The effects of rostral anterior cingulate lesions on avoidance and decision-making processes in rats living in a naturalistic, risky foraging environment

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A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Science

University of Washington

2018

Committee:

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Program Authorized to Offer Degree:

Psychology

University of Washington

Abstract

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In rodents, the rostral segment of the anterior cingulate cortex (rACC) is thought to be primarily involved in affect-related processes and behaviors. However, most rodent studies investigating the rACC measure a limited set of variables over brief periods, possibly providing only a partial picture of its hypothesized functions. The present study employed a longitudinal, ethologically relevant risky-foraging paradigm to further elucidate the rACC's role in affect-related behavior, and in decision-making under adverse conditions. Rats lived in novel chambers consisting of a safe nest zone and "risky" foraging zone where unpredictable foot shocks can be delivered for an extended period of time. Lesions of the rACC had little effect on avoidance of foot shock, but interfered with initial threat-induced foraging suppression. The results of this experiment conflict with findings showing that rACC lesions disrupt avoidance acquisition and decrease the amount of effort animals are willing to exert for food reward.

Introduction

The anterior cingulate cortex (ACC) is a rather elusive structure, as it has been implicated in many diverse functions, including—but not limited to—decision making (Botvinick, 2007; Hillman & Bilkey, 2010; Holec, Pirot, & Euston, 2014; Hyman, Holroyd, & Seamans, 2017; Kennerley, Walton, Behrens, Buckley, & Rushworth, 2006; Schweimer & Hauber, 2005; Shenhav, Botvinick, & Cohen, 2013), foraging behavior (Li et al., 2012; Zhong et al., 2017), attention (Janice L. Muir, 1996; Wu et al., 2017) pain processing [for review; (Papini, Fuchs, & Torres, 2015)], and fear learning/behavior (Etkin, Egner, & Kalisch, 2011). The ACC is a midline cortical structure situated directly above the majority length of the corpus callosum. In the rat, the structure is part of the medial prefrontal cortex along with prelimbic cortex, infralimbic cortex, and the medial precentral area, also known as secondary motor cortex or area FR2 (Krettek & Price, 1977; Van Eden & Uylings, 1985). Historically, the ACC in the rodents has been further subdivided into the rostral anterior cingulate cortex (rACC), which is comprised of perigenual Brodmann's areas 24b, portions of perigenual 24a and caudodorsal area 32, and the caudal anterior cingulate (cACC), which is comprised of portions of postgenual Brodmann's areas 24a and 24b (Vogt & Peters, 1981). The rACC has been described as the affective region of the ACC, as opposed to the cACC being considered the evaluative portion of the ACC (George Bush, 2000; Malin, 2007). Not surprisingly, the rACC has substantial (although sub-nuclei specific) connections with the amygdala (Cassell & Wright, 1986; Krettek & Price, 1977; McDonald, 1991; Sarter & Markowitsch, 1983 ; Sripanidkulchai, Sripanidkulchai, & Wyss, 1984).

Early studies investigating the ACC's contribution to fear learning/behavior have demonstrated that lesions to this area disrupt the acquisition of active avoidance responses to aversive foot shock (Gabriel, Kubota, Sparenborg, Straube, & Vogt, 1991; Kimble and Gostnell, 1968; Peretz, 1960). Later studies have implicated specifically the rACC in conditioned responding to a variety of aversive stimuli. Lesion of the rACC reduce conditioned place avoidance to compartments paired with chemically-induced, inflammatory hind paw pain (Gao, Ren, Zhang, & Zhao, 2004; Johansen & Fields, 2004; Johansen, Fields, & Manning, 2001; Ming Yi, 2011). Similarly, lesion of the rACC (but not cACC) leads to impairment in the consolidation and retrieval of inhibitory avoidance memory in step-through inhibitory avoidance paradigms utilizing shock as the aversive stimulus (Liu, Zheng, & Li, 2009; Malin, 2007).

Research employing classical conditioning fear paradigms, on the other hand, have shown that lesioning of the rACC disrupts the acquisition of conditioned responding to tone paired with hind paw CO_2 laser pulses (Kung, Su, Fan, Chai, & Shyu, 2003), as well as to tone paired with foot shock (Bissiere et al., 2008). Finally, pharmacologically inhibiting rACC NMDA (NR2B) receptors during context fear conditioning attenuates context fear memory, whereas inhibiting protein synthesis in the rACC at time points after context fear conditioning interferes with memory consolidation and reconsolidation (Einarsson & Nader, 2012). From decades of research, it is apparent that this elusive structure plays an important role in several key aspects of associate fear learning. However, while these studies have been essential in shedding light on the rACC's function in fear learning and behavior, the paradigms used are typically conducted in small chambers, are of short duration and measure very specific behaviors. In consequence, the animal's behavioral repertoire faces restriction and other insightful behavioral observations may be occluded (Pellman & Kim, 2016).

The current experiment sought to expand upon the rACC's contributions to fear-related behavior, and to examine its role in decision-making under threatening conditions by utilizing a longitudinal, semi-naturalistic foraging paradigm entitled, the “closed economy” (Fanselow, Lester, & Helmstetter, 1988; Helmstetter & Fanselow, 1993; Hursh, 1980; Kim et al., 2014; Pellman et al., 2015; Pellman, Schuessler, Tellakat, & Kim, 2017). In this paradigm, animals live continuously and generally undisturbed in modified operant chambers partitioned into two major zones: a bedded, “safe” nesting area, and a “risky” foraging area (Fig. 1). The foraging area contains lever(s) which when pressed, and under certain experimenter-defined contingencies, yield food pellets in adjacent feeder ports. The area also contains a water port. After baseline periods of foraging behavior assessment, pseudo-random foot shocks are introduced daily in the foraging zone for an extended period of time. After this period, shock is terminated and post-shock behavior is assessed (Fig. 2).

Given the paradigm’s ability to measure a multitude of variables (avoidance, meal patterns, lever-pressing strategy, etc.) over long periods (1.5-2 months, 23 hours/day), the closed economy presents an attractive way to further parse and characterize the ACC’s wide range of proposed functions and contributions to behavior. Moreover, the design takes a more ethologically relevant approach, as it simulates a recurring and unavoidable problem in nature: approach food and avoid danger (Lima & Dill, 1990; Pellman et al., 2017). By generating a more naturalistic scenario that the brain’s fear system has likely evolved to solve, a more comprehensive set of fear-related behavior becomes measurable (Pellman & Kim, 2016). It is also possible that such ethologically relevant scenarios may distinctively engage neural substrates underlying fear behavior as opposed to more traditional, yet contrived paradigms. It was hypothesized that lesions specific to the rACC would decrease avoidance of shock and suppress foraging beyond that of control animals during the shock phase of the experiment.

Methods

Subjects:

Male Long-Evans rats initially weighing 325-350 g were immediately single-housed in closed economy chambers upon arrival to the animal facility in one of two designated rooms in the Department of Psychology at the University of Washington (accredited by the Association of Assessment and Accreditation of Laboratory Animal Care). Rooms were set to a reverse 12 hour light-dark cycle, and white noise (70 dB) was continuously emitted through speakers to mask outside noise. Both rooms were matched for luminosity and temperature. Control and experimental groups were countered balanced between both rooms. Animals were tested for 23 hours/day, and only removed during the last hour of the light cycle, where they were briefly housed in a separate vivarium (also within the Department of Psychology at the University of Washington and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care) for closed economy room maintenance. While in the vivarium, animals were allowed access to water ad libitum. The overall experiment and procedures were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and reviewed by the University of Washington Institutional Animal Care and Use Committee.

Closed Economy Task:

Dimensions of the closed economy chambers measured 74.3 x 25.4 x 33 cm (length x width x height). The chambers were partitioned into two separate zones: a plastic tray nest area covered with betachip sawdust, and a foraging area with a 32 stainless steel rod (4.5 mm diameter) floor connected to a precision animal shocker (Coulbourn Instrument) for shock administration. Two identical operant levers/feeding ports were situated in the foraging zone; one lever/port located on the chamber wall adjacent to the nest area, and the other on the same wall but further from the nest. A single lick meter/water port was centrally located in between the levers. Infrared cameras were mounted above each chamber (Fire-I B/W Board camera; Unibrain) and connected to a computer for animal tracking. ANY-maze software in communication with the ANY-Maze interface was used to track the animal, measure lever pressing/water licks, implement lever contingencies, operate food dispensers and administer pseudo-random foot shock during the "shock" phase of the experiment.

Upon housing, animals were acclimated to the chamber and shaped to press both levers. Animals were gradually shaped over 12-14 days to lever press 25 times in order to gain access to a continuous reinforcement schedule (FR25-CRF). This compound reinforcement schedule was chosen to simulate effort required to obtain food in nature. The FR threshold was doubled every two days, except when transitioning from 16 to 25 lever presses. A break in the FR25 lever press threshold was counted as a "meal," whereas the amount of pellets obtained after every FR25 threshold break was counted as "meal size." In all cases, lever contingencies were reset if more than one minute elapsed between lever presses. After stable lever pressing at FR25-CRF, baseline foraging behavior was assessed for 7-8 days ("pre-surgery baseline").

Immediately following the initial baseline, subjects underwent sterile survival-surgery over the course of 2 days, and were allowed to recover in closed economy chambers until pre-surgery baseline foraging and locomotor behavior returned (approximately 5-7 days). Afterwards, a post-surgery baseline was conducted for an additional 7 days ("post-surgery baseline"). Following the post-surgery baseline, the shock period began. Pseudo-random foot shocks (0.8 mA, ~ 2 shocks/hour) were delivered daily in the foraging area for 16 days. These shock parameters were chosen to maximize changes in foraging behavior without creating an overly aversive environment for the subjects (Helmstetter & Fanselow, 1993). While the animal was in the foraging zone, shock continued for 10 seconds, or was immediately terminated if the animal retreated to the nest area. Finally, after the 16 day shock period, shock was terminated and a 16 day post-shock extinction (“extinction”) phase ensued, the end of which concluded the experiment.

Surgery:

Rats were randomly assigned to receive electrolytic lesions of the rACC (“lesion” group, n = 4) or sham lesions (“sham” group, n = 3) before the start of the experiment. Animals were anesthetized with a ketamine/xylazine solution (30 mg/kg ketamine and 2.5 mg/kg xylazine, i.p.) before undergoing stereotaxic surgery. For the lesion group, bilateral lesions were made at the following coordinates from bregma: AP + 1.6 mm, ML ± 1.3 mm, DV -3.7 mm and AP + 2.7 mm, ML ± 1.3 mm, DV - 2.9 mm. All lesions were made at 15° angle of entry. Epoxy coated insect pins (size 00; for lesions at AP; +1.6, 1 mm exposed tip, current = 0.5 mA, 20 second duration; for lesions at AP +2.7; 0.5 mm exposed, 0.5 mA, 15 second duration) were used. Animals in the sham group had electrodes lowered 1 mm above lesion targets, but no current was passed.

Histology:

At the conclusion of the experiment, rats were overdosed with Beuthanasia and perfused intracardially with 0.9% saline followed by 10% buffered formalin. Brains were extracted and left overnight in the buffered formalin solution. The following day, brains were transferred to a 30% sucrose solution where they remained until sunk. Brains were then sectioned into 60 µm sections and mounted onto slides with a gelatin mounting medium. Finally, sections were stained with crestyl violet and Prussian blue for histological examination and lesion reconstruction.

Statistics:

All data are presented as daily means ± SEM. Total meals and meal size variables were normalized to account for individual baseline differences in food consumption patterns. Normalized post-surgery, shock, and extinction phase values are expressed as post-surgery values/pre-surgery averages, shock values/post-surgery baseline averages and extinction values/post-baseline averages, respectively. Mann-Whitney U non-parametric tests (2-tailed) were used to compare group distributions of daily values during each phase. For phases lasting longer than one week, the first and second half of each phase (8 days/half; shock and extinction) were analyzed separately. All data were analyzed using custom Python scripts and SPSS 19. All graphs were generated by GraphPad Prism 7.

Results

There were no significant differences in overall locomotor activity (Fig. 3) or foraging behavior (Fig. 6-8) between groups during post-surgery baseline. Likewise, during shock and extinction phases, there were no significant differences between overall locomotor activity. No differences between groups were detected in overall time spent and shocks received in the foraging zone throughout the shock phase (Fig.4-5). However, foraging patterns were markedly different between groups during the first week of shock (Fig.6-7). Specifically, lesioned animals obtained more pellets ($p < 0.001$) and ate more meals ($p = 0.001$) than sham animals. Meal size was not significantly different throughout the experiment (Fig. 8). Interestingly, lesioned animals also made fewer entries into the foraging area during this time ($p = 0.014$), although both groups increased their amount of entries well above post-surgery baseline values (Fig. 9). Following the first week of shock, lesioned animals' foraging pattern resembled that of sham controls, and were no longer significantly different. The majority of animals preferred the proximal levers before shock; thus, lever preference switching during shock was not measurable given the small sample size. No significant differences in avoidance or foraging behavior were detected during extinction. Histological examination revealed lesion damage extending from + 0.70 to + 3.20 mm AP relative to bregma, with the greatest damage occurring between + 2.20 to + 1.6 mm AP (Fig 10). In all cases, secondary motor cortex was damaged.

Discussion

The present study investigated the long-term effects of rostral anterior cingulate lesions on avoidance and decision-making behavior using an ethologically-relevant foraging paradigm. The effects of rACC lesion did not affect overall locomotor or foraging behavior measured during a post-surgery baseline, suggesting no overt motor deficits or alterations in appetitive motivation in the lesion group prior to threat onset. From the initiation of the shock phase, however, differences in foraging patterns emerged. Lesioned animals, although experiencing a slight initial decrement in total pellets consumed, ate considerably more than sham animals during this period. Similarly, they obtained more meals during this period. Overall, lesions appeared to have blunted the effect of diffuse threat on meal pattern reorganization, while avoidance of the threat was largely intact as measured by time spent and shocks received in the foraging region. By the second half of shock, both groups displayed similar foraging behavior, and there were no differences in behavior at any time during the final extinction phase.

In considering the similarity in avoidance between both groups, and in regards to previous literature demonstrating a lack of effect of rostral anterior cingulate lesions on behavior in the elevated plus maze paradigm (Bissiere, McAllister, Olpe, & Cryan, 2006; Li et al., 2012), the present findings argue against a role of the rostral anterior cingulate cortex in innate fear/anxiety. Rather, the deficit appears to be more related to a lack of inhibitory control over appetitive drives normally suppressed in threatening situations. This notion aligns with theories of anterior cingulate cortex being primarily involved in allocating additional cognitive control during conflict and in updating default behavior based on changing environments/outcomes (Ebitz & Hayden, 2016; Shenhav et al., 2013). Thus, it is feasible that lesioned animals failed to exhibit appropriate top-down inhibition of feeding behavior, and in turn failed to adequately adjust during the initial threat onset. While on the surface lesioned animals appeared more efficient than controls in maintaining stable feeding patterns, in nature, such a lack of response to threat would be maladaptive. It is also unlikely that lesions affected overall behavioral flexibility, as there were no deficits seen during extinction.

Less entries into the foraging area compared to sham controls during the first week could have been due to the fact that lesioned animals, who obtained more food overall during that time, were more satiated than sham controls who ate less, and therefore did not have as great a need to enter the region. However, it cannot completely be ruled out that lesioned animals experienced less aversion to the threat of footshock during the first week, and thus engaged in less risk assessment behavior, such as maintaining a stretched posture in the nest while extending their heads into the risky foraging zone (Blanchard, Blanchard, Rodgers, & Weiss, 1990). Indeed, previous closed economy experiments imply that only during the highest levels of fear does feeding become suppressed (Kim et al., 2014). Future closed economy experiments can address this issue by varying shock averseness by means of shock density and/or intensity; if rACC lesions caused decrements in fear and enabled feeding to persist during threat introduction, then perhaps engendering a more fearful situation would overcome lesion effects and produce immediate foraging changes more similar to control animals. Conversely, if lesions did not affect fear but interfered with cognitive control and behavioral switching, then increasing averseness and consequently fear would not immediately suppress foraging to the degree of control animals, and would presumably produce results similar to the current experiment.

Overall these data appear to conflict with findings showing that rostral anterior cingulate lesions decrease free foraging of food pellets in an open arena—especially when a form of adversity is present, such as physical barriers or social threat (Li et al., 2012; Zhong et al., 2017). However, such differential lesion effects may be due to task specificity and lesion type—that is, shorter tests, different threat modalities (an identifiable, physical or social threat), and fiber-sparing excitotoxic lesions. Recent evidence shows that rACC lesioned rats work equally as hard as control rats to obtain food pellets in a lever pressing task using a progressive ratio schedule of reinforcement, implying that rACC lesions may affect foraging behavior only in certain adverse conditions (Hyman et al., 2017). These data also contrasts with older research showing cingulate lesions delay the acquisition of inhibitory avoidance responses—although, in those studies, damage extended well beyond the anterior cingulate cortex (Kimble & Gostnell, 1968; Peretz, 1960). It is unknown whether lesions in the present experiment could have caused deficits in long-term memory retrieval, because animals were never removed from the experimental context for more than one hour per day and thus chronically exposed to context cues.

Limitations of this study include small sample size and permanent inactivation methods. Electrolytic lesioning may result in the destruction of fibers of passage, potentially confounding results. Permanent inactivation could have also led to compensation by other brain areas, which may account for the transient effects seen during shock. Moreover, all experimental animals received damage to secondary motor areas, creating another confound. Future studies of the rACC using the closed economy paradigm aim to implement non-permanent methods of inactivation. To preserve minimal subject-experimenter interaction, and in light of the longitudinal design of the closed economy, traditional cannulated options are not desirable. One attractive solution is to use programmable, refillable minipump-cannula systems for long-term, regimented dosing of compounds to inactivate nuclei. The system also allows one to explore the effects of transient inactivation (or excitation) of the rACC in the same subject over long periods, as opposed to permanent manipulations. Apart from varying shock averseness, future studies may also seek to vary lever contingencies. Perhaps at a higher meal threshold, further differences will emerge between lesion and sham lesion groups.

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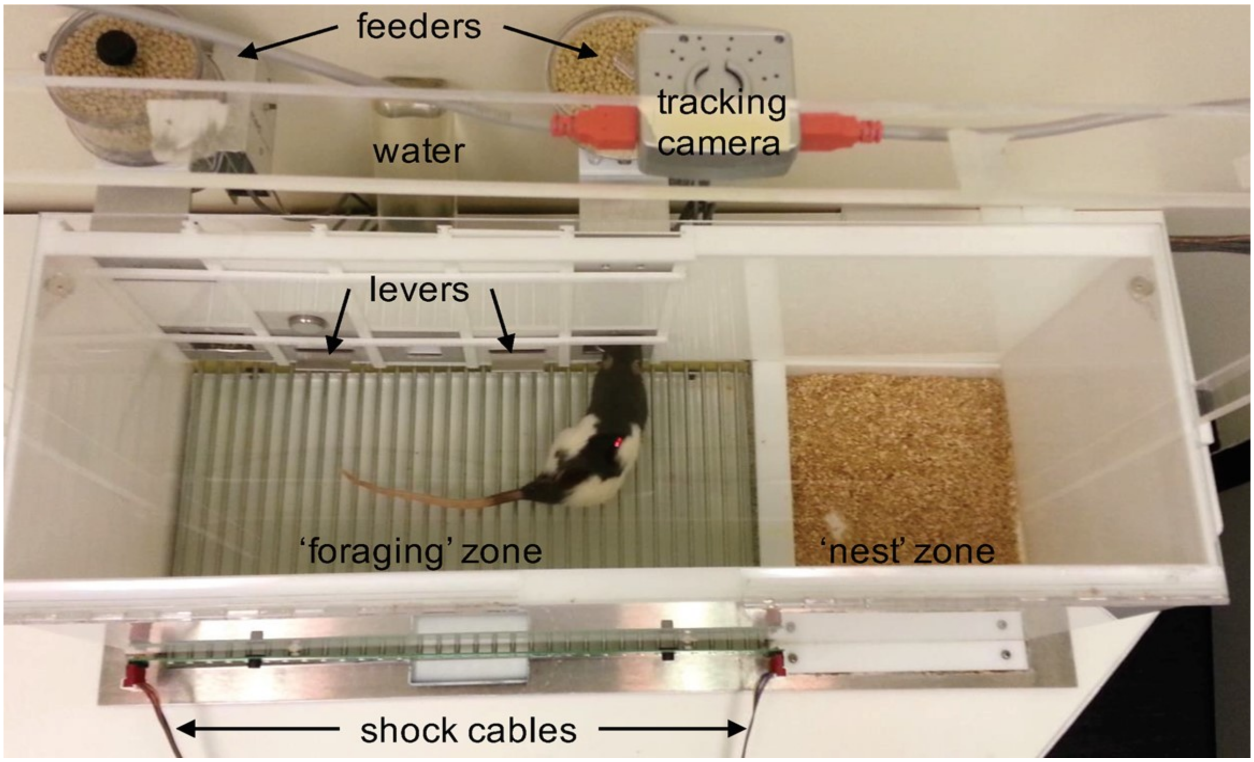

Figure 1. Closed economy chamber design. The apparatus is partitioned into two distinct regions; a safe, bedded nest zone and a risky foraging zone. The foraging zone contains levers/feeder ports (proximal and distal to the nest) for food pellet procurement. The floor of the foraging zone is comprised of a steel rod grid wired to a precision animal shocker (Coulbourn Instrument) for pseudo-random footshock delivery (~ 2/hr). A water port is situated in between levers. Animals are continuously tracked via an infrared cameras mounted atop the chambers, which are connected to a central computer running ANY-maze software.

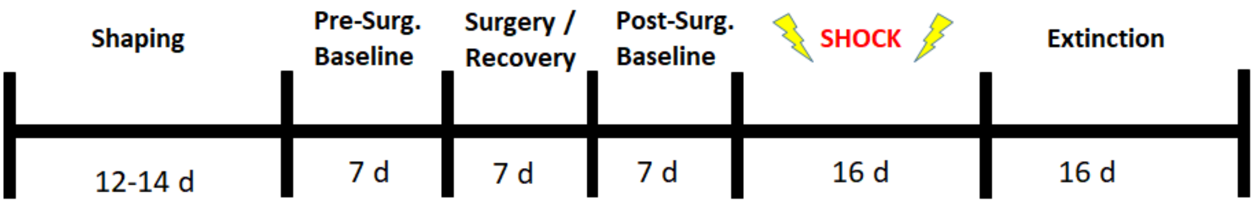

Figure 2. Experimental timeline. Animals were first shaped to lever press at a fixed ratio 25 – continuous reinforcement schedule. After lever contingencies were learned, baseline behavioral assessment occurred for 7-8 days, followed by stereotaxic surgery (rACC lesion: n = 4 or, rACC sham lesion: n = 3). A post-surgery baseline assessment was conducted following recovery before introduction of pseudo-random footshock in the foraging zone. The shock phase lasted 16 days, at which point shock delivery ceased and extinction behavior was measured for an additional 16 days.

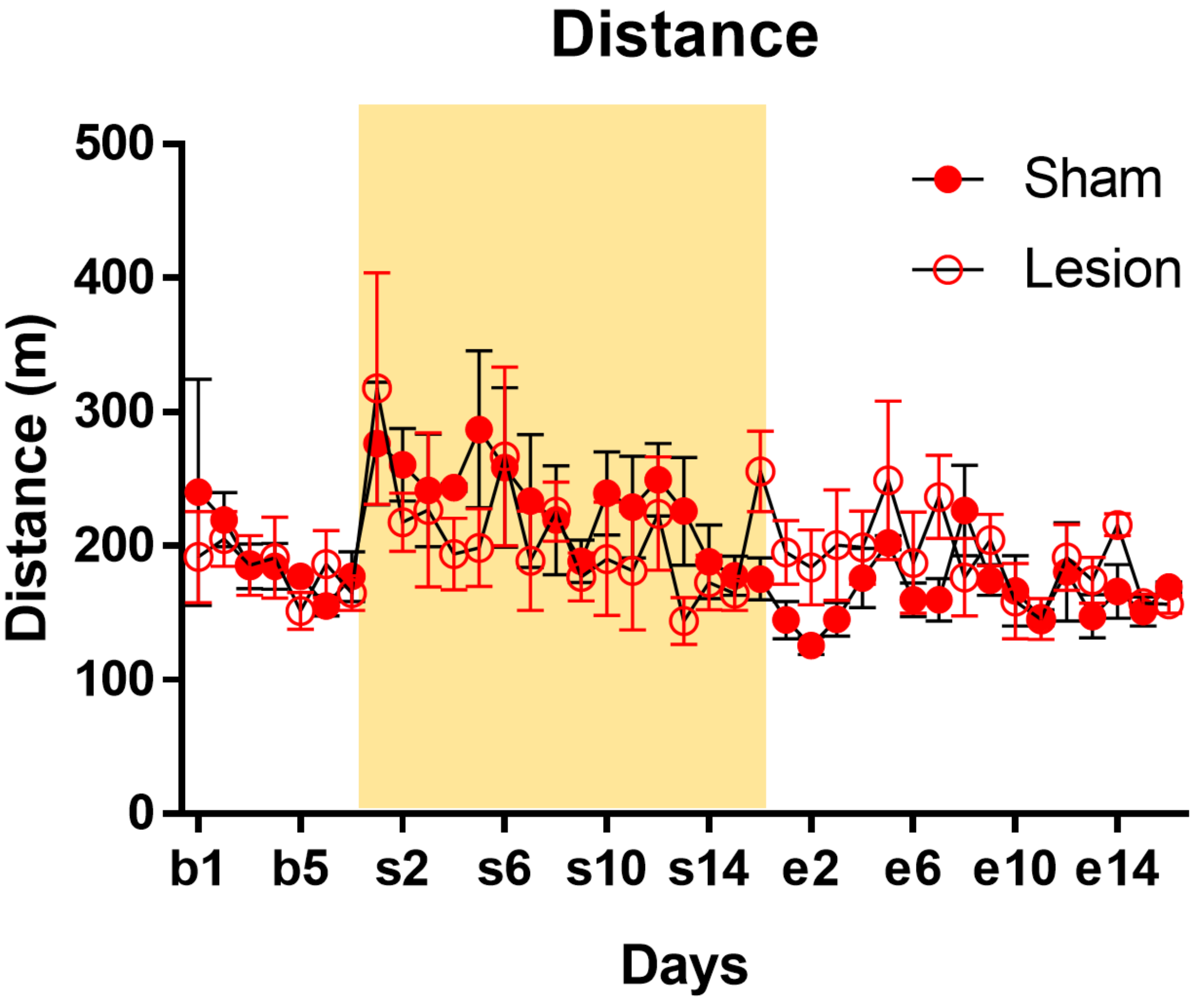

Figure 3. Overall locomotor activity. Overall locomotor activity throughout the experiment as measured by distance travelled in meters. Data points and error bars are daily group means/SEMs, respectively. “b” = post-surgery baseline days, “s” = shock days, “e” = extinction days. Yellow background indicates shock phase.

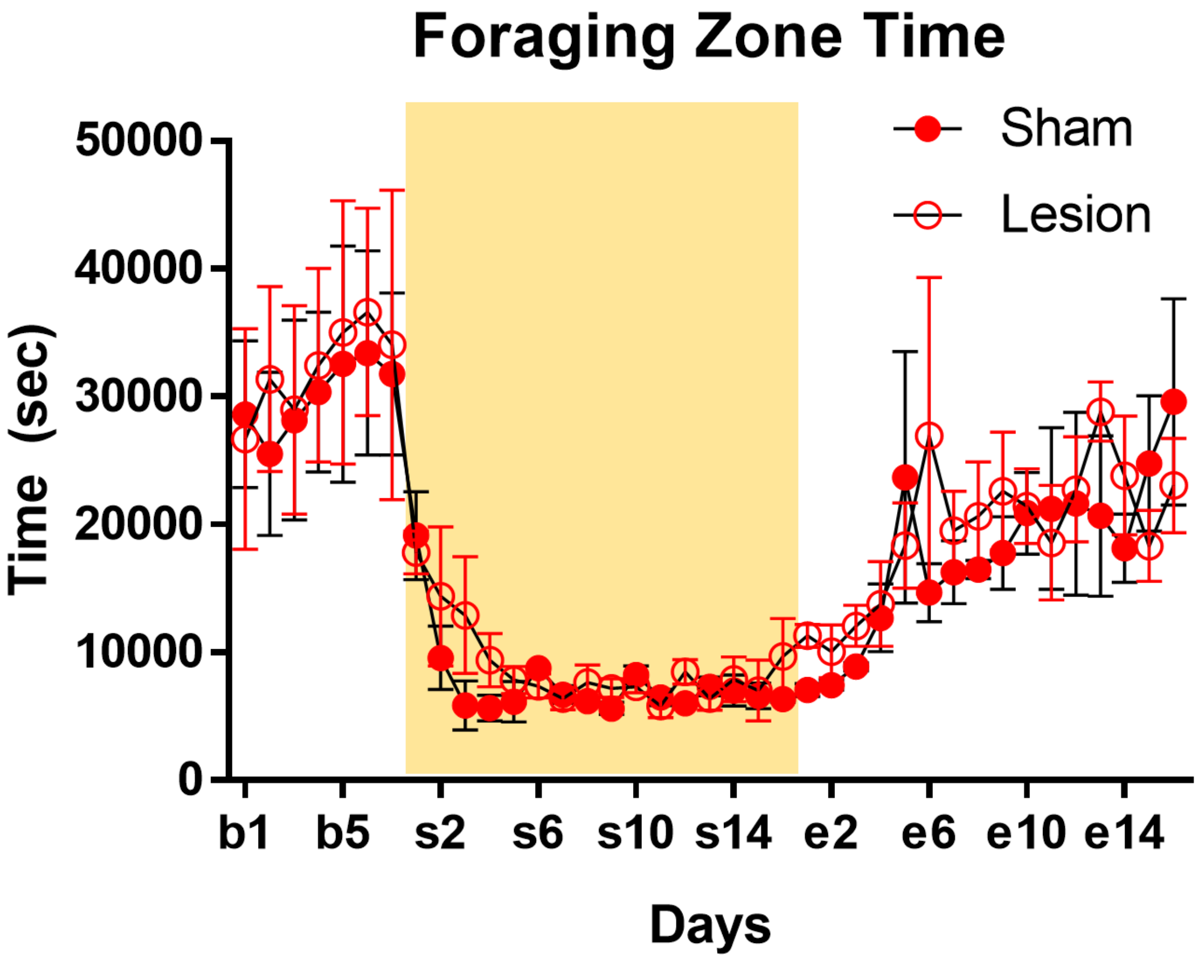

Figure 4. Overall time spent in the foraging area. Overall time spent in the foraging area measured in seconds. Data points and error bars are daily group means/SEMs, respectively. “b” = post-surgery baseline days, “s” = shock days, “e” = extinction days. Yellow background indicates shock phase.

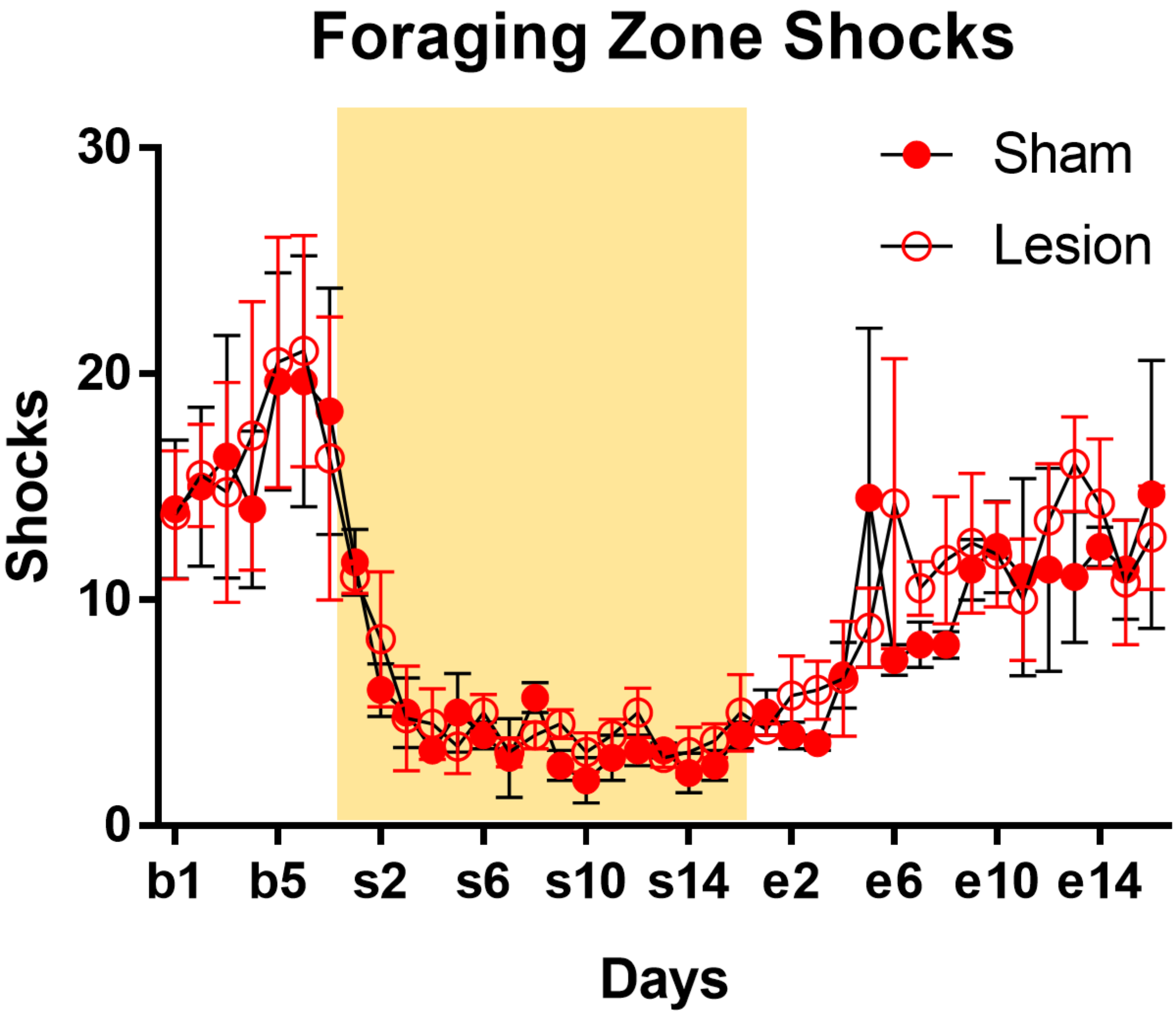

Figure 5. Shocks received. Total shocks received in the foraging zone throughout the experiment. Data points and error bars are daily group means/SEMs, respectively. "b" = post-surgery baseline days, "s" = shock days, "e" = extinction days. *Note that no actual shocks were delivered during baseline or extinction periods*; shock cables during these phases were not attached to the shock grid, and data points during such periods indicate theoretical shocks received based on shock delivery algorithm and animals' position within the foraging area at the time of shock trigger. Yellow background indicates shock phase, in which actual shocks were delivered to animals as described in the methods section.

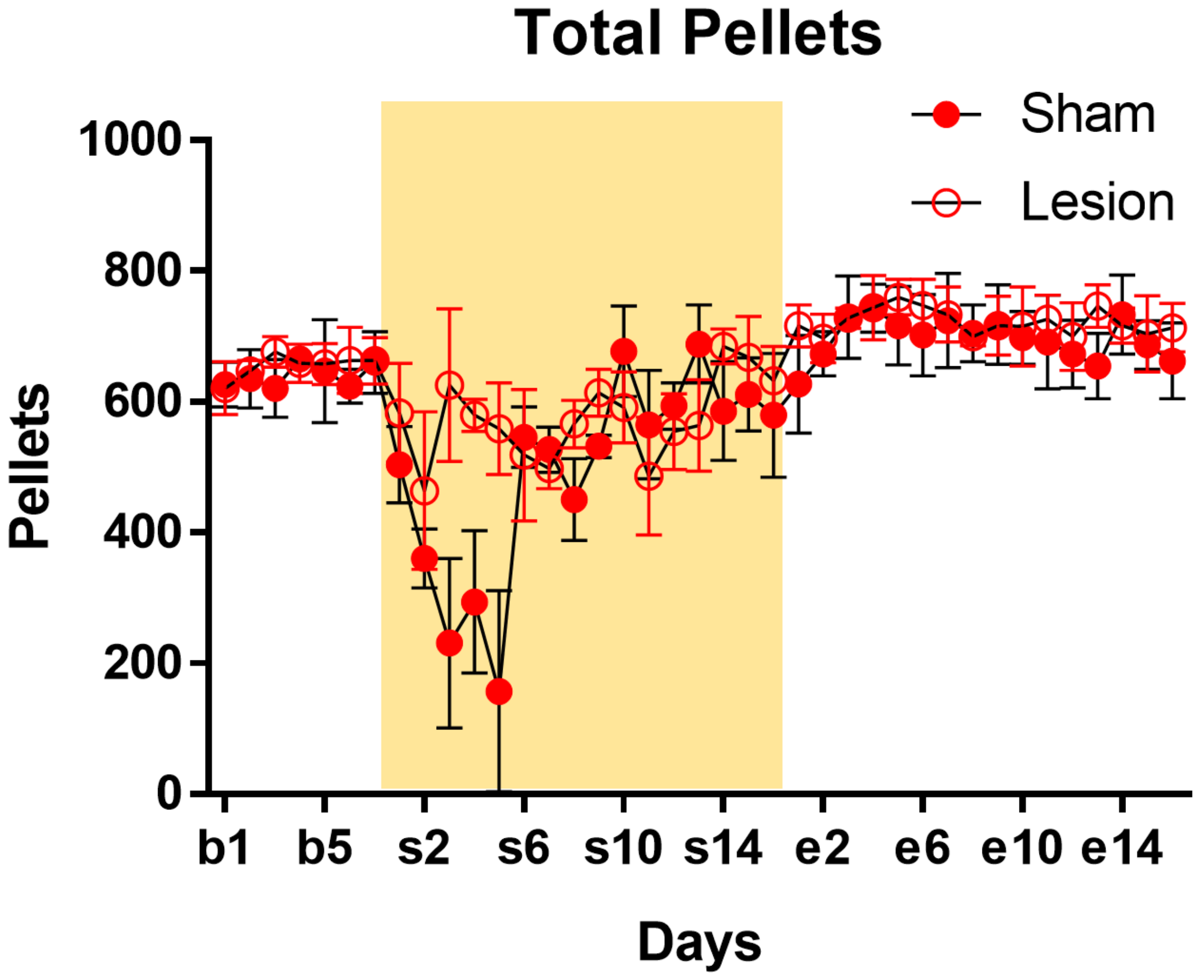

Figure 6. Total pellets consumed. Daily total amount of pellets consumed throughout the experiment. Data points and error bars are daily group means/SEMs, respectively. “b” = post-surgery baseline days, “s” = shock days, “e” = extinction days. Yellow background indicates shock phase.

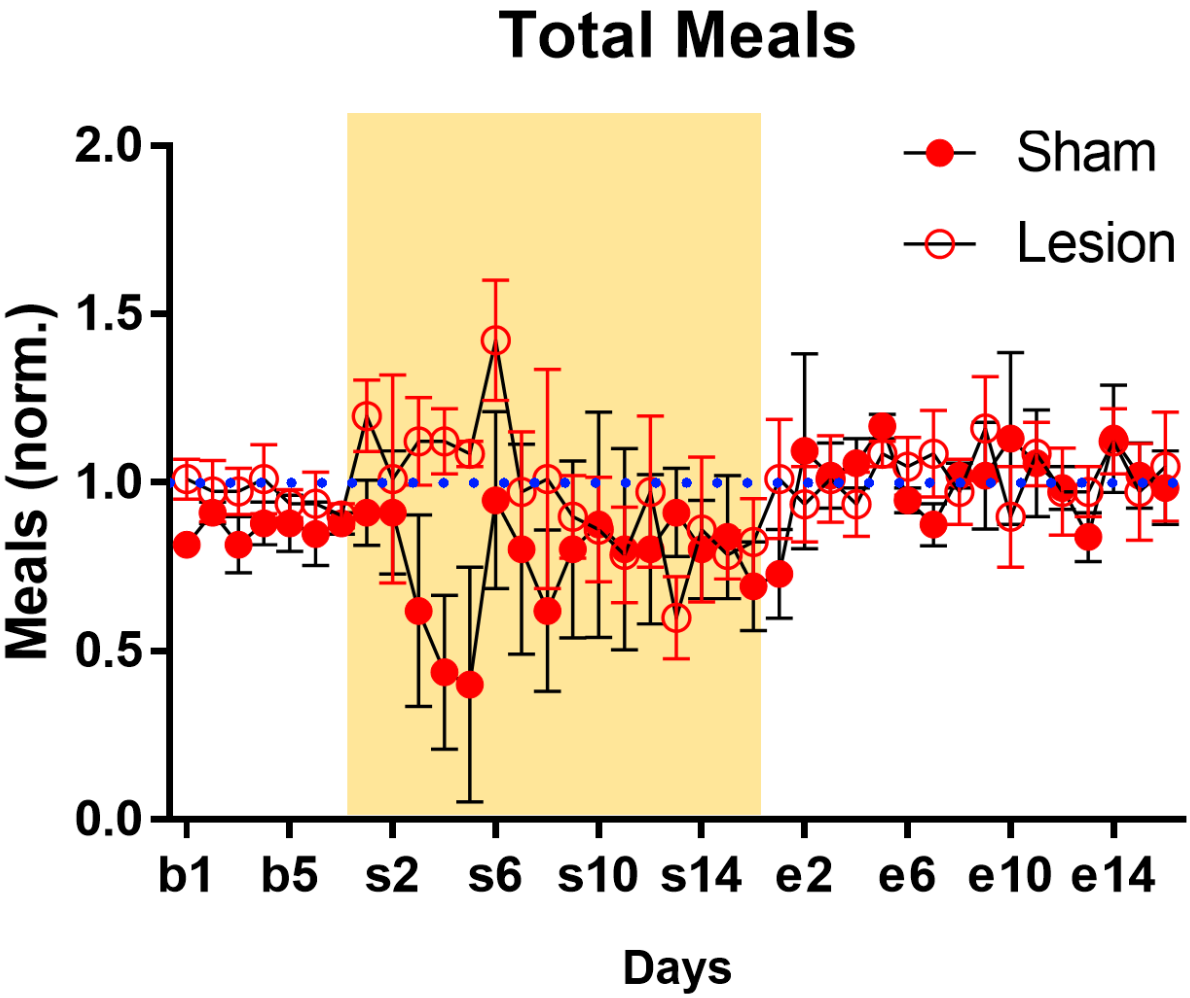

Figure 7. Total meals obtained. Daily amount of meals obtained throughout the experiment, normalized to post-surgery baseline averages. Meals are defined as threshold breaks in the FR25 lever schedule. Data points and error bars are daily group means/SEMs, respectively. “b” = post-surgery baseline days, “s” = shock days, “e” = extinction days. Yellow background indicates shock phase. Blue dashed line indicates baseline.

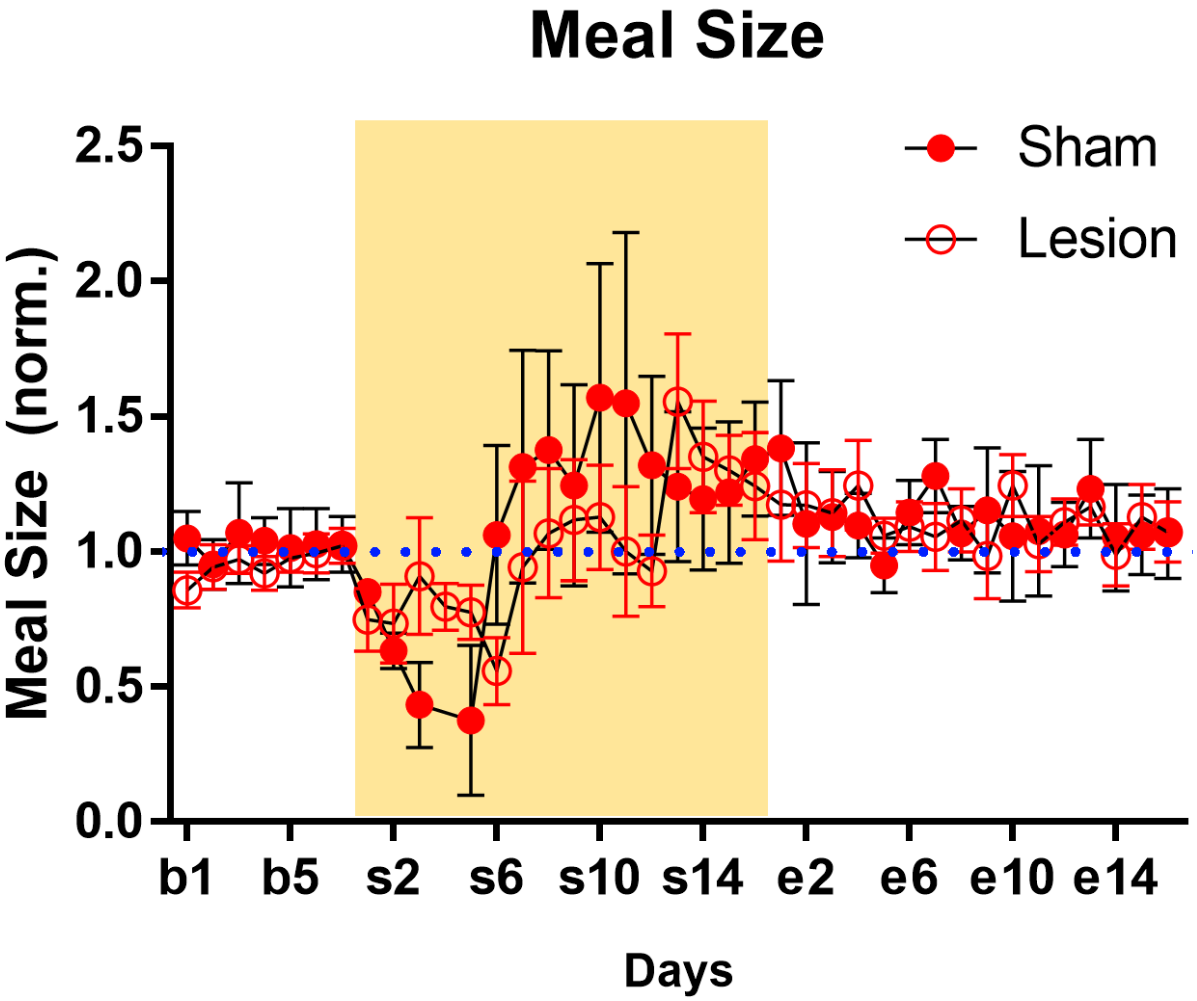

Figure 8. Pellets obtained per meal (meal size). Daily meal size throughout the experiment, normalized to post-surgery baseline averages. Meals size is defined as the number of pellets obtained after each break in FR25 lever schedule. Data points and error bars are daily group means/SEMs, respectively. “b” = post-surgery baseline days, “s” = shock days, “e” = extinction days. Yellow background indicates shock phase. Blue dashed line indicates baseline.

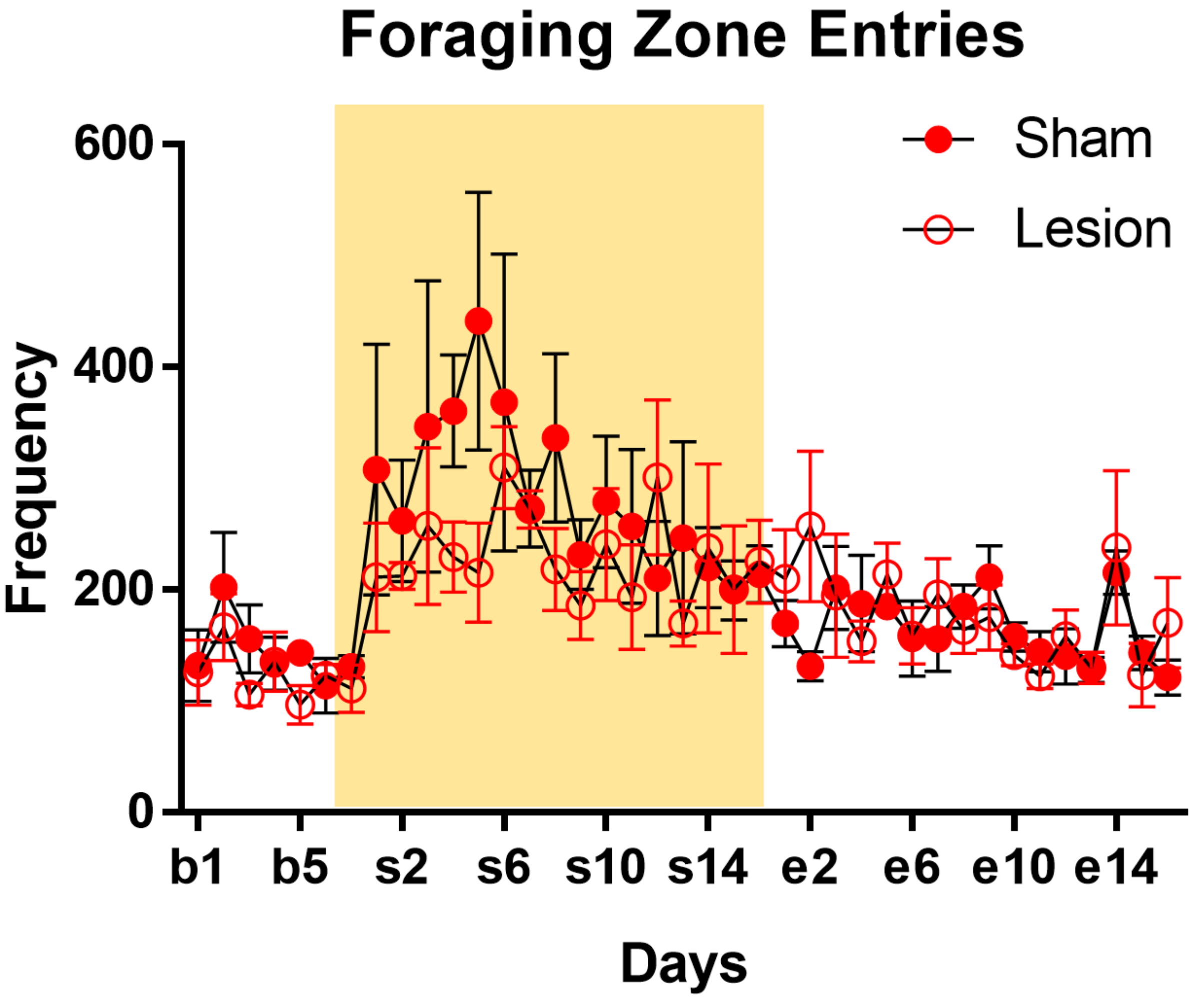

Figure 9. Total entries into the foraging zone. Daily crossings into the foraging zone throughout the experiment. Data points and error bars are daily group means/SEMs, respectively. "b" = post-surgery baseline days, "s" = shock days, "e" = extinction days. Yellow background indicates shock phase.

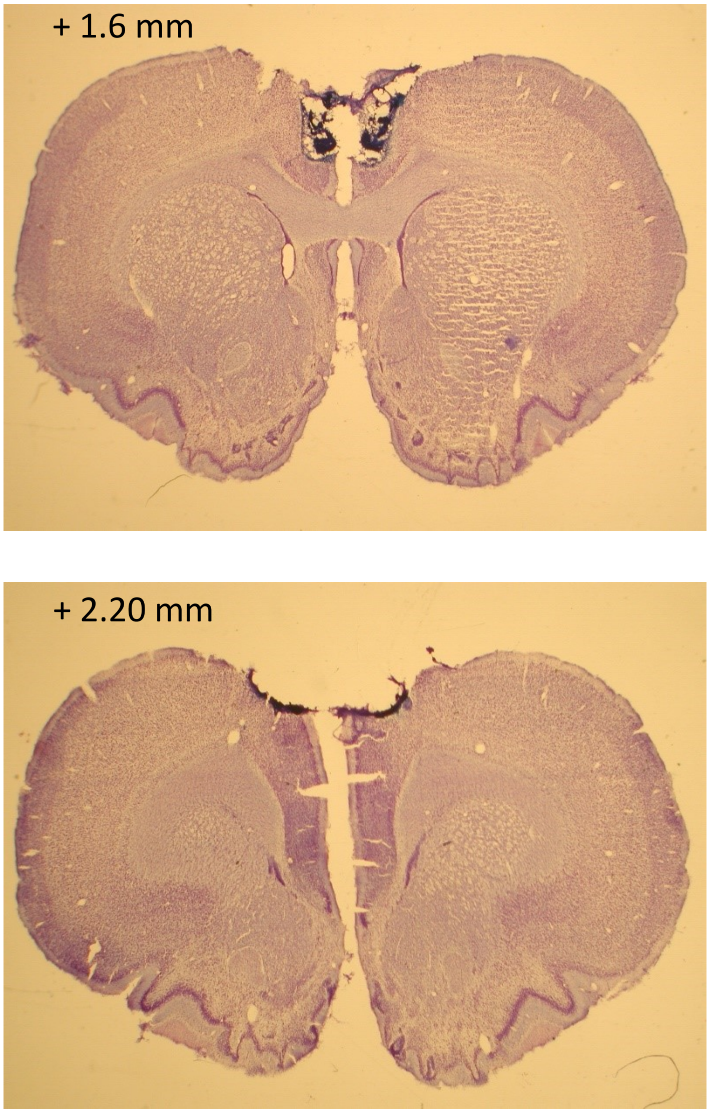

Figure 10. Histology. Representative histology depicting areas of greatest lesion damage (+ 1.6 mm/2.20 mm above bregma).