Effects of a common polymorphism of the mu-opioid receptor gene in binge-like feeding behavior, and the role of estrous stage, in female mice.

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Abstract

A single nucleotide polymorphism (SNP) of the mu opioid receptor gene (*Oprm1* A118G) is associated with an altered susceptibility to drug abuse in humans and rodents. This study explores the interaction between the A118G SNP and binge-like feeding in female mice. It was hypothesized that mice homozygous for the GG allele would have greater binge intake than AA mice in a model of dietary-induced binge-like feeding. Female mice were assigned to one of four feeding groups (two independent variables, chow restriction and binge access): Restrict Binge, Binge, Restrict, and Naïve. The Restrict Binge and Binge feeding groups were given intermittent access to "sweetened fat" (hydrogenated vegetable oil plus 10% sucrose) for a 30-minute period, twice weekly. The "sweetened fat" binge food mimics the macronutrient composition of the types of palatable foods typically consumed by individuals with Binge Eating Disorder (BED). Vaginal cytology was performed on the mice each morning of a binge, in order to control for the effect of estrous stage. Regardless of feeding group and genotype, mice were most frequently in the metestrus phase and least frequently in the proestrus phase on binge days. Previous studies show that rodents in proestrus (highest estrogen level) have reduced food intake due to smaller meal size, whereas increased meal size is observed in metestrus and diestrus stages. Because BED is disproportionately expressed in females, the interaction between this common SNP and estrous cycle in the context of feeding behavior warrants further investigation.

Background

- A single nucleotide polymorphism (SNP) of the mu opioid receptor gene (*Oprm1* A118G) is associated with an altered susceptibility to drug abuse in humans and in rodents.
- Mice homozygous for the GG allele are more likely to engage in binge like feeding behavior than the mice homozygous for the AA allele.
- Humans with the GG variant have a proclivity for highly palatable foods which eventually leads to over eating and obesity.
- Previous studies show that rodents in proestrus (highest estrogen level) have reduced food intake due to smaller meal size, whereas increased meal size is observed in metestrus and diestrus stages
- BED is a common eating disorder in the US which particularly affects females.

Goal of the Research

- Perform vaginal cytology to discover whether estrus stage has an effect on binge like eating behavior.

Methods and Materials

Feeding schedules for binge protocol groups

	Calorie Restriction	Sweetened Fat Access
Restrict-Binge	24 hr (Day 2 & 5)	30 min (Day 3 & 6)
Binge	None	30 min (Day 3 & 6)
Restrict	24 hr (Day 2 & 5)	None
Naïve	None	None

Figure 1. Mice were put in Experimental groups and feeding schedules.

- Female mice homozygous for the SNP and homozygous for the wildtype underwent a 6-week binge-like feeding protocol.
- Mice were randomized to one of four feeding schedules, with no initial differences in body weight.
- The four schedules were Restrict-Binge, Binge, Restrict, and Naïve groups
- Prior to the feeding protocol, mice were exposed to a 24-hour "pre-exposure" to "sweetened fat" consisting of vegetable shortening and 10% sucrose.
- Vaginal cytology was conducted pre- and post- pre-exposure to control for stages of estrous cycle.
- Vaginal Cytology was conducted in the morning prior to binge which allowed binge intake results to be unaffected by the stress from the cytology.

Sample Collection Protocol

- Tip of pipette containing 0.2-0.25mL of saline solution was inserted in the vaginal orifice about 1cm deep.
- Flush vagina a couple of times with saline solution until a cloudy liquid appears in the pipette.
- A drop or two was placed on the slide and the smear was stained in order to examine the stages of Estrous cycle.

Characteristics of the four stages of Estrous Cycle:

Proestrus:
- Identified by the presence of clusters of round, nucleated, epithelial cells

Estrus:
- Discernible by the presence of large numbers of needle-like cornified cells lacking nuclei.

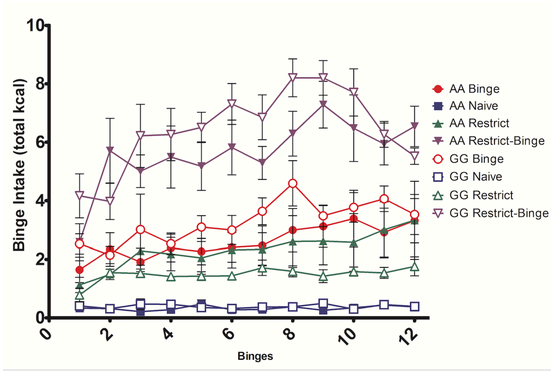
Metestrus:
- Charecterized by a combination of leukocytes, Proestrus and Estrus cells.

Diestrus:
- Infiltrated with immune fighting cells, known as leukocytes.

Results

- Female mice were found to be in metestrus and diestrus phases more frequently compared to proestrus phases.
- From these results, it can be temporarily concluded that estrus phase does not have an effect on binge-like eating behavior.
- Female mice with the SNP are more susceptible in binge like feeding behaviors.

Female binge eating behavior



Estrus phases in female mice with the GG and AA genotypes.

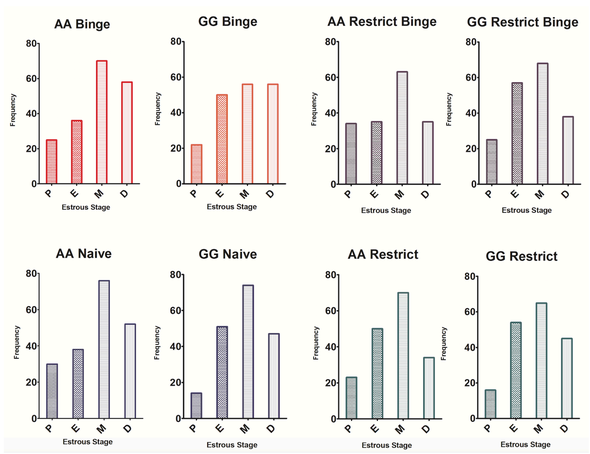


Fig 2. Comparisons of estrus phases in GG and AA female mice show that the estrus stage did not have an effect on binge-like behavior.

Future Direction

- Further studies in the Bello Lab will explore the interaction between this common SNP and the estrous cycle in regards to the binge like feeding behavior, using the binge diet induced feeding model.

Summary & Significance

- The polymorphism in the mu opioid receptor gene (*Oprm1* A118G) is associated with an increased vulnerability to drug and alcohol use.
- BED is commonly associated with an excessive consumption of sweet and fatty foods and higher prevalence of the G/G allele.

Acknowledgements

I would like to thank Dr. Bello for giving me such an amazing opportunity for the academic year and making my first research experience memorable. Additionally, I would like to thank Bryn for being a great mentor to me getting me involved with her project. I would also like to thank Gina for always keeping me on track with my research and all the other grad and undergrad students in keeping a light and friendly environment in the lab. Finally, I would like to extend my gratitude to the Aresty Research Center

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