Input and population specific regulation of lateral habenula neurons by kappa opioid receptors.

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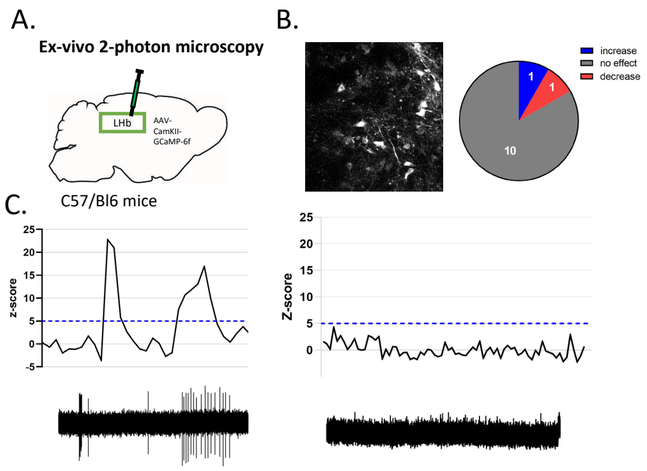
Introduction

The lateral habenula (LHb) is an epithalamic brain region associated with value-based decision making and stress evasion. Increased activity of the LHb is associated with drug addiction and stress-related mood disorders. Dynorphin (DYN)/Kappa opioid receptor (KOR) signaling is an endogenous mediator of stress response in reward circuitry. Previously, we have shown a novel functional role of KOR signaling within LHb. Such that KOR activation has bidirectional effect on LHb neuronal excitability. Here we used an unbiased high- through put approach of GCAMP calcium signaling which verified our previous findings. To identify KOR-expressing projections to LHb we used a viral based retrograde tracing in KOR-Cre mice, where we found that entopenduncular nucleus (EP) is a major KOR- expressing input. EP has been implicated in stress-induced mood disorders and may contribute to aberrant LHb excitability in depressive-like phenotypes. KOR activation significantly reduced LHb action potential generation in a subset of LHb neurons in response to optical stimulation of EP inputs. This suggests input and cell-type specific KOR regulation of LHb neurons. In the future we will explore the effects of early life stress and traumatic brain injury on KOR modulation of LHb activity in projection and input-specific manner.

Methods

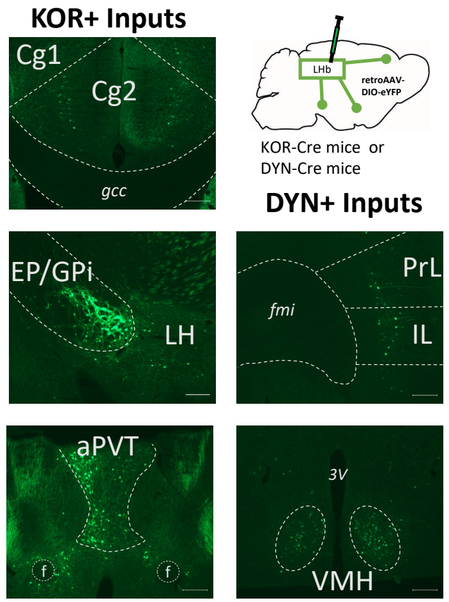
1. **GCaMPs fluorescence.** All data generated from adult male C57BL6 mice with LHb injection of AAV-CamKIIa-GCaMPS-6f and ex-*vivo* calcium fluorescent activity of LHb assessed with 2-photon microscopy (Zeiss). Real-time GCaMPs fluorescence data was recorded from 20X imaging with 3Hz sampling rate. Example traces shown for simultaneous GCaMPs and patch-clamp recording across baseline conditions in an active neuron (10s) and silent neuron (1min). All example fluorescence traces are represented as Z-score=(F-F0)/(stdev(F0)); where F0=baseline. Threshold for fluorescent event was 5 stdev above the baseline (ie z score=5). **KOR agonist (U50,488) concentrations for all experiments = 10uM.** Significance of KOR agonist on GCaMPs fluorescence was set to >30% change in Ave freq at baseline (2min) compared to post-drug application across 2 min bins.
2. **Retrograde Tracing of KOR and Dynorphin inputs into LHb.** Data generated from male KOR-Cre mice (n3) or DYN-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. For each ROI numbers were averaged across 2 consecutive Bregma levels and then across mice.
3. **Ex-vivo optogenetics.** Male Sprague Dawley rats were injected with AAV-CamKII-ChR2-eYFP into the EP/GPi at PN21-PN28. *ex-vivo* electrophysiological recordings conducted at PN40-50. Hyperpolarizing current (Ih+) measured following -50pA current injection, Ih- defined as >-10pA change in current, Ih- defined as <-10pA change in current. Optically evoked action potentials (oAP) were induced by 300us-1ms whole-field light stimulation (350nm). Maximal and 50% max response for each cell was determined during 1Hz stimulation through alteration of LED strength (5-100%). Frequency of stimulation tested at 1-40Hz. Baseline oAP recorded prior to >30min of U50,488 10uM bath application.

GCaMPs fluorescence reflects LHb activity



A. Glutamatergic neurons were isolated through stereotaxic injection of AAV-CamKII-GCaMP-6f viral construct into the LHb of C57/Bl6 mice. **B.** Example GCaMPs expression in LHb-containing slice and pie chart displaying number of neurons with significant increase (blue), decrease (red) or unchanged (gray) fluorescent activity across 10min of stable baseline recording (12 neurons from 1 mouse). **C.** Example simultaneous recording traces of both normalized GCaMPs fluorescence (z-score, top) and corresponding neuronal activity (mV, bottom) from simultaneous patch-clamp recording. **left:** example traces of an active neuron with bursting event and high-frequency train events (10s recording). **right:** example trace of a silent neuron (1min)

KOR-Cre and DYN-Cre retrograde tracing of LHb inputs

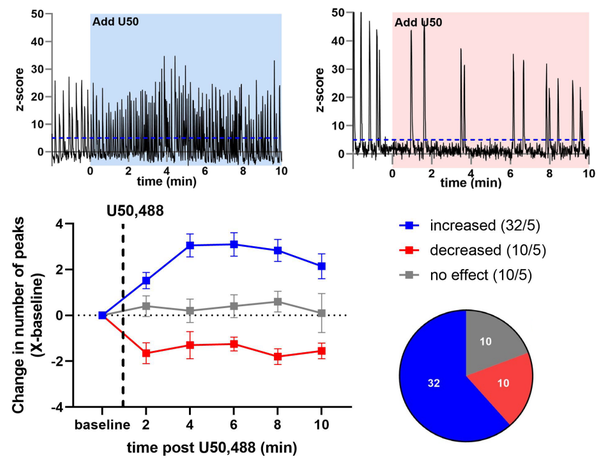


LHb input region		KOR	DYN
Cortex	Cg1	+	-
	Cg2	++	++
	PrL	+	++
	IL	+	++
Striatum	CP	-	-
	NAc	-	-
Pallidum	VP	(+)	-
	BNST	+	-
	EP/GPi	+++	-
Amygdala	BLA	-	-
	cEA	-	-
	Ext. Amg	+	+

LHb input region		KOR	DYN
Thalamus	aPVT	++	-
Hypothalamus	AVPV	+	-
	LPO	(+)	-
	PVN	(+)	-
	LHA	++	
	VMH	+	++
	PH	+	-
	ZI	++	-
Midbrain	PAG	++	-
	IF	++	-
	RLi	+	-
	VTA	+	-

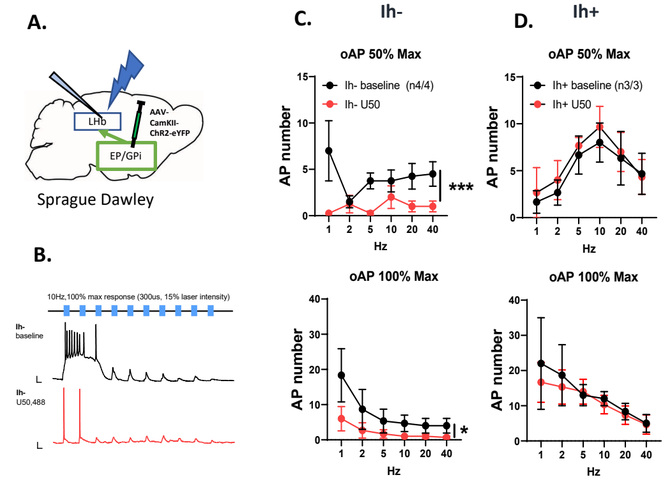
Example labeled regions from KOR-Cre (left column) and Dynorphin-Cre mice with LHb injection with retro-AAV-DIO-eYFP. Scale bar = 200um. Table displays relative gradient of labeling: - none; (+) sparce <5; + medium 5-25; high 25-50; very high >50 across regions of interest across Cortex, Striatum, Pallidum, Amygdala, Thalamus, Hypothalamus, and Midbrain.

KOR agonist alters GCaMPs activity in LHb



A. Example GCaMPs fluorescence traces (z-score) of U50-induced increase [left, blue] and decrease [right, red] number of peaks, 2min baseline and up to 10min post U50,488 drug application. **B.** Graph displaying average normalized change in number of peaks from 2min baseline and across 2 min bins from time of U50,488 application. Pie chart displaying number of neurons with significant increase (32 neurons from 5 mice, blue), decrease (10 neurons from 5 mice, red) or unchanged (10 neurons from 5 mice, gray) fluorescent activity across 10min of recording following U50,488 KOR agonist application.

KOR agonist alters EP → LHb oAP in Ih- but not Ih+ LHb neurons.



A. Experiment summary. Projection-specific inputs from EP → LHb were isolated through stereotaxic injection of AAV-CamKII-ChR2-eYFP viral construct in the EP. **B.** Example recordings of oAP from EP-input optical stimulation at 10Hz at baseline and post-U50,488 application. **C.** U50,488 significantly reduced oAP of Ih- LHb neurons at 50% and 100% maximal response. **D.** U50,488 had no significant effect on EP-evoked oAP generation in Ih+ LHb neurons.

Future Directions

1. Explore molecular identity of Ih- and Ih+ LHb neurons – GABAergic/ Glutamatergic markers (SST, GAD, VGLUT-Cre), retrobeads labeling/tracing.
2. Determine effects of KOR agonist on E/I balance of EP→LHb inputs.
3. Compare effects of KOR agonist on other LHb inputs (mPFC, PAG, VTA, etc)
4. Behavioral effects of silencing EP→LHb inputs (Gi-DREADDs) on KOR-related behaviors.
5. Effects of early life stress on input- and cell type-specific synaptic plasticity and its modulation by KOR signaling.

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No conflicts of interest to declare.