Flee or Freeze: The Differential Role of Amygdala Subregions in Fear and Avoidance

Xinyue Li

A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Science

University of Washington

2024

Committee:

Jeansok J. Kim

Sheri Mizumori

Program Authorized to Offer Degree:

Psychology

University of Washington

Abstract

Flee or Freeze: The Differential Role of Amygdala Subregions in Fear and Avoidance

Xinyue Li

Chair of the Supervisory Committee:

Jeansok J. Kim

Department of Psychology

Fear is a powerful emotion that is crucial to the survival in a complex environment. The amygdala, a key brain region implicated in processing fear, is not homogenous but comprises of several subregions, most notably the central amygdala (CEA) and the basolateral amygdala (BLA). To examine their functional differences, this study utilized a novel naturalistic approach-food avoid predator task and a standard classical fear conditioning task to examine defensive responses in CEA and BLA lesioned rats. Both lesioned groups demonstrated impaired fear responses than sham; however, BLA lesioned animals showed more impairments in active defensive responses (i.e., fleeing), while CEA lesioned animals showed more impairments in passive defensive responses (i.e., freezing). Maladaptation of fear can lead to serious mental illnesses; these findings may elucidate how fear is processed and facilitate the discovery of more effective treatment of disorders such as such as post-traumatic stress disorder (PTSD).

Introduction

Fear is a powerful and salient behavioral and physiological response in both animals and humans, and it can trigger an innate instinct for self-preservation. Fear can also be learned if certain contexts or stimuli have previously been linked to perilous experiences. While fear is adaptive and crucial for survival in a complex environment, it can also become maladaptive when it is prolonged, intense, or paralyzing, ultimately leading to the deterioration of physiological and psychological well-being.

Fear responses are natural physiological reactions that arise upon perceiving danger. It includes an initial acute stress response and defensive and adaptive strategies that follow. There is a plethora of defensive behaviors exhibited across the animal kingdom. Cephalopods, for example, react to threat by ejecting ink as a form of camouflage (Wood et al., 2010). Birds and rodents react to predators with vocal calls out of fear or alarm (Hollén & Radford, 2009; Kim et al., 2010). Other prey animals such as deer freeze to avoid detection (Fentress, 1968). These diverse defensive responses all fall on the spectrum of active or passive avoidance strategies. Active avoidance strategies such as fleeing or attacking may be more effective in avoiding harm or eliminating some overt threats, and in these cases, animals are often demonstrating specific motor responses to escape aversive stimuli. When threats are ambiguous, adopting passive avoidance strategies such as risk assessment and freezing may be more effective in avoiding detection and further evaluating the imminence of danger. These strategies often involve the inhibition of a biologically probable response, such as moving or fleeing (Cimadevilla et al., 2000).

Regardless of the strategy type, the overall purpose of these adaptive behaviors is to react to fear and ensure survival against danger. While some fear response may be instinctive, the

decision to adopt the most effective defensive strategy may depend on many factors, which can include the perceived proximity of the danger, the magnitude of the threat, and how much time is available (Fanselow & Lester, 1988; Tseng et al., 2023; Fanselow, 2022). This perception of danger can also become distorted; fear becomes maladaptive when the perception of the immediacy and the severity of the threat becomes exaggerated. These distortions lead to defensive responses that may be involuntary and intrude upon adaptive responses. In humans, this may lead to stress-related psychiatric disorders such as anxiety, depression, and PTSD. However, the neural circuitry associated with fear and defensive responses remains largely ambiguous, and the maladaptation process of stress is still unclear. Thus, dissecting the neural architecture and elucidating the neural pathways involved is critical for understanding stress-related pathology.

The amygdala is an important structure linking sensory stimuli to fear. Lying deep within the limbic system, this group of nuclei in the medial temporal lobe detects and interprets the threat-related stimulus in the environment. It also underlies emotion and motivation and acts as a hub of both inputs and outputs to and from regions such as the hippocampus, the thalamus, and the prefrontal cortex (Kim et al., 1993; Chudasama et al., 2009; Izquierdo et al., 2005). Circuitries from which the amygdala is involved in are implicated in a wide range of dysfunctional states in humans such as addiction, autism, as well as stress-related psychiatric disorders (Koob et al., 2010; Sato et al., 2023; Haris et al., 2023). Lesions to the whole amygdala have been shown to impair emotional reactions to stimuli; in as early as the 1800s, non-human primate studies revealed that amygdala lesion animals could perceive sensory stimuli but cannot comprehend or react to them (Brown & Schäfer, 1888). The amygdala also plays an important role in related stimuli with positive and negative emotions: in humans, bilateral lesions in the

amygdala are associated with deficits in emotional recognition of faces (Wang et al., 2017). Similarly in rodents, amygdala lesions have also been tied to dysfunctions in emotional learning (Blanchard & Blanchard, 1972; Ledoux et al., 1990). However, the amygdala should not be treated as a homogenous whole. Rather, both the internal circuits within the amygdala and the external circuits its subregions are involved in many be distinct and complex.

Both the basolateral (BLA) and the central nucleus of the amygdala (CEA) regions are involved in the acquisition and expression of fear, thus these were the two subregions of interest in the current study (Kim & Davis, 1993; Killcross et al., 1997; Nadar & LeDoux, 1997; Amorapanth et al., 2000; Goosens & Maren, 2001; Koo et al., 2004; Corbit & Balline, 2005; Martinez et al., 2011). In fear these two subregions could be functionally distinct: it has been suggested that the BLA receives sensory signals while the CEA sends output signals to autonomic and somatomotor centers that govern behavioral responses (LeDoux, 1996; Keifer et al., 2015). The external pathways they are involved in may also be different, so they could be mediating different types of downstream behavioral responses: projections from the BLA can reach the prefrontal cortex, the hippocampus, caudate nucleus, and the nucleus accumbens (NAc), while the CEA projects to the hypothalamus, midbrain, pons and the bed nucleus of the stria terminals (BNST) (Janak & Tye, 2015; Gross et al., 2012).

Amygdala subregions including the CEA and BLA may be mediating parallel but distinct pathways in the detection, integration, and output of defensive behaviors (Silva et al., 2016). Many studies have examined the behavioral effects of selectively manipulating or inactivating these subregions in behavioral paradigms, but the type of stimulus presented (visual, auditory, olfactory) and the type of defensive responses elicited or observed (active or passive avoidance) are factors to be considered when interpreting results. For example, looming aerial stimuli can

trigger innate fear in rodents; such cues are visual, processed lateral (LA) and basomedial amygdala (BMA), a predator-responsive circuit that involves the hypothalamus, and through the periaqueductal grey (PAG) to elicit both freezing and fleeing (Choi & Kim, 2010; Yilmaz., 2013; Zambetti et al., 2019; Gross et al, 2012). Optogenetic inactivation disrupting incoming projections to the BLA was shown to affect innate but not conditioned freezing response to predator odor (Jhang et al., 2018). Optogenetic manipulations of the CEA have demonstrated that it is required for acquiring and expressing conditioned freezing responses, and these studies involve pairing innocuous sensory cues with shocks to examine passive avoidance behavior (Wilensky et al., 2006, Amorapanth et al., 2000, Ciocchi et al., 2010). On the other hand, electrolytic or excitotoxic lesions of BLA, but not CEA, were found to impair performance in active avoidance tasks (Lazaro-Munoz et al., 2010; Choi et al., 2010). Furthermore, direct optogenetic stimulations of lateral and basal amygdala were sufficient for evoking fleeing behaviors in a foraging chamber (Kim et al., 2013; Kim et al., 2018; Kong et al., 2021).

The current study examined the behavioral effects of bilateral CEA and BLA lesions and directly compared these effects on active avoidance (fleeing) and passive avoidance (freezing) in two different behavioral paradigms. We adapted a novel approach-food avoid-predator (AFAP) task where rats would need to leave a safe nest zone and forage for a food pellet in an open foraging zone, and a predator stimulus was triggered before they can reach the reward (Choi & Kim, 2010; Zambetti et al., 2019; Zambetti et al., 2022). Since we expect avoidance and defensive behaviors from the animals, this naturalistic paradigm allows the observation of a full repertoire of behaviors associated with decision-making under threat. Upon completion of the AFAP task, animals then underwent a classical fear conditioning task. In this task, aversive stimuli (footshock) were paired with an odorous context, allowing direct observation of

conditioned freezing responses. It was hypothesized that CEA and BLA lesioned animals will show impaired fear responses compared to sham animals in both tasks, but that BLA lesioned animals will show more impaired fleeing behaviors from the foraging zone in the AFAP task while CEA lesioned animals will show more impaired conditioned freezing behavior during classical fear conditioning. In summary, this study aimed to shed light on the functional heterogeneity of amygdala subnuclei and parse out their differential contributions to the fear response circuitry.

Methods

Subjects

Adult male Long-Evans rats were purchased from Charles River Laboratory (Washington, USA) and maintained on a reverse 12-hr light-dark cycle throughout the duration of the experiment. After lesion surgery, animals were all individually housed and placed on a food deprivation schedule with *ad libitum* access to water to reach 80-85% of their normal weight, which was maintained. Experiments were conducted during the dark phase of the light-dark cycle under red light. All animal experimental facilities and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Washington.

Surgery

Animals were randomly assigned to receive bilateral electrolytic lesion of the CEA (n = 6), BLA (n = 6), or sham (n = 4) surgery. All animals were anesthetized with a ketamine/xylazine cocktail (30 mg/kg ketamine, 2.5 mg/kg xylazine) and mounted onto a stereotaxic instrument (Kopf) where bilateral electrolytic lesions to either the CEA (n = 6), BLA (n = 6), or sham (n = 4) surgeries were performed. Lesions were made using epoxy coated insect pins (#00, 0.5 mm

exposed tip) connected to a precision lesion making device (15s, 0.2 mA; Grass Medical Instrument). Bilateral electrolytic lesions were made using the coordinates in Table 1 below**.**

Stereotaxic coordinates			
	AP	ML	DV
CEA	-2.0, -2.6	±4.2	-8.3
BLA	-2.3, -3.1, -3.8	±5.0, ±5.2, ±5.3	-8.4, -8.8
Sham	0.1 above target coordinates, no currents passed		

Table 1. Coordinates for Bilateral Electrolytic Lesions of the Amygdala. Coordinates were measured from bregma (adapted from Koo et al., 2004).

Apparatus and procedures for AFAP Task

A custom-built semi-naturalistic foraging arena consisted of a "nest zone" (69 cm length × 58–66 cm width × 61 cm height) that opened via an automated sliding gate to reveal a large, expanded foraging area ("foraging zone", 208 cm length × 66–120 cm width × 61 cm height) where 0.5 g food pellets (grain-based; F0171, Bio-Serv) were placed at variable locations. The testing room was kept under red light (11 lux foraging area, 2 lux nest area) with constant white noise (72 dB) playing in the background. Prior to placing each animal, the arena was wiped with 70% ethanol. An ANY-maze software and Ami interface system (Stoelting) was connected to a PC that automatically tracked the animal's position in the arena via a ceiling mounted camera, and triggered an aerial predator stimuli, which was a life-like model owl mounted onto a 92 cm pneumatic air cylinder (Bimba) at the opposite end of the foraging arena and hidden behind a black curtain, plunged downward towards the rat (46 cm/s), then retracted back to it starting position.

Stages of the AFAP task include habituation, baseline, and owl encounter test days, performed sequentially over a total of ~ 12-21 days. The arena was vacuumed, then cleaned and wiped with 70% alcohol solution between animals. Each baseline and owl encounter days

included three trials per day, and animals were allowed a maximum of ~190s to retrieve the pellet from the foraging zone to consume in the nest, after which the gate would automatically close. Latency of pellet retrieval was equivalent to "trial latency" as well as the duration between the gate opening (beginning of trial) and closing (end of trial).

Habituation. Animals were placed in the nest zone of the apparatus for 30 minutes a day for 2 days. For each day 20 pellets (Bio-Serv, 0.5g) were placed in the nest and animals were allowed to consume the pellets.

Baseline. Animals underwent five consecutive days of baseline, with three trials per baseline day. At the beginning of each baseline day, animals were placed in the nest zone with two food pellets. After consuming the two pellets in the nest, the gate opened revealing one food pellet placed at a certain distance from the gate. During the first two days of baseline, the pellets were placed at increasing distances (day 1: 25→50→75 cm, day 2: 50→75→100 cm) from the gate for each trial. Starting from the third baseline day, all pellets were placed at a fixed distance of 100 cm from the gate opening.

Owl encounter stage. After the last baseline day, animals entered the owl encounter stage. For each owl encounter trial, a model owl would lunge towards the animal for ~1s before retracting back behind the curtain once the center of the animals crossed into the "trigger zone" (25 cm before the pellet). There were three owl encounter trials with pellets placed at the 100, 75, and 50 cm distance from nest. Trials were deemed unsuccessful if the rat was not able to retrieve the pellet within the time limit of 190 s, upon which the gate would automatically close. The owl encounter lasted for at least five days and persisted until the animal successfully confronted the owl and retrieved the pellet for three consecutive days.

Contextual Fear Conditioning Task Apparatus and Procedures

Upon the end of the AFAP foraging task, all animals underwent a classical fear conditioning task, during which one training day was followed by one day of contextual testing (test day). The test chamber is an enclosed box consisting of metal grid flooring allowing current (1 s, 1 mA) to pass beneath the animals' paws, above a tray with ammonia scent. Chamber, tray and bars were cleaned between each animal. A camera was mounted on one side of the chamber to record the animal's movements. Movement and freezing (animal remained immobile over a threshold of 2 s) were tracked using the crossing of laser beams. Training day included three minutes of pre-shock baseline followed by three unsignaled shocks (1-s shocks with a 1-min intershock interval). Freezing during each post-shock minute was measured. After the last shock was delivered, the animals remained in the chamber for an additional minute before being placed back in their home cage. On the subsequent contextual fear testing day, animals were placed in the same chamber as the previous day for 8 minutes to monitor the total duration of contextual freezing.

Histology

At the end of the experiment, animals were overdosed with Beuthanasia and transcardially perfused with 0.9% saline followed by 10% buffered formalin. Brains were then extracted and left overnight in the fixative at 4°C. The following morning, the fixative was replaced with 30% sucrose solution. Brains remained in the sucrose solution until fully sunk before being sectioned by a microtome (Leica SM2010R; Leica Biosystems). Transverse, 45 µm sections were collected and mounted onto gelatin-subbed slides for subsequent Cresyl Violet and Prussian blue staining, which was used to visualize the extent of lesion damage. Coordinates and reconstruction of lesion damage was accomplished using The Rat Brain in Stereotaxic

Coordinates (Paxinos & Watson, 1998). Representative lesion histology from CEA and BLA lesion animals are shown in Figure 1.

Statistical analysis

Levene's test or Shapiro-Wilk test was used to test for homogeneity of variances or normality, and if assumptions were satisfied, a univariate analysis of variance (One Way ANOVA) was used for the dependent variable, followed by a post-hoc multiple comparisons test to isolate the respective effects. If the results for the test of normality were significant ($p < .05$), nonparametric tests were used to isolate the differences between the groups. All statistical analysis was performed using the statistical software-package SPSS (IBM) and R version 4.0.1, graphs using GraphPad Prism (GraphPad Software) for figures. Means were shown with error bars indicating standard error of the mean (SEM). All statistical tests were performed with the alpha value set to 0.05.

Results

Fear response during the AFAP task

Baseline. Overall, electrolytic lesions did not impair animals' ability to learn the AFAP task, and only led to differences in owl exposure test days. Specifically, during the baseline phase, all animals quickly learned to search for pellets in the foraging area of the arena and returned to the nesting area upon retrieving a pellet (Figure 2A). However, on owl exposure trials, all lesioned animals showed shorter retrieval latencies across all owl exposure trials compared to sham animals (Figure 3).

Owl exposure test days. Retrieval latencies on the owl baseline trials on the first day of owl tests were similar to the fifth baseline day, which shows before owl exposure, all animals readily left the nest and were motivated to retrieve the pellet (Figures 2A & 2B). Latencies of

retrieval for owl baseline trials increased from baseline levels starting from the second day (following the first owl exposure the day prior) and remained somewhat elevated for both CEA lesioned animals and sham animals. Only the latencies of retrieval for owl baseline trials for BLA lesioned animals remained low (Figure 2B). For analysis of the owl exposure trials, the average latency across five owl exposure days test days were compared between the three groups (Figures 4A, 5A & 6A). A separate analysis was performed for every individual AFAP task test day and groups were compared with each other (Figures 4B, 5B & 6B).

During the first owl exposure trial (100 cm trials) where the distance between the pellet and the nest was the furthest, sham animals and CEA lesioned animals more readily fled from the foraging zone to the nest after triggering the owl. Averaged across the first five owl exposure days, sham animals had the longest retrieval latency to retrieve pellets, while BLA lesioned animals had the shortest retrieval latencies (Figures 4A). Breaking the latencies down by day, BLA lesioned animals were significantly different from sham animals for 4 out of 5 test days, while there was no significant difference between CEA and sham on any test day (Figure 4B).

During the second owl exposure trial (75 cm trials), averaged across the first five owl exposure days, sham animals also showed the longest latency to retrieve pellets (Figure 5A). Both CEA and BLA lesioned animals were significantly difference from sham animals. Further examining the latencies across the 5 test days individually, there were significant differences between BLA and Sham animals on 4 out of 5 days, while CEA lesioned animals and sham animals only differed on the fifth day (Figure 5B).

Averaged across the first five owl exposure days for the third owl exposure trial (50 cm trial) where the pellets were placed the closest to the nest, sham animals also showed the longest latency to retrieve pellets (Figure 6A). In particular, BLA lesioned animals had significantly

shorter average retrieval latencies than CEA lesioned animals or sham animals during the 50 cm trial. Further examining these latencies across individual test days, BLA lesioned animals was again significantly different from sham animals on 4 out of 5 test days, while CEA lesioned animals were only significantly different on the fifth day (Figure 6B).

Overall examining the trend across all the test days, BLA lesioned animals took the shortest average amount of days to successfully retrieve pellets during owl exposure trials; in particular, there was a significant difference between BLA lesioned animals and sham animals (Figure 7A). All BLA lesioned animals succeeded in retrieving all the pellets by day 3 of owl exposure, while CEA lesioned animals and sham animals took up to 14 days in total till all animals in these groups were able to habituate to the owl exposure and retrieve pellets successfully (Figure 7B).

Fear responses during classical fear conditioning

Training day. On the training day of fear conditioning, three minutes of non-shock baseline was followed by a 1-s unsignaled shock and a 1-min post-shock period, repeated two more times. All animals showed little to no freezing to the novel context during the first three baseline minutes (Figure 8A). On average, all three groups of animals showed a significant increase from baseline to post-shock stages during training day (Figure 8B). Freezing increased after the first unsignaled shock periods, as well as after the second and third unsignaled shock period for all animals; however, CEA lesioned animals showed the least amount of increase in freezing in post-shock minutes (Figure 8B).

Contextual test day. Contextual testing was performed on the following day. Animals were placed in the context where they had received unsignaled foot-shocks the previous day and freezing across 8 minutes was observed. Total percent of time spent freezing to the context was

the lowest in CEA lesioned animals compared to the sham animals (Figure 9A). Comparing the time spent freezing over each of the minutes, CEA lesioned animals froze less than sham animals in 7 out of 8 minutes and froze less than BLA animals in 5 out of 8 minutes; in comparison, BLA lesioned animals did not differ significantly from sham animals during any of the minutes (Figure 9B).

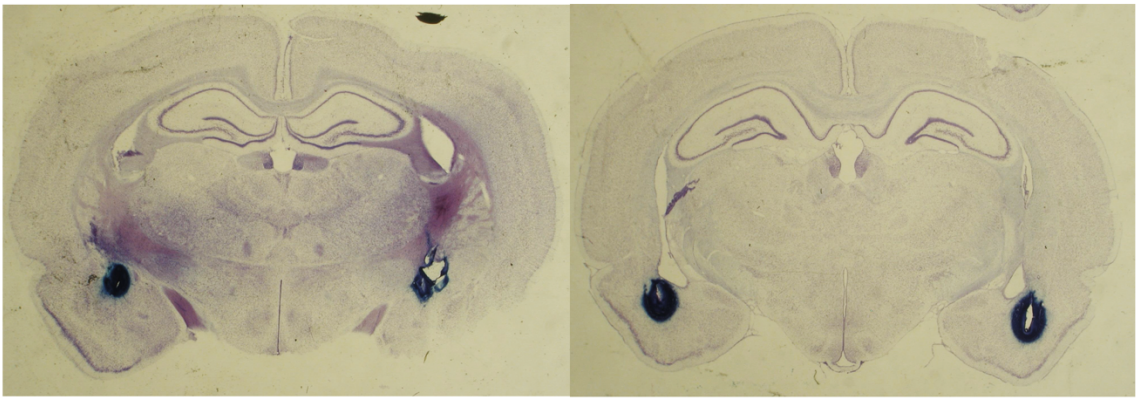

Figure 1. Representative lesion histology from CEA and BLA lesion animals. Left: representative CEA lesion animal. Right: representative BLA lesion animal.

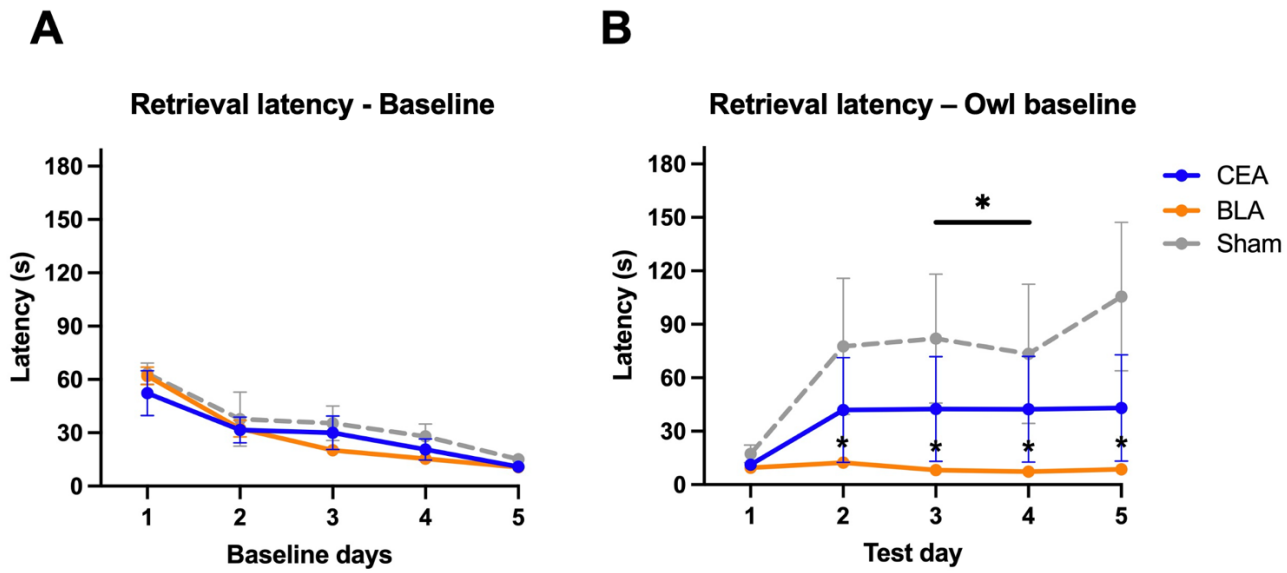

Figure 2. Retrieval Latency During Baseline Days and Pre-Owl Baseline on Test Days. ***(A) Retrieval latency during five baseline days.*** One-way ANOVA revealed that average trial latencies did not differ across groups across baseline days ($F(2, 12) = .27$, $p = .76$). ***(B) Retrieval latency during the pre-owl baseline trials on test days.*** Latencies were averaged across the three trials. Kruskal-Wallis test an overall effect on owl baseline trial latency ($H = 6.603$, $p = .037$). Specifically, owl baseline latencies for the three groups were significantly different from each other on day 3 ($H = 8.00$, $p = .018$) and day 4 ($H = 6.656$, $p = .036$). Significant differences were found between BLA and Sham (Mann-Whitney's U test, $Z = -2.558$, $p = .011$). No significant differences were found between CEA and BLA ($Z = -.801$, $p = .423$), or CEA and Sham ($Z = -1.706$, $p = .088$). While there were no differences in the baseline trials on the first test day prior to owl exposure, BLA significantly diverged from Sham on day 2 ($Z = -2.132$, $p = .033$), day 3 ($Z = -2.558$, $p = .011$), day 4 (-2.558, $p = .011$), day 5 ($Z = -2.566$, $p = .01$); there was no difference found between CEA vs. BLA or CEA vs. Sham on any of the test days. *top denotes $p < .05$ for significant difference across all three groups. *bottom denotes $p < .05$ for significant difference between BLA and Sham.

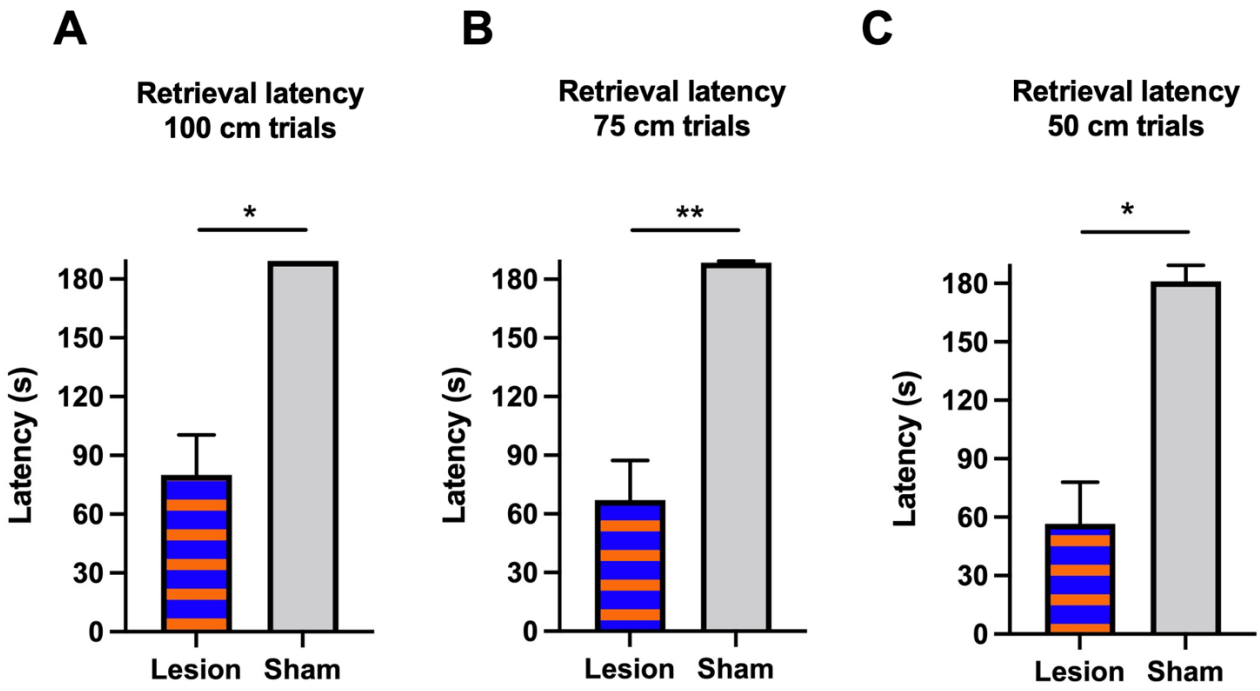

Figure 3. Retrieval Latency During Owl Exposure Trials. Mann-Whitney U test revealed a significant difference between lesion (n = 12) and sham animals (n = 4) for 100 cm trials ($Z = -2.183$, $p = .029$), 75 cm trials ($Z = -2.668$, $p = .008$), and 50 cm trials ($Z = -2.425$, $p = .015$). *denotes $p < .05$ and **denotes $p < .01$ for significance between two groups.

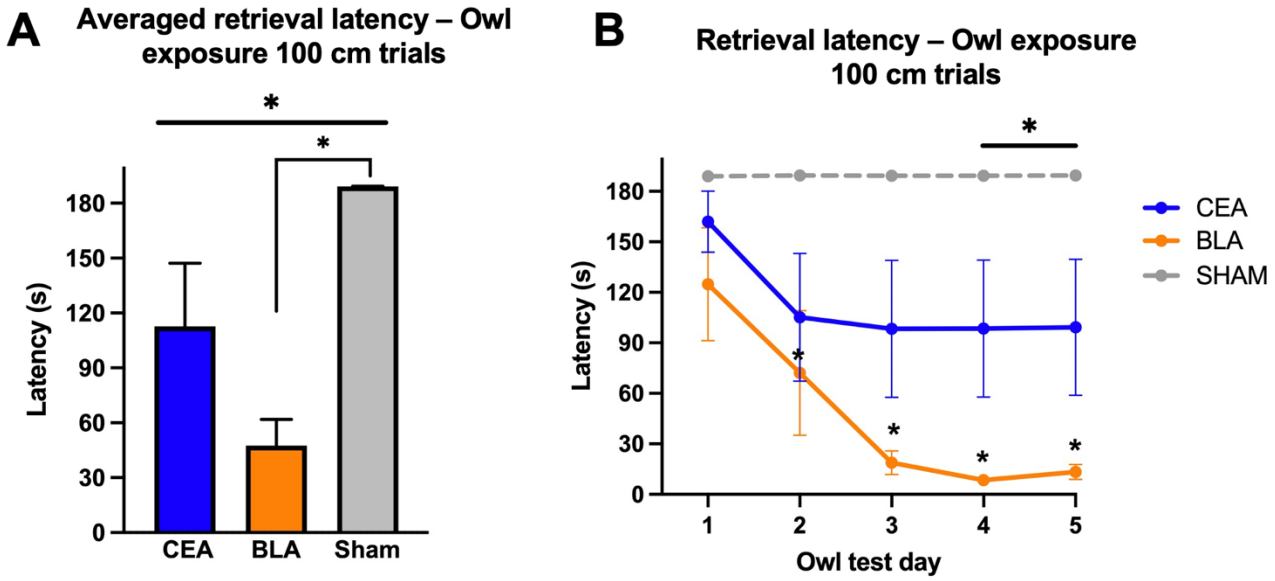

Figure 4. Retrieval latency for 100 cm Owl Exposure Trials. ***(A) Averaged retrieval latency over test days for 100 cm trial by group.*** CEA: M = 112.7 ± 34.53, BLA: M = 47.50 ± 14.33, Sham: M = 189.3 ± .06. Kruskal-Wallis test revealed an overall effect between all three groups (H = 7.264, p = .026); Post hoc Mann-Whitney's U test revealed that there was a significant difference between BLA and Sham (Z = -2.558, p = .011). *top denotes p < .05 for group difference, and *bottom denotes p < .05 for significant difference between BLA and Sham. ***(B) Retrieval latency for 100 cm trials across test days***. Kruskal-Wallis test revealed an overall difference between groups for day 4 (H = 6.915, p = .032) and day 5 (H = 6.390, p = .041). Mann-Whitney's tests revealed BLA and Sham were significantly different on day 2 (Z = -2.032, p = .042), day 3 (Z = -2.566, p = .01), day 4 (Z = -2.558, p = .011), and day 5 (Z = 2.558, p = .011). *top denotes p < .05 for group difference, *bottom denotes difference between BLA and Sham groups.

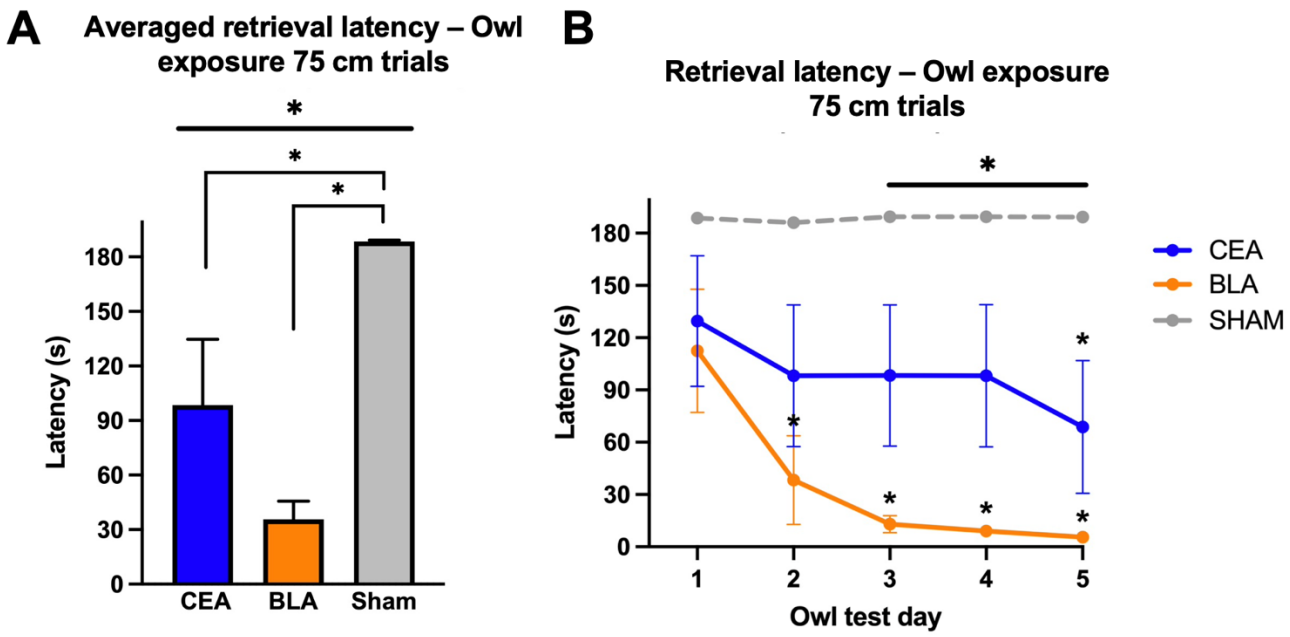

Figure 5. Retrieval latency for 75 cm Owl Exposure Trials. ***(A) Averaged retrieval latency over test days for 75 cm trial by group.*** CEA: M = 98.55 ± 36.14, BLA: M = 35.7 ± 9.92, Sham: M = 188.55 ± .69. Kruskal-Wallis test revealed a significant effect of group on trial latency for the 75 cm trial ($H = 7.647$, $p = .022$). Mann-Whitney post hoc comparisons revealed that there was a significant difference between CEA and Sham ($Z = -2.132$, $p = .038$), and between BLA and Sham ($Z = -2.558$, $p = .011$), but not between CEA and BLA ($Z = -.801$, $p = .423$). *top denotes $p < .05$ for overall difference, denotes $p < .05$ for significance between two groups. ***(B) Retrieval latency for 75 cm trials across test days.*** Kruskal-Wallis test revealed a significant difference between all three groups on day 3 ($H = 7.589$, $p = .022$), day 4 ($Z = 6.232$, $p = .044$), day 5 ($Z = 7.947$, $p = .019$). Further Mann-Whitney's U tests revealed that while there was a significant difference between CEA and Sham on day 5 ($Z = 2.5$, $p = .042$), BLA and Sham were significantly different on day 2 ($Z = -2.558$, $p = .011$), day 3 ($Z = -2.558$, $p = .011$), day 4 ($Z = -2.556$, $p = .01$), day 5 ($Z = -2.558$, $p = .011$). *top denotes $p < .05$ for significant difference across all three groups, *denotes $p < .05$ for difference between a lesion group and sham.

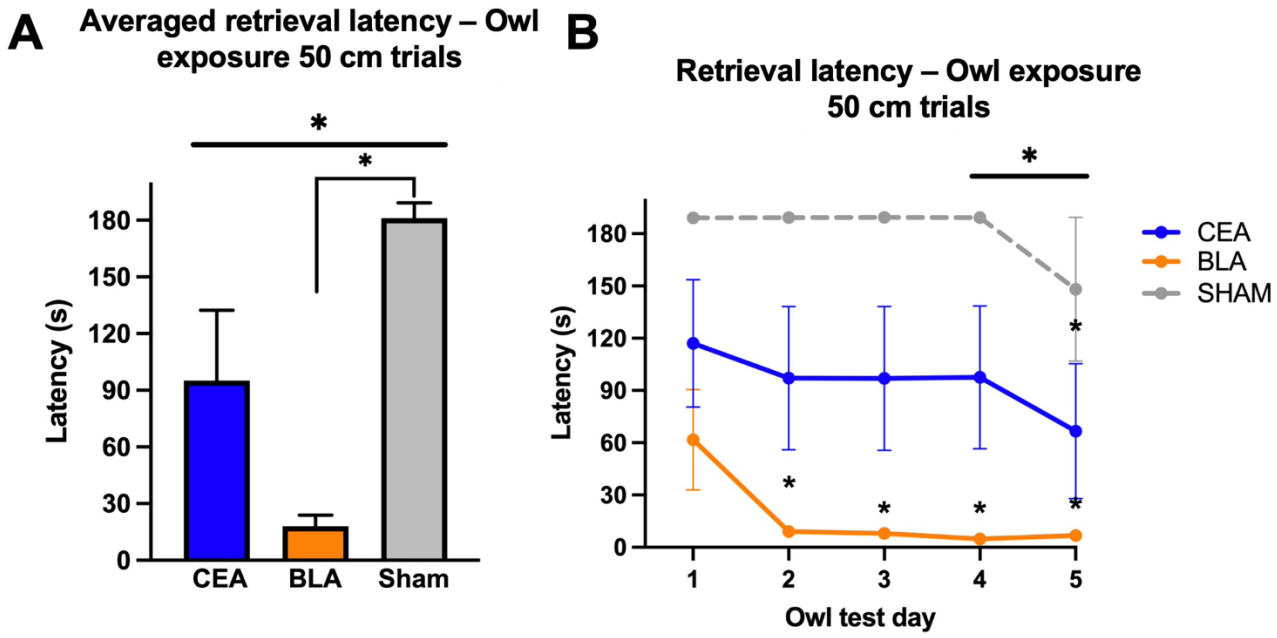

Figure 6. Retrieval latency for 50 cm Owl Exposure Trials. ***(A) Averaged retrieval latency over test days for 50 cm trial by group.*** CEA: M = 95.08 ± 37.27, BLA: M = 18.09 ± 5.74, Sham: M = 181.01 ± 8.26. Kruskal-Wallis test showed a significant difference between all three groups (H = 6.824, p = .033); post hoc Mann-Whitney U test revealed that only BLA and Sham were significantly difference (Z = -2.558, p = .011). *top denotes $p < .05$ for all three groups, *bottom denotes $p < .05$ for significance between two groups. ***(B) Retrieval latency for 75 cm trials across test days***. Kruskal-Wallis test revealed a significant difference between all three groups on day 4 (Z = 7.67, p = .022), day 5 (Z = 7.176, p = .028). Mann-Whitney's U tests revealed a significant difference between CEA and Sham on day 5 (Z = -2.132, p = .033), but BLA and Sham were significantly different on day 2 (Z = -2.556, p = .01), day 3 (Z = -2.558, p = .011), day 4 (Z = -2.574, p = .01), day 5 (Z= -2.558, p = .011). *top denotes $p < .05$ for significant difference across all three groups, *denotes $p < .05$ for difference between a lesion group and sham.

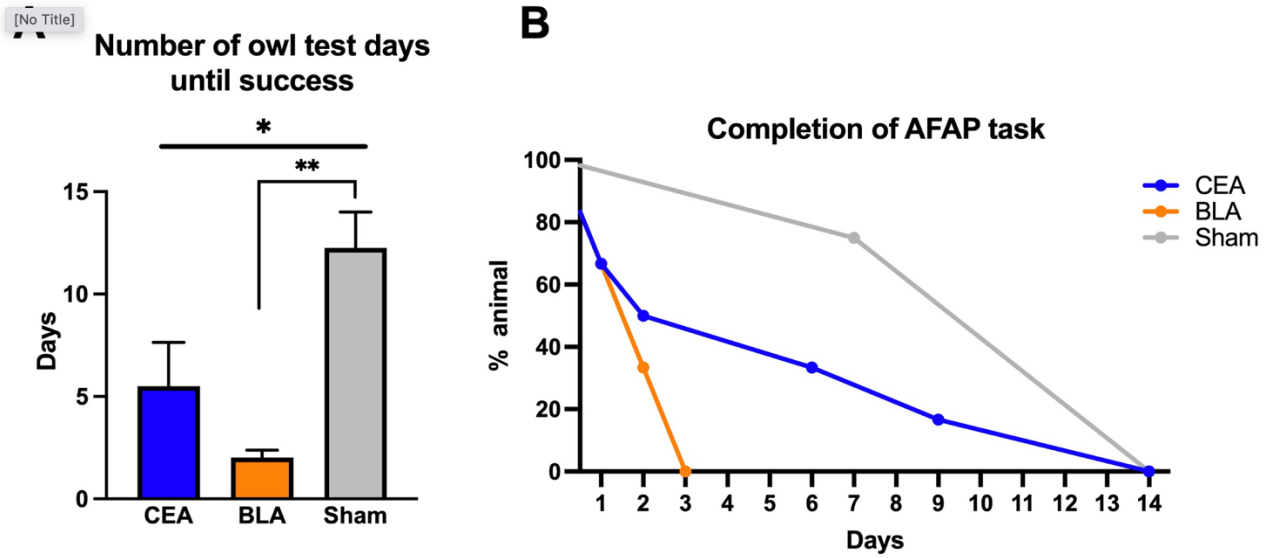

Figure 7. Number of Owl Test Days Till Success on All Owl Exposure Trials. ***(A) Number of Owl Test Days Till Success.*** CEA: M = 5.5 ± 2.14, BLA: M = 2 ± .36, Sham: M = 12.25 ± .1.75. Kruskal-Wallis test revealed an overall difference between all three groups (H = 7.112, p = .029), further Mann-Whitney's U test revealed a significant difference between BLA and Sham (Z = -2.614, p = .009), but not between CEA and Sham (Z = -1.876, p = .061) or CEA and BLA (Z = -.823, p = .411). *denotes p < .05 for significant difference across three groups, and **denotes p < .01 for significant difference between two groups. ***(B) Percentage of Completion of Animals Within Groups.*** BLA animals were the first group to complete all the owl exposure trials within 5 test days. Both CEA and Sham animals took up to 14 days for full completion of all animals within groups.

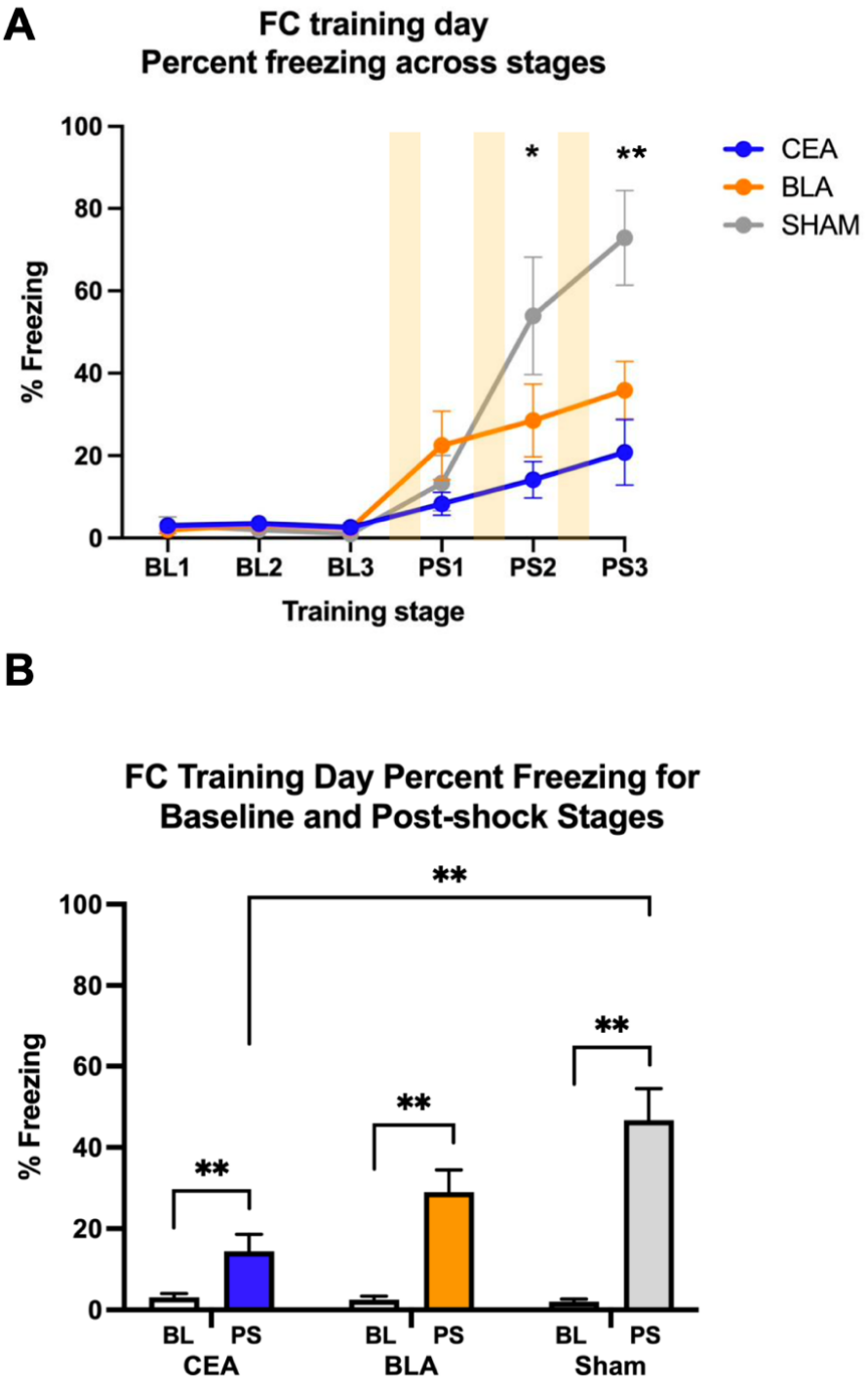

Figure 8. Percent Freezing During Fear Conditioning Training Day.

(A) Percent Freezing Across Stage. Three baseline stages were followed by three repeated sessions of unsignaled shock (unsignaled shock period shown in yellow shade) and post-shock (PS). One-way ANOVA revealed significant effect of group during PS2 ($F(2, 13) = 4.596$, $p = .031$) and PS3 stages ($F(2, 13) = 8.717$, $p = .004$). Tukey's LSD post hoc multiple comparisons

revealed a significant difference between CEA and Sham during PS2 ($p = .01$) and PS3 ($p = .001$), as well as between BLA and Sham during PS3 ($p = .012$). *denotes $p < .05$ and **denotes $p < .01$ for significant difference across all three groups.

(B) Training Day Averaged Freezing Percentage by Group and Stage. *(1) Baseline vs. Post-shock:* Non-parametric Wilcoxon rank-sum tests yielded significant differences between baseline and post-shock stages for CEA ($Z = -3.385$, $p < .001$), BLA ($Z = -4.75$, $p < .001$), and sham ($Z = -3.677$, $p < .001$). ** denotes $p < .01$ for significant difference between averaged baseline and averaged post-shock freezing. *(2) Training day averaged baseline*: One-way ANOVA revealed no significant difference between groups during training day baseline stages for CEA ($M = 3.06 \pm .97$), BLA ($M = 2.52 \pm .83$), Sham ($M = 2.01 \pm .67$), $F(2, 13) = .322$, $p = .73$. *(3) Training day averaged post-shock:* across the three groups, CEA ($M = 14.43 \pm 4.17$), BLA ($M = 28.97 \pm 5.48$), and Sham ($M = 46.71 \pm 15.54$), One-way ANOVA revealed a significant effect of group on post-shock percent freezing ($F(2, 13) = 7.599$, $p = .007$). Post hoc Tukey's HSD multiple comparisons test revealed a significant difference between CEA and Sham ($p = .002$), but not between BLA and Sham ($p = .052$) or CEA and BLA ($p = .072$). ** denotes $p < .01$ for significant difference between two groups.

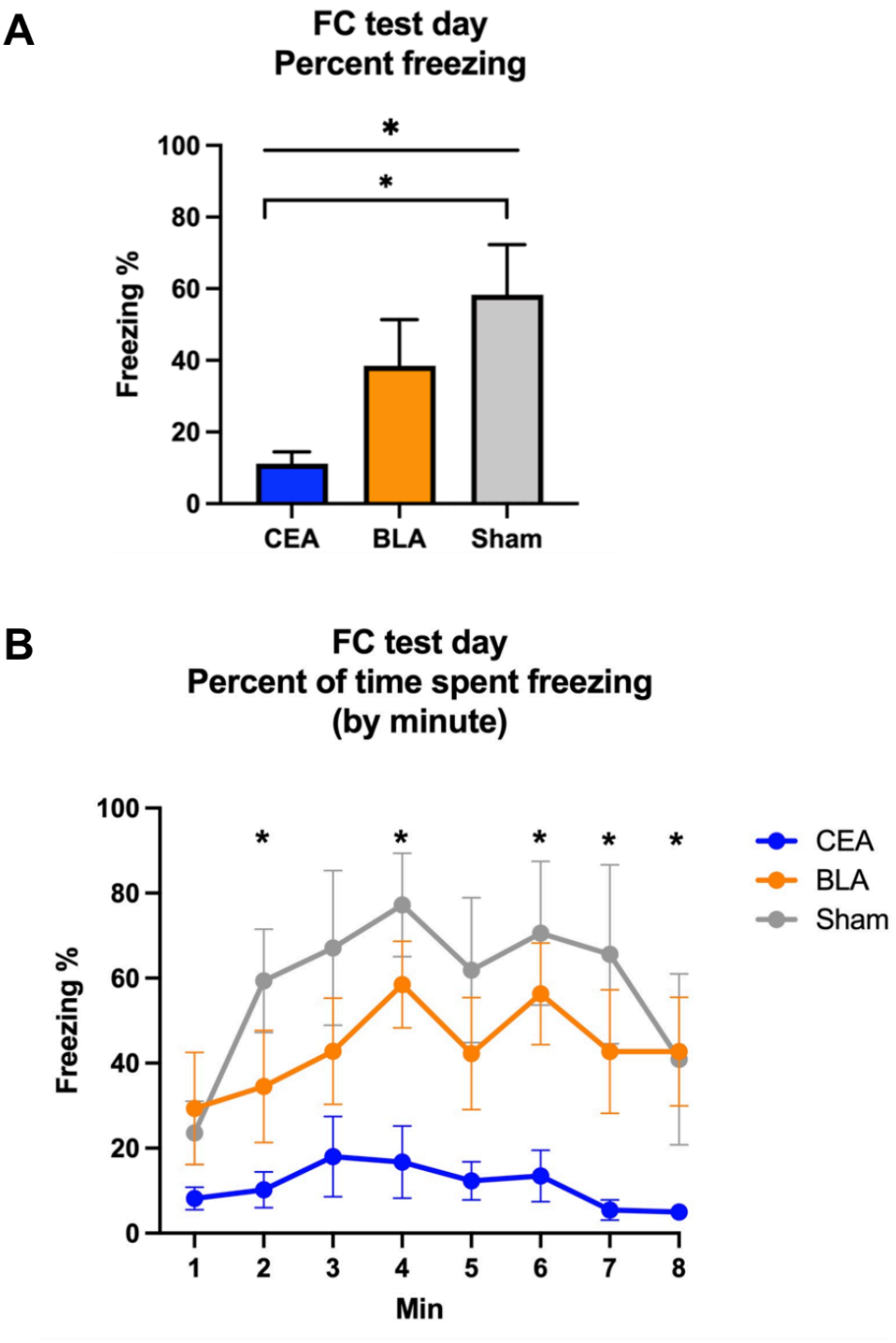

Figure 9. Percent Freezing on Fear Conditioning Test Day.

(A) Fear Conditioning Test Day Percent Freezing. One-way ANOVA was used to determine the difference between the average percent freezing between CEA (M = 11.17 ± 3.31), BLA (M = 38.44 ± 12.89), and Sham (M = 58.28 ± 14.04), revealing a significant effect of group on contextual freezing ($F(2, 13) = 4.732$, $p = .029$). *top denotes $p < .05$ for significant difference between three

groups. Post hoc multiple comparisons test revealed significant differences between CEA and Sham groups ($p = .01$), and no significant differences were found between CEA vs. BLA ($p = .074$) or BLA vs. Sham ($p = .228$). *bottom denotes $p < .05$ for significant difference between two groups. ***(B) Fear Conditioning Test Day Freezing by Minute.*** Kruskal-Wallis H test revealed that there were significant differences between all three groups during minute 2 ($H = 7.806$, $Z = -1.684$, $p = .02$), minute 4 ($H = 6.755$, $Z = -1.687$, $p = .034$), minute 6 ($H = 8.354$, $Z = -2.406$, $p = .015$), minute 7 ($H = 8.665$, $Z = -2.722$, $p = .013$), and minute 8 ($H = 7.382$, $Z = -2.246$, $p = .025$). Mann-Whitney's U test found significant differences between CEA and Sham during minute 2 ($Z = -2.558$, $p = .011$), minute 3 ($Z = -2.132$, $p= .033$), minute 4 ($Z = -2.352$, $p = .019$) minute 5 ($Z = -2.345$, $p = .019$), minute 6 ($Z = -2.352$, $p = .019$), minute 7 ($Z = -2.132$, $p = .033$), minute 8 ($Z = -2.352$, $p = .019$). CEA was significantly different from BLA during minute 6 ($Z = -2.406$, $p = .016$) minute 7 ($Z = -2.722$, $p = .006$), minute 8 ($Z = -2.246$, $p = .025$). There were no significant differences between BLA and sham across any of the minutes. * denotes $p < .05$ for significant difference between all three groups.

Discussion

The present study employed two separate behavioral paradigms, one performed in a food-motivated open field foraging task and the other in a confined chamber with fear-related contextual cues to examine differences in defensive behaviors in animals that received selective lesions in amygdala subregions. These tests were performed to examine the functional roles of the CEA and the BLA, and how these subregions differently moderate the link between sensory cues, fear, and fear-induced behavioral responses.

In this study, animals receiving bilateral lesions to amygdala subregions generally showed stunted fear responses and shorter latencies to retrieve pellet and did not readily flee from the predator stimuli, as well as impaired freezing in contextual fear conditioning. The general impairment in fear responses observed across all lesioned animals in these two tasks echoes previous findings and solidifies the role of the amygdala as a regulator of fear and associated responses. Since the amygdala is an important switchboard structure sending efferent signals to prefrontal cortex, hippocampus, hypothalamus and the midbrain, an unimpaired

amygdala is crucial in interpreting sensory stimuli and connecting them to appropriate avoidance or defensive behavior (Kim et al., 1993; Chudasama et al., 2009; Izquierdo et al., 2005; Heimer & Van Hoesen, 2006). Structural damage to any subregion blocks appropriate self-preservation responses and leads to malfunctions in the fear circuity.

However, if the functional role of amygdala subnuclei was unvaried, lesioned animals should have shown comparable performance in both the AFAP task and conditioned freezing. However, this was not the case in the present study. BLA lesioned animals were more successful at completing the AFAP task compared to CEA lesioned animals but showed more contextual freezing. Specifically, in the AFAP task, animals with BLA lesions showed the most impaired fear responses, demonstrated by the shortest latencies to retrieve the pellets across on all owl exposure trials. These animals showed little to no hesitancy to leave the nest on the day after first exposure to the owl stimuli in the foraging zone and were able to approach and retrieve the food pellet quickly under predator threat. This was not observed in CEA lesioned animals, who showed more hesitancy to approach the pellet after owl exposure and more readily fled the foraging zone. However, during the classical fear conditioning task, CEA lesioned animals spent the least amount of time freezing during both the post-shock periods on training day as well as context testing day, demonstrating more impaired conditioned fear responses when confronted with the context previously associated with foot-shocks. The same level of impairment was not observed in the BLA lesioned animals, and the time they spent freezing was comparable to sham animals.

This reinforces the notion that the amygdala is not a homogenous region; instead, lesions of the BLA and CEA differentially affect downstream defensive behaviors. BLA lesioned animals showed little to no fear or reaction towards the lunging predator during the trial and

quickly proceeded to retrieve the pellet; this suggests that the BLA lesions were more effective at blocking fleeing behavior, thus this subregion may play a more important role in regulating active defensive responses. The cues during the AFAP task were mainly visual and auditory; it was previously proposed that fear towards these types of stimuli involves a pathway that passes through the lateral amygdala (LA) and the basomedial amygdala (BMA), largely bypassing the CEA to reach the dorsolateral PAG to elicit innate defensive responses to predator stimuli (Gross et al, 2012). Conversely, it has also been suggested that projections from the dorsal PAG to the BLA gives rise to innate fear responses; in this study, stimulations of the BLA could directly evoke fleeing behavior in a foraging task (Kim et al., 2013). Nevertheless, both findings corroborate that there exists an important pathway between the PAG and BLA that is crucial for eliciting active defensive responses. If this pathway was disrupted, such as in electrolytic lesions, we would expect impaired fleeing responses in BLA lesioned animals and not CEA lesioned animals, which was what we observed in the current study.

Current findings also suggest that the CEA plays a more important role in mediating conditioned fear or passive defensive behaviors, as seen in the contextual fear conditioning task where CEA lesions more effectively impaired conditioned freezing. This echoes previous findings where abolishing efferent signals from the CEA suppresses motivated behaviors and impairs freezing responses to conditioned stimulus (Ciocchi et al., 2010; Johansen et al., 2011; Goosen & Maren, 2001). These animals also fled the foraging zone more readily in the AFAP task and showed stronger fear responses towards the lunging owl during the trials. This could be attributed to the limited engagement of the CEA in regulating active avoidance responses, whereas the BLA could be playing a more crucial role in promoting active defensive behaviors to escape aversive stimuli. Indeed, studies have shown that BLA stimulations prompted animals to

flee from the foraging zone in a naturalistic foraging task rather than inducing freezing in the arena (Kim et al., 2013).

Results of the present study also reveal future steps to better understand animal's performance under stress. First, it should be noted that electrolytic lesions damage the passage of fibers through the amygdala; partial damage could be sparing some fibers passing through these subregions. Indeed, there were large variances in trial latency during some owl exposure trials within the CEA and BLA groups. A future step would be to examine whether partial damage was associated with the distribution of the behavioral data during the AFAP task. Another future step could include direct optogenetic stimulation of BLA neurons, which should evoke fleeing in the AFAP task sans predator, and direct stimulation of CEA neurons, which should evoke increased conditional freezing. Alternatively, direct optogenetic inhibition of the BLA or the CEA could test whether the behavioral impairments were due to damage to the structure, or due to damage to fibers that pass through these structures. If the selective inhibition does not yield impairments as seen in the current study, then the effects of lesioning could be due to fibers that pass through these subregions rather than disruptions of the neurons within the BLA or CEA.

Secondly, animals exhibited a diverse set of behaviors in the AFAP apparatus, and the observed variables of latency only captured a facet of their entire repertoire; thus, other behavioral variables in these CEA and BLA lesioned animals such as foraging trajectories, latencies to leave the nest and the time spent at the gate could also be analyzed. Furthermore, while the goal of the two behavioral tests was to compare effects of selective lesions in the AFAP task and the classical fear conditioning task, these behaviors did not occur in only one task exclusively. For example, in the AFAP task, freezing was also observed near and inside the gate and the corners of the arena after exposure to owl in these animals, which could reveal interesting

trends in passive avoidance from animals in a naturalistic setting. Rats have been known to exhibit other behaviors related to decision-making with or without stressors such as retreating and vicarious trial-and-error (Blanchard et al., 1989; Redish, 2016; Resulaj et al., 2009). AI pose estimation tools such as DeepLabCut and the SLEAP model may help extract more nuanced behaviors during these AFAP trials to help enhance our understanding of fear and defensive behavior (Mathis et al., 2018; Pereira et al., 2022).

In summary, the current study observed diverging fear responses in differentially lesioned animals and attempts to parse out differential roles of the CEA and BLA and how they influence defensive behaviors. Findings showed that structural damage to either subregion of the amygdala can lead to a disruption of a cumulative cascading pathway promoting defensive behaviors. However, the diverging impairments in active and passive defensive behaviors across the two different tasks suggests that there may be distinct underlying to further examine. Given the heterogeneity of amygdala subregions and their related upstream and downstream systems, a metaparadigm approach should be taken; future studies could ontogenetically stimulate the BLA or CEA and record cell activity from related upstream and downstream structures such as the PAG, prefrontal cortex, or hippocampus, and compare between naturalistic foraging tasks and classical fear conditioning tasks. The different functional significance of these subnuclei warrants attention; in humans, damage to the BLA has been associated with dysfunctions in the processing of sensory cues in disorders such as autism and Kluver-Bucy syndrome (Bauman & Kemper, 2003). Differential functional connectivity of these subregions has also been supported in stress-related psychiatric disorders such as PTSD (Haris et al., 2023). Recognizing and unraveling the complexity of these stress circuitries could help translate the neural correlates of fear across species, further expanding our knowledge of the foundations of stress and anxiety.

References

Amorapanth, P., LeDoux, J. E., & Nader, K. (2000). Different lateral amygdala outputs mediate reactions and actions elicited by a fear-arousing stimulus. *Nature Neuroscience,* 3, 74–79.

Bauman, M. L., & Kemper, T. L. (2003). The neuropathology of the autism spectrum disorders: what have we learned?. *Novartis Foundation symposium*, 251, 112–297.

Blanchard, D. C. & Blanchard, R. J. Innate and conditioned reactions to threat in rats with amygdaloid lesions. *J. Comp. Physiol. Psychol*. 81, 281–290 (1972).

Blanchard, R. J., Blanchard, D. C., & Hori, K. (1989). An ethoexperimental approach to the study of defense. In R. J. Blanchard, P. F. Brain, D. C. Blanchard, & S. Parmigiani (Eds.), *Ethoexperimental approaches to the study of behavior* (pp. 114–136). Kluwer Academic/Plenum Publishers. https://doi.org/10.1007/978-94-009-2403-1_7

Bolles, R. C. (1970). Species-specific defense reactions and avoidance learning. *Psychological Review*, 77(1), 32-48.

Brown, S. & Schäfer, E. (1888). An investigation into the functions of the occipital and temporal lobes of the monkey's brain. *Phil. Trans. R. Soc. B* 179, 303–327

Burwell, R. D., Saddoris, M. P., Bucci, D. J. & Wiig, K. A. (2004). Corticohippocampal contributions to spatial and contextual learning. *J. Neurosci.* 24, 3826–3836.

Ciocchi, S., Herry, C., Grenier, F., Wolff, S. B., Letzkus, J. J., Vlachos, I., Ehrlich, I., Sprengel, R., Deisseroth, K., Stadler, M. B., Müller, C., & Lüthi, A. (2010). Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature*, 468(7321), 277–282.

Cimadevilla, J. M., Kaminsky, Y., Fenton, A., & Bures, J. (2000). Passive and active place avoidance as a tool of spatial memory research in rats. *Journal of Neuroscience Methods*,

102(2), 155-164. https://doi.org/10.1016/S0165-0270(00)00288-0

Choi, J. S., Cain, C. K., & LeDoux, J. E. (2010). The role of amygdala nuclei in the expression of auditory signaled two-way active avoidance in rats. *Learning & Memory*, 17(3), 139–147.

Choi, J. S., & Kim, J. J. (2010). Amygdala regulates risk of predation in rats foraging in a dynamic fear environment. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 21773–21777. https://doi.org/10.1073/pnas.1010079108

Chudasama, Y., Izquierdo, A. & Murray, E. A. Distinct contributions of the amygdala and hippocampus to fear expression: effects of amygdala and hippocampal lesions on emotion. *Eur. J. Neurosci*. 30, 2327–2337 (2009)

Corbit, L. H., & Balleine, B. W. (2005). Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of Pavlovian-instrumental transfer. *Journal of Neuroscience*, 25, 962–970.

Fanselow, M. S., & Lester, L. S. (1988). *In Evolution and Learning* (Eds. Bolles, R. C. & Beecher, M. D.) (pp. 185–212). Lawrence Erlbaum Associates.

Fanselow, M. S. (2022). Negative valence systems: sustained threat and the predatory imminence continuum. *Emerg. Top. Life Sci*. 6, 467–477.

Fentress, J. C. (1968). Interrupted ongoing behaviour in two species of vole (Microtus agrestis and Clethrionomys britannicus). *Animal Behaviour*, *16*(1), 135-153.

Goosens, K. A., & Maren, S. (2001). Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. *Learning & memory*, 8(3), 148-155.

Gross, C. T., & Canteras, N. S. (2012). The many paths to fear. *Nature reviews. Neuroscience,* 13(9), 651–658. https://doi.org/10.1038/nrn3301

Haris, E.M., Bryant, R.A., Williamson, T. Korgaonkar, M. S. (2023) Functional connectivity of amygdala subnuclei in PTSD: a narrative review. *Mol Psychiatry* 28, 3581–3594 (2023).

Heimer, L. & Van Hoesen, G. W. (2006). The limbic lobe and its output channels: implications for emotional functions and adaptive behavior. *Neurosci. Biobehav. Rev.* 30, 126–147

Hollén, L. I., & Radford, A. N. (2009). The development of alarm call behaviour in mammals and birds. *Animal Behaviour*, 78(4), 791-800

Izquierdo, A., Suda, R. K. & Murray, E. A. (2005). Comparison of the effects of bilateral orbital prefrontal cortex lesions and amygdala lesions on emotional responses in rhesus monkeys. *J. Neurosci*. 25, 8534

Jhang, J., Lee, H., Kang, M.S. et al. Anterior cingulate cortex and its input to the basolateral amygdala control innate fear response. *Nature Communications*. 9, 2744 (2018). https://doi.org/10.1038/s41467-018-05090-y

Janak, P., & Tye, K. (2015). From circuits to behaviour in the amygdala. *Nature*, 517(7534), 284–292. https://doi.org/10.1038/nature14188

Keifer, O. P., Jr, Hurt, R. C., Ressler, K. J., & Marvar, P. J. (2015). The Physiology of Fear: Reconceptualizing the Role of the Central Amygdala in Fear Learning. *Physiology* (Bethesda, Md.), 30(5), 389–401.

Kim, J. J., Rison, R. A., & Fanselow, M. S. (1992). Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. *Behavioral Neuroscience*, 107, 1093–8.

Kim, E. J., Kim, E. S., Covey, E., & Kim, J. J. (2010). Social transmission of fear in rats: the role of 22-kHz ultrasonic distress vocalization. *PloS one*, *5*(12), e15077.

Kim, E. J., Horovitz, O., Pellman, B. A., & Kim, J. J. (2013). Dorsal periaqueductal gray-

amygdala pathway conveys both innate and learned fear responses in rats. *Proceedings of the National Academy of Sciences,* 110(36), 14795-14800.

Kim, E. J., Kong, M. S., Park, S. G., Mizumori, S. J. Y., Cho, J., & Kim, J. J. (2018). Dynamic coding of predatory information between the prelimbic cortex and lateral amygdala in foraging rats. *Science advances*, 4(4), eaar7328. https://doi.org/10.1126/sciadv.aar7328

Kong, M. S., Kim, E. J., Park, S., Zweifel, L. S., Huh, Y., Cho, J., & Kim, J. J. (2021). 'Fearful-place' coding in the amygdala-hippocampal network. *eLife*, 10, e72040. https://doi.org/10.7554/eLife.72040

Killcross, S., Robbins, T. W., & Everitt, B. J. (1997). Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. Nature, 388(6640), 377–380.

Koo, J. W., Han, J. S., & Kim, J. J. (2004). Selective neurotoxic lesions of basolateral and central nuclei of the amygdala produce differential effects on fear conditioning. *The Journal of Neuroscience*, 24(35), 7654–7662.

Koob, G., Volkow, N. Neurocircuitry of Addiction. *Neuropsychopharmacol* 35, 217–238 (2010). https://doi.org/10.1038/npp.2009.110

Lazaro-Munoz, G., LeDoux, J. E., & Cain, C. K. (2010). Sidman instrumental avoidance initially depends on lateral and basal amygdala and is constrained by central amygdala-mediated Pavlovian processes. *Biological Psychiatry*, 67(12), 1120–1127.

LeDoux, J. E., Cicchetti, P., Xagoraris, A. & Romanski, L. M. (1990). The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. *J. Neurosci*. 10, 1062–1069

LeDoux, J. E., (1996). *The Emotional Brain*. Simon and Schuster; New York.

Martinez, R. C., Carvalho-Netto, E. F., Ribeiro-Barbosa, É. R., Baldo, M. V. C., & Canteras, N.

S. (2011). Amygdalar roles during exposure to a live predator and to a predator-associated context. *Neuroscience*, 172, 314-328.

Mathis, A., Mamidanna, P., Cury, K.M. et al. DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nat Neurosci* 21, 1281–1289 (2018). https://doi.org/10.1038/s41593-018-0209-y

Nader, K., & Ledoux, J. E. (1997). Is it time to invoke multiple fear learning systems in the amygdala?. Trends in cognitive sciences, 1(7), 241–244.

Pereira, T.D., Tabris, N., Matsliah, A. et al. SLEAP: A deep learning system for multi-animal pose tracking. *Nat Methods* 19, 486–495 (2022).

Ramirez, F., Moscarello, J. M., LeDoux, J. E., & Sears, R. M. (2015). Active avoidance requires a serial basal amygdala to nucleus accumbens shell circuit. *The Journal of Neuroscience*, 35(8), 3470–3477.

Redish A. D. (2016). Vicarious trial and error. Nature reviews. *Neuroscience*, 17(3), 147–159. https://doi.org/10.1038/nrn.2015.30

Resulaj, A., Kiani, R., Wolpert, D. M., & Shadlen, M. N. (2009). Changes of mind in decision-making. *Nature*, 461(7261), Article 7261. doi:10.1038/nature08275

Sato, M., Nakai, N., Fujima, S., Choe, K. Y., & Takumi, T. (2023). Social circuits and their dysfunction in autism spectrum disorder. *Molecular Psychiatry*, 28(11), 3194–3206.

Silva, B. A., Gross, C. T., & Gräff, J. (2016). The neural circuits of innate fear: detection, integration, action, and memorization. *Learning & Memory* (Cold Spring Harbor, N.Y.), 23(10), 544–555. https://doi.org/10.1101/lm.042812.116

Tseng, Y.-T., Schaefke, B., Wei, P., & Wang, L. (2023). Defensive responses: behaviour, the brain and the body. *Nature Reviews Neuroscience*, 24(11), 655–671

Wang, S., Yu, R., Tyszka, J. M., Zhen, S., Kovach, C., Sun, S., Huang, Y., Hurlemann, R., Ross, I. B., Chung, J. M., Mamelak, A. N., Adolphs, R., & Rutishauser, U. (2017). The human amygdala parametrically encodes the intensity of specific facial emotions and their categorical ambiguity. *Nature communication*s, 8, 14821.

Wilensky, A. E., Schafe, G. E., Kristensen, M. P. & LeDoux, J. E. (2006). Rethinking the fear circuit: the central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning. *J. Neurosci*. 26, 12387–12396.

Wood, J.B., Maynard A., Lawlor A., Sawyer E.K., Simmons D., Pennoyer K.E. and Derby C.D. (2010). Caribbean reef squid, Sepioteuthis sepioidea, use ink as a defense against predatory French grunts, Haemulon flavolineatum. *Journal of Experimental Marine Biology and Ecology*. 338, 20–27

Yilmaz, M., & Meister, M. (2013). Rapid innate defensive responses of mice to looming visual stimuli. *Current Biology,* 23, 2011–2015. https://doi.org/10.1016/j.cub.2013.08.015

Zambetti, P. R., Schuessler, B. P., & Kim, J. J. (2019). Sex differences in foraging rats to naturalistic aerial predator stimuli. iScience, 16, 442–452.

Zambetti, P. R., Schuessler, B. P., Lecamp, B. E., Shin, A., Kim, E. J., & Kim, J. J. (2022). Ecological analysis of Pavlovian fear conditioning in rats. *Communications biology*, 5(1), 830. https://doi.org/10.1038/s42003-022-03802-1