

Sex- and Withdrawal-Dependent Proteomic Changes in Nucleus Accumbens and Prefrontal Cortex: Insights into Synaptic Adaptations in Substance Use Disorders

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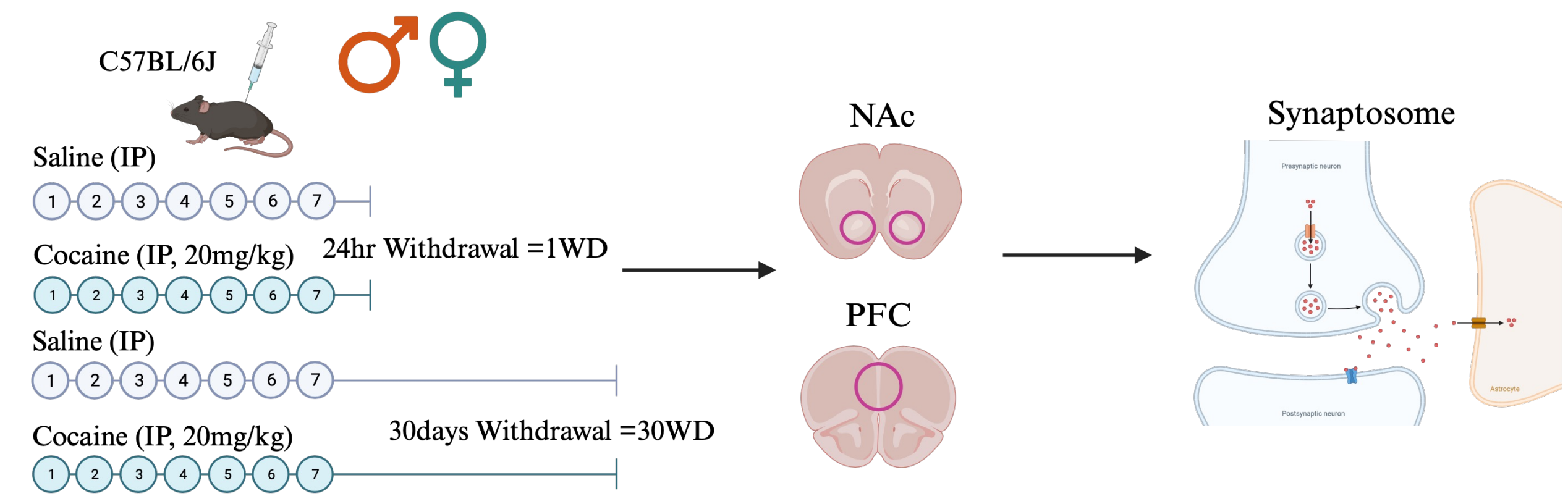


Background

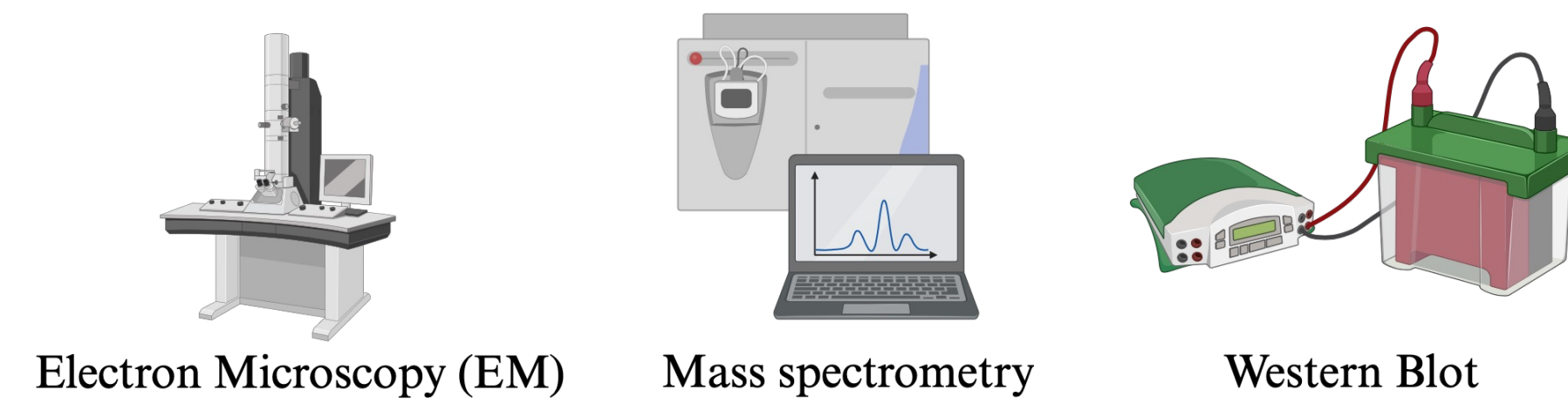
- Dysregulated signaling within reward-processing brain regions, such as the nucleus accumbens (NAc) and prefrontal cortex (PFC), plays a critical role in promoting drug-seeking behavior and relapse.
- Compared to our understanding of transcriptional responses to drugs of abuse, our knowledge about changes in the proteomic landscape of synapses is limited.
- Identifying these changes could reveal more effective targets for substance use disorder (SUD) treatment.
- Goal:** Identify the sex- and withdrawal (WD)-dependent cocaine mediated changes in the synaptic proteome of the NAc and PFC.

Experimental Design

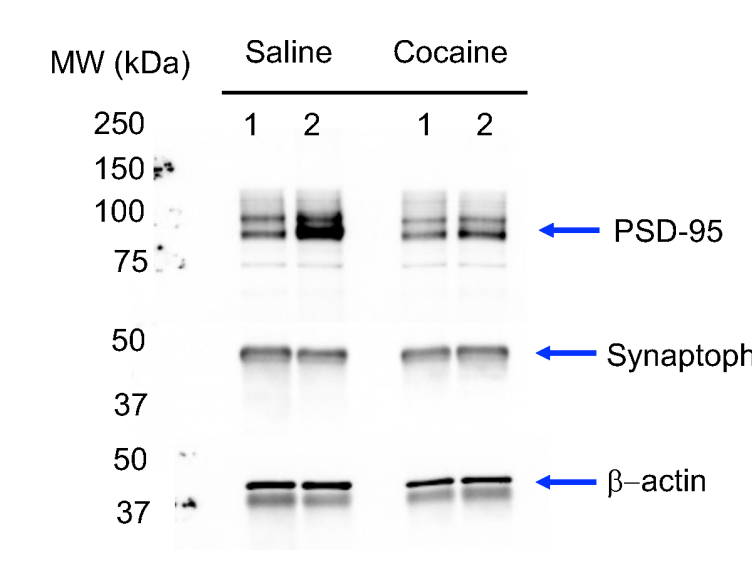
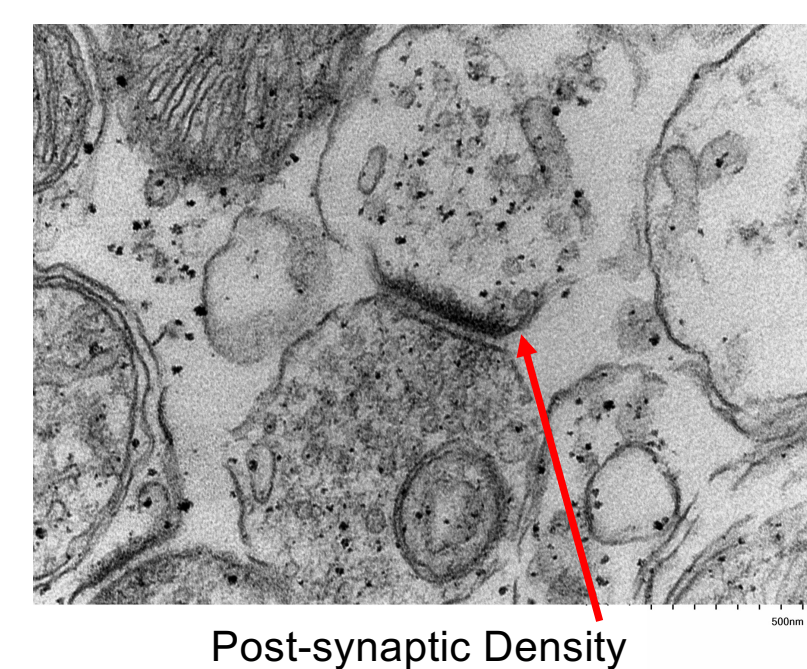
A. Sample Preparation



B. Sample Analysis



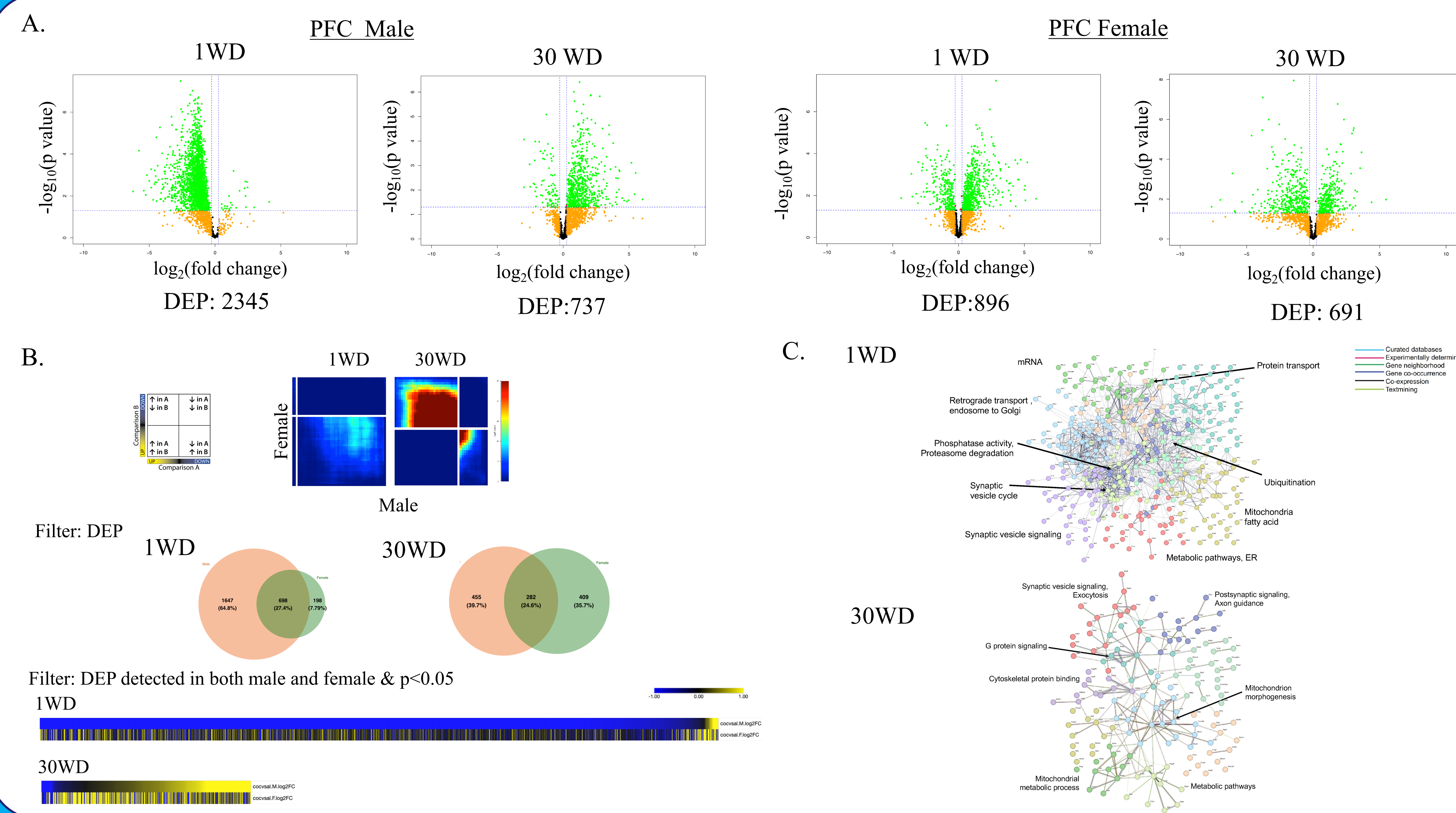
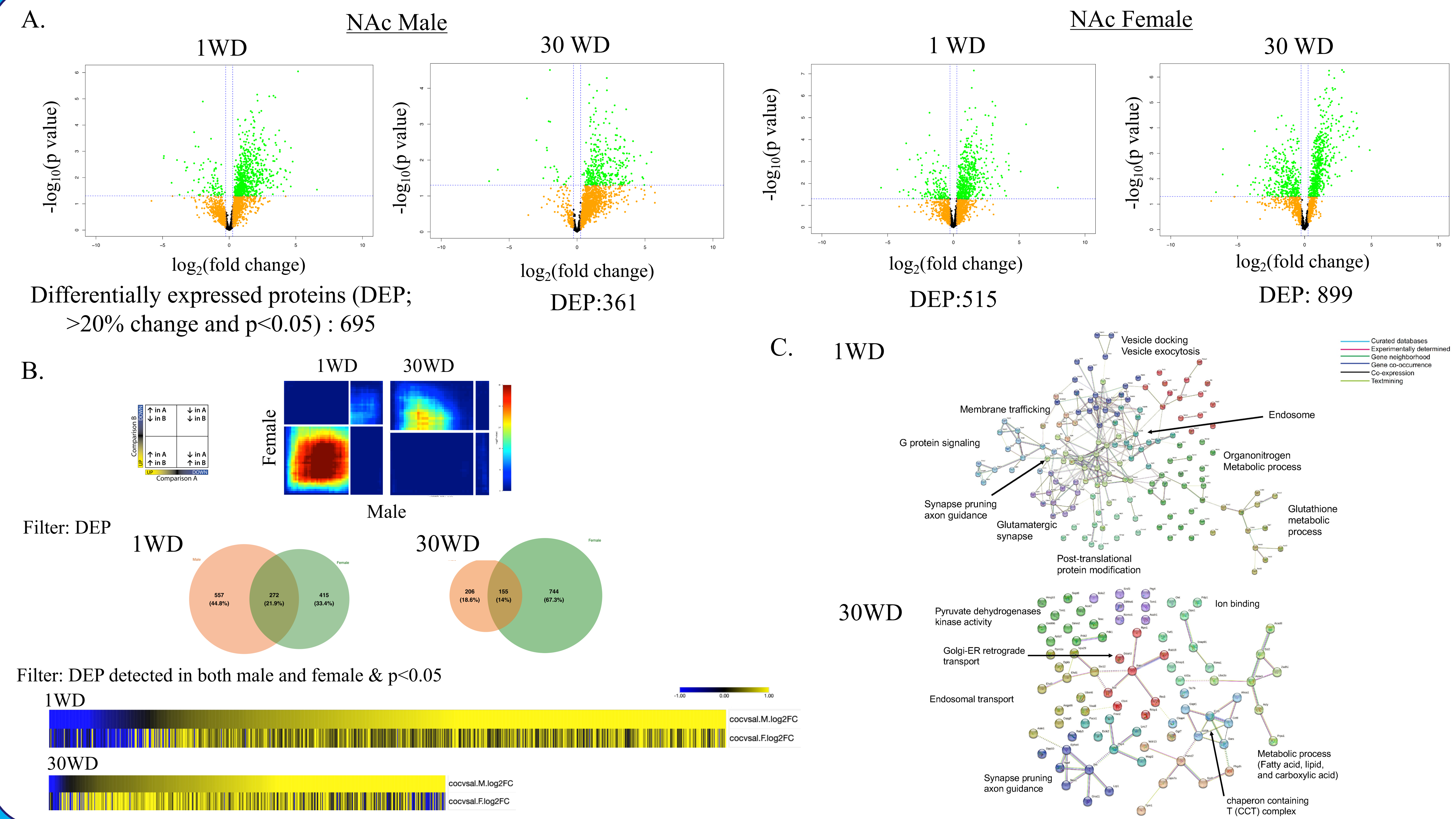
Synaptosome



The quality of NAc and PFC synaptosomes isolated from mice injected with saline and cocaine are assessed using cryo-electron microscopy (cryo-EM) and Western blot analysis.

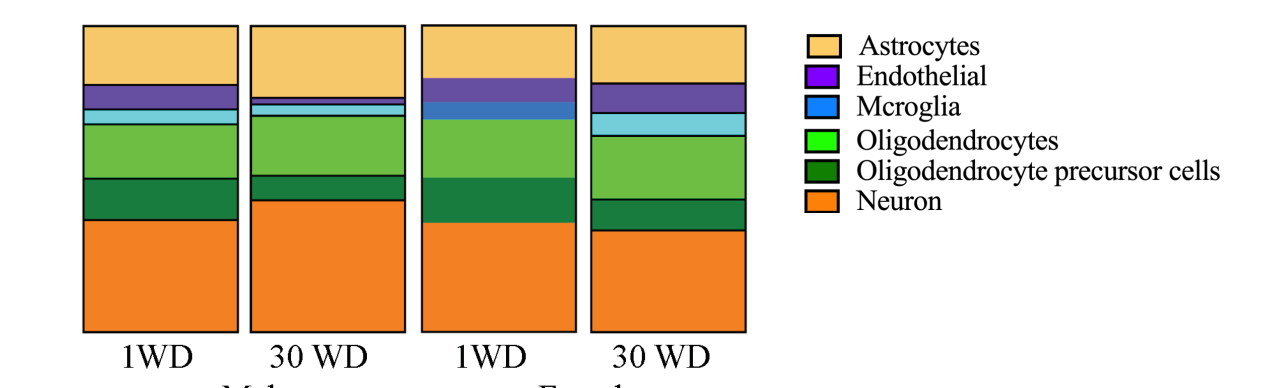
Proteomic Data Analysis

The NAc and PFC synaptosomes undergo analysis using lipid chromatography-tandem mass spectrometry (LC-MS/MS) followed by label-free, data-dependent acquisition (DDA) for untargeted protein quantification at the Yale/NIDA neuroproteomics Center. Two-way ANOVA with type 3 sums of squares is conducted using R to identify differentially expressed proteins.



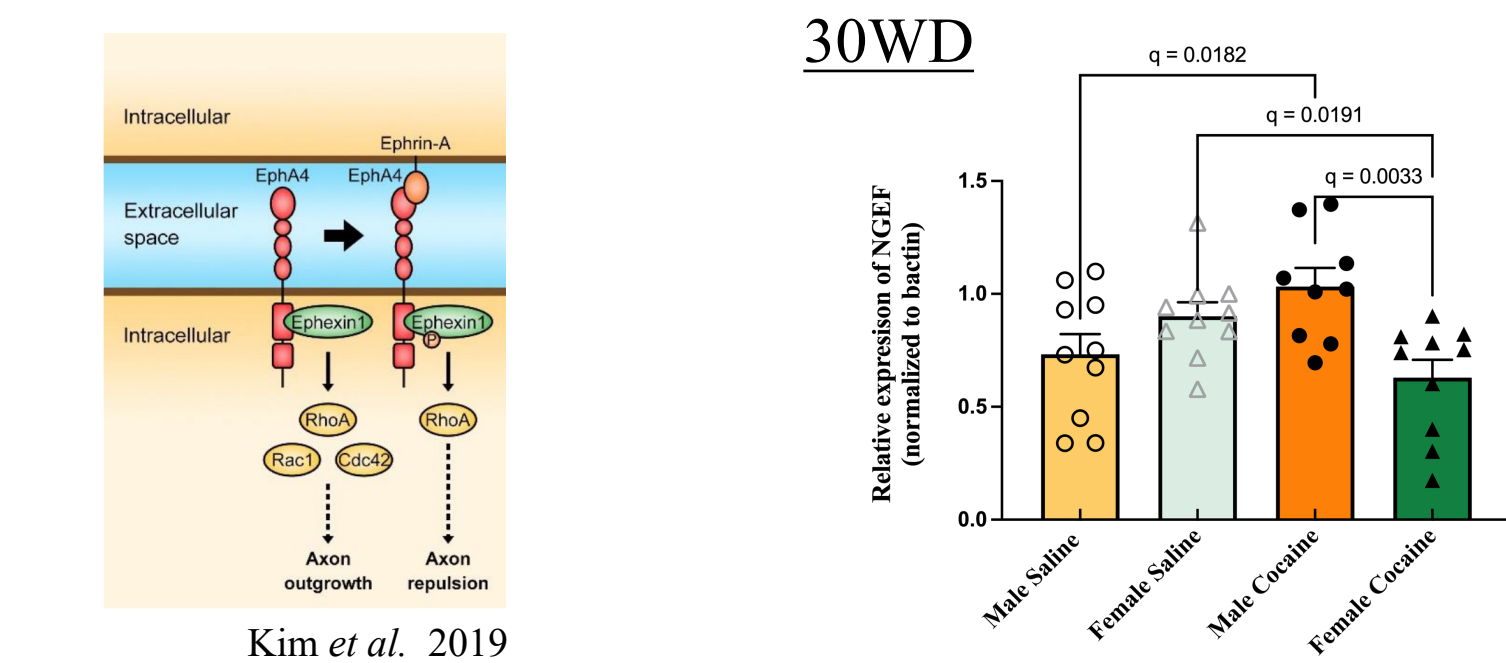
Conclusions & Future Directions

- We conducted a whole-proteome analysis of synapses following the 7-days injection model.
- We identified multiple synapse-enriched proteins that were either induced or repressed in a brain region-, sex-, and WD-time-dependent manner.
- While most of these cocaine-regulated proteins are expressed by neurons, a significant subset is enriched in astrocytes or microglia, consistent with the involvement of these cell types in synaptic processes.



- Interestingly, in the NAc after 30 days of WD, female mice exhibited ~2.5 times more significant proteome changes than male mice. Conversely, in the PFC after 24 hours of WD, male mice displayed ~2.5 times more significant proteome changes, primarily repression, compared to female mice.

- We are currently validating these findings and characterizing particular synaptic proteins in NAc.



- Future goal is to identify novel synaptic protein targets regulated by drugs of abuse, such as cocaine and heroine, in a cell-type and circuit-specific manner, to better understand and develop treatment.

Acknowledgements

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Data were analyzed through the use of R, GraphPad Prism (version 10.0.0 for Mac, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com), and STRING database.

References

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