Conditioned food aversion: A strategy to study disordered eating?

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Introduction

Multiple eating disorders show dramatic onsets during childhood or adolescence, and involve conditioned avoidance to previously accepted foods. Anorexia Nervosa (AN) has the highest fatality rate of any psychiatric disorder. Current animal models of the disease focus on anorexia associated with food restriction, extreme stress, and/or excess physical activity. No current animal model captures the key characteristics of visceral hypersensitivity leading to learned food avoidance, adolescent onset and female dominance. Avoidant Restrictive Food Intake Disorder (ARFID) is slightly more prevalent in males, appears earlier developmentally and in some cases may transition into AN. The purpose of this study was to evaluate sex and age differences in conditioned taste/food aversion (CTA, CFA) to determine if sex differences and ontogenetic pattern resembles either of these two important eating disorders. This study aimed to develop a new rodent model for disordered eating that more accurately reflects certain human phenotypes. By studying the behaviors and brain activations and development associated with this model, we aimed to gain a greater understanding of the biological mechanisms and vulnerability markers for disordered eating. Understanding these biological aspects may lead to the development of better treatments for disorders such as Anorexia Nervosa and ARFID.

Methods

Both studies were conducted with modified CTA/CFA procedures which involved no food or water restriction.

Study 1: Adolescent (PN 28) and adult (PN 70) male and female Sprague Dawley rats were placed in feeding cages containing bedding on Day 0 and given water to drink for habituation. On Day 1 (24 hours later), they were placed in cages, given the nutritional liquid Boost™ and allowed to drink for 1 hr. They were injected with NaCl or LiCl (19, 38 or 80 mg/kg) and left in the cages for 1 hr. Total Boost consumed and incidences of pica (chewing on bedding) were quantitated by an observer blinded to treatment conditions (0-3 for intensity, time-sampled for 1 hr). On Day 2, they were placed again in drinking cages with access to Boost and water. Drinking data are expressed as D2/D1 volume consumed (ml/kg body weight).

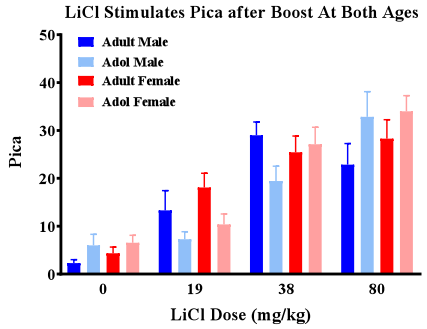
Study 2: Procedure was conducted as described in Study 1, except that on Day 0, rats were given a novel food (Cheerios) for habituation. On Day 1, they were given a second novel food (Froot Loops), allowed to eat for 1 hr and treated with NaCl or LiCl (19 mg/kg) as in Study 1. On Day 2, animals were placed back in feeding cages, and allowed to choose between Froot Loops, or a third novel cereal, Apple Jacks (AJ). CFA is expressed as Froot Loops D2/D1. Data were analyzed by 3-way ANOVA (age / sex / treatment) using statistical package NCSS.

Brains were collected and 40 um sections were stained for c-fos. C-fos expression was automatically quantified by Imaris software.

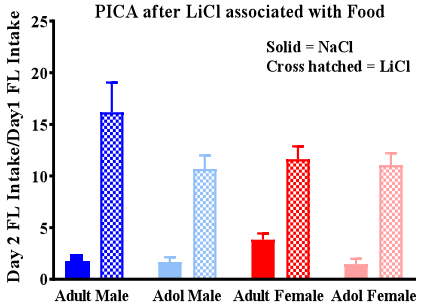
Day 0: Habituation	Day 1: Conditioning		Day 2:Choice Test
1 hour: H_2O in cage	1 hour:Boost Measure Intake	Inject LiCl/NaCl 1 hour Pica observation	1 hour:Boost or water

Day 0: Habituation	Day 1: Conditioning		Day 2:Choice Test
1 hour: Feeding Cage + Cheerios Measure Intake	1 hour: FL Measure Intake	Inject LiCl/NaCl 1 hour Pica observation	1 hour: FL and AJ choice

Results

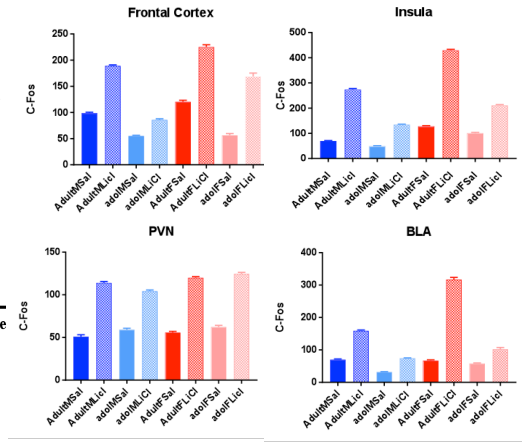


Pica behavior in Adult and Adolescent animals after Boost drinking followed immediately by NaCl or LiCl. Pica was rated for one hour. Results expressed as mean ± SEM. N = 6-13/group p < 0.0001 effect of dose, p < 0.005 age x dose. Adol p < 0.0001 effect of dose Adults p < 0.0001 effect of dose.

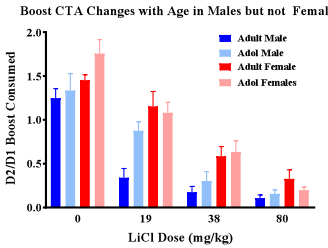


Pica behavior after FL and NaCl or LiCl. On Day 1. Results expressed as mean ± SEM. N= 12-18/group. P < 0.024 effect of age, p < 0.0001 effect of treatment, p < 0.032 age x sex x treatment. Adol p < 0.0001 effect of treatment, Adults p < 0.0001 effect of treatment, p < 0.034 sex x treatment.

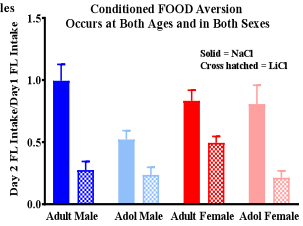
Neuronal Activation in cortex after LiCl is less in adolescents than adults



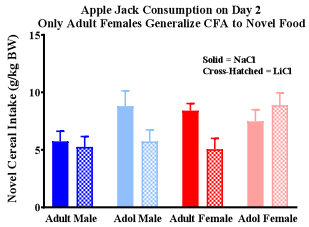
C-Fos expression Quantification: Results expressed as mean ± SEM. N = 4 per group. All: P < 0.0001 effects of sex, age, treatment, age x treatment, sex x treatment, and age x sex x treatment.



CTA in Adult and Adolescent animals drinking Boost 24 hours after Boost followed immediately by NaCl or LiCl. Consumption presented as D2/D1. Results expressed as mean ± SEM. N = 6-13/group. p < 0.0001 effect of sex, p < 0.0001 effect of treatment, p < 0.053 age x sex x treatment. Males p < 0.012 effect of age, p < 0.0001 effect of treatment. Females p < 0.0001 effect of dose. Adult p < 0.0001 effect of sex, p < 0.0001 effect of treatment, p < 0.013 sex x treatment.



CFA in Adult and Adolescent animals eating Froot Loops 24 hours after FL followed immediately by NaCl or LiCl. Consumption presented as D2/D1. Results expressed as mean ± SEM. N = 12-18/group. p < 0.015 effect of age, p < 0.0001 effect of treatment, p < 0.012 age x sex x treatment. Adol: p < 0.004 effect of treatment. Adults: p < 0.0001 effect of treatment, p < 0.04 effect of sex.



Consumption of Novel Food (Apple Jacks) on Day 2. Results expressed as mean ± SEM. N = 12-18/group. p < 0.01 effect of age, p < 0.02 effect of sex, p < 0.001 effect of treatment. Females p < 0.03 effect of age, p < 0.001 effect of treatment.

Conclusions

- **Females show lower CTA/CFA than Males in Adulthood despite high levels of pica in both sexes.**
- **Adolescent Insensitivity to CTA is paralleled by lower pica response to the GI irritant LiCl.**
- **Conditioned FOOD Aversion (CFA) is more robust in adolescence than CTA to fluids.**
- **Distinct developmental changes in CTA/CFA occur in males and females: females acquire generalization of CTA to novel foods between adolescence and adulthood, while males develop more robust CTA to specific illness-associated foods. Adolescent males more neophobic in general.**
- **Generalizability may act as a potential vulnerability marker for ED onset.**
- **Developmental differences in Frontal Cortex: Females show adult-like levels, males remain lower than adults -- region develops earlier in adolescence for females.**
- **PVN: earliest to develop, so adolescent rats express adult levels of c-fos.**
- **BLA c-fos age/sex differences similar to pica -- BLA plays role in nausea sensation itself.**

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