were no other significant group*stimulus effects in the third seed or fourth seed, the right hippocampus.

Connectivity during CS+ presentation in seeds 1 and 2 were also correlated with measures of adaptive and maladaptive avoidance and personality.

In seed 1, connectivity between the left amygdala and the left ventral-medial prefrontal cortex was correlated with maladaptive avoidance (GC3-CS-Δ , r=.594, p=.004, All GCs vs CS-Δ , r=.557, p=.007) overall avoidance, (r=.517, p=.017), but only marginally to adaptive avoidance (r=.393, p=.078). Additionally it and was associated with trait anxiety (r=.445, p=.445) and harm avoidance (r=.602, p=.004). Connectivity between the left amygdala and the right anterior cingulate was also correlated with maladaptive avoidance (GC3-CS-Δ , r=.481, p=.023, All GCs vs CS-Δ , r=.457, p=.033) but not measures of overall avoidance or personality.

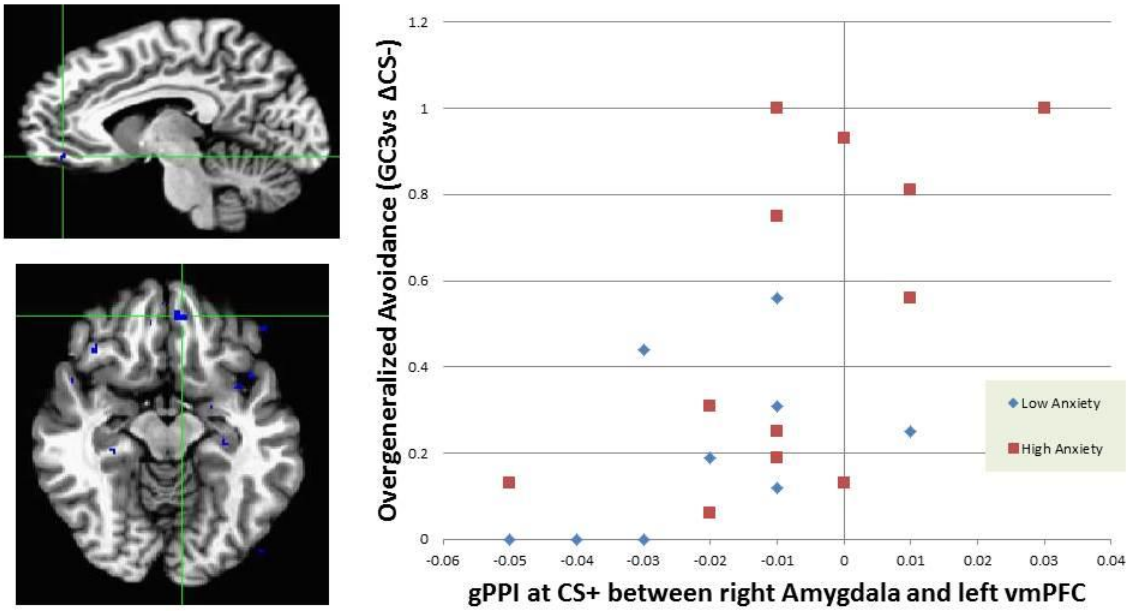

Figure 37: *Left:* Images depicting functional region of interest for generalized psychophysiological interaction (gPPI) for the right amygdala seed. *Right:* Scatterplot showing the relationship between gPPI in the ventral medial prefrontal cortex (vmPFC) during presentation of the conditioned danger cue during Pavlovian trials, and overgeneralized avoidance (as measured by a difference score between the average of the generalization stimuli and the triangular conditioned safety cue). Each individual is color/shape coded for group membership based on trait anxiety on the Spielberger State Trait Anxiety Inventory (red squares are high trait anxiety group, blue diamond are low anxiety group).

In seed 2, the right amygdala, connectivity with the right declive was correlated with maladaptive avoidance using the generalization singe score measure (r=.437, p=.042) and inversely with the degree of discrimination between CS+ and GC3 (r=-.561, p=.007). Connectivity with the right culmen was also inversely with the degree of discrimination between CS+ and GC3 (r=-.469, p=.028). Connectivity with the superior

parietal lobule was also correlated with trait anxiety (r=.447, p=.042) and harm avoidance (r=.444, p=.044). Connectivity with the left middle frontal gyrus was also correlated with maladaptive avoidance using the generalization singe score measure (r=.594, p=.004) and inversely with the degree of discrimination between CS+ and GC3 (r=-.603, p=.003).

Gender Effects

Post-hoc analysis were completed for gender effects due to observation of significant differences in avoidance tendencies across genders during the task. Females avoided more overall ($\bar{x}$=.2634) than males ($\bar{x}$=.10; t(20)=2.644, p=.016). This was driven by increased avoidance to the CS+ (female $\bar{x}$=.74, male $\bar{x}$=.344; t(20)=2.862, p=.01) and GC3 (female $\bar{x}$=.5378 male $\bar{x}$=.1671; t(20)=3.077, p=.006). When entered as a covariate in the overall repeated measures ANOVA, it was a trending effect (F(5,15)=2.608, p=.069) while trait anxiety remained significant (F(5,15)=3.283, p=.034) for the multivariate analysis. However for the within subjects effect, gender was highly significant (F(5,95)=6.729, p<.001) while trait anxiety fell to a trend level (F(5,95)=2.047, p=.079).

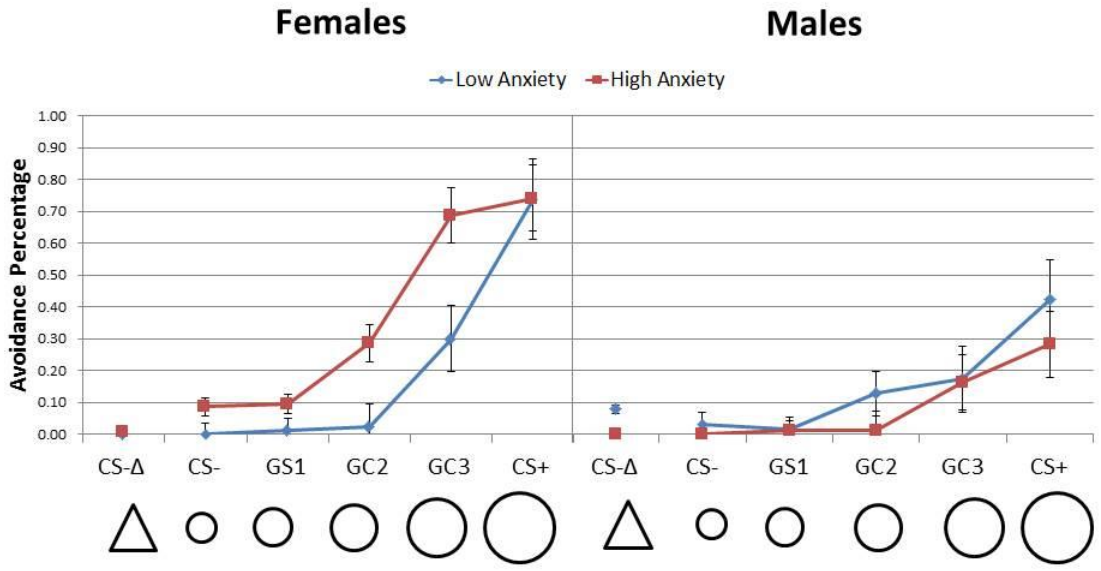

Figure 38: Avoidance as a percentage of trials the long dirt path was chosen for each group during the generalization phase as a function of stimulus type (CS-Δ = Triangular shaped conditioned safety cue; CS- = ring-shaped conditioned safety cue; GC1, GC2, GC3 = generalization stimuli, CS+ = conditioned danger cue),group based on trait anxiety on the Spielberger State Trait Anxiety Inventory (blue = low anxiety, re*d*=high anxiety) and gender (Females displayed on left, males displayed on right).

Gender was therefore entered as a covariate into all of the repeated measures ANOVAs for the Pavlovian and Instrumental analyses.

For Pavlovian trials there was an overall effect of gender in the right precentral gyrus ($F(5,15)=3.206$, $p=.037$), the left precentral gyrus (between subjects $F(5,95)=2.813$, $p=.021$, group*linear $F(1,19)=6.952$, $p=.016$ and group*quadratic $F(1,19)=7.061$, $p=.016$), right anterior cingulate (multivariate $F(5,15)=3.17$, $p=.038$,

anxiety effects no longer significant), posterior insula (between subjects $F(5,95)=2.456$, $p=.039$). It did not reduce significance of trait anxiety in other regions.

For Instrumental trials, there was a significant effect of gender in the right fusiform gyrus (multivariate $F(5,15)=2.991$, $p=.045$), the right postcentral gyrus (Multivariate $F(5,15)==2.842$, $p=.02$, within-subjects $F(5,95)==4.477$, $p=.042$), the right superior temporal gyrus (multivariate $F(5,15)==3.021$, $p=.014$), right lingual gyrus (multivariate $F(5,15)==3.391$, $p=.007$), and the right declive (multivariate $F(5,15)==2.925$, $p=.017$). Again, adding gender as a co-variate did not reduce any previously significant findings by trait-anxiety to a non-significant level.

Discussion

This study replicated the finding from van Meurs et al. 2014 and again demonstrated that this paradigm is capable of eliciting a generalization gradient for behavioral avoidance responses. It also expanded on this result in many important ways. First, it demonstrated that this approach avoidance conflict paradigm is capable of eliciting both Pavlovian and Instrumental generalization gradients in many brain regions implicated in fear conditioning and avoidance studies. Some of these neural gradients are also associated with the behavioral gradients for avoidance and perception of risk. Additionally, it demonstrated differences in generalization of an avoidance response between subjects with high and low trait anxiety with those with high anxiety displaying more maladaptive, but not more adaptive avoidance responses. Moreover, there were differences in many of the neural gradients between those with high and low trait anxiety.

Fear acquisition

Activation in several brain areas were associated with fear conditioning including bilateral anterior insula, bilateral thalamus, bilateral medial frontal, right inferior frontal, right precuneus and right occipital regions. In all of these regions %BOLD signal increases were seen to the CS+. In the right insula there was a strong relationship between the strength of the conditioning in the brain and ratings of perceived risk, consistent with previous work demonstrating the insula as important for fear conditioning and anticipatory processing (Lovero et al. 2009; Nitschke et al. 2006). This is consistent with the view that the insula integrates threat information and relays it to other areas responsible for coordinating responses based on this information, and is therefore sensitive to perceived levels of risk.

Contrary to our hypotheses, none of the regions that were sensitive to conditioning were predictive of later behavioral avoidance decisions. This was unexpected given that fear-potentiated startle during acquisition was predictive of later responses (Lissek et al., in prep). However as we failed to find any differences in amygdala activation or fear inhibition areas during fear acquisition, this could reflect a lack of sensitivity and specificity in our imaging measures.

Pavlovian Generalization Brain Areas

We replicated previous findings of generalization gradients with heightened responding to the CS+ in the bilateral anterior insula, as well as the right dmPFC, and right cingulate cortex (Lissek et al., 2013). This pattern of responding was also found in the bilateral thalamus and caudate. We also replicated previous findings of inverse

gradients in the precuneus. However in each of the regions the activation in the current task was much smaller than in previous studies using the generalization gradient approach. We did not replicate the typical inverse gradient activation in the vmPFC, but did find an inverse gradient in a significantly smaller region sometimes conceptualized as part of the vmPFC, the subgenual Anterior Cingulate Cortex (sgACC). This is not the same region which typically shows the inverse gradient in previous studies, however the sgACC does have direct projections to the bed nucleus of the stria termanalis, the periaqueductal grey, the caudate, and the amygdala (Freedman et al., 2000) as well as the nucleus accumbens (Johansen-Berg, et al., 2008) and thus could play an important role in fear inhibition. Of these regions, only the activation in the left insula differed between those with high and low anxiety, with the high anxiety group demonstrating significantly more generalization. This result is consistent with the literature on the role of the insula in fear conditioning and anticipatory processing and suggests that those with high anxiety are overestimating threat during presentations of the generalization stimuli.

The sgACC region was not associated with anxiety differences, but was inversely related to avoidance adaptive and maladaptive avoidance behavior, adding to the literature on the role of this region in contributing to avoidance behavior. Other studies have found that activation in the sgACC is specifically related to avoidance cluster symptoms in PTSD (Hopper et al., 2009) and is related to harm avoidance in adolescents (Yang et al. 2009). Additionally, the sgACC has been implicated in self-control. One study found that sgACC was activated when individuals made a decision to confront a feared stimulus (Nili et al., 2010), which is consistent with our finding of deactivation

when an avoidance decision is made. One possible cause for overgeneralized avoidance in high anxiety is low self-control in the face of threat. Interesting data to support this ideas comes from the post-experimental questionnaires in our study. The only question in which high and low anxiety subjects difference was how in control they felt when making their decisions, with the high anxiety group feeling less in control.

This study failed to replicate finding of inverse gradients in the hippocampus. The hippocampus, working with sensory cortex, is thought to play an important role in fear generalization. Lesions studies in animals demonstrate greater generalization with either hippocampal or sensory cortical lesions (Bucci et al. 2002; Solomon and Moore, 1975). For this reason, the hippocampus plays a central role in a model of fear generalization proposed by Lissek and colleagues (Lissek et al., 2013). In this model, sensory cortex and the hippocampus work together to compare the current stimulus to previously conditioned cues. It can either complete pattern-matching or pattern-separation, and as the hippocampus is generally more responsive to safety cues in generalization studies, is thought to potentially play more of a role in identifying safety stimuli. The failure to find any activation in the hippocampus during this study could reflect the greater complexity of the task. In previous generalization tasks from our lab, the CSs and GSs are the only stimuli present. The addition of the farmer, his movement, and reaching the garden could account for the difference in this study as there are many more elements to remember that are included in the "baseline" condition in the present study.

This study, consistent with others from our lab, again failed to find any activation in the amygdala. The amygdala is associated with fear activation and expression (Davis,

1992, Ledoux, 2000), but the response in the amygdala habituates quickly (Buchel, Morris, Dolan & Friston, 1998). Consistent with this, there was an activation in the amygdala during the acquisition sequence, however it was only 13 voxels and did not survive the cluster size threshold (Additionally follow up analysis revealed there were no group differences in activations in this region during acquisition, or any relationship to later avoidance).

Some finding that were observed but not predicted were the inverse gradients in the occipital cortex, primary motor cortex, and primary somatosensory cortex. Previous literature does implicate sensory regions in the brain in generalization.Aversive conditioning impairs behavioral indices of discrimination and makes the perceptual threshold wider in the primary sensory cortex for which the aversive stimulus is processed. Moreover, the degree to which individuals found it aversive was related to the amount of generalization demonstrated(Resnik et al. 2012). Additionallylesions in sensory cortex and thalamus increase generalization (Antunes & Moita, 2010; Jarrell et al., 1987). Previous studies from similar generalization gradient paradigms in our lab however have found increased responding to the CS+ in sensory regions, whereas we found greater deactivations in these regions in the current paradigm. Although the direction is different than in previous studies, the results are in line with the overall generalization effect as those with low anxiety showed a trend towards greater discrimination, and thus less generalization in occipital regions compared to those with high anxiety. This is consistent another recent study that suggests overgeneralization in anxiety is driven by changes in stimulus representations in the primary cortex responsible

for processing that stimulus (Laufer et al., 2016). In our study, deactivations in the inferior occipital gyrus were the only responses during Pavlovian conditioning that were associated with overall avoidance behavior, and we found relationships between overgeneralized avoidance and overgeneralized neural activations in other region in the primary visual and inferior occipital cortex. In comparing directly the activations for the CS+ and the closest approximation, the only regions associated with changes in perceived level of risk were the primary visual cortex, right thalamus, and right parahippocampal gyrus. This provides further evidences supporting differences in perceptual processing of the stimuli contributing to overgeneralization.

The only other region that was associated with group differences was in the right precentral gyrus. This region has previously been seen to be most reactive to the CS+ (Dunsmoor et al., 2010) which differs from the current results. However, as with the finding in the sensory cortex, the low anxiety group showed better discrimination and the high anxiety group, more generalization in this region.

In addition to this group difference in the right precentral gyrus, there were deactivations across the entire sample in the bilateral precentral and postcentral gyrus. This is a finding that has not been established in the literature, and the meaning unclear. One possibility is that these reflect a freezing response when an avoidance response is unavailable. Although freezing is controlled by the periaqueductal grey (PAG; LeDoux et al., 1988) which was not observed to be activated during this study, there is evidence that the PAG then coordinates simultaneous responses in the motor and sensory circuits

(Koutsikou et al., 2015). The bilateral deactivation in both sensory and motor regions could therefore reflect a freezing response in the face of the danger cue.

Interestingly, the responses in these regions also showed the largest and most consistent correlations with risk ratings and avoidance behavior, both adaptive and maladaptive. Although the freezing response and the avoidance response are each facilitated by different sub-regions of the amygdala (Killcross, Robbins and Everitt, 1997), with the central nucleus subserving conditioned suppression and the basolateral nucleus implicated in learning avoidance responses, the amygdala could play a central role in coordinating both the hypothesized freezing and avoidance responses. This would explain the high correspondence between this bilateral deactivation and the avoidance response. This result also fits well with a recently developed model of avoidance behavior that is an expansion of a the two-factor model to a neurophysiological two factor model, the Escape from Fear Model (EFF; Cain & LeDoux, 2008). This model ties together neurophysiological findings from classical and instrumental conditioning paradigms. It suggests that it is collaboration between these two distinct neural systems which form the circuit for avoidance behavior. In the model, after Pavlovian acquisition of fear, subsequent presentation of the danger cue activates an "upstream process" . This involves activation in the thalamus and sensory cortex, which then drive activation in the central nucleus of the amygdala and other arousal centers. This produces a negative emotional state, and is representative of the Pavlovian response. In the model, instrumental learning processes are a "downstream process" and information to motivate a behavioral avoidance decision flows from the lateral and basal amygdala to the nucleus

accumbens and eventually to the ventral pallidum and downstream motor systems. Of note, in the current study activations in the primary visual cortex and thalamus were only found in the Pavlovian analysis, consistent with the first factor in the EFF model. Unfortunately, this study did not capture any amygdala activation, and counter to our hypothesis, did not capture any nucleus accumbens activation to provide further support for this model.

Instrumental Neurobiological Regions

When compared to the Pavlovian analysis, similar activations were found in the bilateral insula and dmPFC during the instrumental task, however there was no longer any thalamus, caudate, dmPFC (sgACC) or similar precuneus activations (there were 4 areas of precuneus that were identified, but none of them overlapped with the one identified in the Pavlovian analysis and all were more caudal and dorsal). This differentiation suggests that these latter areas play a role exclusively in Pavlovian processes.

The areas that were sensitive to differences in anxiety were the postcentral gyrus, a region in the cerebellum, and in the precentral sulcus. The region in the postcentral gyrus was a negative gradient and showed very similar results as during Pavlovian conditioning. The continued presence of postcentral gyrus deactivation during Instrumental conditioning is noteworthy, especially given the group differences. The postcentral gyrus has been shown to become deactivated in regions that are non somatotopically related to the anticipation of pain with both PET imaging (Drevets et al., 1995) and fMRI (Porro et al., 2002). Given that none of our deactivations in the post-

central gyrus are in the region of the foot or ankle where the shock is given this alternatively could represent "stealing" of activation by the ankle region resulting in deactivation in other somatosensory cortex (Porro et al., 2002). While it is curious there is no hyper-activation in the ankle region to correspond to this effect, simply attention to pain or attention to sensation in the ankle might have also persisted through baseline conditions, making it difficult to distinguish, and only revealing itself through this stealing effect. The deactivation in the somatosensory cortex is also interesting given the role of the insula. The insula can said to be considered sensory cortex as it enacts a rich representing of internal homeostatic states. However, by also anticipating, it could help regulate sensory cortex to prepare it for pain or other aversive events.

In the precentral sulcus and postcentral gyrus, the high anxiety group showed overgeneralization responses. The area in the cerebellum, the culmen, had a different pattern with the high anxiety group showing no generalization, and perhaps a sensitization effect, and the low anxiety group showing generalization. The role of the cerebellum is processing fear is discussed in further detail later, however the significance of these group differences is less clear as it is not in the same direction as would be hypothesized given the behavioral results.

Between subjects effects showed greater left anterior insula activation in the high anxiety group, and there was a similar pattern of responding in the right anterior insula as well. These results extend the hypothesized role of the insula in supporting anxiety related differences in Pavlovian fear conditioning (Lissek et al. 2013, Kaczurkin et al., in press) to behavioral avoidance as well.

There were several regions during the Instrumental period which were correlated with measures of avoidance. All of the regions associated with avoidance behavior were right lateralized. This fits with the laterality hypothesis for approach and avoidance with avoidance being associated with the right hemisphere (Harmon-Jones, Gable, & Peterson, 2010; Fetterman et al., 2013). Maladaptive avoidance was associated with activations in the right dmPFC, which has been hypothesized to play a role in the appraisal and expression of negative emotions (Etkin, Enger, and Kalisch, 2011) and in fear conditioning (Lissek et al, 2013). The dmPFC is also thought to play a role in selecting actions during decision making, and detecting errors in those actions (Fellows, 2004) and has been proposed as a region in the brain that integrates both approach and avoidance related signals (Aupperle and Paulus, 2010). It was also associated with activations in the dlPFC which is thought to play a role in emotion regulation (Goldin et al., 2008), and also tracks past decisions as well as outcomes of those decisions in order to further optimize future decisions (Barraclough et al., 2004). This is particularly interesting given the shape of the gradient in the high anxiety group, with a nearly inverted U as opposed to the typical generalization gradient. This fits with the idea of more regulation necessary at the middle of the continuum, as less regulation would be required at either end of the continuum (for CS+, not necessary to regulate because mainly choosing to avoid, and no fear related avoidance tendency to regulate for CS-), whereas for ambiguous levels of threat, more regulation is necessary to make the correct response.

Strong correlations were also found in the right superior parietal lobule. These regions have been shown to be active during risky choices (Paulus et al., 2003). This

would fit with the gradient as the low anxiety group actually demonstrated greater activation, and made more "risky" choices to approach, often even when faced with the danger cue.

Several regions in the right precuneus were also significantly related to (and were the strongest and most consistent relationships across different contrasts measuring maladaptive avoidance) avoidance behavior. Volume of the precuneus has been related to trait differences in harm avoidance (Gardini et al., 2009), behavioral inhibition (Fuentes et al, 2012), and severity of social avoidance in social anxiety disorder (Irle et al., 2014). The precuneus has also been shown to be related to avoidance of risk (Roy et al., 2011), and to make prospective plans to guide motor planning to either avoid or approach objects (Lindner et al., 2010). Activations in our study were almost similar to the dlPFC activations, with nearly inverted U-shape gradients in high anxiety patients. This could be reflective of high levels of conflict or uncertainty during decision making to ambiguous stimuli.

Adding uncertainty to the perceptual model of fear conditioning

The perceptual model of fear generalization, which relies on pattern matching from the hippocampus and visual processing areas, has recently been expanded on by another model that incorporates ambiguity-based uncertainty at the middle of the generalization gradient as a factor that drives generalization in addition to the perceptual processes (Onat & Buchel, 2015). They argue that perceptual processing can be dissociated from ambiguity-based uncertainty, and evidence of "hypersharp tuning" in the insula in their study demonstrates that strict perceptual processing cannot underlie all

generalization effects. Although the current study had different methods and did not find evidence for hypersharp tuning in the insula, hypersharp tuning was observed in some structures important for fear conditioning. To measure the degree of tuning, we used the ratio of the first approximation compared to the danger cue after each had been normalized to baseline ΔCS- (that is (GC3- ΔCS-)/(CS+- ΔCS-), with scores closer to 0 representing better discrimination/tuning, and higher scores reflecting more generalization. First there were differences in the behavioral tuning profiles, with better tuning for risk ratings (.514), than for avoidance decisions (.657). Our sample demonstrated worse tuning in the insula (left t=.759, right t=.908), but hypersharp tuning in the bilateral substantia nigra (left t=.441, right t=.457) and the bed nucleus of the stria terminalis (.306). Both of these regions are implicated in Pavlovian conditioning as they receive input from the central nucleus of the amygdala and lesions to either area result in the abolition of the fear potentiated startle reflex (Hitchcock and Davis, 1991). Although not located in the same brain area or using the same measure, evidence for hypersharp tuning is perplexing. If the brain can differentiate at a neuronal level, what then gets in the way of producing a similarly efficient behavioral response?

Onat and Buchel argued that evidence of ambiguity in the infratemporal cortex, which is important for face processing (and in their study faces were used as the conditioned stimuli) as accounting for this difference. In the current study, the activations described above that take the shape of an inverted U, could also serve as markers for ambiguity processing.

Decision Making

Returning to the decision making framework is useful for making sense of the data, and offering some additional hypothesis as to the anxiety related differences in generalization of the avoidance decision in this study. Each of the valuation systems in the decision making framework presented earlier also relies on neural systems. There is overlap between the systems, but there is evidence from various studies that some types of valuation rely more on some neural structures than others (Rangel et al., 2008; van der Meer et al., 2012). One immediate question from this perspective is does there appear to be a specific system that is being used for valuation either across all subjects, or possibly differing by group.

One area involved in decision making is the dorsal striatum. In rodents, one of the major sites for habitual decision making is the dorsolateral striatum, while the dorsomedial striatum is involved in deliberative decisions (Yin and Knowlton 2004). In humans the tail of the caudate is involved in habitual decisions, and the head of the caudate is for deliberative decisions (Kim et al., 2013). There is activation in the caudate head in the study, it is only during the Pavlovian trials, and not correlated with avoidance behavior.

The two brain regions that are thought to be most involved with the deliberation system are the hippocampus and the orbitofrontal cortex (OFC) and vmPFC. It is thought that the hippocampus is needed to do the mental time travel necessary to place yourself in an imagined future (Buckner and Carroll 2007). The OFC and vmPFC are then thought to evaluate these possible futures (O'Doherty et al. 2001; Coricelli et al. 2005). As discussed above, although activations in both of these areas has been found in similar

studies, this study failed to replicate those findings. The dlPFC is another region that has been shown to be involved in outcome valuation in both primates (Wallis and Miller, 2003) and humans (Plassman et al., 2007).

The neural basis of the New Pavlovian system is basolateral amygdala, OFC, and ventral striatum (van der Meer et al., 2012). Activations were not found in any of these regions during either Pavlovian or Instrumental trials. Activations were found however in midbrain dopamine regions during Pavlovian trials, and given the role of dopamine in processing of reward and punishment in the ventral striatum, could reflect part of a dopamine pathway response. The fact that it was only seen during Pavlovian trials is also consistent with the fact that ventral striatum is usually necessary for learning, but not implementation of habit-based instrumental decision tasks (Atallah et al.,2007).There is evidence however that the ventral striatum is involved in all decision systems, and this may not be specific to any one system (Pavlovian: Cardinal et al., 2002; Habit: Atallah et al., 2007, Deliberative: Nicola, 2010).

In addition to the fact that the differenent valuation systems are known to have overlapping neural structures involved, it is also thought that all of these valuation systems will be working simultaneously. These systems are likely often in agreement, however when there is conflict there must be a way to deliberate between the various systems. One way that has been proposed is that whichever system has the least uncertain estimate of the true value will be selected (Daw et al.,2005). One proposed idea of mental illnesses is that control is assigned to the incorrect system, and this has particular relevance for anxiety and ambiguity.

In addition to the valuation systems themselves, there are also certain parameters of a task that can modulate the decision systems, and may have different effects on the

systems. Such moderators are risk and uncertainty/ambiguity (Rangel et al., 2008). There is ample evidence that those with clinical anxiety are aversise to unpredictable/ambiguous events, and this may in fact be the defining feature of anxiety (Grillon, 2008; Grillon et al., 2008). Reaction time data from our task may be helpful in understanding what may be happening as participants make their choices during the task. Specifically, patients with anxiety are very quick to make their decisions of possible risk, and make their avoidance decision when the CS+ and GC3 are presented compared to the low anxiety group. This reflects the high anxieties group unwillingness to tolerate uncertainty, and leads to overgeneralization of the avoidance response. In contrast, the high anxiety group by taking more time to evaluate makes better decisions for performance in the task.

As reviewed earlier, processing of ambiguity can occur in the dlPFC and this is also most likely to influence deliberative valuation. If individuals with high anxiety are viewing the stimuli as more ambiguous, there is less certainty around the value of the choices. The competing New Pavlovian system for individuals with high anxiety may therefore be assigned control, and given the aversive nature of the task and the "hardwired" Pavlovian defense to flee/avoid aversive events, a decision to avoid is made. In contrast, the low anxiety group view the stimuli as less ambiguous, possibly because they spend more time analyzing the stimuli. Their longer reaction times allow them to resolve some of the ambiguity rather than relying and the deliberative system is therefore able to take control and a decision to approach is made.

Additionally, the outcome-valuation system is also modulated by higher order cognitive beliefs. This was shown in human studies where activations in the OFC were determined by experimenter imparted beliefs about if the scent they were smelling was cheddar cheese or a sweaty sock (De Araujo et al., 2005), and activation in the mPFC for wine consumed as part of the study was dependent on beliefs about the price of the wine (Plassmann et al., 2008). This raises several questions about higher order cognitive beliefs in our study. Anecdotal evidence comes from one participants open ended questionnaire. He stated that he did not avoid even when he knew the CS+ was present because as a farmer, he needed to care for his wife and many children, and could not let them go hungry. By using imaginative play he made very few avoidance decisions. This is an interesting finding, and raises questions about the possible clinical use. It is possible that such a strategy using imagination could make exposure therapy more tolerable.

Gender Effects

One of the interesting and unexpected findings from this study was the large effect of gender. Females avoided at significantly higher rates than males, and there were gender differences in several brain regions including bilateral precentral gyrus, anterior cingulate, and posterior insula during Pavlovian conditioning and cerebellum, right postcentral, and various occipital activations during Instrumental conditioning. This is consistent with the emerging literature on sex differences in avoidance.

Females are known to suffer from anxiety disorders at higher rates as males, with nearly twice the risk of developing PTSD (Breslau et al. 1998), and accounting for 55-

60% of individuals with GAD (APA, 2013). It is therefore surprising that the topic has received such little attention. There is inconsistent literature on sex differences in Pavlovian fear conditioning. There is recent evidence that females with PTSD have greater acquisition of fear (Inslicht et al., 2013), and enhanced reactivation of classically conditioned fear (Benson et al., 2014), but females show superior extinction recall (Shivl et al., 2015). The authors asserted that impaired extinction recall is therefore unlikely to underlie the greater higher incidence rates of PTSD in women.

Another pathway to increased rates of disorder could be differences in avoidance. Female rats are quicker to learn avoidance responses (Beck et al. 2010; Avcu et al. 2014) and recently these findings have been extended humans, demonstrating quicker acquisition of avoidance (Sheynin et al. 2014a) which was then replicated and extended showing longer latencies to extinction of the avoidance response in humans as well (Sheynin et al. 2014b).

Neural mechanisms of sex differences in Pavlovian fear conditioning implicate the insula, posterior cingulate cortex, thalamus, and cerebellum in enhanced reactivation of fear (Benson et al, 2014), but the dorsal anterior cingulate activation was correlated with better extinction recall outcomes in females (Shivl et al., 2015). Recently, there have been advances in understanding the role of the cerebellum in contributing to functions other than the motor and sensory-motor integration domain which it is thought to mainly be involved in. One such function is contributing to pain-related emotional learning such as in classical conditioning (Timmann et al., 2010). The cerebellum is not thought to be a primary site of this processing, but rather modulates processing in cortical

brain structures (Bellebaum & Daum, 2011). A recent study found gender differences in the processing of conditioned safety cues in the cerebellum, with females showing enhanced activation in lobules that contribute to somatomotor networks, and men showing activation in lobules that contribute to frontoparietal and ventral attention networks (Labrenz et al. 2015). This is consistent with our results demonstrating gender differences in the cerebellum, precentral and postcentral gyrus. Given that the latter two regions were also significantly associated with avoidance behavior in our task, more research into the relationship between avoidance, gender, cerebellar and somatomotor and somatosensory processing is warranted.

Connectivity

For all regions identified with gPPI, the gradients were marked by severe deviations in connectivity during the presentation of the CS+. This could reflect either great specificity of the amygdala and hippocampus in responding to the danger cue, and another area displaying of hyper-sharp tuning. Alternatively it could be the result of the use of the lighting flash on all CS+ trials, even unreinforced trials, which might have served as a highly salient and memorable cue that influenced the neural responses in a markedly different way from previous studies. In either case, there were still interesting differences between groups and relationships to avoidance that warrant further discussion.

The most striking overall result was that connectivity between the right amygdala and the left vmPFC during the presentation of the danger cue was highly correlated with maladaptive avoidance, and was also associated with trait anxiety and harm avoidance.

This further establishes the connectivity between these regions as crucial for fear-related learning and behavior. Connectivity from the left amygdala to the cerebellum was also related to avoidance and personality measures, adding even more evidence to the role of the cerebellum in fear related processes. Although amygdala activation was not captured during the whole brain analysis, this confirms the role of the amygdala in supporting avoidance behavior and differences in anxiety. This is important given its central role in the processing of fear.

Relationships across Pavlovian and Instrumental Analyses

Several relationships across the Pavlovian and Instrumental trials were observed. First, the relationship between activation in the substantia nigra during Pavlovian conditioning and right dmPFC activation is interesting given the hypothesized role of the dmPFC integrating reward and avoidance motivation and the substantia nigra potentially providing such motivational influence.

Also of note, although both the dmPFC and substantia nigra were positive gradients, the relationship between the two was negative. That is, for lower levels of activation to the generalization stimuli during in the SN during Pavlovian generalization, there was more generalization in the dmPFC. Although the dopaminergic system is clearly implicated in avoidance (Oleson et al., 2012), there is also a vast literature supporting its role in reward processing (Reynolds et al., 2001; Shultz, 1997). This inverse relationship could signify that the substantia nigra was providing reward motivation for approach behaviors, and that in the absence of such motivation, increases in dmPFC activation are seen and lead to avoidance behavior. The caudate, another

positive gradients, also showed similar negative correlations with areas in Instrumental conditioning that were then related to avoidance.

The sgACC during Pavlovian conditioning was related to the cerebellum, and the largest precuneus activation during Instrumental trials. If the precuneus was involved in processing ambiguity and risk as other studies have suggested (Krain et al., 2006), the degree of ambiguity and perceived risk could be directly related to the individuals ability to inhibit fear to stimuli that resemble danger cues. The lack of an association between the precuneus and regions like the insular cortex is also interesting in that it provides an account for avoidance based decisions being related specifically to fear-inhibition mechanisms rather than fear excitation.

There were also relationships between the sensory, and somatosensory regions during Pavlovian conditioning and several areas associated with avoidance. While a direct relationship cannot be ruled out, it also highlights the possibility of other brain regions such as the amygdala, mediating responses during each trial type that have similar gradient shapes due to the mediation.

Limitations and Future Directions

The most significant limitation of this study was the lack of separation between avoided and non-avoided trials. When analyses were attempted with the responses separated, there were not indefinable regions of interest. This has significant ramifications for the interpretation of the results, such that activations, particularly during the Instrumental analysis, cannot be clearly established as to contributing to approach or avoidance motivation. Right sided lateralization of the findings, as well as the paltry

reward for winning should give some confidence that it is more related to avoidance processes, however some of the correlations with novelty seeking or reward dependence challenge this view. Additionally, when all of the avoid trials and non-avoided trials were lumped together, the major flaw is that non-avoidance responses to the ΔCS-, CS- and GC1 are likely be quite different from non-avoidance responses to the GC3 or GC2 which would theoretically require more inhibitory processes. This is potentially why that analysis did not yield many fROIs or significant differences between groups. Future studies must be able to capture the neural response to avoid and non-avoidance responses to threatening stimuli to further this line of work.

Another major methodical question was the large number of regressors. Our results tended to have lower amplitudes of % Bold Signal change that could reflect the large number of variables. Splitting the analysis into two separate analyses for Pavlovian and Instrumental portions and including the other trial type as a regressor of no interest was also not preferable, and could have influenced the observed results in unexpected ways.

Another small methodological issue that could account for some differences were the presence of some of the visual stimuli only for avoided trials (the presence of the chickens coming to eat the crops, the fact that the farmer actually traveled at a quicker pace when taking the long road, and visual attention being directed more to the right side of the screen for avoidance trials only). While the study was kept as comparable to the psychophysiological version as possible, these small details potentially affect the fMRI analysis to a degree that warrants changing in the future.

Conclusions

This study investigated differences in the neurocircuitry of both Pavlovian fear processes and instrumental avoidance decisions in individuals with high and low trait anxiety. There were significant group differences in several regions in each analysis, and there were significant relationships between overgeneralized avoidance behavior and neurally measured overgeneralization. Some findings were consistent with previous research and add to the literature for both the insula as being central to contributing to anticipation of threat and avoidance behavior and primary sensory regions being implicated in overgeneralization of fear. Additional findings that warrant future research include the role of the cerebellum and somatosensory regions on avoidance and its overgeneralization as well as the role of gender.

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Appendix A:

Exclusion Criteria

The exclusion criteria for this study include the following:

1. Participants will be excluded if they are currently using medications that could affect fMRI or physiological measurement (medications that alter central-nervous-system function such as beta-blockers, medications for cardiovascular disease, pain-relieving agents, antitussives, expectorants, centrally acting muscle relaxants, and antiepileptics).

2. Participants from the low-trait anxiety group that are determined by the psychological history questionnaire to have any current or past Axis I psychiatric disorder as will be excluded. High-trait anxiety participants with any non-anxiety Axis I disorder will be excluded (i.e. depression, psychosis, ADHD). Individuals who currently (or during the last six months) meet criteria for substance abuse/dependence will be excluded.

3. Potential participants with a history of any medical or neurological condition which in the investigator's opinion may confound the results of the study will be excluded. Medical and neurological conditions for exclusion include, but not limited to, history of organic mental disorders, seizure, or mental retardation.

4. Participants for whom it may not be safe to enter the high-magnetic field environment will be excluded. The MRI criteria for exclusion include, but may not be limited to, the following:

a. Participants with any type of implanted devices controlled by mechanical, electronic, or magnetic means (e.g., cochlear implants, pacemakers, neural stimulators, biostimulators, electronic infusion pumps).

b. Participants with any type of ferromagnetic implant that could potentially be displaced or damaged, such as aneurysm clips, metallic joints, or metallic skull plates.

c. Females who are pregnant or suspect they may be pregnant.

d. Participants who are claustrophobic and/or exhibit noticeable anxiety about the MRI scanning environment.

e. Participants who have known cardiac or circulatory impairment

f. Participants who have known conditions that can lead to emergency medical care.

g. Participants with any type of implant for which the contents of that implant are unknown (and therefore may contain ferromagnetic or electrically conductive metal). If the participant is unsure whether a particular medical device is MR compatible, they will not be allowed to enter the MRI environment until written confirmation has been obtained from the participant's physician, stating that the device in question is known to be MR compatible, beyond all doubt.

h. Participants who are not willing or able to remove clothing, jewelry, cosmetics, piercings, or devices that contain ferromagnetic or conductive metal. This includes participants who use eyeglasses with metal frames (rather than glasses with plastic frames or contact lenses).

i. Participants who have metal in their person from shrapnel or other kinds of injuries involving metal parts entering the body.

Appendix B:

MRI data preprocessing steps

1. Slice-timing correction. We collect 35 slices within each volume, which are collected in an interleaved order (e.g., slice 0, 2, 4, …, 34, 1, 3, 5, …, 33). Because slices are not collected at the exact same time, there will be slight differences in the amplitude of the signal between slices collected earlier vs. later during the scanning of a single volume. To account for this, we will need to use slice-timing correction, an algorithm that adjusts the amplitude of the signal acquired from each slice to account for the order of slice acquisition within each volume.

2. Motion correction. (e.g., Jenkinson, Bannister, Brady, & Smith, 2002; Oakes et al., 2005). Participant movement during the EPI scans will be corrected by aligning all of the volumes obtained during the experiment to a reference volume (the seventh volume obtained during the first functional imaging scan). Two types of motion are estimated and corrected: translations along the x, y, and z axes and rotations about these axes (pitch, roll, and yaw, respectively). We will use the following AFNI motion correction algorithms during preprocessing: 2dImReg (corrects in-slice motion, especially "nodding," a common source of head movement) and 3dvolreg (corrects out-of-slice motion). Additionally, motion correction parameters will be entered as covariates in the statistical model which allows us to retain subjects with some movement. Subjects with excessive amounts of motion as determined by a predefined threshold (the subject's head moved more than 4.0 mm in any dimension from one volume acquisition to the next) will be excluded from analysis.

3. Brain extraction. We will eliminate non-brain tissues (e.g., bones, meninges) from the image, so that the only BOLD signal analyzed will be from the brain (e.g., Smith, 2002).

4. Distortion correction (e.g., Jenkinson, 2003). Inhomogeneties in the magnetic field lead to distortions in the EPI images, especially at air-tissue boundaries such as near the sinuses which lie directly below the frontal lobes of the brain. Distortion correction is accomplished by taking information about the strength of the magnetic field (obtained with a field-mapping pulse sequence), using an algorithm to estimate the amount of distortion in the EPI images due to differences in the strength of the magnetic field at different locations, and correcting the EPI images using these estimates. If the estimated amount of distortion exceeds a predefined threshold, data from these regions will not be analyzed.

5. Spatial smoothing. Spatial smoothing "blurs" the EPI images by spreading the intensity of each voxel in the image over adjacent voxels. Smoothing is done to reduce high-frequency noise introduced into the data during the scanning procedure and to increase the validity of statistical tests by reducing the total number of tests and improving the normality of error variance (Huettel et al., 2004). Spatial smoothing will be accomplished using a Gaussian spatial filter with a full-width-half-maximum (FWHM) of 4 mm. We will also apply a de-spiking algorithm on a voxel-wise basis to smooth out large deviations in signal (> 2.5 SD from the mean).

6. Temporal filtering (e.g., Huettel et al., 2004). Noise is introduced into the fMRI data by low-frequency drift in magnetic field strength across the course of the scanning session. To reduce the effects of low-frequency drift on the BOLD signal with minimal effect on stimulus-related components of the BOLD signal, we will primarily use a cutoff frequency that is 1.5 times the task frequency. Specifically, we will use a band-pass filtering algorithm (either > 0.011-s or < 0.15-s).

7. Structural-functional co-registration. Structural-functional co-registration refers to the alignment of the pre-processed EPI images with the high resolution structural MRI images. This enables us to identify specific regions of interest and to spatially normalize the images for combining data across subjects, where necessary (Huettel et al., 2004; Jenkinson et al., 2002; Jenkinson & Smith, 2001).

8. Normalization to percent signal change. We will normalize each subject's BOLD signal intensity data to percent signal change using as a baseline each subject's voxel-wise time-series mean.