



Activation patterns in male and female forebrain circuitries during food consumption under novelty

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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 potential danger. Previous work found that novel food hypophagia is enhanced in a novel environment and that males habituate faster than females. The current study's aim was to identify the neural substrates of separate effects of food and context novelty. Adult male and female rats were tested for consumption of a novel or familiar food in either a familiar or in a novel context. Test-induced Fos expression was measured in the amygdalar, thalamic, striatal, and prefrontal cortex regions that are important for appetitive responding, contextual processing, and reward motivation. Food and context novelty induced strikingly different activation patterns. Novel context induced Fos robustly in almost every region analyzed, including the central (CEA) and basolateral complex nuclei of the amygdala, the thalamic paraventricular (PVT) and reuniens nuclei, the nucleus accumbens (ACB), the medial prefrontal cortex prelimbic and infralimbic areas, and the dorsal agranular insular cortex (AI). Novel food induced Fos in a few select regions: the CEA, anterior basomedial nucleus of the amygdala, anterior PVT, and posterior AI. There were also sex differences in activation patterns. The capsular and lateral CEA had greater activation for male groups and the anterior PVT, ACB ventral core and shell had greater activation for female groups. These activation patterns and correlations between regions, suggest that distinct functional circuitries control feeding behavior when food is novel and when eating occurs in a novel environment.

Keywords Novelty · Consumption · Sex differences

Introduction

Novel stimuli are initially treated with weariness or avoidance. This is an adaptive 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. Avoidant behaviors are common among those with anxiety disorders (Salters-Pedneault et al. 2004) and restrictive eating is a core symptom in Anorexia Nervosa and Avoidant/Restrictive Food Intake Disorder (Treasure et al. 2020; Zimmerman and Fisher 2017).

Interactions with new foods are critical because of the potential risk of illness after consumption. A common behavioral reaction to novel foods is *taste neophobia*. In

animals, taste neophobia is defined as lower consumption of a new taste during initial exposures compared to when the taste is familiar and food is considered safe (Lin et al. 2012).

Novelty of the environment also powerfully impacts feeding behavior. Rodents have been shown to have longer latencies to consume food in a novel open field (for review see Ramaker and Dulawa, 2017). However, prior preparations, traditionally, only studied males. Recent work that compared the impact of novel environments on feeding in males and females found sex differences and more pronounced effects in females (Greiner and Petrovich 2020; De Oliveira Sergio et al. 2021). Females had longer latency to approach food in a brightly lit open arena and lower consumption than males (De Oliveira Sergio et al. 2021). Male and female rats that were given a choice of novel and familiar foods in novel or familiar environments, suppressed feeding in a new context in a sex dependent manner (Greiner and Petrovich 2020). In a novel context, males habituated to eating a novel food faster than females, who showed suppressed consumption throughout testing (Greiner and Petrovich 2020). The

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prolonged suppression in females may be relevant to sex differences in avoidant behaviors (Sheynin et al. 2014) and the development of anxiety disorders, Anorexia Nervosa, and Avoidant/Restrictive Food Intake Disorder (Kessler et al. 2012; Treasure et al. 2020; Zimmerman and Fisher 2017). However, there is a significant gap in our knowledge about the neural substrates underlying novelty effects on feeding in males and females.

The current study systematically examined Fos induction in key forebrain regions during the consumption of novel or familiar foods in novel or familiar environments and compared patterns in male and female rats. While the underlying neural circuitry is largely unknown, a specific subset of interconnected cortical, thalamic, striatal, and amygdalar areas are strong candidates for mediating consumption during novelty exposure. The amygdala is essential for emotional learning and memory consolidation, and the basolateral complex nuclei and the central nucleus (CEA) were examined in the current study because they play integral roles in appetitive behavior (reviewed in Cole et al. 2013). The CEA and the basolateral complex nuclei are activated by novel food (Koh et al. 2003; Lin et al. 2012), and bilateral lesions of the CEA in rats eliminated feeding inhibition under fear (Petrovich et al. 2009), while lesions to the basolateral complex nuclei lessened neophobic reactions to novel tastes in familiar environments (Nachman and Ashe 1974; Lin et al. 2009).

Two midline thalamic nuclei that are important for appropriate regulation of avoidance behavior were analyzed, the paraventricular nucleus of the thalamus (PVT) and the nucleus reuniens (RE). The PVT is known for the regulation of food consumption and body weight (Li and Kirouac 2008; Bhatnagar and Dallman 1999), and has been shown to regulate the motivation to eat in a novel environment (Cheng et al. 2018). The RE is necessary for appropriate regulation of avoidance behavior (Linley et al. 2021), and relevant for contextual processing of novel environments, as it is a major link between the medial prefrontal cortex (mPFC) and the hippocampal formation (McKenna and Vertes 2004).

The mPFC is necessary in decision making, particularly in the calculation of risk versus reward (Bechara and Damasio 2005; Ishikawa et al. 2020). It is also critical in regulation of food consumption under cognitive control (learned cues) (Petrovich et al. 2007; Cole et al. 2020) and via opioid stimulation (Mena et al. 2013). This region is also of interest for sex differences in the regulation of feeding behavior in a novel context. The mPFC was engaged differently in males and females during context-induced renewal of responding to food cues (Anderson and Petrovich 2017), and during feeding tests when hunger and fear compete (Reppucci and Petrovich 2018).

The role of the nucleus accumbens (ACB) in appetitive motivation is well known (for reviews see Wise 2000; Kelley 2004; Salamone 1994; Morales and Berridge 2020).

The ACB shell (ACBsh) contains hedonic hotspots that drive the motivation to eat palatable foods (Castro and Berridge 2014; Thompson and Swanson 2010). The agranular insular cortex (AI) processes visceral and taste information (Gogolla 2017). Lesions to the AI blocked conditioned taste aversion behavior (Cubero et al. 1999), while novel taste induced Fos expression in the AI, similar to the patterns in the CEA (Koh et al. 2003).

Here, we determined Fos induction patterns within the above identified areas of interest, in order to outline the neural networks that mediate novelty effects on food consumption and sex differences within these networks. For clarity of neural analysis, rats were given access to only one food during testing, either novel or familiar, and they were tested in either a novel or familiar context. This paradigm design allowed us to separately analyze the effects of sex, context, and food type on consumption and neural activation.

Materials and methods

Subjects

Adult male ($n=32$) and female ($n=32$) Long Evans rats (Charles River Laboratories; Portage, MI), that weighed 225–250 g upon arrival, were individually housed and maintained on a 12-h 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 (mainly from starch), 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. Female estrous cycle was not measured, due to potential stress and confounding effects on feeding behavior.

Apparatus

Half of the animals were tested in a familiar environment (their housing cages; Home Cage) and the other half were tested in a novel environment (behavioral chamber; plexiglass box (30×28×30cm) with grid flooring and a recessed port (3.2×4.2 cm) on one wall; Coulbourn Instruments). Each chamber was enclosed in monolithic rigid foam box. Food was presented in a ceramic bowl.

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 groups in order to test the effects of sex, testing context, and food presented (see Table 1) All groups underwent one 30-min testing session. Prior to testing all rats were food deprived for 20 h, based on the protocol in Greiner and Petrovich 2020. For the test, each rat was presented with a ceramic bowl that contained either 15 g of a familiar food (Rat Chow) or 15 g of a novel food (TestDiet (TD) pellet; 3.4 kcal/g; 21% protein, 13% fat, 67% carbohydrate (all from sucrose); 5TUL 45 mg).

All rats were habituated to transport to the conditioning chamber room, as well as to the ceramic bowls, at least 24 h 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 number of grams consumed per 100 g of body weight.

Histological Procedures

Rats were perfused 90 min 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 0.02 M 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, Billerica, MA; or 1:5,000, 226 003, Synaptic Systems, Gottingen, Germany; the use of each antibody 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.

Table 1 List of experimental groups

		Testing groups	n/group
Familiar context	Familiar food	Females given a familiar food in a familiar context	n = 8
		Males given a familiar food in a familiar context	n = 8
	Novel food	Females given a novel food in a familiar context	n = 8
		Males given a novel food in a familiar context	n = 8
Novel context	Familiar food	Females given a familiar food in a novel context	n = 8
		Males given a familiar food in a novel context	n = 8
	Novel food	Females given a novel food in a novel context	n = 8
		Males given a novel food in a novel context	n = 8

Image acquisition and 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 borders for analysis were determined based on the Swanson rat brain atlas (Swanson 2018). Representative atlas levels and distances from bregma for each analyzed region is documented in the table below (Table 2). Analysis was conducted across the rostro-caudal extent of each subregion of the CEA: capsular (CEAc), lateral (CEAl), and medial (CEAm). Within the basolateral complex, each nucleus was analyzed: anterior basolateral nucleus (BLAa), posterior basolateral nucleus (BLAp), anterior basomedial nucleus (BMAa), posterior basomedial nucleus (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 at 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

Table 2 The rostro-caudal extent of each brain region analyzed

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
AI	AId	10	+2.15
	AIp	22	-0.83

Atlas levels refer to the Swanson rat brain atlas (2018)

each analyzed on a separate representative level. For the AI analysis was conducted on a representative level for the dorsal AI (AId) and posterior AI (AIp). Bilateral images were acquired for all regions, except for the PVT and RE, where both sides were acquired in a single image. 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.

Statistical analysis

Following arrival, males gained weight faster than females, resulting in body weight differences by the time of testing (Table 3). Therefore, all consumption results are reported as grams consumed per 100 g of body weight $([\text{food consumed}(\text{g})/\text{body weight}(\text{g})] \times 100)$.

Consumption results were analyzed using a between-subjects 3-way univariate ANOVA for food type, sex, and testing context. Analysis of subregions and anatomical levels of interest for each region 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 conducted 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 and 26) and posterior (levels 27 and 28) anatomical levels analyzed for each subregion (anterior, aCEAm, aCEAl, and aCEAc; posterior, pCEAm, pCEAl, and 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 ($p = 0.05/3 = 0.017$).

Table 3 Consumption and body weight (mean \pm SD) in grams of each group on the day of the consumption test

			Consumption	Body weight
Familiar context	Familiar food	Females	4.4 \pm 1.3	252 \pm 30
		Males	5.7 \pm 1.2	283 \pm 9.8
	Novel food	Females	3.6 \pm 1.0	232 \pm 4.3
		Males	4.1 \pm 1.9	271 \pm 20
Novel context	Familiar food	Females	2.4 \pm 1.1	249 \pm 14
		Males	3.9 \pm 0.7	285 \pm 20
	Novel food	Females	2.4 \pm 0.7	237 \pm 13
		Males	2.5 \pm 0.9	266 \pm 23

Due to body weight differences, all consumption was analyzed as a percentage of body weight $[(\text{consumption}(\text{g})/\text{body weight}(\text{g})) \times 100]$

Results

Food consumption

Consumption during testing differed based on food type and context familiarity (Fig. 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 $F(1, 52)=0.066$ $p=0.79$).

Fos induction

Central nucleus of the amygdala

Each CEA subregion (medial, lateral, and capsular) had similar increase in Fos induction in rats that were given novel food (Fig. 2) (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, had more Fos positive neurons compared to those tested in a familiar context, and the induction was overall higher for males compared to females (Fig. 2G) (context: $F(1, 52)=8.926$, $p<0.01$; sex: $F(1, 52)=6.449$, $p=0.014$). In the CEA_m, there was no

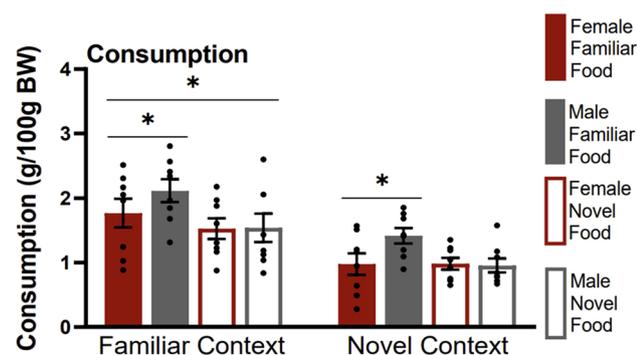


Fig. 1 Food consumption test. The graphs show the amounts of each food that subjects in each testing condition consumed (mean \pm SEM), expressed as grams per 100 g of their body weight (BW). Groups in the familiar context ate more than groups in the novel context. Groups given a familiar food ate more than groups given a novel food. Asterisks indicate significant main effects and are placed above groups with greater consumption ($p<0.05$), asterisk above the longer line represents main effect of context and asterisk above the shorter line represents main effect of food type

effect of context ($F(1, 52)=0.069$, $p=0.79$) and no effect of sex ($F(1, 52)=0.198$, $p=0.66$). In the CEA_l, there was no effect for context ($F(1, 52)=0.287$, $p=0.59$), or for sex ($F(1, 52)=3.6$, $p=0.06$).

Additional analysis examined CEA subregions across rostro-caudal levels. There was greater activation for rats tested in a novel context in CEA_c 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 CEA_l at level 28 ($F(1, 52)=4.87$, $p=0.032$). Of note, the males given a novel food in a familiar context had the greatest number of Fos positive neurons in the CEA_l 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 CEA_l. There were no effects of context for any other parts of the CEA at any rostro-caudal levels ($p>0.05$ for all). 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 CEA_m 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 CEA_l 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 ($p>0.05$ for all).

Basolateral nuclei of the amygdala

Fos induction in the BLA_a and BLA_p was greater for rats tested in a novel context than for rats tested in a familiar context (Fig. 3) (BLA_a: $F(1, 41)=12.534$ $p=0.001$; BLA_p: $F(1, 41)=12.889$ $p=0.001$). There were no effects of food type (BLA_a: $F(1, 41)=0.960$ $p=0.333$; BLA_p: $F(1, 41)=0.076$ $p=0.784$) or sex (BLA_a: $F(1, 41)=1.156$ $p=0.289$; BLA_p: $F(1, 41)=0.38$ $p=0.541$) or any significant interactions in these regions ($p>0.05$ for all).

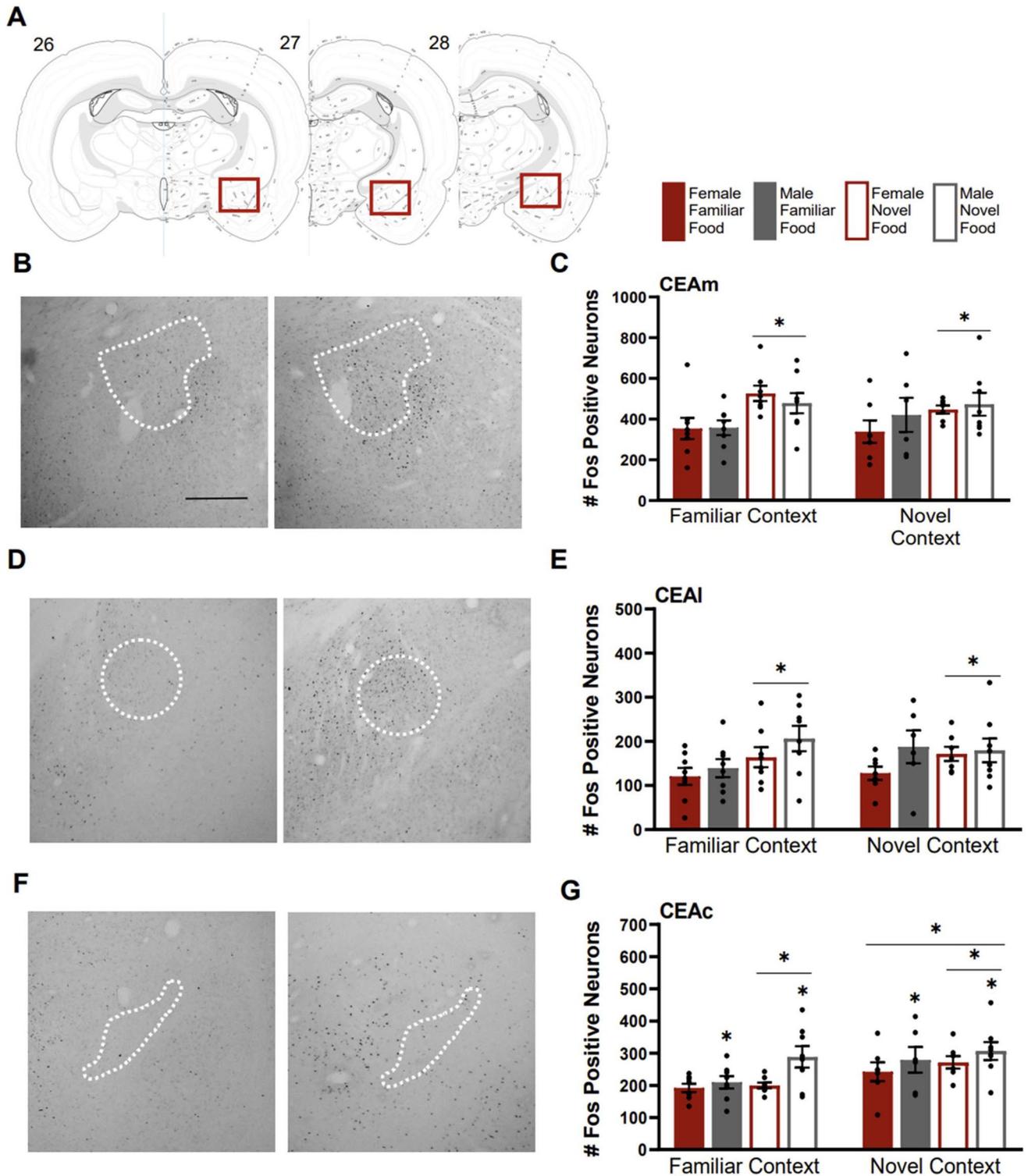
Basomedial nuclei of the amygdala

Fos induction in the BMA_a 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 ($F(1, 41)=12.947$ $p=0.001$) (Fig. 4B, C). There were no effects of sex ($F(1, 41)=0.592$ $p=0.446$) or interactions ($p>0.05$ for all).

The BMA_p had greater Fos induction for rats tested in a novel context (Fig. 4 D-E) ($F(1, 41)=14.813$ $p<0.001$) 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 ($p>0.05$ for all).

Lateral nucleus of the amygdala

The Fos induction in the LA was greater for rats tested in a novel context (Fig. 5) ($F(1, 41)=12.534$ $p=0.001$)



compared to rats tested in a familiar context, but there were no effects of food type ($F(1, 41) = 0.1.108$ $p = 0.299$), sex ($F(1, 41) = 0.242$ $p = 0.625$) or any interactions ($p > 0.05$ for all).

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 (Fig. 6 B-C). Additionally, females given a novel food had

Fig. 2 Previous page: Fos induction in the medial (CEAm), lateral (CEAl), and capsular (CEAc) subregions of the central nucleus of the amygdala. Scale bar in upper left image = 500 μ m. Asterisks indicate significant main effects ($p < 0.05$) and are placed above groups with greater Fos induction, asterisk above the longer line indicates main effect of context, asterisk above the shorter line represents main effect of food type, asterisk alone represents main effect of sex. **A** Atlas templates show anatomical levels analyzed. The red square indicates where tissue images were taken for each region of interest. Templates were adapted from Swanson Atlas (2018). **B** Tissue images stained for Fos of the CEA_m (atlas level 26, right side) for a female tested in a familiar context given a familiar food (left image) and a female tested in a familiar context given a novel food (right image). **C** In the CEA_m, Fos induction (mean \pm SEM) was greater for groups given a novel food than groups given a familiar food. **D** Tissue images stained for Fos of the CEAl (atlas level 28, right side) for a male tested in a familiar context given a familiar food (left image) and a male tested in a familiar context given a novel food (right image). **E** In the CEAl, Fos induction (mean \pm SEM) was greater for groups given a novel food than groups given a familiar food. **F** Tissue images stained for Fos of the CEAc (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). **G** In the CEAc, Fos induction (mean \pm SEM) was greater for groups given a novel food than groups given familiar food, and for groups tested in a novel context than groups tested in a familiar context

greater Fos induction than males given a novel food. There were significant main effects 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 ($F(1, 51) = 5.605$, $p = 0.022$), but no other significant interactions ($p > 0.05$ for all). A Bonferroni post hoc analysis revealed that among novel context tested animals, females had greater Fos induction than males ($p = 0.04$).

The Fos induction in the PVT_p was 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 ($F(1, 51) = 4.006$, $p = 0.051$) (Fig. 6 D-E). There were no main effects of sex ($F(1, 51) = 0.804$, $p = 0.374$), food type ($F(1, 51) = 0.01$, $p = 0.92$) or interactions ($p > 0.05$ for all).

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 (Fig. 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 ($F(1, 50) = 1.213$, $p = 0.28$). In the novel context, females had slightly higher Fos induction than males, and females that were given novel food had higher Fos induction than males given novel food. However, this difference did not reach statistical significance for interactions of context *by* sex ($F(1, 50) = 3.327$, $p = 0.074$) and sex *by* food type ($F(1, 50) = 3.127$, $p = 0.084$). There were no other interaction

effects (context *by* food type $F(1, 50) = 1.475$, $p = 0.23$; context *by* sex *by* food type $F(1, 50) = 0.573$, $p = 0.453$).

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 (Fig. 8B–G) (ACB_c $F(1, 47) = 22.582$, $p < 0.01$; ACB_{dsh} $F(1, 47) = 16.693$, $p < 0.01$; ACB_{vsh} $F(1, 47) = 14.67$, $p < 0.01$). Additionally, Fos induction was greater for females than males in both the ACB_c ($F(1, 47) = 6.829$, $p = 0.012$) and ACB_{vsh} ($F(1, 47) = 6.773$, $p = 0.012$) (Fig. 8B, C and F–G). These sex differences were more obvious in the novel context, however, there was not a significant interaction of sex and context within the ACB_{vsh} ($F(1, 47) = 3.264$, $p = 0.077$), but not ACB_c ($F(1, 47) = 2.386$, $p = 0.128$).

Fos induction in the ACB_{dsh} was similar for both sexes ($F(1, 47) = 0.665$, $p = 0.419$). There were no differences in Fos induction based on food type for any subregion of the ACB (ACB_c $F(1, 47) = 0.441$, $p = 0.51$; ACB_{dsh} $F(1, 47) = 0.125$, $p = 0.725$; ACB_{vsh} $F(1, 47) = 0.562$, $p = 0.457$), and no significant interactions of factors for any ACB subregion ($p > 0.05$ for all).

Medial prefrontal cortex

Fos induction in the PL was greater for rats tested in a novel context compared to a familiar context (Fig. 9 B-C) ($F(1, 49) = 18.539$, $p < 0.001$). In addition, females had slightly higher Fos induction compared to males, however the effect of sex did not reach significance ($F(1, 49) = 3.713$, $p = 0.06$). There were main effects of food type ($F(1, 49) = 2.075$, $p = 0.16$) or any 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 (Fig. 9C, D) ($F(1, 49) = 14.402$, $p < 0.001$). 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 $F(1, 49) = 1.958$, $p = 0.17$; 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 $F(1, 49) = 0.002$, $p = 0.97$).

Agranular insular cortex

Fos induction in the AId was greater for rats tested in a novel context compared to rats tested in a familiar context (Fig. 10B, C) ($F(1, 49) = 4.941$, $p = 0.03$). There were no differences in Fos induction based on sex ($F(1,$

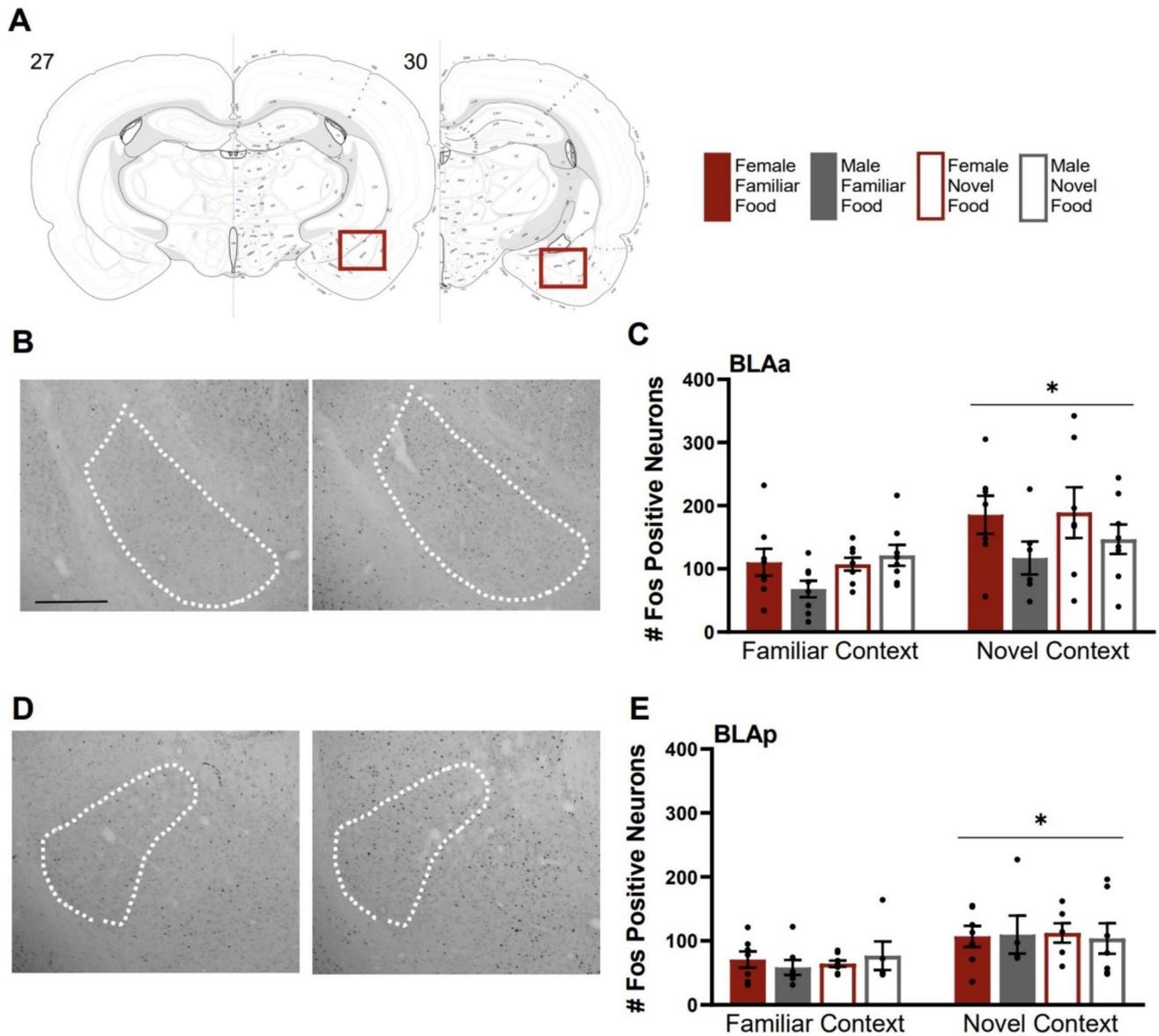


Fig. 3 Fos induction in the anterior (BLAa) and posterior (BLAp) basolateral nuclei of the amygdala. Scale bar in upper left image = 500 μ m. Asterisks indicate significant main effect of context ($p < 0.05$) and are placed above context with greater Fos induction. **A** Atlas templates show anatomical levels analyzed. The red square indicates where tissue images were taken for each region of interest. Templates were adapted from Swanson Atlas (2018). **B** Tissue images stained for Fos of the BLAa (atlas level 27, left side) for a female tested in a familiar context given a familiar food (left image) and a female tested in a familiar context given a novel food (right image). **C** In the BLAa, Fos induction (mean \pm SEM) was greater

for groups tested in a novel context than groups tested in a familiar context. **D** Tissue images stained for Fos of the BLAp (atlas level 30, left side) for a male tested in a familiar context given a familiar food (left image) and a male tested in a novel context given a familiar food (right image). **E** In the BLAp, Fos induction (mean \pm SEM) was greater for groups tested in a novel context than for groups tested in a familiar context. ($F(1, 41) = 12.889$ $p = 0.001$). 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: $F(1, 41) = 0.38$

$49) = 0.214$, $p = 0.65$) or food type ($F(1, 49) = 0.664$, $p = 0.42$) and no significant interactions of factors (context by sex $F(1, 49) = 0.238$, $p = 0.63$; context by food type $F(1, 49) = 0.177$, $p = 0.68$; sex by food type $F(1, 49) = 1.377$, $p = 0.25$; context by sex by food type $F(1, 49) = 0.638$, $p = 0.43$).

Fos induction in the AIP was greater for rats given a novel food compared to rats given a familiar food (Fig. 10C, D) ($F(1, 49) = 6.519$, $p = 0.014$). There were no differences in Fos induction based on sex ($F(1, 49) = 0.472$, $p = 0.50$) or context ($F(1, 49) = 0.676$, $p = 0.42$) and no significant interactions of factors (context by sex $F(1, 49) = 0.577$, $p = 0.45$;

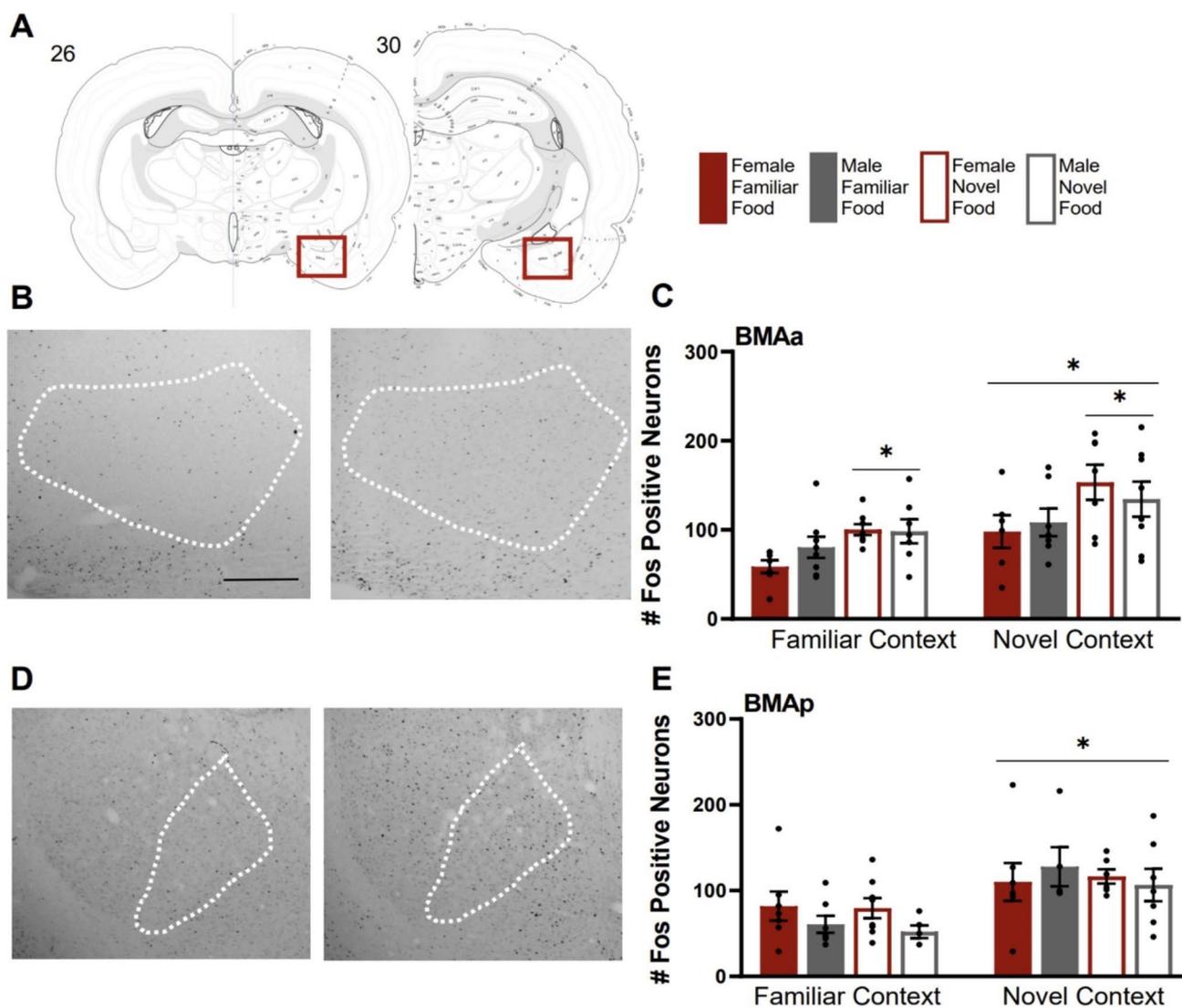


Fig. 4 Fos induction in the anterior (BMAa) and posterior (BMAp) basomedial nuclei of the amygdala. Scale bar in upper left image = 500 μ m. Asterisks indicate significant main effects ($p < 0.05$) and are placed above groups with greater Fos induction, asterisk above the longer line indicates main effect of context and asterisk above the shorter line represents main effect of food type. **A** Atlas templates show anatomical levels analyzed. The red square indicates where tissue images were taken for each region of interest. Templates were adapted from Swanson Atlas (2018). **B** Tissue images stained for Fos of the BMAa (atlas level 26, right side) for a female tested in a novel context given a familiar food (left image) and a female tested

in a novel context given a novel food (right image). **C** In the BMAa, Fos induction (mean \pm SEM) was greater for groups tested in a novel context than for groups tested in a familiar context and greater for groups given a novel food than groups given a familiar food. **D** Tissue images stained for Fos of the BMAp (atlas level 30, left side) for a male tested in a familiar context given a familiar food (left image) and a male tested in a novel context given a familiar food (right image). **E** In the BMAp, Fos induction (mean \pm SEM) was greater for groups tested in a novel context than for groups tested in a familiar context. $p = 0.541$ or any significant interactions in these regions ($p > 0.05$ for all)

context by food type $F(1, 49) = 1.302, p = 0.26$; sex by food type $F(1, 49) = 0.369, p = 0.55$; context by sex by food type $F(1, 49) = 0.67, p = 0.42$.

Correlations of Fos induction between regions

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 (Fig. 11 and Table 4) had significant positive and negative correlations. There were positive correlations between CEA subregions, aCEAI with pCEAm, and pCEAm with pCEAI, as well as between CEA and other regions. The BLAa was positively correlated with pCEAI and pCEAc, the BLAp with the aCEAm and the aCEAc, and the LA with the pCEAc. The RE was negatively correlated

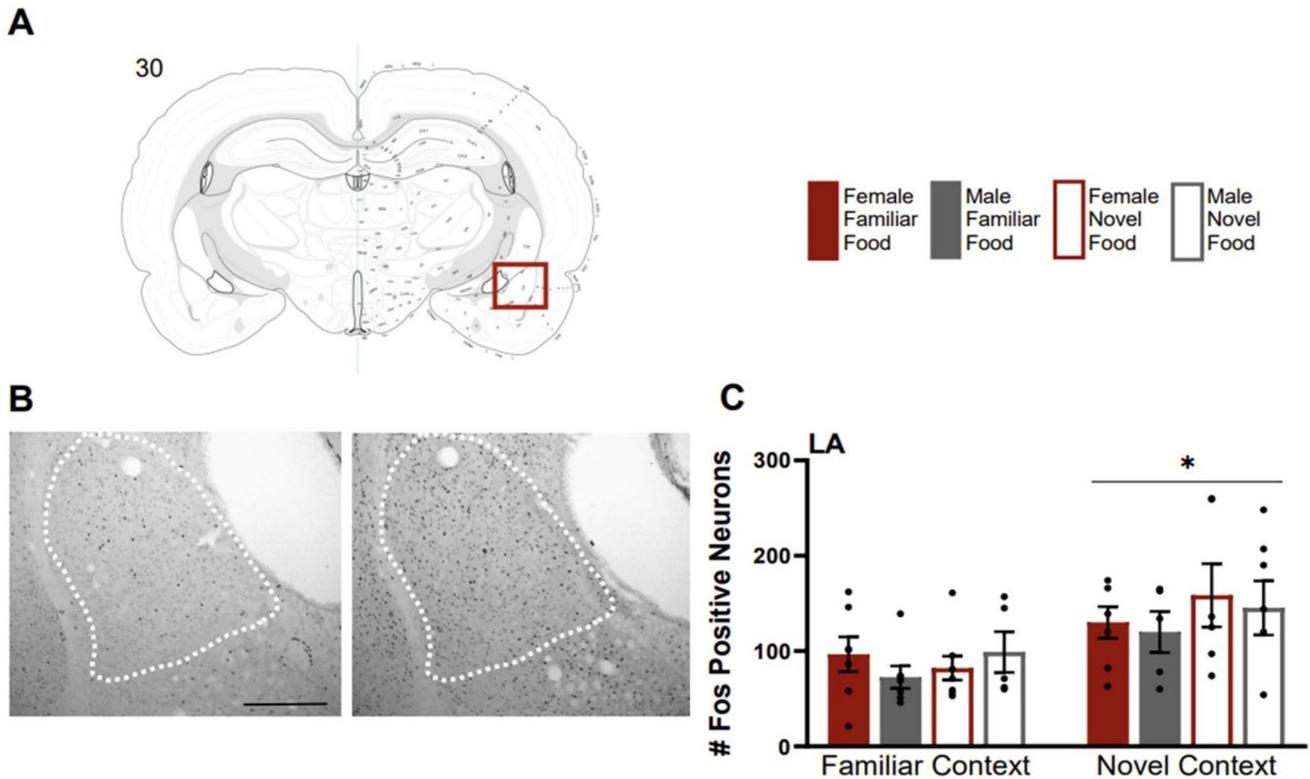


Fig. 5 Fos induction in the lateral nucleus of the amygdala (LA). Scale bar in left image = 500 μm . Asterisks indicate significant main effect of context ($p < 0.05$) and are placed above context with greater Fos induction. **A** Atlas templates show anatomical levels analyzed. The red square indicates where tissue images were taken for each region of interest. Templates were adapted from Swanson Atlas

(2018). **B** Tissue images stained for Fos of the LA (atlas level 30, left side) for a male tested in a familiar context given a familiar food (left image) and a male tested in a novel context given a familiar food (right image). **C** In the LA, Fos induction (mean \pm SEM) was greater for groups tested in a novel context than for groups

with the aCEAl. The ACBvsh was positively correlated with the BMAa. The ILA had two significant correlations with other brain regions, a negative correlation with ACBdsh and a positive correlation with the PL. The AId was positively correlated with the PL and ILA. The AIp was negatively correlated with the ACBdsh and positively correlated with the PL, ILA, and AId.

Males given a familiar food in a familiar context (Fig. 11 and Table 4) 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 PVTp was correlated with the BMAp, the RE with the pCEAm and pCEAc, and ACBdsh with ACBc. The PL was correlated with aCEAm and pCEAc and the ILA with pCEAm, PVTp and RE. The AId was correlated with pCEAl.

Females given a novel food in a familiar context (Fig. 11 and Table 4) had significant positive and negative correlations. There was positive correlation between pCEAl and aCEAm. In the BMA, there were positive correlations between the anterior and posterior parts and between the

BMAp and the pCEAm and BLAp. The RE was positively correlated with the aCEAm. In the ACB, the ACBdsh was negatively correlated with the BLAa and BMAa and the ACBvsh was negatively correlated with the LA. Additionally, the ILA was positively correlated with the RE and PL. The AId was positively correlated with RE and ILA. The AIp was positively correlated with RE, ILA, and AId.

Males given a novel food in a familiar context (Fig. 11 and Table 4) had significant positive and negative correlations. There were positive correlation between pCEAl and pCEAm. There were positive correlations between the anterior and posterior BLA as well as the BMAa with BLAa and BLAp. The LA was positively correlated with and BLAp, BMAp, and BMAp. The PVTp 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 PVTp and PL. The AId was positively correlated with aCEAc.

Females given a familiar food in a novel context (Fig. 12 and Table 5) had significant positive and negative correlations. There were positive correlations between aCEAl and

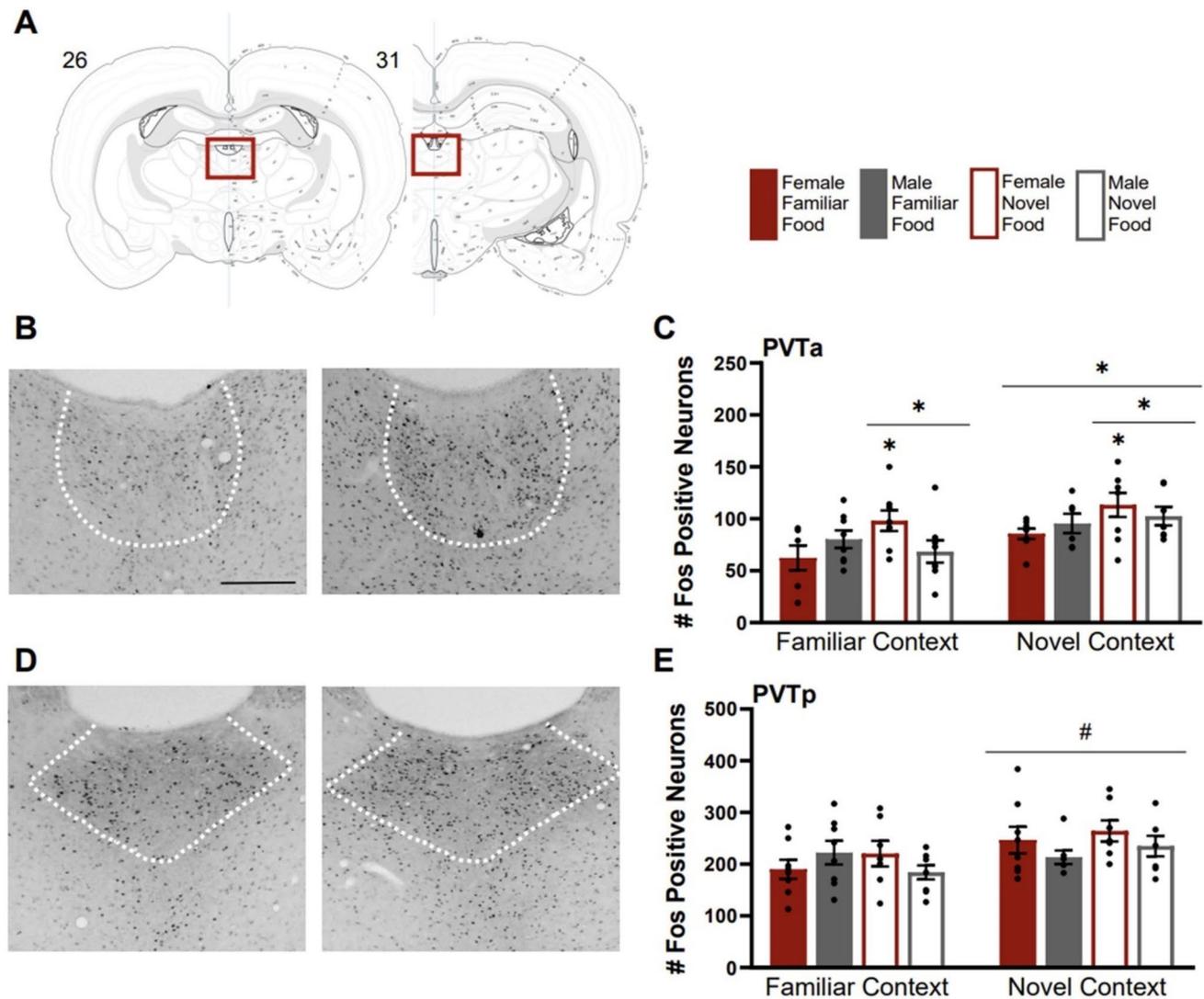


Fig. 6 Fos induction in the anterior (PVTa) and posterior (PVTp) paraventricular nucleus of the thalamus. Scale bar in upper left image = 500 μ m. Asterisks indicate significant main effects ($p < 0.05$) and are placed above groups with greater Fos induction, asterisk above the longer line indicates main effect of context and asterisk above the shorter line represents main effect of food type. Pound symbol indicates $p = 0.051$. **A** Atlas templates show anatomical levels analyzed. The red square indicates where tissue images were taken for each region of interest. Image adapted from Swanson Atlas (2018). **B** Tissue images stained for Fos of the PVTa (atlas level 26, midline) for a male tested in a familiar context given a novel food

(left image) and a female tested in a familiar context given a novel food (right image). **C** In the PVTa, Fos induction (mean \pm SEM) was greater for groups tested in a novel context than for groups tested in a familiar context, greater for groups given a novel food than a familiar food, and greater for females given a novel food than males given a novel food. **D** Tissue images stained for Fos of the PVTp (atlas level 31, midline) for a female tested in a familiar context given a familiar food (left image) and a female tested in a novel context given a familiar food (right image). **E** In the PVTp, higher Fos induction (mean \pm SEM) in the novel context groups did not reach significance ($p = 0.051$)

aCEAm, aCEAc and aCEAm, aCEAc and aCEAI, pCEAI and aCEAc, and pCEAc and pCEAI. The anterior and posterior BMA were positively correlated, and the BMAa was correlated with BLAa and the BMAp with BLAa and BLAp. In the PVT there were negative correlations between PVTa and aCEAm and between PVTp and both aCEAI and aCEAc. The ACBc was positively correlated with BLAa, BLAp, and BMAa and the ACBdsh was positively

correlated with BLAa and ACBc. There were positive correlations between PL and ACBc and between ILA and ACBc, ACBdsh, and PL. The AIa was positively correlated with the ACBc, ACBdsh, PL, and ILA. The AIp was positively correlated with the aCEAm, aCEAc, and RE and negatively correlated with the PVTp.

Males given a familiar food in a novel context (Fig. 12 and Table 5) had only positive correlations. Many CEA

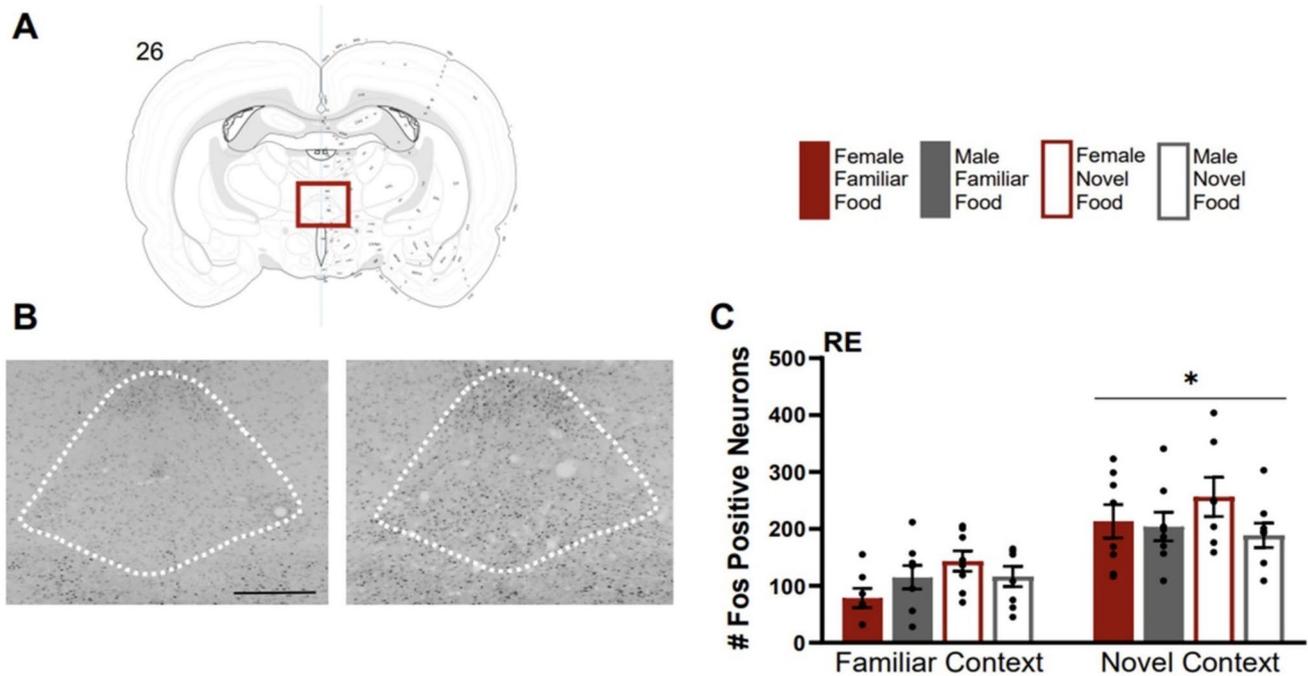


Fig. 7 Fos induction in the nucleus reuniens (RE) of the thalamus. Scale bar in left image = 500 μ m. Asterisks indicate significant main effect of context ($p < 0.05$) and are placed above context with greater Fos induction. **A** Atlas templates show anatomical levels analyzed. The red square indicates where tissue images were taken for each region of interest. Image adapted from Swanson Atlas (2018). **B** Tis-

sue images stained for Fos of the RE (atlas level 26, midline) for a male tested in a familiar context given a familiar food (left image) and a male tested in a familiar context given a novel food (right image). **C** In the RE, Fos induction (mean \pm SEM) was greater for groups tested in a novel context than for groups tested in a familiar context

subregions were correlated, the aCEAl and aCEAm, aCEAc and CEAm, 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 BLAa and BMAa. 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. The AI_d was correlated with aCEAm, aCEAc, pCEAc, PVT_p, PL, and ILA. The AI_p was correlated with the aCEAm, pCEAc, BMA_p, PL, and ILA.

Females given a novel food in a novel context (Fig. 12 and Table 5) had significant positive and negative correlations. There was a negative correlation between anterior and posterior CEAl and CEAl. The anterior and posterior BLA were positively correlated, as well as anterior and posterior BMA. In addition, the BMAa and BMA_p were positively correlated with BLAa and BLA_p. Also, the LA was positively correlated with BLAa. In the PVT, there was a positive correlation between PVT_a and pCEAl, and PVT_p and aCEAl, and a negative correlation between PVT_p and BMAa. The RE was positively correlated with aCEAl and negatively correlated with pCEAl. The ACB_{dsh} was negatively correlated with BLA_p and positively correlated with

PVT_p, while the ACB_{vsh} was positively correlated with the ACB_c. The PL was negatively correlated with aCEAl. Lastly, the AI_p was positively correlated with the LA.

Males given a novel food in a novel context (Fig. 12 and Table 5) 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 BLA_p was correlated with BLAa and BMAa was correlated with BLAa and BLA_p. The LA was correlated with both BLA_p and BMAa. Additionally, there was a correlation between RE and aCEAm. The ACB_{dsh} was correlated with BLAa and with ACB_c and the ACB_{vsh}. The ILA was correlated with aCEAc, ACB_{vsh}, and PL. The AI_d was correlated with the ACB_c. The AI_p was correlated with the aCEAm, aCEAc, pCEAl, and pCEAc.

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 (Greiner and Petrovich 2020), both male and female rats ate less of the novel than familiar food, and the groups tested in a novel context ate much less than those tested in a familiar context. 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.

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 basolateral complex nuclei of the amygdala (BLAa and BLAp, BMAa and BMAP, LA), the central nucleus of the amygdala (CEAc), all subregions of the ACB (core, vsh, dsh), thalamus (PVTa and RE), medial prefrontal cortex (PL and ILA), and the dorsal AI (AId). Robust Fos expression in the basolateral complex nuclei in the novel context condition was expected, given that several of these nuclei are interconnected with the hippocampal formation (HF), including with the entorhinal cortex, which is important for spatial cognition and is a component of the trisynaptic circuit (McDonald 1998), the ventral field CA1 (Cenquizca and Swanson 2007), and the ventral subiculum (SUBv), which has an established role in contextual encoding (Maren, 1999). Of particular interest, the SUBv has been previously found to mediate novel stimulus detection—particularly novel environments (Legault and Wise 2001; Lisman and Grace 2005).

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 and Swanson 2007) and the SUBv (Canteras and Swanson 1992). In addition, contextual information could reach the CEA via multiple relays from the HF (Canteras and Swanson 1992; Cenquizca and Swanson 2007), most notably, via inputs from the medial PFC (Hurley et al. 1991; Messanvi et al. 2023) and BLA.

It is important to note that neuronal activity during feeding in a novel environment may be related to processing of novel contextual information, as well as regulation of behavioral responding. Furthermore, some of the 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

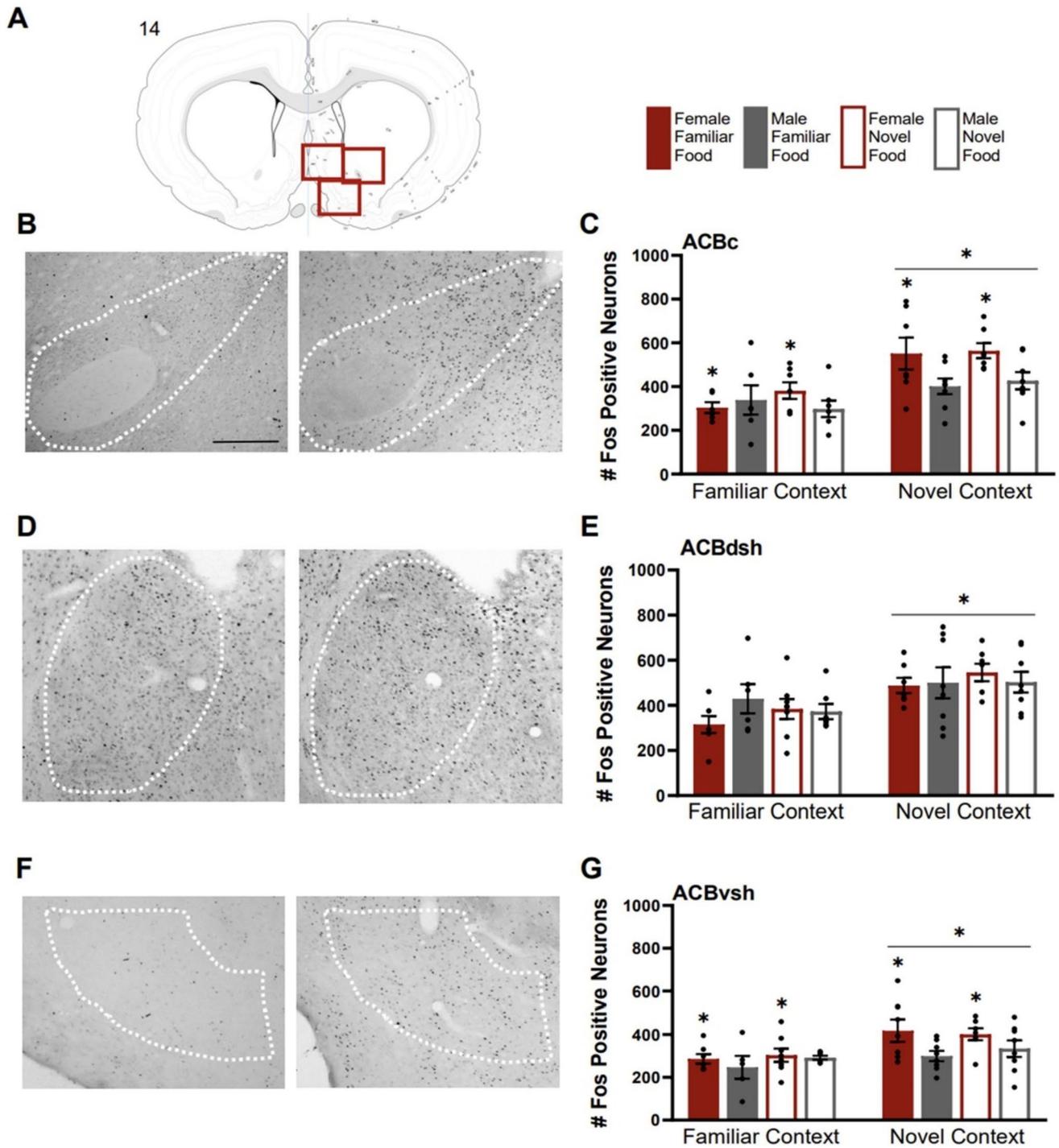
be particularly important for driving appropriate behavioral responding. The BMAa sends substantial projections to the CEA (Petrovich et al. 1996) and the CEA is known to both drive (Douglass et al. 2017) and inhibit (Cai et al. 2014) consumption, which is relevant to current findings since consumption levels varied based on novelty 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 recruited both the BLA (both anterior and posterior) and the CEAc. As the BLA R-spondin 2 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 a novel context was also robust across thalamic, striatal, and cortical areas analyzed. Within the thalamus, the RE and PVTa had higher Fos induction in the novel context condition, and in the PVTp the effect was just above significance. 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 and Vertes 2004). The RE is also critical for the formation and retrieval of distinct contextual memories (Ramanathan et al. 2018) and its inactivation after fear conditioning resulted in a generalized fear-response to novel contexts (Ramanathan et al. 2018).

The PVTa has been shown to control consumption in a novel environment, via the ACB. 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 reduced activity of the PVT to ACB pathway, decreased consumption and food seeking behavior (Ong et al. 2017). It is unclear, however, how this pathway is represented in our findings, where both ACB and PVTa had higher Fos induction in the groups that had suppressed consumption in a novel context. Our methodology was not cell type- 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 a novel context. The ACB mediates motivation for reward and is critical for context-mediated appetitive behavior (Raynolds and Berridge 2008). It receives direct HF input (Groenewegen et al. 1999; Canteras et al. 1992) and inactivation of both the ACB core and shell 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).

The AId had increased Fos induction in groups tested in a novel context. The AId receives direct inputs from the hippocampal field CA1 (Cenquizca and Swanson 2007) and



the basolateral and basomedial nuclei (Kita and Kitai 1990; Petrovich et al. 1996) and sends projections to the basolateral complex nuclei and the central amygdala, particularly strongly to the BLAp and the CEAl (McDonald et al. 1996; Shi and Cassell 1998). Optogenetic inhibition of pathways from the BLA to anterior AI, which includes the AIc and ventral AI, enhanced extinction in a conditioned place preference task (Gil-Lievana et al. 2020), implicating this system

in reward contextual memory. Additionally, neurons in the right anterior AI are sensitive to aversive stimuli, and activation of these neurons suppresses feeding in mice (Wu et al. 2020). Thus, the AIc recruitment in the novel context in the current study may reflect its role in adaptive inhibition of feeding under uncertainty.

Both medial prefrontal cortical regions analyzed, the PL and ILA, had increased Fos induction in the novel context

Fig. 8 Fos induction (mean \pm SEM) in the core (ACBc), dorsal shell (ACBdsh), and ventral shell (ACBvsh) of the nucleus accumbens. Scale bar in upper left image = 500 μ m. Asterisks indicate significant main effects ($p < 0.05$) and are placed above groups with greater Fos induction, asterisk above the line indicates main effect of context and asterisk alone represents main effect of sex. **A** Atlas templates show anatomical levels analyzed. The red square indicates where tissue images were taken for each region of interest. Templates were adapted from Swanson Atlas (2018). **B** Tissue images stained for Fos of the ACBc (atlas level 14, left side) for a male tested in a novel context given a novel food (left image) and a female tested in a novel context given a novel food (right image). **C** In the ACBc, Fos induction (mean \pm SEM) was greater for groups tested in a novel context than for groups tested in a familiar context and greater for females than for males. **D** Tissue images stained for Fos of the ACBdsh (atlas level 14, right side) for a male tested in a familiar context given a novel food (left image) and a male tested in a novel context given a novel food (right image). **E** In the ACBdsh Fos induction (mean \pm SEM) was greater for groups tested in a novel context than for groups tested in a familiar context. **F** Tissue images stained for Fos of the ACBvsh (atlas level 14, right side) for a male tested in a familiar context given a familiar food (left image) and a female tested in a novel context given a familiar food (right image). **G** In the ACBvsh Fos induction (mean \pm SEM) was greater for groups tested in a novel context than for groups tested in a familiar context and greater for females than for males

condition. Both regions receive heavy inputs from the HF (Hoover and Vertes 2007; Cenquizca and Swanson 2007; Messanvi et al. 2023) and can impact the HF via the RE (McKenna and Vertes 2004; Vertes 2002; Hallock et al. 2016). In addition, the mPFC is well positioned to control feeding behavior. One model suggests that local GABAergic inhibition or disinhibition of mPFC glutamatergic projections to the ACBsh and lateral hypothalamus (LHA) controls food consumption (Baldo 2016). In accordance with this model and prior evidence that PFC mu-opioid stimulation drives feeding through activation of the LHA neurons (Mena et al. 2013), some of the Fos induction within the medial PFC in the current study could represent activity of GABA neurons that are shutting down feeding by inhibiting PFC-LHA pathway. Additionally, given the patterns within the ACBsh in the current study, it is possible that PFC mu-opioid activation of LHA was attenuated by ACB AMPA-receptor activation, which could suppress feeding through inhibition of LHA neurons (Mena et al. 2013; Stratford et al. 1998).

Novel food

Novel food, regardless of context, increased Fos induction within the CEA, BMAa, PVTa, and AIp. Novel food induced Fos in fewer regions than novel context. However, the CEA was particularly responsive to food type differences. Novel food recruited all CEA subregions (medial, lateral, and capsular). This matches with previous findings that novel food exposure increases Fos induction in the CEA (Koh et al.

2003). The CEA recruitment may reflect different drives: aversive responding related to novel taste avoidance or appetitive drives and the palatability of the novel food. 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 (Cai et al. 2014; Douglass et al. 2017; McCullough et al. 2018; McCullough et al. 2018).

Stress due to the relative uncertainty of novel food may have recruited populations within the CEAm and CEAl that express corticotropin releasing hormone (CRH; also known as CRF) or somatostatin. Neurons that express CRH are important in stress responding (Bale and Vale 2004) and are largely concentrated within the CEAl (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 and Pitkanen 1998; McCullough et al. 2018). The CEA may be a site where competing drives converge to impact consumption—positive motivation due to food palatability and avoidance due to novelty.

Another consideration is that at least some of CEA recruitment may be due to the palatability of the novel food rather than novelty processing or inhibition of eating. The novel food (TestDiet pellets) used in the current study is high in sucrose, and therefore more palatable than the familiar food (Rat Chow). Palatable foods were previously shown to increase Fos induction in the CEA (Park and Carr 1998; Wu et al. 2014; Parsons et al. 2022) and a subset of CEA neurons that express prepronociceptin (CEA^{Pnoc}) mediate palatable food consumption (Hardaway et al. 2019). These CEA^{Pnoc} neurons are located predominantly in the CEAm and CEAl (Hardaway et al. 2019), and their inhibition reduced the latency to feed in a novel environment as well as consumption in home cage after novelty exposure (Hardaway et al. 2019). In the current study, the CEAm and CEAl had selective Fos induction in response to the novel food.

The only other amygdala region analyzed that was recruited by the novel food was the BMAa. The BMAa heavily innervates the CEA (Petrovich et al. 1996), and the two regions had similar patterns in the current study. Previous work found that damage to this region increased latency to approach food in a novel environment (Lukaszevska et al. 1984). Like the CEA, the BMA regulates 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 suppression of both freezing and anxiety-state behaviors (Adhikari et al. 2015). Therefore, the BMAa recruitment in the current paradigm may be related to an attempt to override

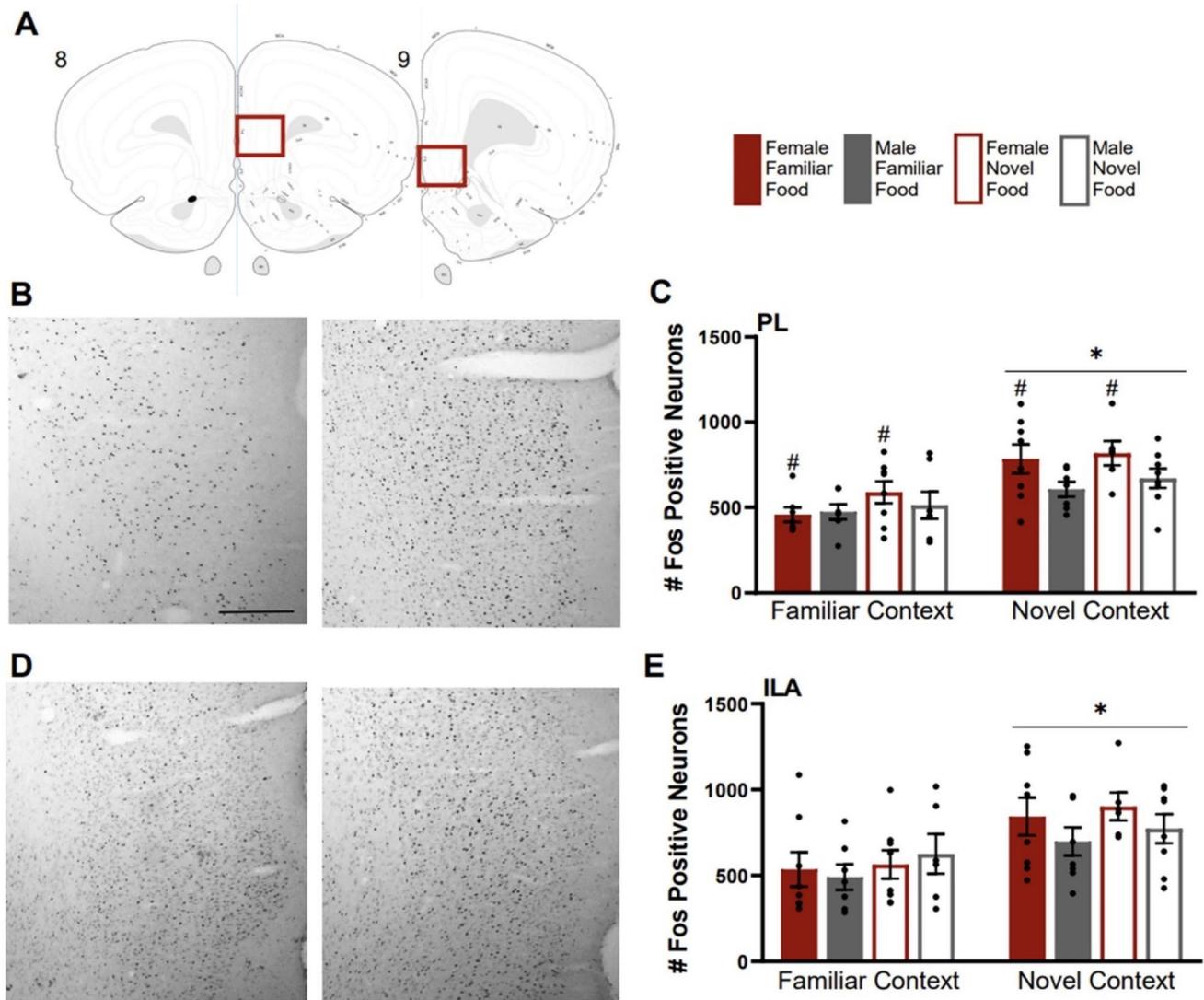


Fig. 9 Fos induction in the prelimbic (PL) and infralimbic (ILA) regions of the medial prefrontal cortex. Scale bar in upper left image = 500 μ m. Asterisks indicate significant main effect of context ($p < 0.05$) and are placed above context with greater Fos induction. Pound symbol indicates $p = 0.06$ for main effect of sex. **A** Atlas templates show anatomical levels analyzed. The red square indicates where tissue images were taken for each region of interest. Image adapted from Swanson Atlas (2018). **B** Tissue images stained for Fos of the PL (atlas level 8, left side) for a female tested in a familiar context given a familiar food (left image) and female tested in a novel

context given a familiar food (right image). **C** In the PL, Fos induction (mean \pm SEM) was greater for groups tested in a novel context than for groups tested in a familiar context. The difference between males and females did not reach significance ($p = 0.06$) OR The difference between males and females was $p = 0.06$. **D** Tissue images stained for Fos of the ILA (atlas level 9, left side) for a male tested in a familiar context given a familiar food (left image) and male tested in a novel context given a familiar food (right image). **E** In the ILA, Fos induction (mean \pm SEM) was greater for groups tested in a novel context than for groups tested in a familiar context

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

The AIP, like the CEAm and CEAl, had selective activation to a novel food only. The AIP projects to the CEA (McDonald 1998; Shi and Cassell 1998), and stimulation of the AIP-CEA pathway induced avoidance behavior and suppressed appetitive responding (Gehrlach et al. 2019). Additional AIP projections to the ACB, were also shown

to inhibit consumption particularly following internal state changes (Gehrlach et al. 2019).

The only other region that had a greater response to novel food was the PVTa. Like the BMAa and the CEAc, the PVTa was recruited by 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

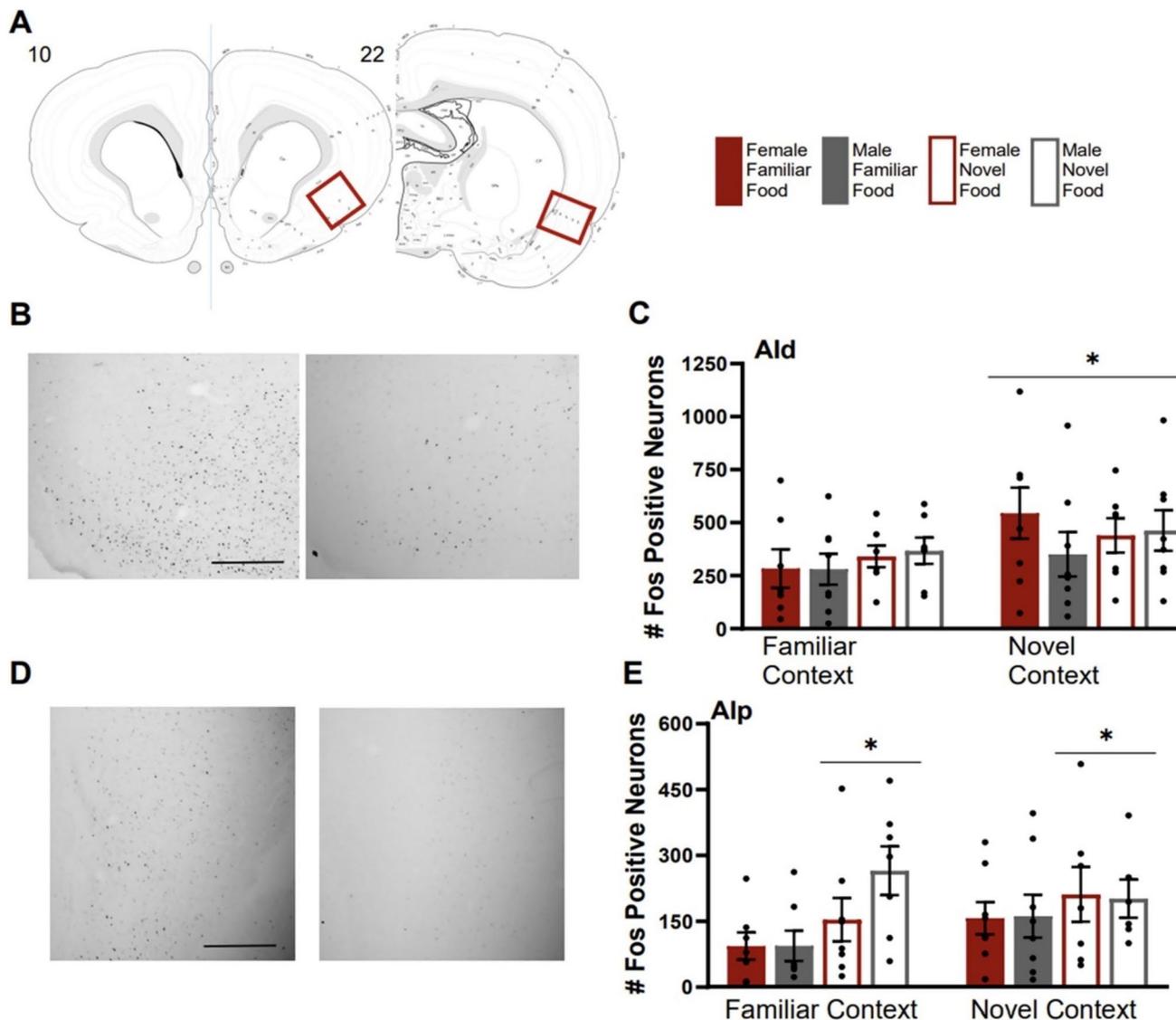


Fig. 10 Fos induction in the dorsal agranular insula (AId) and posterior agranular insula (AIp). Scale bar in upper left image = 500 μ m. Asterisks indicate significant main effects ($p < 0.05$) and are placed above groups with greater Fos induction, asterisk above the longer line indicates main effect of context and asterisk above the shorter line represents main effect of food type. **A** Atlas templates show anatomical levels analyzed. The red square indicates where tissue images were taken for each region of interest. Image adapted from Swanson Atlas (2018). **B** Tissue images stained for Fos of the AId (atlas level 10, left side) for a female tested in a familiar context given a familiar

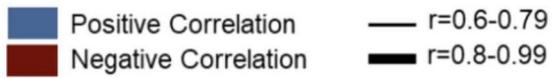
food (left image) and a female tested in a novel context given a familiar food (right image). **C** In the AId, Fos induction (mean \pm SEM) was greater for groups tested in a novel context than for groups tested in a familiar context. **D** Tissue images stained for Fos of the AIp (atlas level 22, right side) for a male tested in a familiar context given a novel food (left image) and a male tested in a familiar context given a familiar food (right image). **E** In the AIp, Fos induction (mean \pm SEM) was greater for groups given a novel food than groups given a familiar food

expression of galanin (*Gal*) (Gao et al. 2020). *Gal*-positive neurons respond to increased arousal states, and their connections to the ILA are implicated in physiological responses 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. There were no group differences within the PVTp due to food type.

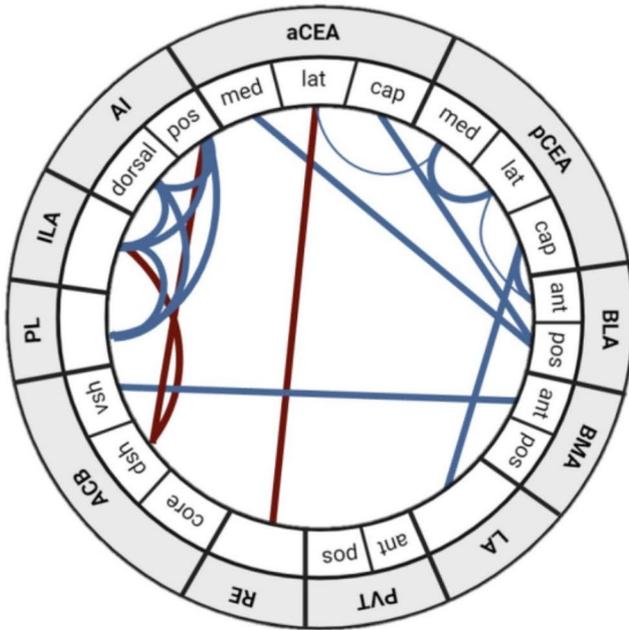
However, the PVTp had higher Fos induction in the novel context condition, which was close to significance.

Sex differences

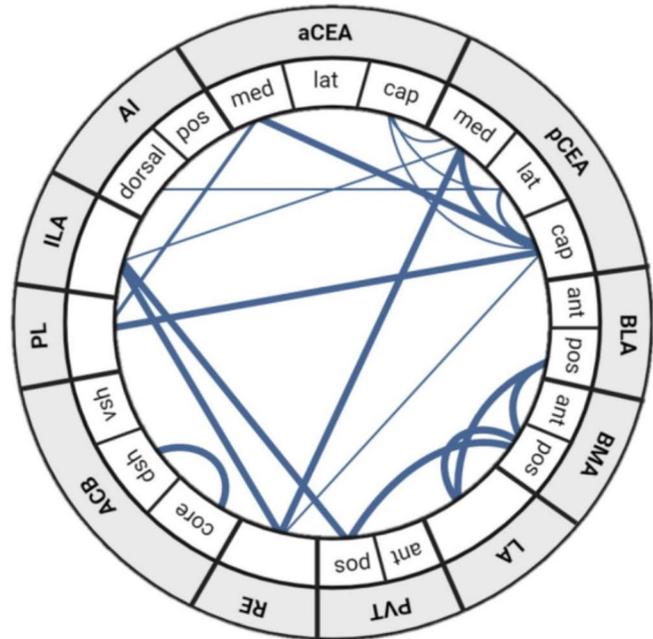
There were sex differences in Fos induction in the PVTa, the core and ventral shell of the ACB, the CEAc, and the posterior part of the CEAl. Additionally, a difference between



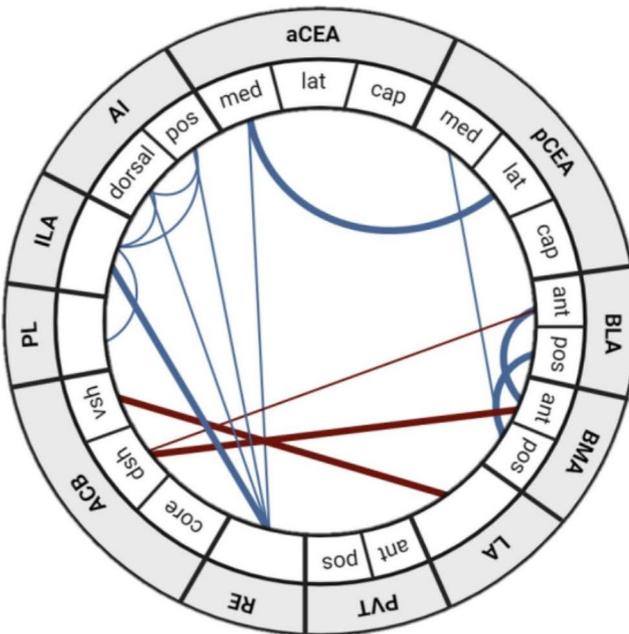
A Familiar Context & Familiar Food Females



B Familiar Context & Familiar Food Males



C Familiar Context & Novel Food Females



D Familiar Context & Novel Food Males

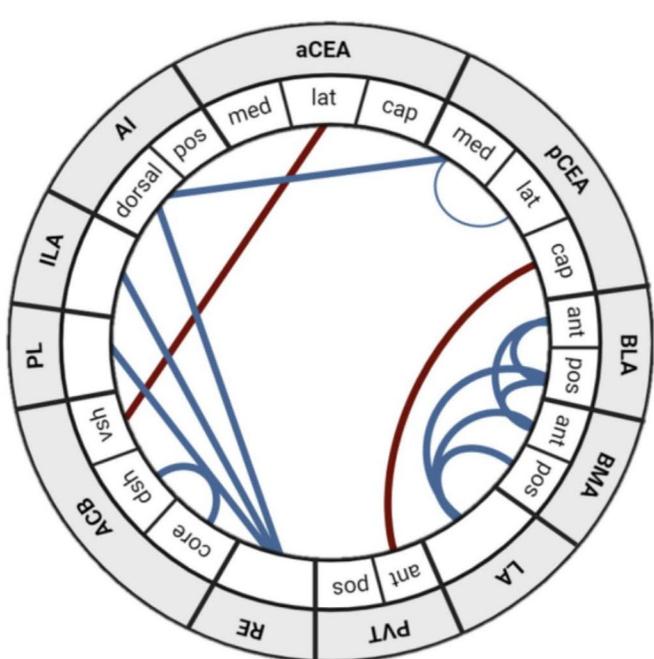


Fig. 11 Diagrams depict pairs of brain regions with significant correlations of Fos induction in familiar context tested groups **A** females given a familiar food in a familiar context, **B** males given a familiar food in a familiar context, **C** females given a novel food in a familiar

context, and **D** males given a novel food in a familiar context. Significant positive correlations are shown with blue connecting lines and significant negative correlations are shown with red connecting lines. The strength of the correlation is indicated by the thickness of the line

Table 4 Significant correlations of Fos induction between regions for groups tested in the familiar context. Correlation results (*r* and *p* values) listed for each pair of regions

Familiar context											
Familiar food						Novel food					
Females			Males			Females			Males		
<i>Regions</i>	<i>r</i>	<i>p</i>	<i>Regions</i>	<i>r</i>	<i>p</i>	<i>Regions</i>	<i>r</i>	<i>p</i>	<i>Regions</i>	<i>r</i>	<i>p</i>
aCEAm-BLAp	0.807	0.028	pCEAm-aCEAc	0.709	0.05	aCEAm-pCEAl	0.826	0.011	pCEAl-pCEAm	0.775	0.024
aCEAl-pCEAm	0.729	0.04	pCEAl-aCEAc	0.791	0.02	aCEAm-RE	0.794	0.019	BLAp-BLAa	0.876	0.022
aCEAl-RE	-0.84	0.038	pCEAc-aCEAm	0.906	0	pCEAm-BMAp	0.761	0.047	BMAa-BLAa	0.869	0.011
aCEAc-BLAp	0.901	0.006	pCEAc-aCEAc	0.781	0.02	BLAa-BMAa	0.818	0.025	BMAa-BLAp	0.934	0.006
pCEAm-pCEAl	0.883	0.004	pCEAc-pCEAm	0.848	0.01	BLAa-ACBdsh	-0.736	0.037	LA-BLAp	0.843	0.035
pCEAl-BLAa	0.742	0.035	pCEAc-pCEAl	0.751	0.03	BLAp-BMAp	0.828	0.021	LA-BMAa	0.942	0.005
pCEAc-BLAa	0.829	0.011	BMAp-BLAp	0.909	0.01	BMAa-ACBdsh	-0.988	0.0001	LA-BMAp	0.823	0.044
pCEAc-LA	0.873	0.01	LA-BLAp	0.827	0.04	LA-ACBvsh	-0.844	0.017	PVTa-pCEAc	-0.9	0.002
BMAa-ACBvsh	0.805	0.029	LA-BMAp	0.912	0.01	RE-ILA	0.816	0.014	ACBdsh-ACBc	0.882	0.009
ACBdsh-ILA	-0.85	0.016	PVTp-BMAp	0.86	0.03	RE-AId	0.718	0.038	ACBvsh-aCEAl	-0.858	0.013
ACBdsh-AIp	-0.81	0.05	RE-pCEAm	0.822	0.01	RE-AIp	0.708	0.049	PL-RE	0.824	0.023
PL-ILA	0.919	0.003	RE-pCEAc	0.761	0.03	PL-ILA	0.742	0.035	ILA-RE	0.952	0.003
PL-AId	0.925	0.008	ACBdsh-ACBc	0.849	0.03	ILA-AId	0.755	0.05	ILA-PL	0.839	0.037
PL-AIp	0.984	0.0001	PL-aCEAm	0.787	0.04	ILA-AIp	0.823	0.012	AId-aCEAc	0.885	0.008
ILA-AId	0.816	0.025	PL-pCEAc	0.813	0.03	AId-AIp	0.779	0.039	AId-RE	0.83	0.021
ILA-AIp	0.921	0.003	ILA-pCEAm	0.786	0.04						
AId-AIp	0.836	0.019	ILA-PVTa	0.853	0.02						
			ILA-PVTp	0.852	0.02						
			AId-pCEAl	0.74	0.04						

sexes in PL activation was close to significance. Sex differences in Fos induction were unexpected given that males and females did not differ behaviorally during the test. This suggests that different neural substrates underlie the same behavior in males and females. It is also possible that the neural activation differences may be predictive of future behavioral sex differences, as males and females differ during habituation to novel contexts (Greiner and Petrovich 2020).

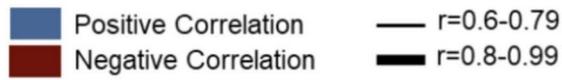
Females had overall greater Fos induction in the ACBc and ACBvsh. Sex-specific role of the ACB in the control of food consumption has been observed before. Projections to the ACB from a sub-population of LHA neurons that produce melanin-concentrating hormone promoted food consumption for males but not females (Terrill et al. 2020).

Within the PVTa, females given a novel food, regardless of context, had greater Fos expression than their male counterparts. There is prior evidence that stress induced activity of the PVTa differs in females. Ovariectomized females without estradiol replacement had higher stress-induced Fos expression in the PVTa compared to those with replacement (Uneyama et al. 2006). There were key differences between that study and the current that do not allow for direct comparison; the stressor used in the prior study was restraint,

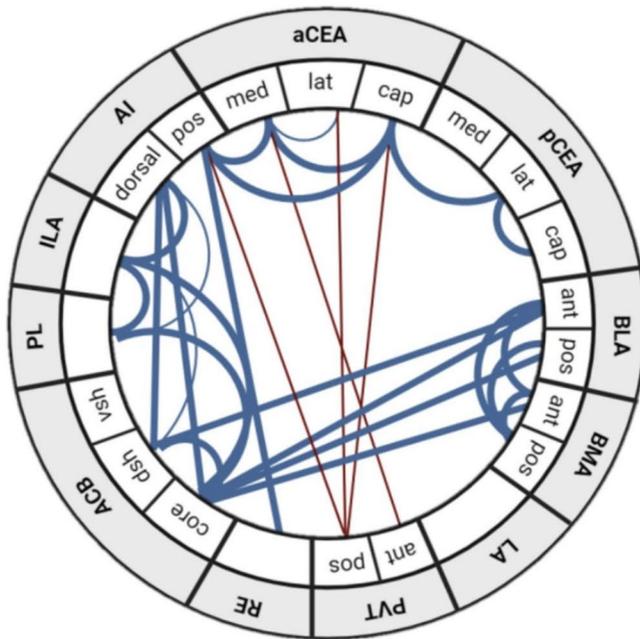
while we used novelty and intact females. Nevertheless, our findings contribute to the evidence of sex-specific responding of PVTa neurons.

In the current study, there was a close to significant sex difference in the PL, where females had higher Fos induction than males. Sex differences in medial PFC recruitment has been identified in two related tasks. Higher Fos induction was found in the PL and ILA during context induced renewal of responding to food cues after extinction, though exclusively in males (Anderson and Petrovich 2017). Another study identified female-specific recruitment of the medial PFC during fear induced hypophagia (Reppucci and Petrovich 2018). However, the differences in behavioral paradigms between these and the current study preclude further comparisons.

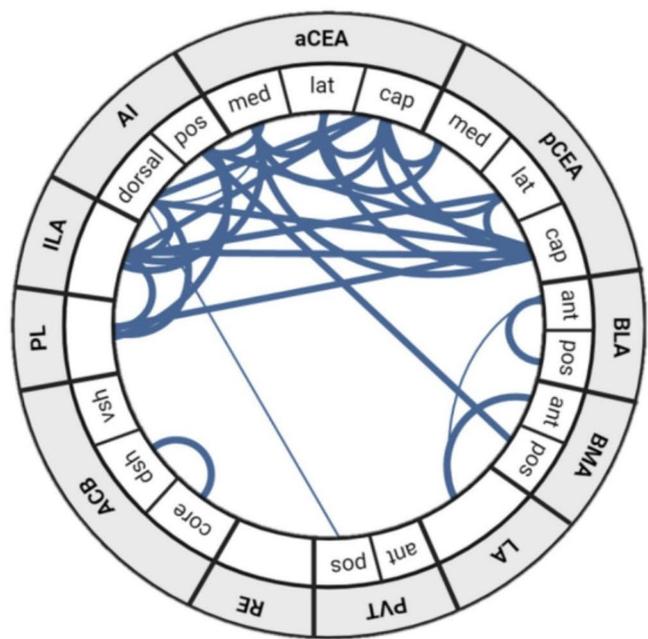
Within the CEA, there were sex differences in two sub-regions. Fos induction in males was overall greater in the CEAc compared to females regardless of food type or testing context, and in the posterior CEAl, the males given a novel food in a familiar context had greater Fos induction than all other groups. These regions receive distinct inputs from the PB, and the PB-CEAl pathway is implicated in visceral information and the PB-CEAc in nociception (Bernard et al. 1993; Bernard and Besson 1990). Therefore, visceral



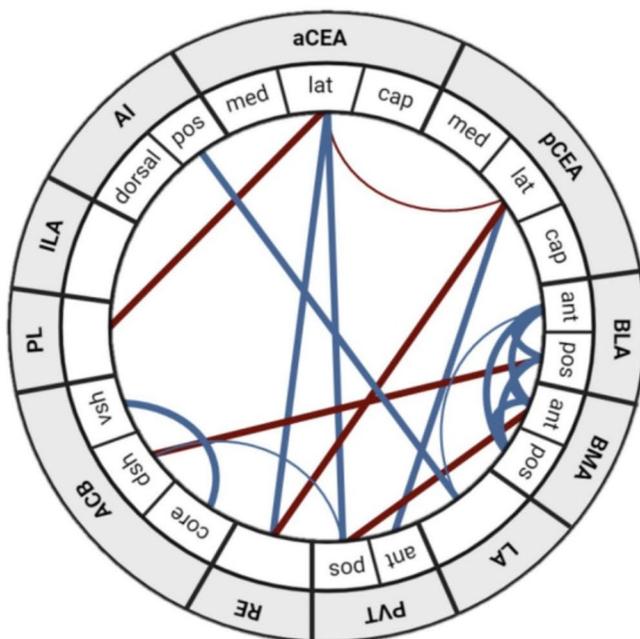
A Novel Context & Familiar Food Females



B Novel Context & Familiar Food Males



C Novel Context & Novel Food Females



D Novel Context & Novel Food Males

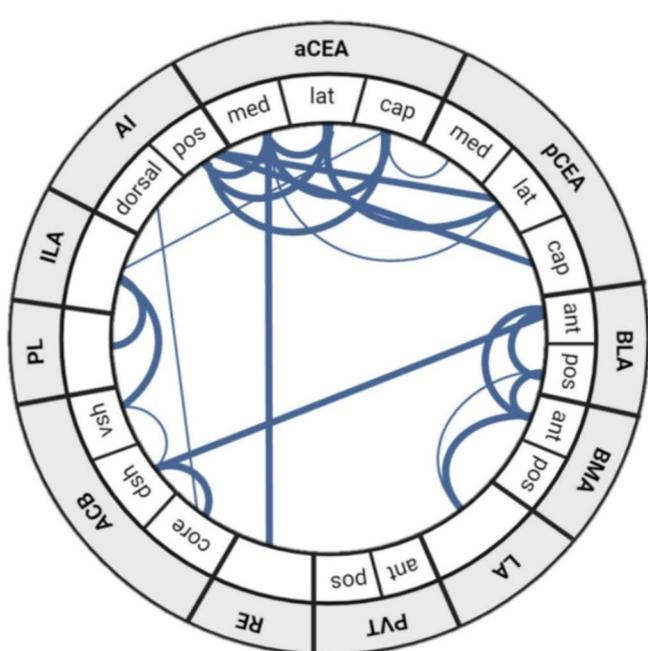


Fig. 12 Diagrams depict pairs of brain regions with significant correlations of Fos induction in novel context tested groups **A** females given a familiar food in a novel context, **B** males given a familiar food in a novel context, **C** females given a novel food in a novel context,

and **D** males given a novel food in a novel context. Significant positive correlations are shown with blue connecting lines and significant negative correlations are shown with red connecting lines. The strength of the correlation is indicated by the thickness of the line

Table 5 Significant correlations of Fos induction between regions for groups tested in the novel context

Novel context											
Familiar food						Novel food					
Females			Males			Females			Males		
<i>Regions</i>	<i>r</i>	<i>p</i>									
aCEAm-aCEAl	0.76	0.047	aCEAl-aCEAm	0.813	0.014	aCEAl-pCEAl	-0.765	0.045	aCEAl-aCEAm	0.837	0.009
aCEAm-aCEAc	0.947	0.001	aCEAc-aCEAm	0.859	0.006	aCEAl-PVTp	0.826	0.022	aCEAc-aCEAm	0.892	0.003
aCEAm-PVTa	-0.763	0.046	aCEAc-aCEAl	0.827	0.011	aCEAl-RE	0.953	0.003	pCEAm-aCEAc	0.783	0.021
aCEAm-AIp	0.833	0.02	pCEAm-aCEAl	0.858	0.029	aCEAl-PL	-0.838	0.037	pCEAl-aCEAm	0.716	0.046
aCEAl-aCEAc	0.827	0.022	pCEAm-aCEAc	0.883	0.02	pCEAl-PVTa	0.823	0.023	pCEAl-aCEAl	0.851	0.007
aCEAl-PVTp	-0.761	0.047	pCEAl-aCEAc	0.828	0.042	pCEAl-RE	-0.903	0.014	BLAp-BLAa	0.899	0.006
aCEAc-pCEAl	0.837	0.019	pCEAc-aCEAm	0.854	0.031	BLAa-BLAlp	0.902	0.005	BMAa-BLAa	0.86	0.013
aCEAc-PVTp	-0.782	0.038	pCEAc-aCEAl	0.812	0.05	BLAa-BMAa	0.963	0.001	BMAa-BLAp	0.878	0.009
aCEAc-AIp	0.858	0.014	pCEAc-aCEAc	0.869	0.024	BLAa-BMAp	0.92	0.003	LA-BLAp	0.766	0.045
pCEAl-pCEAc	0.902	0.005	pCEAc-pCEAl	0.856	0.03	BLAa-LA	0.773	0.042	LA-BMAa	0.879	0.009
BLAa-BMAa	0.926	0.008	BLAp-BLAa	0.832	0.02	BLAp-BMAa	0.872	0.024	RE-aCEAm	0.829	0.021
BLAa-BMAp	0.925	0.024	LA-BLAa	0.777	0.04	BLAp-BMAp	0.883	0.017	ACBdsh-BLAa	0.804	0.029
BLAa-ACBc	0.941	0.005	LA-BMAa	0.935	0.002	BLAp-ACBdsh	-0.831	0.041	ACBdsh-RE	0.864	0.006
BLAa-ACBdsh	0.833	0.04	ACBdsh-ACBc	0.852	0.007	BMAa-BMAp	0.893	0.017	ACBvsh-ACBdsh	0.778	0.023
BLAp-BMAp	0.892	0.042	PL-pCEAc	0.963	0.002	BMAa-PVTp	-0.895	0.016	ILA-aCEAc	0.747	0.033
BLAp-ACBc	0.894	0.018	ILA-aCEAm	0.873	0.005	LA-AIp	0.86	0.028	ILA-ACBvsh	0.831	0.011
BMAa-BMAp	0.938	0.018	ILA-aCEAc	0.824	0.012	PVTp-ACBdsh	0.761	0.047	ILA-PL	0.82	0.013
BMAa-ACBc	0.889	0.018	ILA-pCEAl	0.872	0.024	ACBc-ACBvsh	0.816	0.025	AId-ACBc	0.726	0.042
PVTp-AIp	-0.722	0.043	ILA-pCEAc	0.939	0.005				AIp-aCEAm	0.958	0.003
RE-AIp	0.813	0.014	ILA-PL	0.883	0.008				AIp-aCEAl	0.959	0.003
ACBc-ACBdsh	0.839	0.018	AId-aCEAm	0.74	0.036				AIp-aCEAc	0.961	0.002
ACBc-PL	0.844	0.017	AId-aCEAc	0.829	0.011				AIp-pCEAl	0.895	0.016
ACBc-ILA	0.877	0.009	AId-pCEAc	0.869	0.025				AIp-pCEAc	0.855	0.03
ACBc-AId	0.849	0.016	AId-PVTp	0.754	0.031						
ACBdsh-ILA	0.767	0.044	AId-PL	0.901	0.006						
ACBdsh-AId	0.838	0.019	AId-ILA	0.83	0.011						
PL-ILA	0.806	0.016	AIp-aCEAm	0.814	0.014						
PL-AId	0.739	0.019	AIp-pCEAc	0.934	0.006						
ILA-AId	0.907	0.002	AIp-BMAp	0.817	0.025						
			AIp-PL	0.917	0.004						
			AIp-ILA	0.882	0.004						

Correlation results (r and p values) listed for each pair of regions

sensory processing related to eating during our task, could be the reason why males and females recruited CEAc and CEAl in unique ways.

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 groups. 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 some inputs to the CEA may suppress local connections and activation patterns in distinct subregions (Jolkkonen and Pitkanen 1998).

Groups given a novel food had an additional similarity. Regardless of testing context or sex, these groups had significant positive correlations between BLAa and BMAa. Interestingly, while BMAa was a region with increased Fos induction to a novel food, BLAa was not. Additionally, the two areas are considered to be parts of distinct circuits

within the basolateral complex (Swanson and Petrovich 1998), and the correlation observed is not due to their direct communication, 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, this correlation was not exclusive to males, and was also present in females in the novel context/familiar food condition. Females in the novel/novel condition, instead had a positive correlation between the core and ventral shell. There were no other correlations between ACB subregions in females. These sex differences are interesting because the core and shell have been shown to play opposing roles in a conditioned place preference task with contextual and discrete cues (Ito and Hayen 2011). Given that our paradigm uses both contextual (novel context) and non-contextual (novel food) cues in tandem, it is possible that, coordination between the ACBc and dorsal shell in males but ventral shell in females is necessary in order to drive appropriate appetitive responses.

Another unique pattern in females in the novel/novel condition, was that the PL and ILA were not correlated. The PL and ILA are bidirectionally interconnected (Marek et al. 2018), and activation of PL-ILA pathways enhances fear extinction (Marek et al. 2018). Considering that fear habituation and extinction circuits have been shown to partially overlap, at least in males (Furlong et al. 2016), it is possible that similar neural mechanisms may be recruited during habituation to a potentially dangerous novel stimulus. The lack of correlation between the PL and ILA may indicate enhanced fear/anxiety and poorer habituation to novelty over time in females, as previously observed (Greiner and Petrovich 2020).

Females also had more of the negative correlations, with the greatest number in the novel/novel condition. Most negative correlations for females in the novel/novel condition included the anterior and posterior CEAl, which were negatively correlated with the PL and RE. The PL and RE are interconnected and are involved in a circuit relaying contextual information from the hippocampus (McKenna and Vertes 2004; Vertes 2002), but only PL sends direct pathways to the CEA (Vertes 2004). Negative correlations between CEAl and PL and RE suggest unique contextual processing in the female novel/novel condition.

Both female groups given a familiar food had negative correlations between anterior CEA subregions and one of the midline thalamic nuclei analyzed. The familiar/familiar group had a negative correlation between anterior CEAl and RE, while the familiar food/novel context group had negative correlations between anterior CEAm and PVTa and between both anterior CEAl and anterior CEAc and PVTp. Since there are no direct (monosynaptic) pathways between the RE

and CEA (Vertes et al. 2006; McKenna and Vertes 2004), the activation of one region may be impacting the other via indirect connections. It is also possible that a shared input impacts these regions in opposite ways.

The females in the familiar/familiar condition also had unique negative correlations between the ACBdsh and ILA and between the ACBdsh and AIp. 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 and Carelli 2020). The AIp sends some projections to the ACB shell (Reynolds and Zahm 2005), and a negative correlation between these regions is expected, given that the ACBsh drives motivation to consume foods (Castro et al. 2016) while AIp-ACB stimulation suppresses food consumption (Gehrlach et al. 2019).

Negative ACB shell correlations were present in females in the novel food/familiar context condition as well, though they were exclusively with the amygdala basolateral complex nuclei. Activation of the BLA-ACB pathway is thought to facilitate reward learning (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.

Females in the familiar food/novel context group had negative correlations between the AIp and PVTp. The AIp sends dense projections to the PVTp (Li and Kirouac 2012) and is thought to communicate gustatory and viscerosensory information (Kirouac 2015). The negative relationship between these areas may be indicate suppression of response to physiological hunger for females in a novel context.

Another unique pattern in groups with no novel stimuli, was no overlap in region correlations between females and males in the familiar/familiar groups. That suggests that males and females have distinct processing for food consumption at baseline.

Proposed functional circuitries

Based on the activation patterns found in the current study and known connectivity, we have identified two distinct, functional circuitries within a larger network that may control feeding behavior. The proposed circuitry for control of feeding when consuming a novel food (hereby referred to as the *novel food circuitry*) is shown in Fig. 13. The proposed circuitry for control of feeding in a novel context (hereby referred to as the *novel context circuitry*) is shown in Fig. 14. Within each circuitry we identified regions that were activated differently in males and females.

Each circuitry includes a subset of areas identified that were selectively activated during novel context or novel food exposure and are known to transmit specific information

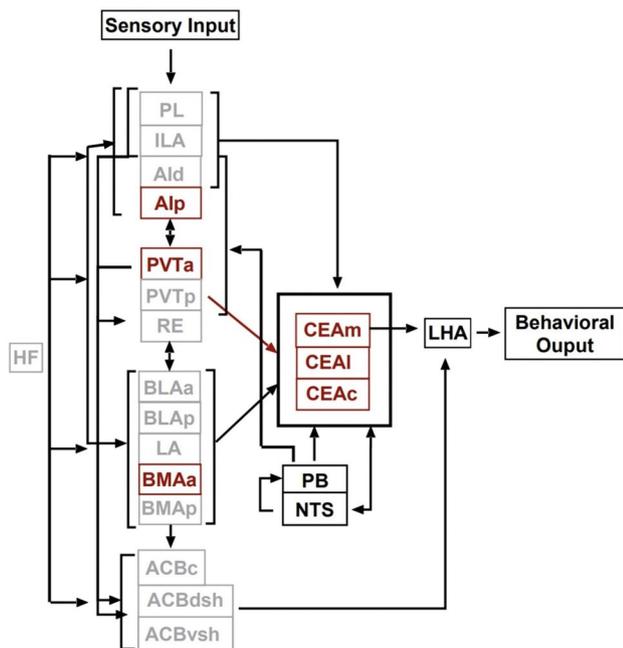


Fig. 13 The diagram shows the proposed circuitry for feeding inhibition in response to a novel food. Areas that were activated in response to a novel food during the consumption test are shown in red. Areas that were not activated are in gray. Included are also regions that were not analyzed in the current study but are anatomically connected within the proposed circuitries (HF, NTS, and PB). For clarity some connections are not shown. Within this circuitry, females had greater activation than males in PVTa and males had greater activation than females in CEAc. Hippocampal formation, *HF*; prefrontal cortex, *PL*; infralimbic cortex, *ILA*; dorsal agranular insular cortex, *Aid*; posterior agranular insular cortex, *Alp*; anterior paraventricular thalamus, *PVTa*; posterior paraventricular thalamus, *PVTp*; nucleus reuniens of the thalamus, *RE*; anterior basolateral amygdala, *BLAa*; posterior basolateral amygdala, *BLAp*; lateral amygdala, *LA*; anterior basomedial amygdala, *BMAa*; posterior basomedial amygdala, *BMAp*; nucleus accumbens core, *ACBc*; nucleus accumbens dorsal shell, *ACBdsh*; nucleus accumbens ventral shell, *ACBvsh*; medial central amygdala, *CEAm*; lateral central amygdala, *CEAI*; capsular central amygdala, *CEAc*; parabrachial nucleus, *PB*; nucleus of the solitary tract, *NTS*; lateral hypothalamus, *LHA*

about context and feeding. We additionally included regions that were not analyzed in the current study but are anatomically connected within the proposed circuitries: the HF, which is critical for contextual processing and encoding, the nucleus of the solitary tract (NTS) and parabrachial nucleus (PB), which are necessary for transmitting gustatory, taste, and visceral sensory information, and the lateral hypothalamus (LHA) which is considered a motivation-cognition interface in the control of feeding behavior (for review see Petrovich 2018).

These two distinct circuitries have key regions of overlap—the PVT, CEA, and BMAa—and we postulate that when both circuitries are active, they have a cumulative impact on the inhibition of feeding. Patterns of activity

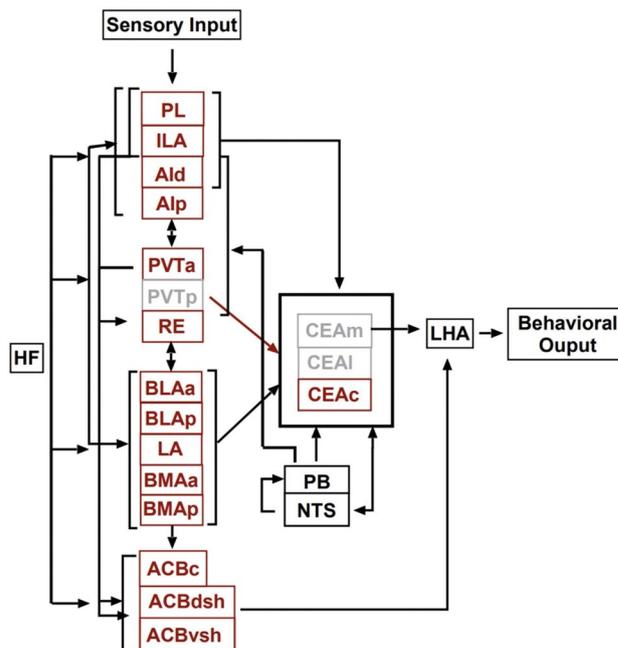


Fig. 14 The diagram shows the proposed circuitry for feeding inhibition in a novel context. Areas that were activated in a novel context are shown in red. Areas that were not activated are in gray. Included are also regions that were not analyzed in the current study but are anatomically connected within the proposed circuitries (HF, NTS, and PB). For clarity some connections are not shown. Within this circuitry, females had greater activation than males in ACBc and ACBvsh and males had greater activation than females in CEAc. Hippocampal formation, *HF*; prefrontal cortex, *PL*; infralimbic cortex, *ILA*; dorsal agranular insular cortex, *Aid*; posterior agranular insular cortex, *Alp*; anterior paraventricular thalamus, *PVTa*; posterior paraventricular thalamus, *PVTp*; nucleus reuniens of the thalamus, *RE*; anterior basolateral amygdala, *BLAa*; posterior basolateral amygdala, *BLAp*; lateral amygdala, *LA*; anterior basomedial amygdala, *BMAa*; posterior basomedial amygdala, *BMAp*; nucleus accumbens core, *ACBc*; nucleus accumbens dorsal shell, *ACBdsh*; nucleus accumbens ventral shell, *ACBvsh*; medial central amygdala, *CEAm*; lateral central amygdala, *CEAI*; capsular central amygdala, *CEAc*; parabrachial nucleus, *PB*; nucleus of the solitary tract, *NTS*; lateral hypothalamus, *LHA*

within the PVT, CEA, and BMAa suggest that they are drivers of eating control, regardless of whether feeding inhibition is due to novel taste or novel context. We speculate that the *novel food circuitry* is a subset of the *novel context circuitry*, and that the main difference is heavy mediation by cortical inputs in the *novel context circuit*. The CEA is a place of convergence of competing drives: appetitive and hedonic information that would increase feeding, and stress, anxiety and arousal information that would suppress feeding (Petrovich 2018). The proposed model suggests that in the *novel food circuitry*, the CEA drives feeding inhibition, whereas in the *novel context circuit* both CEA and ACB drive feeding inhibition. Therefore, when animals consume a novel food in a novel context, both circuitries would be

engaged, and both the ACB and CEA would mediate feeding inhibition.

Implications

The current study identified distinct circuits that underlie food and context novelty processing during consumption. The identified neural circuitries mediate the control of competing motivations and behaviors: appetitive drive to feed and acquire reward versus avoidance of uncertainty. Additionally, we found sex-specific activation patterns that may be predictive of enhanced hypophagia in females during habituation to eating in an uncertain, novel environment. These findings have important implications for future functional studies and our better understanding of neural and behavioral mechanisms underlying maladaptive eating behaviors and psychopathology in each sex.

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Authors contribution GDP conceptualized the study and supervised the experiment and directed the data analysis. EMG and GDP designed the experiments. EMG carried out the experiment. EMG, SJM, and MEW conducted histological preparation and analysis. EMG, SJM, and MEW prepared figures. EMG and GDP wrote the manuscript. All authors read and approved the final manuscript.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval 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.

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