

The Role of Extended Amygdala Corticotropin-Releasing Factor Neurons in Binge Ethanol Drinking

James M. Irving*, Colin J. Maehler, Houman Qadir, Kasey S. Girven, Dennis R. Sparta

Department of Anatomy and Neurobiology, University of Maryland School of Medicine
Program in Neuroscience, University of Maryland, Baltimore

*Direct correspondence to: jirvi001@umaryland.edu

ABSTRACT

Binge drinking has been linked to the progressive dysfunction of multiple organs and is considered a critical first step in the development of alcoholism. Conceptual models of alcoholism and addiction predict that repeated binge consumption of alcohol promotes negative affective states that compel the ethanol to consume increasing amounts of ethanol. Therefore, understanding the specific neural circuit mechanisms involved in chronic binge drinking is critical. Development of better treatments is important as a key component of knowledge on how alcohol abuse alters underlying circuits. Corticotropin-releasing factor (CRF) neurons within the extended amygdala project to reward areas of the brain, and are believed to promote negative affect and binge drinking. However, the precise CRF circuit mechanisms that drive these behaviors remain poorly understood. Here we used CRF-Cre mice to specifically manipulate CRF neuron populations within the central amygdala (CeA) and bed nucleus of the stria terminals (BNST) to determine how chronic binge drinking cycles alter the function of these CRF populations. We hypothesized that the activity of CeA-CRF and BNST-CRF neurons increase, thereby driving chronic binge ethanol consumption. To test this, we first recorded the activity of CRF neurons in the CeA using in vivo electrophysiology and optogenetics to record CRF neurons during multiple binge ethanol drinking sessions. Interestingly, we found a population of CRF neurons that increased their activity in response to binge ethanol consumption. However, we did observe a few subpopulations of phototagged CRF neurons with differential responses to binge ethanol drinking. These data suggest functional subpopulations of CRF neurons within CeA that differentially encode binge drinking behavior. Additionally, ongoing experiments using pharmacogenetic inhibition and optogenetic excitation are testing if CRF activity these nuclei are required or sufficient, respectively, to drive binge ethanol consumption behavior. These experiments have shown overlapping, but divergent roles of BNST and CeA CRF neurons.

HYPOTHESIS

» Changes in activity of CRF neurons in the bed nucleus of the stria terminals (BNST) and central amygdala (CeA) are necessary and sufficient for escalation of binge drinking.

» The projections of both CeA-CRF and BNST-CRF neurons to the ventral tegmental area are responsible for behavior alterations in alcohol consumption

» Endogenous CeA-CRF activity encodes alcohol consumption.

PREDICTIONS

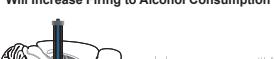
i. Optogenetic Excitation of numerous CRF neuron targets will Increase Alcohol Consumption, including:
 a) CRF Neurons in the CeA
 b) CeA-CRF terminals in the VTA
 c) BNST-CRF terminals in the VTA



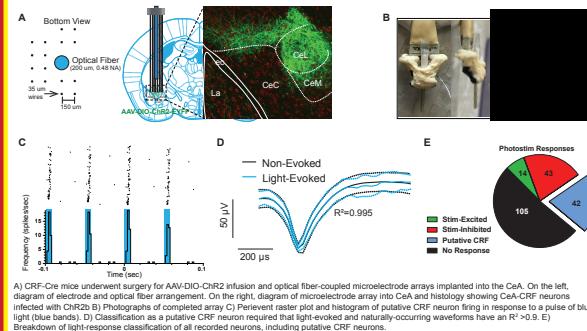
ii. Pharmacogenetic Inhibition of these CRF Neurons Regions Will Decrease Alcohol Consumption



iii. Optically-Identified Putative CRF Neurons Will Increase Firing to Alcohol Consumption

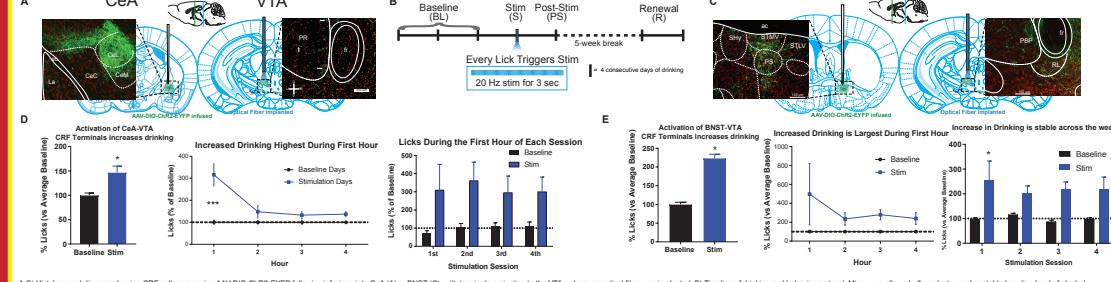


Optical Identification of Putative CRF Neurons

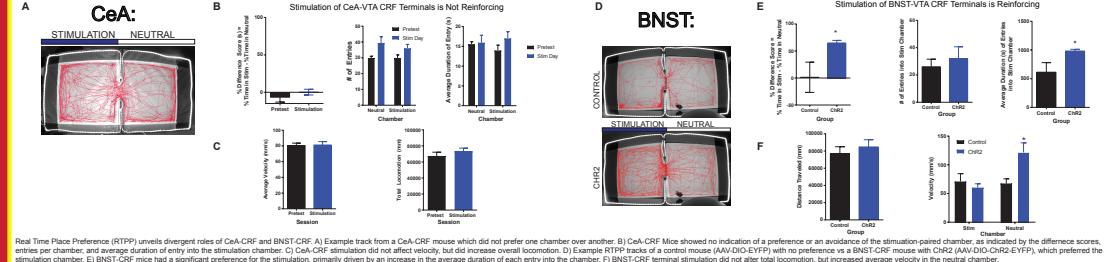


A) CRF-Cre mice underwent surgery for AAV-DIO-CH2 infusion and optical fiber-coupled microelectrode arrays implanted into the CeA. On the left, diagram of electrode and optical fiber arrangement. On the right, diagram of microelectrode array into CeA and histology showing CeA-CRF neurons in the CeA. B) Optical fiber was implanted into the CeA. C) When optogenetic stimulation was applied to the CeA, a putative CRF neuron fired in response to a pulse of blue light (blue bands). D) Classification as a putative CRF neuron required that light-evoked and naturally-occurring waveforms have an $R^2 > 0.8$. E) Breakdown of light-response classification of all recorded neurons, including putative CRF neurons.

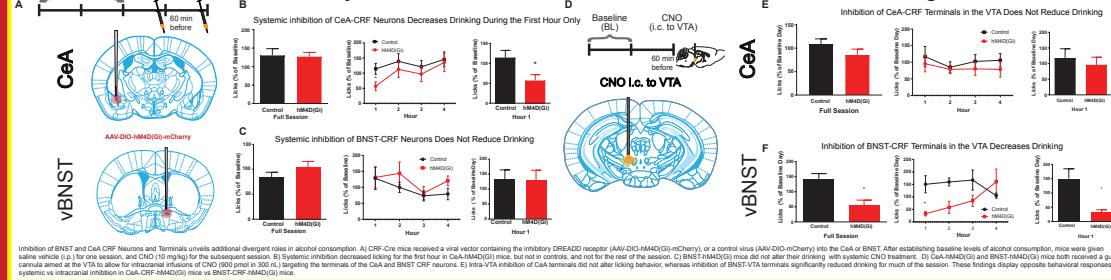
CRF Projections from BNST and CeA Promote Alcohol Consumption



CRF Projections from the BNST to VTA, but not CeA to VTA, are Reinforcing



CRF Projections from the BNST to VTA, but not CeA to VTA, are Reinforcing



CONCLUSIONS

- » Optogenetic stimulation of CeA-CRF neurons in the CeA increased binge drinking following abstinence, but did not affect acute drinking.
- » Optogenetic stimulation of both CeA-CRF and BNST-CRF terminals in the VTA promote drinking.
- » Only BNST-VTA CRF projections are inherently reinforcing.
- » Systemic pharmacogenetic inhibition of CeA-CRF neurons reduces drinking acutely, but inhibition of BNST-CRF terminals in VTA greatly decreases drinking.
- » Systemic pharmacogenetic inhibition of BNST-CRF neurons does not affect drinking, but inhibition of BNST-CRF terminals in VTA greatly decreases drinking.
- » Electrophysiological recordings from putative CRF neurons unveiled multiple subpopulations of CRF neurons with differential responses to alcohol consumption.
- » Our data indicate that CeA-CRF & BNST-CRF neurons play a complex and critical role in binge drinking, with some shared roles, as well as divergent ones.