# *Mesohabenular GABA/Glutamate Projections Balance Postsynaptic Activity and Reinforcement*

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Individual neurons projecting to lateral habenula (LHb) have the surprising capacity to release both GABA and glutamate, including inputs from entopeduncular nucleus as well as from ventral tegmental area (VTA). Indeed, most LHb-projecting VTA neurons express both the vesicular GABA and glutamate transporters, VGAT and VGLUT2. Yet it remains mysterious why neurons would simultaneously release excitatory glutamate with inhibitory GABA onto the same post-synaptic cells. Here we test the hypothesis that the net excitatory vs. inhibitory effect of VTA terminal stimulation depends on the activity state of postsynaptic neurons in LHb. To test this we used optogenetics in mice to stimulate VTA inputs while simultaneously using DREADDs to alter the excitability of postsynaptic neurons in LHb. We injected an intersectional Cre- and FLP-dependent Channelrhodopsin (ChR2) into the VTA of double mutant VGLUT2-Cre, VGAT-FLP mice to express ChR2 selectively in neurons that co-release GABA and glutamate. Optogenetic stimulation of these projections led to net inhibition of neurons in lateral habenula (LHb), and was sufficient to support positive reinforcement in a self-stimulation task, consistent with the role for LHb activity in aversion. Using CRISPR-Cas9 mediated deletion of either VGLUT2 or VGAT in VTA GABA/glutamate neurons, we next found that VTA GABA release and not glutamate release was necessary for self-stimulation behaviors. To simultaneously control postsynaptic LHb excitability, we next expressed either an excitatory (Gq) or inhibitory (Gi) DREADD in VGLUT2-Cre neurons in LHb, then measured ex vivo optogenetic evoked post-synaptic potentials (oPSPs) or in vivo opto-evoked GCaMP fluorescence from VTA GABA/glutamate terminals in LHb. In Gi-expressing LHb, clozapine-n-oxide (CNO) led to hyperpolarization, and caused opto-evoked activity to become more excitatory. Conversely, in Gq-expressing cells CNO led to depolarization, and opto-evoked activity became more inhibitory. These results suggest that the net effects of GABA/glutamate co-release can become either more inhibitory (and thus more rewarding) or excitatory (and thus less inhibitory/less rewarding) depending on the state of the post-synaptic cell. To test this, we chemogenetically manipulated LHb while assaying the reinforcing qualities of VTA GABA/glutamate release using a 2-nosepoke self-stimulation task. Systemic CNO injections decreased self-stimulation in Gi-expressing mice, and increased self-stimulation in Gq-expressing mice. These results suggest that postsynaptic excitability of LHb can dictate whether the effect of GABA/glutamate co-release is net inhibitory or net excitatory, and provide a mechanistic explanation by which activity in VTA neurons that co-release GABA/glutamate can normalize LHb activity and reinforcement behavior by inhibiting LHb when it's hyperactive, and exciting LHb when it's hypoactive. This ability of GABA/glutamate co-release to normalize activity may have broad implications for disorders of imbalanced motivation such as substance use disorder.