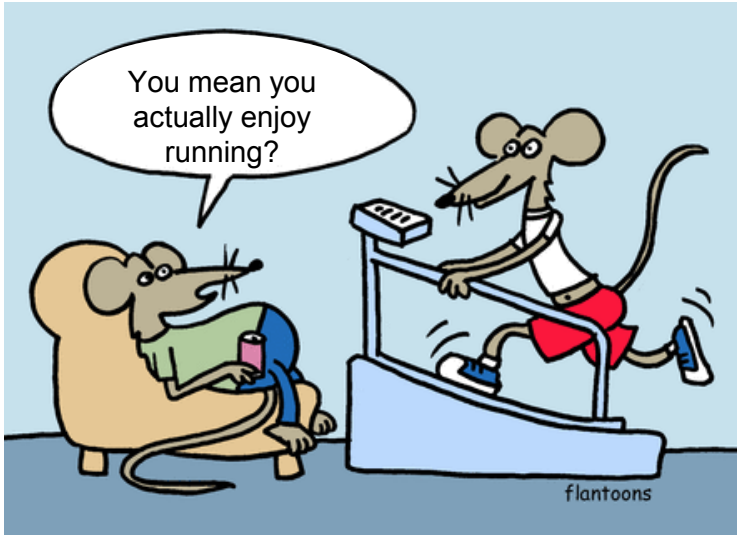
Behavioral assay development to assess preference for exercise relative to palatable food



Physical Activity and Behavior Workgroup
MMPC

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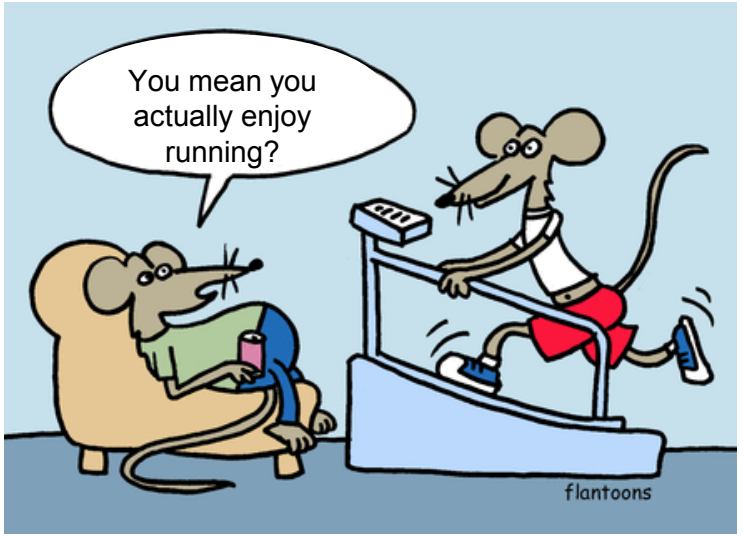
Image modified from: https://scienceabstracts.wordpress.com/2013/01/23/athletic-mice-have-bigger-mid-brains/

Background

- Sedentary lifestyles and increased palatable food consumption contribute to obesity and related metabolic comorbidities.
- Difficult to modify behavior (exercise, diet) and maintain these changes over the long term
- Not all individuals are prone to weight gain in an obesogenic environment, and differences in behavior may contribute to susceptibility vs. resistance (e.g., exercise may be more pleasurable to some, while palatable foods may be more pleasurable to others)
- Understanding of the neural mechanisms that regulate these behavioral differences is needed to optimize therapeutic strategies
- Critical need *to develop and validate a new mouse standardized test for exercise vs. palatable feeding preference*



Strategy

Vs.

- We will first characterize how much wheel-running and palatable drink intake an individual mouse engages in when either reward type is available *alone*. Then we will give concurrent access to both the running wheel and the drink solution for a *limited amount of time* to place the two types of reward in competition with each other, revealing the innate preference of the mouse (or the “ratio” of exercise-to-food).
- Our prediction is that mice that inherently have a relatively greater preference for exercise will be characterized by high amounts of running when the wheel is offered concurrently with palatable drink, whereas mice that inherently prefer the palatable food will be characterized by higher amounts of drink intake when it is offered concurrently with the running wheel.
- Initial experiments to optimize test parameters and validate the approach will be conducted in parallel at Cincinnati and Vanderbilt. Then the approach to will be taught to Davis personnel to ensure it can be readily adopted and replicated by other investigators/sites.

Anticipated outcomes

Exercise-to- Food Preference Test (EFPT) Phenotype	Distance run when offered wheel alone (R1)	Palatable drink intake when offered alone (S1)	When offered both running wheel and palatable drink:		
			Distance run (R2)	Drink intake (S2)	Expected ratio equations
Exercise-preferring	High	Variable (low-to-high)	High (similar to wheel alone)	Lower than drink alone	$\frac{R2}{R1} > \frac{S2}{S1}$
Palatable food-preferring	Variable (low-to-high)	High	Lower than wheel alone	High (similar to drink alone)	$\frac{R2}{R1} < \frac{S2}{S1}$
Similar Exercise/ Palatable food preferences	Variable (low-to-high)	Variable (low-to-high)	Lower than wheel alone	Lower than drink alone	$\frac{R2}{R1} = \frac{S2}{S1}$

Optimizing test parameters in male C57BL6/J mice

- Pre-exposure: 24 h access to running wheel and palatable drink (each alone) to overcome neophobia and confirm palatability
  - Did not consistently drink 0.1% saccharin (only 6/16 mice drank at least 1 ml during 24 hr)
  - Consistently drank 4% sucrose (16/16 mice drank at least 1 ml during 24 hr)
  - Consistently ran on wheel (16/16 mice ran at least 2 km during 24 hr)

- Proceeded to testing wheel running vs. 4% sucrose

Optimizing test parameters in male C57BL6/J mice

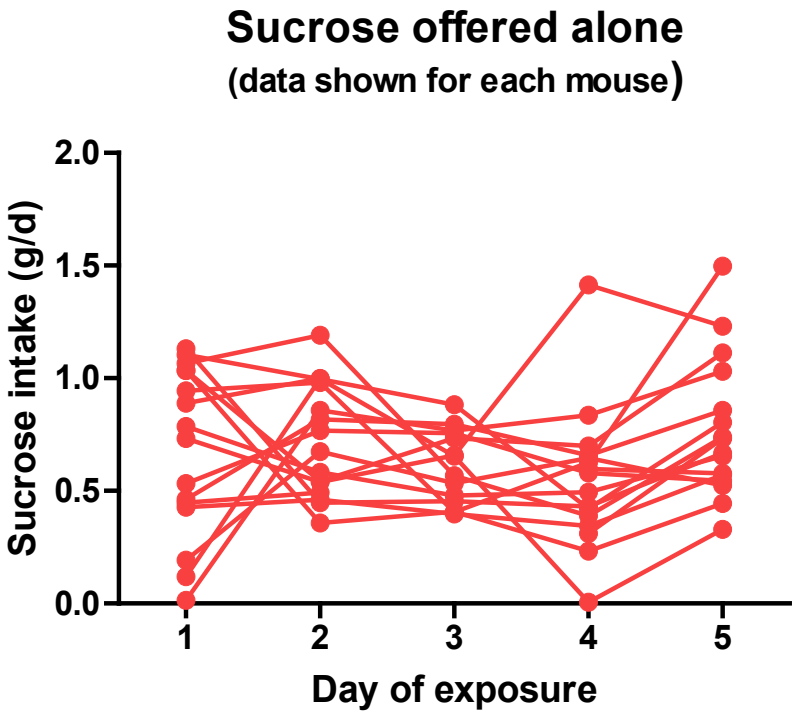
- For phases 1-3: Fasted at beginning of dark cycle to minimize potential confound of recent meals. Order of phases 1 and 2 are counter-balanced.
  - Phase 1: Sucrose alone. Access to a sucrose drink (4%) for 30 min in their home cage at two hours after the onset of the dark. The amount of sucrose consumed is recorded. This will be repeated daily for 5 days to assess stability across days.
  - Phase 2: Wheel alone. Access to running wheel for 30 min and the distance run is recorded. This will be repeated daily for 5 days to assess stability across days.
  - Phase 3: Co-exposure to sucrose and wheel. Access to both the sucrose drink and running wheel for 30 min in their home cage. This will be repeated daily for 4 days to assess stability across days.

Experimental design

Group	Week 1	Week 2	Week 3
Group A (n=8)	Sucrose alone	Wheel alone	Both sucrose and wheel
Group B (n=8)	Wheel alone	Sucrose alone	Both sucrose and wheel

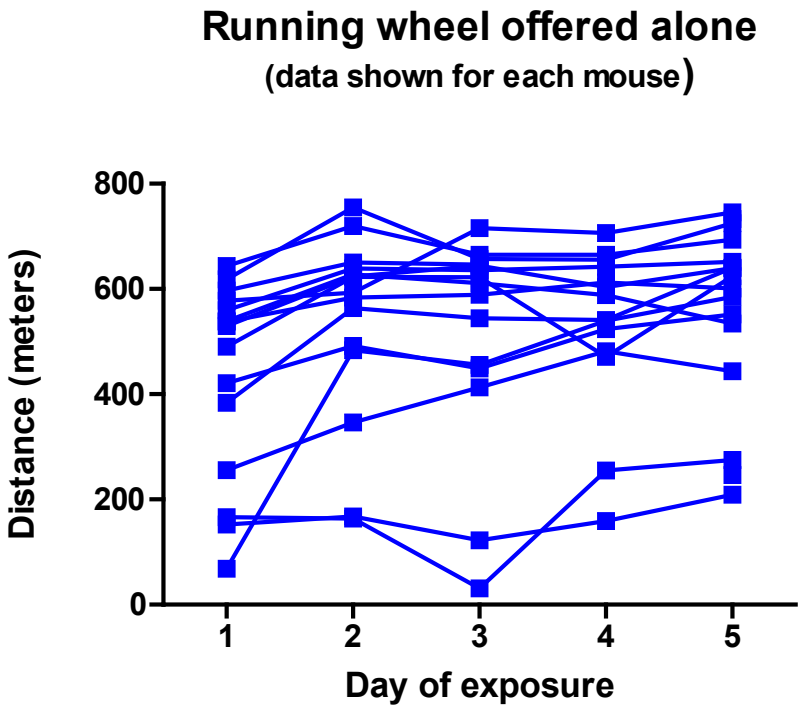
*There were no differences between groups A and B in any end point; data were then pooled across groups.

Phase 1- Sucrose intake when offered alone



⇒ High variability on day 1
⇒ Largely stabilizes on/after day 2
⇒ Large group sizes needed for behavioral studies

Phase 2- Wheel running when offered alone



⇒ High variability on day 1
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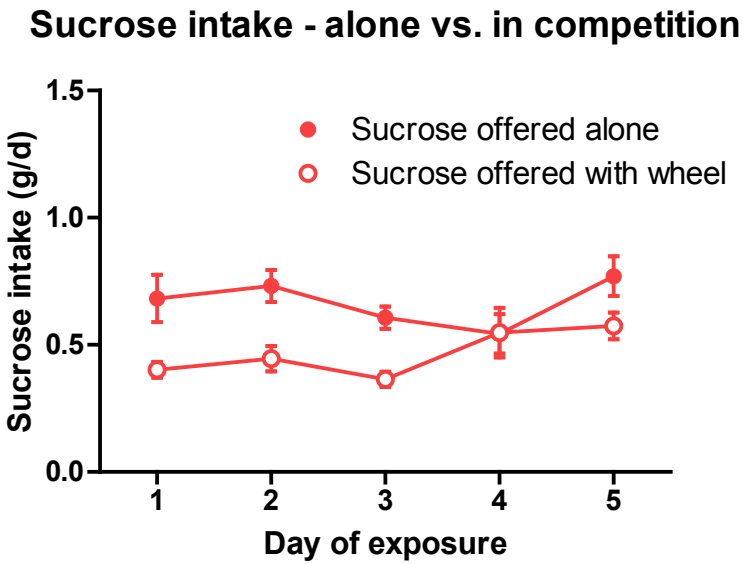
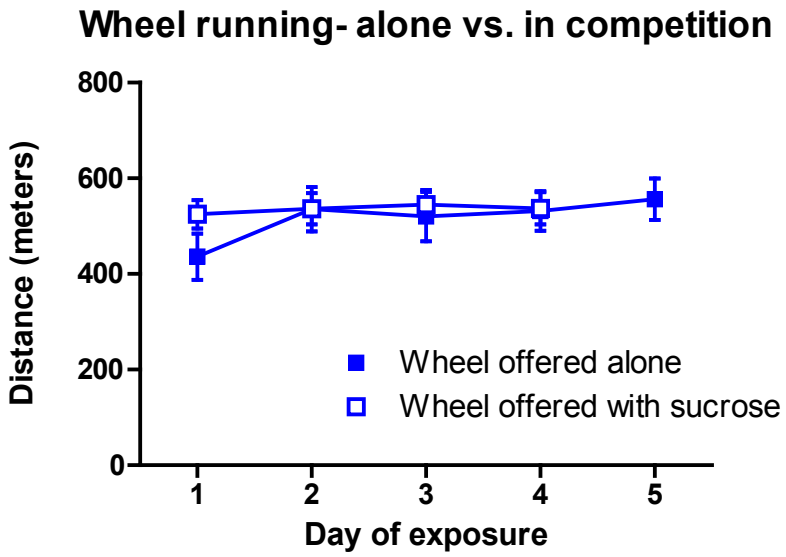
⇒ Large group sizes needed for behavioral studies

Phase 3- Sucrose intake and wheel running when offered in competition

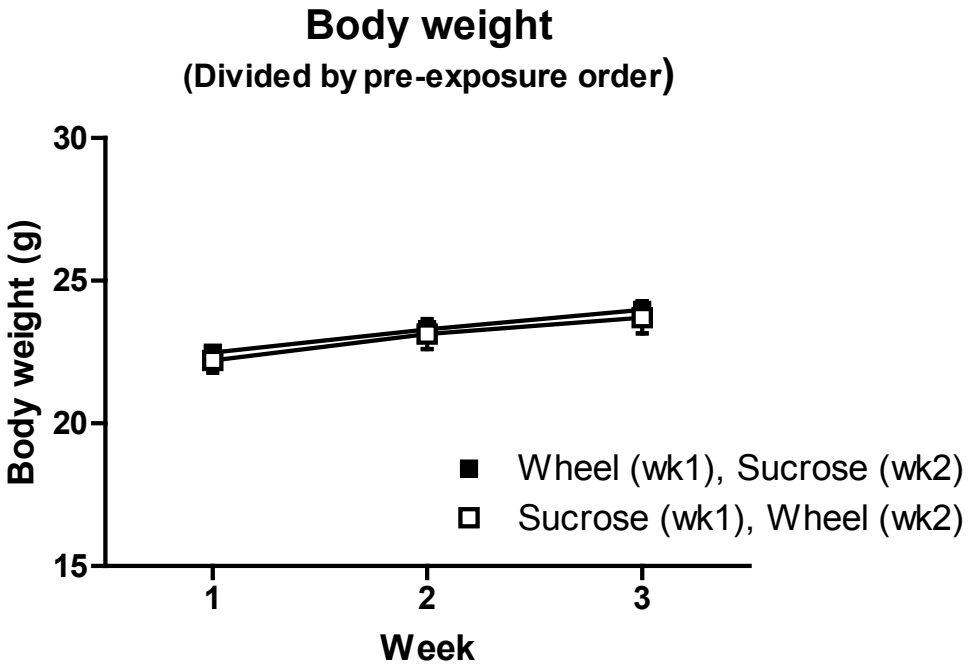




⇒ When offered in competition, wheel running is preserved while sucrose intake is reduced ~50%

⇒ Effect is present as early as day 1 of co-exposure

Body weight



⇒ Suggests sucrose intake and wheel running do not themselves alter body weight when limited to 30 min/day for up to 5 days

⇒ Important for minimizing interpretive confounds when using this assay in metabolic studies

Key aspects of experimental design

1. Pre-exposure (Include to prevent neophobia)
2. Palatable food type (4% sucrose drink works well)
3. Duration and timing of daily session (30-min sessions at 2 hrs after dark onset works well, along with fasting at onset of dark)
4. Order effects (Exposure order for sucrose-alone vs. wheel-alone does not alter results)
5. Body weight (not affected by limited sucrose and running exposure)
6. Number of daily sessions needed
   - 3 days each for exposure to drink and wheel alone (use average of data from days 2 and 3)
   - 1 day for co-exposure

Anticipated outcomes

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Discussion point: R2/R1 ÷ S2/S1 = single number that represents Exercise-to-Food Preference (EFP)

Next step:

- Vary sucrose concentration (1 vs. 10%)
- Sex differences
- Test the shortened paradigm

Longer-term goals:

- Test differences across multiple strains of mice that vary in their inherent preference for exercise vs. palatable food.
- Test differences among many individuals of the outbred Institute of Cancer Research (ICR) mouse stock to identify subsets of individuals with high vs. low exercise-to-food preference.
- Use RNAseq to profile mRNA expression in reward-related brain regions of banked brain samples to test the hypothesis that inter-individual or inter-strain differences in the exercise-to-food preference vary with the extent of neuronal activation, synaptic plasticity, and neuroinflammation in brain reward circuits. In addition, mRNA expression, metabolomics and proteomics analysis of banked peripheral tissues (e.g., blood, muscle, liver) can be performed to test the hypothesis that the phenotype of these tissues varies with exercise-to-food preference.
- Lots of working group discussion on other cool things that this assay can be used for once validated- diet, obesity, aging, circadian influences, etc., as well as importance of working towards future publication of results.

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Potential benefit to the MMPC

This proposal seeks funding to develop a new behavioral testing paradigm designed specifically for use in mice, thereby leveraging the unique opportunities provided by the many available inbred mouse strains and transgenic mouse lines.

If successful, the standardized test developed under this proposal has the potential to provide new services offered by the MMPC to determine and perhaps amend preferences toward palatable feeding and exercise.

Furthermore, this standardized test will provide a platform that can then be used by MMPC customers to ask a number of critical questions, including:

- How does brain reward system activity (e.g., ventral tegmental area, nucleus accumbens, medial prefrontal cortex) differ among individuals that have inherent differences in exercise-to-food preference?
- How do the metabolic adaptations (e.g., hypothalamus/brainstem, muscle, liver, adipose tissue) that occur in response to exercise vary with differences in exercise-to-food preference?
- How does diet alter an individual's exercise-to-food preference?
- Do differences in an individual's exercise-to-food preference predict the propensity to develop diet-induced obesity and its associated comorbidities?
- How does the exercise-to-food preference vary with estrus cycle stage?
- How does the exercise-to-food preference vary with aging?
- How do early life conditions alter the exercise-to-food preference later in life?
- How does chronic stress (or the opposite condition of environmental enrichment) alter the exercise-to-food preference?
- How does ambient temperature (thermoneutrality) affect the exercise-to-food preference?
- How do various genetic or pharmacological interventions alter the exercise-to-food preference?