Circuits and functions of the lateral habenula in health and in disease

Hailan Hu[1,2,3,4,5]✉, *Yihui Cui*[2] *and Yan Yang*[2]

Abstract | The past decade has witnessed exponentially growing interest in the lateral habenula (LHb) owing to new discoveries relating to its critical role in regulating negatively motivated behaviour and its implication in major depression. The LHb, sometimes referred to as the brain's 'antireward centre', receives inputs from diverse limbic forebrain and basal ganglia structures, and targets essentially all midbrain neuromodulatory systems, including the noradrenergic, serotonergic and dopaminergic systems. Its unique anatomical position enables the LHb to act as a hub that integrates value-based, sensory and experience-dependent information to regulate various motivational, cognitive and motor processes. Dysfunction of the LHb may contribute to the pathophysiology of several psychiatric disorders, especially major depression. Recently, exciting progress has been made in identifying the molecular and cellular mechanisms in the LHb that underlie negative emotional state in animal models of drug withdrawal and major depression. A future challenge is to translate these advances into effective clinical treatments.

Reward
Psychologically, 'reward' refers to a positive emotional stimulus, which is reinforcing and promotes repeated responding to obtain the same stimulus.

[1]*Department of Psychiatry of First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China.*

[2]*The MOE Frontier Science Center for Brain Research and Brain–Machine Integration, Zhejiang University School of Brain Science and Brain Medicine, Hangzhou, China.*

[3]*NHC and CAMS Key Laboratory of Medical Neurobiology, Mental Health Center, Zhejiang University, Hangzhou, China.*

[4]*Center for Brain Science and Brain-Inspired Intelligence, Guangzhou, China.*

[5]*Fountain-Valley Institute for Life Sciences, Guangzhou, China.*

✉*e-mail: huhailan@zju.edu.cn*

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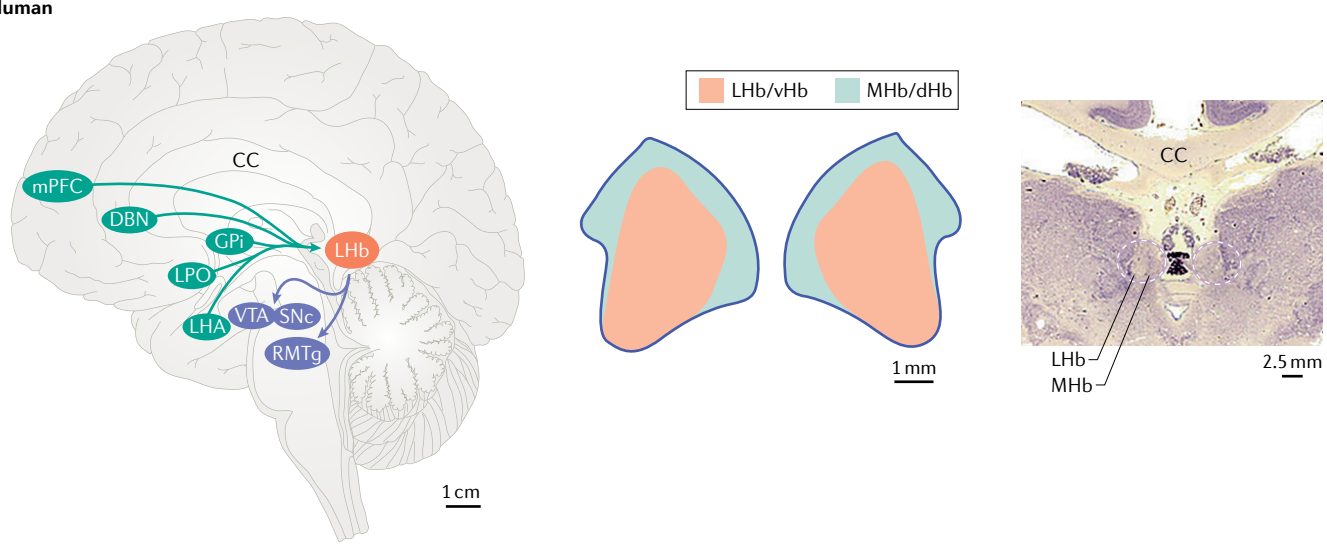
The habenula is an ancient brain structure that is conserved in virtually all vertebrate species[1,2]. In mammals, it consists of a pair of small nuclei located at the posterior–dorsal–medial end of the thalamus and can be divided into the medial habenula (MHb) and lateral habenula (LHb) regions[2] (FIG. 1). In fish and amphibians, the corresponding parts of the mammalian MHb and LHb are the dorsal habenula (dHb) and the ventral habenula (vHb), respectively[3,4] (FIG. 1).

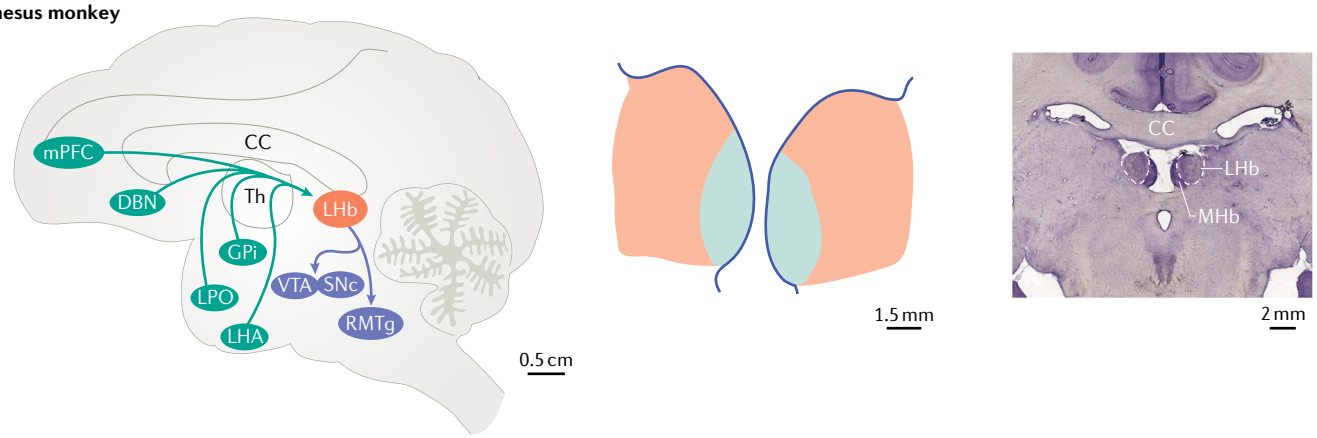
Over the past decade, as reflected in a rapidly growing body of literature, the habenula and, in particular, the LHb, have received a surge in interest. The increasing interest in the LHb can be attributed to three major reasons. First, through direct[5,6] or indirect[7,8] innervations, the LHb is one of the few brain regions that control both the dopaminergic system and the serotonergic system[5–7,9–13]. As these neuromodulatory systems have essential roles in a wide range of motivational, motor and cognitive functions[14], the LHb, as a powerful negative regulator of both systems, may be strategically positioned to regulate these functions[15]. Second, seminal studies from Hikosaka and colleagues revealed a critical function of the LHb in encoding negative reward, in particular negative reward prediction error (RPE; the discrepancy between expected and actual rewards)[13,16,17]. These discoveries provide a conceptual framework for studying LHb function and have been confirmed by a series of lesion and optogenetic studies[18–23]. Third, evidence from human studies and animal models has grown considerably in support of the association of the LHb with multiple psychiatric disorders[21,24–26], especially major depressive disorder (also termed 'major depression')[21,25,26]. Whole-brain screening studies in rodents or zebrafish identified the habenula as the only brain region showing consistently heightened activity in multiple animal models of depression[27–29]. In humans, despite the small size of the habenula, recent technology advances have allowed functional imaging studies of this structure, revealing its hyperactivity in individuals with major depression[30–32]. Moreover, two pilot clinical case studies applying deep brain stimulation to inactivate the LHb reported either full remission[33] or alleviation[34] of major depression, suggesting a potential causative relationship between LHb malfunction and at least some symptoms of depression. Most excitingly, studies in animal models of depression have begun to uncover key molecular and cellular mechanisms in the LHb that shed light on disease cause[35–39]. One of the most promising rapid antidepressant drugs, ketamine, was recently shown to act by suppressing the bursting activity of the LHb neurons[39,40]. These discoveries may inspire the development of new-generation antidepressant treatments.

The aim of this Review is threefold. First, we provide an update on the basic organization of the LHb, including discussion of regional molecular markers, electrophysiological properties, anatomical subdivisions and circuitry connections. In particular, we emphasize optogenetic circuit dissection studies that highlight the specific functions of the upstream input and downstream output pathways of the LHb. Second, we describe the various physiological functions in which the LHb

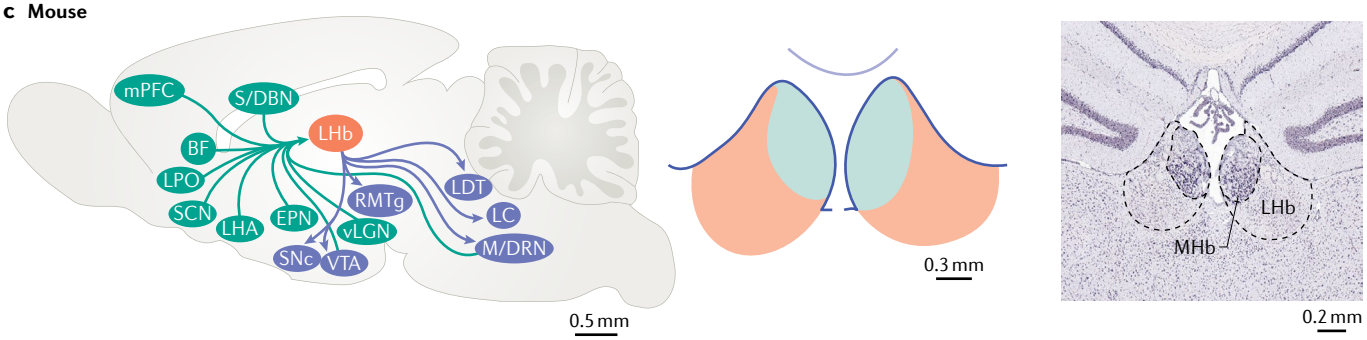
a Human



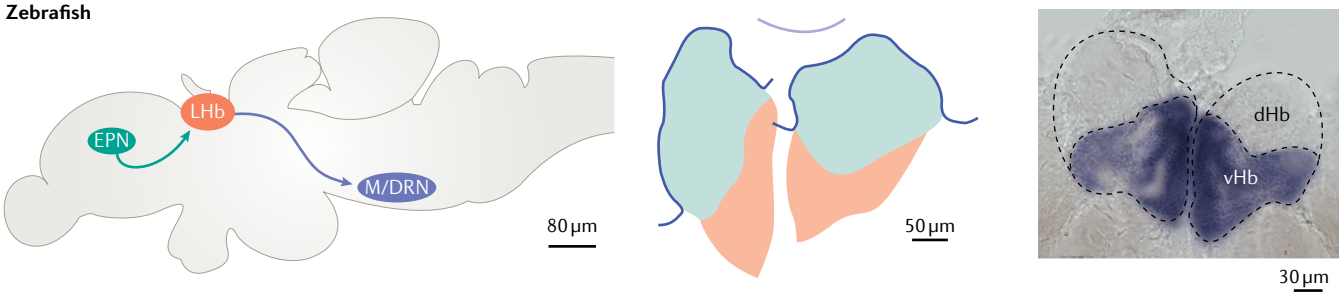
b Rhesus monkey



c Mouse



d Zebrafish



◀ Fig. 1 | **Evolutionary conservation of lateral habenular pathways and anatomical organization of the habenula in vertebrates. a** | Humans. **b** | Rhesus monkeys. **c** | Mice. **d** | Zebrafish. The left-hand column shows homologous circuits across species for the lateral habenula (LHb), with inputs to the LHb in green and outputs of the LHb in blue, on schematic sagittal brain sections. The middle and right-hand columns show schematic coronal sections and coronal histological sections, respectively, of the habenula in different species, detailing the territories of the LHb and the medial habenula (MHb) or equivalent regions. BF, basal forebrain; CC, corpus callosum; DBN, diagonal band nuclei; dHb, dorsal habenula; EPN, entopeduncular nucleus; GPi, globus pallidus internus; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; LHA, lateral hypothalamic area; LPO, lateral preoptic area; M/DRN, medial raphe nucleus or dorsal raphe nucleus; mPFC, medial prefrontal cortex; RMTg, rostromedial tegmental nucleus; SCN, suprachiasmatic nucleus; S/DBN, septum and diagonal band nuclei; SNc, substantia nigra pars compacta; Th, thalamus; vHb, ventral habenula; vLGN, ventral lateral geniculate nucleus; VTA, ventral tegmental area. Left panel of part **a** adapted with permission from REF.[276], Elsevier. Right panel of part **a** adapted with permission from Michigan State University, Brain Biodiversity Bank https://msu.edu/user/brains/brains/human/coronal/2240_cell.html. Left and right panels of part **b** adapted courtesy of Dr Okihide Hikosaka (REF.[277]) Middle panel of part **b** adapted with permission from REF.[45], Frontiers. Left panels of parts **c** and **d** adapted with permission from REF.[278], Elsevier. Right panel of part **c** adapted with permission ©2020 Allen Institute for Brain Science (Allen Mouse Brain Atlas, http://mouse.brain-map.org/experiment/show?id=252. Available from http://mouse.brain-map.org). Right panel of part **d** adapted with permission from REF.[4], SFN.

has been implicated, highlighting its importance in the coding of negative reward signals and in the control of motivated behaviours. Third, we review the relevance of the LHb in major psychiatric disorders. We focus on the molecular and cellular mechanisms leading to aberrant overactivation of LHb neurons in animal models of depression. This leads to a discussion of potential new treatment strategies that may target specifically the neural substrate of LHb hyperactivity (for example, LHb bursts). The MHb is discussed at some points in the Review to make a comparison with the LHb but it is not covered in detail. The detailed molecular and anatomical features and functional roles of the MHb can be found in REFS[41–44].

Anatomical organization

Within the mammalian MHb and LHb subdivisions, profiles of gene expression and patterns of afferent and efferent connectivity are clearly distinct[21,41,45]. Neurons in the MHb mainly use acetylcholine, substance P and glutamate as neurotransmitters[46,47], receive major inputs from the septum and project mostly into the interpeduncular nucleus[1,5]. By contrast, most neurons in the LHb are glutamatergic[41], receive far more distributed afferent inputs from the basal ganglia and limbic forebrain[6,48] and primarily project to the rostromedial tegmental nucleus (RMTg) and midbrain aminergic centres[7,8,49,50]. Not much is known about the extent to which communication occurs between the MHb and the LHb, although some studies indicate a potential medial-to-lateral connection[51,52]. Connecting the left and right habenulae, there are substantial and topographically organized commissural projections[52]. Although the functional implication is still unclear, an interesting feature of the habenula is that it displays left–right asymmetries, which are most noticeable in fish, amphibians and reptiles but are still detectable in birds and mammals[1,2] (FIG. 1).

The LHb itself contains highly heterogeneous functional compartments and neuronal subtypes, which can be categorized by different morphologies, molecular markers and electrophysiological properties[1,41,42,53–55]. In the next section, we discuss the basic organization of the LHb in rodents, in which it has been best studied (FIGS 1,2). We note species differences where they are relevant to the data being discussed, but we do not attempt a detailed description of the comparative neuroanatomy.

Molecular markers and subregions

Anatomically, the LHb is subdivided into the principal medial and lateral subdivisions (LHbM and LHbL) and is further differentiated into at least seven subnuclei[41,42,45,56] (FIG. 2a).

Cells within the LHb are predominantly glutamatergic[41], expressing the mRNA of either vesicular glutamate transporter 2 (VGLUT2)[49] or VGLUT3 (REF.[57]) but not VGLUT1 (REFS[49,57,58]). Functionally, these glutamatergic LHb neurons are heterogeneous in terms of their response to aversive stimuli. As revealed by single-unit recording and juxtacellular labelling in anaesthetized mice, most foot shock-responding LHb neurons are glutamatergic, among which 78% are excited by and the rest are inhibited by foot shocks[59].

Whether the LHb contains GABAergic neurons has been the subject of debate. A small subset of neurons in the LHbM express glutamic acid decarboxylase 2 (GAD2) and GABA transporter 1 (GAT1)[60,61] but it was debated whether they express vesicular GABA transporter (VGAT)[61,62], and thus it was not clear whether they may be able to load GABA and function properly. Two recent studies, however, showed that optogenetic activation of neurons expressing GAD2 or parvalbumin in the LHb locally drives inhibitory responses in nearby cells[63,64], indicating the existence of functional local inhibitory circuit within the LHb.

Various monoamine receptors[41], neuropeptides[65] and calcium-binding proteins[66] exhibit heterogeneous distributions in the LHb (Supplementary Table 1). mRNAs of both D1-like (*Drd5*)[41] and D2-like (*Drd2*)[61] dopamine receptors have been detected in the LHb (Supplementary Table 1). Serotonin type 2c receptor (*Htr2c*) mRNA is expressed much more specifically and intensively in the LHb than transcripts of the other subtypes of serotonergic receptors[67]. The expression of *Drd2* and *Htr2c* mRNA seems complementary in the LHbM and coincides partly in the LHbL, revealing heterogeneity between the LHbM and the LHbL with respect to monoamine regulation[41]. Among calcium-binding proteins, which are often markers of interneurons, calbindin and calretinin appear to be selectively expressed in the parvocellular subnucleus of the LHbM (LHbMPc) and the superior part of the LHbM (LHbMS)[60], whereas expression of parvalbumin tends to be distributed within the medial core (MC) of the LHbM and the LHbMPc[68] (FIG. 2; Supplementary Table 1). Various neuropeptides (including opiates, substance P, vasopressin, somatostatin and neurotensin)[65,69] are present in the LHb and show an enriched distribution in the MC (Supplementary Table 1).

Several interesting markers are differentially expressed in the MHb versus the LHb or enriched at the boundary between the two. One good example is choline *O*-acetyltransferase (CHAT), which is highly abundant in the MHb but absent in the LHb[41] (Supplementary Table 1).

Reward prediction error
(RPE). The difference between the actual outcome of a situation or action and the expected outcome. A positive RPE indicates the outcome was better than expected, whereas a negative RPE indicates it was worse than expected.

Optogenetic
Optogentics involves the use of genetically encoded light-activated proteins (for example, light-sensitive ion channels and pumps) to control the functional parameters (for example, membrane potential and firing rate) of targeted neuronal populations.

Deep brain stimulation
A method that involves chronically implanted electrodes for stimulation of specific subcortical brain areas to treat symptoms of neurological and psychiatric diseases.

Ketamine
An inhibitor of NMDA-type glutamate receptors; it was initially discovered as an anaesthetic drug and was later found to be a rapid-acting antidepressant treatment.

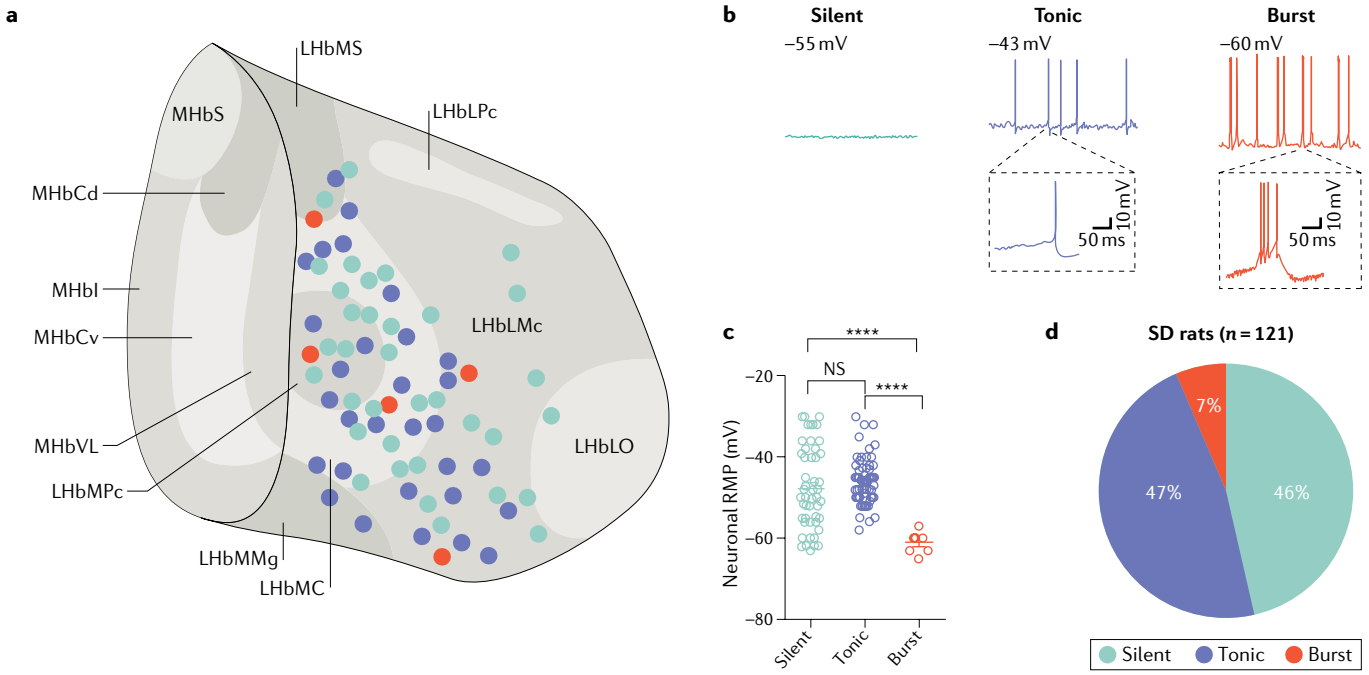


Fig. 2 | **Anatomical divisions and cellular and electrophysiological properties of the LHb. a** | Subdivisions of the rat habenula showing subnuclei within the medial habenula (MHb) and the lateral habenula (LHb). The coloured circles represent the locations of the three types of LHb neurons recorded in parts **b**–**d**. Burst firing neurons are represented by red dots, tonic firing neurons are represented by purple dots and silent neurons are represented by green dots. **b** | Representative traces for the three types of LHb neurons, recorded with whole-cell patch clamp, under unstimulated conditions. **c** | Distribution of resting membrane potentials (RMPs) of the three types of neurons in the LHb of Sprague Dawley (SD) rats. **d** | Pie charts indicating the abundances of the three types of neurons in the LHb of SD rats. LHbLMc, magnocellular part of the lateral LHb (LHbL); LHbLO, oval part of the LHbL; LHbLPc, parvocellular subnucleus of the LHbL; LHbMC, central part of the medial LHb (LHbM); LHbMMg, marginal part of the LHbM; LHbMPc, parvocellular subnucleus of the LHbM; LHbMS, superior part of the LHbM; MHbCd, central and dorsal part of the MHb; MHbCv, central and ventral part of the MHb; MHbI, inferior part of the MHb; MHbS, superior part of the MHb; MHbVL, ventral and lateral part of the MHb; NS, not significant. ****, $P < 0.0001$. Part **a** adapted from REF.[39], Springer Nature Limited, and with permission from REF.[41], Wiley-VCH. Parts **b**–**d** adapted from REF.[39], Springer Nature Limited.

This distribution is consistent with the prominent role of the MHb in acetylcholine signalling[70]. Within the medial boundary subnuclei between the MHb and the LHb, *Tac1* (encoding protachykinin 1, from which substance P is cleaved) mRNA and *Oprm1* (encoding μ-type opioid receptor (MOR1)) mRNA are enriched and partially colocalized[71,72] (Supplementary Table 1). This colocalization pattern is of particular interest given the functional interactions between the MOR1 and substance P signalling in pain and addiction[73]. The expression of two orphan G protein-coupled receptor genes, *Gpr139* (REFS[42,74]) and *Gpr151* (REFS[41,75,76]), which are highly druggable targets, is also enriched at the MHb and LHb boundary. The expression of *Gpr151* (REF.[41]) in the brain is virtually restricted to the habenula[41,42,75] (Supplementary Table 1).

Genes differentially expressed in specific cell populations provide a venue for genetic and functional dissection of habenular subregions. Owing to progress made in the Gene Expression Nervous System Atlas Project (GENSAT), a few neuronal ensembles in LHb subdivisions can now be specifically manipulated in the LHb of mouse strains using genetically engineered Cre–*lox* recombination[21]. Most recently, a series of systematic single-cell transcriptional profiling studies have provided a more thorough gene expression atlas of the habenula[62,77–79]. Several transcriptionally discriminable LHb neuronal subtypes are identified to spatially segregate into anatomical subregions[62,77] and are differentially engaged by aversive stimuli[79]. One study also detailed biomarkers that are enriched in specific LHb pathways and in the passive coping phenotype[78] (Supplementary Table 1). These studies advance our understanding of genetically defined neuronal subtypes in the LHb and open avenues for precisely targeting the LHb and its subregions.

Electrophysiological properties

If we put aside the molecular diversity, LHb neurons are physiologically quite homogenous across different subregions[39,52,80]. In comparison with pyramidal neurons in the cortex or hippocampus, LHb neurons generally have higher input resistances and more-depolarized resting membrane potentials (RMPs)[39,80]. For example, in Sprague Dawley rats, the RMPs of LHb neurons range from −60 to −40 mV, averaging −48 mV (REF.[39]) (FIG. 2b,c). Electrophysiological recordings from the LHb brain slices in vitro revealed that, in addition to silent neurons without discharge, there are two spontaneous activity patterns: the tonic firing type with regular or irregular frequencies and the bursting type displaying clusters of high-frequency spikes[39,53,80] (FIG. 2b). Under normal conditions, silent and tonic firing types each constitute 40–50% of LHb neurons, while bursting neurons account for only a small percentage of the total LHb population (less than 10%) in Sprague Dawley rats[39].

NMDA-type glutamate receptors
(NMDARs). NMDARs are one of the three types of ionotropic glutamate receptors. Calcium flux through the NMDAR is critical in synaptic plasticity, as well as burst firing in several brain regions.

Real-time place aversion
Behavioural tests in which an animal avoids a compartment that was paired with an aversive stimulus (is often mimicked by optogenetic stimulation) in a real-time manner.

Real-time place preference
Behavioural tests in which an animal approaches a compartment that was paired with a rewarding stimulus (is often mimicked by optogenetic stimulation) in a real-time manner.

Resilience
Psychologically, 'resilience' refers to the ability to maintain the original normal physiological and behavioural function in the face of severe stress.

Valences
Psychologically, 'valence' refers to the emotional value associated with a stimulus.

Characteristically, bursting neurons display more hyperpolarized RMPs (approximately −60 mV) than the other two neuronal types[39,80] (FIG. 2c,d). However, in response to membrane hyperpolarization, nearly all LHb neurons are capable of converting into the bursting type[39,80,81]. This voltage dependency is mediated by low voltage-sensitive T-type calcium channels (T-VSCCs)[39,53], which are de-inactivated by a short hyperpolarization below −55 mV and allow calcium entry to initiate bursts[39,82,83]. As T-VSCCs quickly inactivate, NMDA-type glutamate receptors (NMDARs) then take over the relay to drive membrane potential to cross the action potential threshold to sustain the bursts[39]. In addition, LHb bursting is also subject to regulation by a glia-specific potassium channel, Kir4.1, which regulates RMPs by adjusting extracellular potassium levels[38].

Bursting activities in the LHb are also detected in behaving[39,59,78] and anaesthetized[84] animals. However, unlike in LHb brain slices, in which spontaneous neuronal activities are fixed to a single pattern of activity, silent, tonic and bursting activity patterns alternate in the same neurons in vivo in behaving animals[39]. This may reflect the influence of abundant in vivo inputs, which may alter membrane potential and firing pattern. Functionally, the bursting feature of LHb neurons plays a critical role in the depression-like behaviours and the rapid antidepressant response of ketamine in mouse and rat models[39], which is discussed in detail later.

Inputs to the LHb

Through the stria medullaris fibre tract, the LHb receives afferent inputs mainly from the limbic forebrain regions and the basal ganglia, which funnel through largely parallel circuits to reach the LHbM and LHbL, respectively[21,26,48] (FIG. 3). Limbic inputs to the LHb are mostly derived from hypothalamic structures, including the lateral hypothalamic area (LHA), lateral preoptic area (LPO), paraventricular nucleus (PVN), suprachiasmatic nucleus (SCN) and central nucleus of the amygdala (CeA); basal forebrain structures, including the nucleus accumbens (NAc), diagonal band nuclei (DBN), ventral pallidum (VP), lateral septum and medial septum; and bed nucleus of the stria terminalis (BNST) and visual thalamus. Basal ganglia inputs to the LHb mostly originate from the entopeduncular nucleus (EPN; the rodent equivalent of the globus pallidus internus (GPi) in primates), which receives cortical inputs through the striatum. In addition, the LHb also receives direct cortical inputs from the medial prefrontal cortex (mPFC)[85–88] and reciprocal feedback inputs from the monoaminergic centres, including the ventral tegmental area (VTA) and raphe nuclei[6]. These various presynaptic afferents to the LHb contain glutamatergic, GABAergic and sometimes dopaminergic or serotonergic axons[89–93]. Optogenetic studies have begun to reveal the functional role of each of these LHb input pathways, as we review below.

As one of the major glutamatergic inputs to the LHb, the EPN primarily innervates the LHbL[19] and is the most promising source of the negative RPE[94]. EPN neurons that project to the habenula are inhibited by the presence of cues predicting reward, and are activated by negative RPE resulting from reward omission, and in turn bidirectionally modulate activity in the LHb[95]. An interesting feature of the EPN–LHb afferent is that GABA and glutamate are co-released from the same terminals[96–98], although it remains to be resolved whether they exist in the same[96] or different[98] synaptic vesicles. The function of such co-release is not completely clear, but the balance of glutamatergic and GABAergic transmission in the LHb has been implicated in various maladaptive states, including depression and substance abuse[19,96–98].

Another major glutamatergic input to the LHb arises from the LHA. Activation of the LHA–LHb pathway is aversive, as optogenetic stimulation or inhibition of this pathway causes real-time place aversion or real-time place preference, respectively[23,99]. This pathway negatively regulates food consumption[23] and mediates escape behaviour from aversive stimuli, including foot shock and looming visual stimulus[84]. Activity-dependent synaptic plasticity specifically at LHA–LHb excitatory synapses, but not at the medial VTA–LHb or EPN–LHb synapses, mediates the associative learning that instructs avoidance[100].

In addition to the EPN and LHA, the LPO is one of the largest regions with input to the LHb[101]. Although early studies showed that the LPO sends GABAergic inputs into the LHb[102,103], the majority (~75%) of the LHb-projecting LPO neurons are now known to be glutamatergic[104]. Aversive stimuli simultaneously activate both glutamatergic and GABAergic LHb-projecting LPO neurons. However, independent activation of either the glutamatergic or the GABAergic input to the LPO–LHb pathway drives aversion or reward, respectively[104].

Although the VP is predominantly a GABAergic nucleus[105], emerging findings suggest that the VP sends a considerable portion of glutamatergic fibres to the LHb[106–109]. In mice exposed to social defeat stress, neuronal activity is significantly enhanced in a subset of LHb-projecting, parvalbumin-expressing, glutamatergic VP neurons[106]. Silencing these neurons induces resilience to social defeat stress[106]. The glutamatergic and GABAergic VP–LHb projections oppositely encode negative and positive motivational valences and are engaged in driving punishment-avoidance[107–109] and reward-seeking[109] behaviours, respectively (but see REF.[107], in which reward-seeking behaviours were not observed). These results highlight the balance between inhibitory and excitatory VP–LHb afferents in determining the outcomes of motivational behaviour.

Vasopressin-expressing magnocellular neurons in the PVN also send direct glutamatergic inputs into the LHb, specifically to the putative GABAergic neurons located within the LHbM[60]. This group of LHbM neurons may be activated (as indicated by *Fos* expression) by water deprivation[60]. At the behavioural level, water deprivation decreases freezing following predator exposure, as well as immobility time in the forced-swim test[60]. This study provides correlative evidence that the PVN–LHbM pathway may be involved in the regulation of motivational state via thirst, although further experiments are needed to establish a causal relationship.

Other structures in the basal forebrain, including the NAc, lateral septum, medial septum and DBN, also

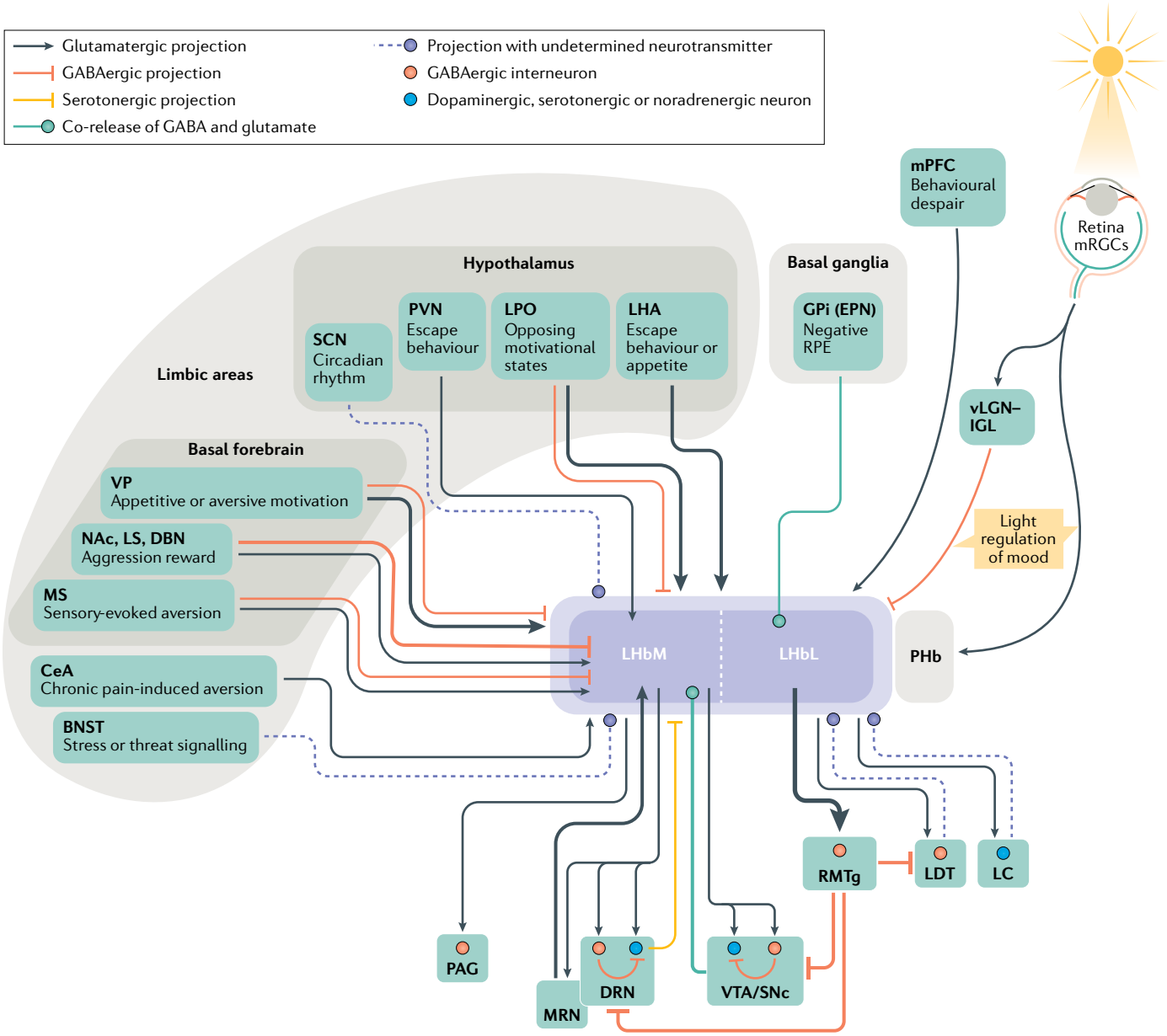


Fig. 3 | **Summary of the afferent and efferent circuitry of the LHb.** The lateral part of the lateral habenula (LHbL) preferentially receives inputs from the globus pallidus internus (GPi; in rodents) or the entopeduncular nucleus (EPN; in primates) of the basal ganglia[19,94–96]. The medial part of the lateral habenula (LHbM) preferentially receives inputs from the limbic areas, including the septum (medial septum (MS)[112] and lateral septum (LS)[22]), diagonal band nuclei (DBN)[22], nucleus accumbens (NAc)[22], paraventricular nucleus (PVN)[60], median raphe nucleus (MRN)[93] and ventral tegmental area (VTA)[134]. The two subregions of the lateral habenula (LHb) also receive inputs from the lateral hypothalamic area (LHA)[23,84,99], lateral preoptic area (LPO)[54,103,104,279], ventral pallidum (VP)[106–109], central nucleus of the amygdala (CeA)[231], bed nucleus of the stria terminalis (BNST)[101,122], suprachiasmatic nucleus (SCN)[122], medial prefrontal cortex (mPFC)[85–88] and locus coeruleus (LC)[101,276]. Melanopsin-expressing retinal ganglion cells (mRGCs) send direct inputs to the perihabenular region (PHb)[114] and indirect inputs to the LHb via the ventral lateral geniculate nucleus and intergeniculate leaflet (vLGN–IGN)[113]. On the output side, LHb neurons preferentially project to GABAergic neurons in the rostromedial tegmental nucleus (RMTg; inhibiting dopaminergic and serotonergic neurons)[7,8,17,123–125], neurons of aminergic nuclei, including dopaminergic and GABAergic neurons in the VTA and substantia nigra pars compacta (SNc)[133–135] and serotonergic and GABAergic neurons in the MRN and dorsal raphe nucleus (DRN)[50,280], and neurons in the LC[5,276], laterodorsal tegmental nucleus (LDT)[138,280] and periaqueductal grey (PAG)[144,212,280]. The thicknesses of lines and arrows indicate the strength of the input and output pathways. Lines and arrows extending into the box for the LHbM or the LHbL indicate that these pathways have confirmed subregion targeting within the LHb. Lines and arrows outside the box indicate that targeting of these pathways within the LHb has not been shown to have subregion specificity. Some inputs to the LHb (for example, the medial preoptic nucleus and the superior colliculus)[101] have not been studied functionally and are not depicted.

Anhedonia
A depression-like phenotype that refers to loss of the ability to experience pleasure from normally rewarding stimuli. In mice and rats, the anhedonia aspect of depression is classically modelled by the sucrose preference test.

provide inhibitory input to the LHb. As suggested by GAD67 expression, most LHb-projecting neurons in the septum (~75%), DBN (~80%) and medial NAc shell (100%) are GABAergic[22]. Activity of the basal forebrain–LHb pathway encodes the valence of aggressive interactions, in particular aggression reward[22]. Some glutamatergic projections are also present in the basal forebrain–LHb pathway. For example, neurons in the medial septum, which can be activated by both sound and touch[110,111], send excitatory inputs to the LHb and transform sensory information into aversive emotion, which can be antagonized by GABAergic input from the same pathway[112].

Among other LHb inputs associated with sensory information, there is a GABAergic input from a visual thalamic region, namely the thalamic ventral lateral geniculate nucleus–intergeniculate leaflet (vLGN–IGL)[113]. These vLGN–IGL neurons receive direct inputs from non-image-forming, melanopsin-expressing retinal ganglion cells (mRGCs) in the eye. This visual pathway may mediate the antidepressant effects of bright light therapy by suppressing LHb activity[113]. The mRGCs also send direct inputs into the perihabenular region (PHb), through which light may directly regulate mood[114].

The reciprocal feedback innervation to the LHb from the VTA represents another input with a co-release mechanism[89,90,98,115–117]. Most LHb-projecting VTA neurons co-express markers for both GABA and glutamate signalling, and co-release GABA and glutamate[89,98,117]. Optogenetic activation of this pathway suppresses the firing of LHb neurons in brain slices and increases the spontaneous firing of VTA dopaminergic neurons in vivo[115], suggesting the existence of a disinhibition loop. Consequently, this activation produces reward-related behavioural phenotypes, including real-time place preference and self-stimulation[115]. Despite the presence of dopamine receptors in the LHb and the tyrosine hydroxylase expression in the LHb-projecting VTA neurons, currently there is no evidence that dopamine is released from this neuronal population[90,115,116]. As local elevation of dopamine or dopamine receptor agonist levels in the LHb can enhance LHb firing[118] and decrease serotonin release in basal ganglia[119], a further exploration of the source of dopaminergic innervation to the LHb is needed[120].

The LHb is also connected in a reciprocal manner to the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN)[5]. The DRN shows a moderate serotonergic innervation to the entire LHb[121] whereas the MRN shows a strong glutamatergic innervation onto the LHbM[93]. By analysis of miniature excitatory postsynaptic currents under constant optogenetic activation of DRN–LHb terminals and paired-pulse ratio of light-evoked responses, it was demonstrated that serotonin projections from the DRN to the LHb suppress its excitability through a presynaptic mechanism[92]. However, bath application of serotonin inhibits glutamatergic transmission in the most LHbL neurons but enhances glutamatergic transmission in most LHbM neurons, probably owing to the heterogeneous expression of serotoninergic receptor subtypes[91]. Optogenetic activation or inhibition of the DRN–LHb pathway alleviates or induces depressive-like behaviours, respectively[92]. Intriguingly, a recent study showed that projections from the MRN to the LHb are mainly glutamatergic, expressing VGLUT2, and trigger bursting activity in LHb neurons when they are optogenetically stimulated in brain slices[93]. Instant photostimulation and long-term chemogenetic activation of this pathway lead to real-time place aversion and anhedonia, respectively[93].

Other than the aforementioned inputs, the LHb also receives innervations from the medial preoptic nucleus, the superior colliculus and the laterodorsal tegmental nucleus (LDT)[101]. However, the functional roles of these pathways have not been characterized. A major species difference in LHb inputs lies in the GPi–EPN input: there are fewer projections to the LHb from this input in monkeys and cats than in rats[122].

Overall, more than a dozen input pathways to the LHb have been identified. Strikingly, activation of many of these pathways leads to similar aversive responses, as revealed by the real-time place aversion assay. Does this reflect the inherent property of the LHb as a hub that integrates different aversive inputs, or is it partly due to the artificial patterns used in optogenetic stimulation? Future studies should aim to identify specific external stimuli that selectively activate these pathways, as well as the physiological firing pattern (for example, firing rate, tonic or bursting) of each pathway on activation. These basic details are urgently needed to guide future optogenetic studies that aim to faithfully connect pathway activities to behavioural consequences.

Outputs from the LHb

Whereas upstream projections into the LHb are diverse in origin, its downstream output is relatively conforming. Through the fasciculus retroflexus, also known as the habenula–interpeduncular tract, efferents from the LHbL send a major projection to the GABAergic RMTg (also known as the tail VTA). The RMTg, in turn, projects to several aminergic neurons[7,8,17,123–125] (FIG. 3), preferentially dopaminergic neurons in the VTA and substantia nigra pars compacta (SNc)[126], to suppress their activity.

The RMTg has a considerable impact on the VTA dopaminergic neurons: stimulation of the RMTg dramatically suppresses the firing of VTA dopaminergic neurons[127,128], whereas inactivation of the RMTg enhances the activity of such neurons[128]. Exposure to aversive stimuli increases the activity of RMTg neurons[7], as well as the excitatory drive from the LHb to RMTg neurons[20]. Functionally, activation of the LHb–RMTg pathway promotes active, passive and conditioned behavioural avoidance[20,78,129,130], reduces effortful behaviours[131] and facilitates the onset of depressive-like behaviour induced by a learned helplessness paradigm[132].

Besides the disynaptic connection through the RMTg, the LHb also sends direct glutamatergic projections to both GABAergic and dopaminergic neurons in the VTA, albeit they are much sparser than the LHb's RMTg projections[49,133–135]. Direct optogenetic activation of the LHb–VTA pathway produces conditioned place avoidance[78,129] and increases despair-like behaviours[78]. However, given that the direct projections are sparse, and that the terminal regions in these two studies also

include the RMTg, which is located at the caudal end of the VTA, it is likely that the LHb–VTA terminal stimulation also activates the RMTg, which may account for the behavioural effects.

The DRN also receives a strong direct innervation from the RMTg[136]. Optogenetic activation of the RMTg–DRN terminals elicits monosynaptic inhibitory responses and dramatically reduces the excitability of DRN neurons in slice recordings[92]. In addition to this main route via the RMTg, the LHbM sends direct glutamatergic projections to both raphe serotonergic neurons and GABAergic neurons, which may, in turn, exert feedforward inhibition onto serotonergic neurons in the raphe[50,136]. In mice, in vivo electrical stimulation of the LHb markedly suppresses neuronal activity in both DRN and MRN neurons[9,10], but whether these raphe neurons are serotonergic remains unknown. In zebrafish, the effect of habenular input to the raphe nuclei is less clear. Using electrophysiological recordings, one study showed that stimulation of the vHb can directly excite putative serotonergic neurons in the MRN[137]. Using whole-brain imaging, another zebrafish study showed a mixture of inhibitory and excitatory (although a net inhibitory) effects from the vHb to the serotonergic neurons of the entire raphe nuclei[29]. Further research, including in vivo recording of tagged serotonergic neurons in response to LHb stimulation, and pathway-specific optogenetic manipulation of the LHb–MRN and LHb–DRN pathways in both rodents and fish, is needed to help clarify the nature of the regulation and function of this pathway.

Other efferent targets of the LHb include the noradrenergic locus coeruleus[5], the cholinergic LDT[138], several thalamic nuclei (including the centromedial, mediodorsal, ventromedial and parafascicular nuclei), the superior colliculus and the dorsal tegmental region[5]. These efferent LHb targets are comparatively much less well characterized than those discussed above. In one functional study, optogenetic activation of the LHb glutamatergic terminals targeting the LDT GABAergic interneurons induced fear-like behaviour in mice, mimicking the effect of predator odour[138].

Similarly as for input pathways, functional studies of the LHb output pathways will also benefit from identifying the physiologically relevant pattern of firing. Although LHb regulation of the dopaminergic system is extensively studied, how the LHb regulates other monoaminergic systems, especially the serotonergic system, remains relatively unclear and requires further investigation.

Physiological functions of the LHb

As a critical node that interconnects forebrain and midbrain aminergic nuclei, and as one of the few brain regions that control both the dopaminergic system and the serotonergic system, the LHb is strategically positioned to integrate the brain's motivational, movement and memory systems. It regulates various essential physiological functions, including reward and aversion[15,21], motor output[15,139], cognitive functions[140,141], sleep and circadian rhythms[142,143], pain[144] and navigation and maternal behaviours[15]. Here, we summarize the role of the LHb in these functions, with particular focus on its critical role in reward and aversion.

Reward behaviour

The ability to seek rewards and avoid punishments is crucial for animals' survival and well-being. Actions associated with reward are facilitated (approach or go) when the actual reward exceeds the predicted one, often referred to as positive RPE. Conversely, actions associated with reward are suppressed (avoidance or no-go) when the actual reward is smaller than that predicted (negative RPE). Thus, RPE is a measure of the discrepancy between reward prediction and outcome, and serves as a powerful learning signal to guide approach or avoidance (go or no-go) behaviour[145].

Consistent with their role in inhibiting dopaminergic neurons, which are prominent for RPE coding[145], LHb neurons encode RPE in the opposite direction to that of dopaminergic neurons: that is, they are activated by reward omission or punishment (negative RPE) and are inhibited by unexpected reward enhancement (positive RPE)[13,16,146]. These observations, initially made in monkeys, were later confirmed in human functional imaging studies, which showed that habenular activities were potentiated in response to either an absence of expected positive feedback[147] or an unexpected negative feedback[148].

Studies in rhesus monkeys showed that negative RPE signals in the LHb are inverted and transmitted by the GABAergic RMTg relay nucleus or local interneurons within the VTA and SNc[13,17] before reaching the dopaminergic neurons, which then send efferents to the striatum to modulate go (via D1 receptors) and no-go (via D2 receptor) behaviours[149]. Of note, however, the LHb may not be an exclusive source of dopaminergic RPE signals. LHb lesion in mice abolishes the negative RPE of VTA dopaminergic neurons caused specifically by reward omission but leaves intact the negative RPE caused by aversive stimuli[150].

The complexity of this system has further increased in the years since the identification of heterogeneity in the roles of dopaminergic neurons in valence coding. According to their cell body locations and input–output pathways, at least two dopaminergic populations — reward-activated and aversion-activated dopaminergic neurons — are segregated in the VTA and SNc[129,151,152]. Besides inhibiting reward-coding dopaminergic neurons[151], the LHb may activate aversion-coding dopaminergic neurons that are located in the medial VTA and project to the mPFC[129]. In addition to reward value, the LHb neurons also encode reward probability and reward magnitude[16,153], which may facilitate sophisticated decision-making to distinguish magnitude and cost differences between different rewards[154].

The role of the LHb in reward processing goes beyond its connection with the dopaminergic system. Unlike the dopaminergic neurons, which signal value changes (that is, RPE), serotonergic neurons have been shown to reflect reward-associated value state; these neurons show changes in their tonic activity after the reward value is updated[153,155,156]. An intriguing hypothesis

proposed that the value change encoded by dopaminergic neurons may relate to motivation or wanting[157], whereas the value state encoded by serotonergic neurons may relate to mood or liking[158]. Thus, through its connections with both the dopaminergic system and the serotonergic system, the LHb may integrate information on both value state and value change, making it an ideal node to control reward-based decision-making and mood[21].

Aversive behaviour

A primary function of the LHb, strongly related to its role in processing negative reward information, is to encode aversive state[15]. In mice and rats various stressors and negative emotional stimuli — such as inescapable foot or tail shock, physical restraint, lithium chloride-induced illness, maternal deprivation and social defeat stress — induce immediate neural activation (indicated by *Fos* expression[159,160] or calcium activity[161]) in the LHb, especially in the LHbM[159]. Electrophysiological recordings in brain slices or in mice in vivo found that spontaneous firing of LHb neurons is increased by maternal deprivation, foot shock or looming visual stimulus[84,162]. In vivo electrophysiological recording in monkeys also showed that unexpected punishment signals, such as an air puff in the eye or cues predicting such punishment, in addition to the omission of an expected reward (so-called disappointment), strongly excite LHb neurons[16].

In addition to directly affecting LHb neuron activity, stress also alters the plasticity of LHb neurons. Stress greatly facilitates long-term potentiation induction in the LHb[163] and impairs endocannabinoid-dependent long-term depression[164]. Such alterations may contribute to long-term changes in LHb activity under chronic stress, leading to depressive-like behaviours.

A causal relationship between LHb activity and aversive state has been established by a series of manipulation studies. Local electrical stimulation or disinhibition with focal microinjections of the $GABA_A$ receptor antagonist bicuculline in the LHb elicits autonomic physiological responses similar to those associated with emotional stress in rats[165]. Moreover, as described earlier, an aversive state can be induced by optogenetic stimulation of the EPN, LHA, VP, medial septum, LPO or VTA excitatory afferents to the LHb[19,23,99,104,107,108,112,117,166], or efferents from the LHb to the RMTg or VTA[20,129], or the LHb itself (using a burst-triggering protocol)[39]. Reciprocally, in rats, inhibition of LHb activity using the designer receptors exclusively activated by designer drugs (DREADD) system[167,168] or local infusion of drugs such as ketamine[39] leads to an antidepressive-like[39,167,168] or antianxiety-like[169] state. In zebrafish, in vivo optogenetic activation of vHb neurons promotes passive coping behaviours, whereas inhibition of these neurons prevents behavioural passivity induced by inescapable shocks[29]. Together, these observations build a compelling case for the function of the LHb in coding stress and aversion.

Although they are not studied as extensively, there is much evidence showing functions of the LHb beyond reward and aversion, which we describe next.

Motor suppression

In response to stress or negative RPE, animals often show suppressed motor activity, which provides self-protection, saves energy and minimizes further exposure to stress[170]. Owing to its role in processing upstream negative RPE and aversive stimuli, as well as its inhibition of the downstream dopaminergic and serotonergic systems, which are strong modulators of motor activity, the LHb is proposed to be a convenient node in motor suppression[15,139]. Indeed, habenula lesions make animals hyperactive, distractible and produce premature motor responses in behavioural tasks[171,172]. However, although optogenetic activation of LHb neurons using a rebound burst protocol (discussed later) reduces swimming and struggling in the forced-swim test, it does not reduce general locomotion in the open field test[39], suggesting that motor control by the LHb may not be generalized but rather may be specifically linked to motivation-related activities.

Memory

The LHb has been implicated in both working memory[140,173] and long-term spatial memory processes[174–176]. Anatomically, the LHb is both directly and indirectly connected to brain areas implicated in memory, including the mPFC, hippocampus, medial septum and basal ganglia[1,26,139]. Neurochemically, the neurotransmitter systems regulated by the LHb, such as the dopaminergic system and the serotonergic system, also critically regulate memory processing[177].

The causal role of the LHb in distinct phases of spatial memory processes was best illustrated in a pharmacological inactivation experiment using the $GABA_A$ receptor agonist muscimol or the AMPA-type glutamate receptor (AMPAR) antagonist cyanquixaline at different stages of the Morris water maze task[178]. Specifically, infusion of these drugs impaired memory performance when they were given before each training session or before the retrieval test but did not impair memory performance when they were given after training. This observation suggests that the LHb is involved in 'online' information processing during memory encoding and retrieval but not in 'offline' processes such as memory consolidation. A similar bilateral inactivation strategy also confirmed the role of the LHb in working memory tasks[140]. Furthermore, disruption of the prelimbic mPFC–LHb connection caused similar working memory deficits[140], suggesting that the LHb relays top-down information from the mPFC, a structure heavily implicated in working memory[179]. Such capacity of the LHb to integrate online information is proposed to be useful in evaluating ongoing situations to control behavioural flexibility[140] (discussed next).

Behavioural flexibility

To cope with their dynamic environments, animals must assess whether ongoing behaviour is appropriate on the basis of information about internal states and external cues, and decide whether they should switch to an alternative behaviour[141]. There is evidence that such behavioural flexibility requires LHb function[141,174,180,181]. The activity of some LHb neurons can track choice

outcome information[146]. In both appetite-motivated and aversion-motivated tasks, lesion or inactivation of the LHb causes deficits in reversing learned behaviours[154,174,180,182]. Specifically, these LHb-lesioned animals can learn an initial strategy but are unable to abandon or update it to an alternative one when needed. Indeed, if we deem the interpretation of prediction errors as the key element for reversal leaning, it seems logical that LHb-mediated monoamine suppression plays a critical role to enforce the behaviour flexibility. At the network level, communication between the mPFC, hippocampus and LHb, possibly mediated by theta co-modulation, is proposed to be essential for behavioural flexibility[141].

Sleep, circadian cycles and anaesthesia

Data from a series of lesion studies have suggested that the habenula, like its co-evolved brain structure, the pineal gland, has a role in the control of the normal sleep–wake cycle, especially of rapid eye movement (REM) sleep[183–186]. Removal of the habenula output (that is, fasciculus retroflexus fibres) in rats reduces both the amount of time spent in REM sleep and its associated atonia[187,188] and induces fragmentation of non-REM sleep[189]. Lesion of the LHb causes similar effects[186,190], as well as a decrease in sleep rebound time after sleep deprivation[186] and shortened duration of hippocampal theta oscillations[190].

The neural mechanism underlying the control of the sleep–wake cycle by the LHb may involve intrinsic and extrinsic aspects. First, the LHb is suggested to be a component of the brain's extended circadian time-keeping system[142,143]. The LHb has its own internal clock and shows a higher average firing rate during the light phase than during the dark phase[183,191]. Even in isolated brain slices that lack feedback from the master circadian pacemaker in the SCN, or inputs from sensory and behavioural states, the LHb is still capable of sustaining daily rhythmic firing in vitro[183]. This regular activity is potentially driven by its cyclic expression of endogenous clock genes[192,193]. Second, the LHb and PHb may be entrained by photic information received directly or indirectly from mRGCs[113,114,194] or circadian signals from the SCN[114]. Finally, dopaminergic and serotonergic neurons[190,195,196], and descending circuits to the brainstem[197,198], may act downstream of the LHb to regulate sleep.

Several psychiatric disorders that have been associated with LHb dysfunction show circadian dependence as well as disrupted circadian rhythm and sleep disturbance[199]. For example, the severity of depression or drug withdrawal symptoms, or the pattern of drug self-administration, can vary with the time of day[199]. Gene expression rhythms are phase-shifted and dysynchronized from external time in patients with depression[200]. Reciprocally, in mice and rats, misalignment of the day–night cycle with dark and light phases induces a depressive-like state[114,201]. Of particular interest, increased REM sleep, which is subject to negative regulation by LHb activity[186,188,190], is a signature of major depression[202]; indeed, REM sleep deprivation can rapidly alleviate depression[203]. The detailed mechanisms underpinning these reciprocal interactions between sleep, LHb circadian cycling and psychiatric conditions remain an interesting area of exploration. Knowledge gained from such research could be translated to improved pharmacological or physical therapies for psychiatric disorders.

Multiple anaesthetic drugs metabolically activate sleep-promoting brain regions and suppress arousal-promoting areas[204], indicating that similar neural circuit mechanisms underlie sleep and anaesthesia[204,205], although these two processes are not identical[206]. Hypnotic doses of anaesthetic drugs (including ethanol, barbiturates, chloral hydrate, ketamine and propofol) have been found to induce *Fos* expression in LHb neurons[189,207,208]. Silencing of LHb using tetanus toxin light chain increased resistance to propofol-induced anaesthesia; by contrast, DREADD-based activation of the LHb facilitates the anaesthetized state[189]. This set of data suggests that the LHb is involved in propofol-induced sedation and unconsciousness. It remains to be tested whether the LHb causally contributes to the action of other anaesthetic drugs.

Pain and analgesia

Some evidence points to a potential role of the LHb in the processing of pain and analgesic signals in animals[144]. Among the known afferents thought to be involved in pain transmission, the LHb receives direct and indirect pain inputs via the LHA[209] or the NAc[210] from the spinal cord[211]. The LHb also sends efferents to canonical pain modulatory regions such as the periaqueductal grey (PAG) and DRN[144,212]. In vivo electrophysiological recordings in anaesthetized rats have shown that LHb neurons respond to peripheral noxious stimuli, mostly with excitatory responses[213]. This pain-induced increase in LHb neural activity can be prevented by systemic injection of morphine[213]. Of interest to morphine-mediated analgesia, expression of *Oprm1* in the brain is highest at the boundary between the MHb and the LHb[214]. Local infusion of morphine directly into the habenula produces analgesia[215,216], raising the possibility that opioid signalling in the habenula contributes to pain relief. However, this morphine infusion and other lesioning studies[217,218] involved the whole habenula, and thus the role of the LHb remains to be pinpointed.

Summary of physiological functions

Taken together, as striking as it is at first glance that so many different physiological functions are embedded in such a tiny brain region, it is rational considering that many of these behaviours are under the governance of monoamines. It is conceivable that more monoamine-controlled behavioural types will be assigned to this region in the future. To this end, on the one hand, we may appreciate why reward and aversion, learning and memory, and behaviour flexibility are all evolutionarily intertwined, and the LHb might be the conserved region to integrate multifaceted upstream inputs to a single type of downstream output, with profound impact on various behaviours. On the other hand, given the multiple anatomically and molecularly defined subregions within the LHb, it is likely that these various functions may be mediated by different LHb subregions or pathways. Facilitated by updated knowledge

Burst firing
Burst firing, or bursting, is an activity pattern of neurons involving clusters of rapid action potential spiking.

from gene profiling and more precise circuit dissection tools, one future goal is to unravel whether LHb neurons that share specific molecular characteristics or circuit connectivity may specifically mediate different physiological functions.

Pathophysiology and dysfunction of the LHb

An increasing body of literature in the past decade suggests that malfunction of the LHb, as the 'antireward' centre, may be causally involved in multiple psychiatric disorders associated with dysregulated reward circuitry, including mood disorders, schizophrenia and substance use disorder (reviewed in REFS[21,24,26,219,220]). The strongest evidence in support of a contribution of the LHb to a psychiatric disorder and the best-illustrated mechanism is perhaps in major depression.

Major depressive disorder

The monoamine hypothesis of depression, proposed more than 50 years ago, states that the underlying basis of depression is a deficiency in brain monoamines[221]. This view has been updated by the modern neural plasticity hypothesis, which suggests that depression results from maladaptive molecular and cellular changes in specific brain regions and/or circuits[222]. Consistent with its activation by aversive emotional stimuli or negative RPE[13,129,150] and its role in suppressing monoaminergic nuclei, the LHb has been strongly implicated in the pathophysiology of major depression[21,26,40]. A study showed that in patients with depression, the activity of the habenula is elevated and shows the most significant covariation with the depression rating across the whole brain[30]. In whole-brain screening for metabolic changes in three rat models of depression, the LHb is the only brain region that shows consistent hyperactivity when the rats are in a depressive-like state[27] (FIG. 4a). More recently, a Ca^{2+} imaging-based whole-brain screening in zebrafish revealed, again, that the only brain region that becomes hyperactive in a passive coping state is the vHb, the fish equivalent of the LHb[29] (FIG. 4b). Furthermore, manipulations enhancing or suppressing LHb activity bidirectionally lead to depressive-like or antidepressant effects, respectively, in mice and rats[36–39]. These observations across multiple species emphasize the evolutionally conserved and uniquely important role of LHb hyperactivity in the origin and manifestation of depression-like behaviours. In the following sections, we review advances in understanding the molecular and cellular mechanisms underlying LHb hyperactivity in depressive-like behaviours, with emphasis on the role of LHb burst firing (FIG. 5a).

LHb bursting in depression and antidepressant treatment. As mentioned earlier, LHb neurons can be divided into three types on the basis of spontaneous activity patterns in ex vivo brain slice recordings: silent, tonic

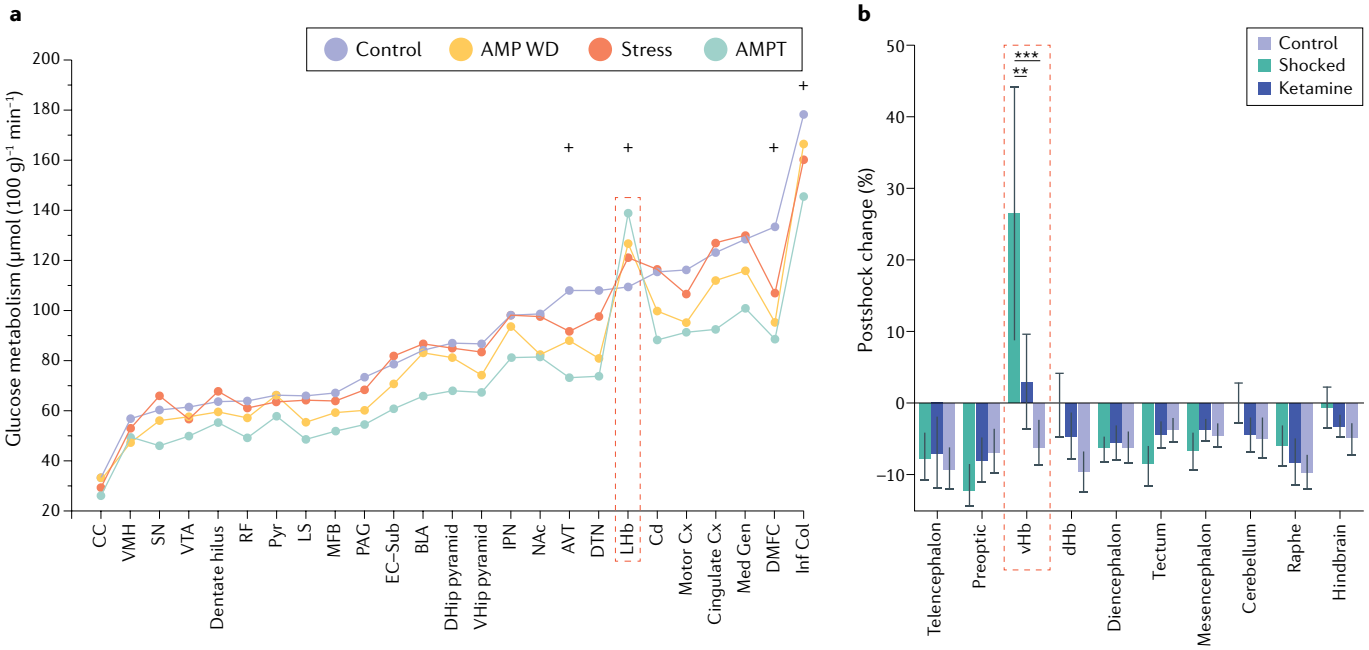
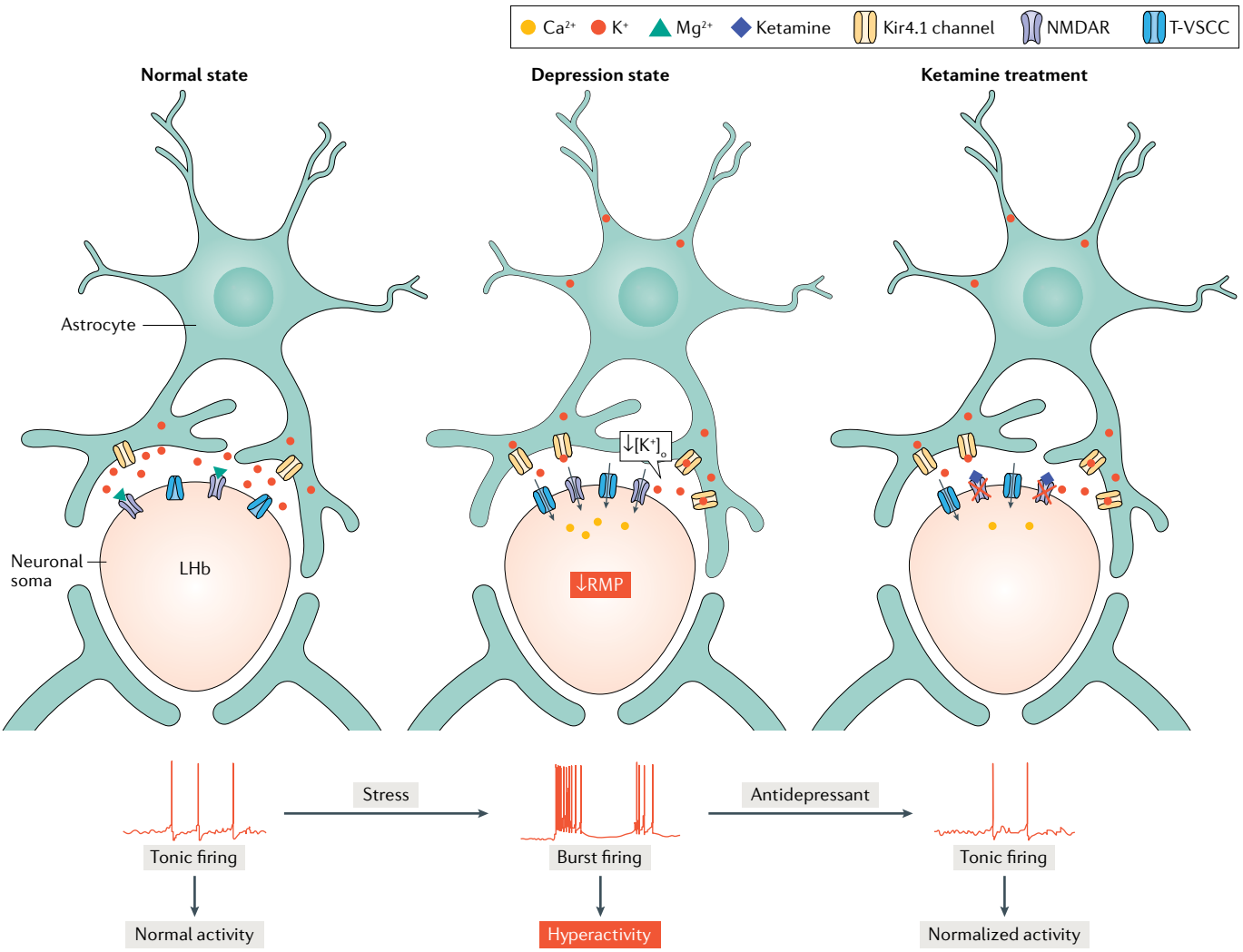
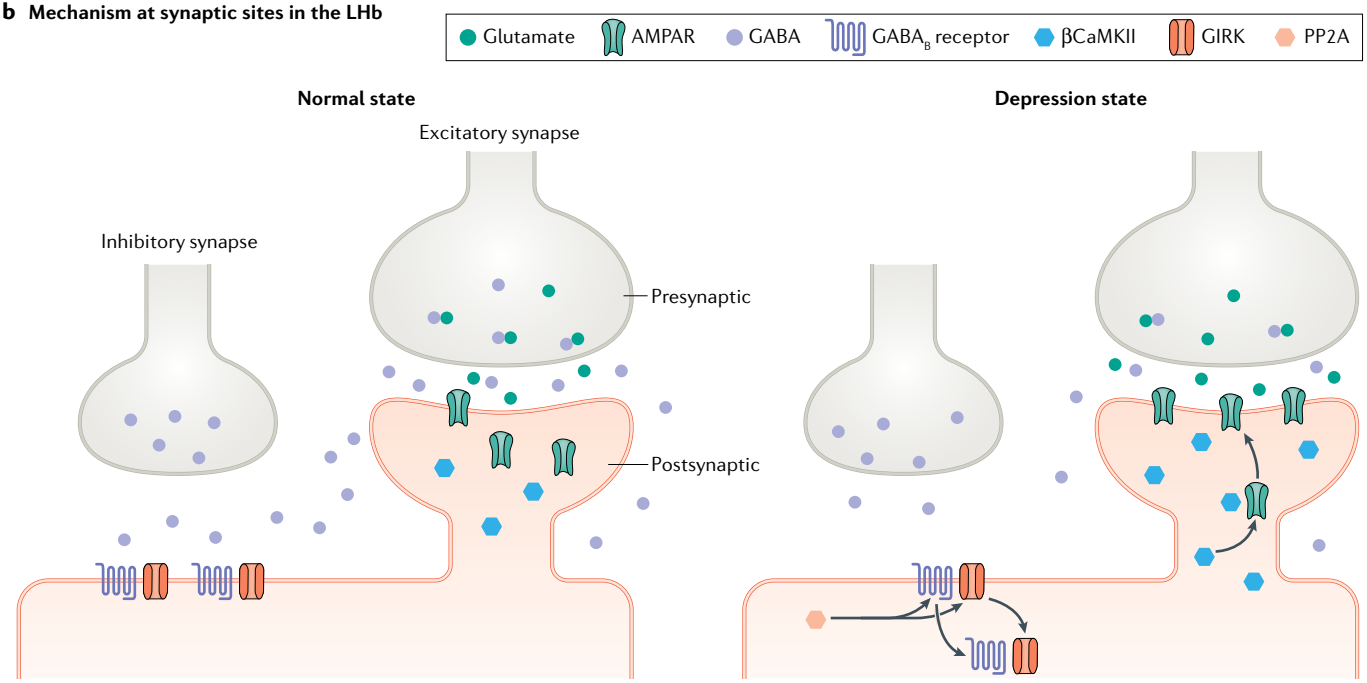


Fig. 4 | **The LHb is the only brain region showing consistent hyperactivity in multiple animal models of depression. a** | Glucose metabolic rates for 25 brain regions in three rat models of depression (amphetamine base withdrawal-induced depression rats (AMP WD), α-methyl-*para*-tyrosine-induced depression rats (AMPT) and chronic stress-induced depression rats) and controls. The lateral habenula (LHb) is the only brain region that shows consistent hyperactivity in a depression-like state. **b** | Whole-brain calcium imaging showing the ventral habenula (vHb) as the only region with hyperactivity in repeatedly shocked zebrafish. AVT, anterior ventral nucleus of the thalamus; BLA, basolateral amygdala; CC, corpus callosum; Cd, caudate; Cx, cortex; dHb, dorsal habenula; DHip pyramid, pyramidal cell layer of the dorsal hippocampus; DMFC, dorsal medial prefrontal cortex; DTN, dorsal tegmental nucleus; EC-Sub, entorhinal and subicular cortex; Inf Col, inferior colliculus; IPN, interpeduncular nucleus; LS, lateral septum; Med Gen, medial geniculate; MFB, medial forebrain bundle; NAc, nucleus accumbens; PAG, periaqueductal grey; Pyr, pyriform cortex; RF, reticular formation; SN, substantia nigra; VHip pyramid, pyramidal cell layer of the ventral hippocampus; VMH, ventral medial hypothalamus; VTA, ventral tegmental area. +, significant differences in all three models from controls; **, $P < 0.01$; ***, $P < 0.001$. Part **a** adapted with permission from REF.[281], SFN. Part **b** adapted with permission from REF.[29], Elsevier.

a **Mechanism at neuron–glia interface at soma in the LHb**



b **Mechanism at synaptic sites in the LHb**



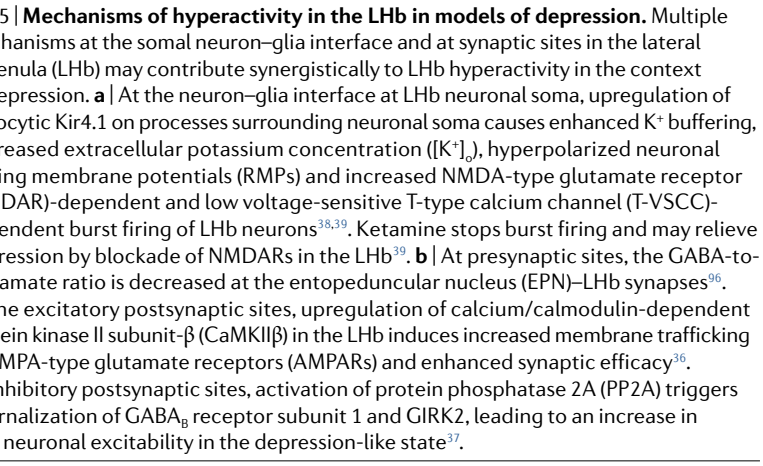


Fig. 5 | **Mechanisms of hyperactivity in the LHb in models of depression.** Multiple mechanisms at the somal neuron–glia interface and at synaptic sites in the lateral habenula (LHb) may contribute synergistically to LHb hyperactivity in the context of depression. **a** | At the neuron–glia interface at LHb neuronal soma, upregulation of astrocytic Kir4.1 on processes surrounding neuronal soma causes enhanced K^+ buffering, decreased extracellular potassium concentration ($[K^+]_o$), hyperpolarized neuronal resting membrane potentials (RMPs) and increased NMDA-type glutamate receptor (NMDAR)-dependent and low voltage-sensitive T-type calcium channel (T-VSCC)-dependent burst firing of LHb neurons[38,39]. Ketamine stops burst firing and may relieve depression by blockade of NMDARs in the LHb[39]. **b** | At presynaptic sites, the GABA-to-glutamate ratio is decreased at the entopeduncular nucleus (EPN)–LHb synapses[96]. At the excitatory postsynaptic sites, upregulation of calcium/calmodulin-dependent protein kinase II subunit-β (CaMKIIβ) in the LHb induces increased membrane trafficking of AMPA-type glutamate receptors (AMPARs) and enhanced synaptic efficacy[36]. At inhibitory postsynaptic sites, activation of protein phosphatase 2A (PP2A) triggers internalization of $GABA_B$ receptor subunit 1 and GIRK2, leading to an increase in LHb neuronal excitability in the depression-like state[37].

firing and burst firing types[38,39,55,81]. The burst firing type constitutes a small percentage of the LHb neuronal population in mice or rats not showing depressive-like behaviour[38,39,55,81], but its proportion is more than doubled or even tripled in several rodent models of depression, including congenitally learned helplessness (cLH) rats[39], chronically stressed mice[39,223] and hemiparkinsonian rats with depressive-like phenotypes[224]. Similar increases in LHb burst firing have since been confirmed in additional mouse and rat models of depression, including maternal deprivation[162], social defeat[113,225] and chronic mild stress[78,113].

Mechanistically, burst firing can potentially intensify LHb output by decreasing synaptic failure and synchronizing the neural network, and thus strengthen the output to downstream targets[226]. Therefore, enhanced bursting in the LHb may cause a stronger suppression of the downstream aminergic reward centres, leading to depression[40,227]. Indeed, an optogenetic rebound bursting protocol used to drive burst firing specifically in the LHb induced depressive-like behaviours in naive C57/B6 mice, including behavioural despair and anhedonia[39], suggesting a causal relationship between LHb burst firing and the induction of depression. Regarding whether tonic firing in the LHb is increased or unchanged in animal models of depression, there are different opinions, which may be caused by differences in how burst firing is defined in vivo[39,78].

Bursting of LHb neurons was found to critically depend on NMDARs[39], which are the major molecular target of the rapid-acting antidepressant ketamine[228]. This raised the interesting possibility that blockade of LHb bursts might underlie the antidepressant effects of ketamine. Indeed, in LHb brain slices, perfusion of either ketamine (at a treatment-relevant dosage) or the more specific NMDAR blocker AP5 (2-amino-5-phosphonopentanoic acid) instantly blocks burst firing[39]. Consistently, intraperitoneal injection of ketamine attenuates the increased LHb burst firing observed in mice or rats with depressive-like symptoms[39,162]. Importantly, local infusion of ketamine or AP5 into the LHb rapidly alleviates depressive-like symptoms in cLH rats within 1 h of treatment[39], recapitulating the effects of ketamine whole-body administration. Suppression of overall LHb neuronal activity by ketamine has also been demonstrated in zebrafish, in which systemic application of a single dose of ketamine reduces behavioural passivity and attenuates a shock-induced increase in vHb activity[29].

Along with NMDARs, burst firing of LHb neurons also depends on the low voltage-sensitive T-VSCCs and a relatively hyperpolarized membrane potential[39]. In line with this, two T-VSCC blockers, ethosuximide and mibefradil, cause rapid-acting antidepressant responses within 1 h in mice and rats similar to those of ketamine[39]. Such antidepressant effects were not observed when ethosuximide was administered in mice 4 or 24 h before the behavioural tests[229]. Possible reasons for this discrepancy (for example, ethosuximide's plasma half-life is 1 h in mice) were discussed in REF.[40]. Moreover, a molecular regulator of LHb neuron RMPs — the astroglial potassium channel Kir4.1 — bidirectionally regulates LHb neuron burst firing and depressive-like behaviours (discussed in more detail later). Collectively, these data provide a new molecular, cellular and circuit-based model for the cause of depression, in addition to unveiling a potential mechanism underlying the rapid-acting antidepressant effects of ketamine[40,227].

Glial regulation of LHb neural activity. Whereas LHb bursts can be regulated by a neuron's intrinsic ionic properties or synaptic inputs[39], our recent work has also revealed a critical role of glial–neuron interaction in regulating the RMPs and bursting activity of LHb neurons in depressive-like behaviours in mice and rats[38] (FIG. 5a).

An unbiased proteomic screen showed that the glial potassium channel Kir4.1 is upregulated in the LHb of cLH rats[36,38]. Intriguingly, immunohistochemistry and electron microscopy experiments revealed that Kir4.1 is present at astrocytic processes that tightly wrap around the soma of LHb neurons[38]. We predicted that within this highly confined extracellular space, the potassium released from actively spiking neurons is cleared by Kir4.1. Consequently, upregulation of Kir4.1 might result in reduced extracellular potassium levels and hyperpolarized neuronal RMPs[38]. Indeed, overexpression of Kir4.1 in LHb astrocytes resulted in neuronal hyperpolarization and increased burst firing, and precipitated depressive-like behaviours in mice. Conversely, knockdown of Kir4.1 expression or suppression of its function in the LHb of cLH rats caused neuronal depolarization, eliminated burst firing and attenuated depressive-like behaviours[38].

Another astrocytic malfunction implicated in the LHb in mouse and rat models of depression is the disturbance of glial glutamatergic transmission. Habenula-specific inhibition of the glial glutamate transporter GLT1 increases the neuronal firing rate in the mouse LHb and induces depressive-like phenotypes[185]. Moreover, overexpression of GLT1 in the LHb alleviates depressive-like behaviours in a rat model of ethanol withdrawal[230]. Although dysfunction of GLT1 in animal models of depression remains to be shown, current loss-of-function and gain-of-function data suggest that modulating GLT1 expression or function can regulate depressive-like behaviours in such models.

Despair
A depression-like phenotype that reflects the feeling that nothing will improve. In mice and rats, the despair aspect of depression is classically modelled by several behavioural paradigms, including the forced-swim test, the tail suspension test and the learned helplessness test.

Synaptic changes in the LHb in depression. Apart from changes in intrinsic properties as described earlier, synaptic mechanisms including both presynaptic and postsynaptic changes have also been described to contribute to the enhanced output of the LHb in mouse and rat models of depression.

On the presynaptic side, although various presynaptic inputs responsible for transforming sensory signals (such as light[113,114], sound[112], touch[112] and pain[231]) to the LHb have been identified, how these inputs may be altered during depression is only beginning to be revealed (FIG. 5b). The VTA-projecting LHb neurons of cLH rats show decreased paired-pulse ratio of glutamatergic responses, suggesting an increased probability of presynaptic release[35]. Consistently, stress-induced increase of release probability has also been reported in the EPN–LHb pathway, which provides a major input to the VTA-projecting LHb neurons[78]. The balance between co-released GABA and glutamate at the synapses from upstream locations (for example, the EPN[96,97,232] and VTA[98]) to the LHb neurons is another presynaptic mechanism subject to modulation by depressive state[21,25,26]. This is exemplified in cLH rats, in which the ratio of EPN–LHb GABAergic to glutamatergic neurotransmission decreases, which is normalized by treatment with selective serotonin reuptake inhibitor-type antidepressants[96].

On the postsynaptic side, two major putative molecular mechanisms in LHb neurons have been discovered: increases in activity of calcium/calmodulin-dependent protein kinase II subunit-β (CaMKIIβ)[36,233] and of protein phosphatase 2A (PP2A)[37] (FIG. 5b). At excitatory synapses, CaMKIIβ regulates the synaptic trafficking of AMPARs[234]. The proteomic screen that identified upregulation of Kir4.1 in the LHb of cLH rats also found that CaMKIIβ is upregulated[36]. Viral-mediated overexpression of CaMKIIβ within the LHb enhances the synaptic efficacy of LHb neurons by inserting AMPARs into synapses, leading to increased spike output and, consequently, behaviours that resemble aspects of human depression. Conversely, downregulation of CaMKIIβ levels or blocking of its activity in the LHb is sufficient to reverse these behaviours[36]. Similar changes in CaMKIIβ were also observed in another animal model of depressive-like symptoms induced by alcohol withdrawal[233]. At GABAergic synapses, PP2A regulates $GABA_B$ receptor signalling[37]. Repeated stress enhances PP2A activity in the LHb, subsequently reducing LHb $GABA_B$ receptor-activated inhibitory currents, leading to increased neuronal excitability and depressive-like behaviours[37].

In summary, multiple mechanisms operating at the neuronal soma, surrounding glia (FIG. 5a) and synaptic sites (including both presynaptic and postsynaptic compartments, and excitatory and inhibitory synapses) (FIG. 5b) can act synergistically to regulate the intrinsic and synaptic properties of LHb neurons to enhance LHb output, leading to depressive-like behaviours. Understanding the molecular underpinnings of these mechanisms might shed light on new treatment strategies. Moreover, whether different mechanisms or different parts of the LHb circuitry are selectively recruited by different types of stress or differentially contribute to distinct behavioural phenotypes of depression[78] are interesting questions to explore.

Substance use disorders

A hallmark of addiction is the compulsion to seek drugs during withdrawal, which is a negative motivational state that has a key role in driving drug relapse[235–237]. The drug withdrawal state is characterized by symptoms often seen in major depressive disorder, such as dysphoria (an intense state of distress), anhedonia and enhanced stress sensitivity[238,239]. Indeed, on the basis of the opposing affective states during drug intake and withdrawal, the theory of an opponent process[240] and the concept of an antireward system were first proposed[241]. The LHb has been hypothesized to be a critical component of the antireward system engaged in the development of drug addiction[242]. This hypothesis is supported by the findings that the LHb is involved in animal models of various substance use disorders and especially in the drug withdrawal state[24,54,243].

The best-studied drug for its effects on the LHb is cocaine[97,232,243–245]. Cocaine injection causes an initial decrease followed by a persistent increase in LHb activity in rats[244]. The time course of this decrease and increase in activity parallels with that of cocaine-induced initial rewarding and later aversive states, respectively[242]. In particular, the increase in LHb firing rate persists for at least 30 min after cocaine injection[244], or even 7 days following the last self-administration[246], coinciding with the timing of the negative affect state. Mameli and colleagues confirmed the hyperactivity of the LHb during cocaine withdrawal[97], and further demonstrated that such LHb hyperactivity mediates a negative affect state[37,247]. They found that, similarly to the situation in mouse models of depression[96], the balance of co-released GABA and glutamate in the EPN–LHb circuit is tilted towards more excitation[97]. Importantly, normalizing this imbalance by either overexpression of a dominant-negative form of AMPAR[248] or of VGAT[97] abolished the negative state, thus preventing stress-induced cocaine reinstatement[97]. In addition, work from the same group demonstrated that morphine withdrawal triggers the release of cytokines from microglia and attenuates glutamatergic transmission in the raphe-projecting LHb neurons while leaving the LHb–VTA transmission unchanged[249]. These results point to the specificity of LHb–raphe circuit in morphine-withdrawal-induced negative state.

Other classes of addictive substances, such as nicotine[250], ethanol[251] and opioids[252], also cause a variety of changes in LHb firing rates, or excitatory and inhibitory synaptic transmission, often with mixed effects on different neural subpopulations, in brain slice recordings. Further functional and behavioural experiments are needed in animal models to understand whether and how the LHb mediates the various impacts of these addictive substances.

Schizophrenia

The dopamine hypothesis of schizophrenia attributes the symptoms of this disorder to an increase in dopaminergic neurotransmission in the brain[253,254]. Given the close

ties between the LHb and the dopaminergic system, the LHb has been implicated in schizophrenia in both patients and animal models[24,255].

Individuals with schizophrenia exhibit large calcifications[256] and altered functional connectivity[257], as well as diminished response to negative RPE[255], in the habenula. In addition, the major treatment for schizophrenia, antagonists of dopamine D2 receptors, increase glucose metabolism in the LHb of mice and rats[258,259]. However, owing to the lack of reliable animal models of schizophrenia and paradigms to induce and monitor schizophrenia-like phenotypes, there is relatively limited evidence regarding the causal role of the LHb in schizophrenia[260], although schizophrenia-related cognitive impairments, including deficits in spatial memory, attention and sensorimotor gating, have been simulated in rats by lesion[172,174] or pharmacological inhibition[154] of the habenula.

Although the dopamine hypothesis may explain the positive symptoms of schizophrenia[261], antipsychotics that target dopamine signalling, such as D2 receptor antagonists, often have negligible effects on the negative and cognitive symptoms of this condition[262], suggesting that schizophrenia is unlikely to be due to simply dysfunction in dopamine signalling. NMDAR antagonists such as phencyclidine and ketamine can induce not only psychotic symptoms but also the negative and cognitive symptoms of schizophrenia[263–266]. These findings gave rise to the glutamate hypothesis of schizophrenia, pointing to a hypofunction of glutamatergic signalling via NMDARs[267–270]. Considering that ketamine exerts opposite effects on schizophrenia and depression, and that individuals with schizophrenia show reductions in RPE responses in the LHb[255], it would be tantalizing to investigate whether NMDAR-dependent burst firing, either within the LHb or in other brain regions, has a role in the cause of schizophrenia[40].

Conclusions and future perspectives

The past decade has seen exponentially growing interest and progress in delineating the neural circuits and functions of the LHb. Detailed investigations on the role of the LHb in reward, aversion, stress and cognitive processing have provided new insights into how dysfunction of the LHb may be implicated in disorders associated with mood and substance use. With the recent discovery that the LHb acts as a primary target of the rapid-acting antidepressant ketamine in mice and rats, this brain region will continue to attract more intense interest. Indeed, we expect more in-depth investigations of the LHb at the molecular, cellular and circuit levels. Here we outline a few emerging questions to be prioritized in future studies.

What is the relationship between the LHb and other brain regions that also regulate negative emotional states and react strongly to stressors, such as the amygdala[271], PVN[272,273] and paraventricular thalamus[274]? Do they act in parallel to specify different components of the stress response or in tandem to complexify response intensity, durability or persistence towards such stressors? Do they undergo similar or unique changes in activity patterns or plasticity during depression? Likewise, how does the LHb work together with other candidate target brain regions of ketamine to orchestrate its rapid and sustained antidepressant effects?

Do subnuclei within the LHb (the LHbM versus the LHbL) have specific functions? Do neighbouring nuclei (the PHb and MHb) have different or shared functions with respect to the LHb? Owing to technological limitations, most current manipulations have not reached subnucleus resolution, and we speculate that several early manipulations of LHb function were not absolutely restricted within the LHb region: virus or drug infusion targeting the LHb can leak into neighbouring areas; and local lesions may cause damage and inflammation beyond the LHb. The development and application of new LHb-specific, or even subnucleus-specific, tools and more meticulously controlled experimental designs are needed to precisely pinpoint the specific role of the LHb and its subnuclei in various functions.

Most importantly, how can our current understanding of LHb firing mechanisms be translated into clinical applications in depression diagnosis and treatment? Understanding the characteristics of LHb firing patterns in depression should help design better deep brain stimulation protocols for quenching its activity. Moreover, many identified key molecules regulating LHb activity, such as NMDARs, T-VSCCs, Kir4.1, CaMKIIβ and PP2A, are highly druggable ion channels or enzymes. These molecules, together with other ion channels or signalling pathways that can diminish LHb hyperactivity (for example, by regulating the RMPs or bursting activities), may provide a wealth of resources for future drug development[40,227]. In particular, recent advances in ultrasound and microbubble-mediated drug delivery technology[275] should soon allow LHb-specific targeting of drugs, antibodies or antisense oligonucleotides and hold great promise as treatments for depression and other LHb-related psychiatric disorders.

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