Identifying behavioral and neural correlates of observational avoidance learning in rats

by

Shannon Ruble

B.S., Kentucky Wesleyan College, 2022

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Psychological Sciences
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2025

Approved by:

Major Professor
Dr. Maria M. Diehl

Copyright

Abstract

Social interactions enrich the lives of individuals while also providing valuable information about the environment and appropriate behaviors. Observational learning, a form of social learning, allows individuals to learn about potential outcomes in an environment without directly experiencing those outcomes. Prior research has investigated how individuals learn fear through observation, but little is known about how individuals learn to avoid danger through observation. We therefore modified the platform-mediated avoidance (PMA) task to understand how rats (Observers) learn to avoid a tone-signaled footshock by observing another rat (Demonstrator) perform the task. We found that pre-exposure to a single footshock enhanced Observers' learning when Observers witnessed a Demonstrator undergoing early, but not late, stages of PMA training. Additionally, we found evidence that Observers may shift from utilizing avoidance strategies after observing early stages of PMA, to utilizing freezing strategies after observing late stages of PMA. To better understand the neural mechanisms underlying observational avoidance learning, we used *c-Fos* immunohistochemistry to quantify neural activation in the anterior cingulate cortex (ACC) and the infralimbic cortex (IL) since prior work has implicated these regions in observational learning and fear and avoidance learning, respectively. Surprisingly, we found no differences in ACC activation between Observers and Demonstrators. Conversely, the IL showed increased activation in shock pre-exposed Observers compared to Demonstrators and shock-naïve Observers. These findings provide insight into how different brain regions may be recruited to perform the same behavior depending on prior experiences.

Table of Contents

List of Figures

List of Tables

Chapter 1 - Introduction

Social interactions enrich the lives of individuals while also providing valuable information about the environment and appropriate behaviors. Observational learning, a form of social learning, allows individuals to learn about potential outcomes in an environment without directly experiencing those outcomes. Observational learning has been highly conserved across species with evidence of this ability demonstrated in nearly all taxa including insects[1,2], fish[3], birds [4,5], rodents[6,7], primates[8–11], and humans[12]. For example, bumblebees develop flower preferences after observing the foraging behaviors of conspecifics[1,2], and human children develop fears of animals after observing others express fear to those animals[13]. Witnessing the outcomes experienced by others eliminates the need for direct learning through trial and error, thus facilitating learning and reducing the risk of harm or missed opportunities. While learning through observation is highly advantageous, this ability is disrupted in individuals affected by certain neuropsychiatric diseases that impair social interaction including autism spectrum disorder[14,15], schizophrenia[16,17], and depression[18] among others. Therefore, studying the behavioral processes and neural substrates of observational learning can aid in understanding social deficits associated with neuropsychiatric disease.

In addition to impaired social interactions, individuals with autism spectrum disorder, schizophrenia, and depression also tend to suffer from heightened fear and avoidance of social situations and are often also diagnosed with anxiety disorders[19–24]. Avoidance is an adaptive behavior that allows individuals to circumvent danger, however, individuals with anxiety disorders may overestimate threats and avoid excessively consequently causing an adaptive behavior to become maladaptive[25]. An estimated 4% of the global population experience some form of an anxiety disorder[26], and individuals with comorbid anxiety disorders have lower recovery rates and

suffer from increased chronicity and symptom severity[27–29]. Maladaptive avoidance behaviors related to anxiety can also be acquired through observation, especially in cases where children learn from parents[30]. Given the prevalence of avoidance symptomology, it is important that we define the behavioral and neural mechanisms mediating avoidance across a multitude of contexts in order to better diagnose and treat individuals with maladaptive avoidance behaviors.

In this study, we designed a task that allowed rats to learn platform-mediated active avoidance by observing another rat perform the task. By studying observational avoidance learning in rats, we are able to determine what behavioral conditions promote avoidance acquisition through observation. We can also identify potential neural targets for promoting observational learning where this ability is impaired or for inhibiting avoidance when it becomes maladaptive. We used immunohistochemistry to characterize neural activation in the anterior cingulate cortex, an area well-established to be involved in observational learning[31–33], as well as the infralimbic cortex, an area known to play a role in extinction of fear and platform-mediated avoidance[34–38]. Examining neural activation in these brain areas provides a first look into how the brain may function during observational learning of platform-mediated avoidance as opposed to learning the task through direct experience.

1.1: Early Studies Investigating Observational Learning

Observational learning was first studied in a laboratory setting in the late 1800s by Edward Thorndike using cats in puzzle boxes. In these experiments, one cat, called an Observer, watched another, called a Demonstrator, perform behaviors such as pulling levers and loosening bolts to escape the puzzle box. The Observer was then placed in the puzzle box and expected to escape in the same way they had witnessed the Demonstrator escape. Thorndike found that the Observer cats

did not learn to escape the puzzle box simply by watching a Demonstrator cat escape the box and concluded that cats could not learn through observation[39]. Experiments using other animal models including primates[40], rats[41], and raccoons[42] replicated the failure to learn through observation, so it was further concluded that observational learning must be specific to humans.

However, the pursuit of evidence supporting observational learning continued. Research conducted in cats[8] and primates[10,11] found that animals could, in fact, learn through observation, but that some tasks were better learned than others due to the ecological relevance of the behaviors to be acquired. For example, the puzzle boxes that Thorndike used in his experiments required cats to learn behaviors that were neither commonly performed by the species nor typically associated with escaping confinement. Conversely, cats readily learned behaviors considered typical of the species such as catching mice and retrieving food from a bottle after witnessing a conspecific perform these behaviors[8]. The issue of ecological relevance extended beyond cats in a laboratory. Robert M. Yerkes found that a gorilla did not learn to use a hammer and nail through observation but readily learned to eat new foods that were previously ignored following observation[10]. The hammer held no ecological relevance for the gorilla, but learning to eat new foods was advantageous for the gorilla because the ability to consume a broader range of foods increases survival likelihood.

While the ecological irrelevance of puzzle boxes likely contributed to Thorndike's failure to document observational learning, his definition of observational learning may have been too strict to find evidence supporting the phenomenon even with an ecologically relevant task. Observational learning was viewed as an all-or-nothing process such that Observers were expected to immediately learn a task with which they had no prior experience by watching an experienced Demonstrator perform the task a single time[43]. Research using monkeys[44] and rats[7] showed that

learning different appetitive tasks through observation resulted in faster learning than learning through direct experience. These findings suggest that observational learning should not only be evaluated as successful or unsuccessful after watching a single session of some task. Rather, changes in learning rate should also be evaluated to truly understand the behavioral and neural processes mediating observational learning.

Herbert and Harsh also considered how individual differences in attention may impact observational learning. They noted that "an observer's act is limited by his perception of the demonstrator's act[45]," pointing out that previous studies did not account for attention allocation of the Observer. If an Observer does not allocate attention to the Demonstrator's performance of the task, then the Observer will not be able to recreate the Demonstrator's behavior later. Indeed, observational learning can be impaired when all or part of the Demonstrator's behavior is visually blocked from the Observer[7,46–48]. These findings highlight the need to limit an Observer's distractions while watching the Demonstrator as well as measure attention in some way.

1.2 Observational Fear Conditioning

Until this point, observational research focused on appetitive learning tasks, but it was unknown whether observational learning could be achieved for other ecologically relevant tasks such as responding to threats. To explore this question, researchers modified Pavlovian and contextual fear conditioning paradigms to allow an Observer to witness a Demonstrator undergo fear conditioning. In Pavlovian fear conditioning, a conditioned stimulus (CS) is paired with an aversive unconditioned stimulus (US), often a footshock[49]. Following the administration of the US, rodents exhibit a fear response known as freezing which is defined as the cessation of all movement except for respiration[50]. After repeated trials, rodents exhibit freezing to the CS making

freezing a conditioned response (CR) to the stimuli that predict the US. In contextual fear conditioning, there is no US predicting the CS. Instead, the context signals to the rodents that the US will be delivered. Following contextual fear conditioning, rodents display the CR of freezing in the fear conditioning context, but the CR is not present in a new context[49]. After witnessing the Demonstrator undergo fear conditioning, Observers' fear responses to either the context or to the CS were measured to assess their fear learning. Observers showed increased fear responses compared to individuals who did not previously witness fear conditioning[48,51–55], thus showing that individuals can learn to fear stimuli that predict danger through observation.

Previous research has shown that rodents need to directly experience the unconditioned stimulus to learn a CS-US association through observation or other social cues. One study showed that rats needed to experience footshocks in order for social cues signaling danger to induce freezing[56]. During observational fear conditioning, shock-exposed Observers increased freezing during CS presentations compared to baseline during a fear recall test while shock-naïve Observers did not[51]. While these and other studies argue that Observers need exposure with the aversive stimulus to learn through observation[51,56–60], some observational learning research argues Observers pre-exposed to shock have already directly experienced the US and is therefore not a true form of observational learning of conditioned fear[48,52]. One observational avoidance study used shock pre-exposure[57], but behavioral results from these animals were not directly compared to animals without shock pre-exposure. Despite the extensive research implementing shock pre-exposure in observational fear, it remains unknown how shock pre-exposure influences observational avoidance.

The Pavlovian and contextual fear conditioning paradigms used in previous observational fear studies are simple, yet effective, ways to examine threat responses in a laboratory setting, but

danger is unavoidable in these tasks. Outside of the laboratory, individuals must learn to avoid danger in addition to reacting to stimuli that predict danger. Learning avoidance through observation, therefore, allows an individual to learn how to cope with threats without directly experiencing those threats. Presley and Riopelle[9] were the first to study observational learning of an active avoidance response using monkeys. Demonstrator monkeys were required to jump over a hurdle to avoid a footshock, and the Observer monkeys were later tested in the same task. Much like the previous work on observational learning in appetitive tasks, avoidance was learned in fewer trials following observation compared to learning through trial and error. These findings were later replicated in cats in the hurdle-jumping task[57] and rats in a shuttle avoidance task[6]. Despite these findings, observational avoidance learning has remained unstudied for over 40 years. Deciphering the behavioral and neural correlates of observational avoidance learning is important for understanding how individuals gather information from their environment to respond accordingly to threats as well as why this ability is impaired in some individuals.

1.3 The Role of the Anterior Cingulate Cortex in Observational Learning

The anterior cingulate cortex (ACC) is implicated in various forms of social learning across multiple species[31–33], therefore making it a prime target for study in observational avoidance learning. Prior work has shown that ACC activity is necessary for observational reward learning[61] as well as observational fear conditioning[48,51,62,63]. Interestingly, the ACC is recruited during observational fear conditioning[48,51,62,63] even though ACC activity is not necessary for fear conditioning through direct experience[48]. The ACC is also not necessary for recalling fear learned through observation. Inactivating the ACC in Observers while watching a Demonstrator undergo fear conditioning inhibited fear recall, but inactivating the ACC during recall did not impair fear

expression[51]. Given that avoidance learning stems from fear conditioning[64], it stands to reason that the ACC would be involved in observational avoidance learning but not in the recall of avoidance.

However, recall of observed pain does involve the ACC. Single-unit recordings showed that a large proportion of neurons that respond to a Demonstrator receiving footshock also respond to directly-experienced pain via laser in the Observer[65]. While this study did not determine whether ACC activity was *necessary* for the recall of observed pain, it did provide insight into how neuronal firing compares between observing and experiencing pain. In avoidance, subjects learn across multiple trials that pain from footshock or other aversive stimuli can be avoided, thus decreasing fear responses like freezing to a CS[66]. As a result, an Observer witnessing avoidance training may not see fear responses. However, if a Demonstrator fails to avoid a trial late in training and receives shock, they will show signs of pain from receiving shock such as emitting alarm calls and jumping or running around the cage. Therefore, the ACC may be active during recall of avoidance learned through observation even though ACC activity is not necessary for recall of observational fear.

1.4 The Role of the Anterior Cingulate Cortex in Active Avoidance

Active avoidance requires subjects to execute an overt response in order to avoid an aversive outcome. In our lab, we use the platform-mediated avoidance (PMA) task in which rodents can avoid a tone-signaled footshock by giving up access to a sucrose reward and stepping onto a designated safe platform[34]. The PMA task was designed as a modification of Pavlovian fear conditioning such that rodents learn the CS-US association, but they are given an opportunity to avoid the aversive US. Early in PMA training, rats exhibit high freezing and low avoidance because they have not yet learned that shocks can be avoided. As training progresses, freezing

decreases and avoidance increases because rats have learned that the shock is occurs only during the final 2 seconds of the tone and can be avoided by stepping onto the platform[34]. Previous work using the PMA task under solitary conditions has shown that projections from the prelimbic cortex (PL) to the ventral striatum (VS) and basolateral amygdala (BLA) bidirectionally control active avoidance. Projections from the PL to the BLA and from the BLA to the VS promote active avoidance while the projection from the PL to the VS inhibits avoidance[67]. The ACC has been shown to project to both the PL[68,69] and the BLA[70–72], therefore making it a key region of interest for studies using the PMA task as well as other active avoidance tasks. Indeed, our lab has recently shown that ACC activity plays a role in the expression of PMA learned under both solitary and social conditions[73].

ACC activity has also been shown to play an important role in other active avoidance paradigms. Lesioning the ACC inhibited the acquisition of active avoidance in rabbits in which subjects had to activate a running wheel to avoid a tone-signaled shock[74,75]. ACC inactivation also impaired the ability of rats to avoid a fast-moving robot that delivered shock if rats were too close to the robot[76]. Given its known role in active avoidance and its connectivity with other brain regions regulating active avoidance such as the PL and BLA, the ACC is a prime target for study in observational avoidance.

1.5 The Role of the Infralimbic Cortex in Active Avoidance

The infralimbic cortex (IL) is typically associated with extinction of fear and avoidance [34–38] while also playing a role in the acquisition of some forms of active avoidance[77–80]. IL inactivation has been shown to have no effect on PMA acquisition but instead impairs PMA extinction[34,35]. Given that we are studying acquisition, not extinction, of avoidance through

observation, we would not expect IL to play a significant role in Observational PMA. However, IL has been shown to be important for selecting an appropriate response in the presence of multiple contingencies[78,81] or when a previously learned behavior is no longer adaptive[81–84].Observational learning requires an individual to transition from simply watching behaviors to performing those behaviors. When we consider the context of observational fear conditioning, subjects must update the previously learned stimulus-outcome association that shock is administered to a conspecific following a cue or in a certain context to the new stimulus-outcome association that shock is administered to themselves. In the context of observational active avoidance acquisition, subjects must make the same contingency transitions while also performing an avoidance response which also requires selecting the appropriate avoidance response instead of freezing. Given the complex transitions between watching and performing behaviors, IL may serve a role in observational learning even when the task to be acquired does not typically recruit IL when learned through direct experience.

Chapter 2 - Methods

2.1 Subjects

All procedures described here were approved by the Institutional Animal Care and Use Committee of Kansas State University in compliance with the National Institutes of Health guidelines for the care and use of laboratory animals.

A total of 159 adult Sprague-Dawley rats (89 males and 70 females) were used in this study. All experimental rats were be bred in-house from breeder rats purchased from Charles River Laboratories. Rats were housed in pairs or groups of three in standard cages and kept on a reverse light-dark cycle (lights off at 0830). Rats were handled at least twice weekly until females weighed 215 g and males weighed 300 g (2-3 months of age) at which point rats entered experiments and were handled daily. Experimental rats were food-restricted to 16-18 g of standard laboratory rat chow per day to maintain at least 85% of their free-feeding weight while being trained to lever-press for sucrose pellets. Some rats were used as homecage controls for this study and were never exposed to the experimental context. Homecage controls were housed in pairs or groups of three with other homecage controls and handled and food restricted as described above.

2.2 Observational PMA

This study employed a new modification of the PMA task that allowed for observational learning of avoidance: Observational PMA. In Observational PMA, an Observer rat witnessed a Demonstrator rat undergo PMA training as previously described[34,73,85]. Demonstrators lever-pressed for sucrose reward on a 30-second variable interval schedule of reinforcement (VI-30) and experienced 9 presentations of a 30-second tone that co-terminated with a 2-second footshock (4kz

tone, 0.4mA shock) with an average inter-trial interval (ITI) of 3 minutes. The Demonstrator could avoid the tone-signaled footshocks by stepping onto a designated safe platform located in the corner of the conditioning chamber; however, this avoidance behavior came at the cost of reward access as the lever could not be reached from the platform. While the Demonstrator performed PMA, the Observer watched an entire training session (9 tone-shock pairings) through a transparent acrylic barrier from an adjacent chamber. The barrier was perforated and allowed the Observer to receive visual, auditory, and olfactory cues from the Demonstrator without interfering with the Demonstrator's performance of the task. The Observer did not have access to a lever, food dish, or platform while watching the Demonstrator to limit distractions and encourage the Observer to focus their attention on the behavior of the Demonstrator. The side of the chamber in which the Demonstrator performed PMA (left or right) was counterbalanced across pairs to control for any potential effect the side of the chamber may have had on behavior.

Prior to assigning rats to be Demonstrators or Observers, all rats underwent lever-press training in the operant boxes used for Observational PMA. Rats underwent lever-press training in a social context with their cagemate occupying the adjacent chamber[85]. Both rats had access to their own lever and food dish during lever-press training, and platforms were present in the box. Lever-press training began with sucrose available on a fixed ratio schedule such that every press produced a sucrose pellet and gradually progressed to sucrose being available on a VI-30 reinforcement schedule. Lever-press training all rats prior to Observational PMA limited the amount of new information an Observer was expected to acquire by watching a Demonstrator while also habituating all rats to the operant boxes as well as being transferred to the experimental room. After reaching a pressing criterion of at least 12 lever-presses per minute, rats were randomly assigned to be Observers or Demonstrators and Observational PMA training began.

Demonstrators were trained in PMA for 10 days (**Figure 1A)**. Across the 10 days of Demonstrator PMA training, there were 2 different groups of Observers that witnessed Early (Day 1) or Late (Day 10) PMA training during an Observation Session. Having Observers witness Early or Late PMA allowed us to determine how the Demonstrator's experience with the task affected an Observer's acquisition of the task. Prior to the Observation Session, some Observers were pre-exposed to footshock while the rest of the Observers remained naïve to the footshock (**Figure 1B, far left**). Shock pre-exposure sessions consisted of a single unsignaled footshock (0.4mA, 2 sec duration) after a 5-minute habituation period. Shock pre-exposed rats were left in the chamber for an additional minute following the footshock and then immediately underwent their Observation Session (**Figure 1B, middle**). The platform was present during the shock exposure session and sucrose was available contingent upon lever-pressing on a VI-30 schedule. Rats were housed with cagemates of the same experimental group (Early versus Late PMA, shock pre-exposed versus shock-naïve) to limit social transfer of information in the homecage between rats experiencing different experimental conditions. Including both shock pre-exposed and shock-naïve Observers in this study allowed us to determine whether Observers needed experience with shock to learn observational avoidance, as has been shown in observational fear learning[51,57].

24 hours after observation, the Observers were placed into the chamber the Demonstrator previously occupied and given a full PMA test session in which they experienced 9 tone-shock presentations themselves (**Figure 1B, far right**). Observers that were used for c-Fos immunohistochemistry were sacrificed 90 minutes after Tone 1 of the Test Session. A group of homecage control rats housed under the same conditions was included in this study for *c-Fos* immunohistochemistry comparisons (described below). These rats were not exposed to the tone or

footshock used in Observational PMA in order to determine a baseline of neural activation in the brain areas of interest.

Figure 1: Experimental Timeline

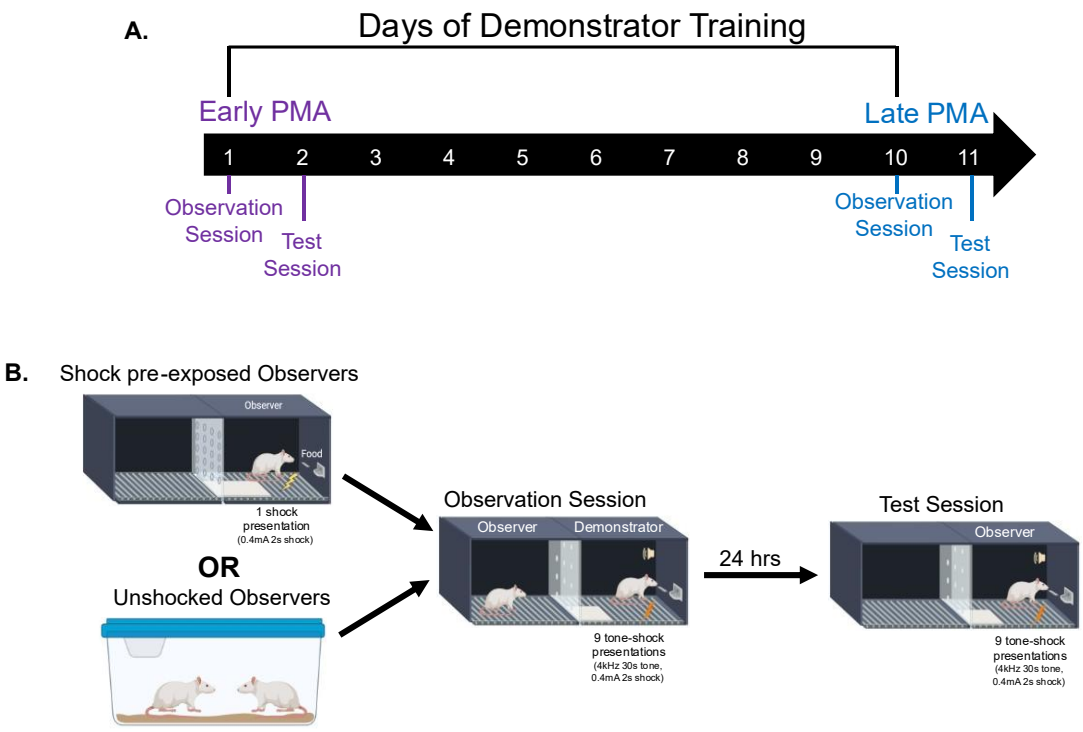

Figure 1: Experimental timeline. (A) Demonstrators are trained in 10 days of PMA. Observers watch either Day 1 (Early PMA) or Day 10 (Late PMA) of Demonstrator training. Observers then undergo their test session 24 hours after they watched the Demonstrator perform PMA. **(B)** Prior to the Observation Session, Observers are either exposed to one unsignaled footshock in the side of the box in which the Demonstrator will later perform PMA or remain unshocked and enter the Observation Session from the homecage. Shock pre-exposed Observers are placed in the box for 5 minutes, receive one footshock, and then remain in the box for 1 additional minute before being removed from the box. During the Observation Session, the Demonstrator receives 9 tone-shock pairings. The Observer does not have access to a platform, lever, or food dish during the Observation Session. 24 hours after the Observation Session, Observers are placed in the side of the chamber the Demonstrator previously occupied and presented with 9 tone-shock pairings themselves.

2.3 Behavioral Data

Behavioral measures of freezing and head orientation toward the Demonstrator chamber were automatically scored using AnyMaze behavioral tracking software during the Observation Session. A rat was oriented towards the Demonstrator chamber when the barrier between the chambers was within a 90-degree visual angle as measured from the tip of the rat's nose (45 degrees to either side of the nose). This range of head orientation was used to account for the fact that rats are prey animals and have a nearly 200-degree field of view, but only approximately 40 to 110 degrees of binocular vision[86,87]. Using a value within but near the high end of this range of binocular vision allowed to capture head direction as a measure of attention without potentially overestimating the amount of time the Observer spent looking at the Demonstrator's chamber.

Freezing and time spent on the platform were automatically scored during the Observer Test Session. Freezing is a commonly used measure of fear that is defined as the cessation of all movement excluding respiration[50]. AnyMaze scored head orientation, freezing, and time spent on the platform during the pretone period (1 min), the tone (30 sec) and the shock period (2 sec). The number of shocks avoided was measured as the rat spending at least 1.75 sec of the 2 sec shock period on the platform. 1.75 sec was used instead of the entire 2 sec shock period to account for the fact that rats must have two points of contact with the grid in order to receive shock. Near the end of the tone and during the shock period, rats often engage in "testing" behavior in which they lunge off the platform toward the sucrose lever and place a paw on the grid to check for shock. When rats engage in testing behavior, the video tracking software may track the rats as off the platform. Because rats can test while still avoiding, using a value slightly less than the full 2 sec shock period allowed us to account for discrepancies between the tracking software and the actual behavior.

2.4 *c-Fos* Immunohistochemistry

A subset of animals from all experimental groups (all Observer groups as well as Early and Late PMA Demonstrators) as well as a group of homecage controls were sacrificed for *c-Fos* immunohistochemistry (IHC). 90 minutes following the presentation of Tone 1 of the Observer Test Session, Observers were deeply anesthetized with sodium pentobarbital (450 mg/kg i.p.) and transcardially perfused with approximately 300 ml of 0.9% saline until all blood was cleared, followed by 500 ml of 4% paraformaldehyde. Demonstrators underwent an additional PMA session on the same day as the Observer Test Session and were perfused in the same manner as the Observers 90 minutes after Tone 1 of this session. Homecage control rats also underwent the same perfusion process without having been exposed to any task parameters as to provide a baseline measurement of neural activation.

Brains were removed from the skull and stored in 4% paraformaldehyde at 4°C for 12 to 24 hours. Brains were then transferred to a 20% sucrose solution in 1X PBS for 24 hours followed by a 30% sucrose solution in 1X PBS for another 48 to 72 hours (4°C). Brains were then gradually cooled to -20°C and sliced into 40 μm thick coronal sections on a cryostat and stored in cryoprotectant at -20°C until the immunohistochemical assay was conducted.

The tissue was washed in 0.01M PBS three times (10 min each) to remove cryoprotectant. Tissue was then blocked in 10% normal goat serum (NGS), 5% bovine serum albumin (BSA), and 0.5% triton-X in PBS at room temperature for 1 hr. Sections were then incubated in *c-Fos* monoclonal primary antibody raised in rabbit (1:3200) at room temperature for 48 hr and then washed in 1X PBS three times (10 min each) to remove excess antibody. The tissue was then incubated in a secondary anti-rabbit antibody (1:500 AlexaFluor 588) at room temperature for 6 hr followed by five 1X PBS washes (10 min each) and a 10 min wash in a

DAPI+1X PBS solution (1:25000 DAPI). The tissue was then washed with PBS two more times (1 min wash followed by 10 min wash) and stored in fresh PBS overnight. Sections were then mounted on slides and coverslipped with Fluoromount-G mounting medium.

2.5 *c-Fos* Imaging and Quantification

Following *c-Fos* IHC, coronal sections were imaged using an Olympus BX63F fluorescence microscope and digital camera at 10x magnification. Individual Z-stack images were stitched together using CellSens software to create an X-Y composite image of the ACC and the infralimbic cortex (IL). Z-stacks were merged to create a single plane in which all *c-Fos* positive cells in the 40 μm thick section could be seen simultaneously. Images were registered to the rat brain atlas using ABBA[88], and quantification of *c-Fos* positive cells was conducted automatically using QuPath[89]. Thresholding for counting was performed with the experimenter blinded to condition. The ACC and IL were delineated to calculate the total area of the region for each rat. Density of *c-Fos* positive cells was calculated by dividing the number of *c-Fos* positive cells by the total area of the brain region. *c-Fos* density was measured in both hemispheres from three non-consecutive slices and averaged to obtain a final *c-Fos* density for each rat [90]. Left and right hemispheres were not marked prior to tissue slicing, so we cannot compare *c-Fos* densities across hemispheres in each rat nor determine an overall difference in neural activation between hemispheres across the rats in this study.

2.6 Statistical Analyses

Behavioral measures of time spent freezing and time on platform were analyzed from the Observers' Test Session using multilevel beta regressions. Head orientation was analyzed from the

Observers' Observation Session using a multilevel beta regression. Beta regressions were used because behaviors were measured as the proportion of each Tone spent performing that behavior, producing data bound between zero and one. Tone, condition (Early or Late PMA), shock pre-exposure status, and all their interactions were specified as fixed effects in the regression models and subject ID was specified as a random effect. Two other regression models were compared to this base model to determine whether the Demonstrator's behavior during the Observation Session or the amount of time the Observer spent oriented toward the Demonstrator during the Observation Session significantly predicted the behavior of the Observer during the Test Session (see Model Comparison section below). Ultimately, the simplest model proved to be the best fit of the data and was thus used for all behavioral models. Tukey's adjusted post-hoc tests were used for individual comparisons.

A Poisson regression was used to analyze the number of shocks avoided with the same fixed effect structure used for the beta regressions. Tukey's adjusted post-hoc tests were used for individual comparisons. Correlations were conducted between Demonstrator behavior during the Observation Session and Observer behavior during the Observer Test Session to understand the relationship between the behaviors the Observer witnesses and the behaviors they later perform in their Test Session. Additionally, correlations were conducted between the head orientation of Observers during the Observation Session and their time on platform or freezing during the Test Session.

A one-way ANOVA was used to analyze the effect of shock pre-exposure on *c-Fos* density in the ACC and IL. Separate ANOVAs were used for Early and Late PMA, and the Demonstrators were also included in these analyses. Tukey's post hoc tests were used for individual comparisons. Single sample t-tests were used to determine whether *c-Fos* density in

each group of Observers or Demonstrators differed significantly from that of the homecage controls. To conduct this analysis, all *c-Fos* density counts were normalized to those of the homecage controls, and it was determined whether *c-Fos* density differed significantly from 0. Correlations were conducted between time spent freezing during the Observer Test Session and *c-Fos* density as well as between time spent on the platform during Observer Test Session and *c-Fos* density in both the ACC and IL. We also conducted correlations between *c-Fos* density in the ACC and *c-Fos* density in the IL for all conditions.

2.7 Model Comparison

Three different beta regression models were compared to determine which predictors best explained the behavioral data (**Table 1,** significant effects shaded in green). Model comparison was performed with time on platform as the outcome variable, and the best model was then used to analyze freezing. The same fixed effect structure was then used in the Poisson regression predicting the number of shocks avoided.

All models used the same random effect structure where the subject ID was the only random effect, thus allowing the intercept of each individual to vary. More complex random effect structures led to non-convergence of the models. Model 1 used the simplest fixed effect structure where shock pre-exposure status (shocked or unshocked), condition (Early or Late PMA), Tone, and all possible interactions were included in the model. Model 2 used all the effects of Model 1 with the addition of the Demonstrator's time on platform as a predictor as well as all of the possible interactions with this added predictor. Model 2 found the same significant effects as Model 1 as well as a significant three-way interaction of shock pre-exposure, condition, and Demonstrator behavior. Model 3 used all of the effects of Model 1 with the addition of head orientation as a

predictor as well as all of the possible interactions with this added predictor. None of the additional effects were significant in Model 3, therefore, it can be concluded that the amount of time that the Observer spent oriented towards the Demonstrator is not a significant predictor of observational avoidance learning. Model 3 was also removed from further comparisons.

Model 1 and Model 2 were the best of the three available models, so AIC values were compared. While Model 2 provides additional insight into how the Demonstrator's behavior influences the Observer's performance during the Test Session, the AIC value for Model 2 (AIC=-2920.8) is higher than that of Model 1 (AIC=-2926.8). This discrepancy in AIC values indicates that the added complexity of Model 2 cannot be justified by the additional amount of variance that the predictor of Demonstrator avoidance accounts for in the model. Indeed, when the significant three-way interaction was probed using Tukey's adjusted post-hoc tests, none of the individual comparisons were found to be significant. These findings indicate that Demonstrator behavior is an important aspect of observational avoidance learning, but also that there may be other potentially more important factors leading to observational learning that we did not measure in this experiment. As such, Model 1 will be used for all data analyses in this experiment moving forward.

Table 1: Model Comparison

Model 1: Simplest effect structure				AIC=-2926.8
Predictor	Estimate	Standard Error	z-value	p-value
Shock pre-exposure	-0.339	0.097	-3.485	<0.001
Tone	0.223	0.019	11.882	<0.001
Condition	-0.002	0.097	-0.024	0.981
Shock pre-exposure* Tone	0.027	0.018	1.529	0.126
Shock pre-exposure* Condition	-0.146	0.097	-1.509	0.131
Tone * Condition	0.003	0.018	0.164	0.870
Shock pre-exposure * Tone * Condition	-0.028	0.018	-1.567	0.117
Model 2: Demonstrator behavior as a predictor				AIC= -2920.8
Shock pre-exposure	-0.398	0.100	-3.990	<0.001
Tone	0.210	0.022	9.704	<0.001
Condition	0.016	0.099	0.162	0.871
Demonstrator Avoidance	0.120	0.159	0.756	0.450
Shock pre-exposure*Tone	0.046	0.021	2.184	0.029
Shock pre-exposure*Condition	-0.182	0.099	-1.834	0.067
Tone*Condition	-0.003	0.021	-0.134	0.893
Shock pre-exposure * Demonstrator Avoidance	-0.122	0.159	-0.768	0.443
Tone * Demonstrator Avoidance	-0.043	0.054	-0.799	0.425
Condition*Demonstrator Avoidance	-0.062	0.159	-0.389	0.698
Shock pre-exposure * Tone * Condition	-0.016	0.021	-0.788	0.431
Shock pre-exposure * Tone * Demonstrator Avoidance	-0.333	0.160	-2.082	0.037
Tone * Condition * Demonstrator Avoidance	-0.076	0.055	-1.388	0.165
Shock pre-exposure * Tone * Condition * Demonstrator Avoidance	0.065	0.054	1.203	0.229
Model 3: Head orientation as a predictor				AIC=-2916.7

Shock pre-exposure	-0.340	0.099	-3.450	<0.001
Tone	0.222	0.019	11.685	<0.001
Condition	0.007	0.098	0.073	0.942
Head Orientation	0.020	0.169	0.120	0.905
Shock pre-exposure * Tone	0.026	0.018	1.477	0.140
Shock pre-exposure * Condition	-0.145	0.098	-1.471	0.141
Tone * Condition	0.002	0.018	0.105	0.916
Shock pre-exposure * Head Orientation	0.060	0.169	0.355	0.723
Tone * Head Orientation	-0.005	0.061	-0.085	0.932
Condition * Head Orientation	0.232	0.169	1.367	0.172
Shock pre-exposure * Tone * Condition	-0.032	0.018	-1.815	0.069
Shock pre-exposure * Tone * Head Orientation	-0.037	0.061	-0.617	0.538
Shock pre-exposure * Condition * Head Orientation	0.184	0.169	1.092	0.275
Tone * Condition * Head Orientation	-0.068	0.061	-1.114	0.265
Shock pre-exposure * Tone * Condition * Head Orientation	0.058	0.061	0.960	0.337

Note: Categorical predictors were effect-coded and continuous predictors were mean-centered

Chapter 3 - Results

3.1 Shock pre-exposure enhances observational avoidance when Observers witness early stages of PMA.

Prior studies have found that shock pre-exposure improves or is necessary for observational fear conditioning[51,59,60,91]; however, it remains unknown how shock pre-exposure may influence observational avoidance learning. In contrast to fear conditioning which can be acquired in a single session, PMA requires multiple days of training to learn[67,73,85,90,92,93]. It can therefore be reasoned that the Demonstrator would display different behaviors (i.e time spent on the platform during the tone, number of shocks avoided, and freezing) for the Observer to watch depending on the number of training days the Demonstrator previously had. To examine how both shock pre-exposure and experience level of the Demonstrator influence Observational PMA, we used a two-by-two design, producing four separate Observer groups: shock pre-exposed and shock-naïve Early PMA Observers, and shock pre-exposed and shock-naïve Late PMA Observers. Avoidance measures of time on the platform during the tone and the number of shocks avoided as well as freezing measures were examined during the Observer Test Session.

The percentage of the tone that Observers spent on the platform during the Test Session was analyzed using a multilevel beta regression with shock pre-exposure status (shocked or unshocked), condition (Early or Late PMA), Tone, and all possible interactions specified as fixed effects. Observer ID was specified as a random effect to account for individual variability. There was a significant main effect of Tone ($p<0.001$) showing that time on platform increases across the session across Observer groups. There was a significant main effect of shock pre-exposure status ($p<0.001$) such that shock pre-exposed Observers spent more time on the platform than unshocked Observers. Tukey's corrected post-hocs on the interaction of shock pre-exposure status

and condition revealed that at Early PMA, shock pre-exposed Observers spent a significantly higher percentage of time on the platform during the tone than unshocked Observers (**Figure 2B**; p=0.003). Shock pre-exposure did not, however, alter the percentage of time spent on the platform when Observers witnessed Late PMA (**Figure 2C**; p=0.492). There were no significant differences in percentage of time on the platform between Observers who witnessed Early versus Late PMA Training (p=0.981) in shock pre-exposed (**Figure 2B compared to 2C**, light triangles; p=0.730) or unshocked Observers (**Figure 2B compared to 2C**, dark circles; p=0.687).

The number of shocks that Observers avoided during the Test Session was analyzed using a Poisson regression with shock pre-exposure status (shocked or unshocked), condition (Early or Late PMA), and their interaction specified as fixed effects. There was again a significant main effect of shock pre-exposure status (p<0.001) such that shock pre-exposed Observers avoided more shocks than unshocked Observers. Tukey's corrected post-hocs on the interaction of shock pre-exposure status and condition revealed that at Early PMA, shock pre-exposure led to an increased number of shocks avoided (**Figure 2D**; p=0.003). At Late PMA shock pre-exposure did not cause a difference in the number of shocks avoided. (**Figure 2E**; p=0.054 There were no differences in number of shocks avoided between Observers who witnessed Early or Late PMA (p=0.374) in either shock pre-exposed (**Figure 2D compared to 2E,** light colors; p=0.628) or unshocked Observers (**Figure 2D compared to 2E,** dark colors; p=0.998).

The percentage of the tone that Observers spent freezing during the Test Session was analyzed using a multilevel beta regression with shock pre-exposure status (shocked or unshocked), condition (Early or Late PMA), Tone, and all possible interactions specified as fixed effects. Observer ID was specified as a random effect to account for individual variability. There was a significant main effect of Tone (p<0.001) showing that freezing increases across the Test

Session across all Observer groups. There were no significant differences in freezing between shock pre-exposed and unshocked Observers at either Early (**Figure 2F**; p=0.549) or Late (**Figure 2G**; p=0.802) PMA. There were also no differences in freezing between Observers who witnessed Early or Late PMA (**Figure 2F compared to 2G**; p=0.398). Taken together, these results show that shock pre-exposure increases avoidance when Observers witness Early, but not Late, stages of PMA without altering freezing responses typically associated with fear.

Figure 2: Observer Test Session avoidance and freezing behaviors.

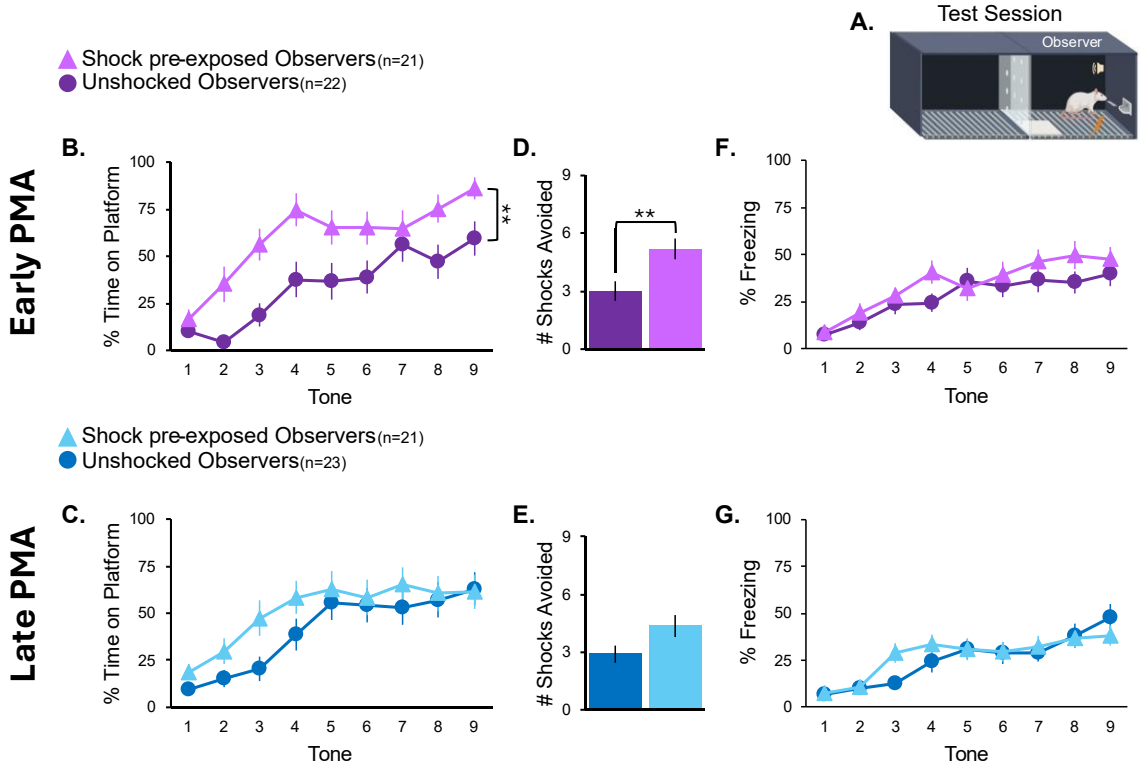

Figure 2: Observer Test Session avoidance and freezing behaviors. (A) Schematic depicting Observational PMA Test Session. **(B-C)** A multilevel beta regression revealed a significant main effect of shock pre-exposure ($p<0.001$). **(B)** Tukey's post-hoc tests showed that shock pre-exposed Observers spent significantly more time on the platform during the tone (p=0.003) at Early PMA. **(C)** There were no differences between shock pre-exposed and shock-naïve Observers at Late PMA. **(D-E)** A Poisson regression revealed a significant main effect of shock pre-exposure on the number of shocks avoided (p<0.001). **(D)** Tukey's post-hoc tests showed that shock pre-exposed Observers avoided significantly more shocks than shock-naïve Observers at Early PMA (p=0.003). **(E)** There were no differences in the number of shocks avoided between shock-naïve and shock pre-exposed Observers at Late PMA. **(F-G)** A multilevel beta regression revealed a significant main effect of Tone (p<0.001). There were no differences in freezing between any of the Observer groups.
*p<0.05, **p<0.01, ***p<0.001

3.2 Observer avoidance is correlated with Demonstrator avoidance during early stages of PMA.

We were next interested in the relationship between the behaviors of the Demonstrator that the Observer witnessed and the Observer's behavior during the Test Session. We conducted correlations between the Demonstrator's behaviors that the Observer witnessed during the Observation Session and the Observer's behaviors during the Test Session 24 hours later. At Early PMA, we found that the Demonstrator's percentage of time on the platform during training was positively correlated with the Observer's percentage of time on the platform during the Test Session when the Observer had shock pre-exposure (**Figure 3A**, light triangles; r=0.529, p=0.014). Conversely, the correlation between Demonstrator and Observer's percentage of time on the platform was negatively correlated when the Observer was shock-naïve (**Figure 3A**, dark circles; r=-0.703, p<0.001). At Late PMA, there were no significant correlations between the Demonstrator's percentage of time on the platform during training and the Observer's percentage of time on the platform during the Test Session in either shock pre-exposed (**Figure 3B**, light triangles; r=-0.040, p=0.865) or shock-naïve Observers (**Figure 3B**, dark circles; r=0.332, p=0.121).

There was not a correlation between the Demonstrator's number of shocks avoided during training and the Observer's number of shocks avoided during the Test Session when Observers had shock pre-exposure at Early PMA (**Figure 3C**, light triangles; r=0.368, p=0.101). A negative correlation was seen between the Demonstrator's number of shocks avoided during training and the Observer's number of shocks avoided during the Test Session in shock-naïve Observers (**Figure 3C**, dark circles; r=-0.571, p=0.005). At Late PMA, there were no significant correlations between the Demonstrator's number of shocks avoided during training and the Observer's number

of shocks avoided during the Test Session in either shock pre-exposed (**Figure 3D**, light triangles; r=0.049, *p*=0.833) or shock-naïve Observers (**Figure 3D**, dark circles; r=0.330, *p*=0.124).

Additionally, there were no significant correlations between the percentage of time that the Demonstrator spent freezing during training and the Observer's freezing during the Test Session at Early PMA (**Figure 3E,** light triangles, r=0.288, *p*=0.205; dark circles, r=-0.107, *p*=0.635). However, at Late PMA, Demonstrator and Observer freezing were correlated when the Observer was shock-naïve (**Figure 3F**, dark circles, r=0.467, *p*=0.025) but not when the Observer had received shock pre-exposure (**Figure 3F**, light triangles, r=0.265, *p*=0.246). Taken together, these findings suggest that the Demonstrator's avoidance behaviors are related to the Observer's avoidance behaviors at Early PMA but that the Demonstrator's freezing is related to the Observer's freezing at Late PMA.

Figure 3: Correlations of Demonstrator Training and Observer Test behaviors.

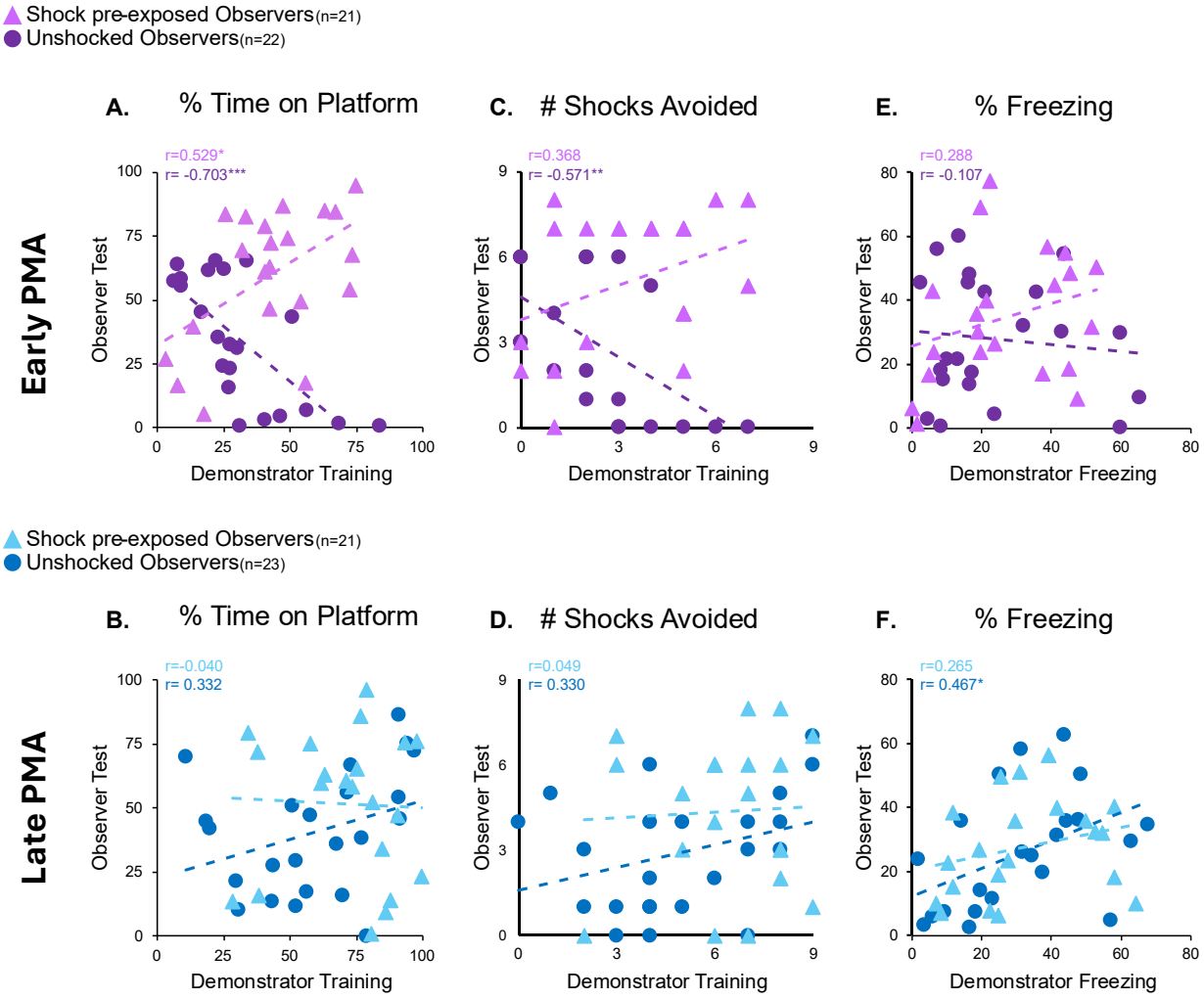

Figure 3: Correlations of Demonstrator Training and Observer Test behaviors. (A) During Early PMA, unshocked Observers exhibit negative correlations between their Test Session time on platform and the Demonstrator's Training time on platform (p<0.001). Shock pre-exposed Observers exhibit positive correlations with their Demonstrator's time on platform (p=0.014). **(B)** During Late PMA, there are no correlations between the Observer's Test session time on platform and their Demonstrator's Training time on platform regardless of shock pre-exposure status. **(C)** During Early PMA, unshocked Observers exhibit negative correlations between their Test Session number of shocks avoided and the Demonstrator's Training number of shocks avoided (p=0.005). Shock pre-exposed Observers do not exhibit correlations with their Demonstrator's number of shocks avoided. **(D)** During Late PMA, there are no correlations between the Observer's Test session time on platform and their Demonstrator's Training time on platform regardless of shock pre-exposure status. **(E)** During Early PMA, there are no correlations between the Observer's Test Session freezing and the Demonstrator's Training freezing. (F) During Late PMA, unshocked Observers show a significant correlation between their Test Session freezing and their Demonstrator's freezing during training (p=0.025). Shock pre-exposed Observers do not exhibit correlations with their Demonstrator's freezing.
*p<0.05, **p<0.01, ***p<0.001

3.3 Head orientation is correlated with avoidance in shock pre-exposed Observers at late stages of PMA.

Prior work has shown that blocking visual information impairs observational learning[7,46–48], so we were interested in how visual attention as measured by head orientation was related to Observational PMA acquisition. The percentage of the tone that Observers spent oriented towards the barrier during the Observation Session was analyzed using a multilevel beta regression with shock pre-exposure status (shocked or unshocked), condition (Early or Late PMA), Tone, and all possible interactions specified as fixed effects. Observer ID was specified as a random effect to account for individual variability. There was a significant main effect of Tone ($p<0.001$) indicating that orientation toward the barrier increases across the Observation Session across Observer groups. There were no differences in the percentage of the tone spent oriented toward the barrier between the Observer groups at Early (**Figure 4B**, light triangles compared to dark circles, $p=0.999$) or Late PMA (**Figure 4C**, light triangles compared to dark circles, $p=0.725$).

We also conducted correlations between the Observer's head orientation during the Observation Session and the Observer's avoidance and freezing during the Test Session 24 hours later. At Early PMA, there were no significant correlations between head orientation and avoidance in either shock pre-exposed (**Figure 4D**, light triangles, r=0.175, $p=0.448$) or shock-naïve Observers (**Figure 4D,** dark circles, r=0.095, $p=0.675$). At Late PMA, shock-naïve Observers showed no correlation between head orientation and time spent on the platform (**Figure 4E**, dark circles, r=0.270, $p=0212$), however, shock pre-exposed Observers showed a positive correlation between head orientation and time spent on the platform (**Figure 4E**, light triangles, r=0.596, $p=0.004$). These results suggest that visual information may be more important for Late PMA compared to Early PMA Observers.

Figure 4: Observation Session head orientation.

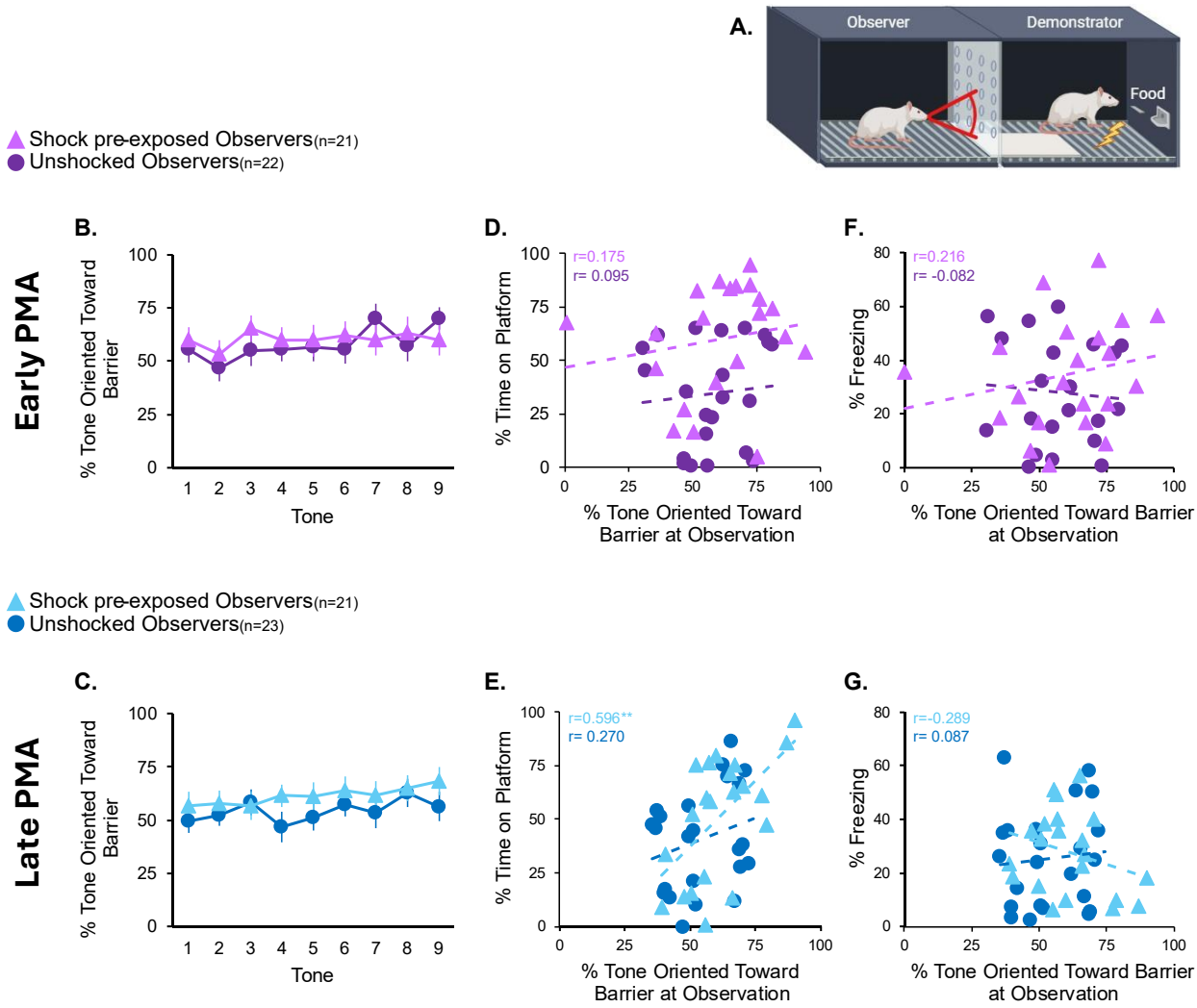

Figure 4: Observation Session head orientation. A) Schematic showing how head orientation was measured during Observational PMA. **B-C)** There were no differences between shock pre-exposed and shock-naïve Observers in the percentage of time oriented towards the barrier at Early or Late PMA. **(D)** At Early PMA, there were no significant correlations between the percentage of time the Observer spent oriented toward the barrier during the Observation Session and their percentage of time on the platform during the test session. **(E)** At Late PMA, there was a significant correlation between the percentage of time that shock pre-exposed Observers spent oriented toward the barrier during the Observation Session and the percentage of time spent on the platform during the Test Session (p=0.004). The same correlation was not significant for shock-naïve Observers. **(F-G)** There were no correlations between the time spent oriented towards the barrier during the Observation Session and Test Session Freezing in any Observer conditions.
*p<0.05, **p<0.01, ***p<0.001

3.4 IL, but not ACC, shows increased activation in shock pre-exposed Observers.

The role of ACC is observational fear conditioning[48,51,62,63] as well as observational reward learning[61] has been well-characterized. We were therefore interested in how neural activation of

the ACC differs between Observers and Demonstrators in Observational PMA. The IL has a well-established role in fear and PMA extinction[34–38], but there is also evidence suggesting that the IL may be important for inhibiting previously learned information to respond to new stimuli or contingencies[82–84]. We hypothesized that the IL would not show increased activation compared to control in any of our experimental groups because we were studying acquisition rather than extinction of PMA. However, we measured neural activation from the Test Session when Observers must transition from watching to performing PMA, introducing the possibility that Observers would show increased IL activation compared to Demonstrators due to the changing contingencies for the Observers.

We measured the density of *c-Fos* positive cells in the ACC and IL of Observers following the Test Session. Additionally, the Demonstrators were given an extra PMA session 24 hours after being observed and were sacrificed for immunohistochemistry following this session. We first normalized *c-Fos* density in both the ACC and IL to that of the homecage controls to better understand how activation differs between our experimental groups and rats that do not undergo Observational PMA as either Observers or Demonstrators. Using a single sample t-test, we compared normalized *c-Fos* density of each group to 0. The shock pre-exposed Late PMA Observers exhibited a trend toward increased ACC *c-Fos* desnity compared to homecage controls (p=0.094). No other experimental groups approached significant differences from controls. Shock pre-exposed Observers that witnessed both Early (p=0.011) and Late PMA (p=0.008) exhibited increased IL *c-Fos* densities compared to those of the homecage controls.

An ANOVA revealed that there were no differences in ACC *c-Fos* density between either Observer group or Demonstrators at Early PMA (**Figure 5B**, p=0.234). There were also no correlations between ACC *c-Fos* density and time spent on the platform during the Test Session

(**Figure 5C**). Conversely, an ANOVA revealed a main effect of group at Late PMA (**Figure 5D**, p=0.036). Tukey's corrected post hocs revealed only trends in the comparisons such that shock pre-exposed Observers trended toward have higher ACC *c-Fos* density than Demonstrators (p=0.061) and shock-naïve Observers (p=0.053). Again, there were no correlations between ACC FOS density and time spent on the platform during the Test Session (**Figure 5E**).

When examining IL *c-Fos* density, an ANOVA showed that there was a significant main effect of group at Early PMA (**Figure 5G**, p=0.024). Tukey's corrected post hocs revealed that shock pre-exposed Observers exhibited increased IL *c-Fos* density compared to Demonstrators (p=0.033) and trended toward increased *c-Fos* density compared to shock-naïve Observers (p=0.059). Shock pre-exposed Observers also showed a negative correlation between IL *c-Fos* density and their time spent on the platform during the Test Session (**Figure 5H**, r=-0.818, p=0.047). A similar pattern of IL activation was observed at Late PMA. An ANOVA showed a significant main effect of group (**Figure 5I**, p=0.009). Tukey's corrected post hocs showed that shock pre-exposed Observers exhibited increased IL *c-Fos* density compared to Demonstrators (p=0.027) and shock-naïve Observers (p=0.012). Unlike Early PMA, however, there were no correlations between IL *c-Fos* density and Test Session time on platform for any of the Late PMA groups (**Figure 5J**).

We then correlated *c-Fos* densities in the ACC and IL to gain insight into how multiple prefrontal brain areas may work together to perform Observational PMA. We found that both Early (**Figure 5K**, r=0.971, p=0.001) and Late (**Figure 5L**, r=0.839, p=0.037) PMA Demonstrators showed correlations between ACC and IL *c-Fos* densities. Shock-naïve Observers at Early PMA exhibited a trend towards a positive relationship between ACC and IL *c-Fos* densities (**Figure 5K**, r=0.856, p=0.064).

Figure 5: Test Session neural activation.

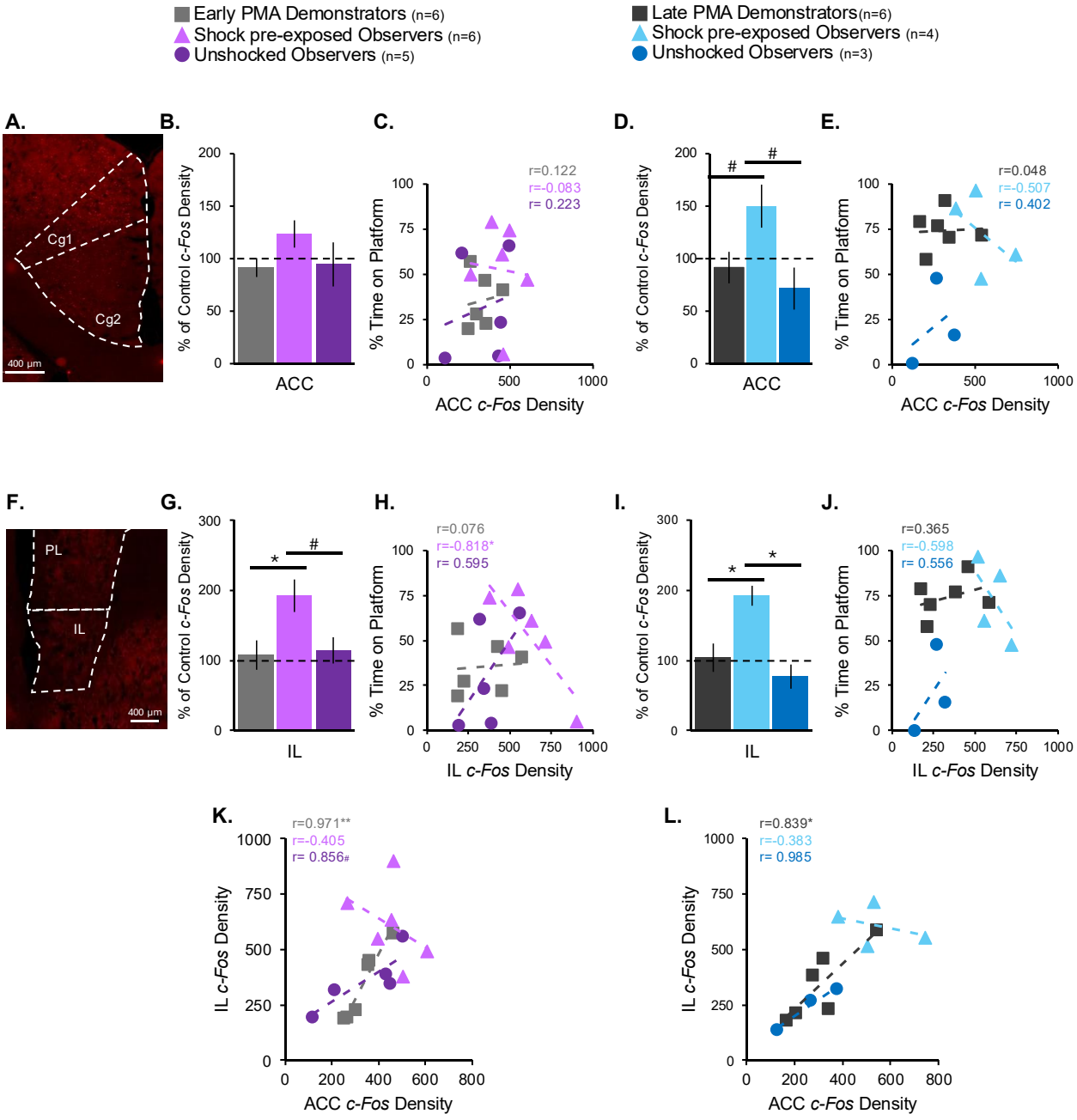

Figure 5: Test Session neural activation. (A) Micrograph showing *c-Fos* expression in ACC. **(B)** There were no differences in ACC *c-Fos* Density between groups at Early PMA **(C)** There were no correlations between ACC *c-Fos* Density and time on platform at Early PMA. **(D)** There were no significant differences in ACC *c-Fos* density between groups at Late PMA. Shock pre-exposed Observers trended toward higher densities than demonstrators (p=0.061) and shock-naïve Observers (p=0.053). **(E)** There were no correlations between ACC *c-Fos* Density and time on platform at Late PMA. **(F)** Micrograph showing *c-Fos* expression in IL. **(G)** Shock pre-exposed Observers had higher *c-Fos* density in IL than Demonstrators (p=0.033) and trended toward higher density than shock-naïve Observers (p=0.059) at Early PMA. **(H)** Shock pre-exposed Observers showed a negative correlation between IL *c-Fos* density and time on platform (r=-0.818, p=0.047). **(I)** Shock pre-exposed Observers had higher *c-Fos* density in IL than Demonstrators (p=0.027) and shock-naïve Observers (p=0.012) at Late PMA. **(J)** There were no correlations between IL FOS Density and time on platform at Late PMA. **(K)** Early PMA Demonstrators showed a correlation between ACC and IL *c-Fos* densities (r=0.971, p=0.001). Shock-naïve Observers trended toward a positive correlation between ACC and IL *c-Fos* densities (r=0.856, p=0.064). **(L)** Late PMA Demonstrators showed a correlation between ACC and IL *c-Fos* densities (r=0.839, p=0.037). Dashed line in B, D, G, I indicates homecage control *c-Fos* density. Data in these panels was normalized to homecage controls.
#p<0.100, *p<0.05, **p<0.01, ***p<0.001

Chapter 4 - Discussion

We developed a new paradigm to study observational avoidance learning and the behavioral conditions that promote Observational PMA. The use of a two-by-two design allowed us to parse apart how the Demonstrator's experience (Early or Late PMA training) as well as how the Observer's prior experiences (shock pre-exposed or shock-naïve) affect Observational PMA acquisition. Overall, we saw that shock pre-exposure improved avoidance in Observers who witnessed Early, but not Late, PMA. We also saw that PMA could be acquired through observation regardless of the experience level of the Demonstrator. We discuss below how Early and Late PMA Observers may utilize different information to produce the same behavioral outcomes as well as provide future directions for directly testing these hypotheses.

We also examined neural activation in two brain areas, the ACC and IL, when Observers and Demonstrators were required to recall information they had learned through observation or through direct experience (Demonstrators) during a Test Session. We surprisingly saw no differences in ACC activation between Demonstrators and Observers despite the known role of the ACC in observational learning. Interestingly, we saw that the IL showed increased activation in shock pre-exposed Observers compared to Demonstrators and shock-naïve Observers. We did not expect to find this difference given that the IL is typically implicated in the extinction of conditioned fear and avoidance, not the acquisition of these behaviors. We address these unexpected neural findings below and discuss future directions for better understanding the roles of ACC and IL in Observational PMA.

4.1 Early stages of Observational PMA show similarities to observational fear conditioning.

Because there is ongoing debate as to whether experience with the aversive stimulus is necessary for observational fear learning[48,51,52,56,59], we exposed half of our Observers to footshock prior to the Observation Session. We saw that shock pre-exposure enhanced avoidance in Observers that witnessed Early PMA while having no effect on freezing (**Figure 2B, 2D, 2F**). The lack of change in freezing indicates that enhanced avoidance learning was not simply a product of increased fear. Some previous studies have shown that acute shock stress enhances active avoidance acquisition, but these studies define "acute stress" as at least 30 shocks compared to the single shock our Observers received[94–96]. As such, it is unlikely that we are capturing an effect of stress enhanced avoidance learning. This is further evidenced by the fact that we did not see an increase in avoidance in the shock pre-exposed Observers at Late PMA (**Figure 2C, 2E**). Future work using the Observational PMA task could also examine behaviors such as lever-press suppression and darting to further understand how fear responses may influence avoidance acquisition through observation.

The shock pre-exposure that we used in this study is similar to the procedure employed by Allsop et al.[51] in which an Observer is placed into the compartment that the Demonstrator will later occupy for 5 minutes and then given one unsignaled footshock. In the previous study, they found that both shock pre-exposed and shock-naïve Observers showed increased cued freezing compared to various control conditions. However, shock-naïve Observers did not significantly increase their freezing from baseline during their Test Session indicating that they did not acquire observational fear to the same extent as the shock-exposed Observers[51]. These results are similar to those that we see here in our Early PMA Observers: shock pre-exposed rats exhibit increased

avoidance compared to shock-naïve Observers. These similarities in results may indicate that Early Observational PMA may be controlled by similar mechanisms that control observational fear conditioning. This conclusion also aligns with previous work showing that early PMA training is similar to classical fear conditioning because rats have not yet learned that the tone-signaled footshocks can be avoided by stepping onto the platform[34,66,73,93].

Similarities in learning mechanisms between Early Observational PMA and observational fear conditioning were also observed in behavioral correlations between Demonstrator and Observer (**Figure 3A**). As the Demonstrator spent more time on the platform, shock pre-exposed Observers also spent more time on the platform. In observational fear conditioning, shock pre-exposed Observers significantly increase their freezing in response to the conditioned stimulus after witnessing the Demonstrator freeze in response to the conditioned stimulus[51]. The shock pre-exposed Observers in both Observational PMA and observational fear conditioning have prior experience with footshock which potentially provides them with additional information regarding the Demonstrator's reaction to footshock and why they increase their avoidance or freezing respectively.

Conversely, as the Demonstrator spent more time on the platform, shock-naïve Observers spent less time on the platform during their Test Session. Because shock-naïve Observers have never experienced footshocks, they may lack insight into why the Demonstrator reacts the way that they do to footshocsk. Indeed, shock-naïve Observers in observational fear conditioning failed to increase their freezing to the conditioned stimulus during their Test Session, potentially indicating that they did not understand why the Demonstrator was freezing[51]. Moreover, shock-naïve Observers may not obtain sufficient task contingency information about the safety of the platform if the Demonstrator stays on the platform for extended periods spanning the tone and ITI

periods of the task. A Demonstrator who spends excessive amounts of time on the platform does not accurately convey that reward-seeking is safe during the ITI and that the footshock is only present during the tone. In the current study, we did not examine time spent on the platform during the ITI, but future work could measure the amount of time that the Demonstrator spends on the platform during the ITI to determine how this impacts Test Session behavior in the Observers. Demonstrators that spend less time on the platform during the ITI would be more likely to be performing avoidance as expected and only going to the platform when the tone is presented. Demonstrators with this pattern of time on platform would theoretically be the most effective Demonstrators and we could expect Observers of these rats to perform the best in Observational PMA.

It is also possible that shock pre-exposure acts as an auto conditioning session in which the Observers learn not only about the pain of the footshock but also about reactive responses to the footshock such as flinching and vocalizing. A prior study showed that rats emit pain squeaks during shock pre-exposure that later enhance their freezing to pain squeaks or aversive ultrasonic vocalizations compared to rats without shock pre-exposure[97]. While the prior study focused on vocalizations, the same phenomenon may apply to other behavioral responses as well. For example, shock pre-exposed Observers may recognize behavioral responses to shock such as jumping or flinching that the Demonstrator may exhibit because those are the same responses they produced when feeling the footshock. Future work should record vocalizations during Observational PMA to determine the role of auditory social information in observational learning. Future work could also compare behavioral shock responses (shock reactivity, jumping, etc.) between the Observers during shock pre-exposure session and the Demonstrators during the

Observation Session to determine whether auto conditioning occurs for behaviors other than vocalizations.

4.2 Late stages of Observational PMA may depend on freezing responses more heavily than Early stages of Observational PMA.

In contrast to our Early PMA results, we did not find behavioral differences in avoidance or freezing between shock pre-exposed and shock-naïve Observers in Late PMA. Fear can be acquired in a single conditioning session whereas PMA takes multiple training sessions to acquire[34,93,92,67]. An Observer can therefore witness the entire conditioning process within a single session in observational fear whereas they cannot in Observational PMA.

In Observational PMA, Late PMA Demonstrators undergo initial training days without an Observer present. We have observed that Late PMA Demonstrators do not alter their behaviors from when they underwent PMA training alone to when they are given an Observer (unpublished data). The Observer's presence does not seem to constitute a contextual change that would cause the Demonstrator to alter their avoidance behaviors during the first few tones of the PMA task. In fact, we see Demonstrators avoiding most shocks at Late PMA when the Observer is present (**Figure 3D**) which limits the amount of task contingency information they can convey to the Observers. Taken together, results from Early and Late PMA Observers suggest that witnessing a Demonstrator receive shocks but also successfully perform avoidance may be the most effective means of acquiring Observational PMA. Early PMA Observers are provided with the tone-shock contingency information whereas Late PMA Observers are provided with tone-avoidance contingency information. According to Mowrer's two factor theory of avoidance[98,99], both of these pieces of information are important for acquiring and maintaining an avoidance response. Future

work could combine multiple video clips of Demonstrators getting shocked with clips of Demonstrators avoiding successfully to create an "ideal" Demonstrator and determine if observing both the tone-shock and tone-avoidance contingencies produces the highest observational avoidance.

While we did not see freezing differences between any of the Observer groups, we did observe a positive correlation between Demonstrator and Observer freezing in the shock-naïve Observers that witnessed Late PMA. It is possible that freezing becomes more nuanced across days of Demonstrator training in a way that makes freezing more informative to Observers. Previous work has shown that the lack of auditory information during freezing can induce freezing in other rats[100]. Interestingly, this effect was only significant when other rats were pre-exposed to footshocks, but we only saw a significant correlation in our shock-naïve Observers. Future work could examine how Observers behave when their Demonstrator is performing specific behaviors like freezing or sitting on the platform. Investigating specific interactions between Observer and Demonstrator behavior such as these could provide insight into how and when social information is most effectively transmitted between individuals.

We did not find any differences between Observer groups in the time that they spent oriented towards the Demonstrator during the Observation Session (**Figure 4B** and **4C**). This lack of difference is not surprising given that all Observers were paired with a non-cagemate Demonstrator which made the Demonstrator a novel social stimulus. Even Observers without shock pre-exposure had been exposed to the operant chamber during lever-press training, so the Demonstrator and tones were novel stimuli, but the context itself was not novel. The Observers also did not have access to a lever, food dish, or platform while they were observing the Demonstrator, which eliminated possible distractions and ensured that the Demonstrator was the

only stimulus to which they would attend. Interestingly, head orientation was correlated with avoidance in only one Observer group: shock pre-exposed Observers at Late PMA (**Figure 4E**). This finding may also support the idea that freezing becomes more informative across days of PMA training. Shock pre-exposed Observers know that harm or danger is a possibility in the context, so tone-induced freezing and cessation of noise from the Demonstrator[100] may cause the shock pre-exposed Observer to orient towards them to also obtain visual information about the potential threat. While we did not see a significant predictive effect of head orientation in the model comparison nor any other significant correlations involving head orientation, previous work has shown that eliminating visual information impairs observational fear learning[7,47,48,46].

4.3 ACC activation does not correlate with Observational PMA.

We hypothesized that the ACC would exhibit increased activation in Observers compared to Demonstrators and homecage controls given the well-established role of ACC in observational learning[31–33]. However, we found that *c-Fos* density in the ACC did not differ from that of homecage controls in any of the Demonstrator or Observer groups, and we did not find any differences between groups within Early or Late PMA (**Figure 5B** and **5D**). We also did not see any correlations between ACC *c-Fos* density and avoidance behavior (**Figure 5C** and **5E**). We did see a trend in the shock pre-exposed Late PMA Observers toward a difference from the homecage control. This same group also showed trends towards differences in ACC *c-Fos* density from Late PMA Demonstrators and shock-naïve Late PMA Observers (**Figure 5D**). It is possible that these trends did not reach significance due to reduced statistical power from tissue loss during freezing. Future work will resolve this issue by running additional rats.

Prior work has shown that there is lateralization in function in the ACC[62,101]. We did not process the brain tissue in a way that allowed us to distinguish between left and right hemispheres, so we are potentially missing an effect of observational learning localized to a single hemisphere. Future tissue processing could be done in a manner that allows for hemispheric comparisons to determine whether our findings replicate the lateralized ACC functionality. We may have also failed to find an effect of observational learning in the ACC given that the ACC also plays a role in PMA acquired through direct experience[73]. Future studies could parse apart the role of ACC in observational learning versus PMA performance by inactivating the ACC during either the Observation or Test Session in Observers and measuring avoidance behaviors.

4.4 Shock pre-exposure increases IL activation.

Despite the role of IL in extinction[34–38], we saw that shock pre-exposed Observers at both Early and Late PMA exhibited increased *c-Fos* densities compared to Demonstrators (**Figure 5G** and **5I**). As mentioned previously, IL does play a role in response discrimination and inhibiting previously learned information to learn new stimulus-outcome associations[78,81–84]. Both shock pre-exposed and shock-naïve Observers must transition from observing to performing PMA thus transitioning from shock being administered to a conspecific to shock being administered to themselves. By that logic, we would expect all Observers to show increased IL activation compared to Demonstrators, but we only saw increased IL activation in the shock pre-exposed Observers. It is possible that the shock pre-exposure session acted as a contextual fear conditioning session and that the shock pre-exposed Observers had to transition from fear conditioning to avoidance rather than from observing to performing PMA. This explanation agrees with prior work

showing that the IL is necessary to inhibit learning processes that are PL-driven, like fear conditioning[37,102], in order to learn and apply new information[84,103,104].

A limitation of the results we obtained from both ACC and IL is that *c-Fos* expression is not specific to any one type of cell. Using this method, we cannot detect activation differences in excitatory versus inhibitory neurons. Additionally, we cannot determine where active neurons are projecting to nor from where they are receiving projections. cFos IHC provides us with information regarding gross activation of brain regions that can then inform later experiments testing the role of specific brain areas or connections between brain areas in Observational PMA. Future work could combine cFos IHC with anatomical tracing techniques to determine where active neurons are sending projections to provide better understanding of the circuitry underlying Observational PMA.

Chapter 5 - Conclusions

Both PMA and observational learning are complex processes that require multiple behaviors as well as recruit a range of brain areas to balance successful avoidance behavior with reward-seeking. We have shown that despite the complexities of PMA, this task can be acquired through observation and that the neural mechanisms mediating Observational PMA may differ from those mediating observational fear conditioning and those mediating PMA learned through direct experience. Future work will use techniques such as DREADDs and optogenetics to determine what brain areas and their projections are necessary for Observational PMA and at which stages of PMA learning. While we included both male and female rats in this study, we did not examine sex differences in order to limit the overall number of animals needed while designing an intricate behavioral paradigm with many experimental groups. Future work will examine sex differences in Observational PMA to determine how males and females may utilize different information or recruit different brain areas to acquire active avoidance through observation.

References

1. Avarguès-Weber A, Chittka L. Observational Conditioning in Flower Choice Copying by Bumblebees (Bombus terrestris): Influence of Observer Distance and Demonstrator Movement. Nascimento FS, ed. *PLoS ONE*. 2014;9(2):e88415. doi:10.1371/journal.pone.0088415

2. Worden BD, Papaj DR. Flower choice copying in bumblebees. *Biol Lett*. 2005;1(4):504-507. doi:10.1098/rsbl.2005.0368

3. Lovén Wallerius M, Johnsson JI, Cooke SJ, Arlinghaus R. Hook Avoidance Induced by Private and Social Learning in Common Carp. *Trans Am Fish Soc*. 2020;149(4):498-511. doi:10.1002/tafs.10246

4. Johnston ANB, Burne THJ, Rose SPR. Observation learning in day-old chicks using a one-trial passive avoidance learning paradigm. *Anim Behav*. 1998;56(6):1347-1353. doi:10.1006/anbe.1998.0901

5. Brodin A, Utku Urhan A. Sex differences in learning ability in a common songbird, the great tit—females are better observational learners than males. *Behav Ecol Sociobiol*. 2015;69(2):237-241. doi:10.1007/s00265-014-1836-2

6. Del Russo JE. Observational learning of discriminative avoidance in hooded rats. *Anim Learn Behav*. 1975;3(1):76-80. doi:10.3758/BF03209103

7. Groesbeck RW, Duerfeldt PH. Some relevant variables in observational learning of the rat. *Psychon Sci*. 1971;22(1):41-43. doi:10.3758/BF03335928

8. Berry CS. An experimental study of imitation in cats. *J Comp Neurol Psychol*. 1908;18(1):1-25. doi:10.1002/cne.920180102

9. Presley WJ, Riopelle AJ. Observational Learning of an Avoidance Response. *J Genet Psychol*. 1959;95(2):251-254. doi:10.1080/00221325.1959.10534265

10. Yerkes RM. The mind of a gorilla. *Genet Psychol Monogr*. 1927;2:1-193.

11. Yerkes RM. Suggestibility in Chimpanzee. *J Soc Psychol*. 1934;5(3):271-282. doi:10.1080/00224545.1934.9921598

12. Bandura A, Ross D, Ross SA. Transmission of aggression through imitation of aggressive models. *J Abnorm Soc Psychol*. 1961;63(3):575-582. doi:10.1037/h0045925

13. Askew C, Field AP. Vicarious learning and the development of fears in childhood. *Behav Res Ther*. 2007;45(11):2616-2627. doi:10.1016/j.brat.2007.06.008

14. Taylor BA, DeQuinzio JA. Observational Learning and Children With Autism. *Behav Modif*. 2012;36(3):341-360. doi:10.1177/0145445512443981

15. Varni JW, Lovaas OI, Koegel RL, Everett NL. An analysis of observational learning in autistic and normal children. *J Abnorm Child Psychol*. 1979;7(1):31-43. doi:10.1007/BF00924508

16. Matthews N, Gold BJ, Sekuler R, Park S. Gesture Imitation in Schizophrenia. *Schizophr Bull*. 2013;39(1):94-101. doi:10.1093/schbul/sbr062

17. Fornito A, Yücel M, Wood SJ, et al. Morphology of the paracingulate sulcus and executive cognition in schizophrenia. *Schizophr Res*. 2006;88(1-3):192-197. doi:10.1016/j.schres.2006.06.034

18. Thoma P, Norra C, Juckel G, Suchan B, Bellebaum C. Performance monitoring and empathy during active and observational learning in patients with major depression. *Biol Psychol*. 2015;109:222-231. doi:10.1016/j.biopsycho.2015.06.002

19. Jacobson NC, Newman MG. Avoidance mediates the relationship between anxiety and depression over a decade later. *J Anxiety Disord*. 2014;28(5):437-445. doi:10.1016/j.janxdis.2014.03.007

20. Trew JL. Exploring the roles of approach and avoidance in depression: An integrative model. *Clin Psychol Rev*. 2011;31(7):1156-1168. doi:10.1016/j.cpr.2011.07.007

21. De La Asuncion J, Docx L, Sabbe B, Morrens M, De Bruijn ERA. Converging evidence of social avoidant behavior in schizophrenia from two approach-avoidance tasks. *J Psychiatr Res*. 2015;69:135-141. doi:10.1016/j.jpsychires.2015.08.008

22. Spain D, Sin J, Linder KB, McMahon J, Happé F. Social anxiety in autism spectrum disorder: A systematic review. *Res Autism Spectr Disord*. 2018;52:51-68. doi:10.1016/j.rasd.2018.04.007

23. Montaser J, Umeano L, Pujari HP, et al. Correlations Between the Development of Social Anxiety and Individuals With Autism Spectrum Disorder: A Systematic Review. *Cureus*. Published online September 7, 2023. doi:10.7759/cureus.44841

24. Peters EA. Motivation and Social Withdrawal in Schizophrenia: Factors Related to Passive Social Withdrawal and Active Social Avoidance. *Masters Thesis UConn*. Published online 2020. https://opencommons.uconn.edu/gs_theses/1538

25. Ball TM, Gunaydin LA. Measuring maladaptive avoidance: from animal models to clinical anxiety. *Neuropsychopharmacology*. 2022;47(5):978-986. doi:10.1038/s41386-021-01263-4

26. World Health Organization. Anxiety disorders.https://www.who.int/news-room/fact-sheets/detail/anxiety-disorders#:~:text=Symptoms%20and%20patterns,impending%20danger%2C%20panic%20or%20doom. 2023.

27. Bruce SE, Yonkers KA, Otto MW, et al. Influence of Psychiatric Comorbidity on Recovery and Recurrence in Generalized Anxiety Disorder, Social Phobia, and Panic Disorder: A 12-Year Prospective Study. *Am J Psychiatry*. 2005;162(6):1179-1187. doi:10.1176/appi.ajp.162.6.1179

28. Klein Hofmeijer-Sevink M, Batelaan NM, Van Megen HJGM, et al. Clinical relevance of comorbidity in anxiety disorders: A report from the Netherlands Study of Depression and Anxiety (NESDA). *J Affect Disord*. 2012;137(1-3):106-112. doi:10.1016/j.jad.2011.12.008

29. Davies MR, Glen K, Mundy J, et al. Factors associated with anxiety disorder comorbidity. *J Affect Disord*. 2023;323:280-291. doi:10.1016/j.jad.2022.11.051

30. Lieb R, Wittchen HU, Höfler M, Fuetsch M, Stein MB, Merikangas KR. Parental Psychopathology, Parenting Styles, and the Risk of Social Phobia in Offspring: A Prospective-Longitudinal Community Study. *Arch Gen Psychiatry*. 2000;57(9):859. doi:10.1001/archpsyc.57.9.859

31. Burgos-Robles A, Gothard KM, Monfils MH, Morozov A, Vicentic A. Conserved features of anterior cingulate networks support observational learning across species. *Neurosci Biobehav Rev*. 2019;107:215-228. doi:10.1016/j.neubiorev.2019.09.009

32. Olsson A, Knapska E, Lindström B. The neural and computational systems of social learning. *Nat Rev Neurosci*. 2020;21(4):197-212. doi:10.1038/s41583-020-0276-4

33. Carcea I, Froemke RC. Biological mechanisms for observational learning. *Curr Opin Neurobiol*. 2019;54:178-185. doi:10.1016/j.conb.2018.11.008

34. Bravo-Rivera C, Roman-Ortiz C, Brignoni-Perez E, Sotres-Bayon F, Quirk GJ. Neural Structures Mediating Expression and Extinction of Platform-Mediated Avoidance. *J Neurosci*. 2014;34(29):9736-9742. doi:10.1523/JNEUROSCI.0191-14.2014

35. Martínez-Rivera FJ, Bravo-Rivera C, Velázquez-Díaz CD, Montesinos-Cartagena M, Quirk GJ. Prefrontal circuits signaling active avoidance retrieval and extinction. *Psychopharmacology (Berl)*. 2019;236(1):399-406. doi:10.1007/s00213-018-5012-7

36. Do-Monte FH, Manzano-Nieves G, Quiñones-Laracuente K, Ramos-Medina L, Quirk GJ. Revisiting the Role of Infralimbic Cortex in Fear Extinction with Optogenetics. *J Neurosci*. 2015;35(8):3607-3615. doi:10.1523/JNEUROSCI.3137-14.2015

37. Sotres-Bayon F, Quirk GJ. Prefrontal control of fear: more than just extinction. *Curr Opin Neurobiol*. 2010;20(2):231-235. doi:10.1016/j.conb.2010.02.005

38. Barad M. Fear extinction in rodents: basic insight to clinical promise. *Curr Opin Neurobiol*. 2005;15(6):710-715. doi:10.1016/j.conb.2005.10.005

39. Thorndike EL. Animal intelligence: An experimental study of the associative processes in animals. *Psychol Rev Monogr Suppl*. 1898;2(4):i-109. doi:10.1037/h0092987

40. Thorndike EL. The mental life of the monkeys. *Psychol Rev Monogr Suppl*. 1901;3(5):i-57. doi:10.1037/h0092994

41. Small WS. An Experimental Study of the Mental Processes of the Rat. *Am J Psychol*. 1900;11(2):133. doi:10.2307/1412267

42. Shepherd WT. Imitation in Raccoons. *Am J Psychol*. 1911;22(4):583. doi:10.2307/1412802

43. Yerkes RM. Do animals imitate one another voluntarily. *Century Mag*. 1909;78:393-403.

44. Warden CJ, Jackson TA. Imitative Behavior in the Rhesus Monkey. *Pedagog Semin J Genet Psychol*. 1935;46(1):103-125. doi:10.1080/08856559.1935.10533146

45. Herbert MJ, Harsh CM. Observational learning by cats. *J Comp Psychol*. 1944;37(2):81-95. doi:10.1037/h0062414

46. Hong EH, Choi JS. Observational threat conditioning is induced by circa-strike activity burst but not freezing and requires visual attention. *Behav Brain Res*. 2018;353:161-167. doi:10.1016/j.bbr.2018.06.034

47. Kavaliers M, Choleris E, Colwell DD. Learning from others to cope with biting flies: Social learning of fear-induced conditioned analgesia and active avoidance. *Behav Neurosci*. 2001;115(3):661-674. doi:10.1037/0735-7044.115.3.661

48. Jeon D, Kim S, Chetana M, et al. Observational fear learning involves affective pain system and Cav1.2 Ca2+ channels in ACC. *Nat Neurosci*. 2010;13(4):482-488. doi:10.1038/nn.2504

49. Curzon P, Rustay N, Bowman K. Cued and Contextual Fear Conditioning for Rodents. In: *Methods of Behavior Analysis in Neuroscience*. 2nd ed. CRC Press/ Taylor & Francis; 2009. https://www.ncbi.nlm.nih.gov/books/NBK5223/

50. Fanselow MS. Factors governing one-trial contextual conditioning. *Anim Learn Behav*. 1990;18(3):264-270. doi:10.3758/BF03205285

51. Allsop SA, Wichmann R, Mills F, et al. Corticoamygdala Transfer of Socially Derived Information Gates Observational Learning. *Cell*. 2018;173(6):1329-1342.e18. doi:10.1016/j.cell.2018.04.004

52. Silverstein SE, O'Sullivan R, Bukalo O, et al. A distinct cortical code for socially learned threat. *Nature*. 2024;626(8001):1066-1072. doi:10.1038/s41586-023-07008-1

53. Lidhar NK, Insel N, Dong JY, Takehara-Nishiuchi K. Observational fear learning in degus is correlated with temporal vocalization patterns. *Behav Brain Res*. 2017;332:362-371. doi:10.1016/j.bbr.2017.06.011

54. Shi T, Feng S, Shi W, Fu Y, Zhou W. A modified mouse model for observational fear learning and the influence of social hierarchy. *Front Behav Neurosci*. 2022;16:941288. doi:10.3389/fnbeh.2022.941288

55. Ito W, Morozov A. Prefrontal-amygdala plasticity enabled by observational fear. *Neuropsychopharmacology*. 2019;44(10):1778-1787. doi:10.1038/s41386-019-0342-7

56. Kim EJ, Kim ES, Covey E, Kim JJ. Social Transmission of Fear in Rats: The Role of 22-kHz Ultrasonic Distress Vocalization. Chapouthier G, ed. *PLoS ONE*. 2010;5(12):e15077. doi:10.1371/journal.pone.0015077

57. John ER, Chesler P, Bartlett F, Victor I. Observation Learning in Cats. *Science*. 1968;159(3822):1489-1491. doi:10.1126/science.159.3822.1489

58. Schneider KN, Sciarillo XA, Nudelman JL, Cheer JF, Roesch MR. Anterior Cingulate Cortex Signals Attention in a Social Paradigm that Manipulates Reward and Shock. *Curr Biol*. 2020;30(19):3724-3735.e2. doi:10.1016/j.cub.2020.07.039

59. Atsak P, Orre M, Bakker P, et al. Experience Modulates Vicarious Freezing in Rats: A Model for Empathy. Ferrari PF, ed. *PLoS ONE*. 2011;6(7):e21855. doi:10.1371/journal.pone.0021855

60. Sanders J, Mayford M, Jeste D. Empathic Fear Responses in Mice Are Triggered by Recognition of a Shared Experience. Lu L, ed. *PLoS ONE*. 2013;8(9):e74609. doi:10.1371/journal.pone.0074609

61. Kurup D. Reward-based observational learning: Importance of social information and anterior cingulate cortex for attention. *Masters Thesis NTNU*. Published online 2021.

62. Kim S, Mátyás F, Lee S, Acsády L, Shin HS. Lateralization of observational fear learning at the cortical but not thalamic level in mice. *Proc Natl Acad Sci*. 2012;109(38):15497-15501. doi:10.1073/pnas.1213903109

63. Keum S, Kim A, Shin JJ, Kim JH, Park J, Shin HS. A Missense Variant at the Nrxn3 Locus Enhances Empathy Fear in the Mouse. *Neuron*. 2018;98(3):588-601.e5. doi:10.1016/j.neuron.2018.03.041

64. LeDoux JE, Moscarello J, Sears R, Campese V. The birth, death and resurrection of avoidance: a reconceptualization of a troubled paradigm. *Mol Psychiatry*. 2017;22(1):24-36. doi:10.1038/mp.2016.166

65. Carrillo M, Han Y, Migliorati F, Liu M, Gazzola V, Keysers C. Emotional Mirror Neurons in the Rat's Anterior Cingulate Cortex. *Curr Biol*. 2019;29(8):1301-1312.e6. doi:10.1016/j.cub.2019.03.024

66. Diehl MM, Bravo-Rivera C, Quirk GJ. The study of active avoidance: A platform for discussion. *Neurosci Biobehav Rev*. 2019;107:229-237. doi:10.1016/j.neubiorev.2019.09.010

67. Diehl MM, Iravedra-Garcia JM, Morán-Sierra J, et al. Divergent projections of the prelimbic cortex bidirectionally regulate active avoidance. *eLife*. 2020;9:e59281. doi:10.7554/eLife.59281

68. Condé F, Maire-lepoivre E, Audinat E, Crépel F. Afferent connections of the medial frontal cortex of the rat. II. Cortical and subcortical afferents. *J Comp Neurol*. 1995;352(4):567-593. doi:10.1002/cne.903520407

69. Jones BF, Groenewegen HJ, Witter MP. Intrinsic connections of the cingulate cortex in the rat suggest the existence of multiple functionally segregated networks. *Neuroscience*. 2005;133(1):193-207. doi:10.1016/j.neuroscience.2005.01.063

70. Bissière S, Plachta N, Hoyer D, et al. The Rostral Anterior Cingulate Cortex Modulates the Efficiency of Amygdala-Dependent Fear Learning. *Biol Psychiatry*. 2008;63(9):821-831. doi:10.1016/j.biopsych.2007.10.022

71. Cassell MD, Wright DJ. Topography of projections from the medial prefrontal cortex to the amygdala in the rat. *Brain Res Bull*. 1986;17(3):321-333. doi:10.1016/0361-9230(86)90237-6

72. Gabbott PLA, Warner TA, Jays PRL, Salway P, Busby SJ. Prefrontal cortex in the rat: Projections to subcortical autonomic, motor, and limbic centers. *J Comp Neurol*. 2005;492(2):145-177. doi:10.1002/cne.20738

73. Ruble S, Payne K, Kramer C, et al. *Social Context Modulates Active Avoidance: Contributions of the Anterior Cingulate Cortex in Male and Female Rats.* Neurobiology of Stress; 2024. https://doi.org/10.1016/j.ynstr.2024.100702

74. Gabriel M. Chapter 23 Functions of anterior and posterior cingulate cortex during avoidance learning in rabbits. In: *Progress in Brain Research*. Vol 85. Elsevier; 1991:467-483. doi:10.1016/S0079-6123(08)62695-3

75. Gabriel M, Kubota Y, Sparenborg S, Straube K, Vogt BA. Effects of cingulate cortical lesions on avoidance learning and training-induced unit activity in rabbits. *Exp Brain Res*. 1991;86(3). doi:10.1007/BF00230532

76. Svoboda J, Lobellová V, Popelíková A, Ahuja N, Kelemen E, Stuchlík A. Transient inactivation of the anterior cingulate cortex in rats disrupts avoidance of a dynamic object. *Neurobiol Learn Mem*. 2017;139:144-148. doi:10.1016/j.nlm.2017.01.003

77. Jiao X, Beck KD, Myers CE, Servatius RJ, Pang KCH. Altered activity of the medial prefrontal cortex and amygdala during acquisition and extinction of an active avoidance task. *Front Behav Neurosci*. 2015;9. doi:10.3389/fnbeh.2015.00249

78. Capuzzo G, Floresco SB. Prelimbic and Infralimbic Prefrontal Regulation of Active and Inhibitory Avoidance and Reward-Seeking. *J Neurosci*. 2020;40(24):4773-4787. doi:10.1523/JNEUROSCI.0414-20.2020

79. Martinez RCR, Gupta N, Lázaro-Muñoz G, et al. Active vs. reactive threat responding is associated with differential c-Fos expression in specific regions of amygdala and prefrontal cortex. *Learn Mem*. 2013;20(8):446-452. doi:10.1101/lm.031047.113

80. Moscarello JM, LeDoux JE. Active Avoidance Learning Requires Prefrontal Suppression of Amygdala-Mediated Defensive Reactions. *J Neurosci*. 2013;33(9):3815-3823. doi:10.1523/JNEUROSCI.2596-12.2013

81. Roughley S, Killcross S. The role of the infralimbic cortex in decision making processes. *Curr Opin Behav Sci*. 2021;41:138-143. doi:10.1016/j.cobeha.2021.06.003

82. Gonzalez ST, Fanselow MS. The role of the ventromedial prefrontal cortex and context in regulating fear learning and extinction. *Psychol Neurosci*. 2020;13(3):459-472. doi:10.1037/pne0000207

83. Barker JM, Taylor JR, Chandler LJ. A unifying model of the role of the infralimbic cortex in extinction and habits. *Learn Mem*. 2014;21(9):441-448. doi:10.1101/lm.035501.114

84. Mukherjee A, Caroni P. Infralimbic cortex is required for learning alternatives to prelimbic promoted associations through reciprocal connectivity. *Nat Commun*. 2018;9(1):2727. doi:10.1038/s41467-018-05318-x

85. Kramer C, Ruble S, Fort TD, West L, Diehl MM. Modifying the platform-mediated avoidance task: A new protocol to study active avoidance within a social context in rats. Melgarejo Da Rosa M, ed. *PLOS One*. 2025;20(4):e0321776. doi:10.1371/journal.pone.0321776

86. Nikbakht N, Diamond ME. Conserved visual capacity of rats under red light. *eLife*. 2021;10:e66429. doi:10.7554/eLife.66429

87. Wallace DJ, Greenberg DS, Sawinski J, Rulla S, Notaro G, Kerr JND. Rats maintain an overhead binocular field at the expense of constant fusion. *Nature*. 2013;498(7452):65-69. doi:10.1038/nature12153

88. Chiaruttini N, Castoldi C, Requie LM, et al. ABBA, a novel tool for whole-brain mapping, reveals brain-wide differences in immediate early genes induction following learning. Published online September 6, 2024. doi:10.1101/2024.09.06.611625

89. Bankhead P, Loughrey MB, Fernández JA, et al. QuPath: Open source software for digital pathology image analysis. *Sci Rep*. 2017;7(1):16878. doi:10.1038/s41598-017-17204-5

90. Bravo-Rivera H, Rubio Arzola P, Caban-Murillo A, Vélez-Avilés AN, Ayala-Rosario SN, Quirk GJ. Characterizing Different Strategies for Resolving Approach-Avoidance Conflict. *Front Neurosci*. 2021;15:608922. doi:10.3389/fnins.2021.608922

91. Kim A, Keum S, Shin H. Observational fear behavior in rodents as a model for empathy. *Genes Brain Behav*. 2019;18(1):e12521. doi:10.1111/gbb.12521

92. Diehl MM, Bravo-Rivera C, Rodriguez-Romaguera J, et al. Active avoidance requires inhibitory signaling in the rodent prelimbic prefrontal cortex. *eLife*. 2018;7:e34657. doi:10.7554/eLife.34657

93. Martínez-Rivera FJ, Sánchez-Navarro MJ, Huertas-Pérez CI, Greenberg BD, Rasmussen SA, Quirk GJ. Prolonged avoidance training exacerbates OCD-like behaviors in a rodent model. *Transl Psychiatry*. 2020;10(1):212. doi:10.1038/s41398-020-00892-5

94. López-Moraga A, Beckers T, Luyten L. The effects of stress on avoidance in rodents: An unresolved matter. *Front Behav Neurosci*. 2022;16:983026. doi:10.3389/fnbeh.2022.983026

95. Koba T, Kodama Y, Shimizu K, et al. Persistent Behavioural Changes in Rats Following Inescapable Shock Stress: a Potential Model of Posttraumatic Stress Disorder. *World J Biol Psychiatry*. 2001;2(1):34-37. doi:10.3109/15622970109039982

96. Brennan FX, Beck KD, Ross RJ, Servatius RJ. Stress-induced increases in avoidance responding: an animal model of post-traumatic stress disorder behavior? *Neuropsychiatr Dis Treat*. 2005;1(1):69-72. doi:10.2147/nedt.1.1.69.52292

97. Packheiser J, Soyman E, Paradiso E, et al. Audible pain squeaks can mediate emotional contagion across pre-exposed rats with a potential effect of auto-conditioning. *Commun Biol*. 2023;6(1):1085. doi:10.1038/s42003-023-05474-x

98. Mowrer OH. Anxiety-reduction and learning. *J Exp Psychol*. 1940;27(5):497-516. doi:10.1037/h0056236

99. Mowrer OH, Lamoreaux RR. Fear as an intervening variable in avoidance conditioning. *J Comp Psychol*. 1946;39(1):29-50. doi:10.1037/h0060150

100. Pereira AG, Cruz A, Lima SQ, Moita MA. Silence resulting from the cessation of movement signals danger. *Curr Biol*. 2012;22(16):R627-R628. doi:10.1016/j.cub.2012.06.015

101. Kim JH, Choi DE, Shin HS. The lateralized LC-NAergic system distinguishes vicarious versus direct fear in mice. *Nat Commun*. 2025;16(1):2364. doi:10.1038/s41467-025-57701-0

102. Giustino TF, Maren S. The Role of the Medial Prefrontal Cortex in the Conditioning and Extinction of Fear. *Front Behav Neurosci*. 2015;9. doi:10.3389/fnbeh.2015.00298

103. Gourley SL, Taylor JR. Going and stopping: dichotomies in behavioral control by the prefrontal cortex. *Nat Neurosci*. 2016;19(5):656-664. doi:10.1038/nn.4275

104. Nett KE, LaLumiere RT. Infralimbic cortex functioning across motivated behaviors: Can the differences be reconciled? *Neurosci Biobehav Rev*. 2021;131:704-721. doi:10.1016/j.neubiorev.2021.10.002