# Heroin-Seeking Behavior and the Synaptic Proteome are Both Regulated by Phospholipase Cgamma1 in the Nucleus Accumbens

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## Introduction

- Drug-induced signaling in the nucleus accumbens (NAc) activates BDNF-TrkB-PLCg1 signaling (**Figure 1**)
  - Acute morphine increases phospho-PLCg1 protein in the NAc (Narita et al., 2003)
  - Chronic morphine upregulates PLCg1 protein in the NAc (Wolf et al., 2007)
- Altering TrkB-PLCg1 signaling in the NAc modulates cocaine-taking behavior (Anderson et al., 2017)
- Overexpressing PLCg1 in the NAc reduces the motivation for cocaine (Anderson et al., 2017) and reduces cocaine-induced increases in dendritic spine density
- Since BDNF-TrkB-PLCg1 signaling is altered by opioids, and can regulate other forms of drug-related behaviors, we investigated if PLCg1 regulated heroin-seeking behavior.
- We also investigated PLCg1's effects on the synaptic proteome.

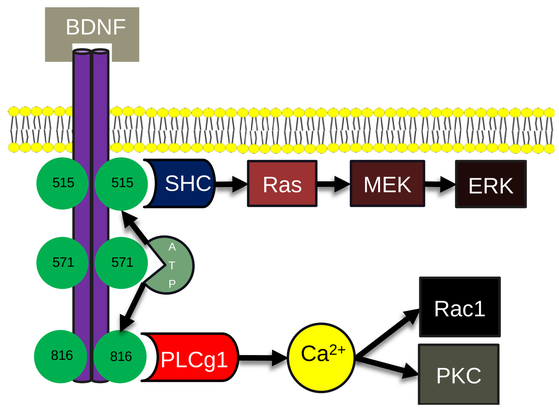


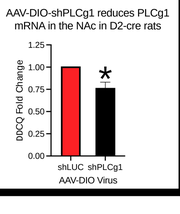

**Figure 1. PLCg1 signaling.** Brain-derived neurotrophic factor (BDNF) can bind to the tyrosine kinase B receptor (TrkB). This causes autophosphorylation of several sites including Tyr515 and Tyr816. Tyr816 phosphorylation can activate PLCg1 and cause changes in downstream signaling.

## Conclusions

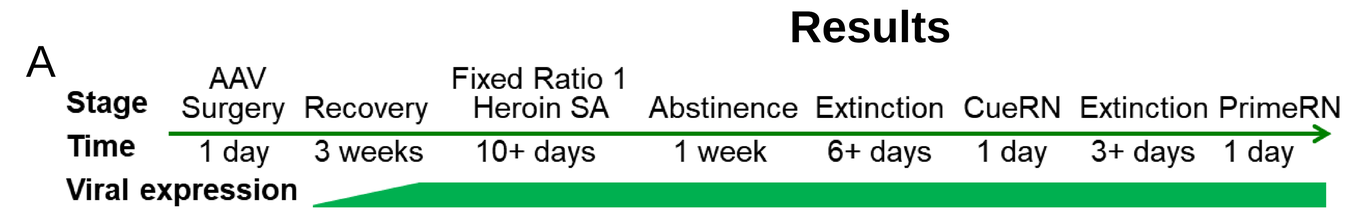
- Endogenous PLCg1 normally reduces heroin-seeking following self-administration
  - Knocking down endogenous PLCg1 increases heroin seeking
- No obvious sex differences were found (data not shown), but these studies are underpowered currently
- Both NAc PLCg1 and heroin regulate the synaptic proteome during drug-seeking behavior
- Using Toppgene and SynGo, the most significant enrichments are in synapses, axons, neuron projections, synaptic signaling, and cytoskeletal protein binding
- NAc PLCg1 and heroin have synergistic effects on arhgap39, gabrb2, nos1 and shank2. These are potential targets for NAc PLCg1's effects on heroin-seeking

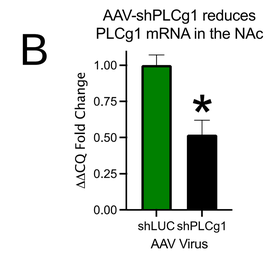
## Future Directions

- Determine which downstream signaling targets of PLCg1 are responsible for these effects
- Examine cell type specific effects of PLCg1 on these behaviors with D1- and D2-cre transgenic rats
- Investigate PLCg1's effects on calcium signaling in the NAc during heroin-seeking




## Results



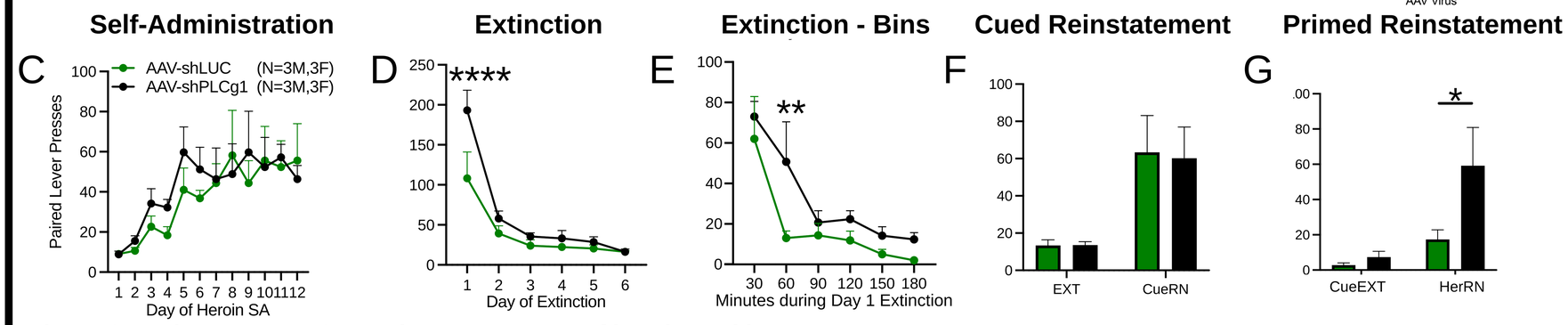
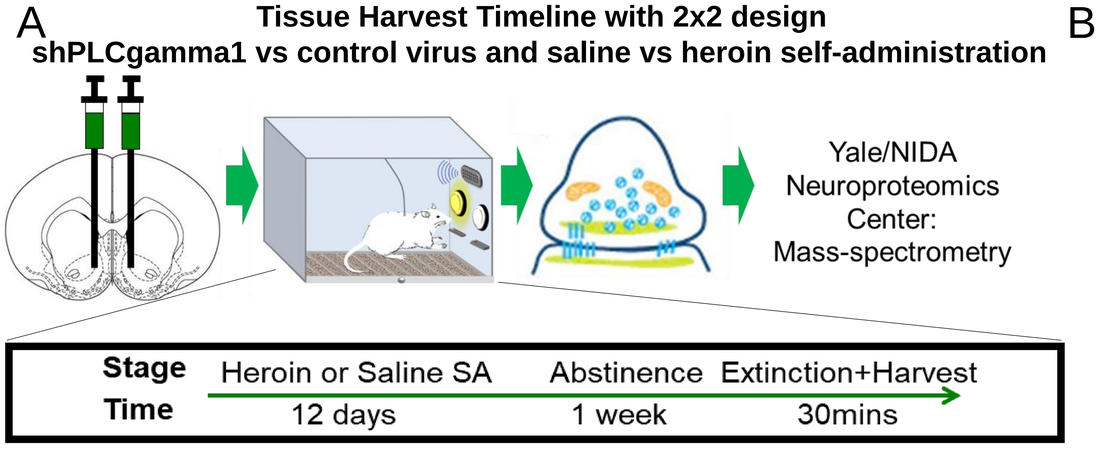




**Figure 2. Endogenous PLCg1 reduces two types of heroin seeking.** **A)** Timeline for C-G. **B)** AAV-shPLCg1 reduces PLCg1 mRNA in the NAc as compared to AAV-shLuciferase. **C)** Sprague-Dawley rats were infused with AAV-shPLCg1or AAV-shLuc in the NAc and 3 weeks later were allowed to self-administer heroin. **D)** Following 7 days of abstinence rats went through extinction for at least 6 days. **E)** Extinction Day 1 paired lever presses in 30-minute bins. **F)** NAc PLCg1 had no effect on cue reinstatement. **G)** NAc shPLCg1 increased heroin-primed reinstatement. Data are expressed as mean +/- s.e.m. *$p < 0.05$, **$p < 0.01$, and ****$p < 0.0001$.



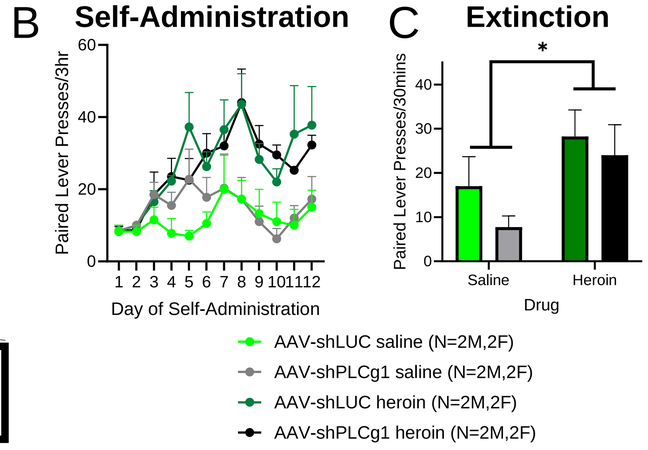
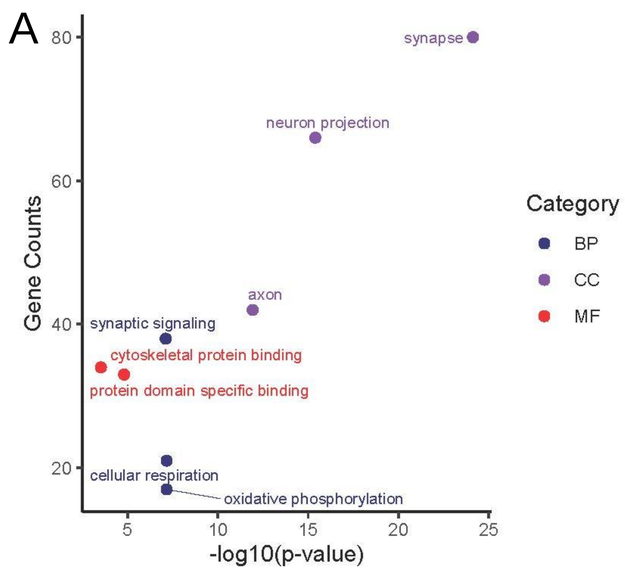
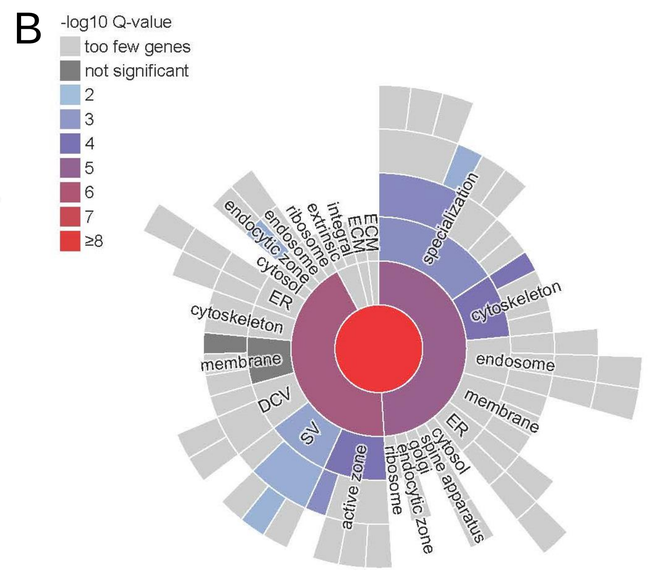




**Figure 3. NAc tissue collection for synaptic proteome mass spectrometry.** **A)** Timeline for B-C. **B)** Sprague-Dawley rats were infused with AAV-shPLCg1or AAV-shLuc in the NAc and 3 weeks later were allowed to administer either saline or heroin. **C)** Following 7 days of abstinence rats went through extinction for 30mins and tissue was then immediately harvested for synaptosomal prep and mass spectrometry. Heroin self-administration rats lever pressed more than saline rats. Data are expressed as mean +/- s.e.m. *$p < 0.05$.



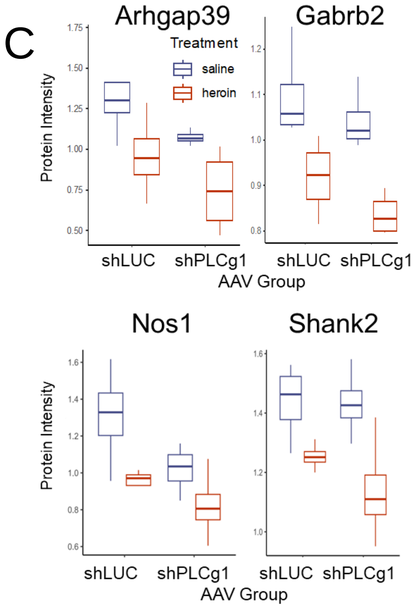




**Figure 4. NAc PLCg1 and heroin regulate the synaptic proteome.** **A)** We detected 1,967 proteins and found 223 differentially regulated proteins in the NAc altered by PLCg1, heroin, or a combination of both. A functional enrichment analysis showed our samples were enriched with synaptic proteins, cytoskeletal protein, and neurons as expected (Biological Processes (BP), cellular components (CC), molecular functions (MF). **B)** We used SynGo and found that these proteins were enriched for pre- and post-synaptic proteins as expected. **C)** Several candidate genes show a synergistic effect of both heroin and PLCg1 knockdown, suggesting that the PLCg1 knockdown may be potentiating some of the effects of heroin on the synaptic proteome. These proteins are also potential candidates for the increases in heroin-seeking observed following our NAc PLCg1 knockdown.