Circuit Specific Modulation of Corticotrophin Releasing Factor in Ventral Tegmental Neurons

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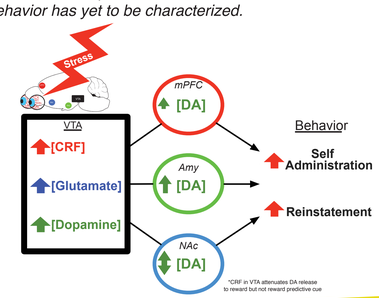
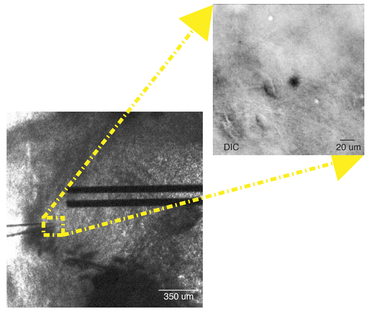
Introduction

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.

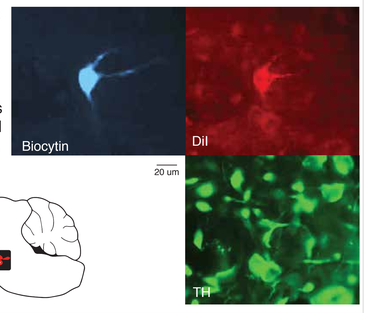


Methods

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



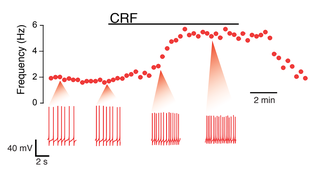
To analyze circuit specific responses of CRF the retrograde fluorescent marker DiI was injected into midbrain terminal regions including, Nucleus Accumbens (NAc), medial prefrontal cortex (mPFC), and amygdala (AMY)



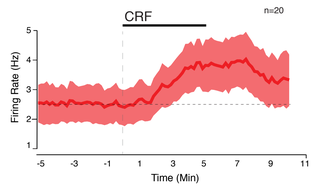
Electrophysiology

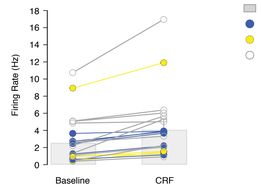
I. CRF increases the firing rate in neurons that are spontaneously active

Single neuron example of CRF induced increase in firing rate



Summary of CRF (100nM to 1uM) effect on firing rate





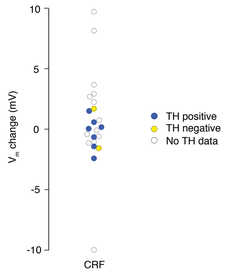
Mean
TH positive
TH negative
No TH data

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

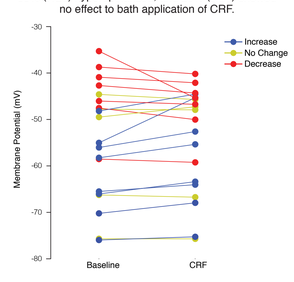
Both TH positive and TH negative neurons showed a consistent increase in firing rate to bath application of CRF in spontaneously active neurons.

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

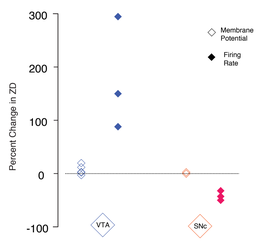
No difference in response in comparing TH content in these neurons.



Summary of responses: 40% (8 of 20) depolarized, 35% (7/20) hyperopolarized, and 25% (5/20) showed no effect to bath application of CRF.



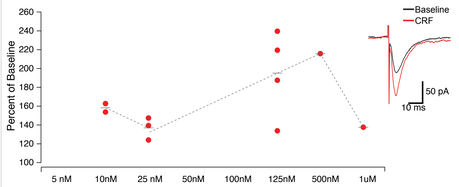
III. Are CRF's excitatory actions mediated by activation of hyperpolarization-activated cyclic nucleotide–gated (HCN) channels?



Not in VTA neurons, however in the SNc the HCN channel blocker ZD 7288 (10nM) does prevent CRF mediated excitations(5 of 5 neurons).

CRF has mixed effects on Glutamatergic EPSCs

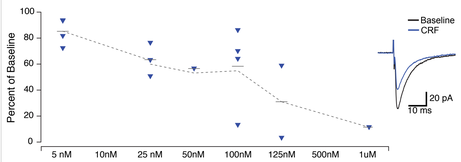
Using Microdialysis, Wang et al. 2005 showed that CRF increases both glutamate and dopamine concentrations in the VTA; further these effects are blocked with a local glutamate antagonist. To explore CRF's relationship with glutamatergic inputs, we investigated CRF's ability to modulate glutamatergic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



Excitations:

Increase in electrically evoked EPSC amplitude was observed at both high and low concentations

11 of 29 Cells (38%)



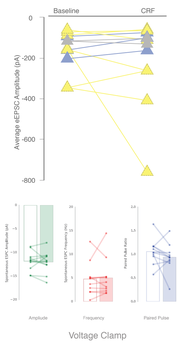
Inhibitions:

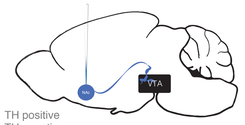
Decrease in evoked EPSC amplitude was observed at both high and low concentations

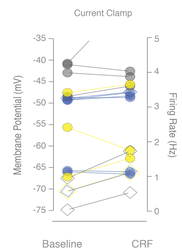
15 of 29 cells (52%)

Do CRF Neuronal Effects Differ by their Projecting Target?

I. NAc Projecting Neurons

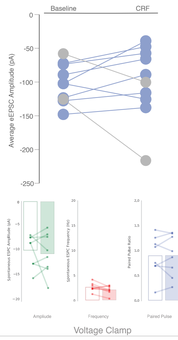


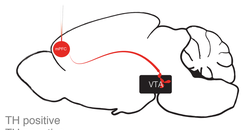


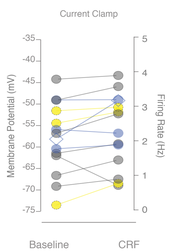


NAc-projecting neurons show both excitations and inhibitions in current clamp and voltage clamp experiments

II. mPFC Projecting Neurons

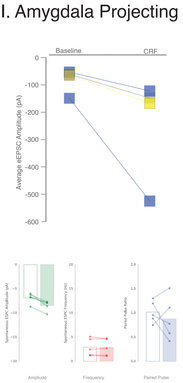


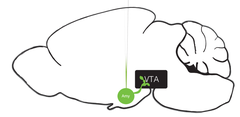


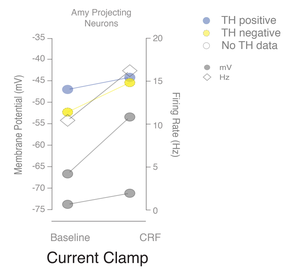


mPFC-projecting neurons show no consistant pattern of response to CRF

III. Amygdala Projecting Neurons







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

Summary

Synaptic Actions of CRF in VTA neurons:

Are heterogeneous, causing both excitations and inhibitions

Targets both dopaminergic and non-dopaminergic neurons

Are excitory in neurons that are already spontaneously active, regardless of projection target

Excites neurons that project to the Amygdala

Furture work is needed to understand how CRF effects behavior based on differences in projection

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Acknowledgements:

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