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Repeated nicotine exposure in adolescent rats: Reduction of medial habenular activity and augmentation of nicotine preference

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HIGHLIGHTS

- Nicotine enhances spontaneous firing activity in the medial habenula.
- Repeated nicotine exposure alters medial habenular activities in adolescent rats.
- Basal firing activity in the medial habenula is reduced in nicotine-injected rats.
- Firing activity upon re-exposure to nicotine is decreased in nicotine-injected rats.
- Nicotine-injected rats show a significant increase in nicotine preference.

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ABSTRACT

Adolescence is a critical period for the initiation of tobacco use. Nicotine not only stimulates brain reward circuits to establish and maintain the tobacco smoking habit, but also produces aversive reactions to nicotine after initial exposure, due to its noxious properties. Although new insights into the mechanisms that regulate nicotine avoidance could result in an advantageous treatment strategy for addiction, little is known about the mechanism of nicotine aversion in adolescence. Because growing evidences suggest that the habenula to interpeduncular nucleus circuitry plays a critical role in nicotine aversion, we investigated the effects of repeated nicotine exposure on the electrical activity of medial habenular neurons in adolescent rats, using extracellular recordings. Nicotine strongly increased the frequency of spontaneous spike activity in the medial habenula of naïve rats. In repeated nicotine-injected rats, we found a reduction in nicotine-induced spontaneous spike frequency, such that these neurons displayed a significantly lower basal activity and reduced spontaneous activity upon re-exposure to nicotine. Moreover, nicotine intake preference in repeated nicotine-injected rats is significantly more increased than that in saline-injected rats. These results demonstrate that repeated phases of nicotine exposure induce a functional switch in the activity of medial habenular neurons in adolescent rats and suggest that medial habenular activity is one of mediators for an inhibitory motivational signal that limits nicotine consumption.

1. Introduction

Drug-seeking behavior and drug self-administration in animals and humans can be triggered by the drugs of abuse themselves. The repeated use of addictive drugs can produce tolerance, dependence, withdrawal, and sensitization to differing degrees, as well as addiction. Nicotine, the addictive component of tobacco, presents a complex motivational profile capable of producing both aversive and highly reinforcing effects [1,2]. Nicotine plays a critical role in establishing and maintaining the tobacco smoking habit because it stimulates brain reward circuitries, most prominently the mesocorticolimbic dopamine system. It is important to note, however, that due to the highly noxious properties of nicotine, most smokers initially have unpleasant smoking experiences. Aversive responses to nicotine appear to play key roles in determining the overall amount of tobacco smoke consumed and patterns of intake; stronger aversive reactions after initial exposure are negatively correlated with the development of habitual tobacco use in first time smokers [3]. Diminished sensitivity of nicotine-related aversion systems in the brain is therefore likely to increase vulnerability to the development of habitual smoking. It may be possible to target such brain circuits to enhance the noxious properties of nicotine, offering a novel treatment strategy to facilitate lower levels of tobacco consumption and increase the ability to cease tobacco smoking altogether.

Nicotine dependence is linked to single nucleotide polymorphisms in the CHRNB4–CHRNA3–CHRNA5 gene cluster encoding the α3β4α5 nicotinic acetylcholine receptors (nAChRs) [4,5]. The expression of the α3β4 and α5 subunit nAChR combination is restricted to a few discrete

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brain areas including the medial habenula (MHb) and interpeduncular nucleus (IPN) [6,7]. Mice lacking α5 subunits in the medial habenulo–interpeduncular (MHb–IPN) tract increase their nicotine consumption at high doses, indicating that MHb–IPN tract through α5 subunits nAChRs controls nicotine intake, triggering an inhibitory motivational signal that acts to limit nicotine intake [8]. Elevated expression of the nAChR β4 subunit increases nicotine aversion in mice by enhancing the activity of the MHb to the IPN and this effect is reversed by viral-mediated expression of the α5 into MHb, suggesting that the balanced activity of β4 and α5 nAChRs in MHb contributes to nicotine aversion [9]. Although the MHb is a pivotal element in the circuitry controlling nicotine-dependent phenotypes, it is still unclear how repeated exposure to nicotine alters neural activity in the MHb and affects habitual nicotine intake in adolescence which is a critical period for the initiation of tobacco use. In this study, we sought to determine the effect of repeated nicotine exposure on nicotine-induced excitability in MHb neurons of adolescent rats and nicotine consumption behavior. To achieve this, we carried out electrophysiological extracellular recordings in MHb slices in saline-injected rats and nicotine-injected rats to characterize the pattern of nicotine-induced neural activity, and examined the total amount of nicotine consumed using a two-bottle choice paradigm.

2. Materials and methods

2.1. Animals

All animal studies were conducted in accordance with the Dankook University ethics committee's guidelines for the care and use of laboratory animals. Sprague–Dawley rats (20–40 days old) were obtained from ORIENT BIO (Seongnam, Korea). Rats were housed in Plexiglas cages (45.72 cm × 22.86 cm × 20.32 cm) with wood bedding in a room with the temperature maintained at 19–21 °C (40–60% humidity), a standard 12-h light/dark cycle (light on 09:00–21:00), and food and water *ad libitum*.

2.2. Nicotine-injected rat model

Nicotine hydrogen tartrate (Sigma-Aldrich) was dissolved in sterile saline. For drug administration, rats were injected subcutaneously at the same time each day with saline or nicotine (0.7 mg/kg free-base) in volumes of 0.1 ml/kg. Injections occurred three times at 12-h intervals from 21:00.

2.3. Slice preparation

Following the final nicotine or saline injection, spontaneous extracellular recordings were performed in coronal MHb slices from rats in each group. To obtain habenular slices, rats were briefly anesthetized with isoflurane and decapitated. Brains were quickly removed and immersed in an oxygenated (95% O_2/5% CO_2), ice-cold modified artificial cerebrospinal fluid (ACSF) solution containing the following (in mM): 201 sucrose, 3 KCl, 1.25 NaH_2PO_4, 3 $MgCl_2$, 1 $CaCl_2$, 26 $NaHCO_3$, and 10 D-glucose. Coronal habenular slices of 400-μm thickness were cut using a vibratome (1000S, TedPella). Before each recording, slices were incubated with warm (30 °C) oxygenated ACSF containing the following (in mM): 126 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 26 $NaHCO_3$, and 10 D-glucose. Following a recovery period of 60 min, slices were left at room temperature. For recording, individual slices were transferred to a submerged-type recording chamber that was fixed to a microscope stage (SZ-STU1, Olympus). The slices were kept at a constant temperature of 27–30 °C and superfused with oxygenated ACSF at a rate of 2–4 ml/min.

2.4. Spontaneous extracellular multi-unit recordings

We recorded spontaneous spikes in the MHb region using a borosilicate glass capillary electrode (O.D. 1.5 mm/I.D. 0.84 mm, WPI) with ACSF connected to a microelectrode AC amplifier (model 1800; A-M Systems Inc.). Recording pipettes were pulled with a horizontal micropipette puller (P-9, Sutter Instrument Company) and polished with a microforge (MF-830, Narishige). Neuronal signals were recorded differentially using an AC-coupled four-channel amplifier at a gain of 1000, with a ground electrode located nearby in the bathing medium. The analog signal was digitized by an A/D converter (Digidata 1322A, Molecular Devices) and collected with pClamp10 software (Molecular Devices). The number of spontaneous spikes was counted using a threshold search method in the pClamp10 software. We measured real spikes that had an amplitude >0.06 mV and any spikes below this threshold were ignored. Control spike amplitude was taken as 0.06–0.5 mV. All histograms were constructed to reflect the number of spikes per 10 s. For normalized number of spikes in Fig. 1, we collected stable spontaneous neuronal activity for 3 min prior to nicotine treatment (control), the peak frequency during the 10 min after the start of nicotine application (excitation), the average frequency for 3 min from the 5 min after the end of nicotine application (reduction), peak frequency from the 10 min after the end of nicotine application to the wash period (re-excitation) and the average frequency for 3 min after 30 min from the start of nicotine application (wash).

2.5. Nicotine preference test: oral nicotine consumption by two bottle free-choice paradigm

Following the first injection of nicotine or saline solution, rats were presented with two bottles at 21:00. One bottle contained water and the other bottle contained a nicotine solution (25 μg/ml) diluted in water. The intake of fluid from each bottle was measured for 36 h. The position of the two bottles alternated every 12 h to exclude any positional preference in the rats. Nicotine preference was calculated as a ratio of the volume of nicotine solution consumed divided by the total fluid intake for each rat.

Nicotine preference (%) = [Nicotine consumption (ml) / Total fluid consumption (ml)] × 100

2.6. Statistical analysis

Data were presented as mean ± standard error of the mean and analyzed using Prism5 software (GraphPad software Inc.). Student's *t*-tests were employed to compare data between the two groups. Significance tests were based on one sample *t*-tests against 50%, which is the reference value meaning indifference with respect to either consumption with Bonferroni–Holm's correction for multiple comparisons. To normalize the number of spikes as a function of time in the extracellular recording data, we divided the number of spikes in the presence of drugs by the basal average value, which was determined as the average number of spikes in the absence of drugs.

3. Results

3.1. Nicotine enhances spontaneous firing activity in the medial habenula of adolescent rats

The MHb has been suggested as a critical element in the circuitry that controls nicotine-dependent behavior [8,9]. We therefore assessed the effect of nicotine on medial habenular neural activity in naïve adolescent rats. To measure any nicotine-induced alteration of spontaneous firing activity in the MHb, we measured extracellular recordings in the MHb slices of naïve rats (Fig. 1). Stable spontaneous neuronal activity was collected for 3 min prior to nicotine treatment (Control). In the control phase, the average firing rate frequency was 0.2–16 Hz. After