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Title: Uncovering Molecular Adaptations in the Addicted Brain: A Focus on Dopamine Genes and Sex Differences

Introduction & Purpose

Addiction is a chronic and relapsing brain disorder that alters neural circuits related to motivation, pleasure, and behaviour control. One of the most studied pathways in addiction is the **dopaminergic system**, which plays a central role in the brain's reward processing. Chronic drug exposure, such as cocaine, significantly disrupts dopamine signalling in the nucleus accumbens (NAc) — a brain region critical for reinforcing addictive behaviour. This project uses publicly available RNA-seq data from mouse brain samples to investigate how gene expression in dopamine-related pathways varies under different biological conditions. We focus on two core questions:

1. Are there significant gene expression differences between **male and female** mice?

2. Are there gene expression differences after **28 days of cocaine withdrawal**?

We selected a set of dopamine-associated genes because they represent critical components in this signalling system:

- *Cartpt*: Cocaine- and amphetamine-regulated transcript, directly activated by stimulant drugs (Lohoff et al., 2008)
- *Drd1a, Drd2, Drd3, Drd4, and Drd5*: Dopamine receptors mediating excitatory/inhibitory signals (Ozburn et al., 2015, Zhang et al., 2004)

These genes are biologically relevant and responsive to drug exposure, making them ideal targets to study sex-based and drug-related expression changes. We analyze this dataset using `ggplot2` to create visual comparisons and apply linear models to test for statistical significance.

Summary of Findings

Linear models and visualization techniques revealed the following key observations:

No significant global differences in dopamine-related gene expression were detected between male and female mice (Figure 1) or between cocaine-exposed and naive mice (Figure 2). The combined model (LogExpression ~ Sex + Condition) yielded a non-significant result (p > 0.7) (Figure 3), suggesting minimal variance explained by these predictors across the entire gene set.

Among individual genes, **Drd3** stood out with a statistically significant increase in expression in the naive group compared to cocaine-withdrawn mice (p < 0.01) (figure 2). This supports the hypothesis that *Drd3* expression is suppressed by prolonged cocaine exposure. Other genes like *Drd2* and *Drd1a* showed upward trends in naive mice, but these did not reach

statistical significance. Sex-based comparisons revealed no consistent patterns in dopamine receptor expression, indicating that sex may not independently drive expression differences in this gene subset under the study's conditions.

These findings partially align with those reported in the original study (LaRese et al., 2019), which found broader sex-specific expression patterns and significant changes induced by cocaine. While my reanalysis did not replicate strong sex-based expression differences across dopamine genes, **the significant suppression of *Drd3* following withdrawal supports the paper's conclusion that cocaine leaves lasting transcriptional changes in reward circuitry** (Lohoff et al., 2008, Zhang et al., 2004) (Figure 4). Additionally, prior research (Hu et al., 2004) has shown that estrogen modulates dopamine receptor expression in females, especially *Drd1* and *Drd2*, although those effects may require more targeted models or hormonal manipulation data to observe.

The observed downregulation of *Drd3* following cocaine exposure suggests that this receptor may play a central role in neuroadaptations underlying addiction. Given *Drd3*'s role in motivation and reward sensitivity, its suppression could reflect a shift toward reduced dopaminergic responsiveness, potentially increasing vulnerability to relapse. The lack of significant sex-based differences in my analysis suggests that sex effects may be more gene- or pathway-specific, or possibly confounded by other variables such as hormonal status or regional heterogeneity within the NAc (Hu et al., 2004).

Practical Implications & Future Directions

Understanding how specific dopamine genes are altered by cocaine exposure could inform the development of pharmacological treatments aimed at restoring dopaminergic tone in individuals recovering from addiction. *Drd3* may represent a promising molecular target for therapeutic modulation. Future studies should explore time-course effects, hormone interactions, and gene-environment interactions using expanded models. A larger sample size and integration with behavioural data would also strengthen inferences about functional outcomes.

Conclusion

This study reinforces the role of dopamine-related genes, especially *Drd3*, in the neuroplasticity of cocaine addiction and highlights the importance of analyzing gene-level differences to uncover subtle but meaningful molecular changes. While sex-based differences were not significant in this dataset, gene-specific effects suggest the need for a more nuanced understanding of addiction's molecular underpinnings, with implications for both theory and intervention.

Reference

Hu, M., Crombag, H., Robinson, T. et al. Biological Basis of Sex Differences in the Propensity to Self-administer Cocaine. Neuropsychopharmacol 29, 81–85 (2004). https://doi.org/10.1038/sj.npp.1300301

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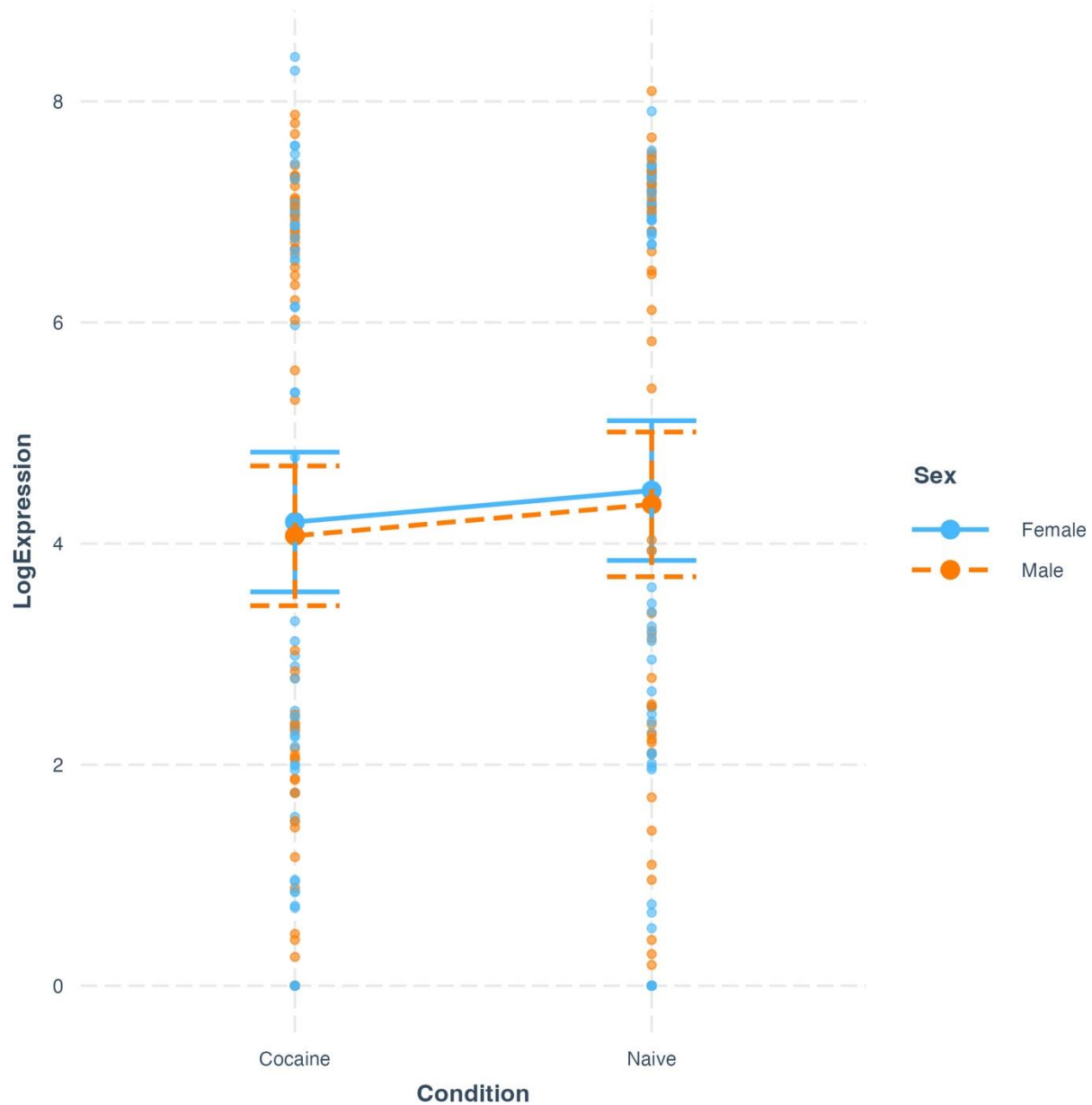
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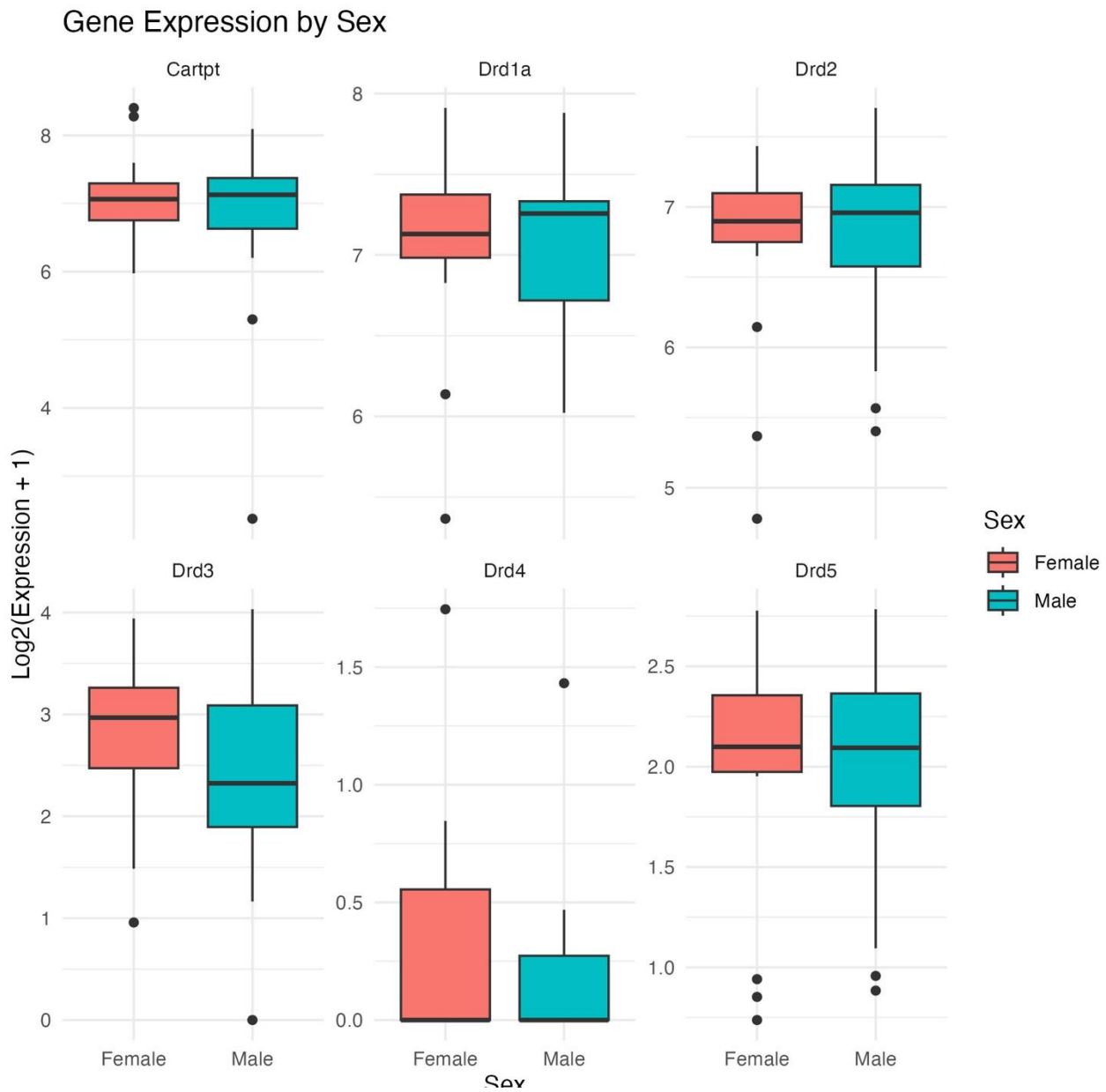
Figure 1. Drd3 shows a mild increase in females.

Although the effect of sex is statistically significant, it is very small and not biologically meaningful in the context of global gene expression (p = 0.00046, R-squared = 0.000013). The sample size is likely driving the statistical significance.
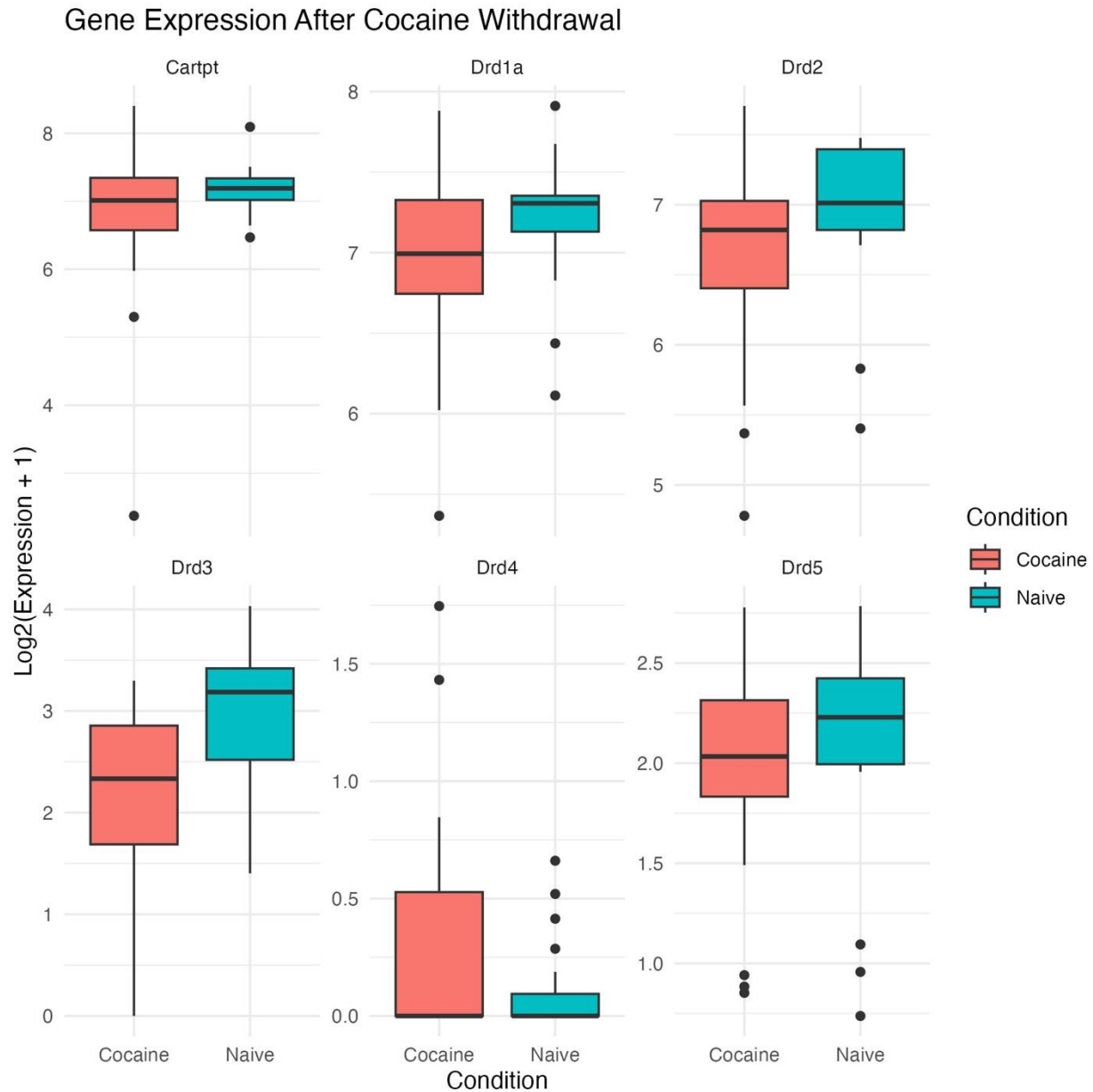
Figure 2. Cocaine withdrawal appears to suppress dopamine receptor gene expression, particularly Drd3, highlighting its potential role in addiction-related neuroplasticity.

There is a statistically significant difference in expression between Naive and Cocaine groups, with Naive mice showing slightly higher gene expression overall ($p < 2e\text{-}16$). This is statistically strong but biologically weak due to a low effect size (R-squared = 0.00085). Drd2 and Drd1a also show reduced levels in the cocaine group. Drd4 is low across all samples, and Drd5 shows mild upregulation in Naïve. Drd3 shows a significant increase in the Naive group, suggesting cocaine withdrawal suppresses its expression

Figure 3. Even when accounting for both predictors, each contributes a statistically detectable but very minimal amount of variance in gene expression.

The differences between sexes and drug conditions exist, but they are not strong enough to account for most of the variation in gene expression (R-squared = 0.003038). The model explained only 0.3% of the variation in gene expression, suggesting these predictors are not meaningful drivers of overall dopamine gene expression.

```
[1] "Drd3"

Call:
lm(formula = LogExpression ~ Sex + Condition, data = dataset)

Residuals:
   Min     1Q  Median     3Q    Max
-2.0153 -0.6317  0.1272  0.5394  1.2122

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    2.3611     0.2162  10.922 5.59e-13 ***
SexMale       -0.3458     0.2518  -1.373  0.17822
ConditionNaive 0.8046     0.2518   3.195  0.00291 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.7858 on 36 degrees of freedom
Multiple R-squared:  0.2551,          Adjusted R-squared:  0.2138
F-statistic: 6.165 on 2 and 36 DF,  p-value: 0.004982
```

Figure 4. Drd3 exhibited a significant increase in expression in naive mice compared to those exposed to cocaine withdrawal (p = 0.0029).

While other dopamine receptor genes (Drd1a, Drd2, Drd4) showed non-significant trends toward differential expression by condition, no gene demonstrated significant sex-based differences. These results support previous findings linking Drd3 downregulation to drug-induced neuroplasticity and highlight it as a candidate for further exploration in addiction-related gene expression changes.