Nature Reviews Neuroscience | AOP, published online 15 October 2014; doi:10.1038/nrn3848

NEUROTRANSMISSION

Transmission takes two

The lateral habenula (LHb) processes reward- and punishment-related information and sends projections to various brain regions. How LHb output is regulated is incompletely understood, but two studies in rodents now reveal that LHb activity is controlled by inputs that co-release glutamate and GABA.

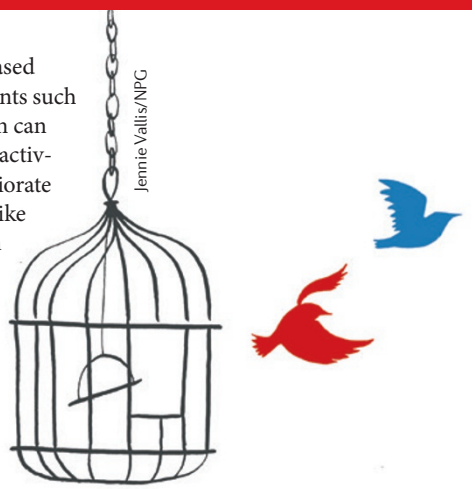
The ventral tegmental area (VTA) is a well-known target region for LHb output, but neurons from the VTA also project back to the LHb. The nature and the function of these VTA–LHb (mesohabenular) neurons are poorly understood; therefore, Morales and colleagues set out to examine their neurotransmitter type in rodents.

Immunolabelling and radioactive *in situ* hybridization experiments in rats revealed that ~80% of these neurons expressed vesicular glutamate transporter 2 (VGLUT2; a marker of glutamatergic neurons) and isoforms of glutamate decarboxylase (GAD; markers of GABAergic neurons), whereas only 30% expressed a dopaminergic neuron marker. Moreover, immunoelectron microscopy in brain slices from transgenic mice in which the fluorescent protein mCherry was expressed in VGLUT2-expressing VTA neurons showed that most VGLUT2-expressing mesohabenular axon terminals also expressed the vesicular GABA transporter (VGAT), which is another GABAergic neuron marker. This technique also showed that individual mCherry-labelled axon terminals formed both asymmetrical (probably excitatory) and symmetrical (probably inhibitory) synapses. Together, these results suggest that mesohabenular neurons signal through glutamate and GABA.

To investigate this assertion, the authors recorded from LHb neurons in brain slices from mice in which the expression of channelrhodopsin2 (ChR2) was placed under the control of the *Vglut2* or *Vgat* promoter. In both cases, light stimulation of ChR2-expressing mesohabenular neurons evoked synaptic currents in LHb neurons that comprised fast inward and slower outward components. NBQX, an AMPA receptor antagonist, selectively blocked the inward current, whereas picrotoxin, a $GABA_A$ receptor antagonist, blocked the outward current, confirming that mesohabenular axon terminals co-release glutamate and GABA.

In their study, Shabel *et al.* examined inputs to the LHb arising from the entopeduncular nucleus (EP), an output region of the basal ganglia. They infected the EP of mice with a ChR2-expressing adeno-associated virus and recorded the responses of LHb neurons to light-induced EP neuron activation. The light-evoked synaptic responses in LHb neurons again had glutamate receptor- and $GABA_A$ receptor-mediated components that could be individually blocked through the use of selective receptor antagonists, and similar results were obtained in mice in which ChR2 was expressed under the *Vglut2* or *Gad67* promoter. Thus, EP neurons also co-release glutamate and GABA in the LHb.

Hyperactivity of the LHb may be associated with depression, and chronic treatment with serotonin-based antidepressants such as citalopram can reduce LHb activity and ameliorate depression-like behaviour in animals. Shabel *et al.* found that, in mice, two weeks of citalopram treatment increased the ratio of $GABA_A$ receptor- to AMPA receptor-mediated LHb neuron synaptic responses following the activation of EP neurons, and that this ratio was decreased in a rat model of depression. The changes in ratio were associated with increased and decreased expression, respectively, of GAD at EP–LHb synapses. Thus, these data indicate that depression may be associated with an imbalance in glutamate–GABA co-transmission at these synapses and that antidepressant treatments may act to restore normal co-transmission.



Together, these two studies show that neurons that co-release GABA and glutamate regulate the activity of the LHb.

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ORIGINAL RESEARCH PAPERS Root, D. H. *et al.* Single rodent mesohabenular axons release glutamate and GABA. *Nature Neurosci.* http://dx.doi.org/10.1038/nn.3823 (2014) | Shabel, S. J. *et al.* GABA/glutamate co-release controls habenula output and is modified by antidepressant treatment. *Science* **345**, 1494–1498 (2014)

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