The Transgenerational Effects of Fetal Opioid Exposure:
An Examination of the Long-Term Neurogenetic and Behavioural Effects

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Context

- **Rates of opioid misuse have increased dramatically since the beginning of the 21st century, fed by overprescription, lack of education, and other socioeconomic challenges (1).**
  - In England, opioid prescriptions increased by 457% between 1998 and 2018 (2).
  - In 2021, 3.3% of the US population struggled with opioid misuse (3).
- **The rise has been paralleled in pregnant women, resulting in more babies being diagnosed with neonatal opioid withdrawal syndrome (NOWS) (4).**
  - In some US states, the percentage of babies born with NOWS is nearly 5% (5).
- **NOWS can have consequences for both mother and child.**
  - Mothers are more likely to miscarry, have a stillbirth, or go into pre-term labor (4).
  - NOWS Typically presents with neurologic and gastrointestinal effects at birth (6).

Aim

Through the use of animal models, this project aims to investigate whether fetal opioid exposure influences offspring **gene expression** in the long term, helping to explain the genetic underpinnings of the long-term effects of opioids on development and behaviour.

Additionally, through looking at offspring's own opioid use patterns, it may be possible to get a further glimpse at how the genes of interest are affected by and related to opioid **addiction**.

Methodology

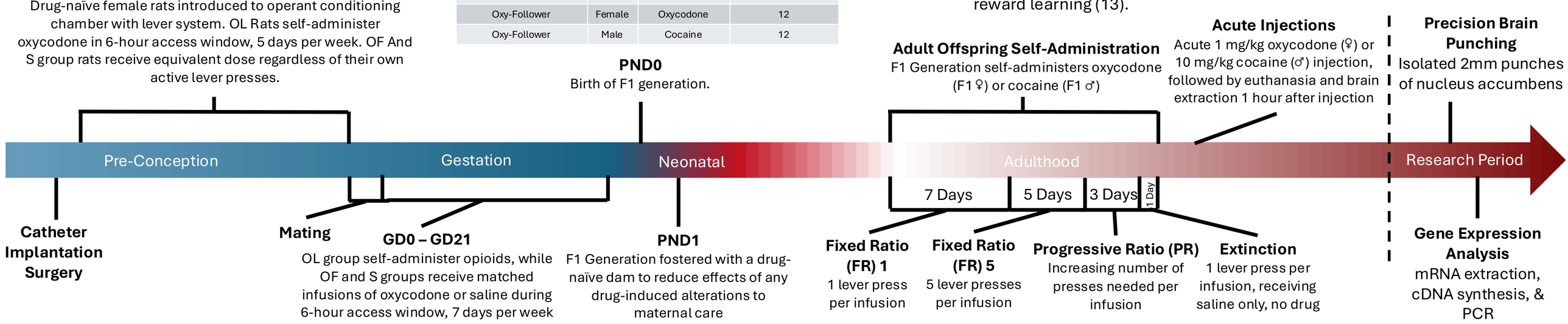
Terminology:
- F0 Generation = Maternal generation
- F1 Generation = Offspring
- Nucleus Accumbens = Brain region associated with pleasure and motivation; implicated in addiction due to high concentration of dopaminoceptive neurons (7)
- Infusion = Intravenous 0.1 mg/kg/infusion of oxycodone. For F1 male cocaine: 0.5 mg/kg/infusion, maximum of 100 infusions per session.

Experimental Groups:
- Oxy-Leader (OL): F0 generation self-administering oxycodone
- Oxy-Follower (OF): F0 Generation receiving equivalent dose of oxycodone as OL, but had no agency over dosage
- Saline (S): F0 Generation receiving equivalent volume of saline.

Subjects:

F0 Drug Administration Group	F1 Sex	F1 Drug Exposure	Number of F1 Subjects
Saline	Female	Oxycodone	12
Saline	Male	Cocaine	11
Oxy-Leader	Female	Oxycodone	13
Oxy-Leader	Male	Cocaine	11
Oxy-Follower	Female	Oxycodone	12
Oxy-Follower	Male	Cocaine	12

Target Genes:
- Arc: Immediate early gene expressed upon synaptic activation and thus a marker of brain activity in response to stimuli (8).
- C-Fos: Immediate early gene, used as a measure of neuronal stimulation/activity (9).
- MeCP2: Epigenetic regulator, key to neuronal maturation and brain development. Recent studies suggest opioids may affect its expression, and thereby the regulation of other genes as well (10, 11)
- Oprm1: Codes for the Mu-Opioid receptor and thus a predictor of sensitivity to opioids and opioid-related addictive behaviour (12).
- Drd2: Codes for dopamine receptor D_2, a receptor which is important to reward learning (13).



Acquisition
Drug-naïve female rats introduced to operant conditioning chamber with lever system. OL Rats self-administer oxycodone in 6-hour access window, 5 days per week. OF And S group rats receive equivalent dose regardless of their own active lever presses.

PND0
Birth of F1 generation.

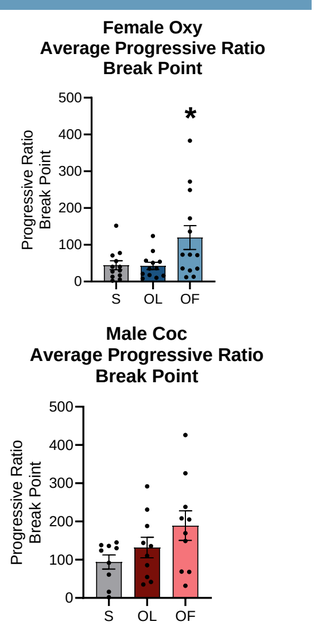
Adult Offspring Self-Administration
F1 Generation self-administers oxycodone (F1 ♀) or cocaine (F1 ♂)

Acute Injections
Acute 1 mg/kg oxycodone (♀) or 10 mg/kg cocaine (♂) injection, followed by euthanasia and brain extraction 1 hour after injection

Precision Brain Punching
Isolated 2mm punches of nucleus accumbens

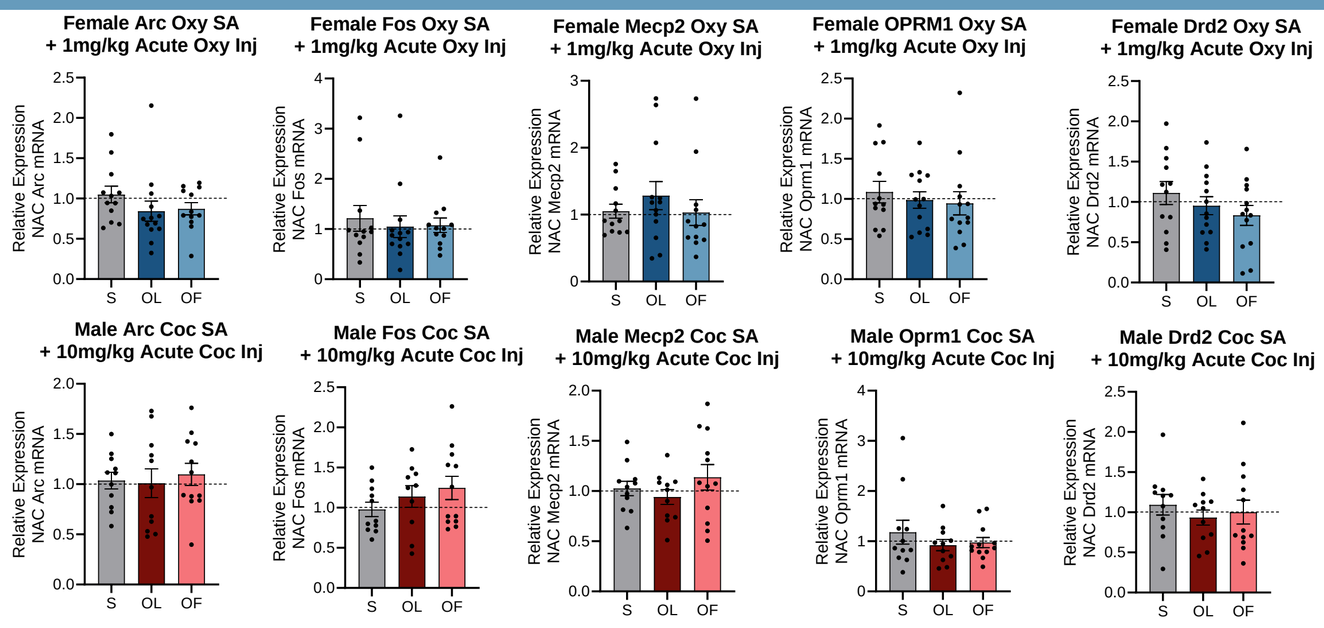
Results

F1 Self-Administration Intake



In females, the **OF offspring showed a significant increase in Oxy intake** compared to those without pre-natal opioid exposure. Males exhibited a similar trend, but non-significantly. Additionally, there is a noticeable, but non-significant, increase in intake between the OF and OL groups, particularly amongst the females.

Relative Gene Expression in the Adult Nucleus Accumbens of the F1 Generation



The data exhibits non-significant differences in expression of the 5 target between groups. This may be as result of utilizing bulk tissue samples for analysis and the varying intake of opioids by different dams and between individual offspring. However, some observations of note are:
- **(1)** that there appears to be reduced expression of immediate early genes Arc and C-Fos as a general effect of fetal opioid exposure in females.
- **(2)** There seems to be an increase MeCP2 expression in OF males, which may be the result of the increased F1 cocaine intake.
- **(3)** Drd2 expression appears to be reduced in females with pre-natal opioid exposure, most notably in offspring of the OF group.
- **(4)** C-Fos Expression in males exposed to opioids in utero seems to be increased, particularly for the OF group.

Conclusions

Lack of **maternal agency over opioid intake** results in significantly increased motivation for opioid acquisition and addictive-like behaviour in the next generation. This underscores the importance of considering agency in reviewing many existing studies, conducting future research, and translating findings to humans.
- Potential decreases in **Arc** and **C-Fos** in females could point to reduced synaptic activation in the NAC correlating to altered adult activity in the region, which could have motivation and reward processing implications. In males, the opposite trend appears true, with increased FOS expression in the NAC, indicating increased synaptic activation and activity. The divergent effects highlight the importance of studying the effects different opioids on genes and behaviour.
- Increase in male **MeCP2** expression appear to be correlated with F1 PR levels. Alterations in MeCP2, with its epigenetic regulator role, could have far-reaching consequences in terms of motivation, mood, and addiction – particularly reinforcing Cocaine use.
- A possible reduction in **Drd2** expression in female offspring could indicate alterations in dopamine processing, and could impact reward processing, affecting propensity for addiction.

Further Investigations

With varying drug intake across and within groups making direct comparisons of relative gene expression challenging, we graphed how **F1 and F0 intake correlated with expression of the different target genes** across experimental conditions. The results of this comparison can be viewed using the provided QR code.

Finally, in the future, looking at regional and cell-type specific effects of fetal opioid exposure on neurogenetics would lend further nuance to this data and combat the confounding effects of multiple cell types within the extracted tissue sample.

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LAIDLAW SCHOLARS

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