

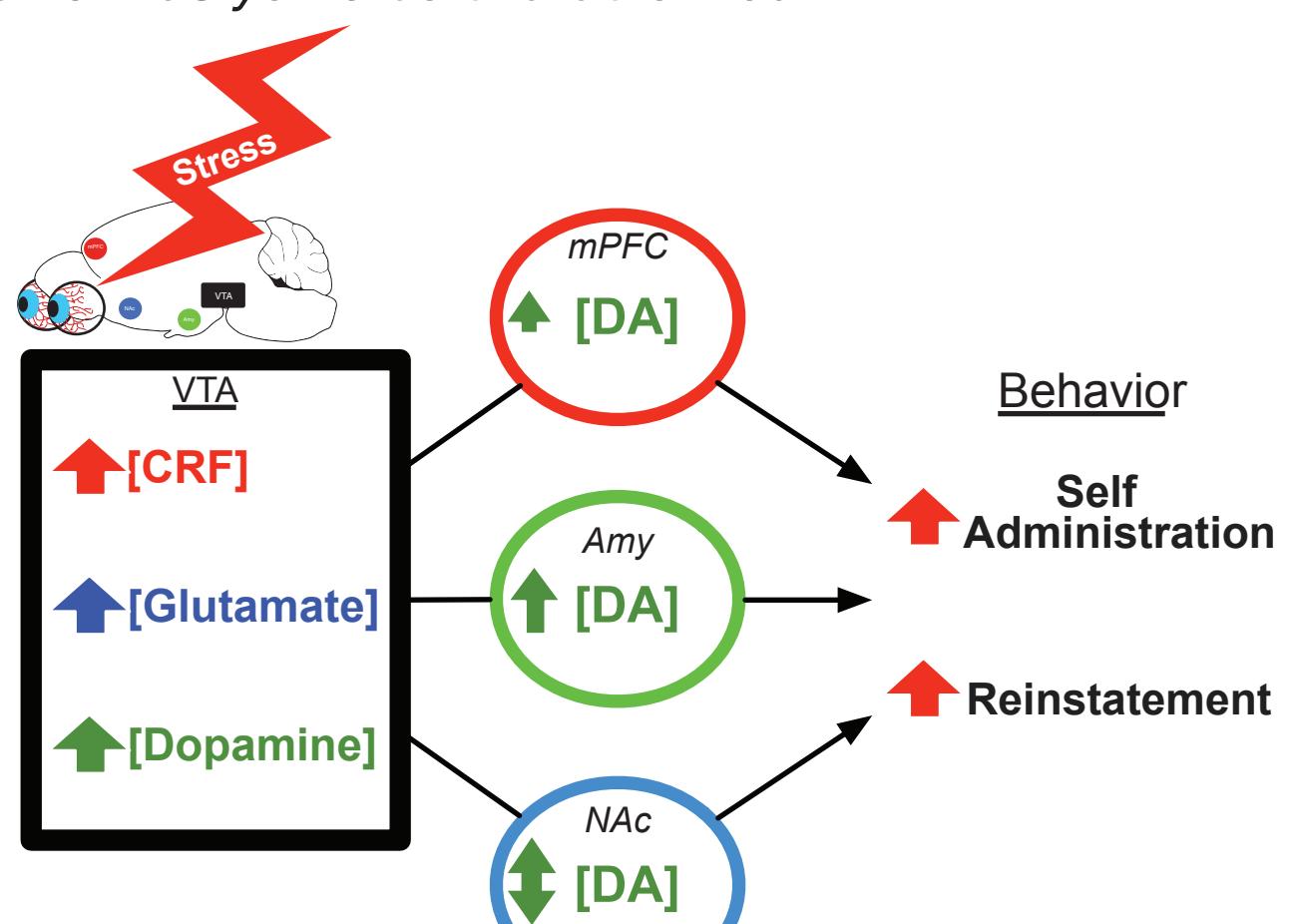
# Circuit Specific Modulation of Corticotrophin Releasing Factor in Ventral Tegmental Neurons

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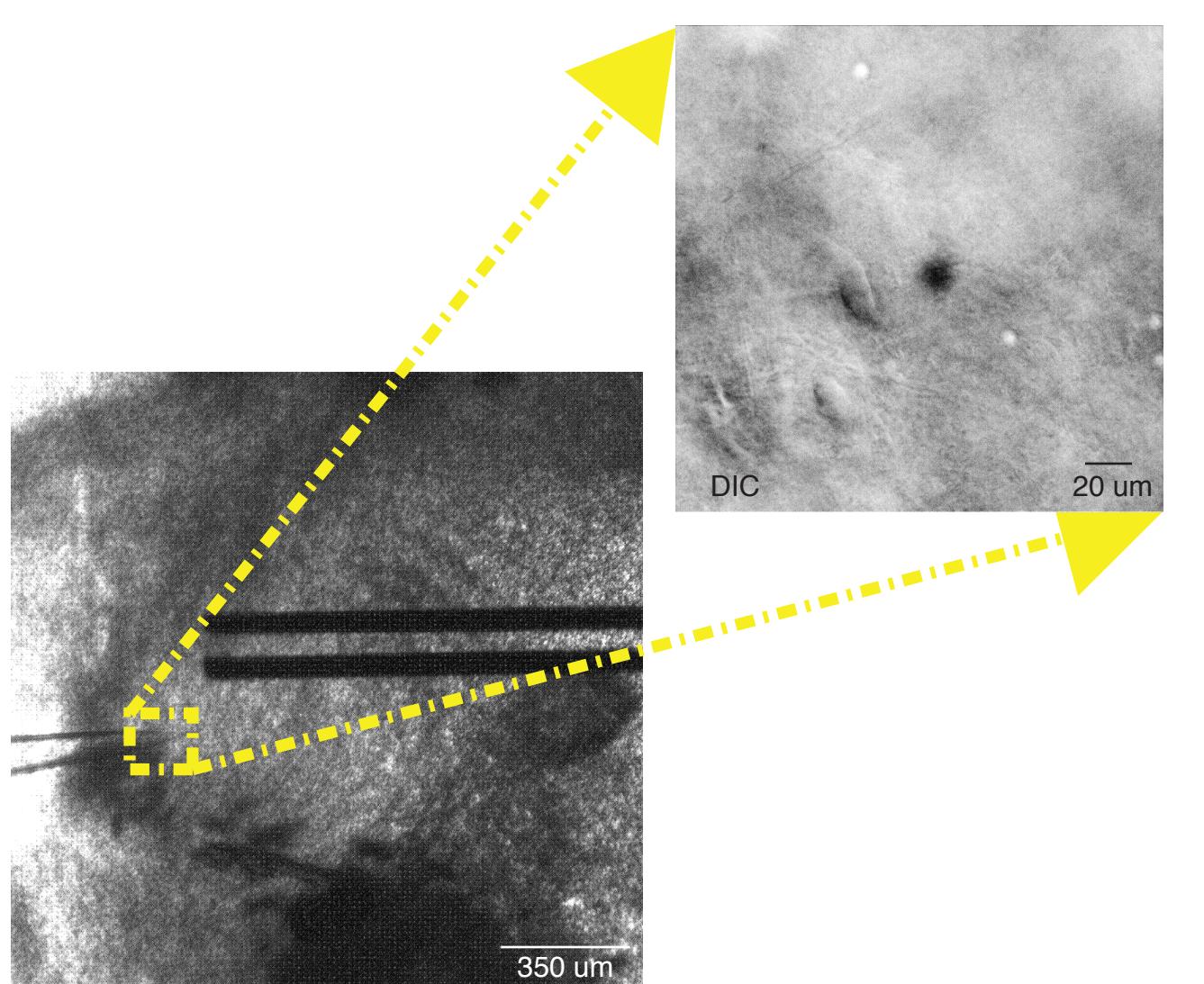
## Introduction

Several rodent models of stress (e.g. footshock, foot pinch, restraint stress, and social defeat stress) increase the activity of VTA neurons and result in an increase in dopamine in the terminal regions of VTA neurons. Corticotrophin releasing factor (CRF) is released in the VTA during stress and is reported to increase the firing rate of dopamine neurons, yet decrease dopamine release in the Nucleus Accumbens. These results suggest that CRF differentially modulates VTA neurons with different projection targets. However, the synaptic action of CRF on specific subsets of VTA neurons and their role in influencing behavior has yet to be characterized.

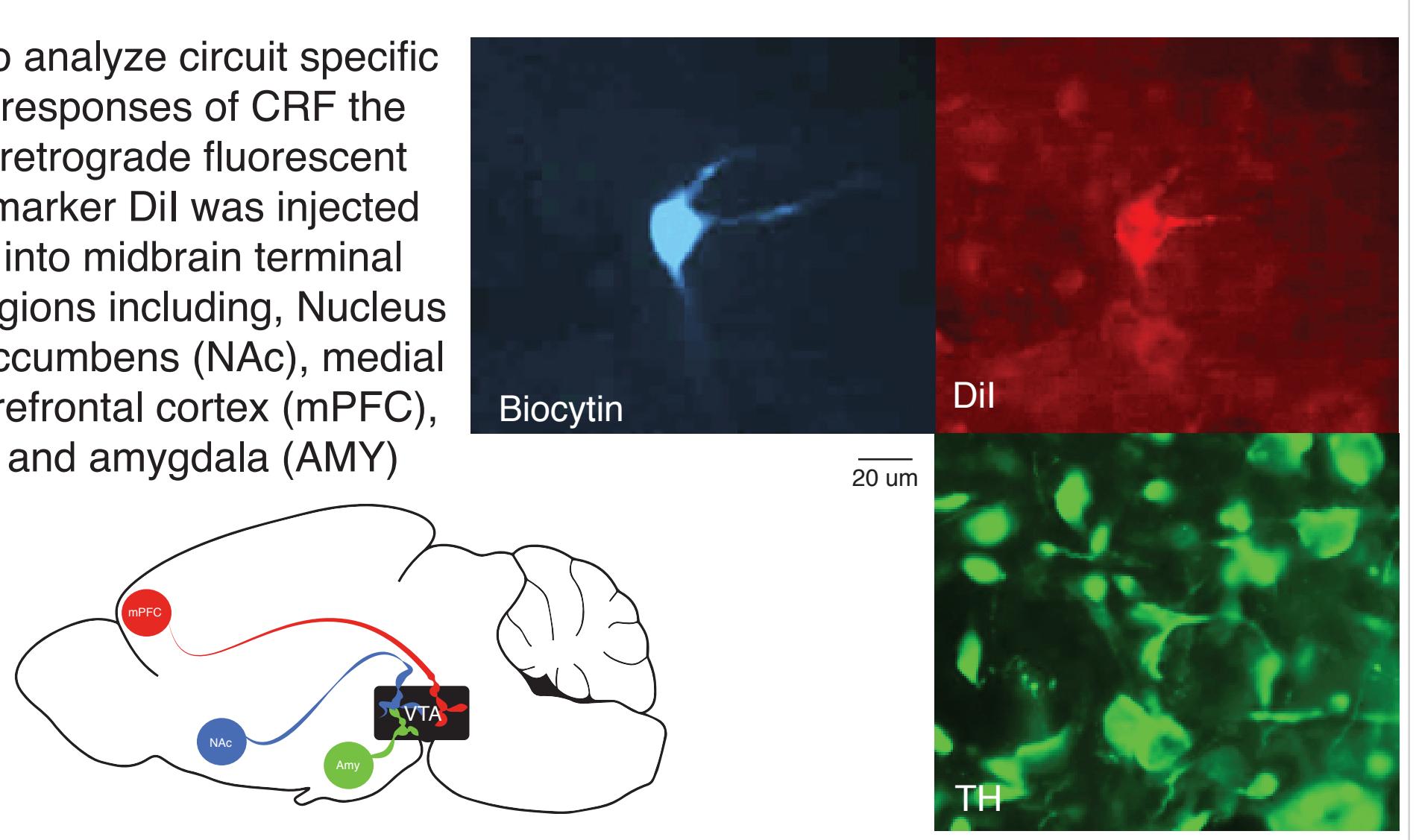


## Methods

We used whole cell patch clamp recordings in VTA neurons *ex vivo* to examine the synaptic actions of CRF. Current clamp was used to assess changes in membrane potential or firing rate. Voltage clamp was used to determine effect on glutamate EPSCs



To analyze circuit specific responses of CRF the retrograde fluorescent marker Dil was injected into midbrain terminal regions including, Nucleus Accumbens (NAc), medial prefrontal cortex (mPFC), and amygdala (AMY)



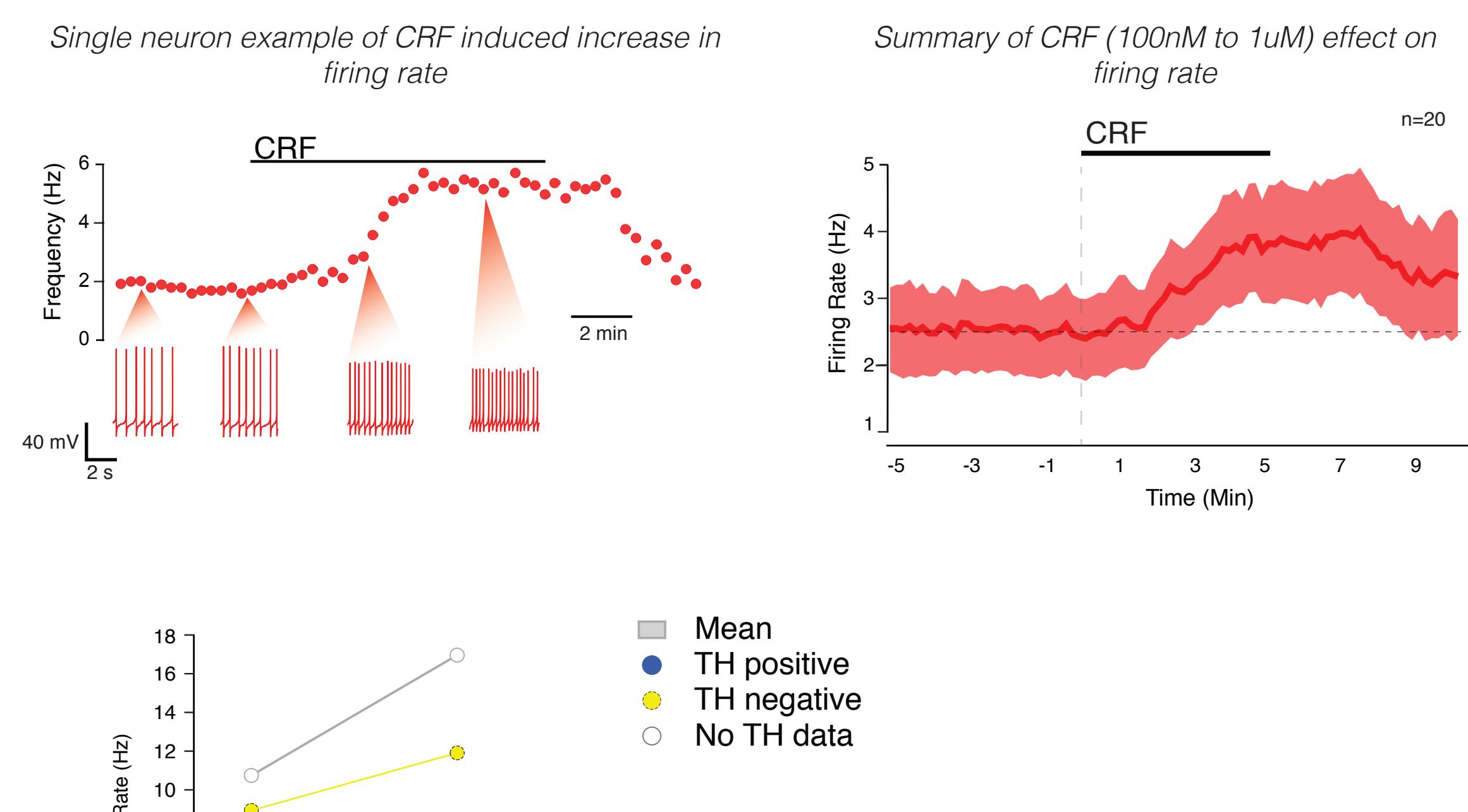
## Acknowledgements:

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## Electrophysiology

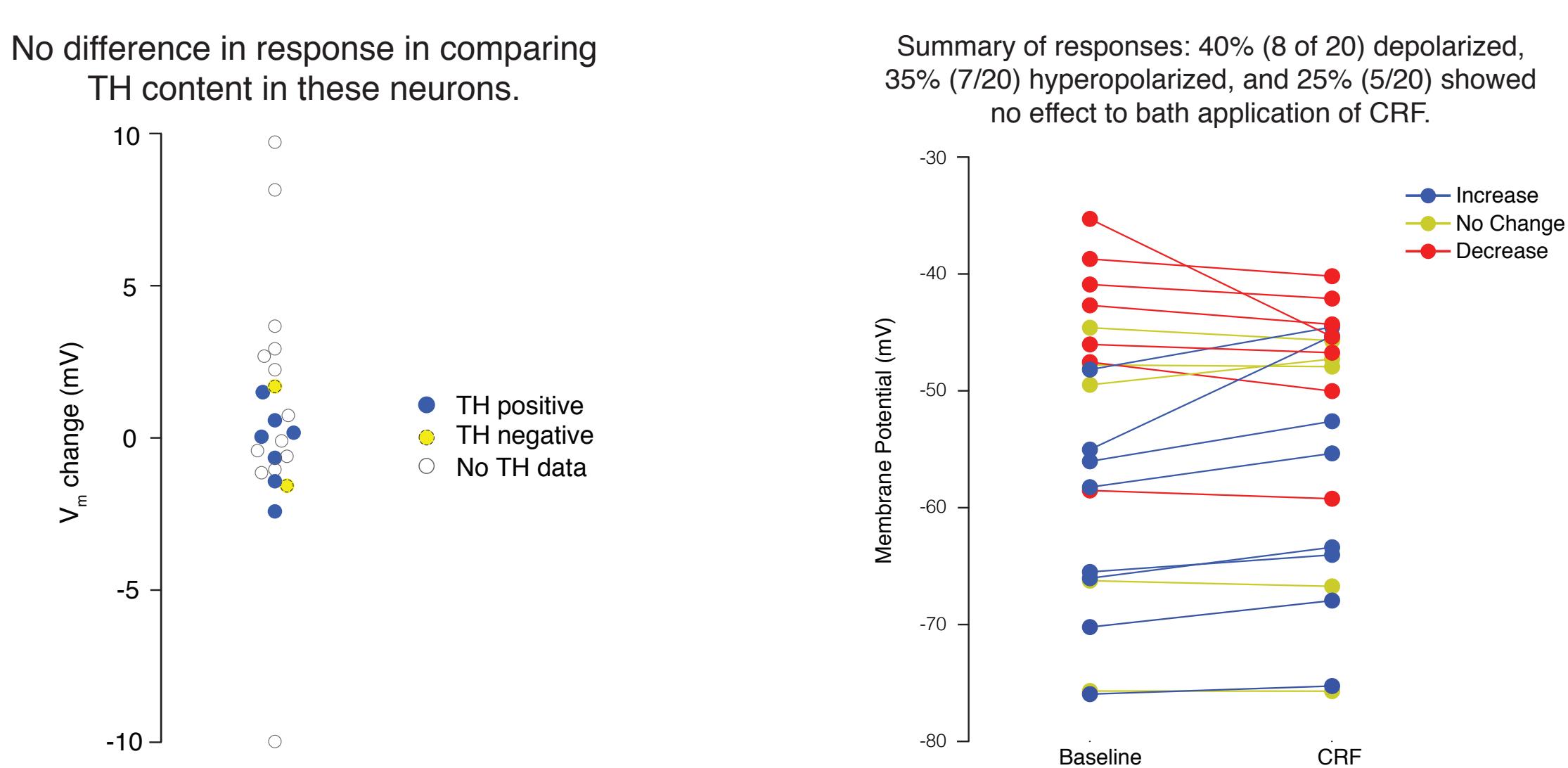
### I. CRF increases the firing rate in neurons that are spontaneously active



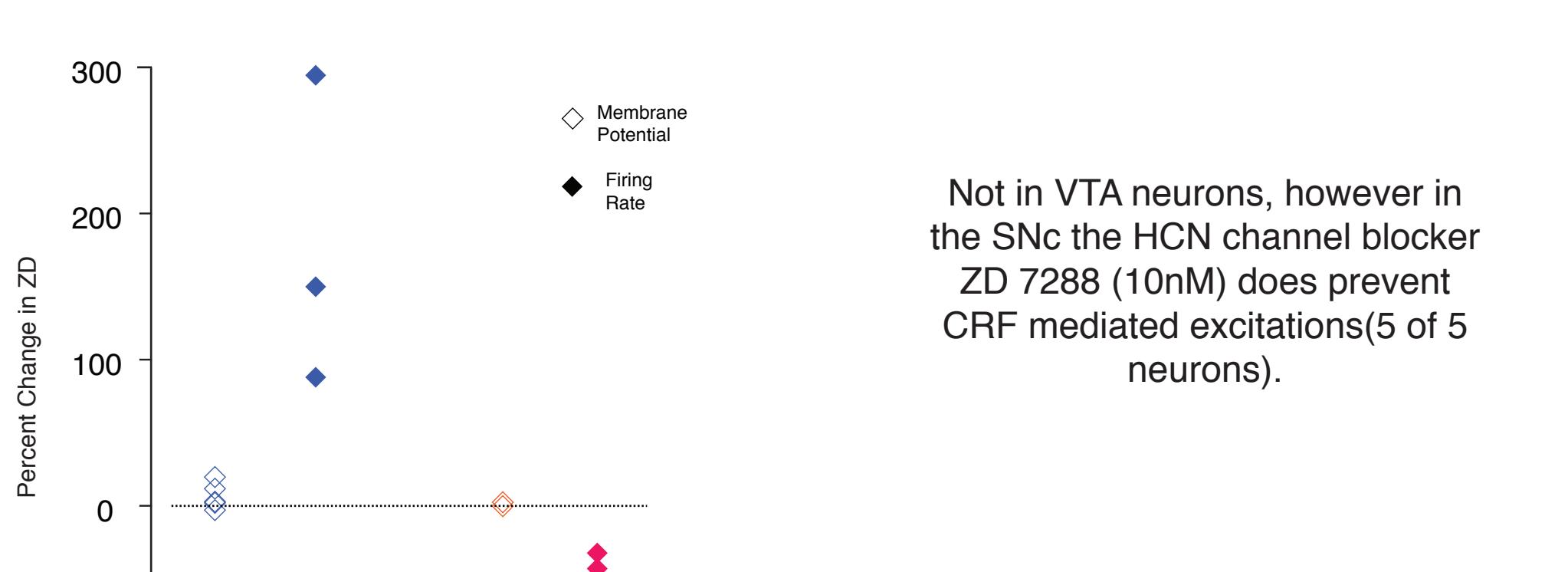
Neurons were filled with biocytin during the recording and later tested for tyrosine hydroxylase (TH) content with immunocytochemistry.

Both TH positive and TH negative neurons showed a consistent increase in firing rate to bath application of CRF in spontaneously active neurons.

### II. Neurons that are quiescent at baseline respond to CRF with either depolarizations or hyperpolarizations.

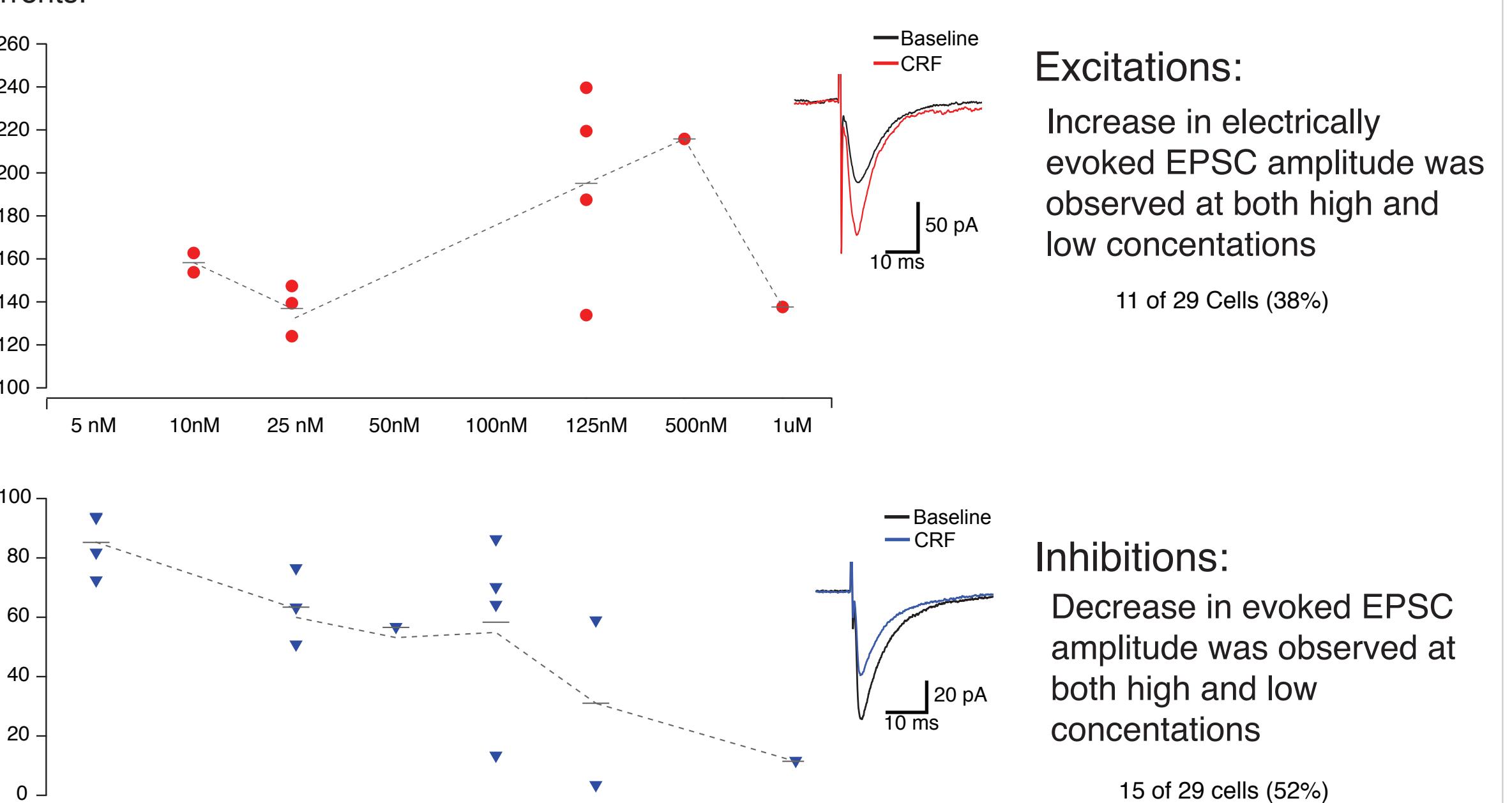


### III. Are CRF's excitatory actions mediated by activation of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels?



## CRF has mixed effects on Glutamatergic EPSCs

Using Microdialysis, Wang et al. 2005 showed that CRF increases both glutamate and dopamine concentrations in the VTA; further these effects are blocked with a local glutamate antagonist. To explore CRF's relationship with glutamatergic inputs, we investigated CRF's ability to modulate glutamatergic excitatory postsynaptic currents.

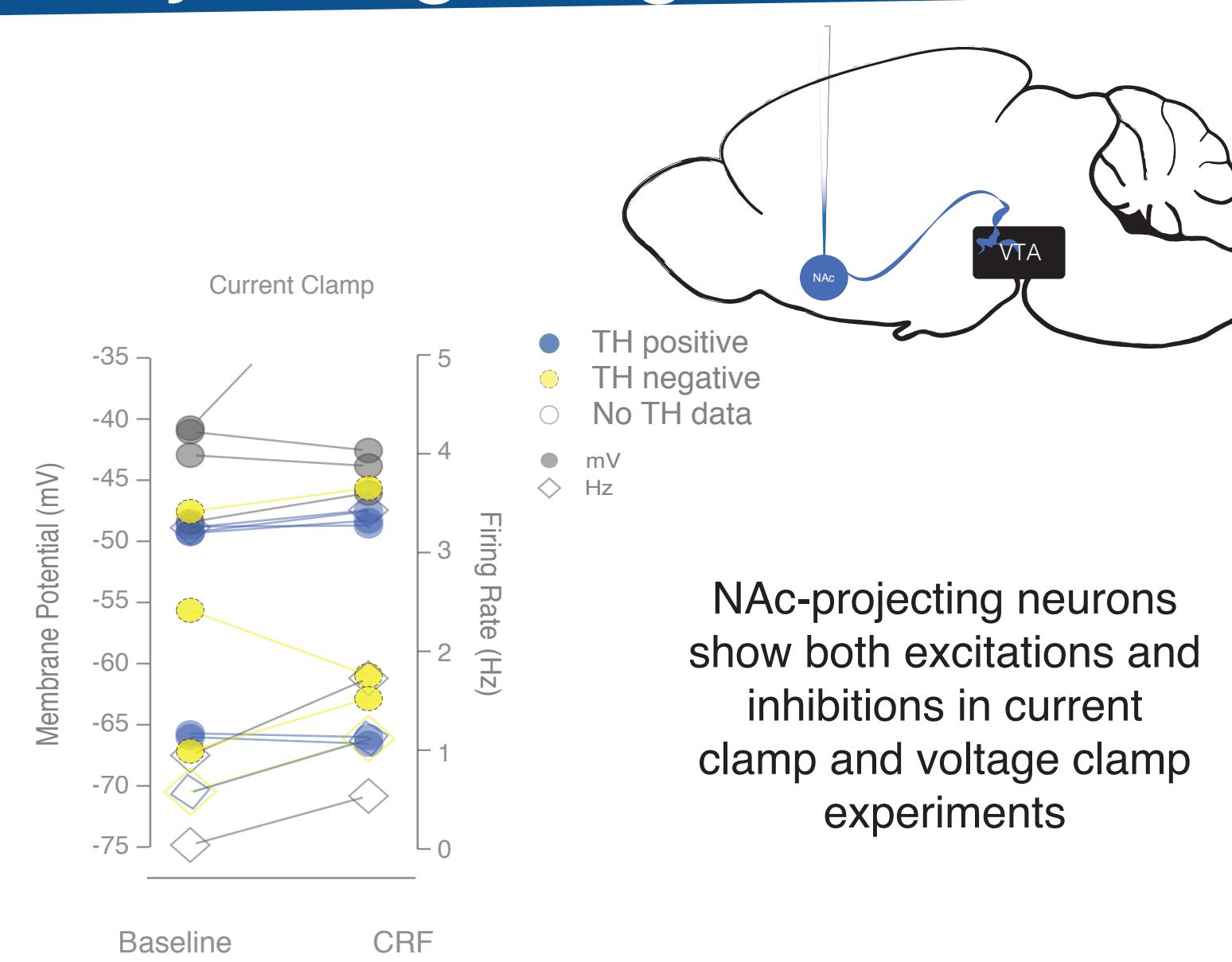


**Excitations:**  
Increase in electrically evoked EPSC amplitude was observed at both high and low concentrations

**Inhibitions:**  
Decrease in evoked EPSC amplitude was observed at both high and low concentrations

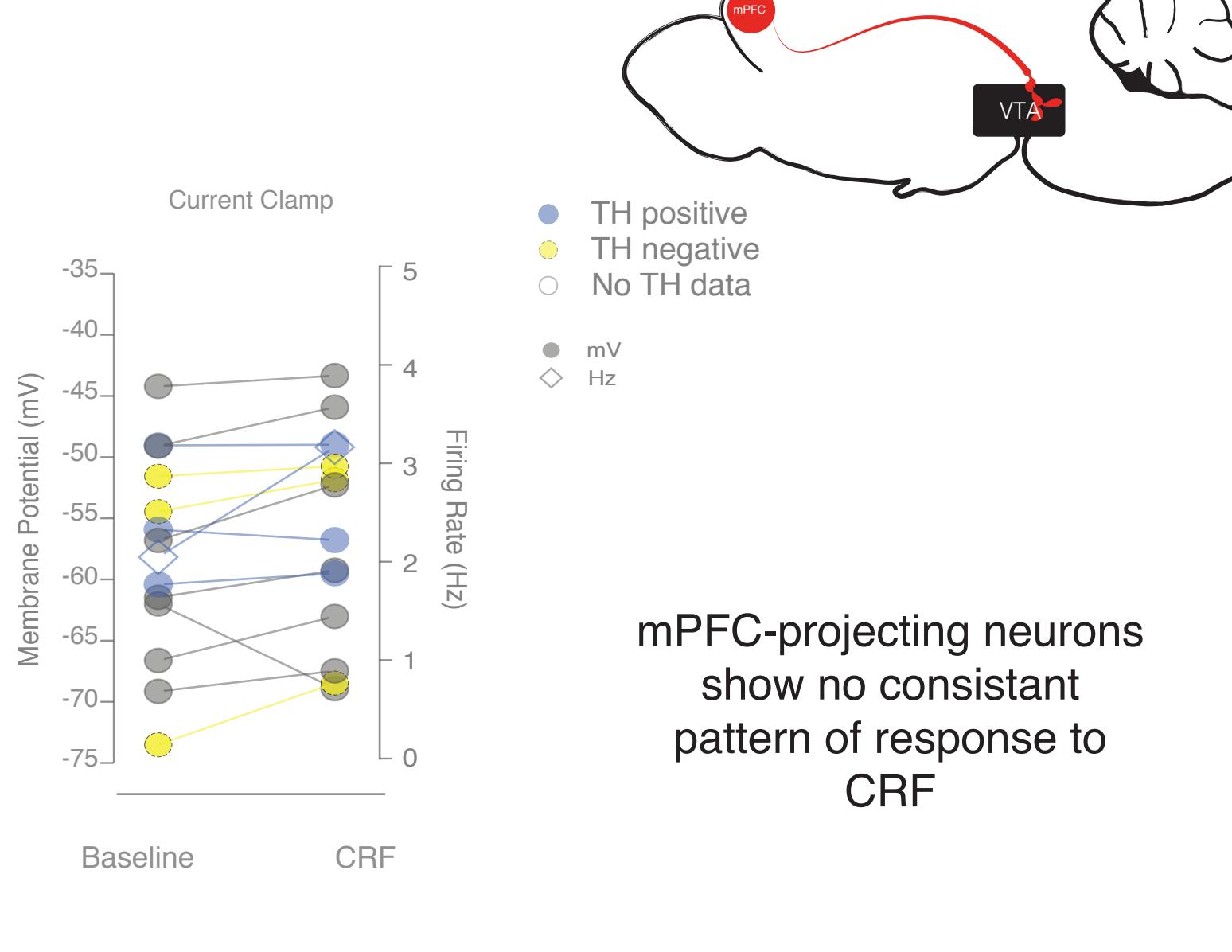
## Do CRF Neuronal Effects Differ by their Projecting Target?

### I. NAc Projecting Neurons



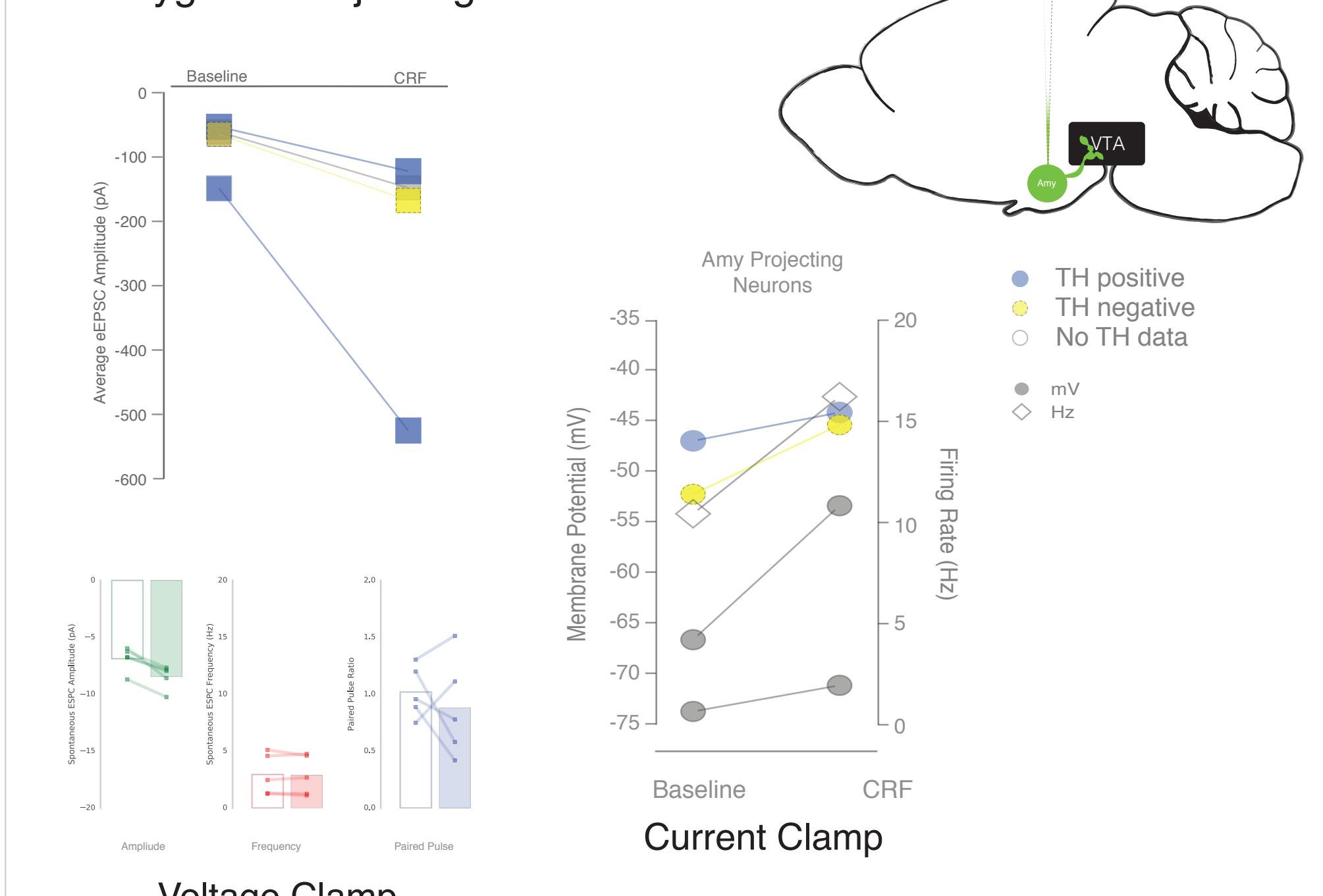
NAc-projecting neurons show both excitations and inhibitions in current clamp and voltage clamp experiments

### II. mPFC Projecting Neurons



mPFC-projecting neurons show no consistent pattern of response to CRF

### III. Amygdala Projecting Neurons



AMY-Projecting neurons are consistently excited by CRF, demonstrated by an increase in firing rate, depolarization, or facilitation of evoked glutamatergic EPSCs.

## Summary

### Synaptic Actions of CRF in VTA neurons:

- Are heterogeneous, causing both excitations and inhibitions
- Targets both dopaminergic and non-dopaminergic neurons
- Are excitatory in neurons that are already spontaneously active, regardless of projection target
- Excites neurons that project to the Amygdala
- Future work is needed to understand how CRF effects behavior based on differences in projection

### References:

- Beuckel, M. J. et al. CRF Enhancement of GIRK Channel-Mediated Transmission in Dopamine Neurons. *J. Neurosci.* 29, 1895–1905 (2009).
- Blackton, J. M. et al. Augmented Cocaine Seeking in Response to Stress or CRF Delivered into the Ventral Tegmental Area Following Long-Access Self-Administration is Mediated by CRF Receptor Type 1 But Not CRF Receptor Type 2. *Trends in Pharmacological Sciences* 31, 1136–1140 (2011).
- Erb, S., Shaham, Y. & Stewart, J. The role of corticotropin-releasing factor and corticosterone in stress- and cocaine-induced relapse to cocaine seeking in rats. *J. Neurosci.* 29, 1136–1140 (2009).
- Erb, S., Hof, F. W. & Bond, A. Chronic Cocaine Enhances Corticotropin-Releasing Factor-Dependent Potentiation of Excitatory Transmission in Ventral Tegmental Area Dopamine Neurons. *Journal of Neuroscience* 29, 6533–6544 (2009).
- Kalivas, P. W., Duffy, P. & Latimer, L. G. Neurochemical and behavioral effects of corticotropin-releasing factor in the ventral tegmental area of the rat. *Journal of Pharmacology and Experimental Therapeutics* 242, 757–763 (1987).
- Leiberman, J. A. & Roth, C. J. Cocaine and corticotropin-releasing factor stimulates catecholamine release in hypothalamus and prefrontal cortex in freely moving rats as assessed by microdialysis. *J. Neurochem.* 60, 602–612 (1993).
- Piazza, P. V. & Le Moal, M. The role of stress in drug self-administration. *Trends in Pharmacological Sciences* 19, 67–74 (1998).
- Shaham, Y. & Stewart, J. Facilitation of Relapse to Cocaine Seeking in Rats by Stress in the Absence of Cocaine. *J. Neurosci.* 20, 559–570 (2000).
- Erb, S., Shaham, Y., Lepp, S., Rieck, V. & Stewart, J. CP-154,526, a selective non-peptide antagonist of the corticotropin-releasing factor attenuates stress-induced relapse to drug seeking in cocaine- and heroin-treatment rats. *Psychopharmacology (Berl.)* 137, 184–190 (1998).
- Taglialero, P. & Morales, M. Synapses between corticotropin-releasing factor-containing axon terminals and dopaminergic neurons in the ventral tegmental area are predominantly glutamatergic. *J. Comp. Neurol.* 506, 616–626 (2007).
- Leiberman, J. A. & Roth, C. J. Cocortropin-releasing factor enhances group II mGluR activation of NMDA receptors in the ventral tegmental area. *Neuron* 39, 401–409 (2003).
- Wenar, M. J., Hopf, F. W., Stuber, G. D., Phillips, P. E. M. & Bond, A. Corticotropin-releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C-dependent enhancement of  $\text{Ca}^{2+}$  current. *J. Physiol.* 562, 2157–2167 (2005).
- Wang, B., You, Z.-B., Rice, K. C. & Wise, R. A. Stress-induced relapse to cocaine seeking: roles for the CRF2 receptor and CRF-binding protein in the ventral tegmental area. *Psychopharmacology (Berl.)* 193, 283–294 (2007).
- Wang, B. et al. Cocaine experience establishes control of midbrain glutamate and dopamine by corticotropin-releasing factor: a role in stress-induced relapse to drug seeking. *Journal of Neuroscience* 29, 5299–5306 (2009).
- Williams, C. L., Buchta, W. C. & Regal, A. C. CRF-R2 and the Heterosynaptic Regulation of VTA Glutamate during Reinforcement of Cocaine Seeking. *Journal of Neuroscience* 30, 10402–10414 (2010).

