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Inactivation of the *Nucl. Accumbens* Core Exerts No Effect on Nicotine-Induced Conditioned Place Preference

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Effects of transient inhibition of the core part of the *nucl. accumbens* (NAcC) by lidocaine on nicotine-induced conditioned place preference in male Wistar rats were examined. Lidocaine (2%) was injected into the NAcC of nicotine-conditioned animals before each nicotine i.p. injection. On the test day, behavior of the animals in a two-compartment apparatus was recorded during 10 min. Results revealed that i.p. injections of nicotine (1.0 or 1.5 mg/kg) induced place preference. Transient lidocaine-induced inhibition of one or both sides of the NAcC did not change place preference but changed the numbers of compartment crossings, rearings, and sniffings. Inhibition of the left part and both parts of the structure reduced sniffing and increased place preference; inhibition of the right part of the nucleus increased the intensity of this phenomena.

Keywords: nicotine, conditioned place preference, lidocaine, core part of the ***nucl. accumbens***, shell part of the ***nucl. accumbens***, rat.

INTRODUCTION

Nicotine addiction results from tobacco smoking and, simultaneously, it is the main reason for the maintenance of this habit. It is now clear that the mesolimbic dopamine (DA) system is the key brain system involved in the nicotine reward and dependence [1, 2]. Dopamine concentration increases after nicotine injection in the *nucl. accumbens* (NAc) [2–5]. This nucleus contains GABAergic medium spiny neurons (more than 90% of the total neuronal population) [6, 7]. The NAc receives excitatory and modulatory DAergic inputs from the cortex and ventral tegmental area, respectively [8, 9].The nucleus is subdivided into the core (C) and shell (Sh) parts according to noticeable anatomical and cytochemical differences [10–12]. There are data that these two parts may play different roles in the formation of a reward relation to nicotine [3, 8, 13]. There are reasons to believe that the Sh part of the NAc after nicotine introduction initiates nicotine rewarding properties, while the C part may play a role in the nicotine conditioning (Pavlovian type) [3]. Our earlier study indicated that an asymmetry between the right and left sides of NAcC may exist in morphine reward [14]. In addition, the asymmetry is also manifested with respect to locomotion activity [14–16]. However, the possible important role of this asymmetry in nicotine reward was not clear. Therefore, our main aim was to evaluate the results of transient switching off of the NAcC by lidocaine when trying to clarify the roles of the left and right halves of the NAcC in the nicotine reward using the place conditioning paradigm

METHODS

Animals. Male Wistar rats (250 ± 20 g, the Pasteure Institute, Tehran, Iran) were used throughout the study (6–8 rats for each experiment). Animals were housed in groups of 4 per cage at a 12/12 h light/dark cycle with food and water available *ad libitum* and randomly allocated to different groups of the experiment.

Drugs. The following drugs were used in the experiments: nicotine hydrogen tartrate salt, lidocaine hydrochloride, and diazepam (Sigma, USA) and ketamine hydrochloride (Alfasan Worden, The Netherlands). The drugs were, if necessary, dissolved in sterile saline before use. Nicotine was i.p. injected in a volume of 1.0 ml/kg, while 2% lidocaine [14] was given intra-NAcC in a volume of 1.0 μl/rat 5 min before the test nicotine injection.

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The characteristic time of action for lidocaine is around 30 min; this time was interfaced with the action of nicotine (10-25 min) [17]. The control groups received saline.

Surgical Procedures. Rats were anesthetized with ketamine (70 mg/kg, i.p.) + diazepam (10 mg/kg, i.p.), and one or two stainless steel 23-gauge cannulas (Stoelting Instruments, USA) oriented to the NAcC were implanted stereotaxically with their tips 0.5 mm above the intended site of lidocaine injection according to the atlas [18]. Stereotaxic coordinates for the NAcC were the following: incisor bar, –3.3 mm, anterior to the bregma, 0.8 mm, lateral to the sagital suture, ±1 mm, and 7.0 mm down from top of the skull. Cannulas were secured to jeweler screws with dental acrylic. After completing the surgery, a dummy stylet was inserted into the guide cannula and left in place until injections were made. Animals were allowed 7 days to recover from surgery and anesthesia. For drug infusion, the animals were gently restrained by hands; the stylets were removed from the guide cannulas and replaced by 30-gauge injection needles (0.5 mm below the tip of the guide cannula). The solutions were slowly administered in a total volume of 1.0 µl/rat (0.5 µl into each side) over a period of 60 sec. Injection needles were left in place for an additional 60 sec to facilitate diffusion of the drugs. The effect of lidocaine as a reversible Na^+ channel blocker is the greatest around 8 min after infusion and may last for up to 30 min.

Place Preference. A two-compartment place preference apparatus (30×60×30 cm, made of wood) [14] was used. Two equal-sized compartments were separated by shading with a removable guillotine door; the compartments were distinguishable by texture and olfactory and visual cues. One of the compartments had a smooth floor, while another one had a nylon white mesh floor. A drop of menthol was placed at the center of the compartment with a textured floor to provide the olfactory difference between the compartments. For visual differences, the compartments were differently striped black on their sides. Under pre-testing conditions, the rats showed no consistent preference for one or another compartment.

Behavioral Testing. Each animal initially received nicotine pretreatment (0.4 mg/kg, i.p.) for three consecutive days. Place conditioning was carried out using an unbiased procedure, with minor changes in the previously described design [19].

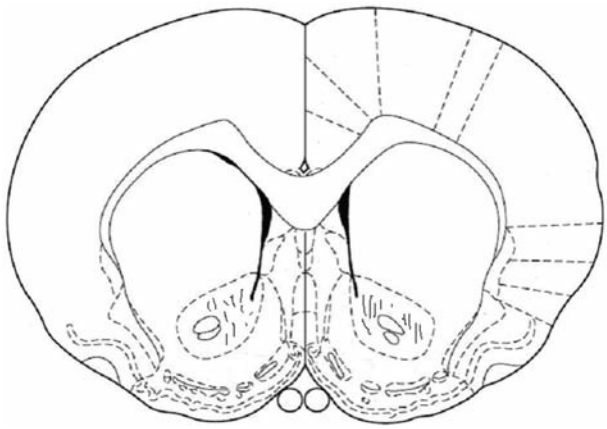
On day 1 (pre-exposure), each rat was placed into the apparatus for 10 min with free access to both compartments, and the time spent by the rat in each compartment was measured. The animals did not show any consistent preference for either compartment.

The conditioning phase consisted of a 3-day schedule of the sessions. Within this phase, animals received three trials in which they experienced the effects of nicotine, while confined in one compartment for 45 min, and three trials in which they experienced the effects of saline, while confined in another compartment also for 45 min. Access to the other compartment was blocked during these days. In addition, nicotine and saline compartments were randomly assigned for each animal in a counterbalanced way. Five minutes before each nicotine injection, 2% lidocaine was injected into the NAcC according to the experimental procedure.

On the 5th day (the preference test day), the partition was removed, and each rat was placed in the middle part of the apparatus where it could access both compartments. Behavior of each animal was digitally videotaped for 10 min. Video files were later analyzed off-line by a person who was not familiar with the experiment. Sniffing and rearings were considered stereotype behavioral phenomena, while compartment crossings were considered a non-stereotype behavior and an indicator of locomotor activity [19]. The total times spent by the animal in each compartment were distinguished and measured [14, 20].

Histology. After the completion of testing, all animals were anesthetized and perfused transcardially with 0.9% normal saline followed by 10% buffered formalin. The brains were removed, blocked, and cut coronally into 40-µm-thick sections through the cannula placements. The tissues were stained with cresyl violet and examined by light microscopy by an unfamiliar observer. Only the animals with correct cannula placements were included in the analysis (Fig. 1).

Data Analysis. All data are expressed as means ± ± s.e.m. The nicotine dose-response relation was analyzed using one-way analysis of variance (ANOVA) followed by the Tukey *post-hoc* test. A three-way analysis of variance (ANOVA) was applied for estimation of the differences between the lidocaine-treated groups considering side, pretreatment, and treatment as factors. When this analysis showed a significant difference, the Tukey HSD test was applied. Differences with $P < 0.05$ were considered significant.

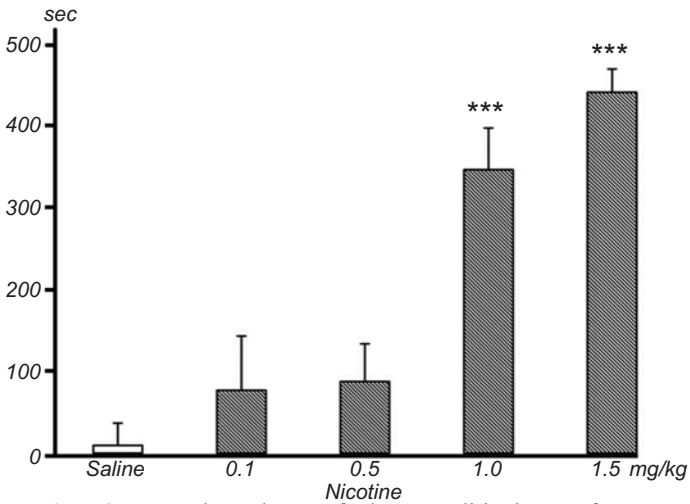


F i g. 1. Location of the cannula tips in the core parts of the *nucl. accumbens*.

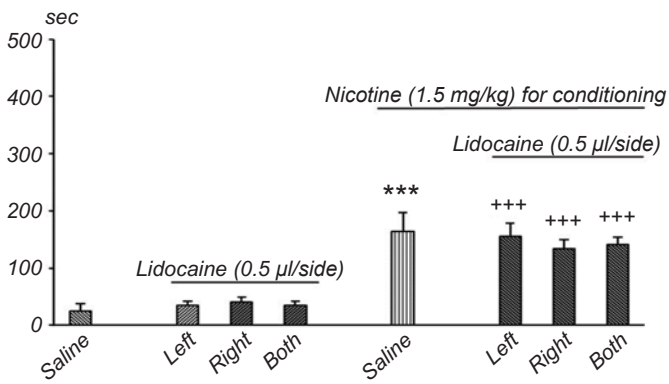
RESULTS

Nicotine Dose-Response Relation. The effects of nicotine on place preference are shown in Fig. 2. Different doses of nicotine (0.1, 0.5, 1.0, or 1.5 mg/kg) were i.p. injected into rats, and two higher doses caused a significant place preference to the drug-paired compartment [$F(5,30) = 3.21$, $P < 0.01$]. Based on these data, the dose of 1.5 mg/kg of nicotine was selected as an effective amount for the rest of the experiments.

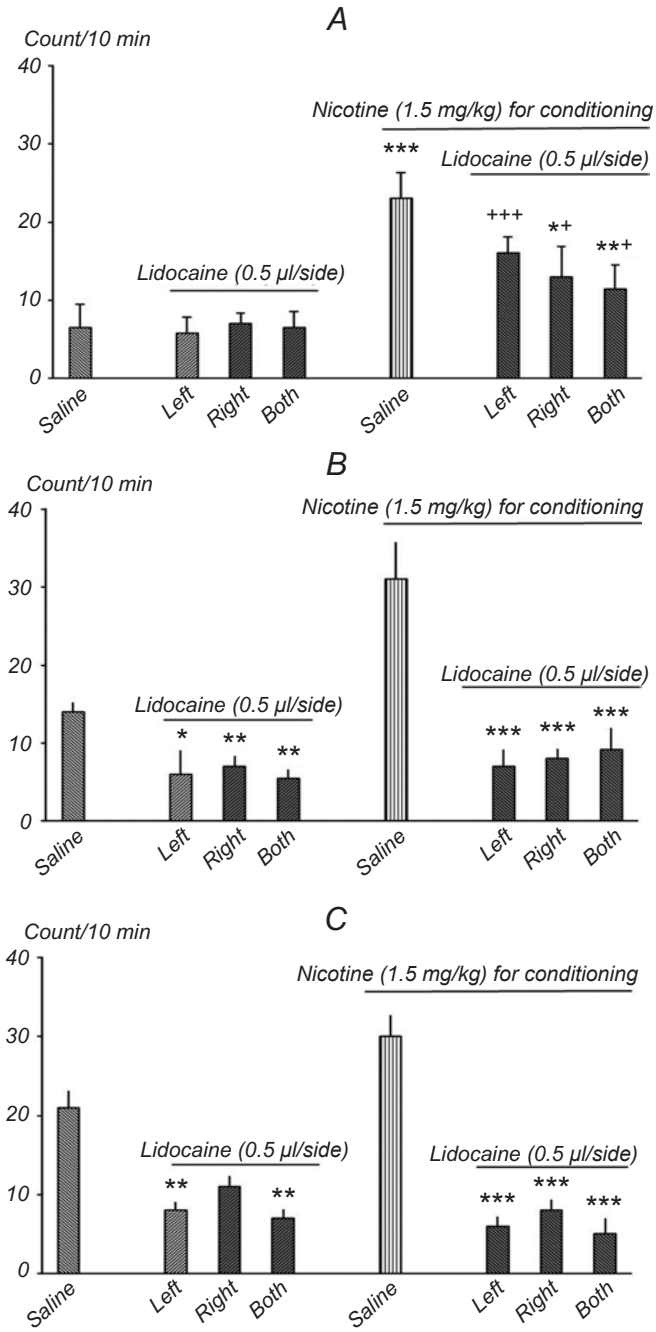
Nicotine Place Conditioning Paradigm at Unilateral and Bilateral Inactivation of the NAcC. In these experiments, the place conditioning paradigm was provided by nicotine (1.5 mg/kg, i.p.) introductions, but 2% lidocaine was injected 5 min before each nicotine administration into either right or left side of NAcC or applied to this structure bilaterally. The control group received sterile saline into their NAcC. Our results showed that place preference for a drug-paired compartment demonstrated no significant changes when the right or left side of the NAcC was pharmacologically inhibited [Three-Way ANOVA within-group comparison: Side effect: $F(5,35) = 0.76$, $P > 0.05$, Pretreatment effect: $F(1, 35) = 1.10$, $P > 0.05$, Treatment effect: $F(5,35) = 3.28$, $P < 0.01$, Side × × Pretreatment × Treatment effect: $F(8, 73) = 4.53$, $P < 0.001$] (Fig. 3). In addition, total compartment crossing was reduced over all groups in comparison with the control significantly [Three-Way ANOVA within-group comparison: Side effect: $F(5,35) = 2.34$, $P < 0.01$, Pretreatment effect: $F(1, 35) = 2.39$, $P < 0.05$, Treatment effect: $F(5,35) = 3.89$, $P < 0.01$, Side × Pretreatment × Treatment effect: $F(8, 73) = 3.65$, $P < 0.001$] (Fig. 4A). As to rearings and sniffing, these behavioral phenomena were suppressed when the left, right, or both sides of the NAcC were inhibited. For rearing: [Three-Way ANOVA within-group comparison: Side effect: $F(5,35) = 2.69$, $P < 0.01$, Pretreatment effect: $F(1, 35) = 3.27$, $P < 0.01$, Treatment effect: $F(5,35) = 2.45$, $P < 0.001$, Side × Pretreatment × Treatment effect: $F(8, 73) = 4.52$, $P < 0.0001$] (Fig. 4B). For sniffing: [Three-Way ANOVA within-group comparison: Side effect: $F(5,35) = 4.68$, $P < 0.001$, Pretreatment effect: $F(1, 35) = 3.61$, $P < 0.01$, Treatment effect: $F(5,35) = 4.12$, $P < 0.01$, Side × Pretreatment × Treatment effect: $F(8, 73) = 4.29$, $P < 0.01$] (Fig. 4C).



F i g. 2. Dose dependence of place conditioning preference induced by nicotine. Horizontal scale) Doses of nicotine, mg/kg; vertical scale) changes in preference, sec. Means ± s.e.m. for 6-8 rats are shown. *** Significant differences from the saline control group, $P < 0.001$.



F i g. 3. Effects of transient inhibition of the core part of the *nucl. accumbens* (NAcC) on nicotine place preference. Animals received lidocaine injections into their left, right, or both NAcCs before 1.5 mg/kg nicotine (i.p.) or 1 ml/kg saline in each conditioning session. Means ± s.e.m. of conditioning score for 7-8 rats are shown. ***Significant difference from the saline control group with $P < 0.001$; $^{+++}$ that from lidocaine control group, $P < 0.001$. Other designations are similar to those in Fig. 2.



F i g. 4. Inhibitory effects of transient inactivation of the core part of the *nucl. accumbens* (NAcC) on dopamine-related behaviors; A) compartment crossing, B) rearing, and C) sniffing, episodes per 10 min. Other designations are similar to those in Fig. 3. Significant differences from the saline control group with $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ and those from the lidocaine control group with $^{+++}P < 0.001$, $^{++}P < 0.01$, $^{+}P < 0.05$ are shown.

DISCUSSION

Our findings showed that i.p. nicotine administration significantly influences place preference in the rats with a previous history of nicotine introductions. These data are in agreement with previous data that rats with a history of nicotine show a clear place preference [21, 22]. Moreover, our data demonstrated the special role of the core part (C) of the NAcC in this regard. Interestingly, transient inactivation of the NAcC did not change the preference to the nicotine-paired compartment in the animals, although the other DA-related behaviors, including compartment entering, rearing, and sniffing, were affected noticeably. These results indicated that the site of action of nicotine for inducing its rewarding properties may be out of the C part of the NAcC, as measured by the place preference technique.

As was mentioned in previous studies, transient inhibition of different parts of the NAcC, as compared with general inhibition, may help to better elucidate the role of this CNS structure in the phenomena related to nicotine drug abuse [14].

Our data showed that nicotine influences place preference in a dose-dependent manner. Our results are in agreement with previous studies showing that subcutaneous (SC) and i.p. administrations of an opioid can induce place preference in both rats and mice [23]. However, our data indicated that nicotine in the dose of 7.5 mg/kg did not induce any place preference, as other investigators insisted [17]. This contradiction seems to be related to the difference in the devices used in our and previous studies [17]. We used a two-compartment apparatus, while Moaddab et al. [17] used a three-compartment apparatus. The effect of the apparatus type on the results obtained within the place conditioning paradigm has been mentioned earlier [23].

Our data also showed that nicotine-treated animals demonstrated increases in their DA-related behaviors, including compartment crossings considered an indicator of the locomotion intensity [19]. There were no reports considering the possible relationship between nicotine place conditioning and DA-related behaviors. It was expected that locomotor activity can directly interact with nicotine place conditioning. In our study, we measured such DA-related behaviors as sniffing and rearing, considered to be good indicators of activity of the mesolimbic DA system [20]. Our results showed that the intensities of all these behaviors increased with increases in the dose of nicotine, except for a high dose of 7.5 mg/kg causing suppression of these behaviors. However, since these types of behavior were not measured in the earlier studies regarding nicotine place conditioning [23], the mentioned studies were not focused on this topic, and no comparison can be made with our results.

Our data demonstrated that transient inactivation of the Sh part of the NAc is sufficient to reduce nicotine place conditioning. This was true when either right or left side of this structure was inactivated. In addition, the same responses were obtained when both sides of the NAcC were inhibited. The Sh part of the NAc was the target of several studies in which various methods (electrophysiological, pharmacological, microdialysis, and application of toxic agents such as 6-hydroxydopamine) were used to reveal the role of this part of the nucleus in the formation of drug dependence [1]. Moreover, locomotion (a nonstereotyped behavior) was also reduced when both sides of the NAcSh were inhibited. It is interesting that the number of sniffing significantly increased in this case. Such stereotyped behaviors were considered striatum-related phenomena dependent on cerebral DA activity [24-28]. It became clear that the DA level increased in the Sh part of the NAc after nicotine administration [13] and decreased during drug-seeking behavior [29]. According to these facts, it can be postulated that a possible decrease in the DA concentration in the NAcC results in reduction of locomotion and rearing observed in our present study. The existence of functional segregation between the ventral and medial Sh parts of the NAc (with a greater activity in the ventral Sh) was found in previous studies [30]. Our experiments were also focused on the ventral portion of the Sh part, and we believe that the role of the medial portion should be investigated in further studies.

An interesting finding regarding inhibition of the NAcSh is a trend toward reduction of all signs measured through the left side, right side, and, finally, both sides. This trend may indicate that the left side of the NAcSh may be less important than the right side in nicotine place preference.

Our results from the C part of the NAc demonstrated that transient inhibition of this part also decreases the time spent in the nicotine-paired side, which, in fact, reflects the importance of precisely this part in nicotine place preference. Moreover, in contrast to the Sh part, the left side of the C of the NAc seems to be more important than the right one in nicotine reward, as transient inhibition of the left part induced more pronounced inhibition of nicotine place conditioning. There are no investigations concerning the effect of each part of the NAcC C on nicotine reward using the place conditioning paradigm. Previous studies showed that neurotoxical destruction of different parts of the NAcC exerted dissimilar effects on responses to nicotine, amphetamine, and cocaine place preference and locomotion [31–34]. Our results also indicated that inhibition of the left side or both sides of the NAcC C reduced total locomotion, but inhibition of right side did not affect this DA-related behavior. Interestingly, this trend can be observed in other DA-related behaviors, including rearing and sniffing. Dopamine-related behaviors are important indicators of the striatal DA function, which are differentially integrated in the Sh and C parts of the NAc [1]. Our experiments indicated that animals with a history of nicotine administration showed different DA-related behaviors; moreover, transient inactivation of the C and Sh parts of the NAc also differentially influenced these behaviors. These findings also emphasized the importance of different parts of the NAcC in this regard. We suggest that these behaviors should be investigated in further experiments related to drug abuse.

Therefore, our results indicated that both parts of the NAc play certain roles in nicotine reward, but the roles of the left C part and the right Sh part are more important. In addition, the right C part and the left Sh part of this nucleus seem to be important for other functions, including sniffing, rearing, and locomotion. It should be noted that the responses we observed are restricted to nicotine; the effects of other abused drugs should be examined separately. Based on these findings, we believe that interventions to the Sh or C parts of the NAc by any means should be accompanied by the results related to both compartments.

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All experiments were conducted in accordance with standard international ethical guidelines and approved by the local Ethical Committee (the Baqiyatallah (a.s.) University Medical Committee on the Use and Care of Animals, 81/021, July 10, 2002).

The authors of this communication, S. B. Hosseini, H. Sahraei, A. Mohammadi, B. Hatef, G. H. Meftahi, D. Chalabi-Yani, H. Alibeig, S. Sadeghi-Gharajehdaghi, M. Ranjabaran, and M. Sadeghi, confirm the absense of any conflict related to comercial or financial interests, to interrelations with organizations or persons in any way involved in the research, and to interrelations of the co-authors.

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