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Deconstructing craving: dissociable cortical control of cue reactivity in nicotine addiction

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Abstract

Background—Cue reactivity, the ability of cues associated with addictive substances to induce seeking and withdrawal, is a major contributor to addiction. Although human imaging studies show that cigarette-associated cues simultaneously activate the insula and the orbitofrontal cortex and evoke craving, how these activities functionally contribute to distinct elements of cue reactivity remains unclear. Moreover, it remains unclear whether the simultaneous activation of these cortical regions reflects coordinated functional connectivity or parallel processing.

Methods—We selectively lesioned the insula or orbitofrontal cortex with the excitotoxin ibotenic acid in mice, and their approach to nicotine-associated cues ($n = 6\text{--}13/\text{group}$) and avoidance of withdrawal-associated cues ($n = 5\text{--}12/\text{group}$) were separately examined in place conditioning paradigms. We additionally tested the role of these two cortical structures in approach to food-associated cues ($n = 6\text{--}7/\text{group}$) and avoidance of LiCl-associated cues ($n = 6\text{--}7/\text{group}$).

Results—Our data show a double dissociation in which excitotoxic lesions of the insula and orbitofrontal cortex selectively disrupted nicotine-induced cue approach and withdrawal-induced cue avoidance, respectively. These effects were not entirely generalized to approach to food-associated cues or avoidance of LiCl-associated cues.

Conclusions—Our data provide functional evidence that cue reactivity seen in addiction includes unique neuroanatomically dissociable elements and suggest that the simultaneous activation of these two cortical regions in response to smoking-related cues does not necessarily indicate functional connectivity.

Keywords

cue reactivity; incentive; nicotine; withdrawal; insula; orbitofrontal cortex

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Introduction

Approach/avoidance is a fundamental principle governing behavior to maximize the survival of organisms. Environmental stimuli exert a powerful control over this dimension of behavior. This process, termed cue reactivity, together with craving as its subjective motivation, is thought to be a significant contributor to addiction (1–4). Ex-smokers report exposure to smoking-related cues and strong craving as factors for lapses (5). In laboratory settings, smoking-related cues evoke craving and smoking (6,7).

Functional imaging studies have consistently demonstrated that in smokers, exposure to smoking-associated cues simultaneously activates, among others, both the insula (INS) and the orbitofrontal cortex (OFC) and evokes subjective reports of craving (8–11). However, it remains unclear whether coordinated activation of these two cortical regions indicates functional connections or independent, parallel processes of the various elements of craving.

This issue is further complicated by the potentially multiple motivational processes of cue reactivity. In experimental set-ups where cues are conditioned with smoking, such cues evoke both desire to smoke and withdrawal (6,7,12). Cue reactivity can be interpreted as indicating that smoking-related cues evoke incentive seeking for nicotine's effects, avoidance of withdrawal, or both (1). Questionnaires used to define craving include elements of both processes (13,14). Moreover, it is not certain whether smokers are aware of the exact motivational state underlying craving and are capable of accurately reporting their real motivational processes.

Cue reactivity can be experimentally established in humans under Pavlovian contingency and such cues are used as classically conditioned stimuli (15–17). Like human cue reactivity, the rodent place conditioning paradigm utilizes Pavlovian conditioning and is thought to model the whole process of cue reactivity --including the impact of nicotine or withdrawal, coding of these effects in association with cues, and retrieval and sustained expression of this memory (18). This paradigm can independently reveal both drug-induced cue approach and withdrawal-induced cue avoidance, by separately pairing cues with nicotine to induce approach (19) or with precipitated withdrawal to induce avoidance (20,21). Moreover, predictive validity has been satisfied for both nicotine-associated cue approach and withdrawal-associated cue avoidance, as anti-craving agents (22,23) attenuate the development of both conditioned behaviors (24–26).

The motivationally complex nature of craving prompted us to investigate the relative roles played by the INS and the OFC in cue-evoked incentive and withdrawal processes. Here, we lesioned the INS or OFC in mice with the fiber-sparing excitotoxin ibotenic acid and separately tested for nicotine cue approach and withdrawal-induced cue avoidance in Pavlovian place conditioning paradigms. Our data indicate that these two cortical regions independently contribute to two distinct motivational processes of nicotine cue reactivity.

Methods and Materials

Animals

Male C57BL/6J mice were used (Jackson Laboratory, Bar Harbor, ME). Surgery was done at the age of 4 weeks and behavioral analysis began at the age of 5 weeks. This age period is the developmental stage at which mice exhibit signs of sexual maturation (27). Mice were maintained on a 14-h light/10-h dark cycle with food and water available *ad libitum* unless otherwise specified. Animal handling and use followed a protocol approved by the Animal Care and Use Committee of the Albert Einstein College of Medicine, in accordance with NIH guidelines.

Drugs

(-)-Nicotine hydrogen tartrate salt (Sigma-Aldrich, St. Louis, MO) for injection was dissolved in physiological saline at a concentration of 0.1 free base mg ml⁻¹ and pH was adjusted to 6.8–7.6. Mecamylamine hydrochloride (Sigma-Aldrich) was dissolved in physiological saline at a concentration of 1.25 free base mg ml⁻¹. LiCl (Sigma-Aldrich) was dissolved in water at a concentration of 40 free base mg ml⁻¹. Nicotine and mecamylamine injections were given subcutaneously (s.c.) at a volume of 2 ml kg⁻¹. LiCl was injected intraperitoneally (i.p.) at a volume of 6 ml kg⁻¹. For the induction of nicotine dependence, alkaline (-)-nicotine free-base solution (200 free base µg ml⁻¹, Sigma-Aldrich) was prepared in 0.33% (wt/vol) saccharin water.

Surgery

Mice were anesthetized with sodium pentobarbital (40 mg kg⁻¹, i.p., Sigma Aldrich) and placed into a stereotaxic apparatus (MyNeuroLab, Richmond, IL). We used the excitotoxin ibotenic acid to produce localized lesions without damaging fibers of passage (28). Ibotenic acid (Sigma-Aldrich) was dissolved in sterile 0.1 M phosphate buffered saline at a concentration of 10 µg µl⁻¹, and an amount ranging from 0.5–2.0 µg was bilaterally injected. Sham mice received bilateral injections of the vehicle. The coordinates used for the targeted regions were as follows: INS, anterior-posterior (AP) 1.98 mm, medial-lateral (ML) 2.25 mm, dorsal-ventral (DV) -3.5 mm; OFC, AP 2.46 mm, ML 0.75 mm, DV -3.0 mm. Separate control groups of mice received lesions 1.5 mm dorsal to the target regions. Animals were allowed 5–7 days to recover from surgery.

Behavioral Testing

The apparatus used was a rectangular Plexiglas box composed of three distinct compartments (29,30). Two large compartments (24.5 cm × 18 cm × 33 cm) had distinguishable visual and tactile cues: one compartment had black-and-white striped walls and a wire mesh floor with 2.1 × 2.1-mm openings and was lit at 3.12 lux; the other compartment had gray walls and a wire mesh floor with 3.7 × 3.7-mm openings and was lit at 1.40 lux. These two large compartments were separated by a central compartment (13 cm × 18 cm × 33 cm). Each large compartment was divided from the center compartment by a guillotine door (18 cm × 37 cm). The guillotine doors were opened 5 cm above the floor, and the mice were allowed to explore the three compartments freely during pre- and post-conditioning test days.

We designed our experiment so that each conditioned behavior was optimally induced. Surgery was done at the age of 4 weeks and behavioral analysis began at the age of 5 weeks. Consistent with the literature (31–37), we found that nicotine induced reliable cue approach in adolescent, but not adult C57BL/6J mice, in our pilot studies. For withdrawal-induced cue avoidance, mice started to receive chronic nicotine administration at 5 weeks of age and were conditioned with withdrawal at 7 weeks of age. We found that withdrawal-induced cue avoidance was most robustly induced at this age (38), consistent with studies reporting that precipitated withdrawal induces robust cue avoidance in rats after, but not during, adolescence (39,40).

For nicotine-induced cue approach (Fig. 1A) and LiCl-induced cue avoidance (Fig. 1A), animals were allowed to explore the apparatus for 15 min on the first day, and time spent in either side compartment was recorded. Like precipitated withdrawal, LiCl induces cue avoidance that is mediated by the brain (41–44). This group was used to test the specificity of lesion effects on avoidance. On the second day, animals received an injection of nicotine (0 and 0.2 free base mg kg⁻¹, s.c.) or LiCl (0 and 240 free base mg kg⁻¹, i.p.) and were immediately confined to either of the two distinct place conditioning compartments in two

30-min sessions at least 5 h apart. This dose of nicotine was chosen because it induces the most robust cue approach under our experimental condition (29,30). Nicotine was always given when animals were confined to the initially non-preferred compartment while LiCl pairings were arranged such that on a group basis, mice showed equal preference for the compartments to be paired with drug and vehicle. The order of injections was counterbalanced between all animals. From days 3–12, animals were allowed to freely explore the apparatus for 15 min per day, and time spent in each side compartment was recorded by an experimenter blinded to the specific treatment.

For food-induced cue approach (Fig. 1B), mice were singly housed and, through limited access to regular food pellets, maintained at approximately 80% free feeding weight. Mice were habituated with 2 ml of food reward (sweetened condensed milk diluted 1:1 with water, America's Choice, Montvale, MA) per day in their home cages for 3 days. The animal's pre-existing bias toward the two compartments was tested for 15 min on the first test day. On days 2–7, mice were confined to one of the compartments for 30 min with either a cup containing 2 ml of food reward or an empty cup, alternating each day. Three conditioning days with food were given, because our pilot studies showed that this number of pairings was needed to induce food cue approach comparable to nicotine cue approach. The order of exposure was counterbalanced between the mice. The food was always placed in the initially non-preferred compartment, and no food was provided when mice were placed in the initially preferred compartment during conditioning. On days 8–17, mice were allowed to freely explore the apparatus for 15 min, with one empty food cup present in each of the two large compartments. An experimenter blinded to the specific treatment recorded time spent in each side compartment.

We followed our published procedure for withdrawal-induced cue avoidance (Fig. 1C) (38). Briefly, mice were singly housed in a home cage with a single bottle containing $200\mu\text{g ml}^{-1}$ nicotine in a 0.33% saccharin solution. Every 3 days, the mice and bottles were weighed and fresh nicotine solution was provided. On the 14th day, animals were pretested in the place conditioning apparatus. On the 15th day, animals received mecamylamine (0 and 2.5 mg free base kg^{-1} , s.c.) immediately before two 30-min sessions at least 5 h apart. This nAChR antagonist has been used to precipitate withdrawal in the place conditioning paradigm in rats and mice chronically exposed to nicotine (20,21). The order of injections, as well as the drug pairings, was counterbalanced between animals so that the animals, as a group, did not show a pre-existing bias to either compartment. From the 16th to 25th day, animals were allowed to freely explore the apparatus for 15 min per day, and time spent in each side compartment was recorded by an experimenter blinded to the specific treatment. Nicotine intake was measured in home cages and the total volume up to the pre-conditioning test day was analyzed.

Histology

Following completion of testing, animals were anesthetized with pentobarbital (62.5 mg kg^{-1} , i.p.) and transcardially perfused with physiological saline followed by 4% paraformaldehyde in 0.1 M Na-K phosphate buffer (PB). The brains were removed and postfixed in 4% paraformaldehyde for 1–2 h and cryoprotected in 20% glycerol in PB overnight. Brains were sliced coronally at 50 μm on a freezing microtome, and sections were kept in PB with sodium azide (0.1%). We used mitogen-associated protein type 2 (MAP2) staining to delineate the extent of lesions. Because this protein is found mainly in dendrites, a lack of MAP2 staining is indicative of neuronal cell death (28,45). Moreover, unlike Nissl staining, this marker does not label glial proliferation within a lesioned area, thereby showing neuronal loss as a clear lack of any staining. Free-floating sections were treated for 10 min with 3% H_2O_2 in 0.01 M Na phosphate buffer containing 0.2% Triton X-100 and 0.9% NaCl (PBS, pH 7.4) and for 30 min with 5% normal goat serum. Following washes

with PBS (3×10 min), sections were incubated in rabbit MAP2 antibody (1:2,000, Millipore, Billerica, MA) for 48 h at 4°C. Sections were washed in PBS (3×10 min), incubated in goat anti-rabbit IgG (1:500, Vector Laboratories, Burlingame, CA) for 1 h, and subsequently in avidin-biotin-peroxidase complex (1:170, Vector Laboratories) for 1 h. Each of these steps was followed by three washes in PBS except for the last wash in PB. Reaction between H_2O_2 (0.003%) and horseradish peroxidase conjugated with biotin oxidized diaminobenzidine (DAB; Sigma-Aldrich, St. Louis, MO) yielded brown deposits.

Statistics

Data were analyzed as the time spent in the drug-paired compartment minus the time spent in the vehicle-paired compartment. All data are presented as the mean $\pm$ standard error of the mean (SEM). Statistical significance was determined by two-way ANOVA followed by the Newman-Keuls *post hoc* test. Minimum significance was set at 5%. The Jarque-Bera test showed that JB values ranged from 0.009–4.41, demonstrating the normality of all groups ($p = 0.111$ – 0.996).

Results

Lesions were centered within each target (see Fig. 2). INS lesions were located within the entire antero-posterior extent (Fig. 2A,C). Lesions of the OFC included, throughout the anterior-posterior extent, the medial, ventral, and lateral subregions (Fig. 2B,D). Because we noted that lesions were often extended along the cannula tracks, dorsally above the target regions, we tested separate groups of mice that received lesions just above the targets; they were mostly located in the primary and secondary motor, primary somatosensory, cingulate, prelimbic, and frontal association cortices (Fig. 2A,B).

Mice with INS lesions showed no approach, but mice with sham (Sham), OFC-lesions, and lesions in areas dorsal to the INS (Dorsal control) did (Fig. 3A; Group, $F(3,30)=3.43$, $p=0.0295$). Moreover, all groups, except for the INS group, increased their preference for a compartment following conditioning with nicotine compared to a pre-conditioning day (see P, pre-conditioning day), and their preference gradually extinguished (Day, $F(10,300)=25.36$, $p<0.0001$; Group $\times$ Day, $F(30,300)=3.28$, $p<0.0001$).

To determine whether the effects of INS lesions can be generalized to natural incentive cue approach, we tested INS-lesioned mice in food-induced cue approach (Fig. 3B). Both sham and INS-lesioned mice increased their preference for a food-paired compartment on the first post-conditioning test day. However, while sham-operated mice maintained cue approach from the second post-conditioning day, INS-lesioned mice did not (Group, $F(1,11)=18.64$, $p=0.0012$; Day, $F(10,110)=11.70$, $p<0.0001$; Group $\times$ Day, $F(10,110)=4.02$, $p=0.0001$).

We next examined the role of these two cortical regions in withdrawal-induced cue avoidance. Precipitated withdrawal established conditioned cue avoidance in Sham mice and mice with lesions to the INS and areas dorsal to the OFC (Dorsal control). By contrast, OFC-lesioned mice failed to show this conditioned behavior (Fig. 3C; Group, $F(3,27)=24.82$, $p<0.0001$). All but the OFC group showed avoidance following conditioning compared to the pre-conditioning day, and gradually decreased this avoidance during post-conditioning test days (i.e., extinction) (Day, $F(10,270)=12.40$, $p<0.0001$; Group $\times$ Day, $F(30,270)=3.39$, $p<0.0001$). All groups of mice consumed indistinguishable amounts of nicotine (Group, $F(3,27)=0.17$, $p=0.9182$; Fig. 3D).

To determine the specificity of the deficit, we tested mice with OFC lesions for LiCl-induced cue avoidance. Compared to the pre-conditioning test day, this drug equally induced cue avoidance in both OFC- and sham-lesioned mice, which equally extinguished during

postconditioning test days (Fig. 3E; Group, $F(1,11)=0.57$, $p=0.46$; Day, $F(10,110)=5.87$, $p<0.0001$; Group x Day, $F(10,110)=0.52$, $p=0.87$).

Discussion

The present study separated cue reactivity into seeking of incentive stimuli and avoidance of withdrawal and demonstrated that the INS and OFC are independently required, through a dissociable manner, for these separate elements of craving. Our finding is consistent with the idea that cue-evoked craving is not a unitary process (13,14,46) and further suggests parallel processing of the two elements of craving at the level of the two cortical regions.

We designed our experiments to optimally induce nicotine-associated cue approach and withdrawal-associated cue avoidance in the place conditioning paradigm. It has been noted that nicotine induces cue approach more reliably in the biased design --in which an initially non-preferred compartment is paired with nicotine --compared to the non-biased procedure (19). Our pilot studies were consistent with this claim and we observed consistent cue approach when nicotine was paired with an initially non-preferred compartment in C57BL/6J mice; the non-biased design failed to establish statistically reliable cue approach in C57BL/6J mice.

The biased design has been criticized on the grounds that it fails to isolate the rewarding effects of drugs and might be contaminated by other effects of drugs (47). However, there is no compelling reason to isolate "reward" alone. First, nicotine preference in humans might include effects other than reward (e.g., reduction of baseline depression or anxiety) (48–50). Second, compared to cue-evoked craving, reward and positive reinforcement might not play a significant role in daily smoking or relapse and might not contribute to motives that uniquely characterize addiction and dependence (51,52). Third, cue-evoked craving includes not only the approach-inducing effects of drugs but also the entire Pavlovian conditioning process (see Introduction).

Withdrawal-induced avoidance has been demonstrated in the non-biased design in rats (20) and in the biased design where mecamylamine-precipitated withdrawal was paired with an initially preferred compartment in mice (21,25). We tested cue avoidance in the non-biased design for two reasons. First, we have reported that robust withdrawal-cue avoidance can be induced in the non-biased procedure (38). Second, because OFC inactivation has been shown to both potentiate and attenuate many cocaine-related effects in self-administration (53–58), we needed a design that would allow us to evaluate both possibilities. If withdrawal is conditioned with an initially preferred compartment in the biased design, any effect other than avoidance would be difficult to detect due to a ceiling effect; if mecamylamine is paired with the initially non-preferred compartment, a floor effect would make it difficult, if not impossible, to reveal any conditioned avoidance.

Because INS lesions impaired nicotine cue approach in the biased design, and OFC lesions impaired withdrawal cue avoidance in the non-biased design, the differential effects might be due to this procedural difference, rather than some functional differences of these two cortical regions in nicotine cue approach and withdrawal cue avoidance. Obviously, our conclusions are limited to these experimental conditions. However, these designs were adapted to optimally induce conditioned behaviors. Moreover, there is no reliable way to make the experimental designs of cue approach and cue avoidance equal. Even if the non-biased (or biased) place conditioning procedure was used for both preference and avoidance, there would remain a number of procedural differences (e.g., nicotine vs. withdrawal and presence vs. absence of chronic nicotine infusion). Nonetheless, the effects of INS and OFC lesions were evaluated within the identical condition and lesion effects were clearly

dissociated within each procedure; INS, but not OFC lesions impaired nicotine cue approach and OFC, but not INS lesions impaired withdrawal cue avoidance. Moreover, the effects of OFC lesions were not specific to the non-biased design, as such lesions impaired withdrawal cue avoidance but not LiCl-induced cue avoidance in the non-biased design. Similarly, the effects of INS lesions on nicotine- and food-cue approach were not identical despite the fact that both were done in the biased design.

INS-lesioned mice did not express nicotine cue approach from the first test day. By contrast, such mice acquired food-cue approach and expressed it normally on the first testing day, but failed to maintain the behavior from the second extinction day. Because the duration and level of nicotine and food cue approach were indistinguishable in sham mice, the subtle difference in the way INS lesions affect these two types of incentive cue approach is unlikely to reflect a difference in the strength of conditioning. One possibility is that the INS is differentially involved in the acquisition and retrieval of nicotine and food cue approach. Alternatively or additionally, INS lesions might have accelerated extinction of nicotine cue approach more rapidly than food cue approach by more rapidly establishing the new learning of cue-no incentive on the first post-conditioning day. More work is needed to ascertain how the INS contributes to the acquisition, expression, and extinction of nicotine and food cue approach.

Our observations that INS lesions eliminated nicotine cue approach, and extinction diminished cue approach over a course of 10 days, support the validity of this rodent behavior as a model of craving. The vast majority of smokers relapse within the first 8 days (59) and experience the most intense craving during this period, but progressively less craving thereafter (60–63). Moreover, cues indicative of cigarettes activate the INS (8–11,64–66). This activation is functionally significant, as smokers with widespread damage, including damage to the INS, are significantly more likely to have less craving and quit smoking (67) (but see (68)). Together with our observation that INS lesions had no effect on withdrawal-induced cue avoidance, it could be suggested that the INS functionally mediates the cue-guided incentive motivation component, but not the avoidance of withdrawal component, of craving.

While the functional role of the INS in other forms of addiction in humans is not well understood, the INS has been shown to contribute to cue reactivity with amphetamine in the place conditioning paradigm (44) and cue-evoked reinstatement of nicotine (69) and cocaine (70) self-administration in rats. Such a role might be embedded in other functions ascribed to this structure, including interoceptive representation (i.e., bodily states), subjective feelings (e.g., emotion), and cognitive functions. Alternatively, incentive motivation might be one additional function to which this region contributes.

The INS is likely to be selectively required for the prolonged expression of cue-food association; it is not critical for acquisition of cue-food association or its acute expression. Expression of cue-food association during extinction could include heterogeneous elements. Cues indicative of food activate the INS in humans (71). Such activation might selectively reflect its role in the maintenance of cue reactivity.

While smoking-related cues activate the OFC and induce craving (8–10,72–75), our data suggest that this activation is functionally required for processing of the cue-evoked withdrawal avoidance component, but not the cue-evoked incentive component, of craving. It should be noted that withdrawal cue avoidance includes a state of dependence, induction of withdrawal, association between cues and withdrawal, and acute and maintained retrieval/expression of this association. Given that abstinence alone also is associated with activation

of this cortical region in smokers (76), a future challenge is to identify the exact process of withdrawal cue reactivity for which this cortical region is functionally required.

As our paradigm does not require reversal or devaluation, such functions of the OFC (77) do not offer a plausible explanation for our finding. The cortical region might additionally be involved in some aspects of withdrawal-associated cue reactivity. Although this region is implicated in processing of cues that signal aversive stimuli in humans (78), mice with OFC lesions showed normal LiCl-induced cue avoidance. This cortical region might not equally contribute to all types of aversive cue learning.

As the functional specification among corticolimbic regions might not be identical between humans and mice, caution is needed in comparing activation of regions in humans and effects of damage to the regions in rodents. With this interpretative caveat, it is still interesting to observe that human and rodent data are not entirely consistent. Among corticolimbic regions, the cingulate cortex is another cortical area that is activated by smoking-associated cues (8,9,11,64–66,72–74,79). However, excitotoxic lesions of this area have little effect on cue approach with cocaine, morphine, or amphetamine in rodents (80). We also observed that six out of eight “dorsal control” mice had lesions in this cortical area (see Fig. 2B) but exhibited normal withdrawal-associated cue avoidance. It remains unclear what functional role the cingulate cortex plays in cue reactivity.

Other corticolimbic regions have been shown to be activated by smoking-related cues. The medial prefrontal region responds to smoking-related cues in smokers (9,75). The rodent medial prefrontal cortex has been implicated in cue approaches with morphine and cocaine (80,81). Smoking-related cues activate the amygdala and hippocampus in smokers (10,11,74,82). In rats, excitotoxic lesions of the basolateral amygdaloid complex impair cue approach induced by amphetamine (83) and cocaine (84,85), and cue avoidance induced by withdrawal in morphine-dependent rats (86). Cocaine-associated cue approach has been shown to be reduced by excitotoxic lesions of the dorsal hippocampus (87). As the role of these structures in nicotine cue reactivity has not been examined in rodents, a future challenge is to fully understand how these structures, together with the INS and OFC, orchestrate craving through cue-evoked incentive motivation and withdrawal in addictions to nicotine and other addictive substances.

Given that pre-existing traits are a determinant for addiction susceptibility (50,88) and cue reactivity is seen in addiction with many other substances (2), our observations provide a framework to further elucidate the way in which pre-existing activity levels of the various cortical regions contribute to susceptibility to many forms of cue reactivity in addiction.

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Figure 1.

Experimental protocol. **A)** Nicotine-induced conditioned cue approach (0 and 0.2 mg free base kg⁻¹, s.c.) and LiCl-induced conditioned cue avoidance (LiCl salt, 0 and 240 mg kg⁻¹, i.p.). An acute nicotine or LiCl injection was not given on the test days. **B)** Food (sweetened condensed milk)-induced conditioned cue approach. Food and an empty cup were alternately given in each compartment during conditioning sessions. **C)** Mecamylamine-induced conditioned cue avoidance (0 and 2.5 mg free base kg⁻¹, s.c.). The black horizontal line indicates exposure to oral nicotine (200 µg free base ml⁻¹) in the home cage. Black box, 15-min drug-free testing session; P, pre-conditioning test; T, post-conditioning test; grey box, 30-min conditioning (C) session.

Figure 2. Schematic of ibotenic acid-induced lesions of the **A)** insula and **B)** orbitofrontal cortex. Red-toned areas represent individual lesions. The darker red areas represent areas lesioned in more than one mouse. Grey areas represent individual lesions of "dorsal control" groups. Representative photographs of coronal sections showing MAP2 staining. Lesioned areas are devoid of staining in the insula (**C**) and the orbitofrontal cortex (**D**). INS, insula; OFC, orbitofrontal cortex; M1, primary motor cortex; M2, secondary motor cortex; S1, primary somatosensory cortex; Cg, cingulate cortex; PrL, prelimbic cortex; FrA, frontal association cortex; CL, claustrum; MO, medial orbitofrontal cortex; VO, ventral orbitofrontal cortex; LO, lateral orbitofrontal cortex. Scale bar: 500 μm.

Figure 3.

Impact of cortical lesions on cue approach and avoidance. **A)** Nicotine-induced cue approach. N=6–13/group. **B)** Food-induced cue approach. N=7 (Sham) and 6 (Insula). **C)** Withdrawal-induced cue avoidance. N=5–12/group. **D)** Total nicotine intake of groups used for withdrawal-induced cue avoidance. OFC, orbitofrontal group; INS, insula group; Cont, control group that received lesions dorsal to the orbitofrontal cortex. **E)** LiCl-induced cue avoidance. N=7 (Sham) and 6 (orbitofrontal). X axis in **A–C** and **E**, pre-conditioning (P) and 10 postconditioning test days (Test day). Y axis in **A**, **C** and **E**, difference in time spent in the drug-paired and saline-paired compartments (sec). Y axis in **B**, difference in time spent in the food-paired and unpaired compartments (sec). # and ## ($p < 0.05$ and $p < 0.01$) represent statistically significant difference from sham group; * and ** represent a statistically significant difference from the pre-conditioning test at 5 and 1% levels, respectively. NS indicates no difference from the pre-conditioning test day.