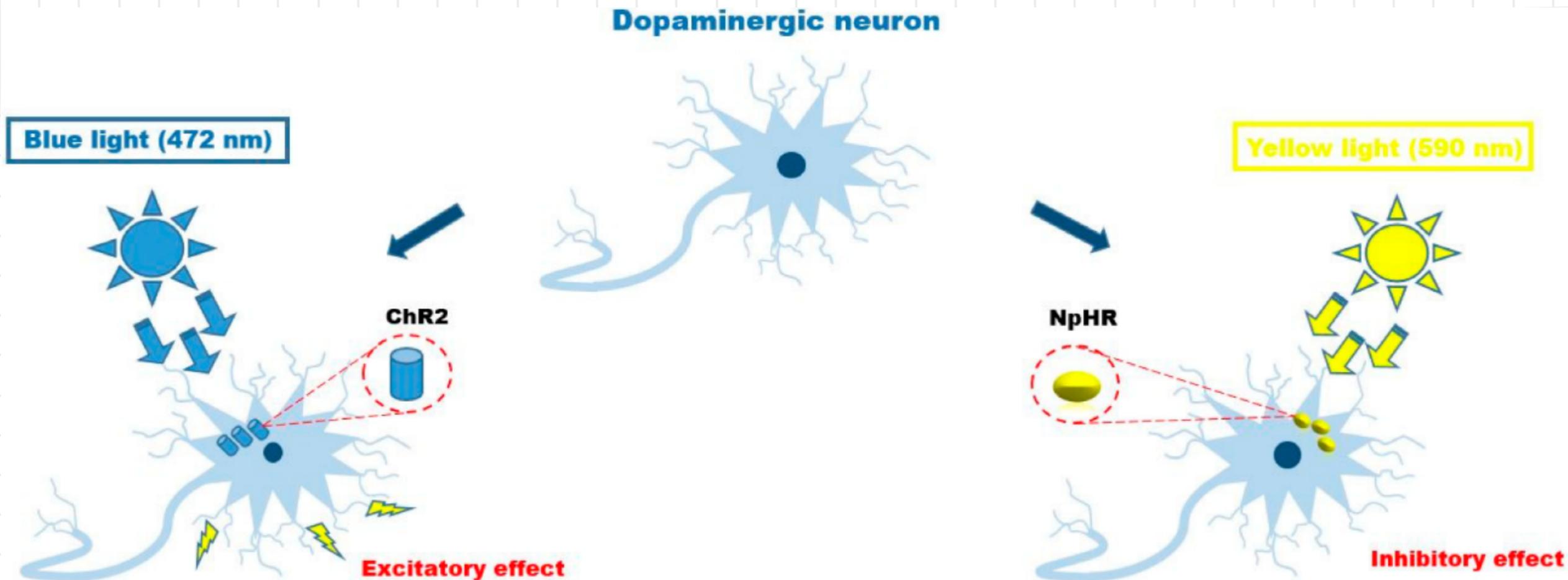


Effects of Medial Prefrontal Cortex-Laterodorsal Tegmental Nucleus Projection Inhibition on Cocaine-Induced Sensitization

Marko Koruna & Stephan Steidl

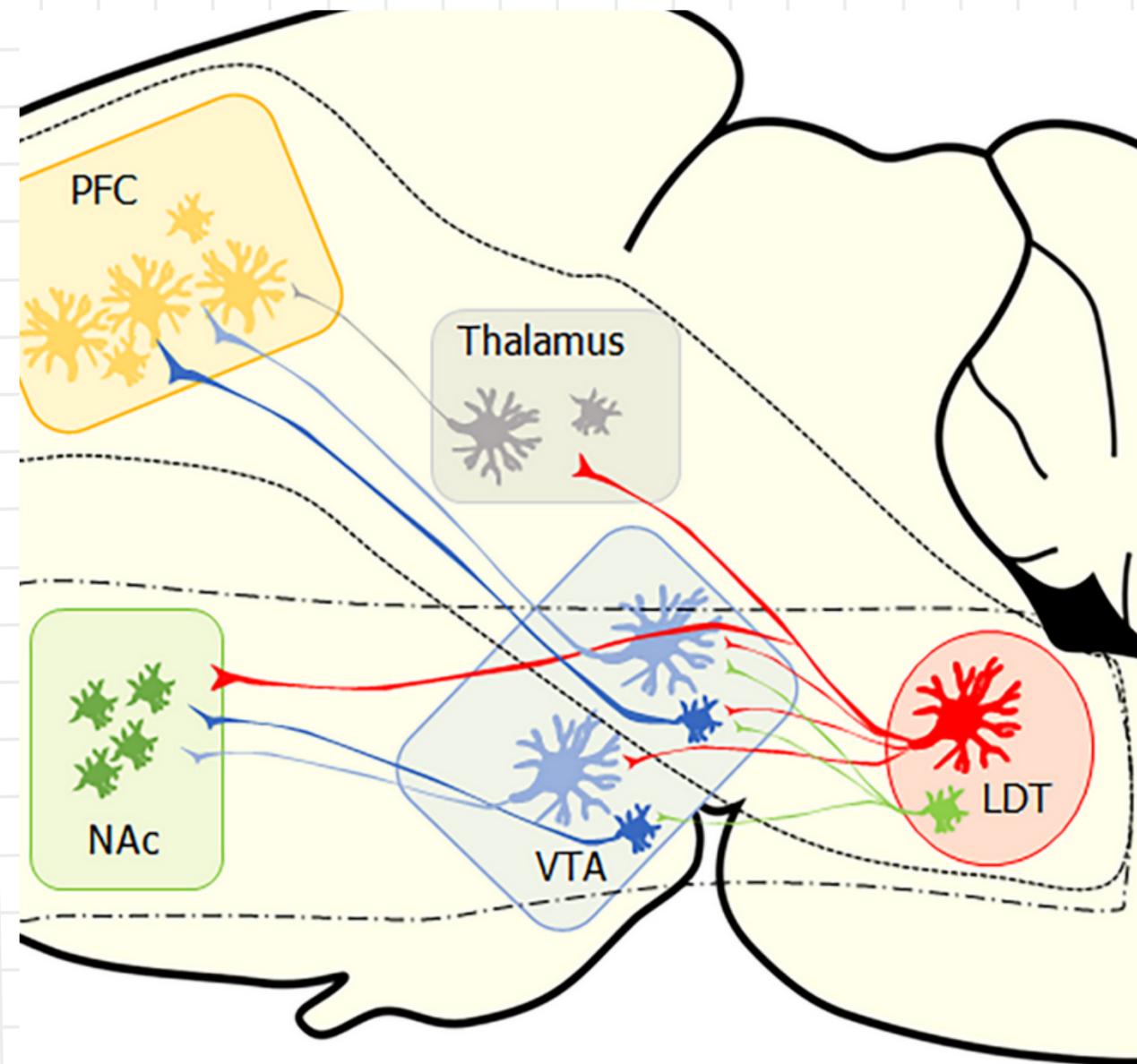
Optogenetics

- Manipulation of the activity of neurons with light
- Uses light-gated ionic pumps called opsins derived from microorganisms (ex: algae)
- Our study uses Halorhodopsin (NpHR) and Enhanced Yellow Fluorescent Protein (eYFP) through viral transfection into mice brains



Spagnuolo G, Genovese F, Fortunato L, Simeone M, Rengo C, Tatullo M. The Impact of Optogenetics on Regenerative Medicine. *Applied Sciences*. 2020; 10(1):173. <https://doi.org/10.3390/app10010173>

Sensitization

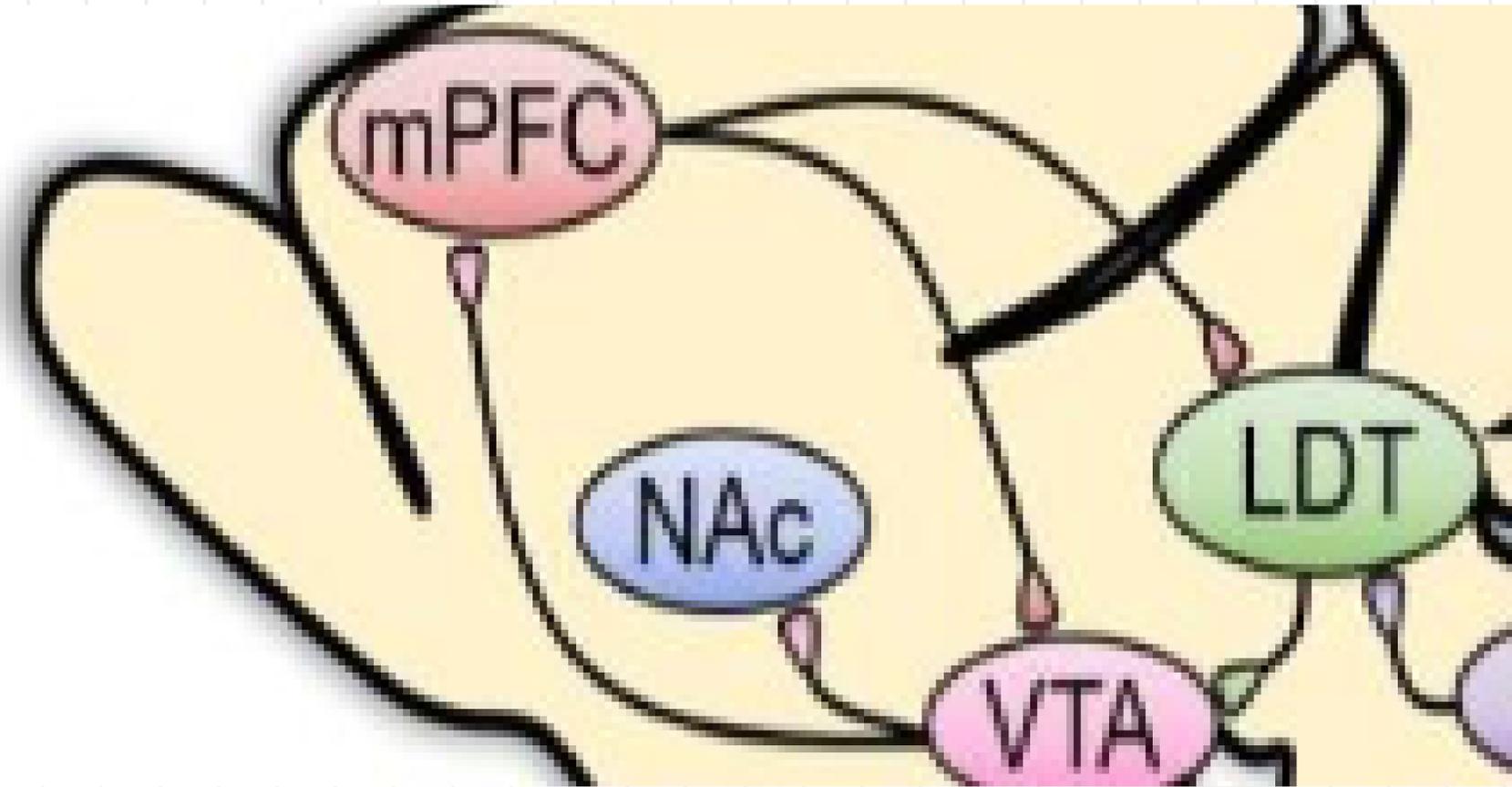


- Sensitization is the heightened ability of drugs of abuse, like cocaine, to activate dopamine neurotransmission increasing locomotor activity and stimulating the reinforcement and reward systems
- The Laterodorsal Tegmental Nucleus (LDTg) serves as an input of neurotransmitters, including glutamate (GLU) to the Ventral Tegmental Area (VTA)
- Our lab has shown previously, that LDTg GLU input to the VTA is important for sensitization. Also, the regulation of midbrain dopamine neuron firing along with the regulation of Nucleus Accumbens dopamine levels are implicated.

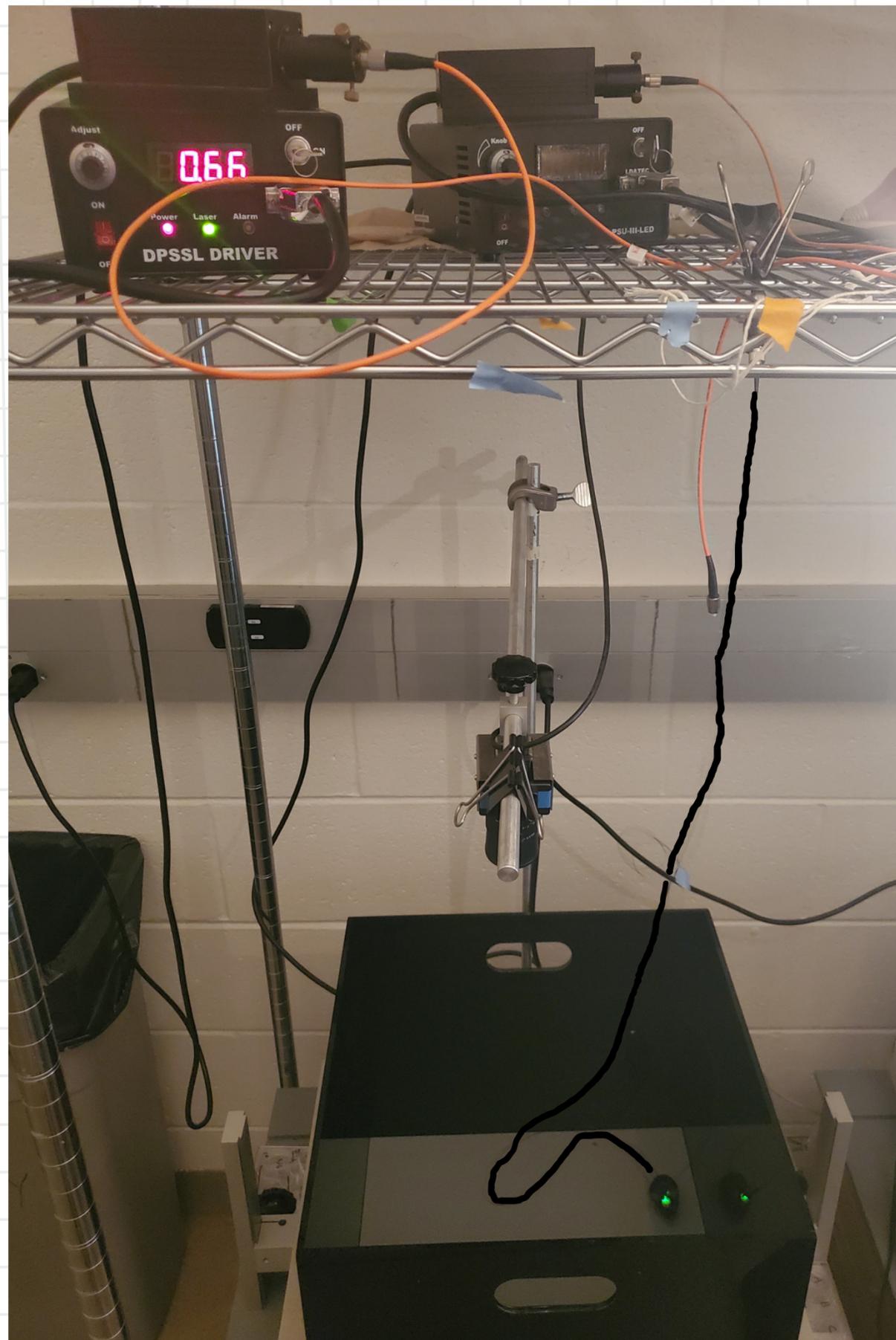
Polli FS, Kohlmeier KA. Prenatal nicotine alters development of the laterodorsal tegmentum: Possible role for attention-deficit/hyperactivity disorder and drug dependence. *World J Psychiatry* 2022; 12(2): 212-235 [PMID: 35317337 DOI: 10.5498/wjp.v12.i2.212]

The Medial Prefrontal Cortex is Critical for the Development of Sensitization

Kaneda, K. (2018). Neuroplasticity in cholinergic neurons of the laterodorsal tegmental nucleus contributes to the development of cocaine addiction. *European Journal of Neuroscience*. doi:10.1111/ejn.13962



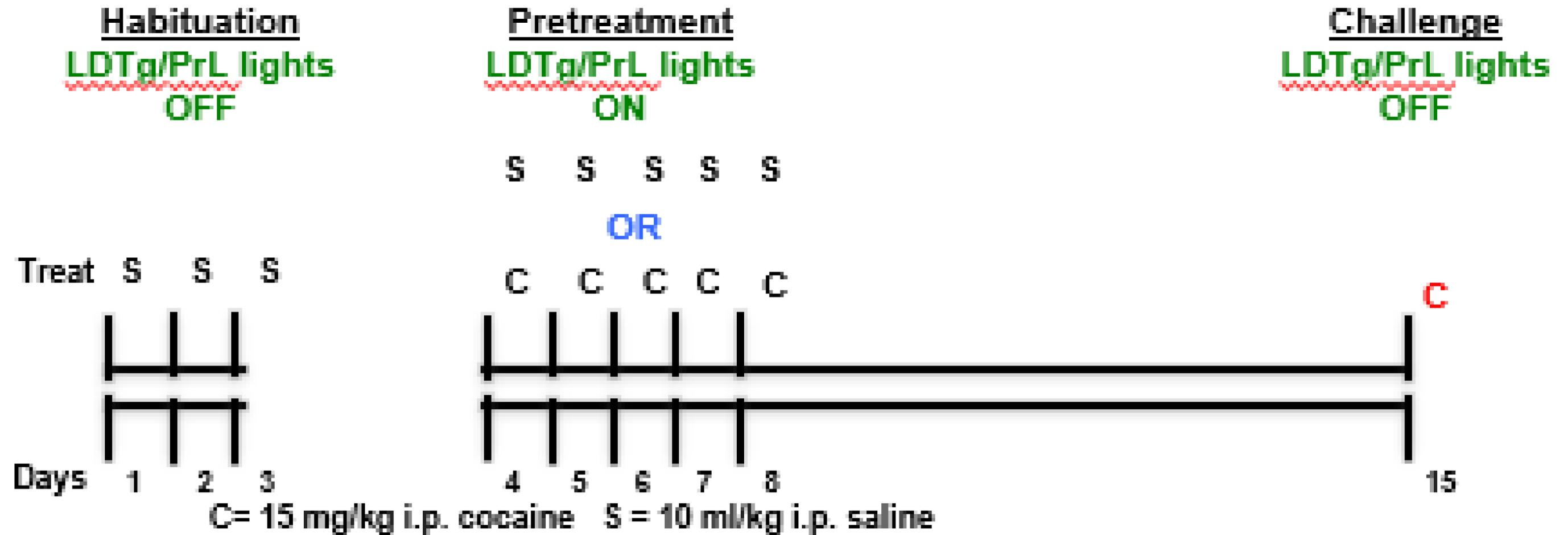
- The medial prefrontal cortex (mPFC) has also been shown through studies involving lesioning of the mPFC to be integral in locomotor sensitization
- It is believed that sensitization induced by cocaine results from increased mPFC excitatory transmissions to its downstream targets, one of which is the LDTg.
- As LDTg is a major input for the VTA, inhibition of mPFC projections to LDTg during cocaine exposure will test the roles of these projections in sensitization development



Behavioral Testing

- Mice are given injections, of saline or cocaine, and placed into open boxes for a one-hour period
- Optical tethers are connected to mice throughout experimental days
- Movement and rearing of mice is tracked through a camera above each box

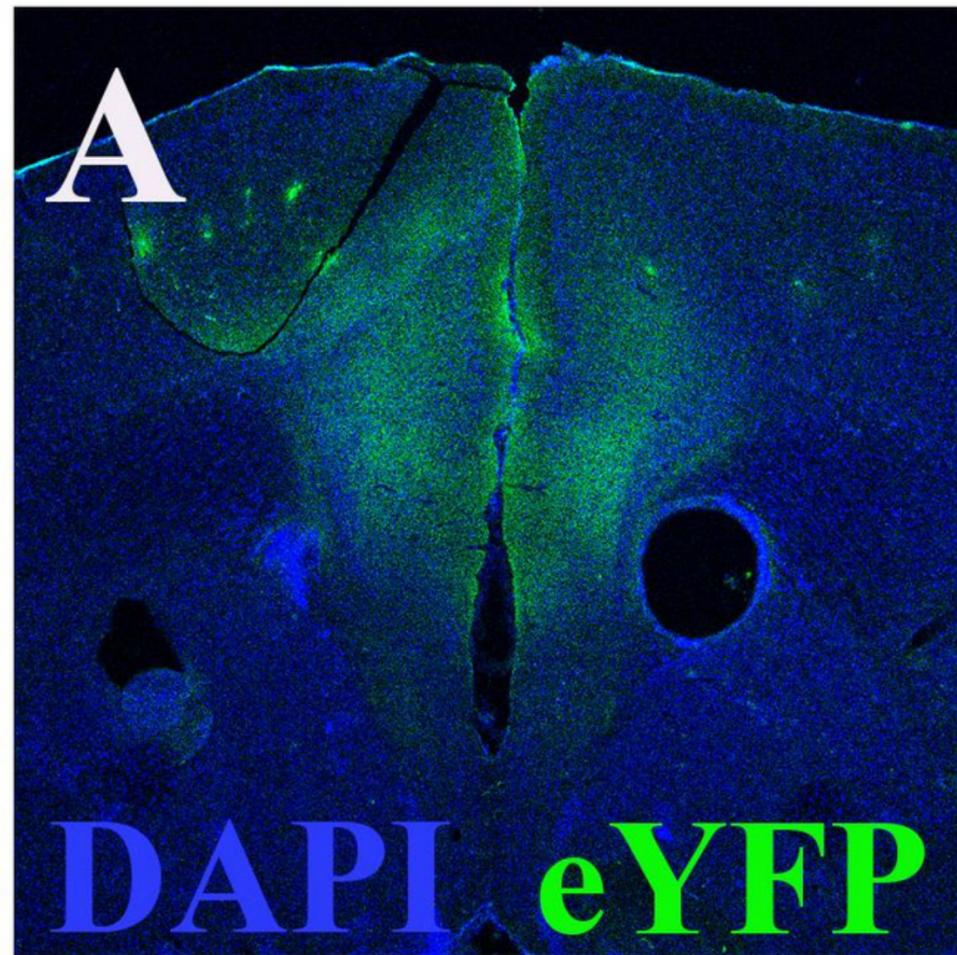
Experimental Timeline



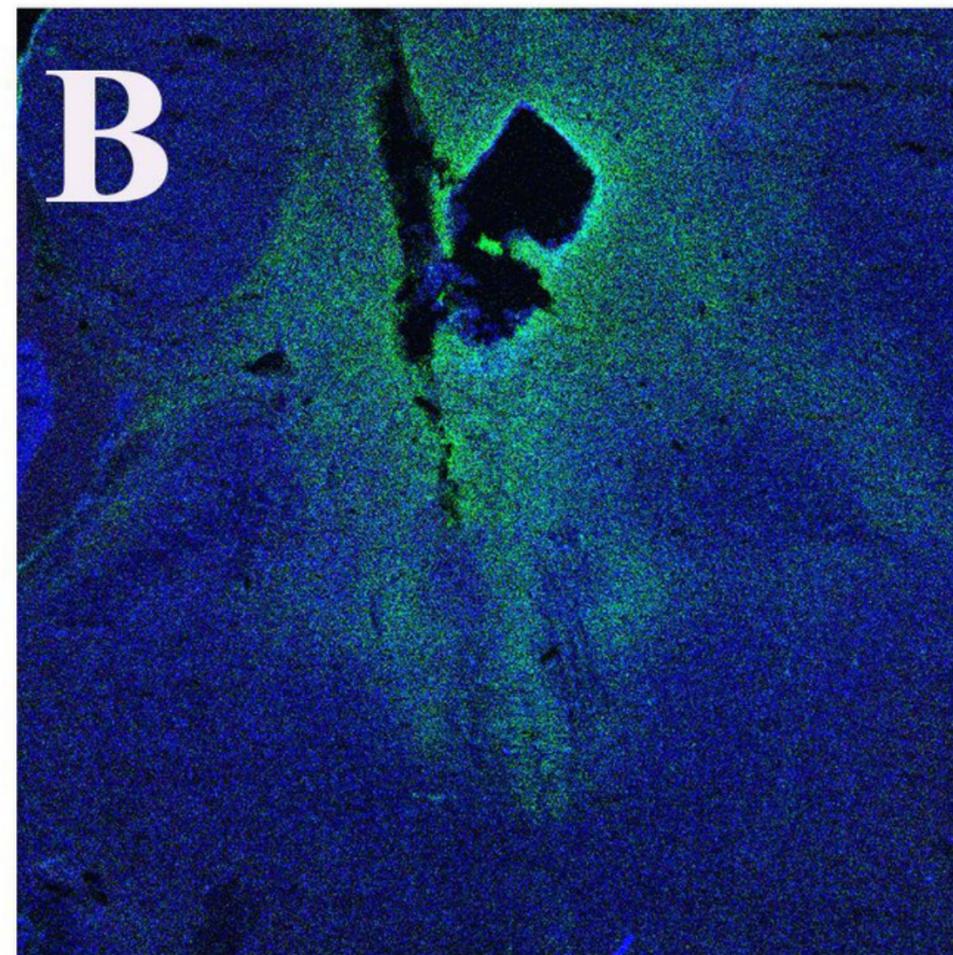
- Two phases of sensitization, pre-exposure and exposure post withdraw period

Sensitization Process

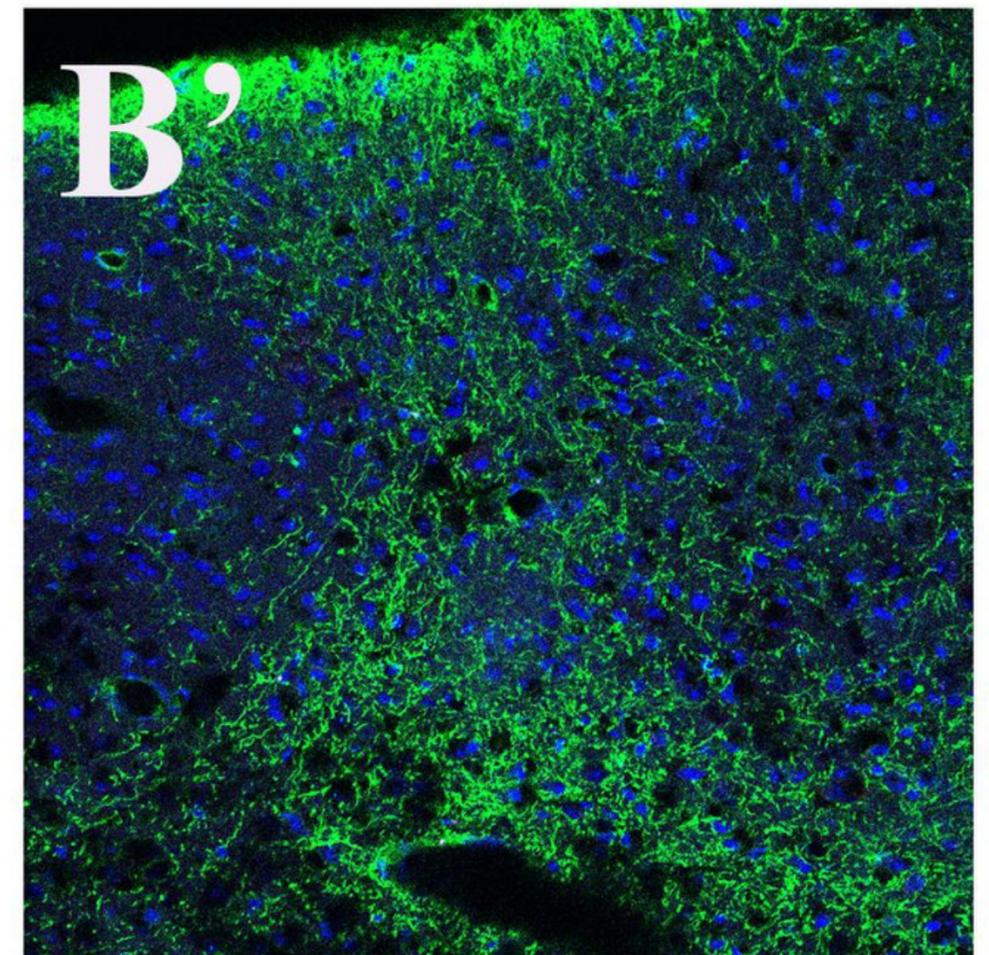
Histology: NpHR+ Neurons in the mPFC following viral transfection



mPFC expressing eYFP

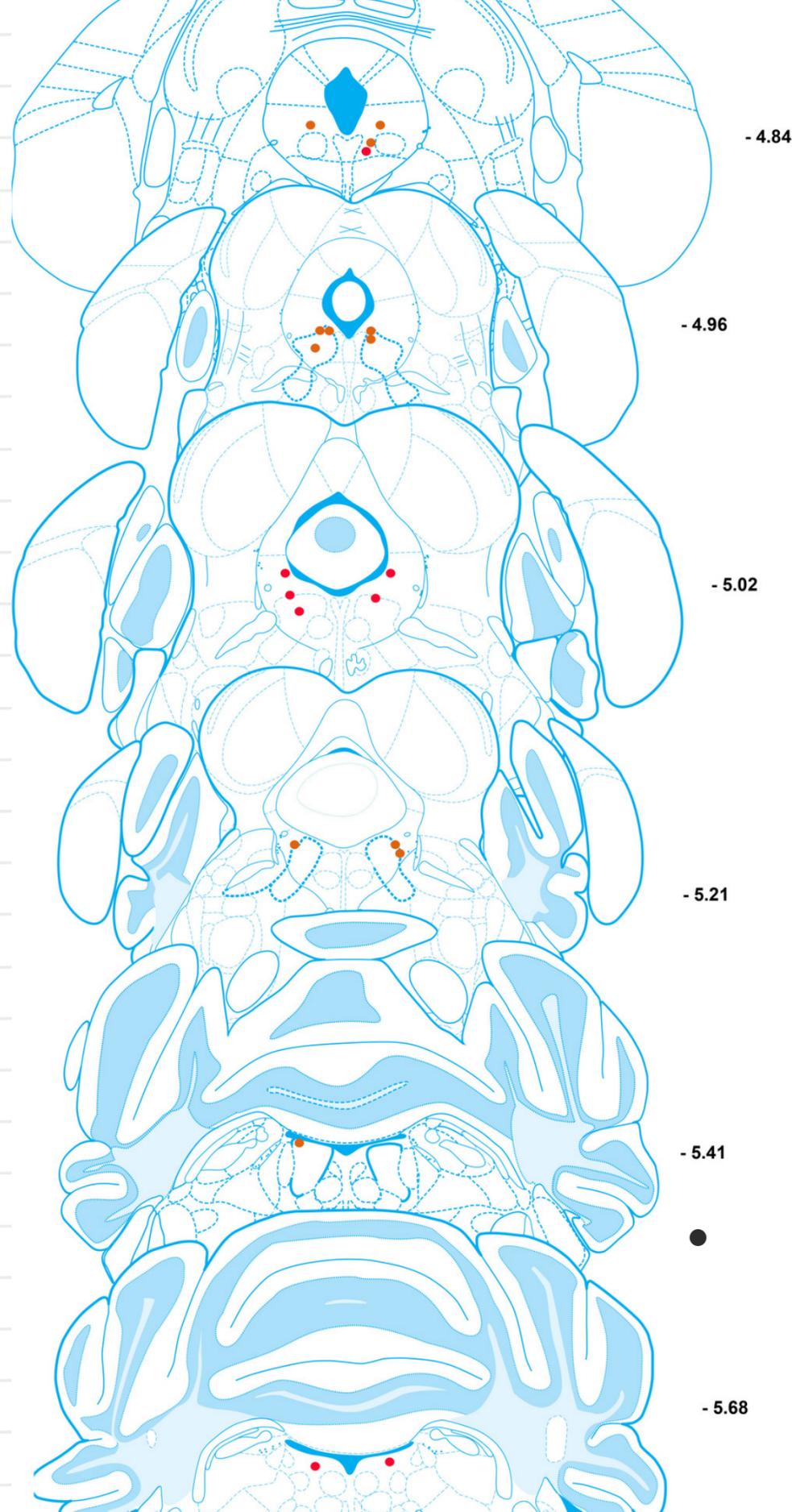


LDTg expressing eYFP

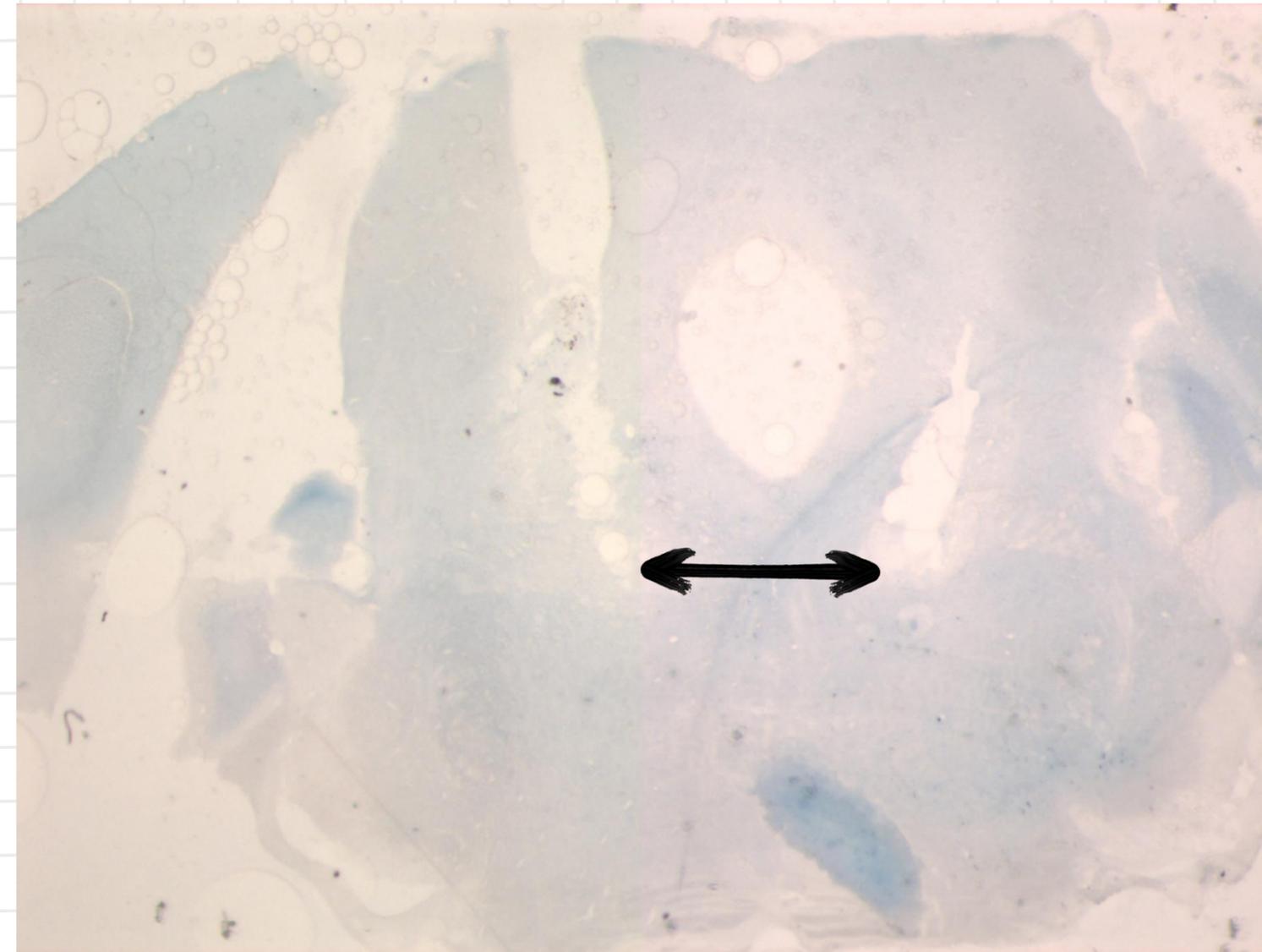


Close up of image B

Histology: Cresyl Violet Staining



- Cresyl Violet is a dye that stains nissl substance in the cytoplasm of neurons purple-blue as seen in the picture to the right



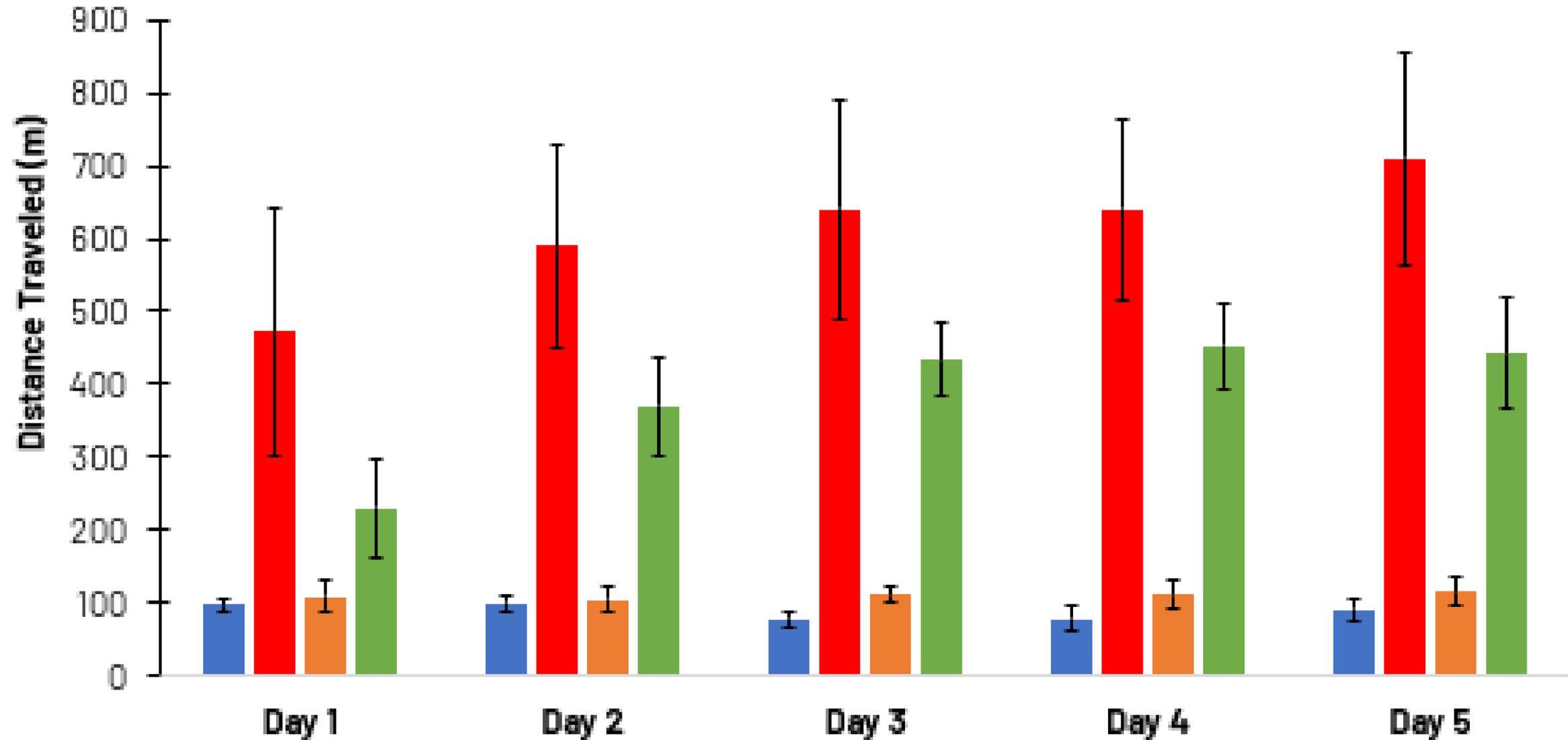
• LDTg probe placements

- Single brain segment showing the probe tracks into the LDTg

Results: Inhibition of PrL projections to LDTg affects acute locomotor activation induced by cocaine

Cocaine Trial Days 1-5

■ eYFP SAL (n=6, F=4, M=2) ■ eYFP COC (n=7, F=3, M=4)
■ NpHR SAL (n=6, F=2, M=4) ■ NpHR COC (n=5, F=2, M=3)



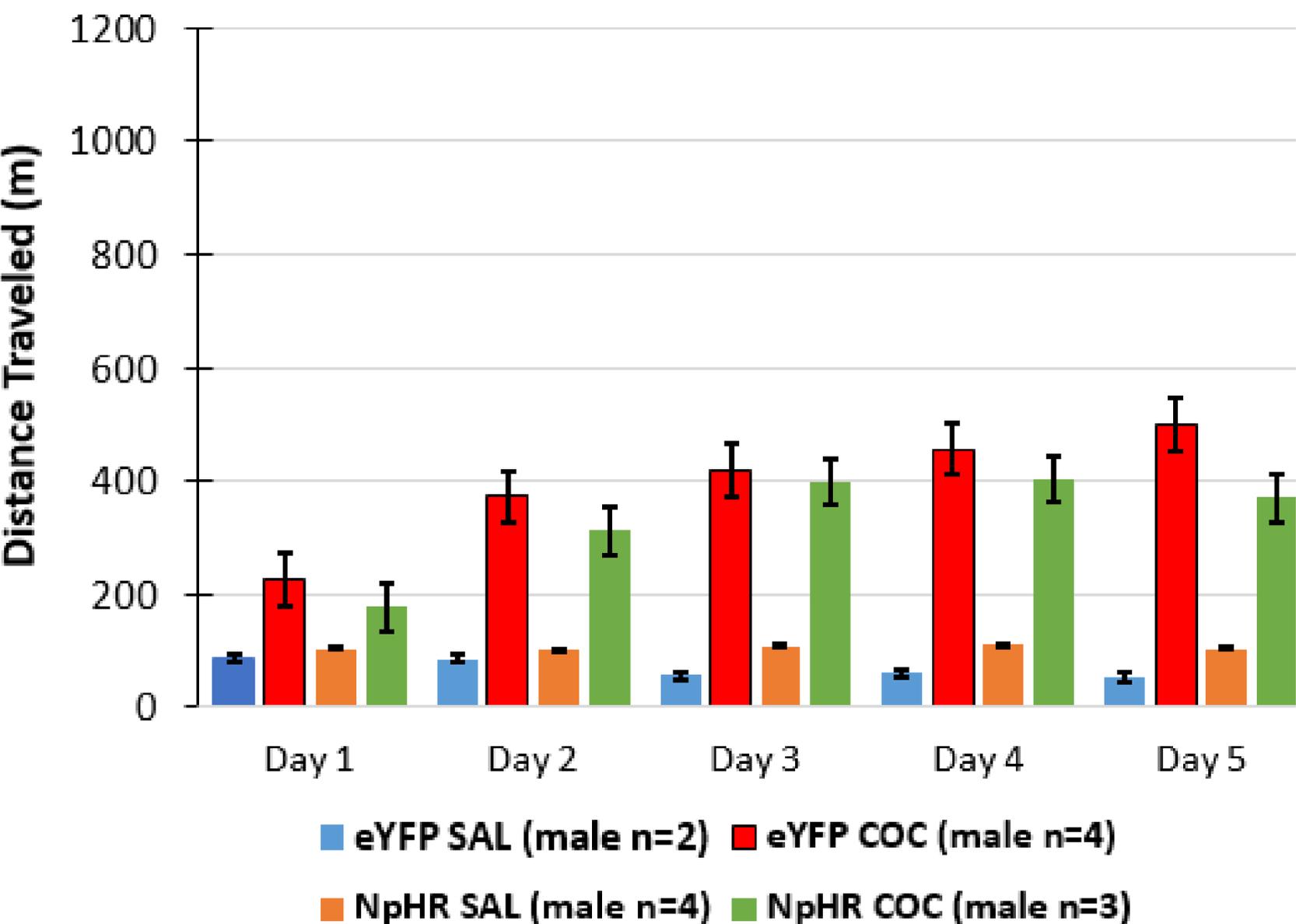
• pvalue within days =
<.001

• pvalue between groups =
0.002

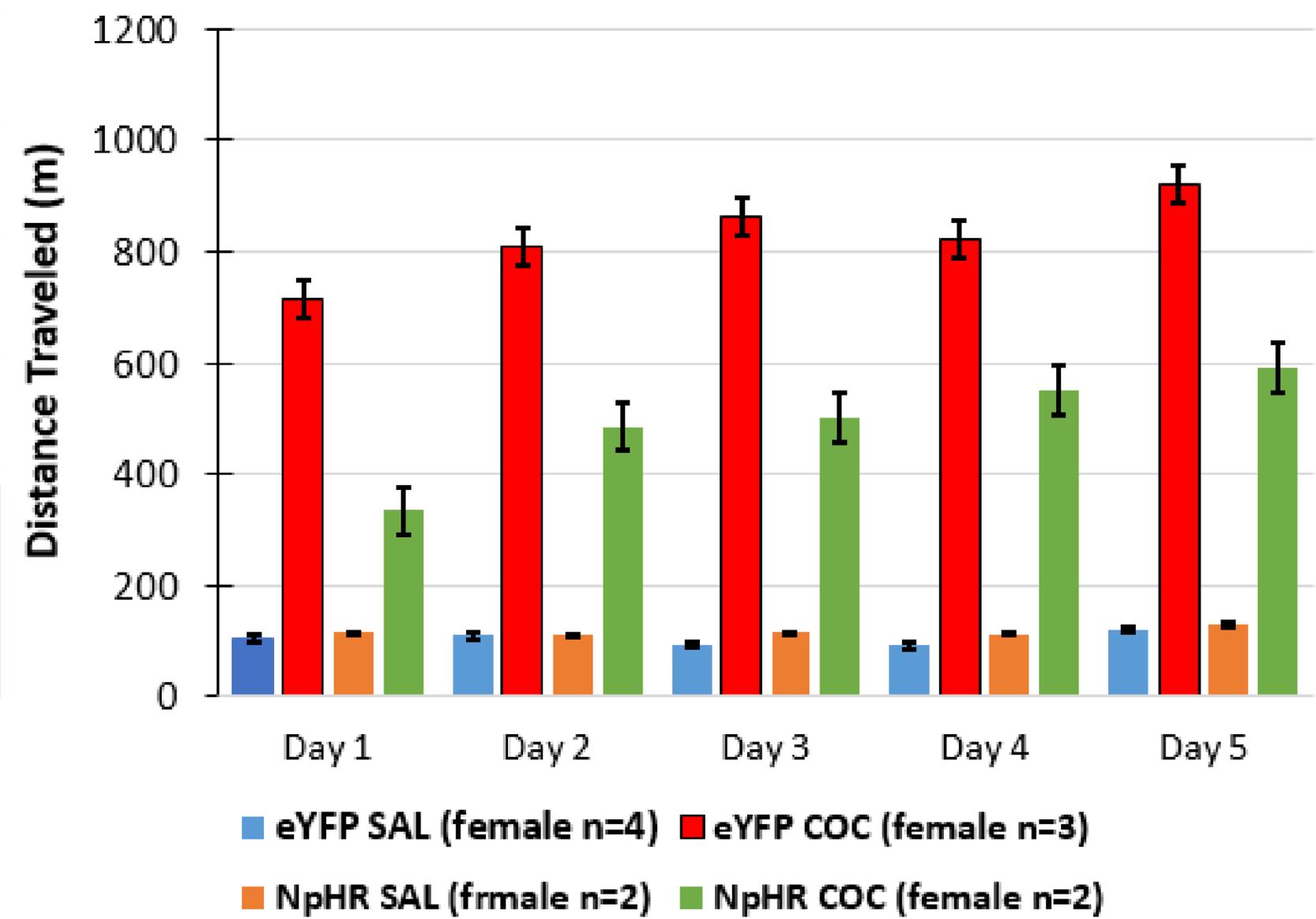
Results: Cocaine Trial Days Sex Comparison

• pvalue between sexes = 0.181

Cocaine Trial Days 1-5 Male Mice

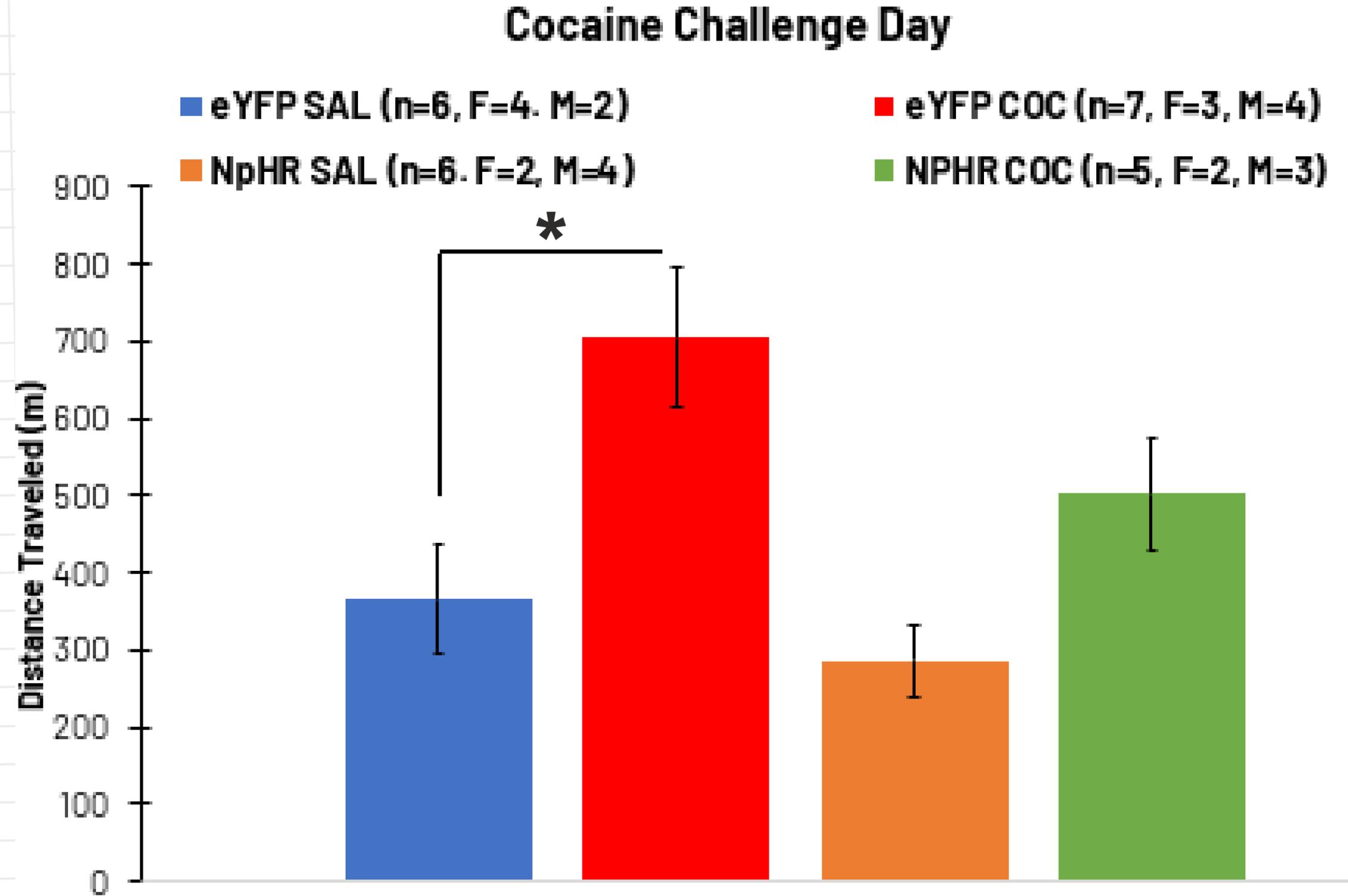


Cocaine Trial Days 1-5 Female Mice



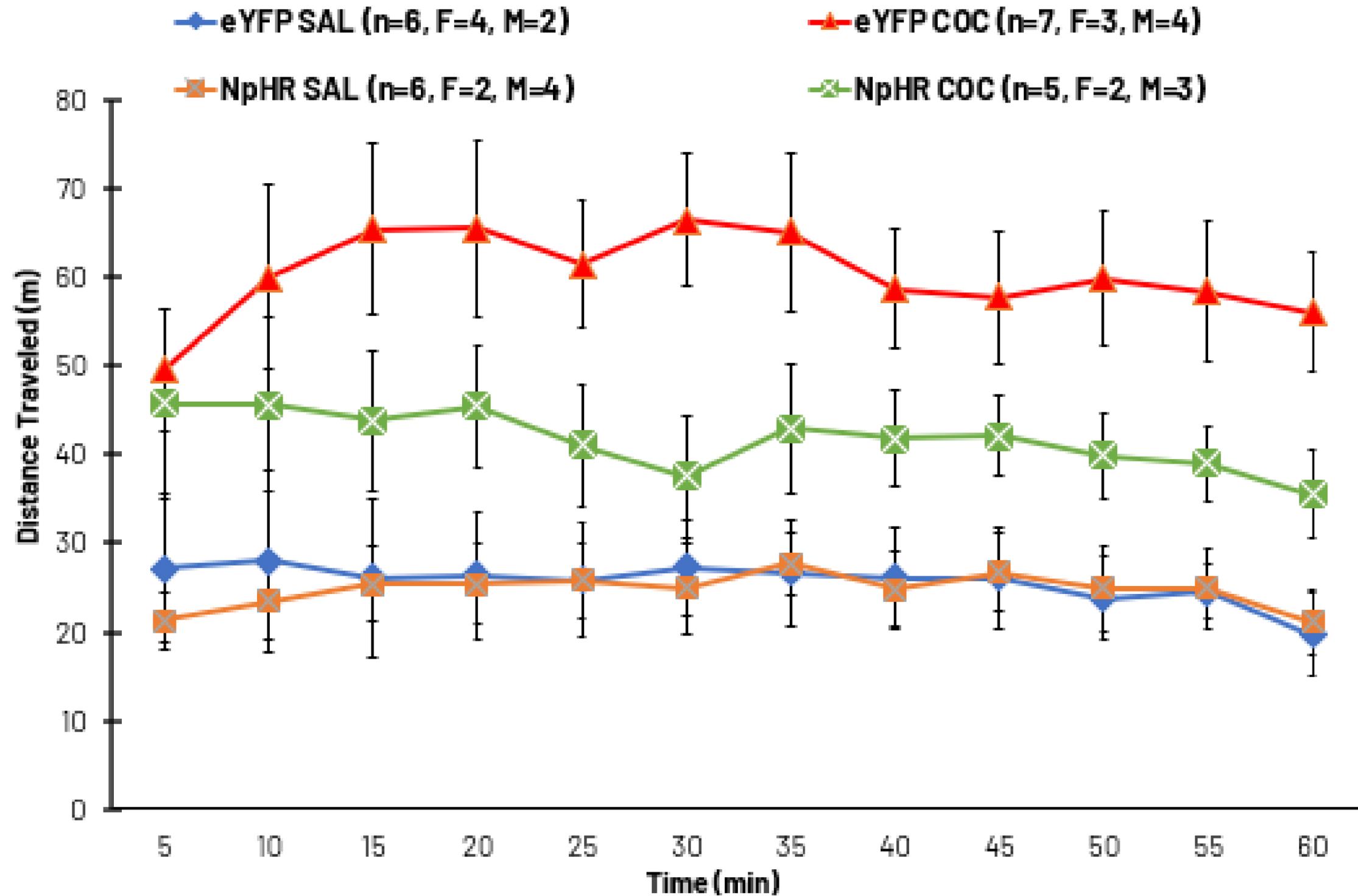
Results: Inhibition of PrL projections to LDTg during repeated cocaine exposures reduces locomotor sensitization

- pvalue for main effect of group = 0.001
- Post-Hoc test pairwise comparison (Fisher's LSD) = EC>ES (pvalue <.001) and NS-NC (pvalue = 0.097)



Results: Cocaine Challenge Timecourse

Cocaine Challenge Timecourse



• pvalue for main effect of group = 0.002

Summary

- Inhibition of PrL projections to the LDTg during repeated cocaine exposures reduces the expression of drug-induced locomotor sensitization on a delayed challenge test
- We propose that cocaine-evoked increases in PrL dopamine results in enhanced PrL excitation of LDTg neurons. This in turn results in excitation of LDTg GLU input to the VTA known to be critical for the development of sensitization

Acknowledgments

- We thank the National Institute on Drug Abuse for generously supply cocaine hydrochloride for these studies. Mulcahy and Provost funding; Kaitlyn Schreiter