# Ephexin1 as a Sex- and Withdrawal-Dependent Regulator of Cocaine Seeking in the Nucleus Accumbens

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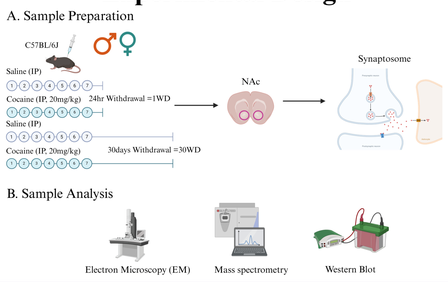
## Background

- Dysregulated signaling within reward-processing brain regions, such as the nucleus accumbens (NAc), plays a key role in drug-seeking behavior and relapse.
- While transcriptional responses to drugs of abuse are well characterized, cocaine-induced changes in the synaptic proteome remain poorly understood.
- Defining these proteomic alterations may reveal novel therapeutic targets for cocaine use disorder.
- Goal: : Identify the sex- and withdrawal (WD)-dependent, cocaine-induced changes in the NAc synaptic proteome to uncover candidate regulators of synaptic remodeling.

## Experimental Design




## Proteomic Data Analysis

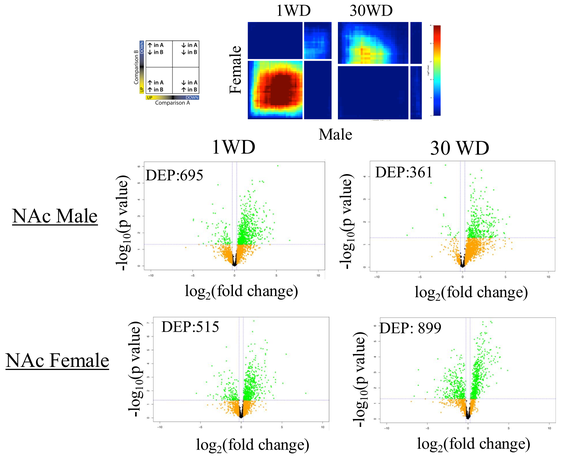



**Figure 1. Sex- and withdrawal-dependent proteomic signatures in NAc synaptosome**s. Label-free LC–MS/MS (DDA) was used to profile NAc synaptosomes at the Yale/NIDA Neuroproteomics Center. Differentially expressed proteins (DEPs) were identified using two-way ANOVA (type III) in R. (A) RRHO comparing male and female NAc proteomes at 1-day (1WD) and 30-day withdrawal (30WD). (B) Volcano plots of proteomic changes at 1WD and 30WD. $N \geq 5$ per condition.

**Acknowledgements**

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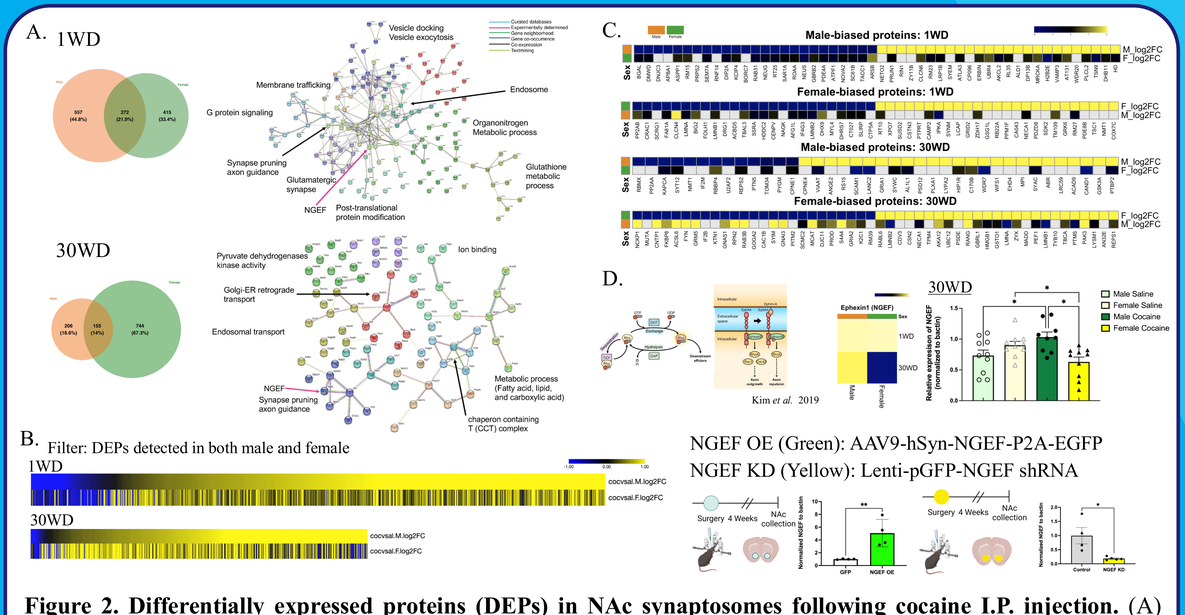


**Figure 2. Differentially expressed proteins (DEPs) in NAc synaptosomes following cocaine I.P. injection.** (A) Venn diagram and STRING network analysis of DEPs identified in male and female mice, highlighting proteins altered at 1WD and 30WD. (B) Heatmap of DEPs shared between sexes at each time point. (C) Heatmap of sex-biased DEPs at each time point, including proteins detected preferentially in one sex. For each sex, the top up –and down-regulated proteins are shown (25 per direction when available). Grey indicates proteins not detected in the indicated sex and/or timepoint. (D) Ephexin1 (NGEF) is identified as a DEP at both withdrawal time points and exhibits a sex-dependent expression pattern. Schematic of NGEF overexpression (OE) or knockdown (KD).




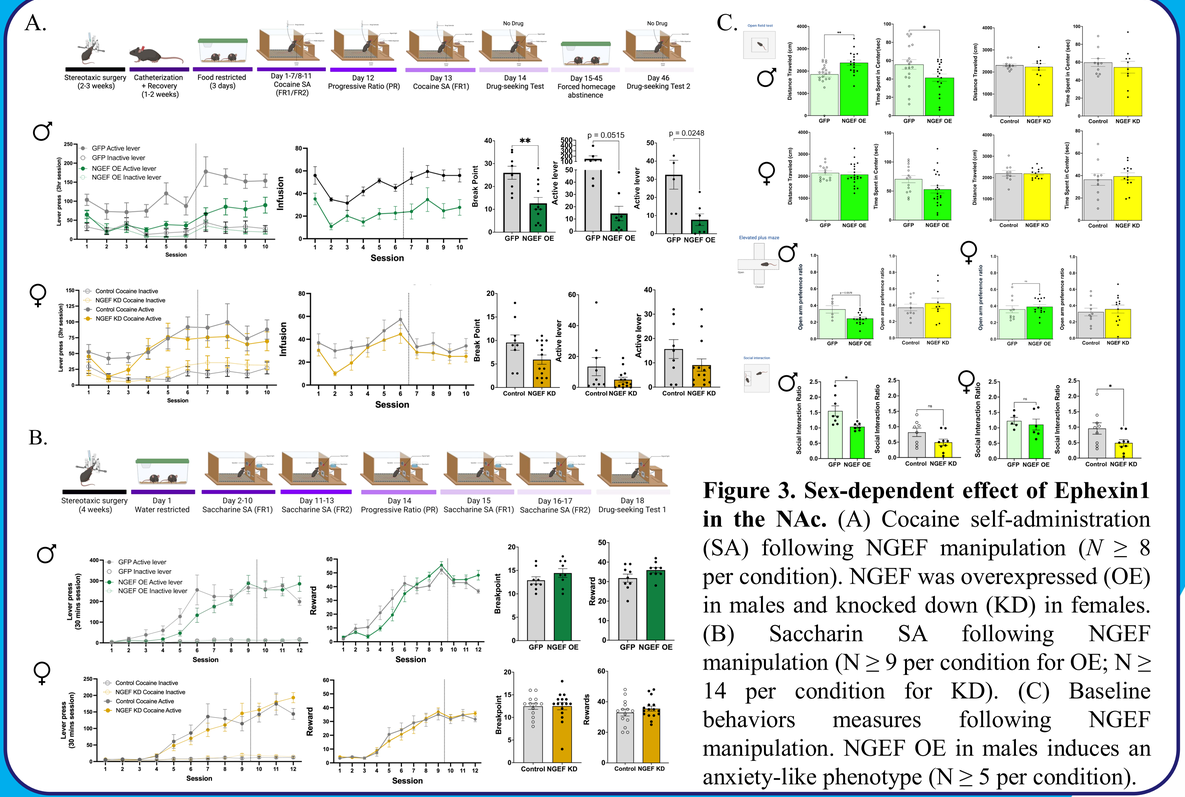


**Figure 3. Sex-dependent effect of Ephexin1 in the NAc.** (A) Cocaine self-administration (SA) following NGEF manipulation ($N \geq 8$ per condition). NGEF was overexpressed (OE) in males and knocked down (KD) in females. (B) Saccharin SA following NGEF manipulation ($N \geq 9$ per condition for OE; $N \geq 14$ per condition for KD). (C) Baseline behaviors measures following NGEF manipulation. NGEF OE in males induces an anxiety-like phenotype ($N \geq 5$ per condition).

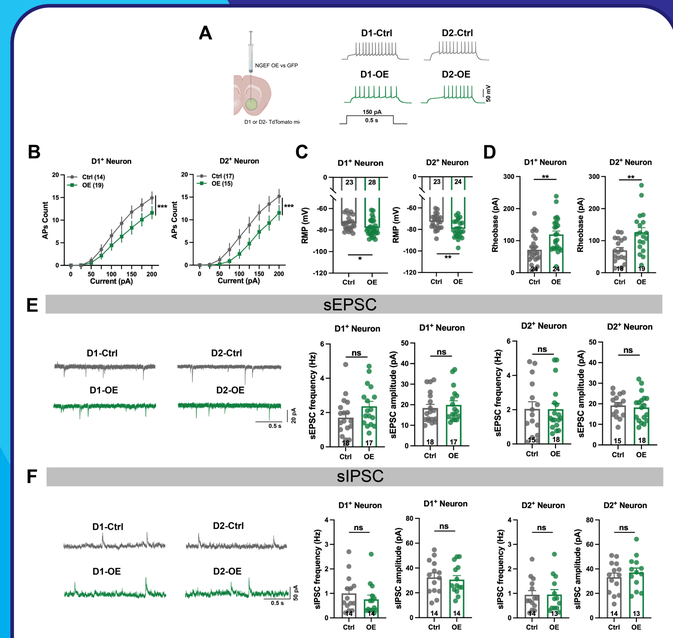


**Figure 4. Whole cell path-clamp recordings. (**A) Experimental design for the *ex vivo* electrophysiology study using Drd1 and Drd2-tdTomato mice. Control virus (Ctrl) expressed GFP. Representative membrane responses from D1- and D2-MSNs to a 150 pA current injection are shown. (B) NGEF OE reduced 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 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 male mice and assess how NGFE regulates intrinsic neural excitability in males.

**References**

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