Corticotrophin Releasing Factor Alters Kappa Opioid Receptor Function in the Ventral Tegmental Area.

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Background

Depending on the degree or type, stress can be associated with either positive (rewarding) or negative (aversive) behavioral outcomes.

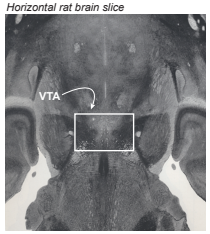
During stress, corticotrophin releasing factor (CRF) is released into the ventral tegmental area (VTA), which increases dopamine (DA) signaling in VTA terminal regions including the nucleus accumbens (NAc) (Wang et al. 2005), DA in the NAc is typically associated with reward.

CRF release also leads to kappa opioid receptor (KOR) activation in brain areas that are involved in stress, resulting in conditioned place aversion (Land et al. 2008, Bruchas et al. 2009). We previously showed that the KOR agonist U69593 selectively hyperpolarizes VTA DA neurons that project to the amygdala or prefrontal cortex, but not DA neurons projecting to the NAc (Margolis et al., 2006; 2008).

Understanding the interaction of CRF and the kappa opioid system may help to dissociate the negative deleterious aspects of stress from those that allow for positive physiological adaptation.

Methods

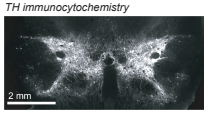
Horizontal rat brain slice

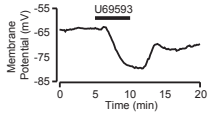


Whole cell ex vivo electrophysiology was used to examine how CRF modulates responses to U69593 in VTA neurons

In current clamp (I=0 pA), VTA neurons were tested for changes in membrane potential to bath applications of U69593, a KOR agonist, and CRF

TH immunocytochemistry





U69593 and CRF were applied by bath perfusion

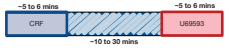
CRF was applied first for 5-6 min and then washed out for at least 10 min before a second U69593 application

I. Biased Sampling

Baseline KOR responses were evaluated with U69593 (1 uM) before application of CRF to confirm KOR responses in a subset of experiments

Comparison of multiple applications of U69593 to CRF then U69593 applicaiton in confirmed KOR responding neurons

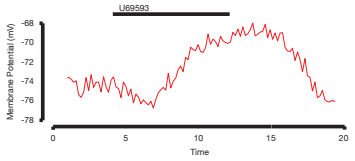
II. Unbiased Sampling



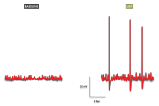
In a seperate set of experiments, cells were blindly sampled from the VTA and tested with CRF followed by U69593 without prior knowledge of kappa responses

Electrophysiology

I. Current Clamp

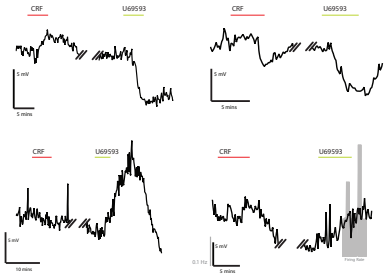


KOR agonist mediates excitation of Vm

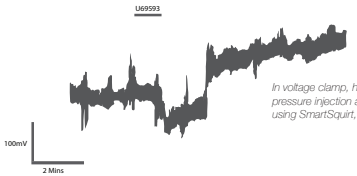


KOR activation causes increases in firing

II. CRF effect does not predict U69593 response



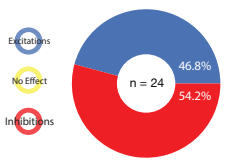
III. Voltage Clamp



In voltage clamp, holding at -60mV, quick pressure injection applications of U69593, using SmartSquirt, induces inward current

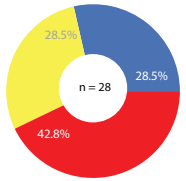
IV. Summary: How often are excitations observed?

Biased Sampling:



46.8% of cells that initially responded to U69593 with an inhibition, responded with an excitation to U69593 after experiencing CRF stimulation

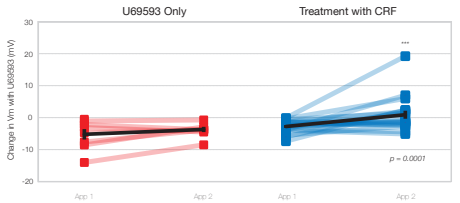
Unbiased Sampling:



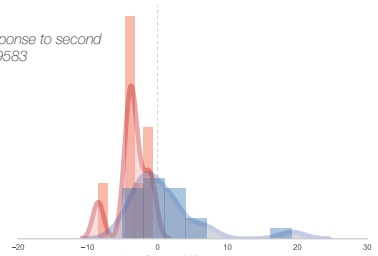
Cells not first tested with U69593, still showed excitations following CRF application. Excitations were less frequent (28%)

Excitations to U69593

I. Comparison of U69593 effects in confirmed KOR responding cells



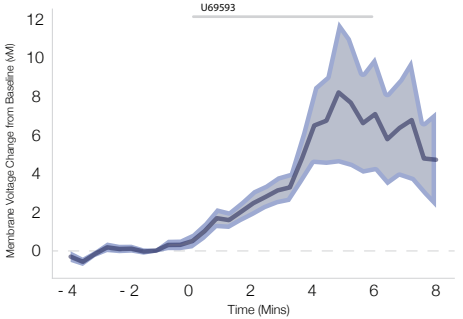
Histograms of response to second application of U69583



Dual application of U69593 compared to U69593 following CRF application in confirmed KOR responsive cells

Size of initial U69593 response not predictive of later excitation (data not shown)

II. Timing of excitatory responses



To observe the kinetics of the excitation, confirmed excitations were averaged together time locked to the start of the drug application. Each individual response was first normalized to zero, by which the mean of the first 4 mins of baseline were substracted from the whole trace. There was a significant increase in membrane potential that began to washout.

Summary

1) CRF exposure robustly altered KOR induced signaling in VTA neurons, causing a switch from inhibition to excitation in a subset of VTA dopaminergic neurons.

2) Excitations did not correlate with magnitude of the baseline U69593 response or the response to CRF

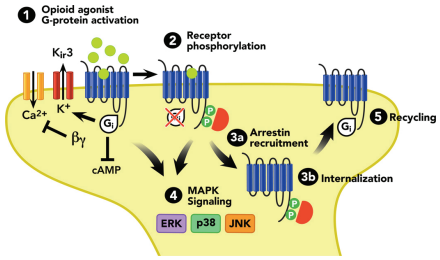
3) Excitations were seen in ~45% of cells that were previously identified with KOR hyperpolarizations and ~25% of neurons that were blindly sampled from the VTA

These results provide insight into how stress may profoundly alter neuronal responses to KOR activation in mesolimbic neurons.

Future Directions

I. Intracellular vs Synaptic Mechanism

Future studies evaluate how CRF stimulates changes within the cell to alter response properties to KOR activation



Credit to: Al-Hasani and Bruchas, 2011

II. Behavioral Relevance

Conditioned Place Preference to U69593 in stressed and CRF pretreated animals

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