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

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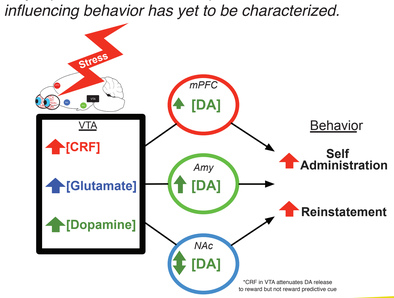
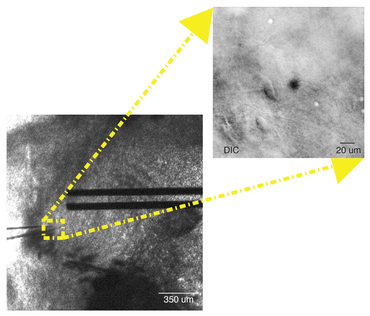
Introduction

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.

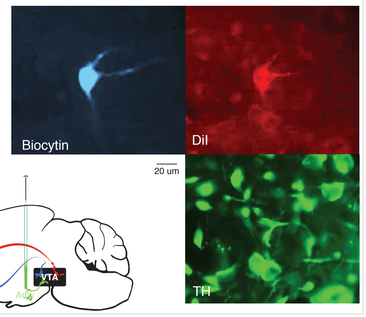


Methods

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.

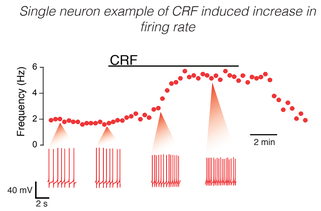


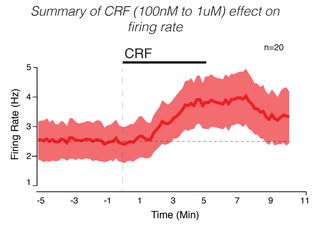
To analyze circuit specific responses of CRF the retrograde fluorescent marker DiI was injected into midbrain terminal regions including nucleus accumbens (NAc), medial prefrontal cortex (mPFC), and amygdala (AMY)

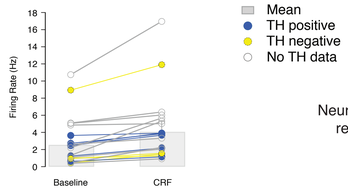


Electrophysiology

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







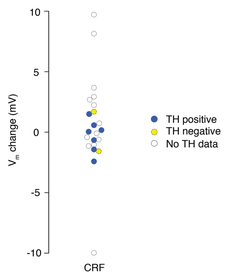
Mean
TH positive
TH negative
No TH data

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

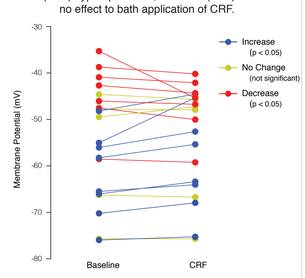
Both TH positive and TH negative spontaneously active neurons showed a consistent increase in firing rate to bath application of CRF.

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

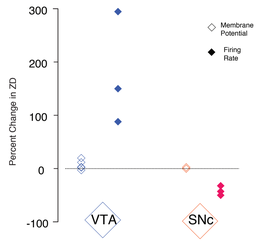
No difference in response in comparing TH content in these neurons.



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



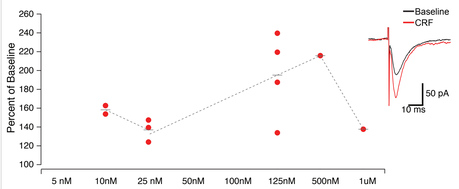
III. Are CRF's excitatory actions mediated by activation of hyperpolarization-activated cyclic nucleotide–gated (HCN) channels?



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

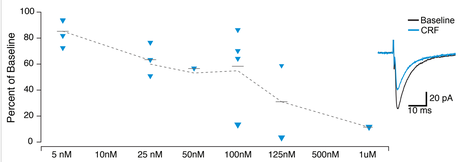
Using microdialysis, Wang et al. 2005 showed that CRF increases both glutamate and dopamine concentrations in the VTA; further, these effects are blocked with a local glutamate antagonist[16]. To explore CRF's modulation of glutamatergic inputs, we investigated CRF's ability to modulate glutamatergic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



Excitations:

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

11 of 29 Cells (38%)



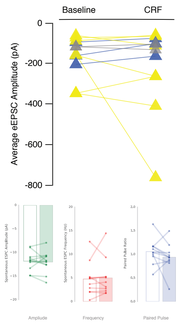
Inhibitions:

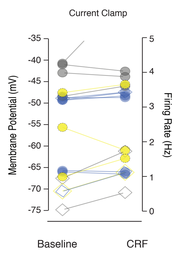
Decrease in evoked EPSC amplitude was observed at both high and low concentations

15 of 29 cells (52%)

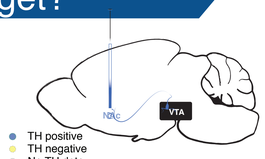
Do CRF Effects Differ by neuronal projection target?

I. NAc-Projecting Neurons



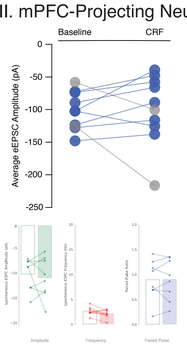


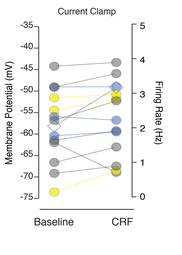
TH positive
TH negative
No TH data
mV
Hz



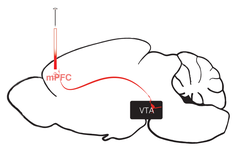
NAc-projecting neurons show both excitations and inhibitions in current clamp and voltage clamp experiments

II. mPFC-Projecting Neurons



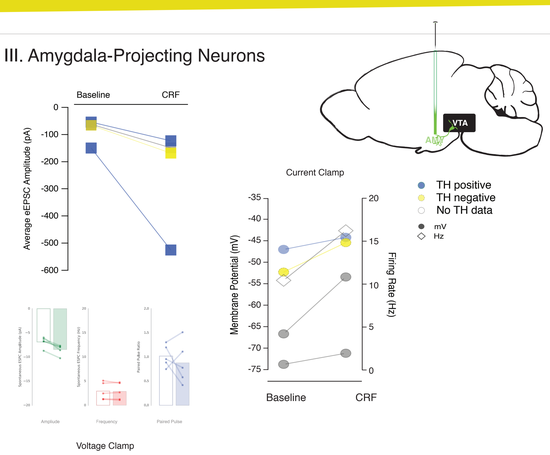


TH positive
TH negative
No TH data
mV
Hz



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

III. Amygdala-Projecting Neurons



TH positive
TH negative
No TH data
mV
Hz

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

References:

1. Anstrom, K.K., Woodward, D.J., 2005. Restraint Increases Dopaminergic Burst Firing in Awake Rats. Neuropsychopharmacology 30, 1832–1840. doi:10.1038/sj.npp.1300730
2. Deutch, A.Y., Lee, M.C., Gillham, M.H., Cameron, D.A., Goldstein, M., Iadarola, M.J., 1991. Stress Selectively Increases Fos Protein in Dopamine Neurons Innervating the Prefrontal Cortex. Cereb. Cortex 1, 273–292. doi:10.1093/cercor/1.4.273
3. Deutch, A.Y., Lee, M.C., Gillham, M.H., Cameron, D.A., Goldstein, M., Iadarola, M.J., 1991. Stress selectively increases fos protein in dopamine neurons innervating the prefrontal cortex. Cereb. Cortex 1, 273–292.
4. Inglis, F.M., Moghaddam, B., 1999. Dopaminergic innervation of the amygdala is highly responsive to stress. Journal of neurochemistry 72, 1088–1094.
5. Jedema, H.P., Moghaddam, B., 1994. Glutamatergic control of dopamine release during stress in the rat prefrontal cortex. Journal of neurochemistry 63, 785–788.
6. Kalivas, P.W., Abhold, R., 1987. Enkephalin release into the ventral tegmental area in response to stress: modulation of mesocorticolimbic dopamine. Brain Research 414, 339–348. doi:10.1016/0006-8993(87)90015-1
7. Kalivas, P.W., Duffy, P., 1995. Selective activation of dopamine transmission in the shell of the nucleus accumbens by stress. Brain Res. 675, 325–328.
8. Korotkova, T.M., Brown, R.E., Sergeeva, O.A., Ponomarenko, A.A., Haas, H.L., 2006. Effects of arousal- and feeding-related neuropeptides on dopaminergic and GABAergic neurons in the ventral tegmental area of the rat. European Journal of Neuroscience 23, 2677–2685. doi:10.1111/j.1460-9568.2006.04792.x
9. Lavicky, J., Dunn, A.J., 1993. Corticotropin-Releasing Factor Stimulates Catecholamine Release in Hypothalamus and Prefrontal Cortex in Freely Moving Rats as Assessed by Microdialysis. Journal of Neurochemistry 60, 602–612. doi:10.1111/j.1471-4159.1993.tb03191.x
10. Piazza, P.V., Le Moal, M., 1998. The role of stress in drug self-administration. Trends in Pharmacological Sciences 19, 67–74. doi:10.1016/S0165-6147(97)01115-2
11. Roth, R.H., TAM, S.-Y., Ida, Y., YANG, J.-X., Deutch, A.Y., 1988. Stress and the Mesocorticolimbic Dopamine Systemsa. Annals of the New York Academy of Sciences 537, 138–147.
12. Thierry, A.M., Tassin, J.P., Blanc, G., Glowinski, J., 1976. Selective activation of the mesocortical DA system by stress. Nature 263, 242–244. doi:10.1038/263242a0
13. Ungless, M.A., Singh, V., Crowder, T.L., Yaka, R., Ron, D., Bonci, A., 2003. Corticotropin-Releasing Factor Requires CRF Binding Protein to Potentiate NMDA Receptors via CRF Receptor 2 in Dopamine Neurons. Neuron 39, 401–407. doi:10.1016/S0896-6273(03)00461-6
14. Wanat, M.J., Bonci, A., Phillips, P.E.M., 2013. CRF acts in the midbrain to attenuate accumbens dopamine release to rewards but not their predictors. Nature Neuroscience 16, 383–385. doi:10.1038/nn.3335
15. Wanat, M.J., Hopf, F.W., Stuber, G.D., Phillips, P.E.M., Bonci, A., 2008. Corticotropin-releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C-dependent enhancement of Ih. The Journal of Physiology 586, 2157–2170. doi:10.1113/jphysiol.2007.150078
16. Wang, B., Shaham, Y., Zitzman, D., Azari, S., Wise, R.A., You, Z.-B., 2005. Cocaine Experience Establishes Control of Midbrain Glutamate and Dopamine by Corticotropin-Releasing Factor: A Role in Stress-Induced Relapse to Drug Seeking. J. Neurosci. 25, 5389–5396. doi:10.1523/JNEUROSCI.0955-05.2005
17. Wise, R.A., Morales, M., 2010. A ventral tegmental CRF–glutamate–dopamine interaction in addiction. Brain Research 1314, 38–43. doi:10.1016/j.brainres.2009.09.101

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