Granular Insular Cortex Inactivation as a Novel Therapeutic Strategy for Nicotine Addiction

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Background: Nicotine is the principal component of tobacco smoke, resulting in addiction, and recent evidence suggests that damage to the insular cortex (insula) disrupts tobacco addiction in human smokers. However, the effect of an inactivation of this structure in an animal model of nicotine addiction has yet to be evaluated.

Methods: To study this question, we investigated the effects of reversible inactivation of the granular insula by local injection of a γ-aminobutyric acid agonists mixture (baclofen/muscimol) on nicotine self-administration (SA) under fixed and progressive ratio and on reinstatement of nicotine seeking induced by nicotine priming or nicotine-associated cues in rats. We also evaluated the effects of granular insula inactivation on food SA and relapse as a control.

Results: The inactivation of the granular insula decreased nicotine SA under both fixed and progressive ratios without affecting the SA of food under the same schedules of reinforcement. This inactivation also prevented the reinstatement, after extinction, of nicotine seeking induced by nicotine-associated cues or nicotine priming without modifying the reinstatement of food seeking.

Conclusions: Our study indicates that the integrity of the granular insula is necessary for exhibiting motivation to take nicotine and to relapse to nicotine seeking but not for consuming food pellets or to relapse for food seeking. Indeed, it might be interesting to study the effect of methods that are able to modulate the activity of the insula—such as repetitive transcranial magnetic stimulation or deep brain stimulation— on tobacco addiction and relapse in humans.

Key Words: Insula, motivation, nicotine, relapse, self-administration

Tobacco smoking results in 5 million deaths/year globally, and this number is expected to double by the year 2025 (1), highlighting the necessity of finding new effective therapies for this addiction.

Nicotine is considered the main component of tobacco smoke, resulting in addiction in humans (2,3), and is self-administered IV in rodents (4–6), nonhuman primates (7,8), and humans (2,9).

The insular cortex, or insula, is a part of the frontal cortex that has been previously implicated in a multitude of behaviors in humans, such as conscious urges, representation of interoceptive states, anxiety, pain, cognition, and mood (10–16).

This structure has also been implicated in addictive behaviors (17, for review). The insula is activated by presentation of cues previously associated with drugs of abuse, such as cocaine and tobacco, and the activity in this structure is positively correlated with craving for these drugs (18–24). Naqvi *et al.* recently found that smokers who sustained damage to the insula exhibited a significant disruption of their smoking addiction (25). However, the type of tobacco smoking-related behavior (motivation to smoke and/or relapse) affected by damage of the insula is not precisely known, and the evaluation of the involvement of the insular cortex in an animal model of these behaviors might help answer this question.

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In rats, the insula can be divided into anterior, posterior, and parietal regions and further into a dorsal granular, an intermediate dysgranular, and a ventral agranular region.

The agranular region of the insular cortex is functionally connected to reward-related areas, such as the prefrontal cortex, the amygdala, and the mediodorsal thalamus; and the dysgranular insular cortex is also connected to the amygdala (26–28). On the contrary, the granular insular cortex (GI) does not send projections to the amygdala (28) and is generally involved in processing basic visceral and gustatory information (29).

Thus, the agranular and dysgranular regions seem better-positioned than the granular part of the insular cortex to underlie high-order cognitive processes that can be associated with addiction to drugs of abuse.

However, two recent studies suggest that the GI is critically involved in drug addiction by showing that its inactivation blocks the expression of an amphetamine-conditioned place preference and that the blockade of hypocretin-1 receptors in the same area reduces nicotine self-administration (SA) in rats (30,31).

To clarify the role of the GI in multiple behaviors related to nicotine addiction, the present study was designed to evaluate the effect of reversible inactivation of the GI on nicotine SA under fixed (FR) and progressive ratio (PR) and on reinstatement of nicotine seeking induced by nicotine priming or cues previously associated with nicotine administration in rats. To control the specificity of the effect of GI inactivation on nicotine-related behaviors, rats have also been tested on food SA under the same schedules and on relapse to food seeking.

Materials and Methods

Animals

Male Long Evans rats (Charles River, Lachine, Quebec, Canada) experimentally naive at the start of the study and initially weighing 250–275 g were used for all experiments. All rats were individually housed in a temperature-controlled environment on a 12-hour reverse light/dark cycle (lights off from 7:00 am to 7:00 PM). Before any experimental manipulation, animals were given

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a minimum of 7 days to habituate to the colony room, during which they were weighed, handled, and received unlimited access to both food and water. All the experimental procedures described in this report were carried out in compliance with the guidelines of the Canadian Council on Animal Care and were reviewed and approved by the institutional Animal Care Committee. All efforts were made to minimize animal suffering and to reduce the number of animals required. Use of a repeated-measures design contributed to the latter.

Initial training procedures and surgical techniques were similar to those previously reported (4,32). Animals were trained to press a lever on a schedule in which each press resulted in the delivery of a 45-mg food pellet (continuous reinforcement, no associated cues). Once trained, each animal was surgically prepared with a chronic IV catheter implanted in the jugular vein; the catheters exited between the scapulae. Surgery was performed under anesthesia induced by xylazine (10 mg/kg IP) and ketamine hydrochloride (75 mg/kg, IP). Incision sites were infiltrated with the local anesthetic bupivacaine (.125%). Buprenorphine was given for postoperative analgesia (.01 mg/kg SC), and a single dose of penicillin (30,000 U, IM) was administered before surgical procedures. Animals were allowed to recover for a 1-week period before drug SA sessions were begun.

Drugs

(−)Nicotine hydrogen tartrate (Sigma-Aldrich, St. Louis, Missouri) was dissolved in saline, the pH was adjusted to 7.0 (± .2), and the solution was filtered through a .22-mm syringe filter (Fisher Scientific, Pittsburgh, Pennsylvania) for sterilization purposes. All nicotine doses are reported as free base concentrations. Nicotine was administered IV in a volume of 100 μL/kg/injection or SC in a volume of 1 mL/kg.

Muscimol (Sigma; .03 nmol/side) and baclofen (Sigma; .3 nmol/side) mixture (Bac/Mus mix) was also dissolved in saline and used for intracranial delivery. The doses of muscimol and baclofen are based on previous reports (33,34). The Bac/Mus mix (.3 and .03 nmol/side, respectively) was chosen to pharmacologically inactivate the GI, because it produces a rapid and prolonged reduction in neuronal activity without inhibiting fibers of passage (35,36).

Acquisition of the Nicotine or Food SA

The SA sessions were carried out in experimental chambers equipped with two levers (Med Associates, St. Albans, Vermont). The start of the session was signaled by the illumination of a house-light; switching off this light indicated the time-out period during which time lever-responding was recorded but had no consequences. Rapid delivery of the self-administered drug (1-sec delivery time) was achieved with Med Associates Model PHM-104 pumps. Unit doses were 100 μL/kg; volume adjustments were used to accommodate interanimal or between-session differences in body weight. Responding on one of the levers (active) resulted in drug delivery when schedule requirements were met, whereas responding on the other lever was recorded but did not produce any change of lights or drug infusion (active levers were counterbalanced). The SA sessions occurred 7 days/week.

In this study, rats acquired nicotine SA under an FR schedule of reinforcement, and the unit dose was 30 μg/kg/infusion of nicotine base. Session duration was 60 min, and the time-out period (switching off the house light and illumination of a cue light above the active lever) after each infusion was 1 min. During the first 5 days of acquisition, each lever press during the time-in period resulted in the delivery of an infusion (FR-1); then the response requirement was increased to FR-2 for 3 days and then to the final value of FR-5 (i.e., animals were required to make five lever presses for each drug infusion) for 5 days.

The apparatus, the stimuli associated with food delivery, and the schedule of the acquisition for the food SA experiments were exactly the same as described in the preceding text, except that the rats received a food pellet (45-mg precision pellets; Bioserv, Laurel, Maryland) instead of a nicotine injection.

Cannulation

After the acquisition phase of the nicotine SA, stereotaxic surgeries to implant microcannulae were done under the regimen of anesthetics, analgesic, and antibiotic described in the preceding text. Brain microcannula guides were prepared from 22-gauge stainless steel needle tubing and fitted with an occluder made of stainless steel suture wire to restrict the entry of foreign material between brain infusions. Guide cannulae were positioned bilaterally to terminate above the histological boundary of the GI; injection cannulae were cut to a longer length to reach the GI. Brain cannulae were anchored with dental acrylic to small screws threaded partially into the skull. The coordinates used to target the GI, relative to bregma, were anteroposterior −.40 mm, lateral ± 4.8 mm, and dorsoventral 4.7 mm, according to the atlas of Paxinos and Watson (1998). Injectors (28 gauge) were cut to reach 1.3 mm beyond the guides (to reach coordinate + 6 mm). The guide cannulae for the GI were implanted in divergent direction at 10° from the vertical.

Rats were allowed 1 week to recover before the reacquisition of the nicotine SA under an FR-5 schedule of reinforcement.

Cortical Inactivation

The injection cannulae were coupled to a 10-μL Hamilton Syringe by a polyethylene tubing (inner diameter .58 mm; Plastics One, Roanoke, Virginia) filled with the Bac/Mus mix or the vehicle (sterile saline) and inserted into the guide cannula after removing the occluder. Rats were first habituated to the procedure of inserting the injection cannulae before performing the testing. We injected .5 μL of the Bac/Mus mix (or sterile saline) over the course of 1 min on each side (driven by a microinfusion pump, Harvard Apparatus, Model 22, South Natick, Massachusetts), 5–10 min before a test session. After the infusion, 1 min was allowed for diffusion, the microinjectors were removed, and occluders were replaced.

Testing Under FR

One week after the brain cannulation, the nicotine or food SA under the FR-5 schedule of reinforcement was reestablished for all rats until stabilization. Rats were considered to have acquired stable nicotine SA when they pressed the active lever more than twice the number of times they pressed the inactive lever and received a minimum of 10 infusions/pellets/1-hour session with <20% variation in the number of infusions/pellets earned/session during two consecutive sessions.

Two groups of rats ($n = 7$ for nicotine SA, and $n = 9$ for food SA) were tested under the FR-5 schedule of reinforcement with preinfusion of the Bac/Mus mix or vehicle (saline) in the GI, in a counterbalanced, within-subject design.

Testing Under PR

After the reestablishment of a stable responding under FR-5 schedule of reinforcement (1 week after the surgery for brain cannulation), two groups of rats ($n = 8$ for nicotine, and $n = 7$ for food) were switched to a PR schedule wherein the response

requirement increased with each successive injection or food pellet delivery. The response requirement progression was based on the formula $5e^{(0.25*[\text{inj. number} + 3])} - 5$, with the first two values replaced by 5 and 10 (modified from Roberts [37]). Thus, the response requirements for successive injections were 5, 10, 17, 24, 32, 42, 56, 73, 95, 124, 161, 208, and so forth. The break point (BP) was defined as the highest ratio completed before the first 30-min period without a response on the active lever in both nicotine- and food-SA.

The PR sessions lasted a maximum of 4 hours. The animals were allowed 10 days of nicotine or food SA on the PR schedule before testing with the infusion of the Bac/Mus mix or vehicle in the GI began. Rats in nicotine SA were also tested in a session where nicotine was substituted with saline (saline substitution condition). A counterbalanced within-subject design was employed for the testing.

Extinction

After completion of testing with the infusion of the Bac/Mus mix or vehicle in the GI on nicotine SA under FR or PR schedule, rats continued on their SA session under their respective schedule for a minimum of five additional sessions to reestablish a stable baseline level of responding for three consecutive sessions. This was followed by an extinction phase for both groups that was conducted by withholding nicotine and its associated cues (the house light remained on during the whole session and there was no presentation of the nicotine-associated cues). Responses on the active or inactive lever were recorded but had no consequences. The criterion for extinction was <20 active lever presses/1-hour session over two consecutive sessions.

Cue-Induced Reinstatement of Nicotine Seeking

Animals that have been tested under the FR schedule were tested for the effect of GI inactivation on cue-induced reinstatement in a counterbalanced, within-subject design (except one rat who did not extinguish, $n = 6$). Testing days were separated by at least three extinction sessions with a stable extinction responding (under the criteria for extinction) over two consecutive sessions. Reinstatement tests were conducted under conditions identical to that of SA, except that (1) a single presentation of the visual cue (light above the active lever on and house-light off for 60 sec) was delivered response-independently immediately at the start of the session, and (2) responses on the active lever (under an FR-5 schedule) resulted in contingent presentation of the cues (light above the active lever on and house-light off for 60 sec) without nicotine availability (no infusions). Responses on the inactive lever were recorded but were without consequence. The testing sessions lasted 1 hour. This procedure of cue-induced reinstatement was chosen because it has been shown to induce a high level of reinstatement (38,39).

Nicotine-Induced Reinstatement of Nicotine Seeking and Food-Induced Reinstatement of Food Seeking

Animals that were tested under the PR schedule were also tested for the effect of GI inactivation on nicotine- or food-induced reinstatement in a counterbalanced, within-subject design ($n = 8$ and $n = 6$, respectively; one rat in each group was eliminated because they never reached extinction criteria).

Testing days were separated by at least three extinction sessions with a stable extinction responding over two consecutive sessions. Nicotine priming consisted of an SC injection of nicotine at .15 mg/kg, 10 min before the test-session. Food priming consisted of noncontingent delivery of two food pellets at the start of the test-session. The Bac/Mus mix or its vehicle was infused in the GI 5–10 min before the nicotine or food priming or 5–10 min before the session for testing on extinction.

Data Analysis

Only rats with correct bilateral placement of the cannulae in the GI were included for data analysis.

For the data on nicotine SA under the FR-5 schedule, the statistical analysis was conducted on the total number of injections received during the 1-hour session, with a Student *t* test with repeated measure.

For the data on nicotine SA under the PR schedule, the statistical analysis was conducted on the total number of injections received during the session before 30 min without any active lever press (BP). Data were subjected to repeated measures analysis of variance (ANOVA), followed by post hoc Dunnett's test for comparisons with the vehicle condition. Reinstatements of nicotine seeking were analyzed by repeated measures ANOVA followed by Newman–Keuls post hoc test for multiple comparisons (dependant variable: active lever presses).

Changes were considered significant when $p < .05$.

Histological Procedures

After the completion of behavioral testing, rats were overdosed with pentobarbital (approximately 350 mg/kg, IP) and then perfused transcardially with 10% formalin. Brains were dissected out, and coronal serial sections (30-μm-thick) were examined for placement. Acceptable histology required that the tip of the injector lie within the GI on each side of the brain.

Results

Effect of GI Inactivation on Nicotine and Food SA Under the FR Schedule

The *t* test performed on the number of nicotine injections showed a significant effect of treatment [$t(6) = 2.74$, $p < .05$], indicating that the infusion of the Bac/Mus mix into the GI significantly reduced nicotine SA compared with vehicle administration in the GI (Figure 1A). The mean (± SEM) active lever presses after vehicle and Bac/Mus mix infusion were 143 ± 18 and 85 ± 22, respectively.

The *t* test performed on the number of food pellets indicated no significant effect of treatment [$t(8) = .4$, $p =$ ns], suggesting that the inactivation of the GI did not modify food SA (Figure 1B). The mean (± SEM) active lever presses after vehicle and Bac/Mus mix infusion were 788 ± 101 and 998 ± 155, respectively.

These results indicate that the inactivation of the GI specifically reduced nicotine SA under FR-5 schedule of reinforcement in rats.

Effect of GI Inactivation on Nicotine and Food SA Under the PR Schedule

The ANOVA performed on the number of nicotine injections that the rats received before 30 min of inactivity (BP) showed a main effect of treatment [$F(2,14) = 9.4$, $p < .01$]; and pairwise comparisons indicated that infusion of the Bac/Mus mix into the GI before the session or substitution of nicotine by saline significantly reduced the BP compared with vehicle administration ($p < .01$ for both comparisons) (Figure 2A). There was no significant difference between the BPs after the infusion of the Bac/Mus mix into the GI or after saline substitution.

The *t* test performed on the BP for food pellets showed no significant effect of treatment [$t(6) = .7$, $p =$ ns], suggesting that

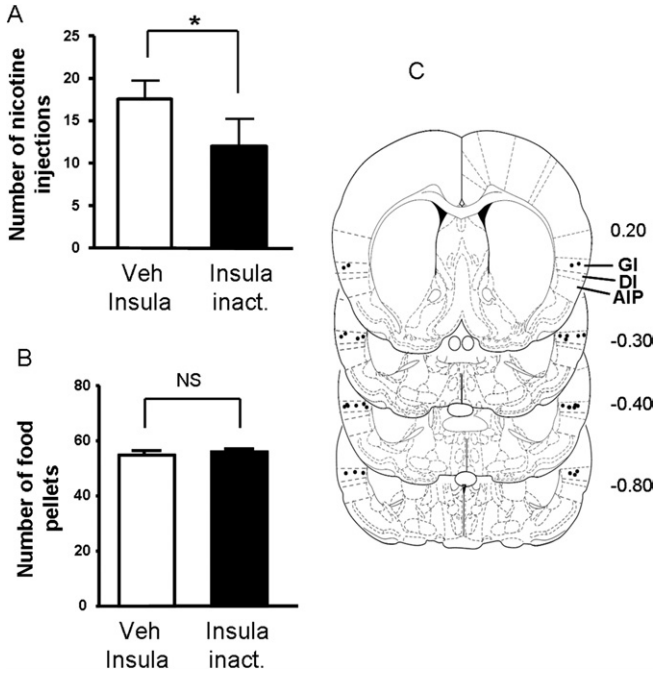

Figure 1. Effects of insula inactivation on nicotine **(A)** or food **(B)** self-administration under a fixed ratio of five lever presses/drug infusion (FR-5) schedule of reinforcement ($n = 7$ and $n = 9$, respectively). **(C)** Histological reconstruction of the injection sites in the insula. Black dots indicate locations of injector tips from the rats that were included in statistical analysis. The number beside each reconstructed image indicates the distance (in millimeters) from bregma. Schematic figure was published in *The Rat Brain in Stereotaxic Coordinates, 4th ed.*, by Paxinos and Watson, Copyright Elsevier (1997) (56). Data are expressed as means (± SEM) of the number of injections or food pellet delivery after infusion of the baclofen/muscimol (Bac/Mus) mix (insula inact.) or vehicle (Veh insula) in the insula. GI, granular insula; DI, dysgranular insula; AIP, posterior agranular insula. *$p < .05$, Student *t* test.

the infusion of the Bac/Mus mix into the GI did not modify motivation for food (Figure 2B).

These results indicate that the inactivation of the GI selectively reduced the motivation to self-administer nicotine.

Effect of GI Inactivation on Cue-Induced Reinstatement

The ANOVA performed on the active lever presses indicated a main effect of treatment [$F(2,10) = 17.5$, $p < .001$]. The post hoc analysis showed that the cue presentation induced a significant reinstatement of the presses on the active lever ($p < .001$ vs. baseline) and that preinfusion of the Bac/Mus mix into the GI significantly decreased this cue-induced reinstatement ($p < .01$ vs. reinstatement under vehicle preinfusion). In addition, there was no difference between the baseline responding (no cue) and the cue-induced reinstatement with preinfusion of the Bac/Mus mix (Figure 3B).

This result suggests that the inactivation of the GI prevented the cue-induced reinstatement of nicotine seeking in rats.

Effect of GI Inactivation on Nicotine- and Food-Induced Reinstatement

The ANOVA performed on the active lever presses of rats previously trained to self-administer nicotine indicated a main effect of treatment [$F(2,14) = 7.3$, $p < .01$], and the post hoc analysis showed that the nicotine priming (.15 mg/kg SC) induced a significant reinstatement of nicotine seeking ($p < .01$ vs. baseline) and that preinfusion of the Bac/Mus mix into the GI significantly decreased this nicotine-induced reinstatement ($p < .05$ vs. reinstatement under vehicle preinfusion). In addition, there was no significant difference between baseline responding and responding during nicotine-induced reinstatement with preinfusion of the Bac/Mus mix (Figure 3A).

The ANOVA performed on the on the active lever presses of rats previously trained to self-administer food pellets indicated a significant main effect [$F(2,10) = 6$, $p < .05$]. The post hoc analysis showed that the food priming induced a significant reinstatement of food seeking after infusion of the vehicle or the Bac/Mus mix into the GI ($p < .05$ for both groups vs. baseline). The infusion of the Bac/Mus mix into the GI did not modify food-induced reinstatement of food seeking (Figure 3C).

Therefore, the inactivation of the GI seems to specifically block the nicotine-induced reinstatement of nicotine seeking in rats.

Effect of GI Inactivation on Inactive Lever Presses

The numbers of inactive lever presses in every experiment are indicated in Table 1.

The *t* test or ANOVAs performed on the numbers of inactive lever presses in all conditions tested showed no statistically significant effect of treatment (Table 1), indicating that the effect of GI inactivation was selective on the active lever as well as the

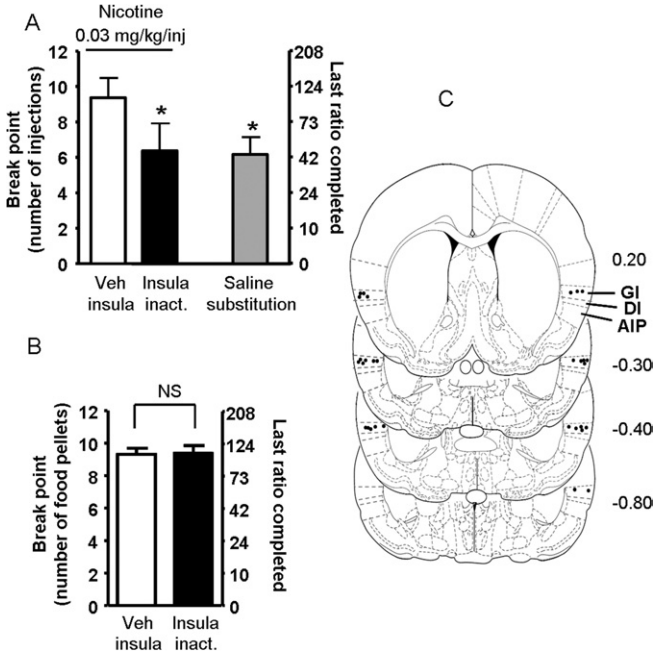

Figure 2. Effects of insula inactivation on nicotine **(A)** or food **(B)** SA under a progressive ratio schedule of reinforcement (wherein the response requirement increased with each successive injection or food pellet delivery; $n = 8$ and $n = 7$, respectively). **(C)** Histological reconstruction of the injection sites in the insula. Black dots indicate locations of injector tips from the rats that were included in statistical analysis. The number beside each reconstructed image indicates the distance (in millimeters) from bregma. Schematic figure was published in *The Rat Brain in Stereotaxic Coordinates, 4th ed.*, by Paxinos and Watson, Copyright Elsevier (1997) (56). Data are expressed as means (± SEM) of the number of injections or food pellets delivery (break point, left y axis) and of the last ratio completed (in number of lever presses, right y axis) during sessions after infusion of the Bac/Mus mix or vehicle in the insula, and during session with saline substitution. Abbreviations as in Figure 1. *$p < .05$; Dunnett's test after significant analysis of variance for repeated measures.

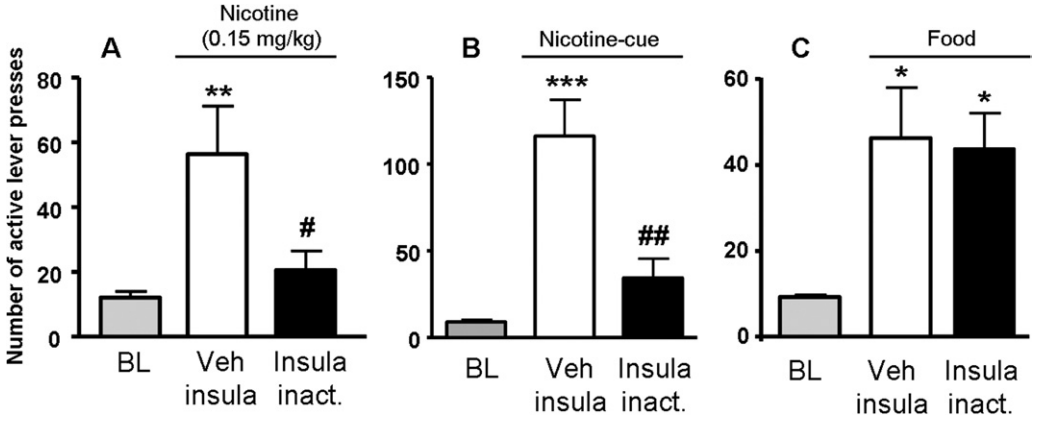

Figure 3. Effect of insula inactivation on **(A)** nicotine- (.15 mg/kg SC, 10 min before the session) or **(B)** cue-induced reinstatement of nicotine seeking tests after extinction ($n = 8$ and $n = 6$, respectively). **(C)** Effect of insula inactivation on food-induced reinstatement of food seeking ($n = 6$). Data are expressed as means (± SEM) of the number of lever presses during baseline conditions (BL) and during sessions with infusion of vehicle (Veh insula) or the Bac/Mus mix in the insula (insula inact.) 5–10 min before nicotine or food priming. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$ versus baseline; $^{\#}p < .05$; $^{\#\#}p < .01$ versus vehicle infusion (Veh insula); Student–Newman–Keuls multiple comparison test after significant analysis of variance for repeated measures.

reinstatements induced by nicotine-associated cue or nicotine priming.

Discussion

We demonstrated in this study that reversible inactivation of the GI (by local infusion of a Bac/Mus mix) decreases nicotine SA under both FR and PR schedules of reinforcement and prevents the reinstatement of nicotine seeking induced by nicotine priming or nicotine-associated cue, without altering food SA or reinstatement of food seeking.

These findings are in line with the fact that human smokers with damage of the insula report a disruption of their addiction to tobacco but no modification in their pleasure or desire to eat or in their intake of food (25). The findings are also in agreement with studies indicating that smoking-associated cues activate the insula and that the activity in the insula is positively correlated with cigarette craving in humans (18–20,40); see Naqvi and Bechara (17) for review.

Effects of GI Inactivation on Nicotine SA Under FR and PR Schedule

The Bac/Mus mix (.3/.03 nmol/side, respectively) has been used to pharmacologically inactivate the GI, because it produces a rapid and prolonged reduction in neuronal activity without inhibiting fibers of passage (35,36). In addition, this dose has been shown to modify drug-related behaviors when injected in several brain areas, without inducing any motor effect (33,34,40).

The inactivation of the GI significantly decreased the number of nicotine injections that the rats received during sessions under FR-5 or PR schedule of reinforcement, suggesting that the GI is implicated in nicotine intake, motivation for nicotine, and in its reinforcing effect (41–43).

These effects of GI inactivation are likely specific for nicotine SA and not a result of a perturbation of general motivational processes, because the infusion of the same Bac/Mus mix in the GI had no effect on food SA under the same schedules of reinforcement.

It has been recently shown in rats that acute administration of nicotine increases Fos expression (a measure of neuronal activity) in hyprocretin neurons of the lateral hypothalamus (LH) (44) and that exposure to amphetamine-associated environmental stimuli (in a conditioned place preference paradigm) increases Fos immunoreactivity in both the GI and hypocretin neurons of the LH (30). In addition, the blockade of hypocretin-1 receptors in the GI reduces nicotine SA under both FR and PR schedules of reinforcement without affecting food SA under the same schedules (31).

Taken together, these data suggest that the hypocretin transmission from the LH to the GI might be critical for the reinforcing effect of nicotine. Indeed, the decreasing effect of GI inactivation on nicotine SA in our study might likely be due to a reduction of the activity of neurons that contain hypocretin-1 receptors in this structure. However, the nature and the target of these neurons remain to be investigated.

Effects of GI Inactivation on Reinstatement of Nicotine Seeking After Extinction

After extinction, both the noncontingent administration of nicotine (.15 mg/kg SC) and the presentation of the nicotine-

Table 1. Inactive Lever Data for All Groups in Nicotine or Food SA (FR-5 and PR), Nicotine- and Cue-Induced Reinstatement of Nicotine Seeking

	Nicotine SA (FR-5)	Nicotine SA (PR)	Nicotine-reinst.	Cue-reinst.	Food SA (FR-5)	Food SA (PR)	Food-reinst.
Baseline			8.1 ± 1	5.9 ± 2.5			5.8 ± 3.3
Vehicle Insula	37 ± 15	47.1 ± 20	16.8 ± 7	7.9 ± 4	38.3 ± 21.3	9.6 ± 2	3.7 ± 2
Insula Inactivation	34.7 ± 8	30.4 ± 18	8.6 ± 3	6.8 ± 3	43.6 ± 30.3	7.6 ± 2.5	7.3 ± 4.7
Saline Substitution		28.5 ± 13					
Statistics	$t(6) = .2, p = ns$	$F(2,14) = 1.3, p = ns$	$F(2,14) = 1.7, p = ns$	$F(2,10) = 2.9, p = ns$	$t(8) = .5, p = ns$	$t(6) = .8, p = ns$	$F(2,10) = 1.8, p = ns$

Data are expressed as mean (± SEM) number of inactive lever presses. SA, self-administration; FR, fixed ratio; PR, progressive ratio; reinst. reinstatement.

associated cues reinstated the active lever pressing behavior, an effect that is considered to reflect reinstatement of nicotine seeking (45,46).

The inactivation of the GI prevented both nicotine priming- and cue-induced reinstatement of nicotine seeking, suggesting a strong implication of this structure in relapse to nicotine seeking. This effect was unlikely to be due to impairment of operant conditioning, because food-induced reinstatement of food seeking was not affected by the inactivation of the GI.

These effects on reinstatement might also be related to an inhibition of the activity of neurons that contain hypocretin-1 receptors in the GI, because this receptor seems implicated in morphine, ethanol, and cocaine seeking in rats (47–50). However, the participation of hypocretin-1 receptors in the GI on reinstatement of nicotine seeking has not yet been investigated.

In conclusion, our study indicated that reversible inactivation of the granular part of the insula decreased the motivation of rats to take nicotine and its reinforcing value but did not affect motivation to consume food pellets. In addition the inactivation of this structure also prevented nicotine priming- and cue-induced reinstatement of nicotine seeking without affecting relapse to food seeking.

Those results indicate that the functional integrity of the GI is necessary for nicotine-related behaviors (but not food-related behaviors) to be exhibited. However, we cannot conclude that the GI plays a selective or special role in the behaviors involved in nicotine addiction, because such a conclusion can only be drawn by comparing the effects obtained in the present study with experiments on the effects of inactivation of other brain areas (such as infralimbic or prelimbic areas) on nicotine-related behaviors.

This conclusion suggests that targeting the insula might represent a new therapeutic way to prevent relapse to tobacco smoking in humans. Indeed, it might be interesting to evaluate the acute and long-term effects of reducing the cortical excitability of the insular cortex in humans by repetitive transcranial magnetic stimulation (51,52) or deep brain stimulation techniques (53–55) on tobacco smoking and cue-induced craving, to determine the therapeutic potential of these approaches.

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1. Proctor RN (2004): The global smoking epidemic: A history and status report. *Clin Lung Cancer* 5:371–376.
2. Le Foll B, Goldberg SR (2006): Nicotine as a typical drug of abuse in experimental animals and humans. *Psychopharmacology* 184:367–381.
3. Rose JE, Corrigall WA (1997): Nicotine self-administration in animals and humans: Similarities and differences. *Psychopharmacology* 130:28–40.
4. Corrigall WA, Coen KM (1989): Nicotine maintains robust self-administration in rats on a limited-access schedule. *Psychopharmacology* 99: 473–478.
5. Markou A, Paterson NE (2001): The nicotinic antagonist methyllycaconitine has differential effects on nicotine self-administration and nicotine withdrawal in the rat. *Nicotine Tob Res* 3:361–373.
6. Donny EC, Caggiula AR, Knopf S, Brown C (1995): Nicotine self-administration in rats. *Psychopharmacology* 122:390–394.
7. Goldberg SR, Spealman RD, Goldberg DM (1981): Persistent behavior at high rates maintained by intravenous self-administration of nicotine. *Science* 214:573–575.
8. Le Foll B, Wertheim C, Goldberg SR (2007): High reinforcing efficacy of nicotine in non-human primates. *PLoS ONE* 2:e230.
9. Sofuoglu M, Yoo S, Hill KP, Mooney M (2008): Self-administration of intravenous nicotine in male and female cigarette smokers. *Neuropsychopharmacology* 33:715–720.
10. Damasio AR, Grabowski TJ, Bechara A, Damasio H, Ponto LL, Parvizi J, Hichwa RD (2000): Subcortical and cortical brain activity during the feeling of self-generated emotions. *Nat Neurosci* 3:1049–1056.
11. Craig AD (2002): How do you feel? Interoception: The sense of the physiological condition of the body. *Nat Rev Neurosci* 3:655–666.
12. Paulus MP, Stein MB (2006): An insular view of anxiety. *Biol Psychiatry* 60:383–387.
13. Hardy SG (1985): Analgesia elicited by prefrontal stimulation. *Brain Res* 339:281–284.
14. Suhara T, Nakayama K, Inoue O, Fukuda H, Shimizu M, Mori A, Tateno Y (1992): D1 dopamine receptor binding in mood disorders measured by positron emission tomography. *Psychopharmacology* 106:14–18.
15. Watanabe M, Kodama T, Hikosaka K (1997): Increase of extracellular dopamine in primate prefrontal cortex during a working memory task. *J Neurophysiol* 78:2795–2798.
16. Goldman-Rakic PS (1998): The cortical dopamine system: Role in memory and cognition. *Adv Pharmacol* 42:707–711.
17. Naqvi NH, Bechara A (2009): The hidden island of addiction: The insula. *Trends Neurosci* 32:56–67.
18. Bonson KR, Grant SJ, Contoreggi CS, Links JM, Metcalfe J, Weyl HL, *et al.* (2002): Neural systems and cue-induced cocaine craving. *Neuropsychopharmacology* 26:376–386.
19. Wang GJ, Volkow ND, Fowler JS, Cervany P, Hitzemann RJ, Pappas NR, *et al.* (1999): Regional brain metabolic activation during craving elicited by recall of previous drug experiences. *Life Sci* 64:775–784.
20. Brody AL, Mandelkern MA, London ED, Childress AR, Lee GS, Bota RG, *et al.* (2002): Brain metabolic changes during cigarette craving. *Arch Gen Psychiatry* 59:1162–1172.
21. McBride D, Barrett SP, Kelly JT, Aw A, Dagher A (2006): Effects of expectancy and abstinence on the neural response to smoking cues in cigarette smokers: An fMRI study. *Neuropsychopharmacology* 31:2728–2738.
22. Franklin TR, Wang Z, Wang J, Sciortino N, Harper D, Li Y, *et al.* (2007): Limbic activation to cigarette smoking cues independent of nicotine withdrawal: A perfusion fMRI study. *Neuropsychopharmacology* 32: 2301–2309.
23. McClernon FJ, Hiott FB, Huettel SA, Rose JE (2005): Abstinence-induced changes in self-report craving correlate with event-related FMRI responses to smoking cues. *Neuropsychopharmacology* 30:1940–1947.
24. Lee JH, Lim Y, Wiederhold BK, Graham SJ (2005): A functional magnetic resonance imaging (FMRI) study of cue-induced smoking craving in virtual environments. *Appl Psychophysiol Biofeedback* 30:195–204.
25. Naqvi NH, Rudrauf D, Damasio H, Bechara A (2007): Damage to the insula disrupts addiction to cigarette smoking. *Science* 315:531–534.
26. Allen GV, Saper CB, Hurley KM, Cechetto DF (1991): Organization of visceral and limbic connections in the insular cortex of the rat. *J Comp Neurol* 311:1–16.
27. Shi CJ, Cassell MD (1998): Cascade projections from somatosensory cortex to the rat basolateral amygdala via the parietal insular cortex. *J Comp Neurol* 399:469–491.
28. Shi CJ, Cassell MD (1998): Cortical, thalamic, and amygdaloid connections of the anterior and posterior insular cortices. *J Comp Neurol* 399: 440–468.
29. Cechetto DF, Saper CB (1987): Evidence for a viscerotopic sensory representation in the cortex and thalamus in the rat. *J Comp Neurol* 262:27–45.
30. Contreras M, Ceric F, Torrealba F (2007): Inactivation of the interoceptive insula disrupts drug craving and malaise induced by lithium. *Science* 318:655–658.
31. Hollander JA, Lu Q, Cameron MD, Kamenecka TM, Kenny PJ (2008): Insular hypocretin transmission regulates nicotine reward. *Proc Natl Acad Sci U S A* 105:19480–19485.
32. Corrigall WA, Coen KM, Zhang J, Adamson KL (2001): GABA mechanisms in the pedunculopontine tegmental nucleus influence particular aspects of nicotine self-administration selectively in the rat. *Psychopharmacology* 158:190–197.

33. McFarland K, Kalivas PW (2001): The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *J Neurosci* 21:8655–8663.
34. Di Ciano P, Everitt BJ (2004): Contribution of the ventral tegmental area to cocaine-seeking maintained by a drug-paired conditioned stimulus in rats. *Eur J Neurosci* 19:1661–1667.
35. Martin JH, Ghez C (1999): Pharmacological inactivation in the analysis of the central control of movement. *J Neurosci Methods* 86:145–159.
36. van Duuren E, van der Plasse G, van der Blom R, Joosten RN, Mulder AB, Pennartz CM, Feenstra MG (2007): Pharmacological manipulation of neuronal ensemble activity by reverse microdialysis in freely moving rats: A comparative study of the effects of tetrodotoxin, lidocaine, and muscimol. *J Pharmacol Exp Ther* 323:61–69.
37. Roberts DS (1992): Self-administration of stimulants and serotonergic systems. *NIDA Res Monogr* 119:136–140.
38. Liu X, Caggiula AR, Yee SK, Nobuta H, Sved AF, Pechnick RN, Poland RE (2007): Mecamylamine attenuates cue-induced reinstatement of nicotine-seeking behavior in rats. *Neuropsychopharmacology* 32:710–718.
39. Forget B, Coen KM, Le Foll B (2009): Inhibition of fatty acid amide hydrolase reduces reinstatement of nicotine seeking but not break point for nicotine self-administration-comparison with CB(1) receptor blockade. *Psychopharmacology* 205:613–624.
40. Peters J, LaLumiere RT, Kalivas PW (2008): Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. *J Neurosci* 28:6046–6053.
41. Markou A, Weiss F, Gold LH, Caine SB, Schulteis G, Koob GF (1993): Animal models of drug craving. *Psychopharmacology* 112:163–182.
42. Arnold JM, Roberts DC (1997): A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. *Pharmacol Biochem Behav* 57:441–447.
43. Paterson NE, Markou A (2005): The metabotropic glutamate receptor 5 antagonist MPEP decreased break points for nicotine, cocaine and food in rats. *Psychopharmacology* 179:255–261.
44. Pasumarthi RK, Reznikov LR, Fadel J (2006): Activation of orexin neurons by acute nicotine. *Eur J Pharmacol* 535:172–176.
45. Shaham Y, Shalev U, Lu L, De Wit H, Stewart J (2003): The reinstatement model of drug relapse: History, methodology and major findings. *Psychopharmacology* 168:3–20.
46. Kalivas PW, McFarland K (2003): Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology* 168:44–56.
47. Harris GC, Wimmer M, Aston-Jones G (2005): A role for lateral hypothalamic orexin neurons in reward seeking. *Nature* 437:556–559.
48. Lawrence AJ, Cowen MS Yang HJ, Chen F, Oldfield B (2006): The orexin system regulates alcohol-seeking in rats. *Br J Pharmacol* 148:752–759.
49. Richards JK, Simms JA, Steensland P, Taha SA, Borgland SL, Bonci A, Bartlett SE (2008): Inhibition of orexin-1/hypocretin-1 receptors inhibits yohimbine-induced reinstatement of ethanol and sucrose seeking in Long-Evans rats. *Psychopharmacology* 199:109–117.
50. Boutrel B, Kenny PJ, Specio SE, Martin-Fardon R, Markou A, Koob GF, de Lecea L (2005): Role for hypocretin in mediating stress-induced reinstatement of cocaine-seeking behavior. *Proc Natl Acad Sci U S A* 102:19168–19173.
51. Chen R, Seitz RJ (2001): Changing cortical excitability with low-frequency magnetic stimulation. *Neurology* 57:379–380.
52. Daskalakis ZJ, Möller B, Christensen BK, Fitzgerald PB, Gunraj C, Chen R (2006): The effects of repetitive transcranial magnetic stimulation on cortical inhibition in healthy human subjects. *Exp Brain Res* 174:403–412.
53. Dostrovsky JO, Levy R, Wu JP, Hutchison WD, Tasker RR, Lozano AM (2000): Microstimulation-induced inhibition of neuronal firing in human globus pallidus. *J Neurophysiol* 84:570–574.
54. Magarinos-Ascone C, Pazo JH, Macadar O, Buño W (2002): High-frequency stimulation of the subthalamic nucleus silences subthalamic neurons: A possible cellular mechanism in Parkinson's disease. *Neuroscience* 115:1109–1117.
55. Kiss ZH, Mooney DM, Renaud L, Hu B (2002): Neuronal response to local electrical stimulation in rat thalamus: Physiological implications for mechanisms of deep brain stimulation. *Neuroscience* 113:137–143.
56. Paxinos G, Watson C (1997): *The Rat Brain in Stereotaxic Coordinates*, *4th ed*. San Diego: Academic Press.