HHS Public Access

Author manuscript

J Neurochem. Author manuscript; available in PMC 2019 September 12.

Published in final edited form as:

J Neurochem. 2017 August ; 142(Suppl 2): 130–143. doi:10.1111/jnc.14008.

The medial habenula and interpeduncular nucleus circuitry is critical in addiction, anxiety, and mood regulation

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Abstract

Abstinence from chronic use of addictive drugs triggers an aversive withdrawal syndrome that compels relapse and deters abstinence. Many features of this syndrome are common across multiple drugs, involving both affective and physical symptoms. Some of the network signaling underlying withdrawal symptoms overlaps with activity that is associated with aversive mood states, including anxiety and depression. Given these shared features, it is not surprising that a particular circuit, the dorsal diencephalic conduction system, and the medial habenula and interpeduncular nucleus (MHb-IPN) in particular, have been identified as critical to the emergence of aversive states that arise both as a result, and independently, of drug addiction. As the features of this circuit continue to be characterized, the MHb-IPN axis is emerging as a viable target for therapeutics to aid in the treatment of addiction to multiple drugs of abuse as well as mood-associated disorders.

Keywords

opioid; alcohol; psychomotor stimulants; medial habenula; interpeduncular nucleus; epithalamus; reward; withdrawal; depression; anxiety; stress

Introduction

Drug addiction is a substantial public health and societal burden, causing over $700 billion in annual costs associated with crime, lost work productivity, and healthcare (National Center for Chronic Disease *et al.* 2014, Sacks *et al.* 2015, Center 2011). Although they have unique pharmacological effects, all addictive drugs share actions on the dopaminergic system that contribute to their rewarding effects (Di Chiara & Imperato 1988). The

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Conflict of interests

All authors declare that they do not have any conflicts of interest to report.

withdrawal syndrome that follows cessation of chronic use has also been identified as having common substrates. Anxiety and mood disorders, the first and second most common psychiatric disorders in the United States (Kessler *et al.* 2005), may also reflect alterations in overlapping circuits. Interestingly, research characterizing the mechanisms underlying addiction, withdrawal, and aversive moods states has implicated a common pathway. The medial habenula (MHb) and interpeduncular nucleus (IPN) are two components of the dorsal diencephalic conduction system (DDC), a highly evolutionarily conserved pathway which, along with the medial forebrain bundle, conveys signals from the limbic forebrain to the midbrain and hindbrain (Bianco & Wilson 2009, Okamoto *et al.* 2012). In higher vertebrates, the DDC has evolved to be a significant pathway by which the forebrain regulates midbrain motivation and reward circuitry (Sutherland 1982). The epithalamic MHb receives inputs from a variety of structures, including the triangular septal nucleus, septofimbral nucleus, ventral tegmental area, and raphe nuclei (Herkenham & Nauta 1977, Lecourtier & Kelly 2007, Phillipson & Pycock 1982). Studies also indicate that the MHb receives inputs from the nucleus accumbens (Sutherland 1982), locus coeruleus and superior cervical ganglion (Gottesfeld 1983), diagonal band nucleus and medial septum (Qin & Luo 2009), as well as the median raphe nucleus (Sutherland 1982, Conrad *et al.* 1974). Some studies suggest that the MHb may project to the pineal body (Ronnekleiv & Moller 1979, Guglielmotti & Cristino 2006), and it may also send sparse efferents to the VTA (Cuello *et al.* 1978), as well as extend *boutons en passant* to the LHb (Kim & Chang 2005). However, the MHb sends its most dense efferent projections to the mesencephalic IPN through the core of the fasciculus retroflexus (FR) (Herkenham & Nauta 1979), which, in turn, sends efferents to a wide variety of mid- and hindbrain structures implicated in regulating affective states. Those structures include the dorsal tegmental nucleus (Shibata & Suzuki 1984), hippocampus (Shibata & Suzuki 1984, Baisden *et al.* 1979, Wyss *et al.* 1979), lateral hypothalamus (Massopust & Thompson 1962, Morley 1986, Kemali & Guglielmotti 1982, Smith *et al.* 1980), ventral tegmental area (Hayakawa *et al.* 1981, Smaha & Kaelber 1973), septum, preoptic area, and nucleus of the diagonal band (Smaha & Kaelber 1973, Morley 1986). Additionally, there are data indicating projections from the IPN to the dorsal and median raphe nuclei (Groenewegen *et al.* 1986, Behzadi *et al.* 1990), as well as the lateral habenula (Massopust & Thompson 1962, Morley 1986). Though the major source of innervation in the IPN arrives from the MHb, there is evidence of afferents arriving from structures including the horizontal limbs of the diagonal band nucleus (Contestabile & Flumerfelt 1981), substantia innominata (Vertes & Fass 1988), infralimbic region of the medial prefrontal cortex (Takagishi & Chiba 1991), preoptic nucleus (Shibata *et al.* 1986), hypothalamic nuclei (Contestabile & Flumerfelt 1981, Hamill & Jacobowitz 1984), supramammillary nucleus (Contestabile & Flumerfelt 1981, Hamill & Jacobowitz 1984), raphe nuclei (Conrad et al. 1974), nucleus incertus (Hamill & Jacobowitz 1984) and dorsal tegmental nucleus (Hamill & Jacobowitz 1984). While some of these anatomical studies were conducted recently, some were conducted many years ago, so corroborative reproducibility experiments are likely warranted, given the availability of more targeted tracer techniques. A summary of these afferent and efferent projections for the MHb and IPN is available in figure 1 and tables 1 and 2.

While both are fairly small anatomical structures, the MHb and IPN host the synthesis and release of a wide variety of neurotransmitters. Studies have identified acetylcholine (McCormick & Prince 1987), substance P (SP) (Burgunder & Young 1989, De Biasi *et al.* 2016, Jackson *et al.* 2015), glutamate and GABA (Qin & Luo 2009), norepinephrine (Gottesfeld 1983), serotonin (Kinsey *et al.* 2001), ATP (Edwards *et al.* 1992, Sperlagh *et al.* 1998), interleukin-18 (Sugama *et al.* 2002), and a host of neuropeptides in the MHb-IPN pathway (McLaughlin *et al.* 2015, Kopp *et al.* 2002). Additionally, the MHb-IPN circuit has been implicated in mechanisms that mediate some of the acute and aversive features of withdrawal from multiple drugs, including alcohol, opiates, nicotine, and other stimulants. This review will focus on studies that specifically implicate the DDC, and the MHb-IPN pathway in particular, in the neurophysiology associated with both addiction and mood-related psychiatric conditions, with an eye towards the possibility of identifying druggable targets within the pathway that may yield therapeutic benefits for the treatment of both sets of conditions.

Alcohol

The pharmacological activity of ethanol encompasses a broad range of targets in the central nervous system, and studies have indicated that the MHb-IPN circuit represents a significant component of its affective and behavioral effects. Local cerebral glucose utilization rates in alcohol-preferring rats are significantly elevated in both divisions of the habenular complex relative to non-preferring rats (Smith *et al.* 2001). Data from our lab have identified an interaction between ethanol and nicotine withdrawal, as well as a role played by nAChRs in the MHb or IPN in ethanol withdrawal. Specifically, intraperitoneal injection of a non-selective nAChR antagonist, mecamylamine, is capable of precipitating a withdrawal syndrome in mice chronically treated with ethanol. Withdrawal symptoms are also exhibited when mecamylamine is infused into the MHb or IPN, but not the ventral tegmental area (VTA) or hippocampus (Perez *et al.* 2015), indicating that nAChR blockade in the MHb-IPN circuit is sufficient to precipitate the ethanol withdrawal syndrome. Another recent finding has identified changes in signaling molecules associated with apoptosis, inflammation, neurodegeneration, and senescence in the habenula, among other structures, following chronic treatment with ethanol (Roux *et al.* 2015). Receptor knock-out studies have shown that neuropeptide Y (NPY) acts as an important regulator of alcohol intake, with knock-out mice exhibiting increased alcohol ingestion relative to wild-type mice (Thiele *et al.* 2002). More recently, it was shown that alcohol-preferring rats exhibit an absence of NPY mRNA in the MHb while non-preferring rats do have a signal of mRNA for the neuropeptide (Hwang *et al.* 2004). Taken together, the literature suggests that activity in the MHb-IPN circuit likely modulates the effects and ingestive behavior of alcohol, as well as its withdrawal symptoms. Variations between individuals in the signaling within this circuit may underlie predispositions to pathological intake patterns.

Opioids

Drug overdose represents the leading cause of accidental death in the United States (Medicine 2016), and addiction to opioids, including both illicit substances, such as heroin, and prescription analgesics, such as oxycodone and fentanyl, drive this epidemic (Prevention

2015). Interestingly, overdose fatalities, sales, and substance use disorder treatment admissions have increased in parallel from 1999 to 2008 (Paulozzi 2014), indicating a significant need for improved treatments for substance abuse disorder and opioid addiction in particular.

One of the densest regions of opioid receptor expression is the DDC, and the MHb, FR, and IPN are particularly rich with expression (Gackenheimer *et al.* 2005, Gardon *et al.* 2014, Zhu *et al.* 1998, Sim-Selley *et al.* 1999). Additionally, there is a plethora of neurotransmitters that the MHb-IPN circuit synthesizes or is sensitive to, including acetylcholine, neurokinins, interleukin-18 (IL-18) (Viswanath *et al.* 2013, Sugama et al. 2002), and purines (Pankratov *et al.* 2009, Pankratov *et al.* 2006, Kanjhan *et al.* 1999) that may be modulated by the activities of opioids. Given the broad efferent targets of the IPN that are known to regulate affect and substance use, including the raphe nuclei, nucleus incertus, lateral septum, lateral dorsal tegmentum (LDTg), and hypothalamus (Sutherland 1982, Ryan *et al.* 2011, Bianco & Wilson 2009, Morley 1986, Gardon et al. 2014), the MHb-IPN circuit is likely an anatomical node, centrally involved in the signaling underlying both acute effects of, and withdrawal from, opioids. Evidence over the past several decades has corroborated such a role. For example, lesions of the MHb have been observed to induce hyperalgesia and increase the analgesic efficacy of morphine (Meszaros *et al.* 1985), and morphine is capable of inducing analgesia when infused directly into the habenular complex (Cohen & Melzack 1985). When evaluating the effects of intracranial self-stimulation of the VTA on opioid release, a unique reduction of endogenous opioid binding was observed in the MHb (Stein 1993). Altered acetylcholinesterase (AChE) activity is observed in the MHb following chronic morphine administration, and precipitation of withdrawal with the opioid receptor antagonist, naloxone, results in altered AChE activity in the IPN (Neugebauer *et al.* 2013). Additionally, chronic morphine administration induced a trend towards increased nAChR expression in the MHb (Neugebauer et al. 2013). Acute administration of 18-methoxycoronaridine (18-MC), an α3β4 nAChR antagonist, reduced signs of naltrexone-precipitated withdrawal from morphine (Rho & Glick 1998), an effect that appears to be mediated by activity in the MHb and IPN (Panchal *et al.* 2005, Taraschenko *et al.* 2007). 18-MC was also observed to reduce morphine self-administration upon intracranial infusion into the MHb or IPN (Glick *et al.* 2006). In the MHb, RSK2, a component of the ribosomal S6 kinase 90kDa family, which act as substrates of extracellular-regulated kinases 1 & 2 to regulate cytosolic and nuclear targets, has been identified as critical to morphine-induced analgesia (Darcq *et al.* 2012). Finally, the LDTg, an efferent target of the IPN, has been shown to exhibit significant increases in vesicular ACh transporter markers following chronic morphine administration (Bajic *et al.* 2015, Gardon et al. 2014). Altogether, these data implicate the DDC, and MHb-IPN circuit in particular, in the signaling underlying some of the acute effects of opioids, as well as aspects of their addictive properties. Furthermore, adaptations in cholinergic components may represent a significant facet of these changes.

Nicotine and Psychomotor Stimulants

The role of the lateral division of the habenular complex (LHb) in regulating dopaminergic activity in the VTA via the rostromedial tegmental nucleus has been established, and represents an important mechanism by which aversion and addiction are modulated (Barrot

et al. 2012, Sanchez-Catalan *et al.* 2016, Jean-Richard Dit Bressel & McNally 2014, Quina *et al.* 2015). Both the LHb and MHb send dense efferent projections through the fasciculus retroflexus, and anatomical studies suggest that projections emerging from the MHb course through the core and those from the LHb through the sheath of this dense fiber bundle (Bianco & Wilson 2009). Degeneration of dopaminergic fibers in the caudate following chronic exposure to psychomotor stimulants like amphetamines, was observed decades ago (Ellison *et al.* 1978). In addition to dopaminergic fibers, similar degeneration has been observed in axons populating the sheath of the FR following chronic exposure to cathinone, cocaine, amphetamine, methamphetamine, and MDMA (Ellison 2002, Carlson *et al.* 2000). In rats treated with cocaine, increased expression of Fos-protein, a marker of neuronal activation, was observed in the MHb associated with cue-induced reinstatement (James *et al.* 2011). Additionally, similar to its interference with opioid-derived reward, 18-MC administration results in reduced methamphetamine self-administration in rats (Glick *et al.* 2000). Once again, direct infusions into the MHb and/or IPN induced similar reductions of self-administration, with what appears to be greater efficacy when infused into the IPN, suggesting that activity in the MHb-IPN circuit likely mediates this effect (Glick *et al.* 2008). Finally, methamphetamine and cocaine have been shown to increase extracellular concentrations of ACh in the IPN, with cocaine inducing a dose-dependent biphasic effect (Hussain *et al.* 2008).

Probably the best-characterized activity of a drug in the MHb-IPN pathway is that of nicotine, perhaps attributable to the considerable density of a variety of nAChRs that populate the structure (Mugnaini *et al.* 2002). Studies have suggested that up 90–100% of MHb neurons express nAChRs, with the majority containing the α3, α4, α5, β2, and/or β4 subunits (Viswanath et al. 2013, Sheffield *et al.* 2000). Some data suggest that approximately 20% of nAChRs in the MHb expressed by neurons that project to the IPN contain the α5 subunit (Picciotto & Kenny 2013, Grady *et al.* 2009). In the IPN, high levels of α2 subunit-containing nAChRs can be found (De Biasi & Salas 2008, Grady et al. 2009), and the distributions of nAChRs composed of specific subunit combinations can help distinguish subnuclei within both the MHb and IPN (Shih *et al.* 2014).

As many reviews of the effects of nicotine in this circuit have been written over the years (McLaughlin et al. 2015, De Biasi *et al.* 2014, De Biasi & Dani 2011, Dani & De Biasi 2001, Jackson et al. 2015), this section will focus on recent advances in characterizing the effects of chronic use and withdrawal from nicotine in the MHb-IPN pathway. For example, it was recently shown that, during withdrawal from nicotine, the spontaneous action potential frequencies in MHb cholinergic neurons are doubled after mice are administered nicotine relative to mice in withdrawal treated with saline (Gorlich *et al.* 2013). Further, these studies demonstrated that the pacemaking activities of MHb cholinergic neurons are determined by the activities of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, and pharmacological inhibition of these HCN channels resulted in the manifestation of nicotine withdrawal-associated behaviors, including both somatic and anxiety-associated symptoms. A study from our lab showed that nicotine enhances the intrinsic excitability of MHb neurons by activating α5- containing nAChRs, which results in the facilitation of neurokinin release onto NK1 and NK3 receptors (Dao *et al.* 2014). Notably, pharmacological blockade of NK1 & NK3 receptors in the MHb of mice

chronically treated with nicotine resulted in the precipitation of somatic symptoms of nicotine withdrawal. Further implicating the α5-containing nAChRs in the MHb in the physiology of nicotine addiction and withdrawal, mice lacking expression of the subunit self-administer doses of nicotine at levels that are aversive to wild-type mice, and virus-mediated re-expression of the subunit in the MHb rescues self-administration to levels resembling those consumed by wild type mice (Fowler *et al.* 2011a). Additionally, mice lacking the α5 nAChR subunit exhibit reduced IPN activation following exposure to nicotine relative to wild type mice, suggesting a significant role played by this subunit in the MHb-IPN pathway in determining the range of nicotine doses capable of facilitating activity in brain reward circuitry (Fowler *et al.* 2011b, Fowler *et al.* 2013). Mice lacking the α2 nAChR subunit exhibit elevations of both glutamate and GABA in the IPN, suggesting that α2-containing nAChRs may participate in the signaling underlying the effects of nicotine (Lotfipour *et al.* 2013). Studies have also demonstrated a significant role played by IPN signaling in somatic symptoms of nicotine withdrawal by the IPN, with GABAergic neuronal activation enhanced by increased glutamate release, perhaps from the MHb (Zhao-Shea *et al.* 2013). This group also showed that pharmacological inhibition of NMDA receptor activation reduced symptoms of withdrawal, suggesting a glutamatergic signal from the MHb playing a significant role in the nicotine withdrawal syndrome. Following chronic nicotine exposure, mecamylamine infusion into the MHb or IPN has been observed to induce anxiety-associated behaviors and symptoms of nicotine withdrawal (Zhao-Shea *et al.* 2015, Salas *et al.* 2009). Given that mecamylamine infusion into nicotine-naïve mice does not result in significant changes in anxiety-associated behavior, the MHb-IPN axis represents a node of neuroplastic adaptations resulting from chronic nicotine exposure that underlie the affective and somatic symptoms of nicotine withdrawal upon cessation.

Accordingly, while not necessarily a direct pharmacological target of most psychomotor stimulants apart from nicotine, the MHb-IPN circuit likely represents a system that modulates the acute effects of psychomotor stimulants, and contributes to signaling underlying withdrawal and relapse. A summary of the signaling associated with drug action in this pathway is available in table 3.

Depression-like behavior

In addition to playing a role in the acute effects of multiple drugs and the manifestation of their withdrawal, the MHb-IPN circuit has been implicated in mood-associated conditions. For example, one group worked to identify brain regions exhibiting activation during uncontrollable stress that then correlated with a subsequent manifestation of helplessness behaviors (Mirrione *et al.* 2014). Using positron emission tomography (PET) with rats, the habenula and lateral septum emerged as central in a network of brain regions, corroborating a role played by these structures in vulnerability to uncontrollable stress (Mirrione et al. 2014). An evaluation of the effects of chronic mild stress in rats on somatostatin (SST) receptor expression and SST release revealed that this signaling system significantly changes in the MHb (Faron-Gorecka *et al.* 2016). In fact, SST2 receptor expression in the MHb, and peripheral SST concentration in plasma, were identified as particularly sensitive biochemical indicators of whether an animal would be stress responsive or non-responsive. An effort to characterize changes in the expression of microRNA (miRNA) species in the MHb and LHb

of rats that underwent learned helplessness identified six miRNAs that were significantly altered (Svenningsen *et al.* 2016). The miRNA species identified are associated with MAPK, neutrophin, and ErbB signaling pathways.

Though the MHb is a relatively small structure, it can be segregated into subnuclei based upon localization of neurotransmitter and transporter protein expression in specific regions. The MHb is glutamatergic, and is often sub-divided into a dorsal sub-region, which expresses SP, and a ventral sub-region, which expresses ACh (Kobayashi *et al.* 2013). Some studies suggest that it can be further divided based on transporter protein and receptor densities (Aizawa *et al.* 2012). Moreover, efferent projections from the MHb target the IPN topographically, with efferents from the dorsal MHb targeting the lateral IPN, those from medial regions of the MHb terminating in the ventral IPN, and those from the lateral MHb terminating in the dorsal IPN (Bianco & Wilson 2009). Given the neurochemical differences in these topographic projections, it is not surprising that studies suggest these anatomical distinctions correspond to different functional roles (Ichijo & Toyama 2015). As altered physical activity and anhedonia are diagnostic indicators of major depressive disorder according to the Diagnostic and Statistical Manual of Mental Disorders (Association 2013), the MHb has been evaluated as a regulator of analogous behaviors like wheel running activity (WRA) in rodents (Hsu & Wang 2014). Working with a genetic ablation model of the dorsal subnucleus of the MHb, reduced WRA and sucrose preference was observed, both of which are indicative of depressive-like behaviors. Interestingly, despite no known direct synaptic connection with dopaminergic populations, mice exhibited a significant preference for optogenetic intracranial self-stimulation of the dorsal MHb (Hsu & Wang 2014). Conversely, inhibition of the terminals arriving in the IPN from the dorsal MHb yielded place aversion. This corroborates studies that characterized metabolic differences in brain regions of rats bred for susceptibility to helplessness, which exhibited significantly increased markers of metabolic activity in both the lateral and medial habenula (Shumake *et al.* 2003). Using the same metabolic profiling method in Holtzman rats, a strain that exhibits susceptibility to stress-evoked helplessness, Padilla and colleagues characterized the effects of a two-week treatment with fluoxetine following a learned helplessness paradigm (Padilla *et al.* 2011). The group identified a protective effect of fluoxetine against depression-like behaviors in the forced swim test relative to rats treated with vehicle. This protective effect correlated with changes in regional metabolic activity in a variety of brain networks, including the habenular complex, IPN, and dorsal raphe nucleus (Padilla et al. 2011). In particular, fluoxetine treatment was associated with comparatively stronger positive correlations with prefrontal regions, including the dorsal, orbital, and prelimbic cortices. The IPN was positively correlated with the lateral orbital cortex in rats treated with fluoxetine, and this correlation was not present in vehicle-treated rats. Finally, metabolic markers in the dorsal raphe become somewhat positively correlated with the habenula in fluoxetine-treated rats, while strongly negatively correlated among the vehicle-treated group.

Due to challenges derived from the small size and sub-cortical location of the MHb-IPN circuit, the majority of studies characterizing its function have been performed in animal models. However, a few studies with humans have corroborated a role played by the circuit in depression. Accumulating literature over the past decade indicates that ketamine may represent a novel pharmacological treatment strategy for major depressive disorder (Han *et*

al. 2016, Burger *et al.* 2016, Zarate *et al.* 2006, Murrough *et al.* 2013). When evaluating changes in cerebral glucose metabolism using PET, 20 unmedicated patients with treatment-resistant major depressive disorder were scanned before and after a ketamine infusion. The habenular complex, among several other brain regions, exhibited reduced metabolism in association with a rapid antidepressant effect of ketamine (Carlson *et al.* 2013). Finally, a post-mortem study of sections from the brains of patients diagnosed with various mood disorders identified significant reductions in the volumes, cell numbers, and mean cell areas in the MHb of depressive patients (Ranft *et al.* 2010).

Anxiety, fear, and stress

While anxiety and fear have been associated for quite some time with anatomical structures including the amygdala, hippocampus, hypothalamus, and periaqueductal gray, recent research has implicated the DDC in these behaviors as well (Okamoto & Aizawa 2013). A genetic lesion study indicates that selective elimination of MHb afferents arriving from two forebrain structures, the triangular septum and bed nucleus of the anterior commissure, results in disrupted anxiety- and fear-associated behaviors (Yamaguchi *et al.* 2013). In particular, lesions of the projections from the triangular septum, which terminate in the ventral subnucleus of the MHb, disrupted anxiety-associated behavior. Conversely, lesions of projections arriving from the bed nucleus of the anterior commissure, which terminate in the dorsal subnucleus of the MHb, disrupted fear-associated behavior. Following both acute and chronic restraint stress in rats, significant elevations of the pro-inflammatory cytokine, IL-18, have been observed in the dorsal MHb (Sugama et al. 2002). Mast cells are another immune system-associated signaling component that appears to be sensitive to environmental stressors and aversive mood states (Georgin-Lavialle *et al.* 2016, Nautiyal *et al.* 2008, Silver & Curley 2013, Frenzel & Hermine 2013). Mice treated with a 3-week behavioral subordination paradigm, either via exposure to an aggressor or placement in a clean cage, exhibited increased numbers of mast cells in the habenula, thalamus, and hypothalamus (Cirulli *et al.* 1998).

When characterizing fear-associated behavior in zebrafish, inhibition or lesion of the dorsal habenula (analogous to MHb in mammals) resulted in elevated freezing behaviors in response to a conditioned fear stimulus (Agetsuma *et al.* 2010, Lee *et al.* 2010). Both control and habenula-disrupted fish froze upon first exposure to an electric shock, but as subsequent shocks were administered, control fish exhibited reduced freezing behavior while those with disrupted habenular function exhibited no such behavioral adaptation. Another study identified behavioral signatures of elevated baseline anxiety in zebrafish following dorsal habenula lesions, including responses to novel environments and alarm substance secretion in response to overhead shadows (Mathuru & Jesuthasan 2013). This has led to the suggestion that reciprocal connectivity between the IPN, raphe nuclei, and dorsal tegmental region may be critical to behavioral responses to stressors. Furthermore, activity in the habenular complex may be an upstream determining factor in selection of behavioral strategies to cope with stressors (Okamoto et al. 2012, Jesuthasan 2012). A unique characteristic of the habenular complex is its asymmetry, with the left habenula larger than its right counterpart (Ahumada-Galleguillos *et al.* 2016, Hetu *et al.* 2016). When this

asymmetry is lost in zebrafish, behavioral assays indicated elevated manifestations of anxiety, as well as elevated cortisol levels in response to stressors (Facchin *et al.* 2015).

While the size of the habenular complex renders current neuroimaging technology incapable of distinguishing the LHb from the MHb in human studies, some studies indicate a role played by the habenular complex in the pathophysiology of bipolar disorder (BD). In a study using high-resolution magnetic resonance imaging (MRI), it was found that patients diagnosed with BD who had either never been medicated, or had been un-medicated for at least two months, exhibited smaller habenular volumes than healthy controls (Savitz *et al.* 2011).

Studies have also implicated the IPN in regulating anxiety and fear. Early studies of the IPN identified a role for the structure in the retention of avoidance conditioning. Rats were trained to perform a jumping response following a visual stimulus to avoid a shock. After a learning period, a group of trained rats were treated with electrolytic lesions of the IPN. Following recovery from the procedure, rats re-learned the task and retention of the response was compared to controls. Rats with IPN lesions exhibited comparatively inferior retention of the response, though exhibited other signatures of a fear reaction, implying a role played by IPN signaling in specific components of fear learning (Thompson 1960). Following chronic nicotine administration, increased corticotropin releasing factor (CRF) synthesis is observed in dopaminergic neurons in the VTA, which appear to send efferents to the ventral IPN (Zhao-Shea et al. 2015). This is accompanied by an increase in CRF1 receptor expression in a particular subnucleus of the ventral IPN, and withdrawal induces release of CRF by the VTA onto ventral IPN neurons. Blockade of CRF1 receptor binding in the IPN was shown to reduce anxiety-associated behavior generated during withdrawal from nicotine. Table 4 presents a compilation of studies characterizing the role of this pathway in mood-associated conditions.

Conclusion

The dorsal diencephalic conduction system is emerging as an important node in the pathophysiology of addiction to multiple drugs, including alcohol, opioids, and nicotine. In addition to this involvement in substance abuse and addiction, the DDC also appears to regulate affect and mood-associated psychiatric conditions. Given the high comorbidity of substance use disorders and psychiatric illnesses, it makes sense that both conditions engage some overlapping pathways. For example, approximately one-third of individuals diagnosed with major depressive disorder also have a substance use disorder (excluding nicotine dependency), with almost half of all patients with depression having a family history of substance use disorders (Davis *et al.* 2008). Individuals with both disorders tend to experience earlier onsets of depression, greater persistence of the disorders, and exhibit more suicide attempts. When considering another addictive drug, nicotine, individuals diagnosed with mental illnesses are approximately twice as likely to smoke tobacco (Lasser *et al.* 2000), and studies have shown that 81.8% of individuals diagnosed with bipolar disorder, and 76.8% diagnosed with generalized anxiety disorder have smoked daily for at least a month (Lasser et al. 2000). Anxiety disorders have been implicated in alcohol, opioid, and stimulant abuse (Vorspan *et al.* 2015). Among adolescents, the majority of substance use

disorders occur among youth with prior psychiatric disorders, with alcohol abuse observed in 17.3% and drug abuse observed in 20% of those diagnosed with prior anxiety disorders (Conway *et al.* 2016). A relationship between anxiety, stress, depression, and other psychiatric conditions and increased nicotine consumption was established many years ago (Lasser et al. 2000, Feldner *et al.* 2007, Morissette *et al.* 2007, Patton *et al.* 1998, Zvolensky *et al.* 2005, Kassel *et al.* 2003, Cougle *et al.* 2010), and a recent study has indicated that this dynamic has not changed, despite declining overall usage over the past 6 decades (Prochaska *et al.* 2016). This relationship even translates to a unique puff volume among those with psychiatric conditions, with smokers who have a history of panic attacks exhibiting increased puff volumes relative to those without histories of panic attacks (Farris *et al.* 2016). Accordingly, mounting data indicates involvement of the DDC, and the MHb-IPN pathway in particular, in both addiction to multiple drugs of abuse and mood-associated conditions, and the MHb-IPN circuit may represent a junction at which signaling underlying both sets of conditions occurs. Given the presence of nAChRs composed of unique subunit compositions in the MHb-IPN pathway (Antolin-Fontes *et al.* 2015), this circuit may yield strategic targets for pharmacological therapeutics to improve treatment outcomes of both sets of conditions.

Acknowledgements

This effort for this work was supported by grants from the National Institutes of Health DA0941, P50CA179546 (MDB), NS21229, DA09411(JAD), and T32DA028874 (IAM) and.

Abbreviations

18-MC	18-methoxycoronaridine
ACh	acetylcholine
AChE	acetylcholinesterase
ATP	adenosine triphosphate
BAC	bed nucleus of the anterior commissure
BD	bipolar disorder
CRF1	Corticotropin-releasing factor
DDC	dorsal diencephalic conduction system
DTg	dorsal tegmentum
FR	fasciculus retroflexus
HA	hypothalamic nuclei
HCN	hyperpolarization-activated cyclic nucleotide-gated channels
Hipp	hippocampus
IL-18	interleukin-18

IL	infralimbic region of the medial prefrontal cortex
IPN	Interpeduncular nucleus
LC	locus coeruleus
LDTg	lateral dorsal tegmentum
LHb	lateral habenula
MDMA	3,4-methylenedioxymethamphetamine
MHb	medial habenula
miRNA	microRNA
MRI	magnetic resonance imaging
MS	medial septum
NAChRs	nicotinic acetylcholine receptors
NBM	Nucleus Basalis of Meynert
NI	nucleus incertus
NK1	neurokinin-1
NK3	neurokinin-3
NMDA	N-methyl-D-aspartate receptor
NPY	neuropeptide Y
PA	Preoptic area
PET	positron emission tomography
Pi	pineal body
RN	raphe nuclei
Sfi	septofimbrial nucleus
SI	Substantia innominate
SP	substance P
SST	somatostatin
TS	triangular septum
VTA	ventral tegmental area
WRA	wheel running activity

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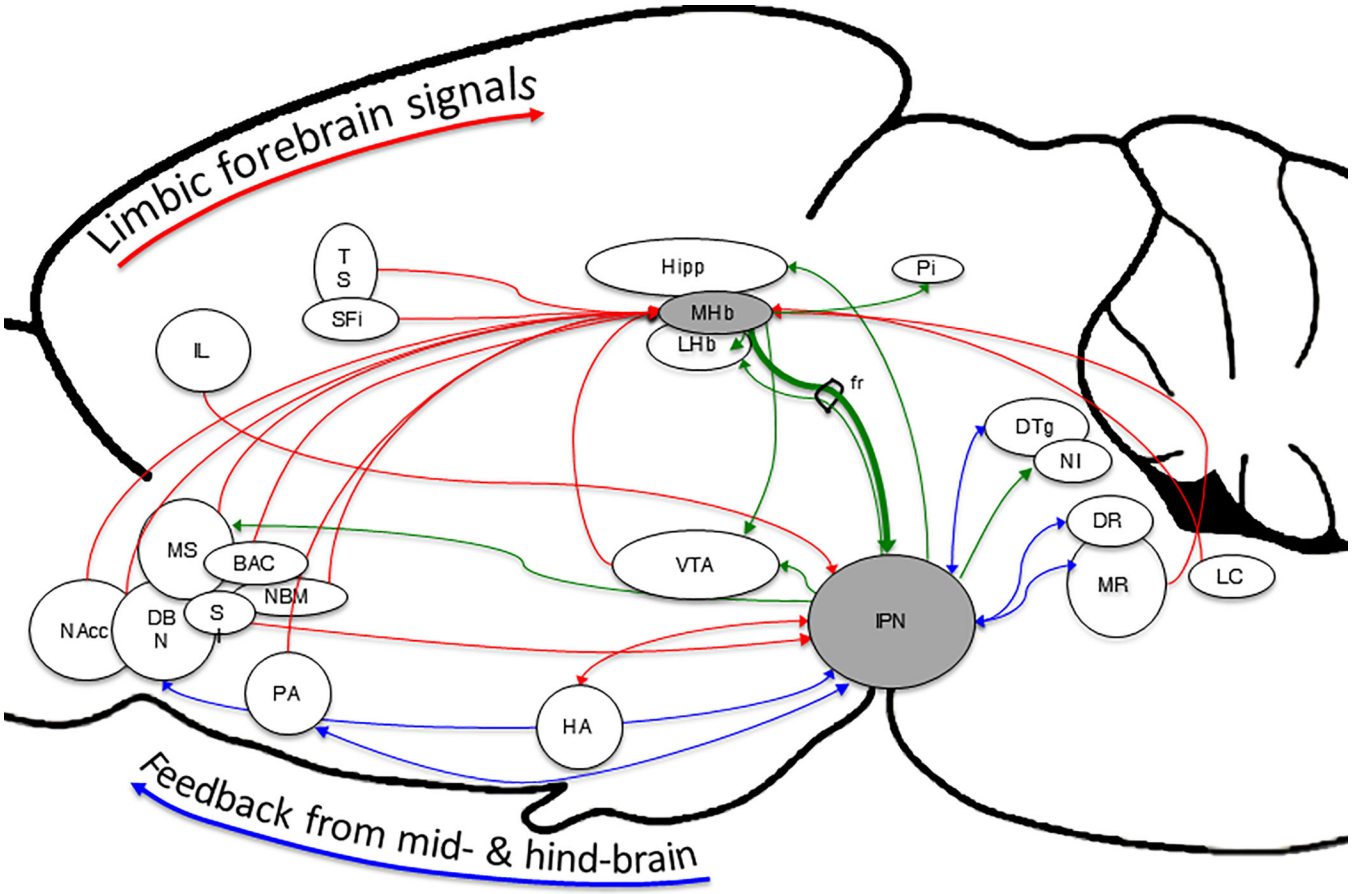

Figure 1.

Afferent and efferent connections of the MHb-IPN pathway. The medial habenula-interpeduncular nucleus pathway unites forebrain limbic with midbrain & hindbrain motivation & reward signaling. The medial habenula receives afferent inputs from a wide variety of forebrain limbic structures, and the interpeduncular nucleus sends efferent projections to a variety of midbrain & hindbrain structures implicated in the neurophysiology underlying addiction and a variety of mood-related psychiatric conditions. Red lines indicate afferent projections to the MHb or IPN, and green lines identify efferent

Table 1.

MHb Afferent & Efferent Connections

Afferent		Efferent	
Structure	**Reference**	**Structure**	**Reference**
Triangular Septum	Herkenham & Nauta, 1977	Interpeduncular Nucleus	Morley, 1986; Herkenham & Nauta, 1979
Medial Septal Nucleus	Qin & Luo, 2009	Pineal Body	Ronnekleiv & Moller, 1979; Guglielmotti & Cristino, 2006
Diagnoal Band Nucleus	Qin & Luo, 2009	Lateral Habenula	Kim & Chang, 2005
Septofimbral Nucleus	Herkenham & Nauta, 1977	VTA	Cuello, *et al.*, 1978
VTA	Herkenham & Nauta, 1977		
Interfascicular Nucleus of the VTA	Phillipson & Pycock, 1982		
Locus Coeruleus	Gottesfeld, 1983		
Superior Cervical Ganglion	Gottesfeld, 1983		
Preoptic Area	Groenewegen, 1986; Herkenham & Nauta, 1977		
Median Raphe Nucleus	Conrad, *et al.*, 1974; Sutherland, 1982		
Nucleus Basalis of Meynert	Herkenham & Nauta, 1977		
Nucleus Accumbens	Sutherland, 1982		

Table 2.

IPN Afferent & Efferent Connections

Afferent		Efferent	
Structure	**Reference**	**Structure**	**Reference**
Medial Habenula	Morley, 1986	Hippocampus	Shibata & Suzuki, 1984; Baisden, *et al.,* 1979; Wyss, *et al.,* 1979
Diagonal Band Nucleus	Contestabile & Flumerfelt, 1981	Lateral Hypothalamus	Massopust, Thompson, 1962; Kemali, Gugliemotti, 1982; Smith, *et al.*, 1980
Substantia Innominata	Vertes & Fass, 1988	Ventral Tegmental Area	Morley, 1986; Hayakawa, *et al.*, 1981; Smaha, Kaelber, 1973
Hypothalamic Nuclei	Contestabile & Flumerfelt, 1981; Hamill & Jacobowitz, 1984	Septum	Morley, 1986; Hayakawa, *et al.*, 1981
Supramammillary Nucleus	Contestabile & Flumerfelt, 1981; Hamill & Jacobowitz, 1984	Preoptic Area	Morley, 1986; Hayakawa, *et al.*, 1981
Dorsal Tegmental Nucleus	Hamill & Jacobowitz, 1984	Diagonal Band Nucleus	Morley, 1986; Hayakawa, *et al.*, 1981
Preoptic Area	Shibata, *et al.,* 1986	Dorsal Raphe Nucleus	Groenewegen, 1986
Infralimbic region of Medial Prefrontal Cortex	Takagishi & Chiba, 1991	Median Raphe Nucleus	Groenewegen, 1986; Behzadi, *et al.*, 1990
Raphe Nuclei	Conrad, *et al.,* 1974	Lateral Habenula	Morley, 1986; Massopust, Thompson, 1962
Nucleus Incertus	Hamill & Jacobowitz, 1984	Dorsal Tegmental Nucleus	Hamill & Jacobowitz, 1984; Groenewegen, 1986

Table 3.

Roles in the actions of drugs

MHb			IPN		
Technique	**Finding**	**Reference**	**Technique**	**Finding**	**Reference**
Local cerebral glucose utilization	Higher in MHb of EtOH-preferring rats	Smith, D. G, *et al.,* 2001	Naloxone-precipitated opioid withdrawal	Altered AChE activity in the IPN	Neugebauer, N. M., *et al.,* 2013
IP nAChR antagonist injection	Precipitates EtOH withdrawal syndrome	Perez, E., *et al.,* 2015	18-MC IPN infusion	Reduced morphine self-administration	Glick, S. D., *et al.* 2006
NPY Receptor knock-out	Knock-out mice ingest more EtOH	Roux, A. *et al.,* 2015	18-MC IPN infusion	Reduced methamphetamine self-administration	Glick, S. D., et al. 2000
In situ hybridization	EtOH-preferring mice lack NPY mRNA in MHb	Thiele, T.E., *et al.,* 2002; Hwang, B.H., *et al.,* 2004	Methamphetamine & cocaine administration	Increased extracellular [ACh]	Hussain, R. J., *et al.,* 2008
MHb lesion	Hyperalgesia, increase analgesic efficacy of morphine	Meszaros, J., *et al.,* 1985			
Intracranial infusion to MHb	Analgesia	Cohen, S. R., *et al.,* 1985			
VTA ICSS	Reduced endogenous opioid binding in MHb	Stein, E. A., *et al.,* 1993			
Chronic morphine administration	Altered AChE activity in the MHb	Neugebauer, N. M., *et al.,* 2013			
Chronic morphine administration	Trend to increased MHb nAChR expression	Neugebauer, N. M., *et al.,* 2013			
18-MC MHb infusion	Reduced morphine self-administration	Glick, S. D., *et al.* 2006			
RSK2 knock-out in MHb	Reduced morphine analgesia	Darcq, E., *et al.,* 2012			
Cocaine cue-induced reinstatement	Increased Fos-protein in MHb	James, M. H. *et al.,* 2011			
18-MC MHb infusion	Reduced methamphetamine self-administration	Glick, S. D., *et al.* 2000			

Table 4.

Roles in Mood Disorders

MHb			IPN		
Technique	**Finding**	**Reference**	**Technique**	**Finding**	**Reference**
PET in stress-treated rats	Hb identified as central in network of brain regions	Mirrione, M. M., *et al.,* 2014	Inhibition of afferent terminals in IPN	Place aversion	Hsu, Y. W., *et al.,* 2014
Chronic mild stress in rats	Altered SST release and SST2 receptor expression in MHb distinguish stress-responsive from non-responsive	Faron-Gorecka, A. *et al.,* 2016	Stress-evoked helplessness treated with fluoxetine in rats	Fluoxetine was protective, correlated with altered metabolic activity in IPN	Padilla, E., *et al.,* 2011
Learned helplessness in rats	Altered miRNA associated with MAPK, neutrophin, and ErbB in MHb & LHb	Svenningsen, K., *et al.,* 2016	Electrolytic lesion of IPN	Inferior retention of conditioned avoidance response	Thompson, R., 1960
Genetic ablation of dorsal MHb	Reduced wheel running activity	Hsu, Y. W., *et al.,* 2014	Chronic nicotine administration	Increased CRF release to ventral IPN	Zhao-Shea, R., *et al.,* 2015
Optogenetic self-stimulation of dorsal MHb	Preference for stimulation	Hsu, Y. W., *et al.,* 2014	Chronic nicotine administration	Increased CRF1 receptor expression in ventral IPN	Zhao-Shea, R., *et al.,* 2015
Rats bred for susceptibility to helplessness	Increased markers of metabolic activity in MHb & LHb	Shumake, J. *et al.,* 2003	Blockade of CRF1 receptor in IPN	Reduced anxiety-associated behaviors during nicotine withdrawal	Zhao-Shea, R., *et al.,* 2015
Stress-evoked helplessness treated with fluoxetine in rats	Fluoxetine was protective, correlated with altered metabolic activity in Hb	Padilla, E., *et al.,* 2011			
PET in depressed patients treated with ketamine	Reduced metabolism in the Hb	Carlson, P. J., *et al.,* 2013			
Post-mortem study of brain sections of patients with depression	Reduced volume, cell number, and mean cell areas in MHb	Ranft, K., *et al.,* 2010			
Elimination of MHb afferents from TS & BAC	Disrupted anxiety-associated behaviors	Yamaguchi, T., *et al.,* 2013			
Acute & chronic restraint stress	Elevated IL-18 in dorsal MHb	Sugama, S., *et al.,* 2002			
Subordination paradigm	Elevated mast cell numbers in the Hb	Cirulli, F., *et al.,* 1998			
Inhibition or lesion of dMHb in zebrafish	Elevated freezing behavior, elevated anxiety, elevated alarm substance secretion	Agetsuma, M., *et al.,* 2010; Lee, A., *et al.,* 2010; Mathuru, A.S., *et al.,* 2013			
MRI of un-medicated patients with bipolar disorder	Reduced Hb volumes	Savitz, J.B., *et al.,* 2011			