NEURAL CIRCUITRIES FOR THE CONTROL OF FEEDING DURING NOVELTY IN MALE AND FEMALE RATS

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Abstract

The influence of novelty on feeding behavior is significant and can override both homeostatic and hedonic drives due to the uncertainty of danger. The potential risks associated with consuming novel foods or consuming foods in a novel environment can lead to avoidance. While it is established that both novel foods and novel feeding environments can reduce or suppress feeding, it remains unclear how these two factors interact with each other to impact consumption and whether there are sex differences. Additionally, the neural mechanisms that underlie the impact of novelty on consumption are not well understood. This dissertation aimed to investigate the behavioral and neural mechanisms of the impact of novelty during food consumption in male and female rats. We first examined the consumption of novel and familiar foods in novel or familiar contexts for male and female rats (Chapter 2). Acutely food deprived rats were tested in either their familiar or novel context and were given two foods, one familiar and one novel. They underwent repeated consumption tests to allow us to track habituation to novelty overtime. Results indicated a robust behavioral sex difference in consumption during habituation. Males habituated to novel foods faster than females who showed suppressed consumption throughout testing. Next, we aimed to determine the neural circuitry mediating consumption of novel foods and feeding in novel environments (Chapter 3). Male and female rats were tested for consumption in either a familiar or in a novel context and were given either a familiar or novel food. Rats were perfused after testing to determine Fos induction. Results revealed increased activation in the novel

context condition within several key areas: the central (CEA) and basolateral complex nuclei of the amygdala, the thalamic paraventricular (PVT) and reuniens nuclei, the nucleus accumbens (ACB), and the medial prefrontal cortex prelimbic and infralimbic areas. Additionally, novel food condition increased activation within the CEA, anterior basomedial nucleus of the amygdala, and anterior PVT. Sex differences in activation patterns were also observed within specific regions. The capsular and lateral CEA had greater activation for male groups and the anterior PVT, ACBv core, and ACB ventral shell had greater activation for female groups. We also investigated different patterns of related regions and the nature of those relationships and found that the CEA is a pivotal hub in our network. Therefore, we investigated the recruitment of specific inputs to the CEA in male and female rats during consumption of a novel food in a novel context (Chapter 4). We used a combination of retrograde tract tracing and Fos induction to determine whether PVTp, ILA, and AId neurons that send direct projections to the CEA were specifically recruited during the consumption test under novelty and whether that activation was sex specific. Results indicated that during consumption of a novel food in a novel context, connections from the PVTp to the CEA were recruited more heavily compared to rats that were consuming familiar food in a familiar context. These results suggest that projections from the PVTp to CEA may be driving the inhibition of feeding during novelty processing. Overall, this dissertation provides valuable insights into the behavioral and neural mechanisms of consumption under novelty and allows us to begin building the circuitry that underlies feeding inhibition during novelty processing.

Table of Contents

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List of Figures & Tables

[1] General Introduction

Initial exposure to novel stimuli in the environment induces avoidant behaviors. Before it can be established whether something is safe or dangerous, novel stimuli are often treated with a level of wariness or avoidance. This is an adaptive behavioral response that allows for evaluation of danger or risk posed by the novel stimulus. However, when these avoidant behaviors become persistent, they can become maladaptive and result in the development of psychopathology. Interactions with new foods are critically important because of the potential risk of illness after consumption. A common behavioral reaction to novel foods is *taste neophobia*. Taste neophobia in animals is defined as lower consumption of a new, possibly dangerous, taste during initial exposures compared to when the taste is familiar and food is considered safe (Lin, et al., 2012). For example, rodents, upon first exposure to a novel saccharin solution, exhibit lower intake compared to later presentations (Lin et al., 2012).

Consumption can be additionally impacted by the relative novelty of the environment. Previous research shows that environmental cues can override physiological hunger signals, with contextual and discrete fear cues that signal danger causing inhibition of food intake in rats even after food deprivation (Reppucci et al., 2013; Petrovich & Lougee, 2011; Petrovich et al., 2009). Novel contexts can also mediate a decrease in appetitive behavior, as mice placed in a novel open field have longer latencies to consume food (Ramaker & Dulawa, 2017; Dulawa & Hen, 2005).

Given that novel contexts have a great impact on consumption of familiar foods, it is important to determine whether there is an interaction between novel contexts and novel foods. It was previously shown that unconditioned fear decreases the consumption of a novel taste (Lin, et al., 2012), suggesting that the effects of a novel context and a novel taste may be cumulative, especially if the underlying mechanisms are related to fear or anxiety states. In that regard, paradigms with novel tastes and contexts have been used

as behavioral models of depression and anxiety (Ramaker & Dulawa, 2017), denoting lower food consumption under these conditions as higher depression or anxiety levels. However, studies into the specific interaction of novel foods and novel environments are lacking.

The few studies that examined the impact of both novel foods and environments on consumption only studied male rats (Ramaker & Dulawa, 2017), neglecting to explore the possibility of behavioral and neural differences between the sexes. Due to the lack of behavioral investigation into the effects of novelty processing on food consumption, the underlying neural circuitry is also unknown. The work outlined in this dissertation aimed to address a significant gap in our knowledge about how novelty impacts feeding behavior in males & females and establish the underlying neural substrates mediating the inhibition of feeding during novelty processing.

As reported in Chapter 2, we first behaviorally characterized novelty effects on food consumption by comparing how male and female rats eat novel and familiar foods in novel and familiar environments. We tracked their consumption across multiple tests until animals were habituated. Due to the lack of investigation into the compounding effects of novel stimuli on consumption, we examined food consumption of novel and familiar foods in either novel or familiar environment. All groups were given access to both the familiar and the novel food to track their preference for each food type throughout testing. This study additionally examined if there are sex differences in consumption during initial novelty exposure or during habituation.

Next, as reported in Chapter 3, we employed a similar behavioral paradigm to determine activation patterns within specific regions of interest during the first novelty exposure. We analyzed activation in the central nucleus of the amygdala (CEA), the amygdala nuclei in the basolateral complex, the paraventricular nucleus and nucleus reuniens of the thalamus, the prelimbic and infralimbic cortex, and the nucleus

accumbens. For clarity of neural analysis, rats were given access to only one food during testing, either novel or familiar, with half of the rats tested in a novel context and half tested in a familiar context. Activity of neurons was determined through Fos induction, a protein product of an immediate early gene *c-fos*, which is commonly used as an indirect measure of neuronal activity. Establishing activation patterns of these regions was the starting point in mapping the neural networks that mediate the effect of novel foods and novel contexts on food consumption, and in determining possible sex differences within these networks.

Our analysis identified the CEA as a core node of the network that mediates consumption during novelty processing and therefore, we investigated the sub-network of its inputs. As described in Chapter 4, we used a retrograde tracer in conjunction with Fos induction to determine the recruitment of specific projections to the CEA during consumption of a novel food in a novel context. We focused our analysis on the major cortical and thalamic inputs to the CEA, the infralimbic cortex, agranular insular cortex, and paraventricular thalamus. Determining the recruitment of these projections to the CEA enabled us to determine the functional connectivity of these regions within the networks that drive feeding inhibition during novelty processing.

The work described in this dissertation established sex differences in consumption of novel food and in novel contexts. We determined the patterns of neural activation across several regions of interest and investigated the recruitment of pathways to the CEA, which served as a key region within our network. Through this work, we were able to define the potential neural circuitry mediating feeding inhibition during novelty processing for male and female rats. The established circuitry will provide important knowledge on the mechanisms of adaptive novelty processing and of maladaptive feeding inhibition present in neuropsychiatric disorders, such as anorexia.

[2] Experiment 1: The effects of novelty on food consumption in male & female rats*

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[2.1] Introduction

Adaptive reactions to novel stimuli in the environment are essential for survival. Before it can be established whether something is safe or dangerous, novel stimuli are often treated with a level of wariness or avoidance. Initially limiting contact with a novel stimulus allows an accurate assessment of threat level. Once assessed, an animal increases contact, if the novel stimulus is considered innocuous, or continues avoidance, if considered harmful. However, when these avoidant behaviors become maladaptive it can lead to the development of psychopathology.

Appropriate response to new foods is essential for survival because consumption of a new food could lead to illness or even death. A common behavioral reaction to a novel food is a decrease in consumption compared to a familiar food, which is defined as taste neophobia (Lin et al., 2012; Mitchell et al., 1980). In addition to lower consumption, animals are slower to approach a novel tastant and to express hedonic orofacial responses compared to when it is familiar (Lin et al., 2012). A novel feeding environment can also have a great effect on consumption (Mitchell et al., 1980). Studies conducted with mice have found that novel context mediated a decrease in appetitive behavior, as mice placed in a novel open field had longer latencies to consume food (Ramaker & Dulawa, 2017; Dulawa & Hen, 2005). Previous research has also shown that environmental cues (both contextual and discrete) that signal danger can override physiological signals and inhibit food intake of a palatable, familiar food in rats after food deprivation (Petrovich & Lougee, 2011; Petrovich et al., 2009; Reppucci et al., 2013). Given that feeding environments can have a great impact on the consumption of familiar

foods, it is important to determine whether there is an interaction between novel contexts and novel foods. Collectively, prior work suggests that there may be compounding effects of multiple inhibitors, such as novel contexts and novel foods. However, studies into the specific interaction of novel foods and novel environments are lacking. Previous studies have also neglected to compare male and female behavior under these conditions.

Male and females show different consumption patterns based on context. Following contextual fear conditioning, males restricted consumption in the fear context whereas females restricted consumption more generally, in the fear and in a neutral context (Reppucci et al., 2013). This generalization across contexts was attributed to possible higher levels of anxiety in females and suggests that context has a greater effect on female feeding behavior. In agreement, sex differences have also been found in context effects on appetitive aspects of feeding behavior, particularly in context-induced renewal of responding to food cues (Anderson & Petrovich, 2015). Overall, previous studies indicate that females and males may have different patterns of consumption in a novel context, particularly in conjunction with the presentation of a novel food.

The current study was designed to determine the impact of novel taste and novel feeding environment independently and together. We characterized feeding behaviors in both sexes to establish whether there are sex differences initially and during habituation to novel taste and novel feeding environment. Food-deprived rats were given access to novel and familiar foods in a familiar or new environment, and their consumption patterns were tracked over eight tests to determine habituation and preferences.

[2.2] Materials & Methods

[2.2.1] Subjects

Adult male (n=16) and female (n=16) Long Evans rats (Charles River Laboratories; Portage, MI), that weighed 225-250g upon arrival, were individually housed and

maintained on a 12-hour light/dark cycle (lights on 06:00). Males and females were housed in the same colony room on separate shelves. After arrival, subjects were allowed one week to acclimate to the colony housing room before behavioral procedures began, during which they had *ad libitum* access to water and standard laboratory chow (Purina Lab Diet Prolab RMH 3000; 3.47 kcal/g; 26% protein, 15% fat, 59% carbohydrates), and were handled daily. All housing and testing procedures were in compliance with the National Institutes of Health Guidelines for Care and Use of Laboratory Animals and approved by the Boston College Institutional Animal Care and Use Committee.

[2.2.2] Apparatus

Half of the animals were tested in their Home Cage and the other half were tested in a novel environment (behavioral chamber; plexiglass box (30x28x30cm) with grid flooring and a recessed food port (3.2 x4.2 cm) on one wall; Coulbourn Instruments). Each chamber is enclosed in monolithic rigid foam box). Food was presented in a ceramic bowl.

[2.2.3] Testing Procedure

Male and female Long Evans rats were tested for consumption of both novel and familiar foods in either a familiar or novel context. The animals underwent 8 identical testing sessions, each lasting 10 minutes. Prior to each test all rats were food deprived for 20 hours. After each test rats were given *ad libitum* access to food for at least 24 hours before the following test. For each testing session, each rat was presented with two identical bowls. One of the bowls contained 15g of a familiar food (Rat Chow) and the other contained 15g of a novel food (Test Diet pellets (TD; 3.44 kcal/g; 21% protein, 13% fat, 67% carbohydrate (all sucrose)).

There were four groups of rats: home cage tested females, home cage tested males, novel context tested females, and novel context tested males. The experiment was

conducted in two identical replications with four rats per condition. All rats were habituated to transport to the conditioning chamber room, as well as to the ceramic bowls, at least 24 hours prior to testing. The weight of both foods was measured following the end of testing to determine how much of each was consumed.

Body weights for each rat was taken in the morning of each test day prior to the testing session. Average body weights were calculated for each group. Due to a suspected technical error on test day 5, body weight measurements for two home cage tested males were replaced with a value calculated by averaging their body weight from the test day before and after.

[2.2.4] Statistical Analysis

For each test, consumption levels of each food by experimental groups were analyzed using a mixed model ANOVA with a within-subject factor of food type (novel, familiar) and a between subject factor of group (home cage tested females, home cage tested males, novel context tested females, novel context tested males) and *post hoc* Bonferroni multiple comparisons following significant main effects. Following ANOVAs with significant interactions, simple effects (Bonferroni adjusted) were calculated. Differences between context and sex were analyzed using *a priori* planned orthogonal contrasts. Total consumption during each test (a sum of both foods, novel and familiar) was also analyzed for each group using a univariate ANOVA and *post hoc* Bonferroni multiple comparisons. Differences between context and sex were analyzed using *a priori* planned orthogonal contrasts.

A significance value of $p < 0.05$ was used for all analyses, except for *post-hoc* analyses in which Bonferroni adjusted alpha level was used ($p=0.05/3=0.017$). Data were analyzed for normality using Shapiro-Wilk test. In instances when the data failed normality test, mixed model ANOVA results were confirmed with a non-parametric test. In order to compare the consumption across groups, the difference in the amounts of

familiar minus novel food consumed was calculated (the difference score) for each test, and the difference scores were compared across groups with a Kruskal-Wallis between-subjects one-way ANOVA. In two comparisons, the results differed from parametric analysis and those are reported in the results section. In addition, the average rate of change of novel food consumption between the first and last test was calculated for each group ([grams consumed T8-grams consumed T1]/[8-1]).

[2.3] Results

Male and female rats were exposed to familiar and unfamiliar foods in either their home cage or a novel context for eight testing sessions. Following arrival, males gained weight faster than females, resulting in body weight differences during testing ($p<0.01$, all tests; Figure 2.1). However, there were no differences in body weight between rats tested at home versus novel environment within the same sex; therefore, all consumption results are reported as grams consumed.

During the Test 1 home cage groups of both sexes ate more of the familiar food compared to the novel, while the novel context tested groups showed no preference and overall suppressed consumption (Figure 2.2, Figure 2.3 A). A

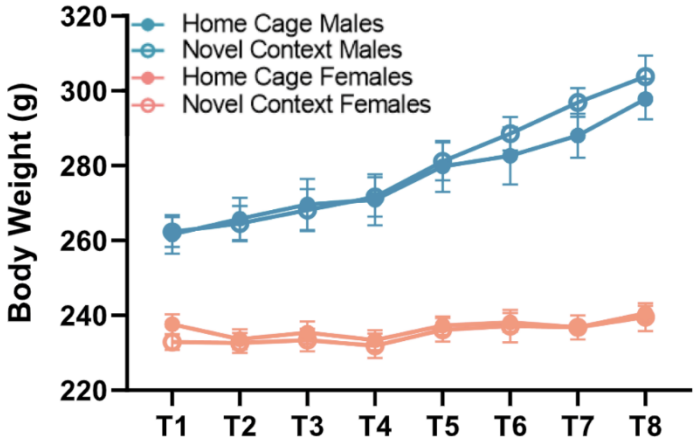


Figure 2.1 *Body weight averages (mean ±SEM) for each group across testing sessions.*

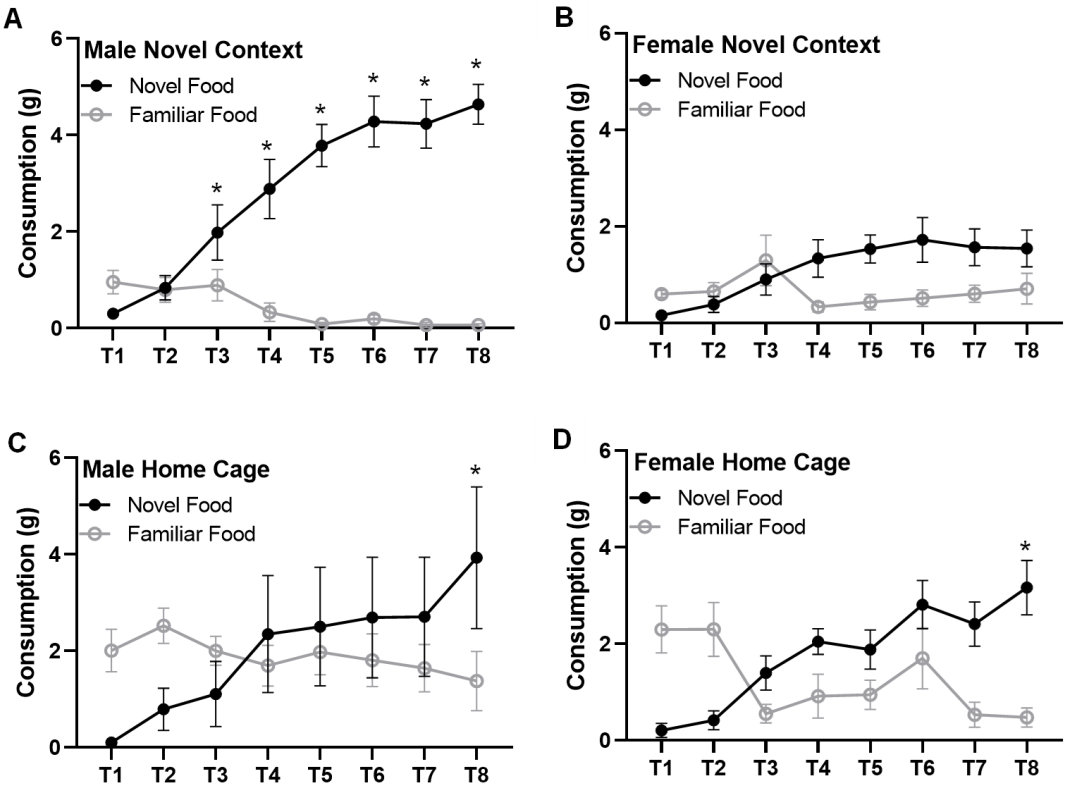
mixed model ANOVA for food type and testing group found main effects of food type and group ($F(1,28)=55.410$, $p<0.001$; $F(3, 28)=4.138$, $p=0.015$) as well as a food type *by* group interaction ($F(3, 28)=6.103$, $p=0.002$). Male and female groups that were tested in home cages showed a higher consumption of familiar food than of the novel food. This

was supported by significant simple effects (Bonferroni adjusted) within each sex ($F(1, 28)=30.99$, $p<0.001$; $F(1,28)=37.395$, $p<0.001$). Group differences found by the ANOVA in consumption of novel food versus familiar food was further supported with a Bonferroni *post hoc,* which found a significant difference in consumption patterns between the home cage tested female and novel context tested female groups ($p=0.022$).

There were overall differences in total consumption in different contexts. *A priori* planned contrasts of context and sex showed a significant difference between groups tested in home cage and groups tested in a novel context in average consumption of each food ($p=0.002$), but no difference of sex ($p=0.6$). A comparison of total food consumption across groups revealed that females tested in novel context ate the least and that was significantly less than males tested in home cage ($p=0.004$; Figure 2.4). The pattern of consumption during Test 2 was similar to that of Test 1. Home cage groups ate more of the familiar than the novel food and novel context groups ate similar low amounts of both foods (Figure 2.2). A mixed model ANOVA with factors for food type and group found a main effect of both food type and group ($F(1,28)=15.312$, $p=0.001$; $F(3,28)=4.247$, $p=0.004$) and a food by group interaction ($F(3, 28)=4.06$, $p=0.016$). Bonferroni post hoc comparison found a significant difference in consumption between home cage tested males and novel context tested females ($p=0.006$). A test of simple effects confirmed that both home cage tested males and females consumed more of the familiar food than the novel food (males $F(1,28)=12.467$, $p=0.001$; females $F(1,28)=14.720$), $p=0.001$). There was no difference in consumption of the two foods in either male or female novel context groups (males, $p=0.93$; females, $p=0.59$). Context based differences in consumption were further supported by an a priori planned significant contrast for testing context ($p=0.001$). The analysis of group differences in total consumption levels (one-way ANOVA; $F(3,28)=5.459$, $p=0.004$) found that males

tested in home cage consumed more than females tested in novel context (p=0.007, Bonferroni adjusted; Figure 2.4). However, non-parametric analysis using Kruskal-Wallis between-subjects one-way ANOVA of the difference scores (see Statistical Analysis) did not yield a significant main effect of group for Test 2 ($x^2(3) = 6.778$, p=0.079.

During Test 3 most rats ate similar amounts of both foods (Figure 2.2, Figure 2.3 B). This was confirmed with an ANOVA that found no main effect of food type or group (F(1,28)=2.99, p=0.095; F(3,28)=1.245, p=0.31). However, there was a significant interaction of food type *by* group (F(3,28)=4.678, p<0.01). Analysis of simple effects revealed that males tested in home cage consumed more of the familiar food than the



novel food (F(1,28)=15.537, p<0.01). *Post hoc* multiple comparisons found no

Figure 2.2 *Familiar & Novel food consumption across tests. Graphs show consumption in grams (mean ± SEM) of each food type for Males tested in a novel context (A), Females tested in a novel context (B), Males tested in home cage (C), and Females tested in home cage (D). Asterisks indicate a significant difference in consumption between food types.*

differences between groups (p>0.05, all). Total consumption was similar across all groups (F(3,28)=2.102, p=0.123; Figure 2.4).

In Test 4 novel context tested males consumed more novel food than familiar food (Figure 2.2 A), whereas all other groups ate similar amounts of both foods (Figure 2.2 B-D, Figure 2.3 C). The results of a mixed model ANOVA revealed a main effect of both food type and group (F(1,28)=7.489, p=0.011; F(3,28)=3.259, p=0.036), but no interaction (F(3,28)=0.733, p=0.54). A Bonferroni *post hoc* comparison yielded group differences in consumption between males tested in home cage and females tested in novel context (p=0.028). Results of a test of simple effects showed males tested in novel context consumed more of the novel than familiar foods (F(3,28)=6.887, p=0.014). Our *a priori* planned orthogonal contrasts, showed no significant difference between contexts (p=0.062), but showed significant difference of sex (p=0.036). Non-parametric analysis did not reveal a greater difference in consumption for novel context males compared to

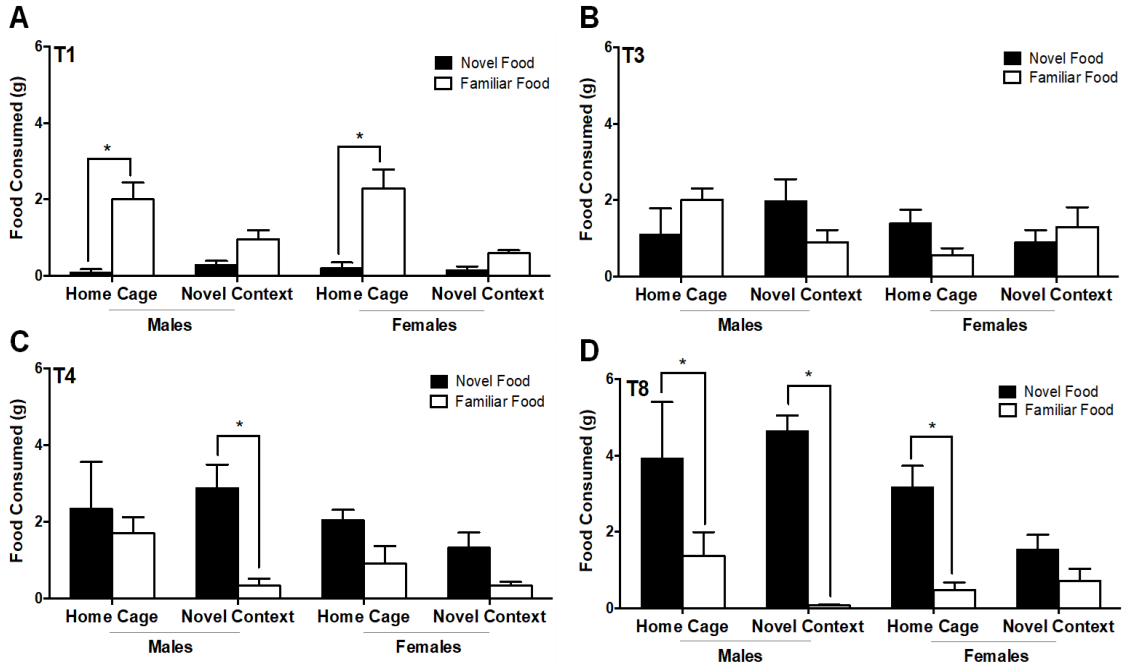


Figure 2.3 *Familiar & Novel food consumption across tests. Graphs show consumption in grams (mean ±SEM) during Test 1(A), Test 3 (B), Test 4 (C), and Test 8 (D). Asterisks indicate a significant difference in consumption between food types.*

other groups until Test 5 (Group: $x^2(3) = 19.409$, $p<0.001$; Dunn-Bonferroni *post hoc*: novel context males vs. novel context females p=0.033, novel context males vs. home cage females p=0.006).

In terms of total consumption, there was an emerging sex difference as females tested in novel context ate less than males tested in home cage (Figure 2.4). A Bonferroni post hoc, following a significant one-way ANOVA for group ($F(3,28)=3.259$, $p=0.036$), confirmed a signficant *d*ifference in consumption between home caged tested males and novel context tested females (p=0.01).

Consumption patterns during Test 5 through 7 were similar to patterns during Test 4; only novel context tested males consumed more of the novel than familiar food (Figure 2.2 A). For Test 5, there was a significant main effect of food type and group ($F(1,28)=11.799$, $p=0.002$; $F(3, 28)=3.99$, $p=0.017$), but no interaction ($F(3,28)=2.495$, $p=0.08$). Bonferroni post hoc comparisons showed group differences between males tested in home cage and females tested in novel context (p=0.021). There were significant simple effects, when food type consumption was examined within each group, for males tested in novel context ($F(1,28)=16.435$, $p<0.001$), but no other group (male home cage, $F(1,28)=0.338$, $p=0.57$; female home cage, $F(1,28)=1.057$, $p=0.31$; female

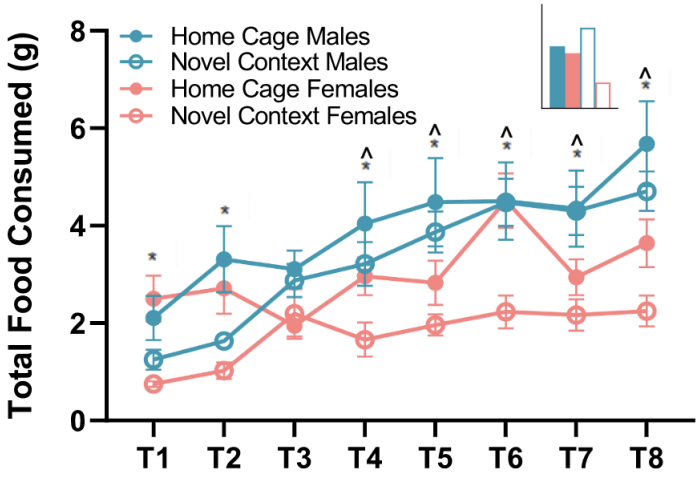


Figure 2.4 *Total food consumed in grams (mean ± SEM) by groups across testing sessions. Asterisks indicate a significant difference in consumption between groups. Symbol ^ indicates a significant difference in consumption between sexes. Bar graph (insert in upper right) shows average rate of change in Novel food consumption across tests.*

novel context, F(1,28)=1.456, p=0.24). Using a priori contrasts, we additionally found significant difference of sex (p=0.003).

In terms of total consumption during Test 5, males consumed more than females within each context (Figure 2.4). Analysis of total consumption revealed a significant main effect of group (F(3,28)=3.99, p=0.017) with significant contrasts for sex within home cage tested groups and novel context tested groups separately (p=0.045; p=0.022).

During Test 6 (Figure 2.2) there was a main effect of both food type and group (F(1, 28)=11.12, p=0.002; F(3, 28)=3.994, p=0.017), but no significant interaction. Bonferroni post hoc comparisons showed a significant group difference between females tested in home cage and females tested in novel context (p=0.049). There were marginally significant group differences between males tested in home cage and females tested in novel context (p=0.051) and between males and females tested in novel context (p=0.055). As in Tests 4 and 5, males tested in novel context continued to consume more novel food than familiar (F(3,28)=13.951, p=0.001). Total consumption differed between sexes in novel context, where males consumed more than females (Figure 2.4). Analysis using *a priori* contrasts, showed significant difference of sex within novel context tested groups (p=0.009). Total consumption analysis also showed that both males and females tested in home cage and males tested in novel context ate significantly more than females tested in novel context (p=0.02, p=0.045, p=0.05, respectively).Similar patterns continued during Test 7 (Figure 2.2) with a main effect of food type and group (F(1,28)=16.896, p<0.001; F(3, 28)=4.170, p=0.015). Bonferroni *post hoc* comparisons showed group differences between males tested in home cage and females tested in novel context (p=0.039) and between males and females tested in novel context (p=0.045). There was also a significant contrast of sex, regardless of context (p=0.002). An analysis of total consumption levels showed that females tested in

novel context ate significantly less overall than males tested in home cage and males tested in novel context (p=0.012, p=0.035, respectively; Figure 2.4). In the final test session, Test 8, all groups except novel context tested females consumed more novel food than familiar (Figure 2.2, Figure 2.3 D). This was confirmed with main effects of both food type and group ($F(1, 27)=25.087$, $p<0.001$; $F(3, 27)=5.936$, $p=0.003$). Novel context tested females ate similar amounts of both foods, differing significantly from consumption patterns of both novel context and home cage tested males (p=0.004, p=0.02). The remaining three groups consumed more novel food than familiar; males tested in home cage ($F(1, 27)=5.233$, $p=0.03$), females tested in home cage ($F(1, 27)=6.628$, $p=0.016$), and males tested in novel context ($F(1, 27)=19.156$, $p<0.001$). *A priori* planned contrasts revealed a significant different between contexts (p=0.001), but no overall difference of sex.

Females tested in novel context had the lowest total consumption compared to all other groups (Figure 2.4). Analysis of total consumption using a one-way ANOVA yielded a significant main effect of group ($F(3,28)=12.771$, $p=0.003$). A *post hoc* Bonferroni test found that females in novel context ate significantly less than males tested in home cage and males tested in novel context (p=0.004, p=0.02, respectively). Similarly, the females tested in novel context had the lowest rate of change in novel food consumption across the first and last test (0.197) compared to all other groups (Novel Context Males: 0.62; Home Cage Females: 0.422; Home Cage Males: 0.477) (Figure 2.4).

[2.4] Discussion

In this study, we investigated how novelty impacts food consumption in males and females. We behaviorally characterized the effects of novel food and novel feeding environment and their interaction by tracking consumption across multiple tests until habituation. To our knowledge, this is the first study to examine the interaction of novel

foods and novel environments in male and female rats. We found sex differences when animals were tested in a novel environment. Female rats tested in a novel context did not habituate to the novel food, or to the new environment, as they consumed small amounts of both foods across all tests. In contrast, all other groups increased consumption of the novel food and by the final testing session all showed preference for the novel food.

In the current study, we chose a palatable novel food to encourage habituation to a novel taste. The novel food (TD pellets) was calorically similar to the familiar food (standard chow), but had high sucrose content, making it sweet tasting. Previously, we compared rats' preference for TD pellets to other high-sugar/high-fat (Oreos, Nabisco), high-sugar/low-fat (Lucky Charms, General Mills), and low-sugar/ high-fat (Cheetos, Frito Lay) palatable foods (Reppucci, 2010). The highest preference score was for TD and the high-sugar/ high-fat food (equal), based on the amounts consumed during 30min tests (5g of single food given, test order counterbalanced). Nevertheless, in the current study, females fed TD pellets in the novel context showed slow and subdued signs of habituation through eight exposures, compared to their male counterparts.

Our results are in agreement with prior studies that examined taste and context exposure separately. Reilly and colleagues outlined the course of taste neophobia, with rats showing lower intake on initial presentations of a novel saccharin solution that increased over time, with the number of licks increasing in cluster size (an index of palatability) across each trial (Lin et al., 2012). Our findings are generally consistent with an increase in preference over time, however we tested rats across multiple sessions and did not observe an emergence of preference for the novel taste until test 4. Additionally, the timeline for increased consumption of the novel food varied based on testing context. Males in the novel context showed preference much earlier (Test 4) than home cage tested groups (Test 8).

A previous study examining the effect of context habituation length on novel food intake found a similar effect. Male rats who were habituated for 5 days instead of 25 were faster to increase novel saccharine solution intake in a preference test (Mitchell et al., 1980). Our males tested in a novel context began to show preference for the novel food faster than the home cage tested males who were tested in a familiar context. Our study also included female rats, who, when tested in home cage, also showed increased preference for novel food by the final testing session. Females tested in a novel context were the only group that did not show that pattern.

Novelty effects on feeding behavior have been previously used as behavioral models of depression and anxiety (Ramaker & Dulawa, 2017). In general, greater cessation of feeding behavior has been considered to indicate greater depression or anxiety. However, there are procedural differences in terms of the type of novelty (food, feeding environment) and in behavioral measures (consumption and/or latency to approach food) (Ramaker & Dulawa, 2017; Dulawa & Hen, 2005). Prior work often used these behaviors to determine the efficacy of anxiolytic drugs, typically in preparation that examined either the effects of novel foods or novel feeding environments. Studies that have examined the effects of novel contexts on consumption have noted longer latencies to consume familiar food, however, this was observed within a single testing session rather than across multiple presentations (Ramaker & Dulawa, 2017; Dulawa & Hen, 2005). In the current study, novel context attenuated overall consumption during initial exposure similarly for males and females, but only males developed a preference for the novel food across multiple testing sessions while females did not.

Fewer studies have examined novelty effects on food consumption in both sexes and potential differences when a novel food is consumed in a familiar versus novel environment. The current results indicate that there is a cumulative effect when novel foods are consumed in a novel environment, and that together they lower total

consumption more than each separately. Previous work showed that unconditioned fear (electric footshock) decreased the consumption of a novel taste (Lin et al., 2012), aligning with our observation that the novelty effects may be cumulative, especially if the underlying mechanisms are related to anxiety or fear states. In our study, the compounding effect of novelty had a stronger impact on females, as females fed a novel food and in a novel context showed sustained low total consumption. While females in novel context increased novel food consumption over time, their consumption was less than half of any other group and they never reached the levels of habituation seen in other groups. Their average rate of change for novel food consumption was also the lowest of any group. Conversely, males tested under the same conditions were the first of any testing group to show increased consumption of the novel food. The average rate of change for novel context tested males was the highest of any group. The faster increase in consumption in males could be due to faster habituation or greater preference for the palatable food or both.

Interestingly, rats tested in a familiar context were slower to increase consumption of the novel food and did not show preference until the final testing session, and average rate of change of novel food consumption was similar for males and females. This delay is likely due to the strong association previously established between home cage (familiar context) and the consumption of their usual rat chow (familiar food). Additionally, slower increase in consumption for home cage males could be related to the greater individual variability for this group. Four of the eight males tested at home never showed any preference for the novel food. These four males also had lower total consumption suggesting that perhaps they experienced higher levels of aversion or stress that drove their food avoidance.

Sex differences in states akin to anxiety or depression may driving the low consumption we observed in females but not males tested in a novel context. In

agreement with this hypothesis, a previous study using a different model of anxiety, social separation, found greater effects on food consumption for females. Following social separation, female Syrian hamsters showed an increased latency to consume food compared to their male counterparts (Shannonhouse et al., 2014). Differences in consumption patterns of male and female rats have been noted, particularly when they are tested in settings that are presumed to induce a state akin to fear or anxiety. In one study, when presented with a tone that signals a footshock (fear cue), female rats maintained inhibition of consumption much longer than males (Petrovich & Lougee, 2011). This aligns with the current finding that the females tested in novel context, instead of habituation, show sustained inhibition of consumption. The difference in the male versus female response to novel food and environment may indicate differences in adaptivity. Sustained low consumption in novel context could serve as protective measure in females. On the other hand, the resistance to habituation overtime can become disadvantageous and even dangerous.

The mechanisms underlying habituation may be related to extinction processes. Fear habituation and extinction circuits have been shown to partially overlap, at least in males (Furlong et al., 2016). For females there is evidence of an effect of estrous cycle on extinction learning, as rats in proestrus (high estradiol) show better extinction (Milad et al., 2009). Therefore, estradiol levels may impact habituation, similar to the effects on extinction. In this study, we did not monitor estrous cycling in females in order to avoid the potentially stressful effects of that procedure on food intake. Interestingly, total consumption for home cage tested females varied across tests in a manner that may suggest differences in cycling estrogen (lower consumption compared to male counterparts in test 5, the same in test 6, and again lower in test 7). We did not observe similar variability in the novel context tested females, however that could be due to their consistent low total consumption. Of note, estrous cycle effects on consumption are

typically observed over a 24-hour period (Eckel et al., 2000). Our testing sessions were short (ten minutes) and may have been too brief to capture an effect of estrous stage on total intake.

In conclusion, our study revealed robust sex differences in food consumption under novelty. Rats of both sexes increased consumption of the novel food overtime in a familiar environment, indicating similar habituation to novel taste and similar preference for the novel palatable food. In a novel environment, males habituated to a novel food faster than females, who showed sustained, suppression of consumption across multiple exposures. These results demonstrated that novel context has a greater effect on female's consumption compared to males. The differences in how novelty impacts consumption in males and females may be relevant to sex differences in avoidant behaviors (Sheynin et al., 2014) in maladaptive circumstances and the development of psychopathology. Research investigating novelty processing can provide insight to underlying behavioral and neural mechanisms and aid in the development of treatment for avoidance-based neuropsychiatric disorders. This behavioral preparation is therefore a valuable model to test neural substrates for adaptive habituation and novelty processing.

[2.5] Acknowledgements

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[2.6] Funding

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[2.7] References

Anderson, L. C., & Petrovich, G. D. (2015). Renewal of conditioned responding to food cues in rats: Sex differences and relevance of estradiol. *Physiology & Behavior, 151*, 338-344. doi:10.1016/j.physbeh.2015.07.035.

Dulawa, S. C., & Hen, R. (2005). Recent advances in animal models of chronic antidepressant effects: The novelty-induced hypophagia test. *Neuroscience & Biobehavioral Reviews, 29*(4-5), 771-783. doi:10.1016/j.neubiorev.2005.03.017.

Eckel, L., Houpt, T. A., Geary, N. (2000). Spontaneous meal patterns in female rats with and without access to running wheels. *Physiology & Behavior*, 70(3-4), 397–405. doi:10.1016/s0031-9384(00)00278-x.

Furlong, Teri M., Richardson, McNally (2016). Habituation and extinction of fear recruit overlapping forebrain structures. *Neurobiology of Learning and Memory*, *128*, 7–16. doi:10.1016/j.nlm.2015.11.013.

Lin, J., Amodeo, L. R., Arthurs, J., & Reilly, S. (2012). Taste neophobia and palatability: The pleasure of drinking. *Physiology & Behavior, 106*(4), 515-519. doi:10.1016/j.physbeh.2012.03.029.

Lin, J., & Reilly, S. (2012). Amygdala–gustatory insular cortex connections and taste neophobia. *Behavioural Brain Research, 235*(2), 182-188. doi: 10.1016/j.bbr.2012.07.040

Milad, M., Igoe, S., Lebron-Milad, K., & Novales, J. (2009). Estrous cycle phase and gonadal hormones influence conditioned fear extinction. *Neuroscience*, 164(3), 887–895. doi: 10.1016/j.neuroscience.2009.09.011

Mitchell, D., Yin, M., & Nakamatsu, K. (1980). Context habituation and taste neophobia: evidence for a cross-modality contrast effect. *Behavioral and Neural Biology*, *29*(1), 117–122. doi: 10.1016/s0163-1047(80)92554-6

Petrovich, G. D., & Lougee, M. A. (2011). Sex differences in fear-induced feeding cessation: Prolonged effect in female rats. *Physiology & Behavior, 104*(5), 996-1001. doi: 10.1016/j.physbeh.2011.06.020

Petrovich, G. D., Ross, C. A., Mody, P., Holland, P. C., & Gallagher, M. (2009). Central, But Not Basolateral, Amygdala Is Critical for Control of Feeding by Aversive Learned Cues. *Journal of Neuroscience, 29*(48), 15205-15212. doi: 10.1523/jneurosci.3656-09.2009

Ramaker, M. J., & Dulawa, S. C. (2017). Identifying fast-onset antidepressants using rodent models. *Molecular Psychiatry, 22*(5), 656-665. doi: 10.1038/mp.2017.36

Reppucci C. J, (2010) Chronic Stress, Food Consumption, and Emotional Behavior in Rats. Master's thesis, Boston College.

Reppucci, C. J., Kuthyar, M., & Petrovich, G. D. (2013). Contextual fear cues inhibit eating in food-deprived male and female rats. *Appetite, 69*, 186-195. doi: 10.1016/j.appet.2013.06.004

Reppucci, C. J., & Petrovich, G. D. (2018). Neural substrates of fear-induced hypophagia in male and female rats. *Brain Structure and Function, 223*(6), 2925-2947. doi: 10.1007/s00429-018-1668-3

Shannonhouse, J. L., Fong, L. A., Clossen, B. L., Hairgrove, R. E., York, D. C., Walker, B. B., . . . Morgan, C. (2014). Female-biased anorexia and anxiety in the Syrian hamster. *Physiology & Behavior, 133*, 141-151. doi: 10.1016/j.physbeh.2014.05.019

Sheynin, J., Beck, K. D., Pang, K. C., Servatius, R. J., Shikari, S., Ostovich, J., & Myers, C. E. (2014). Behaviourally inhibited temperament and female sex, two vulnerability factors for anxiety disorders, facilitate conditioned avoidance (also)

in humans. *Behavioural Processes*, *103*, 228–235. doi: 10.1016/j.beproc.2014.01.003

[3] Experiment 2: Fos induction pattern analysis following novelty exposure during food consumption in male and female rats

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[3.1] Introduction

As documented in Chapter 2, Experiment 1 investigated the consumption patterns of male and female rats exposed to novel and familiar foods in novel or familiar environments and determined that taste neophobia is enhanced in a new context in a sex dependent manner (Greiner & Petrovich, 2020). When tested in a novel context, males habituated to eating familiar and novel foods faster than females, who showed suppressed consumption throughout testing (Greiner & Petrovich, 2020). The prolonged suppression in females may be relevant to sex differences in avoidant behaviors (Sheynin et al., 2014) and the development of psychopathology. However, there is a significant gap in our knowledge about the neural substrates underlying how novelty impacts feeding behavior.

To investigate how novelty effects are mediated in male and female rats, the present study examined Fos induction in key brain regions during the consumption of novel or familiar foods in novel or familiar environments. While the underlying neural circuity is largely unknown, certain brain regions are strong candidates for mediating consumption during novelty exposure. The amygdala is essential for emotional learning and memory consolidation, particularly in fear and appetitive learning. The BLA and CEA both play integral roles in appetitive behavior. Bilateral lesions of the CEA in rats were shown to eliminate feeding inhibition during the presentation of an aversive stimulus (Petrovich et al., 2009). The central nucleus of the amygdala (CEA) and the basolateral amygdala (BLA), in particular, are not only critical for appetitive learning and consumption (Petrovich et al., 2009; Cole et al., 2013), but are also activated in the presence of novel food (Koh et al., 2003; Lin et al., 2012). Additionally, lesions to the BLA lessen

neophobic reactions to novel tastes in familiar environments (Nachman & Ashe, 1974; Lin et al., 2009). Despite both the CEA and BLA being strong candidates for mediating the effects of novelty, their role in driving, mediating, or inhibiting consumption of novel foods in novel contexts has not been investigated.

The paraventricular nucleus of the thalamus (PVT) is a part of the connectional network with CEA and is known for the regulation of food consumption and body weight (Li & Kirouac, 2008; Bhatnagar & Dallman, 1999; Petrovich, 2018). The PVT was part of the recruited network for contextual mediation of appetitive behavior (renewal of responding to food cues) (Anderson & Petrovich, 2017). The PVT also plays a critical role in regulating the motivation to eat in novel environments, with optogenetic activation of the anterior PVT resulting in increased feeding in a novel open field (Cheng et al., 2018).

The nucleus reuniens (RE) is another midline thalamic structure that appears to be necessary for appropriate regulation of avoidance behavior (Linley et al., 2020). Additionally, it serves as a major link between the medial prefrontal cortex (mPFC) and the hippocampal formation (McKenna & Vertes, 2004), which is relevant for contextual processing of novel environments.

The medial prefrontal cortex (mPFC) plays a critical role in decision making, particularly in the calculation of risk versus reward (Bechara & Damasio, 2005). It is also critical for consumption under cognitive control (learned cues) (Petrovich et al., 2007). Prior work in our lab used retrograde tracers to demonstrate that the BLA has extensive projections to both the infralimbic (ILA) and prelimbic (PL) subregions of the mPFC (Reppucci & Petrovich, 2016), suggesting that the mPFC could mediate decision making with information from the amygdala. On the other hand, the mPFC can reach both the CEA (Hurley et al., 1991) and BLA (Gabbott et al., 2005) and these connections have been shown to control behavioral outputs, at least during conditioned fear (Quirk et al.,

2003). Additionally, mPFC functioning differs between males and females during renewal of responding to food cues (Anderson & Petrovich, 2017), making it a key region of interest for sex differences in activation patterns.

The nucleus accumbens (ACB), a key area involved appetitive motivation (Bozarth & Wise, 1981; for review see Salamone, 1994), is functionally connected to the posterior subregion of PVT (Parsons et al., 2007; Dong et al., 2017), mPFC (Sesack et al., 1989; Groenewegen et al., 1999), and BLA (Christie et al., 1987; Brog et al., 1993). Additionally, the ACB shell (ACBsh) contains hedonic hotspots that drive the motivation to eat palatable foods (Castro et al., 2016) particularly due to its projections to the lateral hypothalamus and the parabrachial nucleus (Usuda et al., 1998).

In the present study, Fos induction was used to determine activation patterns within the above identified areas of interest, as a start to mapping the neural networks that mediate novelty effects on food consumption and in determining possible sex differences within these networks. For clarity of neural analysis, rats were given access to only one food during testing, either novel or familiar, with half of the rats tested in a novel context and half tested in a familiar context. This paradigm allowed us to separately analyze the effects of sex, context, and food type for both consumption and neural activation within our proposed network.

[3.2] Materials & Methods

[3.2.1] Subjects

Adult male (n=32) and female (n=32) Long Evans rats (Charles River Laboratories; Portage, MI), that weighed 225-250g upon arrival, were individually housed and maintained on a 12-hour light/dark cycle (lights on 06:00). Males and females were housed in the same colony room on separate shelves. After arrival, subjects were allowed one week to acclimate to the colony housing room before behavioral procedures began, during which they had *ad libitum* access to water and standard Rat chow (Purina

Lab Diet Prolab RMH 3000; 3.47 kcal/g; 26% protein, 15% fat, 59% carbohydrates), and were handled daily. All housing and testing procedures were in compliance with the National Institutes of Health Guidelines for Care and Use of Laboratory Animals and approved by the Boston College Institutional Animal Care and Use Committee.

[3.2.2] Apparatus

Half of the animals were tested in their housing cages (Home Cage) and the other half were tested in a novel environment (behavioral chamber; plexiglass box (30x28x30cm) with grid flooring and a recessed food port (3.2 x4.2 cm) on one wall; Coulbourn Instruments). Each chamber is enclosed in monolithic rigid foam box). Food was presented in a ceramic bowl.

[3.2.3] Behavioral Testing Procedure

Male and female rats were tested for consumption of either a novel or a familiar food in either a novel or familiar environment and, after testing, the brain tissue was collected for later processing. There were eight testing groups in order to test the effects of sex, testing context, and food presented. All groups underwent one 30-minute testing session. Prior to testing all rats were food deprived for 20 hours. For the test, each rat was presented with a ceramic bowl that contained either 15g of a familiar food (Rat Chow) or 15g of a novel food (Test Diet (TD) pellets; 3.4 kcal/g; 21% protein, 13% fat, 67% carbohydrate).

All rats were habituated to transport to the conditioning chamber room, as well as to the ceramic bowls, at least 24 hours prior to testing. The weight of all foods was measured following the end of testing to determine how much was consumed. Body weights for all rats were taken in the morning of test day. Average body weights were calculated for each group. All consumption data is presented as a percentage of grams consumed per body weight.

[3.2.4] Histological Procedures

Rats were perfused 90 minutes after start of testing and brains were harvested. Rats were briefly anesthetized with isoflurane (5%; Baxter Healthcare Corporation, Deerfield, IL), and then deeply anesthetized with an intraperitoneal injection of tribromoethanol (375 mg/kg; Sigma-Aldrich, St. Louis, MO). Rats were then transcardially perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1 M borate buffer. Brains were extracted and post-fixed overnight in a solution of 12% sucrose dissolved in the perfusion liquid, then rapidly frozen in hexanes cooled in dry ice and stored at −80 °C. Brains were sliced in 30-µm sections using a sliding microtome and collected into four adjacent series.

The first series was stained using standard immunohistochemical procedures for visualization of Fos. Free-floating tissue sections were incubated in a blocking solution for 1 h at room temperature to minimize nonspecific binding. The blocking solution contained 0.02M potassium phosphate-buffered saline (KPBS), 0.3% Triton X-100 (Sigma-Aldrich), 2% normal goat serum (S-1000; Vector Laboratories, Burlingame, CA), and 10% non-fat milk (M-0841; LabScientific, Livingston, New Jersey). Then, the tissue was incubated with the primary antibody, anti-*c-fos* raised in rabbit (1:5,000, ABE457, EMD Millipore, Billercia, MA; or 1:5,000, 226 003, Synaptic Systems, Gottingen, Germany; the use of each primary antibody for histological procedures was counterbalanced across training conditions) in the blocking solution for 72 h at 4 °C. The tissue was rinsed in KPBS then incubated with the secondary antibody, biotinylated goat anti-rabbit IgG (1:500; BA-1000; Vector Laboratories) in the blocking solution for 45 min. Subsequently, the tissue was rinsed in KPBS then reacted with avidin–biotin complex (ABC solution; PK-6100; Vector Laboratories) for 45 min. To improve specific binding, this was followed by rinses in KPBS, a second 30 min incubation in the secondary antibody solution, rinses in KPBS, a second 30 min incubation in the ABC solution, and additional rinses in KPBS. To produce a color reaction, the tissue was incubated in a

diaminobenzidine solution (SK-4100; Vector Laboratories) for 1–2 min with constant, manual agitation. Stained tissue was then mounted onto SuperFrost Plus slides (Fisher Scientific, Pittsburgh, PA) and air-dried, followed by drying in an oven at 45 °C overnight. Tissue was then dehydrated through graded alcohols, cleared in xylenes, and coverslipped with DPX (13512; Electron Microscopy Sciences, Hatfield, PA).

The second series was collected into KPBS solution, mounted onto gelatin-subbed slides, and stained with thionin for identification of cytoarchitectonic borders of brain structures, as defined in Swanson's rat brain atlas (Swanson 2018). The remaining series were collected into trays containing a cryoprotectant solution (0.025 M sodium phosphate buffer with 30% ethylene glycol and 20% glycerol) and stored at −20 °C for later use. Brain perfusions, collection, slicing, and length of storage were counterbalanced across training conditions.

[3.2.5] Image Acquisition & Analysis

Images of stained tissue were acquired with an Olympus BX51 light microscope at 10X and attached Olympus DP74 camera using DP2-BSW software (Olympus America Inc, Center Valley, PA). Using the ImageJ software program (NIH), borders for regions of interest were drawn onto the image of the thionin-stained tissue, and then transposed to the image of the adjacent immunohistochemically-stained tissue to allow for semi-automated counting of Fos-positive neurons based on size and circularity measures. Identification of regions and drawn borders for analysis were determined based on the Swanson rat brain atlas (Swanson, 2018). Representative atlas levels and distance from bregma for each analyzed subregion is documented in the table below (Table 3.1). Analysis was conducted across the rostro-caudal extent of each subregion of the CEA: capsular (CEAc), lateral (CEAl), and medial (CEAm). For the cell groups of the basolateral complex (BLC): anterior BLA (BLAa), posterior BLA (BLAp), anterior basomedial nucleus (BMAa), posterior BMA (BMAp); and the lateral amygdala (LA).

Analysis for ACB was conducted for each subregion: core (ACBc), dorsal shell (ACBdsh), and ventral shell (ACBvsh). The PVT was analyzed on a representative anterior (aPVT) and posterior (pPVT) level. Analysis of RE was conducted on a single representative level. The subregions of the mPFC (PL and ILA) were each analyzed on a separate representative level. Bilateral images were acquired for CEA, ACB, mPFC, and BLC and both sides were acquired in a single image for PVT and RE. Images were analyzed for each region of interest; counts from left and right hemispheres were summed for each rat to calculate the total number of Fos-positive neurons per region.

Brain Region	Analyzed Subregions	Representative Atlas Level(s)	Distance from Bregma
CEA	*CEAm*	25, 26, 27, 28	-1.53, -1.78, -2, -2.45
	CEAc		
	CEAl	26, 27, 28	-1.78, -2, -2.45
BLA	*BLAa*	27	-2
	BLAp	30	-3.25
BMA	*BMAa*	26	-1.78
	BMAp	30	-3.25
LA	--	30	-3.25
PVT	*PVTa*	26	-1.78
	PVTp	31	-3.7
RE	--	26	-1.78
ACB	*ACBc*	13	+1.2
	ACBdsh		
	ACBvsh		
mPFC	*PL*	8	+3.2
	ILA	9	+2.8

Table 3.1*: The rostro-caudal extent of each brain region analyzed. Atlas levels refer to the Swanson rat brain atlas (2018).*

[3.2.6] Statistical Analysis

Following arrival, males gained weight faster than females, resulting in body weight differences by the time of testing. Therefore, all consumption results are reported as a percentage of grams consumed per body weight ([food consumed(g)/body weight(g)]X100).

Consumption results were analyzed using a between-subjects 3-way univariate ANOVA for food type, sex, and testing context. Total Fos induction was analyzed using a between-subjects 3-way univariate ANOVA for food type, sex, and context. Analysis of subregions and anatomical levels of interest were analyzed using 3-way multivariate ANOVAs for food type, sex, and context. All significant interactions were followed by Bonferroni *post hoc* analyses.

Bivariate Pearson correlation analysis were done within each testing group to assess the relationship of Fos induction between each subregion analyzed. For this analysis, the CEA was collapsed across the two anterior (levels 25 & 26) and posterior (levels 27 & 28) anatomical levels analyzed for each subregion (anterior, aCEAm, aCEAl, & aCEAc; posterior, pCEAm, pCEAl, & pCEAc). A value of $p<0.05$ was considered significant for all analyses, except for *post-hoc* analyses in which Bonferroni adjusted alpha level was used ($p=0.05/3=0.017$). A value of $p<0.09$ was considered trending.

[3.3] Results

[3.3.1] Consumption

Consumption during testing differed based on food type and context familiarity (Figure 3.1). Male and female rats given a familiar food ate more than male and female rats given a novel food ($F(1,52) = 7.509$ $p = 0.008$) and groups tested in a familiar context had greater consumption compared to groups tested in a novel context ($F(1,52)=26.767$ $p<0.001$) regardless of food type. Male and female groups were similar ($F(1,52)=2.313$ $p=0.13$) and there were no interactions of any factor (sex *by* food $F(1,52)=2.598$ $p=0.11$, sex *by* context $F(1,52)=0.003$ $p=0.96$, food *by* context $F(1,52)=0.356$ $p=0.5$, sex *by* food *by* context $F(1,52)=0.066$ $p=0.79$).

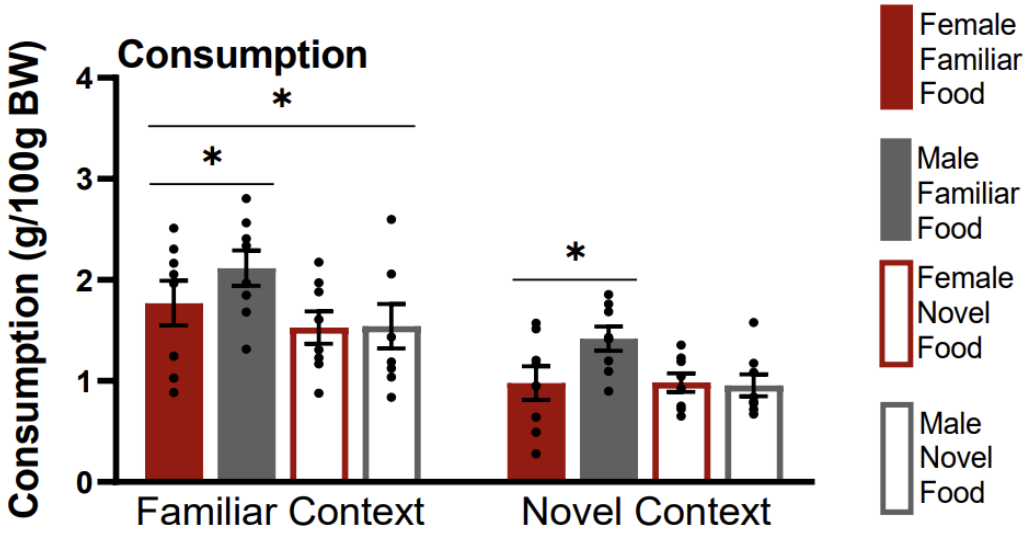

Figure 3.1: *Food consumption test. The graphs show the amounts of each food that subjects in each testing condition consumed, expressed as grams per 100 grams of their body weight. consumed per each gram of the animal's body weight (BW). Asterisks indicate p<0.05.*

[3.3.2] Fos Induction in the Central Nucleus of the Amygdala

Total Fos induction in the CEA was greater for rats that were exposed to a novel food and that was clearly evident during tests in a familiar environment ($F(1,52)=9.246$, $p=0.004$). There were no differences based on the sex of the animal ($F(1,52)=2.344$ $p=0.13$) or testing context ($F(1,52)=0.771$ $p=0.38$) and there were no effects of interactions (sex *by* food, $F(1,52)=0.09$ $p=0.77$; sex *by* context, $F(1,52)=0.224$ $p=0.64$; food *by* context, $F(1,52)=0.983$ $p=0.33$; sex *by* food *by* context, $F(1,52)=0.337$ $p=0.56$).

Each CEA subregion (medial, lateral, and capsular) had similar increase in Fos induction in rats that were exposed to a novel food (Figure 3.2 B-D) (CEAm $F(1,52)=10.196$, $p<0.01$; CEAl, $F(1,52)=4.658$, $p=0.036$; CEAc, $F(1,52)=4.166$, $p=0.046$). In addition, in the CEAc, all rats tested in a novel context, compared to those tested in a familiar context, had more Fos positive neurons, and the induction was higher for males compared to females (Figure 3.2 D) (context: $F(1,52)=8.926$, $p<0.01$; sex: $F(1,52)=6.449$, $p=0.014$; respectively). In the CEAm, there was a trend towards

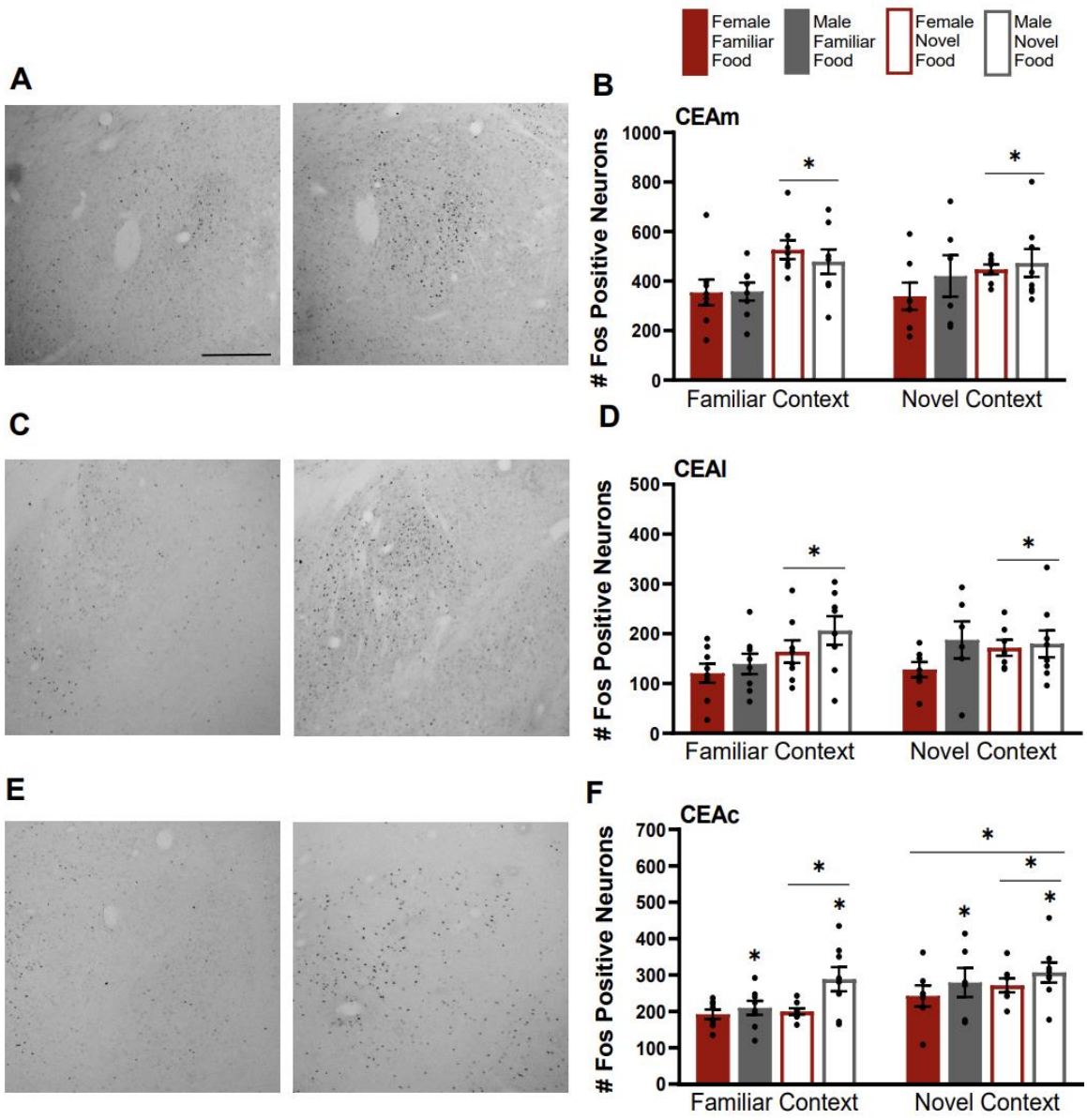

Figure 3.2: *Fos induction in the medial (CEAm), lateral (CEAl), and capsular (CEAc) subregions of the central nucleus of the amygdala.* ***A)*** *Tissue images stained for Fos of the medial central amygdala (atlas level 26, right side) for a familiar context tested female given a familiar food (left image) and a familiar context tested female given a novel food (right image).* ***B)*** *Fos induction for each testing condition in the medial central amygdala.* ***C)*** *Tissue images stained for Fos of the lateral central amygdala (atlas level 28, right side) for a familiar context tested male given a familiar food (left image) and a familiar context tested male given a novel food (right image).* ***D)*** *Fos induction for each testing condition in the lateral central amygdala.* ***E)*** *Tissue images stained for Fos of the capsular central amygdala (atlas level 27, right side) for familiar context tested female given a novel food (left image) and a novel context tested female given a novel food (right image).* ***F)*** *Fos induction for each testing condition in the capsular central amygdala. Asterisks indicate p<0.05. Scale bar in upper left image=500um.*

F(1,52)=0.198, p=0.66). In the CEAl, there was no effect for context, but a trend towards significance for sex (context, F(1,52)=0.287, p=0.59; sex, F(1,52)=3.6, p=0.06).

Additional analysis examined CEA subregions across rostro-caudal levels (as defined in Swanson 2018). There was greater activation for rats tested in a novel context in CEAc at atlas level 25 and 27 (L25, F(1, 52)=5.676, p=0.021; L27, F(1, 52)=6.133, p=0.017) and in the CEAl at level 28 (F1, 52)=4.87, p=0.032). Of note, the males given a novel food in a familiar context had the greatest number of Fos positive neurons in the CEAl of L28 compared to all other groups. This was supported by a between-subjects interaction of context *by* food type *by* sex (F(1,52)=7.459, p=0.009) for the L28 CEAl. There were no effects of context for any other parts of the CEA at any rostro-caudal levels (L25-CEAm, F(1, 52)=0.495, p=0.485; L26-CEAm, F(1,52)=0.068, p=0.796; L26-CEAl, F(1,52)=3.029, p=0.088; L26-CEAc, F(1,52)=3.486, p=0.068; L27-CEAm, F(1,52)=0.229, p=0.634; L27-CEAl, F(1,52)=0.103, p=0.75; L28-CEAm, F(1,52)=0.653, p=0.423; L28-CEAc, F(1,52)=2.912, p=0.094).

There were additional between-subjects effects for food type where rats given a novel food had higher Fos induction compared to rats given a familiar food in the CEAm at level 27 and 28 (L27, F(1, 52)=25.096, p<0.001; L28, F(1, 52)=17.633, p<0.001) and for the CEAl at level 28 (F(1, 52)=4.664, p=0.035). There were no effects for food type in any other part of the CEA (L25-CEAm, F(1,52)=3.778, p=0.057; L25-CEAc, F(1,52)=0.629, p=0.431; L26-CEAm, F(1,52)=0.054, p=.818; L26-CEAl, F(1,52)=0.692, p=0.409; L26-CEAc, F(1,52)=2.763, p=0.102; L27-CEAl, F(1,52)=3.033, p=0.088; L27-CEAc, F(1,52)=3.243, p=0.078; L28-CEAc, F(1,52)=1.574, p=0.215).

[3.3.3] Fos Induction in the Basolateral Nuclei of the Amygdala

Fos induction in the BLAa and BLAp was greater for rats tested in a novel context than for rats tested in a familiar context (Figure 3.3) (F(1,41)=12.534 p=0.001; (F(1,41)=12.889 p=0.001), respectively). There were no effects of food type (BLAa:

F(1,41)=0.960 p=0.333; BLAp: F(1,41)=0.076 p=0.784) or sex (BLAa: F(1,41)=1.156 p=0.289; BLAp: F(1,41)=0.38 p=0.0.541) or any significant interactions in these regions (BLAa: sex *by* food type F(1,41)=1.052 p=0.311; sex *by* context F(1,41)=0.290 p=0.593; food *by* context F(1,41)=0.514 p=0.478; sex *by* food *by* context F(1,41)=0.013 p=0.91; BLAp: sex *by* food type F(1,41)=0.014 p=0.905; sex *by* context F(1,41)=0.251 p=0.619; food *by* context F(1,41)=0.135 p=0.715; sex *by* food *by* context F(1,41)=221 p=0.641).

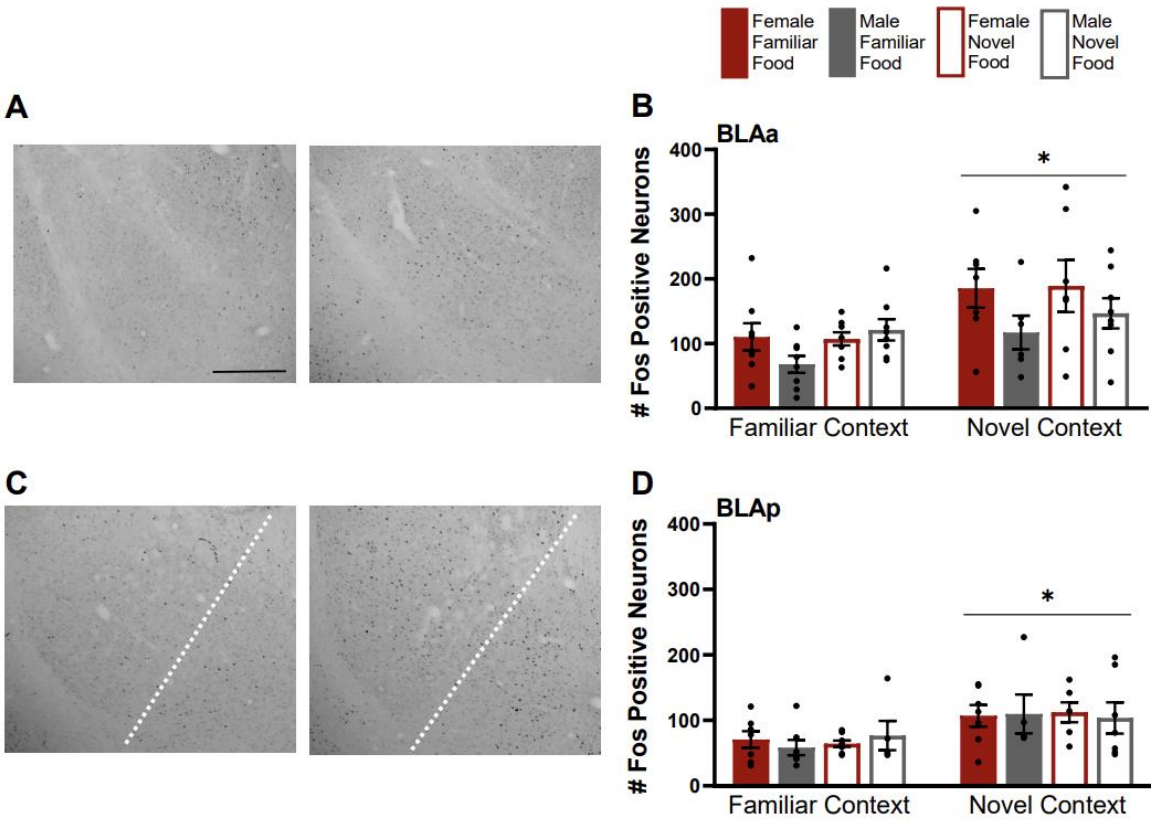

Figure 3.3: *Fos induction in the anterior (BLAa) and posterior (BLAp) basolateral nuclei of the amygdala.* ***A)*** *Tissue images stained for Fos of the anterior basolateral amygdala (atlas level 27, left side) for a familiar context tested female given a familiar food (left image) and a familiar context tested female given a novel food (right image).* ***B)*** *Fos induction for each testing condition in the anterior basolateral amygdala.* ***C)*** *Tissue images stained for Fos of the posterior basolateral amygdala (left of the dotted line) (atlas level 30, left side) for a familiar context tested male given a familiar food (left image) and a novel context tested male given a familiar food (right image). Dotted line indicates a border between the BLAp and BMAp.* ***D)*** *Fos induction for each testing condition in the posterior basolateral amygdala. Scale bar in upper left image=500um. Asterisks indicate p<0.05.*

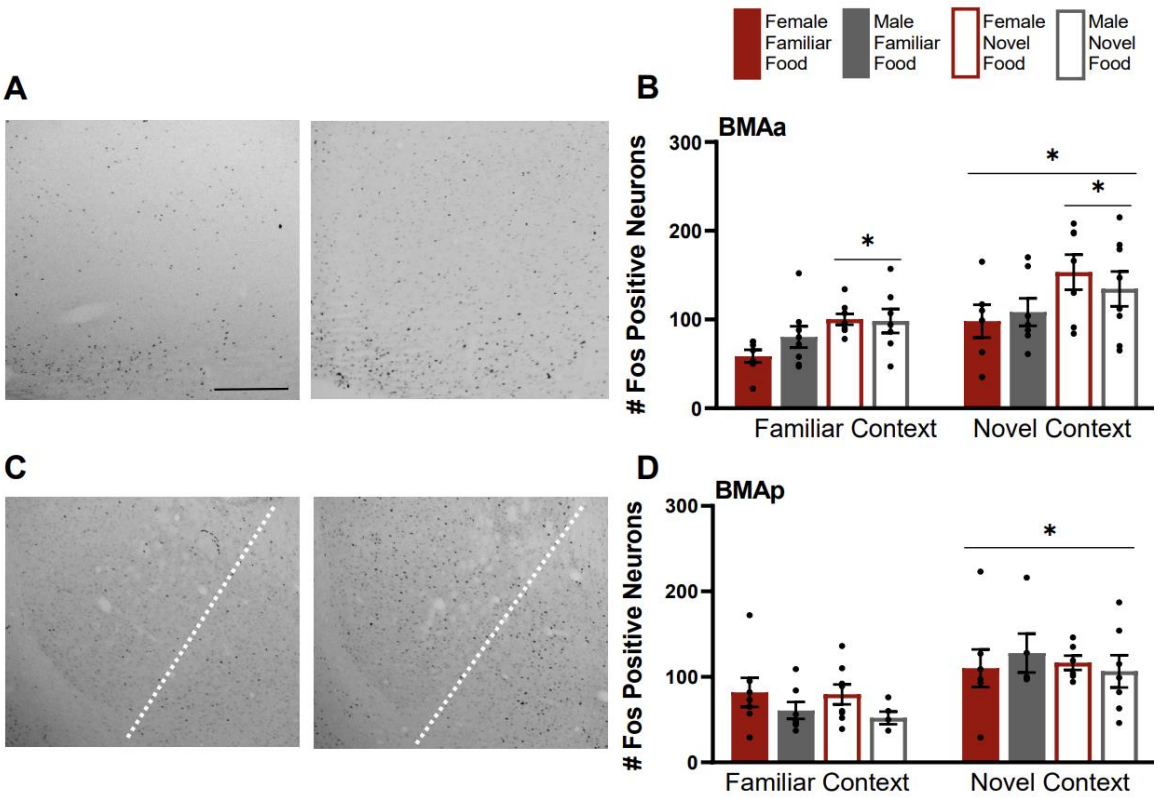

Figure 3.4: *Fos induction in the anterior (BMAa) and posterior (BMAp) basomedial nuclei of the amygdala.* ***A)*** *Tissue images stained for Fos of the anterior basomedial amygdala (atlas level 26, right side) for a novel context tested female given a familiar food (left image) and novel context tested female given a novel food (right image).* ***B)*** *Fos induction for each testing condition in anterior basomedial amygdala.* ***C)*** *Tissue images stained for Fos of the posterior basomedial amygdala (right of the dotted line) (atlas level 30, left side) for a familiar context tested male given a familiar food (left image) and a novel context tested male given a familiar food (right image). Dotted line indicates a border between the BLAp and BMAp.* ***D)*** *Fos induction for each testing condition in the posterior basomedial amygdala. Scale bar in upper left image=500um. Asterisks indicate p<0.05.*

[3.3.4] Fos Induction in the Basomedial Nuclei of the Amygdala

Fos induction in the BMAa was greater for rats tested in a novel context compared to rats tested in a familiar context ($F(1,41)=9.408$ $p=0.004$) and for rats given a novel food compared to those given a familiar food ($F(1,41)=12.947$ $p=0.001$) (Figure 3.4 A). There were no effects of sex ($F(1,41)=0.592$ $p=0.446$) or interactions (sex *by* food type $F(1,41)=0.193$ $p=0.663$; sex *by* context $F(1,41)=0.620$ $p=0.436$; food *by* context $F(1,41)=0.165$ $p=0.686$; sex *by* food *by* context $F(1,41)=0.059$ $p=0.81$).

The BMAp had greater Fos induction for rats tested in a novel context (Figure 3.4 B) ($F(1,41)=14.813$ $p<0.001$) compared to rats tested in a familiar context, but had no effect of food type ($F(1,41)=0.431$ $p=0.515$), sex ($F(1,41)=0.929$ $p=0.341$), or any interactions (sex *by* food type $F(1,41)=0.673$ $p=0.417$; sex *by* context $F(1,41)=1.951$ $p=0.17$; food *by* context $F(1,41)=0.026$ $p=0.873$; sex *by* food *by* context $F(1,41)=183$ $p=0.873$).

[3.3.5] Fos Induction in the Lateral Amygdala

The Fos induction in the LA was greater for rats tested in a novel context (Figure 3.5) ($F(1,41)=12.534$ $p=0.001$) compared to rats tested in a familiar context, but had no effect of food type ($F(1,41)=0.1.108$ $p=0.299$) or sex ($F(1,41)=0.242$ $p=0.625$) or any interactions (sex *by* food type $F(1,41)=0.021$ $p=0.887$; sex *by* context $F(1,41)=0.004$ $p=0.951$; food *by* context $F(1,41)=0.755$ $p=0.39$; sex *by* food *by* context $F(1,41)=193$ $p=0.663$).

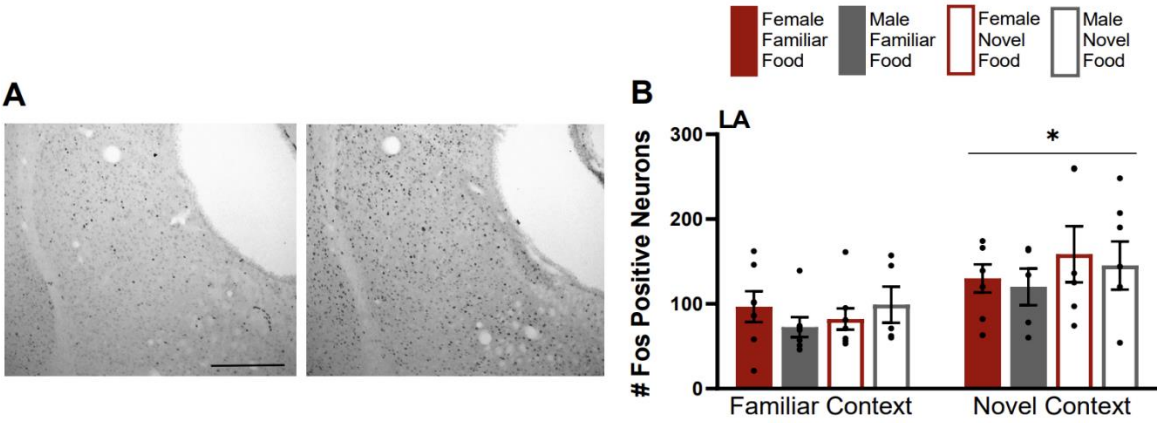

Figure 3.5: *Fos induction in the lateral nucleus of the amygdala (LA).* ***A)*** *Tissue images stained for Fos of the lateral (atlas level 30, left side) for a familiar context tested male given a familiar food (left image) and a novel context tested male given a familiar food (right image).* ***B)*** *Fos induction for each testing condition in the lateral amygdala. Scale bar in left image=500um. Asterisks indicates p<0.05.*

[3.3.6] Fos Induction in the Paraventricular Nucleus of the Thalamus

The Fos induction in the PVTa was greater for rats tested in a novel context compared to those tested in a familiar context and those given a novel food compared to

a familiar food (Figure 3.6 A-B). Additionally, females given a novel food had greater Fos induction than males given a novel food. Statistical analysis revealed a significant main effect of food type ($F(1,51)=4.149$, $p=0.047$) and context ($F(1,51)=9.355$, $p=0.004$), but not sex ($F(1,51)=0.157$, $p=0.69$). There was a significant interaction of food type *by* sex ($F(1,51)=5.605$, $p=0.22$), but no other significant interactions (context *by* food type $F(1,51)=0.115$, $p=0.74$; sex *by* context $F(1,51)=0.19$, $p=0.66$; context *by* sex *by* food $F(1,51)=0.573$, $p=0.453$). A Bonferroni *post hoc* analysis revealed that among novel context tested animals, females had greater Fos induction than males ($p=0.04$)

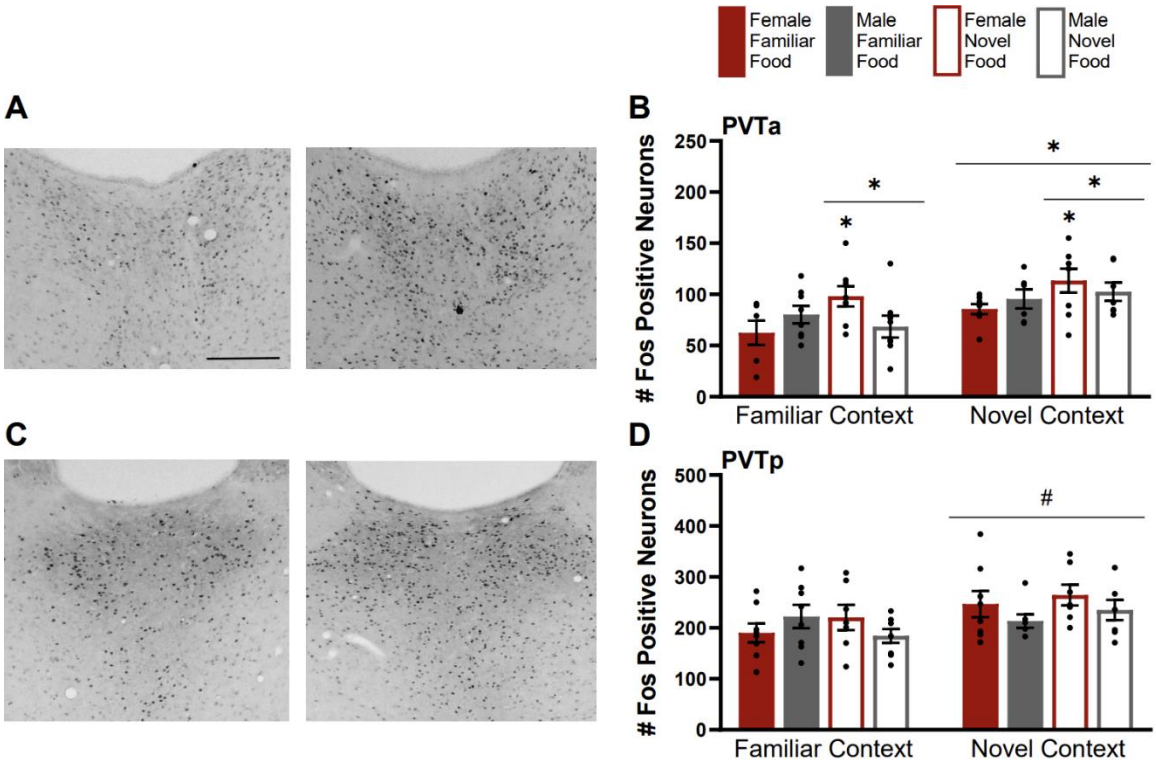

Figure 3.6: *Fos induction in the anterior (PVTa) and posterior (PVTp) paraventricular nucleus of the thalamus.* ***A)*** *Tissue images stained for Fos of the anterior paraventricular thalamus (atlas level 26, midline) for a familiar context tested male given a novel food (left image) and a familiar context tested female given a novel food (right image).* ***B)*** *Fos induction for each testing condition in the anterior paraventricular*

The Fos induction in the PVTp was generally greater for rats tested in a novel context compared to a familiar context, however statistical analysis yielded results

slightly above the level of significance for a main effect of context (F(1,51)=4.006, p=0.051) (Figure 3.6 C-D). There were no significant differences in Fos induction for the factors of sex (F(1,51)=0.804, p=0.374) or food type (F(1,51)=0.01, p=0.92) and no significant interaction of factors (context *by* sex F(1,51)=0.088, p=0.77; context *by* food type F(1,51)=0.023, p=0.88; sex *by* food type F(1,51)=2.846, p=0.098; context *by* sex *by* food type F(1,51)=0.434, p=0.51).

[3.3.7] Fos Induction in the Nucleus Reuniens of the Thalamus

Fos induction in the RE was greater for rats tested in a novel context than rats tested in a familiar context (Figure 3.7) (F(1,50)=35.977, p<0.01), but there were no differences based on food type (F(1,50)=2.013, p=0.17), or main effect of sex (F(1,50)=1.213, p=0.28). Additionally, females tested in a familiar context appeared to show greater Fos induction when consuming a novel food compared to a females in a novel context that consumed a familiar food. This difference was not apparent for male groups tested in a familiar context. Statistical analysis found no significant interactions to support this difference (context *by* food type F(1,50)=1.475, p=0.23; context *by* sex *by* food type

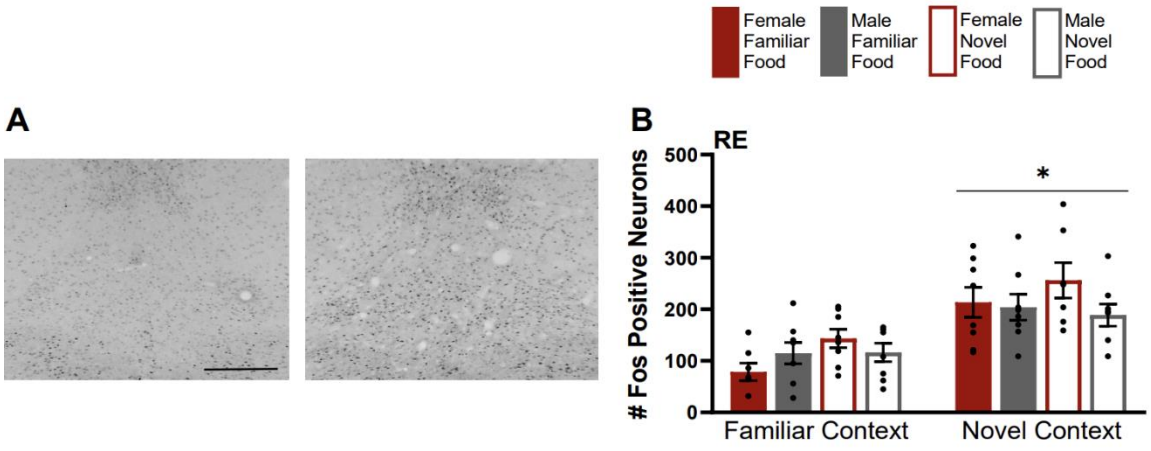

Figure 3.7: *Fos induction in the nucleus reuniens (RE) of the thalamus.* ***A)*** *Tissue images stained for Fos of the nucleus reuniens (atlas level 26, midline) for a familiar context tested male given a familiar food (left image) and a familiar context tested male given a novel food (right image).* ***B)*** *Fos induction for each testing condition in the nucleus reuniens. Scale bar in left image=500um. Asterisks indicate p<0.05.*

F(1,50)=0.573, p=0.453); however, interactions of context *by* sex (F(1,50)=3.327, p=0.074) and sex *by* food type (F(1,50)=3.127, p=0.084) were close to significance.

[3.3.8] Fos Induction in the Nucleus Accumbens

Fos induction was greater for animals tested in a novel context than for animals tested in a familiar context in all three subregions of the ACB (Figure 3.8 A-C) (ACBc F(1,47)=22.582, p<0.01; ACBdsh F(1,47)=16.693, p<0.01; ACBvsh F(1,47)=14.67, p<0.01). Additionally, Fos induction was greater for females than males in both the ACBc (F(1,47)=6.829, p=0.012) and ACBvsh (F(1,47)=6.773, p=0.012) (Figure 3.8 A & C). Fos induction in the ACBdsh was similar for both sexes (F(1,47)=0.665, p=-.419) and there was no difference in Fos induction based on food type for any subregion of the ACB (ACBc F(1,47)=0.441, p=0.51; ACBdsh F(1,47)=0.125, p=0.725; ACBvsh F(1,47)=0.562, p=0.457).

Sex differences were more obvious in the novel context, , with novel context tested females appearing to show greater Fos induction than their male counterparts. However, statistical analysis found only a trend for interaction of sex and context within the ACBvsh (F1,47)=3.264, p=0.077), and no other significant interactions of factors for any ACB subregion (context *by* sex, ACBc F(1,47)=2.386, p=0.128, ACBdsh F(1,47)=1.28, p=0.264; context *by* food type, ACBc F(1,47)=0.053, p=0.819, ACBdsh F(1,47)=0.15, p=0.7, ACBvsh F(1,47)=0.004, p=0.951; sex *by* food type, ACBc F(1,47)=0.3, p=0.586, ACBdsh F(1,47)=1.015, p=0.319, ACBvsh F(1,47)=0.909, p=0.345; context *by* sex *by* food type, ACBc F(1,47)=0.781, p=0.381, ACBdsh F(1,47)=0.119, p=0.732, ACBvsh F(1,47)=0.080, p=0.779).

[3.3.9] Fos Induction in the medial Prefrontal Cortex

Fos induction in the PL was greater for rats tested in a novel context compared to a familiar context (Figure 3.9 A) (F(1,49)=18.539, p<0.001). In addition, females had slightly higher Fos induction compared to males, however the effect of sex did not reach

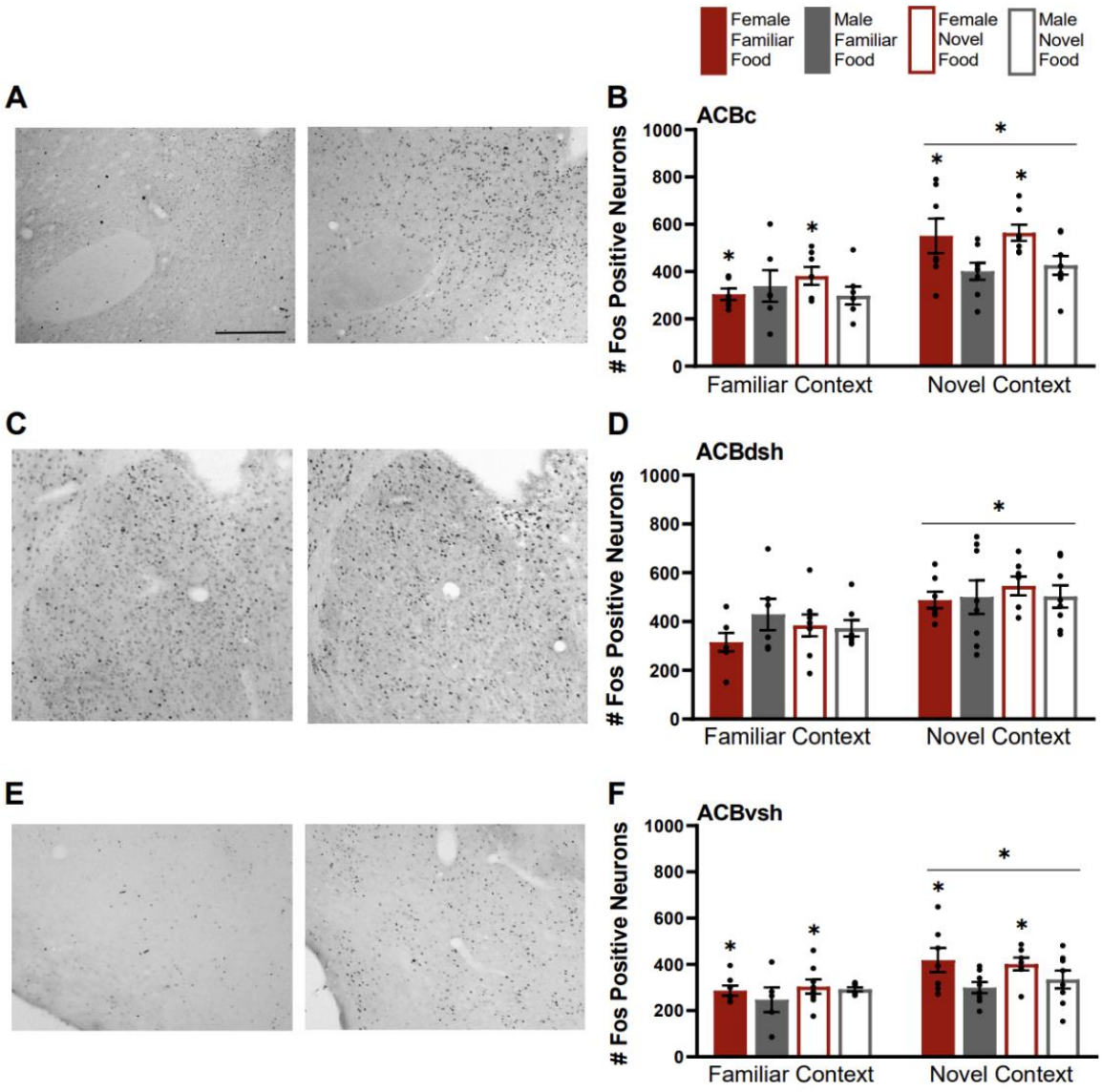

Figure 3.8: *Fos induction in the core (ACBc), dorsal shell (ACBdsh), and ventral shell (ACBvsh) of the nucleus accumbens.* ***A)*** *Tissue images stained for Fos of the nucleus accumbens core (atlas level 14, left side) for a novel context tested male given a novel food (left image) and a novel context tested female given a novel food (right image).* ***B)*** *Fos induction for each testing condition in the nucleus accumbens core.* ***C)*** *Tissue images stained for Fos of the nucleus accumbens dorsal shell (atlas level 14, right side) for a familiar context tested male given a novel food (left image) and a novel context tested male given a novel food (right image).* ***D)*** *Fos induction for each testing condition in the nucleus accumbens dorsal shell.* ***E)*** *Tissue images stained for Fos of the nucleus accumbens ventral shell (atlas level 14, right side) for a familiar context tested male given a familiar food (left image) and a novel context tested female given a familiar food (right image).* ***F)*** *Fos induction for each testing condition in the nucleus accumbens ventral shell. Scale bar in upper left image=500um. Asterisks indicate* $p<0.05$.

significance (F(1,49)=3.713, p=0.06). There was no main effect of food type (F(1,49)=2.075, p=0.16) or any significant interactions of factors (context *by* sex F(1,49)=0.701, p=0.41; context *by* food type F(1,49)=0.25, p=0.88; sex *by* food type F(1,49)=0.703, p=0.41; context *by* sex *by* food type F(1,49)=0.331, p=0.57).

Fos induction in the ILA was greater for rats tested in a novel context compared to a familiar context (Figure 3.9 B) (F(1,49)=14.402, p<0.001). There were no differences in Fos induction based on sex (F(1,49)=0.897, p=0.348) or food type (F(1,49)=2.028, p=0.161) and no significant interactions of factors (context *by* sex F(1,49)=1.958,

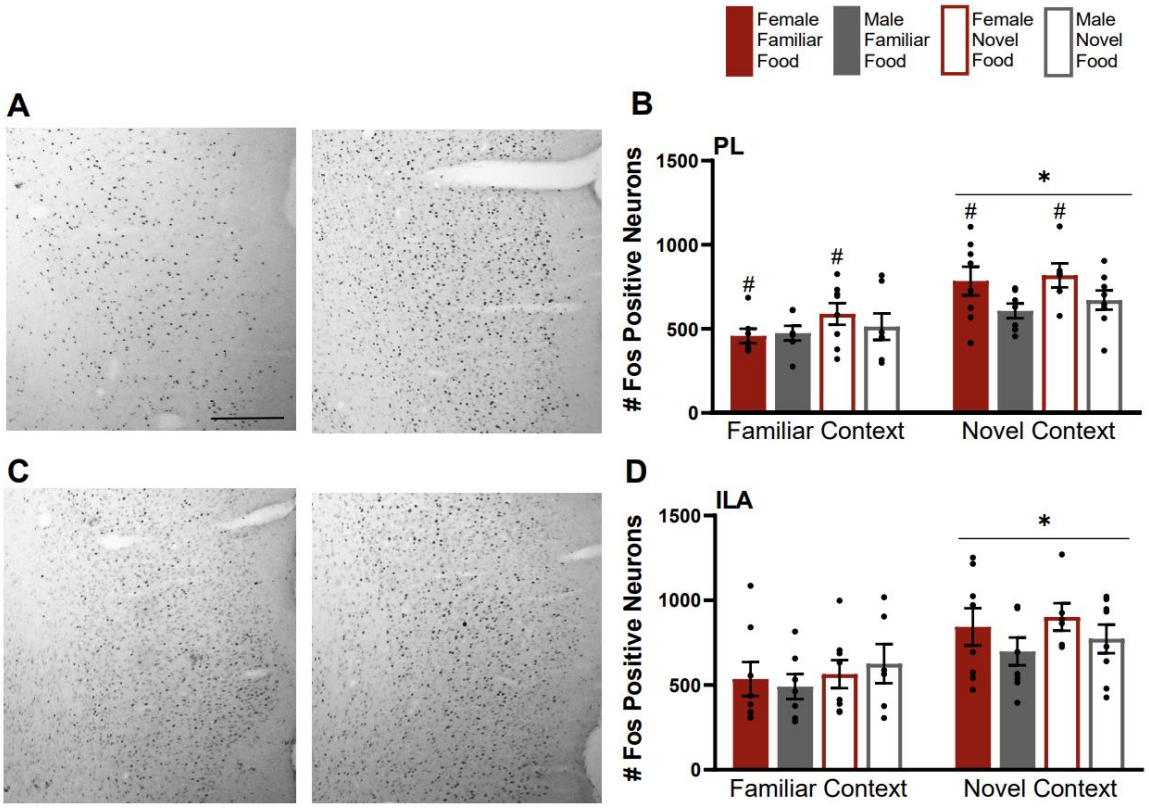

Figure 3.9: *Fos induction in the prelimbic (PL) and infralimbic (ILA) regions of the medial prefrontal cortex.* ***A)*** *Tissue images stained for Fos of prelimbic cortex (atlas level 26, leftt side) for a familiar context tested female given a familiar food (left image) and novel context tested female given a familiar food (right image).* ***B)*** *Fos induction for each testing condition in the prelimbic cortex.* ***C)*** *Tissue images stained for Fos of the infralimbic cortex (atlas level 28, left side) for a familiar context tested male given a familiar food (left image) and novel context tested male given a familiar food (right image).* ***D)*** *Fos induction for each testing condition in the infralimbic cortex. Scale bar in upper left image=500um. Asterisks indicate p<0.05. Pound symbol indicates p=0.06.*

p=0.17; context *by* food type F(1,49)=0.019, p=0.89; sex *by* food type F(1,49)=0.196, p=0.66; context *by* sex *by* food type F(1,49)=0.002, p=0.97).

[3.3.10] Fos Induction Correlations

Bivariate Pearson correlations were conducted within each testing group, to examine the relationship of Fos induction between regions of interest. Females given a familiar food in a familiar context (Table 3.2; right/above the diagonal) had significant positive and negative correlations. There were positive correlations between CEA subregions, aCEAl with pCEAm, and pCEAm with pCEAl as well as between CEA and other regions. The aBLA was positive correlated with pCEAl and pCEAc, the pBLA with the aCEAm and the aCEAc, and the LA with the pCEAc. The RE was negatively correlated with the aCEAl. The ACBvsh was positively correlated with the aBMA. The ILA had two significant correlations with other brain regions, a negative correlation with ACBdsh and a positive correlation with the PL.

Males given a familiar food in a familiar context (Table 3.2; left/below the diagonal) had only positive correlations. There were correlations between CEA subregions, pCEAm and aCEAc, pCEAl and aCEAc, as well as pCEAc and aCEAm, aCEAc, pCEAm, and pCEAl. The BMAp was correlated with the BLAa and the LA with the BLAp and BMAp. The pPVT was correlated with the pBMA, the RE with the pCEAm and pCEAc, and ACBdsh with ACBc. The PL was correlated with aCEAm and pCEAc and the ILA with pCEAm, pPVT and RE.

Females given a novel food in a familiar context (Table 3.3; right/above the diagonal) had positive correlation between pCEAl and aCEAm. In the BMA, there was a positive correlation between the aBMA and the aBLA and there were positive correlations between the pBMA and the pCEAm and pBLA. The RE was positively correlated with the aCEAm. In the ACB, the ACBdsh was negatively correlated with the aBLA and

Familiar Context & Familiar Food			aCEA			pCEA			BLA		BMA		LA	PVT		RE	ACB			PL	ILA
			med	*lat*	*cap*	*med*	*lat*	*cap*	*ant*	*pos*	*ant*	*pos*	*mid*	*ant*	*pos*	*mid*	*core*	*dsh*	*vsh*	*mid*	*mid*
aCEA	*med*	r		0.292	0.684	0.647	0.511	0.407	0.487	.807*	-0.098	0.353	0.151	-0.596	-0.374	-0.384	0.210	-0.600	-0.170	0.021	0.474
		p		0.483	0.061	0.083	0.196	0.317	0.221	0.028	0.817	0.437	0.747	0.158	0.362	0.452	0.690	0.154	0.716	0.964	0.235
	lat	r	0.489		-0.118	.729*	0.665	0.270	0.218	0.091	-0.452	0.108	-0.026	0.369	-0.327	-.837*	0.149	-0.462	-0.041	0.446	0.521
		p	0.219		0.780	0.040	0.072	0.517	0.604	0.846	0.261	0.819	0.956	0.416	0.429	0.038	0.778	0.297	0.930	0.316	0.186
	cap	r	0.614	0.589		0.261	0.199	0.372	0.490	.901**	-0.170	0.681	0.360	-0.631	-0.490	0.072	-0.184	-0.188	-0.506	-0.183	0.132
		p	0.105	0.124		0.533	0.637	0.364	0.218	0.006	0.688	0.092	0.428	0.128	0.218	0.892	0.728	0.686	0.246	0.694	0.756
pCEA	*med*	r	0.571	0.496	.709*		.883**	0.687	0.679	0.712	0.022	0.334	0.538	-0.074	-0.098	-0.76	0.603	-0.299	0.207	0.121	0.36
		p	0.139	0.211	0.049		0.004	0.060	0.064	0.073	0.959	0.464	0.213	0.874	0.817	0.078	0.205	0.515	0.656	0.795	0.381
	lat	r	0.659	0.647	.791*	0.548		0.496	.742*	0.754	-0.165	0.565	0.254	0.252	-0.046	-0.494	0.406	0.128	-0.115	-0.152	0.124
		p	0.076	0.083	0.019	0.160		0.211	0.035	0.050	0.696	0.187	0.582	0.585	0.914	0.319	0.424	0.785	0.806	0.746	0.770
	cap	r	.906**	0.559	.781*	.848**	.751*		.829*	0.516	0.246	0.411	.873*	-0.210	0.193	-0.31	0.793	-0.049	0.296	-0.32	-0.200
		p	0.002	0.150	0.022	0.008	0.032		0.011	0.236	0.556	0.359	0.010	0.651	0.647	0.555	0.060	0.917	0.519	0.483	0.635
BLA	*ant*	r	-0.436	-0.493	-0.171	-0.476	-0.206	-0.521		0.726	0.033	0.701	0.593	0.033	0.172	-0.052	0.556	0.348	-0.043	-0.595	-0.348
		p	0.280	0.215	0.686	0.234	0.625	0.186		0.065	0.938	0.079	0.160	0.944	0.684	0.921	0.252	0.444	0.927	0.158	0.398
	pos	r	-0.488	-0.171	0.354	0.557	0.049	-0.025	0.435		0.121	0.707	0.38	-0.632	-0.045	0.248	0.090	0.243	-0.004	-0.143	0.145
		p	0.326	0.746	0.491	0.251	0.926	0.963	0.389		0.797	0.076	0.401	0.179	0.924	0.687	0.885	0.643	0.994	0.788	0.756
BMA	*ant*	r	0.132	-0.555	0.212	-0.230	-0.089	-0.001	0.487	0.477		-0.265	0.573	-0.390	0.681	0.795	0.490	0.213	.805*	-0.053	-0.161
		p	0.778	0.196	0.648	0.620	0.849	0.999	0.268	0.416		0.566	0.179	0.387	0.063	0.059	0.323	0.647	0.029	0.911	0.703
	pos	r	-0.511	-0.108	0.322	0.287	0.140	-0.181	0.672	.909*	0.258		0.202	0.101	0.074	0.328	-0.208	0.452	-0.295	-0.240	-0.259
		p	0.300	0.838	0.534	0.582	0.791	0.731	0.144	0.012	0.675		0.664	0.849	0.875	0.590	0.737	0.368	0.570	0.647	0.575
LA	*mid*	r	-0.461	-0.417	0.155	0.142	0.136	-0.194	0.707	.827*	0.479	.912*		-0.506	0.322	-0.226	0.722	-0.088	0.472	-0.193	-0.212
		p	0.358	0.410	0.769	0.788	0.798	0.713	0.117	0.042	0.414	0.011		0.306	0.482	0.714	0.169	0.869	0.344	0.714	0.649
PVT	*ant*	r	-0.347	-0.148	0.191	0.154	-0.257	-0.191	0.591	0.717	0.352	0.659	0.418		0.376	-0.247	-0.060	0.673	-0.165	-0.199	-0.664
		p	0.400	0.727	0.651	0.716	0.540	0.650	0.123	0.109	0.439	0.155	0.410		0.406	0.688	0.924	0.143	0.755	0.705	0.104
	pos	r	0.195	0.073	0.220	0.429	0.226	0.295	0.120	0.676	0.047	.860*	0.803	0.285		0.649	0.424	0.722	0.736	-0.294	-0.59
		p	0.644	0.864	0.600	0.289	0.590	0.478	0.777	0.141	0.920	0.028	0.054	0.493		0.163	0.402	0.067	0.059	0.522	0.127
RE	*mid*	r	0.640	0.354	0.295	.822*	0.284	.761*	-0.640	0.134	-0.315	-0.229	-0.281	-0.152	0.439		-0.375	0.751	0.279	-0.317	-0.55
		p	0.088	0.389	0.477	0.012	0.495	0.028	0.088	0.801	0.492	0.662	0.589	0.720	0.276		0.534	0.085	0.592	0.540	0.258
ACB	*core*	r	0.089	0.465	0.723	0.773	0.256	0.437	-0.486	0.784	-0.124	0.674	0.335	0.515	0.685	0.560		0.011	0.582	-0.455	-0.354
		p	0.867	0.352	0.104	0.072	0.625	0.386	0.328	0.117	0.843	0.212	0.581	0.296	0.134	0.248		0.984	0.225	0.365	0.491
	dsh	r	-0.211	0.002	0.331	0.570	-0.249	0.118	-0.196	0.756	0.427	0.565	0.345	0.788	0.540	0.423	.849*		0.068	-0.607	-.849*
		p	0.688	0.997	0.522	0.238	0.634	0.823	0.710	0.139	0.473	0.321	0.569	0.063	0.269	0.403	0.032		0.885	0.149	0.016
	vsh	r	0.070	0.368	0.555	0.821	0.052	0.426	-0.464	0.595	-0.174	0.239	-0.015	0.608	0.115	0.705	0.806	0.813		0.194	0.017
		p	0.911	0.542	0.332	0.088	0.933	0.475	0.431	0.405	0.826	0.761	0.985	0.277	0.854	0.183	0.099	0.094		0.677	0.970
PL	*mid*	r	.787*	0.738	0.498	0.604	0.624	.813*	-0.501	-0.149	-0.235	0.094	-0.223	-0.135	0.691	0.679	0.627	0.291	0.169		.919**
		p	0.036	0.058	0.256	0.151	0.134	0.026	0.252	0.811	0.654	0.880	0.719	0.773	0.086	0.094	0.258	0.635	0.831		0.003
ILA	*mid*	r	0.493	0.038	0.248	.786*	0.336	0.720	-0.195	0.805	-0.084	0.685	0.722	0.135	.853*	.852*	0.708	0.772	0.702	0.553	
		p	0.261	0.936	0.592	0.036	0.461	0.068	0.675	0.100	0.874	0.202	0.168	0.773	0.015	0.015	0.181	0.126	0.298	0.198	

males bottom left	0.8 to 1	0.6 to 0.79	n.s.	0.6 to 0.79	0.8 to 1	females top right

negative

Table 3.2: *Correlation of Fos induction between regions for rats tested in a familiar context & given a familiar food. Correlations results (r and p values) for males are featured left/below the diagonal and for females right/above the diagonal. Significant positive correlations are shown in green for males and blue for females. All negative correlations are additionally outlined in red.*

aBMA and the ACBvsh was negatively correlated with the LA. Additionally, the ILA was positively correlated with the RE and PL.

Males given a novel food in a familiar context (Table 3.3; left/below the diagonal) had positive correlation between pCEAl and pCEAm. There were positive correlations

Familiar Context & Novel Food			aCEA			pCEA			BLA		BMA		LA	PVT		RE	ACB			PL	ILA
			med	*lat*	*cap*	*med*	*lat*	*cap*	*ant*	*pos*	*ant*	*pos*	*mid*	*ant*	*pos*	*mid*	*core*	*dsh*	*vsh*	*mid*	*mid*
aCEA	*med*	r		0.605	0.337	0.451	.826*	0.11	-0.065	0.295	-0.485	0.345	0.022	-0.185	0.028	.794*	0.357	0.420	-0.076	0.317	0.388
		p		0.112	0.415	0.262	0.011	0.795	0.878	0.521	0.270	0.449	0.963	0.661	0.948	0.019	0.432	0.300	0.859	0.444	0.343
	lat	r	0.674		-0.274	0.277	0.578	0.224	-0.330	0.099	-0.453	0.024	-0.727	-0.032	0.386	0.364	0.441	0.536	0.643	0.093	0.010
		p	0.067		0.511	0.507	0.133	0.594	0.424	0.833	0.307	0.960	0.064	0.940	0.345	0.376	0.322	0.171	0.086	0.826	0.980
	cap	r	0.320	0.162		-0.25	0.306	-0.43	-0.169	-0.047	-0.005	-0.267	0.373	-0.327	-0.385	0.451	-0.041	0.021	-0.564	-0.230	0.304
		p	0.440	0.702		0.546	0.461	0.285	0.689	0.920	0.992	0.563	0.410	0.429	0.346	0.262	0.930	0.960	0.146	0.584	0.464
pCEA	*med*	r	0.299	0.114	-0.18		0.356	0.472	0.588	0.644	0.338	.761*	0.360	-0.157	-0.276	0.089	-0.077	-0.398	-0.108	0.120	-0.05
		p	0.471	0.788	0.665		0.387	0.238	0.125	0.118	0.458	0.047	0.428	0.710	0.508	0.834	0.870	0.328	0.798	0.778	0.910
	lat	r	0.125	-0.153	0.275	.775*		0.003	-0.344	0.635	-0.413	0.492	-0.209	-0.396	-0.291	0.542	0.499	0.499	0.084	0.162	0.289
		p	0.769	0.718	0.509	0.024		0.994	0.404	0.126	0.357	0.262	0.653	0.332	0.484	0.165	0.254	0.208	0.843	0.702	0.487
	cap	r	0.478	-0.062	0.69	-0.3	-0.06		-0.031	0.533	0.201	0.548	0.151	0.168	0.374	0.305	0.018	-0.083	-0.184	0.641	0.509
		p	0.231	0.885	0.058	0.471	0.882		0.942	0.218	0.666	0.203	0.747	0.690	0.362	0.463	0.969	0.844	0.663	0.087	0.197
BLA	*ant*	r	-0.480	-0.322	0.394	-0.515	-0.064	0.202		-0.059	.818*	0.244	0.620	0.091	-0.254	-0.316	-0.482	-.736*	-0.254	-0.185	-0.419
		p	0.276	0.482	0.382	0.237	0.892	0.664		0.900	0.025	0.599	0.138	0.831	0.544	0.446	0.273	0.037	0.543	0.661	0.302
	pos	r	-0.670	-0.434	0.272	-0.113	0.275	-0.107	.876*		0.396	.828*	0.197	-0.485	-0.550	0.087	0.302	-0.135	-0.241	0.266	0.256
		p	0.145	0.390	0.602	0.831	0.598	0.840	0.022		0.437	0.021	0.672	0.270	0.201	0.853	0.561	0.772	0.603	0.564	0.580
BMA	*ant*	r	-0.458	-0.410	0.588	-0.182	0.373	0.241	.869*	.934**		0.107	0.415	-0.044	-0.525	-0.529	-0.672	-.988**	-0.357	-0.463	-0.339
		p	0.301	0.362	0.165	0.695	0.409	0.602	0.011	0.006		0.839	0.413	0.926	0.226	0.222	0.144	0.000	0.431	0.296	0.458
	pos	r	0.047	0.273	0.409	0.368	0.463	0.013	0.536	0.636	0.636		0.421	-0.507	-0.33	0.145	0.122	-0.078	-0.203	0.557	0.324
		p	0.930	0.600	0.421	0.472	0.355	0.981	0.273	0.174	0.175		0.347	0.245	0.466	0.757	0.818	0.869	0.663	0.194	0.478
LA	*mid*	r	-0.183	-0.114	0.671	-0.021	0.388	0.314	0.805	.843*	.942**	.823*		-0.281	-0.336	0.156	-0.527	-0.551	-.844*	0.229	0.316
		p	0.729	0.829	0.145	0.969	0.448	0.545	0.054	0.035	0.005	0.044		0.542	0.461	0.739	0.283	0.200	0.017	0.621	0.489
PVT	*ant*	r	-0.183	0.368	-0.524	0.356	0.123	-.900**	-0.119	0.078	-0.233	0.207	-0.200		0.319	-0.155	0.510	-0.103	-0.120	0.255	-0.236
		p	0.665	0.369	0.183	0.387	0.772	0.002	0.799	0.884	0.615	0.695	0.705		0.441	0.714	0.242	0.807	0.777	0.542	0.574
	pos	r	-0.532	-0.534	-0.319	0.143	0.190	-0.378	-0.241	0.038	-0.014	-0.26	-0.280	0.034		0.361	-0.112	0.467	0.330	0.476	0.34
		p	0.175	0.173	0.441	0.736	0.653	0.356	0.603	0.943	0.977	0.622	0.591	0.936		0.380	0.810	0.243	0.425	0.233	0.411
RE	*mid*	r	0.401	-0.203	0.590	0.323	0.598	0.674	0.218	0.153	0.445	0.511	0.605	-0.543	-0.049		0.195	0.525	-0.301	0.580	.816*
		p	0.325	0.630	0.124	0.435	0.118	0.067	0.639	0.772	0.317	0.301	0.203	0.164	0.909		0.676	0.181	0.469	0.132	0.014
ACB	*core*	r	0.341	-0.088	0.638	-0.372	0.103	0.673	-0.105	-0.322	0.032	-0.443	-0.068	-0.682	-0.078	0.396		0.69	0.010	0.403	0.051
		p	0.454	0.851	0.123	0.411	0.826	0.098	0.823	0.534	0.946	0.378	0.898	0.092	0.867	0.380		0.087	0.984	0.370	0.914
	dsh	r	-0.091	-0.487	0.364	-0.543	-0.041	0.503	0.020	-0.157	0.100	-0.655	-0.150	-0.669	0.167	0.198	.882**		0.433	0.429	0.434
		p	0.847	0.268	0.422	0.207	0.931	0.249	0.966	0.766	0.832	0.158	0.776	0.101	0.721	0.670	0.009		0.284	0.289	0.282
	vsh	r	-0.698	-.858*	0.106	-0.574	-0.039	0.156	0.451	0.424	0.471	-0.396	0.138	-0.416	0.386	0.040	0.399	0.753		-0.264	-0.427
		p	0.081	0.013	0.822	0.178	0.934	0.738	0.310	0.402	0.286	0.437	0.795	0.354	0.393	0.932	0.376	0.051		0.528	0.291
PL	*mid*	r	0.561	0.102	0.368	0.137	0.097	0.675	0.095	-0.139	0.109	0.442	0.310	-0.512	-0.153	.824*	0.105	-0.126	-0.310		.742*
		p	0.190	0.827	0.417	0.770	0.835	0.096	0.839	0.793	0.815	0.380	0.550	0.240	0.743	0.023	0.823	0.788	0.499		0.035
ILA	*mid*	r	0.112	-0.501	0.399	0.394	0.522	0.595	0.016	0.142	0.352	0.413	0.516	-0.638	0.631	952**	0.200	0.166	0.141	.839*	
		p	0.833	0.312	0.433	0.440	0.289	0.213	0.975	0.820	0.493	0.489	0.373	0.173	0.179	0.003	0.704	0.753	0.790	0.037	

males bottom left | 0.8 to 1 | 0.6 to 0.79 | n.s. | 0.6 to 0.79 | 0.8 to 1 | females top right

negative

Table 3.3: *Correlation of Fos induction between regions for rats tested in a familiar context & given a novel food. Correlations results (r and p values) for males are featured left/below the diagonal and for females right/above the diagonal. Significant positive correlations are shown in green for males and blue for females. All negative correlations are additionally outlined in red.*

between the anterior and posterior BLA as well as the aBMA with aBLA and pBLA. The LA was positively correlated with and pBLA, pBMA, and pBMA. The pPVT was negatively correlated with pCEAc. The ACBdsh was positively correlated with ACBc and the ACBvsh was negatively correlated with aCEAl. The PL was positively correlated with RE and the ILA with both pPVT and PL.

Novel Context & Familiar Food			aCEA			pCEA			BLA		BMA		LA	PVT		RE	ACB			PL	ILA
			med	*lat*	*cap*	*med*	*lat*	*cap*	*ant*	*pos*	*ant*	*pos*	*mid*	*ant*	*pos*	*mid*	*core*	*dsh*	*vsh*	*mid*	*mid*
aCEA	*med*	r		.760*	.947**	0.311	0.736	0.61	0.481	0.868	0.521	0.622	0.650	-.763*	-0.609	0.588	0.661	0.259	-0.107	0.59	0.695
		p		0.047	0.001	0.498	0.059	0.146	0.335	0.057	0.289	0.188	0.235	0.046	0.147	0.165	0.106	0.574	0.819	0.163	0.083
	lat	r	.813*		.827*	0.487	0.554	0.421	0.039	0.265	-0.051	0.196	0.472	-0.561	-.761*	0.311	0.151	-0.242	-0.344	0.210	0.308
		p	0.014		0.022	0.268	0.197	0.347	0.941	0.667	0.923	0.710	0.423	0.190	0.047	0.498	0.746	0.600	0.450	0.651	0.501
	cap	r	.859**	.827*		0.358	.837*	0.677	0.518	0.807	0.440	0.606	0.709	-0.599	-.782*	0.587	0.669	0.254	0.035	0.652	0.718
		p	0.006	0.011		0.430	0.019	0.095	0.293	0.099	0.382	0.203	0.180	0.155	0.038	0.166	0.100	0.582	0.941	0.113	0.069
pCEA	*med*	r	0.558	.858*	.883*		0.482	0.677	0.144	0.126	0.104	0.042	0.069	-0.497	-0.092	-0.28	0.086	-0.125	0.201	-0.167	-0.17
		p	0.250	0.029	0.020		0.273	0.095	0.786	0.839	0.845	0.937	0.912	0.257	0.845	0.544	0.855	0.790	0.666	0.721	0.715
	lat	r	0.725	0.786	.828*	0.796		.902**	0.582	0.792	0.465	0.202	0.572	-0.485	-0.525	0.619	0.740	0.412	0.379	0.750	0.600
		p	0.103	0.064	0.042	0.058		0.005	0.225	0.110	0.353	0.701	0.314	0.269	0.226	0.138	0.057	0.358	0.402	0.052	0.154
	cap	r	.854*	.812*	.869*	0.728	.856*		0.583	0.607	0.543	0.203	0.043	-0.596	-0.359	0.3	0.635	0.264	0.424	0.469	0.323
		p	0.031	0.050	0.024	0.101	0.030		0.224	0.277	0.266	0.699	0.945	0.158	0.429	0.513	0.125	0.567	0.343	0.288	0.480
BLA	*ant*	r	0.068	-0.197	0.035	-0.354	-0.451	-0.157		0.711	.926**	.925*	0.353	-0.147	-0.350	0.196	.941**	.833*	0.760	0.688	0.727
		p	0.873	0.640	0.934	0.491	0.370	0.766		0.178	0.008	0.024	0.560	0.780	0.496	0.709	0.005	0.040	0.080	0.131	0.102
	pos	r	-0.052	-0.462	-0.327	-0.644	-0.637	-0.182	.832*		0.839	.892*	0.562	-0.713	-0.107	0.484	.894*	0.580	0.055	0.700	0.702
		p	0.911	0.297	0.474	0.241	0.248	0.769	0.020		0.076	0.042	0.325	0.176	0.865	0.408	0.041	0.305	0.931	0.188	0.186
BMA	*ant*	r	0.102	0.074	0.268	-0.480	-0.644	-0.374	0.703	0.395		.938*	0.293	-0.379	-0.256	0.289	.889*	0.752	0.555	0.059	0.449
		p	0.810	0.861	0.520	0.335	0.168	0.465	0.052	0.380		0.018	0.632	0.402	0.580	0.529	0.018	0.085	0.253	0.900	0.312
	pos	r	0.638	-0.005	0.489	0.079	0.381	0.669	0.349	0.268	0.182		0.585	-0.343	-0.35	-0.055	0.722	0.468	0.179	0.257	0.679
		p	0.123	0.991	0.266	0.899	0.527	0.216	0.442	0.561	0.696		0.300	0.505	0.493	0.918	0.105	0.349	0.734	0.623	0.138
LA	*mid*	r	0.328	0.235	0.268	-0.527	-0.571	-0.051	.777*	0.635	.935**	0.213		-0.190	-0.177	0.556	0.630	0.683	0.085	0.780	0.813
		p	0.473	0.611	0.561	0.361	0.315	0.935	0.040	0.125	0.002	0.647		0.760	0.776	0.331	0.255	0.203	0.892	0.120	0.094
PVT	*ant*	r	-0.032	0.597	0.063	0.170	0.160	-0.227	-0.550	-0.675	-0.038	-0.718	-0.156		0.223	-0.276	-0.282	0.134	0.351	-0.006	-0.102
		p	0.952	0.211	0.906	0.830	0.840	0.773	0.258	0.141	0.943	0.108	0.768		0.595	0.508	0.541	0.775	0.439	0.989	0.810
	pos	r	0.258	0.460	0.548	0.196	0.124	0.035	0.005	-0.383	0.684	0.008	0.460	0.500		-0.386	-0.329	0.077	0.070	-0.127	-0.41
		p	0.537	0.251	0.160	0.710	0.815	0.948	0.990	0.397	0.062	0.986	0.299	0.313		0.345	0.472	0.869	0.881	0.764	0.311
RE	*mid*	r	0.441	0.215	0.378	0.261	0.739	0.416	-0.357	-0.535	-0.545	0.409	-0.572	0.123	-0.077		0.545	0.400	-0.062	0.582	0.631
		p	0.322	0.643	0.403	0.617	0.093	0.412	0.431	0.274	0.206	0.421	0.235	0.844	0.870		0.206	0.374	0.895	0.130	0.093
ACB	*core*	r	0.185	0.495	0.376	0.632	0.540	0.294	0.048	-0.382	0.093	-0.370	-0.010	0.589	0.284	0.207		.839*	0.597	.844*	.877**
		p	0.662	0.212	0.358	0.179	0.269	0.572	0.911	0.398	0.827	0.414	0.983	0.219	0.496	0.657		0.018	0.157	0.017	0.009
	dsh	r	0.022	0.380	0.370	0.377	0.248	-0.105	-0.020	-0.589	0.329	-0.395	0.000	0.747	0.612	0.175	.852**		0.732	0.733	.767*
		p	0.959	0.353	0.367	0.461	0.636	0.844	0.963	0.164	0.426	0.381	0.999	0.088	0.107	0.707	0.007		0.061	0.061	0.044
	vsh	r	0.211	0.587	0.490	0.571	0.164	-0.052	-0.431	-0.632	0.074	-0.327	-0.142	0.586	0.523	-0.133	0.222	0.474		0.396	0.344
		p	0.617	0.126	0.218	0.236	0.756	0.922	0.286	0.128	0.862	0.474	0.762	0.221	0.183	0.776	0.596	0.236		0.379	0.450
PL	*mid*	r	0.635	0.352	0.685	0.570	0.762	.963**	-0.025	-0.020	-0.215	0.768	0.028	-0.462	0.029	0.386	0.018	-0.234	-0.309		.806*
		p	0.126	0.438	0.090	0.238	0.078	0.002	0.957	0.970	0.643	0.075	0.958	0.434	0.950	0.392	0.969	0.614	0.500		0.016
ILA	*mid*	r	.873**	0.673	.824*	0.599	.872*	.939**	-0.194	-0.322	-0.014	0.717	0.101	-0.022	0.361	0.622	0.048	-0.016	0.175	.883**	
		p	0.005	0.067	0.012	0.209	0.024	0.005	0.646	0.482	0.973	0.070	0.829	0.967	0.379	0.136	0.910	0.970	0.678	0.008	

males bottom left | 0.8 to 1 | 0.6 to 0.79 | n.s. | 0.6 to 0.79 | 0.8 to 1 | females top right

negative

Table 3.4: *Correlation of Fos induction between regions for rats tested in a novel context & given a familiar food. Correlations results (r and p values) for males are featured left/below the diagonal and for females right/above the diagonal. Significant positive correlations are shown in green for males and blue for females. All negative correlations are additionally outlined in red.*

Females given a familiar food in a novel context (Table 3.4; right/above the diagonal) had positive correlations between aCEAl and aCEAm, aCEAc and aCEAm, aCEAc and aCEAl, pCEAl and aCEAc, and pCEAc and pCEAl. The anterior and posterior BMA were correlated, and the aBMA was positively correlated with aBLA and the pBMA with aBLA,

Novel Context & Novel Food			aCEA			pCEA			BLA		BMA		LA	PVT		RE	ACB			PL	ILA
			med	*lat*	*cap*	*med*	*lat*	*cap*	*ant*	*pos*	*ant*	*pos*	*mid*	*ant*	*pos*	*mid*	*core*	*dsh*	*vsh*	*mid*	*mid*
aCEA	*med*	r		0.483	-0.440	-0.586	-0.638	-0.11	-0.345	-0.141	-0.543	-0.320	0.108	-0.616	0.426	0.597	-0.134	-0.056	0.278	-0.12	-0.198
		p		0.272	0.323	0.167	0.123	0.822	0.449	0.790	0.265	0.536	0.838	0.141	0.340	0.210	0.774	0.905	0.547	0.827	0.707
	lat	r	.837**		-0.627	0.095	-.765*	-0.111	-0.434	-0.316	-0.727	-0.260	-0.076	-0.401	.826*	.953**	0.459	0.397	0.401	-.838*	-0.417
		p	0.009		0.132	0.839	0.045	0.813	0.330	0.542	0.101	0.618	0.887	0.372	0.022	0.003	0.300	0.378	0.372	0.037	0.410
	cap	r	.892**	0.705		0.409	0.754	0.571	0.071	0.258	0.218	0.424	-0.004	0.405	-0.503	-0.573	0.314	0.029	0.353	0.492	0.338
		p	0.003	0.051		0.362	0.050	0.180	0.880	0.622	0.678	0.402	0.994	0.368	0.250	0.235	0.493	0.952	0.437	0.322	0.513
pCEA	*med*	r	0.620	0.680	.783*		0.247	0.685	-0.352	-0.061	-0.154	0.175	-0.410	0.254	0.128	-0.14	0.501	0.365	0.278	-0.271	0.181
		p	0.101	0.063	0.021		0.593	0.089	0.439	0.909	0.770	0.740	0.420	0.583	0.784	0.787	0.253	0.421	0.546	0.603	0.732
	lat	r	.716*	.851**	0.572	0.639		0.311	0.165	0.032	0.423	0.151	-0.419	.823*	-0.681	-.903*	0.042	-0.096	0.034	0.187	-0.046
		p	0.046	0.007	0.138	0.088		0.497	0.724	0.952	0.404	0.775	0.409	0.023	0.092	0.014	0.929	0.837	0.942	0.722	0.931
	cap	r	0.602	0.686	0.455	0.189	0.678		-0.700	-0.410	-0.585	-0.382	-0.724	0.168	0.120	-0.27	0.235	0.339	0.483	-0.11	0.316
		p	0.114	0.061	0.258	0.654	0.064		0.080	0.420	0.223	0.455	0.104	0.719	0.798	0.610	0.611	0.458	0.273	0.829	0.542
BLA	*ant*	r	-0.428	-0.564	-0.249	-0.491	-0.525	0.070		.902**	.963**	.920**	.773*	-0.097	-0.682	-0.208	-0.244	-0.626	-0.590	0.495	-0.080
		p	0.338	0.187	0.591	0.263	0.227	0.882		0.005	0.001	0.003	0.042	0.818	0.092	0.692	0.598	0.133	0.164	0.318	0.880
	pos	r	-0.255	-0.363	-0.020	-0.195	-0.231	0.234	.899**		.872*	.883**	0.664	-0.490	-0.711	-0.123	-0.209	-.831*	-0.451	0.380	-0.106
		p	0.581	0.423	0.966	0.675	0.618	0.613	0.006		0.024	0.008	0.104	0.265	0.113	0.844	0.692	0.041	0.370	0.528	0.865
BMA	*ant*	r	-0.348	-0.488	-0.136	-0.132	-0.376	-0.067	.860*	.878**		.893*	0.552	0.073	-.895*	-0.661	-0.221	-0.637	-0.614	0.415	-0.120
		p	0.444	0.267	0.772	0.778	0.406	0.887	0.013	0.009		0.017	0.257	0.877	0.016	0.225	0.674	0.174	0.195	0.413	0.820
	pos	r	-0.008	0.136	0.047	0.144	0.216	0.545	0.619	0.726	0.710		0.66	-0.257	-0.64	-0.080	0.155	-0.578	-0.202	0.442	-0.031
		p	0.986	0.771	0.920	0.757	0.641	0.206	0.138	0.065	0.074		0.106	0.578	0.174	0.898	0.769	0.230	0.701	0.456	0.961
LA	*mid*	r	-0.229	-0.294	-0.097	-0.169	-0.358	0.018	0.717	.766*	.879**	0.674		-0.723	-0.170	0.446	-0.130	-0.355	-0.328	0.784	0.528
		p	0.621	0.522	0.836	0.718	0.431	0.969	0.070	0.045	0.009	0.097		0.067	0.748	0.452	0.805	0.489	0.526	0.117	0.361
PVT	*ant*	r	-0.342	-0.253	-0.473	-0.488	0.088	0.226	0.344	0.181	0.095	0.366	-0.258		-0.254	-0.680	0.235	0.307	0.165	-0.173	-0.137
		p	0.452	0.585	0.284	0.267	0.851	0.626	0.504	0.732	0.857	0.476	0.622		0.583	0.137	0.612	0.503	0.724	0.743	0.796
	pos	r	-0.290	-0.302	-0.510	-0.725	-0.424	-0.149	0.223	0.110	0.320	0.17	0.677	-0.154		0.779	0.404	.761*	0.481	-0.407	0.214
		p	0.528	0.510	0.242	0.065	0.343	0.750	0.671	0.836	0.536	0.748	0.140	0.742		0.068	0.368	0.047	0.275	0.423	0.684
RE	*mid*	r	.829*	0.572	0.752	0.326	0.366	0.508	-0.196	-0.261	-0.337	-0.124	-0.317	-0.068	-0.323		0.400	0.347	0.341	-0.799	-0.48
		p	0.021	0.179	0.051	0.475	0.419	0.244	0.674	0.571	0.460	0.791	0.489	0.898	0.532		0.432	0.501	0.509	0.105	0.409
ACB	*core*	r	0.104	-0.069	0.154	-0.326	-0.267	0.490	0.743	0.652	0.462	0.463	0.524	0.057	0.187	0.355		0.627	.816*	-0.168	0.004
		p	0.806	0.871	0.716	0.430	0.523	0.218	0.055	0.113	0.296	0.296	0.227	0.903	0.688	0.434		0.132	0.025	0.751	0.995
	dsh	r	-0.046	-0.307	0.197	-0.212	-0.518	0.098	.804*	0.703	0.569	0.246	0.551	-0.214	-0.020	0.205	.864**		0.643	-0.093	0.459
		p	0.915	0.459	0.639	0.615	0.189	0.817	0.029	0.078	0.182	0.596	0.200	0.644	0.966	0.659	0.006		0.119	0.860	0.360
	vsh	r	0.425	0.109	0.693	0.339	-0.018	0.267	0.496	0.644	0.521	0.310	0.520	-0.447	-0.209	0.386	0.622	.778*		-0.128	0.033
		p	0.293	0.797	0.057	0.412	0.966	0.523	0.257	0.119	0.231	0.499	0.232	0.315	0.653	0.393	0.099	0.023		0.809	0.950
PL	*mid*	r	0.34	0.287	0.492	0.286	-0.088	0.351	0.546	0.404	0.405	0.509	0.478	-0.218	-0.139	0.594	0.688	0.676	0.635		0.712
		p	0.409	0.490	0.215	0.492	0.836	0.394	0.204	0.369	0.367	0.243	0.277	0.639	0.767	0.160	0.059	0.065	0.090		0.112
ILA	*mid*	r	0.620	0.404	.747*	0.467	0.099	0.335	0.265	0.331	0.372	0.360	0.534	-0.558	0.005	0.595	0.552	0.576	.831*	.820*	
		p	0.101	0.321	0.033	0.244	0.816	0.417	0.565	0.469	0.411	0.427	0.217	0.193	0.992	0.159	0.156	0.135	0.011	0.013	

males bottom left | 0.8 to 1 | 0.6 to 0.79 | n.s. | 0.6 to 0.79 | 0.8 to 1 | females top right

negative

Table 3.5: *Correlation of Fos induction between regions for rats tested in a novel context & given a novel food. Correlations results (r and p values) for males are featured left/below the diagonal and for females right/above the diagonal. Significant positive correlations are shown in green for males and blue for females. All negative correlations are additionally outlined in red.*

pBLA. In the PVT there were negative correlations between aPVT and aCEAm and between pPVT and both aCEAl and aCEAc. The ACBc was positively correlated with aBLA, pBLA, and aBMA and the ACBdsh was positively correlated with aBLA and ACBc.

There were positive correlations between PL and ACBc and between ILA and ACBc, ACBdsh, and PL.

Males given a familiar food in a novel context (Table 3.4; left/below diagonal) had only positive correlations. Many CEA subregions were correlated, the aCEAl and aCEAm, aCEAc and aCEAm, aCEAc and aCEAl, pCEAm and aCEAl, pCEAm and aCEAc, pCEAl and aCEAc, pCEAc and aCEAm, pCEAc and aCEAl, pCEAc and aCEAc, and pCEAc and pCEAl. The anterior and posterior BLA were correlated, and the LA was correlated with the aBLA and aBMA. The core and dorsal shell of the ACB were correlated. Additionally, the PL was correlated with pCEAc and the ILA with aCEAm, aCEAc, pCEAl, pCEAc, and PL.

Females given a novel food in a novel context (Table 3.5; right/above the diagonal) had a negative correlation between anterior and posterior CEAl and CEAl. The anterior and posterior BLA were corelated, as well as anterior and posterior BMA. In addition, the BMAa and BMAp were correlated with BLAa and BLAp. Also, the LA was positively correlated with aBLA. In the PVT, there was a positive correlation between aPVT and pCEAl, a positive correlation between pPVT and aCEAl, and a negative correlation between pPVT and aBMA. The RE was positively correlated with aCEAl and negatively correlated with pCEAl. The ACBdsh was negatively correlated with pBLA and positively correlated with pPVT, while the ACBvsh was positively correlated with the ACBc. Lastly, the PL was negatively correlated with aCEAl.

Males given a novel food in a novel context (Table 3.5; left/below the diagonal) had only positive correlations. The CEA subregions had correlations between aCEAl and aCEAm, aCEAc and aCEAm, pCEAm and aCEAc, pCEAl and aCEAm, and pCEAl and aCEAl. The pBLA was correlated with aBLA and aBMA was correlated with aBLA and pBLA. The LA was correlated with both pBLA and aBMA. Additionally, there was a correlation between RE and aCEAm. The ACBdsh was correlated with aBLA and with ACBc and

the ACBvsh. The ILA was correlated with aCEAc, ACBvsh, and PL.

[3.4] Discussion

Here, we determined recruitment of several forebrain areas when rats consumed either a novel or familiar food in a novel or familiar context. We analyzed Fos induction in amygdalar, thalamic, striatal, and cortical regions known to be important for appetitive responding, contextual processing, and motivation. Our behavioral preparation was designed to determine separate effects of food and context novelty on both consumption and neuronal activity in each sex. During the food consumption test, similar to previous behavioral findings reported in Chapter 2 (Greiner & Petrovich, 2020), both male and female rats ate less of the novel than familiar food. Both male and female rats tested in a novel context ate less of both foods, novel and familiar. Novel context and novel food conditions induced Fos within several regions of interest. Novel context induced Fos robustly in almost every region analyzed, while novel food induced Fos in fewer regions. Some regions analyzed were also differentially recruited in males and females.

[3.4.1] Novel Context

Novel context, as the most salient stimulus, induced robust Fos expression in almost every region analyzed. Rats in the novel context condition had increased Fos induction in all the regions of the basolateral complex (BMAa & BMAp, LA, BLAa & BLAp), the central nucleus of the amygdala (CEAc), all subregions of the ACB (core, vsh, dsh), thalamus (PVTa & RE), and medial prefrontal cortex (PL & ILA. Robust Fos expression in all of the basolateral complex nuclei in the novel context condition was expected, given that several of these nuclei are interconnected with the hippocampal formation (HF). The entorhinal cortex, which is important for spatial cognition, and a component of the trisynaptic circuit, has bidirectional connections with the BMAp, BLAp, and LA (McDonald & Mascagni, 1997; Swanson & Kohler, 1986; Wyss, 1981). Additionally, ventral subiculum (vSUB), which has an established role in contextual encoding (Maren,

1999), has projections to both the BMAp (Canteras & Swanson, 1992), which projects back (Krettek & Price, 1977; Petrovich et al., 1996), and LA (Cullinan et al., 1993; McDonald & Mascagni, 1997). The LA, BLA (Ottersen, 1982; Van Groen & Wyss, 1990) and BMAp (McDonald, 1982) also receive projections from CA1, with the LA, innervating the CA1 in return (Pikkarainen et al., 1999; Petrovich et al., 2001). Of note, the BMAa and BLAa, where we observed increased Fos induction in novel context conditions, do not have substantial inputs to the HF (Petrovich et al., 2001), but receive some inputs from CA1 (Cenquizca & Swanson, 2007). Additionally, the BMAa receives input from the ventromedial PFC (Adhikari et al., 2015) which could relay information from the HF.

It is important to note that increased neuronal activity during feeding in a novel environment is not likely due only to contextual information, but also in mediating appropriate behavioral responding within the novel context. As mentioned previously, the vSUB is connected to several amygdala nuclei where we found Fos induction in a novel context, namely the CEAc, BMAp, and LA The connectivity with the vSUB is of particular interest because it has been previously found to mediate novel stimulus detection—particularly novel environments (Legault & Wise, 2001; Lisman & Grace, 2005). Therefore, the patterns we observe among these regions could be specific to the fact that the context is novel. Additionally, the activation and connectivity of these regions suggest that they are sharing information.

Within the CEA, Fos induction in novel context tested groups was specific to the CEAc. This finding is interesting because the CEAc receives substantial inputs from the CA1 (Cenquizca & Swanson, 2007) and the ventral subiculum (vSUB) (Canteras & Swanson, 1992). In addition, contextual information could reach the CEA via multiple relays from the HF (Canteras & Swanson, 1992; Cenquizca & Swanson, 2007), most notably, via inputs from the medial PFC (Hurley et al., 1991; Messanvi et al., 2023) and BLA.

Furthermore, some of these amygdala regions could serve as integrators of novel information. The BMAa and CEAc were the only amygdala areas analyzed that responded to both novel context and novel food, which suggests that these regions are processing novelty generally. They could also be controlling feeding in response to novelty rather than responding to specific food or context information. This convergence of novelty processing may be particularly important for driving appropriate behavioral responding. The BMAa sends substantial projections to the CEA (Petrovich et al., 1996) and the CEA is known to both drive (Douglass et al., 2017) and inhibit (Cai et al., 2014) consumption, which is relevant to our behavioral findings since consumption levels varied based on condition. The uncertainty of a novel context may also induce responding within safety or defensive circuits. This would align with our findings that novel context exposure also increased Fos induction within the BLA (both anterior and posterior) as well as within the CEAc. As the BLA R-spondin 2 (Rspo2+) expressing neurons that inhibit appetitive behavior, and elicit defensive behavior, project to the CEAc (Kim et al. 2016).

Increased neuronal activity for groups tested in novel context was also robust across thalamic, striatal, and cortical areas analyzed. Within the thalamus, both of the midline areas that we analyzed, the PVTa and RE, had higher Fos induction in the novel context condition. The recruitment of the RE in a novel context is consistent with its role in contextual memory and novel context encoding. The RE functions as a major thalamic relay for the transfer of information from the medial PFC to the hippocampus (Ferraris et al., 2021; McKenna & Vertes, 2004). The RE is also critical for the formation and retrieval of distinct contextual memories (Ramanathan et al., 2018) and inactivation of RE after fear conditioning resulted in a generalized fear-response to novel contexts (Ramanathan et al., 2018).

The PVTa has been shown previously to control consumption within a novel environment (Cheng et al., 2018). Activation of PVTa neurons that project to the ACB increased consumption in a novel context (Cheng et al., 2018) and activation of PVT GLP-1 receptors, which reduces activity of the PVT to ACB pathway, resulted in a decrease in consumption and food seeking behavior (Ong et al., 2017). The Fos induction we observed in the ACB would support activation of PVTa-ACB pathway; however, it is unclear how this pathway is represented by our behavior findings. In our study, there was Fos induction in both ACB and PVTa in groups that had decreased consumption within a novel context. Our methodology was not cell- or pathway-specific and thus we cannot determine which circuits are represented by the overall activity within the PVTa and ACB.

All subregions of the ACB had increased Fos induction in the novel context condition. The ACB is well positioned to mediate behavioral responding in the novel context. The ACB mediates motivation for reward and is critical for context-mediated appetitive behavior. It receives direct HF input (Groenewegen et al., 1999; Canteras et al., 1992) and inactivation of the both the ACB core and shell results in impaired context-induced reinstatement (Fuchs et al., 2008). Additionally, connections to ACB shell from the BLA are required for active avoidance (Ramirez et al., 2015).

Both medial prefrontal cortical regions analyzed, the PL and ILA, had increased Fos induction in the novel context condition. Both regions are heavily interconnected with the HF (Gabbott et al., 2005; Hoover & Vertes, 2007; Messanvi et al., 2023) and are known for relaying contextual information to the RE (Vertes & Crane, 1996; Vertes 2002). Sex differences in medial PFC recruitment has been identified in two related tasks. Prior work has found higher Fos induction in the PL and ILA during contextual renewal of responding to food cues, though exclusively in males (Anderson & Petrovich, 2017). Another study identified female-specific recruitment of the medial PFC during fear

induced hypophagia, that correlated with feeding (Reppucci & Petrovich, 2018). In the current study, there was a close to significant sex difference in the PL where females had higher induction than males. However, the difference in behavioral paradigms between these studies does not allow for direct comparison in patterns of activation.

The medial PFC may play a crucial role in controlling feeding behavior in the novel context condition. One proposed model suggests that glutamatergic projections from PFC to both ACBsh and the LHA are regulated by GABAergic inhibition or disinhibition (Baldo, 2016). Previous work has found that PFC mu-opioid stimulation drives feeding behavior through activation of the neurons within the lateral hypothalamus (LHA) (Mena et al., 2013). Therefore, some of the Fos induction within the medial PFC that we observed could represent activity of GABA neurons that are inhibiting activity within PFC to LHA pathway that promotes feeding behavior. Additionally, given the increased Fos induction we observed within the ACBsh, it is possible that PFC mu-opioid activation of LHA is being attenuated by ACB AMPA-receptor stimulation, which could suppress feeding through inhibition of cells within the LHA (Mena et al., 2013; Stratford et al., 1998).

[3.4.2] Novel Food

Novel food, regardless of context, increased Fos induction within all CEA subregions (medial, lateral, and capsular), BMAa, and PVTa. Novel food induced Fos in fewer regions than novel context. However, the CEA was particularly responsive to food type differences. All CEA subregions had increased Fos induction for groups given a novel food. This matches with previous findings that novel food exposure increases Fos induction in the CEA (Koh et al., 2003). This Fos induction may reflect different drives; appetitive drives that are influenced by the hunger state of the animal (after food deprivation) or the palatability of the novel food used, or aversive responding related to novel taste avoidance. The CEA has diverse neuronal cell types, which have different

roles in the control of feeding, and the methodology used in the current study could not differentiate between them. Different neuronal cell types are not evenly distributed across each CEA subregion. Therefore, the activity observed across the CEA subregions may not have been in response to the same aspect of the stimulus.

Neurons that express protein kinase c-delta (PKCd) may be among the Fos-positive cell populations observed within the CEA. The CEA neurons that express PKCd are activated by and mediate anorexigenic signals and are also required for the inhibition of feeding (Cai et al., 2014). However, the CEA is also known to promote appetitive behaviors, potentially through the inhibition of neurons in the parabrachial nucleus (PB) that are involved in the processing of gustatory signals. Activation of the CEA serotonin receptor 2a (HTR2a) containing neurons that project to the PBN increased food consumption even in sated rats (Douglass et al., 2017). PKCd expressing neurons are mostly located within the CEAc and CEAl (Cai et al., 2014; McCullough et al., 2018) and HTR2a neurons are almost exclusively located within the CEAl (Douglass et al., 2017; Kong & Zweifel, 2021).

Another consideration is that the increased Fos induction within the CEA may be due to the palatability of the novel food rather than novelty processing or inhibition of eating. The CEAm and CEAl were the two CEA subregions that had selective Fos induction in response to a novel food only. The novel food (Test Diet pellets) used in our behavioral preparation are high in sucrose, making it more palatable than the familiar food (Rat Chow) used. Palatable foods were previously shown to increase Fos induction in the CEA (Park & Carr, 1998, Wu et al., 2014; Parsons et al., 2022) and a subset of CEA neurons that express prepronociceptin (Pnoc) mediate palatable food consumption (Hardaway et al., 2019). These Pnoc cells are located predominantly in the CEAm and CEAl (Hardaway et al., 2019), and it was found that inhibition of Pnoc neurons reduced

the latency to feed in a novel environment as well as consumption in home cage after novelty exposure (Hardaway et al., 2019).

Outside of appetitive responding, stress responses due to the relative uncertainty of novel food may have recruited populations within the CEAm and CEAl, namely, neurons that express corticotropin releasing factor (CRF; also known as CRH) and neurons that express somatostatin. Neurons that express CRF are involved in stress responding and are largely concentrated within the CEAl, with additional populations within the CEAm and few within the CEAc (Marchant et al., 2007, McCullough et al., 2018). Neurons expressing somatostatin are involved in defensive and fear responses (Yu et al., 2016) and are found in much greater density in the CEAl and CEAm than CEAc (Jolkkonen & Pitkanen, 1998; McCullough et al., 2018). The CEA may be a site where two competing drives converge to impact consumption—positive motivation due to food palatability and avoidance due to novelty.

The only other amygdala region analyzed that exhibited increased Fos induction to novel food is the BMAa. The BMAa heavily innervates the CEA (Petrovich et al., 1996), and the two regions had similar patterns. Previous work indicates that lower consumption or latency to approach food is associated with BMA inhibition rather than activation (Lukaszewska et al., 1984), however that study was conducted exclusively within a novel context, did not investigate females, and did not look at the effects of novel food.

Like the CEA, the BMA is implicated in regulating fear and anxiety responding (Rajbahndari et al., 2021; Amano et al., 2011; de Andrade et al., 2012), including physiological stress responses to social novelty (Mesquita et al., 2016). Additionally, BMA neurons that receive input from the ventromedial PFC are associated with suppressing both freezing and anxiety-state behaviors (Adhikari et al., 2015). Therefore, the Fos induction that we observed within the BMAa may be related to an attempt to

override neophobic responding to satisfy physiological needs, given that our animals were food-deprived at the start of testing.

The only other region that had a greater response to novel food was the PVTa. Like the BMAa and the CEAc, the PVTa exhibited increased Fos induction to both novel food and novel context. The patterns of activation within these regions suggests that they are a network that responds to novelty, regardless of whether it is food or context. The PVTa is distinguished by higher expression of galanin (*Gal*) (Gao et al., 2020). *Gal*-positive neurons respond to increased arousal states, and their connections to ILA are implicated in regulating physiological responding to increased arousal (Gao et al., 2020). Therefore, neurons within the PVTa may have been recruited due to the arousal induced by novelty. Interestingly, the PVTa and PVTp differed in their activation patterns in the current study. The PVTp did not show differences in Fos induction food type. However, the PVTp did have higher Fos induction in the novel context condition, which was close to significance.

[3.4.3] Sex Differences

Sex differences in Fos induction were present in the PVTa, the core and ventral shell of the ACB, the CEAc, and the posterior part of the CEAl. Females had overall greater Fos induction in the ACBc and ACBvsh. Sex-specific responding of the ACB to food consumption has been observed before, with projections to the ACB from of a sub-population of lateral hypothalamus neurons that produce melanin-concentrating hormone promoting consumption of food for males but not females (Terrill et al., 2020). As stated previously, we did not observe any behavioral sex differences within this study. All rats consumed less of a familiar food or when eating in a novel context, regardless of sex. However, as reported in Chapter 2, (Greiner & Petrovich, 2020) behavioral differences between males and females emerge during habituation, where females consume much less than their male counterparts in the novel context.

Within the PVTa, females given a novel food, regardless of context, had greater expression of Fos than their male counterparts. There is prior evidence for sex-dependent differences in stress induced activity of the PVTa. Ovariectomized females without estradiol replacement had higher stress-induced increased Fos induction in the PVTa compared to females with replacement (Uneyama et al., 2006). There were key differences between our studies that do not allow for direct comparison, namely that the stressor used in the prior study was restraint, while we used novelty and that we used intact females. Nevertheless, our findings still contribute to the evidence of sex differences in responding of PVTa neurons.

Within the CEA, there were sex differences in two subregions. Fos induction in males was overall greater in the CEAc compared to females regardless of food type or testing context, and in the the posterior CEAl the males given a novel food in a familiar context had greater Fos induction than all other groups. The difference in visceral sensory information received and processed within each subregion could be the reason why males recruit CEAc and CEAl in unique ways. The PB sends distinct projections to each of the CEA subregions, with the PB-CEAl pathway implicated in visceral processes and the PB-CEAc implicated in nociception (Bernard et al., 1993; Bernard & Besson 1990).

Another consideration for the overall sex differences observed in the CEAc, ACBc, and ACBvsh is that they may be related to hunger-state of the animal. All animals in our preparation were acutely food deprived and it is possible that males and females have different activation of these regions due to deprivation state. Previous work has found differences in Fos induction in the CEAm based on deprivation state that was sex dependent (Parsons et al., 2022). Additionally, the ACB has previously exhibited increased activation during food deprivation) in both sexes. (Parsons et al., 2022; Carr, 2011)

[3.4.4] Network Activation Patterns

The analyses of correlations in Fos induction patterns between our regions of interest, found distinct patterns within each group as well as common patterns across conditions. Overall, the CEA was the most correlated with other regions and across its subregions, and this was most apparent in rats that were given a familiar food. The CEA subregions were inter-correlated much less in groups given a novel food compared to groups given a familiar food in the same context. These patterns suggest that in the presence of novel food distinct inputs to the CEA produce activation patterns that independently activate different subregions. In addition, considering that the CEA has substantial connections between its subregions (Jolkkonen & Pitkanen, 1998), it is possible that communication between the areas during the presence of novel food is more complex, with differing patterns of local inhibition and disinhibition, which cannot be detected by linear correlational analysis.

Groups given a novel food had an additional similarity in their correlation patterns. For every group given a novel food, regardless of testing context or sex, there was a significant positive correlation between BLAa and BMAa. Interestingly, while BMAa was a region with increased Fos induction to a novel food, BLAa was not. Additionally, the BMAa only sends very light projections to the BLAa (Petrovich et al., 1996) and the two areas are considered to be parts of distinct circuits within the basolateral complex (Swanson & Petrovich, 1998). Therefore, the correlation observed is not due to direct communication between the two regions, but more likely due to parallel functioning systems.

Another overlap between groups occurred in males. All males, regardless of testing conditions, had a positive correlation between core and dorsal shell of the ACB. However, it is important to note that, while this correlation was common in all male groups, it was not exclusive to males. A positive correlation between ACBc and ACBdsh was additionally observed in females who were tested in a novel context and given a

familiar food. Therefore, the only group where ACB subregions were not correlated was females given a novel food in a novel context.

The positive correlation between ACBc and ACBdsh is particularly interesting given that the core and shell have been shown to play opposing roles in appetitive behaviors. During appetitive learning, the ACBc uses cue-related information to drive responding, while the ACB shell uses contextual information (Ito & Hayen, 2011). Given that our paradigm uses both contextual (novel context) and non-contextual (novel food) cues in tandem, it is possible that, communication between the ACBc and ACBdsh is necessary in order to drive appropriate appetitive responses. However, the inputs from core to shell are heavier than from shell to core (van Dongen et al., 2005) and their balance may be important during habituation to novel foods in novel context.

The females given a novel food in a novel context were the only novel context tested group where PL and ILA were not correlated. The PL and ILA are interconnected with one another, however the PL to ILA connection is denser than ILA to PL (Marek et al., 2018). Activation of PL to ILA enhances fear extinction (Marek et al., 2018) and fear habituation and extinction circuits have been shown to partially overlap, at least in males (Furlong et al., 2016). While our paradigm is not aversive conditioning, it is possible that there are similar mechanisms used in habituating to a potentially dangerous novel stimulus. Therefore, the lack of correlation between the PL and ILA for females given a novel food in a novel context could be indicative of poorer habituation to novelty over time as observed in Chapter 2 (Greiner & Petrovich, 2020).

Another difference between the sexes within the correlation results is that most of the negative correlations were in the female groups, with the greatest number in females given a novel food in a novel context. Most negative correlations for females that consumed a novel food in a novel context included the CEAl. The anterior and posterior CEAl were negatively correlated with each other and with the PL and RE, respectively.

The PL and RE are interconnected and are involved in a circuit relaying contextual information from the hippocampus (Vertes & Crane, 1996; Vertes 2002), but only PL has direct anatomical connections to the CEA (Vertes, 2004). The negative correlations between the anterior and posterior regions of the CEAl is in line with the Fos induction results, as the patterns of activation differ rostro-caudally within the lateral subregion.

Both female groups given a familiar food had negative correlations between anterior CEA subregions and one of the midline thalamic nuclei analyzed. For females given a familiar food in a familiar context, there was a negative correlation between anterior CEAl and RE, while females given a familiar food in a novel context had negative correlations between anterior CEAm and PVTa and between both anterior CEAl and anterior CEAc and PVTp. Since there are no direct anatomical connections between the RE and CEA, the activation of one region is not directly causing the silencing of another; however, it is possible that these regions are impacted in opposite ways by a shared input.

The females given a familiar food in a familiar context also had a negative correlation between ACBdsh and the ILA. Sex specific responding has been found in the ILA to ACB shell pathway. Stimulation of this pathway suppressed conditioned taste aversion in males only, but increased sucrose preference for both sexes (Hurley & Carelli, 2020). Negative correlation between these regions in females given a familiar food in a familiar context may be because the drive to consume in their condition is driven by physiological needs (a result of food deprivation) rather than hedonic drives. Negative ACB shell correlations were present in the other familiar context tested female group as well, though they were exclusively with regions within the basolateral complex (ACBdsh and BLAa, ACBdsh and aBMA, ACBvsh and LA). Activation of the ACB by the BLA is thought to facilitate reward learning (Amir et al., 2015, Dieterich et al., 2021). An inverse relationship in activity between these two regions could suggest that BLA may be active

in other circuits, potentially driving aversive responding to the novel food, rather than stimulating reward responding through the ACB.

Another interesting pattern observed in females who were tested in a familiar context and given a familiar food, was that the BLAa was positively correlated with two subregions of the posterior CEA (lateral and capsular) and the BLAp was positively correlated with two subregions of the anterior CEA (medial and capsular). This anterior to posterior and posterior to anterior topography between the BLA and CEA could implicate different functional connectivity that varies rostro-caudally. The BLA has very distinct functions and connectivity in the anterior and posterior portion. Of note, the BLAa has very little direct connects with the CEA compared to the BLAp (Swanson & Petrovich, 1998; Pitkanen et al., 1997). Additionally, the BLAp projects to areas, such as the lateral hypothalamus (Petrovich et al., 2001; Hintiryan et al., 2021), that are integral to mediating appetitive behavior, where the BLAa does not.

As another point of interest regarding conditions with no novel stimuli, there was no overlap in region correlations between females and males tested in a familiar context and given a familiar food. That suggests that males and females have differential processing for food consumption at baseline.

[3.4.5] Conclusions

In conclusion, our study revealed behavioral and neural differences in male and female rats exposed to novel foods and contexts compared to familiar. We replicated behavioral findings that male and female rats limit their consumption when the food is novel or when presented with food in a novel context. Fos induction analysis of several key regions of interest revealed different patterns in response to food novelty, context novelty, and the sex of the animal. In areas where we found differences in Fos patterns, novel stimuli induced more Fos than familiar. Novel context induced Fos within at least one subregion of all areas analyzed and novel food induced Fos in the CEA, BMAa, and

PVTa. Of note, the CEAm and CEAl were the only two areas analyzed that had increased Fos induction to a novel food without also having increased Fos induction to a novel context. There were also sex differences in Fos induction and the patterns varied between regions. Within the CEAc and pCEAl male groups exhibited greater Fos induction and within the ACBc, ACBvsh, and aPVT greater Fos induction was observed in female groups.

Fos induction patterns we observed in the novel context condition could represent several types of processing within each region. Many regions with increased activation in a novel context, namely the CEA, ILA, PL, ACB, PVTa, and majority of the basolateral complex, receive inputs from the hippocampal formation that would allow for integration of contextual information within a novel context (Cenquizca & Swanson, 2007; McDonald & Mascagni, 1997; Swanson & Kohler, 1986; Wyss, 1981; Petrovich et al., 1996; Ottersen, 1982; Van Groen & Wyss, 1990; Pikkarainen et al., 1999; Petrovich et al., 2001; Gabbott et al., 2005; Hoover & Vertes, 2007). The RE has been previously established as critical for forming distinct contextual memories (Ramanathan et al., 2018) However, increased neuronal activity in a novel context may reflect activity in other circuits as well. The relative uncertainty of a novel context may also induce responding within safety or defensive circuits, such as those found between BLA and CEAc (Kim et al. 2016). Furthermore, Fos induction could be the result of connections from multiple inputs and some regions may serve as integrators of novel information, particularly regarding regions that responded to both novel context and novel food like CEAc, BMAa, and PVTa.

While novel food induced Fos in fewer regions than the novel context, CEA exhibited robust activity in all subregions in groups given a novel food. This suggests that the CEA subregions mediate different aspects of novelty processing, with novel contextual information focused in the CEAc and food information focused in the CEAm and CEAl.

Sex differences in Fos induction was surprising given that males and females did not differ behaviorally during the test. The neural activation differences may be predictive of future behavioral sex differences, as previously observed during habituation to novel foods and novel contexts (Greiner & Petrovich, 2020). However, it is unclear if these neural differences would persist throughout habituation. It is additionally possible that different neural substrates underlie the same behavior in males and females. Further work would be required to determine the course of activity within these regions as the animals habituate to novelty.

Overall, these results address a gap in our knowledge about the neural substrates underlying how novelty impacts feeding behavior. The result identified distinct circuits that underlie novelty processing during consumption and established sex differences in activation patterns that are potentially predictive of behavioral sex differences in habituation. The delineated circuities will allow for better of understanding of the neural mechanisms that mediate the way environmental factors influence appetitive behaviors.

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[3.7] References

Adhikari, A., Lerner, T. N., Finkelstein, J., Pak, S., Jennings, J. H., Davidson, T. J., Ferenczi, E., Gunaydin, L. A., Mirzabekov, J. J., Ye, L., Kim, S.-Y., Lei, A., & Deisseroth, K. (2015). Basomedial amygdala mediates top-down control of anxiety and fear. *Nature*, *527*(7577), 179–185. https://doi.org/10.1038/nature15698

Amir, A., Kyriazi, P., Lee, S. C., Headley, D. B., & Paré, D. (2019). Basolateral amygdala neurons are activated during threat expectation. *Journal of neurophysiology*, *121*(5), 1761–1777. https://doi.org/10.1152/jn.00807.2018

Anderson, L. C., & Petrovich, G. D. (2017). Sex specific recruitment of a medial prefrontal cortex-hippocampal-thalamic system during context-dependent renewal of responding to food cues in rats. *Neurobiology of Learning and Memory*, *139*, 11-21.

Baldo BA. Prefrontal Cortical Opioids and Dysregulated Motivation: A Network Hypothesis. *Trends Neurosci*. 2016;39(6):366-377. doi:10.1016/j.tins.2016.03.004

Bechara, A., & Damasio, A. R. (2005). The somatic marker hypothesis: A neural theory of economic decision. *Games and Economic Behavior, 52*(2), 336-372.

Bernard J-F, Alden M, Besson J-M (1993) The organization of the efferent projections from the pontine parabrachial area to the amygdaloid complex: A Phaseolus vulgaris leucoagglutinin (PHA-L) study in the rat. *J Comp Neurol, 329*: 201–229.

Bernard, J. F., & Besson, J. M. (1990). The spino(trigemino)pontoamygdaloid pathway: electrophysiological evidence for an involvement in pain processes. *Journal of neurophysiology*, *63*(3), 473–490. https://doi.org/10.1152/jn.1990.63.3.473

Bhatnagar, S. & Dallman, M. F. (1999). The paraventricular nucleus of the thalamus alters rhythms in core temperature and energy balance in a state-dependent manner. *Brain Research, 851(1-2)*, 66-75.

Bozarth, M. A., & Wise, R. A. (1981). Intracranial self-administration of morphine into the ventral tegmental area in rats. *Life sciences*, *28*(5), 551–555. https://doi.org/10.1016/0024-3205(81)90148-x

Brog, J. S., Salyapongse, A., Deutch, A. Y., & Zahm, D. S. (1993). The patterns of afferent innervation of the core and shell in the “accumbens” part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *Journal of comparative neurology*, *338*(2), 255-278.

Cai H, Haubensak W, Anthony TE, Anderson DJ (2014). Central amygdala PKC-δ(+) neurons mediate the influence of multiple anorexigenic signals. *Nature Neuroscience. ,* 17, 9, 1240-8. doi: 10.1038/nn.3767.

Canteras, N. S., & Swanson, L. W. (1992). Projections of the ventral subiculum to the amygdala, septum, and hypothalamus: A PHAL anterograde tract-tracing study in the rat. *The Journal of Comparative Neurology, 324*(2), 180-194.

Carr K. D. (2011). Food scarcity, neuroadaptations, and the pathogenic potential of dieting in an unnatural ecology: binge eating and drug abuse. *Physiology & behavior*, *104*(1), 162–167. https://doi.org/10.1016/j.physbeh.2011.04.023

Castro DC, Berridge KC. Opioid hedonic hotspot in nucleus accumbens shell: mu, delta, and kappa maps for enhancement of sweetness "liking" and "wanting". J Neurosci. 2014 Mar 19;34(12):4239-50. doi: 10.1523/JNEUROSCI.4458-13.2014. PMID: 24647944; PMCID: PMC3960467.

Cenquizca, L. A., & Swanson, L. W. (2007). Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. *Brain Research Reviews, 56*(1), 1-26.

Cheng, J., Wang, J., Ma, X., Ullah, R., Shen, Y., & Zhou, Y. (2018). Anterior Paraventricular Thalamus to Nucleus Accumbens Projection Is Involved in Feeding Behavior in a Novel Environment. *Frontiers in Molecular Neuroscience, 11*.

Christie, M. J., Summers, R. J., Stephenson, J. A., Cook, C. J., & Beart, P. M. (1987). Excitatory amino acid projections to the nucleus accumbens septi in the rat: a retrograde transport study utilizingd [3H] aspartate and [3H] GABA. *Neuroscience*, *22*(2), 425-439.

Cole, S., Powell, D. J., & Petrovich, G. D. (2013). Differential recruitment of distinct amygdalar nuclei across appetitive associative learning. *Learning & Memory*, *20*(6), 295-299.

Cullinan, W. E., Herman, J. P., & Watson, S. J. (1993). Ventral subicular interaction with the hypothalamic paraventricular nucleus: Evidence for a relay in the bed nucleus of the Stria terminalis. *The Journal of Comparative Neurology*, *332*(1), 1–20. https://doi.org/10.1002/cne.903320102

de Andrade, J. S., Abrão, R. O., Céspedes, I. C., Garcia, M. C., Nascimento, J. O. G., Spadari-Bratfisch, R. C., Melo, L. L., da Silva, R. C. B., & Viana, M. B. (2012). Acute restraint differently alters defensive responses and fos immunoreactivity in the rat brain. *Behavioural Brain Research*, *232*(1), 20–29. https://doi.org/10.1016/j.bbr.2012.03.034

Dieterich, A., Floeder, J., Stech, K., Lee, J., Srivastava, P., Barker, D. J., & Samuels, B. A. (2021). Activation of basolateral amygdala to nucleus accumbens projection neurons attenuates chronic corticosterone-induced behavioral deficits in male mice. *Frontiers in behavioral neuroscience*, 17.

Dong, X., Li, S., & Kirouac, G. J. (2017). Collateralization of projections from the paraventricular nucleus of the thalamus to the nucleus accumbens, bed nucleus of the stria terminalis, and central nucleus of the amygdala. *Brain structure & function*, *222*(9), 3927–3943. https://doi.org/10.1007/s00429-017-1445-8

Douglass, A. M., Kucukdereli, H., Ponserre, M., Markovic, M., Gründemann, J., Strobel, C., . . . Klein, R. (2017). Central amygdala circuits modulate food consumption through a positive-valence mechanism.

Ferraris, M., Cassel, J. C., Pereira de Vasconcelos, A., Stephan, A., & Quilichini, P. P. (2021). The nucleus reuniens, a thalamic relay for cortico-hippocampal interaction in recent and remote memory consolidation. *Neuroscience and biobehavioral reviews*, *125*, 339–354. https://doi.org/10.1016/j.neubiorev.2021.02.025

Fuchs, R. A., Ramirez, D. R., & Bell, G. H. (2008). Nucleus accumbens shell and core involvement in drug context-induced reinstatement of cocaine seeking in rats. *Psychopharmacology*, *200*(4), 545–556. https://doi.org/10.1007/s00213-008-1234-4

Gabbott, P. L., Warner, T. A., Jays, P. R., Salway, P., & Busby, S. J. (2005). Prefrontal cortex in the rat: Projections to subcortical autonomic, motor, and limbic centers. *The Journal of Comparative Neurology, 492*(2), 145-177.

Gao, C., Leng, Y., Ma, J., Rooke, V., Rodriguez-Gonzalez, S., Ramakrishnan, C., Deisseroth, K., & Penzo, M. A. (2020). Two genetically, anatomically and functionally distinct cell types segregate across anteroposterior axis of paraventricular thalamus. *Nature Neuroscience*, *23*(2), 217–228. https://doi.org/10.1038/s41593-019-0572-3

Greiner, E. M., & Petrovich, G. D. (2020). The effects of novelty on food consumption in male and female rats. *Physiology & Behavior, 223*, 112970. doi:10.1016/j.physbeh.2020.112970

Groenewegen, H. J., Wright, C. I., Beijer, A. V., & Voorn, P. (1999). Convergence and segregation of ventral striatal inputs and outputs. *Annals of the New York Academy of Sciences*, *877*, 49–63. https://doi.org/10.1111/j.1749-6632.1999.tb09260.x

Hardaway JA, Halladay LR, Mazzone CM, Pati D, Bloodgood DW, Kim M, Jensen J, DiBerto JF, Boyt KM, Shiddapur A, Erfani A, Hon OJ, Neira S, Stanhope CM, Sugam JA, Saddoris MP, Tipton G, McElligott Z, Jhou TC, Stuber GD, Bruchas MR, Bulik CM, Holmes A, Kash TL. Central Amygdala Prepronociceptin-Expressing Neurons Mediate Palatable Food Consumption and Reward. *Neuron*. 2019 Jun 5;102(5):1037-1052.e7. doi: 10.1016/j.neuron.2019.03.037 Hurley, K. M., Herbert, H., Moga, M. M., & Saper, C. B. (1991). Efferent projections of the infralimbic cortex of the rat. *The Journal of Comparative Neurology, 308*(2), 249-276.Knight, R. T. (1996). Contribution of human hippocampal region to novelty detection. *Nature,383*(6597), 256-259.

Hintiryan, H., Bowman, I., Johnson, D. L., Korobkova, L., Zhu, M., Khanjani, N., ... & Dong, H. W. (2021). Connectivity characterization of the mouse basolateral amygdalar complex. *Nature communications*, *12*(1), 2859.

Hurley, S. W., & Carelli, R. M. (2020). Activation of Infralimbic to Nucleus Accumbens Shell Pathway Suppresses Conditioned Aversion in Male But Not Female Rats. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *40*(36), 6888–6895. https://doi.org/10.1523/JNEUROSCI.0137-20.2020

Hurley, K. M., Herbert, H., Moga, M. M., & Saper, C. B. (1991). Efferent projections of the infralimbic cortex of the rat. *The Journal of comparative neurology*, *308*(2), 249–276. https://doi.org/10.1002/cne.903080210

Jolkkonen, E., & Pitkanen, A. (1998). Intrinsic connections of the rat amygdaloid complex: Projections originating in the central nucleus. *The Journal of Comparative Neurology, 395*(1), 53-72. doi:10.1002/(sici)1096-9861(19980525)395:13.0.co;2-g

Kim J, Pignatelli M, Xu S, Itohara S, Tonegawa S. (2016). Antagonistic negative and positive neurons of the basolateral amygdala. *Nature Neuroscience*, 19, 12, 1636-1646. doi: 10.1038/nn.4414.

Koh, M. T., Wilkins, E. E., & Bernstein, I. L. (2003). Novel Tastes Elevate c-fos Expression in the Central Amygdala and Insular Cortex: Implication for Taste Aversion Learning. *Behavioral Neuroscience, 117*(6), 1416-1422.

Kong, M. S., & Zweifel, L. S. (2021). Central amygdala circuits in valence and salience processing. *Behavioural brain research*, *410*, 113355. https://doi.org/10.1016/j.bbr.2021.113355

Krettek, J. E., & Price, J. L. (1977). Projections from the amygdaloid complex and adjacent olfactory structures to the entorhinal cortex and to the subiculum in the rat and cat. *The Journal of Comparative Neurology*, *172*(4), 723–752. https://doi.org/10.1002/cne.901720409

Legault, M., & Wise, R. A. (2001). Novelty-evoked elevations of nucleus accumbens dopamine: Dependence on impulse flow from the ventral subiculum and glutamatergic neurotransmission in the ventral tegmental area. *European Journal of Neuroscience*, *13*(4), 819–828. https://doi.org/10.1046/j.0953-816x.2000.01448.x

Li, S., & Kirouac, G. J. (2008). Projections from the paraventricular nucleus of the thalamus to the forebrain, with special emphasis on the extended amygdala. *The Journal of Comparative Neurology, 509(1)*, 136-140.

Lin, J., Roman, C., Andre, J. S., & Reilly, S. (2009). Taste, olfactory and trigeminal neophobia in rats with forebrain lesions. *Brain Research, 1251*, 195-203.

Lin, J., Roman, C., Arthurs, J., & Reilly, S. (2012). Taste neophobia and c-Fos expression in the rat brain. *Brain Research, 1448*, 82-88.

Linley, S. B., Athanason, A. C., Rojas, A. K. P., & Vertes, R. P. (2021). Role of the reuniens and rhomboid thalamic nuclei in anxiety-like avoidance behavior in the rat. *Hippocampus*, *31*(7), 756–769. https://doi.org/10.1002/hipo.23302

Lisman, J. E., & Grace, A. A. (2005). The hippocampal-VTA loop: Controlling the entry of information into long-term memory. *Neuron*, *46*(5), 703–713. https://doi.org/10.1016/j.neuron.2005.05.002

Lukaszewska, I., Korczynski, R., Kostarczyk, E., & Fonberg, E. (1984). Food-motivated behavior in rats with cortico-basomedial amygdala damage. *Behavioral Neuroscience*, *98*(3), 441–451. https://doi.org/10.1037/0735-7044.98.3.441

Marchant, N. J., Densmore, V. S., & Osborne, P. B. (2007). Coexpression of prodynorphin AND corticotrophin-releasing hormone in the rat central amygdala: evidence of two distinct endogenous Opioid systems in the lateral division. *The Journal of Comparative Neurology, 504*(6), 702-715. Doi:10.1002/cne.21464

McCullough KM, Morrison FG, Hartmann J, Carlezon WA Jr, Ressler KJ. Quantified Coexpression Analysis of Central Amygdala Subpopulations. *eNeuro*. 2018 Feb 6;5(1).0010-18.2018. doi: 10.1523/ENEURO.0010-18.2018.

McDonald, A.J. (1982), Neurons of the lateral and basolateral amygdaloid nuclei: A golgi study in the rat. J. Comp. Neurol., 212: 293-312. https://doi.org/10.1002/cne.902120307

McDonald, A.J. and F. Mascagni (1997) Projections of the lateral entorhinal cortex to the amygdala: A Phaseolus vulgaris-leucoagglutinin study in the rat. *Neuroscience 77*:445–459.

McKenna, J. T., & Vertes, R. P. (2004). Afferent projections to nucleus reuniens of the thalamus. *The Journal of comparative neurology*, *480*(2), 115–142. https://doi.org/10.1002/cne.20342

Mena, J. D., Selleck, R. A., & Baldo, B. A. (2013). Mu-opioid stimulation in rat prefrontal cortex engages hypothalamic orexin/hypocretin-containing neurons, and reveals dissociable roles of nucleus accumbens and hypothalamus in cortically driven feeding. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *33*(47), 18540–18552. https://doi.org/10.1523/JNEUROSCI.3323-12.2013

Marek, R., Xu, L., Sullivan, R. K. P., & Sah, P. (2018). Excitatory connections between the prelimbic and infralimbic medial prefrontal cortex show a role for the prelimbic cortex in fear extinction. *Nature neuroscience*, *21*(5), 654–658. https://doi.org/10.1038/s41593-018-0137-x

Messanvi, K. F., Berkun, K., Perkins, A., & Chudasama, Y. (2023). Parallel Pathways Provide Hippocampal Spatial Information to Prefrontal Cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *43*(1), 68–81. https://doi.org/10.1523/JNEUROSCI.0846-22.2022

Mesquita, L. T., Abreu, A. R., de Abreu, A. R., de Souza, A. A., de Noronha, S. R., Silva, F. C., Campos, G. S., Chianca, D. A., & de Menezes, R. C. (2016). New insights on amygdala: Basomedial amygdala regulates the physiological response to

social novelty. *Neuroscience*, *330*, 181–190. https://doi.org/10.1016/j.neuroscience.2016.05.053

Nachman, M., & Ashe, J. H. (1974). Effects of basolateral amygdala lesions on neophobia, learned taste aversions, and sodium appetite in rats. *Journal of Comparative and Physiological Psychology*, *87*(4), 622–643. https://doi.org/10.1037/h0036973

Ong, Z. Y., Liu, J. J., Pang, Z. P., & Grill, H. J. (2017). Paraventricular Thalamic Control of Food Intake and Reward: Role of Glucagon-Like Peptide-1 Receptor Signaling. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *42*(12), 2387–2397. https://doi.org/10.1038/npp.2017.150

Ottersen, O. P. (1982). Connections of the amygdala of the rat. IV: Corticoamygdaloid and intraamygdaloid connections as studied with axonal transport of horseradish peroxidase. *The Journal of Comparative Neurology*, *205*(1), 30–48. https://doi.org/10.1002/cne.902050104

Park TH, Carr KD. (1998) Neuroanatomical patterns of fos-like immunoreactivity induced by a palatable meal and meal-paired environment in saline- and naltrexone-treated rats. *Brain Res*., 14, 805(1-2),169-80. doi: 10.1016/s0006-8993(98)00719-7.

Parsons, W., Greiner, E., Buczek, L., Migliaccio, J., Corbett, E., Madden, A. M., & Petrovich, G. D. (2022). Sex differences in activation of extra-hypothalamic forebrain areas during hedonic eating. *Brain structure & function*, *227*(8), 2857-2878.

Parsons, M. P., Li, S., & Kirouac, G. J. (2007). Functional and anatomical connection between the paraventricular nucleus of the thalamus and dopamine fibers of the nucleus accumbens. *The Journal of comparative neurology*, *500*(6), 1050–1063. https://doi.org/10.1002/cne.21224

Petrovich, G. D. (2018). Feeding behavior survival circuit: Anticipation & competition. *Current Opinion in Behavioral Sciences, 24*, 137-142

Petrovich G. D., Canteras N. S., Swanson L. W. (2001). Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. *Brain Research Review,* 38:247–289.

Petrovich, G. D., Risold, P. Y., & Swanson, L. W. (1996). Organization of projections from the basomedial nucleus of the amygdala: A phal study in the rat. *The Journal of Comparative Neurology*, *374*(3), 387–420. https://doi.org/10.1002/(sici)1096-9861(19961021)374:3<387::aid-cne6>3.0.co;2-y

Petrovich, G. D., Ross, C. A., Holland, P. C., & Gallagher, M. (2007). Medial prefrontal cortex is necessary for an appetitive contextual conditioned stimulus to promote eating in sated rats. *Journal of Neuroscience*, *27*(24), 6436-6441.

Petrovich, G. D., Ross, C. A., Mody, P., Holland, P. C., & Gallagher, M. (2009). Central, But Not Basolateral, Amygdala Is Critical for Control of Feeding by Aversive Learned Cues. *Journal of Neuroscience, 29*(48), 15205-15212. doi: 10.1523/jneurosci.3656-09.2009

Pikkarainen, M., Ronkko, S., Savander, V., Insausti, R., & Pitkanen, A. (1999). Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the hippocampal formation in rat. *The Journal of Comparative Neurology*, *403*(2), 229–260. https://doi.org/10.1002/(sici)1096-9861(19990111)403:2<229::aid-cne7>3.0.co;2-p

Pitkänen, A., Savander, V., & LeDoux, J. E. (1997). Organization of intra-amygdaloid circuitries in the rat: An emerging framework for understanding functions of the

amygdala. *Trends in Neurosciences*, *20*(11), 517–523. https://doi.org/10.1016/s0166-2236(97)01125-9

Quirk, G. J., Likhtik, E., Pelletier, J. G., & Paré, D. (2003). Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. *Journal of Neuroscience*, *23*(25), 8800-8807.

Rajbhandari, A. K., Octeau, C. J., Gonzalez, S., Pennington, Z. T., Mohamed, F., Trott, J., Chavez, J., Ngyuen, E., Keces, N., Hong, W. Z., Neve, R. L., Waschek, J., Khakh, B. S., & Fanselow, M. S. (2021). A basomedial amygdala to intercalated cells microcircuit expressing PACAP and its receptor PAC1 regulates contextual fear. *The Journal of Neuroscience*, *41*(15), 3446–3461. https://doi.org/10.1523/jneurosci.2564-20.2021

Ramanathan, K. R., Ressler, R. L., Jin, J., & Maren, S. (2018). Nucleus reuniens is required for encoding and retrieving precise, hippocampal-dependent contextual fear memories in rats. *The Journal of Neuroscience, 38* (46), 9925-9933. https://doi.org/10.1523/JNEUROSCI.1429-18.2018

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

Reppucci, C. J., & Petrovich, G. D. (2016). Organization of connections between the amygdala, medial prefrontal cortex, and lateral hypothalamus: a single and double retrograde tracing study in rats. *Brain structure & function*, *221*(6), 2937–2962. https://doi.org/10.1007/s00429-015-1081-0

Reppucci, C. J., & Petrovich, G. D. (2018). Neural substrates of fear-induced hypophagia in male and female rats. *Brain Structure and Function, 223*(6), 2925-2947. doi: 10.1007/s00429-018-1668-3

Salamone J. D. (1994). The involvement of nucleus accumbens dopamine in appetitive and aversive motivation. *Behavioural brain research*, *61*(2), 117–133. https://doi.org/10.1016/0166-4328(94)90153-8

Savander, V., Go, C.-G., Ledoux, J. E., & Pitkänen, A. (1995). Intrinsic connections of the Rat Amygdaloid Complex: Projections originating in the basal nucleus. *Journal of Comparative Neurology*, *361*(2), 345–368. https://doi.org/10.1002/cne.903610211

Sesack, S. R., Deutch, A. Y., Roth, R. H., & Bunney, B. S. (1989). Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *The Journal of comparative neurology*, *290*(2), 213–242. https://doi.org/10.1002/cne.902900205

Sheynin, J., Beck, K. D., Pang, K. C., Servatius, R. J., Shikari, S., Ostovich, J., & Myers, C. E. (2014). Behaviourally inhibited temperament and female sex, two vulnerability factors for anxiety disorders, facilitate conditioned avoidance (also) in humans. *Behavioural Processes*, *103*, 228–235. doi: 10.1016/j.beproc.2014.01.003

Stratford TR, Swanson CJ, Kelley A. Specific changes in food intake elicited by blockade or activation of glutamate receptors in the nucleus accumbens shell (1998). *Behav Brain Res, 93*,1-2, 43-50. doi: 10.1016/s0166-4328(97)00140-x. PMID: 9659985.

Swanson, L. W. (2018). Brain maps 4.0—Structure of the rat brain: An open access atlas with global nervous system nomenclature ontology and flatmaps. *Journal of Comparative Neurology*, *526*(6), 935-943. https://doi.org/https://doi.org/10.1002/cne.24381

Swanson, L. W., & Kohler, C. (1986). Anatomical evidence for direct projections from the entorhinal area to the entire cortical mantle in the rat. *The Journal of Neuroscience*, *6*(10), 3010–3023. https://doi.org/10.1523/jneurosci.06-10-03010.1986

Swanson, L. W., & Petrovich, G. D. (1998). What is the amygdala? *Trends in Neurosciences*, *21*(8), 323-331.

Terrill SJ, Subramanian KS, Lan R, Liu CM, Cortella AM, Noble E, Kanoski SE (2020) Nucleus accumbens melanin-concentrating hormone signaling promotes feeding in a sex-specifc manner. Neuropharmacology 178:108270

Usuda, I., Tanaka, K., & Chiba, T. (1998). Efferent projections of the nucleus accumbens in the rat with special reference to subdivision of the nucleus: biotinylated dextran amine study. *Brain research*, *797*(1), 73-93.

Van Dongen, Y. C., Deniau, J. M., Pennartz, C. M. A., Galis-de Graaf, Y., Voorn, P., Thierry, A. M., & Groenewegen, H. J. (2005). Anatomical evidence for direct connections between the shell and core subregions of the rat nucleus accumbens. *Neuroscience*, *136*(4), 1049-1071.

Van Groen, T., & Wyss, J. M. (1990). Extrinsic projections from area CA1 of the rat hippocampus: Olfactory, cortical, subcortical, and bilateral hippocampal formation projections. *The Journal of Comparative Neurology*, *302*(3), 515–528. https://doi.org/10.1002/cne.903020308

Vertes R. P. (2002). Analysis of projections from the medial prefrontal cortex to the thalamus in the rat, with emphasis on nucleus reuniens. *The Journal of comparative neurology*, *442*(2), 163–187. https://doi.org/10.1002/cne.10083

Vertes R. P. (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse (New York, N.Y.)*, *51*(1), 32–58. https://doi.org/10.1002/syn.10279

Vertes, R. P., & Crane, A. M. (1996). Descending projections of the posterior nucleus of the hypothalamus: Phaseolus vulgaris leucoagglutinin analysis in the rat. *The Journal of comparative neurology*, *374*(4), 607–631. https://doi.org/10.1002/(SICI)1096-9861(19961028)374:4<607::AID-CNE9>3.0.CO;2-5

Vertes RP, Hoover WB (2008) Projections of the paraventricular and paratenial nuclei of the dorsal midline thalamus in the rat. *J Comp Neurol 508*:212–237.

Wright CI, Groenewegen HJ (1996) Patterns of overlap and segregation between insular cortical, intermediodorsal thalamic and basal amygdaloid afferents in the nucleus accumbens of the rat. *Neuroscience* 73:359–373. doi:10.1016/0306-4522(95)00592-7 pmid:8783254

Wu, Q. Lemus, M.B., Stark, R., Bayliss, J.A., Reichenbach, A., Lockie, S.H., Andrews Z.B., (2014). The temporal pattern of cfos activation in hypothalamic, cortical, and brainstem nuclei in response to fasting and refeeding in male mice. *Endocrinology, 155* (2014), 840-853

Wyss, J.M (1981). An autoradiographic study of the efferent connections of the Entorhinal Cortex in the rat. *The Journal of Comparative Neurology*, *199*(4), 495–512. https://doi.org/10.1002/cne.901990405

Yu, K., Garcia da Silva, P., Albeanu, D. F., & Li, B. (2016). Central amygdala somatostatin neurons gate passive and active defensive behaviors. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, *36*(24), 6488–6496. https://doi.org/10.1523/JNEUROSCI.4419-15.2016

[4] Experiment 3: Recruitment of cortical and thalamic projections to the central amygdala in the control of feeding behavior under novelty

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[4.1] Introduction

As described in Chapter 2, we found that a novel context induced lower intake of a novel and familiar food, and the effect was sex dependent. In novel context, males habituated to novel foods faster than females, who showed suppressed consumption throughout testing (Greiner & Petrovich 2020). As outlined in Chapter 3, we determined activity within several key brain regions during consumption of a novel food or in novel context. We found that novelty robustly recruited CEA. Novel food, increased Fos induction in all subregions of the central amygdala (CEA). Novel context increased Fos induction within the capsular region of the CEA. These results indicated that the CEA is involved in processing novelty of both foods and environments. We also found that novel context induced Fos in two regions known to project to the CEA, the paraventricular thalamus (PVT) (Li & Kirouac, 2008) and infralimbic cortex (ILA) (Hurley et al., 1991).

Additionally, our analysis revealed that activation within the CEA during consumption under novelty was heavily intercorrelated with activation of other regions of interest, and across its subregions. These findings strongly implicate CEA processing and its communication with other brain areas in mediating feeding inhibition during novelty processing. The current study aims to establish if such communications are via direct inputs to the CEA. We focused our analysis on projections from major cortical and thalamic inputs, including both the PVT and ILA.

Another region that sends inputs to the CEA, the agranular insula (AI) had elevated levels of Fos induction after exposure to a novel taste (Bermudez-Rattoni, 2014). However, that activation pattern was examined in the context of conditioned taste aversion, rather than to a neutral, or rewarding, novel stimulus. The CEA receives

substantial inputs from the dorsal region of the agranular insula (AId) (McDonald et al., 1996). The anterior AI, which encompasses both AId and the ventral AI (Shi & Cassell, 1998), is thought to play a role in hedonic processes (Peng et al., 2015) and activation of specific connections from AId to CEA suppresses consumption (Zhang-Molina et al., 2020). However, how this pathway may be recruited for feeding inhibition during novelty processing is unknown.

The CEA inputs from the PVT, the ILA, and the AId could control CEA output to specifically impact appetitive behavior. Even though these regions are strong candidates for mediating the effects of novelty on food consumption, their connections with the CEA and whether each pathway is similarly or differentially activated has yet to be examined. Additionally, it is completely unknown if these inputs control food consumption under novelty differently in males and females.

To establish the CEA circuitry that mediates the inhibition of food intake under novelty, the present study used a combination of retrograde tract tracing and Fos induction. We sought to determine whether PVT, ILA, and AI neurons that send direct projections to the CEA are specifically recruited during the consumption test under novelty and whether that activation was sex specific. Male and female rats received injections of the retrograde tracer, FluoroGold (FG), to retrogradely label neurons that send direct projections to the CEA. Rats were then tested for consumption in either familiar or novel condition and Fos induction was assessed within retrogradely-labeled neurons, in order to establish activity in these pathways.

[4.2] Materials & Methods

[4.2.1] Subjects

Adult male (n=24) and female (n=24) Long Evans rats (Charles River Laboratories; Portage, MI), that weighed 200-250g upon arrival, were individually housed and maintained on a 12-hour light/dark cycle (lights on 06:00). Males and females were

housed in the same colony room on separate shelves. After arrival, subjects were allowed one week to acclimate to the colony housing room before surgical procedures began, during which they had *ad libitum* access to water and standard laboratory chow (Purina Lab Diet Prolab RMH 3000; 3.47 kcal/g; 26% protein, 15% fat, 59% carbohydrates), and were handled daily. All housing and testing procedures were in compliance with the National Institutes of Health Guidelines for Care and Use of Laboratory Animals and approved by the Boston College Institutional Animal Care and Use Committee.

[4.2.2] Surgical Procedure

Animals were deeply anesthetized with isoflurane (5%; Baxter Healthcare Corporation, Deerfield, IL, USA), while under anesthesia, animals received unilateral stereotaxically placed infusions into the CEA of 0.1uL 4% Fluorogold (FG Fluorochrome LLC, Denver, CO) delivered at a rate of 0.1uL/min for 1 minute (relative to bregma anterior-posterior [AP]:-2.0mm, mediolateral [ML]: +/- 3.8mm, dorsoventral [DV]: -7.5mm). The injector remained in the site for 6 minutes post-infusion to allow for the diffusion of FG. A 10 µl Hamilton syringe with 32 gauge cannula driven by a motorized stereotaxic injector (Stoelting, Wood Dale, IL) was used to deliver microinjections. Stereotaxic surgeries were performed according to the procedures for aseptic technique in survival surgery and postoperative care approved by Boston College IACUC. Behavioral experiments started two weeks after surgery to allow for recovery and sufficient transport of the tracer.

[4.2.3] Apparatus

Half of the animals were tested in their housing cages (Home Cage) and the other half were tested in a novel environment (behavioral chamber; plexiglass box (30x28x30cm) with grid flooring and a recessed food port (3.2 x4.2 cm) on one wall;

Coulbourn Instruments). Each chamber is enclosed in monolithic rigid foam box). Food was presented in a ceramic bowl.

[4.2.4] Behavioral Testing Procedure

After recovery from surgery, male and female rats were tested for consumption of either a novel food in a novel environment or a familiar food in a familiar environment. After testing, the brain tissue was collected for later processing. There were four testing groups, in order to test the effects of sex and novelty on consumption. All groups underwent one 30-minute testing session. Prior to testing all rats were food deprived for 20 hours. For the test, each rat was presented with a ceramic bowl that contained either 15g of a familiar food (Rat Chow) or 15g of a novel food (Test Diet (TD) pellets; 3.4 kcal/g; 21% protein, 13% fat, 67% carbohydrate).

All rats were habituated to transport, to the conditioning chamber room, as well as to the ceramic bowls, at least 24 hours prior to testing. The weight of all foods was measured following the end of testing to determine how much was consumed. Body weights for all rats were taken in the morning of test day. Average body weights were calculated for each group. All consumption data is presented as a percentage of grams per body weight

[4.2.5] Histological Procedures

Rats were perfused 90 minutes after start of testing and brains were harvested. Rats were briefly anesthetized with isoflurane (5%; Baxter Healthcare Corporation, Deerfield, IL) and then given a lethal dose of Fatal-Plus (0.1mL/100g body weight, Vortech Pharmaceuticals; Dearborn, MI) was administered intraperitoneally. Rats were then transcardially perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1 M borate buffer. Brains were extracted and post-fixed overnight in a solution of 12% sucrose dissolved in the perfusion liquid, then rapidly frozen in hexanes cooled in dry ice

and stored at −80 °C. Brains were sliced in 30-µm sections using a sliding microtome and collected into four adjacent series.

The first series was stained using standard immunohistochemical procedures for visualization of Fos and fluorogold. Free-floating tissue sections were incubated in a blocking solution for 1 h at room temperature to minimize nonspecific binding. The blocking solution contained 0.02M potassium phosphate-buffered saline (KPBS), 0.3% Triton X-100 (Sigma-Aldrich), 2% normal goat serum (S-1000; Vector Laboratories, Burlingame, CA), and 10% non-fat milk (M-0841; LabScientific, Livingston, New Jersey). Then, the tissue was incubated with the primary antibody, anti-*fluorogold* raised in rabbit (1:20,000, ABE457, EMD Millipore, Billercia, MA) in the blocking solution for 72 h at 4 °C. The tissue was rinsed in KPBS then incubated with the secondary antibody, biotinylated goat anti-rabbit IgG (1:500; BA-1000; Vector Laboratories) in the blocking solution for 45 min. Subsequently, the tissue was rinsed in KPBS then reacted with avidin–biotin complex (ABC solution; PK-6100; Vector Laboratories) for 45 min. To improve specific binding, this was followed by rinses in KPBS, a second 30 min incubation in the secondary antibody solution, rinses in KPBS, a second 30 min incubation in the ABC solution, and additional rinses in KPBS. To produce a color reaction, the tissue was incubated in a diaminobenzidine solution (SK-4100; Vector Laboratories) for 1–2 min with constant, manual agitation. The tissue then underwent a second round of staining to label for c-fos, using the same procedure above, but with primary antibody anti-c-fos raised in Guinea Pig (1:60,000, 226 308, Synaptic Systems, Gottingen, Germany) and secondary antibody biotinylated goat anti-guinea pig IgG (1:500; BA-7000-1.5; Vector Laboratories) Stained tissue was then mounted onto SuperFrost Plus slides (Fisher Scientific, Pittsburgh, PA) and air-dried, followed by drying in an oven at 45 °C overnight. Tissue was then dehydrated through graded

alcohols, cleared in xylenes, and coverslipped with DPX (13512; Electron Microscopy Sciences, Hatfield, PA).

The second series was collected into KPBS solution, mounted onto gelatin-subbed slides, and stained with thionin for identification of cytoarchitectonic borders of brain structures, as defined in Swanson's rat brain atlas (Swanson, 2018). The remaining series were collected into trays containing a cryoprotectant solution (0.025 M sodium phosphate buffer with 30% ethylene glycol and 20% glycerol) and stored at −20 °C for later use. Brain perfusions, collection, slicing, and length of storage were counterbalanced across training conditions.

[4.2.6] Image Acquisition & Analysis

Images of stained tissue were acquired with an Olympus BX51 light microscope at 20X and attached Olympus DP72 camera using DP2-BSW software (Olympus America Inc, Center Valley, PA). Using the ImageJ software program (NIH), unilateral images of regions of interest were taken of immunohistochemically-stained tissue to allow for manual counting of neurons double labeled for *c-fos* & FG as well as neurons singled labeled for FG (Figure 4.1). A proportion of FG cells that were double-labelled for Fos was calculated for each animal ([number of cells double labelled for Fos and FG/Total cells labelled for FG]X100). Cells single-labelled for FG from each region were expressed as a percentage of total cells single-labelled for FG across all three regions of interest ([# of

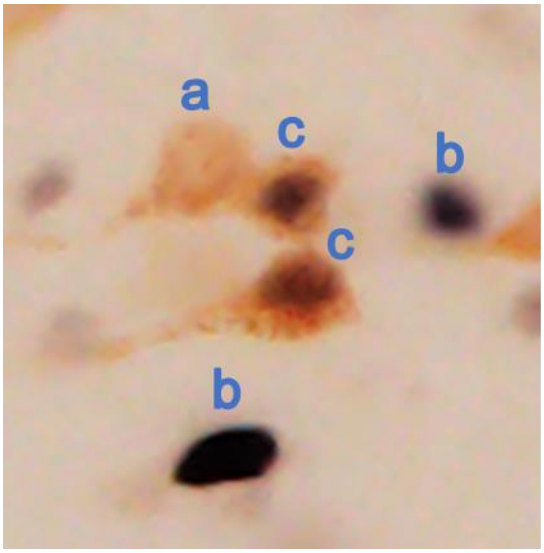

Figure 4.1: *Image shows representative types of labeled neurons. Lowercase letters indicate a representative of each type of labeled neuron: single-labeled FluoroGold (FG) (a), single-labeled Fos (b), and double-labeled FG and Fos (c).*

FG-only cells in region/# of FG-only cells in all three regions]X100). Identification of regions was determined via the Swanson rat brain atlas (Swanson, 2018). Analysis was conducted for ILA, pPVT, & AId using a single representative anatomical level for analysis of each region. The ILA was analyzed at level 9, pPVT was analyzed at level 31, and Aid was analyzed at level 14 as determined by the Swanson rat brain atlas (2018).

[4.2.7] Statistical Analysis

Following arrival, males gained weight faster than females, resulting in body weight differences during testing. Therefore, all consumption results are reported as a percentage of grams consumed per body weight ([food consumed(g)/body weight(g)]X100).

Consumption results were analyzed using a between-subjects 2-way univariate ANOVA for sex and testing condition. Differences in pathway activation (indicated by percentage of double-labelled neurons) for all regions of interest was analyzed using a between-subjects 2-way multivariate ANOVA for sex and testing condition. Differences in number of inputs from each region of interest to the CEA for males and females (calculated as proportion of total FG observed that was represented by inputs from ILA, PVTp, and AId individually) was analyzed using a mixed effect ANOVA for sex and region. All significant interactions were followed by Bonferroni *post hoc* analyses. A value of $p<0.05$ was considered significant for all analyses, except for *post-hoc* analyses in which Bonferroni adjusted alpha level was used ($p=0.05/3=0.017$). Data were analyzed for normality using Shapiro-Wilk test.

[4.3] Results

[4.3.1] Consumption

Male and female rats in a novel condition consumed less than those in a familiar condition (Figure 4.2). A two way ANOVA with the factors of sex and testing condition

(novel, familiar) revealed a main effect of condition ($F(1,42)=15.411$, $p<0.01$), but no main effect of sex ($F(1,42)=0.04$, $p=0.84$) or a significant interaction of the factors ($F(1,42)=2.413$, $p=0.13$)

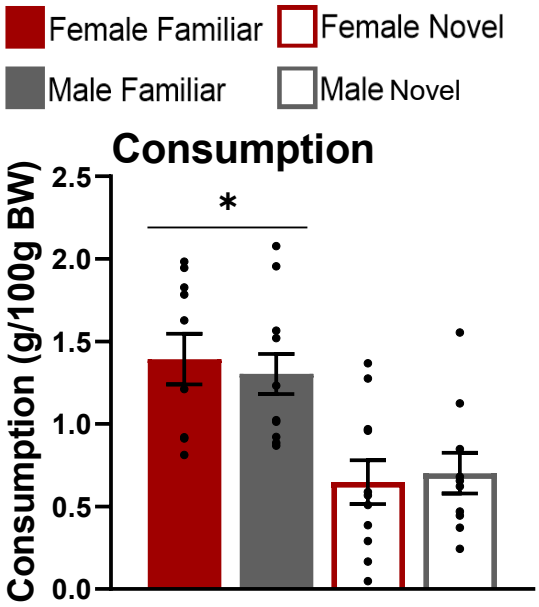

Figure 4.2: *Food consumption test. The graph shows the amounts of each food that subjects in each testing condition consumed, expressed as grams per 100 grams of their body weight (BW). Asterisks indicate p<0.05.*

[4.3.2] Recruitment of the Posterior Paraventricular Thalamus Projections to Central Amygdala

In the PVTp, rats tested in the novel condition had a greater percentage of total number of FG neurons that were double labelled for Fos (FG+Fos) compared to the familiar condition (Figure 4.3). A two-way ANOVA with the factors of sex and testing condition revealed a main effect of condition ($F(1,18)=6.523$, $p=0.02$), but no main effect of sex ($F(1,18)=1.487$, $p=0.238$) or a significant interaction of factors ($F(1, 18)=0.054$, $p=0.819$).

[4.3.3] Recruitment of Infralimbic Cortex Projections to Central Amygdala

In the ILA, there was no difference between testing groups in the percentage of total number of FG cells that were double labelled for Fos between testing groups (Figure 4.4). A two-way ANOVA with the factors of sex and testing condition yielded no main effects of condition ($F(1,20)=1.202$, $p=0.286$) or sex ($F(1,20)=0.839$, $p=0.371$) and no significant interaction of factors ($F(1,20)=0.278$, $p=0.604$).

[4.3.4] Recruitment of Dorsal Agranular Insula Projections to Central Amygdala

There was no difference in the percentage of total number of FG cells double labelled for Fos in the AId between testing groups (Figure 4.5). A two-way ANOVA with

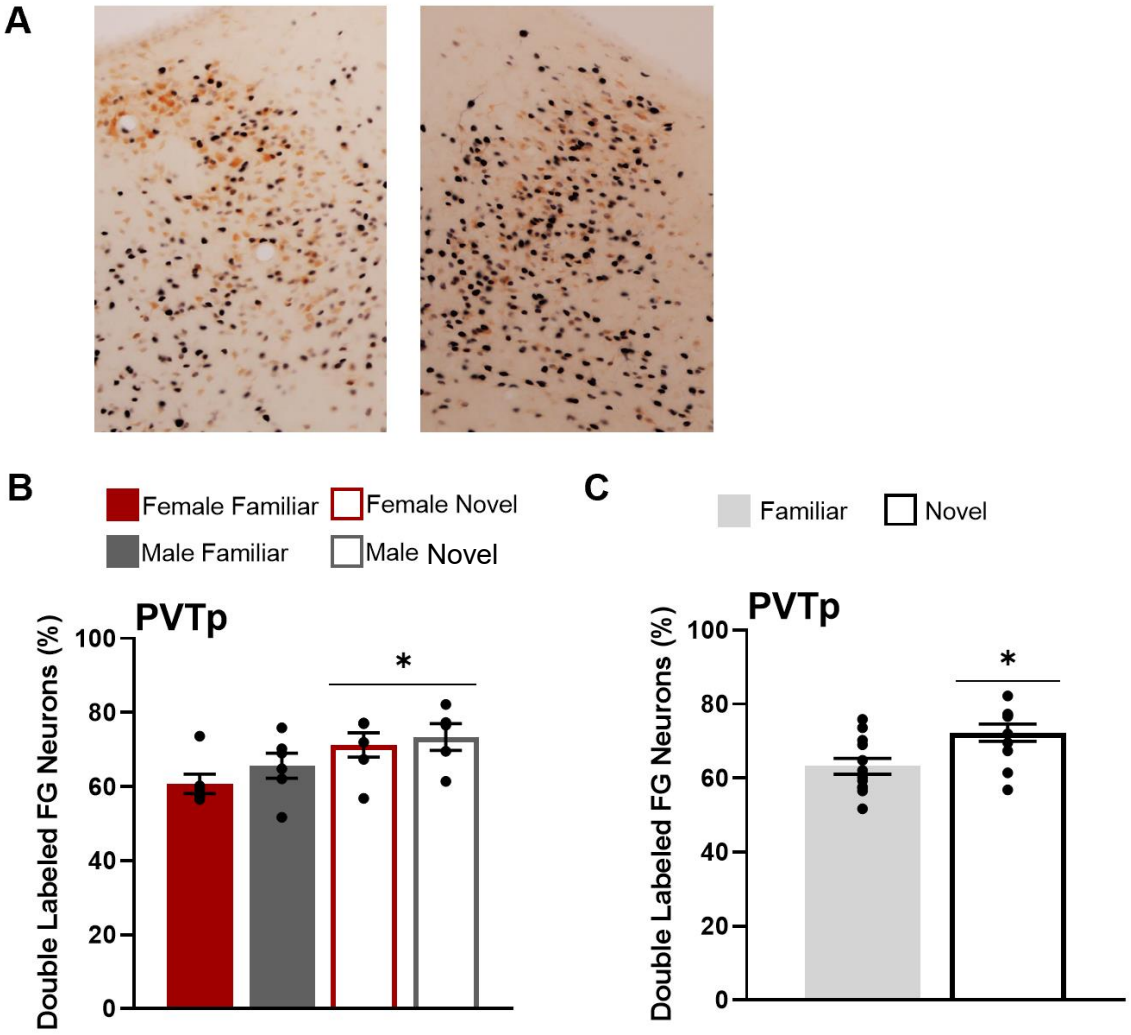

Figure 4.3: *Fos induction in the PVTp neurons that project to the CEA. A) Tissue stained for Fos and FluoroGold (FG) in the PVTp (atlas level 31) for familiar (left) and a novel condition (right). B) Percentage of PVTp FluoroGold neurons double labelled for Fos for each testing condition. C) Percentage of FluoroGold neurons within the PVTp doubled labelled for Fos collapsed across sexes for each condition (familiar & novel). Asterisk indicates p<0.05.*

the factors of sex and testing condition yielded no main effects of condition ($F(1,19)=0.824$, $p=0.375$) or sex ($F(1,19)=0.608$, $p=0.445$) and no significant interaction of factors ($F(1,19)=0.001$, $p=0.977$).

[4.3.5] Proportion of Projection s to the Central Amygdala

The AId had a greater proportion of cells labelled with FG than ILA and PVTp. The number of projections from AId, ILA, and PVTp was calculated as a proportion of total

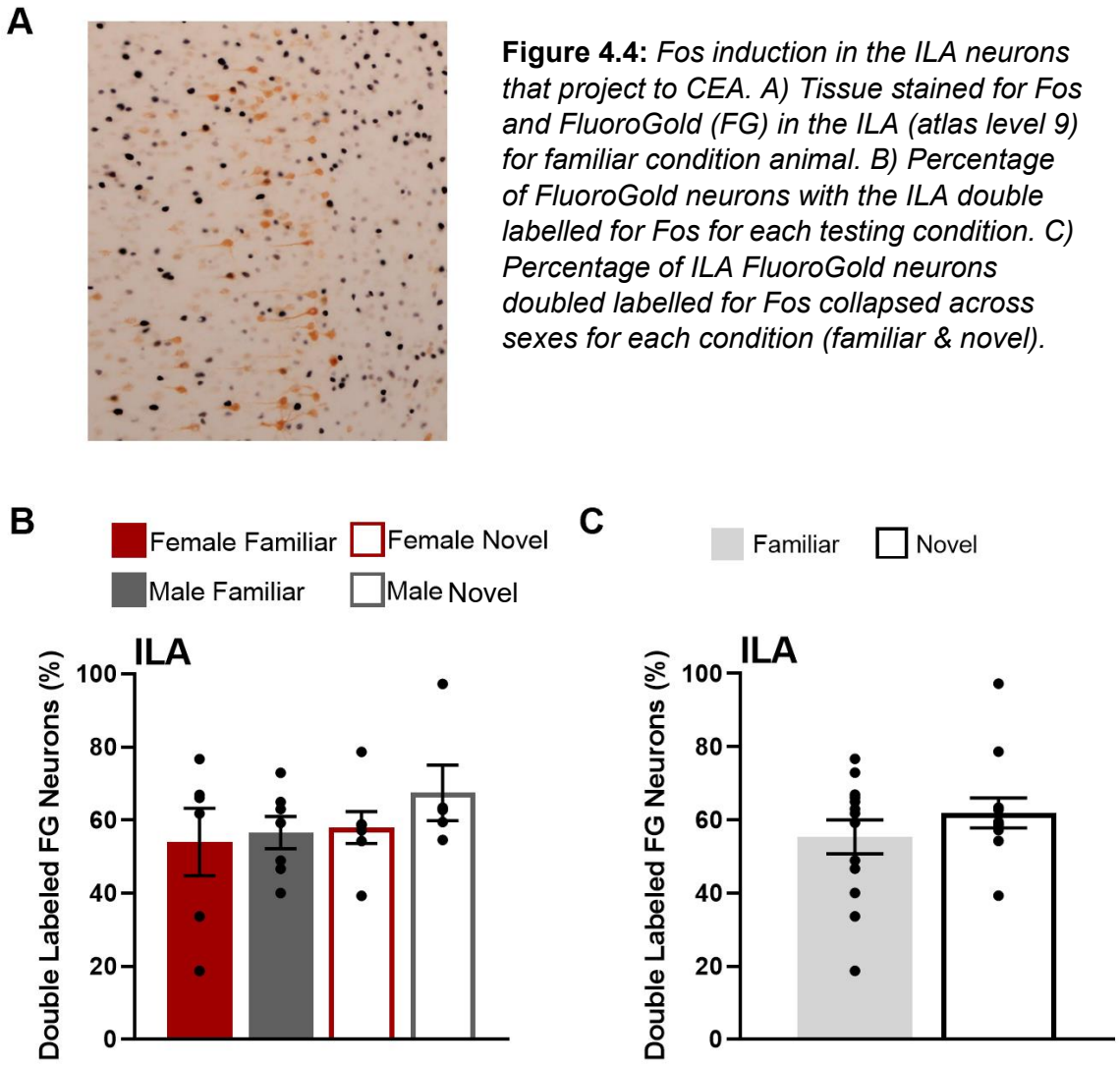

Figure 4.4: *Fos induction in the ILA neurons that project to CEA. A) Tissue stained for Fos and FluoroGold (FG) in the ILA (atlas level 9) for familiar condition animal. B) Percentage of FluoroGold neurons with the ILA double labelled for Fos for each testing condition. C) Percentage of ILA FluoroGold neurons doubled labelled for Fos collapsed across sexes for each condition (familiar & novel).*

FG observed in all three regions (Figure 4.6). A mixed-model ANOVA with the between subjects factor of sex and the within-subjects factor of brain region, revealed a main effect of region ($F(2,51)=17.78$, $p<0.001$), but no main effect of sex ($F(1,51)<0.001$, $p=0.9987$) and no significant interaction of factors ($F(2,51)=2.531$, $p=0.0896$). A Bonferroni post hoc analysis revealed that the proportion of cells labelled with FG in the AId was significantly greater than the ILA ($p=0.001$) and pPVT ($p=0.0017$). The proportion of cells labelled with FG in the pPVT and ILA were not significantly different from each other ($p=0.7959$).

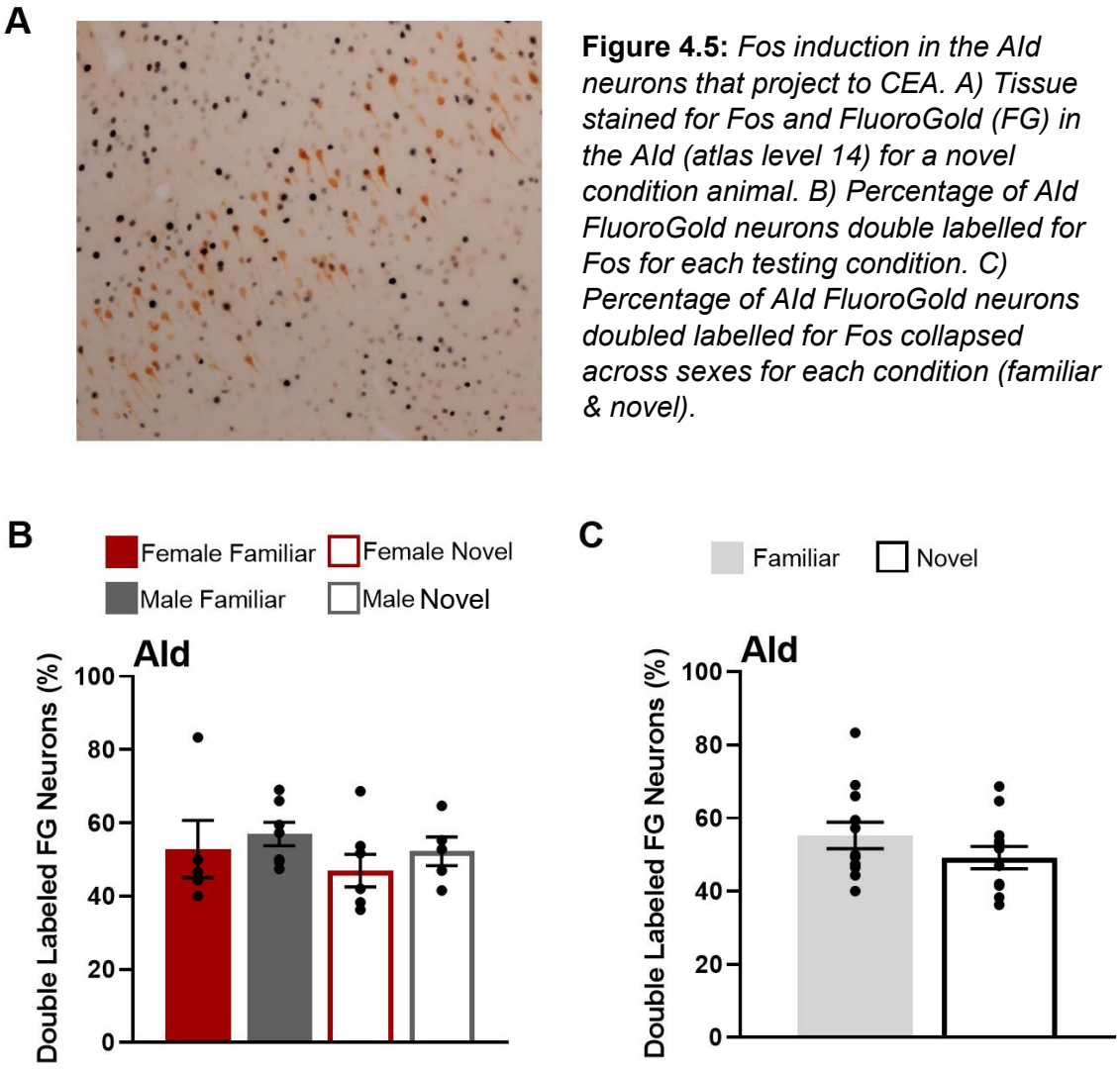

Figure 4.5: *Fos induction in the AId neurons that project to CEA. A) Tissue stained for Fos and FluoroGold (FG) in the AId (atlas level 14) for a novel condition animal. B) Percentage of AId FluoroGold neurons double labelled for Fos for each testing condition. C) Percentage of AId FluoroGold neurons doubled labelled for Fos collapsed across sexes for each condition (familiar & novel).*

[4.4] Discussion

The present study examined the recruitment of specific CEA pathways during food consumption under either familiar or novel conditions. Our previous findings established the CEA as a central hub for mediating consumption during novelty processing (Chapter 3). Therefore, we investigated the recruitment of projections from the ILA, PVTp, and AId, as major cortical and thalamic inputs to the CEA. Food deprived male and female rats were tested either in a novel context with novel food access or in a familiar context with a familiar food. Animals in the novel condition ate less than animals in the familiar condition and males and females had similar consumption patterns under the same

conditions. Injection of the retrograde tracer FluoroGold (FG) into the CEA prior to behavior allowed us to pair Fos induction and anatomical tract tracing to determine the activation patterns of neurons that project to the CEA. Analyses of neurons that were positive for both FG and Fos (FG+Fos) were conducted within the ILA, pPVT, and Aid. Comparison of testing group revealed that animals in the novel condition had greater activation of pPVT neurons that project to the CEA than animals in the familiar condition. Projections from the AId and ILA to CEA were similarly activated in all groups. Additionally, we compared the contribution of each target to the total inputs and found that the greatest proportion of inputs to the CEA, among the regions analyzed, came from the AId.

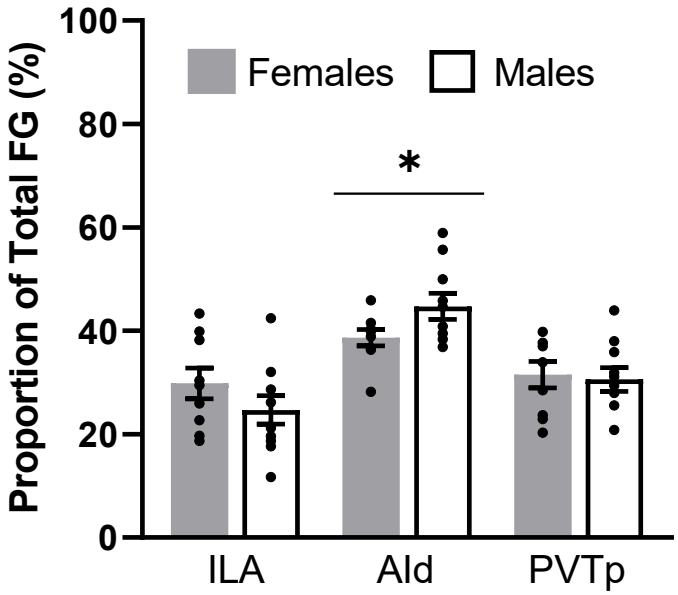

Figure 4.6: *Proportion of inputs to the CEA, from the ILA, AId, and PVTp, for males and females. The y-axis shows the percent of the total number of FluoroGold (FG) labeled neurons summed across the three regions. Asterisk indicates p<0.05.*

Consumption results in the current study aligned with the behavioral findings reported in both Chapter 2 (Greiner & Petrovich, 2020) & 3, such that novel foods and contexts induced lower food intake. Similar behavior of males and females during the first presentation of novel foods was also consistent with previous results. A distinction from previous behavioral paradigms was that here we did not include a condition where animals were given a familiar food in a novel context or a novel food in a familiar context. Therefore, the pathway recruitment investigation was focused on the effect of novel context and novel food simultaneously, rather than either stimulus individually.

Our analysis of FG labelling within the PVTp, ILA, and AId confirmed projections from these regions to the CEA. The PVTp is known to send dense projections to the CEA, which is the main amygdala target for the PVTp (Li and Kirouac, 2008; Vertes and Hoover, 2008; Unzai et al., 2015). The ILA is the major mPFC input to the CEA (Hurley et al., 1991). Majority of ILA inputs to the CEA terminate within the medial CEA (Beckstead, 1979; van der Kooy 1984; Room et al., 1985; Bienkowski & Rinaman, 2012), which was targeted by our tracer injections. Projections from AId to CEA are also well established. Several anatomical tract tracing studies using both retro- and anterograde labelling have found connections between AI regions and the CEA (Saper, 1982; Allen et al., 1991; Otterson, 1982). Though, it is important to note that both Saper (1982) and Otterson (1982) focused their findings on projections from the AIp. The work of Allen and colleagues (1991) investigated both AIp and anterior AI regions, which contains AId (Shi & Cassell, 1998), confirming connections between the AId and CEA, that our findings align with.

Our study provides additional anatomical specificity to these AId connections. Previous work analyzed connections to the anterior AI to the amygdala as a whole and therefore could not distinguish precise locations of origin for the AI projections to CEA. In the current study, the labelling was selective to AId and was concentrated in layer 2-3, with sparse labelling in layer 1 and 5. Projections to CEA from AId layer 2-3 implicates viscerosensory processing information (Gilbert & Wiesel, 1979). Previous work with the AIp found that most layer 2-3 projections terminated in the basolateral and lateral amygdala regions, whereas CEA received projections from layer 5 (Otterson, 1982). Our findings indicate a potential difference in projections from AId and AIp, as it appears that the CEA is, almost exclusively, receiving early sensory processing information from the AId.

We found greater recruitment of the PVTp-CEA pathway for rats that consumed food in the novel condition compared to the familiar condition, and this recruitment did not differ between the sexes. Projections from the PVT to CEA are largely associated with driving fear and anxiety behaviors (Chen & Bi, 2019; Do Monte et al., 2015; Penzo et al., 2015). Novelty effects on feeding behavior have been previously used as behavioral models of depression and anxiety (Ramaker & Dulawa, 2017), implicating anxiety circuits in potentially mediating novelty induced consumption avoidance. Greater recruitment of the PVTp to CEA pathway could drive avoidance behavior and result in the inhibition of feeding in the novel condition. Previous work has found that stimulation of neurons in the PVTp that project to the CEA reduces time in open arms on an elevated plus maze, which is typically interpreted as greater anxiety responding (Chen & Bi, 2019; Pliota et al., 2020). Additionally, stimulation of PVTp increases avoidance of the center of an open field (Li et al., 2009; Li et al., 2010).

In contrast to selective recruitment of the CEA-projecting PVTp neurons, total Fos induction in the PVTp did not differ based on testing condition in our prior study (reported in Chapter 3). Similarly, a prior study did not find changes in overall activation in PVTp during feeding suppression in a novel context (Cheng et al., 2018). The different findings in overall PVTp versus pathway specific PVTp-CEA activations patterns could indicate that a similar number of a different group of neurons within PVTp are recruited in the novel and control condition. For example, while CEA projecting neurons are recruited under novel conditions, other, non-CEA projecting PVTp neurons may be recruited under familiar conditions. For example, the PVTp sends projections to the bed nucleus of the stria terminalis (Shin et al., 2008; Li & Kirouac, 2008), which could promote feeding behavior through GABAergic projections to the parabrachial nucleus and the periaqueductal gray (Luskin et al., 2021; Hao et al., 2019; Dong & Swanson 2004; Dong & Swanson 2004b). Distinct groups of PVTp neurons send pathways to the

bed nucleus of the stria terminalis and to the central nucleus and a third group sends collateral projections to both (Dong et al., 2017). Therefore, it is possible that the PVTp was engaged in this circuit during consumption under familiar conditions as opposed to the recruitment of projections to the CEA under novel conditions, resulting in similar total activation of PVTp during consumption in both circumstances.

The current study found similar recruitment of AId-CEA pathway across conditions. This finding was unexpected, as we predicted that the taste differences between the novel food and familiar food, and the unfamiliarity of the novel taste, would result in different activation of inputs from the AId to the CEA. The insular cortex, including AId, processes taste and visceral information (Jasmin et al., 2004; ; Chen et al., 2011; ; for review see: Moraga-Amaro & Stehberg, 2012) and insular cortex connections with CEA mediate appetitive and aversive responding (Haaranen et al., 2020; Gehrlach et al., 2019; Schiff et al., 2018; Wang et al., 2018; Zhang-Molina et al., 2020). The AId was activated following exposure to a novel taste (Koh et al., 2003; Bermudez-Rattoni, 2014). Importantly, activation of the projections from AId to CEA suppressed consumption and appetitive behavior, even in rats that were food deprived (Zhang-Molina et al., 2020). In the current study, even though rats consumed different amounts of two distinct tasting foods in the novel and familiar conditions, the AId-CEA was recruited similarly. It is possible that distinct AId-CEA pathways were recruited in each condition. However, due to the limitations of the method, we could not determine if the same or different populations of of AId-to-CEA projecting neurons were recruited across conditions. In addition, the same AId neurons could innervate multiple cell types within the CEA (Zhang-Molina et al., 2020). The CEA contains neurons that both drive (HTR2a) (Douglass et al., 2017) and suppress feeding (PKCd) (Cai et al., 2014) and the AId sends excitatory projections to the CEA that do not target a single cell-type (Schiff et al., 2018; Cai et al., 2014; Douglass et al., 2017; Zhang-Molina et al., 2020).

The current study also found similar recruitment of the ILA-CEA pathway based on testing condition. The ILA is well known for extinction learning and memory recall, particularly in regard to fear conditioning (Milad & Quirk, 2002; Burgos-Robles et al., 2009; Rozeske et al., 2015), implicating it as a site that drives the cessation of aversive responding. Specifically, projections from the ILA to CEA are thought to play a role in inhibiting anxiety-like responding (Chen et al., 2021). The ILA is also implicated in mediating feeding behavior as a part of the ventromedial prefrontal cortex (Petrovich et al., 2007; Anderson & Petrovich 2018). Our previous findings, reported in chapter 3, found an increase in overall ILA activation when rats consumed food in a novel context. However, the present findings indicate that the increased activity we observed is not due to a greater recruitment of projections to the CEA. The ILA could be mediating novelty processing and feeding inhibition through other projections, such as projections to the nucleus accumbens that have been shown to regulate avoidance behavior (Schwartz et al., 2017). In addition, similar to AId-CEA pathways, the ILA-CEA pathways may innervate multiple cell types within the CEA.

To determine the number of inputs to CEA among the regions analyzed we calculated the proportion of total FG observed that was represented by inputs from ILA, PVTp, and AId individually. Among our regions of interest, we found that the greatest proportion of total number of neurons that send inputs to the CEA originated in the AId (approximately 40%). The ILA and PVTp represented a similar portion of inputs to the CEA (approximately 30% each). There were no differences between males and females as they had a similar proportion of inputs to CEA from these three areas.

In summary, our findings provide evidence that CEA inputs are recruited differently during consumption of a novel food in a novel context than during consumption of a familiar food in a familiar context. We identified specific recruitment of pPVT to CEA that corresponds to the behavioral differences in consumption under novel and familiar

conditions and suggests that this pathway is important for feeding inhibition during novelty processing. We additionally found that the CEA receives greater proportion of its inputs from AId than the pPVT or ILA. These findings add to our understanding of the neural circuit mechanisms underlying novelty processing during consumption and provide deeper anatomical specificity to our knowledge of CEA circuitry.

[4.5] Acknowledgements

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[4.7] References

Allen GV, Saper CB, Hurley KM, Cechetto DF (1991). Organization of visceral and limbic connections in the insular cortex of the rat. *J Comp Neurol* 311: 1–16.

Beckstead, R.M. (1979) Autoradiographic examination of corticocortical and subcortical projections of the mediodorsal projection (prefrontal) cortex in rat. J. *Comp. Neurol., 184*, 43-62.

Berendse HW, Galis-de-Graaf Y, Groenewegen HJ (1992) Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. *J Comp Neurol, 316*: 314–347.

Bermudez-Rattoni, F. (2014). The forgotten insular cortex: its role on recognition memory formation. *Neurobiology of learning and memory*, *109*, 207-216.

Bernard J-F, Alden M, Besson J-M (1993) The organization of the efferent projections from the pontine parabrachial area to the amygdaloid complex: A Phaseolus vulgaris leucoagglutinin (PHA-L) study in the rat. *J Comp Neurol, 329*: 201–229.

Bienkowski, M.S., Rinaman, L. Common and distinct neural inputs to the medial central nucleus of the amygdala and anterior ventrolateral bed nucleus of stria terminalis in rats. *Brain Struct Funct* **218**, 187–208 (2013). https://doi.org/10.1007/s00429-012-0393-6

Bloodgood, D.W., Sugam, J.A., Holmes, A. *et al.* Fear extinction requires infralimbic cortex projections to the basolateral amygdala. *Transl Psychiatry* **8**, 60 (2018).

Burgos-Robles A, et al. Amygdala inputs to prefrontal cortex guide behavior amid conflicting cues of reward and punishment. *Nat. Neurosci.* 2017;20:824–835.

Cai, H., Haubensak, W., Anthony, T. E., & Anderson, D. J. (2014). Central amygdala PKC-δ(+) neurons mediate the influence of multiple anorexigenic signals. *Nature neuroscience*, *17*(9), 1240–1248. https://doi.org/10.1038/nn.3767Caruana, F., Jezzini, A., Sbriscia-Fioretti, B., Rizzolatti, G., & Gallese, V. (2011). Emotional and social behaviors elicited by electrical stimulation of the insula in the macaque monkey. *Current Biology*, *21*(3), 195-199.

Chen, M., & Bi, L. L. (2019). Optogenetic long-term depression induction in the PVT-CeL circuitry mediates decreased fear memory. *Molecular neurobiology*, *56*, 4855-4865.

Chen, X., Gabitto, M., Peng, Y., Ryba, N. J., & Zuker, C. S. (2011). A gustotopic map of taste qualities in the mammalian brain. *Science*, *333*(6047), 1262-1266.

Chen, Y. H., Wu, J. L., Hu, N. Y., Zhuang, J. P., Li, W. P., Zhang, S. R., Li, X. W., Yang, J. M., & Gao, T. M. (2021). Distinct projections from the infralimbic cortex exert opposing effects in modulating anxiety and fear. *The Journal of clinical investigation*, *131*(14), e145692.

Condé F, Maire-Lepoivre E, Audinat E, Crépel F (1995) Afferent connections of the medial frontal cortex of the rat. II Cortical and subcortical afferents. *J Comp Neurol, 352*: 567–593.

Do-Monte, F. H., Quinones-Laracuente, K., and Quirk, G. J. (2015). A temporal shift in the circuits mediating retrieval of fear memory. *Nature* 519, 460–463.

Dong, X., Li, S., & Kirouac, G. J. (2017). Collateralization of projections from the paraventricular nucleus of the thalamus to the nucleus accumbens, bed nucleus of the stria terminalis, and central nucleus of the amygdala. *Brain structure & function*, *222*(9), 3927–3943. https://doi.org/10.1007/s00429-017-1445-8

Dong, H. W., & Swanson, L. W. (2004). Organization of axonal projections from the anterolateral area of the bed nuclei of the stria terminalis. *The Journal of comparative neurology*, *468*(2), 277–298. https://doi.org/10.1002/cne.10949

Dong, H. W., & Swanson, L. W. (2004). Projections from bed nuclei of the stria terminalis, posterior division: implications for cerebral hemisphere regulation of defensive and reproductive behaviors. *The Journal of comparative neurology*, *471*(4), 396–433. https://doi.org/10.1002/cne.20002

Douglass, A. M., Kucukdereli, H., Ponserre, M., Markovic, M., Gründemann, J., Strobel, C., . . . Klein, R. (2017). Central amygdala circuits modulate food consumption through a positive-valence mechanism.

Gehrlach, D. A., Dolensek, N., Klein, A. S., Roy Chowdhury, R., Matthys, A., Junghänel, M., ... & Gogolla, N. (2019). Aversive state processing in the posterior insular cortex. *Nature neuroscience*, *22*(9), 1424-1437.

Gilbert, C., Wiesel, T. Morphology and intracortical projections of functionally characterised neurones in the cat visual cortex. *Nature* 280, 120–125 (1979). https://doi.org/10.1038/280120a0Greiner, E. M., & Petrovich, G. D. (2020). The effects of novelty on food consumption in male and female rats. *Physiology & Behavior, 223*, 112970. doi:10.1016/j.physbeh.2020.112970

Gray TS (1990) The organization and possible function of amygdaloid corticotropin-releasing factor pathways. In: De Souza EB, Nemeroff CB (eds.). Corticotropin-releasing factor: basic and clinical studies of a neuropeptide. CRC Press, Inc., Florida.

Haaranen, M., Schäfer, A., Järvi, V., & Hyytiä, P. (2020). Chemogenetic Stimulation and Silencing of the Insula, Amygdala, Nucleus Accumbens, and Their Connections Differentially Modulate Alcohol Drinking in Rats [Original Research]. *Frontiers in Behavioral Neuroscience*, *14*.

Hao, S., Yang, H., Wang, X., He, Y., Xu, H., Wu, X., ... & Wang, H. (2019). The lateral hypothalamic and BNST GABAergic projections to the anterior ventrolateral periaqueductal gray regulate feeding. *Cell Reports*, *28*(3), 616-624.

Hurley, K. M., Herbert, H., Moga, M. M., & Saper, C. B. (1991). Efferent projections of the infralimbic cortex of the rat. *The Journal of comparative neurology*, *308*(2), 249–276. https://doi.org/10.1002/cne.903080210

Jasmin L, Granato A, Ohara PT. 2004. Rostral agranular insular cortex and pain areas of the central nervous system: a tract-tracing study inthe rat. J Comp Neurol 468:425–440.

Koh, M. T., Wilkins, E. E., & Bernstein, I. L. (2003). Novel Tastes Elevate c-fos Expression in the Central Amygdala and Insular Cortex: Implication for Taste Aversion Learning. Behavioral Neuroscience, 117(6), 1416–1422.

Krieger MS, Conrad LCA, Pfaff DW (1979) An autoradiographic study of the efferent connections of the ventromedial nucleus of the hypothalamus. *J Comp Neurol, 183*: 785–816.

Krukoff TL, Harris KH, Jhamandas JH (1993) Efferent projections from the parabrachial nucleus demonstrated with anterograde tracer Phaseolus vulgaris leucoagglutinin. *Brain Res Bull, 30*: 163–172.

LeDoux JE, Farb C, Romanski LM (1991) Overlapping projections to the amygdala and striatum from auditory processing areas of the thalamus and cortex. *Neurosci Lett*, 134: 139–144.

Li, S., & Kirouac, G. J. (2008). Projections from the paraventricular nucleus of the thalamus to the forebrain, with special emphasis on the extended amygdala. *The Journal of Comparative Neurology, 509(1)*, 136-140.

Li Y, Li S, Sui N, Kirouac GJ (2009). Orexin-A acts on the paraventricular nucleus of the midline thalamus to inhibit locomotor activity in rats. *Pharmacol Biochem Behav*, *93*(4):506-14.

Li, Y., Li, S., Wei, C., Wang, H., Sui, N., Kirouac, G.J. (2010). Orexins in the paraventricular nucleus of the thalamus mediate anxiety-like responses in rats. *Psychopharmacology 212*, 251–265.

Likhtik, E. & Paz, R. Amygdala-prefrontal interactions in (mal)adaptive learning. *Trends Neurosci.* **38**, 158–166 (2015).

Luskin, A. T., Bhatti, D. L., Mulvey, B., Pedersen, C. E., Girven, K. S., Oden-Brunson, H., ... & Bruchas, M. R. (2021). Extended amygdala-parabrachial circuits alter threat assessment and regulate feeding. *Science Advances*, *7*(9), eabd3666.

Luskin MB, Price JL (1983) The laminar distribution of intracortical fibers originating in the olfactory cortex of the rat. *J Comp Neurol*, 216: 292–302.

Mcdonald, A. J., Mascagni, F., & Guo, L. (1996). Projections of the medial and lateral prefrontal cortices to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat. *Neuroscience*, *71*(1), 55-75.

Milad, M. R., & Quirk, G. J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature*, *420*(6911), 70-74.

Moraga-Amaro, R., & Stehberg, J. (2012). The Insular Cortex and the Amygdala: Shared Functions and Interactions. The Amygdala - A Discrete Multitasking Manager. doi: 10.5772/48495

Ono T, Luiten PGM, Nishijo H, Fukuda M, Nishino H (1985) Topographic organization of projections from the amygdala to the hypothalamus of the rat. *Neurosci Res*, 2: 221–239.

Ottersen, O.P. (1982), Connections of the amygdala of the rat. IV: Corticoamygdaloid and intraamygdaloid connections as studied with axonal transport of horseradish peroxidase. J. *Comp. Neurol., 205*: 30-48.

Peng Y., Gillis-Smith S., Jin H, Tränkner D, Ryba NJP, Zuker CS. (2015) Sweet and bitter taste in the brain of awake behaving animals. *Nature, 527*, 512-515

Penzo, M.A., Robert, V., Tucciarone, J., De Bundel, D., Wang, M., Van Aelst, L., Darvas, M., Parada, L.F., Palmiter, R.D., He, M., Huang, Z.J., Li, B. (2015). The paraventricular thalamus controls a central amygdala fear circuit. *Nature* 519, 455–459.

Petrovich GD, Risold PY, Swanson LW (1996) Organization of projections from the basomedial nucleus of the amygdala: A PHAL study in the rat. *J Comp Neurol*, 374: 387–420.

Pitkänen, A., Pikkarainen, M., Nurminen, N., & Ylinen, A. (2000). Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. *Annals of the New York Academy of Sciences*, *911*, 369–391. https://doi.org/10.1111/j.1749-6632.2000.tb06738.x

Pliota, P., Böhm, V., Grössl, F., Griessner, J., Valenti, O., Kraitsy, K., ... & Haubensak, W. (2020). Stress peptides sensitize fear circuitry to promote passive coping. *Molecular psychiatry*, *25*(2), 428-441.

Ramaker, M. J., & Dulawa, S. C. (2017). Identifying fast-onset antidepressants using rodent models. *Molecular Psychiatry, 22*(5), 656-665. doi: 10.1038/mp.2017.36

Room, P., F.T. Russchen, H.J. Groenewegen, and A.H.M. Lohman (1985) Efferent connections of the prelimbic (area 32) and infralimbic (area 25) cortices: An anterograde tracing study in the cat. *J. Comp. Neurol*., 242, 40-55.

Rozeske, R. R., Valerio, S., Chaudun, F., & Herry, C. (2015). Prefrontal neuronal circuits of contextual fear conditioning. *Genes, Brain and Behavior*, *14*(1), 22-36.

Saper, C.B. (1982), Convergence of autonomic and limbic connections in the insular cortex of the rat. *J. Comp. Neurol., 210*: 163-173.

Schiff, H. C., Bouhuis, A. L., Yu, K., Penzo, M. A., Li, H., He, M., & Li, B. (2018). An insula–central amygdala circuit for guiding tastant-reinforced choice behavior. *Journal of Neuroscience*, *38*(6), 1418-1429.

Schwartz, N., Miller, C., Fields, H.L. (2017). Cortico-Accumbens Regulation of approach-avoidance behavior is modified by experience and chronic p(Greiner & Petrovich, 2020)ain. *Cell Reports 19*, 1522–1531.

Shin, J. W., Geerling, J. C., & Loewy, A. D. (2008). Inputs to the ventrolateral bed nucleus of the stria terminalis. *The Journal of comparative neurology*, *511*(5), 628–657.

Unzai, T., Kuramoto, E., Kaneko, T., & Fujiyama, F. (2015). Quantitative analyses of the projection of individual neurons from the midline thalamic nuclei to the striosome and matrix compartments of the rat striatum. *Cerebral Cortex*, *27*(2), 1164-1181. https://doi.org/10.1093/cercor/bhv295 van der Kooy, D., J.F. McGinty, L.Y. Koda, C.R. Gerfen, and F.E. Bloom (1982) Visceral cortex: A direct connection from prefrontal cortex to the solitary nucleus in rat. *Neurosci. Lett., 3*, 123-127.

Vertes RP, Hoover WB (2008) Projections of the paraventricular and paratenial nuclei of the dorsal midline thalamus in the rat. J Comp Neurol 508:212–237.

Wang, L., Gillis-Smith, S., Peng, Y., Zhang, J., Chen, X., Salzman, C. D., Ryba, N. J. P., & Zuker, C. S.. (2018). The coding of valence and identity in the mammalian taste system. *Nature*, *558*(7708), 127–131. https://doi.org/10.1038/s41586-018-0165-4

Wu, Y., Chen, C., Chen, M., Qian, K., Lv, X., Wang, H., Jiang, L., Yu, L., Zhuo, M., & Qiu, S.. (2020). The anterior insular cortex unilaterally controls feeding in response to aversive visceral stimuli in mice. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-14281-5

Zhang-Molina, C., Schmit, M. B., & Cai, H. (2020). Neural Circuit Mechanism Underlying the Feeding Controlled by Insula-Central Amygdala Pathway. *iScience*, *23*(4), 101033.

[5] General Discussion

[5.1] Summary of Findings

The overall aim of the work presented in this dissertation was to determine the behavioral and neural activation patterns of rats processing novelty during food consumption. Prior work on the effect of novelty on appetitive behavior, examined novel foods and novel contexts separately (Lin et al., 2012; Ramaker & Dulawa, 2017; Dulawa & Hen, 2005). Therefore, our specific interests were in the simultaneous processing of both types of novel stimuli. Additionally, we placed importance on the investigation of both sexes, as females had been largely absent from previous studies. As reported in Chapter 2 (Greiner & Petrovich, 2020), we designed a novel behavioral preparation to test how male and female rats respond when given a choice between novel and familiar food in a novel or familiar context. To encourage habituation, rats were food deprived prior to testing and the novel food used was palatable. Our experiment uncovered a robust behavioral sex difference in consumption during habituation to eating in a novel environment. During the first presentation of a novel food rats ate less of it than a familiar food, and initial exposure to food in novel context induced lower intake of novel and familiar food for both sexes. However, following multiple exposures, males tested in a novel context habituated to novel foods faster than females tested in a novel context, who showed suppressed consumption throughout testing.

After establishing a behavioral profile, we sought to determine the neural circuitry mediating consumption during the first novelty exposure. As reported in Chapter 3, we used Fos induction to infer about activity of neurons during the test. We adjusted our behavior paradigm so that rats only received one food, novel or familiar, during testing in either a novel or familiar context. Despite that change, our results replicated behavioral findings from Chapter 2 (Greiner & Petrovich, 2020) that rats' consumption patterns are different in a familiar versus novel environment. Rats ate less of the novel food in a

familiar context and ate less of both novel and familiar food in a novel context. Additionally, males and females ate similarly in line with our previous findings, where we did not observe a behavioral sex difference until later exposures.

The Fos induction analyses reported in Chapter 3 found increased activation in the novel context and novel food condition within several cortical, thalamic, and striatal areas analyzed. Our findings revealed that distinc brain regions were recruited based on the novelty of the food, the novelty of the context, and the sex of the animal. The circuitry activated for novel context was the largest. Groups tested in a novel context induced Fos within the medial prefrontal cortex (PL, ILA), thalamus (PVTa, RE), cortical regions of the amygdala (BLA, LA, BMA), and the striatum (ACB, CEAc). In addition, we found sex differences in activation patterns within the PVTa, core and ventral shell of the ACB, and the lateral and capsular CEA. Males had greater overall activation in the CEAc while the females had greater overall activation in the ACBc, and ACBvsh. In the PVTa females had greater activation in response to a novel food compared to a familiar, where males did not. Additionally, in posterior CEAl males given a novel food in a familiar context had the greater activation than any other testing group.

Additionally, we investigated the relationship of activity between regions for each testing group and found distinct patterns. The CEA was the most correlated with other regions. However, groups given a novel food exhibited a decrease in CEA subregion inter-correlations (both rostrally and caudally) compared to groups given a familiar food. There was an overlap in Fos induction correlation between ACBc and ACBdsh in all male groups and between BLAa and BMAa for all groups given a novel food. Females exhibited a much greater number of negative correlations in Fos induction between regions, across all groups. Females tested in the novel condition had the greatest number, mostly between the CEAl and another region.

Following identification of recruitment patterns, we investigated specific pathways in male and female rats during consumption of a novel food in a novel context. We focused our investigation on CEA inputs given our findings in Chapter 3 that the CEA was activated during consumption of a novel food and consumption in a novel context and there were sex differences in activation patterns within this region. Specifically, we examined the recruitment of projections to the CEA from cortical and thalamic areas. As outlined in Chapter 4, we made further changes to our behavioral paradigm, limiting testing groups to only familiar stimuli (food and context) or only novel stimuli to focus on the effect of both novel context and novel food simultaneously. Behavioral results were consistent with previous findings reported in Chapter 2 and 3, that rats in the novel condition ate less than those in the familiar condition, and that males and females were similar. We found that more connections from the PVTp to the CEA were recruited in the novel condition compared to the familiar condition. These findings suggest that the PVTp inputs might be causal to different activation of CEA subregions during novel context exposure and novel food consumption.

[5.2] Proposed Networks

Based on the activation patterns we found, we are proposing 2 functional circuitries—one for the inhibition of feeding behavior in a novel context and one for the inhibition of feeding when consuming a novel food. While these are identified as two distinct circuitries they have key regions of overlap—the PVT, CEA, & BMAa—and we are postulating that when both circuitries are active, they have a cumulative impact on the inhibition of feeding. Patterns of activity within the PVT, CEA, and BMAa suggest that they are drivers of eating control, regardless of whether feeding inhibition is driven by novel taste or novel context.

The areas included in each of the proposed circuitries, which are described below, are largely based on our findings reported in Chapter 3, whether they exhibited greater

activation during novel food consumption or during consumption in a novel context. Within each circuitry we identified regions that were activated differently in males and females. We also found greater recruitment of PVTp neurons that project to CEA (Chapter 4) during consumption of a novel food in a novel context and have therefore included it in both of our proposed circuitries. We additionally included regions that were not analyzed in the current study but are anatomically connected within the proposed circuitries: the hippocampal formation (HF), which is critical for contextual processing and encoding (Wood et al., 1999; Komorowski et al., 2009; Maren et al., 2013), the nucleus of the solitary tract (NTS) and parabrachial nucleus (PB), which are necessary for transmitting gustatory, taste, and visceral sensory information to the CEA (Norgren, 1976), and the lateral hypothalamus (LHA) which is critical for the control of feeding (Hoebel and Teitelbaum, 1962; Stuber & Wise, 2016; Petrovich, 2018).

The circuitry proposed to control the inhibition of feeding during novel food consumption (hereby referred to as the novel food circuitry) is shown in Figure 5.1. The main difference between the proposed circuitries is that the novel food circuitry lacks the heavy mediation by cortical inputs present in the novel context circuit. The PVTa, BMAa, and CEA can all receive gustatory information from the brainstem. Taste information from the NTS through the PB can reach CEA (Norgren, 1976; Bernard et al., 1993) the PVT (Krout & Loewy, 2000; Li & Kirouac, 2012), and the BMAa (Bernard et al., 1993; Swanson & Petrovich 1998). These inputs may activate palatability sensitive neurons within the CEA, which would, in turn, promote feeding behaviors. However, the CEA is a place of convergence that is likely integrating both hedonic information that would increase feeding and stress or arousal information that would suppress feeding (Petrovich, 2018).

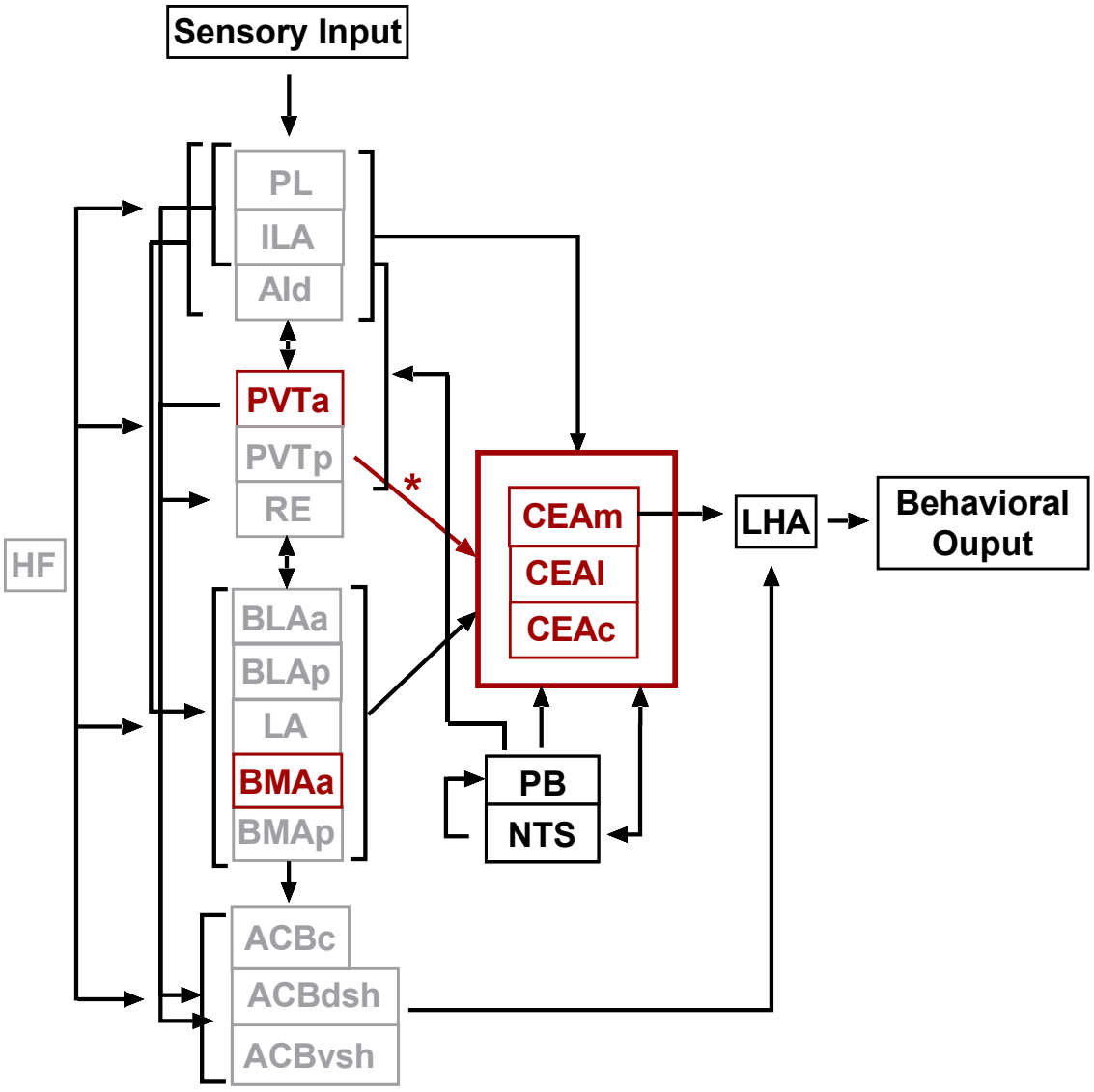

Figure 5.1: *The diagram shows the proposed circuit for feeding inhibition in response to a novel food. Areas that were activated in response to a novel food are shown in red. Areas that were not activated are in gray. Asterisk indicates that the pathway was activated. For clarity some connections not shown. Within this circuit, females had greater activation than males in PVTa and males had greater activation than females in CEAc.*

The PVTa was activated more during novel food consumption and that activation was higher in females compared to males (Chapter 3). The PVTa is known for detecting arousal states (Gao et al., 2020) and exhibits greater activation during increased arousal. Therefore, the activation we observed may have been related to greater state of

arousal due to food novelty for females than for males. Additionally, less recruitment of the PVT may allow for greater recruitment of CEAc neurons which we found in males.

The PVTa does not have substantial direct connections to CEA and therefore it likely communicates information through projections to the PVTp (Vertes & Hoover, 2008) and BMAa (Vertes & Hoover, 2008) which both project to CEA (Petrovich et al., 1996; Vertes & Hoover, 2008, Moga et al., 1995). Additionally, as reported in Chapter 4, we found that the PVTp to CEA pathway was recruited more heavily for animals in the novel context and novel food condition. Previous work has shown that the connections between the PVT and CEAl are necessary for fear learning and expression (Penzo et al., 2015). The BMAa, has been shown to mediate stress responses (Rajbahndari et al., 2021; Amano et al., 2011; de Andrade et al., 2012), could regulate avoidance behavior through the CEA Therefore, the potential threat of novelty could activate the PVTp-CEA pathway and initiate inhibitory feeding circuits from the CEA.

Interestingly, a negative relationship between activation of ACB shell and the amygdala areas in both males and females given a novel food in a familiar context. Females had a negative relationship between BMAa and ACBdsh and males had a negative relationship between CEAl and ACBvsh, suggesting that, during novel food consumption in a familiar context, ACB activity is suppressed. This finding supports our proposition that the CEA is the main driver of feeding inhibition within the novel food circuitry, rather than both CEA and ACB as proposed in the novel context circuitry.

The circuitry proposed to control the inhibition of feeding in a novel context (hereby referred to as the novel context circuitry) is shown in Figure 5.2. The outlined circuitry includes a subset of areas identified in Chapter 3 that were activated during novel context exposure and are also known to transmit specific information about context and feeding. The first is the connection between the HF, PFC, and RE. Contextual information from the HF and inputs from cortical sensory areas to the PFC that

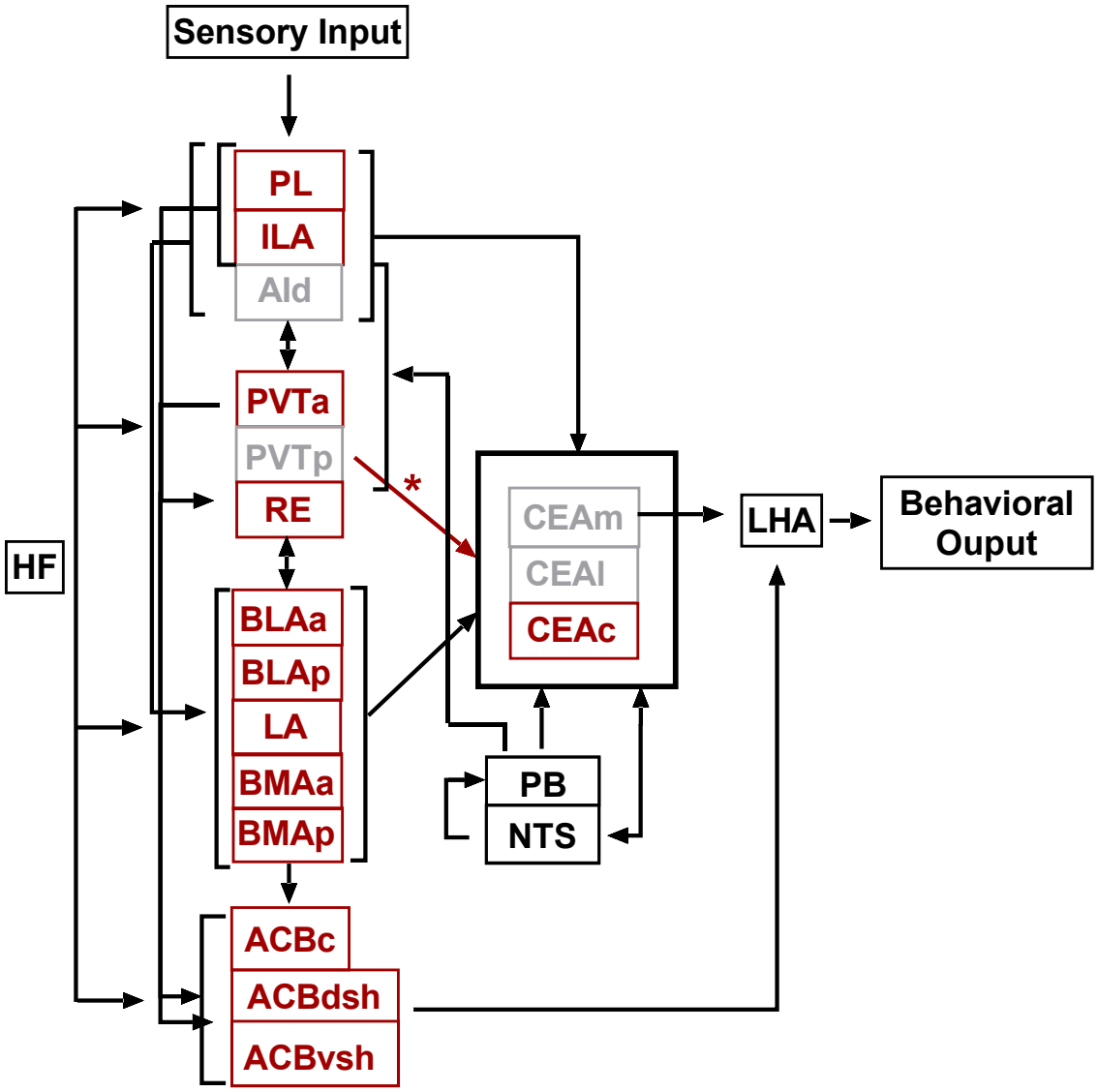

Figure 5.2: *The diagram shows the proposed circuitry for feeding inhibition in a novel context. Areas that were activated in a novel context are shown in red. Areas that were not activated are in gray. Asterisk indicates that the pathway was activated. For clarity some connections are not shown. Within this circuit, females had greater activation than males in ACBc & ACBvsh and males had greater activation than females in CEAc.*

communicate the relative novelty of the context could activate connections to both the RE and ACB shell. The PFC communicates contextual information from the HF to the RE (Vertes & Crane, 1996; Vertes 2002), which allows the RE to engage in contextual processing. Additionally, in the control of feeding, PFC glutamatergic projection to ACB

shell activates inhibitory, GABAergic, projections to the LHA (Baldo, 2016; Mena et al., 2017; Stratford et al., 1998). Novel sensory and contextual information may also trigger mu-opiod mediated suppression of GABAergic interneurons in the PFC, resulting in disinhibitory activation of projections to the ACB shell as outlined in Baldo (2016). The ACB shell, in turn, would be able to inhibit the LHA, allowing for suppression of feeding while in a novel context.

The ACB receives input from other areas, that were also activated, within the proposed circuitry. The basolateral complex, specifically the BLA (Kelley et al., 1982), projects to the ACB. We found positive correlations between BLA and ACB core and dorsal shell activation for females given a familiar food in a novel context. The BLA could be relaying contextual information, as it receives inputs from HF. However, the ACB receives direct HF inputs as well (Groenewegen et al., 1999).

The BLA receives inputs from vmPFC and AId, which could impact decision making and visceral information (McDonald et al., 1996; Shi & Cassell, 1988) and may result in activating BLA defensive or stress circuits that could inform ACB regions of the potential danger of the novel context. Connections between the vmPFC and BLA are particularly important for reward restraint (Ishikawa et al., 2020), to prevent animals from engaging in appetitive behaviors when there is a potential risk. The resulting BLA projections to ACB shell could reinforce inhibition of LHA, but also engage ACB mediation of non-feeding behaviors. Activation of the ACB can drive defensive behavior (Reynolds & Berridge, 2001) and the BLA connections to ACB shell are required for active avoidance (Ramirez et al., 2015). Additionally, the BLA connections to the ACB shell are critical in regulating the switch between consumption and exploratory behaviors (Millan et al., 2021).

The ACB is not the only path to feeding inhibition within the proposed novel context circuitry. The central amygdala circuitry was previously proposed to underlie resolving

competition between feeding versus threat avoidance (Petrovich, 2018). The CEA is known to mediate the inhibition of feeding (Petrovich et al., 2009; Cai et al, 2014; Zhang-Molina et al., 2020) potentially through inhibitory connections to the LHA (Swanson & Petrovich, 1998) and through local inhibition of neurons that project to the PB (Douglass et al., 2017). The CEA, like in the proposed novel food circuitry, is likely once again operating as an area of convergence, receiving inputs from several cortical and thalamic areas within our proposed circuit. The CEA can receive decision making information from the vmPFC, specifically the ILA, (Bechara & Damasio, 2005; Hurley et al., 1991), taste and visceral sensory information from AId (Shi & Cassell 1998; McDonald et al., 1999; Saper 1982), contextual information from CA1 in the HPF (Cenquizca & Swanson, 2007), arousal and threat information from PVT (Kirouac 2021; Li & Kirouac, 2008; Bhatnagar & Dallman, 1999), and emotional state information from the basolateral complex (Pitkanen et al., 1997; Swanson & Petrovich, 1998; Petrovich et al., 1996; Canteras et al., 1992; Canteras et al., 1995; Otterson 1982). The convergence of this information onto the CEA, and the great diversity of cell types among CEA neurons (O'Leary et al., 2022; Jolkkonen & Pitkanen, 1998), makes it uniquely situated to integrate important contextual and physiological information to then appropriately mediate appetitive behavior.

One subregion of the CEA was uniquely activated in a novel context. The CEAc is the only CEA subregion where we found increased Fos induction to a novel context. The CEAc is part of a known defensive circuit with the BLA and receives input from BLA respondin2 neurons that elicit defensive behaviors and inhibit appetitive behaviors (Kim et al., 2016). Importantly, the PVTp synapses on CEAc neurons (Li & Kirouac, 2008; Moga et al., 1995), and we found (Chapter 4) that the PVTp-CEA pathways was recruited more in the novel condition (food and context) compared to familiar. This connection is also a key site for potentially driving behavioral sex differences. We found

(Chapter 3) that the CEAc has greater activation for males, regardless of testing condition. Additionally, females given a familiar food in a novel context had a negative correlation between activity in the PVTp and CEAc where males did not. The greater recruitment of CEAc neurons by males in general, could indicate fewer inputs from PVTp and that other inputs may be counteracting PVTp inputs.

Based on activation patterns and connectivity we have identified two distinct, functional circuits that exist within a larger network in the control of feeding under novelty. We speculate that the novel food circuitry is a subset of the novel context circuitry. In the novel food circuitry, only the CEA is driving feeding inhibition, whereas in the novel context circuit feeding inhibition is driven by both CEA and ACB. Therefore, when animals consume a novel food in a novel context, we postulate, that both circuitries would be engaged, and both the ACB and CEA would mediate feeding inhibition.

[5.3] Methodological Limitations

There are some methodological limitations that should be taken into consideration when interpreting the results outlined in this dissertation. Within our neural analysis, Fos induction was not cell-specific, which prevented us from establishing whether the observed activation patterns within each region, under different conditions, are represented by the same group of cells. Moreover, it is unclear what different cell sub-types are being recruited or whether the observed activity reflects excitation, inhibition, or disinhibition. Similarly, in chapter 3 activation patterns, were not pathway-specific, which limits our understanding of how different areas are interacting with one another to drive activity. Additionally, the pathways in chapter 4 may be connecting multiple cell types between regions, and thus it is unclear whether the activity observed is a part of a singular pathway or multiple, separate pathways. Within our behavioral paradigm, the

absence of a no-food condition means that consumption and novelty processing cannot be completely disentangled.

[5.4] Implications

Our establishment of behavioral and neural effects of novelty on feeding is important for determining how these processes function in health and disease. While initial avoidance of novel stimuli can be adaptive due to the uncertainty of safety—especially when it relates to an activity as essential to survival as consumption—when these avoidant behaviors become persistent, they can become maladaptive. This is especially concerning when dealing with avoidance-linked disorders with high mortality rates, like anorexia (10-15%) (Wildes et al. 2011; Arcelus et al., 2011), where women have a three times higher lifetime prevalence rate (0.9%) than men (0.3%) (Crow et al., 2009).

Much of the previous work investigating appetitive behavior in a novel context or in response to a novel food tested the effects of anxiolytic drugs, using novelty exposure as an anxiety provoking stimulus (Ramaker & Dulawa, 2017; Dulawa & Hen, 2005). Anxiolytic drug administration was able to attenuate neophobic responding in these studies (Dulawa & Hen, 2005), indicating that novelty potentially operates as a stressor and can result in an anxiety-like state. Therefore, our findings could have important implications for how consumption is mediated under stress conditions and help to identify brain areas where males and females may be differentially impacted by anxiety-provoking stimuli.

Eating disorders occur disproportionality more in women and yet, prior to the work documented here, there had been little investigation into novel food consumption in female animal models. Sex differences in food consumption patterns are well documented, particularly when rats are tested in settings that are presumed to induce a state akin to fear or anxiety. In a prior study (Petrovich & Lougee, 2011), when rats were presented with a conditioned fear cue during consumption, male rats were faster to

consume amounts comparable to controls than female rats. These effects may be the result of a higher anxiety state in female rats due to slower extinction. Fear habituation and extinction circuits have been shown to partially overlap, at least in males (Furlong et al., 2016).

The most robust behavioral and neural effects were due to novel context in both sexes, and it appears that novel foods and novel contexts are likely being processed in a cumulative manner. However, the sex differences we observed in our behavior suggest that novel contexts differentially impact male and female consumption during habituation. The sex differences that emerged after repeated testing sessions were unexpected since both displayed similar patterns of behavior for the initial exposures. The divergence that occurred overtime suggest that males and females may be recruiting different neural circuits or recruiting the same circuits in distinct ways. Females may have sustained activity in the novel context circuitry longer than males, resulting in slower habituation.

In the circuitries above, we identified areas that males and females were differentially recruiting during initial exposure to novelty that may also underlie the behavioral differences we observed during habituation (as reported in Chapter 2 (Greiner & Petrovich, 2020). It is possible that through multiple exposures, activation patterns could change in distinct ways for males and females. Further investigation would be required to establish how the proposed circuitries change throughout habituation to novel foods and novel contexts.

This work is also informative for the neural circuitry underlying the control of feeding and motivation for reward, particularly in how motivational drives compete with stressors. The animals within our paradigm were food deprived and the novel food presented was highly palatable, which should lead to an increase in appetitive behavior. However, animals still exhibited decreased consumption because novelty, and the potential stress

associated with it, was sufficient to override these physiological and hedonic drives. We previously identified hedonic circuitries using the same palatable food in both hungry and sated rats (Parsons et al., 2022), which partially overlaps with the proposed novel food and context circuitries. Additionally, within the hedonic circuit, we identified sex differences in activation in PVT, CEA, and ACB (Parsons et al., 2022) which is relevant to the current findings. Future work would be needed to determine how these two networks may interact.

In summary, the findings reported in this dissertation advance our understanding of the neural mechanisms underlying novelty processing during consumption in male and female rats. After uncovering behavioral sex differences during habituation to novelty, we identified key brain regions underlying novelty processing during consumption. We proposed two circuits, for the inhibition of feeding in a novel context and for the inhibition of feeding when consuming a novel food. These circuits are important for determining how the control of feeding is regulated under the influence of novelty and other stressors, and how the neural substrates causal to sex differences may be differentially impacted. Ultimately, our work may have greater implications for understanding the onset of maladaptive eating behaviors and psychopathology.

General References

Amano, T., Duvarci, S., Popa, D., & Paré, D. (2011). The fear circuit revisited: contributions of the basal amygdala nuclei to conditioned fear. *Journal of Neuroscience*, *31*(43), 15481-15489.

Arcelus J, Mitchell AJ, Wales J, Nielsen S. Mortality rates in patients with anorexia nervosa and other eating disorders. A meta-analysis of 36 studies. *Arch Gen Psychiatry*. 2011 Jul;68(7):724-31.

Baldo BA. Prefrontal Cortical Opioids and Dysregulated Motivation: A Network Hypothesis. *Trends Neurosci*. 2016;39(6):366-377. doi:10.1016/j.tins.2016.03.004

Bechara, A., & Damasio, A. R. (2005). The somatic marker hypothesis: A neural theory of economic decision. *Games and Economic Behavior, 52*(2), 336-372.

Bernard J-F, Alden M, Besson J-M (1993) The organization of the efferent projections from the pontine parabrachial area to the amygdaloid complex: A Phaseolus vulgaris leucoagglutinin (PHA-L) study in the rat. *J Comp Neurol, 329*: 201–229.

Bhatnagar, S. & Dallman, M. F. (1999). The paraventricular nucleus of the thalamus alters rhythms in core temperature and energy balance in a state-dependent manner. *Brain Research, 851(1-2)*, 66-75.

Cai, H., Haubensak, W., Anthony, T. E., & Anderson, D. J. (2014). Central amygdala PKC-δ(+) neurons mediate the influence of multiple anorexigenic signals. *Nature neuroscience*, *17*(9), 1240–1248. https://doi.org/10.1038/nn.3767

Canteras, N. S., Simerly, R. B., & Swanson, L. W. (1995). Organization of projections from the medial nucleus of the amygdala: a PHAL study in the rat. *The Journal of comparative neurology*, *360*(2), 213–245. https://doi.org/10.1002/cne.903600203

Canteras, N. S., & Swanson, L. W. (1992). Projections of the ventral subiculum to the amygdala, septum, and hypothalamus: A PHAL anterograde tract-tracing study in the rat. *The Journal of Comparative Neurology, 324*(2), 180-194.

Cenquizca, L. A., & Swanson, L. W. (2007). Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. *Brain Research Reviews, 56*(1), 1-26.

Crow, S.J., Peterson, C.B., Swanson, S.A., Raymond, N.C., Specker, S., Eckert, E.D., Mitchell, J.E. (2009) Increased mortality in bulimia nervosa and other eating disorders. *American Journal of Psychiatry* 166**,** 1342-1346.

de Andrade, J. S., Abrão, R. O., Céspedes, I. C., Garcia, M. C., Nascimento, J. O. G., Spadari-Bratfisch, R. C., Melo, L. L., da Silva, R. C. B., & Viana, M. B. (2012). Acute restraint differently alters defensive responses and fos immunoreactivity in the rat brain. *Behavioural Brain Research*, *232*(1), 20–29. https://doi.org/10.1016/j.bbr.2012.03.034

Douglass, A. M., Kucukdereli, H., Ponserre, M., Markovic, M., Gründemann, J., Strobel, C., . . . Klein, R. (2017). Central amygdala circuits modulate food consumption through a positive-valence mechanism.

Dulawa, S. C., & Hen, R. (2005). Recent advances in animal models of chronic antidepressant effects: The novelty-induced hypophagia test. *Neuroscience & Biobehavioral Reviews, 29*(4-5), 771-783. doi:10.1016/j.neubiorev.2005.03.017.

Furlong, Teri M., Richardson, McNally (2016). Habituation and extinction of fear recruit overlapping forebrain structures. *Neurobiology of Learning and Memory*, *128*, 7–16. doi:10.1016/j.nlm.2015.11.013.

Gao C, et al., Two genetically, anatomically and functionally distinct cell types segregate across anteroposterior axis of paraventricular thalamus. *Nat Neurosci*, 2020. 23(2): p. 217–228.

Greiner, E. M., & Petrovich, G. D. (2020). The effects of novelty on food consumption in male and female rats. *Physiology & Behavior, 223*, 112970. doi:10.1016/j.physbeh.2020.112970

Groenewegen, H. J., Wright, C. I., Beijer, A. V., & Voorn, P. (1999). Convergence and segregation of ventral striatal inputs and outputs. *Annals of the New York Academy of Sciences*, *877*, 49–63. https://doi.org/10.1111/j.1749-6632.1999.tb09260.x

Hoebel, B. G., and Teitelbaum, P. (1962). Hypothalamic control of feeding and self-stimulation. *Science* 135, 375–377. doi: 10.1126/science.135.3501.375

Hurley, K. M., Herbert, H., Moga, M. M., & Saper, C. B. (1991). Efferent projections of the infralimbic cortex of the rat. *The Journal of comparative neurology*, *308*(2), 249–276. https://doi.org/10.1002/cne.903080210

Ishikawa, J., Sakurai, Y., Ishikawa, A., & Mitsushima, D. (2020). Contribution of the prefrontal cortex and basolateral amygdala to behavioral decision-making under reward/punishment conflict. *Psychopharmacology*, *237*, 639-654.

Jolkkonen, E., & Pitkanen, A. (1998). Intrinsic connections of the rat amygdaloid complex: Projections originating in the central nucleus. *The Journal of Comparative Neurology, 395*(1), 53-72. doi:10.1002/(sici)1096-9861(19980525)395:13.0.co;2-g

Kelley AE, Domesick VB, Nauta WJH (1982) The amygdalostriatal projection in the rat—an anatomical study by anterograde and retrograde tracing methods. *Neuroscience*, 7, 615-630.

Kim J, Pignatelli M, Xu S, Itohara S, Tonegawa S. (2016) Antagonistic negative and positive neurons of the basolateral amygdala. *Nat. Neurosci.,* 19,1636–1646.

Kirouac, G. J. (2021). The Paraventricular Nucleus of the Thalamus as an Integrating and Relay Node in the Brain Anxiety Network [Review]. *Frontiers in Behavioral Neuroscience*, *15*. https://doi.org/10.3389/fnbeh.2021.627633

Komorowski, R. W., Manns, J. R., & Eichenbaum, H. (2009). Robust conjunctive item–place coding by hippocampal neurons parallels learning what happens where. *Journal of Neuroscience*, 29(31), 9918-9929.

Krout KE and Loewy AD (2002) Parabrachial nucleus projections to midline and intralaminar thalamic nuclei of the rat. *Journal of Comparative Neurology*, 428(3): p. 475–494.

Li, S., & Kirouac, G. J. (2008). Projections from the paraventricular nucleus of the thalamus to the forebrain, with special emphasis on the extended amygdala. *The Journal of Comparative Neurology, 509(1)*, 136-140.

Li S and Kirouac GJ (2012) Sources of inputs to the anterior and posterior aspects of the paraventricular nucleus of the thalamus. *Brain Struct Funct*, 217(2): p. 257–73.

Lin, J., Amodeo, L. R., Arthurs, J., & Reilly, S. (2012). Taste neophobia and palatability: The pleasure of drinking. *Physiology & Behavior, 106*(4), 515-519. doi:10.1016/j.physbeh.2012.03.029.

Maren, S., Phan, K. L., & Liberzon, I. (2013). The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nature reviews. Neuroscience*, *14*, 6, 417–428. https://doi.org/10.1038/nrn3492

McDonald AJ, Mascagni F, Guo L. (1996) Projections of the medial and lateral prefrontal cortices to the amygdala: a *Phaseolus vulgaris* leucoagglutinin study in the rat. *Neuroscience,* 71:55–75.

Mcdonald, A. J., Shammah-Lagnado, S. J., Shi, C., & Davis, M. (1999). Cortical afferents to the extended amygdala. *Annals of the New York Academy of Sciences*, *877*(1), 309-338.

Mena, J. D., Selleck, R. A., & Baldo, B. A. (2013). Mu-opioid stimulation in rat prefrontal cortex engages hypothalamic orexin/hypocretin-containing neurons, and reveals

dissociable roles of nucleus accumbens and hypothalamus in cortically driven feeding. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *33*(47), 18540–18552. https://doi.org/10.1523/JNEUROSCI.3323-12.2013

Millan, E. Z., Lim, J. H., Power, J. M., & McNally, G. P. (2021). Rapid appetitive transitions are sculpted by amygdala to accumbens pathways. *bioRxiv*, 2021.2004.2011.435530. https://doi.org/10.1101/2021.04.11.435530

Moga MM, Weis RP, and Moore RY(1995) Efferent projections of the paraventricular thalamic nucleus in the rat. *J Comp Neurol*, *359*, 2, 221–38.

Norgren, R. (1976), Taste pathways to hypothalamus and amygdala. *Journal of Comparative Neurology*, 166: 17-30. https://doi.org/10.1002/cne.901660103

O'Leary, T. P., Kendrick, R. M., Bristow, B. N., Sullivan, K. E., Wang, L., Clements, J., Lemire, A. L., & Cembrowski, M. S. (2022). Neuronal cell types, projections, and spatial organization of the central amygdala. *iScience*, *25*(12), 105497. https://doi.org/10.1016/j.isci.2022.105497

Otterson OP (1982) Connections of the amygdala of the rat. IV. Corticoamygdaloid and intra- amygdaloid connections as studied with axonal transport of horseradish peroxidase. J Comp Neurol 205:30–48

Parsons, W., Greiner, E., Buczek, L., Migliaccio, J., Corbett, E., Madden, A. M., & Petrovich, G. D. (2022). Sex differences in activation of extra-hypothalamic forebrain areas during hedonic eating. *Brain structure & function*, *227*(8), 2857-2878.

Penzo, M.A., Robert, V., Tucciarone, J., De Bundel, D., Wang, M., Van Aelst, L., Darvas, M., Parada, L.F., Palmiter, R.D., He, M., Huang, Z.J., Li, B. (2015). The paraventricular thalamus controls a central amygdala fear circuit. *Nature* 519, 455–459.

Petrovich G. D. (2018). Feeding Behavior Survival Circuit: Anticipation & Competition. *Current opinion in behavioral sciences*, *24*, 137–142. https://doi.org/10.1016/j.cobeha.2018.09.007

Petrovich, G. D., & Lougee, M. A. (2011). Sex differences in fear-induced feeding cessation: Prolonged effect in female rats. *Physiology & Behavior, 104*(5), 996-1001. doi: 10.1016/j.physbeh.2011.06.020

Petrovich, G. D., Risold, P. Y., & Swanson, L. W. (1996). Organization of projections from the basomedial nucleus of the amygdala: A phal study in the rat. *The Journal of Comparative Neurology*, *374*(3), 387–420. https://doi.org/10.1002/(sici)1096-9861(19961021)374:3<387::aid-cne6>3.0.co;2-y

Petrovich, G. D., Ross, C. A., Mody, P., Holland, P. C., & Gallagher, M. (2009). Central, But Not Basolateral, Amygdala Is Critical for Control of Feeding by Aversive Learned Cues. *Journal of Neuroscience, 29*(48), 15205-15212. doi: 10.1523/jneurosci.3656-09.2009

Pitkänen, A., Savander, V., & LeDoux, J. E. (1997). Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. *Trends in neurosciences*, *20*(11), 517–523. https://doi.org/10.1016/s0166-2236(97)01125-9

Rajbhandari, A. K., Octeau, C. J., Gonzalez, S., Pennington, Z. T., Mohamed, F., Trott, J., Chavez, J., Ngyuen, E., Keces, N., Hong, W. Z., Neve, R. L., Waschek, J., Khakh, B. S., & Fanselow, M. S. (2021). A basomedial amygdala to intercalated cells microcircuit expressing PACAP and its receptor PAC1 regulates contextual fear. *The Journal of Neuroscience*, *41*(15), 3446–3461. https://doi.org/10.1523/jneurosci.2564-20.2021

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

Ramaker, M. J., & Dulawa, S. C. (2017). Identifying fast-onset antidepressants using rodent models. *Molecular Psychiatry, 22*(5), 656-665. doi: 10.1038/mp.2017.36

Reppucci, C. J., Kuthyar, M., & Petrovich, G. D. (2013). Contextual fear cues inhibit eating in food-deprived male and female rats. *Appetite, 69*, 186-195. doi: 10.1016/j.appet.2013.06.004

Reynolds, S. M., & Berridge, K. C. (2001). Fear and feeding in the nucleus accumbens shell: rostrocaudal segregation of GABA-elicited defensive behavior versus eating behavior. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *21*(9), 3261–3270. https://doi.org/10.1523/JNEUROSCI.21-09-03261.2001

Saper, C.B. (1982), Convergence of autonomic and limbic connections in the insular cortex of the rat. *J. Comp. Neurol., 210*: 163-173.

Shi CJ, Cassell MD (1998. b). Cortical, thalamic, and amygdaloid connections of the anterior and posterior insular cortices. *Journal of Comparative Neurology* 399: 440–468.

Stratford TR, Swanson CJ, Kelley A. (1998). Specific changes in food intake elicited by blockade or activation of glutamate receptors in the nucleus accumbens shell. *Behav Brain Res, 93*,1-2, 43-50. doi: 10.1016/s0166-4328(97)00140-x. PMID: 9659985.

Stuber, G. D., and Wise, R. A. (2016). Lateral hypothalamic circuits for feeding and reward. *Nat. Neurosci*. 19, 198–205. doi: 10.1038/nn.4220

Swanson, L. W., & Petrovich, G. D. (1998). What is the amygdala? *Trends in Neurosciences*, *21*(8), 323-331.

Vertes R. P. (2002). Analysis of projections from the medial prefrontal cortex to the thalamus in the rat, with emphasis on nucleus reuniens. *The Journal of comparative neurology*, *442*(2), 163–187. https://doi.org/10.1002/cne.10083

Vertes, R. P., & Crane, A. M. (1996). Descending projections of the posterior nucleus of the hypothalamus: Phaseolus vulgaris leucoagglutinin analysis in the rat. *The Journal of comparative neurology*, *374*(4), 607–631. https://doi.org/10.1002/(SICI)1096-9861(19961028)374:4<607::AID-CNE9>3.0.CO;2-5

Vertes RP, Hoover WB (2008) Projections of the paraventricular and paratenial nuclei of the dorsal midline thalamus in the rat. *J Comp Neurol 508*:212–237.

Wildes, J. E., Ringham, R. M., & Marcus, M. D. (2010). Emotion avoidance in patients with anorexia nervosa: initial test of a functional model. *The International journal of eating disorders, 43*(5), 398–404.

Wood, E. R., Dudchenko, P. A., & Eichenbaum, H. (1999). The global record of memory in hippocampal neuronal activity. *Nature*, 397(6720), 613-616.

Zhang-Molina, C., Schmit, M. B., & Cai, H. (2020). Neural Circuit Mechanism Underlying the Feeding Controlled by Insula-Central Amygdala Pathway. *iScience*, *23*(4), 101033.