Sex differences in food anticipatory activity are unrelated to circulating sex hormones

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A Kellogg Honors College Capstone Project (Timothy Huddy) and SEES Scholars Project (Antonio Aguayo) mentored by Andrew Steele

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Abstract

Studies of anticipation of scheduled feeding in mice typically utilize males preferentially. Recent studies have demonstrated a sex difference in food entrainment with males showing higher amplitude food anticipatory activity (FAA) relative to females. We replicated these results in inbred C57BL/6J mice, finding that females were both slower to entrain and expressed less FAA than males. We hypothesized that circulating sex hormones were responsible for the differences in activity: either that androgens promote or estrogens interfere with FAA. Much to our surprise, and in contradiction to another recent study (Li, *et al.*, 2015, Hormones and Behavior 70:38-46), we observed no differences between ovariectomized and intact females and castrated male mice showed equal FAA to intact males. Our results suggest that circulating sex hormones are not responsible for the difference in male and female expression of FAA rhythms and that sex differences in brain development may account for this difference. Future studies will investigate the sexual dimorphisms in the male and female brain to gain insight into which brain structures modulate FAA.

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 of light hit the retina and are then interpreted by the brain to attune 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 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.

Sexual dimorphism in FAA has potential for showing how food entrainment occurs. Female mice show weaker magnitude FAA and later onset of FAA when compared to males. We speculated that circulating sex hormones may explain the reduced ability of female mice to entrain to feeding- perhaps because the 4-5 day estrous cycle in female mice is a strong physiological rhythm.

Methods

16 male and 16 female mice were purchased from Jackson labs with 8 males having been castrated and 8 females having been ovariectomized. For each of the 4 groups of mice, a weight of food equal to 60% of their normal calorie intake was established. Starting on day 0 of the experiment, mice had all of their food taken away. From then on, the only food they received was a food pellet of 60% their normal calorie intake given to them at the same time daily. They were fed at ZT6 so the food availability was directly in 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.

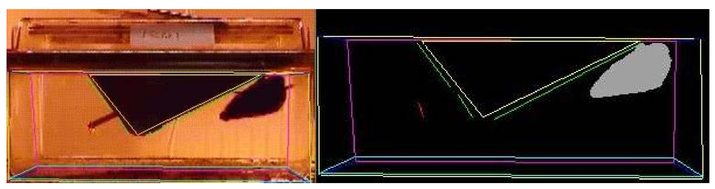

Figure 1. HomeCageScan simulated computer view of a mouse hanging from wire rack

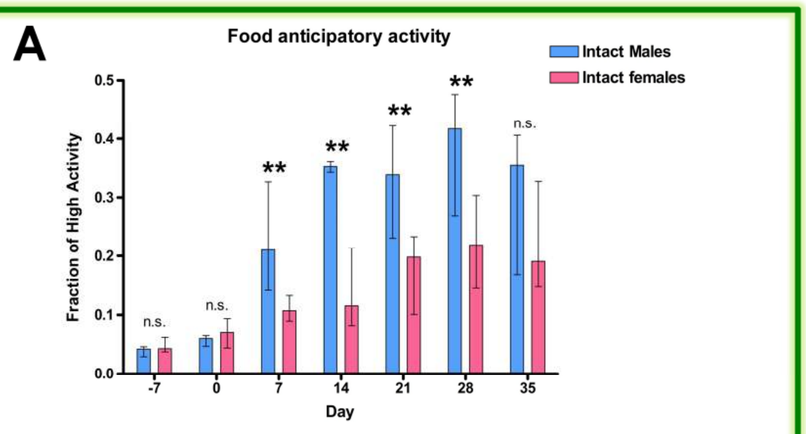

Figure 2. Bar graphs showing FAA as fraction of total daily activity. Bars are shown as median and interquartile range, and stats were done with 1-tailed Mann-Whitney U test(* denotes $p<.05$ and ** denotes $p<.01$).

Panel **A** shows males having significantly higher FAA than females on the most important days of food entrainment. Panels **B** and **C** show that removal of circulating sex hormones does not significantly change the FAA of males or females enough to rectify the sexual dimorphism displayed in panel **A**.

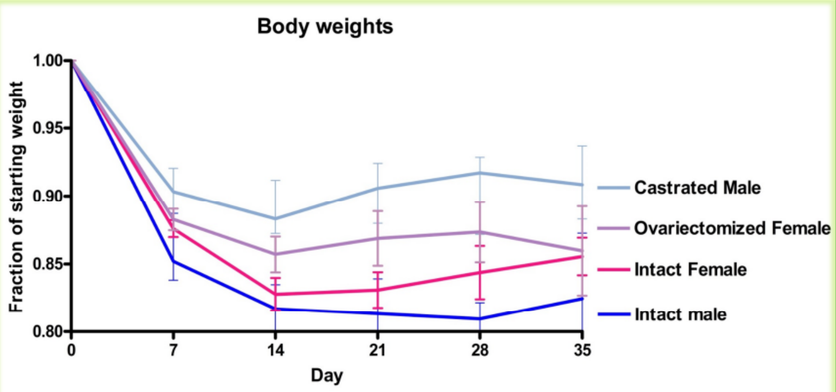

Figure 3. Weight loss for each group is shown as a fraction of each group's starting weight. Both castrated males and ovariectomized females started day 0 at about 3 grams lighter than the intact mice. Weight loss stabilizes between days 14 and 21 for most mice. The amount of weight loss observed is not considered sufficient to induce torpor that might interfere with activity.

Acknowledgements

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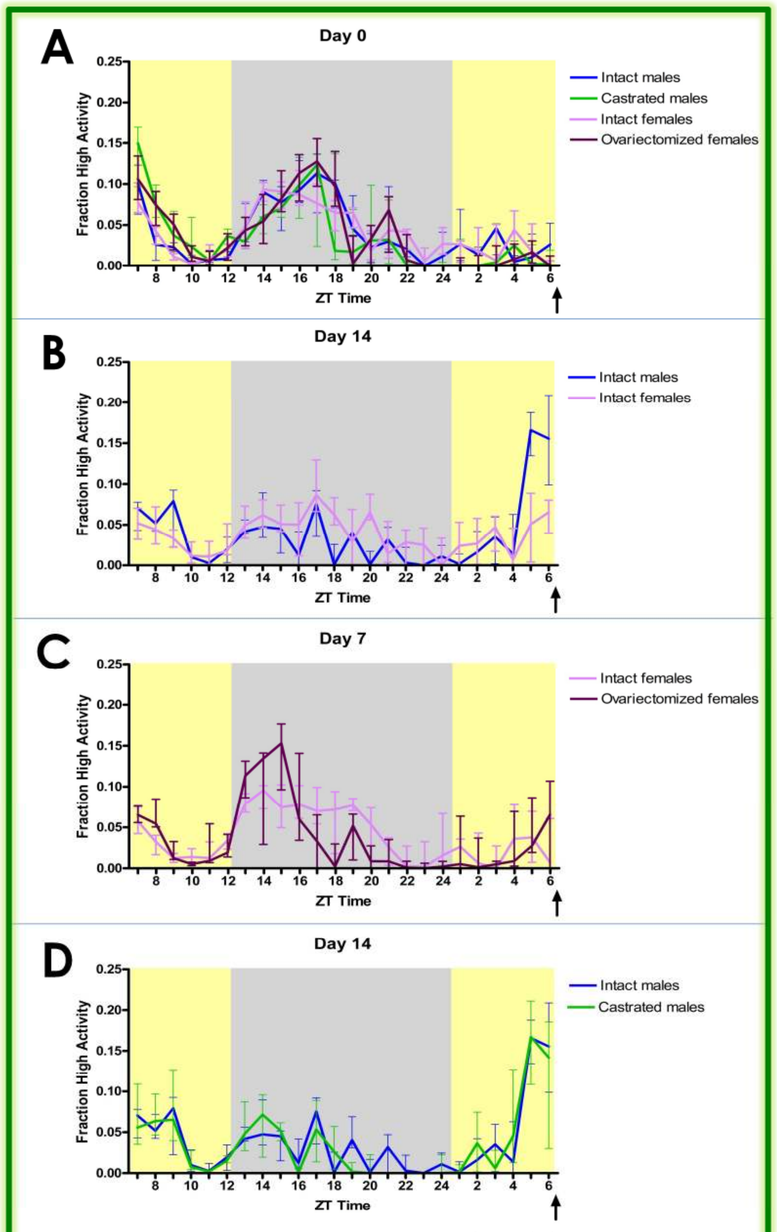
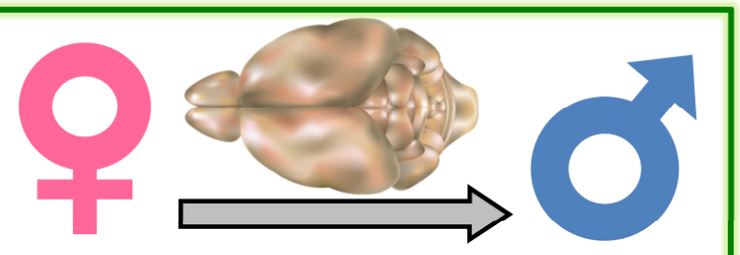


Figure 4. Each panel shows a plot of activity observed in a 24-hour recording. The light availability pattern is shown in the background of each graph with the grey being the 12-hour window when the mice have no light. The mice are fed at ZT 6, indicated by the arrow in each panel. The sum of activity in the last 3 hours of the line plots we generated were used to create the FAA bar graphs in Figure 2.

Panel **A** shows day 0 for all groups. No food entrainment has happened at this point, and all the mouse groups show high nocturnal activity and no FAA. Panel **B** shows male mice at day 14 strongly anticipating feeding (ZT 4-6) with reduced nocturnal activity. Females are lagging in the circadian rhythm shift. Panels **C** and **D** each show different days in which gonadectomized mice show activity very similar to that of their intact counterparts.

Conclusion and Future Direction

We have determined that the effect of circulating sex hormones is not sufficient to explain the magnitude of the sexual dimorphism in FAA. In addition to regulating physiology of mature mice, sex hormones also affect the development of the brain. Some behaviors and neural pathways are sex-specific or at least heavily biased to one sex. To test if differences in brain development are the cause for the sexual dimorphism in FAA, we will be masculinizing the brains of female mouse pups. Aromatase-expressing neurons in the developing brain are able to affect sexual differentiation and can change the brain's developmental fate if extra sex hormones are injected shortly after birth (Wu, *et* al., 2009, Cell 139: 61–72).



We hope that masculinization of the female mouse brain increases female FAA to that of normal male mice.