Sex differences in circadian food entrainment: Observing the role of sex chromosome (X) copy number

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Kellogg Honors College Capstone Project

Abstract

In an effort to provide insights on the biological basis for sex differences seen in eating disorders and other obesity related diseases; our lab uses mouse-model experiments to better characterize and compare biological sex differences seen in circadian entrainment to meal time. Circadian rhythms influence mammalian behavior and physiology and these circadian rhythms can change through the activation of a food entrainable oscillator via calorie restriction and set mealtime. In an attempt to better understand the factors influencing the observed sex differences, our lab has tested a variety of variables including age, sex hormones, and receptor projection by measuring food entrainment; however, these sources appear to not contribute to the observed sex differences. Currently, we are observing the role of sex chromosome copy number. We have obtained male mice with mutated Y chromosomes (Sry gene), rendering them gonadal females (XY-). We can rescue the Sry mutation by transgenic expression of Sry from an autosome (non-sex chromosome) to generate and compare XY-, SryTg and XX SryTg mice. From these comparisons, we hope to discern whether or not the observed sex differences are due to sex chromosome copy number.

Introduction

The circadian rhythm of activity and physiology in mammals is most heavily influenced by the 24-hour light-dark cycle. Zeitgebers (environmental timing signals) in the form of photons hitting the retina which are then interpreted by the brain attuning rhythm to light availability. The suprachiasmatic nucleus (SCN) is critical in mediating this process. However, there is not only one circadian system in mammals, other robust zeitgebers also exist. Mammals can acquire circadian rhythms in response to cyclic food availability, as evidenced by change in activity rhythm when feeding cycle is decoupled from light cycle.

FAA is an activity peak preceding scheduled feeding that is independent of light cycle rhythm. Mice with a lesioned SCN cannot attune circadian rhythm to light availability but are still able to show FAA in response to cyclic food availability. Our goal is to understand the neural circuitry of food-entrained oscillation that operates independently of the relatively well-characterized light-entrained oscillation.

Methods

In order to assess the role of sex chromosome copy number, 4 different genotypes: XX, XXSRY, XY-SRY, and XY- underwent our lab's standard FAA experimental conditions at the same time each day feeding 60% of their normal calorie intake. Starting on day 0 of the experiment, the mice received a food pellet of 60% of their normal calorie intake given to them at the same time daily. They were fed at ZT5 so the food availability occurred close to the middle of the light availability window in which mice are usually dormant (they are nocturnal). Mice were recorded on the same day each week for 24 hours. Video recordings were analyzed by computer vision in the HomeCageScan program that quantified activities including grooming, jumping, walking, and hanging for each hour of recording.

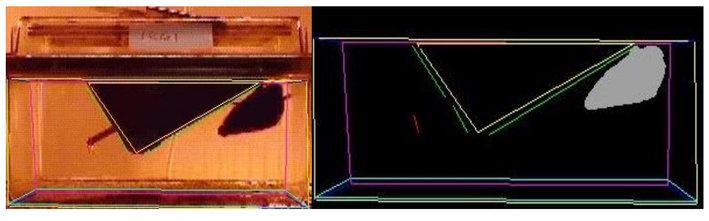

Figure 5.
HomeCageScan Simulated Computer view of a mouse hanging from a wire bar

Female C57BL/6J mice show less food anticipatory activity than males

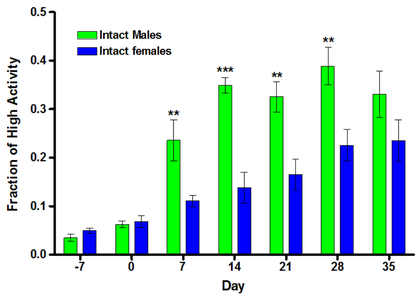

Figure 1.
Intersex comparison in food anticipatory activity. Normalized high activity 3 hours preceding scheduled meal relative to total activity over 35 days of calorie restriction; normalized mean +/- SEM of high activity. For weeks 1-4 on calorie restriction, there is a very significant difference between male and female mice (**p<0.01; *** p<0.001, Mann Whitney).

Removal of gonads does not modulate FAA in either males or females

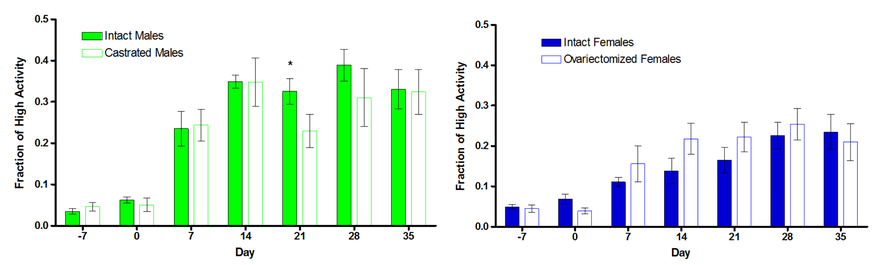

Figure 2.
Intrasex comparison in food anticipatory activity between intact and gonadectomized counterparts. Normalized high activity 3 hours preceding scheduled meal relative to total activity over 35 days of calorie restriction; normalized mean +/- SEM of high activity. No significant difference between intact and gonadectomized mice within respective sex.

Neonatal estrogen exposure to "masculinize" female brains does not alter FAA

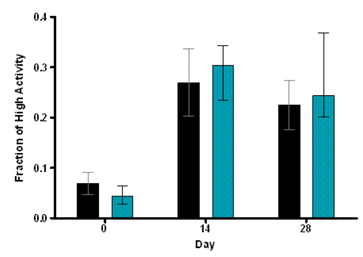

Figure 3.
Estrogen injection into the developing mouse brain masculinizes aromatase receptor projection within the striatum which modulates mating, urination, and aggression behaviors (Wu et al. 2010). Depicted is a comparison of estrogen injected and control female mice; normalized high activity 3 hours preceding scheduled meal relative to total activity over 28 days of calorie restriction; normalized mean +/- SEM of high activity. No significant difference between injected and control females.

Sex chromosome copy number does not appear to modulate FAA

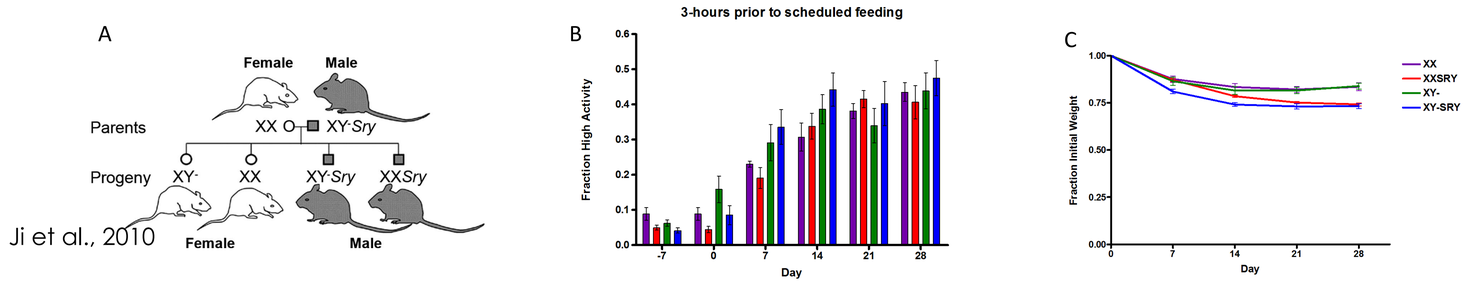

Figure 4.
A) Pictorial description of mouse line used, abstracted from Ji et al. 2010. Genetic variants allow for comparison of sex chromosome copy number as well as SRY presence. B) Normalized high activity 3 hours preceding scheduled meal relative to total activity over 28 days of calorie restriction; normalized mean +/- SEM of high activity. Comparing the four groups, appears to have no difference. Significance will be assessed with ANOVA when n exceeds 4. C) Fraction initial weight over experiment relative to calorie restriction start.

Sex differences in FAA are not observed in older (9 months) mice

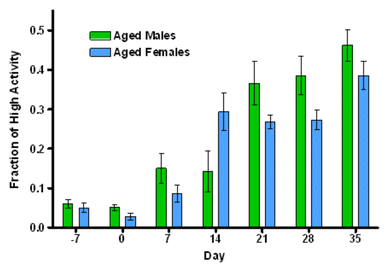

Figure 5.
Intersex comparison of 9 month old mice in food anticipatory activity. Normalized high activity 3 hours preceding scheduled meal relative to total activity over 35 days of calorie restriction; normalized mean +/- SEM of high activity. No significant difference between intact males and intact females at 9 months of age.

Results and Future Direction

Given our results, it appears that sex chromosome copy number is not responsible for the biological sex differences seen with respect to FAA. The data we have processed to achieve this conclusion has a sample size of n=4 so we are adding a few more mice for a sample size of n=10.

After testing gonadal sex hormones, aromatase receptor projection, sex chromosome copy number, and apparent modulation by age; we have observed that biological sex differences in behavior are more complex than can be explained by traditional avenues of biological sex comparison.

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