Circuit Specific Modulation by Corticotrophin Releasing Factor in Ventral Tegmental Area Neurons

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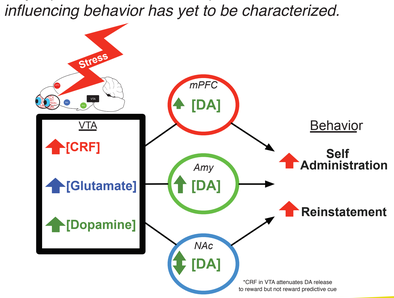
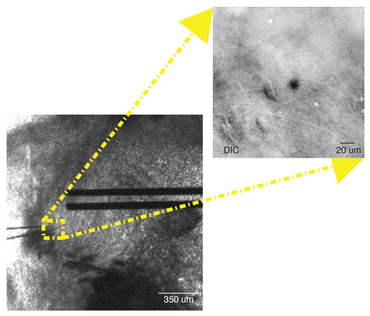
Introduction

Several rodent models of stress (e.g. footshock, foot pinch, restraint stress, and social defeat stress) increase the activity of VTA neurons[1,8,13,14] and result in an increase in dopamine in the terminal regions of VTA neurons[2-7,9,11,12,17]. Corticotrophin releasing factor (CRF) is released in the VTA during stress and is reported to increase the firing rate of dopamine neurons[1,13,14], yet decrease dopamine release in the nucleus accumbens[15]. 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.

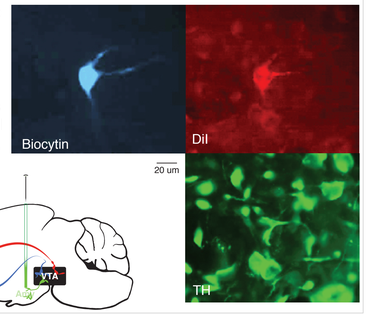


Methods

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.



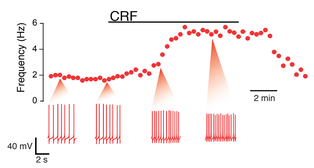
To analyze circuit specific responses of CRF the retrograde fluorescent marker DiI was injected into midbrain terminal regions including nucleus accumbens (NAc), medial prefrontal cortex (mPFC), and amygdala (AMY)



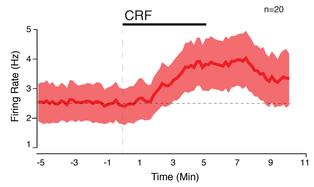
Electrophysiology

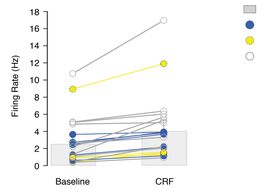
I. CRF increases the firing rate in neurons that are spontaneously active

Single neuron example of CRF induced increase in firing rate



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





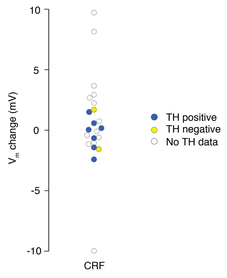
Mean
TH positive
TH negative
No TH data

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

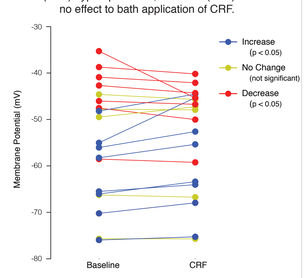
Both TH positive and TH negative spontaneously active neurons showed a consistent increase in firing rate to bath application of CRF.

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

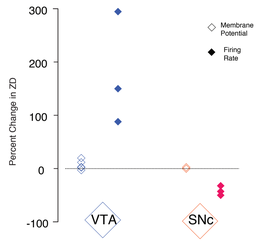
No difference in response in comparing TH content in these neurons.



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



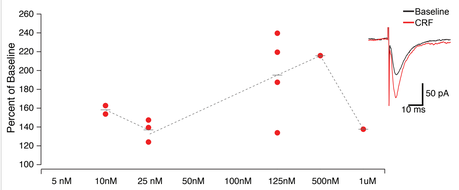
III. Are CRF's excitatory actions mediated by activation of hyperpolarization-activated cyclic nucleotide–gated (HCN) channels?



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

CRF has mixed effects on Glutamatergic EPSCs

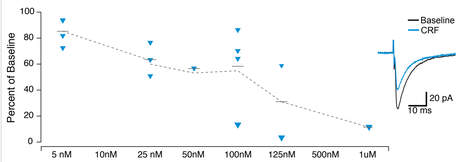
Using microdialysis, Wang et al. 2005 showed that CRF increases both glutamate and dopamine concentrations in the VTA; further, these effects are blocked with a local glutamate antagonist[16]. To explore CRF's modulation of glutamatergic inputs, we investigated CRF's ability to modulate glutamatergic excitatory postsynaptic currents.



Excitations:

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

11 of 29 Cells (38%)



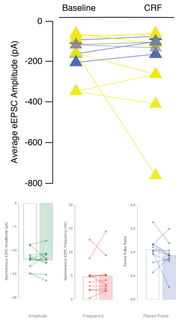
Inhibitions:

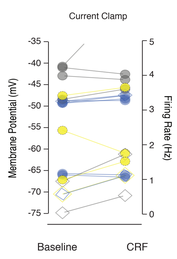
Decrease in evoked EPSC amplitude was observed at both high and low concentations

15 of 29 cells (52%)

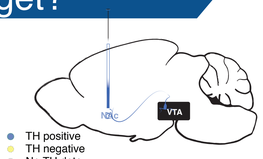
Do CRF Effects Differ by neuronal projection target?

I. NAc-Projecting Neurons



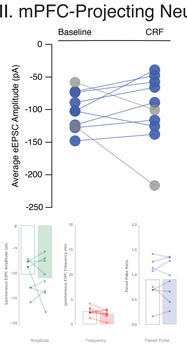


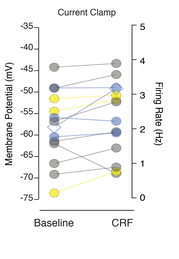
TH positive
TH negative
No TH data
mV
Hz



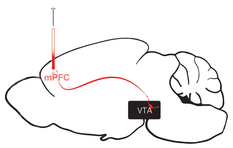
NAc-projecting neurons show both excitations and inhibitions in current clamp and voltage clamp experiments

II. mPFC-Projecting Neurons



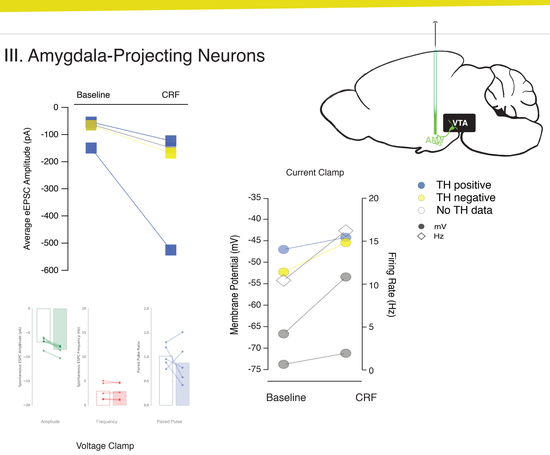


TH positive
TH negative
No TH data
mV
Hz



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 increased amplitude of evoked glutamateric EPSCs.

Summary

Synaptic actions of CRF in VTA neurons:

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

Are heterogeneous in quiescent neurons causing both excitations and inhibitions in both dopaminergic and non-dopaminergic neurons projecting to NAc and mPFC

Universally excite neurons that project to the amygdala

Future work is needed to understand how modulation of these different projections contribute to CRF-induced behavioral effects

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