Intrinsic CRF neurons in LHb regulate anxiety-like behavior.

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Introduction

The lateral habenula (LHb) is associated with value-based decision making and increased LHb activity is associated with stress-related mood disorders through its negative modulation of dopamine-mediated reward. Previously, we have shown a novel functional role of CRF signaling within rat LHb which may mediate aberrant stress response following exposure to an early life adversity. Exogenous CRF increased LHb intrinsic excitability through postsynaptic CRFR1 (but not CRFR2) and PKA signaling. In contrast to effects of exogenous CRF, blockade of CRFR1 but not CRFR2 increased LHb intrinsic excitability suggesting an endogenous inhibitory CRF/CRFR1 tone in male rats. We then used viral-based anterograde and retrograde tracing in CRF-Cre male mice and found CRF+ neurons within the LHb (LHb^{CRF}) in addition to other sources from the paraventricular nucleus of hypothalamus (PVN), periaqueductal grey (PAG), and paraventricular nucleus of thalamus (PVT), and bed nucleus of stria terminalis (BNST). Given the possibility that intrinsic LHb^{CRF} neurons may provide the inhibitory CRF tone, we isolated LHb^{CRF} neurons with a Cre-dependent excitatory Gq-DREADD in male and female CRF-Cre mice and chemogenetically activated LHb^{CRF} neurons using the novel DREADD ligand (JHU37160) in the light-dark box test (LDT). Activation of LHb^{CRF} neurons promoted an anxiolytic behavior in LDT in male but not female mice. This represents a potential novel local LHb^{CRF} circuit that mediates the anxiolytic actions of CRF and may contribute to the sexual dimorphism in CRF signaling within the LHb. In future studies we will identify the precise neurochemical nature, synaptic connectivity, activity, and function of LHb^{CRF} neurons and their contribution to anxiety-related behaviors in male and female mice.

Methods

1. **Slice electrophysiology.** Data generated from male mice (Fig 1) and male rats (Fig 2). Fig 1. intrinsic excitability was assessed through blockade of fast-synaptic signaling and CRF was bath applied to LHb-containing slices for upto 20-30min.
2. **Retrograde Tracing CRF-Cre inputs into LHb.** Data generated from male CRF-Cre mice (n3) with intra-LHb injection of retrograde-AAV-DIO-eYFP. Mice were perfused and brains sectioned at 25um with ~150um between slices ranging from Bregma 2.10 to -4.36. All sections were scanned using Zeiss Axioscan as 10X images. Labeled neurons were counted across regions of interest (ROI) identified using Allen Brain Atlas and Franklin & Paxinos Mouse atlas. Zeiss-AxioScan images were aligned to Allen Brain Atlas using Aligning Big Brains and Atlases (ABBA) and FIJI programs. For each region of interest the sum of labeled cells from three consecutive sections (Bregma +/- 0.12mmm) containing the ROI were counted and then averaged across 3 mice. Data is presented as percent of cells out of the total cells across all ROIs (361 cells total).
3. **Chemogenetic activation of LHb CRF neurons and Light Dark Test (LDT).** Male and female CRF-Cre mice were bilaterally injected with AAV-DIO-hM3D(Gq)-mCh into the LHb. Following 4 weeks for stable viral expression mice underwent light/dark test. 30-min prior to behavioral testing, mice were injected i.p. with 0.3mg/kg DREADD-specific agonist JHU37160. Locomotor activity and time spent in light (750lux) or dark (0lux) sides of the tests apparatus were monitored through infrared tracking and ANYMAZE software.

CRF-Cre retrograde tracing and identification of intrinsic LHb^{CRF} neurons

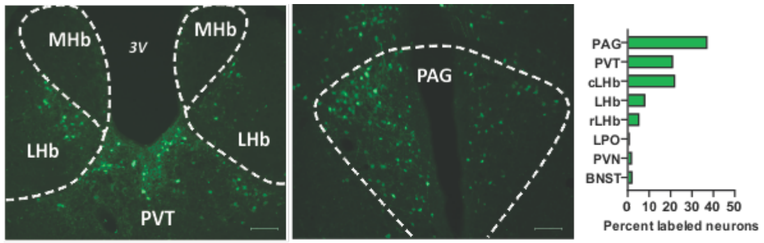

Fig 3. **Retrograde tracing of CRF+ inputs to LHb.** rAAV-DIO-eYFP was injected into the LHb of CRF-Cre male mice and retrograde labeled cell bodies were counted across sections. For each region of interest (ROI) analyzed: the sum of labeled cells from three consecutive sections (Bregma +/- 0.12mm) containing the ROI were counted and then averaged across 3 mice. Data is presented as percent of cells out of the total cells across all ROIs (361cells). ROIs: bed nucleus of the stria terminals (BNST, Bregma -0.1), paraventricular hypothalamic nucleus (PVN, Bregma -0.82); lateral preoptic area (LPO, Bregma -0.1), rostral Lateral habenula (rLHb, Bregma -1.22); lateral habenula (LHb, Bregma -1.58); caudal lateral habenula (cLHb, Bregma -1.94), periventricular thalamic nucleus (PVT, Bregma -1.84), Periaquaductal grey (PAG, Bregma -2.7). Scale bar 100µm

CRF inhibitory tone in male rats

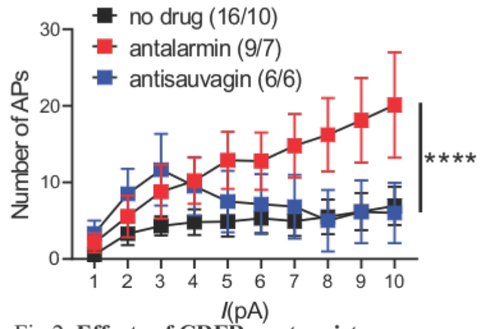

Fig 2. **Effects of CRFR anatgonists on intrinsic excitability of LHb neurons in male rats.** CRFR1 antagonist (1µM antalarmin) but not CRFR2 anatgonist (1µM antisauvagin) increased intrinsic excitability (with fast synaptic transmission blocked) of LHb neurons in slices from male rats. Numbers in graph represent number of cells/rats; F (2, 280) =17.70, P<0.0001; ANOVA.

Sex diff in LHb^{CRF} excitability

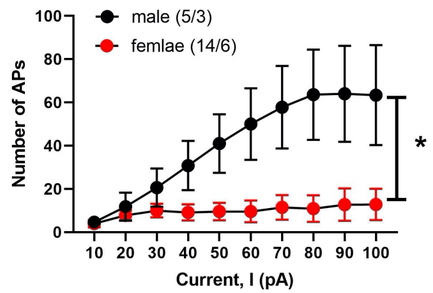

Fig 1. **Sex difference in baseline LHb^{CRF} neuron excitability in CRF-Td reporter mice.** LHb neuron excitability (AP generation, intact synaptic transmission) was assessed across depolarizing current steps. LHb^{CRF} neurons from female mice were significantly less excitable compared to male LHb^{CRF}. Numbers in graph represent number of cells/mice; 2-way ANOVA, effect of sex: F (1,17)=8.12 p<0.05; intensity x sex interaction: F(9,153)=10.38, p<0.0001.

Chemogenetic activation of intrinsic LHb^{CRF} neurons regulate anxiety-like behavior

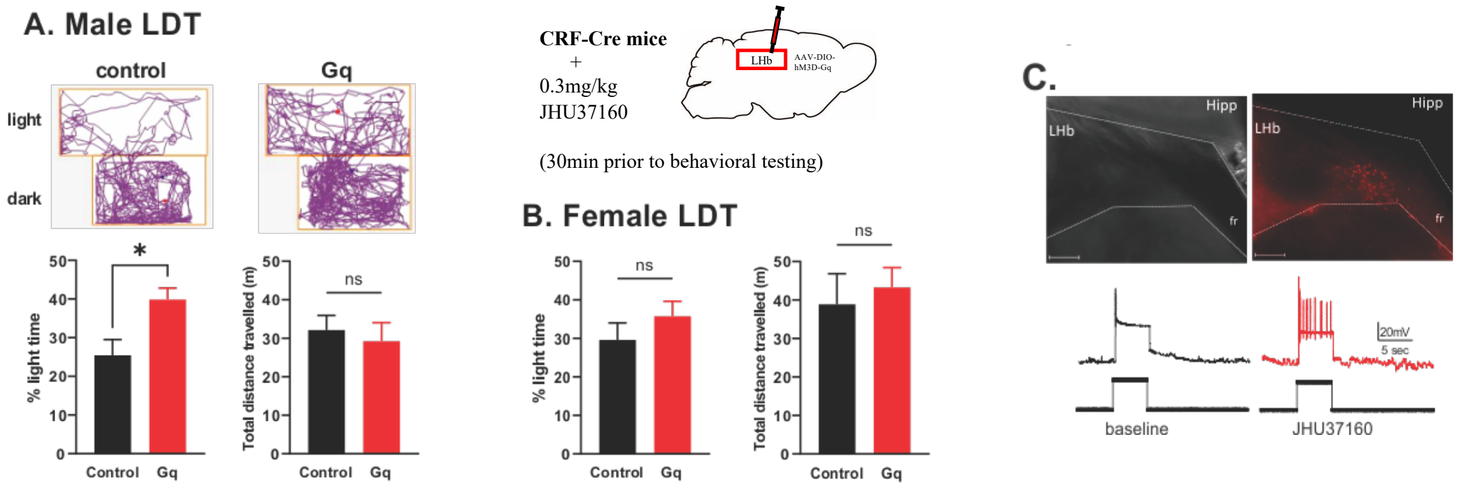

Fig 4. **Effects of chemogenetic activation of LHb CRF neurons in Light/Dark test (LDT) in male and female CRF-Cre mice.** CRF-Cre mice were injected with either Cre-dependent control virus (control) or DIO-hM3D(Gq)-mCherry (Gq) into the LHb 4 weeks prior to behavioral testing (control male n4, Gq male n3; control female n6, Gq female n7). 30min prior to behavioral testing, mice were injected i.p. with 0.3mg/kg DREADD-specific agonist JHU37160. Gq activation of LHb^{CRF} neurons significantly increased precent time in light side of apparatus (anxiolytic-like behavior) in male (A) but not female (B) without altering total locomotor activity. Student t-test, *p<0.05. C. depicts images of a sagittal LHb slice from a Gq-DREADD mouse under IR-DIC (left) and fluorescence (right) with representative traces of depolarization induced action potential generation to 80pA current step before and after addition of JHU37160 (100nM) in the bath.

Future Directions

1. Explore molecular identity of LHB^{CRF} neurons
2. Test for intrinsic circuitry (optogenetic isolation) and projection-specificity of LHb^{CRF} neurons.
3. Effects of Chemogenetic Gi DREADD inactivation of LHB^{CRF} neurons on LDT, elevated plus maze behaviors

Acknowledgments

NIH Grants:
NIDA RO1-DA039533
NINDS R21 -NS120628
No conflicts of interest to declare.