

Short communication

Chronic infusions of mecamlamine into the medial habenula: Effects on nicotine self-administration in rats

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ABSTRACT

The habenula is an epithalamic structure through which descending connections go from the telencephalon to the brainstem, putting it in a key location to provide feedback control over the ascending projections from the brainstem to the telencephalon. The medial habenula has a high concentration of nicotinic receptors. We assessed the role of medial habenular nicotinic receptors for nicotine self-administration (SA) in female young adult Sprague-Dawley rats. The rats had bilateral chronic infusion cannulae placed into the medial habenula nucleus. Each cannula was connected to a slow delivery osmotic minipump to chronically infuse mecamlamine (100 µg/side/day) or vehicle for four consecutive weeks. The rats were tested for nicotine SA for the first two weeks of mecamlamine infusion. Then, they had one week of enforced abstinence, during which they had no access to the nicotine SA. Finally, they had one week of resumed nicotine SA access. There was a significantly differential mecamlamine effects in animals with lower and higher pretreatment baseline nicotine SA. Rats with lower baseline nicotine SA levels showed a nearly significant mecamlamine-induced reduction in SA while those with higher baseline levels of SA showed a significant mecamlamine-induced increase in nicotine SA. This study determined that medial habenular nicotinic receptors are important for nicotine reinforcement. Baseline level of performance makes a crucial difference for the involvement of habenular mechanisms in nicotine reinforcement with nicotinic activation being important for maintaining nicotine self-administration for those with lower levels of baseline self-administration and the opposite effect with subjects with higher levels of baseline self-administration.

1. Introduction

The habenular nuclei are epithalamic structures, which provide feedback important for communication from the telencephalon back to the brain stem. The habenula is situated at a key location in connecting basal ganglia and the limbic system with the brainstem [10] and controls important processes in reinforcement [11]. The medial habenula is the portion that contains dense concentration of a variety of different nicotinic receptor subtypes [3]. $\alpha 3\beta 4$ subunit-containing nicotinic receptors have been found to dominate function in rat medial habenula neurons [15]. However, the importance of $\alpha 5$ containing nicotinic receptors in the habenula for controlling nicotine self-administration has also been shown [7]. Decreases in habenular $\alpha 5$ containing nicotinic receptors removed the aversive effects of higher doses of nicotine, potentiating higher levels of nicotine self-administration [7]. Nicotinic receptors in the medial habenula are important for self-administration of

addictive drugs such as methamphetamine and morphine [8,9].

Neurons in the habenula are sensitive to excitotoxic damage. Ellison et al. found that nicotine can cause significant neurotoxic damage to the habenula [2]. Similar effects are seen with other stimulants [1,4–6]. This neurotoxic action of nicotine and stimulant drugs on habenular neurons may be a source of persisting alterations in control of behavioral response that underlies persistent addiction.

Nicotinic innervation of the habenula is not only involved in controlling reactions to drugs of abuse, they are also key in cognitive function. We have previously found that infusion of mecamlamine into the lateral habenula significantly impaired spatial working memory [17].

The current study was conducted to determine the role of medial habenular nicotinic receptor activation in the control of nicotine self-administration. Mecamlamine was used as a general nicotinic antagonist because as a non-specific channel blocker it antagonizes all nicotinic

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receptor subtypes.

2. Methods

2.1. Subjects

Young adult female Sprague-Dawley rats (2–3 months of age) (Taconic Lab, Germantown, NY, USA) were used in the present study. Females were used in this study to be able to directly compare with effects in females in our previous studies. Animals were individually housed in a temperature-controlled vivarium room located adjacent to the nicotine self-administration testing room. The individual housing was needed so that the rats would not chew on each other's catheters. Animals were maintained on a 12:12 reverse light-dark cycle (lights off at 7:00 a.m.) so that experimental sessions occurred during the active part of the rats' diurnal cycle. Animals were given ad lib access to water at all times excluding experimental sessions and were fed daily 20–30 min after the completion of their experimental session and were maintained at approximately 85% of free-feeding weight to facilitate the operant responding. Animals progressively and healthily gained weight throughout the study. At the end of the study catheter patency tests were conducted and any animals with an obstructed catheter was dropped from the study.

Nicotine bitartrate solutions were prepared biweekly in sterilized isotonic saline. The dose used for self-administration (0.03 mg/kg/infusion) was calculated as a function of the nicotine free base weight. The pH of the nicotine solution was adjusted to 7.0 using NaOH and the solution was filtered in a Nalgene filter (Nalgene Nunc International, Rochester, NY, USA) for sterilization. Between sessions all nicotine was kept in a dark refrigerator. Mecamylamine was dissolved in artificial cerebral spinal fluid for the brain infusion to deliver 100 µg/side/day in a volume of 6.48 µl/day.

2.2. Nicotine self-administration

Before the start of nicotine self-administration sessions, all animals were trained to lever press in a standard dual-lever experimental chamber (Med Associates, St. Albans, VT, USA) for food reinforcement (45 mg pellets, Bio-Serv, Flemington, NJ, USA). Each chamber was equipped with two levers (one active, one inactive), two cue lights located directly above each lever, a house light, and a tone generator. After lever pressing was established, animals had three sessions of lever pressing for food under a fixed ratio (FR) 1 schedule of reinforcement with a cue light indicating the active lever. Following the completion of their final training session with food reinforcement, animals were anesthetized with a mixture of ketamine (60 mg/kg) and dormitor (15 mg/kg) and a catheter (Strategic Application Inc., Libertyville, IL, USA) was implanted into their jugular vein. The jugular catheter was attached to a harness that could be tethered to the infusion pump during experimental sessions. Animals were given a minimum of 24 h to recover from surgery before experiencing nicotine self-administration sessions.

Following surgery, animals were given 5 experimental sessions where a correct lever press resulted in the delivery of a nicotine infusion on a fixed ratio (FR) 1 schedule of reinforcement, and the activation of a feedback tone for 0.05 s. Each infusion was followed by a one-minute period where the cue lights went out, the house light came on and correct responses were recorded but not reinforced.

The catheters were flushed daily, before the experimental sessions, with a 100 U/ml heparinized saline solution. After the completion of a test session nicotine remaining in the port was removed and a 0.3-ml sterile lock solution containing 500-U/ml of heparinized saline and 8-mg/ml of gentamicin was infused (American Pharmaceutical Partners, Schaumburg, IL, USA). At the end of the study catheter patency was verified with a barbiturate sedation challenge.

2.3. Stress manipulation

The effect of inducing stress before the nicotine self-administration session was tested with confinement in a Plexiglas tube (9 cm internal diameter). Before half of the sessions all of the rats went through a behavioral stress procedure of confinement for a 30-min. period before nicotine self-administration. Before the other half of the sessions all of the rats did not undergo the stress procedure. Thus, stress was a within subjects factor with each subject serving as its own control. This was done to test the hypothesis that the habenula is a key part of the neural circuit responding to stress and that disruption of habenular feedback from the telencephalon to the brainstem would impair the quenching of the stress response [12].

2.4. Medial habenular mecamylamine chronic infusion

Young adult female Sprague-Dawley rats were given access to IV nicotine self-administration. After training for nicotine self-administration on an FR1 schedule (0.03 mg/kg/infusion) for five sessions, they were implanted with bilateral infusion cannulae directed to the medial habenula using the Pellegrino Atlas (1979) [14]. Mecamylamine (100 µg/side/day) (N = 14) or the aCSF vehicle infusions (N = 12) were made from a slow delivery (0.27 µl/h) osmotic minipump (Alzet model 2004, Durect, Inc, Cupertino, CA, USA) for four consecutive weeks. The rats were anesthetized with ketamine (65 mg/kg) and dormitor (15 mg/kg) IP. Using a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) bilateral infusion cannulae were placed in the lateral habenular nuclei. From bregma for anterior-posterior and medial-lateral and from the cortical surface for dorsal-ventral, the stereotaxic coordinates for the medial habenula were ±0.4 mm medial-lateral, −2.4 mm anterior-posterior, and −4.7 mm dorsal-ventral [14]. The cannulae connected via PE tubing to the minipump (one per side) to deliver mecamylamine HCl (Sigma, St. Louis, MO, USA). The cannulae were positioned into the medial habenula, and secured using a screw and wire structure. They were then covered with a dental cement cap to prevent outside damage. The rats were given access to nicotine for two weeks after surgery. Then there was a one-week hiatus followed by a final week of resumed access.

2.5. Data analysis

Analysis of variance was used to assess the statistical significance of the data. The dependent measure was nicotine infusions per session. Mecamylamine was a between-subjects factor. Only animals with bilateral placements of cannulae within the medial habenula and patent IV catheters throughout the study were included in the analyses. In addition, the rats were divided into low and high levels of nicotine self-administration based on a median split of lower and higher self-administration during the initial training period which took place before the onset of the mecamylamine treatments. The repeated measure was week of treatment and stress vs. no stress conditions. In all cases, the threshold for significance was set at $p < 0.05$.

3. Results

All of the rats included in the analysis were verified by histological techniques after the end of the study to have the infusion cannulae on both sides within the target area in the medial habenula (Fig. 1) and patent IV infusion catheters for nicotine self-administration. The pre-treatment nicotine self-administration in sessions 4–5 of baseline training was low responder control = 5.40 ± 1.48 , N = 5, high responder control = 17.07 ± 1.81 , N = 7, low responder mecamylamine = 4.77 ± 1.13 , N = 9, high responder mecamylamine = 21.50 ± 2.81 , N = 5 (Fig. 2).

There was a significantly differential effect of mecamylamine in animals with lower and higher pretreatment baseline nicotine SA scores.

Chronic Bilateral Medial Habenular Infusions

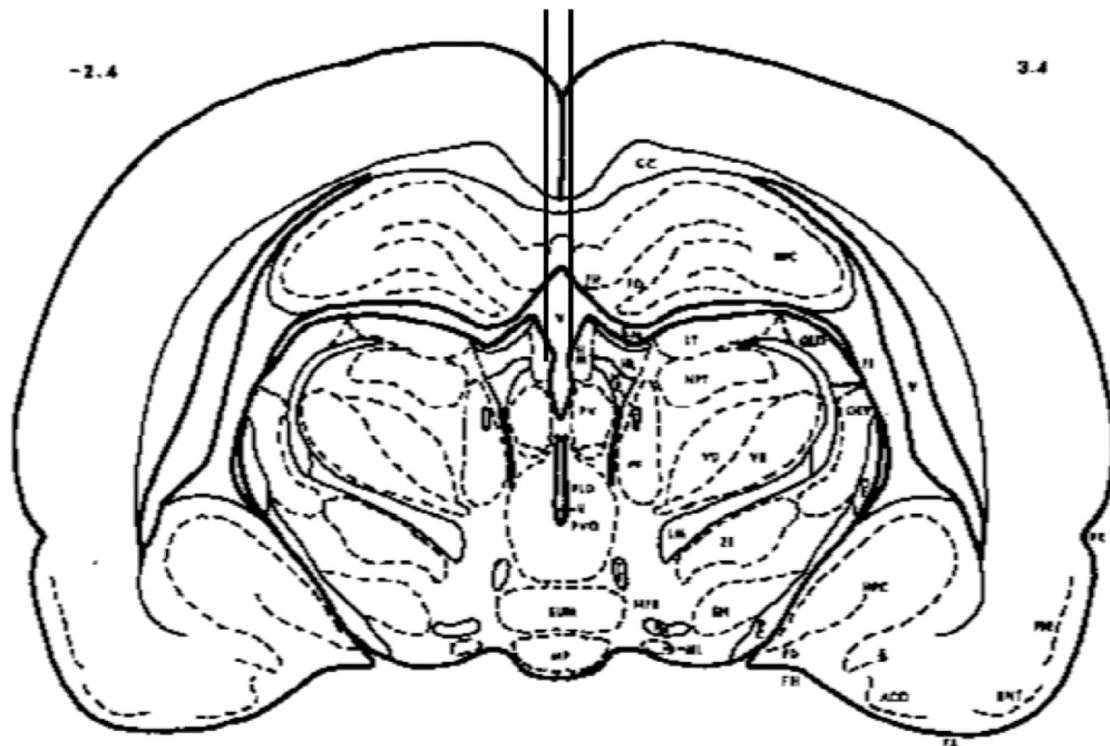


Fig. 1. Site of chronic infusion of mecamylamine into the medial habenular nucleus [14].

Chronic Mecamylamine Infusions into the Medial Habenula IV Nicotine Self-Administration

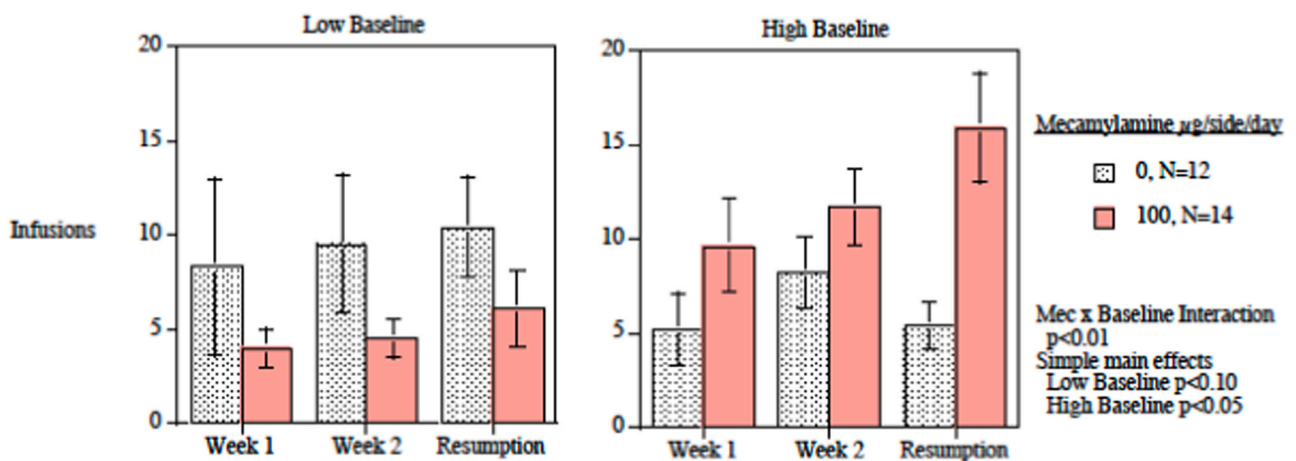


Fig. 2. Effects of chronic bilateral mecamylamine infusion into the medial habenular nuclei for four weeks on IV nicotine self-administration in rats with low vs. high baseline levels of nicotine self-administration, infusions per session (mean \pm sem). There was a significant two-way interaction of mecamylamine treatment \times low vs. high baseline response ($p < 0.01$). Follow-up tests of mecamylamine effects in each of these groups showed divergent effects of medial habenular mecamylamine infusions which caused a significant ($p < 0.05$) increase in nicotine self-administration in rats with higher initial nicotine self-administration, but not in rats with lower initial responding which actually showed a trend toward lower nicotine self-administration with medial habenular mecamylamine treatment ($p < 0.10$). Low responder control $N = 5$, high responder control $N = 7$, low responder mecamylamine $N = 9$, high responder mecamylamine $N = 5$.

The two-way interaction of chronic mecamylamine treatment \times low vs. high baseline response was significant ($F(1,22) = 8.08, p < 0.01$). The effect of chronic habenular infusion on nicotine self-administration was selective depending on the baseline pre-treatment level of responding. Follow-up tests of the simple main effects of habenular mecamylamine in low and high baseline responders averaged over the three phases of treatment (week 1, week 1 and resumption) were made. low vs. high baseline response groups. In the higher initial baseline group, chronic bilateral infusion of 100 $\mu\text{g}/\text{side}/\text{day}$ of the nicotinic antagonist mecamylamine in the medial habenula caused a significant ($F(1,22) = 5.07, p < 0.05$) increase in nicotine self-administration relative to controls. Chronic mecamylamine infusion into the medial habenula in the lower initial baseline group did not increase nicotine self-administration. In contrast, in this group there was a trend ($F(1,22) = 3.09, p < 0.10$) to decrease nicotine self-administration with chronic mecamylamine treatment. Interestingly, the control vehicle-treated rats with higher levels of self-administration during baseline training and initial acquisition of nicotine, before the onset of medial habenular infusions, had lower self-administration after infusion of aCSF into medial habenula.

The stress manipulation did not have a significant main effect on nicotine self-administration. Neither were any of the interactions of stress \times treatment significant.

4. Discussion

Results of the study support the conclusion that nicotinic receptors in the medial habenula play a role in nicotine self-administration. However, the role that they play appears to be different for individuals with higher vs. lower rates of nicotine self-administration. Blocking medial habenular nicotinic receptors by mecamylamine moderately lowered nicotine self-administration in rats with lower rates of nicotine self-administration during baseline training. In contrast, with the high responders mecamylamine infusions in the medial habenula significantly increased nicotine self-administration with the rates continually increasing throughout the four weeks of chronic mecamylamine infusion. This study suggests that the neural substrates underlying higher and lower levels of nicotine self-administration may differ, particularly with regard to the role of nicotinic receptors in the medial habenula.

The rats with the higher baseline nicotine self-administration may have learned to self-administer nicotine more quickly than those in the lower baseline group which continued to increase nicotine self-administration into the treatment phase of the study. We previously showed that infusion of mecamylamine into the habenula significantly impaired spatial working memory [17]. The continued acquisition of nicotine self-administration into the treatment phase of the study by the lower baseline group may have been blocked by mecamylamine into the medial habenula. In contrast, in the higher baseline group that already learned nicotine self-administration during the pretreatment baseline phase, the chronic medial habenular mecamylamine blockade of the aversive effects of nicotine may have potentiated further increases in nicotine self-administration.

Fowler et al. [7] found that $\alpha 5$ containing nicotinic receptors in the medial habenula are particularly important for controlling nicotine self-administration. Deletion of $\alpha 5$ nicotinic receptors caused increased nicotine self-administration by limiting the aversive effects of higher doses of nicotine. Re-establishing $\alpha 5$ nicotinic receptors in the medial habenula reversed this effect. Mecamylamine is a nonspecific channel blocker of nicotinic receptors and would be an antagonist at $\alpha 5$ -containing $\alpha 4\beta 2$ nicotinic receptors as well as other receptor subtypes when infused into the medial habenula, though mecamylamine has been found to have lower potency at $\alpha 5$ -containing $\alpha 4\beta 2$ nicotinic receptors than $\alpha 4\beta 2$ receptors that do not contain $\alpha 5$ subunits [13]. This may have been a critical mechanism by which it significantly increased nicotine self-administration. Given that medial habenular mecamylamine infusion only had this effect of increasing nicotine self-administration in rats with higher initial response, points to differing habenular participation

in lighter and heavier nicotine using subjects.

One curious finding in this study was that the rats with higher acquisition of nicotine had lower nicotine self-administration after implantation of the bilateral local brain infusion cannulae into the medial habenula. One possibility is that the ketamine anesthesia had a persisting effect reducing nicotine self-administration. We have previously shown that lower ketamine doses significantly reduce nicotine self-administration in rats [16]. It may be the case that higher ketamine doses used for surgery cause a more long-lasting effect of reducing nicotine self-administration. If so, it is interesting that this effect is preferential for higher level responders. Also, potentially important is that if ketamine had this effect, it can be reversed by mecamylamine infusions in the medial habenula. Both ketamine and mecamylamine have antagonist effects at NMDA glutamate receptors, so their common effects at NMDA receptors in the medial habenula is unlikely to be the source of the reversal. Other possibilities include ketamine effects on NMDA glutamate receptors elsewhere in the brain since it was administered systemically, mecamylamine effects on nicotinic receptors in the medial habenula or the asynchrony with which the two drugs were given, with the acute systemic ketamine anesthesia first and the chronic medial habenular mecamylamine infusion later. In a previous study, we have found that in female Sprague-Dawley rats, ketamine, at a sub-anesthetic dose significantly reduced nicotine self-administration in high, but not low responding rats [16]. Ketamine anesthesia was used in both that study and the current one. Sorting out the possible mechanisms for ketamine interactions with mecamylamine will take further research.

The current study found that nicotinic receptors in the medial habenula are important control points for nicotine self-administration. The differential effects of chronic mecamylamine infusions on nicotine self-administration in low and high baseline performers suggests that low and high baseline performers likely have different neural substrates for controlling nicotine self-administration. Rats with higher rates of self-administration may have been limited by aversive effects of high levels of nicotine, which would have been blocked by mecamylamine. In contrast the low self-administering rats may have been limited by insufficient reward from nicotinic receptor activation. Human light and heavy tobacco smokers may likewise have differing substrates for maintenance of their smoking and may benefit from different therapeutic treatments to facilitate smoking cessation. Further comprehensive studies are needed to fully understand the role of nicotine receptors in the medial habenula on nicotine addiction.

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CRediT authorship contribution statement

All of the authors substantively contributed to this project and agree to its publication. EDL: Design of study, data analysis, interpretation, writing, CW: Conduct of study, data gathering, SS: Conduct of study, data gathering, JJ: Conduct of study, data gathering, AP: Conduct of study, data gathering, AHR: Design of study, interpretation, writing, JER: Design of study, interpretation, writing.

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