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Review

The behavioral relevance of a modular organization in the lateral habenula

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SUMMARY

Behavioral strategies for survival rely on the updates the brain continuously makes based on the surrounding environment. External stimuli—neutral, positive, and negative—relay core information to the brain, where a complex anatomical network rapidly organizes actions, including approach or escape, and regulates emotions. Human neuroimaging and physiology in nonhuman primates, rodents, and teleosts suggest a pivotal role of the lateral habenula in translating external information into survival behaviors. Here, we review the literature describing how discrete habenular modules—reflecting the molecular signatures, anatomical connectivity, and functional components—are recruited by environmental stimuli and cooperate to prompt specific behavioral outcomes. We argue that integration of these findings in the context of valence processing for reinforcing or discouraging behaviors is necessary, offering a compelling model to guide future work.

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Asking about the lateral habenula (LHb) to ChatGPT 3.5 provides the following output: "a tiny but powerful part of our brain that helps us with feelings, decisions, and learning from experiences. It is like a control center for our emotions and motivation, affecting how we feel about things and the choices we make." This basic definition is representative of the broad conceptual and experimental opportunities that this structure offers to neuroscientists across various fields. Indeed, whether interests lie in decision-making, emotions, social relations, instincts, and learning or in investigating the bases of depression, addiction, or pain, the LHb is likely to have caught attention. The LHb bears a significant influence on all these processes, raising the question of how such a small structure within the epithalamus contributes to them. Does it operate based on a unique common computation or function, such as defining valence processing in the brain? Is it an interface structure connecting and broadcasting information across independent functional networks, like a switchboard controlling emotional responses and stress-related pathways?

In this review, we offer our perspective on the function of the LHb. The present work, however, does not aim to provide an exhaustive overview of the findings supporting the relevance of this brain region, considering the existence of several comprehensive and high-quality published works.[1–4] Instead, our focus will be on combining past and new findings to generate an updated conceptual framework where the LHb works through different interacting modules (Figure 1). The observations made at the level of the LHb molecular code, anatomical connections, and functional responses of LHb neurons—representing the proposed LHb modules—should be now integrated, rather than treated separately. This will provide a comprehensive conceptual framework of a modular organization in the LHb instrumental for efficient, and behaviorally relevant processing. Thus, our objective is to offer a synthetic yet compelling discussion that inspires fresh views as well as future experiments and research avenues.

The working model we propose draws from a wealth of studies utilizing diverse model organisms, molecular, functional, and anatomical neuroscience investigations, as well as behavioral approaches. We begin with a comparative analysis of the LHb. Discussing the evolutionary aspects is fundamental to integrate the concept of a modular organization across species. Elaborating on the developmental trajectories is instead key to understand the genesis of such a modular organization. Complementary to these aspects, the latest findings on the LHb molecular signatures will be then discussed and integrated within the knowledge on evolution and development. Following this, we delve into the integration of LHb within various neural circuits, exploring its connections to the rest of the central nervous system. Finally, we link the activity dynamics of LHb neurons capable of driving discrete aspects of instinctive and learned behaviors (with an emphasis on aversion, negative valence, and negative affect) to end with a conceptual framework bridging the patterns of LHb activity across physiological and pathological conditions.

THE LHb MOLECULAR CODE THROUGH EVOLUTION AND DEVELOPMENT

The habenula[5,6] serves as a central component of the dorsal diencephalic conduction system (DDCS) connecting the stria medullaris (the axonal bundle entering among others the LHb)

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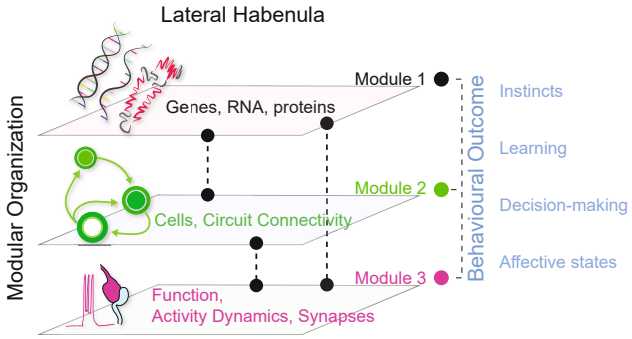

Figure 1. The emergence of a modular organization in the LHb converging to behavioral outcomes

Shown are modules of organization within the LHb, from genes to RNA and proteins (module 1), cells and circuit anatomy (module 2), and function expressed as activity and synaptic function (module 3). Each module connects one another representing crosstalk across modular features. It is through modular interactions that LHb-dependent decision-making, affective states, sociability, instincts, learning, or pathological phenotypes are expressed.

and the fasciculus retroflexus (the bundle of fibers exiting the LHb and neighboring nuclei) that functions as a conduit of information to downstream structures. In this section, recent work directed at understanding the evolutionary, developmental, and molecular features of the LHb will be presented. To complement this aspect, a discussion will follow around the lack of consensus in the identification of LHb-specific markers across species, and how this impacts the knowledge about the general evolutionary aspects of LHb as well as the generation of specific genetic tools useful for the neuroscience field.

Conservation of LHb across vertebrates

The habenula is present in virtually all groups of vertebrate species: jawless vertebrates (hagfishes and lampreys), cartilaginous fishes, teleosts, amphibians, reptiles, birds, rodents, and humans[7,8] (Figure 2A).

Studies aiming at describing the morphology and cytoarchitecture of the habenula stem predominantly from rodents and zebrafish. The habenula in rodents presents a morphological subdivision into lateral and medial components (LHb and MHb), while in zebrafish the habenula comprises a ventral and a dorsal territory. The LHb in rodents and the ventral habenula (vHb) in zebrafish (Figure 2A) are considered homologous structures based on the conservation of a major output to the raphe and the similar expression of a cadherin family gene, *Pcdh10*.[10] Lately, the use of single-cell RNA sequencing (scRNA-seq) techniques further corroborated these results.[11–13] This homology extends to two other animal models representative of the teleost family, the Atlantic salmon and the European eel, which share a common cytoarchitecture and gene expression pattern with the zebrafish. Interestingly, these two species possess an additional asymmetric subnucleus in the dorsal habenula (dHb) that is instead absent in the zebrafish.[14]

These data suggest that even within phylogenetically closer species, the habenula may follow different evolutionary pathways. Leveraging technologies such as scRNA-seq, targeting discrete cell types within the habenula, or even studying them across species could offer compelling information about the evolutionary trend of this structure.

A key evolutionary feature of the habenula is the asymmetry between the left and the right hemispheres (i.e., laterality; Figure 2A). Asymmetry varies across taxa and concerns properties including anatomical organization, neuropil or neuronal density, laterality, or even differential gene expression. For instance, in some species, the right counterpart of the habenula is bigger than the left (lamprey and hagfish), whereas the reverse is observed in others (cartilaginous fishes and teleosts). Although asymmetry remains subtle in a variety of mammalian species, albino mice and moles show clear asymmetric LHb.[15,16] The use of scRNA-seq in C57BL/6J mice unmasked asymmetry features in this case from a transcriptomic standpoint and identified differential gene expression between the left (11 genes) and the right (5 genes) habenula.[17] Finally, asymmetry was also described in the human LHb, with the left LHb described larger than the counterpart hemisphere. Furthermore, the LHb was proportionally enlarged in humans compared with other vertebrate species.[18] Altogether, this highlights that asymmetry is a feature conserved across species, including humans, yet a thorough understanding of its contribution to function or behavior remains elusive.

While asymmetry in the LHb of mammals remains uncharted territory, the published findings set an important ground for future studies to revive this stream of research, and firmly understand its functional and behavioral relevance. An important advance in the field is represented by the ongoing improvements of spatial and temporal resolution in human functional magnetic resonance imaging (fMRI) combined with data analysis techniques to capture more detailed information about brain activity. Considering the importance of habenula in fundamental human encoding and pathologies, such developments in human brain imaging may help define whether habenula asymmetry represents a contributing factor in these processes.

Developmental patterns of LHb neurons

One way to refine our understanding of the evolution of LHb is to integrate the developmental processes across species. When considering the mouse, LHb neurons developmentally stem from the prosomere 2 alongside MHb and thalamic neurons. A recent work employed scRNA-seq throughout embryonic (E) and post-natal (P) developmental stages (E11–E18, P4, P7, and adulthood) in *Brn3a-tau-LacZ* transgenic mice to specifically sort habenula neurons, and among them, ~55% originated from the LHb.[17] The message stemming from these results is that LHb populations acquire most of their molecular identity during a restricted period of embryogenesis. Indeed, molecular comparison between the developmental (E11–E18) and adult LHb datasets revealed that most LHb neurons already acquired their molecular identity at ~E18 and that this identity is retained in adulthood.[11,13,17]

In the case of zebrafish, the homologous dHb (dHb for MHb) and vHb (vHb for LHb) do not instead originate from the same progenitor cells.[19] During the shaping of the vHb, a functional effector of the Wnt pathway, Tcf7l2, is required for neurons to properly develop and migrate.[19] The use of scRNA-seq unraveled that virtually all vHb neuronal cell types are present at 10 days post fertilization (dpf) and from there persist into

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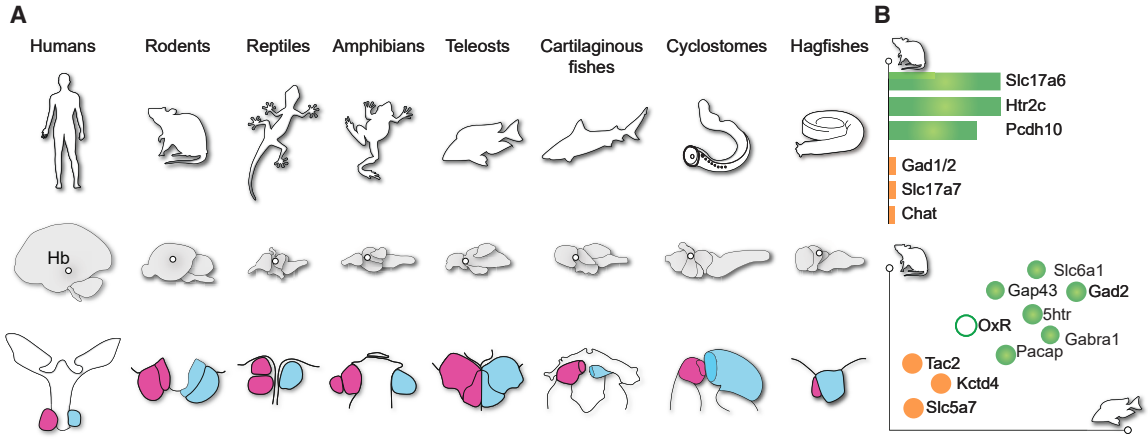

Figure 2. The evolutionary and molecular landscape of the habenula

(A) The habenula is conserved throughout evolution and present in humans, rodents, and teleosts. Among others, asymmetry is an evolutionary feature of the habenula. Each panel at the bottom corresponds to a coronal section of the epithalamus with the habenula (represented as the white spot in the brains) highlighted in colors (blue and magenta) to accentuate the asymmetric nature of this structure in specific species (schematic modified from Concha and Wilson[9]). (B) Single-cell sequencing highlights genes with high expression (green) or low expression (orange) in the LHb. A fraction of those genes in LHb of rodents are conserved in the vHb of zebrafish (open green circle; information on the orexin receptor is available only in mice).

adulthood.[12] A step forward in this knowledge is provided by studies addressing more specifically habenula developmental trajectories. At dpf 5, most of the vHb neurons already acquired their molecular identity, which is defined by the expression of the neuropeptide gene *Kiss1*.[19] While *Kiss1* expression was corroborated in other teleost species,[14] *Kiss1* was not detected in the mouse LHb.

Hence, unlike other brain regions that, at least in rodents, have clear molecular signatures, the habenula (and especially LHb) lacks well-defined and conserved genetic markers that exclusively identify its cells. Therefore, the idea of elaborating a mouse transgenic line using a specific LHb marker remains an open challenge.

The discrepancy between mouse LHb and zebrafish vHb neuronal identity calls for a comparison with an outgroup allowing to identify potential ancestral traits possibly lost in either one of these two species. The study of species belonging to the sister group of bony fish, namely cartilaginous fish, may provide an evolutionary rationale for those differences. In the catshark, an animal model representative of this group, neuronal differentiation starts at early stages of habenula morphological individualization, initially on the left side as in the zebrafish,[20] but the broad subdomain organization and the asymmetries of the structure become morphologically visible later, at embryonic stage 31.[21] This suggests that habenula in the catshark acquires most of its molecular identity already at this embryonic stage. Moreover, both development and asymmetry of habenula within this species are dependent on the Nodal pathway.[22] Indeed, when this signal is pharmacologically inhibited at early developmental stages, individuals fail to develop asymmetry in either part of the habenula. The Nodal pathway is similarly required for habenular asymmetry elaboration in the lamprey, a member of cyclostomes (or agnathans), but not in the zebrafish, where it rather establishes the direction of asymmetry.[23] These results support that the development of the habenula diverged from ancestral mechanisms during the evolution of bony fishes. Further work on elucidating molecular identities and homologies between habenula neuronal populations across species should provide valuable information on the evolutionary trend of this structure. This will be informative to understand not only LHb genesis but also to have insights on its adaptations and functional significance. Although caution should be used in translating findings obtained from animal models to the human condition, recent single-nucleus RNA-seq from the human habenula demonstrated transcriptomic changes associated with schizophrenia that were similar to those found in rodents.[24] This highlights the benefit of comparative studies related to the molecular code, allowing predictions likely relevant to the human condition.

In conclusion, studies uncovering the molecular mechanisms controlling aspects of habenula development highlighted the remarkable level of heterogeneity in genes and signaling pathways across species. This knowledge remains nevertheless limited and scattered across different species thereby hindering the possibility of drawing clear temporal trajectories or generating solid genetic tools. The information available shall now ignite discoveries, especially concerning the development of habenula subdomains characterized by discrete anatomical/functional features. Finally, understanding how disturbances of such developmental processes can mediate disruptions in normal brain function underlying aspects of human disease remains a valuable research ground to be explored.

Defined molecular programs yet unresolved function

To precisely study and manipulate brain function and behavior, laboratories leverage both developmental features (time at which specific genes are expressed) and the molecular identity (the landscape of diverse gene expression) of specific neuronal populations to achieve cell-type-specific targeting. For instance, numerous zebrafish or mouse transgenic lines have proven instrumental for such approaches. Until recently the molecular code defining LHb neurons remained elusive. However, recent studies using scRNA-seq in both mouse and zebrafish have shed light on

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this topic[11–13,25] (Figure 2B). In the mouse, manual dissection of the habenula together with sequencing tools revealed formerly known markers of MHb and LHb and further provided a compelling list of valid candidate marker genes. A striking point emerging from these analyses is the major heterogeneity of LHb molecular identity. Moreover, the genes found within the LHb neurons are also expressed in the surrounding areas (*Pcdh10* and *Gap43* in paraventricular thalamus; *Slc6a7* in MHb), whereas others are limited to restricted subpopulations of the LHb. Therefore, the LHb molecular code does not appear to be conveyed by single gene expression, but rather by a combination of several markers. In addition, in terms of cross-species homology, the integration of single-cell transcriptomes of habenula neurons of zebrafish[12] and mouse[11] together with cluster analysis confirmed a transcriptional homology between mouse LHb and zebrafish vHb, previously proposed on only a few genes.[10] The existence of such a molecular atlas urges the community to employ it as a powerful tool in bridging the gap between molecular identity, connectivity, and functional responses.

The consensus as of today is that LHb neurons are excitatory because of the substantial and homogeneous expression of the vesicular glutamate transporter VGluT2.[11,13] In addition to this, manipulations of smaller clusters that specifically express the glutamate decarboxylase 2 (GAD2) enzyme, serotonin receptor subtypes, the *Pacap* gene, or specific orexinergic receptors have also proven to have functional and behavioral relevance.[26–30] Notably, the expression of several of these genes is shared in rodents and zebrafish, indicative of the conservation of the molecular module across species (Figure 2B). It remains unclear, however, to which extent such genes or "genetically identified" neuronal populations are specific for the screened behaviors (aggression and ethanol intake, for instance). Indeed, these genetically targeted neurons may all be VGluT2 expressing, but do they express other sets of genes equally? A more refined understanding of the LHb molecular module, along with its establishment during development, will be beneficial to tailor connectivity and functional experiments to ultimately probe its behavioral repercussions.

THE LHb: A LOGISTIC HUB FOR INFORMATION FLOW

The identification of a molecular module suggests diversity in the expression of genetic markers in the LHb, despite the homogeneous presence of VGluT2. Is the molecular code serving purposes other than function? One possibility is that the molecular landscape supports specialized circuit connectivity. This may mediate the appropriate assembly of the DDCS that contains stria medullaris, habenula, and fasciculus retroflexus, thereby connecting the cognitive-emotional basal forebrain to the modulatory monoamine areas of the brainstem.[31–33]

In the following sections, we will examine the anatomical organization of the LHb focusing mostly on results stemming from rodents,[1–4,34,35] and we will do so from a perspective of stimuli integration, namely sensorial versus limbic (Figures 3A and 3B).

Anatomical substrate for the LHb integration of sensory stimuli

When summarizing the functional relevance of LHb, it is widely held that this structure exhibits an increase in neuronal firing following various aversive stimuli (nociceptive, pressure, and visual).[36–41] One interpretation of this function is that the LHb may integrate external aversive sensory stimuli by receiving cortical or thalamic information, facilitating environmental perception, and subsequently triggering behavioral actions.

Neurons in the prefrontal cortex (PFC) compute, at least partly, sensorial and especially aversive stimuli as well as negative affect via the control of a complex network of brain structures.[42] Studies using neural circuit manipulation approaches and intersectional viral strategies indicate a direct anatomical connection between the PFC and the LHb. Indeed, anterograde viral constructs injected into the PFC reveal axons in the LHb,[43–45] while complementary retrograde labeling within the LHb labels neurons in the cortex.[43,44] These studies also highlight that the anterior insular and cingulate cortices of rats send axons to the LHb. Recordings in acute brain slices show that PFC axons in LHb are excitatory, releasing glutamate onto postsynaptic neurons. However, the properties of neurotransmitter release, its efficacy in modulating postsynaptic firing, and through which receptors assembly this may occur remain elusive. In addition, an important consideration is the substantial innervation from these cortical areas onto thalamic regions surrounding the LHb. Thus, manipulation strategies, especially done *in vivo*, need careful tailoring to avoid nonspecific targeting that could lead to misinterpretations of behavioral data. These findings should encourage future studies to employ intersectional approaches, molecular tools, and functional methods to refine our understanding of how cortical inputs modulate LHb activity during the integration of environmental information.

Signals transmitted by light through the retina wield significant influence over both behavior and emotional states. Furthermore, light can impact neuronal activity within the LHb, correlating with changes in affective states.[46,47] In the retina, a subset of M4-type retinal ganglion cells (RGCs) innervates GABAergic neurons in the ventral lateral geniculate nucleus (vLGN). These neurons, in turn, project directly to the LHb, providing inhibitory control over LHb cells.[48] Alongside the visual thalamus, the thalamic reticular nucleus, responsible for integrating information between the cortex and dorsal thalamus, also sends inhibitory projections to the LHb. This inhibition arises from thalamic somatostatin (Sst)—and from parvalbumin-expressing neurons in the thalamic reticular nucleus.[49] Finally, thalamic inputs also originate from the medial dorsal thalamus, where a mix of excitatory and inhibitory signals converge onto the LHb.[50]

These anatomical pathways collectively imply that environmental sensory stimuli likely access the LHb through sensory integrative components. However, this represents only a fraction of our understanding regarding how diverse sensory inputs converge onto the LHb. Integrating knowledge across species might be a strategy to open future investigations on the network embedding the LHb. For instance, habenular circuits in zebrafish integrate both visual and olfactory informations. While RGCs convey visual signals to the left habenula through a multisynaptic circuit,[51] sensory information impinging onto mitral cells in the olfactory bulb reaches, via a monosynaptic connection, the right habenula.[52] This olfaction processing is hereby asymmetrical, supporting the relevance of evolutionary features for specific

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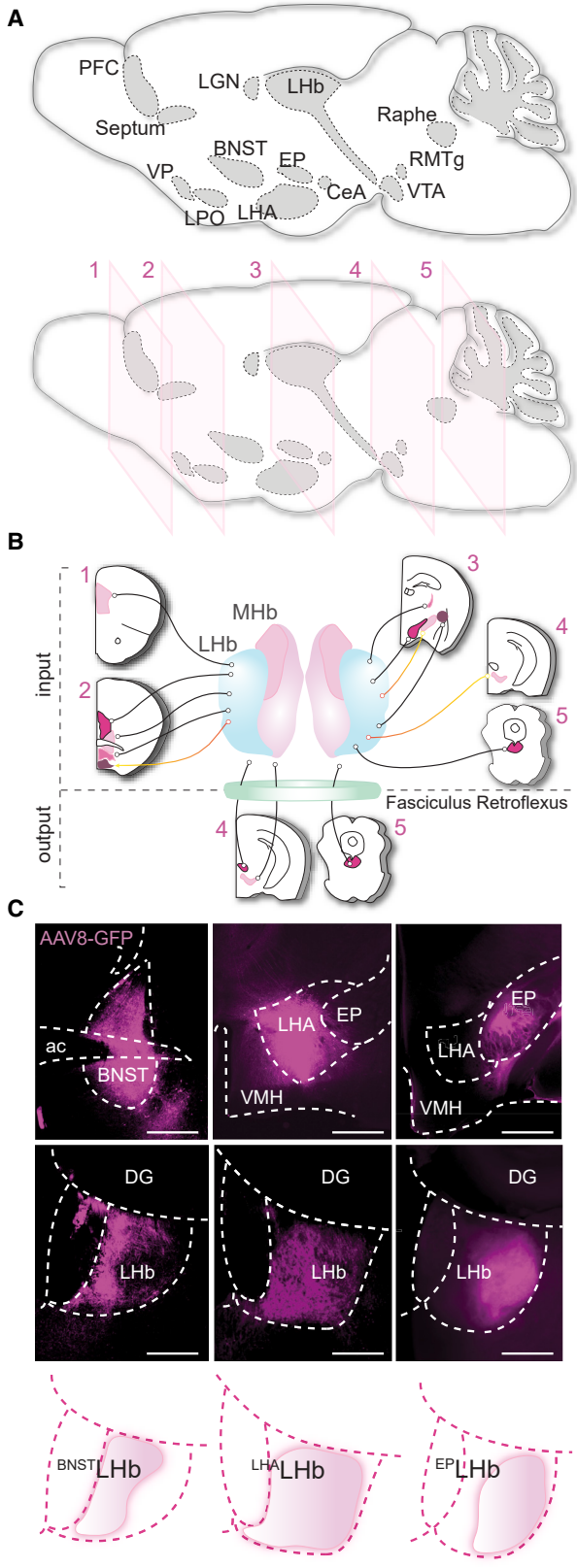

Figure 3. Anatomical integration of the LHb within brain circuitries

(A) The LHb is embedded in a network of both sensory and limbic brain structures. The schematic at the bottom reflects the coronal planes in (B).

(B) Coronal sections including the sensorial and limbic structures that innervate and receive axons from the LHb. Notably, nuclei including the EP, VTA, and VP are capable of providing both glutamate and GABA through both independent axons or axons able to co-release both transmitters (Glu/GABA co-release). The integrated signal in the LHb is then broadcasted to neuromodulatory centers, namely dopamine VTA and serotonin raphe.

(C) An anatomical feature of discrete inputs innervating the LHb is their specificity to stay limited to defined territories. While hypothalamic axons invade entirely the LHb, EP axons remain limited in the lateral portion of the LHb, sparing the medial aspect, which is instead specifically innervated by axons emerging from the BNST (scale bars, 500 μm, top; 300 μm, bottom).

connectivity and function.[53,54] Whether and how this, or other modalities, are also processed in the LHb of mammals should be tested in the future. Furthermore, functional studies aiming to depict the activity dynamics of cortical and thalamic projections could illuminate the temporal encoding of these inputs during specific stimulus presentations. Additionally, leveraging omics approaches in conjunction with viral tools could provide insights into the specific LHb populations receiving and integrating precise cortical or thalamic information.

Anatomical substrate for the LHb integration of limbic information

While the literature on cortical and thalamic innervation remains limited, the majority of inputs mapped onto the LHb originate from limbic structures and areas of the basal forebrain (Figures 3A and 3B).

Neurons from the lateral hypothalamus (LHA) extensively innervate the LHb territory.[55] These LHA axons cover virtually the entire LHb and release glutamate, enhancing action potential firing.[36,55–57] While LHA-to-LHb neurotransmission is predominantly excitatory, the neighboring lateral preoptic area (LPO) presents distinctive features in its axonal projections. LPO neurons innervating the LHb are glutamatergic and GABAergic.[58,59] Indeed, ultrastructural studies revealed that independent populations of LPO glutamatergic and GABAergic neurons converge onto single LHb cells.[59] Such a mixed neurotransmitter release is also observed in central amygdala (CeA) projections to the LHb. Upon viral vector injection expressing Channelrhodopsin-2 fused with mCherry into the CeA of Sst-Cre mice, a significant number of mCherry-positive fibers were detected in the LHb.[60] Light-induced activation of CeA terminals in the LHb triggered both excitatory and inhibitory postsynaptic currents. Whether such synaptic events stem from independent neurons or neurons releasing both neurotransmitters remains, however, unknown.

A projection onto the LHb originates as well from the bed nucleus of the stria terminalis (BNST), a structure that contributes to valence encoding, sociability, and motivation, among other functions.[61,62] BNST axons, in contrast to LHA projections, are confined to a specific area of the LHb, namely the medial LHb territory (Figure 3C). This territorially distinct projection releases glutamate onto LHb neurons and evokes reliable action potential firing.[25,63] The medial septum (MS), another element of the limbic system that regulates emotions, navigation, and memory, projects to the LHb through distinct

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neurotransmission: glutamatergic MS neurons excite LHb neurons, while GABAergic Sst-expressing MS neurons efficiently inhibit the LHb.[64,65] This has led to the proposition that the MS-to-LHb pathway transforms bottom-up sensory signals into emotions and actions. Hence, these studies revealed two fundamental features of afferent innervation to the LHb: the ability of a given projection to both excite and inhibit LHb neurons, and the capacity to target anatomically segregated LHb territories. This highlights a precise organizational structure of input connectivity (Figure 3C). Finally, a mesohabenular projection emerges from the ventral tegmental area (VTA). A combination of anatomical tracing and electron microscopy led to the identification that rat and mouse VTA axons in LHb, rather than conveying dopamine signaling, co-express VGluT2 and vesicular GABA transporter (VGAT) proteins and, surprisingly, establish symmetric and asymmetric synapses onto LHb neurons.[66] This finding supports therefore not only opposing neurotransmission from the same structure but also the principle of co-release of glutamate and GABA.[67]

In addition to limbic structures involved in motivation and emotions, the LHb also receives inputs from the basal forebrain, reflecting its relevance in action selection or decision-making. The entopeduncular (EP) nucleus serves as an output region of the basal ganglia, primarily associated with motor control and movement regulation.[68] The EP represents a major source of inputs to both primate[69,70] and rodent[32] LHb. In contrast to BNST innervation (enriched in the LHb medial territory), EP axons specifically innervate the lateral territory of LHb.[71–73] Sst-positive (Sst+) EP neurons projecting to the LHb express the molecular machinery for glutamate and GABA release[73,74] (Figure 3B). Optical stimulation of EP Sst+ axons expressing excitatory opsins led to glutamate/GABA co-release, which in turn generated compound synaptic currents in LHb neurons through ionotropic glutamate and GABA receptors.[72,74,75] Two proposed models explain the co-release in LHb: the first one suggests that at this synapse, glutamate and GABA are packaged into the same vesicles.[73,76] The second posits that they are segregated into different pools independently released from the same terminal, as supported by evidence from vesicular transporter distribution in LHb.[75] Notably, research on this LHb innervation has drawn interest for its control of behavior and its implication in disease models (see sections on the multiscale habenular plasticity determinant for behavior and implication for disease research). Another input from the basal forebrain deserving attention is the ventral pallidum (VP). The expression of different chemo- and optogenetic tools in the VP highlighted both excitatory and inhibitory innervation in the LHb.[77,78] Notably, the VP population projecting to the LHb expressed both parvalbumin and the dopamine receptor D3[79,80] (Figures 3B and 3C).

Together, these anatomical and functional data underscore the diverse connectivity controlling LHb neurons, highlighting the relevance of this module. Two aspects emerge from such a module and deserve attention when conceptualizing future LHb studies. One is the definition of the territory that axonal terminals specifically innervate, and the other is the assessment of the neurotransmission type these axons provide. Both are fundamental features to be examined. An open challenge emerging from the studies discussed above is to determine how inputs from different structures are organized at the single-neuron level.

Do independent nuclei project their axons onto specific compartments across the dendritic tree of a single LHb neuron? Are synapses multipartite, receiving information from numerous inputs? These and other questions related to the connectivity module, the fundamental LHb connectome, and the integration properties of each LHb cell remain open for future studies employing a combinatorial approach merging biophysics, viral tools, microscopy, and molecular studies.

Detection of neuromodulatory components

The synaptic excitation and inhibition onto LHb neurons originate from various structures with refined territorial specificity or intricate mechanistic complexity. Apart from this "fast transmitter" release, cells within the LHb also integrate "slow neurotransmission" from modulators including serotonin, dopamine, and acetylcholine[81–84] (Figure 3B).

The use of anterograde tracing methods revealed the presence of median and dorsal raphe fibers across the entire LHb.[85] Studies employing transgenic mice expressing Cre-recombinase under the SERT promoter (serotonin transporter) provide evidence that serotonin cells in the dorsal raphe nucleus (DRN) send prominent axonal innervation to the LHb.[65,86–88] In addition, serotonin bath application in acute brain slices diminished both glutamatergic and GABAergic release within the LHb. This indicates a functional impact of serotonin.[72,73] However, direct evidence supporting the physiological relevance of serotonin release is yet to be provided. Notably, the median raphe (MR) harbors a glutamate-releasing cell population providing inputs to the LHb.[89] This highlights multiplexed signals emerging from the raphe—fast and slow neurotransmission—which may differently tune LHb activity.

Exploring the release dynamics, the potential control over specific cell types, or the receptors involved in this raphe-to-LHb projection is crucial for future investigation.

The LHb also receives a significant projection from the VTA with axons expressing VGluT2 and GAD2 as well as the enzyme essential for dopamine production, tyrosine hydroxylase (TH).[90–92] Indeed, an initial estimation proposed that ~30%–50% of LHb-projecting VTA neurons could release dopamine.[90,92] However, Stamatakis et al.[93] challenged this view by placing carbon-fiber microelectrodes in the LHb to measure dopamine release in mice expressing channelrhodopsin-2 in TH neurons of the VTA. Surprisingly, optical stimulation of TH fibers in the LHb failed to elicit dopamine release despite the expression of dopamine receptors within this structure. Instead, a rather important GABAergic projection was highlighted from the VTA, although genetic caveats should be kept in mind in this instance.[93,94] The TH innervation and its function within the LHb remains a debated topic, raising questions on whether the presence of dopamine receptors is meant for alternative inputs or signaling. The possibility that noradrenaline would signal in the LHb through these receptors is a potential avenue for future investigation.[95]

Recent discoveries described the release of acetylcholine within the LHb as viral-based expression of biosensors revealed cholinergic transmission in LHb upon exposure to stimuli of a

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different nature.[81] These results corroborate the observation that carbachol can alter LHb neuronal activity through muscarinic receptors.[96] However, a comprehensive understanding of the anatomy behind cholinergic innervation in the LHb remains elusive. The same applies to several neuropeptides that could be released in the LHb, given the expression of specific receptors in this region (orexin, neuropeptide Y, vasopressin, and corticotropin-releasing factor, for instance). A more detailed comprehension of the neuromodulatory signaling in the LHb is crucial as it might influence stimulus encoding, synaptic function, and specific behaviors, which could have relevance for human pathologies.

The fasciculus retroflexus: An LHb megaphone to the ventral brain

While upstream projections into the LHb originate from diverse sources, LHb axons run ventrally through the fasciculus retroflexus[97,98] (Figure 3B). The knowledge of homogeneous VGluT2 expression in LHb is complemented by the description of a small subset of GAD2-positive neurons. Transcriptomic evidence indicates that this specific cell population can co-express markers for both GABA and glutamate as well as orexin receptors.[26,28,50] Ultimately, the GAD2-expressing population releases glutamate to its downstream target neurons.[30] Consequently, by integrating signals from various inputs, the LHb processes and subsequently excites downstream targets (Figure 3B).

LHb axons innervate the midbrain and further extend caudally to the hindbrain.[99] LHb projections from the lateral territory primarily provide excitation to inhibitory neurons in the rostromedial tegmental nucleus (RMTg) or the tail of the VTA.[71,100] The RMTg, in turn, projects its axons predominantly to inhibit dopamine neurons in the VTA and substantia nigra pars compacta.[101–105] Additionally, this LHb population directly innervates GABAergic and dopaminergic neurons in the VTA.[71,106] Conversely, medially located LHb neurons predominantly control dopaminergic neurons in the VTA.[71,97] This dopaminergic population responds with excitation upon optical activation of LHb synaptic terminals in the midbrain.[98,107]

A subset of LHb axons reaching the midbrain through the fasciculus retroflexus extends posteriorly to innervate the dorsal raphe and MR[99,108] (Figure 3B). Similar to midbrain connectivity, LHb neurons control raphe neurons via a direct projection and an indirect pathway through the RMTg.[71,109] Neurons within the medial LHb territory directly send glutamatergic projections to both raphe serotonergic and GABAergic neurons. Consequently, *in vivo* electrical stimulation of the LHb markedly suppresses neuronal activity in raphe nuclei.[110] However, the exact molecular nature of both LHb and raphe neurons and their connectivity remain elusive.

While the dopamine and serotonin systems represent extensively studied LHb targets, they are not the sole outputs stemming from the LHb. Other efferent targets include the noradrenergic locus coeruleus or the cholinergic laterodorsal tegmentum.[99,111] However, the connectivity and functional properties of these pathways remain comparatively less well characterized than those discussed previously.

Altogether, the complex anatomical organization embedding the LHb, its diversity in the modalities that control the function of this nucleus, and the feature of co-release of fast neurotransmitters make this structure highly attractive for many fields in neuroscience. The development of tools for a more precise dissection of circuit connectivity and molecular targeting will allow further expansion of the existing LHb network. This understanding could be applied across different species, enabling crosstalk between the molecular and connectivity modules of the LHb (see section on the LHb molecular code through evolution and development). The intense innervation of midbrain and hindbrain anticipates that the LHb can control, mediate, and adjust various motivated behaviors—this is the topic discussed in the next sections.

THE MULTISCALE HABENULAR PLASTICITY DETERMINANT FOR BEHAVIOR

The molecular and connectivity modules of LHb are certainly instrumental for guiding behaviors, as demonstrated when employing genetic ablation or lesioning approaches.[112,113] Complementary to this, functional components including ion channels or synaptic players (composing the functional module) are equal pillars to define the behavioral relevance of brain cells. One approach to understand how these functional components contribute to behavior is to investigate whether they undergo adaptations after experience. This is particularly relevant as individuals of every species are exposed to a broad array of experiences throughout their lifespan. Indeed, such experiences profoundly impact neural circuit function and structure by modifying the strength of synaptic transmission (synaptic plasticity) and shaping the molecular landscape of the brain. These adaptations are fundamental to generate very rapid behavioral responses to external stimuli, such as avoiding an imminent threat or seeking a reward, as well as guiding future behaviors through learning-related processes, all of which are crucial for survival (Figures 4A–4D).

The development of paradigm-shifting technologies, including optogenetics, has laid the foundation for fundamental advances in understanding how different brain regions contribute to regulating adaptive behaviors, and among them, the LHb has emerged as a critical node.[114] A growing body of evidence has shown that exposure to different experiences tunes neuronal activity, induces short- and/or long-lasting synaptic adaptations, and triggers transcriptional changes within the LHb. These habenular circuits modifications allow individuals to interact dynamically with their environment but also to reinforce behaviors directed toward approaching rewards and avoiding dangers.

In the next section, we provide a discussion of the immediate computations LHb neurons perform in response to different stimuli and an update about how these responses are more heterogeneous than previously considered. Next, an outline of how aversive and rewarding experiences induce synaptic changes along with transcriptional modifications within the LHb will be provided. Finally, we will integrate these concepts with a series of manipulation studies highlighting the relevant contribution of LHb to a wide range of adaptive behaviors.

The LHb code at the subsecond timescale

LHb neurons in humans, nonhuman primates, rodents, and teleosts exhibit predominantly excitatory responses to aversive

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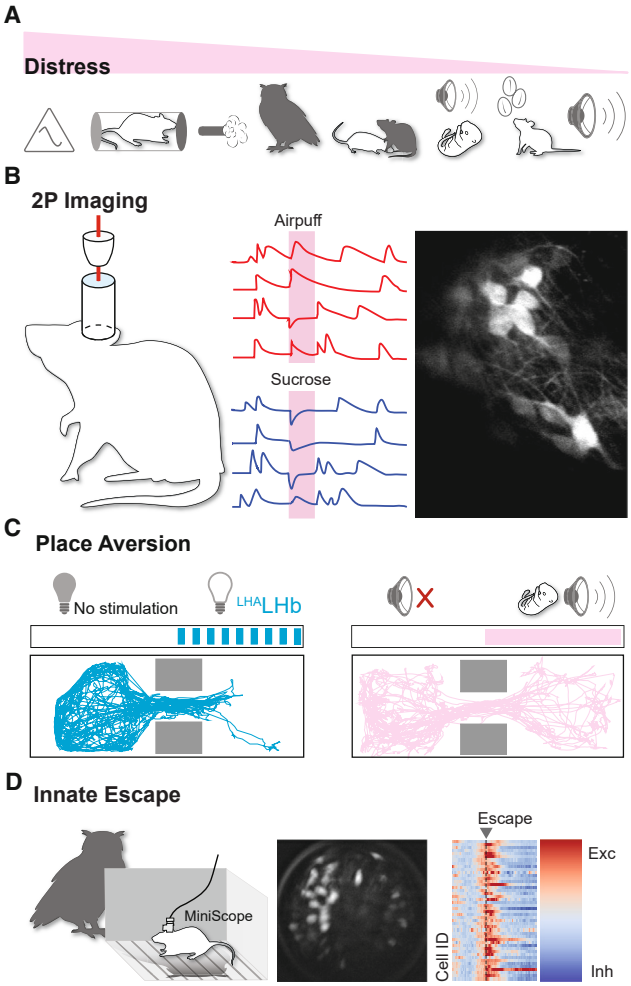

Figure 4. Linking LHb function and behavior reveals specialized features of aversion and reward encoding

(A) The LHb responds to different stimuli and experiences through a gradient of physical distress (the pink bar indicates distress intensity from high to low of the following stimuli: electrical shock, restrained experience, airpuff, predator exposure, social aggression, newborn distress, aversive food, and acoustic stimulus).

(B) Two-photon (2P) imaging approaches (among others) unraveled diversity in the neuronal responses to both aversive stimuli, such as airpuffs, and rewarding experiences (sucrose).

(C) LHb activity translates into actions that relate to aversive behaviors. Both optogenetic excitation of LHA terminals within the LHb and exposure to newborns in distress lead to real-time place aversion.

(D) Behavior and imaging of single neurons corroborated a contribution of the LHb for instinctive behaviors including defensive actions that appear in response to predator-like stimuli.

stimuli (unexpected and expected), their predictive cues, or the omission of expected rewards. Conversely, LHb neurons are generally inhibited by rewards or their predictive cues.[36,38,41,115,116]

Electrophysiology *in vivo* as well as *in vivo* recording of calcium dynamics have shown that a large proportion of LHb neurons exhibit subsecond excitation in response to various aversive experiences, including footshock, airpuff, social aggression, and a looming stimulus mimicking a predator attack.[26,27,36,38,41,117] In addition, LHb neurons are also activated by newborn distress vocalization[25] (Figures 4A and 4C). Thus, LHb neurons are detectors of negatively valenced stimuli independent of their nature. Notably, these cells might not only encode stimulus valence but also salience as evidenced by their responses to neutral auditory stimuli[81,118] (Figure 4A).

Recent studies using techniques that enable to gain access to single-cell resolution challenged the widely accepted model of aversion-excitation and reward-inhibition in the LHb. Single-unit recordings and two-photon (2P) calcium imaging have shown that LHb neurons display excitatory and inhibitory responses to footshock, radiant heat, and airpuff, as well as its predictive cues[81,119–121] (Figure 4B). Remarkably, this diversity in the encoding of aversive events also extends to neuronal responses to rewards.[41,77] Altogether, these studies unveil a remarkable heterogeneity in the response of LHb neurons to aversive and rewarding events.

The neurobiological basis underlying this heterogeneity remains elusive, although it could be explained by different non-mutually exclusive hypotheses. First, aversion-excited and -inhibited neurons could be embedded into independent LHb neuronal circuits characterized by either input-specific connectivity and/or specific projection targets. Anatomical reconstructions of 2P microscopy recorded cells as well as juxtacellular labeling indicate that aversion-excited and -inhibited neuronal populations are anatomically segregated. Excited cells are largely located in the lateral territory of LHb, while the inhibited population tends to cluster in the medial region of LHb.[81,120] Interestingly, as detailed in the section the LHb: a logistic hub for information flow, afferent and efferent connections of the LHb also exhibit a topographical organization.[25,63,72] Second, and in addition to the connectivity-based hypothesis, the heterogeneity in responses could stem from differences in the molecular expression pattern of excited and inhibited neurons. However, validating these hypotheses remains challenging due to the current limitations in identifying neuronal populations depending on their dynamic responses to stimuli. Technological advances in labeling, sequencing, and manipulating neurons based on specific dynamics will be crucial to bridge this knowledge gap.

In addition, the behavioral relevance of such diverse responses is an aspect that remains unexplored. It is perhaps unlikely that any excitatory signaling within the LHb mediates aversion and inhibition homogeneously signals reward. Whether these opposing processes could instead mediate distinct aspects of very specific adaptive innate or learned behaviors merits further investigation.

The traces of experience in the LHb

Different experiences and states trigger short- and long-lasting activity-dependent modifications in synaptic strength as well as in gene expression. These adaptations allow the brain to incorporate transient experiences into persistent traces ultimately shaping subsequent adaptive behaviors.[122]

Substantial experimental evidence indicates that LHb neurons undergo extensive experience-dependent synaptic adaptations, which are causally linked to specific behavioral outcomes (Figure 5). Acute exposure to aversive conditions leads to both presynaptic and postsynaptic adaptations in LHb neurons. While

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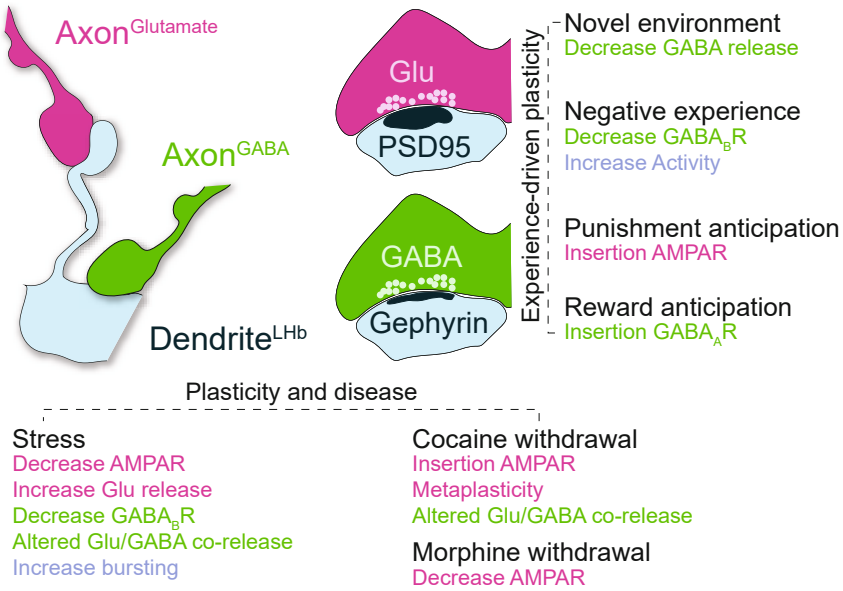

Figure 5. Adaptations in LHb after experience and in disease

LHb neurons receive axons releasing glutamate or GABA as well as terminals capable of releasing both neurotransmitters (Glu/GABA co-release). Overview of the variety of plastic changes occurring after different experiences as well as after stress or drug withdrawal. Such plastic adaptations encompass neurotransmitter release probability and the level of expression of postsynaptic receptors, both of which have repercussions on activity patterns.

exposure to a novel environment in social isolation reduces GABA release probability through presynaptic $GABA_B$ receptors[77] and early life stress as well as inescapable and unpredicted footshock triggers internalization of $GABA_B$ receptors ultimately weakening GABA-mediated transmission.[123,124] While these studies indicate a relevant function of $GABA_B$ receptors within the LHb, basic information on the receptors' assembly or interaction with scaffolding proteins or their signaling pathway remains unknown.[125]

A similar type of experience affects as well excitatory neurotransmission. Footshock exposure reduces LHb AMPA receptors (AMPARs), an adaptation sufficient and required for the expression of stress-related cognitive deficits in a reward-seeking task.[63] On the other hand, similar paradigms can enhance glutamate release probability LHb neurons.[61] These discrepancies might be grounded on experimental conditions, timing of the synaptic assessment, or might be even related to inter-species differences (mice versus rats). Importantly, these studies showed a consistent increase in the output firing of LHb neurons after inescapable shocks.[61] This observation is further corroborated by other studies whereby enhanced glutamate release probability from LHb to midbrain was observed after footshock exposure.[98]

Finally, synaptic adaptations underpin aspects of learning processes. Repeated pairing of a tone with aversive stimuli such as footshock, airpuff, quinine, or rewarding stimuli, such as sucrose, progressively modulates LHb neuronal activity in response to the predictive cue.[41,77,81,126] This occurs along with strengthening of LHb excitatory synapses mediated through increased postsynaptic AMPAR levels in the case of cue-punishment association but through increased postsynaptic $GABA_A$R function when cue-reward associations take place.[77,126] Notably, manipulating the stability of either postsynaptic AMPAR or $GABA_A$R sets causalities between plasticity and anticipatory behaviors. This illustrates how persistent synaptic traces in LHb, after transformation of a neutral stimulus into a predictive one, regulate fundamental learning processes. Learning-mediated reshaping of neuronal function is arguably grounded on changes happening at all synapses/neurons of a given structure. Studies based on 2P imaging, immediate-early genes (IEGs), or scRNA-seq can now be conducted to tackle these questions and decipher learning encoding in the LHb with higher cellular precision.[127–129]

Some steps forward have been made in this direction. After experience, the induction of IEGs expression (genes rapidly and transiently induced upon cellular activation) controls synapses, neuronal activity, and ultimately animal behavior.[130] Studies based on c-Fos labeling, an IEG widely used as a proxy of neuronal activation, show that LHb neurons are consistently activated by a broad array of stressors including footshock, restraint, lithium chloride-induced illness, and predator odor.[111,131,132] The extent of the c-Fos-positive population is however limited, raising the question of whether this labeling reflects neuronal activity or other types of signaling. Notably, diverse aversive stimuli induce similar c-Fos expression patterns in the LHb,[132] which raises the question of whether they engage the same or transcriptionally distinct neuronal populations. The latest methodological advances in genetic profiling have begun to shed some light on this matter. Antibody-guided chromatin tagmentation sequencing (Act-seq) and scRNA-seq have identified transcriptionally defined LHb neuronal clusters that are differentially activated following footshock exposure in mice.[11] In addition, sequencing of ribosome-associated RNAs in the rat LHb revealed that forced swim stress induces divergent gene expression profiles in LHb neurons depending on its output projections.[29] Transcriptomic analyses of stimulus-activated or -inhibited LHb subsets can potentially reveal stimulus-specific molecular signatures, offering a way to target-observe-manipulate relevant LHb neuronal clusters. As of now, there are important technical limitations to transcriptomically segregate inhibited versus excited cell populations, particularly in the LHb, where c-Fos has not demonstrated yet to represent a proxy for neuronal activation. This type of approach is representative of the relevance of integrating information from multiple modules to understand LHb function. Thus, systematically combining information related to the molecular signatures with connectivity and function will be key to underpin the neurobiological substrates in the context of decision-making, motivation as well as psychiatric disorders. Further studies aimed at finding better

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reliable markers of neuronal activity or synaptic plasticity in the LHb might help to address this challenge and elucidate whether discrete LHb neuronal ensembles differentially compute environmental stimuli and regulate distinct facets of adaptive behaviors.

Causalities between LHb function and behavior

Studies employing optogenetic, chemogenetic, and pharmacological manipulations have provided compelling evidence for causalities between plasticity within the LHb and adaptive behaviors (Figures 4C, 4D, and 5). These encompass behavioral flexibility, sociability, aggression, parenting but especially a wealth of aversive- and reward-related innate or learned behaviors.[3,4,40,133]

Optogenetic stimulation targeting different glutamatergic afferents onto the LHb drives robust real-time place aversion. Among these are inputs from the LHA, the VTA, and the EP[36,55,57,66,72] (Figure 3C). Similarly, the activation of LHb terminals projecting onto the VTA or RMTg produces aversion.[98,107] On the other hand, optogenetic inhibition of either LHA-to-LHb or LHb-to-VTA projections results in real-time place preference.[55,121] In addition, activation or inhibition of EP-to-LHb projections bidirectionally modulates reinforcement in a reward-conditioning task.[134] While these observations are instrumental to bridge LHb activity and motivation, they leverage optogenetic protocols that do not necessarily recapitulate physiological patterns of neuronal activity. *In vivo* recordings in behaving animals may inspire future experiments to causally link better refined LHb manipulations and behavior.

External threats engage defensive behaviors such as escape or freezing.[135] A large body of evidence indicates that the LHb can orchestrate threat-driven innate and learned behaviors in rodents as well as teleosts. *In vivo* recordings of calcium dynamics while simulating a predator attack (looming; Figure 4D) revealed opposing LHb neuronal responses contributing to different threat-induced actions. Indeed, while increased calcium dynamics occurred during escape, reduced calcium signal was time-locked with immobility.[117] Representative of the relevance LHb has on threat-related behaviors, chemogenetic inhibition of LHA-to-LHb projections impaired escape responses to the looming stimulus.[36] These effects extend to other aversive stimuli such as footshock, since inhibition of the same circuit also impairs escape and avoidance.[36,126] Notably, a similar behavioral contribution of the vHb was observed in the zebrafish. Inhibition of synaptic transmission within vHb impairs escape behavior upon exposure to shocks while optogenetic vHb manipulation modulates coping behaviors in a paradigm of repeated shocks.[10,115,136] Thus, not only the molecular and anatomical LHb modules are recurrent in different species, but these data offer a scenario in which the LHb functional module may also control comparable behaviors throughout evolution.

How the LHb shapes instead reward-related behaviors remains less clear. LHb inhibition or lesioning in primates, mice, and rats impairs their ability to discriminate between different reward probabilities.[113,137,138] Furthermore, impairing $GABA_AR$ function limits cue-reward association.[77] Finally, the LHb also contributes to decision-making processes as well. In rats, LHb inactivation renders animals indifferent to the cost of a behavioral reward in a probabilistic discounting task, while in nonhuman primates, LHb electrical stimulation influences decisions to gain differentially valued rewards.[113,137]

The LHb also contributes to behavioral flexibility, as its inhibition caused deficits in reversal learning paradigms.[139] In addition, the LHb modulates interactions with conspecifics. Chemogenetic activation of LHb neurons or PFC terminals within the LHb reduced social preference,[43] whereas inhibition of LHb-to-raphe projections decreased sociability.[140] LHb neurons also modulate aggressive social interactions. Inhibition of GABAergic basal forebrain inputs to LHb triggered aggression-based conditioned place preference in mice,[141] supporting the notion that this neural pathway controls the valence of aggressive interactions. Furthermore, glutamatergic LHb neurons projecting to the DRN promote aggressive arousal after social instigation, shaping the aggressive behavior.[142,143] In the zebrafish, inhibition of vHb-to-raphe projections modulated submissive-to-dominant interactions, notably rendering the submissive fish unable to take proper behaviors to prevent further attacks by the dominant fish.[144] Moreover, the LHb has emerged as a critical node for shaping adult-newborn interactions. Optogenetic activation of BNST terminals within the LHb promoted pup retrieval in virgin female mice, whereas silencing this pathway suppressed it.[25] Notably, selectively activating BNST-receiving LHb cells led to real-time place aversion, similar to that produced by newborn distress calls (Figure 4C), highlighting that activity in these cells encodes negative affect and pup retrieval.

Altogether, the LHb emerges as a multifaceted brain structure crucial to orchestrate a wide range of behaviors. Although important progress has been made, many questions remain unanswered regarding the biological mechanisms that explain how the LHb regulates such a vast behavioral repertoire. Does the molecular diversity in LHb translate into functionally different cell types, or the behavioral diversity is based on anatomically segregated cell clusters? Is it mediated by specific input and output connectivity? How do the dynamic and heterogeneous responses of LHb neurons to distinct stimuli contribute to behavioral complexity? Studies demonstrating the existence of specific markers for defined LHb populations or making use of tools labeling LHb neuronal subsets based on their activity are missing. Thus, molecular identity-based and activity-dependent manipulations of discrete LHb circuits, combined with recent computational tools are needed to unravel how LHb coordinates specific aspects of adaptive behaviors.

IMPLICATION FOR DISEASE RESEARCH

The exponentially growing interest in LHb is anchored on the numerous discoveries related to its implication in psychiatric conditions, including mood disorders, schizophrenia, and substance use disorder.[1,3,4,97,145,146] Considering the plethora of excellent review articles on this topic, here, we will focus on contextualizing the results within the framework of diseases, incorporating the various modules depicted in the above sections (molecular, connectivity, and functional) (Figure 5).

The LHb contributes to the pathophysiology of major depression, a disorder characterized by low motivation and passive coping behaviors.[3] In individuals with mood disorders, the

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habenula shows elevated activity, significantly covarying with depression ratings across the entire brain.[147] Recently, activity screening in zebrafish revealed that the vHb becomes hyperactive in a passive coping state and repeated social defeat paradigms.[115,148] This finding is corroborated by experiments manipulating LHb activity in rodents, where both gain and loss of function bidirectionally generated or alleviated the depressive-like state.[61,123,149] Extensive research in the recent years has provided new anatomical, molecular, and cellular insights within the LHb pertaining to the neurobiology of depression.[48,61,65,72,150] Particularly noteworthy are recent advancements highlighting the relevance of burst-firing modalities in LHb neurons as well as their dependence on *N*-methyl-d-aspartic acid receptors and astrocyte function. The latter is of particular interest in the context of depression. LHb astrocytes maintain LHb neurons resting membrane potential and tune burst firing.[151] Notably, astrocytic overexpression of the Kir 4.1 potassium channel in the LHb hyperpolarized and increased burst firing of LHb neurons, subsequently leading to depressive-like states.[151] Kir4.1 knockdown, by contrast, depolarized LHb neurons and reduced burst firing, limiting depression. This has a particular translational value as a single dose of a Kir4.1 inhibitor reversed the Kir4.1 overexpression-driven depressive phenotype and functions as a rapid-onset antidepressant.[152] Future studies shall address whether LHb astrocyte-neuron crosstalk in the context of depression follows any circuit-specific rule or whether distinct molecular programs define astrocyte addressing to precise LHb neuronal populations.[153]

Recent studies unraveled biophysical mechanisms underlying the rapid-acting antidepressant effects of ketamine.[151,154] The growing knowledge on LHb burst activity in depression may suggest the existence of a "depression engram" within this brain region. Is there a specific LHb neuronal subset that changes in its burst activity to mediate a depressive phenotype? Further experiments are required to determine whether a modular logic across molecular, anatomical, and functional features underlies the participation of LHb in the depressive state.[150,155] Finally, understanding whether the LHb and its burst capacity are the core targets of every class of antidepressant, or whether novel pharmacological treatments should be tailored to act on these targets, is a pressing need to advance knowledge on treatment diversity.

The depressive state is also a feature of drug addiction, particularly during withdrawal where individuals experience physical and affective symptoms including dysphoria (intense distress), anhedonia, and enhanced stress sensitivity.[156,157] Withdrawal periods after cocaine or opiate exposure induce remodeling of LHb synapses and signaling processes.[71,80,97,100,140,158–160] Accordingly, causality experiments have demonstrated that restoring basal synaptic function using optical approaches or molecular tools enables the prevention or the rescue of the withdrawal phenotype[71,80,140] (Figure 5). Different phases of drug addiction promote plasticity at synaptic, genetic, and epigenetic levels.[161] It is plausible that such modifications also occur in the LHb. However, this aspect remains largely unexplored. Furthermore, most of these studies used passive exposure to drugs, limiting our understanding on how the LHb may shape drug seeking or drug intake despite negative consequences, a hallmark of addiction.[162] Refined behavioral paradigms that better recapitulate the disease should represent an initial step to clarify how the LHb modules adapt during withdrawal and at which temporal scale.

Finally, and related to asymmetry as a conserved feature of habenula across species, analyses of postmortem brain samples revealed asymmetry in human habenula. Remarkably, such an asymmetry is grounded on the left LHb volume.[18] Advances in neuroimaging provided initial evidence of a perturbed LHb asymmetry in patients affected by schizophrenia and eating disorders.[163–165] This needs an intense investigation yet opens the possibility that an ancestral feature conserved across species might underlie aspects of human disease as well.

Overall, the recent progress in defining LHb dysfunction in disease is exciting. Translating these advances into effective clinical treatments is an ongoing objective in the field.[166]

CONCLUDING REMARKS

The proposal for a modular organization of the LHb arises from technological advancements that have enhanced our understanding of its relevance to fundamental behaviors and diseases (Figure 1). The proposed interconnected modules encompass (1) molecular heterogeneity, (2) anatomical complexity, and (3) diversity in functional responses. Integrating these distinct modules sets a framework for a deeper exploration of the LHb.

Several studies leveraged Cre-driver mouse lines to observe or manipulate LHb function.[26,27,29,167] These studies highlighted specific markers defining LHb cell subpopulations, yet how they integrate into the LHb network remains unknown. A recent study identified a neuronal subset in the LHb innervated by BNST axons and characterized its transcriptome.[25] However, it is unclear whether the described molecular code represents a static snapshot of these neurons or if it undergoes dynamic regulation. The use of activity-dependent approaches including Act-seq is one strategy that may complement the existing molecular module.[11] Another relevant source of inspiration for experimental programs emerges from the results obtained by the Allen Institute and its mapping of the location and intensity of gene expression throughout the central nervous system.[168] Implementation of such a database and tools may provide further insight into the molecular signatures that define the LHb. Finally, information about the molecular code in the LHb stems in large part from studies in teleosts and during development. In contrast, knowledge about these processes in mammals has only recently started to be addressed. The LHb circuit connectivity is instead substantial and compelling in rodents. An effort to uniform this knowledge across species remains a challenge, yet it needs to be a fundamental future objective to ultimately define a comprehensive and evolutionarily relevant organization of the LHb.

From a behavioral perspective, various structures releasing glutamate onto LHb neurons—including LHA, EP, VTA, and CeA—induce place aversion. However, does this imply they serve identical purposes?

This knowledge gap could potentially be bridged by integrating the modular organization into future studies, thereby clarifying the functional role of subsets of LHb neuronal populations. This extends to the potential relevance of non-neuronal populations. Astrocytes and microglia in LHb participate in diseased

states.[140,151] To what extent do they shape innate or learned behaviors? Do they do so by modulating neuronal features? The use of the latest developed tools may open novel streams of research addressing these topics. Furthermore, understanding the different developmental programs—identifying when specific cell types emerge, the temporal formation of networks, and the appearance of distinct behavioral aspects—will offer crucial missing insights.

Advancing methodologies to record and manipulate the LHb, alongside computational tools and AI-based behavioral analyses, is an essential step forward in the field. Recognizing a modular organization within the LHb will be pivotal in enhancing our understanding of its role in both health and disease.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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