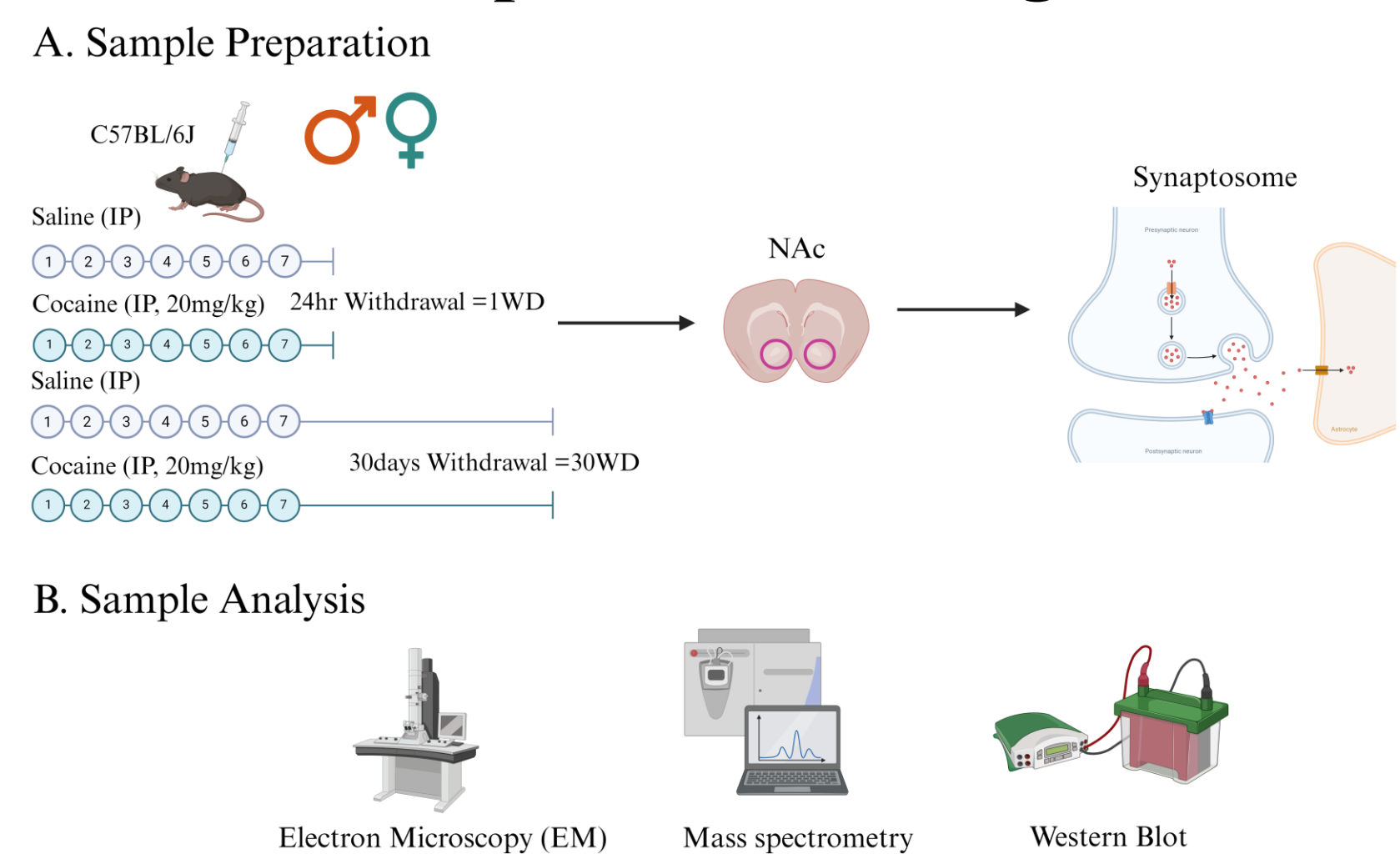


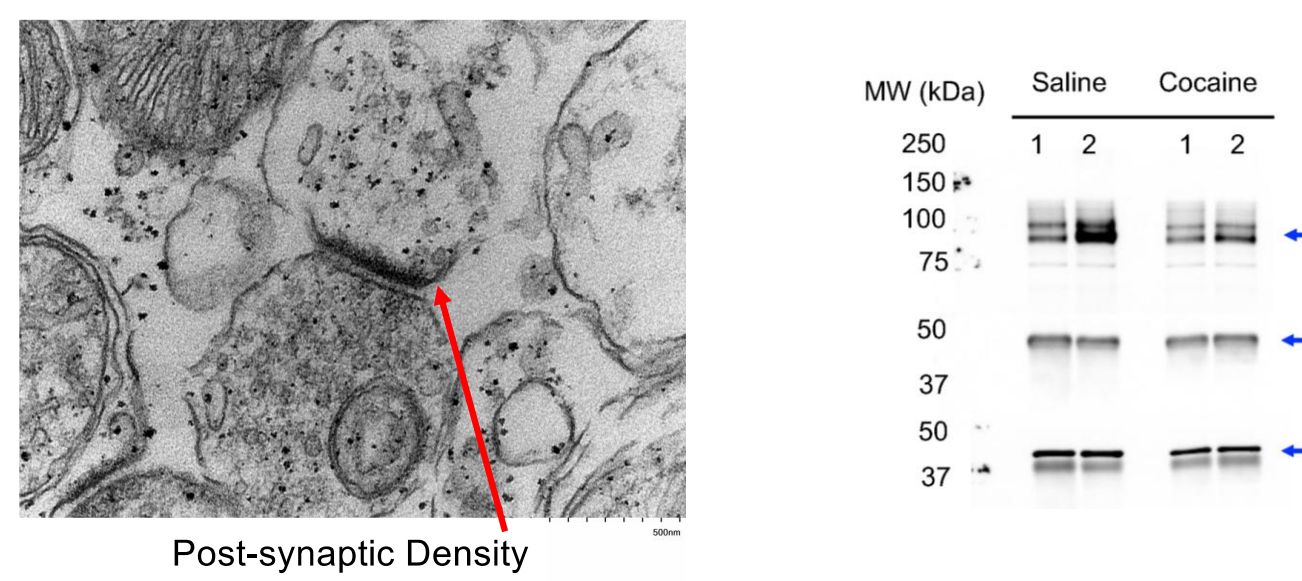
Background

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

Experimental Design



Synaptosome



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

Proteomic Data Analysis

The NAc synaptosomes are analyzed 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. A two-way ANOVA with type III sums of squares via R is used to identify differentially expressed proteins.

References

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Acknowledgements

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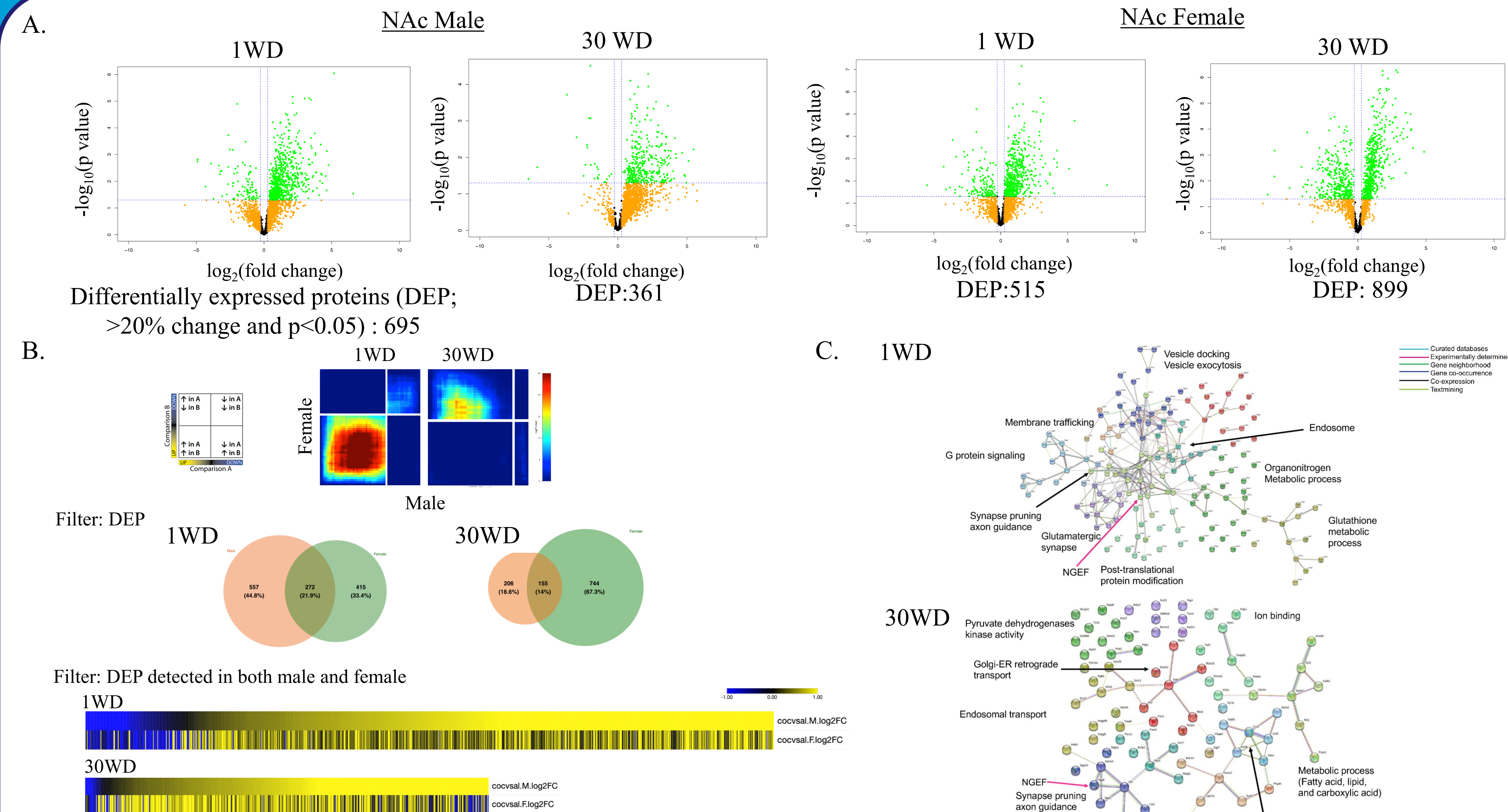


Figure 1. Proteomic Data of NAc Synaptosomes after cocaine I.P. injection. (A) Volcano plot analysis identifying differentially expressed proteins in the NAc of male and female mice at 1WD and 30WD timepoints (N=min. 5 per condition). (B) RRHO plot, Venn diagram, and heatmap depicting the detected proteins in NAc synaptosomes, highlighting those that are differentially expressed. (C) STRING analysis of differentially expressed proteins detected in both males and females.

NGEF (Ephexin1)

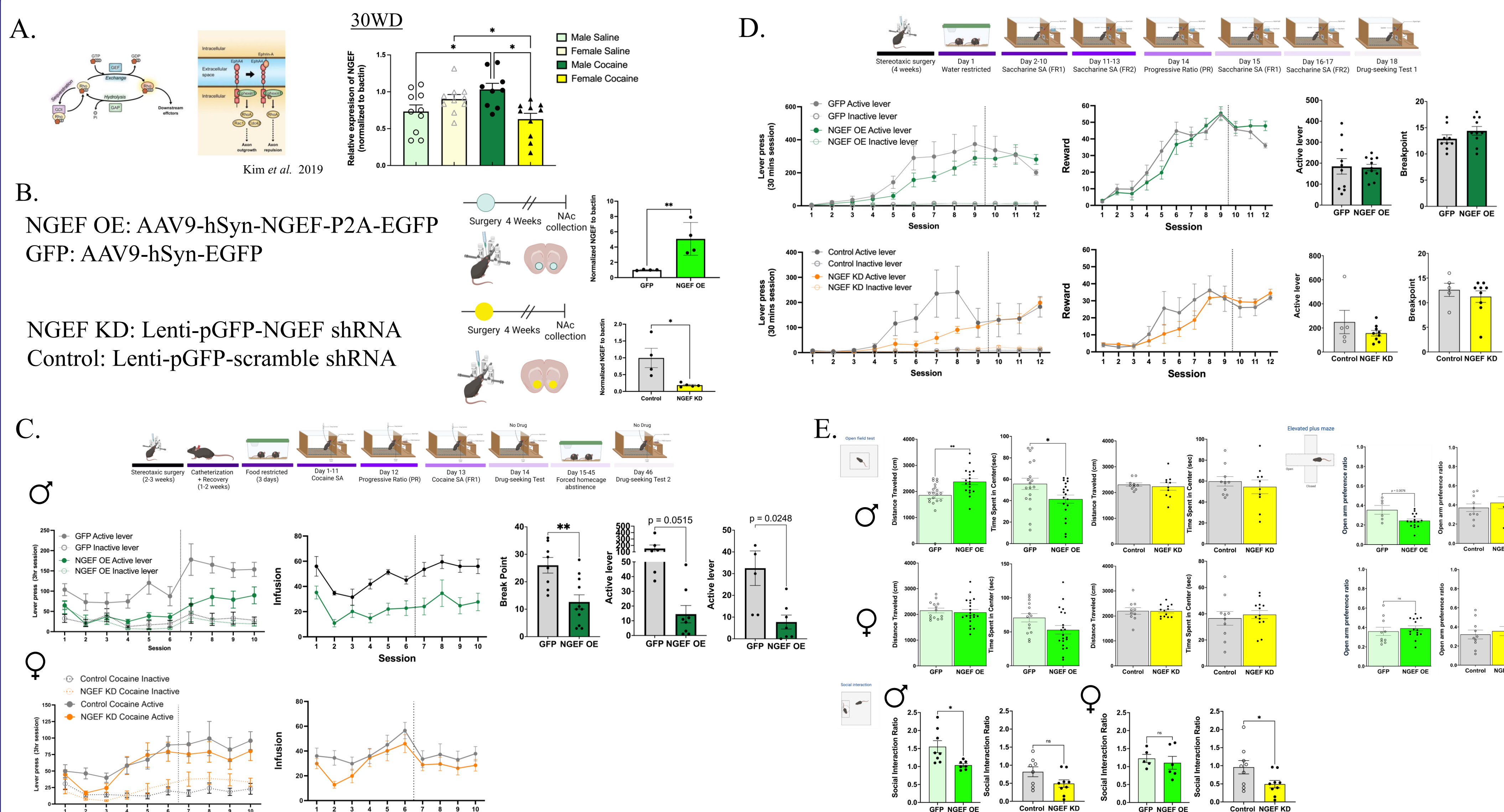


Figure 2. Sex-Specific Effect of NGEF in the NAc. (A) NGEF is a guanine nucleotide exchange factor that interacts with Rho GTPases. (B) Schematic of NGEF overexpression (OE) or knockdown (KD) in the NAc. (C) Cocaine self-administration (SA) following NGEF manipulation (N= min.8). NGEF OE in males and KD in females. (D) Saccharin SA following NGEF manipulation (N= min.5). NGEF OE in males and KD in females. (E) Baseline behaviors after NGEF manipulation. NGEF OE in males produces an anxiety-like phenotype (N= min. 5).

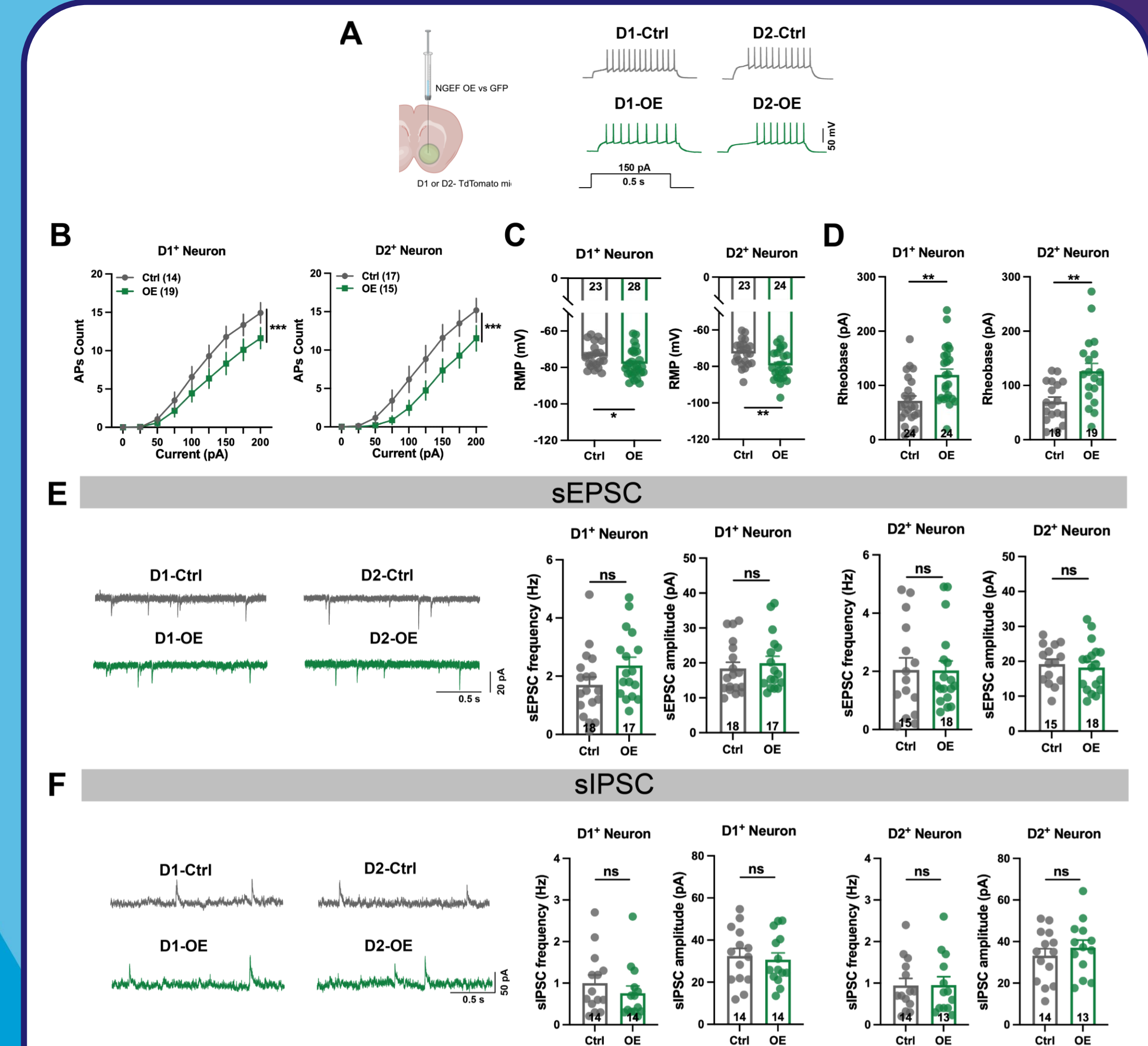


Figure 3. Whole cell path-clamp recordings. (A) Experimental design for the *ex vivo* electrophysiology study using Drd1 and Drd2-tdTomato mice. Control virus (Ctrl) expressed GFP. Representative membrane responses from D1- and D2-MSNs to a 150 pA current injection are shown. (B) NGEF OE reduced the number of evoked action potentials across increasing depolarizing current steps in both D1- (left) and D2-MSNs (right), indicating decreased excitability. (C) Resting membrane potential was more hyperpolarized in NGEF OE. (D) Rheobase was increased by NGEF OE. (E and F) Representative voltage-clamp recordings from NAc D1- or D2-MSNs showing sEPSCs (E) and sIPSC (F), with or without NGEF OE. NGEF OE did not alter frequency or amplitude of sEPSCs or sIPSCs in either MSNs.

Conclusions & Future Directions

- A 7-day cocaine injection model identified synapse-enriched proteins regulated in a sex- and withdrawal (WD) time-dependent manner.
- Proteomics revealed NGEF (Ephexin1) as a sex- and WD-dependent target: increased in both sexes at 1WD; increased in males but decreased in females at 30WD.
- NGEF OE reduced cocaine intake and cocaine seeking, while NGEF KD did not alter cocaine intake compared to controls.
- Neither NGEF OE nor NGEF KD affected natural reward seeking in males or females, respectively.
- In males, NGEF OE increased anxiety-like behavior and locomotor activity while reducing social interaction.
- In females, NGEF KD didn't change locomotion or anxiety-like behavior but decreased social interaction.
- Electrophysiology demonstrated that NGEF OE reduced NAc neuronal excitability without altering synaptic input.
- Future direction:** Determine how NGEF KD influences cocaine-seeking behavior in female mice and assess how NGEF regulates intrinsic neural excitability in males.

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