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A Functional Role of the Amygdala in Rats Living in a Semi-Naturalistic Risky Environment

Earnest S. Kim

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Earnest Shimmoung Kim

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Reading Committee:

Jeansok Kim, Chair

Sheri Mizumori

Jaime Diaz

Program Authorized to Offer Degree:

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University of Washington

Abstract

A Functional Role of the Amygdala Revealed in Rats Living in a Semi-Naturalistic Risky Environment

Earnest Shimmoung Kim

Chair of Supervisory Committee:

Professor Jeansok J. Kim

Department of Psychology

There is considerable evidence that the amygdala is involved in the processing and expression of emotions, particularly fear. It would therefore be plausible to expect that amygdala-coded fear would also influence voluntary decisions on feeding and foraging in animals living in dangerous environments. The studies presented in this dissertation explore a functional role of the amygdala in rats living in a semi-naturalistic "closed economy" setting where all nutritional resources are procured at the risk of receiving random shocks in a foraging area. The first study revealed that amygdala-coded fear influences the animal's voluntary feeding behavior, but the amygdala is not necessary for the animal's voluntary avoidance of the foraging area. The second study showed that the amygdala is not necessary for either feeding or foraging behavior when the danger is signaled by a cue (predictable fear environment). The final study explores how amygdala-coded fear influences circadian rhythms in rats and how the amygdala interacts with the superchiasmatic nucleus to anticipate times of danger and safety.

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Chapter I. Background and Introduction

The function of fear

In nature, a predatory encounter can be considered the most substantial event in an organism's life. The resulting cost of failure to either defend or escape a predatory threat ends the fitness of that organism. Foraging for necessary resources, such as food and water, increases the risk of predation. Therefore, the animal must balance the needs of survival with the cost of predation to maximize fitness (Choi & Kim, 2010). Many have proposed a dual function of fear to address this balance: 1) defend against imminent danger by executing innate "species-specific defense reactions" (SSDR) such as fleeing or fighting during a predatory encounter and 2) taking on protective "antipredator defensive strategies" (ApDS) such as changes to feeding and foraging patterns before or after predatory encounters (Blanchard & Blanchard, 1990; Bolles, 1970; Fanselow, Lester, & Helmstetter, 1988). Therefore, it can be inferred that the functional significance of fear and the underlying brain structures is to protect an organism from threats in a hostile environment.

Human vs. animal fear

Contemporary models of fear and anxiety in humans have utilized ethological perspectives to construct models of fear related disorders (Aupperle & Paulus, 2010; Mobbs et al., 2009). Human fear and anxiety behaviors closely mirror those observed in animals such as avoidance, escape, vocalizations, and vigilance and many common neural correlates have been identified between animals and humans in the fear response (Lang, Davis, & Ohman, 2000). Furthermore, various and similar classes of pharmacological agents have been effective in both humans and animals to treat fear and anxiety related disorders (Blanchard, Griebel, Henrie, &

Blanchard, 1997). Consequently, many have understood fear related disorders such as phobias, anxieties, depression and panic attacks as activations of defensive or protective behaviors at inappropriate times and places of safety. Contemporary understandings of fear have centered around how these erroneous assessments of danger and belief in imminent threat, despite considerable threat distance, has been thought to arise from maladaptive learned fear responses (Lang et al., 2000). Consequently, much research has gone into understanding the neural changes associated with fear learning and memory and various attempts have been made to reverse learning related changes, but these produced ambivalent results (Han et al., 2009; Kindt, Soeter, & Vervliet, 2009; Nader, Schafe, & Le Doux, 2000). It is generally agreed, however, that translating an erasure of memory method in animals into an effective therapeutic strategy in humans remains elusive and may not be possible. Therefore, therapeutic strategies have centered around relearning or re-exposing learned fear stimuli under safe conditions to extinguish fear responses (Bouton, 1988; Stephen Maren, 2011; Norberg, Krystal, & Tolin, 2008; Quirk et al., 2010) However, the results of extinction learning has also been ambivalent with some strategies showing promise, while others have been difficult to implement (Quirk & Mueller, 2008). Part of the challenge has been identifying the neural structures underlying extinction learning that adequately inhibit fear memories. Despite the challenges of translating fear models in a clinical setting, much research continues to understand the fear circuit in a laboratory setting.

The fear circuit

Defensive behavior has always been thought to be species and context specific. Frequently in rats and other rodents, an immobile posture (i.e. freezing) will be undertaken in the presence of a variety of predators in an enclosed context. However, in the same rats, if given a chance to avoid the predator, the rat or rodent will often choose to do so. A number of brain

regions have been identified as important in executing these fear related behaviors of freezing and avoidance. Aversive information is generally thought to converge in the lateral or basolateral amygdala (BLA), which in turn sends excitatory projections to the central nucleus of the amygdala (CeA). The CeA, the major output area of both conditioned and unconditioned fear, is then thought to trigger autonomic responses in the hypothalamus, hormonal responses in the bed nucleus of the stria terminalis, (BNST) and defensive behaviors via the periaqueductal gray (PAG) (de Oca & Fanselow, 2004; Kim et al., 2013). While a number of lesion, stimulation, and recording studies have implicated this traditional fear circuit using fear conditioning, recent evidence has shown a more complex picture of the fear circuit. For example, the CeA, known traditionally as an output structure for the expression of fear, has been found to undergo fear related plasticity and be essential for the learning of fear (Wilensky, Schafe, Kristensen, & LeDoux, 2006). The dorsal PAG, known traditionally as an output of the CeA, was recently found to send unconditioned fear input signals *to* the BLA (Kim et al., 2013). Finally, the BNST was found to be able to support contextual fear memories with amygdala damage (Poulos, Ponnusamy, Dong, & Fanselow, 2010). Taken together, this suggests not only that the flexibility of the fear circuit is due to the multiple pathways for innate and conditioned fear, but the ability of the fear circuit to integrate and participate in various ways depends on the environmental demands.

The amygdala, fear, and decision making

Conventionally, the function of the amygdala has long been investigated as involving emotion, particularly fear related processes. A substantial body of evidence both from animal and human studies have shown that the amygdala is involved in the acquisition, expression, and recall of conditioned fear and anxiety like behaviors (Kim & Jung, 2006; Knight, Smith, Cheng,

Stein, & Helmstetter, 2004; LeDoux, 2003). However, recent evidence has shown that the amygdala also participates in decision making, especially those involving ambiguity and risk (Bechara & Damasio, 2005; Gupta, Koscik, Bechara, & Tranel, 2011). Amygdalar neurons have been found to respond to a number of different decision variables, including basic reward and aversive stimuli and updating evaluations of changing values of conditioned stimuli (Morrison & Salzman, 2010). Subsequently, theories of the amygdala's role in decision making have been suggested to be similar to a value encoder, generated by autonomic responses to emotional stimuli that create "somatic states" that guide downstream decision making (Gupta, Koscik, Bechara, & Tranel, 2012; Morrison & Salzman, 2010). However, recent evidence has also suggested that amygdala neurons carry information about choice irrespective of value (Grabenhorst, Hernádi, & Schultz, 2012). Taken together, the responses of amygdala neurons in various choice tasks show a wide role of the amygdala since it participates not only in emotional processes but voluntary decisions, especially those involving risk and reward. Although the amygdala has been implicated in a number of different emotional and cognitive tasks, traditionally, the amygdala has been investigated in the learning and memory of fear. Two primary paradigms have been used to understand the role of the amygdala in the learning and memory of fear: instrumental and Pavlovian fear conditioning.

Instrumental fear conditioning paradigm

In instrumental fear conditioning, the aversive US experience is contingent upon a specific response emitted by the animal (e.g., moving from bright to dark compartment). As a function of this response-stimulus association formation, the animal learns to perform (active avoidance) or inhibit (passive avoidance) the response that would avert reoccurrence of the previous aversive experience. Much research has gone into the role of the amygdala in inhibitory

avoidance tasks. It is generally agreed that lesions to the lateral amygdala lead to deficits in the learning of inhibitory avoidance tasks, but not the memory of those tasks after learning has been established (Parent, Tomaz, & McGaugh, 1992). Specifically, post-training lesions have not attenuated the memory of the avoidance response and post-training drug manipulations of a variety of receptors in the amygdala either enhance or impair the memory on inhibitory avoidance tasks (McGaugh, Cahill, & Roozendaal, 1996). These results have highlighted the importance of the amygdala in modulating memory formation occurring elsewhere in the brain (McGaugh, 2004). Despite abundant research into the amygdala's role in consolidation, others have argued that the primary role of the amygdala is to associate fearful stimuli with their environmental cues and store those associations *within* the amygdala (LeDoux, 2003). These conclusions have been drawn from research using the Pavlovian fear conditioning paradigm to investigate fear learning and memory.

Pavlovian fear conditioning paradigm

Pavlovian fear conditioning involves pairing a neutral conditioned stimulus (CS) such as a tone or a light, with an aversive unconditioned stimulus (US) such as an electric shock that produces an unconditioned fear response (UR). Through repeated CS-US pairings, the CS comes to elicit a conditioned fear response (CR) that is frequently similar to the UR. Pre-training and post training lesions to the lateral amygdala has significantly attenuated conditioned fear responses using auditory fear conditioning (Ledoux, Romanski, & Xagoraris, 1990). Additionally, plasticity related changes in the amygdala have been shown after fear conditioning that has been thought to underlie fear learning and memory (Chapman, Kairiss, Keenan, & Brown, 1990; Clugnet & LeDoux, 1990; Stephen Maren, 2005) From these results, the Pavlovian

fear conditioning paradigm has pointed to the amygdala as necessary not only for the learning of fear, but the storage of fear memories.

Closed economy paradigm

While differences in behavioral procedures may led to contrasting views on the amygdala's role in learned fear, both classical and instrumental fear conditioning paradigms involve the removing animals from their home cages and transporting and placing them in experimental chambers to assess particular fear responses (e.g., freezing, avoidance) for short durations (ranging from seconds to minutes). Hence, neither paradigm has addressed alterations to continuous behavior associated with fear and amygdalar manipulations. A promising behavioral approach to address this gap is to revert back to predator prey paradigms in a 'closed economy (Hursh, 1980) in which animals dwell in individual chambers over uninterrupted time and forage for resources while navigating in a dangerous environment (i.e., the risk of experiencing footshocks) (Fanselow et al., 1988)(Figure 2.1). Pavlovian and instrumental conditioning paradigms have shown initial "snapshots" of the amygdala's role in species specific defensive reactions like freezing and avoidance to imminent threats (McNaughton & Corr, 2004), but have yet to show the amygdala's role in antipredator defensive strategies to ongoing threats. There are many common examples how animals utilize behavioral strategies to avoid or reduce potential danger (Lima, Steven & Dill, Lawrence, 1990). However, how fear related structures like the amygdala guide decisions on various defensive behavioral strategies remains to be investigated.

The approach avoidance conflict in the closed economy

The animals in the closed economy are living in a situation where there is a safe nesting region next to an adjacent risky foraging area where all nutritional resources must be procured by pressing a lever. These animals can be thought of as in an approach avoidance conflict. Animals are drawn towards the lever for the need for food, but they fear the region when the area becomes randomly electrified. Consequently, the animal must balance its own positive metabolic goals with risky potentially negative outcomes of getting shocked. The central issue of the 3 studies in this dissertation discuss whether the amygdala is involved in the outcome of these approach avoidance decisions involving feeding and foraging. In the first study, two experiments show the role of the amygdala to modulate decisions on feeding and foraging behavior but not to avoidance during unpredictable shock. These results give evidence for the presence of an amygdala based fear or "emotional" fear, which traditionally determines reflex and emotional responses, to also guide decisions on feeding. In addition, non-amygdala based fear or "extra-amygdalar" fear might mediate avoidance responses through cortico-striate circuitry. The second study investigates this dual fear system under predictable shock, to see how the role of the amygdala changes when a signaled threat can be learned. The results of this study give evidence for possible compensatory circuits that can mediate learning to an ongoing threat.

The final study presented in this dissertation involves a more complex competing motivation. Unsignaled and signaled footshocks were introduced only during the dark phase of the rats light dark circadian cycle, where rats normally have the greatest feeding and activity. The introduction of unsignaled but not signaled footshocks reversed nocturnal feeding to diurnal feeding and showed arrhythmic locomotor activity. Additionally, rats anticipated the termination of the shock period by increasing feeding behavior and activity before the onset of the light phase. Lesions to the amygdala and superchiasmatic nucleus (SCN) prevented the fear induced

changes to feeding, activity, and anticipatory behavior. These results suggest that amygdala-dependent fear, together with the SCN can act as a fear entrained oscillator to reverse circadian behavior and anticipate times of danger and safety. This final study raises the intriguing possibility that there are clock genes in the amygdala that can dissociate feeding schedules from photic zeitgebers to time lock feeding behaviors to inactive phases to avoid environmental threats.

Chapter II. The Amygdala Guides Fear Associated Decisions to Feeding and Foraging Patterns.

Introduction

In natural environments, animals seeking resources (such as food and water) face potential perils associated with foraging behavior, namely predation. Thus, animals must balance their needs with risks by utilizing adaptive behavioral strategies. One such strategy is hypothesized to be provided by the fear system by adapting foraging behaviors in the presence of environmental threats.

Contemporary amygdalar models of fear are largely based on classical conditioning (conditioned fear response) and instrumental conditioning (avoidance response) experiments where the subject's fear behaviors are assessed for relatively brief periods of time (e.g., seconds-to-minutes). Whereas the conditioned fear studies postulate that the amygdala is the locus of fear memory, the avoidance learning studies suggest that the amygdala modulates the strength of fear memory formation occurring elsewhere in the brain. It remains unknown, whether amygdala coded fear can guide decisions on feeding and foraging behavior when living uninterrupted in adverse settings.

The present study sought to address these issues by investigating the role of the amygdala in a 'closed economy' paradigm, where rats resided in individual chambers consisting of a safe nest and a risky foraging area. Specifically, while pressing a lever to acquire food pellets, the animals were vulnerable to receiving either unsignaled or signaled footshocks delivered randomly throughout the day. Results indicate that rats significantly alter decisions regarding feeding behavior to unsignaled shocks and avoided the foraging area. Rats with amygdalar lesions, however, did not change their foraging decisions to feed, but were able to avoid the

foraging area. These results suggest that while the amygdala is not necessary for lasting changes in avoidance behavior, it is necessary to guide decisions on defensive feeding behavior under risky conditions.

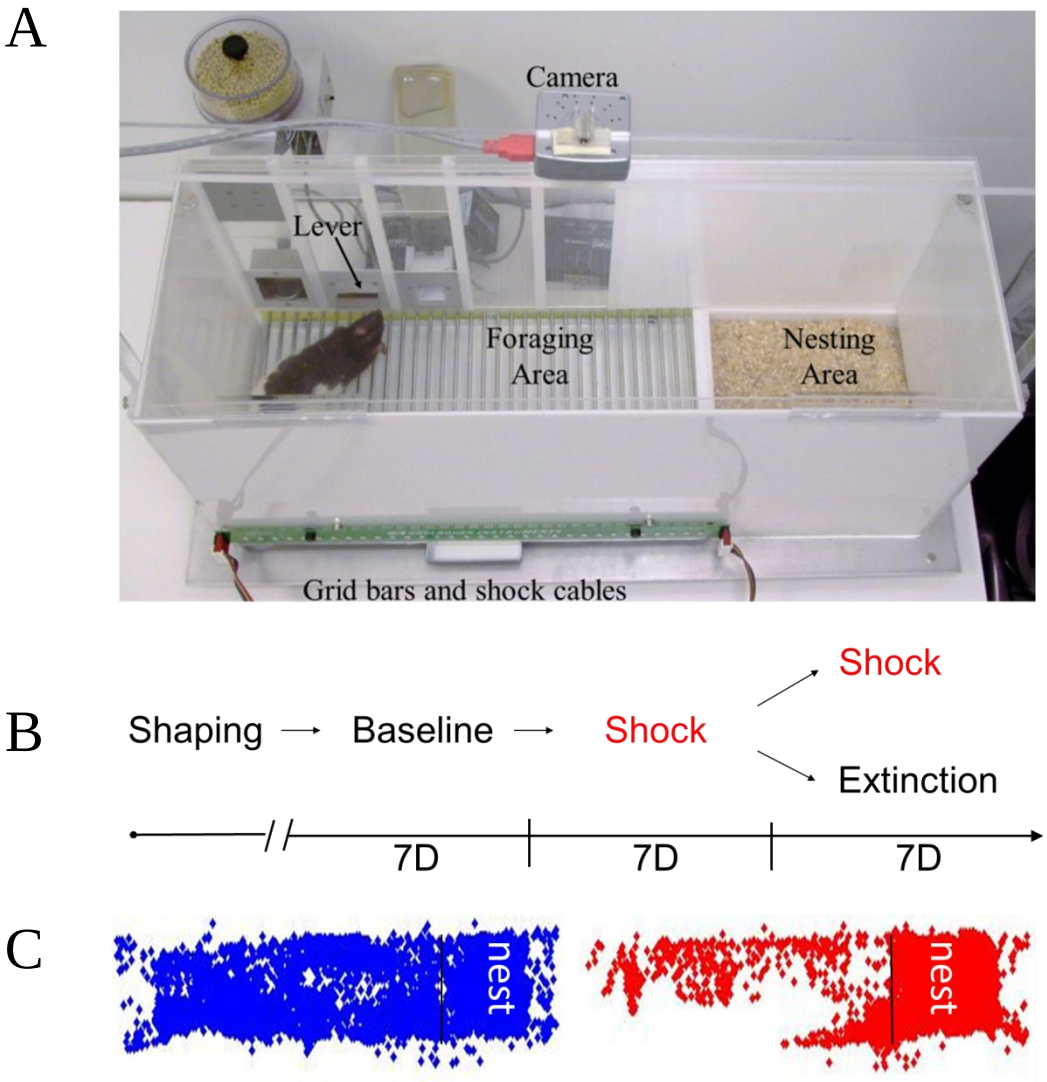


Figure 2.1. **(A)** The closed economy apparatus. The live-in apparatus of a safe nest and a risky foraging area where the animal had to press a lever to obtain food pellets and have access to water. A camera mounted above the chamber tracked the animal's movement continuously. **(B)** Diagram of experimental procedure through 7 days of each phase. During shaping, baseline, and extinction phases, the shock cables were disconnected. **(C)** Representative visit plots from an Intact animal during baseline day 7 (*left*) and shock day 7 (*right*)

Methods

Subjects. Male Charles River Long Evan rats (initially weighing 275-300 g) were individually housed in one of eight 'closed economy' chambers (Fig. 2.1) located in a climate-controlled vivarium (accredited by the Association for Assessment and Accreditation of Laboratory Animal Care) that was on a reverse 12-hr light/dark cycle (lights on at 19:00 hrs.). All experiments were performed in strict compliance with the University of Washington Institutional Animal Care and Use Committee guidelines.

Surgery. Under anesthesia (30 mg/kg ketamine and 2.5 mg/kg xylazine, i.p.), rats received either bilateral electrolytic lesions (Pre-shock lesion group) or bilateral implantations of lesion electrodes (Post-shock lesion group) to their amygdala (from bregma: AP -2.5; ML $\pm$4.2/5.0; DV -8.4/8.6 mm). Lesions were made by passing constant current (1 mA, 10 sec; Grass Medical Instrument, Quincy, MA) through epoxy-coated inset pins (#00, ~0.75 mm tip exposed). The Intact group was unoperated controls in Experiment 1 but was sham controls in Experiment II,. Sham controls were given the same surgical procedure as Pre-shock lesions but current was not delivered.

Apparatus. Closed economy chambers were custom-built from Plexiglas with the following dimensions: 74.3 cm x 25.4 cm x 33 cm (length x width x height). Each chamber consisted of a 'foraging' arena (54 cm x 25.4 cm) and a 'nest' (20.3 cm x 25.4 cm). The floor of the nesting area was filled with sawdust, while the floor of the foraging area was composed of 32 stainless-steel rods (4.5 mm diameter) wired to a precision animal shocker (Coulbourn Instruments, Allentown, PA). As can be seen in Figure 1, a pellet receptacle-dispenser, a lever and a water bottle (Med Associates, Fairfax, VT) were accessible 47, 39 and 30 cm, respectively, from the nest. In the two lever experiment, another lever and pellet receptacle-dispenser was affixed 13

and 22 cm, respectively, from the nest. The ANY-maze video tracking system (Stoelting Co., Woodale, IL) was used to track the animal's movement, via a Fire-I B/W Board Camera (Unibrain Inc., San Ramon, CA) placed above each closed economy apparatus, and to control all input/output devices connected to an AMi interface (Stoelting Co.).

Experimental procedure. The Pre-shock lesion and Control groups went through successive phases of baseline, shock and extinction, whereas the Post-shock lesion group went through baseline, shock and post-shock lesion phases. All phases were 7 days and the animals' behaviors were continuously recorded except for a 1 hour break (every 1-2 days) during which the chamber and bedding pan (underneath the shock floor) were cleaned and the food and water were refilled.

Experiment I: Unpredictable shocks and foraging behavior. During the baseline phase, rats were shaped to press the lever to attain pellets (45 mg dustless precision pellet; Bio-Serv, Frenchtown, NJ) at a fixed ratio 50-continuous reinforcement (FR50-CRF) schedule by gradually increasing the lever pressing schedule (i.e., FR1-CRF, FR5-CRF, FR10-CRF, FR20-CRF, FR30-CRF, FR40-CRF, FR50-CRF). During each FR-CRF schedule, if the animal did not make sequential level pressings within one minute, then the FR-CRF requirement was reset. After 7 days of stable baseline meal patterns were recorded at the FR50-CRF schedule, the animals were switched to the shock phase where 2 unsignaled footshocks were presented randomly every hour regardless of the animal's location (nest or foraging area). If the animal was in the nest, the shock immediately turned off; if the animal was in the foraging area, the shock stayed on until the rat escaped to the nest (or a maximum of 10 sec). Following 7 days of shock, the Post-shock lesion rats were given electrolytic lesions under light halothane anesthesia (Brunzell & Kim, 2001; Kim, Clark, & Thompson, 1995) during the cleaning break and underwent 7 additional

days of shock. In contrast, Pre-lesion and control rats underwent 7 days of extinction where footshocks were no longer presented.

Experiment II: Unpredictable shocks and two lever preference. Pre-shock lesion and Intacts rats underwent 10 daily sessions of baseline, shock and extinction, except two levers (one closer to the nest than the other) were available for procuring food pellets, both on a CRF schedule. All animals displayed a stable bias to one of the levels during the baseline days; hence their lever selections were normalized by dividing the preferred lever presses with the total lever presses for each day

Histology. At the completion of testing, animals were overdosed with Buthanesia and perfused intracardially with 0.9% saline followed by 10% buffered formalin. The brains were removed and stored in 10% formalin overnight and then kept in 30% sucrose solution until they sank. Transverse 50 μm sections were taken through the extent of the lesion, mounted on gelatin-coated slides, and stained with cresyl violet and Prussian blue dyes.

Statistical Analyses. The daily meal frequency, pellet consumption, number of shocks received, and time spent in the foraging area were normalized to the mean baseline values of each animal. The normalized values were analyzed by paired or independent *t* tests, and one-way repeated measures ANOVA where appropriate.

Results

(a) Experiment I. Amygdaloid fear is necessary for meal and water suppression, but not avoidance of dangerous places.

To investigate whether the amygdala is involved in fear associated changes to feeding and foraging behavior, rats with lesions to the amygdala (Pre-shock lesions (n=9), rats with implanted lesion electrodes (Post-shock lesions (n=8)), and unoperated Intacts (n=9) were placed

in a closed economy (Figure 2.1). All animals were shaped to a chained fixed ratio 50-continuous reinforcement (FR50-CRF) schedule and were allowed to control the meal frequency, total pellets, and total water intake each day (see methods). Once stable baseline was reached, foraging behavior was recorded daily in a closed economy for 7 days. After the 7th day, on average, 2 footshocks were introduced randomly each hour in the foraging area. Animals could avoid the shock by remaining in the nesting area. After the 7th day of shock, Pre-shock lesions and Intacts were run for 7 days on extinction conditions, while the Post-shock lesions were given electrolytic lesions, and put back in the closed economy for another seven days of shock.

Intacts. During baseline, controls spent on average 7.9 hrs. (*SD* = 2.97 hrs.) of their time in the foraging area per day, about 16% (1.3 hrs.) of which was spent lever-pressing for food. Intacts consumed on average 6.42 (*SD* = 2.70) meals per day with an average meal size of 116 pellets (*SD* = 17.0).

Upon the introduction of shock, Intacts fairly rapidly reduced the amount of time they spent in the foraging zone (Figure 2.2). On the last day of the shock phase, controls averaged about 32% (2.06 hrs.) of their baseline values of time in the foraging area ($t_8 = 13.72$, $p < .001$). By reducing the time in the foraging zone, Intacts effectively decreased the amount of shocks received (Theoretical shocks baseline day 7 vs. actual shocks phase 2 day 7: $t_8 = 6.58$, $p < .001$) (Figure 2.3A). In addition to reducing the time in the foraging zone, Intacts responded to aversive stimuli by changing meal patterns as previously reported (Fanselow et al., 1988). By the 7th day of unsignaled shock, meal frequency reduced to 45% of baseline values ($t_8 = -4.31$, $p = .003$) with no significant change to average meal size (Last 3 days of shock: $t_8 = -0.96$, $p = .342$) due to decreased total pellet consumption (Last 3 days of shock: $t_8 = -4.12$, $p < .001$) (Figure 2.3C). Intacts also decreased water consumption during the shock phase ($t_7 = -2.62$, $p = .03$)

(Figure 2.3B). Despite decreased water and food intake these changes did not comprise body weight due to sufficient intake throughout the shock phase ($t_8 = 1.45$, $p = .19$) (Figure 2.3D).

During the extinction phase, Intacts *gradually* increased the amount of time they spent in the foraging area toward pre shock values (Day 7 of shock vs. Day 7 of extinction: $t_8 = -3.55$, $p = .007$). The depression of meal frequency, however, recovered mostly within the first day of extinction. (paired t-test of % recovery of Time in Foraging Zone vs. Meal Frequency on day 2 of Phase 3: $t_8 = 5.69$, $p < .001$) (Figure 2.2). This suggests that fear only suppresses behavior in the absence of a competing motivation such as hunger, a point to which will be returned below.

Pre-shock lesions. Prior to the introduction of shock, animals were given amygdala lesions and placed in the closed economy. Pre-shock lesions did not differ from other groups in time in foraging area, meal frequency, total pellet intake, total water intake, or body weight during the baseline phase. Upon the introduction of shock, Pre-shock lesions decreased the amount of time in the foraging area (% Time in Foraging Zone last day of Phase 2 as a % of baseline period < 100%, $t_8 = 8.70$, $p < .001$) but to a lesser extent than controls (% Time in Foraging Zone for Intact vs. Pre-shock animals, last day Phase 2: $t_{16} = 4.15$, $p = .001$). However, Pre-shock lesions did not suppress their meal frequency (Day 7 shock: $t_8 = -.499$, $p = .631$), total pellets, (Day 7 shock: $t_8 = 1.680$, $p = .132$), or total water intake (Day 7 shock: $t_4 = .296$, $p = .782$).

During the extinction phase Pre-shock lesions *immediately* increased the time in the foraging area toward pre shock values, presumably due to the absence of amygdala coded fear (Day 7 shock vs Day 1 of extinction: $t_8 = 2.59$, $p = .03$). However, Intacts gradually increased the time in the foraging area (Day 1 Extinction Intacts vs. Pre-shock lesions: $t_{16} = 4.59$, $p < .001$).

Post-shock lesions. A functional role of the amygdala to suppress food and water intake but not foraging avoidance during unpredictable aversive stimuli can be concluded by the above results. However, some have reported that rats with amygdala lesions after learning are able to avoid fearful contexts due to consolidation like mechanisms (McGaugh, 2004). In order to test this theory of how consolidation effects foraging zone avoidance and meal patterns, a group of animals were run that were not lesioned until *after* receiving 7 days of shock. These animals were then run for an additional 7 days of shock in the closed economy.

By the 7th day of shock, Post-shock lesions, like Intacts, reliably reduced the time spent in the foraging area ($t_7 = 19.84$, $p =<.001$) (Figure 2.2A) that became asymptotic toward the end of the 1st shock phase (one-way repeated measures ANOVA 4th-7th day: $F_{(3,21)} = 2.35$, $p = .10$). The meal frequency, total pellets, and total water intake also showed reliable depression compared to baseline similar to controls by the 7th day (Meal Frequency: $t_7 = 6.88$, $p < .001$, Total pellets: $t_7 = -2.628$, $p = .03$, Total Water: $t_7 = -3.74$, $p = .007$) (Figure 2.3). Upon lesioning the amygdala, there was a small non-significant increase in time spent in the foraging zone (Normalized % Time in Foraging Zone last day of Phase 2 vs. first day of Phase 3: $t_{13} = 2.06$, $p = .06$). However, despite remaining on shock, the meal frequency, total pellets, and total water intake increased toward pre-shock values. (Normalized % last day of Phase 2 vs. first day of Phase 3: Meal Frequency: $t_7 = 4.93$, $p = .002$, Total pellets: $t_7 = 2.71$, $p = .03$, Total Water: $t_7 = 3.81$, $p = .007$) (Figure 2.3). These results suggest that amygdala fear is not necessary to mediate avoidance of a shocked area after sufficient learning has taken place, but amygdala fear *is necessary* for shock mediated depression to meal and water getting behavior.

Time Budgets. All rats during unsignaled shock decreased the amount of time spent in the foraging zone. However, Pre-shock lesions during shock Phase II and Post-shock lesions during

Phase III consumed normal amounts of food despite a significant decrease in time spent in the foraging area. It would therefore, be interesting to note if lever pressing behavior during shock was different for amygdala intact and damaged animals. The time budgets graph in Figure 2.4 shows that amygdala lesioned animals *increased* the rate of lever pressing during shock (Pre-shock and Post-shock Lesion groups combined $t_{16} = 2.90$, $p < .01$). In contrast, the amygdala intact animals *decreased* the rate of lever pressing during shock compared to baseline ($t_{16} = 3.95$, $p = .001$). This suggests that the behavior of lesioned animals when motivated for food were pressing unencumbered by fear, while amygdala intact animals, presumably afraid of receiving shocks even while procuring food, pressed slowly and therefore earned less amounts of food.

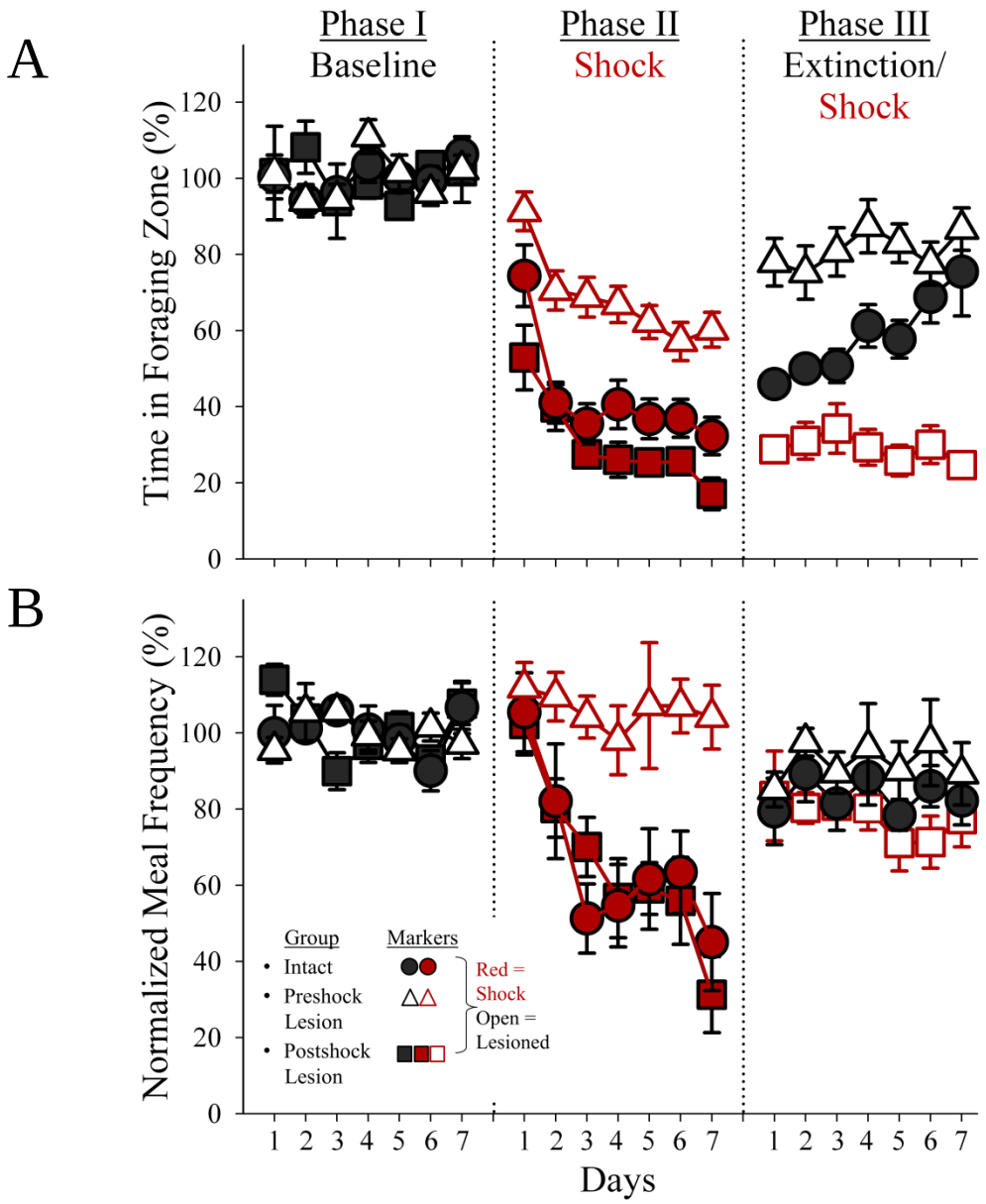


Figure 2.2. **(A)** Percent time spent in the foraging zone. Each animal's daily score was normalized to the mean across the baseline days (Phase 1). ● = Intact group: rats that went through baseline, shock, and extinction phases. ■ = Post-shock lesion group: rats that went through baseline and shock phases, then taken out momentarily and given amygdalar lesions, and then give another 7 days of shock. △= Pre-shock lesion group: rats that receive amygdalar lesions, then go through baseline, shock, and extinction phases. Open markers indicate amygdala lesions; red markers and lettering indicate random shocks. **(B)** Daily meal frequency. The number of meals earned each day was normalized to the mean across the baseline sessions.

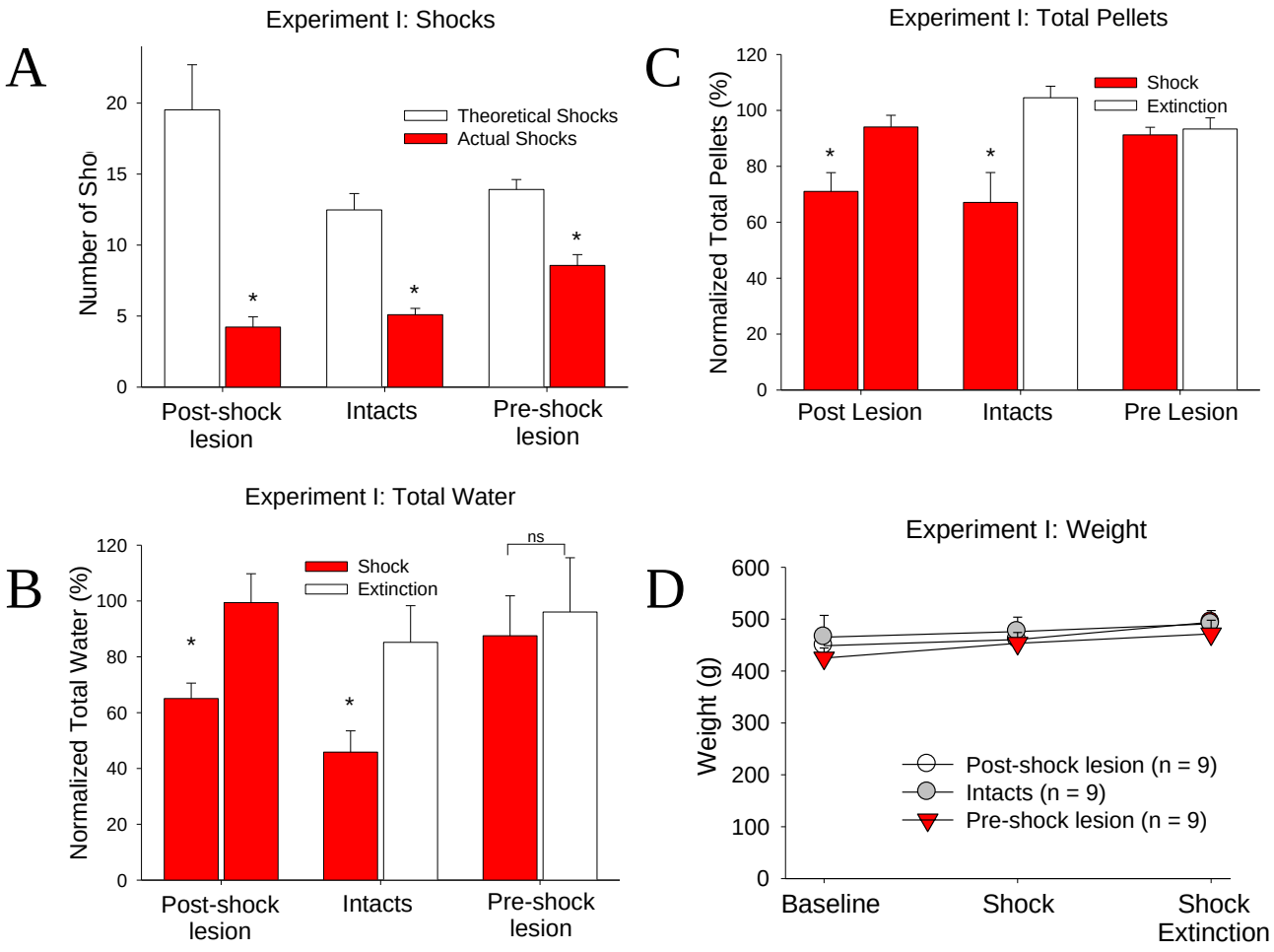


Figure 2.3. **(A)** Number of theoretical (shock cables disconnected during baseline) and actual shocks received. All groups decreased the number of shocks received. **(B)** Normalized total water licks for Experiment I. Post-shock lesion and Intacts decreased water licks during shock. However, upon lesioning the amygdala the Post-shock lesion group recovered to baseline values. The Pre-shock lesion group did not change total water licks from baseline to shock. **(C)** Normalized total pellets for all groups displayed a similar pattern as normalized water licks. **(D)** Despite differences in total pellet intake, all groups increased their body weight through the 3 phases.

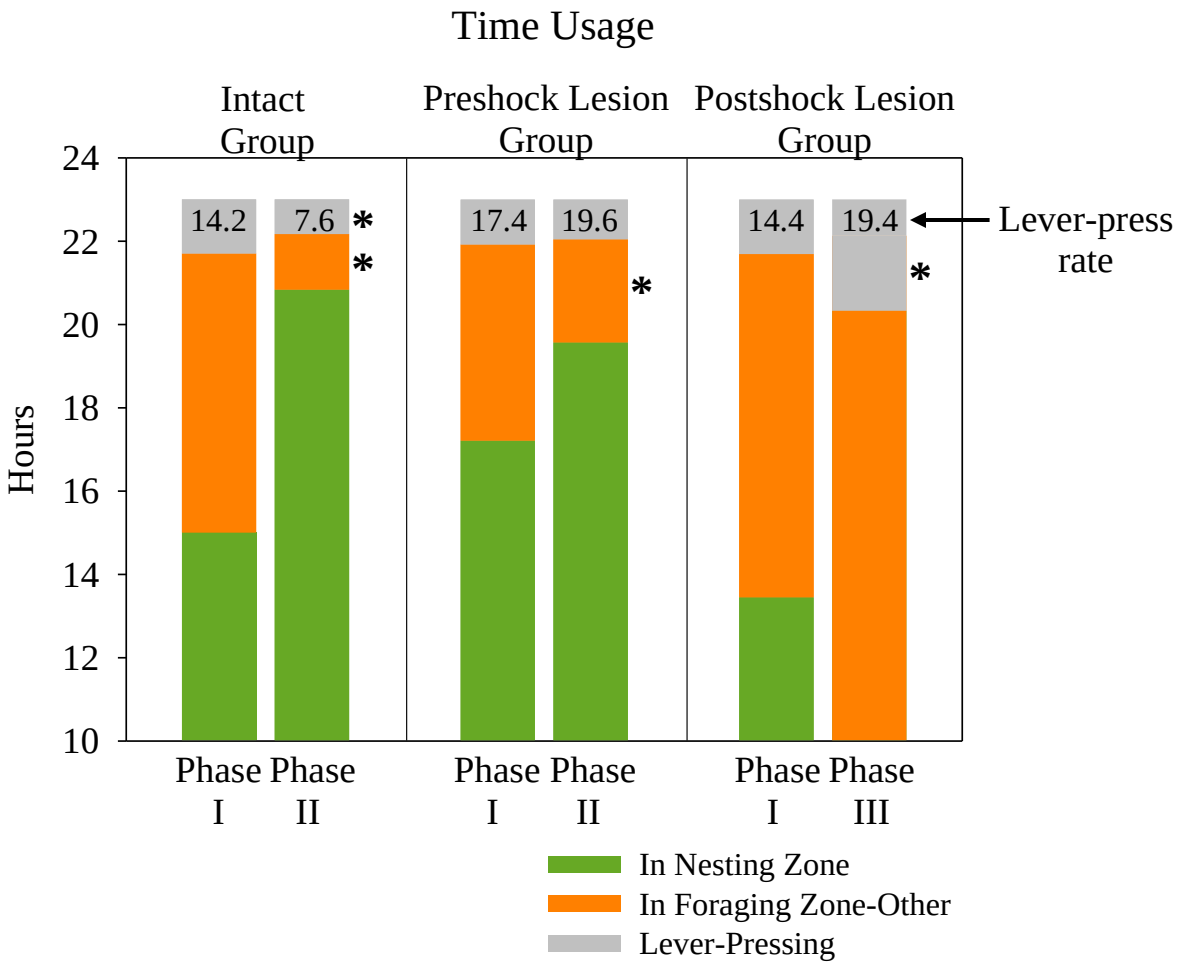


Figure 2.4. Time distribution of Intact and Lesion groups from Experiment 1. Total time per day sums to only 23 h because there were no measurements during enclosure cleaning. Each bar is divided into nesting zone time, non-pressing foraging zone time, and lever-pressing time, taking scores from Phase I (Baseline) Phase II (shock day 7), and Phase III (day 1, Post-shock Lesion group). Time in the foraging zone significantly decreased from baseline to shock phases for all groups (Intact group, $t_8 = 6.71$, $p < .001$; Pre-shock Lesion group, $t_8 = 6.64$, $p < .001$; Post-shock Lesion group, $t_7 = 6.28$, $p < .001$). The 'lever pressing rate' (numbers inside bars) was calculated as the total lever presses/total lever pressing time). The Intact group animals significantly decreased the rate of lever pressing during shock compared to baseline ($t_{16} = 3.95$, $p = .001$). In contrast, the two lesioned groups, which had slightly decreased their *time* lever-pressing, increased their *rate* of pressing (for Pre-shock and Post-shock Lesion groups combined $t_{16} = 2.90$, $p < .01$; for the Post-shock Lesion group alone $t_8 = 3.25$, $p < .01$; for Pre-shock Lesion group, mean rate increased slightly (see figure), but not significantly ($t_8 = 1.22$, $p = .25$)).

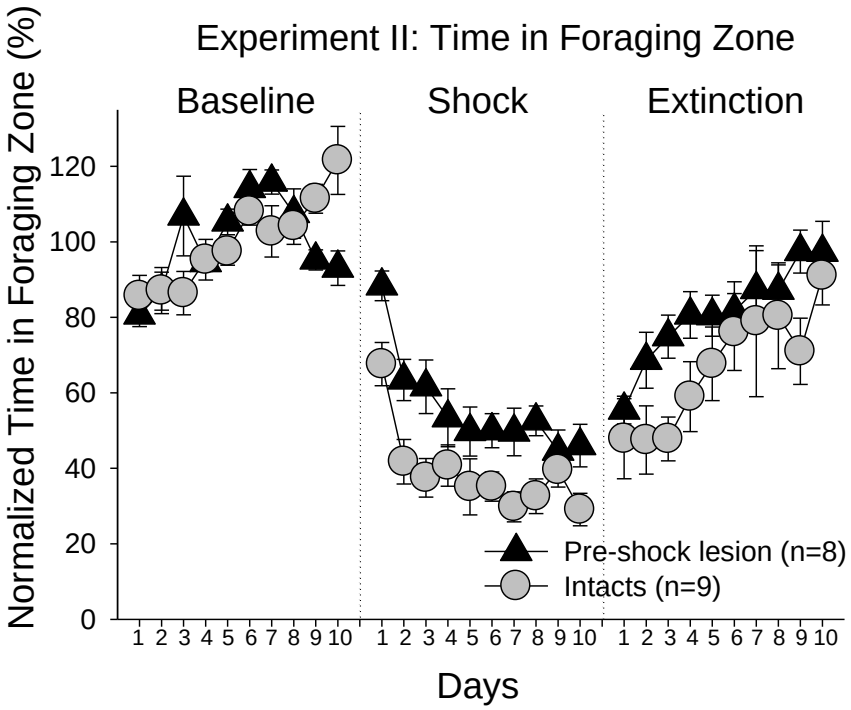
(b) **Experiment II. Amygdaloid fear is necessary for decisions of food getting behavior.**

In order to distinguish whether the above results were due to fear motivated suppression of hunger or if amygdala fear is involved in decisions involving strategic food getting behavior, the following 2-lever experiment was run. Animals with lesions to the amygdala (Pre-shock lesions, n = 8) and animals with sham lesions (Intacts, n = 9) were run identical to the Exp. I, but an additional nearer lever was added along with a change to a continuous reinforcement schedule of food (See Methods).

Upon the introduction of shock, Intacts and Pre-shock lesions reduced the time in the foraging zone (% decrease last day of shock: Intacts: $t_8 = -16.47$, $p < .001$, Pre-lesions: $t_8 = -9.59$, $p < .001$) (Figure 2.5A). Similar to Exp. 1, Intacts had a stronger reduction to time in the foraging zone then lesions (Last day Intacts vs Pre-shock lesions: $t_{16} = -2.39$, $p = .03$). Prior to shock, both groups had strong lever preferences for either the near or far (relative to nest) lever, and about an equal numbers of animals preferred each lever (Figure 2.6). Upon the introduction of shock, Intacts initially vacillated from one lever to another and switched their initial preferences ($\chi^2_1 = 14.4$, $p < .001$) (Figure 2.6). The choice behaviors of Pre-shock lesions were very different. Pre-shock lesions *continued* to prefer the same lever that they had during the baseline phase prior to the introduction of shock (Figure 2.6). Furthermore, Intacts that had initially favored the distal lever farthest away from the nesting area began to favor the near lever toward the end of the shock phase. The mean increase in percentage of near lever presses for the 5 Intacts that initially preferred the far lever was 77.6% (*SD*: 25.8). However, the mean increase in percentage of near lever presses for the 5 Pre-shock lesions was 8.3% (*SD*: 10.8) (Intacts: near vs. far lever, $t_8 = 3.39$, $p = .01$, Pre-lesions: near vs. far lever, $t_8 = 0.25$, $p = .81$, Intacts vs. Pre-shock lesions initially preferring far lever, $t_8 = 5.49$, $p = .001$) . These results indicate that

animals with intact amygdalae developed a preference for the lever closest to the safe nesting area, while the lesioned animals continued to choose their initial baseline lever preference.

A



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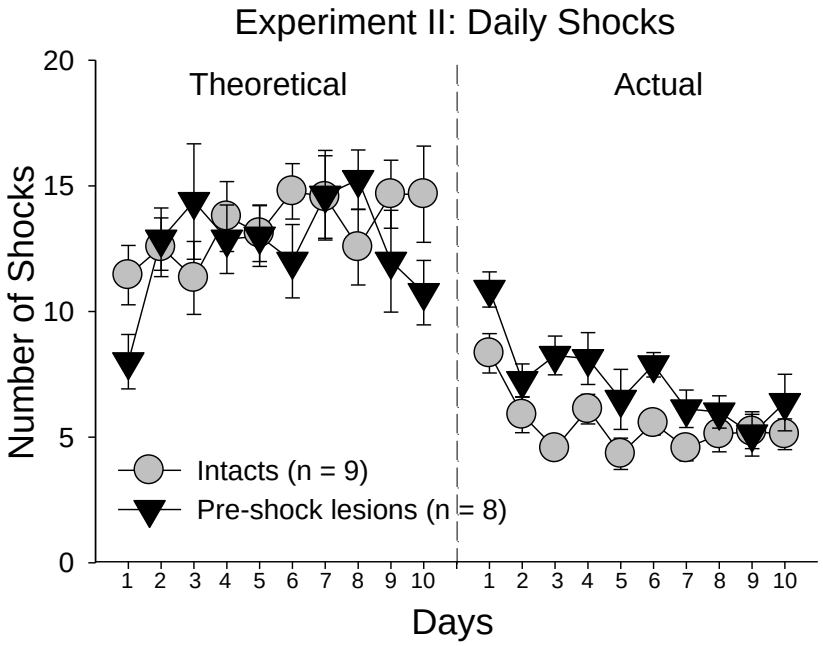

Figure 2.5. **(A)** Time in foraging zone for Pre-shock lesions and Intact rats. Both groups decreased time in the foraging zone, but Intact rats showed a stronger depression Pre-shock lesion rats. **(B)** Theoretical and actual shocks received during baseline and shock phases. Both groups decreased the number of shocks received as days progressed, but Intact rats showed a stronger

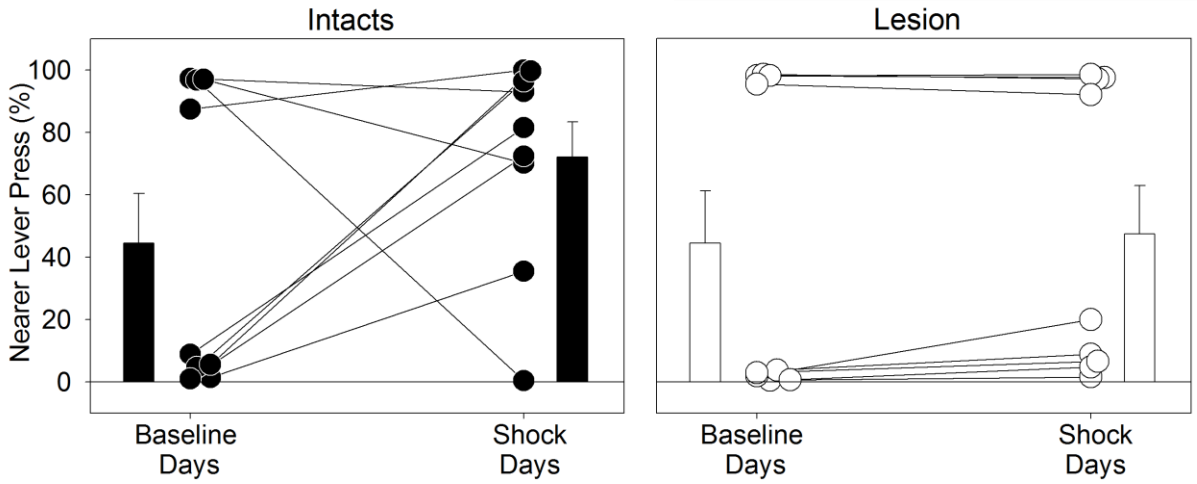

Figure 2.6. Initially, animals had strong lever preferences that were approximately random. On baseline days animals almost always pressed their preferred lever. When random shock was introduced, intact animals modified their behavior in the direction of pressing the lever nearer the safe region. However, lever choice was unaffected by shock in the amygdala-lesioned animals.

Table 2.1. Time spent in foraging zone and pellets earned in Experiment II.

	Time in Foraging Zone				Feeding				(Foraging time-feeding)	
	% Differences		t(df),	p	% Differences		t(df),	p	t(df),	p
Intact	66.7	± 3.8	17.8(8)	< .001	21.7	± 6.8	3.2(8)	= .01		
Pre	51.4	± 4.9	10.5(8)	< .001	5.9	± 1.3	0.2(8)	< .82	(49.9)	
$\frac{\text{Pre}}{\text{Intact}} \times 100$	77.1				27.2				5.8(16)	< .001

Values are mean (±SEM) percent decreases of scores for the last 5 days of the shock phase relative to the mean baseline scores for each animal.

Comparison of Experiment I and II: Avoidance vs Meal pattern behavior.

In Experiment I and II, both results show strong support for a selective role of the amygdala in decisions involving meal getting behavior, but not avoidance of dangerous places. To further analyze the degree of how these variables might be modulated by amygdala lesions, meal getting behavior is compared with time in the foraging zone (Figure 2.7, Table 2.1). This figure clearly shows that amygdala removal shows relatively little shock-produced depression in meal getting behavior, but has little effect on time in the foraging zone (tests of normalized % shock-induced depression of Time in Foraging Zone vs. depression of Meal frequency: Exp I: Pre-shock group, $t_{16} = 4.6$, $p < .001$; Post-shock group, $t_{14} = 5.98$, $p < .001$; Exp II: $t_{16} = 5.23$, $p < .001$). To put this comparison into a graded continuum of how fear, as indexed by a normalized percentage of time in the foraging zone, changes the meal getting behavior of amygdala lesions, Figure 2.8 was constructed. All animals from all days and phases from both experiments are included to show how time in the foraging zone correlates with meal getting behavior. While values at the right are mostly from the baseline phase before shock and from the end of the extinction phase, values at the far left come mostly from the end of the shock period. Intermediate values come from animals midway through the shock and beginning of extinction phases. Values on the y axis are from lever pressing normalized to baseline values. First, animals with lesions to the amygdala mostly do not change their lever pressing behavior no matter how afraid they are of the foraging area. There is a small but significant decrease in lever pressing due to shocks (regression coefficient of lesioned animals, $\beta_{lesions} = .503$, $p < .01$). The animals with intact amygdalae however, are very different. Lever pressing is little depressed and identical to lesions when foraging zone avoidance is intermediate to low, but when foraging zone avoidance is very high, the lever pressing of intact animals becomes substantially depressed (regression

coefficients for Intacts vs. Lesions; $\beta_{lesions}/\beta_{intacts} = 2.16$, Intacts vs. Controls last two bins food depression, $t_{123} = 5.32$, $p < .001$). (Figure). This indicates that the amygdala is necessary for suppression of meal getting behavior when fear becomes high, but not for foraging zone avoidance.

In order to test whether repeated administration of shocks desensitized animals, Exp II animals were fear conditioned after closed economy testing in a separate context and tested 24 hours later. Intacts exhibited robust freezing during the context test, while Pre-shock lesions showed severe impairment ($t_{15} = 11.220$, $p < .001$ (Figure 2.9).

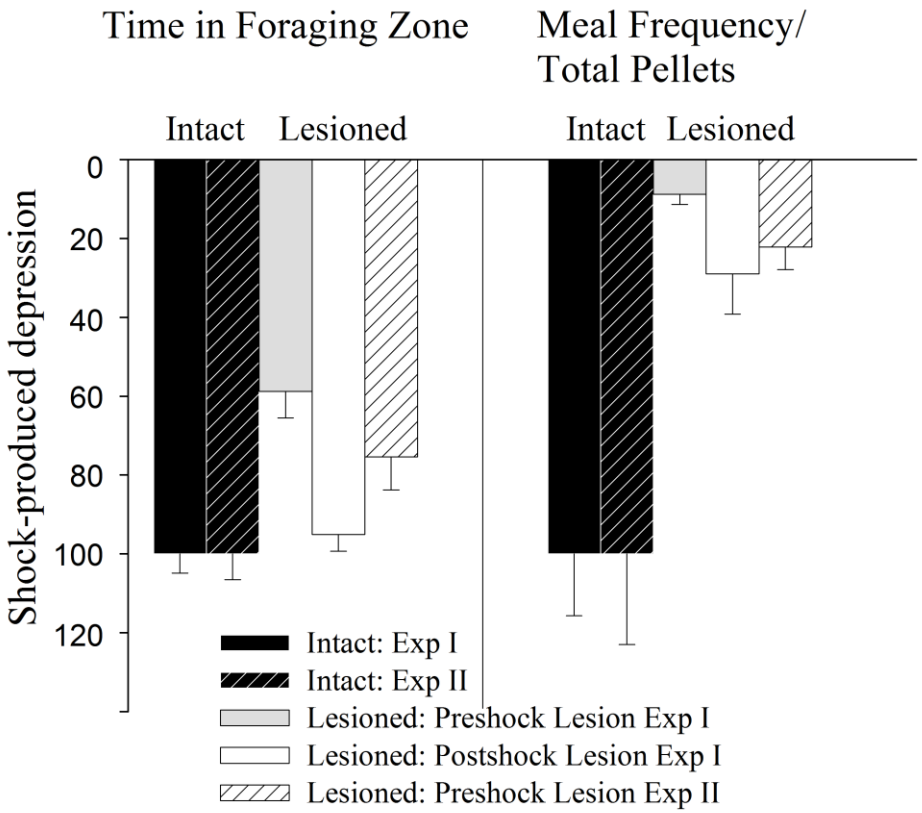

Figure 2.7. Amygdala lesion effects on shock-produced depression of time spent in foraging zone and feeding in Experiments I and II**.** Percent time in foraging zone and meal frequency (Exp I, Fig. 2) or Total Pellets (Exp II, Table 1) scores were normalized so that the intact condition value would be 100%. For Experiment I, Intact control scores are last Phase 2 day scores for both Intact and Post-shock lesion animals (which had not yet been lesioned), and lesion scores are last Phase 2 day scores for the Pre-shock lesion group and first Phase 3 day scores for the Post-shock lesion group (first lesioned day). For Experiment II both Intact scores and Lesioned scores are mean values over the last 8 days of Phase 2. Statistics were done on each group separately (see text) from those of the Pre-shock lesion group. It should be noted that amygdalectomy did not reduce foraging zone avoidance at all in Post-shock lesion animals, in which avoidance was already well learned by the time they were lesioned.

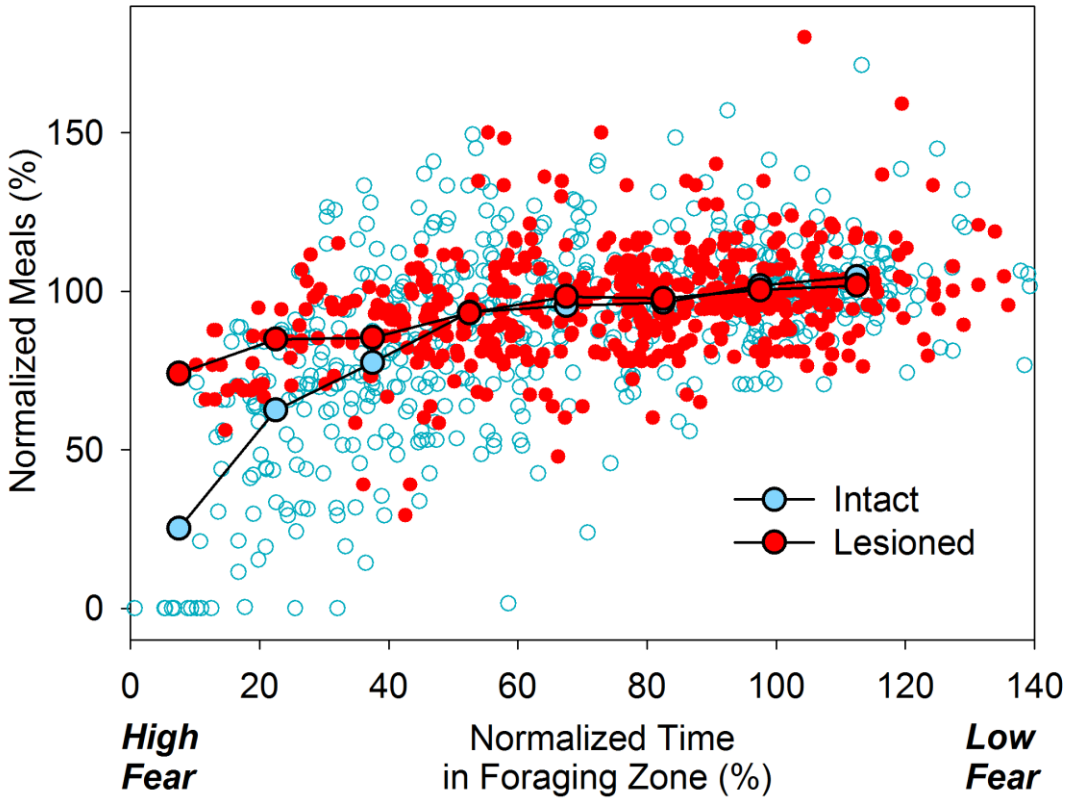

Figure 2.8. Relationship between percent time in foraging zone and meals/pellets earned**.** Daily scores for each animal in Experiments I and II are represented in a scatter plot. Intact and Lesioned animals are represented in blue and red, respectively. Large markers show averages for 15% wide bins on x-axis.

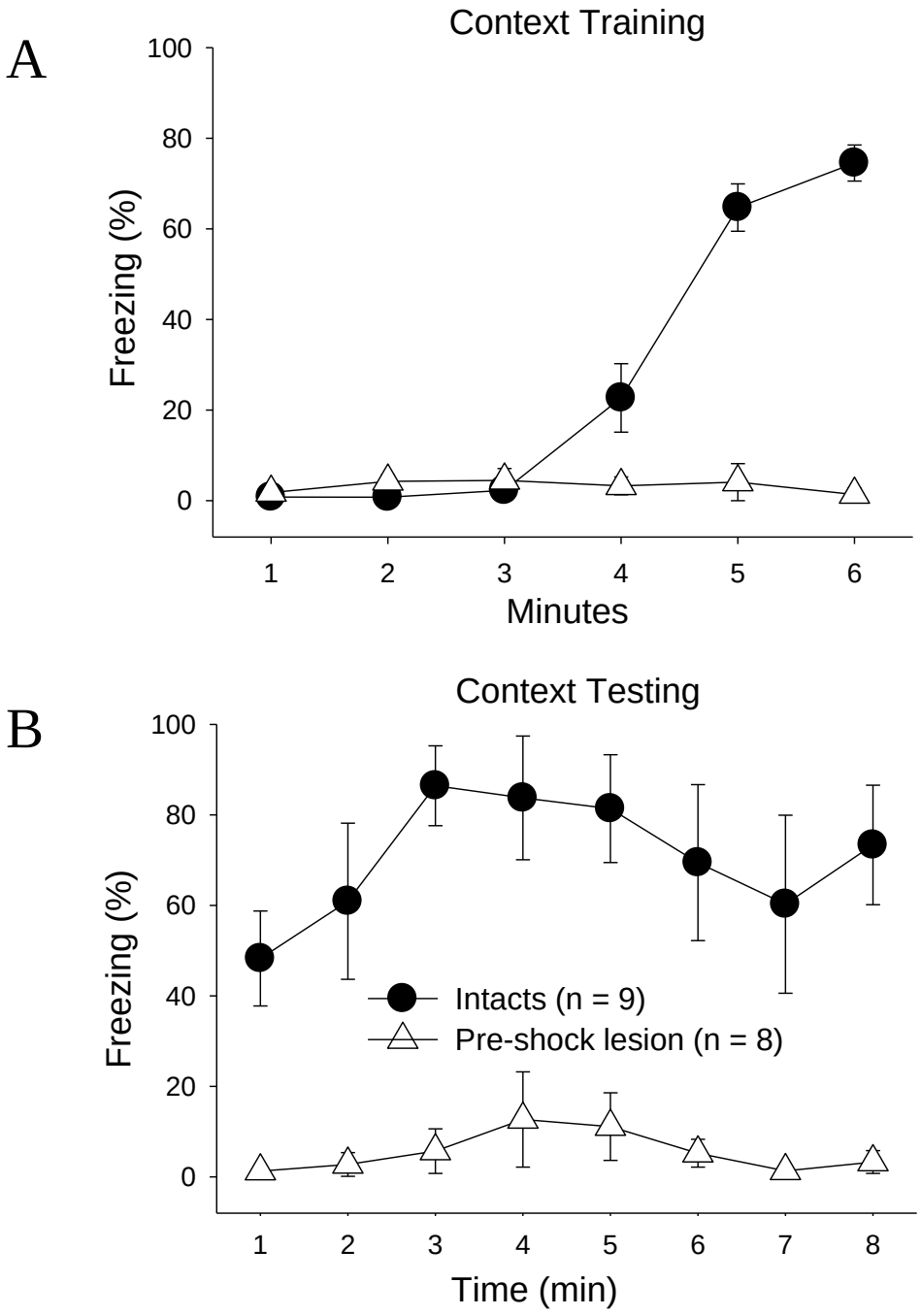

Figure 2.9. Closed economy procedure did not desensitize rats to shock. Intacts displayed robust freezing during training **(A)** and testing 24 hours later **(B)**, but Pre-shock lesions showed severe impairments.

Discussion

The present study investigated whether the amygdala is necessary for fear-induced defensive decisions regarding to meal getting behaviors of rats living in a semi-naturalistic risky environment. In Experiment 1, we found that pseudo-randomly presented unsignaled footshocks decreased the animals' meal frequency, total pellet intake, and total water intake while maintaining body weight. These decreases in feeding behavior while maintaining body weight have been suggested to be defensive strategies against predation risk (Fanselow et al., 1988; Helmstetter & Fanselow, 1993). We found that the amygdala plays a central role in these adaptive behaviors in the presence of environmental threats. Rats with lesions to their amygdala did not change their feeding behavior and rats that had decreased their feeding behavior due to shock recovered to baseline during the 2nd shock phase after receiving amygdalar lesions. Some have suggested that these decreases to meal intake are simply the result of fear motivated suppression of hunger and not strategic voluntary decisions about meal getting behavior. However, Experiment 2, gives sufficient evidence under a 2 choice lever task where one lever is closer to the safe nesting area and one lever farther from the nesting area, that while Intact controls switched their preference to the closer safe lever during unsignaled shock, amygdala lesioned animals did not switch their preference from baseline. The present study raises the intriguing possibility that under conflicting motivational states of hunger and fear, fear structures, such as the amygdala, can guide decisions to alternate or modify approach behaviors that are motivationally significant (i.e. food lever) , but not to general avoidance. The behavior of amygdala lesioned animals show this idea very well. Even under high emotional (fear) states where avoidance behavior is very strong, approach meal getting behavior is little changed. This failure to change strategies under threat could be thought of as a maladaptive perseverative

behavioral foraging strategy. Reward related perseverative behavior with amygdala lesions has been investigated previously (Kemble & Beckman, 1970; White, 1971). However, how amygdala lesions generate perseverative foraging behavior has not been fully elucidated. Many studies have shown perseverative behavior with prefrontal cortex lesions (Morgan, Romanski, & LeDoux, 1993; Morgan, Schulkin, & Ledoux, 2003; Sotres-Bayon, Bush, & LeDoux, 2004) and have highlighted "top down" prefrontal to amygdala regulation of emotions. However, few studies have investigated "bottom up" amygdala to prefrontal cortex circuits that might contribute to a failure to switch behavioral strategies to new risky contingencies. Further studies need to characterize the role of the amygdala and other fear related structures to highlight the functional aspects of fear in mediating alternate defensive strategies to situations of ongoing threat.

Although feeding behavior was modulated by a functioning amygdala, we did not see this modulation by the amygdala to avoidance learning or memory. In both experiments, Intact rats showed a 70-80% reduction in time spent in the foraging area by the end of the shock phase compared to the baseline phase. Interestingly, Pre-shock lesions also showed a 40-50% reduction in the foraging area by the end of the shock phase. These results indicate that like Intacts, Pre-shock lesions were also able to learn the association between the foraging area and footshock. However, this association between the context and footshock was stronger with a functioning amygdala during learning in the shock phase as outlined by the Post-shock lesion animals in Experiment 1. There has been considerable evidence that rats can learn contextual fear with a damaged amygdala given overtraining (Maren, 1999; Ponnusamy, Poulos, & Fanselow, 2007). Recent evidence has shown the bed nucleus of the stria termanalis receives input from the hippocampus and can compensate for fear learning with a damaged amygdala and undergo fear

related plasticity (Poulos et al., 2010). However, pre-training lesions of the amygdala have consistently shown deficits in fear responses compared to controls (Campeau & Davis, 1995; M. Kim & Davis, 1993; Maren, 1998). These results have been hypothesized to mean that primary pathways dominate during learning, while less efficient secondary pathways can compensate when primary pathways are blocked (Fanselow, 2010; Ponnusamy et al., 2007). In the present study, our results confirm that pre-training lesions to the amygdala impaired, but did not prevent behavioral performance to passive avoidance. However, post-training lesions did not impair passive avoidance memories (Figure 1, post-lesion group). This suggests that fear related behaviors can become amygdala independent over time (i.e. inhibitory avoidance) and consolidate behavioral performance to other structures. The environmental conditions and behavioral paradigms that determine how overtraining influences the consolidation of aversive behavior has yet to be fully investigated. Further studies need to characterize how different brain regions participate in the fear experience, particularly in situations when that fear is experienced repeatedly. Recent fMRI studies have shown that a repeated exposure to a threat changes the activity of fear related structures over time (Mobbs et al., 2010). The results of this paper show that overtraining has a differential effect on the amygdala dependence of aversive behaviors.

The primary goal of this study was to explore how the amygdala might influence choices about meal getting behavior in the presence of danger. Our results show a functional role of the amygdala to guide defensive voluntary decisions concerning environmental threats. More precisely, the amygdala alters decisions of foraging only when those decisions involve a strong competing motivational component, such as the need to feed, but not to general voluntary avoidance. This suggests that maladaptive perseverative behavior under conflicting motivational states could be due to impaired processing in the amygdala. Understanding how the amygdala

helps to adapt in situations of ongoing threat presents an avenue of research of how emotional responsiveness to danger guides decisions toward adaptive strategies.

Chapter III. Is the Amygdala necessary for Aversive Learning and Memory?

Introduction

Despite many years of research, the notion that the amygdala is required for fear learning and is the locus of the fear engram remains contentious (Cahill, Weinberger, Roozendaal, & McGaugh, 1999). Lesions of the amygdala before and after Pavlovian fear conditioning have been shown to abolish conditioned fear responses such as freezing, potentiated startle, and 22-kHz ultrasonic vocalizations (Fanselow & Ledoux, 1999; Johansen et al., 2010; Ledoux et al., 1990). Additionally, fear induced plasticity in the amygdala has been shown to be necessary for fear learning (Gale et al., 2004; Lee, Choi, Brown, & Kim, 2001; Stephen Maren, 2003; Muller, Corodimas, Fridel, & LeDoux, 1997). However, the results from aversive instrumental conditioning studies have been less clear. Pre-training and post-training lesions of the amygdala have not abolished inhibitory avoidance learning or memory and selective drug infusions into the amygdala during the learning time window has been discovered to modulate the strength of memory (Ferry, Roozendaal, & McGaugh, 1999; Liang et al., 1982; Parent, Quirarte, Cahill, & McGaugh, 1995). Some have postulated these differences to procedurally different fear related tasks between classical vs. instrumental fear conditioning (Kim & Jung, 2006; Ledoux, 2000). Others have shown the existence of compensatory circuits that undergo plasticity with amygdala lesions that is sufficient for the fear response (Poulos et al., 2010). Still others have shown that amygdala lesion deficits found in both inhibitory avoidance and Pavlovian fear conditioning can be mitigated with over training (Parent et al., 1992).

Therefore, it would be interesting to note the role of the amygdala in aversive learning and memory in the context of a naturalistic paradigm involving feeding and foraging. Would monitoring the animal continuously under these closed economy settings reveal a different

picture of amygdala's role to fear learning and memory? Additionally, would voluntary decisions to feeding and foraging be different from aversive stimuli that could be adequately predicted and therefore avoided? To test this, 2 groups of rats, one with bilateral amygdalar lesions (Pre-lesions, n = 8), and sham controls (Controls, n = 7) were observed continuously in a closed economy setting. Rats were gradually shaped to a FR50:CRF schedule (see chapter II) and allowed to control the meal frequency and total pellet intake each day. Upon 8 days of stable baseline, rats were presented with ~48 coterminating light-footshock pairings daily for 8 days (light: 9 seconds; footshock: 0.8mA, on until the animal escapes to the nesting area or 10 sec maximum). The introduction of footshock did not alter voluntary decisions on feeding intake for Controls or Pre-lesions. While, there were no differences to signaled shock for feeding, there were initial deficits by Pre-lesions to voluntary avoidance learning and memory. Controls decreased escape latencies and increased the number of active avoidance responses during the light cue within 2 days, while rats with amygdalar lesions displayed severe deficits in active avoidance responses and escape latencies. However, Pre-lesions largely recovered deficits by the 8^{th} day of testing. These results suggest that although the amygdala is necessary for the rapid associative fear learning and memory, with repeated exposure to environmental threats, compensatory mechanisms can subserve aversive learning and memory

Methods

Subjects. Male Charles River Long Evan rats (initially weighing 275-300 g) were individually housed in eight 'closed economy' chambers (Fig. 2.1) in a climate-controlled vivarium (accredited by the Association for Assessment and Accreditation of Laboratory Animal Care) on a reverse 12-hr light/dark cycle (lights on at 19:00 hrs.). All experiments were performed in strict

compliance with the University of Washington Institutional Animal Care and Use Committee guidelines.

Surgery. Under anesthesia (30 mg/kg ketamine and 2.5 mg/kg xylazine, i.p.), rats received either bilateral electrolytic lesions (Pre-lesion group, n = 8) to their amygdalae (from bregma: AP -2.5; ML $\pm$4.2/5.0; DV -8.4/8.6 mm) by passing constant current (1 mA, 10 sec; Grass Medical Instrument, Quincy, MA) through epoxy-coated inset pins (#00, ~0.75 mm tip exposed) or had lesion electrodes inserted 1mm above, except current was not delivered to their amygdalae (Controls, n = 7)

Apparatus. The experimental chambers were the same as described in Experiment I of Chapter II, but a light module (Med Associates, Fairfax, VT) was placed just above the lever. The ANY-maze video tracking system (Stoelting Co., Woodale, IL) was used to track the animal's movement, via a Fire-I B/W Board Camera (Unibrain Inc., San Ramon, CA) placed above each closed economy apparatus, and to control all input/output devices connected to an AMi interface (Stoelting Co.).

Experimental procedure. The Pre-lesion and Control groups went through successive phases of baseline, shock and extinction similar to Exp. I. All phases were 8 days and the animals' behaviors were continuously recorded except for a 1 hour break (every 1-2 days) during which the chamber and bedding pan (underneath the shock floor) were cleaned and the food and water were refilled.

Rats were shaped to a FR50-CRF schedule as previously described in Exp. 1 of Chapter II. During each FR-CRF schedule, if the animal did not make sequential level pressings within one minute, then the FR-CRF requirement was reset. After achieving a stable baseline of 8 days, meal patterns were recorded at the FR50-CRF schedule. The animals were switched to the shock

phase 2 signaled footshocks on average were presented randomly every hour regardless of the animal's location (nest or foraging area). If the animal was in the nest, the light immediately turned off; if the animal was in the foraging area, the light stayed on for 9 seconds at which time the animal could escape to the nesting area and not receive shocks. If the animals stayed in the foraging area for longer than 9 seconds, a 0.8 mA shock came on and stayed on with the light until the animal escaped to the nesting area or for a maximum of 10 additional seconds at which both stimuli coterminated.

Histology. At the completion of testing, animals were overdosed with Buthanesia and perfused intracardially with 0.9% saline followed by 10% buffered formalin. The brains were removed and stored in 10% formalin overnight and then kept in 30% sucrose solution until they sank. Transverse 50 μm sections were taken through the extent of the lesion, mounted on gelatin-coated slides, and stained with cresyl violet and Prussian blue dyes.

Statistical Analyses. The daily meal frequency, pellet consumption, number of shocks received, and time spent in the foraging area were normalized to the mean baseline values of each animal. The normalized values were analyzed by paired or independent *t* tests, and one-way repeated measures ANOVA where appropriate.

Results

To investigate whether the amygdala influences foraging behavior in a predictable fear environment, animals were tested under signaled conditions in a closed economy. Two groups of animals with lesions to the amygdala (Pre-lesions, n = 8) and sham controls (Controls, n = 7) were put on a FR50:CRF schedule for 8 days. The meal frequency and time spent in the foraging area were normalized to each rats baseline average.

Upon the introduction of signaled shock, Controls and Pre-lesions did not differ in their meal frequency from baseline or from each other (% Baseline of Controls: $t_6 = -1.76$, $p = .13$, % Baseline of Pre-lesions: $t_7 = -0.76$, $p = .47$, Controls vs Pre-lesions: $t_{13} = 0.27$, $p = .79$). Similarly, Controls and Pre-lesions did not change their total pellet intake (Baseline day 7 vs. shock day 1: Controls, $t_6 = 0.84$, $p = .43$, Pre-lesions, $t_7 = 0.38$, $p = .71$) (Table 3.1).

Amygdala modulation of passive vs. active avoidance

Though feeding patterns were not different between groups under signaled shock, Controls and Pre-lesions differed in their magnitude of passive and active avoidance responses. While both Controls and Pre-lesions decreased the time spent in the foraging area (% Baseline of Controls: $t_6 = -9.71$, $p < .001$, % Baseline of Pre-lesions: $t_7 = -2.71$, $p = .04$), Controls avoided the foraging zone more than Pre-lesions (Controls vs Pre-lesions last 3 days: $t_{13} = 2.28$, $p = .04$) (Figure 3.1A). Similarly, Controls learned to avoid the shock by escaping to the nesting area during the light cue by the second day (1^{st} vs 2^{nd} day of shock: $t_6 = 7.08$, $p < .001$). However, Pre-lesions had severe deficits on the first day compared to Controls (Pre-lesions vs Controls: $t_{13} = 4.77$, $p < .001$). But surprisingly, Pre-lesions also learned to avoid the shock by the 8^{th} day (1^{st} day vs 8^{th} day of shock: $t_7 = -9.70$, $p < .001$) although they were not as successful as Controls (Pre-lesions vs Controls 8^{th} day: $t_{13} = 2.95$, $p = .01$) (Figure 3.1B).

Avoidance and shock escape latency

To further investigate learning related differences between Pre-lesion and Controls, the avoidance and escape latencies were measured. Controls had a mean avoidance latency of 3.96 seconds (*SD*: 0.38) while Pre-lesions had a mean latency of 5.01 seconds (*SD*: 0.73). Controls had a significantly lower avoidance latency then Pre-lesions ($t_{14} = 3.62$, $p = .003$), but both groups did not change throughout the 8 days of shock (Figure 3.2B).

The escape latency had a similar profile. Controls had a mean escape latency of 0.80 seconds (*SD*: 0.12) while Pre-lesions had a mean escape latency of 1.51 seconds (*SD*: 0.16). Controls had a significantly lower escape latency then Pre-lesions ($t_{13} = 3.60$, $p = .003$), but similar to the avoidance latency, both groups did not change throughout the 8 days of shock (Figure 3.2C).

Table 3.1: Mean (±SEM) values of feeding and shock related variables

	Controls			Pre-lesions		
Variable	Baseline	Shock	Extinction	Baseline	Shock	Extinction
Total Pellets	786 ± 42.1	763 ± 44.0*	819 ± 53.5	743 ± 41.4	720.4 ± 33.3	720 ± 29.4
Meal Freq.	9.3 ± 0.8	8.7 ± 1.0	7.0 ± 0.9	9.2 ± 2.3	8.0 ± 1.4	7.0 ± 1.9
Weight	470 ± 20.7	501 ± 23.1	532 ± 29.2	436 ± 12.5	473 ± 13.9	501 ± 21.0
Shocks	13 ± 0.7 †	2.4 ± 0.2 *	-	11.9 ± 1.2 †	5.4 ± 0.4 *	-

* $p < .05$ compared to baseline (Bonferroni test), † Theoretical number of shocks

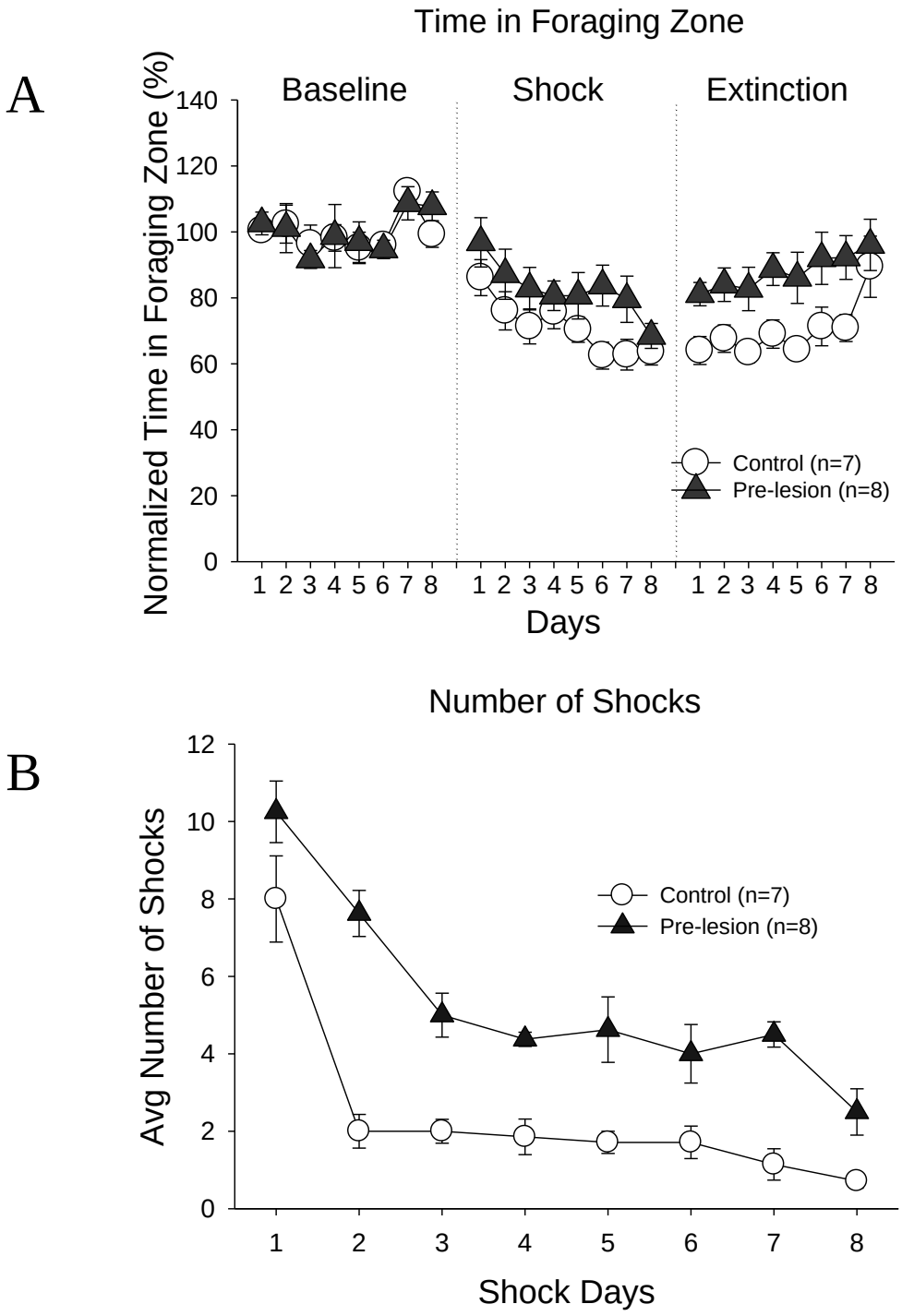

Figure 3.1.Time in the foraging zone and number of shocks per day. **(A)** Time in foraging zone significantly decreases during shock for both Pre-lesions and Controls. **(C)** Number of shocks experienced by Pre-lesions and Controls under signaled shock. Controls learned to associate the shock and light by the 2nd day, while Pre-lesions had severe deficits which attenuated with continuous testing.

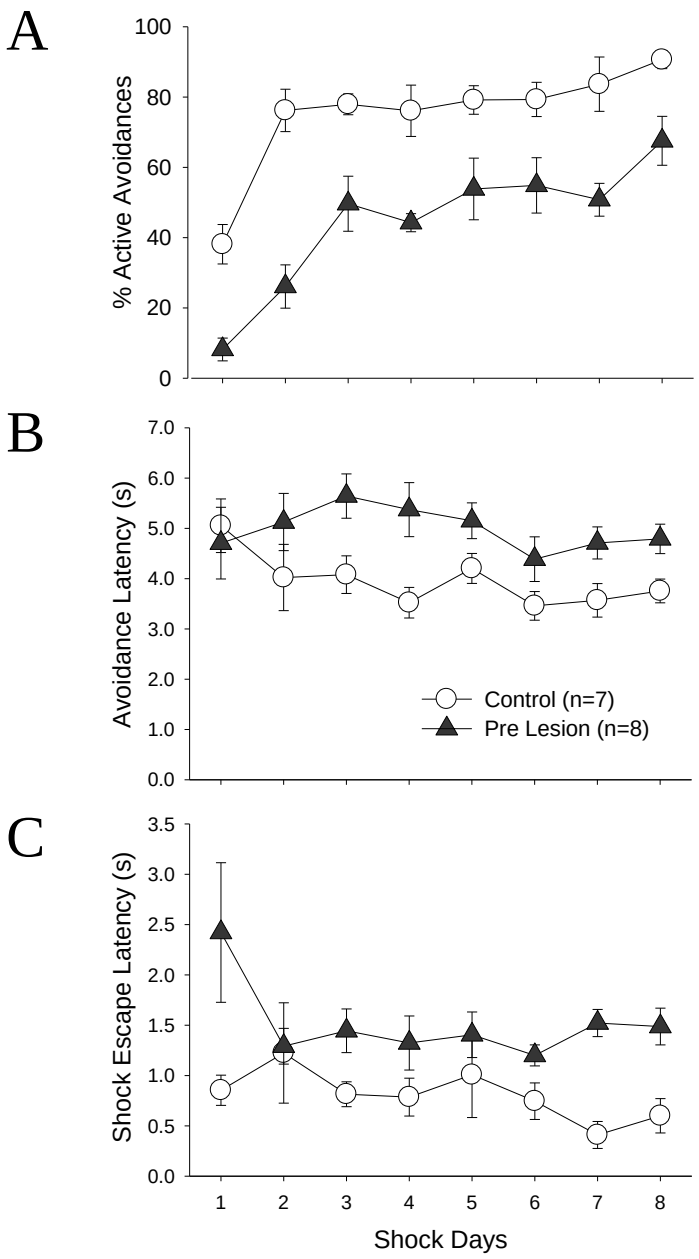

Figure 3.2. Signaled shocks and active avoidance learning. **(A)** Percentage of successful active avoidance responses during the shock phase for Controls and Pre-lesions. (*B*) Avoidance latencies during the 9 second light cue before shock for Controls and Pre-lesions during the shock phase. **(C)** Escape latencies after shock onset for Controls and Pre-lesions during the shock phase.

Avoidance latency when working for food.

In order to gauge how fear changes hunger motivated behavior more directly, the avoidance latency while working for food in the foraging area was compared to the avoidance latency of animals just exploring in the foraging area. The mean avoidance latency for controls not pressing for food by the 8th day of shock was 3.23 seconds (*SD*: 0.29) which was not significantly different from Pre-lesions ($t_{13} = 0.78$, $p = .49$). This increased significantly to 4.81 seconds (*SD: 0.40*) when working for food (Not pressing vs Pressing Avoidance: $t_{12} = 2.44$, $p = .03$) (Figure 3.2) The amygdala lesioned animals had greater increase of avoidance latency to 5.99 seconds (*SD: 0.69*) when working for food, but this was not significantly different then controls ($t_{11} = 1.28$, $p = .22$).

To determine if amygdalar lesions were sufficient to block classical fear conditioning and if there were sensitization effects due to over-training, all animals went through auditory fear conditioning in a separate room after the closed economy testing. Controls exhibited robust freezing during the tone test, while Pre-lesions showed severe impairment ($t_{13} = 5.098$, $p < .001$) (Figure 3.5).

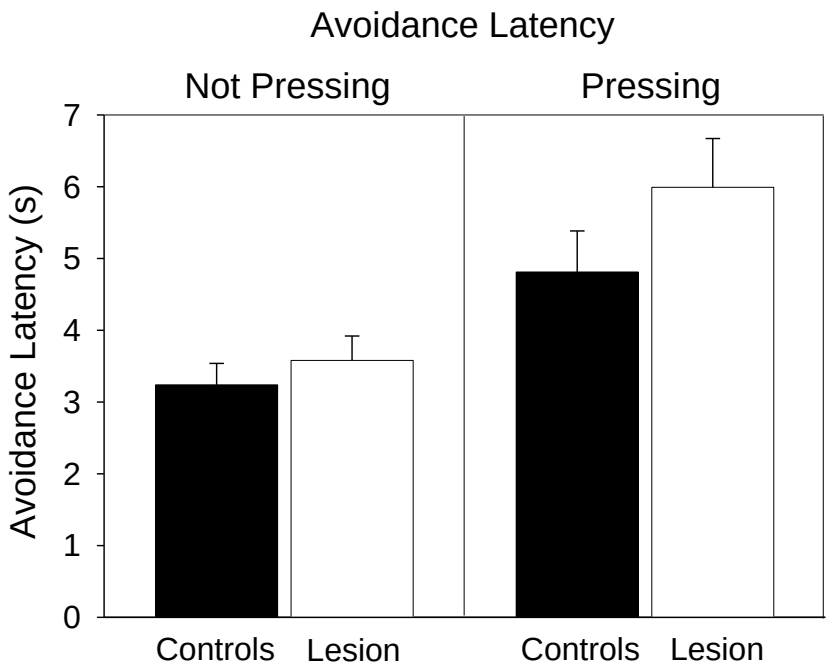

Figure 3.3 Avoidance latency while pressing and not pressing for food. Lesioned animals consistently had a higher avoidance latency then Controls during pressing.

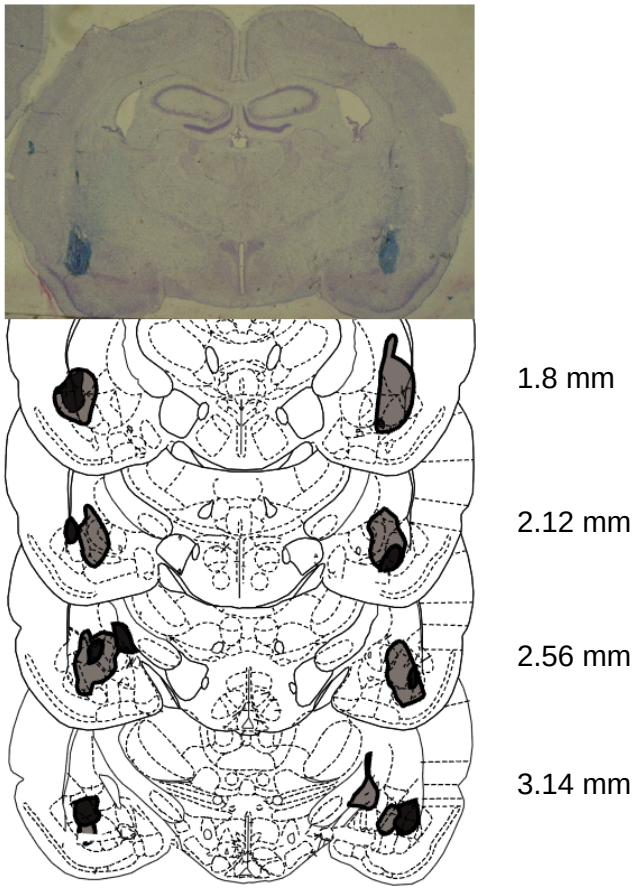

Figure 3.4. Histology. Photomicrograph and histological reconstruction of smallest (dark- shaded) and largest (light-shaded) electrolytic lesions of all lesioned animals for Experiment 1-3 in chapters II and III.

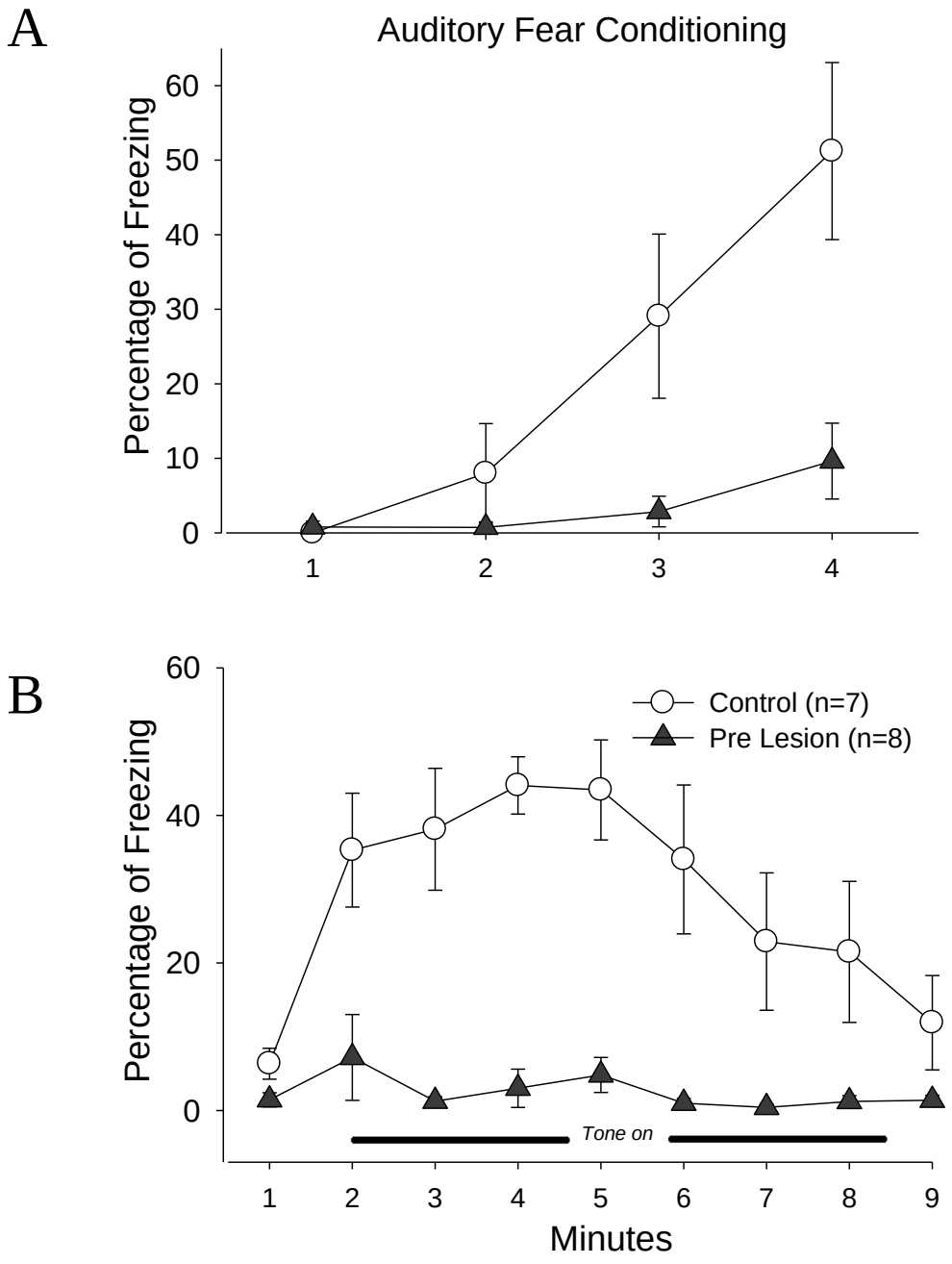

Figure 3.5. Auditory fear conditioning. Controls displayed robust freezing during training **(A)** and testing 24 hours later **(B)**, but Pre-lesions showed severe impairments.

Discussion

The present study investigated whether the amygdala influences foraging behavior in a predictable fear environment. How does learning to predict an aversive experience influence voluntary decisions on feeding and foraging? During the first day of signaled shock, Controls were only escaping the shock during the light cue about 40% of the time, which increased to 80% by the 2nd day. However, during this period and throughout shock, Controls maintained baseline daily meal frequency and total pellet intake. Surprisingly, this indicates that learning to associate the light with shock did not influence decisions on feeding. Consistent with this, is the behavior of Pre-lesions through shock. Pre-lesions had severe deficits learning to associate the cue with shock that did not recover until the 7th and 8th day. However, Pre-lesions also maintained baseline meal frequency and total pellet intake throughout shock. This suggests signaled environments do not impact decisions on feeding as unsignaled environments, and the amygdala has a stronger influence on voluntary decisions involving feeding and foraging behavior under risk of shock in unpredictable than predictable environments. A large number of studies have suggested that an unsignaled environment can create a generalized or sustained fear that increases the magnitude of the fear experience (Davis, Walker, Miles, & Grillon, 2010; Grillon & Morgan, 1999). If the unpredictable fear environments in Chapter 2 produced a state of anxiety in the rats and subsequent greater amygdalar activity, this could suggest how a functional amygdala had a stronger influence on adaptive behavior in the unpredictable fear environment compared to the predictable environment.

Although voluntary decisions involving feeding behavior were not dependent on a functioning amygdala in a signaled fear environment, amygdala dependence was seen to initial voluntary avoidance learning and memory. While, Control rats significantly increased the

number of active avoidance responses by the 2nd day, Pre-lesions still had severe deficits. However, both groups increased the number of active avoidance responses with continuous testing. A similar pattern was seen with passive avoidance, shock escape, and avoidance latency. This suggests that the association between a signal and context is stronger with a functioning amygdala during shock.

There has been considerable evidence that fear associative learning requires synaptic changes in the amygdala (Kim & Jung, 2006; Maren & Quirk, 2004). However, amygdala mediated learning influences decisions on feeding and foraging has not been directly investigated. The present results suggest that voluntary decisions on feeding are not changed to a signaled threat or modulated by a functioning amygdala. Indeed, some have speculated whether signaled threats in avoidance studies are ethologically valid (Bolles, 1970). Predators do not intentionally signal or give cues before attacking prey. Therefore, it would seem natural that the amygdala would not participate in influencing decisions on defensive feeding behavior in a signaled closed economy.

The primary goal of this study was to explore how the amygdala might influence feeding and foraging decisions about continuous behavior in a signaled fear environment. The results show that signaled environments do not change highly motivated feeding behavior, and deficits in voluntary avoidance responses with amygdala lesions can be largely recovered with continuous testing.

Chapter IV. Fear associated changes to Circadian Rhythms.

Introduction

There have been many behavioral mechanisms identified to avoid predatory threat, including changing the timing or amount of activity when foraging (Lima & Dill, 1990). It has long been hypothesized that small mammals, including rats are largely nocturnal due to the difficulty of various predators to locate prey during the night. However, there have been few studies directly measuring predation risk and nocturnality in a laboratory setting. A study by Fenn and Macdonald (1995) showed a population of rats that became diurnal to avoid predation by nocturnal red foxes. When the same rats were placed into a fox proof enclosed chamber, the rats reverted back to nocturnal behavior (Fenn & Macdonald, 1995). Therefore, it would seem plausible that animals can assess predation risk and can alter decisions about periods of feeding and foraging activity to adapt to circumstances involving a predatory threat. However, it remains to be seen, what structures are involved in these adapative responses.

The superchiasmatic nucleus (SCN) has long been considered the master internal clock of the brain. By synchronizing the activity and rest cycle with the environmental lighting conditions, the SCN is thought to have optimized biological fitness by clocking daily activities to maximize the likelihood of attaining resources during times of safety and to minimize the chances of encountering threats. Furthermore, the SCN has been found to be necessary to coordinate and maintain pheripheral rhythms outside the SCN. For example, lesion, pharmacological and genetic manipulations of the SCN have been shown to alter circadian rhythms in various pheripheral tissues (Eckel-Mahan et al., 2008; Reppert & Weaver, 2001, 2002; Schibler & Sassone-corsi, 2002; Stephan & Zucker, 1972). Consequently, many have proposed a heirarchal model of circadian organzation with the SCN (Dibner, Schibler, & Albrecht, 2010).

Although lighting conditions have been the most dominant cue for entraining circadian rhythms, recent evident has shown that in certain circumstances non photic stimuli can entrain peripheral rhythms by dissociating them from the central rhythm (Hamsters & Mistlberger, 1992; F. K. Stephan, Swann, & Sisk, 1979; Stokkan, Yamazaki, Tei, Sakaki, & Menaker, 2001). Evidence is emerging that emotional states associated with anxiety, depression and stress can impact circadian temperature and cortisol rhythms in humans and locomotor activity in rats (Gorka, Moryl, & Papp, 1996; Meerlo, van den Hoofdakker, Koolhaas, & Daan, 1997; Zerssen et al., 1985). However, whether circadian rhythms can entrain to periodic aversive events has not been directly investigated.

To test this, circadian behaviors of male Long Evan rats maintained on an LD (12-h light/12-h dark) cycle were monitored in a live-in chamber comprised of safe nesting and risky foraging areas (Figure 4.1). Initially, animals pressed a lever to procure food pellets in the foraging area and entrained to the environmental conditions for at least 7 stable baseline days. Afterwards, animals were subjected to (i) 14 days of 'Unsignaled' footshocks (0.8 mA; pseudo-random 2 shocks/hr.) which were delivered only during the dark phase of the LD cycle, and (ii) 14 days of 'Signaled' footshocks (a 9-sec light cue preceding the footshock) continuously during the dark cycle (counterbalanced orders). During the unsignaled footshock, rats reversed their normal circadian feeding behavior (i.e., they exhibited more feeding behavior during the light phase than the dark phase), showed arrhythmic locomotor activity. Importantly, these feeding and activity changes were predominantly around the transition from the dark to the light phase. In contrast, rats during the signaled footshock, conserved their normal circadian feeding (i.e., the feeding behavior was observed mostly during the dark phase) and rhythmic locomotor activity and showed less robust anticipatory behavior. Lesioning the amygdala and SCN prevented the

fear induced changes to feeding time and eliminated anticipatory feeding and activity. These results suggest a fear induced timing oscillator dependent on the amygdala and the SCN to predict periods of danger.

Methods

Subjects. Male Charles River Long Evan rats (initially weighing 275-300 g) were individually housed in eight 'closed economy' chambers (Figure 4.1) in a climate-controlled vivarium (accredited by the Association for Assessment and Accreditation of Laboratory Animal Care) on a reverse 12-hr light/dark cycle (lights on at 01:00 hr.). All experiments were performed in strict compliance with the University of Washington Institutional Animal Care and Use Committee guidelines.

Surgery. Under anesthesia (30 mg/kg ketamine and 2.5 mg/kg xylazine, i.p.), rats received either bilateral electrolytic lesions to their amygdalae (Amy lesion group, n = 8: from bregma: AP -2.5; ML $\pm$4.2/5.0; DV -8.4/8.6 mm), the SCN (SCN lesion group, n = 8: from bregma: AP –1.3; ML $\pm$ 0.3; DV –9.1) or had lesion electrodes inserted 1mm above the amygdala or the SCN (groups were combined because of no statistical differences), except current was not delivered (Controls, n =). For the amygdala lesion group, lesions were made by passing constant current at 1mA for 10 seconds (Grass Medical Instrument, Quincy, MA) through epoxy-coated insect pins (#00, ~0.75 mm tip exposed). For the SCN lesion group, lesions were made by passing constant current at 1.75 mA for 17.5 seconds (Grass Medical Instrument, Quincy, MA) through epoxy-coated insect pins (#00, ~.25mm tip exposed) (Zucker, 1972)

Apparatus. The experimental chambers were the same as described in Chapter III. The ANY-maze video tracking system (Stoelting Co., Woodale, IL) was used to track the animal's movement, via a Fire-I B/W Board Camera (Unibrain Inc., San Ramon, CA) placed above each

closed economy apparatus, and to control all input/output devices connected to an AMi interface (Stoelting Co.).

Experimental procedure. The lesion and control groups were shaped to press the lever for food and entrained to the lighting conditions (as confirmed through an actogram) for 10-14 days. After stable food and activity measures, successive phases of baseline and shock that were 7 and 14 days, respectively, were given. Two shocks on average at 0.8 mA (Coulbourn Instruments, Whitehall, PA), were given randomly in the foraging area every hour in either the dark or light cycles. If the animal was in the nest, the shock immediately turned off; if the animal was in the foraging area, the shock stayed on until the rat escaped to the nest (or a maximum of 10 sec). All experiments began at the beginning of the dark cycle and behavioral data was continuously recorded except for a 1 hour break (every 2-3 days) at the end of the light cycle during which the chamber and bedding pan (underneath the shock floor) were cleaned and the food and water were refilled.

Experiment I: Fear associated circadian changes to food and activity. There were 2 unoperated groups that were run through each phase of Experiment 1. One group of animals (Unsig/sig, n = 8), after stable baseline, were given unsignaled shock *only* during the dark cycle for 14 days then given signaled shock during the dark cycle for 14 days. Signaled shock was the same procedure as in Chapter III. Another group of animals, (Sig/unsig, n = 8) were run similar to the Unsig/sig group except the order was reversed to 14 days of signaled shock followed by 14 days of unsignaled shock in the dark cycle.

Experiment II: Amygdala modulation of circadian rhythms. There were 3 groups that were run through Experiment II. One group of animals had bilateral lesions to the amygdala (Amy lesions, n = 8), another group had bilateral lesions to the SCN (SCN lesions, n = 8), and the last

group of animals were sham controls (Controls, n = 4). After recovery from surgery, all animals were shaped as in Experiment I. After stable baseline and entrainment, all groups were run through 14 days of unsignaled shock during the dark cycle followed by 14 days of unsignaled shock during the light cycle, except for the Amy lesion group, which was only run through 14 days of unsignaled shock during the dark cycle.

Histology. At the completion of testing, animals were overdosed with Buthanesia and perfused intracardially with 0.9% saline followed by 10% buffered formalin. The brains were removed and stored in 10% formalin overnight and then kept in 30% sucrose solution until they sank. Transverse 50 μm sections were taken through the extent of the lesion, mounted on gelatin-coated slides, and stained with cresyl violet and Prussian blue dyes.

Statistical Analyses. The daily pellet consumption, number of shocks received, and time spent in the foraging area were normalized to the mean baseline values of each animal. The normalized and raw values were analyzed by paired or independent *t* tests, and one-way repeated measures ANOVA where appropriate.

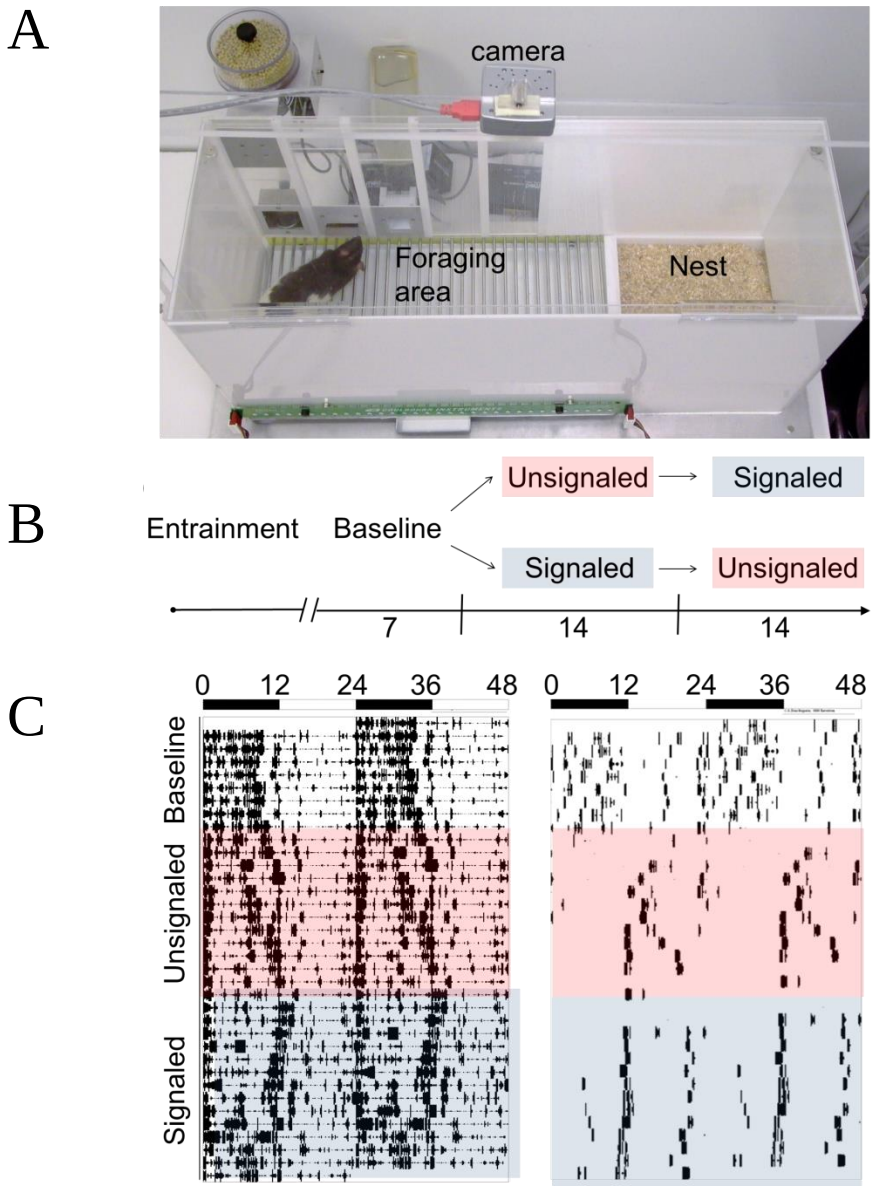

Figure 4.1. **(A)** Picture of closed economy apparatus. **(B)** Diagram of experimental procedure through each phase of Experiment I. Entrainment took between 7-10 days, while baseline, shock phases were 14 days each. **(C)** (*left*) Representative actogram of animals through baseline unsignaled shock followed by signaled shock. (*right*) Rastorplot of feeding behavior of same animal through unsignaled and signaled shock.

Results

Experiment I: Fear associated circadian changes to food and activity

To investigate whether predictable or unpredictable fear can alter circadian rhythms of feeding and activity, 2 groups of animals were tested under signaled and unsignaled conditions in a closed economy (Figure 4.1). One group of animals (Unsig-sig, n = 8) were run through 7 days of baseline, followed by 14 days of unsignaled shock, followed by 14 days of signaled shock. Another group of animals (Sig-unsig, n = 8), were run through the same amount of days but were run through signaled shock followed by unsignaled shock. All shocks were presented randomly at a rate of 2 per hour in the foraging area *only* during the dark cycle were activity and feeding are maximal in rats. Before baseline was acquired, both groups were shaped and entrained to the lighting conditions, as confirmed by an actogram (Figure 4.1).

During baseline, the Unsig-sig group consumed on average 493 pellets (*SD*: 85.0) during the dark phase and 170 pellets (*SD*: 29.0) during the light phase (Figure 4.2). This nocturnal feeding behavior was phase locked to nocturnal daily activity (Figure 4.2 & 4.3). Upon the introduction of unsignaled shock in the dark phase, rats reversed their normal circadian feeding behavior from nocturnal feeding to diurnal feeding (Last 5 days of unsignaled shock light vs. dark feeding: $t_{78} = 3.83$, $p < .001$) (Figure 4.2). Unsignaled shock also decreased locomotor activity during the dark phase and increased during the light phase to show an arrhythmic like behavior (Last day of unsig shock L vs. D phase: $t_7 = 1.22$, $P = .261$) (Figure 4.3). In order to more closely examine how these shock induced changes to feeding and activity was distributed through time, a temporal landscape of feeding and activity during unsignaled and signaled shock was constructed (Figure 4.5). These heat maps revealed a striking pattern of feeding and locomotor activity through time. Upon the onset of unsignaled shock, animals began to feed and

show strong activity around the switch from the dark to the light phase. This crepuscular like behavior continued during signaled shock. In contrast, the introduction of signaled shock during the dark phase after baseline showed a different distribution of feeding and activity. Animals under signaled shock continued to eat and be active during the dark phase, but showed restricted feeding and activity bouts that were distinctly different from the continuous activity and feeding during baseline. In order to examine these changes more closely, 24 hour waveforms of feeding and activity were constructed using the last 5 days of each phase (Figure 4.6).

Rats displayed anticipatory locomotor activity 2.67 hours before the onset of the light phase that continued to increase and subsided by 1.5 hours after the light phase onset (repeated measures ANOVA: main effect of hrs.: $F_{(16, 64)} = 9.65$, $p < .001$) (Figure 4.7B, red line). This shock induced anticipatory activity was significantly different from baseline activity (Baseline vs. shock at 11.2 h: $t_{10} = 3.23$, $p = .005$) and this pattern continued from the unsignaled to signaled transition (repeated measure ANOVA: main effect of hrs.: $F_{(11, 44)} = 4.49$, $p < .001$) (Figure 4.7B, blue line). It is of interest to note that by the 14th of day of unsignaled shock, rats spent 27 % of their dark phase activity in the foraging area (Figure 4.3 & 4.4). This suggests that rats were predominantly spending their time moving around in the nest and anticipating the switch from the dark to the light phase.

Rats showed also showed anticipatory feeding 1 hour before the onset of the light phase that continued to increase and subsided by 4 hours after the light phase onset (repeated measures ANOVA: main effect of hrs.: $F_{(7, 28)} = 2.72$, $p = .028$) (Figure 4.7A). This anticipatory feeding was significantly different from baseline feeding (Baseline vs. shock at 11.8 h $t_{10} = 5.89$, $p < .001$). These effects persisted during the unsignaled to signaled footshock transition showing anticipatory feeding and activity 2.4 hrs. before onset of the light phase (repeated measures

ANOVA: main effect of hrs.: $F_{(11, 44)} = 2.45$, $p = .01$) (Figure 4.7 A). In contrast, during the baseline to signaled footshock days, rats quickly learned to avoid the footshock by the light cue and thereby maintained their circadian feeding and activity and did not show anticipatory activity (Figure 4.7B, right and 4.8). However, rats did show anticipatory feeding behavior that was significantly different from baseline 30 minutes before the onset of the light phase (Figure 4.7A, right). From the signaled to unsignaled footshock transition, the animals again reversed their normal circadian feeding behavior and displayed anticipatory activity and feeding behavior before the onset of the light phase. (Activity: repeated measures ANOVA: main effect of hrs.: $F_{(11, 44)} = 14.92$, $p < .001$; Feeding: repeated measures ANOVA: main effect of hrs.: $F_{(11, 44)} = 4.63$, $p < .001$) (Figure 4.7A & B, right).

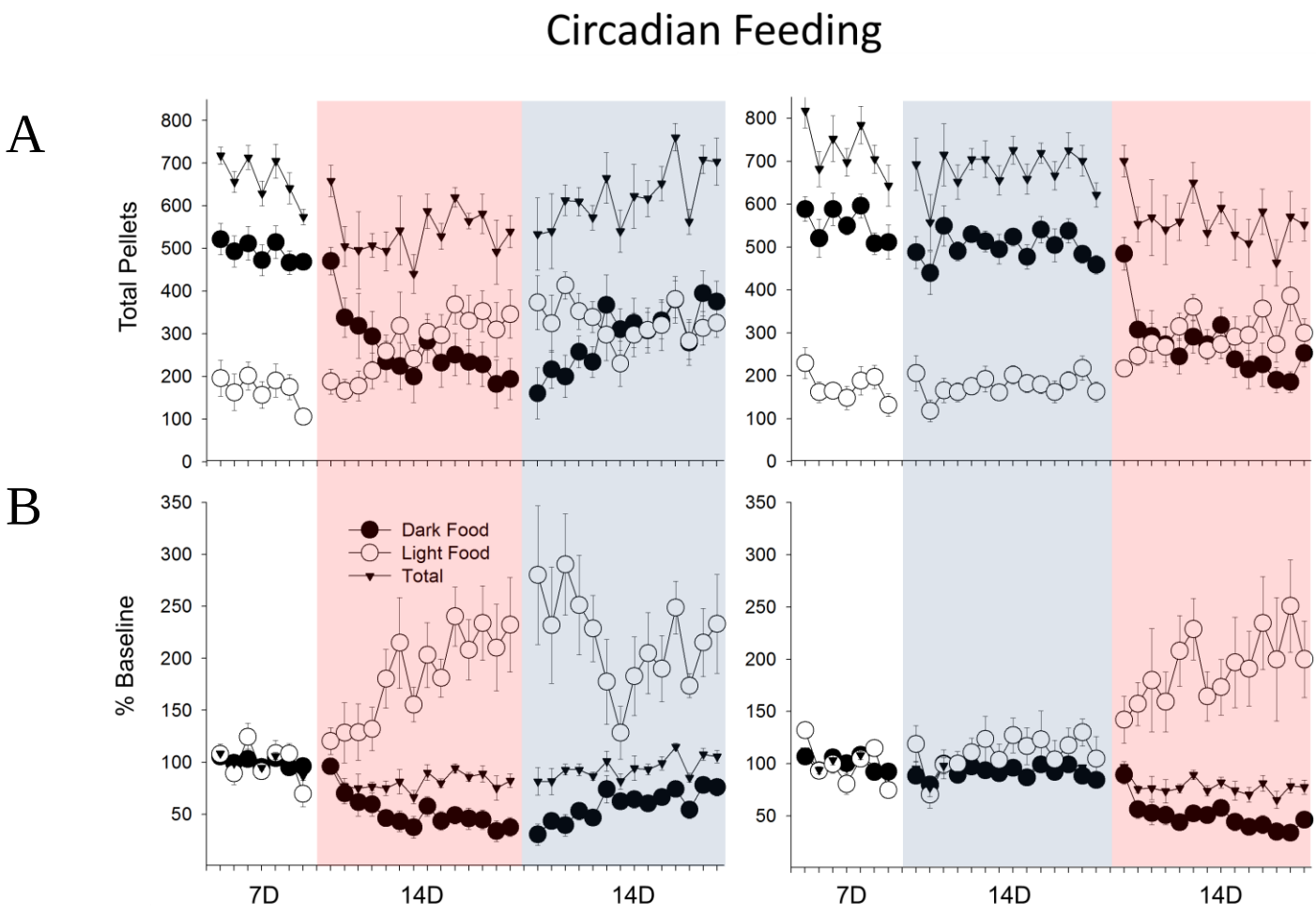

Figure 4.2. Unsignaled shock, but not signaled shock during the dark phase reverses nocturnal feeding to diurnal feeding **(A)** (*left*) Circadian feeding through unsignaled (pink) to signaled (blue) shock phases. (*right*) Circadian feeding through signaled to unsignaled shock phases. **(B)** Normalized feeding behavior from baseline was constructed to show percentage of change to each phase.

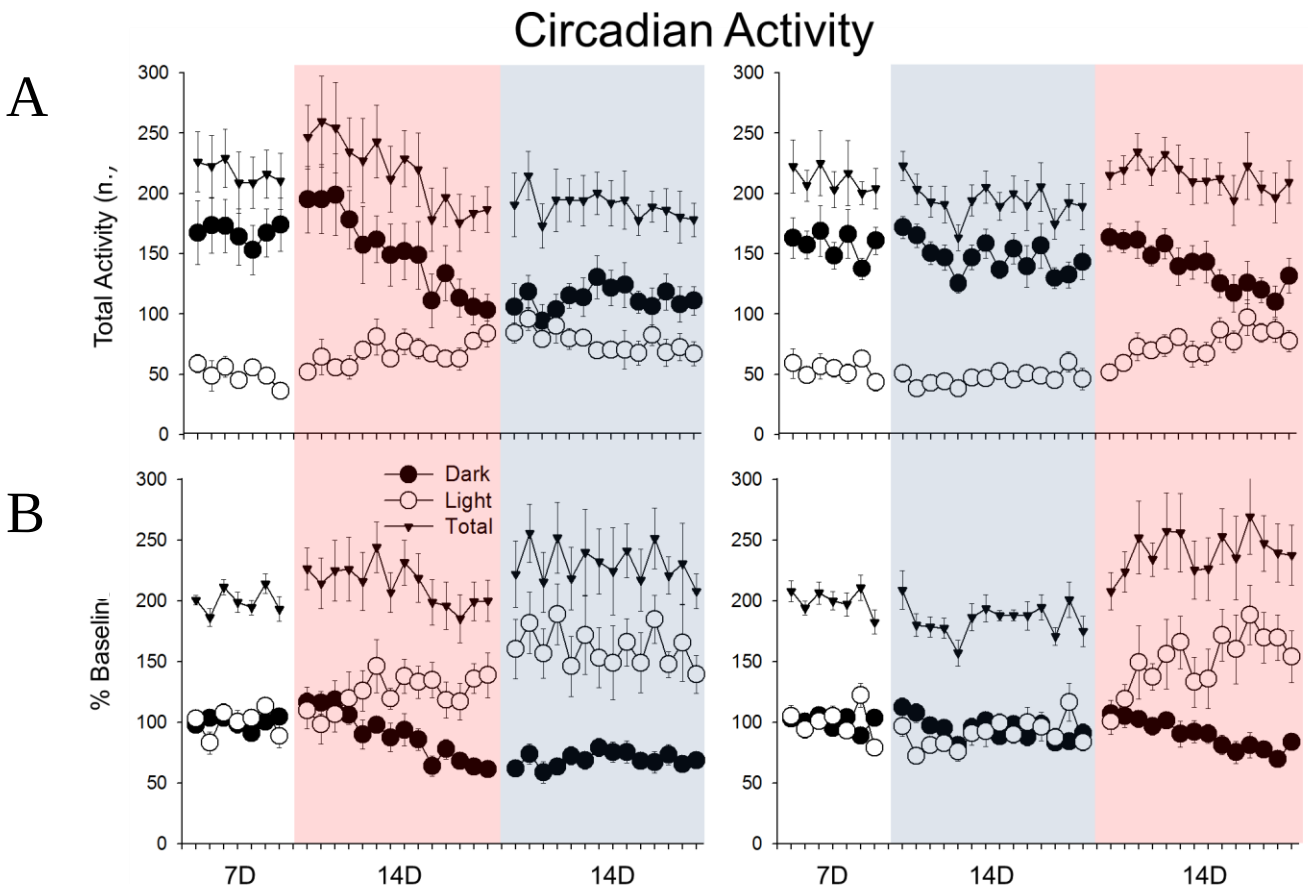

Figure 4.3. Unsignaled, but not signaled shock during the dark phase produces arrhythmic like behavior **(A)** (*left*) Circadian activity through unsignaled (pink) to signaled (blue) shock phases. (*right*) Circadian activity through signaled to unsignaled shock phases. **(B)** Normalized activity behavior from baseline was constructed to show percentage of change to each phase.

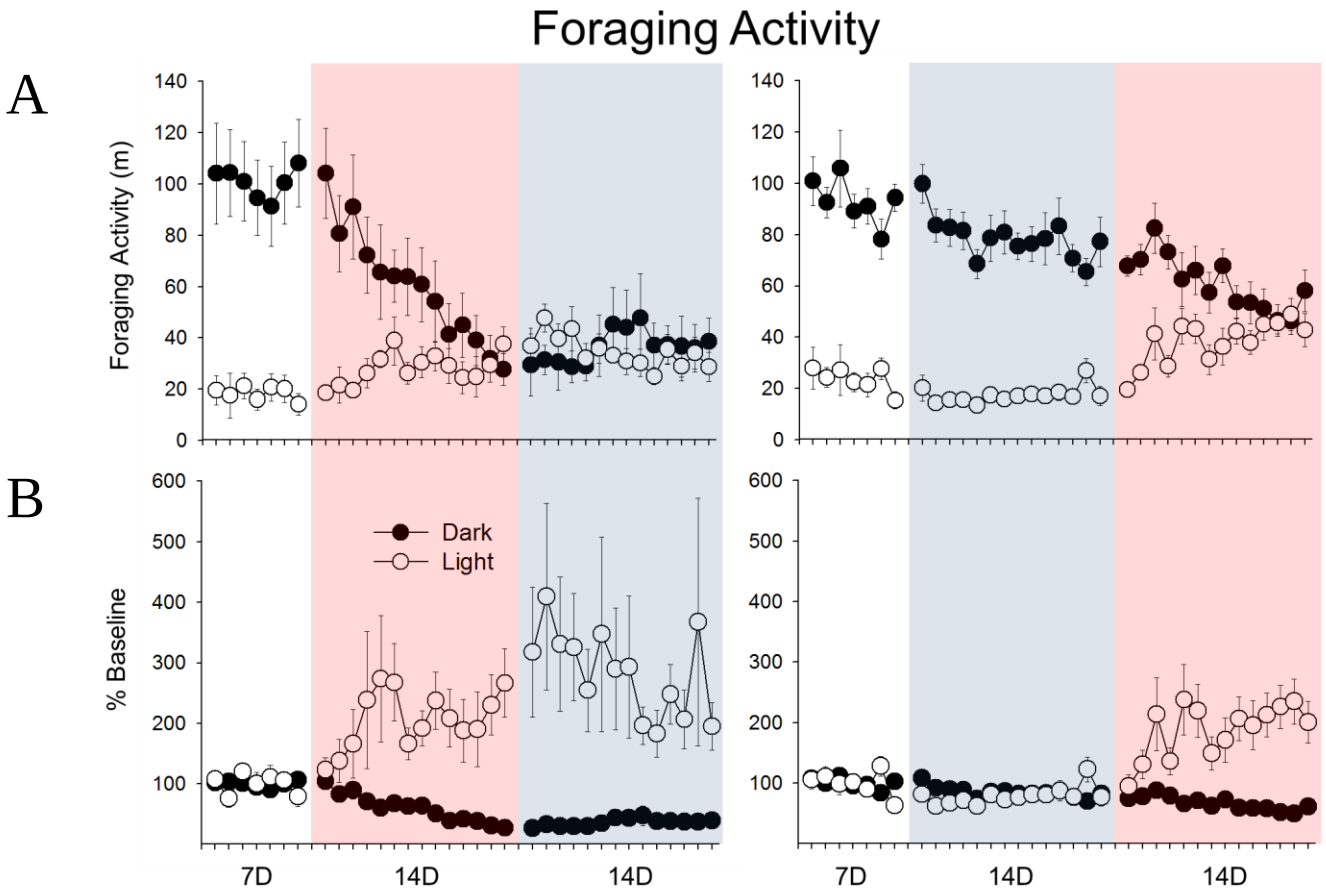

Figure 4.4. Foraging activity significantly decreases to unsignaled but not signaled shock. **(A)** (*left*) Foraging activity through unsignaled (pink) to signaled (blue) shock phases. (*right*) Foraging activity through signaled to unsignaled shock phases. **(B)** Normalized activity behavior from baseline.

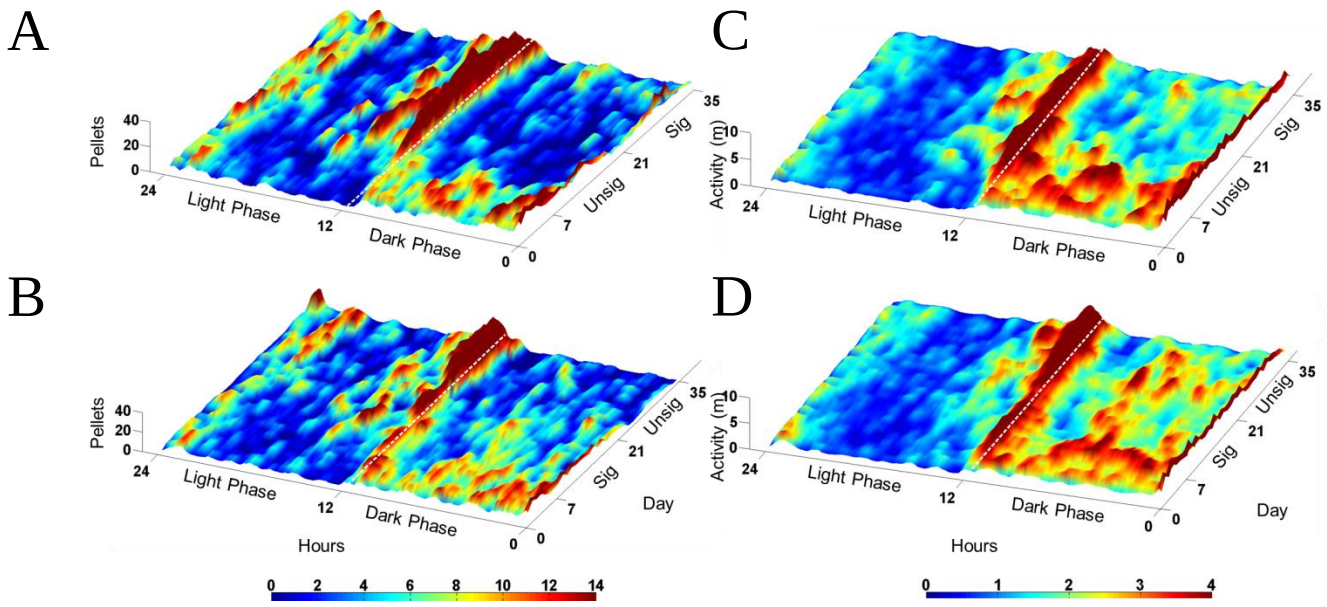

Figure 4.5. Temporal landscape of feeding and activity. **(A)** Feeding behavior during unsignaled is centered around the shift between the dark to light phase. This feeding behavior continues to unsignaled to signaled shock transition **(B)** Feeding behavior of signaled to unsignaled shock rats show feeding to be predominant at the phase shift during unsignaled but not signaled shock **(C)** Locomotor activity is robust around the dark to light shift in the baseline to unsignaled transition but not to the baseline to signaled transition **(D)**.

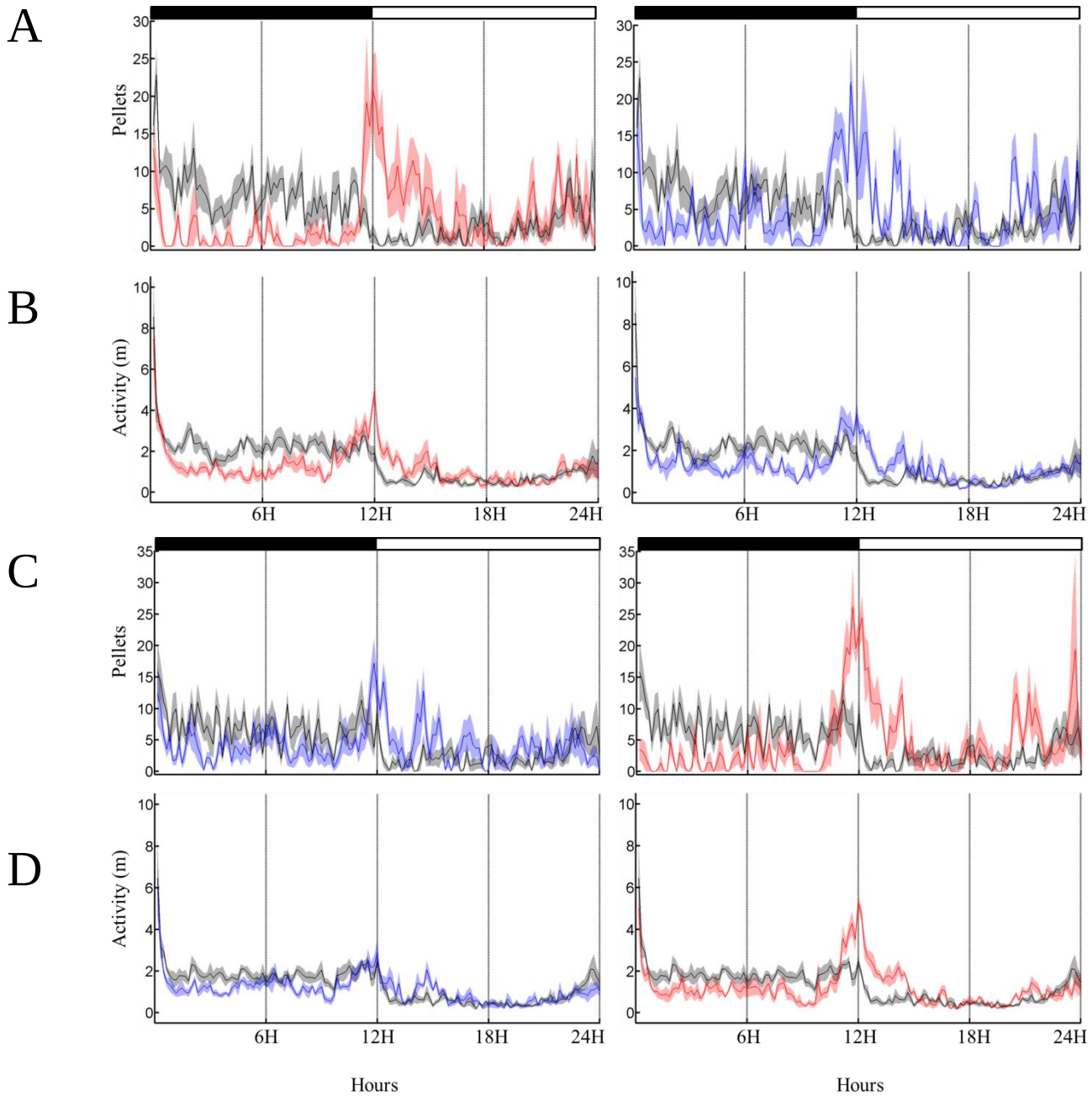

Figure 4.6. 24 hour waveforms of activity and feeding for the last 5 days of baseline (*black*), unsignaled shock (*red*), and signaled shock (*blue*). **(A)** (*left*) Feeding waveform shows unsignaled shock in dark phase causes anticipatory feeding 1-2 hours before shock offset that continues on in signaled shock (*right*) **(B)** Activity waveforms shows a similar anticipatory activity during unsignaled shock but about 2-3 hours before shock offset.

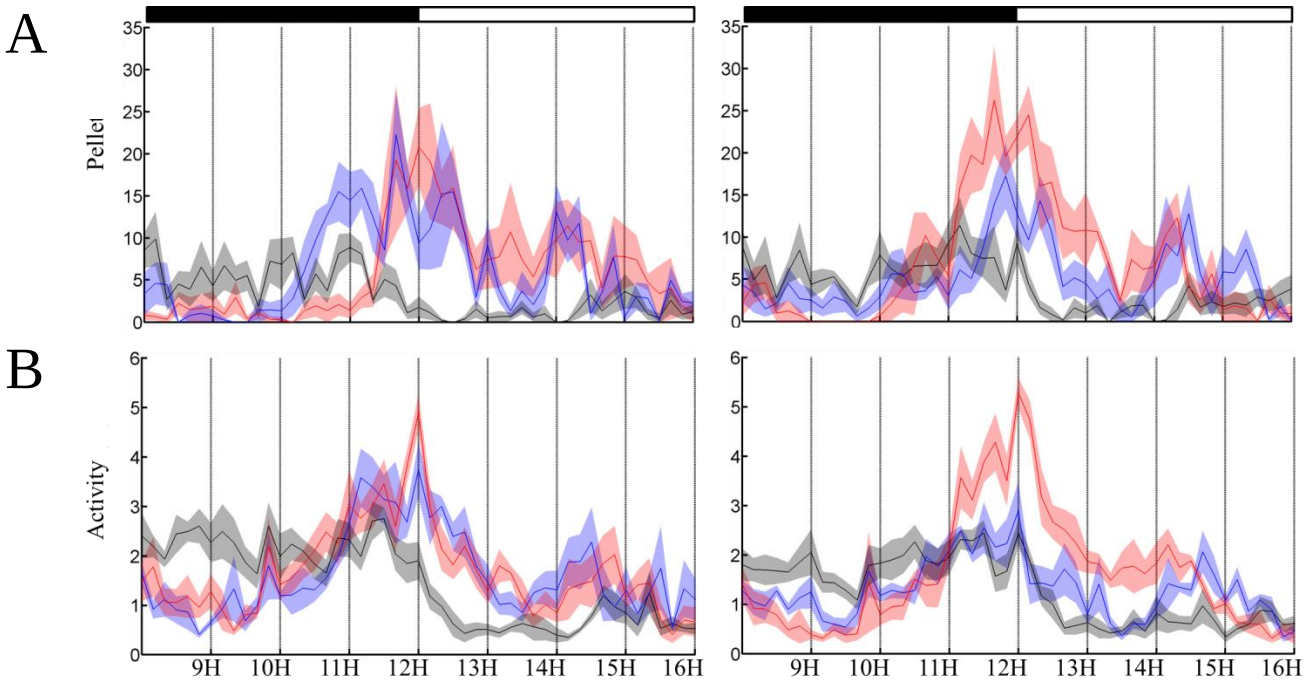

Figure 4.7. Waveforms of feeding and activity 4 hours before and after the dark to light transition (12 hour mark) for the last 5 days of baseline (*black*), unsignaled shock (*red*), and signaled shock (*blue*). **(A)** (*left*) Feeding waveform shows the unsignaled to signaled shock transition in the dark phase causes anticipatory feeding 1-2 hours before the light phase. When the order is reversed to a signaled to unsignaled transition (*right*) the feeding waveform shows a similar robust anticipatory behavior to unsignaled shock, but signaled shock shows reduced anticipatory feeding **(B)** Locomotor activity waveforms shows a similar anticipatory behavior for the unsignaled to signaled shock transition (*left*) and for the signaled to unsignaled transition (*right*).

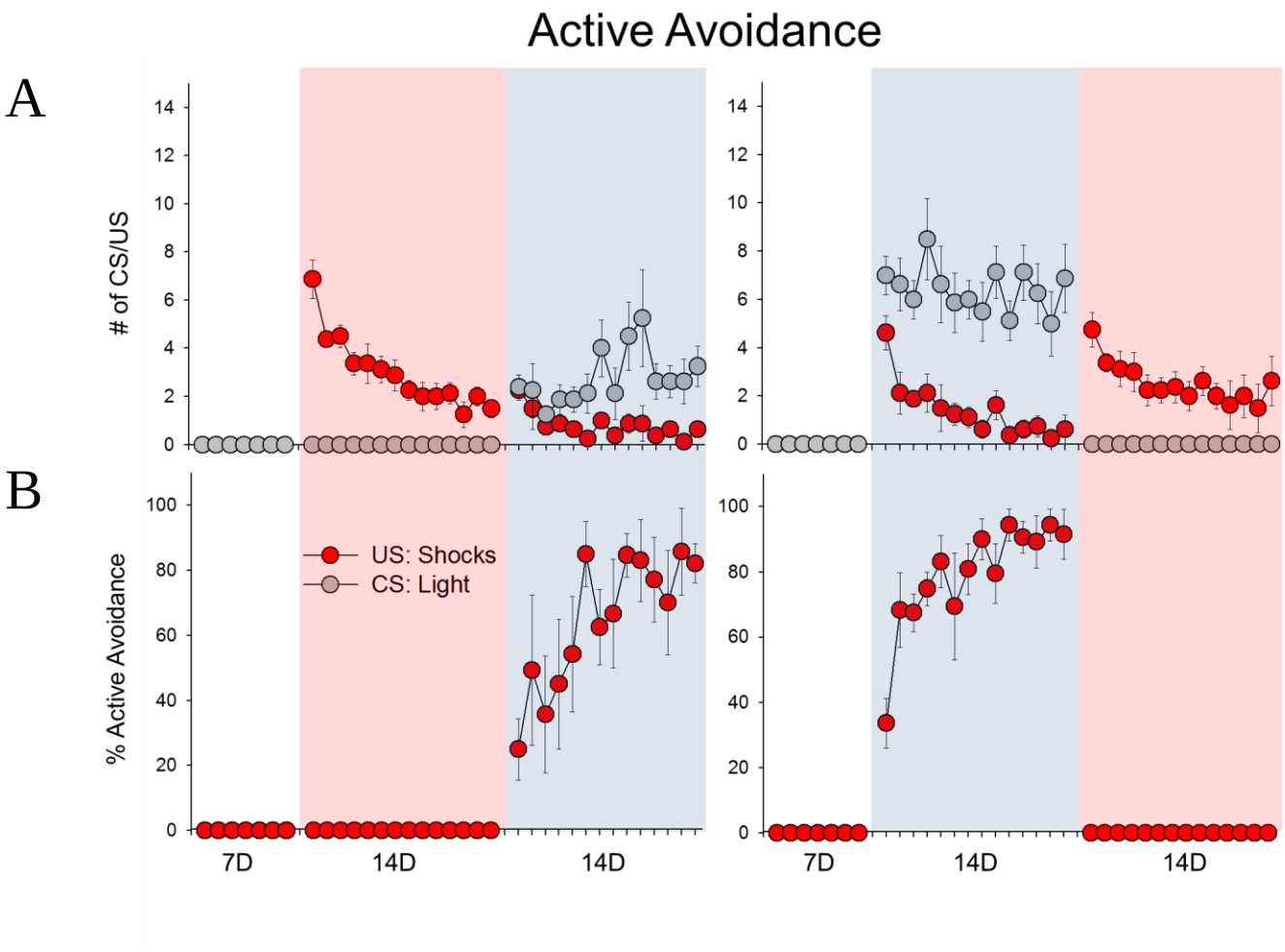

Figure 4.8. Number of US-shock (*red*) and CS-light (*grey*) presentations through signaled (*blue*) and unsignaled (*pink*) shock. **(A)** Number of light and shock presentations. **(B)** Percent of successful active avoidances through shock.

Experiment II: Amygdala and SCN dependent changes to circadian rhythms.

In order to examine whether these feeding and activity changes were dependent on known timing and fear related structures, the effects of nocturnal unsignaled footshocks were examined in SCN and amygdala (AMYG) lesioned rats. During baseline, amygdala lesioned rats' circadian activity and feeding were indistinguishable from those of sham control rats (D/L Baseline Sham vs D/L Baseline AMYG lesioned: $t_8 = .719$, $p = .50$) (Figure 4.9). However, SCN lesioned rats displayed arrhythmic activity and feeding during baseline, though the SCN lesion rats favored feeding in the dark phase slightly more than the light phase (Dark vs. light feeding: $t_{12} = 2.95$, $p = .01$) (Figure 4.10, 4.11). During the nocturnal footshock days, amygdala lesioned rats did not shift their circadian activity and feeding behavior toward the light phase and preserved their total activity and feeding intake (Figure 4.9, 4.11). In contrast, SCN lesioned rats, while maintaining arrhythmic activity, decreased feeding in the dark phase and exhibited more frequent light phase feeding behavior (Last 5 days D vs L: $t_{12} = 2.55$, $p = .03$) (Figure 4.11). However, this light phase feeding behavior was markedly different then sham controls. While sham controls increased feeding during the light phase compared to baseline due to unsignaled shock in the dark phase (L phase baseline vs shock: $t_3 = 4.42$, $p = .02$), SCN lesion rats did not show this pattern. In contrast, SCN lesioned rats maintained light phase feeding and only decreased dark phase feeding (L phase baseline vs shock: $t_6 = 1.82$, $p = .118$; D phase baseline vs shock: $t_6 = 4.61$, $p = .004$) (Figure 4.10).

To assess the degree of flexibility of SCN lesioned rats to reverse feeding to shock, the SCN lesion rats were given an additional 14 days of shock presented only during the light phase. Similar to unsignaled shock in the dark phase, the SCN lesioned maintained dark phase feeding and decreased light phase feeding (D phase feeding: shock in D vs shock in L: $t_6 = 1.84$, $p = .115$;

L phase feeding: shock in D vs. shock in L: $t_6 = 3.37$, $p = .02$) (Figure 4.10, 4.11). Furthermore, SCN lesion rats did not show any anticipatory feeding or activity before the dark to light phase transition (Figure 4.12). These results suggest that both the SCN and the amygdala are necessary to reverse circadian feeding rhythms from nocturnal to diurnal feeding and to show fear related anticipatory feeding and activity.

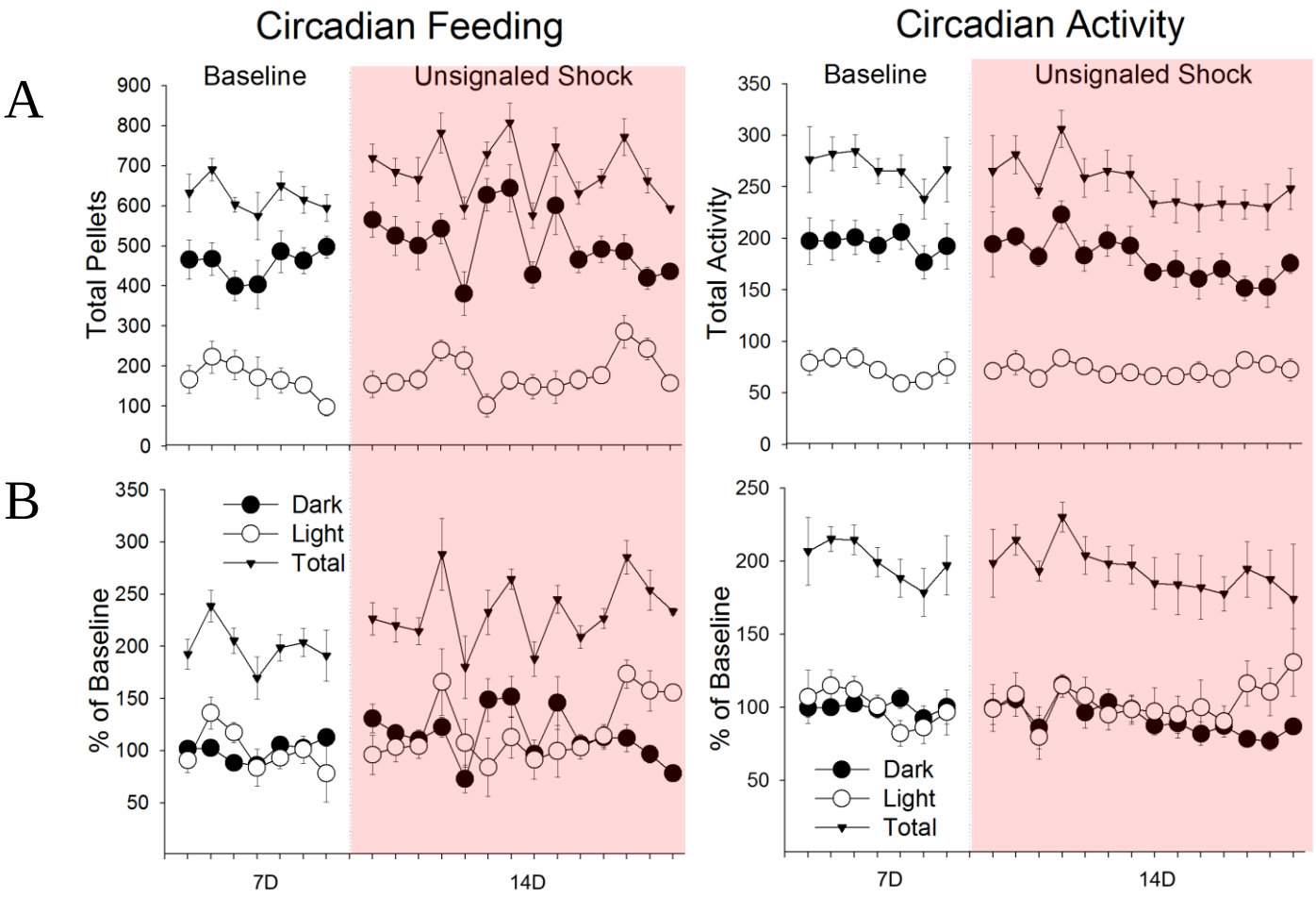

Figure 4.9. Circadian feeding and activity of amygdala lesioned rats. **(A)** (*left*) Circadian feeding and activity (*right*) does not change to unsignaled shock. **(B)** Normalized graphs show feeding and activity does not change to unsignaled shock from baseline.

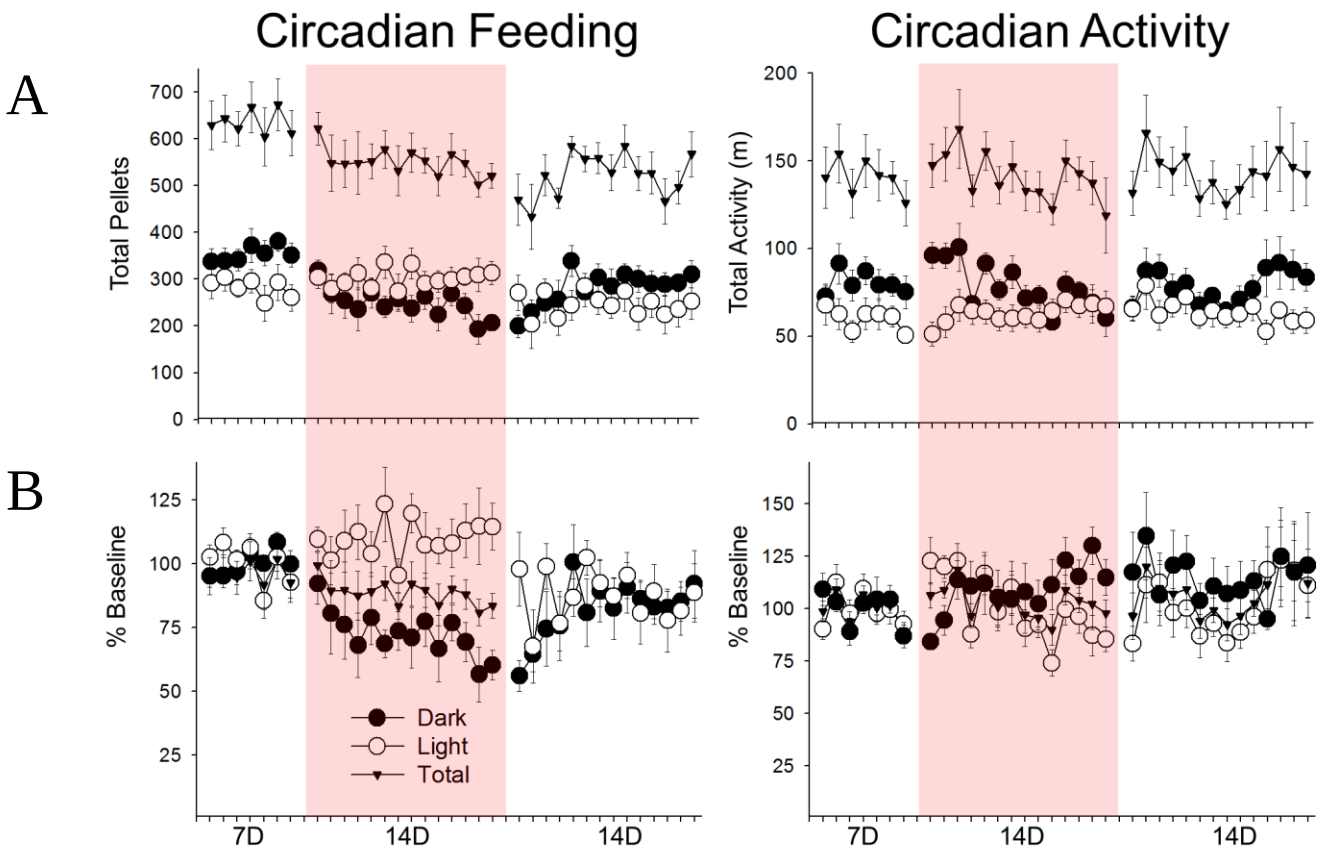

Figure 4.10. Circadian feeding and activity of SCN lesioned rats through unsignaled shock in dark phase (pink) followed by unsignaled shock in light phase **(A)** (*left*) Circadian feeding and activity (*right*) is initially arrhythmic slightly favoring the dark phase, that reverses to shock. **(B)** Normalized graphs show slight feeding and activity feeding and activity changes to unsignaled shock from baseline.

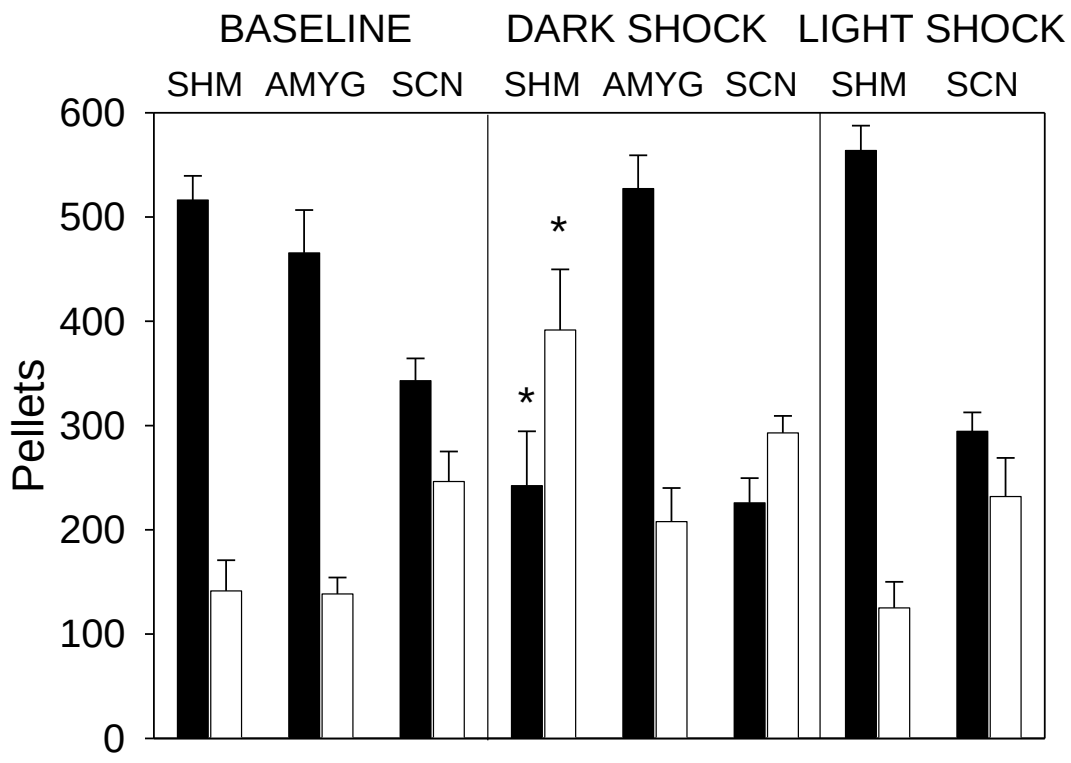

Figure 4.11. Bar graph of last 5 days of circadian feeding of sham control (SHM), amygdala lesioned (AMYG), and superchiasmatic lesioned (SCN) rats during dark (black) and light (white) phases. While SHM group reverses feeding to unsignaled shock, amygdala lesioned animals maintain circadian feeding. The SCN lesion group reverses feeding from baseline during unsignaled shock in the dark phase that reverses back to baseline during unsignaled shock in the light.

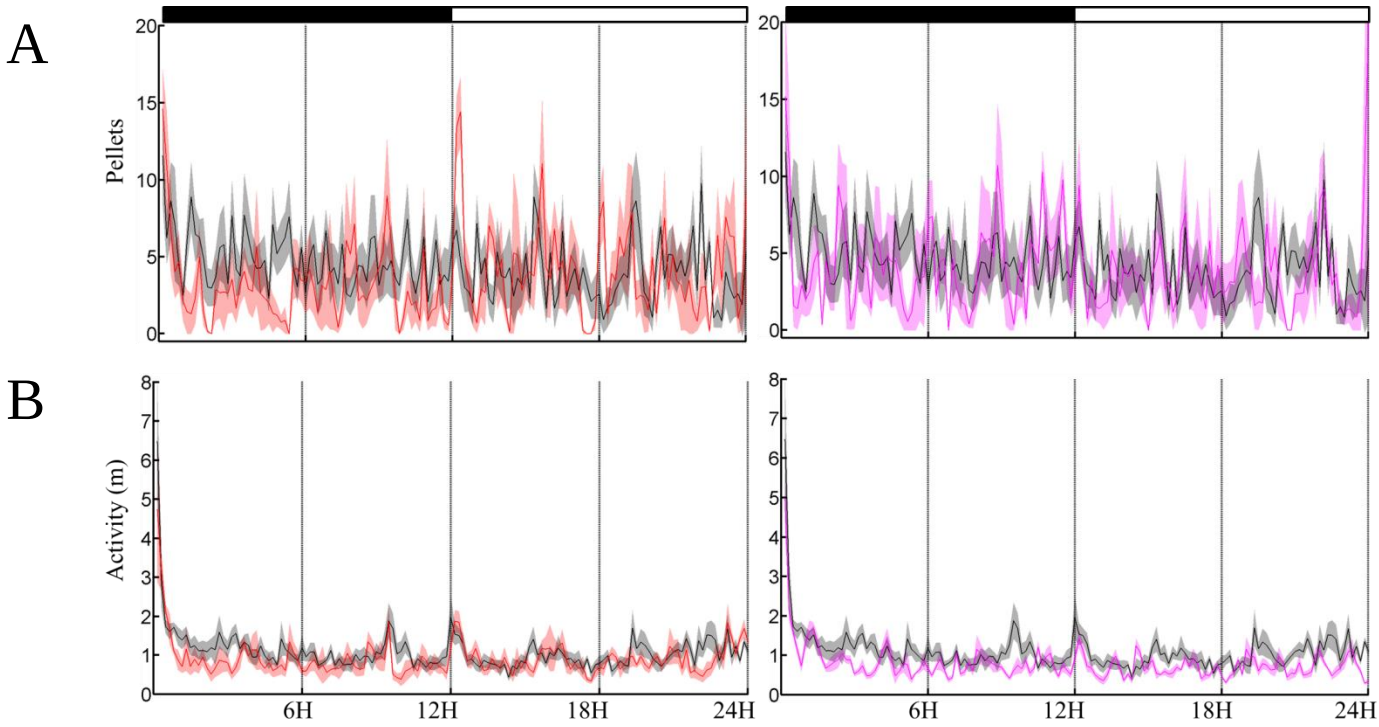

Figure 4.12. Waveforms of SCN lesioned rats for last 5 days of baseline (*black*) and unsignaled shock during dark (*red*) and light (*magenta*) phases. SCN lesioned rats show arrhythmic feeding **(A)** and activity **(B)** throughout dark (*left*) and light (*right*) shock phases. Additionally, SCN lesioned animals do not show anticipatory feeding or activity in dark or light shock phases.

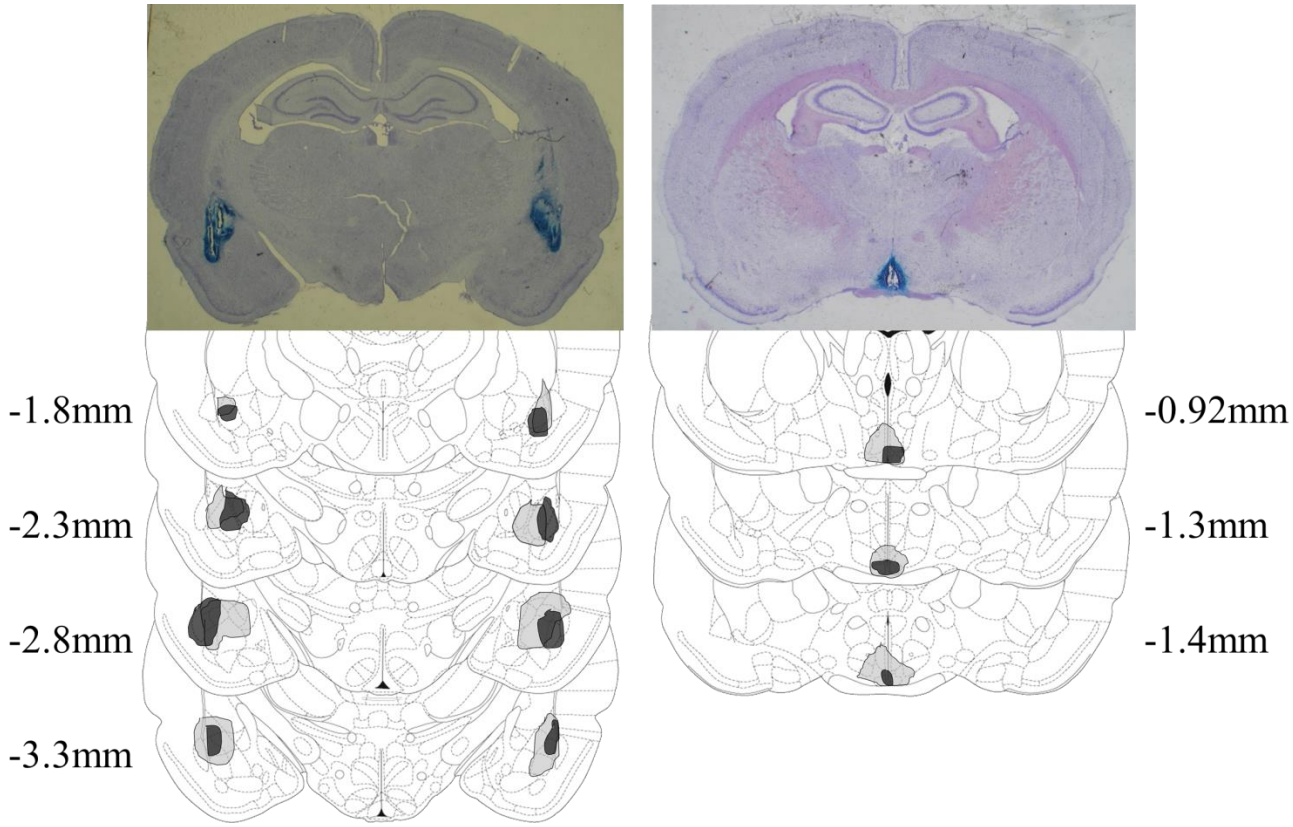

Figure 3.3. Histology. Photomicrograph and histological reconstruction of smallest (dark- shaded) and largest (light-shaded) electrolytic lesions of all amygdala (*left*) and SCN (*right*) lesioned animals. Anterior posterior coordinates were aligned to the hippocampus for amygdala reconstructions and the optic chiasm for the SCN reconstructions.

Discussion

The external lighting conditions have long been known to be the "time giver" of biological rhythms. Through specialized retinal ganglion cells, light information travels to the SCN where it entrains a circadian light dark rhythm that then functions like a central pacemaker for all other peripheral rhythms. It would be interesting to note then how circadian rhythms would adapt to a situation in which one part of the external lighting conditions was associated with danger? Would the activity and rest cycle provided by the central rhythm of the SCN continue to guide nocturnal feeding and foraging or would a peripheral rhythm sensitive to the risk of getting shocked, compete for circadian behavior? Recent evidence has shown in certain circumstances peripheral rhythms can disassociate from the central rhythm of the SCN (Hamsters & Mistlberger, 1992; Stokkan et al., 2001). These results suggest the flexibility of the mammalian circadian system to decouple from central rhythms and adapt to environmental challenges. The present results investigated a unique situation where an animal is driven to nocturnal feeding and activity by innate central rhythms, but also driven by fear to avoid the foraging region due to the risk of getting shocked. These animals can be thought of as being in an approach-avoidance conflict. They are drawn to the foraging region to procure food, but must avoid the time spent in the foraging area. In contrast, during the light phase, the animals are guided to rest and diminish feeding by internal rhythms, but are driven by the opportunity to procure resources without the risk of getting shocked. Therefore, the animal must balance its own need of activity and rest cycles with the decision to approach or avoid resources in times of danger and safety. The primary investigation of this paper is whether the amygdala influences the outcome of these decisions.

Based on both animal and human literatures, the amygdala has been long known to participate in emotional processes, especially those involving conditioned or learned fear (Fanselow & Ledoux, 1999; Kim & Jung, 2006)). It would be therefore be plausible that the amygdala should enter into decisions about when to feed and forage if particular lighting conditions have been associated with danger. The results presented here show that if unsignaled shock is presented in the dark phase, amygdala dependent fear reversed circadian feeding rhythms from nocturnal feeding to diurnal feeding. Rats also decreased activity during the dark phase and increased activity to the light phase to display an arrhythmic like behavior. One can suppose that these changes to feeding and foraging were due to Pavlovian like processes. Indeed, there has been some evidence to show non photic cues, through associative learning, can induce phase shifting and entrain circadian rhythms (Amir & Stewart, 1996). However, upon receiving unsignaled shocks in the dark phase, rats displayed anticipatory activity and feeding up to 2 hours *before* the transition to the light phase. If the reversal of circadian feeding and arrhythmic activity were strictly due to Pavlovian-like processes it would be difficult to understand why rats would increase anticipatory activity before the onset of a phase with diminished risk of getting shocked. Therefore, these results suggest the existence of a "fear entrained oscillator" whose function is to keep track of times of danger and safety. Recent evidence has shown the existence of clock genes in the central nucleus of the amygdala (CeA) and the basolateral amygdala (BLA) to exhibit daily rhythms of expression of a clock gene *Per 2* normally under the control of the master clock, the SCN. These clock genes have been found to be sensitive to hormonal changes that normally accompany emotional states (Lamont, Robinson, Stewart, & Amir, 2005). It would therefore be plausible that clock genes in the amygdala, sensitive to changes in emotional state, can anticipate times of danger and safety. The results of Experiment 2 show that very well.

Amygdala lesion rats did not change circadian feeding or activity patterns or show any anticipatory behavior.

It would be interesting to note whether the amygdala itself or the amygdala interacting with the SCN to be necessary to reverse circadian feeding and activity. Experiment 2 shows that SCN lesion rats with intact amygdale displayed arrhythmic feeding and activity during baseline. However, upon the presentation of shocks in the dark, rats did not increase feeding during the non-shocked phase like sham controls, but predominately decreased feeding during the shock phase. Additionally, SCN lesioned rats could not anticipate the termination of the dark phase and consumed pellets after the transition to the light phase. These results suggest that both the SCN and the amygdala are necessary to adapt to fear related changes to circadian behavior. Recently, both the dorsomedial nucleus of the hypothalamus (DMH) and the SCN have been suggested to participate in a multiple oscillatory system to regulate food anticipatory activity (FAA) found with restricted feeding schedules (Acosta-Galvan et al., 2011). Perhaps, the SCN interacting with the amygdala is another oscillatory system, to adapt to situations where resources must be gathered under some restricted time window involving a predatory threat.

In so much that unsignaled shock caused reversal of feeding patterns and arrhythmic locomotor activity, signaled shock preserved nocturnal feeding and activity. Much research has gone into the differences between the phasic or temporary fear found in signaled environments vs. the sustained fear in unsignaled environments (Davis et al., 2010; Miles, Davis, & Walker, 2011). Perhaps the rats in the signaled shock environment do not change their feeding and activity patterns because phasic responses to fear can be attenuated by avoiding shocks and thereby maintain nocturnal behavior. In contrast, unsignaled shock could create a sustained fear response that then could compete with central rhythms to induce diurnal feeding and foraging.

The primary goal of this study was to explore how amygdala coded fear can compete for circadian behaviors with an internal central rhythm (i.e. SCN) dictated by the external lighting conditions. Our observations suggest an intriguing possibility that the amygdala and the SCN may interact as a fear entrained oscillator to adapt to nocturnal predatory threats by predicting foraging times of danger and safety.

Chapter V. General Conclusions

Summary of results

The studies presented in this dissertation explored a functional role of the amygdala toward guiding decisions on feeding and foraging under the risk of danger. In the first study, rats that received unpredictable shock in the foraging area altered decisions involving feeding intake and feeding location. In contrast, amygdala damaged rats did not change feeding strategies, but were still able to avoid dangerous places. These results highlight a unique role of the amygdala to alter decisions of highly motivated behavior involving resources (i.e. food) under an unpredictable predatory threat. However, the amygdala was not necessary for voluntary avoidance of dangerous places in the absence of a competing motivation. The results of Chapter III highlight this very well. Under a signaled environment, rats with a damaged amygdala were eventually able to voluntarily avoid the shock during the signal presentation, suggesting the existence of compensatory fear circuits. However, the avoidance latency when pressing for food was much higher compared to the avoidance latency of controls that were pressing for food. Additionally, the pressing rate of amygdala lesioned animals in Experiment 1 in Chapter I increased with unpredictable shock, but the pressing rate of intact animals decreased to shock. Taken together this suggests amygdala's role to modify approach behaviors of highly motivated behavior, but not exclusively to avoidance.

The final study in this dissertation involves a similar role of the amygdala to alter the timing of feeding and foraging patterns and thereby minimize predatory threat without compromising total caloric intake. Rats with amygdalar lesions did not reverse circadian feeding or show any anticipatory behavior. However, rats with SCN lesions were able to decrease intake to shock, but were not able to anticipate periods of danger and safety. These results suggest the

existence of a fear entrained oscillator comprised of both the amygdala and the SCN to adapt circadian behaviors to minimize predatory threat.

Theories on emotion in decision making with the closed economy

The amygdala has long been known to participate in emotion, particularly fear related processes. There have been many studies to show amygdala's contribution to conditioned fear using Pavlovian or Instrumental conditioning paradigms. However, recent research in humans and rats has also shown the amygdala to be involved in decision making, especially those involving risk (Brand, Grabenhorst, Starcke, Vandekerckhove, & Markowitsch, 2007). Individuals with isolated amygdala damage lack autonomic responses to emotional stimuli involving reward and punishment (Gupta et al., 2011). Individuals also do not react aversively to monetary loss, and have abnormal responses to betrayals of trust (Koscik & Tranel, 2011). Taken together, it has been hypothesized that the function of the amygdala in decision making is to provide immediate emotional responses to significant environmental circumstances and inform higher cortical areas such as the ventral medial prefrontal cortex (VMPFC) to guide decision making (Bechara & Damasio, 2005). However, the results of this dissertation show a slightly different picture of amygdala's role in decision making. Rats with amygdalar lesions continued to show emotional responses to shock by displaying voluntary aversive behavior. It was only during the procurement of a highly motivated resource (i.e. food) where the rats showed the greatest emotional deficits and subsequent disregard for the risk of danger. These results highlight a possible role of the amygdala during maladaptive decisions where the cost of a highly motivated behavior is disregarded. Indeed, compulsive behavior in the presence of an increasing cost of punishment is a hallmark characteristic of addiction (Pelloux, Everitt, & Dickinson, 2007). In fact, there have been many studies implicating the amygdala in drug addiction and relapse

(See, Fuchs, Ledford, & Mclaughlin, 2003). Current theories of the amygdala and addiction focus on the learned associations in pairing a cue with the reward. Since, the amygdala has long been known to participate in appetitive as well as aversive Pavlovian conditioning, much research has focused on the drug associated cues formed during learning, that have been thought to play a critical role in relapse to drug seeking behavior (Everitt et al., 1999). However, few studies have investigated amygdala's role in decision making involving drug seeking behavior in the face of punishment. Perhaps amygdala neurons encoding the risk of danger are suppressed and cannot compete with highly motivated behavior such as compulsive drug seeking. Or perhaps amygdala neurons themselves are not encoding the risk of danger adequately to inform higher decision areas. Future research is needed to better understand the relationship of how the amygdala and higher cortical areas involved in decision making interact during competing motivations that involve a risky highly motivated behavior.

Fear's contribution to abnormal biological rhythms

Most living organisms have daily cycles in behavior and physiology that are coordinated by a central oscillator. However, in certain circumstances, environmental stimuli have been known to decouple peripheral rhythms from the central rhythm and thus create abnormal circadian profiles. The results of Chapter IV show that amygdala coded fear can create abnormal circadian rhythms of feeding and activity. Abnormal rhythms have been found not only in fear related disorders such as stress, but addictions, cancers, and individuals with mental illness (McClung, 2007; Sephton, Sapolsky, Kraemer, & Spiegel, 2000; Souêtre et al., 1989). Perhaps, the abnormalities in circadian rhythms found in many mood disorders is the result of a competition between an adaptive response to aversive stimuli and the environmental lighting conditions. Or perhaps, instead of environmental zeitgebers, the abnormal rhythms are found at

the genetic level (Falcón & McClung, 2009). Whatever the case, it is clear that changes in emotional state have a direct impact on biological rhythms. However, few studies have been reported investigating fear related structures and biological rhythms. Part of the challenge has been that no direct projections from the amygdala or other fear related structures to the SCN have been currently identified. Areas such as the lateral hypothalamus and the paraventricular nucleus of the thalamus (PVN) has been suggested to connect circadian timing information with motivational aspects of behavior, but this has not been directly investigated (Moga, Weis, & Moore, 1995; Price, 2003; Watts, Swanson, & Sanchez-Watts, 1987). Although no afferent projections from the amygdala have been found in the SCN, efferent projections to the amygdala from the SCN have been identified using various techniques (Stephan, Berkley, & Moss, 1981; Swanson & Cowan, 1975). Perhaps under normal conditions, the SCN regulates the clock gene rhythm in the amygdala. However, upon experiencing aversive stimuli, perhaps clock genes in the amygdala can decouple from the SCN and anticipate danger to allow for the gathering of resources under safe conditions. Future research needs to elucidate a functional role of clock genes in fear related structures.

The closed economy and theories of memory systems

Contemporary theories on the brain's memory systems include the compartmentalizing of information to specialized structures or a competition between primary and secondary circuits that can compensate in the event of a failure (Fanselow, 2010; Squire, 1992). However, recent data have given evidence for more complex interactions between memory systems (Kim & Baxter, 2001). In the closed economy, some behaviors like instrumental avoidance responses showed a transient dependence on the amygdala (i.e. during acquisition) that was later consolidated. However, other behaviors, like decisions on feeding, continued to be amygdala

dependent. Still other behaviors, such as the anticipatory feeding and activity were dependent on both the amygdala and the SCN. These results suggest the flexibility of multiple systems to interact in diverse ways that depend on the environmental circumstances. Perhaps this dynamic and fluid structure between multiple memory systems is one avenue of how an organism adapts to environmental challenges.

Concluding remarks

The results of this dissertation utilized a closed economy paradigm to investigate a functional role of the amygdala in a risky foraging environment. The amygdala was found to be necessary to guide decisions on highly motivated behavior and to anticipate periods of danger, but not to decisions to avoid an aversive experience. Future work needs to outline how the amygdala and other fear structures work together to guide the delicate balance between the needs of survival with the cost of predation.

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VITA

Earnest Kim was born in Los Angeles, California, but currently resides in Seattle, Washington. He received his Bachelor of Arts in Chemistry from Carleton College in Northfield, Minnesota. In 2014, he earned a Doctor of Philosophy in Psychology at the University of Washington