Acute Stress Decreases Glutamate Transmission through Endocannabinoid-CB1 Receptors in the Female Rat Dorsomedial Hypothalamus

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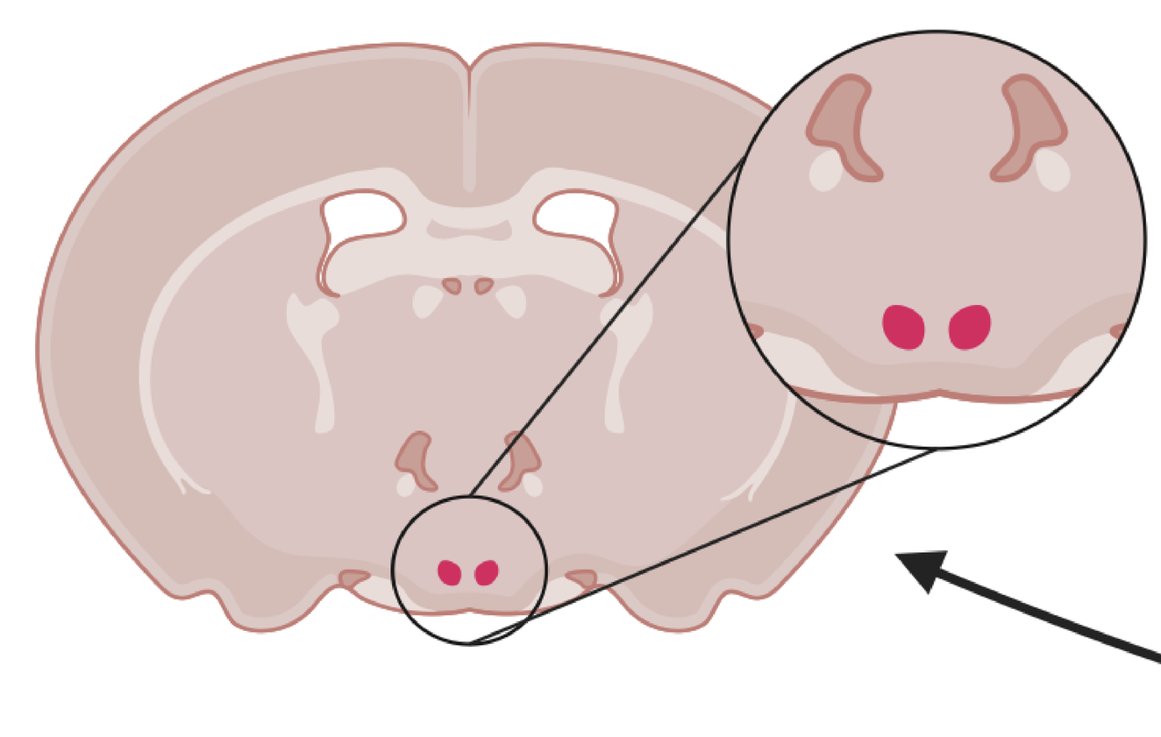
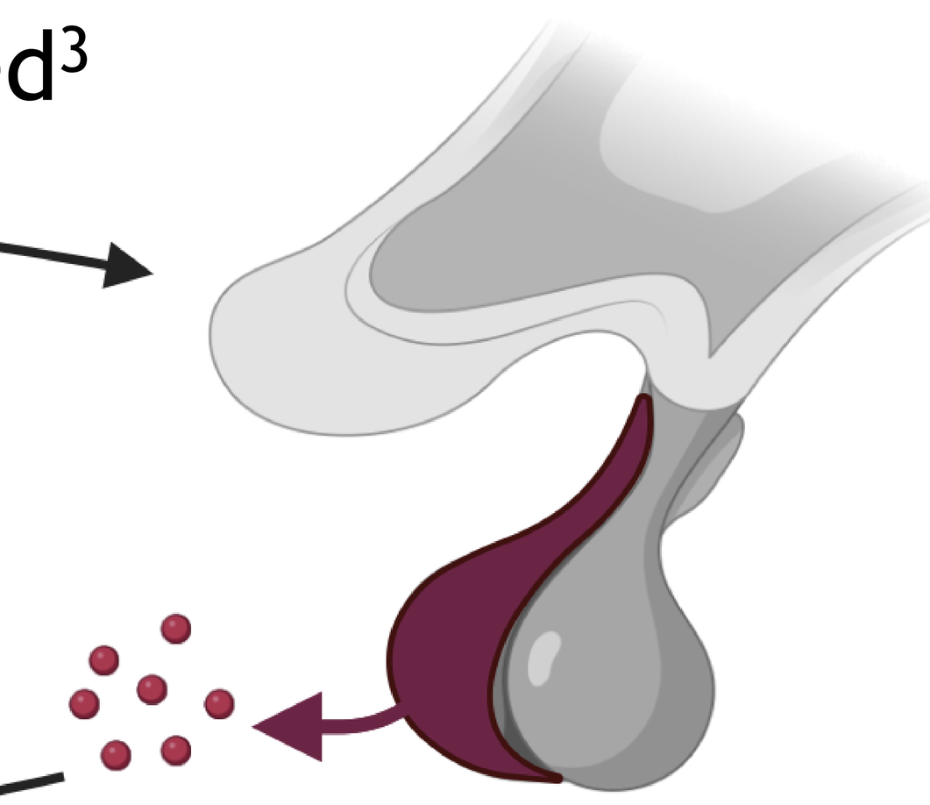
BACKGROUND

The **dorsomedial hypothalamus (DMH)** is a brain region involved in appetite and body weight regulation[1], and the stress response[2]



Using glutamate and GABA, the DMH projects to the paraventricular nucleus where **stress hormones** are produced[3]

DMH neurons have receptors for **stress hormones**[4]



Previous work by **Sarah Wilson** (unpublished) found that **acute stress** in ***male*** rats:

- Did **not change** the strength of glutamate synapses (compared to long-term depression seen in naïve *males*)

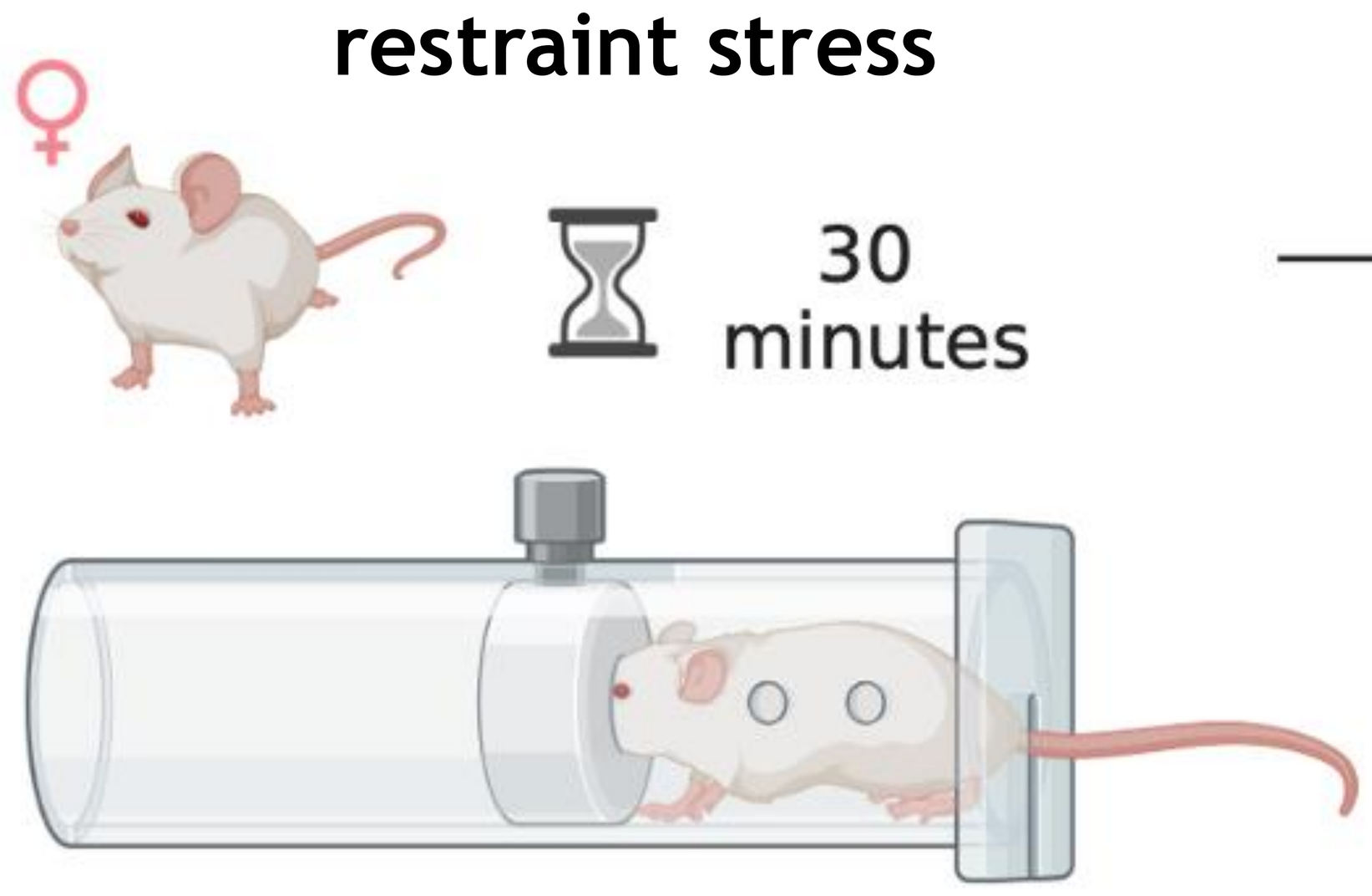
The relationship between eating behaviours and stress is complex. Risk factors for increased food consumption under stress include being female, overweight, or having a history of food restriction[5]

How **acute stress** in ***females*** affects **glutamatergic DMH transmission** to ultimately influence appetite is **unknown**

METHODS

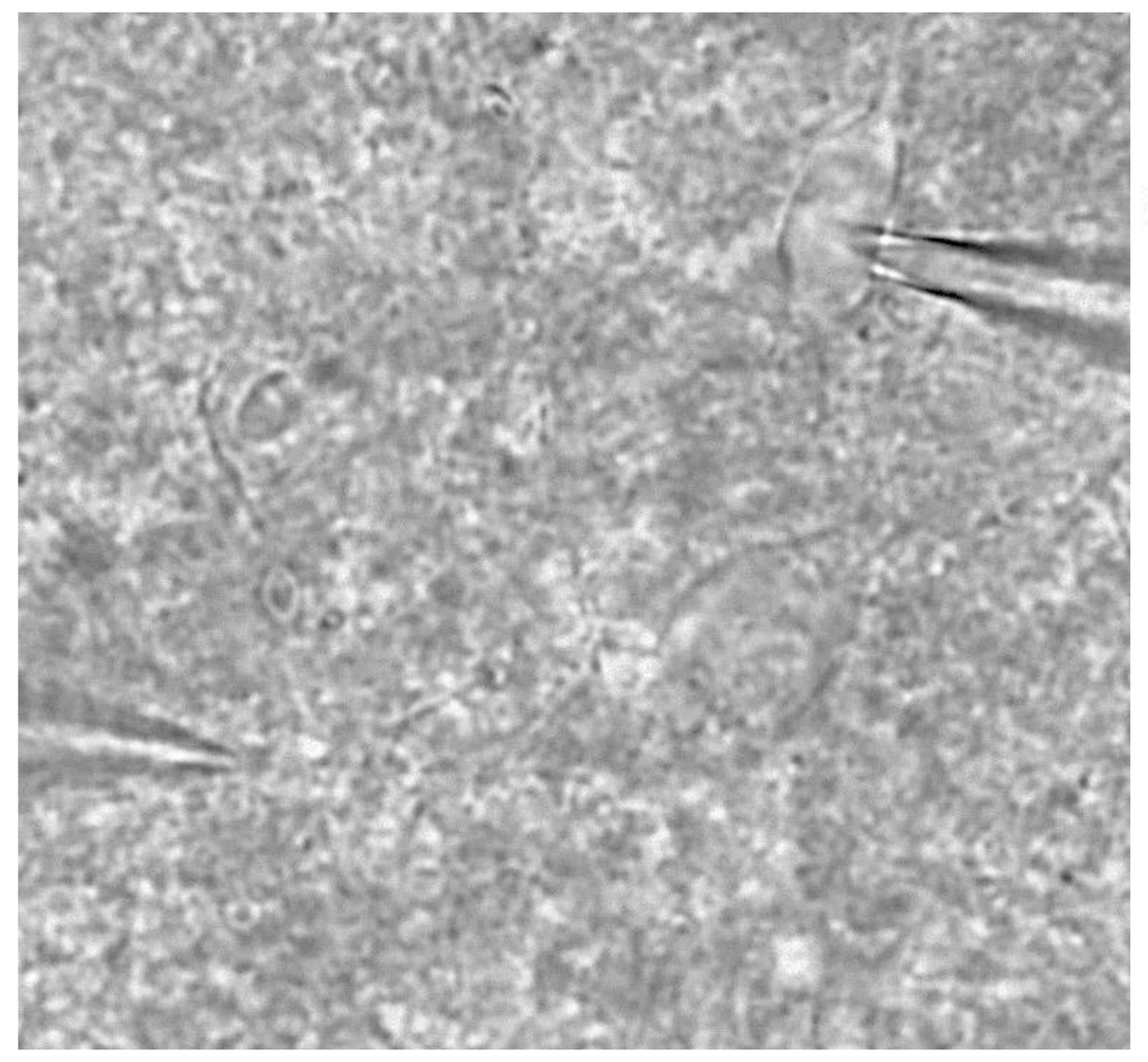
All experiments were performed according to **protocol #104140** approved by the **Mount Allison University Animal Care Committee** in accordance with the **Canadian Council on Animal Care Guidelines**

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



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

Immediately following brain removal, a carotid **blood sample** was collected



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

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

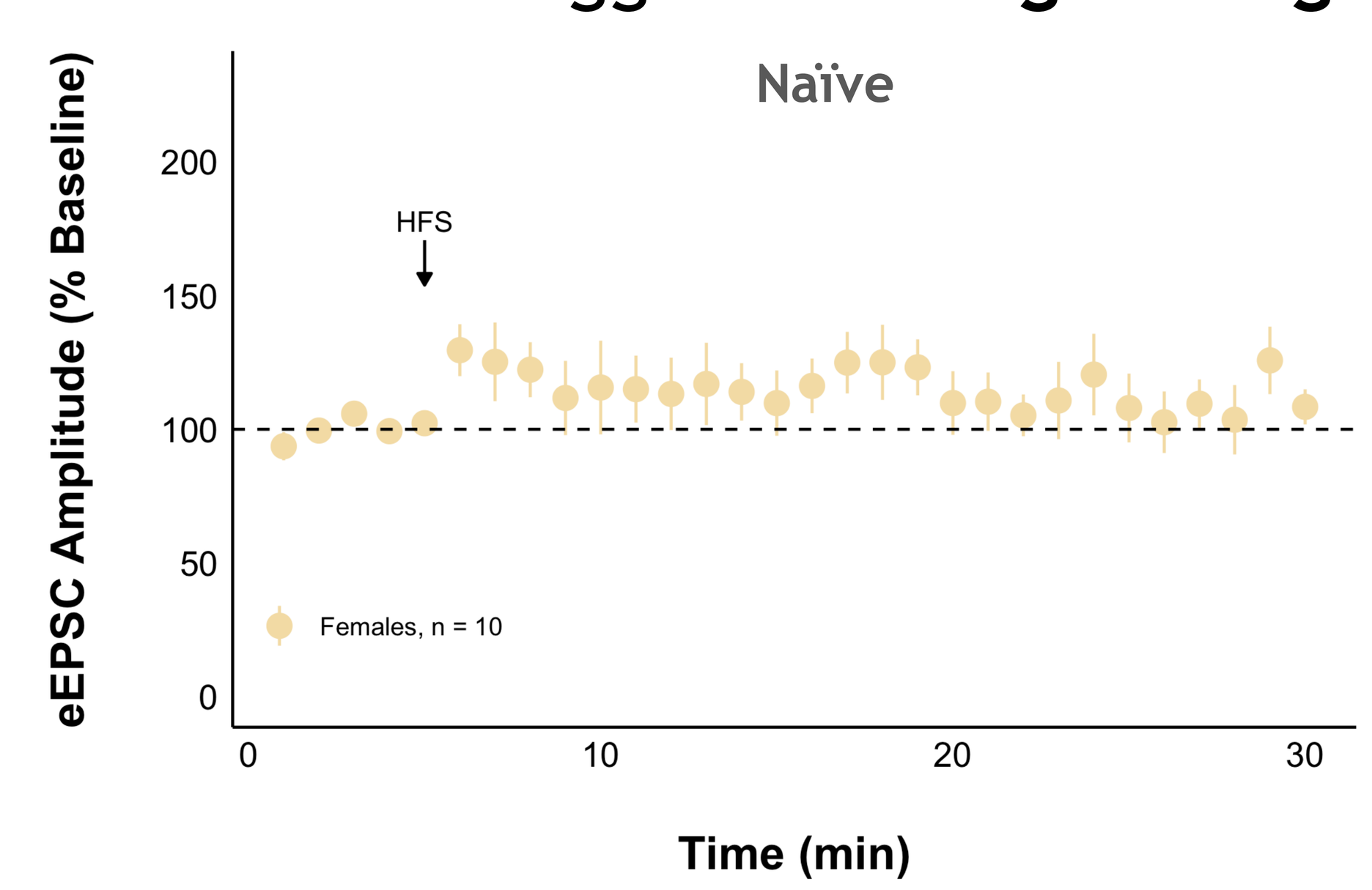
Living neurons were recorded from before and after high frequency stimulation (HFS)

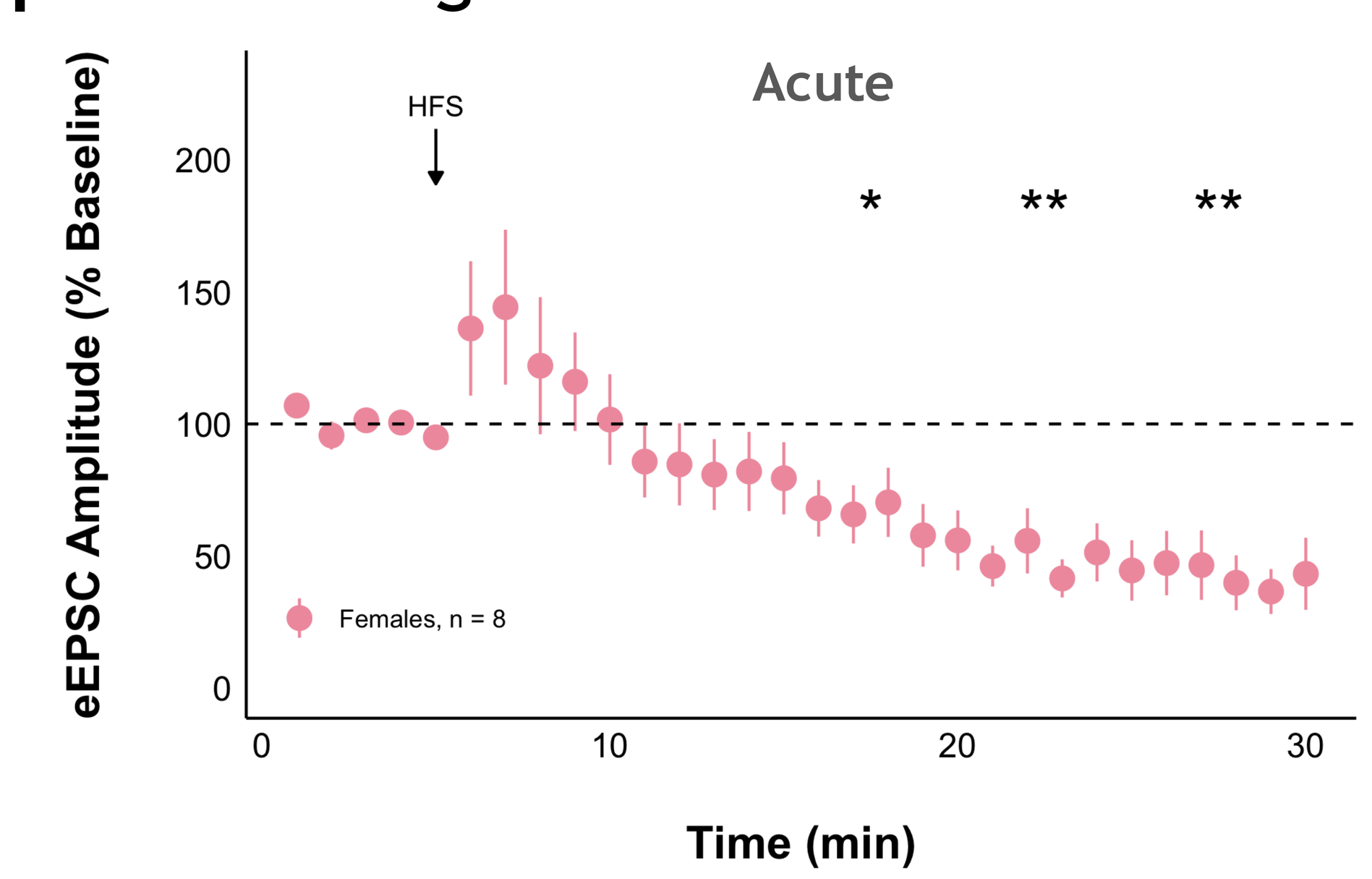
100 Hz for 4 seconds, repeated twice, 20 seconds apart

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

RESULTS

Acute stress triggered a **long-lasting depression** in glutamate transmission

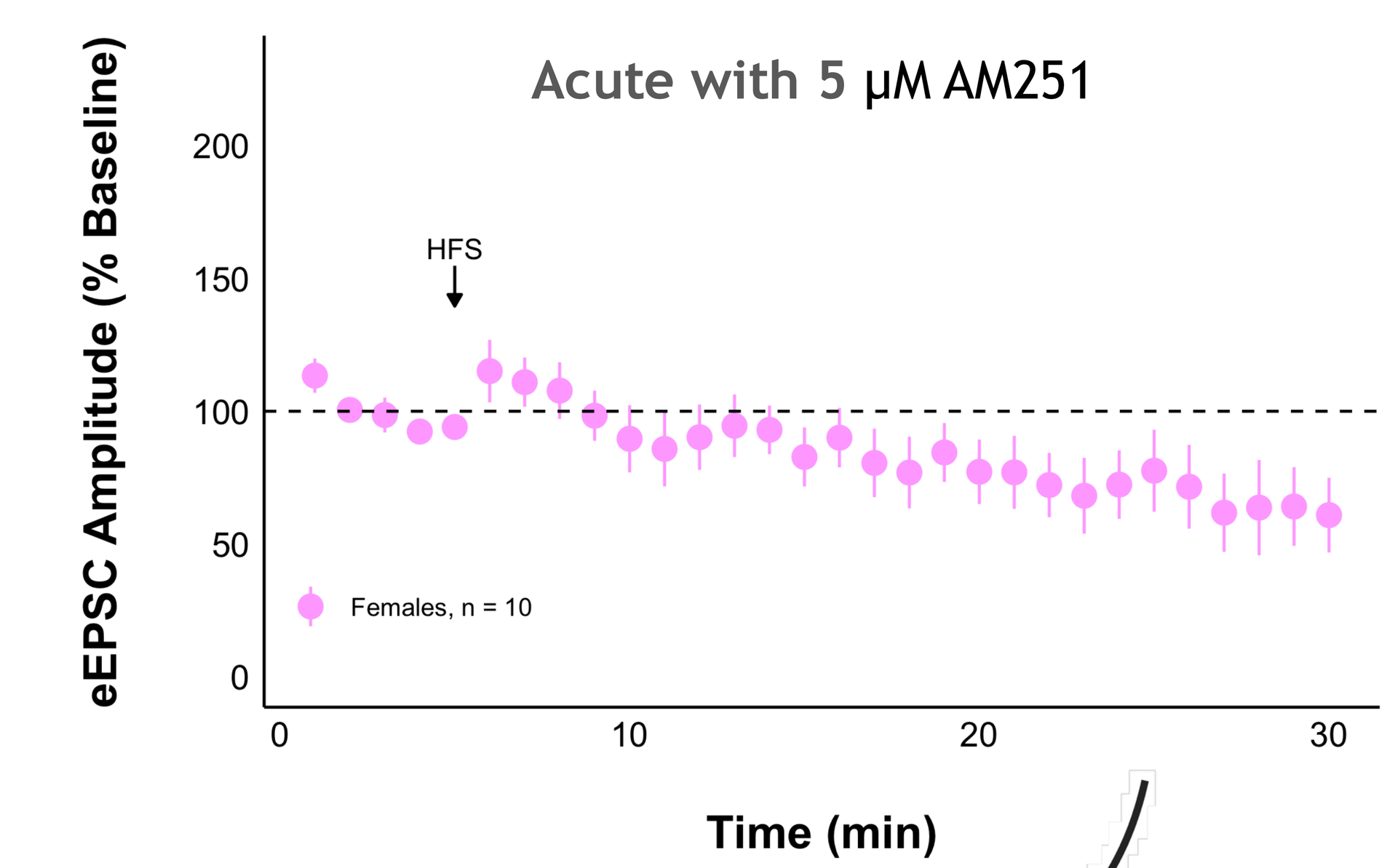




Naïve data was collected by Lara Swart. A paired t-test was used to compare each 5-minute interval to the 5-minute baseline period. * = p-value < 0.05, ** = p-value < 0.01

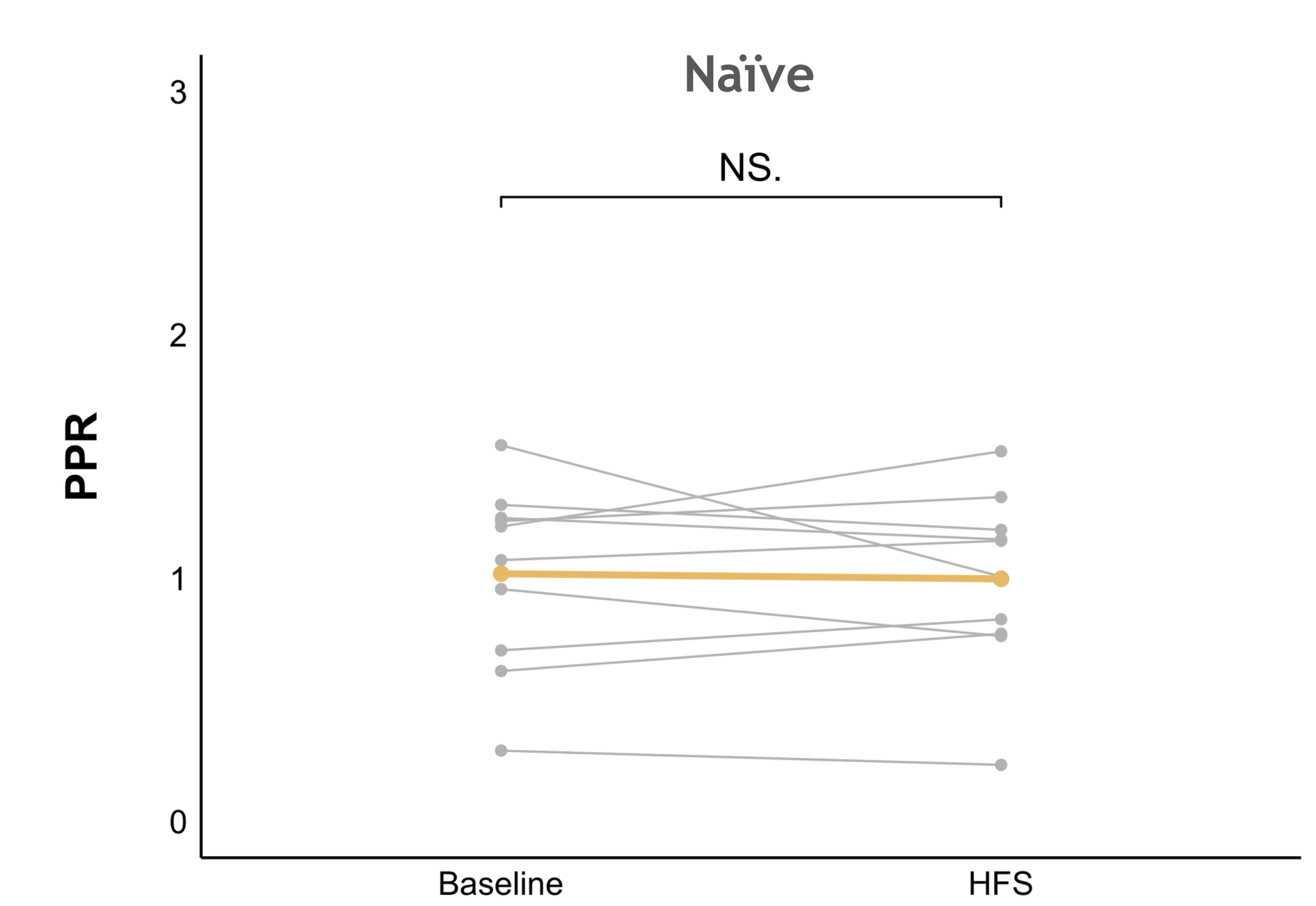
What causes this decrease?

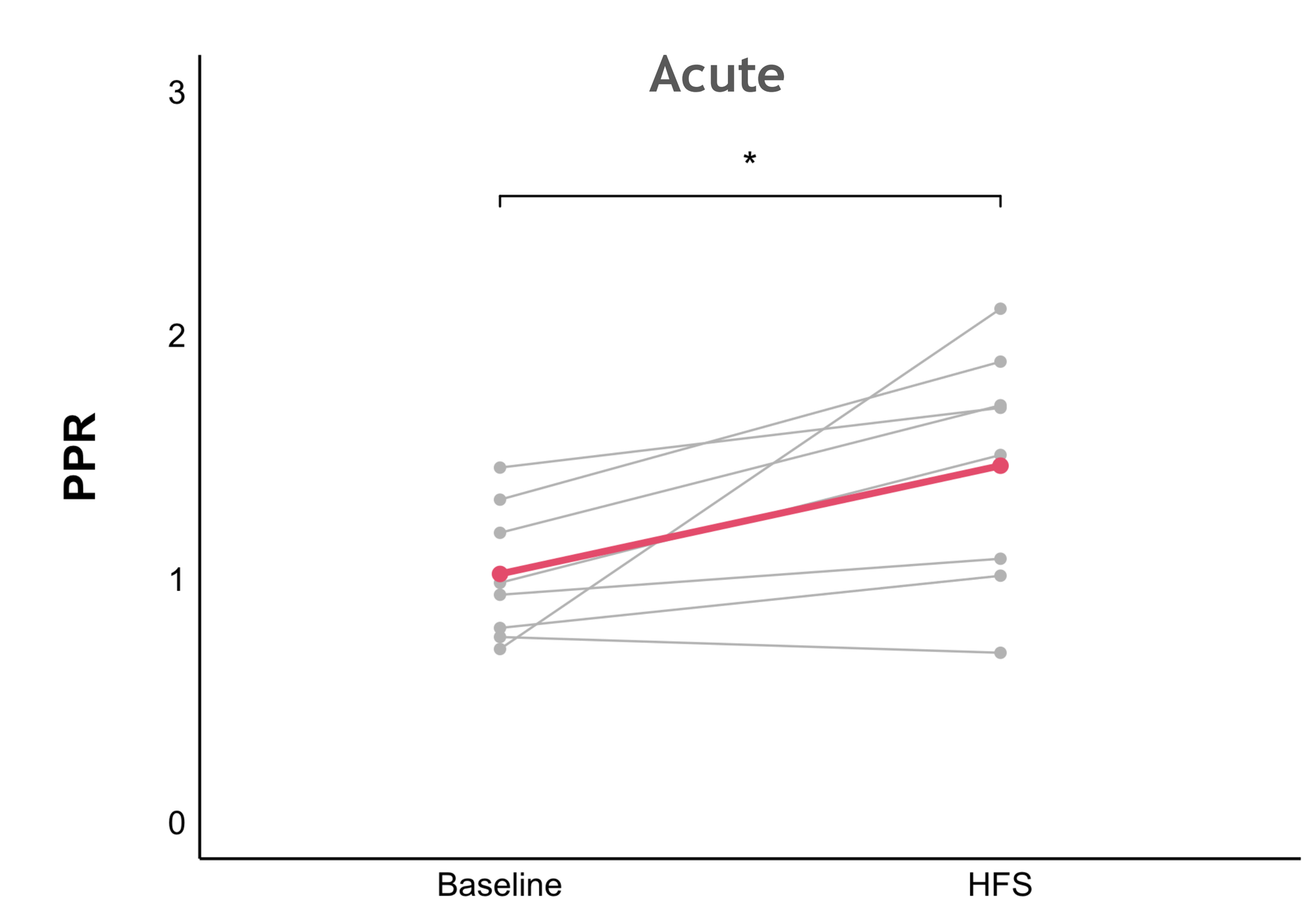
Endocannabinoids can trigger a long-lasting decrease in glutamate release

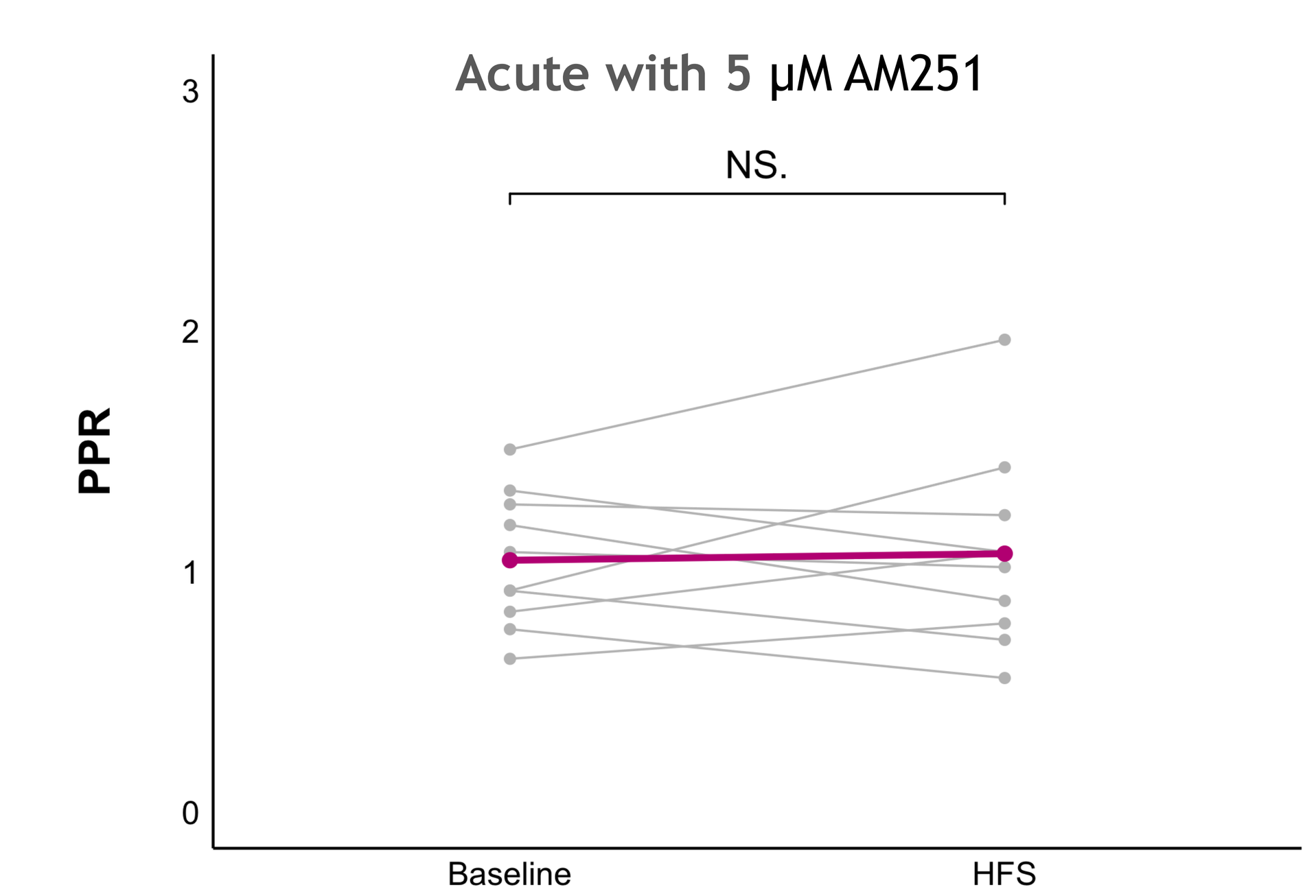


Is the **decrease** due to a presynaptic decrease in glutamate onto DMH neurons during **acute stress**?

The PPR **significantly increased**, indicating a lower probability of glutamate release onto DMH neurons, but there is no change when **Endocannabinoid-CB1 receptors** are **blocked**







The PPR (calculated as P2/P1) compares the amplitude of two evoked currents and is inversely proportional to the probability of neurotransmitter release. A paired t-test was used to compare between baseline and HFS for each group. * = p-value < 0.05

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Schematic Images created using BioRender.

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CONCLUSIONS & FUTURE DIRECTIONS

Future work aims to determine:

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

Stress response mechanisms have not adapted to our high stress society and landscape of high calorie, highly palatable foods[7]. **Women** are particularly vulnerable to **disordered eating** behaviours when **stressed**[5], for which the neurophysiological basis is unclear. Yet, female research subjects remain underrepresented, which this research aims to address.