Identifying Sources of Dynorphin Inputs to the BNST that are Recruited by Social Stress

LSU Health NEW ORLEANS
School of Medicine
Department of Physiology

Katherine Copenhaver, Linh Ha, Evan Doré, Rishith Vaddavalli, Tiffany Wills, and Rajani Maiya

Louisiana State University School of Health Sciences, Department of Physiology

NIH National Institute on Alcohol Abuse and Alcoholism

Introduction

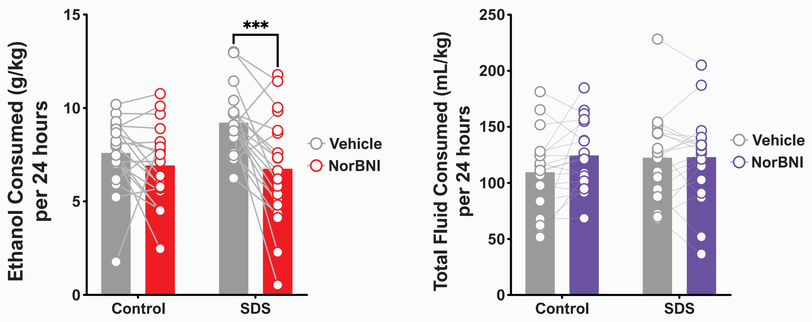
Social Stress and Alcohol Consumption

- Repeated social stress is a risk factor for anxiety, depression and development of substance use disorder (SUD)
- Those who drink alcohol in negative social contexts to reduce social anxiety more likely to meet DSM criteria for an AUD
- Repeated social stress in rodent models leads to escalated drug intake and relapse to drug seeking of a variety of drugs including cocaine and alcohol
- Preliminary data from the Maiya lab show that repeated social defeat stress (SDS) leads to robust increases in alcohol consumption and preference in both males and female C57BL/6J mice

The Dynorphin-Kappa Opioid Receptor System

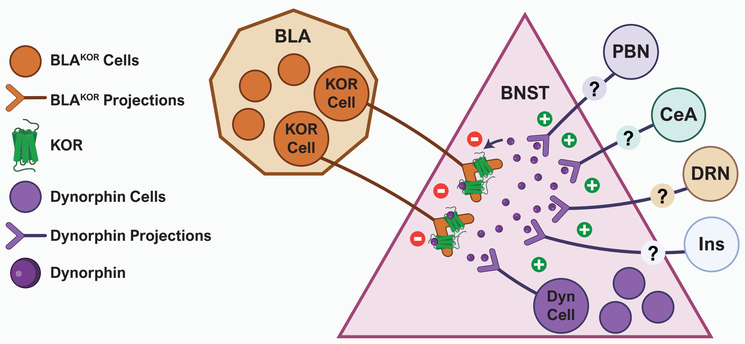
- Dynorphin is a neuropeptide whose expression/release is increased by stress in a variety of limbic and motivation related brain regions
- Dynorphin binds to the kappa opioid receptor (KOR) and has an inhibitory effect on neuronal activity
- Dynorphin-KOR binding interactions have been implicated in neural processes by which stressful events precipitate alcohol and drug use

KOR Antagonism in the BNST Selectively Reduced Alcohol Consumption in Stressed Male Mice



NorBNI infusions into the BNST significantly attenuate alcohol consumption (A). B) Total fluid consumption was not affected. Two-Way RM ANOVA followed by Sidak's post-test, ****, P< 0.001, n = 19/group).

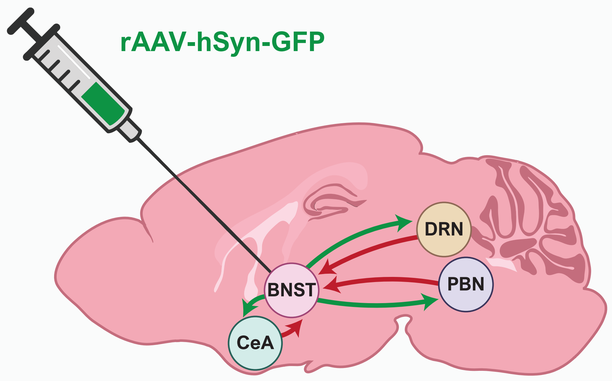
Potential Dynorphin Inputs to the BNST



We predicted that stress causes dynorphin release within the BNST and this dynorphin acts on KORs located on BLA cell terminals in the BNST. We identified five potential sources of dynorphin in the BNST: dynorphin expressing cells within the BNST itself and four regions that contain dynorphin expressing cells that project to the BNST: the central amygdala (CeA), dorsal raphe (DRN), Insular cortex (Ins), and the parabrachial nucleus (PBN)

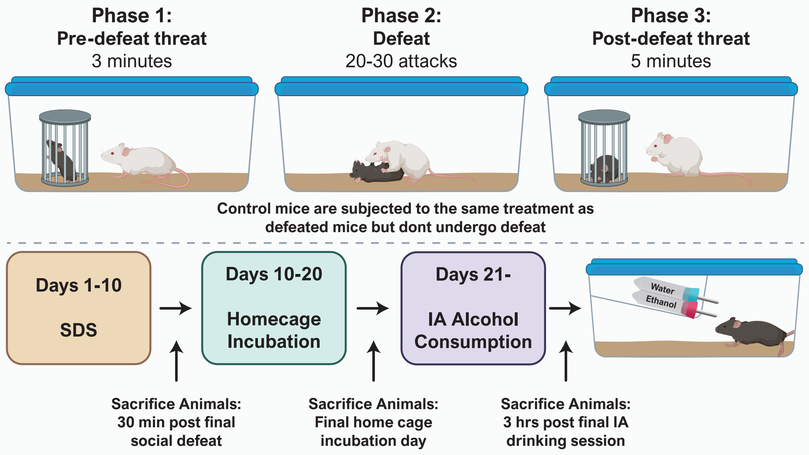
Methods and Experimental Approach

Retro AAV Labeling of Inputs to the BNST

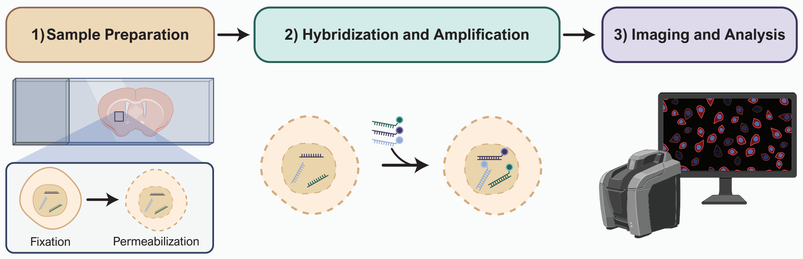


Male C57BL/6J mice received rAAV-hsyn-GFP bilateral injections into the BNST 15 days prior to the beginning of the study. The rAAV travels presynaptically from the BNST to label cells in brain regions that input to the BNST with GFP, enabling us to visualize these inputs (N=24)

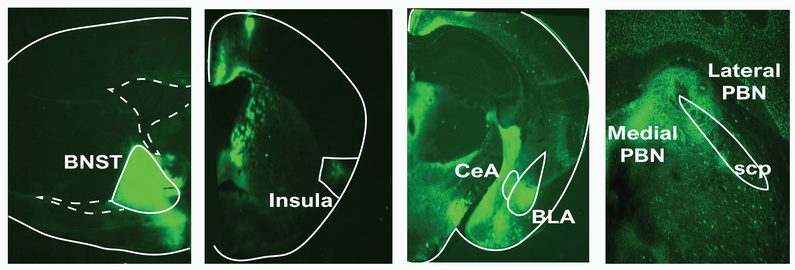
Social Defeat Stress (SDS) and Intermittent Access (IA) Alcohol Consumption Procedure



RNAScope Analysis

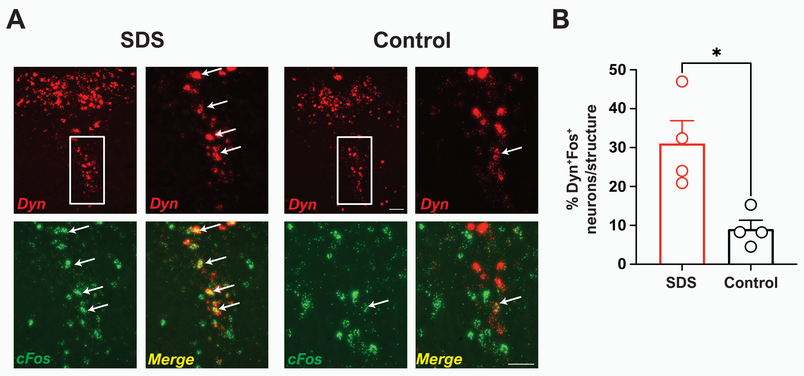


Results



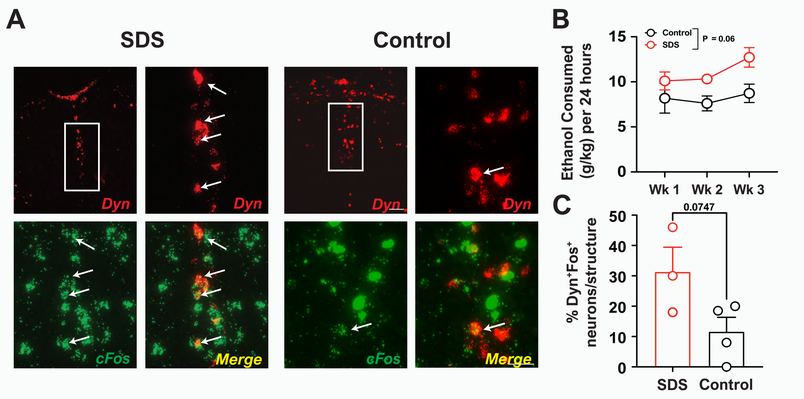
rAAV-hsyn-GFP was injected bilaterally into the BNST of male C57BL/6J mice in experimental and control groups. Brains were extracted 30 minutes post final SDS session and frozen unfixed coronal sections were collected and imaged for GFP. (N=2)

Repeated SDS leads to activated DRN^{Dyn} cells



SDS activates subpopulations of DRN^{Dyn} neurons. A) SDS led to robust induction of cFos in DRN^{Dyn} neurons in the caudal aspects of DRN compared to unstressed controls. B) Quantification of results in A. *, P<0.05, Student's t-test, N = 4 mice/group. Scalebar = 50 µm

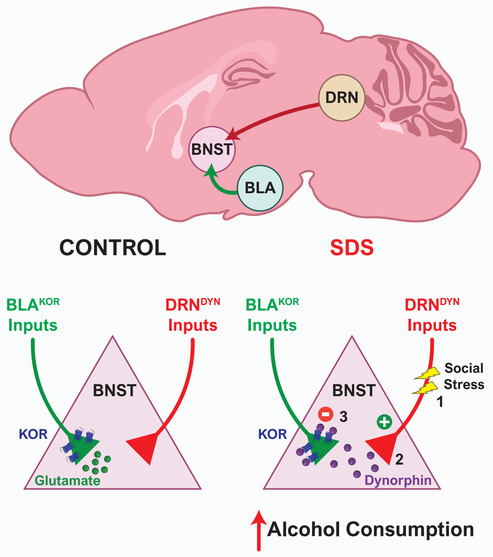
Post-stress activation of DRN^{Dyn} cells



Expression of Dyn and cFos within the DRN for male C57BL/6J mice three hours post day 10 of the SDS procedure. Arrows indicate cell bodies with co-localization of both Dyn and cFos signal. (A). Ethanol consumed during IA alcohol consumption procedure was measured for both SDS and control conditions (B). SDS condition showed a strong trend of increased colocalization of Dyn and cFos expression in cells of the DRN. (C)

Conclusions

- DRN^{Dyn} cells were strongly activated activated 30 mins post-stress compard to unstressed controls
- SDS led to a strong trend towards increased alcohol consumption
- DRN^{Dyn} cells showed increased Fos activation 4 weeks post-stress, 3h after alcohol bottle was introduced
- This work strongly implicates DRN^{Dyn} cells as potential source of Dyn in the BNST that is recruited by SDS



Schematic illustrating KOR modulation of BLAKOR inputs to the BNST. 1) Repeated SDS activates DRNDyn cells and causes Dyn release in the BNST (2). 3) Dyn activates KORs located on BLA terminals in the BNST leading to neuronal inhibition and escalated alcohol intake.

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