

A Subregion of Insular Cortex Is Required for Rapid Taste-Visceral Integration and Consequent Conditioned Taste Aversion and Avoidance Expression in Rats

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Abstract

Postingestive signals are important for shaping appetitive and consummatory responses, but the brain mechanisms required to assimilate interoceptive events with those at the frontlines of ingestion (taste-guided) are poorly understood. Here, we investigated whether an insular cortex (IC) region, which receives viscerosensory input, including gustatory, is required to modify taste-elicited consummatory reactions in response to a real-time interoceptive change using a serial taste reactivity (TR) test where the rats' oromotor and somatic reactions to intraoral (IO) infusions of sucrose were periodically assessed over 45 min following lithium chloride (LiCl) administration. Results showed that neurally-intact rats shifted from an ingestive repertoire to an aversive one as LiCl took effect. Overall, this hedonic shift was delayed in rats with bilateral neurotoxic IC lesions. Rats with greater neuronal loss in posterior gustatory IC displayed fewer aversive reactions to sucrose following this initial LiCl injection. We further assessed whether the failure to integrate interoceptive feedback with ongoing taste-guided behavior impaired acquisition and/or expression of conditioned aversion and/or avoidance in these same rats. Although, as a group, LiCl-injected rats with IC lesions subsequently avoided the sugar in a 48-h two-bottle test, their preference for sucrose was significantly greater than that of the LiCl-injected neurally-intact rats. Overall lesion size, as well as proportion of the posterior gustatory and/or anterior visceral IC were each associated with impaired avoidance. These findings reveal new roles for the posterior gustatory and anterior visceral ICs in multisensory integrative function.

Key words: brain mapping; food reward; gustatory cortex; interoception; taste hedonics; visceral cortex

Significance Statement

Adaptive eating and drinking behaviors require that the brain incorporates interoceptive state information into appropriate taste-guided appetitive and consummatory responses in real time and over the long-term through learning. Here, we show for the first time that loss of function in a subregion of insular cortex (IC) that receives both general visceral and gustatory sensory inputs hinders rapid adjustments to consummatory behaviors in response to a negative interoceptive event. We further show that this primary integrative deficit precludes robust adaptive avoidance behavior that normally safeguards against enduring the same visceral consequences again. Collectively, the findings yield new insights into the neural organization of taste-interoceptive integration in IC, which subserves key aspects of short-term and long-term control of ingestive behavior.

Introduction

Survival depends in part on the ability to rapidly detect, integrate, and respond to sensory events associated with eating and drinking. The insular cortex (IC), which receives both exteroceptive and interoceptive inputs and is highly interconnected with motivational effector circuits, has long been implicated in these types of convergent processes that enable adaptive ingestive decisions (Krushel and van der Kooy, 1988; Allen et al., 1991; Fontanini and Katz, 2006, 2009; Parkes et al., 2015; Gogolla, 2017; Gehrlach et al., 2020; Vincis et al., 2020; Boughter and Fletcher, 2021). Conditioned taste avoidance (CTAvoid) is the most well-studied example of this phenomenon. In CTAvoid, the orosensory properties of an ingested substance (e.g., flavor; conditioned stimulus; CS) become associated with its negative interoceptive consequences (e.g., nausea; unconditioned stimulus; US) such that future encounters with that flavor recall the negative event and prevent its ingestion again (Garcia et al., 1955). Over the last 40 years, numerous studies have shown that the gustatory IC (GC) is necessary to successfully express a CTAvoid (Yamamoto et al., 1980; Braun et al., 1982; Lasiter and Glanzman, 1982; Dunn and Everitt, 1988; Bermudez-Rattoni and McGaugh, 1991; Rosenblum et al., 1995, 1997; Naor and Dudai, 1996; Nerad et al., 1996; Schafe and Bernstein, 1998; Cubero et al., 1999; Fresquet et al., 2004; Roman and Reilly, 2007) and recent studies have further attributed this to a specific IC subregion comprising the contiguous posterior GC and anterior visceral cortex (pGC/aVC; Schier et al., 2014, 2016).

However, CTAvoid, like many ingestive phenomena, is mechanistically complex. In order to successfully reject a flavor CS, the brain must (1) detect the orosensory CS; (2) detect the negative US; (3) associatively link the CS and US; (4) consolidate the association into memory; (5) recall it from memory when the CS is reintroduced; and (6) execute an appropriate response (Spector et al., 1988; Reilly and Bornovalova, 2005; Schier and Spector, 2019; Fig. 1). Disruption in any of these functionally distinct processes would lead to a similar outcome: failure to avoid ingesting the solution at test. Thus, one aim of the present study was to clarify which of these CTAvoid-related events the pGC/aVC is involved in. Based on a prior study, this IC subregion is not necessary to detect the taste CS and

interoceptive US (steps 1–2; Schier et al., 2016), but whether it is required to integrate and consolidate these inputs (steps 3–4) and whether that relates to subsequent deficits in CTAvoid expression (steps 5–6) are unknown.

Moreover, in addition to CTAvoid, USs can foster a fundamentally different type of learned response to the CS: conditioned taste aversion (CTAver; Pelchat et al., 1983; Parker, 2003; Schier et al., 2019). Whereas taste avoidance prevents the anticipated negative outcome, taste aversion refers to the negative hedonic evaluation of the taste stimulus, which itself limits ingestion. The difference in these processes is evident in how animals respond to different types of visceral events associated with food. For example, whereas food poisoning will alter a food's palatability such that its flavor is rendered aversive (i.e., CTAver), lactose malabsorption will cause animals to avoid consuming lactose-containing substances, but without affecting its hedonic appeal (i.e., CTAvoid; Pelchat et al., 1983; Parker, 2003; Schier et al., 2019). Failure to avert the CS ingestion in preference and intake tests does not reveal which of these motivational processes is affected by a given experimental manipulation. But, rodents, like humans, display distinct stereotypic oromotor and somatic responses to positively-valenced and negatively-valenced taste stimuli (Grill and Norgren, 1978) and measurement of these responses in a taste reactivity (TR) test provides a readout on the hedonic appeal of a taste stimulus, in the absence of goal-directed motives like avoidance.

To determine whether the pGC/aVC, which is necessary for CTAvoid, is also required to adaptively shift the hedonic value of the taste CS in response to the emetic event (CTAver), we used the serial TR paradigm (Spector et al., 1988). In the serial TR pairing session, lithium chloride (LiCl) is injected to induce nausea and then a novel tastant, sucrose, is infused into the oral cavity while oromotor and somatic reactions are recorded. As the LiCl takes effect, intact rats rapidly shift TR from an ingestive repertoire to an aversive one. Therefore, here, we assessed whether loss of function in pGC/aVC disrupted the online integration of interoceptive cues with incoming taste information while minimizing the memory requirement. Next, because the coincident presentation of the taste CS and the LiCl US in this initial pairing session is sufficient to condition an aversion to the CS in intact rats (Spector et al., 1988), we tested whether loss of function in pGC/aVC results in subsequent CTAver and/or CTAvoid deficits (Fig. 2).

Materials and Methods

Subjects

Adult male Sprague Dawley rats, weighing 330–420 g at the start of the experiment, were singly housed in polycarbonate shoebox cages with *ad libitum* access to rodent chow [Teklad Rodent Diet (Envigo, Indianapolis) for phase 1, Purina 5001 for phase 2] and deionized water. The climate-controlled colony room was set to 12/12 h light/dark cycle. The experiment was run in two phases ($n = 48$ for phase 1, $n = 30$ for phase 2). Phase 1 was conducted at Stetson University and phase 2 was conducted at the University of

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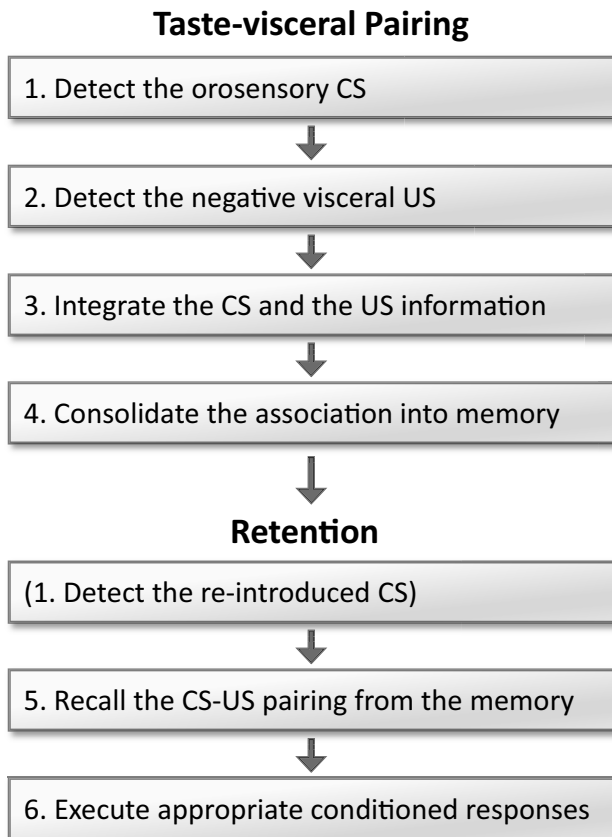


Figure 1. Principal processes to establish and express CTA_{avoid} and/or CTA_{aversion}. To successfully learn CTA_{avoid/aversion}, an animal must detect the orosensory CS, detect the negative visceral event, assimilate those sensory events, and consolidate the association into memory. Then, to later express the learning when re-introduced to a CS, the subject must detect the CS, recall the previous association from memory, and perform the conditioned response. Failure to accomplish any of these pairing and retention steps, would impair the ultimate expression of the learned avoidance/aversion. CS, conditioned stimulus; US, unconditioned stimulus.

Southern California. All behavioral procedures and schedules were identical between the phases. The experiment was performed in successive replications during each phase. All surgical and behavioral procedures were approved by the Stetson University (phase 1) and the University of Southern California (phase 2) Animal Care and Use Committees.

Surgery

Stereotaxic lesions

After at least 5 d of acclimation to the colony room, rats were subdivided into four surgical groups (IC₂ lesion, IC₃ lesion, IC₂+IC₃ lesion, or Sham-operated control, see below; Table 1). Lesions were targeted to the IC₂ region as defined by Schier et al. (2014), which spans 0.2–1.2 mm anterior to bregma and includes the pGC and overlying anterior VC, and the IC₃ region, which spans the VC from –0.8 to +0.2 mm from bregma, or both regions (IC₂+IC₃). The number of animals and corresponding stereotaxic coordinates used for each group and phase are listed in Table 1. All the lesions were systematically mapped postmortem by an experimenter blinded to the experimental treatments and only the animals that met the lesion criteria were included for the final analyses (see below). Stereotaxic surgeries were performed under sterile conditions with anesthesia [ketamine hydrochloride (125 mg/kg) and xylazine (5 mg/kg) mixture for phase 1; isoflurane (5% induction rate, ~2.5–3% maintenance rate) for phase 2]. After the rat was completely anesthetized, the incision area on the scalp was shaved and cleaned with 70% ethanol and betadine. The rat’s head was secured in a stereotaxic apparatus with blunt ear bars. An incision was made in the skin to expose the skull. The skull was leveled against bregma and lambda by adjusting the bite bar. Small holes were made in the skull above the target coordinates on each side using a micro hand drill. A 1- μ l Hamilton syringe filled with either phosphate buffered saline (0.1 M PBS, for sham surgeries) or ibotenic acid (IBO; 20 mg/ml in 0.1 M PBS, for lesion surgeries) was connected to a glass micropipette tip (outside diameter ~50 μ m) with a paraffin wax seal. Using bregma

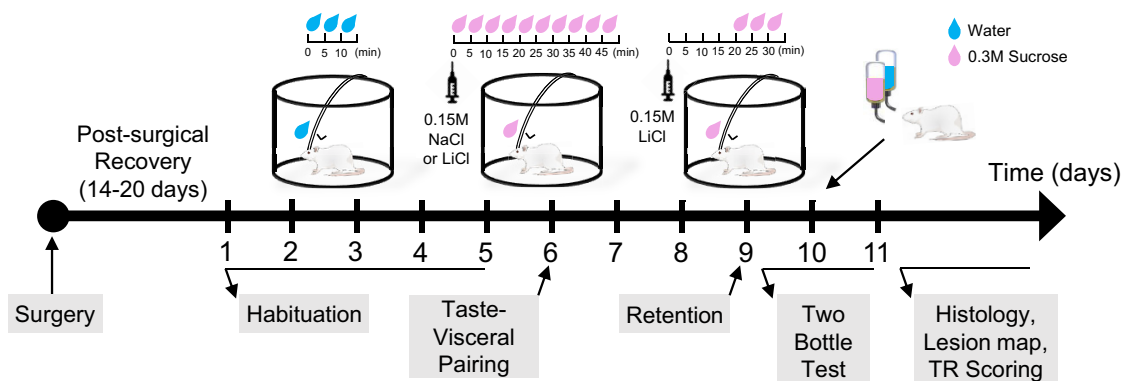


Figure 2. The experimental design and timeline. The experimental timeline showing when (in days) each of the behavioral tests was conducted beginning with habituation, labeled below the black horizontal arrow. The injection and infusion schedule within each session is shown above the diagram. TR tests were conducted in a TR chamber and two-bottle tests were performed in the home cage. Following the completion of two-bottle test, histologic analyses including lesion mapping were completed and TR videos were scored in a blinded fashion. NaCl, Sodium Chloride; LiCl, Lithium Chloride; TR, taste reactivity, M, Molar, Min, Minute.

Table 1: Lesion groups, training groups, and stereotaxic coordinates in each phase

Phase/Ns	Lesion Group	CTA Group/Ns	Stereotaxic target coordinates ⁺							
			AP	ML	IC ₂ DV	Vol. (μl)	AP	ML	IC ₃ DV	Vol. (μl)
Phase 1 N = 29	Sham IC ₂ +IC ₃ (PBS)	NaCl (N = 5)	+0.6	±5.7	-6.6, -6.7	0.18	-0.5	± 6.2	-7.0	0.18
		LiCl (N = 6)								
	Bilateral IC ₂ (IBO)	NaCl (N = 0)	+0.6	±5.7	-6.6, -6.7	0.18			N/A	
		LiCl (N = 3)								
Bilateral IC ₃ (IBO)	NaCl (N = 2)			N/A		-0.5	± 6.2	-7.0	0.18	
	LiCl (N = 3)									
	Bilateral IC ₂ +IC ₃ (IBO)	NaCl (N = 3)	+0.5	± 5.9	-6.6, -6.7	0.18	-0.5	± 6.2	-7.0	0.18
Phase 2 N = 18	Sham IC ₂ +IC ₃ (PBS)	NaCl (N = 4)	+0.6	±5.8	-6.7	0.1	-0.5	± 5.9	-7.0	0.1
		LiCl (N = 5)								
	Bilateral IC ₂ (IBO)	NaCl (N = 0)	+0.6	±5.8	-6.7	0.1			N/A	
		LiCl (N = 3)								
Bilateral IC ₃ (IBO)	NaCl (N = 0)			N/A		-0.5	± 5.9	-7.0	0.1	
	LiCl (N = 0)									
	Bilateral IC ₂ +IC ₃ (IBO)	NaCl (N = 0)	+0.6	±5.8	-6.7	0.1	-0.5	± 5.9	-7.0	0.1
		LiCl (N = 6)								

Sample sizes (N) include animals that underwent surgery, met the lesion criteria and completed all behavioral tests. ⁺ All stereotaxic coordinates were based on the Paxinos and Watson stereotaxic atlas (6th Edition). CTA, conditioned taste aversion; IC, insular cortex; NaCl, sodium chloride; LiCl, lithium chloride; AP, anterior-posterior; ML, medial-lateral; DV, dorsal-ventral; Vol, volume; PBS, phosphate buffered saline; IBO, 20 mg/ml ibotenic acid in PBS; LiCl, Lithium chloride; NaCl, Sodium chloride.

as a reference point, the glass micropipette was positioned at the target coordinate (Table 1). PBS or IBO was slowly infused in two or three microinfusions (volumes listed in Table 1), separated by an ~2-min interval to allow diffusion into the tissue. Infusions were done bilaterally. After all injections were administered, cranial holes were filled with hemostatic sponge.

Intraoral (IO) cannulation

Immediately following the stereotaxic surgery, the anesthetized rat was placed in a supine position for bilateral IO cannula implantation. IO cannulation was performed according to the procedures adapted from (Phillips and Norgren, 1970; Grill and Norgren, 1978). Briefly, polyethylene tubing (PE 100) was inserted just anterolateral to the second maxillary molar and tunneled under the facial muscles to an exit site at the top of the head. The tubes were outfitted with a metal connector pin and anchored to the skull with microscrews and dental cement. After the cement head cap was fully cured, the skin was closed around it with single stay sutures. During surgery and for 3 d after surgery, rats were given analgesic (Carprofen, 5 mg/kg, sc) and antibiotic (Enrofloxacin, 2.3 mg/kg, sc). Powdered chow and/or wet chow mash were provided during the 14–20 d of recovery.

Behavioral procedures

TR: habituation, CTA acquisition, and retention

The TR chamber comprised a cylindrical chamber with a transparent Plexiglas floor. The infusion tubing ran from a 10-ml syringe in an external infusion pump (Harvard Apparatus) through a single channel stainless steel swivel mounted at the top of the TR chamber. The free end of the tubing was encased in a spring tether and connected

to the rat's IO cannula at the connector pin. This system allowed the rat to move freely around the chamber, while preventing it from accessing the infusion line tubing. A camera was mounted on a tripod aimed at an angled mirror below the chamber (phase 1) or was mounted directly below the chamber (phase 2). After recovery from the surgery, rats were habituated to the TR chamber across 5 d (10 min/d). On the first habituation day, the rat was simply placed in the chamber. On the second day, the rat was connected to the tubing, but no infusions were made. On days 3–5, the rat was connected to the tubing and received infusions of deionized water (1 ml/min) for 30 s every 5 min (min 0, 5, and 10). At the end of each 10-min session, the rat was returned to the home cage.

Rats from each surgical group were further subdivided into treatment groups for the taste-visceral pairing session (Table 1). On day 6, rats received an intraperitoneal injection of either 0.15 M LiCl (13.33 ml/kg) to induce visceral malaise or isomolar NaCl (13.33 ml/kg) as a control directly before the start of the session. Then the rat was placed in the TR chamber and its IO cannula was connected to the infusion line. Sucrose (0.3 M) was IO infused at a rate of 1 ml/min for 30 s (from the first oromotor response) every 5 min, beginning at minute 0, for a total of 10 infusions. After the last infusion at minute 45, the rat was disconnected from the tubing and returned to its home cage. The rats' oromotor and somatic responses to sucrose during each infusion period were video recorded and analyzed later offline (see below).

Three days later, rats were given a TR retention test to determine whether the sucrose-LiCl pairing yielded a CTAver. In this test, all rats were intraperitoneally injected with 0.15 M LiCl (13.33 ml/kg), placed in the chamber and connected to the infusion line (min 0). Starting at minute

20, 0.3 M sucrose was infused for 30 s at a rate of 1 ml/min every 5 min up to minute 30 (total of three infusions). After the last infusion, the rat was returned to its home cage. The rats' oromotor and somatic responses to sucrose during each infusion period were video recorded and analyzed later offline (see below). The LiCl was injected during this test because it has been shown that to observe the full display of the conditioned response, the visceral state during conditioning and testing must be concordant (Spector et al., 1988).

Two-bottle preference test (24, 48 h)

After the TR retention test, two bottles were placed on the home cage to measure CTAvoid. One bottle was filled with deionized water and the other one was filled with 0.3 M sucrose. After 24 h, the amount of each solution that the rat consumed was recorded (by weight, to the nearest 0.1 g). The bottles were refilled with fresh sucrose solution and deionized water and the position of two bottles on the home cage was switched. After another 24 h, the amount consumed from each bottle was recorded.

Behavioral analyses

TR

Before scoring, videos recorded during the serial TR taste-visceral pairing and retention sessions were zoomed and cropped to include the rats' face and forelimbs using Sony Movie Studios software. A subset of videos spanning the initial taste-visceral pairing session (min 5, 15, 25, and 35) and all three videos from the TR retention session were viewed in slow motion (frame by frame) while individual ingestive and aversive oromotor and somatic responses were counted by an experimenter blinded to the experimental history of the subjects. These responses were categorized into four stereotypic ingestive TR behaviors and four stereotypic aversive TR behaviors, as described previously (Spector et al., 1988) and outlined in Extended Data Figure 4-4. Ingestive TR behaviors included: tongue protrusion, lateral tongue protrusion, mouth movement, and paw lick. Aversive TR behaviors included: gape, chin rub, head shake, and forelimb flail. Raw counts were used for each of these behaviors, except paw lick. The duration of paw licking was recorded in seconds and then multiplied by 6 to get an estimated count. Passive drips were recorded but not included in the final scores. The counts of individual ingestive behaviors were summated for a total ingestive TR score. Likewise, the counts of individual aversive behaviors were summated for a total aversive TR score. In some cases, the face or paws were obscured; these were categorized as no data and the duration was noted. For videos that had >1 s of no data, individual behavior categories of the ingestive and aversive TR responses were summed and corrected separately for lost time by dividing each score by the scorable time and then multiplying that by 30 which is the duration of a single infusion. Then, the counts of individual ingestive behaviors and aversive behaviors were summated, respectively, to derive a total ingestive and aversive TR score.

Two-bottle preference

Based on the intake records (in grams), sucrose preference over the first 24 h and entire 48-h period was calculated by dividing sucrose intake over the total solution intake (sucrose + water) and multiplying by 100.

Histology and lesion mapping

Histology

The rats were euthanized by overdose with an intraperitoneally injected euthanasia agent containing sodium pentobarbital at the end of the experiment. Transcardial perfusion was performed with saline followed by 4% paraformaldehyde in 0.1 M PBS (PFA). Then, the skull was placed on a stereotaxic apparatus and leveled to match dorsoventral coordinates at bregma and lambda by adjusting the bite bar. Using a hand drill, a band of skull parallel to but slightly anterior to lambda was removed to expose the brain. The brain was then blocked by moving a blade mounted on a stereotaxic holder from left to right. This blocking method was done to ensure that each brain was sectioned in the correct stereotaxic plane. The remaining skull pieces were manually removed and the brain was stored in 4% PFA at 4°C until sectioning. The brains were sliced with vibratome (Leica, VT1200) in serial 50- μ m coronal sections and mounted on gelatin-coated slides. The slides were dried, Nissl stained with thionin and coverslipped to visualize cytoarchitecture and lesion in and around the IC.

Lesion mapping

The lesion mapping system developed by Schier et al. (2016) enables a thorough and quantitative reconstruction of lesion size and location in 50- μ m brain sections onto 2D Microsoft Excel grid. The IC in each coronal section is delineated into three layers (GI: granular, DI: dysgranular, AI: agranular, dorsal to the rhinal fissure anterior to AP 0.0 mm) and each layer is equally divided into three columnal grids along the mediolateral axis. The same segmentation is repeated for the areas dorsal to IC (D) and ventral to the rhinal fissure (V). Note that AI is included in V at AP levels posterior to 0.0 mm. For D and V, the area mapped is equivalent to the dorsoventral distance occupied by the GI, DI, and AI layers in IC in that same section. The left and right hemisphere are separately mapped. Brain sections were examined under 1.25 \times magnification to assess the extent of neuronal loss. In each grid cell for each brain section, the extent of cellular loss was scored on a trinary scale as complete (entire region devoid of cells; red), partial (at least half of the region, but less than the entire region, devoid of cells; yellow) or none (less than half of the region devoid of cells; white) and color-coded onto the 2D Excel grid. On the Excel grid, each row represents a single 50- μ m coronal section from anterior to posterior. Each column is a subdivision of IC (GI, DI, AI) and surrounding area (D, V) which is further divided into five medial-to-lateral subcolumns. These five subcolumns comprise the medial to lateral thirds of insular cortical tissue (three columns), claustrum which is medial to IC (1 column), and the area medial to the claustrum (1 column). Representative images of the

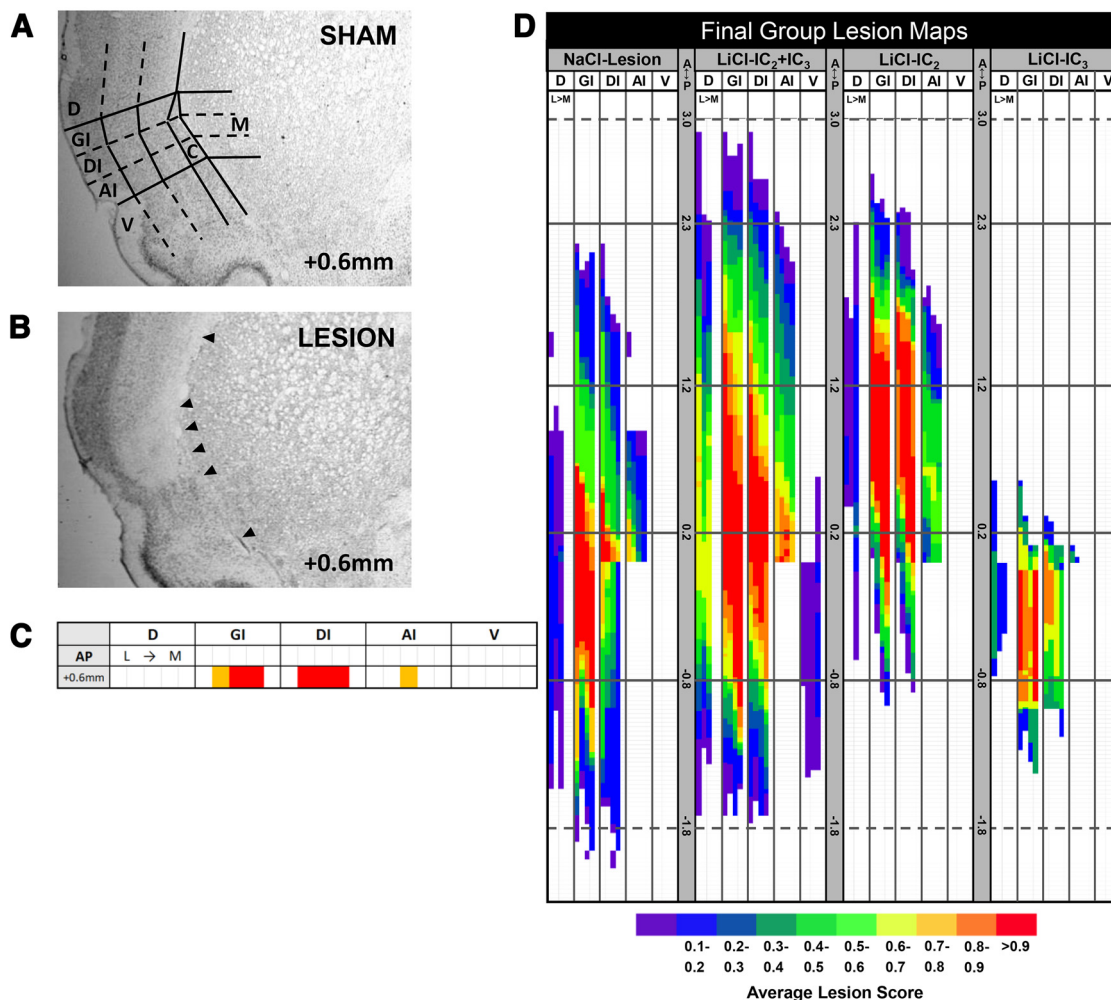


Figure 3. Representative photomicrograph of brain sections with delineated grids of IC and surrounding region and group-wise overlap lesion maps in a color-coded manner on 2D grids. A sham (A) and a lesion brain section (B) at AP coordinate of +0.6 mm are shown. Black arrowheads indicate the approximate borders of each layer in B. An example of the corresponding color-coded lesion map for B is shown in panel C. The overlap maps (D) show the average lesion scores in color scale for the NaCl-lesion (including NaCl-IC₃ and NaCl-IC₂+IC₃), LiCl-IC₂, LiCl-IC₃, and LiCl-IC₂+IC₃ groups. Solid and dotted lines indicate different AP levels relative to bregma including IC₂ borders (+1.2 and +0.2 mm) and IC₃ borders (+0.2 and -0.8 mm). Representative lesion maps and corresponding photomicrographs of brain sections of IC₂, IC₃, and IC₂+IC₃ lesion are shown in Extended Data Figures 3-1, 3-2, and 3-3, respectively. A, anterior to bregma; P, posterior to bregma; D, dorsal to the granular layer (GI); GI, granular IC; DI, dysgranular IC; AI, agranular IC (dorsal to the rhinal fissure); V, ventral to rhinal fissure; C, claustrum (medial to GI, DI, and AI); M, medial to claustrum; LiCl, Lithium chloride; NaCl, Sodium chloride; IC, Insular cortex.

delineated grids on brain sections and 2D plotting on excel grid are shown in Figure 3A-C. Lesions were mapped and scored by an experimenter blind to the subjects' surgery and treatment conditions. Fixation and histology cause some shrinkage in the brain tissue. Therefore, to ensure that the lesion maps were in register with the stereotaxic atlas and with one another (Paxinos and Watson, 2007), a landmark correction procedure was applied as follows. The AP level of coronal sections at key histologic landmarks were recorded on the Excel spreadsheet. These landmarks included the anterior start of the striatum, the joining of the corpus callosum, the disappearance of the indusium griseum below the corpus callosum, the rostral joining of the anterior commissure, the emergence of the CA3 field in

hippocampus, among others. Once these landmarks were noted, the number of sections between one landmark and the next landmark was counted and divided into the actual stereotaxic difference represented in the atlas. This gave a correction factor for each 50-μm section between the two particular landmarks. In essence, the relative AP distance that a single 50-μm section represented in the corresponding row on the mapping grid was determined by multiplying the section thickness (50 μm) by the local correction factor. This also allowed us to get a better estimate of the AP level relative to the two flanking landmarks. Once the entire lesion in both hemispheres was mapped separately and the AP scale was corrected, a third map (symmetry) was generated by comparing the lesion scores of each grid cell from

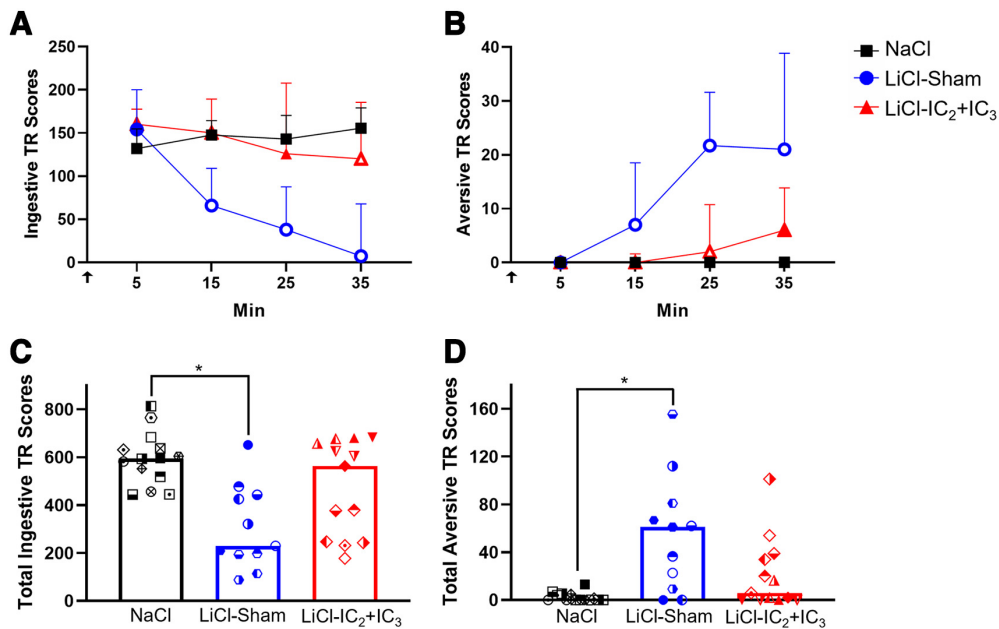


Figure 4. Lesions in IC_2 and IC_3 disrupted the adaptation of oromotor taste responses to sucrose following intraperitoneal LiCl injections on the taste-visceral pairing session. Categories and definitions of oromotor and somatic reactivity behaviors are shown in Extended Data Figure 4-4. Because there were no differences among NaCl-injected rats with sham or IC_2 and/or IC_3 lesion, they were combined into one group (data shown in Extended Data Figs. 4-1 and 4-5). **A, B**, Median (+ Semi-IQR) values of ingestive (**A**) and aversive (**B**) TR scores induced by 30-s IO sucrose infusions across the initial taste-visceral pairing session, plotted as a function of time following the intraperitoneal injection of either NaCl ($n = 14$) or LiCl ($n = 11$ for Sham; $n = 13$ for IC_2+IC_3 lesion). Black arrow indicates the approximate time of the intraperitoneal injection. Open symbols represent time points that the respective LiCl-injected groups were statistically different from the NaCl group ($p \leq 0.05$). **C, D**, Median values of total ingestive (**C**) and aversive (**D**) TR scores during taste-visceral pairing session plotted with different symbols for individual animals in each group. Rats are assigned to the same symbols in each figure. Statistical significances are adjusted based on Benjamini-Hochberg false discovery rate for multiple comparisons and indicated as asterisks. Statistics are in Tables 3-5. LiCl-injected rats with IC_2 or IC_3 lesion were not included because of low sample sizes but their behavioral performances are in Extended Data Figures 4-2 and 4-3. LiCl, Lithium chloride; NaCl, Sodium chloride; TR, Taste reactivity.

two hemispheres and assigning the lower one to the corresponding grid cell in the symmetry map. Total lesion score was calculated by adding the total value of all grid cells in each rat's symmetry map, regardless of where the lesion was located. Region of interest lesion scores (e.g., IC_2 or IC_3) were calculated as the total value of all grid cells in the designated region divided by the total number of cells in that region on the symmetry map to get a proportion of the area with lesion. For IC_2 and IC_3 , this included grid cell values in AI, DI, and GI, above the rhinal fissure.

Finally, to compare lesion size and placement across individual rats or groups of rats, all rats' symmetry lesion maps were extended and put on the same AP scale. To accomplish this, the AP value for each row was rounded to the nearest multiple of 10. Then, each of those rows was subdivided into 10- μm rows. For example, if the corrected AP distance of a row was designated as 81 μm , then it was rounded to 80 μm and further subdivided into eight 10- μm rows. To generate group-wise lesion maps across rats meeting a specified criterion (see below), individual symmetry maps were aligned along these 10- μm -scaled AP coordinates. The proportion of rats in that group with lesion in each grid cell was calculated and converted to color scale to visualize areas of lesion

overlap among rats in each group. Final group lesion maps are shown in Figure 3D.

Data analyses

Percentage of the subregions of interest (i.e., IC_2 , IC_3) with lesion were calculated for each rat as described above. Only rats with $\geq 50\%$ bilateral lesion in IC_2 and IC_3 were included in the IC_2+IC_3 group. Rats with $\geq 50\%$ bilateral lesion in IC_2 , but $< 50\%$ cell loss in IC_3 were included in the IC_2 group. Rats with $\geq 50\%$ bilateral lesion in IC_3 , but $< 50\%$ lesion in IC_2 were included in the IC_3 group. Based on these lesion criteria, rats were divided into three groups for the main statistical analyses— NaCl-injected control, LiCl-injected Sham, LiCl-injected IC_2+IC_3 lesion. Because there were no differences among NaCl-injected rats with Sham or IC_2 and/or IC_3 lesion, they were combined into one group (data shown for comparison purposes in Extended Data Fig. 4-1). Any rat whose lesion score did not meet these criteria was excluded from statistical analyses. Because of low sample sizes, rats that met the IC_2 or IC_3 lesion criteria were not included in these main group-wise analyses but see Extended Data Figures 4-2 and 4-3. Because we observed non-normal distributions in some of

Table 2: Ingestive or aversive TR scores at successive time points versus minute 5 baseline for each group on the taste-visceral pairing session

Ingestive scores			
	Na	Sham-Li (q* = 0.033)	IC ₂ +IC ₃ -Li (q* = 0.0167)
Minute 5 vs 15	0.54	0.083	0.34
Minute 5 vs 25	0.178	0.024+	0.110
Minute 5 vs 35	0.178	0.024+	0.002+
Aversive scores			
	Na	Sham-Li (q* = 0.05)	IC ₂ +IC ₃ -Li (q* = 0.033)
Minute 5 vs 15	0.313	0.008+	0.313
Minute 5 vs 25	0.313	0.004+	0.016+
Minute 5 vs 35	0.56	0.004+	0.016+

Corresponds to Figure 4A,B. Wilcoxon signed-rank tests were conducted and the significance levels within each group were adjusted based on Benjamini-Hochberg false discovery rate for multiple comparisons (q*; Benjamini and Hochberg, 1995). Values with a plus symbol (+) are statistically significant after correction. IC, Insular cortex; Li, Lithium chloride; Na, Sodium chloride.

the behavioral data, nonparametric Mann-Whitney tests were used to determine group-wise statistical differences. Wilcoxon signed rank tests were used for within-group paired comparisons. The p-values were corrected for multiple comparison with the Benjamini-Hochberg false discovery correction procedure (Benjamini and Hochberg, 1995). In order to examine patterns of behavior across the multiple behavioral assessments, each individual rat's behavior performance was converted to a robust Z-score standardized against the median and deviation of the LiCl Sham group's performance on the respective behavioral measurements. Spearman's rank correlations were used to determine whether overall lesion size

Table 3: Comparison of ingestive TR scores between groups at select time points across the taste-visceral pairing session

Minute 5, n.s.			
	Na	Sham-Li	IC ₂ +IC ₃ -Li
Na		0.8825	0.4802
Sham-Li			0.2828
Minute 15, q* = 0.0333			
	Na	Sham-Li	IC ₂ +IC ₃ -Li
Na		0.0011+	0.7093
Sham-Li			0.0104+
Minute 25, q* = 0.0167			
	Na	Sham-Li	IC ₂ +IC ₃ -Li
Na		<0.0001+	0.2555
Sham-Li			0.1021
Minute 35, q* = 0.0333			
	Na	Sham-Li	IC ₂ +IC ₃ -Li
Na		0.0004+	0.008+
Sham-Li			0.4498

Corresponds to Figure 4A. Significance level was adjusted based on Benjamini-Hochberg false discovery rate for multiple comparisons (q*; Benjamini and Hochberg, 1995). Values with a plus symbol (+) are statistically significant after correction. n.s. = none significant. IC, Insular cortex; Li, Lithium chloride; Na, Sodium chloride.

Table 4: Comparison of aversive TR scores between groups at select time points across the taste-visceral pairing session

Minute 5, n.s.			
	Na	Sham-Li	IC ₂ +IC ₃ -Li
Na		0.1748	0.3259
Sham-Li			>0.9999
Minute 15, q* = 0.0167			
	Na	Sham-Li	IC ₂ +IC ₃ -Li
Na		0.0021+	0.3845
Sham-Li			0.0446
Minute 25, q* = 0.05			
	Na	Sham-Li	IC ₂ +IC ₃ -Li
Na		<0.0001+	0.0146+
Sham-Li			0.0385+
Minute 35, q* = 0.0167			
	Na	Sham-Li	IC ₂ +IC ₃ -Li
Na		0.0007+	0.0501
Sham-Li			0.0689

Corresponds with Figure 4B. Significance level was adjusted based on Benjamini-Hochberg false discovery rate for multiple comparisons (q*; Benjamini and Hochberg, 1995). Values with a plus symbol (+) are statistically significant after correction. n.s. = none significant. IC, Insular cortex; Li, Lithium chloride; Na, Sodium chloride.

(including IC, D, V, claustrum and the region medial to claustrum) and/or lesion in specific parts of IC were related to behavioral performance (robust Z-score). All LiCl-injected rats with lesion in IC₂, IC₃, and IC₂+IC₃ were included in these correlational tests and no correction procedure for multiple comparison was applied.

Results

Lesion and groups

Only rats that had at least 50% bilateral lesion of IC₂ and IC₃ were included in the main group-wise analyses and compared with both the histologically verified Sham controls injected with LiCl and the group injected with NaCl during the initial taste-visceral pairing session. Group sample sizes are included in Table 1. Group-wise lesion topographic maps are shown in Figure 3D. Representative IC₂+IC₃, IC₂, IC₃ lesion maps and photomicrographs are found in Extended Data Figures 3-1, 3-2, 3-3.

Table 5: Comparison of total ingestive or aversive TR score between groups for the taste-visceral pairing session

Total ingestive score, q* = 0.0167			
	Na	Sham-Li	IC ₂ +IC ₃ -Li
Na		0.0002+	0.2555
Sham-Li			0.0350
Total aversive score, q* = 0.0167			
	Na	Sham-Li	IC ₂ +IC ₃ -Li
Na		0.001+	0.0479
Sham-Li			0.0590

Corresponds to Figure 4C,D. Significance level was adjusted based on Benjamini-Hochberg false discovery rate for multiple comparisons (q*; Benjamini and Hochberg, 1995). Values with a plus symbol (+) are statistically significant after correction. IC, Insular cortex; Li, Lithium chloride; Na, Sodium chloride.

Table 6: Correlation between lesion size or proportion lesion and performance in LiCl-injected rats

		Correlation statistics					
		Taste-visceral pairing		TR retention		Two-bottle test	
		Total ING	Total AVS	Total ING	Total AVS	24 h	48 h
Overall lesion	<i>r</i>	0.3857	−0.4145	0.07662	−0.05735	0.4613	0.3495
	<i>p</i>	0.0842	0.0618	0.7413	0.8050	0.0307*	0.1108
IC ₂ +IC ₃	<i>r</i>	0.3143	−0.4770	0.1364	−0.2274	0.5528	0.4218
	<i>p</i>	0.1653	0.0288*	0.5556	0.3214	0.0076**	0.0506
IC ₂	<i>r</i>	0.3655	−0.3776	0.1094	−0.1043	0.4394	0.3596
	<i>p</i>	0.1033	0.0915	0.6367	0.6528	0.0407*	0.1003
Anterior half of IC ₂	<i>r</i>	0.3425	−0.3480	0.1239	−0.1358	0.3628	0.2455
	<i>p</i>	0.1286	0.1222	0.5925	0.5571	0.0970	0.2709
Posterior half of IC ₂	<i>r</i>	0.2990	−0.4456	0.1368	−0.1984	0.4994	0.3845
	<i>p</i>	0.1879	0.0429*	0.5543	0.3885	0.0180*	0.0773
IC ₃	<i>r</i>	0.1786	−0.3077	0.05716	−0.1356	0.3716	0.2926
	<i>p</i>	0.4385	0.1748	0.8056	0.5578	0.0886	0.1864
Anterior half of IC ₃	<i>r</i>	0.1700	−0.3164	0.07296	−0.2073	0.4604	0.3777
	<i>p</i>	0.4612	0.1622	0.7533	0.3673	0.0311*	0.0831
Posterior half of IC ₃	<i>r</i>	−0.01301	−0.07670	−0.08203	0.06534	0.1081	0.09451
	<i>p</i>	0.9554	0.7411	0.7237	0.7784	0.6321	0.6757

Spearman's rank-order correlation was conducted (*r*: Spearman's correlation coefficient, *p*: statistical significance); *p* values with an asterisk (*) indicate statistically significant correlations. IC, Insular cortex; Ing, Ingestive; Avs, Aversive.

Behavioral data from rats with $\geq 50\%$ bilateral lesion in IC₂ or IC₃ are shown in Extended Data Figures 4-2 and 4-3 for comparison but were not included in the overall group-wise analyses because of the limited sample sizes.

Behavior

Extensive lesions in IC₂+IC₃ disrupted taste-guided oromotor and somatic adaptive responding to LiCl-induced malaise

First, we asked whether rats with extensive IC₂+IC₃ lesions capably alter TR and somatic behaviors elicited to the CS (IO sucrose infusions) as LiCl takes effect in an initial CS-US pairing session. At 5 min after the initial intraperitoneal injection of LiCl or NaCl, all groups displayed comparably high levels of ingestive TR, with virtually no aversive responses (Fig. 4A,B). Whereas the NaCl-injected group sustained high levels of ingestive responding and low levels of aversive responding across the entire

CS-US pairing session, the LiCl-injected Sham group reduced its ingestive responses and increased its aversive responses at minutes 25 and 15, respectively, compared with the minute 5 baseline levels. On the other hand, the LiCl-injected IC lesion group was slower to diverge from baseline (Fig. 4A,B; Table 2). Group-wise analyses further showed that by minute 15 and throughout the remainder of the session LiCl-injected Sham rats significantly reduced their ingestive responses and increased aversive responses to sucrose relative to the NaCl-injected group. The LiCl-injected IC lesion group failed to alter their ingestive and aversive response patterns at the key inflection points observed for the LiCl-injected Sham group (minutes 25 and 15, respectively; Tables 3, 4). The LiCl-injected IC lesion group suppressed its ingestive responses and increased its aversive responses later in the session (at minutes 35 and 25, respectively) relative to the NaCl-injected group (Fig. 4A,B; Tables 3, 4). When scores were summed across all four IO infusion time points, the LiCl-

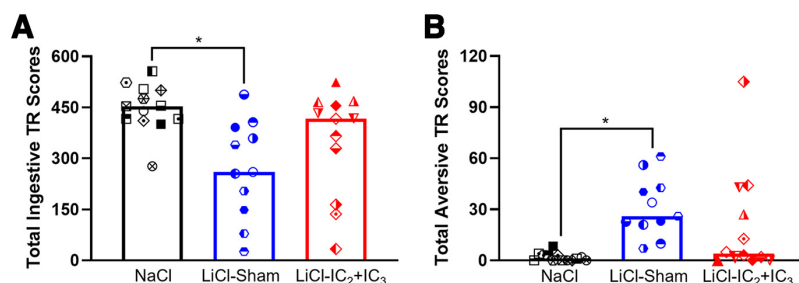


Figure 5. Lesions in IC₂ and IC₃ attenuated, but did not completely prevent, conditioned taste aversion on the TR retention test. Median cumulative ingestive (A) and aversive (B) TR scores in response to 30-s IO sucrose infusions during retention are plotted with different symbols for individual animals in NaCl-injected group (*n*=13), LiCl-injected sham-operated control group (*n*=11), and LiCl-injected IC₂+IC₃ lesion group (*n*=12). Statistical significances are adjusted based on Benjamini–Hochberg false discovery rate for multiple comparisons and indicated as asterisks. Statistics are in Table 7. Ingestive and aversive TR scores in this retention test are plotted as a function of time following the intraperitoneal injection of either NaCl or LiCl in Extended Data Figures 5-1, 5-2, and 5-3. IC, Insular cortex; TR, Taste reactivity; LiCl, Lithium chloride; NaCl, Sodium chloride.

Table 7: Comparison of total ingestive or aversive TR score between groups on the retention test

Total ingestive score, $q^* = 0.0167$		
Na	Sham-Li	IC ₂ +IC ₃ -Li
Na	0.0004+	0.1728
Sham-Li		0.1321
Total aversive score, $q^* = 0.0167$		
Na	Sham-Li	IC ₂ +IC ₃ -Li
Na	<0.0001+	0.0712
Sham-Li		0.0764

Corresponds to Figure 5A,B. Significance level was adjusted based on Benjamini–Hochberg false discovery rate for multiple comparisons (q^* ; Benjamini and Hochberg, 1995). Values with a plus symbol (+) are statistically significant after correction. IC, Insular cortex; Li, Lithium chloride; Na, Sodium chloride.

injected Sham group displayed significantly fewer ingestive and more aversive responses than the NaCl-injected group (Fig. 4C,D; Table 5). The LiCl IC₂+IC₃ group exhibited an intermediate level of cumulative ingestive and aversive TR, which were not different from the NaCl group or the LiCl Sham group (Fig. 4C,D; Table 5). Because there was considerable behavioral variability within each group on this CS-US pairing session (see Fig. 4C,D), Spearman’s rank-order correlations were conducted to determine whether total lesion size or lesion size within IC subregions of interest were associated with a failure to adapt TR in response to LiCl injections. These analyses showed that more extensive neuronal loss in IC₂+IC₃, and more specifically within the posterior half of IC₂, was linked to a more tempered display of aversive reactivity (Table 6). Extended Data Figures 4-2A and 4-3A show the comparative performance of small samples of LiCl-injected rats with lesions localized to either IC₂ or IC₃ on this taste-visceral pairing session.

Extensive lesions in IC₂+IC₃ attenuate the subsequent avoidance of a substance associated with LiCl

TR test. Next, we assessed whether rats with IC₂+IC₃ lesions who previously received IO sucrose paired with LiCl acquired conditioned responses to the orosensory CS. To do this, all rats were tested for their taste-guided

responses to IO sucrose starting 20 min after an intraperitoneal injection of LiCl. This design was based on a prior study that showed rats do not acquire an aversion to an IO-infused stimulus if the negative visceral state precedes the first IO infusion by at least 20 min (Spector et al., 1988). In other words, rats only acquire a CTAver when the IO infusate is experienced before and/or during the onset of the LiCl-induced malaise. Second, this same study found that even neurally-intact rats display a relatively weak conditioned aversion to IO sucrose after the single serial TR pairing session. But, if the conditioned rats are put back into the negative visceral state produced by LiCl (by injecting LiCl 20 min before IO infusion), then aversive responding is significantly bolstered. Therefore, here, all rats were primed with LiCl 20 min before the IO infusions to determine whether the LiCl-IC₂+IC₃ lesion group displayed weaker aversive responses than the LiCl-Sham group on the postpairing test. Indeed, the Sham-operated rats previously treated with LiCl maintained significantly reduced total ingestive response and increased total aversive response to IO sucrose, as compared with the NaCl-injected control rats (Fig. 5A,B; Table 7), showing that the previous sucrose-LiCl pairing yielded a learned taste aversion. The LiCl-injected group with IC₂+IC₃ lesions, on the other hand, displayed high total ingestive and low total aversive TR to IO sucrose, which did not statistically differ from that of the NaCl-treated control rats or the LiCl-treated Sham rats. In fact, whereas the LiCl-injected Sham rats displayed consistent reductions in ingestive TR and increases in aversive TR across the retention test, the LiCl IC₂+IC₃ rats only showed significant reduction in ingestive TR at minute 30 (Extended Data Fig. 5-1). We noted that a subset of animals in LiCl IC₂+IC₃ lesion group appeared to be normal in terms of shifting their taste responses to sucrose highlighting the behavioral heterogeneity within this group. Yet, neither total lesion size nor the extent of lesion within any of the IC subregions of interest analyzed was found to be associated with TR on this retention test (Table 6).

Two-bottle avoidance test. Next, all rats were tested for their avoidance of sucrose in an *ad libitum* two-bottle

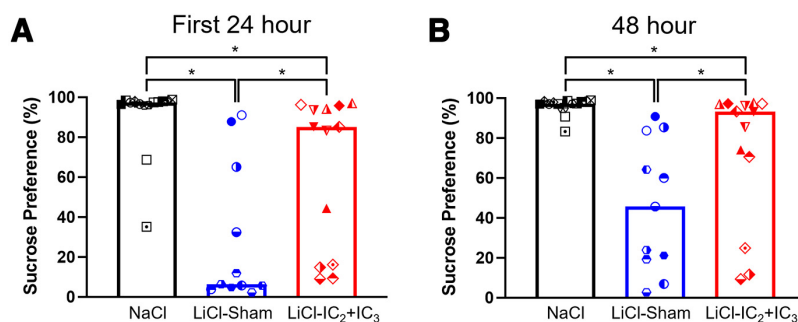


Figure 6. Lesions in IC₂ and IC₃ impaired CTAvoid on the two-bottle choice retention test. Median percent sucrose preference over water during the first 24-h (A) or total 48-h (B) two-bottle choice test are plotted with different symbols for individual animals in NaCl-injected group ($n = 14$), LiCl-injected sham-operated control group ($n = 11$) and LiCl-injected IC₂+IC₃ lesion group ($n = 13$). Statistical significances are adjusted based on Benjamini–Hochberg false discovery rate for multiple comparisons and indicated as asterisks. Statistics are on Table 8. Median values of sucrose intake (g) during the first 24 or 48 h are plotted in Extended Data Figure 6-1, with statistics in Extended Data Figure 6-3. Two subgroups of LiCl-injected IC₂+IC₃ group with the greatest and the least impairment were compared for their lesion sites in Extended Data Figure 6-2. IC, Insular cortex; TR, Taste reactivity; LiCl, Lithium chloride; NaCl, Sodium chloride.

Table 8: Comparison of sucrose preference between groups on the two-bottle choice test

First 24 h, $q^* = 0.05$			
	Na	Sham-Li	IC ₂ +IC ₃ -Li
Na		<0.0001+	0.0005+
Sham-Li			0.0088+
Full 48 h, $q^* = 0.05$			
	Na	Sham-Li	IC ₂ +IC ₃ -Li
Na		<0.0001+	0.0045+
Sham-Li			0.0183+

Corresponds to Figure 6. Significance level was adjusted based on Benjamini–Hochberg false discovery rate for multiple comparisons (q^* ; Benjamini and Hochberg, 1995). Values with a plus symbol (+) are statistically significant after correction.

choice test (vs water) in the home cage. In the first 24 h, the NaCl-injected control group preferred sucrose to water, whereas the LiCl-injected Sham group largely avoided sucrose (Fig. 6A; Table 8; Extended Data Fig. 6-1A). During the first 24 h and across the 48-h test, the LiCl-injected rats with IC₂+IC₃ lesions actually preferred sucrose, with a preference score that was significantly higher than that of their Sham counterparts. Similar patterns were observed over the full 48 h of the test (Fig. 6B; Table 8; Extended Data Figs. 6-1B, 6-3). Overall lesion size was positively correlated with 24-h sucrose preference, and this appeared to be mainly because of the extent of neuronal loss across IC₂ and IC₃, especially in the posterior IC₂ and anterior IC₃ subregions. Conditioned sucrose avoidance of LiCl-treated IC₂ or IC₃ groups is shown for comparison in Extended Data Figures 4-2D and 4-3D.

Individual rats with IC₂+IC₃ lesions that exhibit impaired avoidance generally display impairments on the prior measures of taste aversion

To examine behavioral patterns across the taste-visceral pairing and retention tests of individual rats, group median performance across each phase is displayed in Figure 7, left column, with the standardized behavioral scores of individual LiCl-treated rats in Sham or IC₂+IC₃ lesion groups in Figure 7, middle and right-hand columns. The rats in each group were sorted according to their robust Z-score on the first 24-h two-bottle test (high/most impaired to low/least impaired) and plotted accordingly (left to right) for each behavioral measure. The LiCl IC₂+IC₃ rats that were significantly impaired on the two-bottle test tended to be impaired on earlier measures including TR on the pairing and retention tests (Fig. 7, right column), while no such systematic pattern was seen in the LiCl Sham group (Fig. 7, middle column).

Discussion

Schier et al. (2014) showed that the posterior part of IC, encompassing the posterior gustatory zone and the anterior viscerosensory zone (IC₂+IC₃), is required to properly avoid solutions previously paired with the emetic agent, LiCl. In particular, the deficit was most consistently found in the rats that had lesions spanning the entire IC₂ region (Schier et al., 2016). The precise process(es) disrupted by

posterior IC lesions to produce such a deficit, however, remained unclear. The present findings showed that, by and large, lesions in the IC₂+IC₃ region interfered with the ability to rapidly express changes in taste-guided behaviors to sucrose in response to the coincident aversive effects of LiCl. This, in turn, generally weakened the ability to later avoid the sugar in the two-bottle choice test conducted over 24 and 48 h in the home cage. Taken together, these results provide novel evidence that the IC₂+IC₃ region contributes to isochronous adjustments to taste-guided behaviors according to the change in visceral state, which may contribute to subsequent deficits in CTAvoid.

The roles of IC₂+IC₃ in conditioned taste aversion

The failure to express a conditioned taste aversion/avoidance could be related to deficits in primary sensory processing, including for the taste CS and/or interoceptive US, integrative processing, memory, executing a response or some combination thereof (Fig. 1; Spector et al., 1988). The fact that lesions in IC₂+IC₃ generally perturbed the ability to progressively shift behavioral responses in accord with the emergence of a negative interoceptive state in the serial TR test, which has minimal, if any, memorial demands narrows the search for mechanism to the processes at work during the initial taste-visceral pairing session. Prior work showed that rats with lesions encroaching IC₂ are not ageusic to suprathreshold concentrations of sucrose and show normal unconditioned oromotor and somatic responses to sucrose, though whether more posterior lesions would produce taste deficits in these contexts is unknown (Bales et al., 2015; King et al., 2015). Thus, three main possibilities remain. The delay to suppress ingestive TR and/or express aversive TR after the initial intraperitoneal injection of LiCl could reflect either a blunted sensitivity to the visceral stimulus or a failure to integrate the two sources of sensory input to make the adaptive responses. Prior studies found that rats with bilateral lesions in IC₂ and/or IC₃ were slower to acquire a learned taste avoidance than the LiCl-injected Sham and Unilateral lesion controls (Schier et al., 2014, 2016). Although the animals with these lesions did ultimately acquire learned taste avoidance in a short-term intake test after two stimulus pairings, it was tenuous at best for a subset of rats with extensive IC₂ lesions (Schier et al., 2016). That is, after demonstrating competence in avoiding the CS in short (15 min) single bottle test, these rats ultimately failed to avoid consuming the CS in a longer, 48-h, two-bottle test. The slower initial learning and the expedited extinction could stem from diminished interoceptive and/or CS-US integrative processing. That being said, Schier et al. (2014, 2016) also found that lesions in this IC region interfered with the ability to avoid a solution that was associated with LiCl before the lesion. Thus, at the very least, the contribution of this region to CTAvoid is not limited to real time viscerosensory and/or integrative processes.

A parsimonious possibility is that this region is involved in expressing adaptive taste-guided behaviors in the context of ongoing interoceptive and/or associated events.

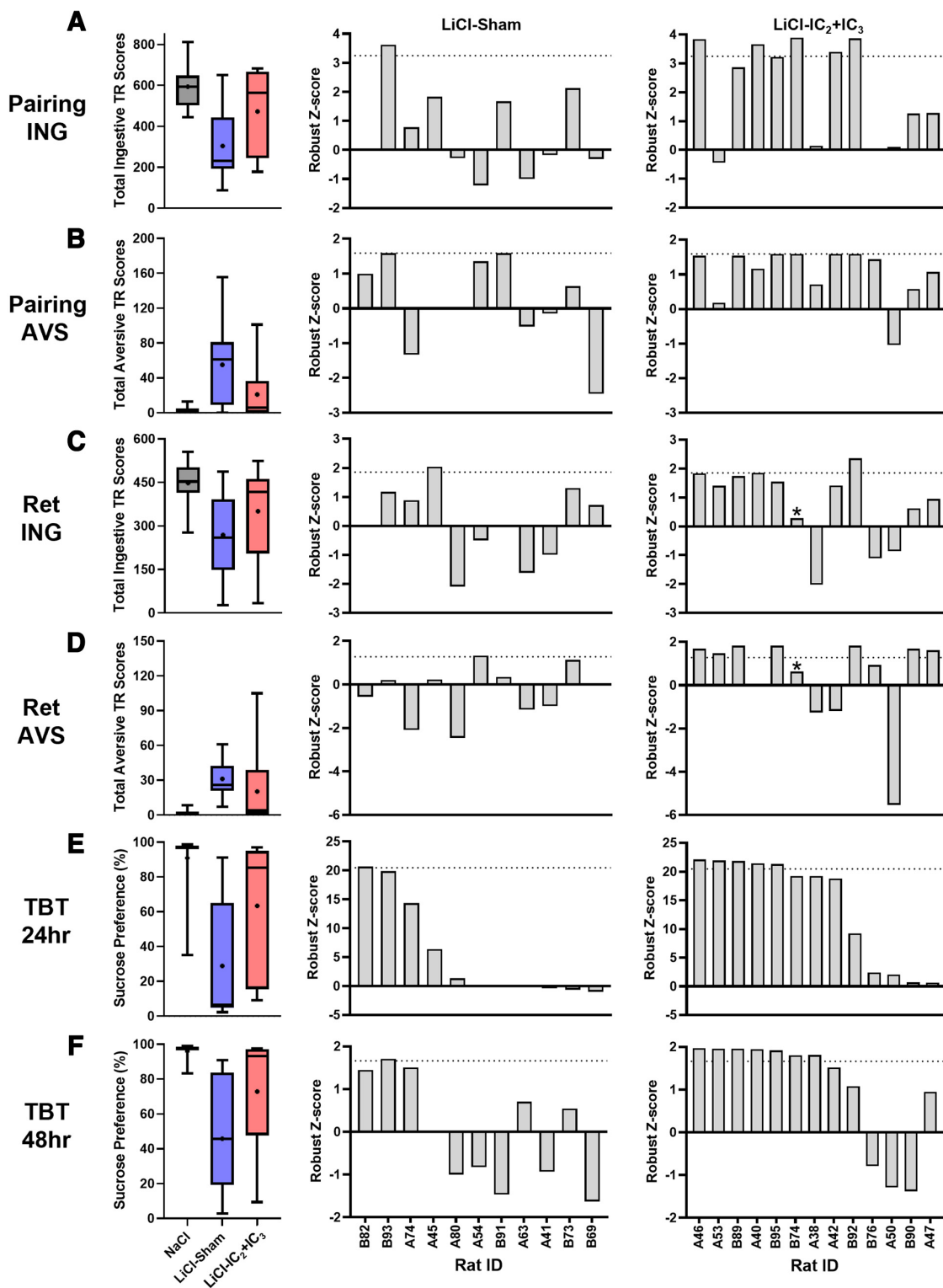


Figure 7. Group performance comparison and standardized behavior scores of individual animals in LiCl-given groups. Group-wise comparison (left) and individual animals' performance in LiCl-sham group (middle) and LiCl-IC₂+IC₃ group (right) for total ingestive (A) and aversive TR (B) in the stimulus pairing session, total ingestive (C) and aversive TR (D) in retention and sucrose preference during the first 24 h (E) and 48 h (F) in two-bottle test. Left column, Box and whisker plots of TR scores or sucrose preference scores for all NaCl-injected or LiCl-injected groups included in the statistical analyses on each of the main behavioral measures (top to bottom). The + within each box represents the group mean. Each group is labeled at the bottom of F. Middle and right columns, The animals are arranged in order of high to low sucrose preference in 24-h two-bottle test and the robust Z scores of individual

continued

animals in each test are plotted to visualize their performance. Their IDs are denoted on the *x*-axis at the bottom of **F**: the IDs of animals in phase 1 start with “A” and the animals in phase 2 start with “B.” For aversive TRs (**B**, **D**), the signs of Z scores are reversed so that the positive values indicate more impairment in their performance. Asterisk above the bar indicates incomplete data. Ret, retention; TBT, two-bottle test; ING, ingestive TR; AVS, aversive TR. In Extended Data Figure 7-1, LiCl-given animals with IC₂ or IC₃ lesion are included and standardized behavior scores of all LiCl-given lesion animals are plotted. IC, Insular cortex; TR, Taste reactivity; LiCl, Lithium chloride; NaCl, Sodium chloride.

The decision to consume or not to consume a particular substance involves many factors, such as weighing the cost-benefit of that action against the current physiological state, other competing drives, other resources, the food's conditioned/unconditioned reward value, and the predicted consequences of its consumption. The types of deficits associated with loss of function in IC₂+IC₃ region could stem from a weakened ability to flexibly switch hardwired, unconditioned taste-guided behaviors in light of discrepant physiological or experiential sources of information. A recent study (Livneh et al., 2020) found that the activity of some IC neurons reflects not only the current physiological status like water or energy depletion, but corresponds to the expected consequences of cues associated with water or food. This supports the hypothesis that insular cortical neurons might be involved in integrative function of current status with cue-predicted outcomes. It is possible that the lesions encompassing IC₂+IC₃ interrupted such function of IC, which led to impaired performance in the present study in terms of the ability to shift toward rejective responses under the influence of the LiCl injection. Such a deficit could also explain why, in the previous studies, animals with lesions in the IC₂ region were unable to avoid a solution that was associated with LiCl before the lesion (Schier et al., 2014, 2016). It will be important to determine whether such a role is specific to LiCl-based conditioned taste aversion or it extends to other types of ingestive behaviors or indeed other types of USs (Geddes et al., 2008; Schier et al., 2019). After all, associative pairings are known to render taste-responsive neurons in the GC responsive to the predictive stimulus and silencing GC during the decision phase of a taste-guided two response operant choice task, but not during the taste stimulus itself, significantly disrupted correct response selection (Vincis and Fontanini, 2016; Vincis et al., 2020).

The association of a taste stimulus with LiCl-induced malaise can cultivate two different types of learned responses: conditioned taste aversion and/or avoidance (Pelchat et al., 1983; Parker, 2003; Schier et al., 2019; Schier and Spector, 2019). Because previous studies examining the role of posterior IC in CTA used intake measurements, it had remained unclear whether the lesion-induced impairments were related to a diminished ability to avoid consuming a taste solution that was associated with LiCl (appetitive or goal-directed deficit) or a failure to alter taste-guided oromotor and somatic repertoires (consummatory deficit). In general, rats with IC₂+IC₃ lesions in the present study showed deficits in both domains. The fact that these rats with cortical lesions displayed a diminished capacity to shift their taste-guided reactions in the initial taste-LiCl pairing

session suggests that this region of IC may play an important role in acutely revising responses linked to hedonic evaluation. One question that still remains is whether the failure to avoid the taste CS in the two-bottle test was because of this consummatory deficit or whether there were additional appetitive deficits produced by the lesion. The types of behavioral deficits observed here generally recapitulate those observed following lesions of the gustatory zone of the parabrachial nucleus (gPBN) using a similar paradigm (Spector et al., 1992; Reilly et al., 1993; Grigson et al., 1998). Provided gPBN sends projections to IC via the ventroposteromedial nucleus of the thalamus, it seems likely that these two regions form a key circuit underlying taste-interoceptive integration. Future work targeting these gPBN to IC projections in a specific manner will be illuminative. Moreover, given previous studies have shown impaired avoidance of a tastant that was paired with LiCl before the lesion, future studies should investigate whether IC₂+IC₃ is required to express taste-guided consummatory changes under these conditions.

Sources of variability

There was a considerable amount of behavioral variability, even within our LiCl-injected Sham group, in terms of the extent to which sucrose-elicited TR was shifted or sucrose preference was blunted in the present study. The serial TR paradigm used here was designed to allow us to observe ongoing changes in taste-guided behaviors in response to the gradual onset of a LiCl-induced toxicosis. The trade-off is that some of these task parameters, like limiting taste stimulation to ten ~30-s presentations, using a submaximal LiCl dose, providing only a single taste-visceral pairing session, and shifting interoceptive and physical contexts between conditioning and testing, would be expected to curtail associative learning or sustained expression of that learning. Nevertheless, on a group-wide basis, the approach revealed significant differences among rats with IC₂+IC₃ lesions and the Sham controls.

Even within our group of rats with confirmed extensive lesion in both IC₂ and IC₃, we had a subset that was severely impaired and a subset that was essentially unaffected by the lesion. In general, this did not appear to be test-dependent. In other words, the LiCl-injected rats with IC₂+IC₃ lesions that were deemed impaired in their ingestive or aversive TR in the initial pairing session were more or less impaired on the remaining test sessions (Fig. 7, right column). This overall effect was even more pronounced when the behavioral data of LiCl-treated rats with lesions only in IC₂ or IC₃ are also considered (Extended Data Fig. 7-1). Nevertheless, lesion size and/

or topography may contribute to the variability in deficits. Schier et al. (2016) noted that the “lesion hotspot” was most commonly found in IC₂. Interestingly, we had a subset of rats in the present study that had massive lesions in IC₂, but these rats were not overtly impaired on any of our measures. Moreover, we had a subset of rats with extensive lesion in IC₂ only, and although the small sample size precluded statistical comparisons, qualitatively speaking this group appeared competent on these various behavioral measures (Extended Data Fig. 4-2), as did rats with extensive lesions limited to IC₃ (Extended Data Fig. 4-3). Correlational analyses (Table 6) indicated that the proportion of IC₂+IC₃ and posterior IC₂ with lesion were significantly negatively correlated with both aversive TR in the initial pairing session and sucrose preference in the 24-h two-bottle retention test. The proportion of lesion in anterior IC₃ was significantly negatively related to sucrose preference in the 24-h two-bottle retention test. Lesion size in anterior IC₂ or posterior IC₃ was not significantly correlated with any behavioral performance. In addition, we compared the lesion sites of two subgroups from the LiCl-IC₂+IC₃ group showing the greatest impairment (“Top 5”) and the least impairment (“Bottom 4”) in the 24-h two-bottle avoidance test. While they both had a full lesion in IC₂-IC₃ border, we noticed that “Top 5” had more complete lesion in IC₂ compared with the “Bottom 4” (Extended Data Fig. 6-2), perhaps especially in GI and AI. Therefore, we conclude that, in general, extensive lesion in IC₂, including the posterior IC₂ and IC₂-IC₃ border, is required to induce the functional deficits we observed. We cannot exclude the possibility that there are simply individual differences in the functional topography of the IC such that a lesion in the same anatomic location interferes with adaptive taste-guided responding in one rat, but not in another. More studies are needed to refine the specific area, neuronal subtypes, and/or extent of lesion in IC sufficient to disrupt CTA and related processes.

In the present study, loss of function in the posterior IC (IC₂+IC₃) severely disrupted the ability to modify taste-guided oromotor and somatic reactions in the face of an ongoing change in visceral state produced by LiCl and resulted in an impaired ability to avoid the associated taste stimulus in subsequent retention test. The results provide new insights into the neural organization of taste-visceral integrative processes that underlie conditioned taste aversion and avoidance.

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