Extinction of fear-cue induced inhibition of eating in male and female rats: Activation of brainstem nuclei

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Introduction

Motivated behaviors—ingestive, defensive, reproductive, and foraging behaviors—are essential for the survival of the individual and the species (Swanson, 2004). Such behaviors are controlled by both internal physiological signals as well as external environmental signals. In certain instances, environmental controls can override physiologic or homeostatic cues to either stimulate or inhibit a given behavior; specifically, studies of feeding behavior demonstrate that environmental factors can induce food intake when sated and inhibit food intake when food-deprived (Weingarten, 1983; Stroebele and De Castro, 2004; Popkin et al., 2005; Petrovich and Gallagher, 2007; Petrovich and Lougee, 2011).

Previously Petrovich and colleagues used Pavlovian fear conditioning methods (Maren, 2004) to show that food-deprived male and female rats will inhibit food consumption when presented with a discrete conditioned stimulus that signals danger, such as a tone previously paired with footshocks (Petrovich and Lougee, 2011; Petrovich et al 2009). This paradigm for fear cue-induced inhibition of feeding was based on well-established fear conditioning protocols (Davis, 1992; Fendt and Fanselow, 1999; LeDoux, 2000; Maren, 2001). Aversive conditioning was used to modulate feeding behavior by presenting tone(Conditioned stimulus, CS)-footshock(Unconditioned stimulus, US) pairings

during training, then testing consumption during tone-only presentations to food-deprived rats (Petrovich and Lougee, 2011). The fear-cue can effectively override physiological signals that would typically result in robust feeding. Also, it has previously been shown that this short-term fear-cue induced anorexia persists longer in females than it does in males (Petrovich and Lougee, 2011).

Using this established fear cue-induced inhibition of eating paradigm, the present investigation examined activation of brainstem nuclei. The brainstem has previously been implicated in integrating oral and gastrointestinal signals to issue motor circuit control of meal size and brainstem neurons have been shown to play a role in controlling energy expenditure in relation to food deprivation (Grill, 2006). Our focus was specifically on the dorsal motor nucleus of the vagus nerve (DMX) and the nucleus of the solitary tract, both of which are implicated in behavioral responses to taste, as well as regulating hunger and satiety cues (Grill, 2006; Johnstone et al, 2006).

Additionally, we studied the sex differences in the behavioral responses and the brain activation patterns during extinction of fear-cue induced inhibition of eating, as sex differences have previously been found in the control of food intake, and have also been observed with eating and anxiety disorders (Geary and Lovejoy, 2008; Striegel-Moore et al, 2009). Studying female rats, in addition to male rats, is a significant step towards understanding the neurobiological underpinnings of these disorders, especially given that they disproportionately affect women yet are still underrepresented in the literature (McCarthy, et al., 2012; Zucker and Beery, 2010).

In the current study, we trained male and female rats in alternating appetitive (where rats consumed food pellets) and aversive sessions (where half of the rats received tone-shock pairings). Food-deprived rats were then tested for fear-cue induced inhibition of eating in the presence of the tone. We also explored the activation patterns of brainstem nuclei during the third test, in order to examine the role of the brainstem during the extinction of fear-cue induced inhibition of feeding.

Materials and Methods

Subjects. Thirty-two experimentally naïve, male and female Long-Evans rats (Charles River Laboratories; Portage, MI) were used in the experiment. Rats were two months old when they arrived to the colony and the body weight range was 225-250g. Rats were individually caged and maintained on a 12 h light/dark cycle and given *ad libitum* access to standard laboratory chow (LabDiet 5P00, Prolab, RMH 3000) and water, except as otherwise noted. Female and male rats were housed in separate housing rooms. Rats were acclimated to the colony rooms for one week and handled daily prior to any behavioral training. Body weights and vaginal smears were obtained every weekday. Due to a fire alarm disruption during testing, 14 of the rats were removed from the study. All housing and testing procedures were in compliance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals, and approved by the Boston College Animal Care and Use Committee.

Apparatus. Training and testing were conducted in a set of eight identical behavioral chambers (30 x 28 x 30 cm; Coulbourn Instruments, Allentown, PA) located in a behavioral room that was different from the colony housing rooms. Each chamber had aluminum tops and sides, a transparent Plexiglas back and front, a grid floor, and was enclosed in an isolation cubicle (79 x 53 x 53 cm; Coulbourn Instruments, Allentown, PA) composed of monolithic rigid foam walls, which isolate the chamber from ambient sound and light. A ventilation fan, located on the back of each isolation cubicle, provided masking noise (55 dB). Each chamber had a "house light" (4W). Video cameras controlled by Digital Video Security System Digital Video Recorder software program (Coulbourn Instruments, Allentown, PA) were mounted on the back wall of each isolation cubicle to record behavior during training and testing. Each chamber contained a recessed food cup (3.2 x 4.2 cm).

Behavioral training procedure. The behavioral training protocol consisted of 9 sessions conducted on separate days: 6 appetitive sessions (S1, S2, S4, S6, S8, S9) and 3 aversive sessions (S3, S5, S7). The appetitive and aversive training sessions took place in two distinct contexts (Context A and B, respectively). Context A was used for appetitive sessions and consisted of Plexiglas flooring, a house light, closed chamber doors and the odor of 1% acetic acid. Context B was used for aversive sessions and consisted of grid flooring, open chamber doors, no house light, a tee-pee shaped Plexiglas insert, and the odor of 5% ammonium hydroxide. For each session, rats were placed on a cart and transported from the colony room to the behavioral training room. Water was

available *ad libitum* in the home cages throughout training. Prior to the start of the behavioral procedure, rats were familiarized with food pellets (formula 5TUL, 45 mg, TestDiet, Richmond, IN) by being given ~1g food pellets in their home cages.

Prior to each appetitive session rats were food deprived for 22 hours. During appetitive sessions, rats were placed inside the behavioral chamber, equipped with 7g of food pellets in the food cup. Rats were allowed to consume food for 10 minute in the chambers. After 10 minutes, rats were removed from the chambers, placed in their home cages, and transported from the training room to the colony room. The food remaining in the food cup was weighed and the amount consumed was calculated. Rats were given *ad libitum* access to lab chow for at least 24 hours between consecutive food-deprivations (between S1 and S2, and S8 and S9), and before the start of the aversive sessions.

Prior to each aversive session rats were allowed *ad libitum* access to lab chow and water for at least 24 hours. During the first 10-minute long aversive session, rats were placed in the aversive context (Context B) but no shocks were administered. During the other two 10-minute long aversive sessions (S5 and S7), half of the male and female rats received tone-shock pairings (conditioned groups; n = 5 females, and n = 4 males) while the other half of the rats received tone-no shock presentations (control groups; n = 4 females and n= 5 males). The conditioned groups received 2 tone (75 dB; 2 kHz, 60s) presentations (variable inter-trial interval, 4 minutes ± 50%), each immediately followed by an electric footshock (1 mA, 1s each; Precision Adjustable Shocker, Coulbourn

Instruments, Allentown, PA) while the control groups received 2 tone presentations and no shocks. Following each aversive session, rats were removed from the chambers, placed in their home cages on a cart and transported from the training room to the colony rooms.

Food consumption tests. Rats were food deprived for 22 hours prior to each of the 3 tests and were allowed *ad libitum* access to water during this time. For each test, rats were placed on a cart in their home cages and transported from the colony room to the testing room. Testing occurred in Context A. This context had previously been used for consumption training during appetitive sessions, thus allowing for us to isolate the effects of the tone from the confounding effects of the aversive training context. Rats were placed in the behavioral chamber with 7 g of food pellets in the food cup for 10 minutes. During the tests, the tone CS (75 dB, 2 kHz, 60s) was presented four times (at minute 1, 3, 5, and 7). No footshocks were administered during the test. Following the test, rats were taken out of the behavioral chambers, placed into their home cages and transported on a cart back to the colony rooms. The food remaining in the food cup was weight and the amount consumed was calculated.

Vaginal smears. After one week of acclimation and daily handling, female rats were examined daily (excluding weekends) by a vaginal lavage procedure to determine the estrous cycle stage. The vaginal smears were obtained and placed on glass slides, and samples were observed under a microscope to identify the estrous cycle stage. The procedure was applied to ensure that the female rats

were showing normal cycling. However, due to a small sample size, the estrous cycle stage was not used as a variable in the analyses.

Behavioral observations. Videos recorded during the testing sessions were watched to observe and record the freezing behavior of the rats. A species-specific defensive behavior, freezing is characterized by the absence of all movement, with the exception of breathing motions (Bolles 1970). An observer who was "blind" with respect to the training group and sex of the rats during behavioral assessments determined freezing behavior. The observer recorded the freezing behavior of the rat during the entire duration of each CS (60s). The sum of all observations (a total of 4 minutes during the 10 minute test) represented the "total time" in the text, and the percentage of this time rats spent exhibiting freezing behavior during that period was calculated.

Histological procedure. To observe patterns of brain activation during fear-cue induced inhibition of eating rats were perfused at the end of the experiment. The perfusion procedure began 90 minutes after the start of the third and final test session. Rats were briefly anesthetized with isoflurane, and then deeply anesthetized with an intraperitoneal injection of tribromoethanol. Rats were then perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde in 0.1M borate buffer. The brains were removed and post-fixed in the fixative solution with 12% sucrose for 24 hours. The brains were then rapidly frozen in hexanes cooled in dry ice, and stored at -80°C. The brains were sliced on a sliding microtome into four series.

Immunohistochemistry. Two tissue series were collected into trays containing cryoprotectant solution and stored at -20°C for later immunohistochemical processing. The other two tissue series were collected into a 0.02M potassium phosphate buffered saline (KPBS) solution; one of these series was stained with thionin for identification of nuclear borders and rat brain atlas levels (Simmons & Swanson, 1993; Swanson, 2004), and the other was stained for visualization of Fos. Brain perfusions, collection, slicing, and storage were counterbalanced across the experimental conditions (experimental, control). Additionally, tissue processing was conducted in pairs (experimental, control) in the same staining tray to balance for possible minute solution differences.

Processing for c-Fos. One tissue set underwent immunohistochemical processing for c-*Fos*, an immediate early gene that is considered an indirect mark of recent neuronal activity. The tissue first underwent 1 hour incubation at room temperature in a blocking solution containing 0.02M potassium buffered saline (KPBS), 0.3% Triton X-100 (Sigma Aldrich, St. Louis, MO), 2% normal goat serum (S-1000; Vector), and 10% non-fat milk to reduce nonspecific binding before being treated with primary antibodies. The tissue was then incubated for 72 hours in the blocking solution described above and the primary antibody: c-Fos antibody raised in rabbit (1:2000; SC-52; Santa Cruz Biotechnology Inc., Santa Cruz, CA). Following rinses in KPBS, the tissue was then incubated for 45 minutes in a KPBS solution containing 0.3% Triton X-100 (Sigma-Aldrich, St. Louis, MO), 2% normal goat serum (S-1000, Vector), and the biotinylated secondary antibody against rabbit (1:500; BA-1000, Vector). The tissue was then subsequently

treated with Avidin-Biotin complex (ABC kit, PK6100; Vector) for 45 minutes. This was followed by a second 30-minute incubation in the secondary antibody solution, and a second 30-minute incubation in the ABC solution to improve specific binding. To produce a color reaction, the dish was then manually agitated in a diaminobenzidine solution (DAB kit, SK-4100; Vector) for two minutes.

Image acquisition and analysis. After the processed tissue was mounted onto slides and coverslipped, it was examined under a microscope (Olympus BX51), which was attached to a digital camera (Olympus DP72) and images were taken using the associated DP2-BSW software which was run by a PC computer. Images of the tissue were taken at two different anatomical levels of the brainstem in order to sample from both a rostral (atlas level 66/67) and a caudal (atlas level 71) area of the chosen structures (Swanson, 2004). At both levels, images of the nucleus of the solitary tract (NTS) and the dorsal motor nucleus of the vagus nerve (DMX) were taken and analyzed bilaterally.

Counting Fos-positive neurons. Using the ImageJ program, anatomical borders for the NTS and DMX were drawn onto the Nissl-stained image, and then transposed to the adjacent DAB-stained tissue to allow for an automated counting of Fos-positive neurons within the chosen structures: the NTS and DMX, at both anatomical levels.

Statistical analysis. Data were analyzed using analysis of variances (ANOVAs), and independent samples *t*-tests within SPSS. In all cases, $p \leq 0.05$ was considered significant.

Results

Training. During appetitive training, we measured the amount of food pellets that rats ate during each session and found that all rats consumed considerable amounts during training.

Overall, male rats (experimental and control groups) ate more than the female rats (experimental and control groups). During the Appetitive Sessions 5 and 6 (which followed the last aversive session) experimental male and female rats ate fewer pellets than their control group counterparts.

Two-way ANOVAs of consumption during appetitive training with sex and training condition as factors revealed a significant effect of experimental condition

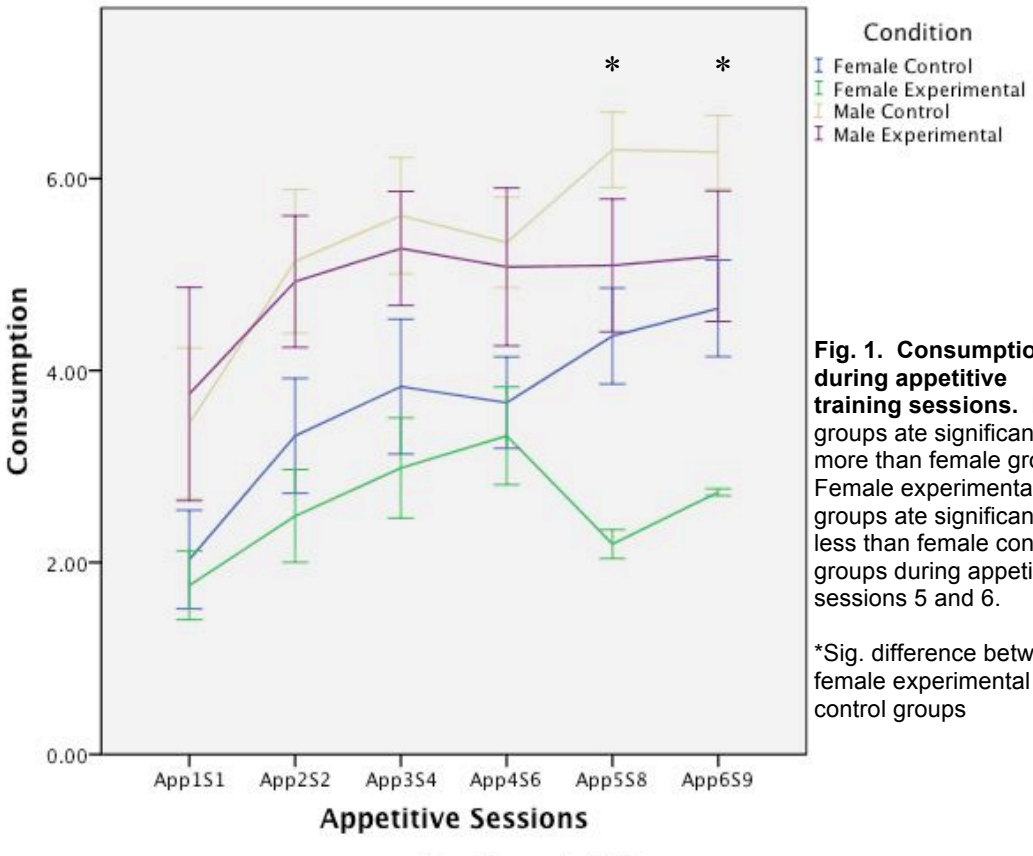

Fig. 1. Consumption during appetitive training sessions. Male groups ate significantly more than female groups. Female experimental groups ate significantly less than female control groups during appetitive sessions 5 and 6.

*Sig. difference between female experimental and control groups

during appetitive sessions 5 and 6 ($F(1,18) > 12.11$, $p < 0.05$ both). There was a

significant effect of training condition on consumption during Appetitive Sessions 5 and 6 ($F(1,18) > 12.12$, $p < 0.01$, both).

Post hoc within-sex independent samples test were run which further revealed that the female rats in the experimental group consumed significantly less than females in the control group during Appetitive Sessions 5 and 6 ($t(1,7) > 3.79$, $p < 0.05$, both); the two male groups were not statistically different ($p > 0.05$).

Testing. We tested the consumption of food-deprived rats in Context A, a context that had not been previously associated with the aversive fear cues. Rats were allowed to eat food pellets as the aversively conditioned CS (tone) was played 4 times during each of three extinction test sessions.

Food consumption. We measured consumption during testing and found that, overall, male rats ate more than female rats. Also, during the first test, male and female experimental rats ate less than their male and female control group counterparts. This inhibition extinguished across the test sessions so that by the third test male experimental rats were consuming pellets in amounts that were closer to the amounts consumed by their control counterparts.

A two-way repeated measures ANOVA across the test days revealed a within-subjects significant main effect of test day, and significant within-subjects

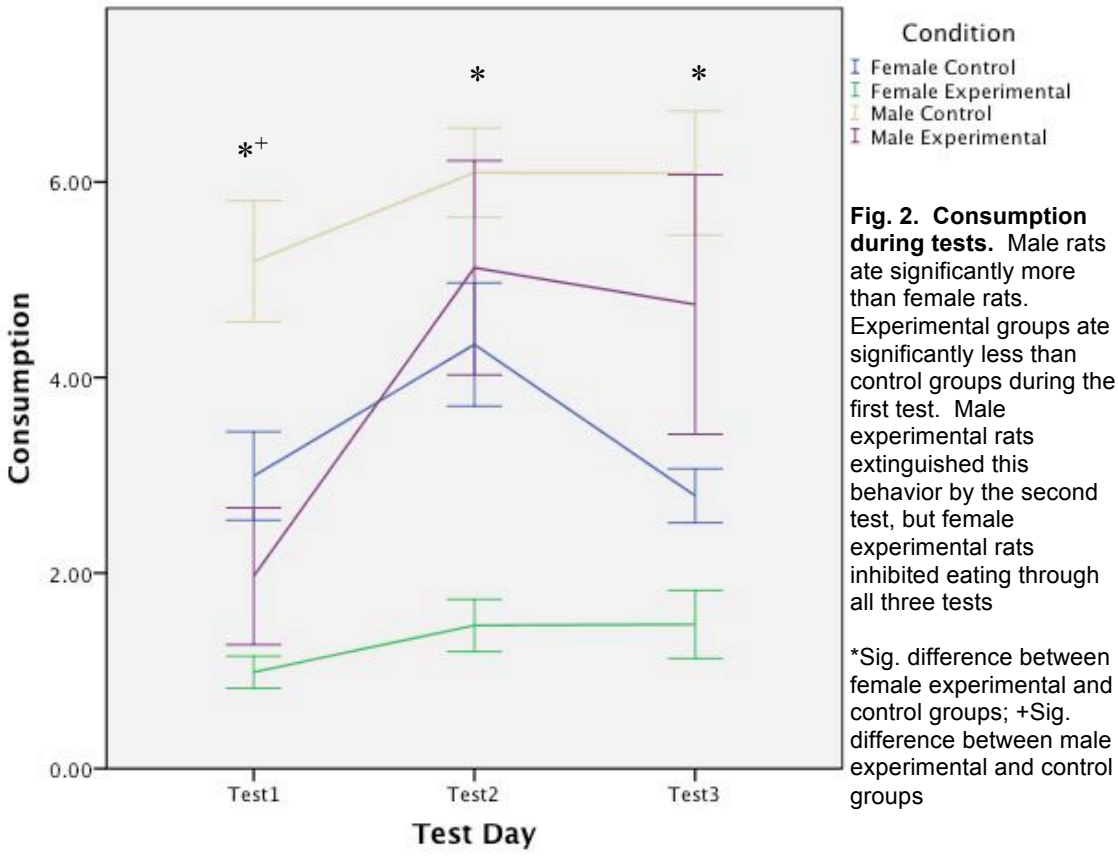

Fig. 2. Consumption during tests. Male rats ate significantly more than female rats. Experimental groups ate significantly less than control groups during the first test. Male experimental rats extinguished this behavior by the second test, but female experimental rats inhibited eating through all three tests

*Sig. difference between female experimental and control groups; +Sig. difference between male experimental and control groups

interactions of test day by sex, test day by condition, and test day by sex by condition ($F(2,28) < 29.619$, $p < 0.05$ all). Additionally, there were significant between-subjects main effects of both sex and condition ($F(1,14) < 18.55$, $p < 0.05$, both).

Post-hoc within-sex independent samples t-tests revealed that these effects were driven by a significant inhibition of intake by the female experimental rats compared to the female control rats during all three test days ($t(1,7) > 2.97$, $p < 0.05$ all). In contrast, the male experimental rats significantly inhibited intake

compared to the male control rats during the first test only ($t(1,7) = 3.45$, $p < 0.05$).

Freezing. Freezing behavior was also analyzed as a species-specific fear response during tests. Male and female experimental groups froze more than the male and female control groups, but only during Test 1.

A two-way repeated measures ANOVA across the test days revealed a within-subjects significant main effect of test day and significant interaction of test day by condition ($F(2,28) < 13.76$, $p < 0.05$ both), and a significant between-subjects main effect of condition ($F(1,14) = 18.55$, $p < 0.05$). There was no significant effect of sex ($p > 0.05$).

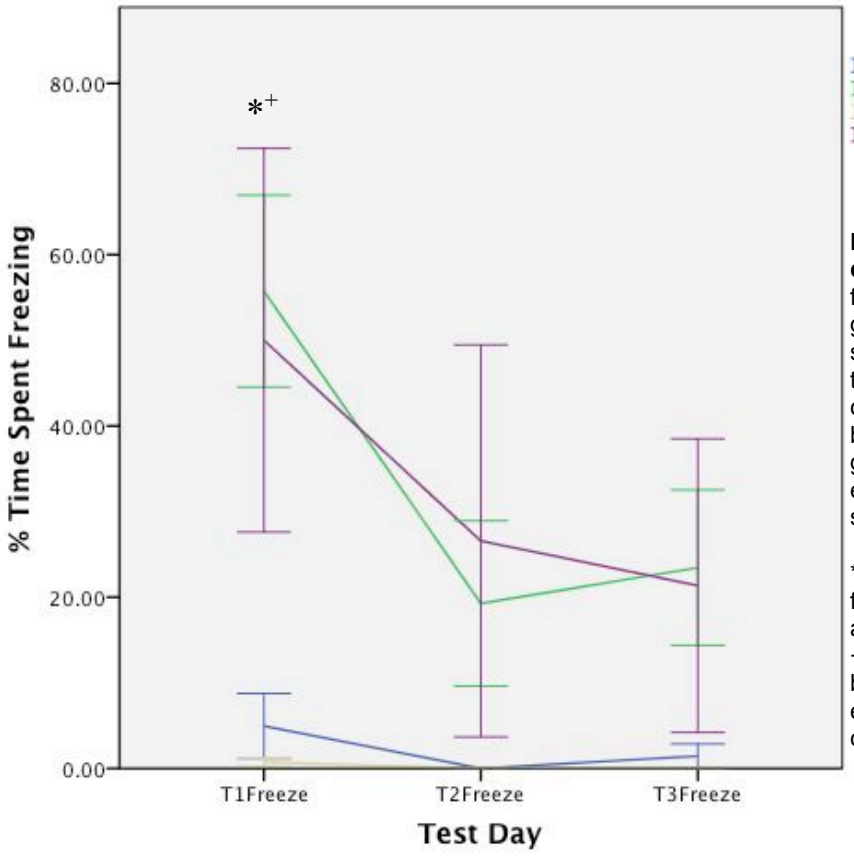

Fig. 3. Freezing during tests. Male and female experimental groups froze significantly more than their control group counterparts. Freezing behavior in experimental groups began to extinguish by the second test.

*Sig. difference between female experimental and control groups; +Sig. difference between male experimental and control groups

Post-hoc within-sex independent samples t-tests revealed that female experimental rats exhibited significantly more freezing behavior than female control rats and male experimental rats froze more than male control rats during the first test only ($t(1,7) > 4.88$, $p < 0.05$, both).

Fos-expression during Test 3. We found more Fos-positive neurons in the brainstem areas in the males compared to the females, then investigated the overall effect further and found that female control rats had more Fos-positive

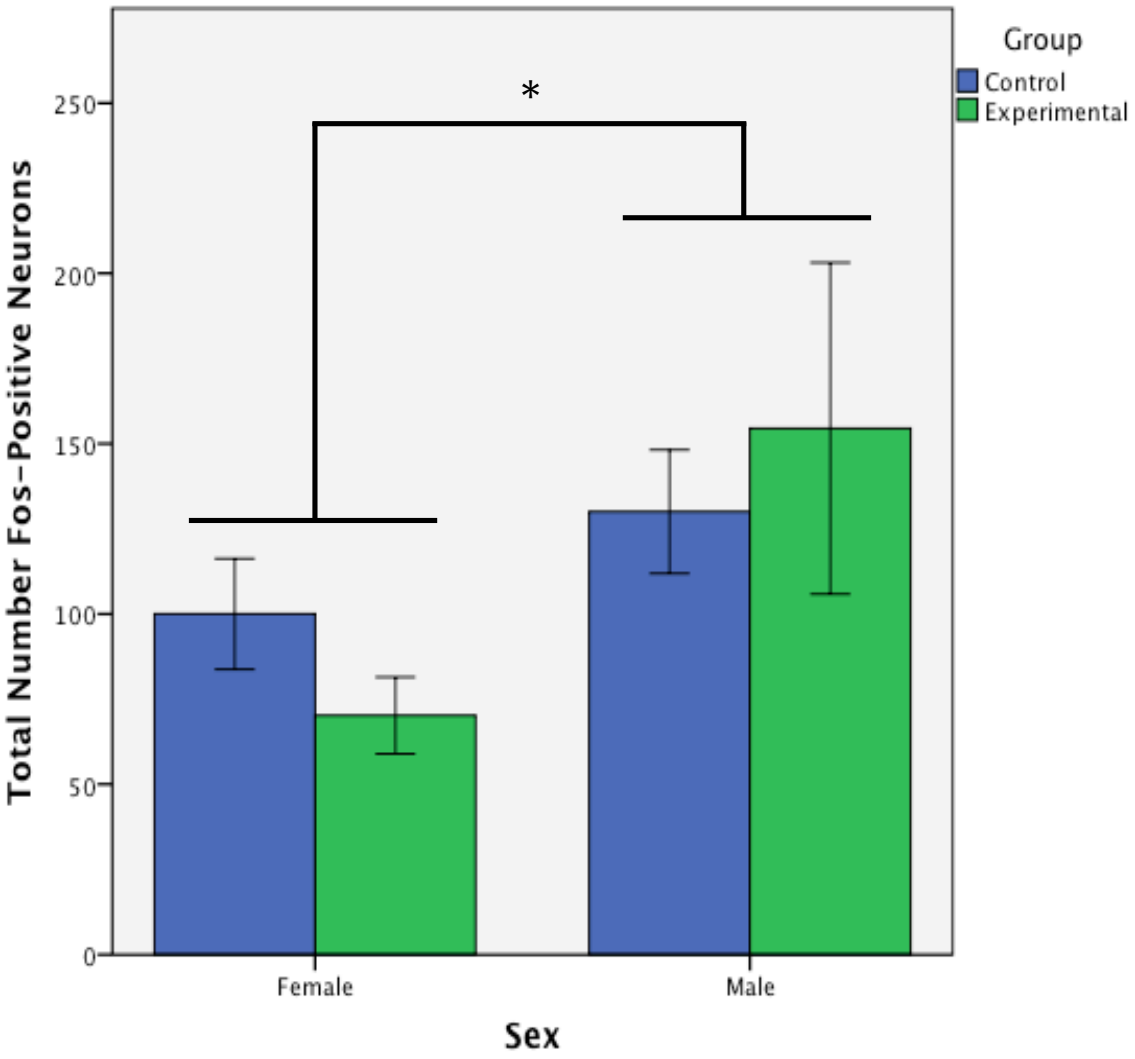

Fig. 5. Total number of Fos-positive neurons in the NTS and DMX across levels 66/67 and 71 (Swanson, 2004). Male rats had higher levels of Fos-positive neurons than female rats, regardless of training condition.

*Sig. difference between male and female groups

neurons caudally (at atlas level 71) than did female experimental rats. Additionally, within this region female control rats had more Fos-positive neurons specifically in the NTS than did female experimental rats.

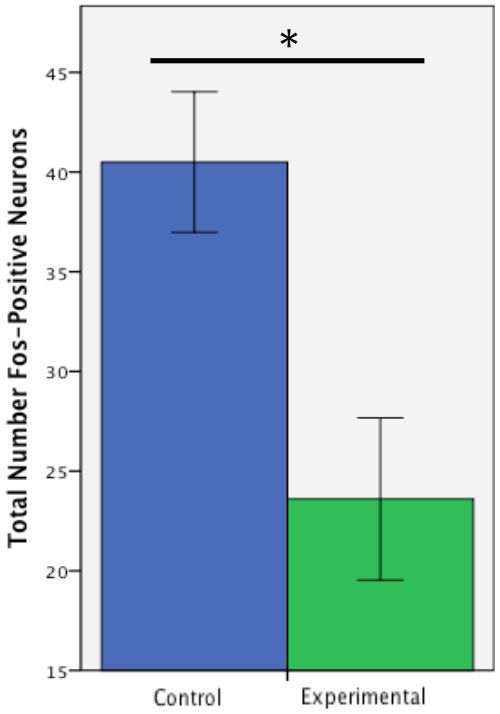

Fig. 4A. Total number Fos-positive neurons at level 71 in female rats (Swanson, 2004). Female control rats showed higher levels of Fos-positive neurons at level 71.

*Sig. difference between female experimental and control groups

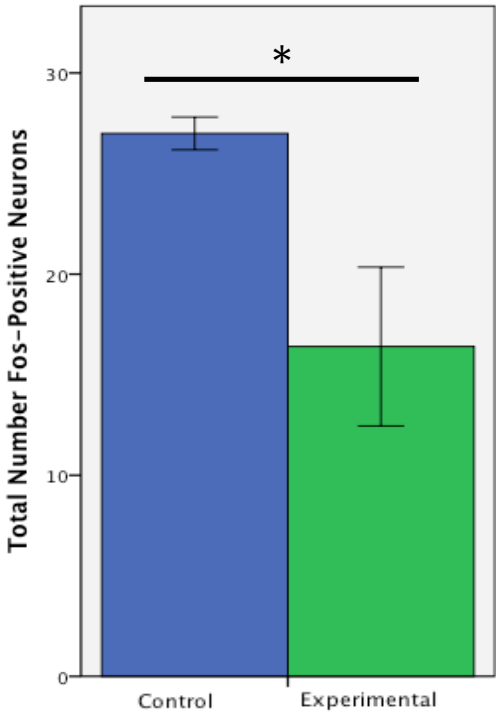

Fig. 4B. Total number Fos-positive neurons in NTS at level 71 in female rats (Swanson, 2004). Female control rats showed higher levels of Fos-positive neurons in the NTS at level 71.

*Sig. difference between female experimental and control groups

A two-way ANOVA revealed a main effect of sex for overall Fos in the NTS and DMX across the two rostro-caudal levels ($F(1,18) = 4.99$, $p < 0.05$).

An independent samples *t*-test revealed an effect of group in females at level 71 ($t(1,7) = 3.04$, $p < 0.05$). There was no significant difference of group in

males at this level ($p > 0.05$). Independent samples *t*-tests of the two brainstem regions at level 71 revealed an effect of group in the NTS of females ($t(1,7) = 2.34$, $p = 0.05$). There was no difference of group in males in the NTS at this level ($p > 0.05$). The number of Fos-positive neurons in the DMX was statistically similar at this level for the experimental and control groups of the same sex ($p > 0.05$).

Discussion

This study broadly aimed to investigate the activation of brainstem nuclei during extinction of a fear-cue induced inhibition eating. Additionally, we hoped to identify sex differences in behavioral effects and brainstem activation patterns.

After behaviorally training and testing male and female rats with a fear-cue paradigm we found that, throughout the experiment, male rats consistently consumed more than female rats, regardless of experimental condition, which is consistent with the baseline consumption levels of each sex during training. During the last two appetitive training sessions (which followed three sessions of aversive training), control groups consumed significantly more than their experimental counterparts, indicating that the shock experience during aversive training sessions had a general inhibitory effect on consumption, evidenced in the appetitive contexts. This group difference in consumption was statistically significant for females, suggesting they may be more susceptible than males to generalized effects due to the aversive experience with footshocks.

Both male and females rats in the conditioned group showed strong inhibition of feeding compared to the same sex controls during the first test when rats were played the aversively conditioned tone. This inhibition of intake is especially interesting given that during tests all rats were both food-deprived and placed in a previously appetitively conditioned context, conditions which normally lead rats to consume large amounts of food. By the second test, male experimental rats were consuming amounts of pellets that were statistically similar to the male control rats. In contrast, female experimental rats significantly inhibited their intake compared to female control rats during all three tests. Interestingly, this sex-difference was not found with the freezing behavior of male and female rats. Both experimental groups were freezing significantly more than control groups during the first test. This behavior extinguished at the same rate for both sexes, and there were no same-sex group differences in freezing during the second or third test.

We then investigated brainstem activation during the third test, when male and female conditioned rats had extinguished freezing behavior, but when conditioned females still continued to suppress food intake. The areas of the brainstem that were specifically examined were the nucleus of the solitary tract (NTS) and the dorsal motor nucleus of the vagus nerve (DMX).

Following the third test, rats were sacrificed and their brains were analyzed for Fos expression as an indirect marker of neuronal activity. We found that across rostral caudal sampling regions of the brainstem, there were higher levels of activity, as measured by higher numbers of Fos-positive neurons, in the NTS

and DMX of males compared to females. The effect of sex on overall activity of these brainstem nuclei follows a pattern similar to that of consumption; male rats consumed more and showed higher levels of brainstem nuclei (NTS and DMX) activity than did female rats.

A further exploration of the specificity of brainstem nuclei activity revealed that female control rats showed more activity caudally, and specifically in the NTS, than did female experimental rats. There was no such significant difference between male experimental and control groups. This effect corresponds to the consumption results in that female control rats were still consuming significantly more than female experimental rats by the third test, whereas male control rats only consumed significantly more than male experimental rats during the first test.

The brainstem is the final output for behavior, and as such can provide key information for the current research on feeding behavior and brain activation patterns. The results presented here suggest a key role for the brainstem in extinguishing inhibition of eating following a fear-cue paradigm. The NTS and DMX have been associated with mediating the cardiovascular correlates of fear and the current study supports previous findings that these particular nuclei could be related to our behavioral observations (Amano et al., 2011). Additionally, combining the results presented in this study with research on the brain activation patterns in the central nucleus of the amygdala (CeA) as well as hypothalamic orexin neurons could illuminate the functional details of the network that mediates fear-cue induced inhibition of eating using a paradigm established here.

The results further suggest differentiation of these activation patterns based on sex. The prolonged cessation of eating in female rats compared to male rats has important implications for research on anorexia nervosa, given that such disorders are known to disproportionately affect women over men. In terms of its diagnosis, anorexia nervosa is defined by the fear of weight gain, thus its inherent association with fear provides a basis for experimental study (Klein and Walsh, 2004). Previous research hypothesizes that the disorder is linked with the rapid acquisition of conditioned fear behavior as well as a heightened resistance to the extinction of associative fear learning (reviewed in Strober, 2003), which is interesting in relation to the current study as the results here suggest effects of sex on the extinction of fear-cue induced inhibition of feeding.

Future research could include an analysis of brainstem nuclei activation patterns during Test 1, before rats extinguish feeding inhibition, to better understand how activation of the brainstem may change throughout the course of testing. Another avenue of future research could focus on the roles of the NTS and DMX after a re-exposure to the aversive context. Additionally, the activation patterns of other brainstem areas, such as the area postrema could be investigated as it has also been shown to be associated with hunger and satiety regulation (Johnstone et al, 2006).

In conclusion, we have found that there are distinct patterns in both behavior and brainstem nuclei activation during the extinction of fear-cue induced inhibition of feeding and that additionally, there are notable sex differences in these effects. Continued research on these patterns is key to a better

understanding of the behavioral and neurobiological basis of feeding behavior as well as eating disorders.

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References

Amano, T., Duvarci, S., Popa, D., & Paré, D. (2011). The fear circuit revisited: Contributions of the basal amygdala nuclei to conditioned fear. *The Journal of Neuroscience 31*(43). 15481-15489. doi:10.1523/jneurosci.3410-11.2011.

Bolles R C. (1970) Species-specific defense reactions and avoidance learning. *Psychol. Rev.* 71. 2-48.

Geary, N., Lovejoy, J. Sex differences in energy metabolism, obesity, and eating behavior. Sex differences in the brain from genes to behavior. New York: Oxford University Press; 2008. p. 253-74.

Grill, H. J., (2006). Distributed Neural Control of Energy Balance: Contributions from Hindbrain and Hypothalamus. *Obesity, 14*(Supplement). doi:10.1038/oby.2006.312

Johnstone, L. E., Fong, T. M., & Leng, G. (2006). Neuronal activation in the hypothalamus and brainstem during feeding in rats. *Cell Metabolism*, *4*(4), 313-321, doi:10.1016/j.cmet.2006.08.003

Klein, D.A. & Walsh, B.T. (2004). Eating disorders: Clinical features and pathophysiology. *Physiol Behav*, *81*(2), 359-374.

Maren, S. (2001). Neurobiology of Pavlovian fear conditioning. *Annual Review of Neuroscience, 24*(1), 897-931. doi:10.1146/annurev.neuro.24.1.897

McCarthy, M.M., Arnold, A.P., Ball, G.F., Blaustein, J.D., & De Vries, G.J. (2012). Sex difference in the brain: The not so inconvenient truth. *J Neurosci, 32*(7), 2241-2247.

Petrovich, G. D., & Gallagher, M. (2007). Control of food consumption by learned cues: A forebrain–hypothalamic network. *Physiology & Behavior, 91*(4), 397-403. doi:10.1016/j.physbeh.2007.04.014

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

Popkin, B. M., Duffey, K., & Gordon-Larsen, P. (2005). Environmental influences on food choice, physical activity and energy balance. *Physiology & Behavior, 86*(5), 603-613. doi:10.1016/j.physbeh.2005.08.051

Simmons, DM, Swanson, LW. The Nissl stain. Neurosci Protocols. 1993 050-12-01-07.

Striegel-Moore, R.H., Rosselli, F., Perrin, N., DeBar N., Wilson, G.T., May, A., & Kraemer, H.C. (2009). Gender difference in the prevalence of eating disorder symptoms. *International Journal of Eating Disorders*, *42*(5). 471-474.

Strober, M. (2004). Pathologic fear conditioning and anorexia nervosa: On the search for novel paradigms. *International Journal of Eating Disorders 35*(4) 504-508. doi:10.1002/eat.20029

Stroebele, N., De Castro, JM. (2004). Effect of ambience on food intake and food choice. *Nutrition, 20*, 821-838.

Swanson, L.W. (2004) Brain Maps: Structure of the Rat Brain: Third Edition, Elsevier, Inc., United Kingdom.

Weingarten, H. (1983). Conditioned cues elicit feeding in sated rats: A role for learning in meal initiation. *Science, 220*(4595), 431-433. doi:10.1126/science.6836286

Zucker, I., & Beery, A.K. (2010). Males still dominate animal studies. *Nature 465*(7299), 690.