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Anxiety and nicotine dependence: Emerging role of the habenulo-interpeduncular axis

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Abstract

While innovative modern neuroscience approaches have aided in discerning brain circuitry underlying negative emotional behaviors including fear and anxiety responses, how these circuits are recruited in normal and pathological conditions remains poorly understood. Recently, genetic tools that selectively manipulate single neuronal populations have uncovered an understudied circuit, the medial habenula (mHb)-interpeduncular (IPN) axis, that modulates basal negative emotional responses. Interestingly, the mHb-IPN pathway also represents an essential circuit that signals heightened anxiety induced by nicotine withdrawal. Insights into how this circuit interconnects with regions more classically associated with anxiety and how chronic nicotine exposure induces neuroadaptations resulting in an anxiogenic state, may thereby provide novel strategies and molecular targets for therapies that facilitate smoking cessation, as well as, anxiety relief.

Keywords

Anxiety; fear; medial habenula; interpeduncular nucleus; nicotine withdrawal

Anxiety disorders and their comorbidity with nicotine dependence

Negative emotional behaviors and reactions to either threatened or neutral stimuli establish the fundamentals of fear- and anxiety-related responses, respectively (see Box 1). Exaggerated and persistent negative emotional responses instigate pathological states of anxiety, one of the most common and costly neuropsychiatric disorders recognized in developed societies [1, 2]. Despite huge progress in our understanding of the neural circuits governing anxiety disorders (ADs), the incidence of ADs remains 18% among adults and has lifetime persistence for years [3, 4]. While these clinical data reveal that there are still

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Conflict of Interest

The authors declare that they have no conflict of interest.

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gaps in our approach towards identifying what triggers an anxiety episode, it remains essential to unveil why, once anxiety is initiated, an individual becomes susceptible to experiencing subsequent anxiety events. Noticeably, ADs represent a risk factor for developing other psychiatric conditions, such as drug addiction [5]. ADs are highly comorbid with drug abuse, particularly with nicotine dependence [6, 7]. Nicotine, the major addictive component of tobacco, binds to and activates/desensitize nicotinic acetylcholine receptors (nAChRs) to elicit neural responses (see Box 2). Acutely, nicotine can induce a relief of anxiety and sustain tobacco use through positive emotional reinforcement (see also [8, 9]). The activation of circuits mediating the anxiolytic effects of nicotine may be considered an attempt at self-medication in people with ADs [10]. However, long-term nicotine exposure produces neuronal adaptations in several brain regions [11]. Thus, when individuals try to quit smoking, cessation of cigarette use precipitates the so-called withdrawal syndrome, with stronger affective responses in people sensitive to experiencing anxiety [12, 13]. It may be feasible that people with ADs experience more severe withdrawal symptoms because common neural circuits could underlie anxiety in both basal conditions and during nicotine withdrawal. A better understanding of this conceivable shared circuitry may not only provide novel strategies for the treatment of pathologies associated with anxiety, but also, for more efficacious smoking cessation therapies.

Box 1

Definitions

Fear is induced by an imminent and identifiable threat and elicits behaviors such as flight, freezing and fight (defensive attack) depending on the imminence of the incoming threat and the possibility of escape (defensive distance).

Anxiety is elicited by an unidentifiable and un-localizable potential threat and induces the animal to suspend ongoing behaviors and increase the levels of arousal for the assessment of potential danger.

Definitions adapted from [75].

Box 2

Neuronal nicotinic acetylcholine receptors (nAChR)

Neuronal nAChRs are pentameric ligand-gated ion channels that are activated by endogenous cholinergic ligands (i.e. ACh), as well as the tertiary alkaloid, nicotine. There are eleven mammalian neuronal nAChR subunits $\alpha 2$ - $\alpha 7$, $\alpha 9$, $\alpha 10$ and $\beta 2$ - $\beta 4$ that co-assemble to form functional heteropentamers consisting of various combinations of α and β subunits or homopentamers exclusively made of α subunits. The different nAChRs subunit compositions determine their biophysical and pharmacological functions (for detailed review see [76]). Of note, the mHb-IPN axis expresses a variety of nAChR subunits including the $\alpha 3$, $\alpha 5$ and $\beta 4$ that are associated with nicotine dependence susceptibility in humans [77, 78]. In addition, expression of nAChRs containing the $\alpha 5$ and/or the $\beta 4$ subunit within the mHb-IPN circuit not only play a role in somatic

withdrawal, but also modulate acute nicotine intake, presumably through balancing the rewarding and aversive properties of the drug [14, 15, 79].

The medial habenula (mHb) – interpeduncular nucleus (IPN) axis, a circuit consisting of neurons of the epithalamic mHb that project to the IPN of the midbrain, has a uniquely high density of nAChRs in the brain and controls acute nicotine responses [14–17]. Whereas most studies on the regions regulating negative emotional behavior have focused on the traditional circuits of anxiety, including the prefrontal cortex or amygdala, emerging data indicate the mHb-IPN circuit may exert a significant role as well. Here we review how, with the aid of new technologic developments and genetic tools, the mHb-IPN circuit and its upstream and downstream connections have been functionally linked to basal fear- and anxiety-related responses to environmental threat. In addition, dysregulation of this circuit during chronic nicotine exposure underlies nicotine withdrawal behavior, including increased anxiety and aversion.

The mHb-IPN axis in basal negative emotional responses

The mHb belongs to the habenular complex, an epithalamic structure phylogenetically conserved throughout vertebrate evolution [18]. In mammals, two bilateral mHb nuclei receive input from the limbic forebrain through the septum and relay information, via axon bundles that make up the fasciculus retroflexus (fr), to the IPN. In turn, the IPN innervates monoaminergic midbrain/hindbrain centers [19]. The mammalian mHb can be roughly subdivided into the dorsal (dmHb) and ventral (vmHb) parts with differentiated neuronal identities, segregated neural input from the septum, and specific pattern of projections towards the IPN [20]. The dmHb comprises mainly neurons expressing the neuropeptide substance P (SP) that receive input from the bed nucleus of the anterior commissure (BAC) and project ipsilaterally to the most lateral part of the IPN (Figure 1). In contrast, the vmHb essentially contains cholinergic and/or glutamatergic neurons that receive input from the triangular septum (TS) and send axon projections to the central portion of the IPN (Figure 1). Conversely, the IPN is a non-homologous brain structure that has been subdivided into 3 unpaired interpeduncular sub-nuclei (apical (IPA), rostral (IPR) and central (IPC)) and 4 paired sub-nuclei (dorsolateral (IPDL), dorsomedial (IPDM), lateral (IPL) and intermediate (IPI)) (Figure 1). The majority of IPN neurons express the glutamate decarboxylase (GAD) enzyme and, presumably synthesize and release the neurotransmitter (γ)-aminobutyric acid (GABA), although glutamatergic and serotonergic neurons have also been identified within IPN sub-regions in rodents [21]. Anatomically, the location of the mHb and its afferents to the IPN allows for the integration and conveyance of emotional and cognitive information from septo-hippocampal circuits, to areas involved in response processing and reward signaling. As a result, the mHb-IPN axis may act as a boundary controlling emotional behavior in a memory/experience-dependent manner. Given the nature of ADs, which arise from disrupted cognitive integration and processing of emotional drives [22], it is not surprising that the septo-habenulo-interpeduncular circuitry ascertains a putative neurobiological substrate for the pathogenesis of anxiety.

Over the past three decades, pharmacological and lesion studies in rodents have implicated the septo-habenulo pathway in anxiety- or fear-related responses [19, 23]. However, because of technical limitations, these studies did not identify precise underlying neural mechanisms that contribute to negative emotional behavior in these circuits. The use of modern innovative genetic tools in model organisms has opened the possibility to specifically manipulate neuronal components of this circuit, thereby providing new data on how it regulates negative emotional behavior. For example, studies in zebrafish with selective genetic inactivation of the dorsal habenula lateral (dHbl), the putative orthologue of the mHb in mammals, have demonstrated a role for the dHbl-IPN circuit in the response to fear. Mutant zebrafish failed to switch from a freezing response towards an increase in turning and agitation (flight response), after consecutive presentations to a conditioned fear stimulus, a learned transition typically detected in wild-type fish [24]. This pioneering work indicated that silencing of the dHbl-IPN pathway might disrupt the weighing of potential threats against previously learned associations, one of the neural underpinnings for anxiogenesis. Moreover, inactivation of the same pathway in zebrafish, exaggerated responses to multiple mildly natural stressors (without a previous learned association)[25]. In line with these results, ablating the putative orthologue of the mammalian septum that projects to the habenula in zebrafish larvae enhanced anxiety-like behavior and fear learning [26]. Based on these data, anxiety- and fear-related responses are likely controlled by the septo-habenulo-interpeduncular connection, a circuit phylogenically conserved between fish and mammalian systems.

In mice, two topographically segregated pathways, the BAC→dmHb and the TS→vmHb within the septo-habenulo-interpeduncular circuit (Figure 1) appear to play specialized roles in controlling fear- vs. anxiety- related emotional behaviors, respectively [27]. Elegant work by Yamaguchi *et al.* demonstrated that the BAC→dmHb neural transmission is critical for fear responses, while the TS→vmHb neural connection controls anxiety but not fear learning [27]. In this study, removal of vmHb cholinergic/glutamatergic excitatory stimulation to the IPN, reduced baseline anxiety-like responses, opposing most observations in the zebrafish model, whereas inactivation of mHb→IPN input actually heightened anxiety. Interestingly, a prominent feature of vmHb cholinergic neurons is a dual mode of neurotransmission; these neurons can co-release acetylcholine (ACh) or glutamate (Glu), depending on the stimulation pattern, with tonic activity favoring Glu release and phasic, high frequency stimulation inducing ACh release [28]. Furthermore, cholinergic/glutamatergic mHb neurons exhibit spontaneous pace-making activity that enables them to generate tonic trains of action potentials (APs), independently of afferent input [29, 30]. These neurons also have a phenotypic diversity of neuronal populations that robustly express a variety of nAChRs, suggesting that nicotinic receptor signaling in vmHb cholinergic/glutamatergic neurons could regulate anxiety-like behavior. To test this hypothesis, Pang *et al.* selectively expressed “gain-of-function” $\alpha 4$ nAChR subunits in vmHb cholinergic/glutamatergic neurons of adult mice. Boosting vmHb cholinergic signaling and activity with an agonist-hypersensitive nAChR increased baseline anxiety levels [31], consistent with the findings of Yamaguchi *et al.* A separate study found that ablation of post-mitotic vmHb neurons in mice mildly raised levels of anxiety-like behavior [32]. These results taken together suggest that vmHb cholinergic/glutamatergic signaling may be necessary to

modulate anxiety-like responses with increased activity of cholinergic/glutamatergic vmHb neurons associated with a heightened anxiety-like state, and decreased activity of cholinergic/glutamatergic vmHb neurons being linked with decreased anxiety. In contrast, IPN cholinergic signaling arising from the vmHb might also control fear memory. The genetic deletion of the cannabinoid receptor 1 in mHb neurons enlarged cholinergic signaling in the IPN, attenuating fear expression [33]. Although the collective data support a critical role of mHb cholinergic neurons in mediating basal anxiety and fear-related responses, the precise physiological mechanism underlying these behaviors remains unclear. It is likely that the balance between mHb glutamatergic and cholinergic signaling in the IPN distinctively controls negative emotional behavior, a detail that requires further investigation.

The mHb-IPN axis in nicotine withdrawal-induced negative emotional responses

Research into the comorbidity between emotional psychopathology and cigarette smoking has often been focused on anxiety responses associated with drug abstinence [7]. Cessation of cigarette use after prolonged exposure to nicotine triggers a withdrawal syndrome that significantly contributes to the low success in quitting [34]. Withdrawal symptoms have been divided into three main categories: 1) somatic (or physical) symptoms including sweating, bradycardia, or gastrointestinal discomfort, 2) affective symptoms involving anxiety and depressed mood, and 3) cognitive deficits such as difficulty concentrating [35]. Heightened anxiety when attempting to quit smoking is one of the major causes of smoking relapse [36]. As such, it is essential to investigate the neuroanatomical bases of anxiety during nicotine withdrawal, as well as the neuroadaptations induced by chronic nicotine that underlie the anxiogenic effects of withdrawal.

As in humans, rodents chronically exposed to nicotine exhibit withdrawal signs upon cessation, allowing the use of these species to better understand the neurobiology and plasticity elicited by nicotine that underlie withdrawal-associated behaviors. Nicotine withdrawal effects are essentially mediated through nAChR signaling, as blocking nAChRs with antagonist is sufficient to precipitate withdrawal [37, 38]. Noticeably, the mHb-IPN axis is highly sensitive to nicotine because of the dense expression of multiple nAChR subtypes in mHb cholinergic/glutamatergic neurons and neurons within the IPN [39]. In addition, this pathway mediates nicotine withdrawal signs. For instance, microinjection of the non-subtype selective nAChR antagonist mecamylamine into the IPN, but not into adjacent regions including the ventral tegmental area (VTA), precipitates both somatic [40] or affective signs [41] in mice chronically exposed to nicotine. Interestingly, mecamylamine infusion into the IPN of nicotine-naïve animals does not significantly affect anxiety-like behavior [41], suggesting that chronic nicotine drives neuroadaptations in IPN efferent projections or within the IPN itself that increase sensitivity of nAChR signaling.

Interestingly, nicotine withdrawal increases activity of the IPN in a sub-region-selective manner. Among the IPN sub-regions, work from our laboratory has demonstrated the IPR and IPI are highly activated during nicotine withdrawal (Figure 2) [41, 42]. Photoactivation of the IPR by channelrhodopsin expression in IPN GAD2-positive GABAergic neurons is

sufficient to trigger somatic withdrawal signs, even in nicotine-naïve mice, but fail to elicit anxiety-like behaviors [42]. In contrast, pharmacological activation of the IPI enhances anxiety-related responses [41]. Based on these results, the IPR and IPI may distinctively contribute to specific components of withdrawal-associated behaviors, with the IPI being responsible for the anxiogenic element of nicotine abstinence.

Mechanistically, how does chronic nicotine exposure induce activation of the IPN during cessation, triggering withdrawal symptoms? One prominent neuroadaptation likely involves nicotinic receptors themselves. Long-term nicotine exposure causes an up-regulation of nAChR signaling, underlying the development of nicotine dependence and withdrawal [43–45]. Within the mHb-IPN circuit, nicotine differentially affects nAChR function and output of cells in specific sub-regions of the vmHb [46]. In particular, up-regulated $\alpha 6/\alpha 4$ nAChR signaling in mHb cholinergic/glutamatergic neurons underlies heightened anxiety during nicotine withdrawal (Figure 3) [31]. Chronic nicotine exposure elicits a functional up-regulation of nAChRs containing the $\alpha 6/\alpha 4$ subunit in at least a sub-population of vmHb cholinergic/glutamatergic neurons, presumably, increasing cholinergic activation of these neurons during withdrawal, ultimately contributing to increasing excitatory input into the IPN. Indeed, infusion of an $\alpha 6$ nAChR selective antagonist into the mHb of nicotine-dependent mice alleviates anxiety-like behavior during withdrawal [31]. As mentioned above, vmHb cholinergic neurons co-release Glu and ACh in the IPN to activate post-synaptic neurons [28], which may contribute to distinct behavioral responses depending on the balance between both neurotransmitters. Gorlich *et al.* [29] showed that blockage of hyperpolarization-activated cyclic nucleotide-gated (HCN) pacemaker channels in mHb neurons triggered both somatic and affective withdrawal signs in nicotine-naïve mice. Blocking HCN and spontaneous generation of APs could imbalance the Glu and ACh release in the mHb-IPN axis, precipitating nicotine withdrawal symptoms. In support of this idea, several lines of evidence postulate that nicotine withdrawal is associated with an IPN increase in Glu release from mHb neurons. Briefly, IPN infusion of a N-methyl-D-aspartate (NMDA) receptor antagonist or pharmacological blockade of IPN neurotransmission from the mHb alleviates both somatic and affective nicotine withdrawal symptoms [41, 42]. Moreover, optogenetic silencing of mHb cholinergic/glutamatergic transmission in the IPN relieves anxiety in nicotine-dependent mice, during drug withdrawal [41]. Together, these data indicate that, under conditions of nicotine abstinence, the IPN is activated through mHb signaling (most notably via excitatory glutamatergic input), triggering an anxiety-like response. The distinct regulatory pathways of IPN glutamatergic signaling may pass through a mechanism whereby ACh is able to control the release frequency of Glu at habenular synapses and hence, the behavioral responses to nicotine [47].

Cessation from chronic exposure to a drug of abuse, including nicotine, has long been hypothesized to cause an over-activation of the brain stress circuitry, engaging elevations in anxiety levels [48]. Stress responses are primarily regulated by the corticotrophin releasing factor (CRF) neuropeptide, that binds to and activates the cognate G-protein-coupled receptors CRF type 1 (CRF1) and type 2 (CRF2)[49]. The extra-hypothalamic CRF stress system within the amygdala has been shown to couple to negative emotional behaviors and anxiety associated with nicotine withdrawal [50], although the complete circuit and mechanistic bases for the CRF system in the anxiogenic effects of nicotine withdrawal still

remain largely unknown. Two recent publications reveal that a meso-interpeduncular circuit consisting of a population of VTA dopaminergic (DAergic) neurons able to synthesize and release CRF, project to the ventral portion of the IPN. These neurons participate in the negative affective manifestations of nicotine withdrawal [41, 51]. Grieder *et al.* showed that chronic nicotine exposure up-regulated CRF expression in this population of DAergic neurons located in the posterior VTA (pVTA) [51, 52]. Withdrawal from nicotine caused the synthesized CRF peptide to be released both into the pVTA itself and the IPN (Figure 3). Thereafter, CRF activated pVTA CRF1 receptors, which were necessary for aversion and anxiety during nicotine withdrawal. In line with these results, our laboratory elucidated that the CRF synthesized and released in VTA DAergic neurons was critical for the activation of the IPI sub-region during nicotine withdrawal, triggering an anxiety-like response [41]. IPI neurons densely express CRF1 receptors that actively respond to the CRF peptide (Figure 2B). Moreover, chronic nicotine up-regulated CRF1 receptors on IPI neurons, which, during withdrawal, raised glutamatergic signaling and amplitude in the IPN, presumably via mHb input (Figure 3). Consequently, blocking activation of CRF1 receptors in the IPN was sufficient to alleviate anxiety during nicotine withdrawal. The findings of these two studies are particularly interesting because they identify a novel neuronal connection that provides a direct link between reward and aversion circuits. Furthermore, both data implicate the CRF1 receptors as therapeutic targets to facilitate smoking cessation, by alleviating the negative emotional effects caused by nicotine abstinence.

The mHb-IPN axis and the classical anxiety circuitry

As detailed above, the habenulo-interpeduncular circuit is emerging as a critical mediator in modulating negative emotional behavior, suggesting that it is a significant component of the brain's network controlling fear and anxiety. Thus, it is vital that future experiments focus on mapping how this pathway integrates and impacts other known, better-characterized circuits that regulate emotional responses.

In recent years, the technology of optogenetics used in mice has led to an unprecedented leap in our knowledge of the brain circuitry responsible for producing anxiety-like behaviors (Figure 4). Reciprocal circuits between the amygdala, bed nucleus of the stria terminalis (BNST), VTA and prefrontal cortex (PFC) govern anxiety-like responses (for detailed review see [53]). These brain regions receive and integrate information from the thalamus and cortex that is assigned a positive or negative valence that then dictates a behavioral response. When mice anticipate aversive conditions, the basolateral amygdala (BLA) neurons projecting to the central amygdala (CeM) and ventral hippocampus (vHPC) respond preferentially over positive valence cues to the nucleus accumbens (NAc)[54]. Optogenetic manipulation of these BLA efferents to the CeM and vHPC is sufficient to influence anxiety-like behaviors [55, 56]. Although the BLA projection to the CeM can drive anxiety-like behaviors, these behaviors are short-term and are present alongside an environmental cue. In contrast, the BNST promotes long-term vigilance and anxiety-like behavior which is more similar to pathological anxiety [1]. The BNST receives innervation from the BLA and can drive and suppress separable features of anxiety [57]. BNST afferents in the VTA promote anxiogenic behavior when Glu is released and anxiolytic behavior when GABA is released via optogenetic stimulation [58]. Additionally, the presumably DAergic afferents

from the VTA in the medial prefrontal cortex (mPFC) are sufficient to drive anxiety-like behaviors when depolarized with high frequency phasic optogenetic stimulation [59]. The mPFC also has reciprocal connections with the vHPC and the amygdala and these circuits are associated with fear learning and are thought to be dysregulated in ADs [60]. The vHPC in turn, projects to the lateral septum (LS) to convey an anxiety signal mainly via hypothalamic areas [61]. These circuits are modulated by dopamine, norepinephrine, serotonin and CRF. Currently, selective serotonin reuptake inhibitors, serotonin and norepinephrine reuptake inhibitors or benzodiazepines are usually the first prescribed anxiolytic medications to target these neural connections [62].

How might the mHb-IPN circuitry inter-connect with other anxiety circuitry? While there are few first-order connections between the mHb-IPN and other anxiety circuitry, most afferents and efferents, particularly those that arise from and innervate the IPN, have not been functionally characterized (Figure 4). At the level of the mHb, the majority of mHb neurons project to the IPN. However, a subpopulation of mHb neurons may innervate the lateral habenula (lHb) [30]. The lHb consists of glutamatergic neurons that are activated by aversive or unrewarding predictive cues [63]. Most lHb neurons terminate in the GABAergic tail of the VTA [64] and provide inhibitory control over DAergic cells of the VTA [65]. The existence of a direct synaptic link between the mHb and lHb may be important in promoting behavioral avoidance and controlling emotional behavior, although the functional relevance of this connection has yet to be explored.

The IPN receives information from the septum (via the mHb), similar to the basolateral amygdala (BLA) and bed nucleus of the stria terminalis (BNST) [27], although a direct connection between the IPN and BLA or BNST has not been reported in the literature. The IPN may affect the amygdala circuitry as well as the rest of the known anxiety circuitry via modulation of other brain regions. For example, the IPN densely innervates and has reciprocal connections with the dorsal raphe (DR) and median raphe (MnR) nuclei, which can modulate anxiety in the aforementioned anxiety-controlling brain areas [53, 66]. Interestingly, the IPN also receives presumably noradrenergic afferents from the locus coeruleus (LC), that could modulate anxiety [67].

Another possibility might include direct connections between the IPN and cortical regions. There is evidence that the infralimbic cortex, a subregion of the mPFC, innervates the IPN [68]. In addition, the IPN has efferents in the entorhinal cortex (EC) [66]. Mice undergoing contextual fear conditioning while the BLA connection to the EC is optogenetically inhibited failed to learn defensive freezing behavior in response to audio cues [69]. This circuitry relays on the BLA connection to vHPC through the EC. The IPN efferents to the EC could modulate the BLA's control of the vHPC and ultimately elicit emotional responses. The IPN is also known to project directly to the HPC [66]. Lesion of the fr in rats decreases theta power of hippocampal theta rhythms, which controls anxiety behaviors and mood [70]. The IPN may accomplish theta rhythm modulation through its efferents in the HPC or reciprocal connections with other areas known to affect theta rhythm. These regions include the septum, MnR and the nucleus incertus (NI) [71].

Reciprocal projections between the IPN and midbrain areas such as the lateral dorsal tegmental nucleus (LDTg) or periaqueductal grey (PAG) may play important roles in the expression of emotional behavior [72]. Optogenetic activation of either PAG glutamatergic neurons [73] or LDTg GABAergic neurons [74] can trigger freezing behavior, which would be implicated in anxiety-related disorders. Additionally, the IPN may regulate the VTA and mPFC activity via its projections to the LDTg. Alternatively, and more recently, a subset of presumably DAergic neurons in the VTA have been implicated in the modulation of anxiety via the release of CRF into the IPN in conditions of chronic nicotine intake in the mouse [41].

The function of IPN efferents and afferents remains very poorly characterized. Future experiments should focus on using opto- and chemogenetic approaches to test the neurotransmitter systems and behaviors signaled and processed by mHb-IPN neuronal connectivity.

Concluding Remarks

The mHb-IPN axis is an important mediator of negative emotional states of the brain such as fear and anxiety, most notably during abstinence from chronic nicotine exposure. Under baseline conditions (i.e. in the absence of chronic drug exposure), anxiety state can be modulated by genetic inactivation of the septo-habenulo-interpeduncular circuit, as shown both in zebrafish and rodent animal models. While a phylogenetic functional conservation of this circuit appears to be maintained from fish to mammals, whether the mammalian brain has dedicated two separate neuronal pathways with dissociated control over fear- and anxiety-associated behaviors requires further investigation (see Outstanding Questions box). Indeed, the neuronal transmission arising from mHb cholinergic neurons is able to influence both types of emotional responses. Given these neurons have a dual-mode of cholinergic/ glutamatergic signaling, disrupted synchronization of ACh and Glu neurotransmitter release appears to be key to instigate exaggerated anxiety or fear-related behaviors (see Outstanding Questions box). A comprehensive understanding of the exact molecular mechanisms that shift the dynamics of this system may provide valuable insights into the neurobiology of an anxiety-like behavior.

Outstanding Questions Box

- Under what physiological conditions does the mHb-IPN axis control anxiety- and fear- related responses?
- Does the mHb-IPN circuit play a more critical role under basal conditions or in response to exogenous stressors, such as abstinence from drug use?
- Ventral mHb neurons can co-release ACh and glutamate. Do the two neurotransmitter systems distinctively regulate negative emotional behavior?
- How do different sub-regions and/or neural populations within the mHb and IPN nuclei uniquely contribute to anxiogenic responses, especially those associated with nicotine withdrawal?

- If DAergic neurons from the VTA shift their signaling mechanisms towards CRF neurotransmission upon chronic nicotine exposure, to modulate the negative effects of nicotine withdrawal, what cause the shift in “neuronal identity”?
- How do the afferents and efferents of the mHb-IPN axis inter-connect with other anxiety circuitry to regulate negative emotional responses?

Prolonged exposure to nicotine induces targeted plasticity particularly in habenulo-meso-interpeduncular synapses. After chronic nicotine exposure the nicotinic system readjusts and, consequently, leads to a disruption in the homeostatic integrity of the habenulo-interpeduncular circuitry. During withdrawal, anxiogenic responses occur as a result of a bias in the system towards excessive glutamatergic signaling in IPN afferents, implying an over-activation of this nucleus (Figures 2 and 3). In particular, activation of neurons within the IPI sub-region triggers the anxiogenic component of nicotine cessation, through the recruitment of an extra-hypothalamic stress system involving CRF signaling. One important clue as to how these IPI neurons interact and impact with other neuronal circuits may provide a better perspective of the complex inter-connected brain areas governing emotional behaviors (see Outstanding Questions box). Work towards identifying unique markers among defined populations within IPI neurons could potentially facilitate therapeutic intervention to ameliorate anxiety in individuals attempting to quit smoking.

Significant interaction exists between anxiety sensitivity and the severity of nicotine withdrawal symptoms [12]. Likely, this is attributed to the overlapping neural circuits underlying anxiety both in basal and nicotine withdrawal conditions. In the present review we highlighted how components of the habenulo-interpeduncular circuit may represent the common neural substrates underpinning anxiety, especially when arising from nicotine abstinence. Understanding of these circuits, their similarities and connections to the IPN can provide novel therapeutic strategies for treatments geared toward alleviating anxiety, as well as facilitating smoking cessation.

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Trends Box

- The application of modern genetic technologies that selectively manipulate neural components of the septo-habenular-interpeduncular (IPN) circuit has begun to reveal how, under baseline conditions, this circuit is responsible for controlling emotional behavior including anxiety- and fear-related responses.
- The substantial comorbidity between anxiety disorders (ADs) and nicotine dependence may be attributed to overlapping neural networks involving the medial habenula (mHb)-IPN circuit. Long-term exposure to nicotine induces targeted plasticity within this circuit that underlies heightened levels of anxiety during nicotine abstinence.
- Optogenetic tools provide valuable insight into how highly interconnected brain networks govern anxiety-like responses. Mapping how the mHb-IPN pathway integrates into these better-characterized circuits regulating emotions may unmask the neural underpinnings of ADs.

Figure 1. Neural connectivity of the septo-habenulo-interpeduncular axis

(A) Schematic representation showing the topographic connections from the TS (orange) and the BAC (green) projecting to the ventral and dorsal sub-regions of the mHb, respectively. MHb neuron axon bundles assemble to form the fr and terminate in an ipsilateral manner in the IPN. DmHb SP⁺ efferents (red) innervate the more lateral part of the IPN, whereas vmHb ACh/Glu⁺ efferents (blue) innervate IPN central regions. ACh, acetylcholine; BAC, bed nucleus of the anterior commissure; Chat, choline acetyltransferase; dmHb, dorsal medial habenula; eYFP, enhanced yellow fluorescent protein; fr, fasciculus retroflexus; IPC, interpeduncular nucleus central; IPDL, interpeduncular nucleus dorsolateral; IPDM, interpeduncular nucleus dorsomedial; IPI, interpeduncular nucleus

intermediate; IPL, interpeduncular nucleus lateral; IPN, interpeduncular nucleus; IPR, interpeduncular nucleus rostral; mHb, medial habenula; TS, triangular septum; SP, substance P; vmHb, ventral medial habenula.

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Figure 2. Activation of IPN sub-regions during nicotine withdrawal

(A) Photomicrographs illustrating c-Fos immunolabeling in coronal sections taken from control or chronic nicotine-treated mice given an intraperitoneal (i.p.) injection of saline or mecamylamine (Mec) (to precipitate withdrawal, Mec, 3 mg kg⁻¹). Activation of the IPR and IPI sub-regions are represented in red and green colors, respectively. (B) Photomicrograph illustrating fluorescence in situ hybridization (FISH, top) with control probe (Scramble, left) or CRF1 receptor probe (right) and counterstained with DAPI for nuclei visualization, in mouse coronal sections containing the IPN. Note a robust CRF1 receptor signal is predominantly localized in the IPI sub-region of the IPN. Photomicrograph of coronal sections from chronic nicotine-treated mice given a CRF infusion (300 ng) into the IPN (bottom). Sections are double-labeled for CRF1 receptor mRNA (using FISH, red) and c-Fos protein (using immunohistochemistry (IF), green). Merged sections imaged at 10X magnification are shown (middle). Scale bar: 200 μm. Outside sections shown at 63X magnification illustrate localization of c-Fos in CRF1 receptor-expressing IPI neurons. Scale bar: 20 μm.

Figure 3. Mechanistic model of increased somatic and affective signs (anxiety) during nicotine withdrawal

(A) The IPN receives glutamatergic and cholinergic inputs from vmHb cholinergic/glutamatergic (Chat⁺) neurons. Chronic nicotine exposure functionally up-regulates $\alpha 6/\alpha 4$ subunit-containing nAChRs in vmHb Chat⁺ neurons (red arrows) and induces CRF synthesis (CRF⁺) in a population of VTA DAergic neurons that innervate the ventral IPN (red arrows), including the IPI. In addition, chronic nicotine increases expression of the CRF1 receptor in GABAergic neurons (GABA⁺) of the IPI (red arrows). (B) During nicotine withdrawal, increased nicotinic receptor signaling through up-regulated nAChRs may boost mHb cholinergic/glutamatergic neuronal activity, a process which might also be regulated by HCN channels in Chat⁺ vmHb neurons. In addition, CRF from the VTA is released and activates the up-regulated CRF1 receptors on GABAergic IPI neurons. CRF also increases spontaneous excitatory postsynaptic currents (sEPSC) frequency indicating increased Glu release, which stems from mHb inputs. Putative CRF1 receptors located in presynaptic mHb terminals could possibly contribute to the enhanced vmHb glutamatergic signaling. Increased Glu release activates IPR GABAergic neurons as well, triggering somatic withdrawal signs. *Adapted from* [29], [31] and [41]. Note that for clarity we have drawn vmHb presynaptic input innervating only the IPR but it is well known that the cholinergic/glutamatergic axons from vmHb neurons terminate both in the dorsal and central IPN. ACh, acetylcholine; Chat, choline acetyl-transferase; CRF, corticotrophin releasing factor; CRF1,

corticotrophin releasing factor receptor 1; DAergic, dopaminergic; GABA, γ -aminobutyric acid; Glu, glutamate; HCN, hyperpolarization-activated cyclic nucleotide-gated channel; IPI, interpeduncular nucleus intermediate; IPN, interpeduncular nucleus; IPR, interpeduncular nucleus rostral; mHb, medial habenula; nAChRs, nicotinic acetylcholine receptors; NMDA, N-methyl-D-aspartate; VTA, ventral tegmental area; vmHb, ventral medial habenula.

Figure 4. Circuitry of the mHb-IPN axis and its connections to anxiety-integrated circuits

A sagittal view of a rodent brain including the mHb-IPN axis together with neural circuits implicated in anxiety-related behaviors. mHb and IPN efferent/afferents are depicted by blue or black line. Blue lines indicate non-reciprocal connections to/from the mHb-IPN axis. Black lines represent reciprocal first-order connections with the mHb-IPN axis. Gray lines and regions indicate anxiety/fear circuitry without first-order connection to the mHb-IPN axis. Importantly, the mHb-IPN axis is poised to regulate fear/anxiety behaviors through direct connections with brain areas classically associated with these behaviors (i.e. HPC and mPFC), as well as through indirect, second-order connections. Of note, the majority of mHb-IPN efferents/afferents depicted have not been functionally characterized and their contribution to affective behavior is not known. Arrows represent projection into a region; blunt ends indicate projection from a region that is not reciprocal. Lines with arrows on either end are reciprocal connections between regions. BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central amygdala; DR, dorsal raphe nucleus; DTg, dorsal tegmental nucleus; HPC, hippocampus; IPN, interpeduncular nucleus; LC, locus coeruleus; LDTg, lateral dorsal tegmental nucleus; LH, lateral hypothalamus; IHb, lateral habenula; LS, lateral septum; MD, medial dorsal nucleus of the thalamus; mHb, medial habenula; mPFC, medial prefrontal cortex (IPN may receive input from infralimbic cortex); MnR, median raphe nucleus; MS, medial septum; NAc, nucleus accumbens; NDB, nucleus of the diagonal band; NI, nucleus incertus; PAG, periaqueductal gray/central gray; PBG, parabigeminal nucleus; SI, substantia innominata; SUM, supramammillary nucleus; VTA, ventral tegmental area; VTg, ventral tegmental nuclei of Gudden.