Sex-specific dysfunction of cognitive and habit circuits underlying opioid self-administration

Abbi Ouimet, Rebecca Pupp, Matthew Hearing
Department of Biomedical Sciences, Marquette University, Milwaukee, WI

Introduction

- America is currently facing the Opioid Epidemic. 130 Americans die every day from an opioid overdose (cdc.gov). Monthly overdoses grew dramatically during the pandemic making this crisis more pressing than ever.
- Opioids are mainstays for clinical pain management despite known risk of dependence, abuse, and ultimately addiction, even when used as prescribed (HHS.gov).
- Biological sex differences in addiction vulnerability exist for numerous illicit substances. Women tend to escalate drug use and exhibit uncontrollable drug-taking on a faster timeline than men (Becker and Hu, 2008; Becker et al., 2012)
- The prefrontal cortex plays a critical role in top-down inhibitory control of "bad" behavior and cognitive control (e.g., cognitive flexibility). A prevailing view in the addiction field is drug taking during the early stages of use is "goal directed", controllable, and facilitated by the PFC.
- Deficits in cognitive flexibility produced by opioid self-administration in mice occurs more rapidly in females than in males and these deficits are driven by a hypoactive state in prelimbic region of the PFC (Anderson et al., 2021).
- More recent pilot data in our lab suggests in females, impaired function of the PrL-PFC aligns with habit-like drug seeking and increased strength of connections in the DLS.
- It's important to understand how opioid use transitions to compulsive drug taking and if it varies across sex to dictate prescription use and protect post-drug pain management behaviors.

Methods

Chemogenetics/DREADDs
To determine the relationship between neural activity and control over drug-taking, neural activity in the PrL-PFC or aDLS was inhibited using a viral-mediated approach to express chemogenetic receptors called Designer Receptor Exclusively Activated by Designer Drugs. These receptors are similar to G protein coupled receptors that are normally expressed in the brain to reduce activity/firing of cells but have been genetically modified to only be responsive to a small molecule drug (agonist) called clozapine-N-oxide (CNO), which can be injected to complete the inhibition of the desired brain region.

Surgical Procedures
In the first step of this experiment, mice underwent a craniotomy in a stereotaxic apparatus for an intra-cranial infusion of an adeno-associated virus (AAV) into either the PrL-PFC or the aDLS to express this inhibitory DREADD (AAV5-hm4Di) followed by a surgical procedure to implant an intravenous catheter for drug delivery via self-administration.

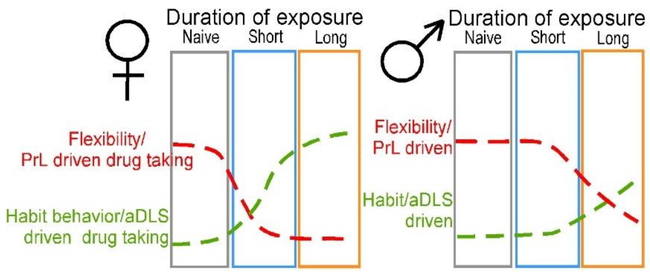
Remifentanil Self-administration
Following a 7-day post-surgery recovery period, mice underwent 14 (or 30 days) of remifentanil self-administration. To start, each mouse went through a fixed ratio lever training schedule, in which Ensure was used as a reward for active lever presses. After passing, mice spent three hours per day in the operant boxes, where there is an active and inactive lever. Pressing the active lever will allow for self-administration of remifentanil, pressing the inactive lever will have no effect. This model's drug addiction in humans. All mice will receive an intraperitoneal injection of saline on day 14 and 30 of self-administration to habituate them to acute stress related to injections. Then they will receive an injection of CNO (2.0 mg/kg) on day 15 and 31 of drug self-administration.

Control Mice
Control mice underwent nearly the same procedures as the remifentanil mice except no drug is administered during their time spend in the operant boxes. Mice are given the same options to press either an active or inactive lever, except pressing of the active lever gives them an Ensure food reward.

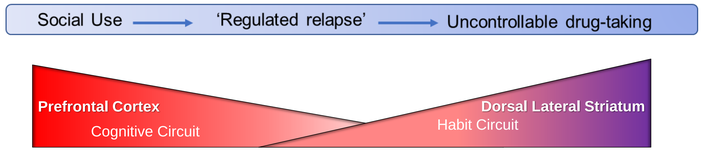
Drop Fixing
Following self-administration, the mice are euthanized, and their brains are drop-fixed into PFA for 1 day, then PBS for 2 days to fix the tissue. Brains are then sliced using a vibratome and examined microscopically for analysis.

Results

Hypothesis/Working Model



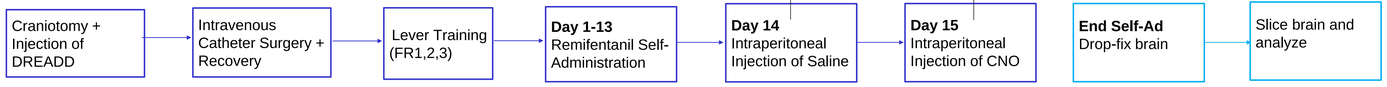
Gradual dysfunction of the prefrontal cortex drives addiction



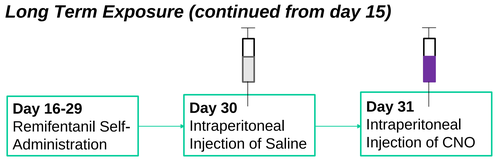
Overall Hypothesis: Dysfunction (reduced activity) in the PrL-PFC occurs faster in females and leads to reduced flexibility in decision making which aligns with reduced control of the PrL-PFC over drug taking (red line) and increased control by habit circuits (green line)

Experimental Timeline

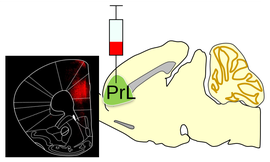
Short Term Exposure



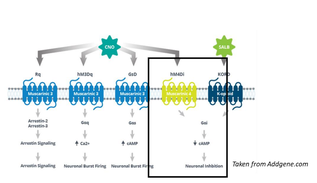
Long Term Exposure (continued from day 15)



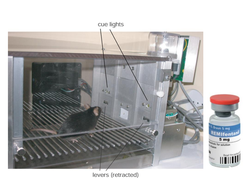
Intra-Cranial DREADD Infusion



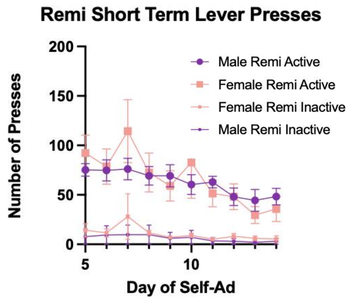
Inhibitory DREADDs

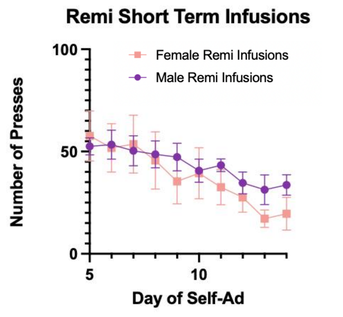


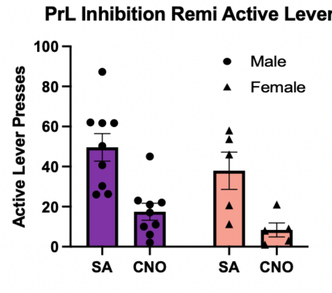
Remifentanil Self-Administration

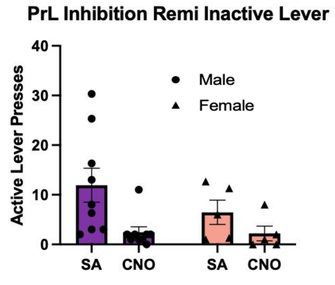


PrL Inhibtion in Remi Mice Short Term

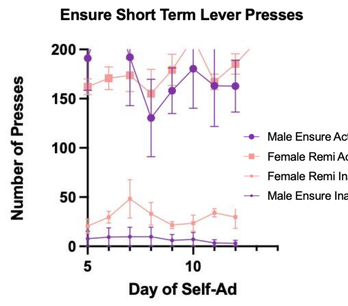


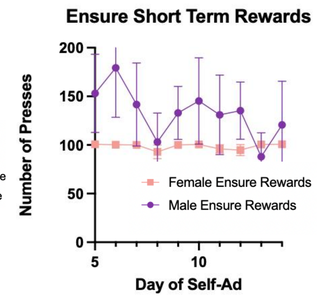


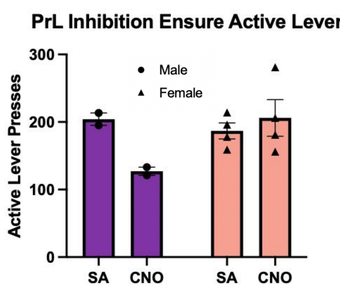


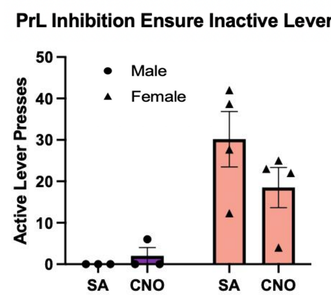


PrL Inhibtion in Ensure Mice Short Term









Model Predictions

Short Term Exposure (14 days)

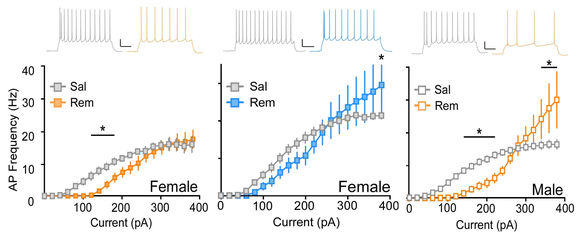
- After short term exposure, males but not females will display decreased drug intake with inhibition of the PrL.
- After short term exposure, inhibition of the DLS will reduce drug seeking in females but not males.
- Inhibition of the PrL will reduce non-drug reward seeking in both males and females following short-term access.

Long Term Exposure (30 days)

- After long term exposure, females will display no change in drug intake with inhibition of the PrL, whereas drug seeking will still be reduced in males.
- After long term exposure, females but not males will display decreased drug intake with inhibition of the DLS

Conclusions

- Following 2 weeks (short term exposure) of remifentanil self-administration, inhibition of the PrL in both males and females resulted in decreased drug intake. Data suggest that drug-seeking remains reliant on PrL circuits in both sexes with short-term use, and that the switch to habit may require more prolonged exposure (see below data).



- Inhibition of the PrL did not alter seeking behavior for a non-drug appetitive reward (Ensure). This suggests that the PrL either doesn't play a role in natural/food seeking behavior or that food seeking becomes more habitual on a shorter timescale.

Future Directions

•Focus on inhibiting the DLS in both males and females to determine its effect in habitual drug taking.

•Extend timelines of both remifentanil mice (inhibiting both the PrL and DLS in both males and females) as well as control mice to allow self administration to continue for 4 weeks in order to study long term exposure.

Funding

- Marquette University Honors Program Summer Research Fellowship
- NIH/NIDA