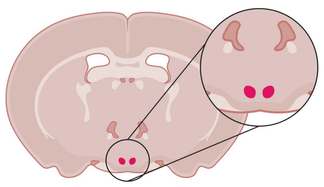
Acute Stress Decreases Glutamate Transmission in the Female Rat Dorsomedial Hypothalamus

Ruby Muzzatti, Sarah Wilson, Lara Swart, Dr Karen Crosby
Department of Biology, Mount Allison University, Sackville, New Brunswick, Canada

NSERC CRSNG

BACKGROUND

Stress response mechanisms have not adapted to our high stress society and landscape of high calorie, highly palatable foods[1]. **Women** are particularly vulnerable to **disordered eating** behaviours when **stressed**[2], for which the neurophysiological basis is unclear. Yet, female research subjects remain underrepresented.

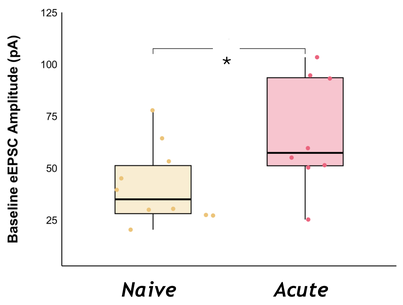


The **dorsomedial hypothalamus (DMH)** is a brain region involved in appetite and body weight regulation[3], and the stress response[4]. Some DMH neurons increase appetite and have receptors for **stress hormones**[5]. How stress affects DMH neurons is unclear.

How acute stress in ***females*** affects **glutamatergic** transmission in the DMH is unknown.

RESULTS

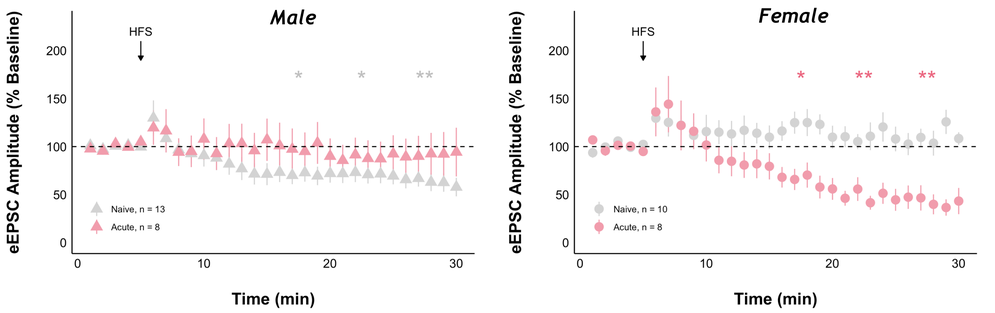
How does acute stress in ***females*** affect baseline glutamate transmission?



An unpaired *t*-test was to compare between groups.
* = *p*-value < 0.05

There is a significant **increase** in eEPSC amplitude.

What about long term changes?

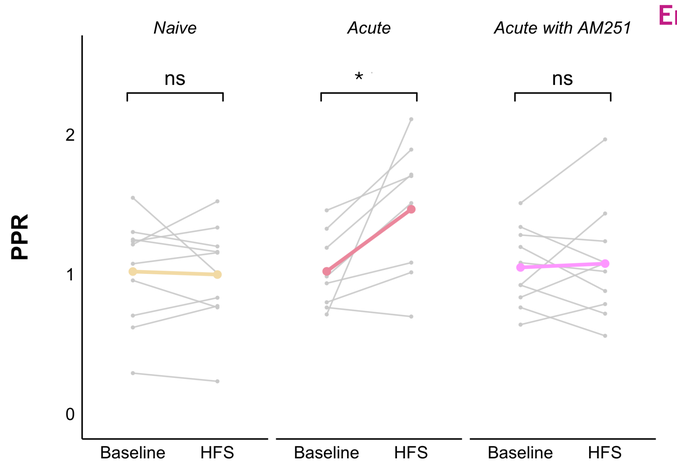


Naive female data collected by Lara Swart, male data collected by Sarah Wilson.
A paired *t*-test was used to compare each 5-minute interval to the 5-minute baseline period.
* = *p*-value < 0.05, ** = *p*-value < 0.01

Acute stress in ***females*** triggered a **long-lasting depression** in glutamate transmission compared to naïve, but **not in *males***.

Is the depression due to a presynaptic decrease in glutamate onto DMH neurons during acute stress?

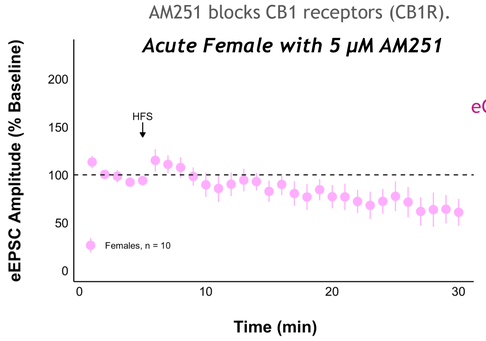
What causes the ***female*** depression?

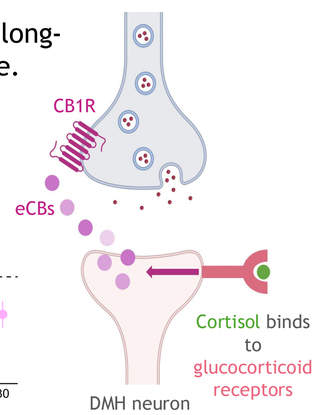


A paired *t*-test was used to compare between baseline and post HFS.
The PPR is calculated as the evoked current amplitude of peak 2/peak 1.

Endocannabinoids (eCBs) can trigger a long-lasting decrease in glutamate release.

AM251 blocks CB1 receptors (CB1R).





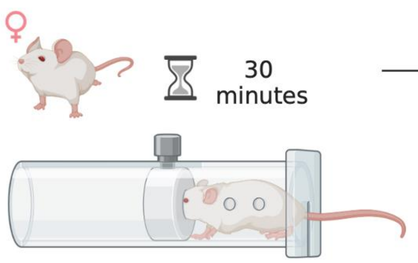
When eCB-CB1 receptors are **blocked** there is no longer a long-lasting decrease in glutamatergic current amplitude under acute stress.

The PPR **significantly increased**, indicating a lower probability of glutamate release onto DMH neurons under acute stress, but there is no change in PPR when eCB-CB1 receptors are **blocked** under acute stress.

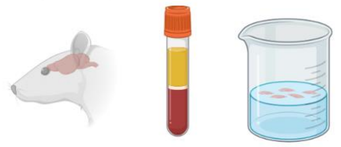
METHODS

Experiments were performed according to **protocol #104140** approved by the **Mount Allison University Animal Care Committee** in accordance with the **Canadian Council on Animal Care Guidelines**

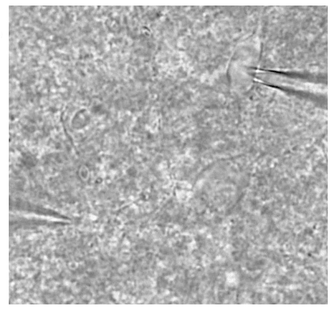
Young, ***female*** Sprague-Dawley rats were exposed to a single **restraint stress**



They were anesthetized, euthanized, and their **brains** were quickly **removed**



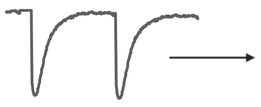
A **blood sample** was collected



Recording electrode inserted into **DMH neurons**, and a stimulating electrode into surrounding tissue to evoke **excitatory** postsynaptic currents (eEPSC) at 0.2 Hz

250 µm coronal brain slices containing the **DMH** were kept alive in oxygenated **artificial cerebrospinal fluid** kept at 32.5 °C

50 µM picrotoxin was applied to observe **glutamate** synapses



Living neurons were recorded from before and after high frequency stimulation (HFS) to observe long-lasting changes

HFS Protocol: 100 Hz for 4 seconds, twice, 20 seconds apart

Data analysis was performed using patchclampplotteR by **Christelinda Laureijs**[6]

CONCLUSIONS

- There are sex differences in glutamate transmission in the rat DMH.
- ***Females*** who are acutely stressed have more glutamate transmission onto DMH.
- There is a long-lasting decrease in glutamate current amplitude and probability of presynaptic glutamate release under acute stress.
- The decreases in eEPSC amplitude and presynaptic glutamate release during acute stress are **no longer significant** when endocannabinoid-CB1 receptors are blocked.

FUTURE DIRECTIONS

Future work aims to determine:

- The effect of chronic (repeated) stress (**work in progress**) on the ***female*** rat DMH.
- How corticosterone is involved in the change in glutamate transmission under acute stress.
- The effect of stress on neuronal excitability.

REFRENCES
Schematic Images created using BioRender.
1. Dallman MF, Pecoraro NC, La Fleur SE, et al. Glucocorticoids, chronic stress, and obesity. In: *Progress in Brain Research*. Vol 153. Elsevier; 2006:75-105. doi:10.1016/S0079-6123(06)53004-3
2. Adam TC, Epel ES. Stress, eating and the reward system. *Physiology & Behavior*. 2007;91(4):449-458. doi:10.1016/j.physbeh.2007.04.011
3. Bellinger LL, Bernardis LL. The dorsomedial hypothalamic nucleus and its role in ingestive behavior and body weight regulation. *Physiology & Behavior*. 2002;76(3):431-442. doi:10.1016/S0031-9384(02)00756-4
4. Crosby KM, Bains JS. The intricate link between glucocorticoids and endocannabinoids at stress-relevant synapses in the hypothalamus. *Neuroscience*. 2012;204:31-37. doi:10.1016/j.neuroscience.2011.11.049
5. Cintra A, Fuxe K, Wikstro¨m AC, Visser T, Gustafsson JA. Evidence for thyrotropin-releasing hormone and glucocorticoid receptor-immunoreactive neurons in various preoptic and hypothalamic nuclei of the male rat. *Brain Research*. 1990;506(1):139-144. doi:10.1016/0006-8993(90)91210-8
6. Laureijs C. *patchclampplotteR: Plot and Analyze Raw Patch Clamp Electrophysiology Data* [R package]. Version 0.1.0. 2025

ACKNOWLEDGMENTS
I want to thank my supervisor, Dr Crosby, for her continued guidance and support. I want to express my gratitude to Jackie Jacob-Vogels for her dedication to and passion for incredible animal care, from which I have learned so much.
This project was supported by a Mount Allison University Independent Student Research Grant (ISRG) and a Natural Science and Engineering Research Counsil of Canada (NSERC) Undergraduate Student Research Award (USRA).

Mount Allison UNIVERSITY