Food Preference in Male Sprague Dawley Rats Based on Neural Mechanism Reconfiguration Following Exercise Contributing to Long Term Body Weight Maintaining Effects

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Abstract

Exercise has shown to affect the homeostatic centers within the hypothalamus and has shown to alter food preference through modification of neural reward networks. The experiments in this study investigated how exercise effects food preference consisting of three mono-macronutrients diets of sugar, fat, and protein in male Sprague Dawley (SD) Rats. The experimental group was exercised on treadmills during weekdays only, and the sedentary group was the negative control. Our hypothesis was exercise reduced preference for palatable food such as the sugar and fat diets. Our results showed all rats in both groups to have a preference for the fat diet initially before exercise was implemented. During the workout protocol, exercised rats showed a better glucose metabolization according to the intraperitoneal glucose tolerance test (IPGTT) and an improvement in endurance. Post exercise protocol, the exercise group exhibited a significantly reduced preference to sugar diet, without a significant difference in the caloric intake of both chow diet and food preference diet, and a greater resistance to weight gain. The results were concurrent with FOS-B Immunohistochemical staining of the hypothalamic arcuate nucleus (ARC), where the exercised rats displayed a greater neural activation, indicating potential neural circuit reconfiguration. The goals of future investigations would be to identify the phenotype of the neurons activated.

Keywords: food preference, macronutrients, treadmills, glucose tolerance, weight maintenance, Immunohistochemical staining

Introductions

Behavioral responses to physiological demands have underlying neural circuits, analysis of which give an understanding of how neural networks work to navigate a complex environment. Exercise is a physiological stress that can produce behavioral responses like a preference to a diet, due to shifts in neural mechanisms that deal with hunger. However, Exercise has shown to have beneficial effects on both physical and mental health.

Within the hypothalamic arcuate nucleus (ARC) , two well studied neural populations are defined by the expression of either agouti-related peptide (AgRP / hunger inducing) or proopiomelanocortin (POMC / hunger inhibiting)-derived peptides, that control feeding behavior. Additionally, there are critical circuits in the brain that dictate behavior. The Lateral Parabrachial Nucleus (LPBN) is involved in mediating taste and reward function and have shown to have connection to dopaminergic neurons in the ventral tegmental area (VTA), which is a major source of dopamine producer in the brain and a strong correlation to reward processing and motivation. The VTA project into the nucleus accumbens (NAc), which is responsible for dopamine signaling.

Previous studies have supported the idea of a decrease in palatable diet after exercise. A clinical study consisting of 46 overweight/obese adults (16 males/30 female) were part of 12-weeks exercise intervention. Following the intervention, the results revealed compared to those the exercised adults show a reduced wanting to be high-fats compared to the non-exercising group, (Beaulieu et al, 2020). Another study experimented wheeling running effect on diet preference in SD rats. The rats were split into four groups — a sedentary group, one with a high fat diet and another with a high sucrose diet, a two-wheel running group, one with a high fat diet and another with a high sucrose diet. The result of the study following 6 days of exercise and 1 weeks off showed that exercised rats displayed a reduced preference for high fat and high sucrose diets compared to sedentary rats, (Moody et al 2015). However, the option of a protein diet was not given.

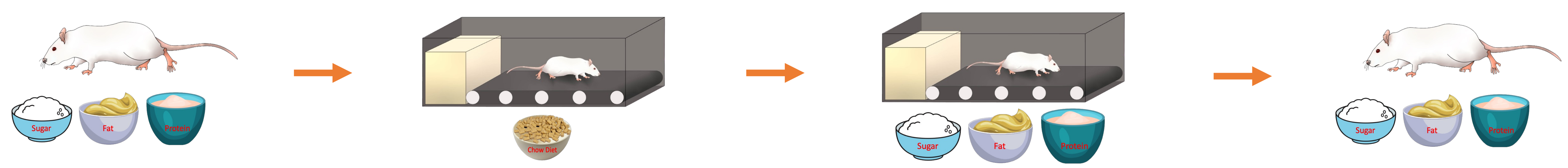
Our hypothesis is that exercised rats will have a reduction for palatable diets such as the fat and sugar diets compared to sedentary rats. The hypothesis was tested in our experiment analyzing the difference between baseline food preference diet and post exercise food preference in male Sprague Dawley Rats with sedentary rats serving as a negative control. The test subjects were male Sprague Dawley Rats due to their docile nature, additionally, rats display better differentiation in specific behaviors making it easier for analysis and all male subjects allow for removal of con-founding variables. Overall, the projects would allow us to evaluate how the brain influences motivation (eating) to respond to physiological needs (effect of exercising) in relation to how the brain sense, process, and prioritize sensory information (type of food) to guide behavior (food preference).

Methods

Male Sprague Dawley Rats separated into two groups:
- Exercised Rats - experimental group
- Sedentary Rats - negative control group

Important Notes:
- Food preference diet are sugar diet (3.3 kilocalories per gram), fat diet (6.9 kilocalories per gram), and protein diet (3.2 kilocalories per gram)
- Exercise only occurred on weeks days
- During the 6 weeks time period intraperitoneal glucose tolerance test (IPGTT)[6] was done on both rat groups. Glucose is intraperitonially injected and blood samples are extracted at different times to analyze glucose levels.

Exercise group	Food preference diet and no exercise	Chow diet and Progressive exercise training from week 1 to week 6 respectively • Range of speeds: 8 meters per minute to 20 meters per minute • Range of exercise session: 30 minutes to 50 minutes	Food preference diet and exercise based on week 6 progressive training protocol	Food preference diet and no exercise
	18 days	6 weeks	2 weeks	2 weeks
Sedentary group	Food preference diet and no exercise	Chow diet and sedentary session in the treadmills equivalent to exercise time in the experimental group in corresponding weeks	Food preference diet and equivalent sedentary session time	Food preference diet and no exercise

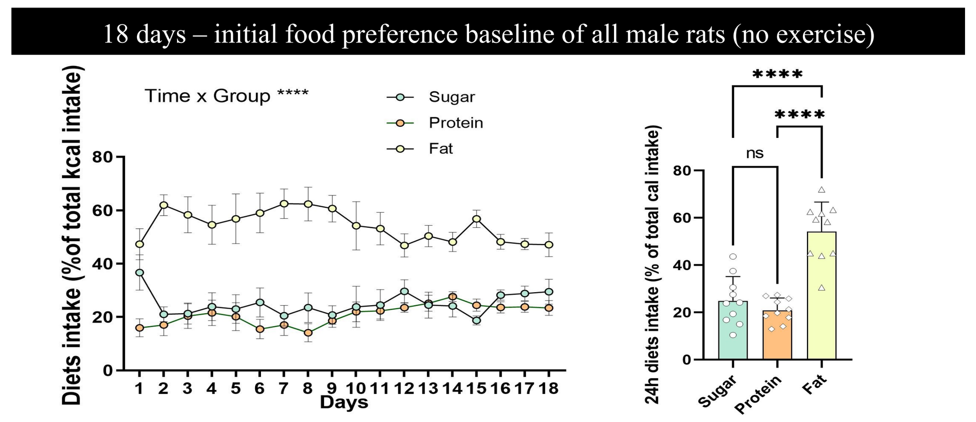


Following the first part of the experiment

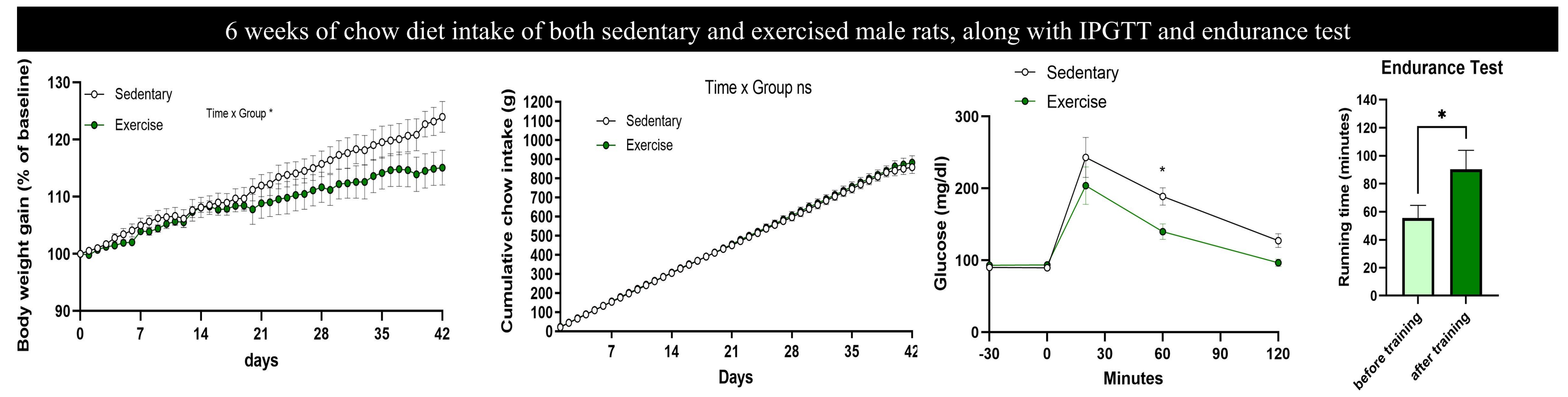
- Perfusion and fixation
- Immunohistochemical microscopy to detect FOS-B marker of chronic neural activation[3,7] in sedentary versus exercised rats

Results

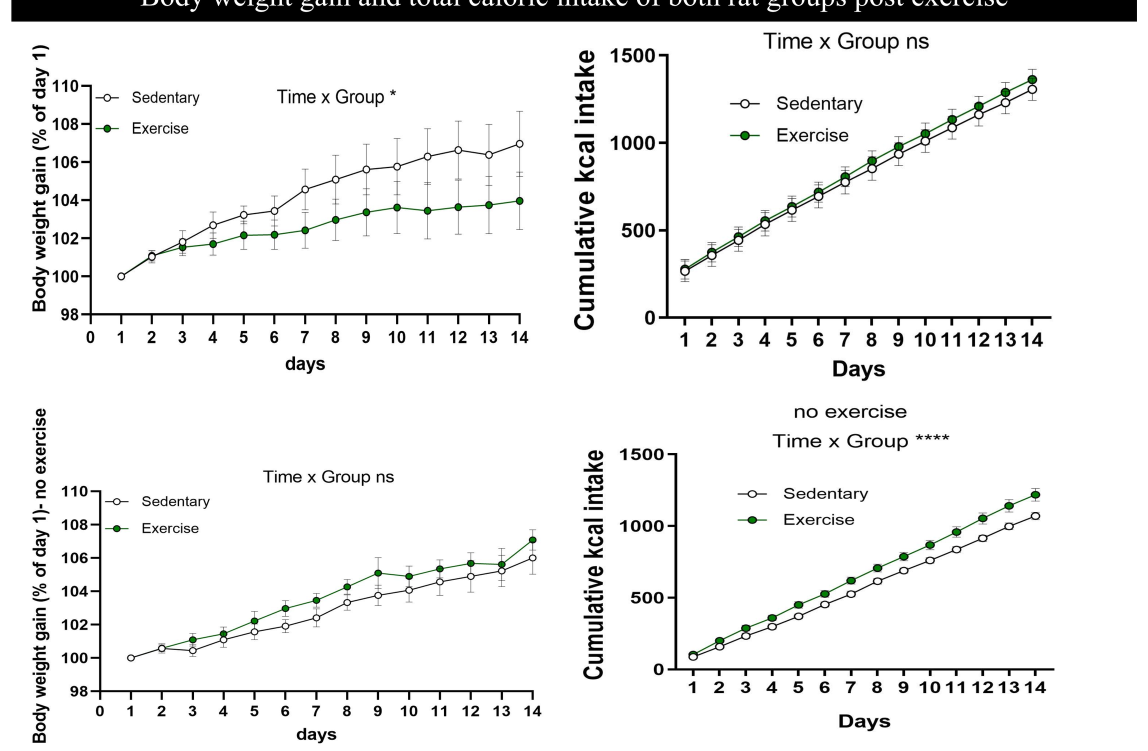
18 days – initial food preference baseline of all male rats (no exercise)



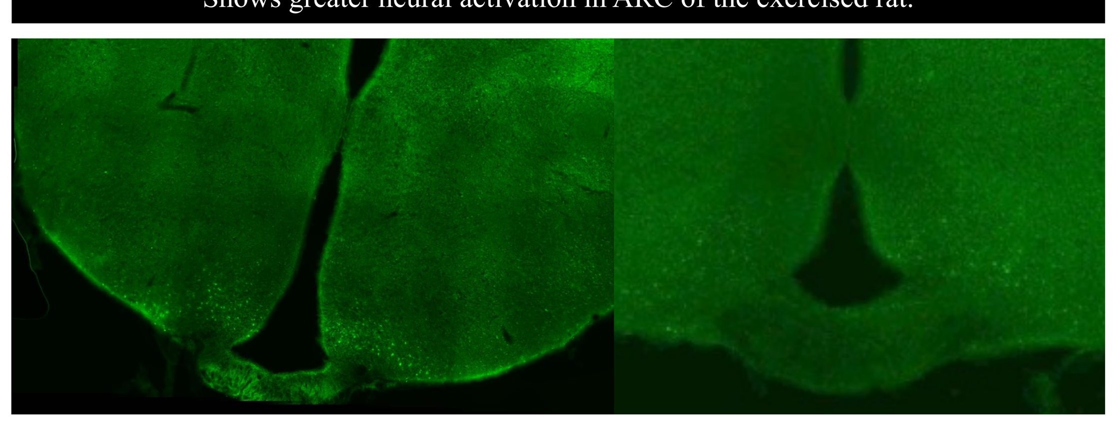
6 weeks of chow diet intake of both sedentary and exercised male rats, along with IPGTT and endurance test



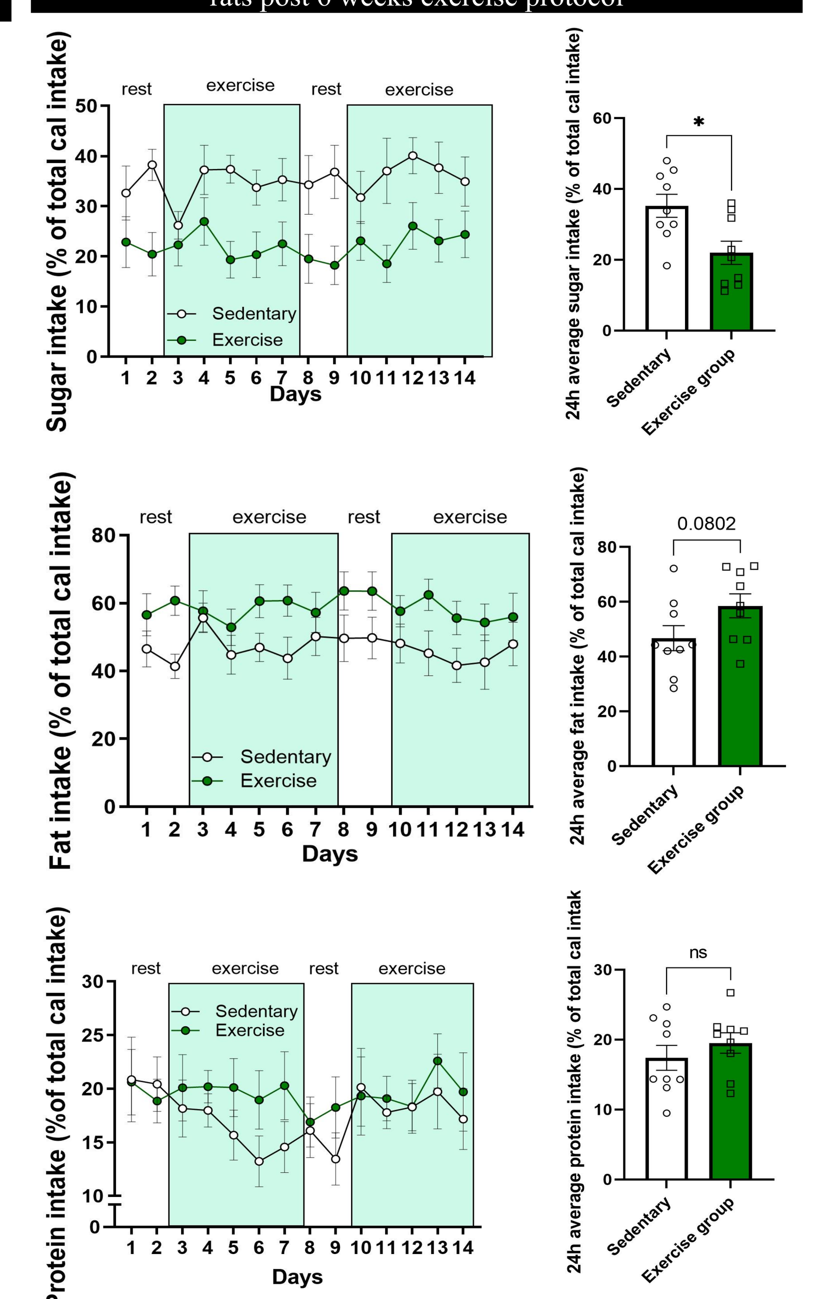
Body weight gain and caloric intake of both rat groups post 6 weeks exercise protocol V.S. Body weight gain and total caloric intake of both rat groups post exercise



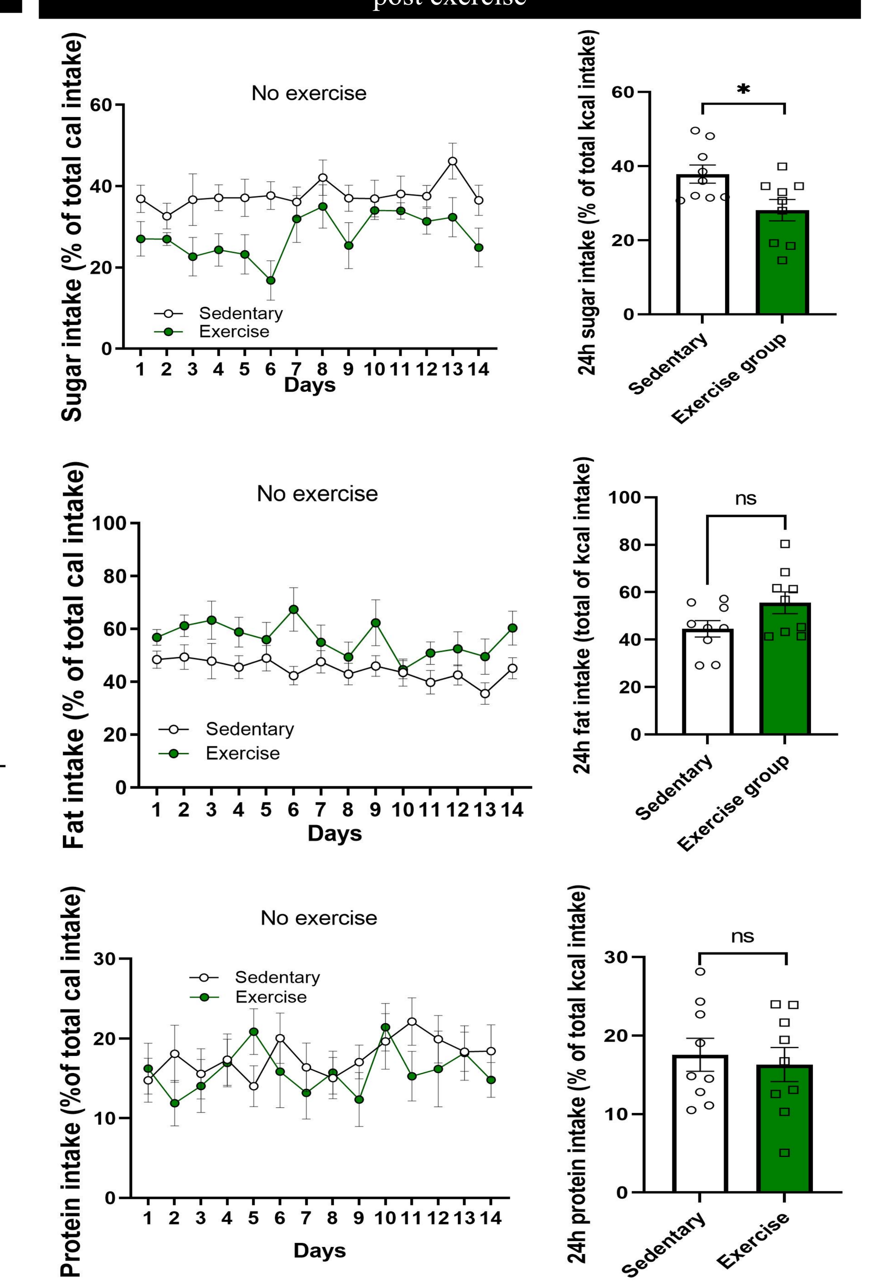
FOS – B Immunohistochemical Staining of exercised rat (right) V.S. sedentary rat (left) near the hypothalamic arcuate nucleus (ARC).
Shows greater neural activation in ARC of the exercised rat.



2 weeks - Food preference diet in exercised rats vs sedentary rats post 6 weeks exercise protocol



2 weeks - Food preference diet in exercised rats vs sedentary rats post exercise



Conclusion

Initial preference (18 days / food preference diet / no exercise)
- food preference in all male Sprague Dawley rats showed a significant preference toward fat diet over sugar and protein diet and no significant preference between the latter two diets

During the 6-week exercise protocol (42 days / chow diet / exercise)
- No significant difference in cumulative chow intake between sedentary and exercised rats
  - A significant difference was seen in body weight gain between exercised rats and sedentary rats
  - IPGTT shows a significant difference in blood glucose level after 60 minutes. A lower blood glucose level in exercised rats after 60 minutes indicates better glucose metabolization.
- Added endurance test, shows a significant increase in endurance in exercised rats after training

2 weeks post 6 weeks exercise protocol (14 days / food preference diet / exercise)
- Exercised rats significantly continued to gain less weight than sedentary rats
  - Even with no significant difference in caloric intake of preference diets
- Significant difference in sugar diet preference only; exercised rats showed reduced preference to sugar diet than sedentary rats
- No significant difference in preference of fat diet or protein diet between the exercised rats and the sedentary rats

2 weeks Post Exercise (14 days / food preference diet / no exercise)
- Exercised rats significantly continued to gain less weight than sedentary rats
  - Even with no significant difference in caloric intake of preference diets
- Significant difference in sugar diet preference only; exercised rats showed reduced preference to sugar diet than sedentary rats
- Similar results to previous post 6-week exercise protocol

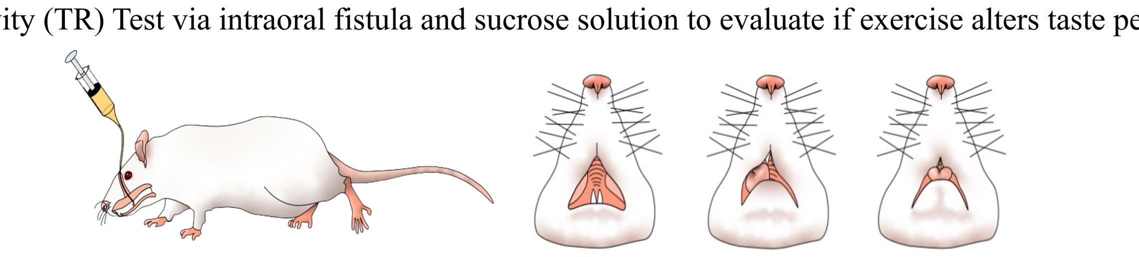
Immunohistochemical staining[5] of FOS-B of hypothalamic arcuate nucleus (ARC) in both an exercised rat and sedentary rat, showed greater neural activation in the ARC region of an exercised rat compared to a sedentary rat.
- Possible implications of neural circuit reconfiguration since the ARC contains critical neural nodes involved in the regulation of feeding behaviors

Future Direction and Challenges

Investigate the neuronal mechanism behind changes in food preference occurring following exercise:

1. The results from this experiment showed neural activity in the hypothalamic arcuate nucleus but is unknown whether the activated neurons in the ARC were POMC and NPY/AgRP
   - Specific Immunohistochemical staining to evaluate the phenotype of the neurons activated

2. Taste Reactivity (TR) Test via intraoral fistula and sucrose solution to evaluate if exercise alters taste perception



3. Introduction of anorectic treatment (causes loss of appetite) along with exercise to determine alteration in food preference

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References

1. Beaulieu, K., Hopkins, M., Gibbons, C., Oustric, P., Caudwell, P., Blundell, J., & Finlayson, G. (2020). Exercise training reduces reward for high-fat food in adults with overweight/obesity - PubMed. *Medicine and Science in Sports and Exercise*, *52*(4). https://doi.org/10.1249/MSS.0000000000002205

2. Bell, B. B., Harlan, S. M., Morgan, D. A., Guo, D.-F., & Rahmouni, K. (2018). Differential contribution of POMC and AgRP neurons to the regulation of regional autonomic nerve activity by leptin. *Molecular Metabolism*, *8*. https://doi.org/10.1016/j.molmet.2017.12.

3. Bullitt, E. (1990). Expression of c-fos-like protein as a marker for neuronal activity following noxious stimulation in the rat - PubMed. *The Journal of Comparative Neurology*, *296*(4). https://doi.org/10.1002/cne.902960402

4. Goldstein, N., McKnight, A. D., Carty, J. R. E., Arnold, M., Betley, J. N., & Alhadeff, A. L. (2021). Hypothalamic detection of macronutrients via multiple gut-brain pathways. *Cell Metabolism*, *33*(3). https://doi.org/10.1016/j.cmet.2020.12.018

5. Im, K., Mareninov, S., Diaz, M. F. P., & Yong, W. H. (2019). An introduction to Performing Immunofluorescence Staining. *Methods in Molecular Biology (Clifton, N.J.)*, *1897*. https://doi.org/10.1007/978-1-4939-8935-5_26

6. International Mouse Phenotyping Consortium . (n.d.). *Intraperitoneal glucose tolerance test (IPGTT) Protocol - IMPReSS*. IMPC. Retrieved August 24, 2022, from https://www.mousephenotype.org/impress/ProcedureInfo?action=list&procID=53

7. Madsen, Bolwig, & Mikkelsen. (n.d.). Differential Regulation of c-Fos and FosB in the Rat Brain After Amygdala Kindling. *Cellular and Molecular Neurobiology*, *26*(1), 87–100. https://doi.org/10.1007/s10571-006-9202-1

8. Moody, L., Liang, J., Choi, P. P., Moran, T. H., & Liang, N.-C. (2015). Wheel running decreases palatable diet preference in Sprague-Dawley rats. *Physiology & Behavior*, *150*. https://doi.org/10.1016/j.physbeh.2015.03.019