Review Article

Lateral Habenula Contribution in Nicotine Addiction: Focus on Dopamine, GABA and Serotonin Interactions

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Abstract

Compelling evidence has shown a pivotal role of dopaminergic function in drug addiction. Recently, the Habenula (Hb) has attracted a great deal of attention as another target for nicotine in the brain because of its role in regulating dopamine (DA), gamma-aminobutyric acid (GABA) and serotonin (5-HT) systems. Nicotine acts binding to acetylcholine receptors that are widely distributed in the brain. Interestingly, the receptor subtypes that mediate nicotine withdrawal responses are highly expressed in the Hb. Moreover, the block of habenular nicotinic receptors in animals chronically treated with nicotine enhances withdrawal responses once nicotine is discontinued. Furthermore, it has been shown how a high dose of nicotine can cause massive degeneration almost exclusively in the medial habenula (MHb) and its output tract, the fasciculus retroflexus. Thus, symptoms associated with nicotine withdrawal may be caused by dysfunctions of the Hb output. Therefore, Hb might be of fundamental importance in the expression of nicotine reinforcing properties and withdrawal. Here, we will focus on the role of the lateral habenula (LHb) on nicotine modulation of DA function and we will evaluate LHb interaction with the rostromedial tegmental nucleus (RMTg), a GABAergic area, and the serotonergic raphé nuclei. Furthermore, as LHb has high density expression of 5-HT_{2C} receptors, these subtypes might be important in the control of its neuronal activity and output to the midbrain monoaminergic and GABAergic systems.

Keywords

Nicotine, Drug Addiction, Habenular Nuclei, Dopamine, GABA, 5-$HT_{2C,}$ electrophysiology.

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Introduction

Tobacco addiction represents a serious social problem with major impacts on public health, representing the second-leading cause of death in the world. The number of yearly deaths in Malta attributable to smoking has risen by 28% from 289 in 1987 to 372 in 2008 as estimated by the Department of Health Information and Research (DHIR, 2008), i.e. approximately one death a day. The economic burden that tobacco use incurs to public health care services is high on Malta's financial agenda. Thus, there is an urgent need for a better understanding of the neurobiological bases of nicotine action, dependence and withdrawal, and new and more efficacious therapy for smoking cessation.

Nicotine acts by binding to nicotinic acetylcholine receptors (nAChRs) that are widely distributed throughout the central nervous system, as well as in the periphery. These receptors are pentameric structures assembled from 5 sub-units arranged around a central water-filled pore to form a receptor belonging to the superfamily of ligand-gated ion channels.[1-4] A wide variety of subtypes of nAChRs arise from the combination of the different subunits that compose the channel-receptor complex. In the mammalian central nervous system, nAChRs are composed by the combination of seven different α (α2–7, α9) and three β (β2-4) subunits to form either homomeric (α7) or heteromeric (α4β2) receptors.[5,6] This subunit composition represents a major factor in determining pharmacological and functional properties of these receptors. For instance, the α4 and β2 containing nAChR, the most represented in the mammalian brain, is characterized by the highest binding affinity to nicotine as well as by high sensitivity to upregulation following nicotine administration.[7,8] The inclusion of the α5 subunit in these receptors is able to modify their properties, such as enhancement of receptor expression, reduction of ligand-mediated upregulation and facilitation of receptor closure.[9,10] Moreover, some additional subunits can participate in the formation of these high affinity nAChRs specifically in some brain areas. Thus, α6 and β3 subunits have been shown to be included in α4β2 nAChRs to generate high-affinity receptors specifically in the basal ganglia, including the ventral tegmental area (VTA) and substantia nigra (SN), representing a relevant evidence for Parkinson's disease.[11,12] Finally, nAChRs assembled with the same subunits but in different stoichiometries show differences in their functions and pharmacological properties. For example, it has been shown that, while all α4β2–containing nAChRs of different stoichiometry bind nicotine with the same high affinity, only the $(\alpha4)_2(\beta2)_3$ nAChR shows the highest sensitivity to upregulation by nicotine[13].

The neural substrate for nicotine dependence and addiction is represented by the mesocorticolimbic neural system of the mammals' midbrain. One of the key components is the dopaminergic (DAergic) system that originates in the VTA and projects to the nucleus accumbens (NAcc) and medial prefrontal cortex (mPFC). This system has long since been implicated in playing a pivotal role in mediating and processing environmental rewards, as indicated by the earliest studies involving intracranial electrical self-stimulation.[14] The DAergic system has been involved in the reinforcement of reward-directed behaviours and likewise in the acquisition of behaviours that are inappropriately reinforced by addictive drugs like nicotine.[15,16] Thus, blocking of DA terminal release in the NAcc, by either localized lesion or DA antagonists administration, decreases nicotine self-administration in rats.[17] In the VTA, nAChRs are localized both post-synaptically on DA neurons and pre-synaptically on glutamatergic and GABAergic terminals innervating DAergic neurons.[18] Nicotine, inhaled with tobacco smoke, reaches a concentration that is enough to increase firing rate and burst firing of DA neurons by binding to nAChRs expressed on these neurons.[19] These receptors are mainly α4β2 which, as described previously, have a high binding affinity and undergo fast desensitization, usually within few minutes following nicotine administration. Simultaneously, α7 containing nAChRs, located pre-synaptically on glutamatergic terminals, are activated by nicotine boosting the glutamatergic drive of VTA DA neurons. These receptors, despite the α4β2, are not desensitized by nicotine because of their low binding affinity for this agonist, thus resulting in a long lasting effect together with long-term potentiation (LTP) induction on glutamatergic afferents.[20,21] Finally, nicotine induces desensitization of β2-containing nAChRs receptors expressed on VTA GABA interneurons leading to a reduction of their inhibitory influence on DA neurons.[19,22-24]

It is now well know how VTA DAergic neurons can respond to events of opposite hedonic valence with a change of their neuronal activity.[25,26] In particular, these neurons are known to inhibit their firing activity in response to aversive stimuli or events with negative motivational values, such as reward omissions. Since the neural activity of DA neurons depends on the balance between excitatory and inhibitory inputs of its afferents, it is important to know the neural pathway encoding of these motivational values in order to better understand how hedonic information is processed and thus to have a more complete understanding of how addictive drugs

like nicotine can exert their effects. Recently, a great deal of attention has been attracted by the lateral habenula (LHb). The habenular complex is an epithalamic structure involved in anxiety, stress, depression, schizophrenia, divided into a medial (MHb) and lateral (LHb) portion. The former receives afferent innervations from limbic structures and projects to the interpeduncular nucleus, while the latter receives its input mainly from the basal ganglia and sends efferent projections to the DAergic and serotonin (5-HT)-containing neurons of the VTA and the dorsal raphé (DR), respectively.[27-29] Both morphological and functional evidence clearly demonstrated the existence of an inhibitory control exerted by the LHb over VTA DA neuronal activity.[30,31] Most importantly, recently published data obtained on monkeys showed how LHb neurons respond to reward related signals in an opposite manner to DA neurons of the VTA. In particular, LHb neurons have been shown to encode for aversive stimuli with a phasic increase of their firing rate that, in turn, corresponds to a DA neurons inhibition in the VTA.[32,33] Since LHb efferents to the VTA are mainly glutamatergic, this implies that such inhibition is not direct but rather involves a multi-synaptic pathway. Thus, recent evidence identified the presence of an area located just posterior to the VTA, indicated as the rostro-medial tegmental nucleus (RMTg) or tail of the VTA, which receives a massive glutamatergic afferent input from the LHb and sends GABAergic projections to the VTA.[34-39] Morphological data also showed how this area receives convergent afferent innervations from various areas located within both the forebrain and brainstem, thus suggesting a role in integrating these influences with the dominant LHb input and in modulating both ascending and descending pathways output.[39] In fact, this area is activated by aversive and inhibited by rewarding stimuli, similarly to the LHb, suggesting its involvement in integrating reward-related information and passing it onto VTA DA neurons. Moreover, it has been shown that RMTg neurons can be modulated by systemically administered drugs of abuse, like cocaine or methamphetamine, and they are also strongly activated by nicotine.[40]

Nicotine modulation of later habenula neuronal activity

Nicotine administration produces positive effects, as demonstrated by nicotine intravenous self-administration behaviours observed in different mammals species like rats, mice and non human primates.[17,41,42] Chronic nicotine exposure leads to neuroadaptations that include tolerance and up-regulation, thus leading to a new homeostatic condition which requires a constant concentration of nicotine to be maintained. When nicotine intake is discontinued, a withdrawal syndrome emerges which is characterized by negative somatic and affective symptoms like irritability and anxiety. The avoidance of these negative symptoms associated with drug withdrawal is responsible for the drug seeking behaviours and motivates nicotine use, representing the main cause of relapse following discontinuation of the tobacco smoking habit.

Since it has been shown that the LHb plays an important role in processing aversive, negative sensory inputs, it is likely that this structure plays an important role in mediating withdrawal symptoms. Furthermore, it has been shown that chronic exposure to nicotine induces degeneration of *fasciculus retroflexus'* axons, the main output of the habenular complex,[43] thus further suggesting an involvement of this structure in nicotine addiction. So far, there are no functional data illustrating how LHb neuronal activity is affected by nicotine administration. Some preliminary data we have obtained in our laboratory show that nicotine is able to increase the neuronal activity of LHb neurons when injected systemically (Figure 1a). The intra-venous administration of single bolus of different doses of nicotine elicited an increase of basal firing rates characterized by a fast onset and followed, at the highest doses, by a complete silencing of the neurons, probably because of the intervention of depolarization block mechanisms. When nicotine was injected as cumulative doses, recorded neuronal activities displayed an inverted-U-shape type of response (Figure 1b), with an initial increase at the lowest doses followed by a reduction of their firing rates that, in some cases, reached a complete silencing. Finally, nicotine administration directly in the LHb was able again to increase neuronal firing rates of a sub-population of the recorded neurons (Figure 1c), thus demonstrating the presence of nAChRs in the LHb and showing that the neuronal activation, induced by systemic administration of nicotine, is partially mediated by the activation of habenular nAChRs.

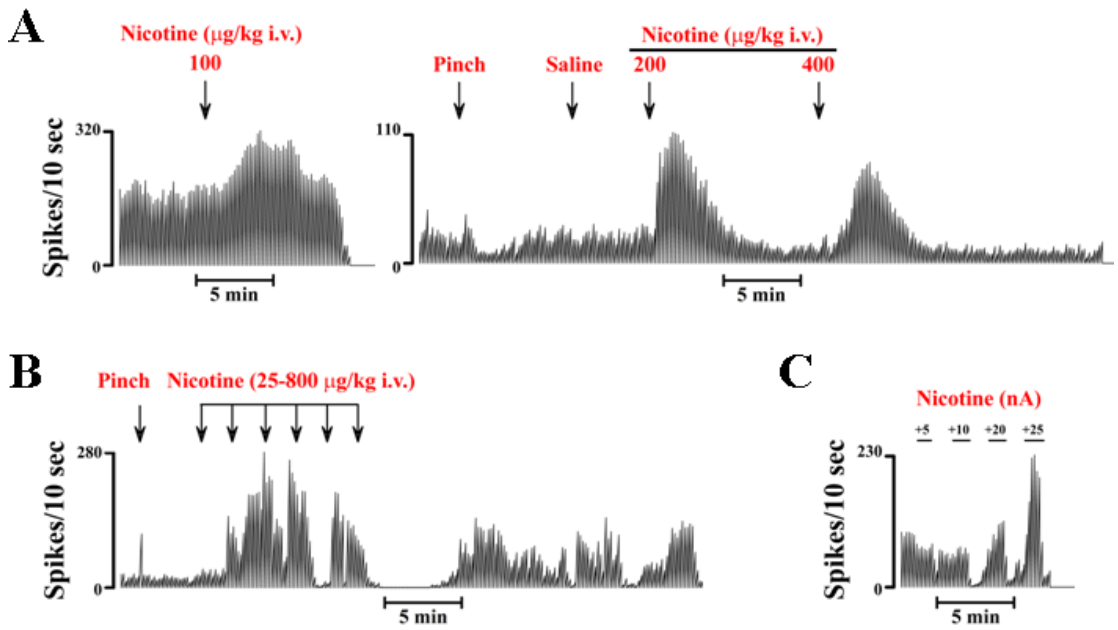

Figure 1

Nicotine increases LHb neuronal activity following systemic or local administration in the LHb. Rate meters show nicotine induced changes in basal firing rate of LHb neurons recorded *in vivo* on anaesthetized rats using the single unit extracellular recording technique. Each column in a trace represents the number of action potentials (spike) fired in a 10 seconds time bin. A) Effect of intravenous injection of single bolus of nicotine. Arrows indicate the time of injections of either nicotine or saline. Nicotine injection elicited a fast on-set increase of firing rate, followed by a slow decrease. B) Intra-venous injection of cumulative doses of nicotine elicited a inverted-U-shaped response in firing rate. C) Microionthophoretic administration of nicotine in the LHb increased basal firing rates in a current (dose) dependent manner; bars indicate time and duration of nicotine ejection in the extracellular space of the recorded neuron.

Lateral habenula, rostro tegmental area and raphé nuclei interactions

As stated above, a part from the LHb innervation, the RMTg receives afferents from both the DR and, to a lesser extent, the median (MR) raph nuclei.[39] Moreover, LHb and DR reciprocal innervations have been proved on both a morphological and functional basis.[44,45] Thus, the modulatory control of the serotononergic system over the neural circuit involving the LHb, RMTg and midbrain DA neurons might represent an important substrate for nicotine addiction.

The 5-HT/DA interaction has been demonstrated to largely depend upon the different 5-HT receptor sub-types expressed by the targeted neurons. A particular emphasis has been placed on the role of the 5-HT receptor subtype 2C (5-HT_{2C}) in modulating the activity of the midbrain DAergic system[46-49], highly expressed in VTA/SNc and in other areas receiving DA-ergic innervation. Several studies have demonstrated the efficacy of 5-HT_{2C} selective activation in blocking the stimulatory action of nicotine on midbrain dopamine function, as well as its behavioural effects,[50-52] thus suggesting a role for this receptor as a potential target in treating nicotine dependence.[53] Since the presence of 5-HT_{2C} mRNA has been demonstrated in the LHb[54,55] and our preliminary data show 5-HT_{2C} immunoreactivity at the level of the RMTg, these two areas may play an important role in 5-HT_{2C} block of the nicotine-induced DAergic hyperactivation. Therefore, the LHb and RMTg might play a central role in the brain reward circuitry.

Conclusions

Although mounting research findings on nicotine have revealed many of its effects, we are far from a full understanding of the complete scenario. The habenula complex has been shown to affect DA, 5-HT and GABAergic systems activity, suggesting it might play a role in nicotine action in the CNS. However, the extent to which the habenula may contribute to the neurobiological action of nicotine still needs to be fully addressed. The evidence reviewed in this paper shows a potential pivotal role for 5-HT in the modulation of the neural circuitry involving the LHb, RMTg and VTA DA system. Thus, 5-HT system may represent a good candidate for new smoking cessation therapies. In particular, the 5-HT_{2C} receptors have already been proved to counteract the effects elicited by nicotine on the DA system and, because of its distribution within the LHb-RMTg-VTA circuitry, it

may represent an important pharmacological target in treating nicotine addiction and alleviating withdrawal symptoms associated with smoking cessation.

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