Sex differences in habituation to novel food and novel context: Examination of recruitment of central and basolateral complex nuclei of the amygdala

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Novel foods and novel environments both impact consumption, but their interaction is poorly understood, especially how this interaction varies across habituation and by sex. Prior studies found that placement in a novel context suppressed consumption of a novel food across habituation in a two-choice paradigm with familiar food, and there were neural correlates in the amygdala of consumption under novelty during the first exposure. The current study extended these findings using a paradigm with only a novel food. We placed adult male and female rats in a novel or familiar environment and measured their consumption of a novel, palatable food across four habituation sessions and a final test session. We collected brain tissue after the test session to measure Fos induction with immunohistochemistry during the final exposure to novelty. Fos induction was measured in the central nucleus of the amygdala and the nuclei of the basolateral complex. We found that placement in a novel context suppressed consumption of a novel food at every time point. During the test, Fos induction was elevated in groups tested in the novel context in the medial part of the central nucleus and all nuclei of the basolateral complex except the anterior part of the basolateral nucleus despite the test being the fifth exposure to the novel stimuli. Parts of the central nucleus and nuclei of the basolateral complex showed sex-specific elevations in Fos induction in females regardless of the testing context. Correlations of Fos induction across regions showed that novel context tested groups had similarly elevated Fos induction throughout the central nucleus and basolateral complex, unlike their familiar context tested counterparts. Females had more

correlations of Fos induction than males regardless of testing context. These results demonstrated that habituation to eating a novel food is prolonged in a novel environment compared to a familiar environment. Notably, Fos induction remained high in the novel context groups after multiple exposures to novelty. These behavioral and neural findings demonstrate that unfamiliar environments remain salient throughout the process of habituation.

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[1] Introduction

Interactions with novel foods and environments disrupt typical patterns of food consumption. An initial wariness towards the consumption of a novel food, referred to as taste neophobia, is common across species (Lin et al., 2012a). A new food source could offer nutrients, but could also cause illness. Novel contexts can also lead to uncertain outcomes, especially if food is presented in the new environment. While taste neophobia and restriction of eating in a novel environment are adaptive responses, maintenance of such avoidant behaviors can be linked to neuropsychiatric disorders. One example of such a disorder is anorexia nervosa, which is associated with low novelty seeking and a high fear of uncertainty (Frank et al., 2012; Klump et al., 2000). Other eating disorders which have restricted food consumption as a symptom likely share intolerance of novelty as a central feature, though research into less diagnosed disorders is quite limited. People with avoidant restrictive food intake disorder (ARFID) are intolerant of novelty, especially in patients with low interest in food or who feel food is aversive (Norris et al., 2021; Treasure et al., 2020; Zimmerman & Fisher, 2017). Determining the neural mechanisms associated with reactions to novelty after habituation is an important step in improving treatments for restrictive eating disorders.

While the initial effects of novelty on ingestive behavior during the first presentation are well documented, less research has characterized such reactions across multiple presentations. A common set of findings when assessing feeding in a novel context is that the latency to approach food increases, and the amount of food consumed decreases (Dulawa & Hen, 2005; Ramaker & Dulawa, 2017). Compounding effects have been demonstrated, where a novel environment slowed habituation to novel food across

multiple testing sessions, more so for females than for males (Greiner & Petrovich, 2020). While males habituated and increased their consumption of the novel food in the novel environment, females' consumption remained low, showing sustained suppression of consumption. The slow habituation occurred even though the novel food was very palatable, and went against the strong hedonic drive females have previously shown towards the same food in a familiar context, where they consumed large amounts regardless of if they were fasted or not (Buczek et al., 2020).

Females have also been shown to suppress feeding in response to learned aversive cues more than males do. Post-fear conditioning, males decrease food consumption in the aversive context only, while females suppressed feeding in both the aversive and a neutral context (Reppucci et al., 2013). Similar sex differences were found during extinction of a discrete fear cue, where females were much slower than males to increase feeding (Petrovich & Lougee, 2011). Females' feeding behavior is therefore more susceptible to aversive stimuli. It is necessary to then determine how an uncertain novel context impacts habituation to a novel food in males versus in females.

Though there is limited research on the brain substrates underlying habituation to eating in a novel environment, the amygdala is a strong candidate. The amygdala was tagged as having a specific role in consumption in a novel environment decades ago, based on lesions of the corticomedial area (Sclafani et al., 1970). Since the amygdala is not a single structural or functional unit (Swanson & Petrovich, 1998), investigation of the subnuclei of the amygdala separately is necessary.

The central nucleus of the amygdala (CEA) is vital for feeding behavior, and can either promote or suppress feeding when appropriate. The CEA is critical for the

suppression of feeding during tests with learned fear cues (Petrovich et al., 2009), and is also activated during cue-food conditioning (Cole et al., 2013). The number of Fos-positive neurons increased in the CEA when rats consumed a novel food in a novel context for the first time (Greiner et al., 2023). The CEA contains several cell types which may reduce consumption under novel circumstances. PKCδ neurons are required for the inhibition of feeding due to administration of anorectic substances like CCK or LiCL (Cai et al., 2014), as well as for control of conditioned fear behaviors (Haubensak et al., 2010). Corticotropin-releasing hormone (CRH) neurons mediate contextual conditioning and approach behaviors towards food (Britton et al., 1982; Kreifeldt et al., 2022; Pitts & Takahashi, 2011; Pitts et al., 2009).

While the CEA is frequently associated with inhibition of feeding, serotonin expressing GABAergic neurons also mediate food as a reward to increase consumption (Douglass et al., 2017). The CEA is a substantial component of circuitries necessary for feeding, behavioral control, and survival (Fadok et al., 2018; Janak & Tye, 2015; Petrovich, 2018a). One goal of this study is to determine if the CEA remains important to processing of a novel food after habituation, and if the role of the CEA is the same in a novel versus a familiar context.

The basolateral complex nuclei of the amygdala are also of interest in determining the circuitry underlying suppression of feeding under novelty. The basolateral nucleus (BLA) is important for taste neophobia, as rats with BLA lesions consume the same amount of a cider vinegar solution whether it is novel or familiar to them (Gómez-Chacón et al., 2012). Infusion of CRH into the BLA but not the CEA reduced feeding in a manner similar to reductions in feeding observed after exposure to a stressor (Jochman et

al., 2005). Compelling evidence connects the BLA with acquisition of a positive incentive towards food during Pavlovian conditioning (Hatfield et al., 1996), and the anterior BLA in particular was associated with cue-food learning both early and late in training (Cole et al., 2013). Few studies have evaluated if the BLA is specifically recruited during novel context processing, so determining the extent to which the BLA mediates consumption after habituation to a novel context is another goal of this study.

The role of the basomedial amygdala (BMA) in habituation to novel feeding is far less established. The BMA is important for recognizing whether an environment is safe or should induce anxiety, and that is accomplished through integration of signals from the medial prefrontal cortex (mPFC) (Adhikari et al., 2015). There is additional evidence about the role of the BMA in novelty processing. BMA disinhibition blocks typical increases in heart rate during a social novelty test (Mesquita et al., 2016). Only one prior study evaluated the role of the BMA in feeding under novel circumstances. In that study, rats with lesions of the BMA and adjacent corticomedial nucleus of the amygdala had a longer latency to eat in a novel context, but the lesions spanned across the cortical amygdala and the BMA, which limits the assessment of the specific location of these effects (Lukaszewska et al., 1984).

The lateral amygdala (LA) has a similar gap in research on its role in both feeding and novelty. Traditionally, the LA is discussed as a site of synaptic plasticity underlying fear conditioning (Rodriques et al., 2004). Lesions of the LA led to increased approach towards novel stimuli, a trait shared with lesions of the BLA and BMA (Misslin & Ropartz, 1981). The current study aims to evaluate if the LA is activated in habituation to novelty context during consumption.

The goal of this thesis was to compare male and female rats during habituation to eating in a novel, uncertain environment, and determine activation of amygdala nuclei after habituation. This was accomplished with evaluation of Fos induction. Rats were first habituated to eating a novel, palatable food in either a familiar or novel context for four presentations, and consumption was measured for each exposure. After habituation, rats were tested for consumption during a fifth exposure to the novel food in either a novel or familiar context, and brain tissue was collected for Fos analysis. We hypothesized that females tested in the novel context would habituate slower than all other groups, based on a prior finding in a similar preparation (Greiner & Petrovich, 2020). Questions remain about whether an initially novel feeding environment remains relevant after multiple presentations, and if males and females differ in their behavioral and neuronal responses. Utilizing a Fos induction approach provides valuable functional activity maps to reveal which amygdala nuclei process novelty after habituation, and if there are sex differences.

[2] Methods

[2.1] Subjects

Adult male (n=16) and female (n=16) Long Evans rats (Charles River Laboratories; Portage, MI), aged 60 days, males weighing 250-300 grams and females weighing 200-240 grams upon arrival, were individually housed on single sex shelves. Rats were maintained on a 12-hour light/dark cycle, with lights on at 06:00. Subjects were given one day to acclimate to the colony room before 5 days of daily handling under conditions of *ad libitum* water and standard laboratory chow (Purina Lab Diet Prolab RMH 3000; 3.47 kcal/g; 26% protein, 15% fat, 59% carbohydrates). For each sex, an

equal number of rats were assigned to either the familiar (home cage) or novel context condition, which yielded four groups: home cage males, home cage females, novel context males, and novel context females. The experiment was conducted in two identical replications, each with 16 rats, and 4 per condition for a total of 32 rats. All testing procedures were in compliance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals, and were approved by the Boston College Institutional Animal Care and Use Committee.

[2.2] Apparatus

Half of the animals were tested in their home cage in the colony room and the other half were tested in a novel behavioral chamber (Coulbourn Instruments, Whitehall, PA; 30x28x30 cm). Behavioral chambers had grid flooring and a recessed food port (3.2x4.2 cm) on one wall. The front panel of the behavioral chambers was made of clear plexiglass, and the other walls were metal. Behavioral chambers were housed in individual isolation cubicles (Coulbourn Instruments, Whitehall, PA). Subjects were tested in the same behavioral chamber each session. Food was presented in a ceramic bowl placed in the back right corner of the chamber. For rats tested in their home cage, food was placed in the front right corner of their cage.

[2.3] Testing Procedure

Subjects were tested for consumption of a novel food in either a novel or familiar environment. Rats were habituated to transport to the room housing the behavioral chambers and the ceramic food bowls separately, with at least an hour in their colony room in between, 24 hours before the first habituation session. Male and female rats were acutely food deprived for 20 hours prior to each of the 4 habituation sessions and the final

test session, with *ad libitum* access to water at all times. Subjects were given a minimum of 24 hours of *ad libitum* access to standard chow before the next instance of food deprivation. Each habituation session was 10 minutes long, and the test session was 20 minutes long. During the habituation and test sessions, rats were presented with a ceramic bowl with 15g of novel Test Diet pellets (TD; 3.44 kcal/g; 21% protein, 13% fat, 67% carbohydrate (all from sucrose)). The remaining food was weighed following the end of each session to calculate the amount of TD consumed. Body weight measurements were taken at least an hour before the start of each session.

[2.4] Histological Procedures

Subjects were perfused 90 minutes after the start of the 20-minute test session. Rats were anesthetized with isoflurane (5%; Baxter Healthcare Corporation, Deerfield, IL) then were given an intraperitoneal injection of Fatal Plus (390 mg/ml pentobarbital sodium; Vortech Pharmaceuticals, Dearborn, MI). Rats were transcardially perfused with 0.9% saline then 4% paraformaldehyde in 0.1M borate buffer. After extraction, brains were post-fixed overnight in a solution of 12% sucrose dissolved in the paraformaldehyde perfusion solution. Brains were blocked in two sections and frozen in hexanes chilled in dry ice, and were then stored at -80° C.

Brains were sliced in 30-µm sections using a sliding microtome in four adjacent series, two in a 0.02 potassium phosphate-buffered saline (KPBS), and two in a cryoprotectant solution (0.025 M sodium phosphate buffer with 30% ethylene glycol, 20% glycerol). The second series in KPBS was used for Nissl staining for identification of cytoarchitectonic borders. Tissue was mounted onto gelatin-coated slides before staining with thionin (Fisher Scientific, Waltham, MA). Both series in cryoprotectant

solution were stored at -20 °C for later use. The first KPBS series was used for immunohistochemical identification of Fos.

Free-floating tissue was incubated at room temperature for 1 hour in a blocking solution composed of KPBS, 0.3% Triton X-100 (Sigma-Aldrich, St. Louis, MO), 2% normal goat serum (S-1000; Vector Laboratories, Burlingame, CA), and 10% non-fat milk (M0841; Fisher Scientific, Waltham, MA**)** to reduce non-specific binding. Tissue was then incubated for 72 h at 4 °C in the primary antibody, anti-*c-fos* in rabbit (1:5000, 226 008; Synaptic Systems, Göttingen, Germany), in the blocking solution. Tissue was rinsed in KPBS then incubated with the secondary antibody, biotinylated goat anti-rabbit IgG (1:500; BA-1000-1.5; Vector Laboratories, Burlingame, CA) in the blocking solution at room temperature for 45 minutes. Tissue was rinsed in KPBS then placed in avidin-biotin complex (ABC solution; PK-6100; Vector Laboratories, Burlingame, CA) for 45 minutes at room temperature. Tissue was then rinsed with KPBS followed by another 30-minute incubation in the recycled secondary antibody solution. Tissue was rinsed with KPBS, then incubated in the recycled ABC solution followed by another KPBS rinse. Tissue was then incubated in a 3,3'-diaminobenzidine solution (SK-4100; Vector Laboratories, Burlingame, CA) for 2 minutes on a rotator at room temperature. Following staining, tissue was mounted on SuperFrost Plus slides (Fisher Scientific, Pittsburgh, PA) and air-dried overnight. Tissue was further dried in a 45 °C oven overnight. Dried tissue was dehydrated through graded alcohols before being cleared in xylenes and coverslipped using DPX (13512; Electron Microscopy Sciences, Hatfield, PA).

[2.5] Image Acquisition and Analysis

Images were obtained at 10X using an Olympus BX51 microscope with an attached Olympus DP74 camera using cellSens imaging software (Olympus America Inc., Center Valley, PA). Fos-positive cells were counted using ImageJ software (NIH, Bethesda, MD). Regions of interest were identified and borders were drawn onto thionin-stained tissue, and these borders were transposed to the matching Fos-stained tissue. Fos-positive neurons were counted automatically and were limited based on circularity and size. Neurons were counted bilaterally, and counts from the left and right hemispheres were summed for each rat and reported as the number of Fos-positive neurons for each region. Regional borders for analysis were determined based on the fourth edition of the Swanson rat brain atlas (Swanson, 2018).

In the amygdala, a total of 8 distinct cell groups were analyzed for Fos induction: three parts of the CEA, and five nuclei of the basolateral complex (**Table 1**). In the CEA, the medial, lateral, and capsular parts were analyzed on Swanson atlas levels 26 and 28. Data for these cell groups were combined across levels 26 and 28. The BMAa was analyzed on level 26, the BMAp on level 30, the BLAa on level 27, the BLAp on level 30, and the LA on level 30.

Due to unavailability of tissue or damaged tissue, subjects were excluded from analyses. Seven subjects were excluded from analyses of the CEA, resulting in the following group sizes: home cage males n=7, novel context males n=6, home cage females n=6, novel context females n=6. Six subjects were removed from analyses in the BMA, resulting in the following groups: anterior (home cage males n=7, novel context males n=6, home cage females n=7, novel context females n=6), posterior (home cage males n=6, novel context males n=6, home cage females n=6, novel context females

n=8). Seven subjects were removed from analyses in the BLAa, and sex were removed from analyses in the BLAp, resulting in the following groups: anterior (home cage males *n*=6, novel context males *n*=5, home cage females *n*=6, novel context females *n*=8), posterior (home cage males *n*=6, novel context males *n*=6, home cage females *n*=6, novel context females *n*=8). Six subjects were removed from analyses in the LA, resulting in the following group sizes: home cage males *n*=6, novel context males *n*=6, home cage females *n*=6, novel context females *n*=8).

[2.6] Statistical Analysis

Consumption results were analyzed as grams consumed per 100g of body weight to normalize for body weight differences between males and females. Consumption results for each habituation session and the test session were analyzed separately using a two-way ANOVA for sex and context. A repeated-measures multivariate ANOVA was used to analyze consumption data across habituation sessions 1-4 by sex, context, and session. Fos induction by region was analyzed using a two-way ANOVA for sex and context. ANOVAs were followed up with Bonferroni multiple comparisons when appropriate. Rate of change from the first to last habitation session was calculated for each subject using the following formula: [(consumption per body weight session 4-consumption per body weight session 1)/(4-1)]. Pearson correlations were used to compare Fos induction between regions. A p-value of ≤ 0.05 was considered significant across all analyses, except for *post-hoc* analyses in which Bonferroni adjusted alpha levels were used. In addition, p-values between 0.05 and 0.1 were noted.

[3] Results

The body weights of the male rats were higher than those of the female rats, but there were no differences between the rats of the same sex in the novel versus familiar context groups at any habituation session or test (**Table 2**). On average, rats gained a small amount of weight across the experiment (**Figure 1**).

[3.1] Novel food consumption during habituation in a novel or familiar environment

Across the four habituation sessions, all groups increased their consumption of the novel food, but rats in the novel context consistently consumed less than those tested in the familiar environment (**Figure 2A**). A repeated measures two-way ANOVA of food consumption across habituation found a significant within-subjects main effect of habituation session ($F(3,28)= 51.046$, $p<0.001$), but no significant interactions (Session*Sex: $F(3,28)=1.113$, $p=0.348$); Session*Context: $F(3,28)=0.398$, $p=0.755$; Session*Sex*Context: $F(3,28)=0.745$, $p=0.528$). The same analysis found a significant between-subjects effect of context ($F(1,28)=11.451$, $p=0.002$), but no main effect of sex ($F(1,28)=1.709$, $p=0.202$) or interaction ($F(1,28)=0.322$, $p=0.575$). In addition, a test of within-subjects contrasts found significant differences in consumption between the first and second sessions ($F(1,28)=63.757$, $p<0.001$) and the second and third sessions ($F(1,28)=11.712$, $p=0.002$), but not between the third and fourth habituation sessions ($F(1,28)=1.92$, $p=0.177$). To further evaluate habituation to the novel food across sessions, the rate of change between the first and last habituation sessions was calculated. Numerically, the lowest rate of change was in females tested in the novel context and the highest in the females tested in their home cage, however, that difference was not statistically significant (Context: ($F(1,28)=0.519$, $p=0.477$); Sex: ($F(1,28)=0.005$, $p=0.947$); Sex*Context: ($F(1,28)=1.313$, $p=0.262$) (**Figure 2B**).

During the first habituation session, Habituation 1, there was lower consumption in the groups that were tested in the novel context, and females (**Figure 3A**). A two-way ANOVA found main effects for context ($F(1,28)=12.838$, $p=0.001$) and sex ($F(1,28)=6.978$, $p=0.013$), but no sex by context interaction ($F(1,28)=0.52$, $p=0.477$).

During Habituation 2, rats tested in the novel context ate less than those tested in the familiar context (**Figure 3B)**. A two-way ANOVA found a main effect of context ($F(1,28)=6.362$, $p=0.0176$), but no effects of sex ($F(1,28)=0.4496$, $p=0.5080$) or interaction ($F(1,28)=0.345$, $p=0.561$). Similar patterns of consumption continued during Habituation 3 and 4, with rats tested in the novel context consuming less than those tested in the familiar context (**Figure 3C-D**). Analyses of consumption during Habituation 3 & 4 found main effects of context (H3: $F(1,28)=5.491$, $p=0.0264$; H4: $F(1,28)=8.265$, $p=0.0076$), but no effects of sex (H3: $F(1,28)=0.007$, $p=0.934$; H4: $F(1,28)=2.181$, $p=0.151$ or interactions (H3: $F(1,28)=0.458$, $p=0.504$; H4: $F(1,28)=0.652$, $p=0.426$)).

[3.2] Novel food consumption after habituation

During the test, rats tested in the novel context consumed less food, and females consumed less than males (**Figure 4**). There was a main effect of context ($F(1,28=6.290$, $p=0.0182$), and the effect of sex was below 0.1 ($F(1,28)=3.259$, $p=0.0818$), but there was no effect of sex by context interaction ($F(1,28)=0.004$, $p=0.952$).

[3.3] Fos induction after habituation to novel food and novel context

Fos induction was measured during the test, which was the subjects' fifth exposure to the novel food and novel context.

Central Nucleus of the Amygdala. Fos induction was higher in the CEAc and CEAm in females, and in the CEAm in the novel context groups (**Figure 5A-C).** A two-way

ANOVA of the number of Fos positive neurons found a main effect of sex in the CEAm ($F(1,21)=5.631$, $p=0.027$) and the CEAc ($F(1,21)=5.692$, $p=0.027$), but not in the CEAl ($F(1,21)=1.357$, $p=0.257$). The p value for the main effect for context was below 0.1 in the CEAm ($F(1,21)=3.55$, $p=0.073$), but not in the CEAl ($F(1,21)=2.654$, $p=0.118$) or CEAc ($F(1,21)=0.026$, $p=0.874$). There were no effects of interaction between sex and context in any subregion (CEAl: ($F(1,21)=2.191$, $p=0.154$); CEAc: ($F(1,21)=0.238$, $p=0.631$); CEAm: ($F(1,21)=2.518$, $p=0.127$)).

Basolateral Nucleus of the Amygdala. Fos activation in the BLA varied between the anterior and posterior parts. There was a higher number of Fos positive cells in the novel context groups and in females in the BLAp, while the pattern in the BLAa was more complex due to a crossover interaction effect (**Figure 6 A-B)**. In the BLAa, in females, there was more Fos induction in the novel compared to familiar context, but in males there was less Fos induction in the novel compared to familiar context. This was confirmed with a two-way ANOVA that found a sex by context interaction $F(1,19)=4.598$, $p=0.045$), but no significant main effects of sex ($F(1,19)=0.104$, $p=0.75$) or context ($F(1,19)=0.021$, $p=0.887$). In the BLAp, there were significant main effects of sex ($F(1,19)=4.701$, $p=0.043$) and context ($F(1,19)=4.95$, $p=0.038$), but no effect of interaction ($F(1,19)=0.505$, $p=0.486$).

Basomedial Nucleus of the Amygdala. Fos activation patterns in the BMA were similar in the anterior and posterior parts, with more Fos positive neurons in the novel context groups and in females (**Figure 7 A-B**). In the BMAa, a two-way ANOVA found significant main effects of sex ($F(1,19)=9.837$, $p=0.005$) and context ($F(1,19)=10.845$, $p=0.004$), and the interaction p value was below 0.1 ($F(1,19)=3.153$, $p=0.092$). In the

BMAp, a two-way ANOVA found main effects of sex ($F(1,19)=4.669$, $p=0.044$) and context ($F(1,19)=19.099$, $p<0.001$), but no interaction ($F(1,19)=0.82$, $p=0.377$).

Lateral Nucleus of the Amygdala. In the LA, there was higher Fos induction in females and rats tested in the novel context (**Figure 8**). A two-way ANOVA found significant main effects of sex ($F(1,19)=6.104$, $p=0.023$) and context ($F(1,19)=8.462$, $p=0.009$) with no interaction ($F(1,19)=0.025$, $p=0.875$).

Correlations of Fos Induction Between Amygdala Nuclei. Correlation analyses were conducted to examine the relationships between Fos induction across different nuclei in each group. There were more correlations in females and groups tested in the novel context. All significant correlations for all groups were positive. Females tested in the novel context had 20 correlations, while males tested in the novel context had 13 correlations. Females tested in the familiar context had 14 correlations, while males tested in the familiar context had 7 correlations.

Females tested in the novel context had the most correlations between brain regions of any group (**Table 3**). Fos induction in the CEAm was correlated with the values in the CEAl, BLAa, BLAp, BMAa, BMAp, and LA. The CEAl was correlated with the BLAa, BMAa, BMAp, and LA, while the CEAc was correlated with the BLAa and LA. The BLAa was correlated with the BLAp, BMAa, and LA. The BLAp was correlated with the BMAa, BMAp, and LA. The BMAa was correlated with the BMAp and LA.

Males tested in the novel context had fewer correlations than females tested in the novel context, but more than males in the familiar context (**Table 3**). The CEAm was correlated with both the CEAl and CEAc, the BLAp, BMAp, and LA. The CEAl was

correlated with the CEAc, BMAa, and BMAp. The CEAc was correlated with the BLAp and BMAp. The BLAa was correlated with the BMAp, the BLAp with the LA, and the BMAa with the BMAp.

Females tested in the familiar context had fewer correlations than females tested in the novel context (**Table 4**). Fos induction in the CEAm was correlated with the CEAc, BLAa, BLAp, BMAa, and LA. There were no correlations with the CEAl. The CEAc was correlated with the CEAm, BLAa, BLAp, and BMAa. The BLAa was correlated with the BLAp, BMAa, and LA. The BLAp was correlated with the BMAa and LA, and the BMAa with the LA. There were no correlations with the BMAp.

Males tested in the familiar context had the fewest correlations between brain regions (**Table 4**). The CEAm was correlated with the CEAl and CEAc. The CEAl was correlated with the BMAa and BLAa, and the CEAc with the LA. The BLAa was correlated with the BMAa, and the BLAp with the BMAp.

[4] Discussion

In this study, we investigated how habituation to consumption of a novel food varied between a novel and familiar environment, whether there were sex differences across habituation, and the neural correlates of these differences. Neophobic responses to a new food source are well established (Lin et.al., 2012a; Greiner & Petrovich, 2020). However, less is known about how novel environments interact with novel foods, as most studies about taste neophobia tested effects only in a familiar environment (Lin et al., 2012b; Mitchell et al., 1980). In the current study, we directly compared habituation to a novel food in a novel versus a familiar context.

Through tracking across multiple presentations of the novel TD pellets, we measured changes in consumption across habituation. As the TD pellets are highly

palatable due to their high sucrose content and rats were food deprived prior to each habituation session, the TD pellets should be viewed as a desirable food source (Buczek et al., 2020). We assessed Fos induction during the fifth and final presentation of TD in order to evaluate areas which are known to be recruited during the first exposure to novelty, focusing on the central nucleus and basolateral complex of the amygdala (Greiner et al., 2023).

Different groups of male and female rats were given novel food in either a novel or familiar environment. Presentation of the same novel food in a novel or a familiar environment allowed us to determine behavioral and neural differences due to the context. We found that the familiar and novel context had different impacts on consumption of the novel food and regional Fos activation.

[4.1] Behavioral Findings

Habituation to eating the novel food was slower for rats fed in the novel context compared to rats fed in the familiar environment. All groups increased their consumption across the habituation sessions, but those tested in the novel context did so at a much slower rate. Male and female rats tested in the novel context ate less than their home cage counterparts at every timepoint. The low intake of TD pellets in the novel context indicates that in addition to the initially low consumption due to the novel taste which is universal across contexts, the novel environment caused further inhibition of eating which was sustained. This is consistent with a prior study that offered a choice between novel (TD pellets) and familiar (regular rat chow) food and found robust inhibition of consumption of both foods in the novel compared to familiar context (Greiner & Petrovich, 2020). That study also demonstrated sex differences in habituation to

consumption in the novel context, with females showing more sustained neophobia in a novel context than males (Greiner & Petrovich, 2020).

Current behavioral results indicate that a distinct shift occurs between early and late habituation. Rats habituated to the taste of the novel food by the third exposure, indicated by the stable consumption patterns between habituation sessions 3 and 4. The transition from session 3 and 4 is a turning point for consumption across our paradigms, as shown by rats consuming more novel (TD) than familiar (chow) food at that point in two-choice paradigms (Greiner & Petrovich, 2020). This pattern suggests that rats were able to recognize the safety and palatability of the food by session 3. In the current study, consumption in the novel context was lower than consumption in the familiar context at every habituation session, showing that the novel context maintains influence on consumption patterns longer than the novel taste does.

It is apparent that the novel feeding context influences habituation to a novel food, despite the palatability of that food increasing motivation towards consumption. Importantly, the novel context did not prevent habituation, as consumption in the novel context still increased. Our findings confirm prior work which found habituation to a novel saccharin solution was slower in a novel environment compared to the home cage (De la Casa & Díaz, 2013). Together, the current and prior results demonstrate that the effect of novel context is consistent across liquid and solid diet, and caloric or nutrient free foods. Placement in a novel context suppresses eating behavior generally, regardless of palatability, form, or nutritional content. Given that novel context altered habituation in the current study, it is necessary to specifically define what habituation is. Habituation has been compared to extinction of conditioned fear (McSweeney & Swindell, 2002).

Habituation and extinction are both processes which reflect behavioral adjustments across multiple sessions. Behaviorally, conditioned fear is reduced by both conditioned stimulus extinction and unconditioned stimulus habituation (Furlong et al., 2016). Habituation and extinction also require similar brain regions, including the amygdala, hippocampus, and infralimbic cortex (Furlong et al., 2016; Knapska et al., 2012).

Novel contexts can inhibit eating to a similar degree as an aversive context. Rats placed into a new context suppressed their consumption of a saccharin solution to the same degree as rats placed back in a context in which they were previously shocked, though this effect was only tested in males (Vicente & De la Casa, 2021). In the current study, rats ate less in the novel context compared to the familiar during all 5 exposures. Rats therefore remain sensitive to their feeding environment even after they habituate to novel taste. In that regard, a prior study found that when rats were presented with a familiar sucrose solution in a novel context, they suppress their consumption (Honey et al., 1992). The current study suggests that even during the fifth exposure to novelty, rats were still evaluating how safe or unsafe the novel context was.

There were sex differences in the current study. Both sexes consumed less in the novel context compared to their familiar context counterparts, but females were more strongly affected by the novel context than males. Females overall ate less during the first habituation session, and females tested in the novel context had numerically the lowest consumption during each habituation session, and the lowest rate of change in consumption across sessions. Conversely, females tested in their home cage had numerically the highest rate of change. By the fourth habituation session, males tested in the novel context consumed about 80% of the amounts that males tested in the familiar

context consumed, while females tested in the novel context consumed only 61% of the amounts that females tested in the familiar context consumed. These patterns are consistent with a prior study that found that females in the novel context suppressed their consumption throughout 8 habituation sessions (Greiner & Petrovich, 2020). There were procedural differences between the two studies that may have contributed to the less pronounced sex differences found here. In the prior study, rats were given a choice between familiar and novel foods, while in the current study only novel food was given.

The lower consumption in females is not caused by low motivation towards palatable foods. At baseline, females may have a stronger drive for palatable food than males, in line with a prior finding that sated females ate similarly large amounts of TD pellets as hungry females did (Buczek et al., 2020). Females tested in the familiar context quickly increased their consumption of the palatable food, indicating high motivation towards palatable foods. Therefore, the low consumption in females tested in the novel context is caused by the novel context. At least for females, placement in a novel, uncertain context is salient enough to counter their drive towards palatable food in a sustained manner. Males also suppressed their consumption due to the novel context, though less pronounced.

Findings across different aversive paradigms indicate that females suppress their food consumption more and for longer than males. Females suppressed their consumption longer than males during extinction to a tone which previously predicted a footshock (Petrovich & Lougee, 2010). In another study, females not only suppressed consumption in the context associated with the shocks, but also in a neutral context never associated with aversive stimuli, showing generalized suppression (Reppucci et al., 2013). In the

current behavioral paradigm, the novel context did not contain any aversive cues, and there were no discrete cues to signal the presence of food. Even without specific aversive cues, the uncertainty of a new environment can affect consumption similarly to aversive cues as discussed above, based on a prior study in males (Vicente & De la Casa, 2021). In the current study, the uncertainty of the novel context had stronger and more sustained effects in females, similar to the prolonged effects previously found with learned cues and contexts (Petrovich & Lougee, 2010; Reppucci et al. 2013).

Animal responses to novel stimuli are often considered as a trait, with opposite reactions of avoidance versus approach in tests for anxiety and depression compared to tests for drug addiction. Reactions to novel stimuli are used to assess anhedonia in rodent models. Chronic administration of antidepressants reduces latency to feed and increases feeding overall in novel environments (Bodnott et al., 1988; Dulawa & Hen, 2005). Rats can be bred to express high or low levels of novelty seeking behavior. Rats bred to have low locomotor activity in a novel environment are more susceptible to anhedonia, a major symptom of depression (Stedenfeld et al., 2011). In contrast, rats who are high in novelty seeking choose higher concentrations of ethanol (Pelloux et al., 2015), and will compulsively self-administer cocaine (Belin et al., 2010). Our paradigm emphasizes how the influence of novel stimuli can interact with individual susceptibility to alter the length and intensity of neophobia. We found that though the novel context robustly suppressed consumption in both sexes, that effect was more pronounced females, potentially related to higher anxiety in females.

[4.2] Fos Induction Analysis

The clear differences across habituation in the familiar compared to novel context conditions suggests that different neural substrates may underly the sustained reductions in consumption. We examined Fos induction in the central nucleus and basolateral complex nuclei of the amygdala and found robust activation in the novel context condition. These regions were robustly activated during the first exposure to consumption of a novel food in a novel context (Greiner et al., 2023). In the central amygdala, Fos was selectively elevated in the CEAm, but not other parts of the CEA, in the novel context groups. In the basolateral complex, the novel context increased Fos activation in all nuclei, except the BLAa. Females had higher Fos induction than males in all regions that responded higher to the novel context, although sex by context interactions were not significant. In addition, Fos induction was also higher in the CEAc of females.

[4.2.1] CEA Fos Induction

In the current study, Fos expression in the CEA varied by subregion depending on context and sex. Fos expression in the CEAm was higher in females and rats of both sexes tested in the novel context, while in the CEAc it was overall greater in females. Fos induction in the CEAl was similar across all groups. All parts of the CEA receive inputs from CA1, a region vital for contextual memory (Cenquizca & Swanson, 2007). CA1 Fos induction analysis is ongoing for this study. The CEAm is unique anatomically positioned to control feeding behavior through multiple networks (Petrovich, 2018a; Petrovich 2018b). The current finding that the CEAm was recruited more in rats tested in the novel context and in females suggests that it is important in mediating the behavioral response to novel contextual stimuli in later sessions. The CEA receives inputs from all basolateral complex nuclei, directly or indirectly (Petrovich et al., 1996; Pitkänen et al.,

2000), and all these regions, except the BLAa, had greater Fos induction in the novel context condition. The CEAm also receives heavy projections from the CEAc and moderate projections from the CEAl, neither of which showed greater Fos activation in novel context groups (Jolkkonen & Pitkänen, 1998; Petrovich & Swanson, 1996). The CEA projects to the lateral hypothalamus (LHA), which is well known to regulate feeding (Petrovich, 2018b). All three subdivisions send pathways to the LHA, but the greatest number of projecting neurons are in the CEAm and in a distinct part of the CEAl (Reppucci & Petrovich, 2015). The LHA is necessary for motivation and memory for consumption (Petrovich et al., 2002; Sharpe et al., 2017; for review see Petrovich, 2018b), and it is positioned within a circuit with the CEA and PFC which further emphasizes the importance of this projection (Reppucci & Petrovich, 2015). The CEAm also receives input from the nucleus of the solitary tract (NTS) which processes gustatory information (Bienkowski & Rinaman, 2012). Therefore, the CEAm is a major source of convergence from the amygdala, hindbrain, and cortical areas, and the Fos patterns in the current study indicate that it may be a vital region for the control of consumption during habituation to novelty.

The prior study that examined activation patterns during the first exposure to novelty found higher Fos induction in all parts of the CEA in rats given novel food and in the CEAc in rats tested in a novel context and in males (Greiner et al., 2023). That study also found higher Fos in the caudal CEAl (atlas level 28) in the novel context groups of both sexes (Greiner et al., 2023). The current findings indicate that activity patterns change across the CEA subregions during habituation to novel food and novel context.

CEA neurons are predominantly GABAergic, but contain different neuropeptides (Sun & Cassell, 1993; Swanson & Petrovich, 1998) and different subgroups were likely captured among Fos positive neurons in the current study. As cell-specific methods were not employed, it is not possible to determine which types of neurons were activated. Nevertheless, somatostatin and substance P are potential candidates. The CEAm and CEAl both contain somatostatin neurons (McCullough et al., 2018), a cell population which has been linked to defensive behaviors in the CEAl (Yu et al., 2016). The CEAm also has substance P containing neurons, which the CEAc and CEAl both lack (Cassell et al., 1986). Substance P was originally conceptualized in regard to noxious stimuli (DeVane, 2001), though this view has been expanded to consider its influence in memory and stress as well (Hasenöhrl et al., 2000). Infusions of substance P into the CEA increased latency to re-enter the dark chamber after an unavoidable shock in a passive avoidance test (Kertes et al., 2009). Though there were no shocks administered in our paradigm, the reduction in consumption in the novel context groups is an avoidant behavior. It is unknown whether avoidant behaviors related to novelty and shocks are mediated through overlapping or different circuitries, but substance P neurons in the CEAm are potential candidate cell type.

Another candidate cell type in the CEA are the corticotropin-releasing factor/hormone (CRH) neurons. The CEAm contains some CRH neurons, however CRH neurons are contained mostly in the CEAl (Pomrenze et al., 2015; Wang et al., 2011). Prior work showed the caudal CEAl was more activated in the novel context during the first exposure to novelty, suggesting that CRH neurons may mediate the initial hesitation towards consuming novel food in a novel environment (Greiner et al., 2023). However, in

the current study, the number of Fos positive neurons in the CEAl was similar across all groups, so CRH neurons may no longer play a significant role after habituation to novelty.

[4.2.2] BMA Fos Induction

Fos activation patterns in the BMA were consistent between the anterior and posterior parts. Within both regions, Fos induction was higher in the novel context groups and in females. Higher Fos induction in females was most notable in females tested in the novel context. Due to these similar patterns, the BMAa and BMAp will sometimes be discussed together, and referred to as the BMA.

Higher Fos induction in the BMA in novel context condition suggests that this region is involved in processing contextual information during habituation and may be causal to sustained inhibition of feeding. The BMA mediates contextual conditioning (Rajbhandari et al., 2021), however it is an understudied structure in regard to novelty processing. One study found that disinhibition of the BMA during a social novelty paradigm reduced the heart rate increases caused by introduction of an intruder rat (Mesquita et al., 2016). Early electrolytic lesion studies of the BMA found increased exploration of a novel context and an increased latency to eat in a novel environment (Lukaszewska et al., 1980; Lukaszewska et al., 1984). Assuming that pyramidal neuron activity sustains the hypophagia, these findings appear to run counter to our findings of increased Fos activation in the BMA in novel context groups. However, these results are difficult to compare directly to our study because the lesions included the BMA as well as the cortical amygdala, only males were tested, and Fos induction could be in pyramidal neurons or interneurons.

Placement of rats into a novel context is an uncontrollable stressor, and the BMA is important in paradigms that use uncontrollable stress. Male mice subjected to uncontrollable footshocks had higher BMA activity along with fewer escape attempts (Ineichen et al., 2021). The BMA has bilateral connections with the PFC, most heavily with the infralimbic region (IL) (Condé et al., 1995; Petrovich et al., 1996). Activation of PFC-BMA circuits increased the time spent in the open arms in the elevated plus maze, as well as exploratory behavior in the open field test (Adhikari et al., 2015). PFC Fos analysis for the current study is in progress. Additionally, the BMA has bilateral connections with the hippocampal formation (Cenquizca & Swanson 2007; Petrovich et al., 1996). It is reasonable to assume that elevated Fos in the BMA during habituation in novel context groups may be due to its communications with the PFC and hippocampal formation.

In a prior study, there was also higher Fos induction in the BMA in rats placed in a novel context for the first time (Greiner et al., 2023). Together that finding and the current data suggest that the increased BMA activity is sustained during habituation to a novel context. The current data also indicate that in females that area is more engaged than in males.

[4.2.3] BLA Fos Induction

Within the BLA, there were overt differences in regional activation. While the BLAp had a Fos activation pattern consistent with the BMAa and BMAp, with more Fos induction in the novel context groups, the BLAa did not. The BLAa had an interaction, where the females tested in the novel context had higher Fos induction compared to their familiar context counterparts, while males showed the opposite pattern.

Few studies distinguish between the BLAa and the BLAp, much like how the BMA is treated. This is in contrast with the very distinct connections which clearly delineate between the BLAa and BLAp. Most notably, the BLAa lacks significant inputs to the hippocampal formation compared to the BLAp (Petrovich et al., 2001; Petrovich & Swanson, 1998; Pitkänen et al., 1997). The hippocampal ventral field CA1 projects most heavily to the BMA, and moderately to very restricted parts of the BLAa and the BLAp (Cenquizca & Swanson, 2007). Similarly, the ventral subiculum only sparsely innervates the BLA and LA, and instead densely innervates the BMAp. In addition, while both the BLAa and BLAp are connected with the prelimbic and infralimbic cortical areas, these connections are distinct across dorsoventral medial PFC (Hurley et al., 1991; Reppucci & Petrovich, 2015), while in mice, only the BLAa is significantly innervated by the PFC (Hintiryan et al., 2019). Fos analysis alone cannot determine if the differences in hippocampal connectivity influenced the separate patterns in the BLAa and BLAp.

Studies of the BLA and feeding under novel conditions suggest that the region may partially control consumption in the novel context. Electrolytic lesions of the BLAa increased latency to eat familiar food in a novel environment (Fitzgerald & Burton, 1981). Based on this finding, when intact, the BLAa reduces food approach behaviors in a novel context. As this study did not include females, these findings cannot directly explain the interaction effect in the BLAa in the current study. Without an existing body of literature about novelty and consumption in the BLAa to compare to, our findings suggest a new role for the BLA which appears to be sex-specific.

In a prior study, rats tested in a novel context had higher Fos activation in the BLAa and BLAp during the first exposure to novelty (Greiner et al., 2023). The current

data indicate that during habituation to novel context, the high activity is sustained within the BLAp but not within the BLAa. In addition, the current study found higher Fos induction in the BLAp of females, which was most notable in the group tested in the novel context. The results of that prior and current study suggest that the BLAp may be necessary for habituation to eating in novel place and together with the BMA is a part of sex-specific circuitry.

[4.2.4] LA Fos Induction

Fos induction patterns in the LA followed the pattern of increased Fos activation in groups tested in the novel context. The specific role the LA plays in processing novelty is poorly defined. Electrolytic lesions of the LA greatly decreased neophobic responses to both novel environments and novel objects in a familiar environment (Misslin & Ropartz, 1981). The LA is necessary for contextual conditioning, mediated through its bilateral connections with the hippocampal formation (Hintiryan et al., 2019; Maren & Hobin, 2007; Petrovich et al., 2001). These inputs are differentially recruited during retrieval of a fear memory versus an extinction memory, with fear memory relying more heavily on the hippocampal inputs (Knapska et al., 2023). Analysis of CA1 is planned, and correlations between these regions would confirm if inputs to the LA vary by the novelty of the context in a similar manner. The majority of neurons in the LA show context-specific changes in firing rates after fear memory extinction (Hobin et al., 2003), which is in line with the increased Fos activation in the novel context groups during habituation in the current study. The LA has been most commonly studied in auditory fear conditioning rather than contextual conditioning (LeDoux et al., 1990; Quirk et al., 1995; Repa et al., 2001). This role in fear conditioning is often linked to synaptic plasticity within the

region (Blair et al., 2001; Doyère, 2007). It is possible that such synaptic plasticity and firing rate changes are occurring during habituation, another form of learning.

Similar to the BLA and BMA, during the first exposure to novelty, Fos induction was elevated in the LA of groups tested in the novel context (Greiner et al., 2023). The current data indicate that the activity within the LA is sustained during habituation to novel context. Interestingly, unlike the BLA and BMA, the patterns were similar for both sexes.

[4.3] Correlational Analyses

In addition to examining the recruitment of each region independently, another major aim of this study was to determine if different networks were recruited during habituation in a familiar versus a novel environment. To accomplish this, we analyzed correlations of Fos induction between amygdalar regions. Novel context groups had more correlations than familiar context groups, and females had more correlations than males. Specific discussion of correlations by region will follow after a brief overview of group patterns.

Females tested in the novel context had the most correlations between the brain regions analyzed. In that group, the LA had more correlations compared to any other group. Males tested in the novel context had sparse LA correlations, suggesting that LA networks are recruited in a sex-specific way under novelty. There were sparser BMAp than BMAa correlations, though most regions which correlated with the BMAp correlated with the BMAa, except for the BLAa. Correlations with CEAm were also notable, as the number of correlations was high, and males and females had similar correlations. The similar correlations in rats of both sexes tested in the novel context

suggest that novelty activates CEAm relevant networks similarly regardless of sex, unlike the LA.

Males tested in the novel context had fewer correlations than females tested in the novel context, though the patterns were similar. Males and females in the novel context had consistent CEAm correlations, with fewer CEAc and CEAl correlations. Compared to females, males tested in the novel context had more correlations with the BMAp than the BMAa. The BMAa and BMAp were robustly activated in the novel context in both sexes, so these correlational differences may indicate that these regions are functioning within distinct networks in males and females. These sex differences in BMAa and BMAp correlations patterns may be related to differences in consumption during the test and higher sensitivity of females to context novelty post-habituation.

Females tested in a familiar environment had more correlations than males tested in a familiar environment. Most of their correlations were with the CEAm and BMAa, with sparser LA and BLA correlations. They had fewer intra-CEA correlations, but a similar pattern of CEAm correlations as the novel context groups of both sexes. The BMAp and CEAl were not correlated with any other region, which was unique to females tested in their home cage.

Males tested in the familiar context had the fewest number of correlations of all groups. There were fewer CEAm correlations than any other group. Like females in the same condition, there were no BMAp correlations, but the CEAl in males was correlated with the BMAa but not in females.

Substantial Fos induction and correlation patterns in both sexes in the novel context condition indicate that the novel context remains salient even after habituation.

This is especially notable in the CEAm and BMAp, which were significantly correlated only in the novel context groups. These patterns suggests that these regions might be driving the sustained reductions in consumption. Correlations with the BMAp are unique to the novel context groups. These correlations are with regions that the BMAp directly innervates, including the BMAa, BLAp, and the CEA (Petrovich et al., 1996). It is notable that the BMAp receives direct inputs from the hippocampal formation (Canteras et al., 1992; Cenquizca & Swanson, 2007). This suggests that the BMAp may coordinate activity within the amygdala based on contextual information processed in the hippocampus.

In the current study, correlation patterns suggest sex-specific networks. There were correlations that were unique to each sex, regardless of the testing context. In females, the BMAa correlations were similar in the familiar and novel context groups, and there were few intra-CEA correlations. In contrast, males had more intra-CEA correlations than females regardless of context. Males also had few correlations with the LA or BLAa. The similarities of correlations within each sex, regardless of context, suggests that males and females may have different amygdala functioning at baseline or during feeding.

[5] Conclusion

Current behavioral and neural results demonstrate that novel environments prolong habituation to novel food compared to familiar environments, and the effect is more pronounced in females than males. The sustained avoidance that continues after the safety and palatability of the food was established could become maladaptive.

The novel context is not processed identically in males and females, demonstrated by neural differences and a slower rate of change in consumption in the novel context. The high number of correlated regions in females tested in the novel context, especially with the BMAp, suggests that the amygdala is functioning more like a single unit in females than in males when under the stress of novelty.

Further research into which regions in the amygdala are controlling the behavioral outputs, versus those that are activated due to local inputs, during habituation to novelty is needed to understand the functional circuitry. Gaining a more specific interpretation of novelty processing during consumption is important to understand how novel stimuli become familiar, in addition to potential applications to maladaptive feeding inhibition in eating disorders.

[6] Tables and Figures

Brain Region	Representative Atlas Level(s)	Distance from Bregma
CEAm	**26, 28**	**-1.78, -2.45**
CEAc		
CEAl		
BMAa	**26**	**-1.78**
BMAp	**30**	**-3.25**
BLAa	**27**	**-2.00**
BLAp	**30**	**-3.25**
LA	**30**	**-3.25**

Table 1. List of all brain regions analyzed and sampling location. Atlas levels refer to the Swanson rat brain atlas (2018).

	Habituation 1	Habituation 2	Habituation 3	Habituation 4	Test
Familiar Context Males	305±10	305±11	310±11	313±10	329±13
Novel Context Males	298±13	298±13	302±14	308±14	325±17
Familiar Context Females	216±2	214±1	215±2	216±1	220±2
Novel Context Females	216±4	215±4	215±4	216±5	224±5

Table 2. Average body weights of the rats on each habituation session and test day (mean ± SEM).

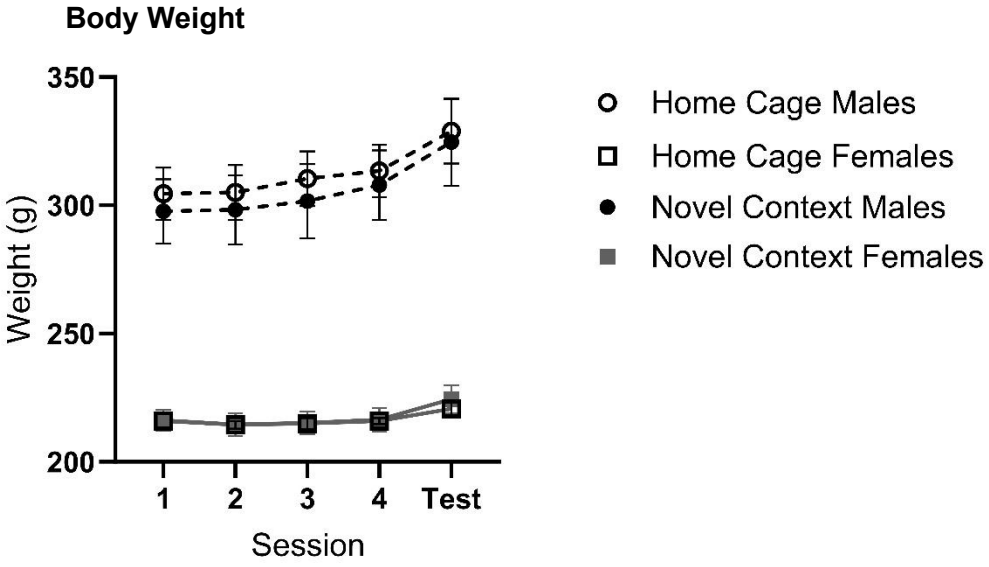


Figure 1. Graph shows body weights on the day of each session during the experiment as group averages ± SEM. Circles represent males, and squares represent females. Filled shapes represent groups tested in the novel context, and open shapes represent groups tested in the familiar context.

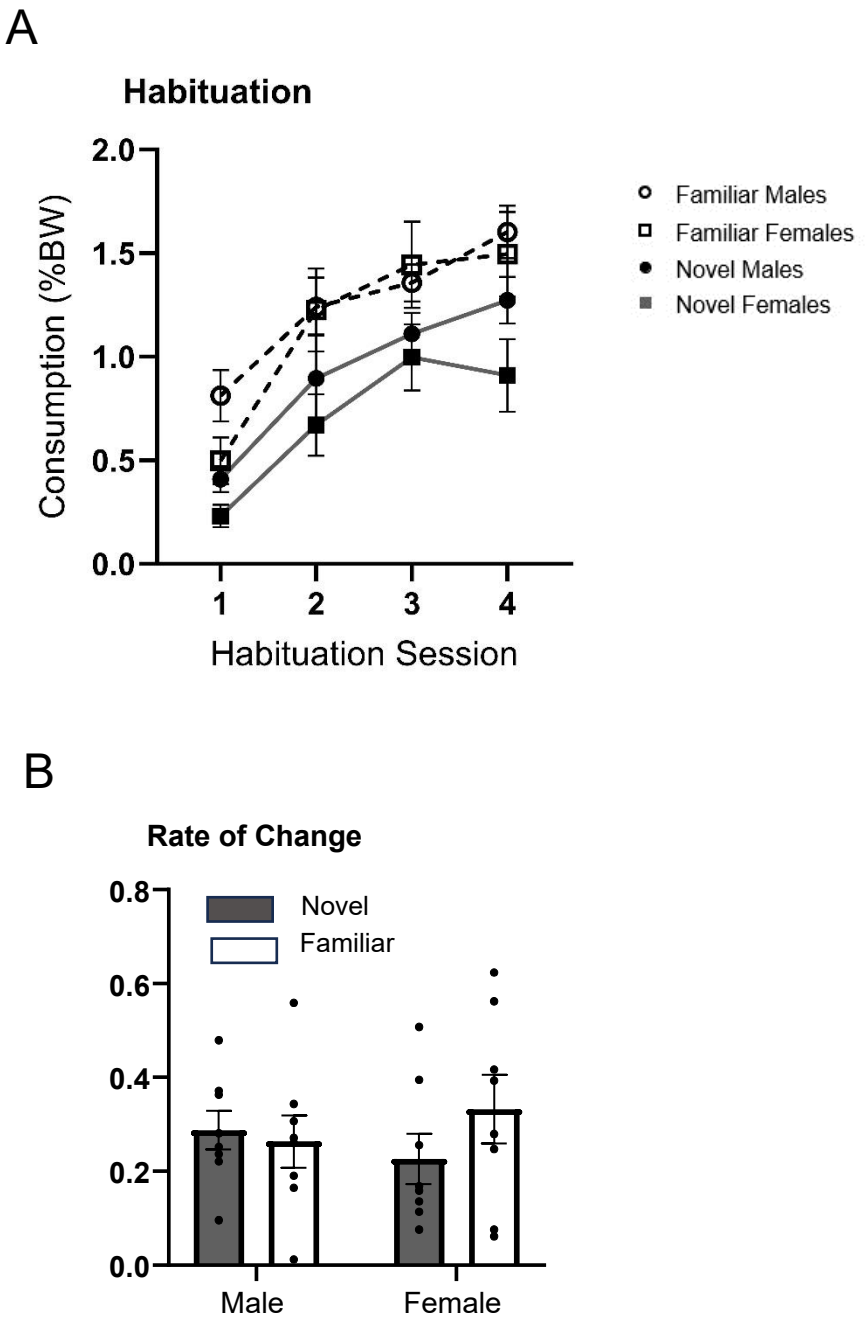


Figure 2. Novel food consumption during habituation sessions. **A**. Line graph shows grams consumed per body weight (mean ± SEM). Circles represent males, and squares represent females. Filled shapes represent groups tested in the novel context, and open shapes represent groups tested in the familiar context. **B**. Bar graph shows rate of change across habituation (mean ± SEM).

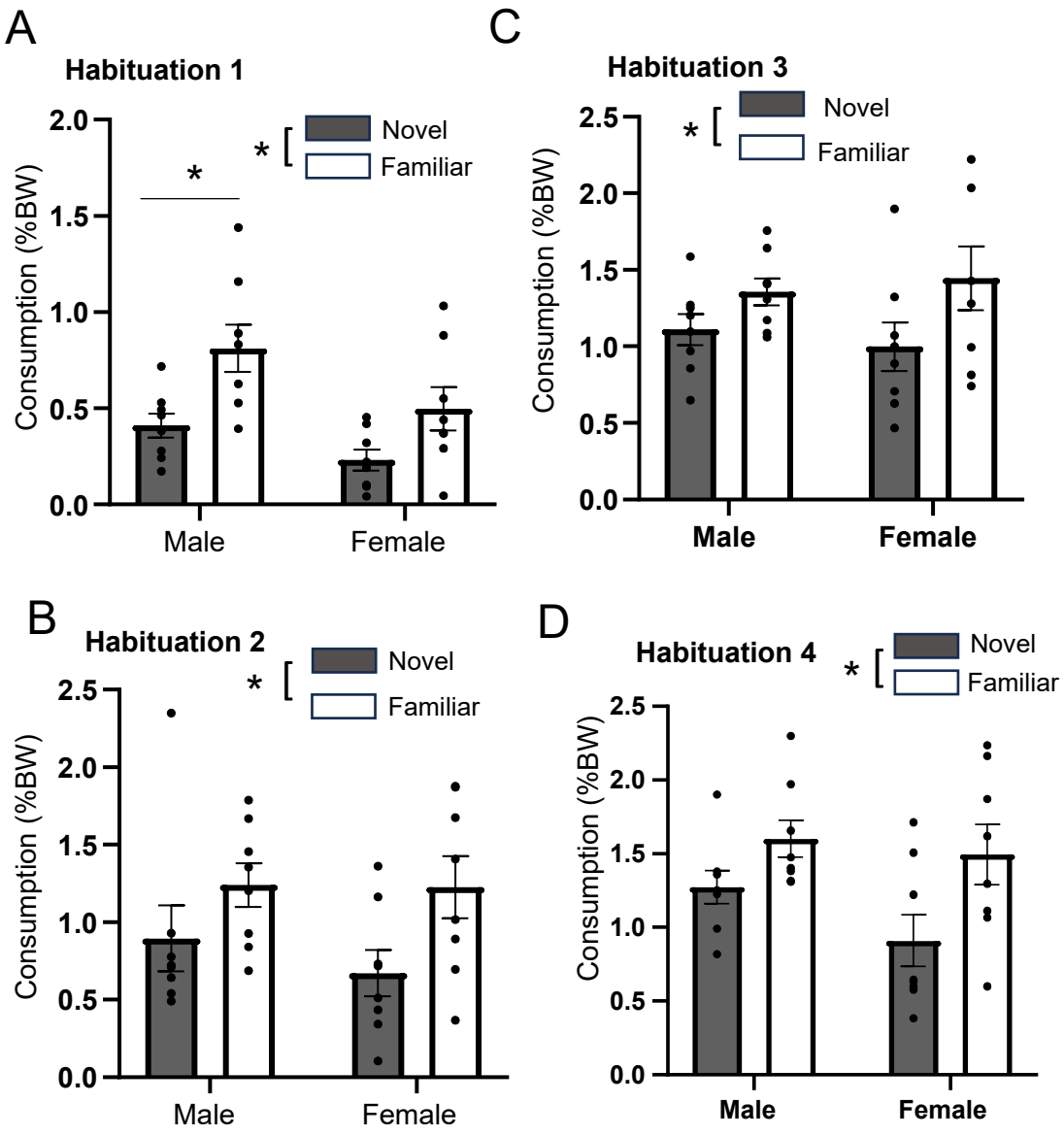


Figure 3. Novel food consumption during habituation sessions. Filled shapes represent groups tested in the novel context, and open shapes represent groups tested in the familiar context. Symbol above line indicates main effect of sex. Symbols next to legends indicate main effect of context. **A**. Bar graph shows novel food consumption during the first habituation session. **B**. Bar graph shows novel food consumption during the second habituation session. **C**. Bar graph shows novel food consumption during the third habituation session. **D**. Bar graph shows novel food consumption during the fourth habituation session. All graphs show grams consumed per 100g body weight (mean ± SEM). (*$p<0.05$).

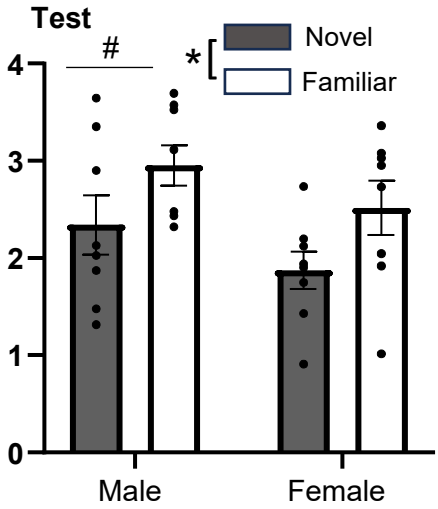
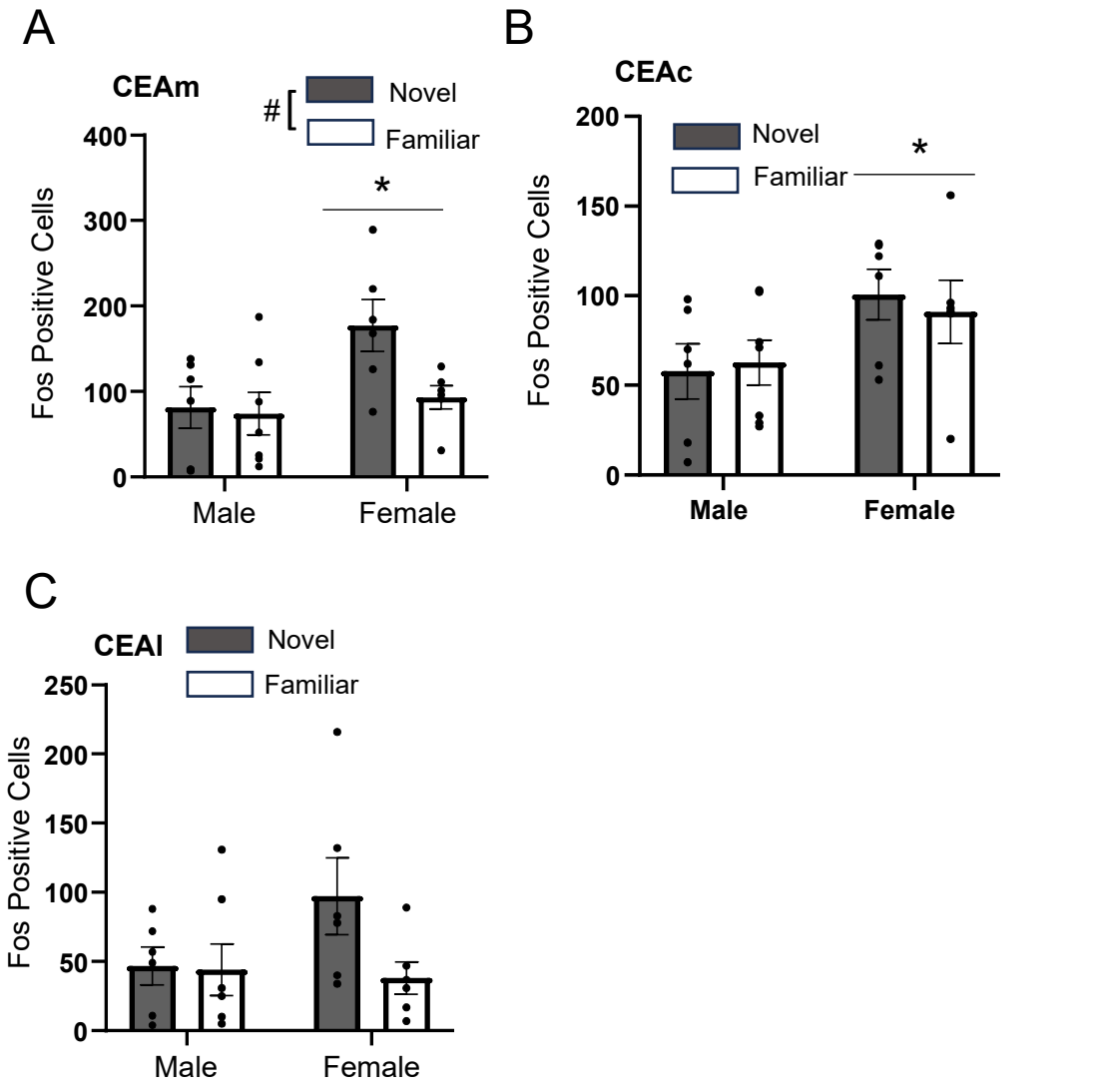
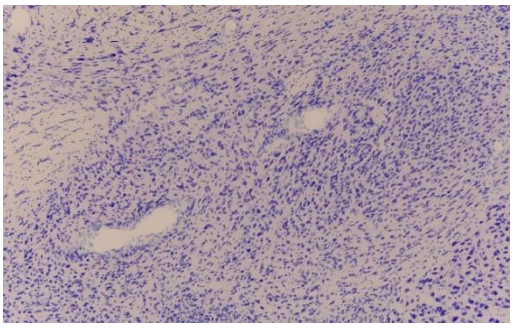


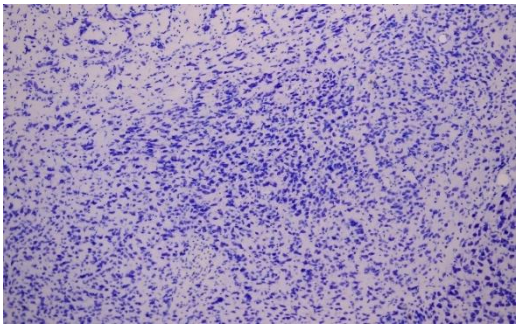
Figure 4. Consumption of novel food during the test. Filled shapes represent groups tested in the novel context, and open shapes represent groups tested in the familiar context. Symbol above line indicates main effect of sex. Symbol next to legend indicates main effect of context. Graph show grams consumed per 100g body weight (mean ± SEM) (*$p<0.05$; #$p<0.1$).


A
CEAm
Novel
Familiar
#
*
Fos Positive Cells
400
300
200
100
0
Male
Female
B
CEAc
Novel
Familiar
*
Fos Positive Cells
200
150
100
50
0
Male
Female
C
CEAl
Novel
Familiar
Fos Positive Cells
250
200
150
100
50
0
Male
Female

D

E





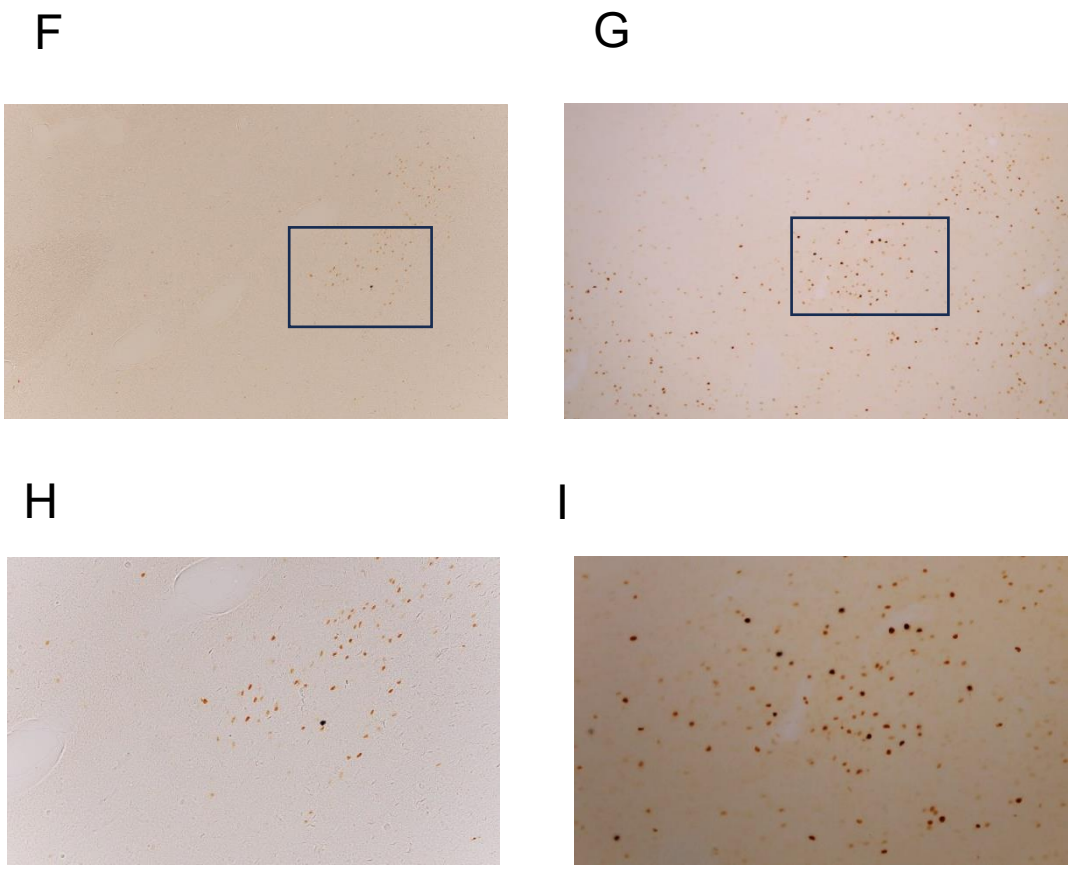


Figure 4. Fos positive cells in the CEA. Filled shapes represent groups tested in the novel context, and open shapes represent groups tested in the familiar context. Symbols above line indicate main effect of sex. Symbol next to legend indicates main effect of context. **A**. Bar graph shows Fos positive cells in the medial division of the CEA. **B**. Bar graph shows Fos positive cells in the capsular division of the CEA. **C**. Bar graph shows Fos positive cells in the lateral division of the CEA. **D**. Nissl-stained tissue showing CEA in a familiar context tested female (atlas level 26, right side). **E**. Nissl-stained tissue showing CEA in a novel context texted female (atlas level 26, right side). **F**. Tissue stained for Fos in CEA in the section adjacent to the tissue in D. Box shows area magnified in panel H. **G**. Tissue stained for Fos in CEA in the section adjacent to the tissue in E. Box shows area magnified in panel I. **H**. Magnified image of tissue stained for Fos from box in panel F. Contrast of image in H was increased. **I**. Magnified image of tissue stained for Fos from box in panel G. Image has contrast increased. Contrast of image in I was increased. Graphs show grams consumed per 100g body weight (mean ± SEM) (*$p<0.05$; #$p<0.1$).

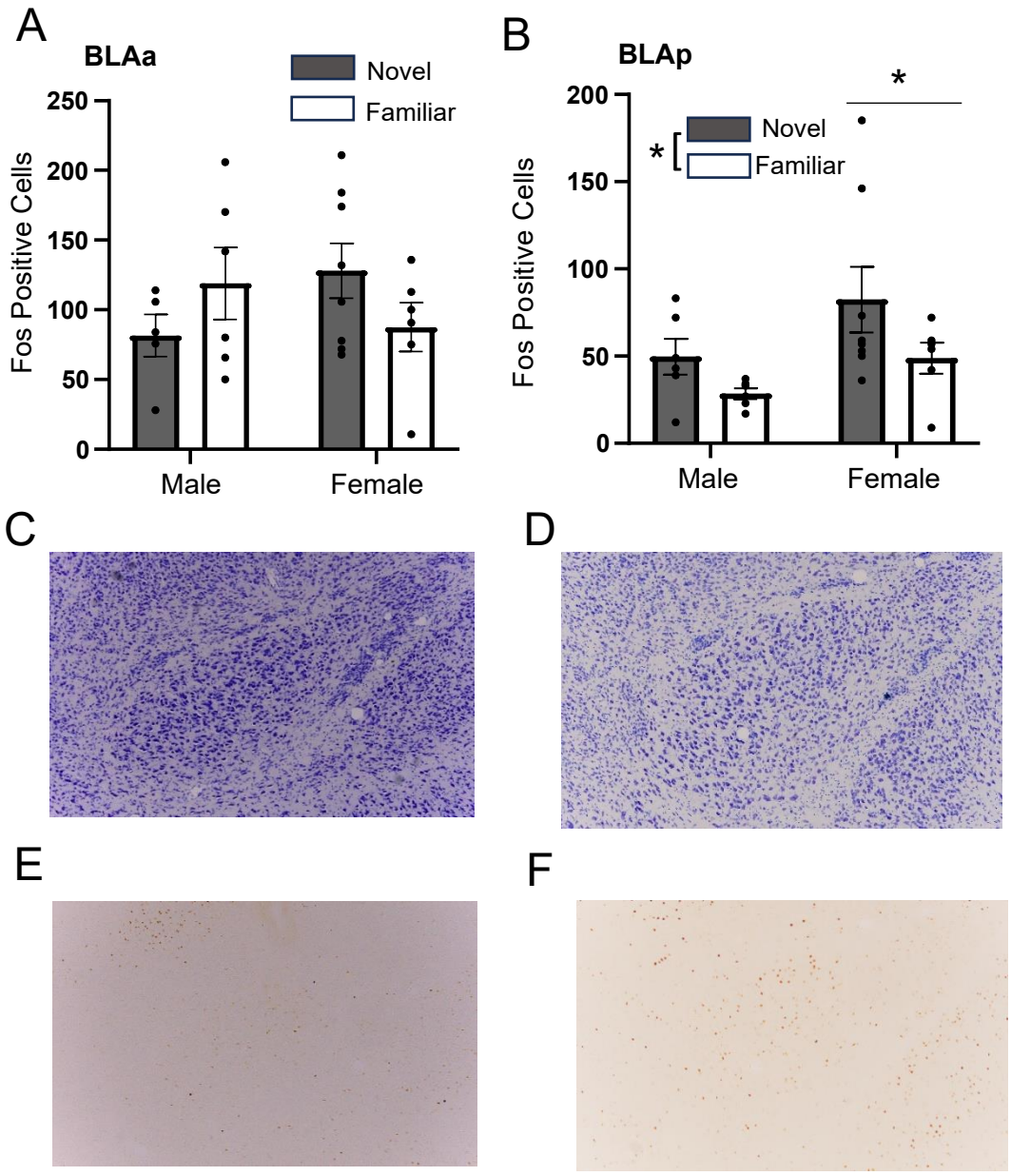


Figure 6. Fos positive cells in the BLA. Filled shapes represent groups tested in the novel context, and open shapes represent groups tested in the familiar context. Symbol above line indicates main effect of sex. Symbol next to legend indicates main effect of context. **A**. Bar graph shows Fos positive cells in the anterior division of the BLA. **B**. Bar graph shows Fos positive cells in the posterior division of the BLA. **C**. Nissl-stained tissue showing BLAa in a novel context tested male (atlas level 27, right side) **D**. Nissl-stained tissue showing BLAa in a novel context tested female (atlas level 27, right side). **E**. Tissue stained for Fos in BLAa in the section adjacent to the tissue in C. **F**. Tissue stained for Fos in BLAa in the section adjacent to the tissue in D. Graphs show grams consumed per 100g body weight (mean ± SEM) (*$p<0.05$).

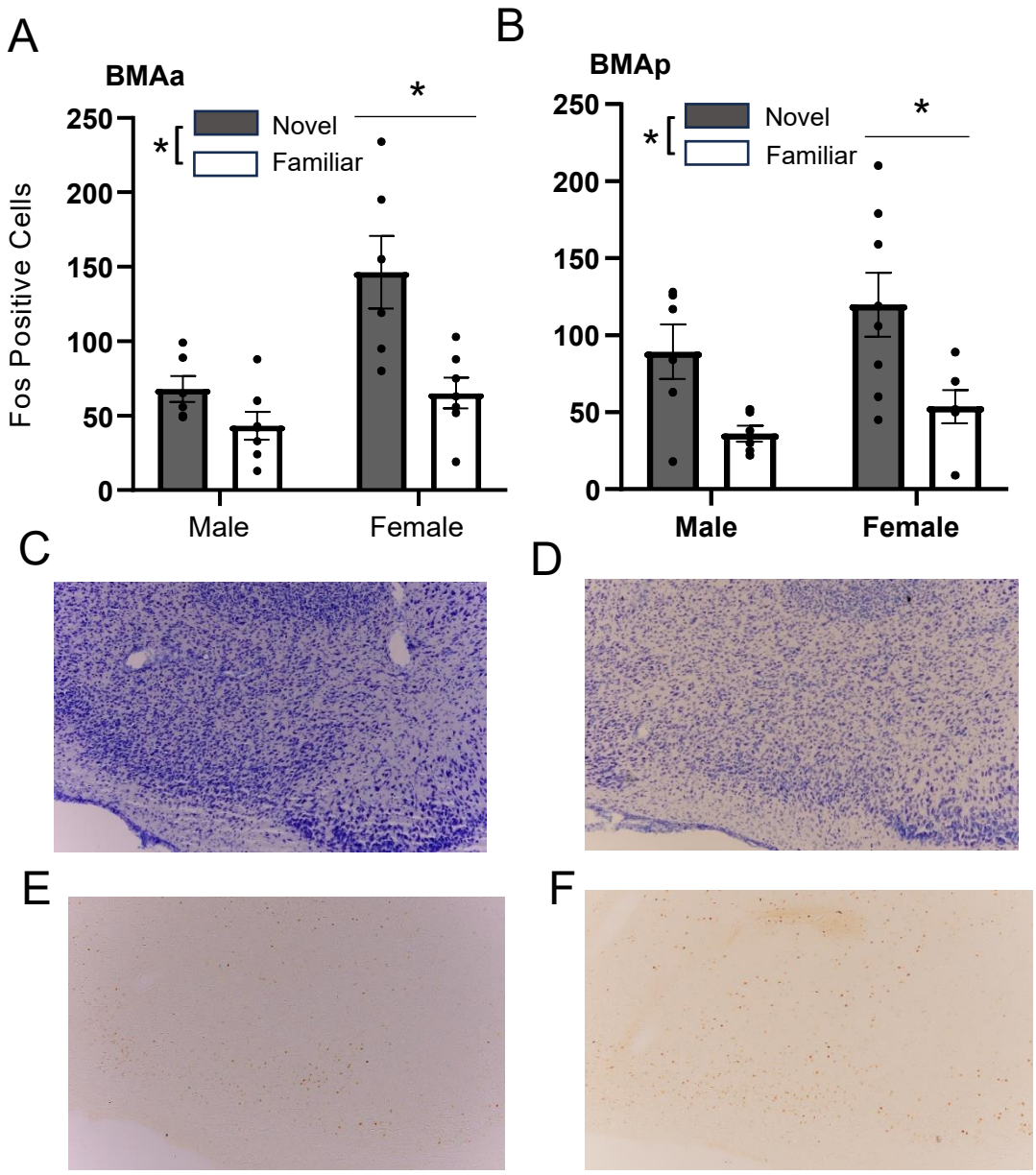


Figure 7. Fos positive cells in the BMA. Filled shapes represent groups tested in the novel context, and open shapes represent groups tested in the familiar context. Symbols above lines indicate main effect of sex. Symbols next to legends indicate main effect of context. **A**. Bar graph shows Fos positive cells in the anterior division of the BMA. **B**. Bar graph shows Fos positive cells in the posterior division of the BMA. **C**. Nissl-stained tissue showing BMAa in a novel context tested male (atlas level 26, right side). **D**. Nissl-stained tissue showing BMAa in a novel context tested female (atlas level 26, right side). **E**. Tissue stained for Fos in BMAa in the section adjacent to the tissue in C. **F**. Tissue stained for Fos in BMAa in the section adjacent to the tissue in D. Graphs show grams consumed per 100g body weight (mean ± SEM) (*$p<0.05$).

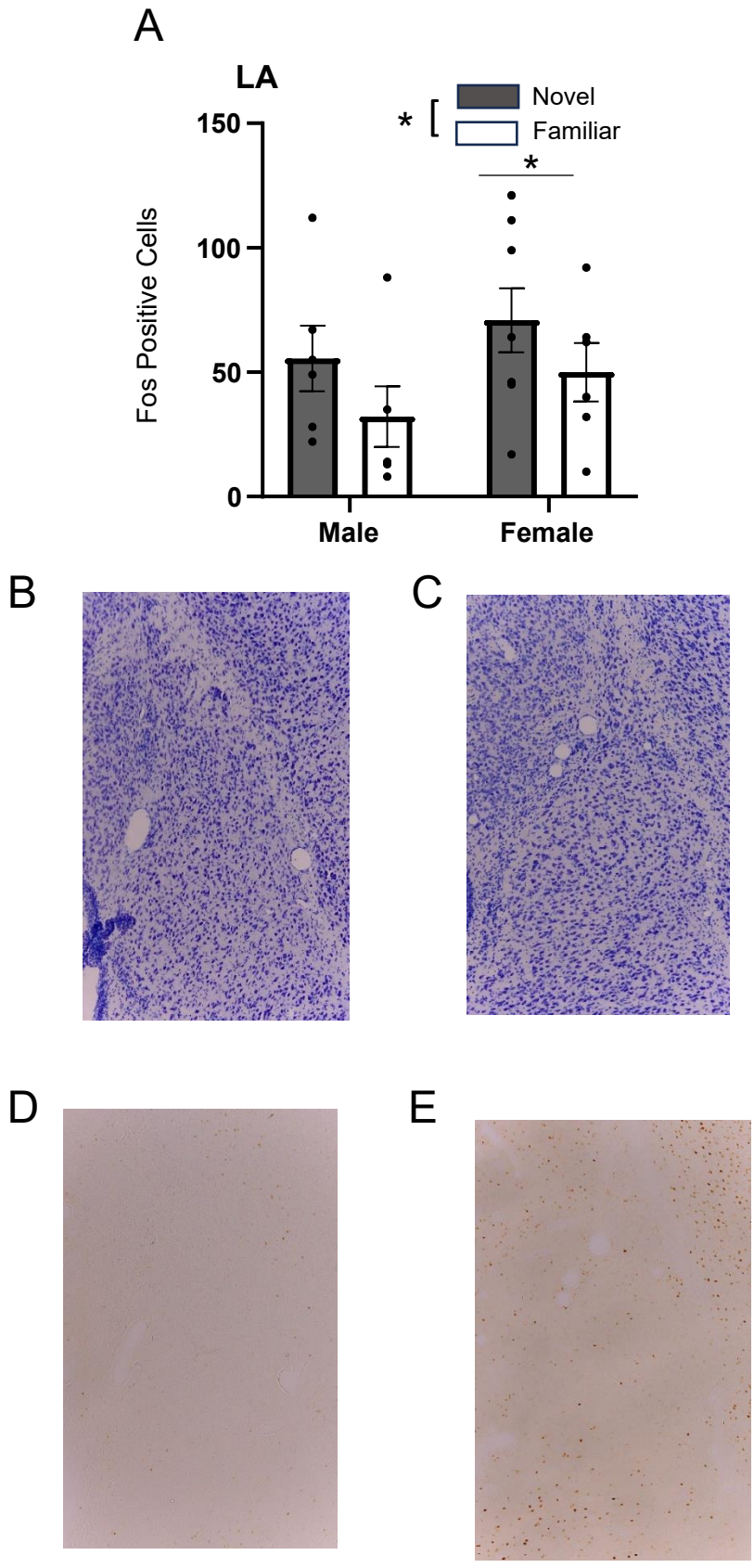

A
LA
Novel
Familiar
*
*
150
100
50
0
Fos Positive Cells
Male
Female
B
C
D
E

Figure 8. Fos positive cells in the LA. Filled shapes represent groups tested in the novel context, and open shapes represent groups tested in the familiar context. Symbol next to legend indicates main effect of context. **A**. Bar graph shows Fos positive cells in the LA. **B**. Nissl-stained tissue showing LA in a familiar context tested female (atlas level 30, right side) **C**. Nissl-stained tissue showing LA in a novel context tested female (atlas level 30, right side). **D**. Tissue stained for Fos in LA in the section adjacent to the tissue in B. **E**. Tissue stained for Fos in LA in the section adjacent to the tissue in C. Graphs show grams consumed per 100g body weight (mean ± SEM) (*$p<0.05$).

		CEAm	CEAl	CEAc	BLAa	BLAp	BMAa	BMAp	LA
CEAm	r		.881*	0.717	.864*	.882*	.908*	.946*	.963*
	p		0.021	0.109	0.027	0.020	0.012	0.004	0.002
CEAl	r	.868*		0.559	.812*	0.680	.955*	0.734	.886*
	p	0.025		0.249	0.050	0.138	0.003	0.097	0.019
CEAc	r	.929*	.984*		.903*	0.688	0.724	0.566	0.809
	p	0.007	0.000		0.014	0.131	0.104	0.242	0.051
BLAa	r	0.593	0.722	0.703		.730*	.939*	0.609	.904*
	p	0.215	0.105	0.119		0.040	0.001	0.109	0.002
BLAp	r	.858*	0.663	0.776	0.634		0.700	.843*	0.706*
	p	0.029	0.152	0.070	0.176		0.053	0.009	0.050
BMAa	r	0.364	0.742	0.615	0.651	0.068		0.680	.869*
	p	0.478	0.091	0.194	0.162	0.899		0.063	0.005
BMAp	r	0.764	.919*	.887*	.927*	0.639	0.795		0.502
	p	0.077	0.010	0.018	0.008	0.172	0.058		0.205
LA	r	.853*	0.509	0.627	0.297	0.793	-0.069	0.409	
	p	0.031	0.303	0.183	0.567	0.060	0.896	0.421	

Table 3. Table shows correlations of Fos induction between brain regions in novel context tested groups. Males are shown in the bottom left, below diagonal and females are shown in the top right, above diagonal. Correlations $p<0.1$ are highlighted in orange for males and green for females. Asterisks denote correlations $p<0.05$.

		CEAm	CEAl	CEAc	BLAa	BLAp	BMAa	BMAp	LA
CEAm	r		0.541	0.803	.958*	.862*	.904*	0.602	.869*
	p		0.268	0.054	0.003	0.027	0.013	0.206	0.025
CEAl	r	.821*		0.372	0.322	0.242	0.280	0.517	0.122
	p	0.023		0.468	0.534	0.644	0.590	0.294	0.818
CEAc	r	0.749	0.668		0.791	.929*	0.768	0.538	0.632
	p	0.053	0.101		0.061	0.007	0.074	0.271	0.179
BLAa	r	0.710	0.730	0.293		.915*	.972*	0.602	.909*
	p	0.114	0.099	0.573		0.011	0.001	0.206	0.012
BLAp	r	-0.337	0.468	0.231	-0.135		.870*	0.675	0.750
	p	0.514	0.349	0.660	0.798		0.024	0.142	0.086
BMAa	r	0.397	0.730	0.252	0.761	0.452		0.514	.884*
	p	0.377	0.062	0.586	0.079	0.368		0.297	0.019
BMAp	r	0.239	0.694	0.727	0.297	0.772	0.576		0.247
	p	0.649	0.126	0.102	0.582	0.072	0.232		0.637
LA	r	0.329	0.242	.934*	0.051	0.309	-0.057	0.693	
	p	0.525	0.643	0.006	0.923	0.551	0.915	0.127	

Table 4. Table shows correlations of Fos induction between brain regions in familiar context tested groups. Males are shown in the bottom left, below diagonal and females are shown in the top right, above diagonal. Correlations $p<0.1$ are highlighted in orange for males and green for females. Asterisks denote correlations $p<0.05$.

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