Symposium

Mu-Opioid Receptor in Habenula Circuits as a Substrate for Psychiatric Disorders and Addiction

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The habenula is a critical regulator of affect, negative reinforcement, avoidance, and coping behavior. It is thus unsurprising that accumulating evidence supports pivotal roles for habenular circuits in the expression of major psychiatric illnesses including depression and addiction. Though high levels of mu-opioid receptors (MORs) were identified in the habenula more than a generation ago, the functional effects of MORs on habenular circuits remained largely unexplored due to challenges of selectively targeting this small and heterogeneous brain structure. In this SfN mini-symposium report, we highlight studies that resolve how MOR is distributed across medial and lateral habenular subnuclei and how MOR expression maps onto molecularly defined habenular cell types. We further describe some of the habenula-intersecting circuits that express MORs and how MOR signaling modulates neural activity and neurotransmission within these unique habenular circuits and cell types. Going beyond cellular-level effects of MOR signaling, we illustrate how neural activity in the habenula can influence affect and behaviors associated with opioid analgesia, opioid seeking, opioid dependence, and opioid withdrawal. Drawing on works outside the opioid field, we suggest commonalities through which drugs of abuse may influence habenula circuits to induce states of negative affect that contribute to continued drug use or relapse. Finally, we discuss how better understanding the molecular and functional properties of habenular cell types and circuits can identify new druggable targets, such as the orphan receptors GPR151 and GPR139, for the treatment of pain, mood disorders, or opioid and other substance use disorders.

Introduction

The medial (MHb) and lateral habenula (LHb) are critical to the regulation of affect, avoidance, and coping behaviors and play pivotal roles in the expression of depression and addiction (Fig. 1). It has long been known that the habenula contains high levels of mu-opioid receptors (MORs), and recent studies have identified some of the functional effects of acute and chronic opioids on habenula cells and synapses. Several reviews have detailed the anatomy, connectivity, and functions of the habenula in rodents and humans (Diaz et al., 2011; Aizawa et al., 2012; Namboodiri et al., 2016; Boulos et al., 2017; Hu et al., 2020; Ables et al., 2023). Here we focus on features of MHb and LHb most relevant for understanding how the actions of MORs on habenular circuits contribute to addiction and affective states.

Medial Habenula: Anatomy and Neurochemistry

The MHb sits at the center of a compact but highly selective limbic network with distinctive cytoarchitecture, neurochemistry, and connectivity that support its roles in nicotine and opioid dependence, mood regulation, and stress-related behaviors. It receives most of its input from septal and basal forebrain nuclei—with sparser monoaminergic input from brainstem centers providing feedback from downstream targets (Herkenham and Nauta, 1977; Phillipson and Pycock, 1982; Gottesfeld, 1983; Qin and Luo, 2009; Boulos et al., 2017; Szonyi et al., 2019). MHb can be divided into subnuclei which differ in gene expression, transmitter profile, and connectivity (Qin and Luo, 2009; Aizawa et al., 2012; Lee et al., 2019; Ables et al., 2023). The MHb is composed of glutamatergic neurons that project almost exclusively to the interpeduncular nucleus (IPN) via the fasciculus retroflexus (Herkenham and Nauta, 1977; Carlson et al., 2001). This projection is topographically organized such that dorsal MHb preferentially innervates more lateral regions of IPN, whereas ventral MHb targets rostral and central IPN

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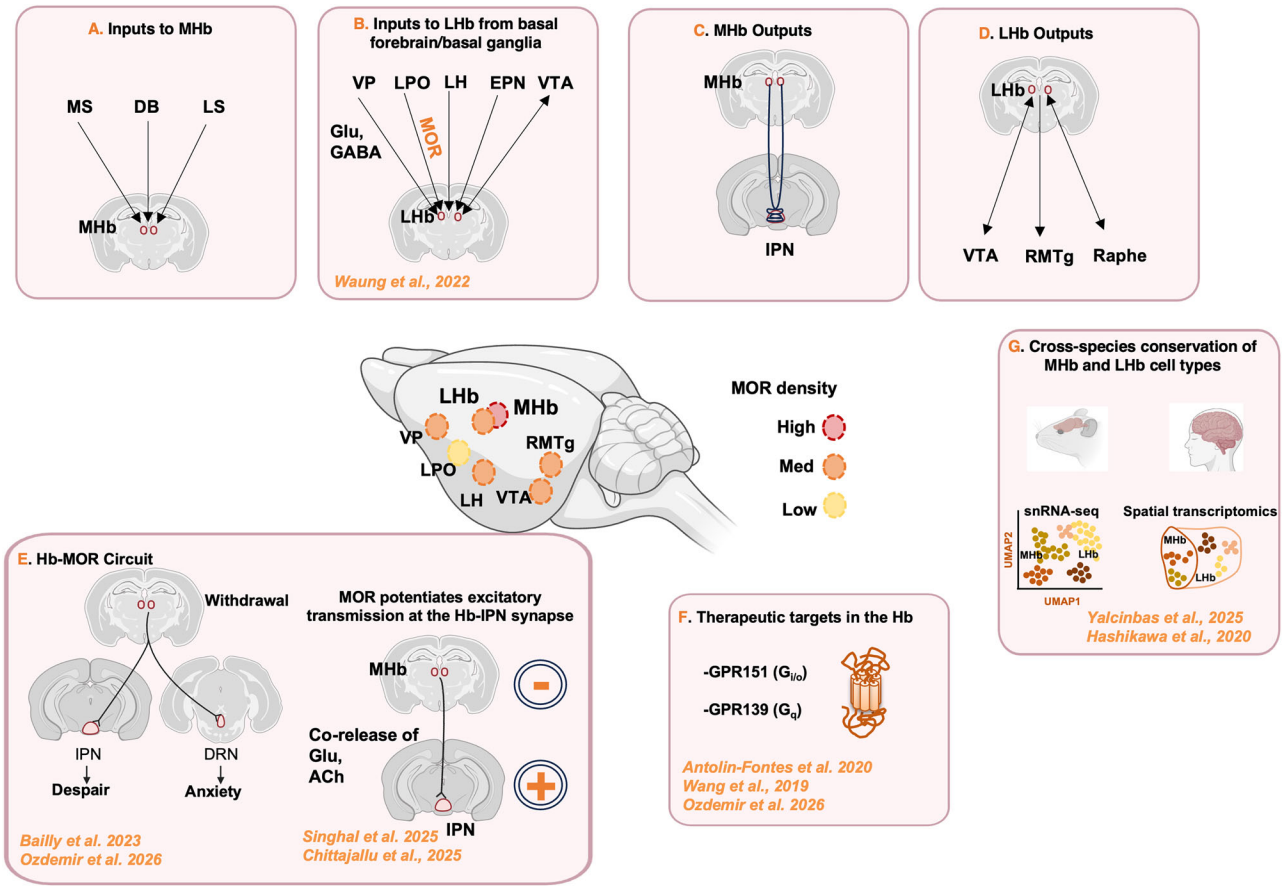


Figure 1. Mu-opioid receptor expression, cellular organization, and circuit functions of the habenula. Schematic overview of recent advances in understanding mu-opioid receptor (MOR) signaling in the medial (MHb) and lateral habenula (LHb). ***A–D***, Key afferent and efferent connectivity of the MHb (***A***, inputs; ***C***, outputs) and LHb (***B***, inputs; ***D***, outputs). The MHb receives inputs primarily from the medial septum (MS), diagonal band (DB), and lateral septum (LS) and projects almost exclusively to the interpeduncular nucleus (IPN). The LHb integrates glutamatergic and GABAergic inputs from forebrain and basal ganglia structures, including the ventral pallidum (VP), entopeduncular nucleus (EPN), lateral hypothalamus (LH), lateral preoptic area (LPO), and ventral tegmental area (VTA), and projects to the VTA, rostromedial tegmental nucleus (RMTg), and dorsal raphe nucleus (DRN). The expression of MOR is highest at the MHb–LHb border. ***E***, Left, Functional organization of MOR+ habenular circuits. Hb–MOR contributes to withdrawal-associated aversion and despair-like behaviors and MOR+ projections to the DRN regulate anxiety-like and affective behaviors. Right, Activation of MORs in MHb neurons decreases spontaneous firing while engaging a hyperpolarizing conductance in the IPN but induces a noncanonical facilitatory effect on the corelease of glutamate (Glu) and acetylcholine (ACh) from MHb terminals. ***F***, Habenula-enriched orphan G-protein-coupled receptors (GPCRs), including GPR151 and GPR139, represent promising therapeutic targets for selectively modulating opioid-sensitive habenular circuits involved in pain, opioid withdrawal, and addiction. ***G***, Single-nucleus RNA sequencing (snRNA-seq) and spatial transcriptomics reveal molecularly distinct but evolutionarily conserved neuronal populations across the MHb and LHb in mice and humans, providing genetic access to discrete opioid-responsive cell types.

subregions. The IPN is composed principally of GABAergic neurons that make both local and distal connections to midbrain and brainstem centers, shaping neural activity within serotonergic raphe and dopaminergic ventral tegmental area (VTA) pathways (Molas et al., 2017).

Neurochemically, MHb neurons are remarkable for transmitter corelease and for high levels of receptors targeted by drugs of abuse. Multiple lines of evidence show that MHb neurons are uniformly glutamatergic with broad expression of the vesicular glutamate transporters VGLUT1 and VGLUT2. These can be divided into two major neurochemical classes: ventral/lateral neurons that corelease acetylcholine (ACh) and glutamate at IPN terminals and dorsal glutamatergic neurons that express substance P (*Tac1*; Ren et al., 2011; Aizawa et al., 2012; Gorlich et al., 2013; Frahm et al., 2015; Quina et al., 2017). MHb neurons express high levels of several nicotinic ACh receptor (nAChR) subunits including α3, α4, and β4. MHb neurons also express $GABA_B$ receptors, MORs, and cannabinoid CB1 receptors (Lee et al., 2019; Ables et al., 2023).

In summary, the MHb is a neurochemically specialized relay that channels limbic information to brainstem monoaminergic centers via the IPN (Fig. 1*A*,*C*). Its distinctive cholinergic–glutamatergic circuitry and dense expression of nAChRs and G-protein-coupled receptors (GPCRs) place it in a unique position to influence reward and mood systems, contributing to addiction vulnerability, processing of aversive experiences, and regulation of stress responses and affect.

Lateral Habenula: Anatomy and Neurochemistry

The efferent and afferent connections of the LHb were carefully reviewed by Zahm and Root (2017). While at least half a dozen cytological subregions of the LHb have been identified, at the connectivity level the LHb is roughly organized in a gradual medial-lateral gradient. Broadly speaking, it is possible to conceptualize the medial part of LHb as being connected to more "limbic" brain regions and the lateral part of LHb to more "pallidal" structures (Herkenham and Nauta, 1977; Zahm and Root, 2017).

The most studied inputs to the LHb include the entopeduncular nucleus (EPN), several regions of the hypothalamus, VTA, and ventral pallidum (VP). There is also a relatively abundant

input from the lateral septum (LS) which is understudied but notable as a brain region with high MOR expression (French and Siggins, 1980; Simon et al., 2025; Fig. 1*B*). These inputs are composed of both glutamatergic and GABAergic synaptic connections. Unlike most other brain regions, the LHb is innervated by some axons that release both glutamate and GABA, such as axons arising from the EPN and VTA (Root et al., 2014; Shabel et al., 2014; Xu et al., 2022). In other afferents, glutamate and GABA are released from separate populations of convergent neurons, including those arising from VP, lateral preoptic area of the hypothalamus (LPO), and lateral hypothalamus (Barker et al., 2017; Faget et al., 2018; Groos et al., 2025). These two different configurations are intriguing given the possibility that MOR activation can presynaptically modulate neurotransmitter release.

The LHb also receives notable inputs from monoaminergic brain centers including raphe nuclei, VTA, and locus ceruleus (LC). Serotonergic input from the raphe has been shown to inhibit GABA/glutamate inputs from EPN and reduce LHb burst firing (Vertes et al., 1999; Shabel et al., 2012; Flanigan et al., 2023; Mondoloni et al., 2024). There is also dense input to LHb from VTA, but from primarily nondopamine neurons that release glutamate and/or GABA (Swanson, 1982; Hnasko et al., 2012; Root et al., 2014; Taylor et al., 2014; Yoo et al., 2016; Warlow et al., 2025). Perplexingly, some of these LHb-projecting VTA neurons express transcripts for the dopamine neuron marker Tyrosine hydroxylase (*Th*) but may not express other dopamine markers or release detectable dopamine from their axons in LHb (Li et al., 2013; Stamatakis et al., 2013). On the other hand, dopamine receptors are expressed in the LHb and, particularly the dopamine D4 receptor, may be targeted by norepinephrine released by axons from the LC (Khaled et al., 2014; Root et al., 2015). As many LC neurons express MOR (Van Bockstaele et al., 1996), this input may be modulated by opioids.

LHb neurons have local glutamatergic collaterals and project distally to many brain regions (Herkenham and Nauta, 1977; Quina et al., 2015). This broad projection pattern is particularly intriguing given evidence that the frequency of axon collateralization is low (Goncalves et al., 2012; Petzel et al., 2017). These parallel, widespread efferents represent a striking contrast to the more focused projection of MHb neurons to IPN. Widely studied LHb projection targets include the rostromedial tegmentum (RMTg), VTA, and dorsal raphe nucleus (DRN; Zahm and Root, 2017; Fig. 1*D*). That the VTA and DRN are also innervated by the GABAergic RMTg suggests that these connections are highly organized both anatomically and with respect to timing of action potential activity. Thus, while anatomically small, the LHb connects to a large number of forebrain and brainstem areas, underlying its potent role in the regulation of mood, affect, and behavior.

Habenula Cell Types

Recent advances in single-cell (sc) and single-nucleus (sn) RNA sequencing as well as translating ribosome affinity purification (TRAP-seq) approaches have advanced our understanding of the extensive cellular and molecular heterogeneity of the MHb and LHb across vertebrate species, including zebrafish (Pandey et al., 2018), mice (Gorlich et al., 2013; Hashikawa et al., 2020; Wallace et al., 2020), and humans (Yalcinbas et al., 2025). Consistent with prior work, sc/snRNA-seq studies show that the Hb is primarily composed of glutamatergic neurons expressing *Slc17a6* (VGLUT2). As described above, the MHb contains multiple populations of glutamatergic neurons including ventrally located neurons that coexpress cholinergic markers (*Chat, Slc18a3, Slc5a7*) and dorsally located neurons that coexpress substance P (*Tac1*). Subclustering of MHb neurons suggests at least 5–6 transcriptomically distinct and topographically organized subtypes in rodents, and three subtypes in humans, that include cholinergic and substance P neurons. Each of these genetically defined MHb subtypes expresses a unique combination of marker genes for neuropeptides (e.g., *Cck*), transcription factors (e.g., *Pou4f1*), and G-protein-coupled receptors (e.g., *Gpr151*). These Hb subtypes are also functionally distinct. For example, Hashikawa and colleagues demonstrated that exposure to an acute aversive stimulus engages specific MHb and LHb subtypes and that different MHb subtypes (marked by expression of either *Spon1* or *Cck*) show unique electrophysiological properties.

The LHb also contains 4–6 genetically defined glutamatergic subpopulations delineated by unique combinations of marker genes and distinct topographical organization (Hashikawa et al., 2020; Wallace et al., 2020). While the electrophysiology and function of these molecular subtypes are less well understood, Wallace and colleagues used monosynaptic retrograde tracing combined with single-molecule fluorescence in situ hybridization to demonstrate that LHb subtypes are organized along the medial-lateral axis according to their projection targets to the VTA and DRN. For example, neurons expressing *Gpr151* localize to lateral LHb and project onto VTA and RMTg GABAergic neurons. Neurons expressing *Vgf* and *Htr2c* are in the most medial region of the LHb and project to the DRN. Human LHb cell types show similar molecular diversity and topographical organization with several medial LHb subtypes expressing conserved marker genes such as *HTR2C* (Yalcinbas et al., 2025).

Cross-species analyses demonstrated convergence of many molecularly defined cell types between zebrafish and rodents (Hashikawa et al., 2020) and rodents and humans (Yalcinbas et al., 2025). While transcriptomically defined cell types in LHb and MHb appear to be largely conserved across species, more work is needed to understand whether the connectivity and functions of these genetically defined cell types are also conserved. One species divergence may be the presence of rare neurons within the dorsal Hb of the zebrafish that express GABAergic markers (*Gad1b*, *Gad2*, *Slc32a1*; Pandey et al., 2018). Though some work suggests that there may be a small population of LHb neurons that express a subset of GABAergic markers in rodents, it remains unclear if these neurons have the capacity to release GABA (Flanigan et al., 2020; Quina et al., 2020; Waung et al., 2022). Molecular profiling studies of the rodent and human Hb have not identified populations of GABAergic neurons, but this may be due to undersampling as rare populations can be missed by sn/scRNAseq approaches.

Taken together, single-cell and spatial transcriptomics studies have revealed the molecular and anatomical heterogeneity of MHb and LHb subtypes and established key molecular markers that can be harnessed for genetic access to these cell populations to better understand their electrophysiology, connectivity, and function. These cross-species datasets can also be leveraged to computationally predict interacting cell types and nominate conserved disease-relevant cell-type and circuit-specific signaling pathways that can be functionally tested in model systems (Fig. 1*G*).

Mu-Opioid Receptor Expression and Signaling in Habenula Circuits

The habenula has long been known as a region with intense MOR binding (Herkenham and Pert, 1980; Mansour et al., 1987) and

mRNA expression (Mansour et al., 1994). However, early studies could not clearly distinguish between MHb and LHb. More recent techniques and higher resolution imaging reveal MOR protein and mRNA localized to both regions (Gardon et al., 2014; Popat et al., 2026). Across species, transcripts encoding MOR are expressed in both MHb and LHb, especially at the border between the two subdivisions. In the human Hb, *OPRM1* is most highly expressed in MHb cholinergic neurons and in two different LHb subtypes (Yalcinbas et al., 2025). In mice, *Oprm1* is expressed in a *Gpr151*-expressing LHb population and in a subset of transcriptionally defined cholinergic and *Tac1*-expressing MHb neurons (Hashikawa et al., 2020).

Electrophysiologically, MOR activation in MOR-expressing MHb neurons decreases spontaneous firing rate ex vivo and activates a hyperpolarizing conductance in a subset of MOR-expressing IPN neurons (Chittajallu et al., 2025; Singhal et al., 2025; Fig. 1*E*). MHb axon terminals in the IPN also respond to MOR activation, but with an unusual impact for opioid receptors. Here, MOR activation increases the synaptic release of glutamate and acetylcholine. A similar effect was observed after $GABA_B$ receptor activation on *Chat*-expressing MHb terminals in IPN (Zhang et al., 2016), demonstrating that at least two G_i-coupled GPCRs can drive noncanonical facilitatory effects at this synapse. This sets up an intriguing opportunity for endogenous opioid peptides to have very different impacts on activity in this circuit when released in the MHb versus the IPN.

In the LHb, MOR expression is diffuse, with greater abundance in the posterior half of the structure (Popat et al., 2026). Patterns of protein detected with immunocytochemistry suggest that a substantial proportion of MOR expression in the LHb is on fibers, likely including axonal innervation. This is consistent with whole-cell electrophysiological recordings showing that somatodendritic MOR activation produces a modest hyperpolarization in only ~1/3 of LHb neurons (Margolis and Fields, 2016). Presynaptic MOR control of glutamatergic inputs onto LHb neurons was also detected in a minority of neurons, but inhibition of neurotransmitter release in these cases was pronounced. MOR activation also inhibits GABA release onto most LHb neurons, although the effect size is somewhat modest. Interestingly, there was a convergence of pre- and postsynaptic effects: neurons that received glutamatergic inputs that were inhibited by MOR activation also showed a postsynaptic outward (inhibitory) current. The strong MOR-induced inhibition of glutamate release is relatively specific to inputs arising from the LPO, with little to no MOR inhibition of other glutamatergic inputs (Waung et al., 2022). Thus, at this specific circuit connection, MORs both presynaptically remove excitatory drive and postsynaptically inhibit activity.

Together, these anatomical and functional findings lay the groundwork for understanding how MORs in MHb and LHb contribute to a variety of behaviors.

Habenula in Pain, Opioid Seeking, and Dependence

Habenular circuits sit at the nexus of pain processing and emotional valence, positioning them to influence the transition from opioid-induced euphoria to both the aversive emotional states and physical pain associated with opioid use disorder (OUD). Indeed, recent evidence suggests the Hb is critical for regulating both the analgesic effects of MOR agonists and the aversive, negative affective states that emerge during opioid dependence and withdrawal (Boulos et al., 2020; Waung et al., 2022; Ozdemir et al., 2026). The MHb–IPN is a well-established mediator of nicotine withdrawal, aversion, anxiety, and stress-induced coping behavior (Molas et al., 2017; Klenowski et al., 2022). Studies have revealed anatomically and molecularly distinct MHb–IPN subcircuits that regulate aversion, resilience, and despair-like states (Yoo et al., 2021; Handa et al., 2025; Wang et al., 2025; Lin et al., 2026). The LHb also contributes to affect by encoding "disappointment" or negative reward prediction errors (Matsumoto and Hikosaka, 2007; Bromberg-Martin and Hikosaka, 2011), its hyperactivity is widely recognized as a core mechanism underlying depressive disorders (Li et al., 2011; Kaye and Ross, 2017; Yang et al., 2018b), and LHb modulation is being explored as a therapeutic target for clinical depression (Sartorius et al., 2010; Yang et al., 2018a). The LHb can translate aversive stimuli into behavioral adaptations by exerting strong control over the monoaminergic midbrain systems via divergent projections to VTA and DRN, both directly and indirectly through the RMTg (Jhou et al., 2009). Within this circuitry, specific efferent pathways contribute to distinct facets of depressive behaviors: LHb–DRN projections mediate passive coping and perseverative reward-seeking behaviors during stress (Coffey et al., 2020), whereas RMTg and VTA projections drive behavioral avoidance and dopamine suppression following reward omission (Proulx et al., 2014; Tian and Uchida, 2015).

MOR agonists exert analgesic effects by acting directly within the LHb but do not produce reward in the absence of pain (Waung et al., 2022). A critical input driving the aversive experience of pain originates in the LPO. Glutamatergic LPO projections to the LHb are excited by noxious stimuli and preferentially inhibited by MOR agonists. Optogenetic inhibition of these projections also produces pain relief without generating rewarding effects in the absence of pain, demonstrating a decoupling of opioid analgesia and reward. Pain also induces downstream negative affect and comorbid depressive symptoms that are modulated by a DRN to central amygdala to LHb circuit. Inhibition of this pathway precipitates depression-like behaviors while activation reduces them (Zhou et al., 2019). Molecularly, intracellular signaling via the Ser/Thr kinase RSK2 within the Hb is required to mediate MOR-induced analgesia, highlighting a specific downstream signaling cascade (Darcq et al., 2012).

One key aspect of OUD is a characteristic negative emotional state during chronic use, withdrawal, and abstinence. Avoidance of withdrawal is a principal driver of relapse (Welsch et al., 2020). A transition from opioid-induced positive reinforcement to negative affect can happen rapidly, even within a single opioid-taking session. During oral fentanyl self-administration, animals transition from a "loading" phase (characterized by positive 50 kHz vocalizations) to a "maintenance" phase (characterized by negative 22 kHz vocalizations). Photometry recordings have shown that LHb reactivity to fentanyl-associated cues is amplified while in this negative affective state (Coffey et al., 2023).

The LHb and MHb govern distinct facets of the withdrawal syndrome via separate output pathways. MOR signaling in *Chnrb4*-expressing MHb neurons normally functions to limit aversion, and when MOR is genetically deleted from *Chnrb4*-expressing neurons, systemic opioid antagonist administration is no longer aversive (Boulos et al., 2020). Further, this knock-out mitigates somatic signs of opioid withdrawal. Optogenetic stimulation of MOR-expressing habenular neurons projecting to the IPN triggers behavioral despair. It is intriguing that this type of stimulation may generate activity similar to what happens when MORs are activated on MHb terminals in the IPN (Singhal et al., 2025). Conversely, optogenetic stimulation of MOR-expressing habenular neurons that project to the DRN triggers anxiety-like behaviors (Bailly et al., 2023; Fig. 1*E*). In

mice, high doses of the opioid receptor antagonist naloxone induced conditioned place avoidance (CPA) and increased intracellular calcium in MOR-expressing habenular neurons in opioid-naive mice (Ozdemir et al., 2026). In opioid-dependent mice, a lower dose of naloxone was sufficient to drive similar responses. Chemogenetic inhibition of MOR-expressing habenular neurons prevented naloxone-induced CPA in both opioid-naive and opioid-dependent mice and attenuated somatic withdrawal signs in dependent mice, indicating increased sensitivity and functional relevance of these neurons during opioid withdrawal. Directly downstream of MHb, ablation of nucleus incertus-projecting IPN GABA neurons similarly blunted naloxone-induced CPA in dependent mice (Liang et al., 2024).

While the MHb influences somatic withdrawal and generalized aversion via the IPN, LHb output to raphe nuclei modulates specific behavioral deficits during withdrawal (Clerke et al., 2021). Withdrawal from opioids caused a depressive-like behavior state driven by aberrant excitatory transmission in DRN-projecting LHb neurons. Morphine withdrawal also decreased the ratio of AMPA to NMDA receptor-mediated currents when stimulating excitatory inputs onto raphe- but not VTA-projecting LHb neurons (Valentinova et al., 2019). Chemogenetic inhibition of this connection produces social deficits that are similar to those observed during opioid withdrawal. Conversely, inhibiting raphe-projecting LHb neurons under highly stressful, nondrug conditions promotes resilience and increases active coping (Coffey et al., 2020), suggesting the behavioral impact of inhibiting the LHb projection to the raphe is highly dependent upon behavior state.

Chronic opioid exposure induces profound structural and transcriptomic alterations in the entire Hb. In humans that had OUD and used heroin, significant volumetric reductions and decreased neuronal cell counts are observed across both MHb and LHb compared with healthy controls (Muller et al., 2021). At the molecular level, chronic fentanyl self-administration in rats triggered transcriptional adaptations in the Hb including upregulation of genes associated with synaptic transmission and ionic conductance and downregulation of genes governing extracellular matrix, myelination, and glial support (Magnard et al., 2025).

Recent studies also underscore the essential role of non-neuronal cells in Hb function. In fact, the depression of glutamatergic synaptic transmission onto raphe-projecting LHb neurons that is observed during morphine withdrawal is dependent on neuroimmune signaling (Valentinova et al., 2019). Morphine withdrawal induces structural adaptations in local microglia and provokes the release of tumor necrosis factor-alpha (TNF-α). This cytokine acts on neuronal TNF receptor 1 (TNF-R1) in the LHb to reduce AMPAR transmission; deleting TNF-R1 in the LHb rescues the social deficits induced by morphine withdrawal. Glial-neuronal interactions are also essential in the MHb. The adult MHb relies on glial cells to tune neuronal activity via unconventional excitatory glycinergic NMDA receptors, a mechanism that is required for the expression of negative affective states and lithium-induced CPA (Otsu et al., 2019).

Parallels to Nicotine and Other Abused Substances

Opioid addiction shares some core biological and behavioral features with nicotine dependence and with other substance use disorders, despite differences in pharmacology. As described above, the MHb–IPN pathway is enriched in α3, α5, and β4 nAChRs encoded by the *CHRNA5–CHRNA3–CHRNB4* gene cluster and is recognized as a major substrate for nicotine-induced aversion, withdrawal, and anxiety-like states (Fowler et al., 2011; Frahm et al., 2011; Antolin-Fontes et al., 2015). Human genetic studies converge on this pathway: common and rare variants in *CHRNA5*, and to a lesser extent in *CHRNB4* and *CHRNA3*, are associated with nicotine dependence risk and with vulnerability to alcohol and cocaine use disorders (Bierut, 2009; Hallfors et al., 2013; Haller et al., 2014; Lassi et al., 2016; Wills et al., 2022). The best characterized risk allele, *CHRNA5* rs16969968, increases risk for heavy smoking and related morbidity, and knock-in mice carrying *Chrna5* or *Chrnb4* risk variants show increased nicotine intake and decreased aversion (Fowler et al., 2011; Frahm et al., 2011; Slimak et al., 2014; Husson et al., 2020). Manipulating α5/β4-containing nAChRs in the MHb–IPN axis changes both somatic and affective nicotine-withdrawal signs as well as nicotine intake, indicating that this circuit normally constrains consumption. Importantly, the *Chrna5* risk variant that promotes nicotine dependence also enhances morphine reward in female mice and has been linked to alcohol use phenotypes, suggesting that a partial loss of function of α5-containing nAChRs represents a shared susceptibility mechanism across nicotine, opioid, and alcohol addiction (Husson et al., 2020; Brynildsen et al., 2022; Wills et al., 2022). Human imaging work also reports altered habenular connectivity in opioid users carrying *CHRNA5* rs16969968, further underscoring the translational relevance of this pathway (Curtis et al., 2017).

Recent work shows that opioid receptors are expressed on these same habenular pathways. Specifically, MORs are highly enriched in a subset of MHb neurons that express β4-containing nAChRs, and conditional deletion of *Oprm1* in this β4-positive population reduces naloxone-induced place aversion and both morphine- and mecamylamine-precipitated withdrawal, with little effect on morphine reward (Boulos et al., 2020). Further, intra-MHb or intra-IPN administration of the α3β4 nAChR antagonist 18-methoxycoronaridine decreases morphine self-administration and withdrawal in rodents (Glick et al., 2000, 2006, 2008; Panchal et al., 2005). Interestingly, chronic nicotine shifts glutamatergic and GABAergic signaling within the MHb–IPN pathway and gradually biases the circuit from encoding low-dose nicotine aversion toward permissive, high-level intake (Fowler et al., 2011; Frahm et al., 2011; Antolin-Fontes et al., 2015; Wills et al., 2022).

The cannabinoid system adds a further axis of convergence. Anatomical and functional studies reveal robust CB1 receptor expression on MHb axon terminals in the IPN and on GABAergic inputs to the MHb from the medial septum. CB1 receptor signaling on MHb axon terminals selectively inhibits cholinergic transmission and modulates the expression of aversive memories (Hsu et al., 2014; Soria-Gomez et al., 2015). Because MHb neurons corelease glutamate and acetylcholine at IPN synapses, changes in glutamate and ACh release from terminals enriched with MOR and CB1 are expected to alter activation of presynaptic nAChRs on habenular terminals and postsynaptic nAChRs in the IPN, thereby tuning circuit responses to both nicotine and opioids (Antolin-Fontes et al., 2015).

Taken together, these findings position the Hb as a generalized "withdrawal hub" through which overuse of opioids, nicotine, cannabinoids, alcohol, and stimulants drive dysphoria, anxiety, and relapse. They further suggest that targeting receptors expressed in the habenula may provide therapeutic strategies that span multiple substance use disorders.

Orphan GPCRs as Therapeutic Targets

One promising category of therapeutic targets that have received recent attention is orphan GPCRs that are highly expressed in

the Hb (Lorente et al., 2025; Fig. 1*F*). Not only are these receptors enriched in specific subpopulations of habenular neurons, but their expression is also quite low outside of the Hb, raising the possibility that the incidence of unwanted side effects from pharmacologically targeting these receptors will be low. Two examples, GPR151 (Broms et al., 2015; Antolin-Fontes et al., 2020; Allain et al., 2022; Ibanez-Tallon et al., 2026) and GPR139 (Wang et al., 2019; Ozdemir et al., 2026), illustrate how Hb-enriched receptors may interact with opioid-sensitive circuits.

GPR151 is preferentially expressed in MHb and LHb neurons compared with the rest of the CNS. It localizes along axons and synaptic terminals where it is enriched near the active zone and synaptic vesicles and is also found in the somatodendritic region of Hb neurons (Broms et al., 2015; Antolin-Fontes et al., 2020). GPR151 is $G_{i/o}$-coupled and preferentially engages $G_{\alpha o}$, lowers cAMP, and acts as a brake on glutamatergic transmission and synaptic plasticity. Knock-out studies indicate that GPR151 contributes to Hb-dependent behaviors, including responses to nicotine and opioids (Antolin-Fontes et al., 2019, 2020) and social reward (Allain et al., 2022). Specifically, in GPR151 knock-out mice, there is a reduction in the aversive impact and motivational "off-signal" for nicotine and opioids, producing blunted sensitivity, but increased self-administration. Reexpression of GPR151 in habenular neurons returned self-administration to control levels, demonstrating that its role in drug reinforcement is circuit specific rather than a generalized developmental consequence of the knock-out. Work on social reward showed that both MOR and GPR151 in the Hb contribute to social preference. Deletion of either in *Chrnb4*-expressing neurons decreased social interactions with novel mice compared with controls, supporting the idea that inhibitory GPCR signaling in these structures may dampen aversive tone and permit rewarding social interaction. This provides a conceptual framework in which GPR151 may tune habenular MOR neurotransmission and influence vulnerability to addictive behaviors across drug classes.

GPR139 provides a complementary example. It is an orphan GPCR also enriched in the Hb and a limited number of other brain regions that contribute to motivation and reward processing (Wang et al., 2019). GPR139 actions can oppose MOR signaling, including through activation of G_q-dependent pathways. This makes GPR139 particularly attractive in the context of opioid dependence, where therapeutic goals may include the reduction of withdrawal-associated distress. Indeed, new findings show that pharmacological blockade of GPR139 can decrease the aversive impact of naloxone-precipitated morphine withdrawal (Ozdemir et al., 2026).

A model may thereby be emerging where GPR151 sets the synaptic gain of aversion-signaling habenular pathways in a manner that cooperates with MOR, whereas GPR139 acts as an opposing "anti-opioid" system. This conceptual framework supports a strategy in which orphan GPCRs that are coexpressed with MOR could be targeted to modulate specific negative-affect circuits in the context of pain, addiction, or mood disorders. By avoiding the broad physiological and behavioral effects associated with the direct targeting of MOR across central and peripheral systems, such a strategy may minimize off-target effects that limit the clinical utility of MOR agonists and antagonists.

Concluding Remarks

The studies reviewed here support an updated view of the Hb in opioid biology. Rather than acting as a uniform aversion center, the Hb contains molecularly, anatomically, and functionally distinct subcircuits through which MOR signaling shapes emotional state and addiction-related behavior. In the MHb, MOR-enriched pathways projecting to IPN regulate aversion, withdrawal, and drug dependence. In the LHb, distinct neuronal ensembles may encode punishment, safety, drug seeking, and outcome evaluation, providing a circuit substrate through which subjects weigh whether to suppress or persist in opioid-seeking behaviors despite negative consequences. Across both subdivisions, MOR may act as a state-dependent regulator of negative affect.

A central concept emerging from recent work is that opioid dependence may alter the gain of habenular circuits. In opioid-naive subjects, endogenous opioid signaling may constrain habenular output that amplifies aversive experiences and thereby shape emotional homeostasis. But during dependence and withdrawal, habenular neurons may be more readily excited, driving enhanced aversive learning and negative affective states. This framework provides a circuit-level explanation for why withdrawal-associated distress is such a powerful driver of relapse. Recent advances in single-cell and spatial transcriptomics, circuit-specific manipulations, and human molecular profiling have revealed a rich diversity of habenular cell types and signaling pathways. These findings suggest that nAChR subtypes, CB1s, and Hb-enriched orphan GPCRs are distributed in patterns that make interactions with MORs likely.

Despite this progress, several fundamental questions remain. Which MHb and LHb cell types express functional MORs across species, and how are these populations organized within defined circuits? What are the relative contributions of somatic, presynaptic, and projection-specific MOR signaling to habenular function, and how are these altered by chronic opioid exposure? An important challenge will be to determine whether MOR-expressing habenular neurons participate in distinct ensembles encoding punishment, safety, withdrawal, drug seeking, or relapse. Finally, the extent to which molecular and circuit mechanisms identified in animal models are conserved in the human Hb and how genetic risk variants and individual differences in habenular connectivity contribute to OUD, depression, and related affective disorders remain largely unknown.

The broader implication is that MOR-expressing habenular circuits may provide a tractable entry point for understanding the emotional burden of OUD. In this context, orphan GPCRs and other Hb-enriched signaling systems are particularly promising because they may allow circuit-selective modulation of opioid-sensitive pathways while avoiding the broader liabilities associated with direct manipulation of MOR signaling. Addressing these questions will be essential for translating mechanistic insights into new therapeutic strategies targeting negative affect, withdrawal, and relapse vulnerability.

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