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Ondansetron blocks LiCl-induced conditioned place avoidance but not conditioned taste/flavor avoidance in rats

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Abstract

The ability of an experimental agent to support conditioned taste/flavor avoidance (CT/FA) in rats often is interpreted as sufficient evidence that the agent produced a state of malaise or nausea. Paradoxically, however, CT/FA also is induced by certain drugs that support conditioned preferences in rats, suggesting that CT/FA is insufficient to reveal a negative hedonic state. The present study tested the hypothesis that the anti-nausea drug ondansetron (OND) would block the ability of nauseogenic lithium chloride (LiCl) to support conditioned place avoidance (CPA), without attenuating LiCl-induced CT/FA. After pre-treatment with either OND or vehicle, rats were conditioned with i.p. injection of 0.15M LiCl containing 2% saccharin (LiCl+sac) on conditioning day 1, and with 0.15M NaCl alone on conditioning day 2. Rats were confined to a distinct chamber of a CPA apparatus after each conditioning injection. In other rats, OND or vehicle pre-treatment was followed by NaCl+sac on conditioning day 1, and LiCl alone on day 2. Subsequent testing revealed that OND blocked the ability of LiCl to support CPA. Conversely, in the same rats, OND did not alter the ability of LiCl to condition avoidance of 0.2% sac solution during a 60 min bottle test. In a separate experiment, a sensitive 2-bottle choice test was used to confirm that OND pretreatment does not reduce the ability of LiCl to support CT/FA. These results support the view that CPA is an additional useful tool to reveal the experience of malaise and nausea in rats, whereas CT/FA demonstrated in bottle intake tests is insufficient for this purpose.

Indexing terms

lithium chloride; ondansetron; nausea; malaise; conditioned avoidance

INTRODUCTION

Visceral malaise is a clinically relevant, multidimensional sensory experience with negative affective components, and lithium chloride (LiCl) is widely used in experimental settings for studying its central neural underpinnings. Systemically administered LiCl promotes nausea and vomiting in humans and other emetic species [1], and supports conditioned oral rejection responses (i.e., gaping) that are stereotypical hallmark signs of nausea in rats and mice, as in other non-emetic and emetic species [2–4].

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In addition to oral rejection responses, LiCl supports robust conditioned taste/food avoidance (CT/FA) in rodents [5]. CT/FA paradigms typically involve conditioning trials in which dehydrated rats or mice voluntarily consume water containing a novel taste/food (conditioned stimulus, CS) from a bottle shortly before a test or control agent (unconditioned stimulus, US) is administered. Subsequent CT/FA testing involves single-bottle intake tests or 2-bottle choice tests to assess the presence or absence of conditioned avoidance of the taste/food CS. Such experimental paradigms are relatively simple to execute, and are widely used in academic and commercial research settings.

A demonstration of CT/FA often is interpreted as sufficient evidence that the US drug or agent in question has nauseogenic and, therefore, aversive properties. However, several groups have reported the paradoxical finding that certain drugs that support appetitive behavior and conditioned place preference (e.g., morphine, cocaine) also support CT/FA [6–9]. Tellingly, although the anti-emetic, anti-nauseogenic 5-HT₃ receptor antagonist ondansetron (OND) significantly attenuates the ability of LiCl to elicit conditioned oral rejection responses in rats, OND does not reduce conditioned avoidance of LiCl-paired tastes or flavors in bottle intake tests [3,6,10,11]. These findings challenge the widely-held view that CT/FA paradigms involving bottle intake tests are sufficient to reveal nauseogenic properties of experimental agents.

In addition to its robust ability to induce conditioned oral rejection responses, LiCl supports conditioned place avoidance (CPA) [3,11–13], a sensitive and widely accepted measure of aversive or negative hedonic state in rodents [14]. For many researchers, the use of CPA paradigms to assess the nauseogenic properties of experimental drugs could be a practical alternative to the more complex assessment of gaping and other behaviors that characterize conditioned oral rejection responses. To our knowledge, however, no one has reported whether anti-nausea treatments such as OND are able to block or attenuate the ability of LiCl to support CPA, which would reinforce the view that CPA can be used as an additional behavioral test to index nausea and malaise in rodents. The present study was designed to test the hypothesis that OND can attenuate or block the ability of LiCl to support CPA, without attenuating the ability of LiCl to support CT/FA.

MATERIALS AND METHODS

All procedures conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and were approved by the University of Pittsburgh Animal Care and Use Committee. Data from 39 adult male Sprague-Dawley rats (Harlan Laboratories; 230–310 g BW) are included in this report. Rats were singly housed in hanging wire cages, with ad libitum access to water and pelleted rat chow (Purina, St. Louis, MO) unless otherwise noted. Colony rooms were maintained at 22–23°C and kept on a 12:12 hr light-dark cycle, with lights on from 0700–1900 hr.

The first experiment was designed to simultaneously condition place and taste avoidance in the same rats via a single dose of LiCl, and to determine whether the anti-nauseogenic drug OND interferes with the ability of LiCl to condition either avoidance response. For this purpose, we used a moderate dose of LiCl (0.15M, 1% BW volume) that has been reported to support robust CPA [12] and CT/FA [15] in rats. We used a rather unusual approach to pair the US (i.e., LiCl or NaCl) with a taste CS, based on the concept of “intravascular taste” [16,17]. For this purpose, the US was administered i.p. in a solution containing 2% saccharin (sac) in a 1% BW volume, similar to a previous report [17]. We reasoned that sac absorbed into the bloodstream after i.p. injection would access and stimulate oral taste receptors to provide a coincident temporal pairing of sac taste with the systemic consequences of the co-administered US. Assuming that sac absorption into the circulation after i.p. injection is gradual and perhaps

incomplete, the 2% injection concentration is consistent with a desired intravascular sac concentration of approximately 0.2% after absorption and dilution within a total blood volume of approximately 17 ml [18]. By using this approach, taste conditioning could occur simultaneously with place conditioning, as described further, below.

Experiment 1: Ability of OND to attenuate LiCl-induced conditioned place and/or taste avoidance

The CPA apparatus comprised a 24" deep rectangular box with a removable pair of central dividing walls. When the walls were removed during pre-conditioning exploration and during the final CPA test, rats had free access to three internal chambers of the box: one 12" × 12" end region with a white floor and 3 black walls, a middle 12" × 6" region with a metal floor and two gray walls, and a second 12" × 12" end region with a black floor and 3 white walls. Rats were confined to one of the two distinct end chambers during conditioning trials, when the central dividing walls were in place. Rats were placed into the CPA apparatus at approximately the same time each day (1000–1200 hr) for pre-conditioning exploration, for conditioning, and for final CPA testing, as detailed below. The apparatus was cleaned with a mild odor-neutralizing cleanser and dried thoroughly between rats.

Initial Exploration—Each rat was removed from its home cage in the colony room, carried in a transfer tub to the adjacent testing room, and then placed into the center of the open CPA apparatus. The experimenter then left the room. After initial exploratory behavior was videotaped for 5 min, the experimenter re-entered the room, retrieved the rat from the apparatus, and returned it to its home cage. Videotapes were later scored to determine the amount of time spent by each rat within each of the 3 CPA regions. Data collected during this initial exploration period indicated that the group of rats used in this study (n=24) spent approximately 15% more time within the end chamber that had black walls compared to the end chamber with white walls (see Results). Thus, we used a biased conditioning procedure, such that LiCl injections always were paired with the black-walled chamber during subsequent CPA conditioning, whereas NaCl injections were paired with the white-walled chamber. The smaller central area was "neutral", in that rats had access to it only during the initial exploration and final CPA testing phases.

Simultaneous CPA and CTA Conditioning—As summarized in Table 1, each rat received a pre-treatment and a treatment injection on each of two conditioning days that were spaced 2–3 days apart. On conditioning day 1, rats were pre-treated by i.p. injection of either OND (0.2 mg/kg BW, 1.0 ml; groups 1 and 3) or 0.15M NaCl vehicle (1.0 ml; groups 2 and 4), and then returned to their home cage. Thirty min later, rats were treated by i.p. injection (1% BW) of either 0.15M LiCl containing 2% sac (LiCl+sac; groups 1 and 2), or 0.15M NaCl +sac (groups 3 and 4), and then immediately placed into a confined end chamber of the CPA apparatus. After 60 min in the chamber, rats were returned to their home cage. On conditioning day 2, rats received the same pre-treatment injection as on day 1, then were injected 30 min later with either NaCl alone (groups 1 and 2) or LiCl alone (groups 3 and 4) before being placed into the opposite confined end chamber of the CPA apparatus for 60 min.

CPA Testing—Two to three days after the second conditioning session, rats were placed individually into the open CPA apparatus (i.e., center walls removed) and allowed to explore for 5 minutes, with their behavior videotaped for later analysis of time spent within each of the three chambers. All videotapes were scored by a single researcher who was blind to experimental conditions. For each rat, the total time (sec) spent within each end chamber during the 5 min CPA test was expressed as a percentage of the time spent within each chamber by the same rat during the pre-conditioning exploration period. These two values (i.e., one for the saline-paired chamber and one for the LiCl-paired chamber) were used to compute a

“difference score” for each rat. Difference scores were analyzed by two-way ANOVA, with pre-treatment injection (OND vs. vehicle) and saccharin pairing condition (LiCl+sac vs. NaCl+sac) as independent factors.

CTA testing—Two or three days after CPA testing, rats were assessed for their voluntary intake of sac (0.2% in water) in a 60 min single bottle access test. Each rat’s water bottle was removed from their home cage for 8 hrs during the light period, beginning at 0900 hr. A similar bottle containing sac solution was returned to the cage at 1700 hr. Bottles were weighed at the beginning and end of the 60 min access period to determine the volume of sac solution consumed (i.e., 1.0 g = 1.0 ml). Results were analyzed by two-way ANOVA, with pre-treatment injection (OND vs. vehicle) and saccharin pairing condition (LiCl+sac vs. NaCl+sac) as independent factors.

Experiment 2: Ability of OND to attenuate LiCl-induced conditioned flavor avoidance in a 2-bottle choice test

A sensitive 2-bottle choice paradigm [19] was used in a new group of rats (n=15) to further test the hypothesis that nausea and malaise are unnecessary to support conditioned avoidance of tastes/flavors previously paired with LiCl treatment. Flavor exposure during CT/FA training and testing was conducted near the end of the light cycle of the photoperiod, between 1600 and 1800 hr.

Conditioning day 1—All rats (n=15) were water deprived for 22 hr, then injected i.p. with 1.0 ml of either 0.15M NaCl vehicle (n=7) or OND (0.2 mg/kg BW; n=8). Half of the rats within each subgroup were then presented with a single bottle of novel almond-flavored tap water to drink, and the others with a single bottle of novel vanilla-flavored water (0.5% McCormick brand almond or vanilla extract). The left-right position of the drinking bottle on each cage was switched after 15 min, with cumulative intake recorded at the 30 min time point. Thirty minutes after the end of this initial single flavor exposure session, each rat was injected i.p. with either 0.15M NaCl or 0.15M LiCl (2% BW). Plain tap water was returned 30 min later, and rats had ad libitum water access for the next 48 hr.

Conditioning day 2—Two days after conditioning day 1, rats were again water deprived for 22 hr. Each rat received the same pre-treatment injection (i.e., NaCl or OND) before flavor exposure as on conditioning day 1, followed by 30 min single bottle access to the alternate novel flavor (i.e., vanilla or almond, depending on which flavor the rat consumed on conditioning day 1). Bottle position on each cage was switched after 15 min and cumulative intake was recorded at 30 min. Thirty minutes after the end of this second single-bottle flavor exposure session, each rat was injected i.p. with the opposite agent received on conditioning day 1 (i.e., 0.15M NaCl or 0.15M LiCl, 2% BW). Plain water was returned 30 min later, and rats had ad libitum water access for 48 hr.

Two-bottle choice test—Two days after conditioning day 2, rats were water deprived for 22 hr, and then given 30 min simultaneous access to two bottles of flavored water. One bottle contained the saline-paired flavor (either almond or vanilla) and the other containing the alternate LiCl-paired flavor. The volume consumed by each rat from each bottle was recorded after 30 min of access, with bottle positions switched at the 15 min time point. Rats then were returned to ad libitum water access.

Multivariate ANOVA was used to test for differences in the volumes of each paired flavor consumed during the 2-bottle choice test. Flavor preference ratios displayed by each rat were averaged within each pre-treatment injection group to obtain group preference ratios (mean $\pm$ SE) for intake of saline-paired vs. LiCl-paired flavors, with or without OND pre-treatment.

Preference ratios close to 1.0 (e.g., 50%:50%) indicate a lack of avoidance of either paired flavor during the test. Outcomes indicating nonequivalent preference ratios (e.g., 3.0, 75%:25%) were interpreted as evidence for conditioned avoidance of the flavor represented by the lower value in the ratio. Flavor preference ratios were analyzed by ANOVA, with pretreatment injection (OND vs. vehicle) as the independent factor.

RESULTS

Experiment 1: Ability of LiCl to support simultaneously condition place and taste avoidance

During 5 min of pre-conditioning exploration, rats ($n=24$) spent approximately 15% more time in the black wall/white floor end chamber ($136.4 \text{ sec} \pm 4.8$) compared to time spent in the opposite white wall/gray floor end chamber ($115.6 \text{ sec} \pm 4.7$). Although this difference was not large, it was significant (paired t-test, $P = 0.03$). Rats spent relatively less time in the smaller central chamber ($48.2 \text{ sec} \pm 3.1$).

Rats that were pre-treated with saline vehicle before conditioning (groups 2 and 4; total $n=12$) subsequently avoided the LiCl-paired chamber during the 5 min CPA test (left side of Fig. 1), whereas such avoidance was absent in OND-pretreated rats (groups 1 and 3; total $n=12$; right side of Fig. 1). Two-way ANOVA confirmed a significant effect of pre-treatment, such that difference scores in vehicle-treated rats (66.54 ± 9.13) were markedly larger than in OND-treated rats [8.19 ± 6.80 ; $F(1,20)=40.7$, $P < 0.001$]. However, there no significant effect of saccharin pairing condition [i.e., LiCl+sac vs. NaCl+sac] on difference scores [$F(1,20)=0.003$, $P = 0.959$], and no significant interaction between pre-treatment agent and saccharin pairing condition [$F(1,20)=0.781$, $P = 0.387$]. Thus, data were combined for OND-pretreated rats and vehicle-pretreated rats for illustration. As shown in Figure 1, vehicle-pretreated rats spent approximately 34% less time in the LiCl-paired chamber during the CPA test compared to time spent there during pre-conditioning exploration, and spent approximately 32% more time in the NaCl-paired chamber. Conversely, OND-pretreated rats did not significantly alter the time spent in either chamber during the CPA test. Thus, OND effectively blocked the ability of LiCl to support CPA.

Saccharin intake test—Despite the effect of OND to block LiCl-induced CPA, in the same rats OND did not interfere with the ability of LiCl to promote conditioned avoidance of 0.2% sac solution in a 60 min single-bottle access test (Fig. 2). Rats that received i.p. LiCl+sac during CPA conditioning after OND (group 1, $n=6$) or vehicle pre-treatment (group 2, $n=6$) consumed approximately 58% less sac in the 60 min single bottle test compared to intake by group 3 OND-or group 4 vehicle-pretreated rats that received i.p. NaCl+sac during CPA conditioning (Fig. 2). Two-way ANOVA revealed a significant effect of sac pairing condition (i.e., LiCl+sac vs. NaCl+sac) on intake during the bottle test [$F(1,20)=44.0$, $P < 0.001$], but no significant effect of pre-treatment injection [OND vs. vehicle; $F(1,20)=0.90$, $P = 0.36$], and no significant interaction between the two factors [$F(1,20)=0.03$, $P = 0.88$].

Experiment 2: Ability of OND to attenuate LiCl-induced conditioned flavor avoidance in a 2-bottle choice test

As expected, rats ($n=15$) consumed similar volumes of each novel flavor during initial (i.e., single bottle) 30-min flavor exposure (vanilla, $12.4 \pm 0.6 \text{ ml}$, range 10–18 ml; almond, $11.5 \text{ ml} \pm 0.9 \text{ ml}$, range 6–16 ml). Fluid intakes during initial flavor exposure did not differ significantly as a function of flavor (i.e., vanilla vs. almond) or presentation order, and also did not differ as a function of the pre-treatment injection (i.e., NaCl vehicle or OND) administered to each rat just before novel flavor presentation on conditioning days.

Figure 3 presents the volumes of each paired flavor consumed by rats within each pre-treatment group during the 2-bottle choice tests (values within bars), and the resulting group preference ratios. Rats within each pre-treatment group (i.e., NaCl vehicle, $n=7$; OND, $n=8$) demonstrated marked and significant avoidance of the flavor previously paired with LiCl injection ($P < 0.001$ within each group; paired samples t -test), with the magnitude of avoidance quite similar between groups (Fig. 3). Multivariate ANOVA revealed no significant effect of pre-treatment condition on intake of either the LiCl-paired flavor [$F(1,13)=0.12$, $P = 0.73$] or the saline-paired flavor [$F(1,13)=1.68$, $P = 0.22$] during the 2-bottle choice test. ANOVA also confirmed that there was no significant effect of pre-treatment on the resulting flavor preference ratios [$F(1,13)=0.3$, $P = 0.59$].

DISCUSSION

Rats and mice are commonly used in academic and corporate research settings to investigate the physiological and behavioral effects of pharmacological agents with therapeutic potential. For example, a drug that suppresses food intake in rats is a potential candidate for fighting obesity in humans, but not if the drug works because it produces visceral malaise. In this regard, the nauseogenic potential of a particular drug or agent often is initially assessed in the laboratory by using CT/FA testing paradigms in rodents. Indeed, with no known exceptions, experimental agents that produce nausea and malaise (such as LiCl) also support CT/FA in rats and mice. Thus, if the drug in question does not support CT/FA, it probably is not nauseogenic. However, if the drug in question does support CT/FA, then it may or may not be nauseogenic. The results of the present study indicate that when OND is used to reduce or block LiCl-induced nausea/malaise, LiCl no longer supports CPA but still maintains its ability to support robust CT/FA.

Our results support prior reports by Linda Parker, Cheryl Limebeer and colleagues [3,6,10, 11], indicating that CT/FA by itself is insufficient to reveal aversive body state in rats. Instead, CT/FA may reflect the “signal properties” of experimental treatments that report a change in physiological state [20,21]. This state-change report would occur via engagement of central visceral sensory systems, even if the change is not accompanied by nausea or malaise. Any novel change in physiological state may signal danger to a rat and thereby support CT/FA, even if the change is ultimately rewarding and supports appetitive behavior. Balleine and colleagues proposed that a second conditioning process takes place in which the hedonic significance of the taste, context, or other set of cues changes, and this process may depend on rewarding or aversive effects that occur as a consequence of the drug-induced change in physiological state [20,21].

The anti-emetic, anti-nauseogenic 5-HT₃ receptor antagonist OND has been reported to block “gaping” and rejection responses to orally infused tastes/flavors that were previously paired with LiCl treatment in rats, without attenuating LiCl-induced CT/FA in bottle tests [3,6,10, 11]. The present study used two very different conditioning and testing paradigms to confirm the inability of OND to attenuate LiCl-induced CT/FA in bottle tests. The first paradigm involved i.p. injection of sac mixed with either LiCl or NaCl in order to expose rats to the taste of sac subsequent to its systemic absorption, without any motor requirements for them to consume sac from a bottle. We reasoned that this conditioning paradigm could be viewed as similar to intra-oral administration of a taste stimulus during CT/FA conditioning. Others have argued that intra-oral administration can produce effects that differ from those obtained when conditioning involves voluntary bottle intake of the taste CS [22]. Our results revealed a significant avoidance of sac during single-bottle intake tests after previous LiCl+sac i.p. Rats pre-treated with OND or vehicle before LiCl+sac displayed similar sac avoidance during the bottle intake test, although in the same rats OND blocked the simultaneously conditioned LiCl-induced place avoidance (CPA). Although the i.p. sac administration approach used in these experiments was rather unusual, we confirmed the inability of OND to attenuate LiCl-induced

CT/FA using a more standard 2-bottle choice test. Thus, conditioning method did not affect the results; OND did not attenuate LiCl-induced CT/FA during single-bottle access or 2-bottle choice tests. These results confirm previous reports in which different conditioning and testing methods were used [3,6,10,11].

A previous study investigated the ability of OND to attenuate LiCl-induced CPA, but in that study OND was administered just before CPA testing, after conditioning was complete [10]. The authors concluded that OND does not modify the LiCl-induced expression of a previously established CPA response, whereas our results indicate that OND administered prior to conditioning does block the ability of LiCl to establish CPA. Although the unconditioned nauseogenic effects of LiCl treatment predominate during acute exposure, its conditioned effects promote avoidance behavior in CPA tests [10,12,23]. The CPA test, which can be automated, examines classically conditioned aversive effects of a wide range of stimuli, including non-nauseogenic stimuli such as mild electrical shock [24,25] and predator odor [26]. Place conditioning is most often used with rats and mice to study the positive (rewarding) or negative (aversive) motivational effects of objects or experiences [14]. There are no motor requirements during US exposure, during which the internal state produced by the situation or agent is paired with an environmental context. CPA is a distinct and readily observable behavior that reflects a conditioned emotional response resulting from a Pavlovian association between a context and an aversive state. The actual conditioned response may be an internal aversive state (i.e., conditioned malaise), and the observed avoidance responses may be behaviors that are facilitated or promoted in the presence of that aversive state [25,27]. In our experiments LiCl-induced CPA was blocked by pre-treatment with the anti-nausea drug OND administered prior to conditioning. This result is consistent with previous reports that OND pretreatment attenuates CPA induced by precipitated withdrawal from morphine or nicotine, which otherwise produces an aversive state of visceral malaise [28,29].

In conclusion, the results reported here support the view that CPA is useful as an additional index of negative hedonic state in rats, including LiCl-induced nausea, whereas CT/FA can occur even when the nauseogenic properties of the US (i.e., LiCl) are antagonized during conditioning. Thus, experimental assessment of CPA should be considered as a potentially useful additional tool to complement assays of oral rejection (taste reactivity), gaping, pica, and other behavioral measures when one requires an index of nausea/malaise and aversive hedonic state.

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Literature Cited

1. Otto B, et al. Endocrine correlates of acute nausea and vomiting. *Autonomic Neuroscience: Basic and Clinical* 2006;129:17–21.
2. Breslin PAS, Spector AC, Grill HJ. A quantitative comparison of taste reactivity behaviors to sucrose before and after lithium pairings: a unidimensional account of palatability. *Behavioral Neuroscience* 1992;106:820–836. [PubMed: 1332732]
3. Parker LA, Limebeer CL. Conditioned gaping in rats: a selective measure of nausea. *Autonomic Neuroscience: Basic and Clinical* 2006;129:36–41.
4. Parker LA, Rana SA, Limebeer CL. Conditioned nausea in rats: assessment by conditioned disgust reactions, rather than conditioned taste avoidance. *Canadian Journal of Physiology and Pharmacology* 2008;Volume:198–209.
5. Bernstein, IL. Flavor aversion, in *Smell and taste in health and disease*. Getchell, TV., et al., editors. New York: Raven Press; 1991. p. 417–428.

6. Parker LA. Taste avoidance and taste aversion: evidence for two different processes. *Learning and Behavior* 2003;31(2):165–172. [PubMed: 12882375]
7. Parker LA. LSD produces place preference and flavor avoidance but does not produce flavor aversion in rats. *Behavioral Neuroscience* 1996;110(3):503–508. [PubMed: 8888996]
8. Berger BD. Conditioning of food aversions by injections of psychoactive drugs. *Journal of Comparative and Physiological Psychology* 1972;81(1):21–26. [PubMed: 5074304]
9. Cappell H, LeBlance AE, Endrenyi L. Aversive conditioning by psychoactive drugs: effects of morphine, alcohol and chlordiazepoxide. *Psychopharmacologia* 1973;29:239–246. [PubMed: 4702275]
10. Limebeer CL, Parker LA. The antiemetic drug ondansetron interferes with lithium-induced conditioned rejection reactions, but not lithium-induced taste avoidance in rats. *Journal of Experimental Psychology: Animal Behavior Processes* 2000;26(4):371–384. [PubMed: 11056879]
11. Limebeer CL, et al. Exposure to a context previously associated with nausea elicits conditioned gaping in rats: a model of anticipatory nausea. *Behavioural Brain Research* 2008;187:33–40. [PubMed: 17897732]
12. Tenk CM, Kavaliers M, Ossenkopp K-P. Dose response effects of lithium chloride on conditioned place aversions and locomotor activity in rats. *European Journal of Pharmacology* 2005;515:117–127. [PubMed: 15899481]
13. Limebeer CL, Hall G, Parker LA. Exposure to a lithium-paired context elicits gaping in rats: a model of anticipatory nausea. *Physiology and Behavior* 2006;88:398–403. [PubMed: 16737724]
14. Cunningham CL, Gremel CM, Groblewski PA. Drug-induced conditioned place preference and aversion in mice. *Nature Protocols* 2006;1(4):1662–1670.
15. Nachman M, Ashe JH. Learned taste aversions in rats as a function of dosage, concentration, and route of administration of LiCl. *Physiology and Behavior* 1973;10:73–78. [PubMed: 4697023]
16. Bradley RM, Mistretta CM. Intravascular taste in rats as demonstrated by conditioned aversion to sodium saccharin. *Journal of Comparative and Physiological Psychology* 1971;75(2):186–189. [PubMed: 5087375]
17. Bures J, Buresova O. Conditioned taste aversion to injected flavor: differential effect of anesthesia on the formation of the gustatory trace and on its association with poisoning in rats. *Neuroscience Letters* 1989;98(3):305–309. [PubMed: 2542844]
18. Lee HB, Blaufox MD. Blood volume in the rat. *The Journal of Nuclear Medicine* 1985;26(1):72–76.
19. Deutsch JA, Hardy WT. Cholecystokinin produces bait shyness in rats. *Nature* 1977;266:196. [PubMed: 859599]
20. Balleine BW, Dickinson A. Instrumental performance following reinforcer devaluation depends upon incentive learning. *The Quarterly Journal of Experimental Psychology* 1991;43B:279–296.
21. Balleine BW, Garner C, Dickinson A. Instrumental outcome devaluation is attenuated by the anti-emetic ondansetron. *The Quarterly Journal of Experimental Psychology* 1995;48B:235–251. [PubMed: 7568998]
22. Schafe GE, Thiele TE, Bernstein IL. Conditioning method dramatically alters the role of amygdala in taste aversion learning. *Learning and Memory* 1998;5:481–492. [PubMed: 10489263]
23. Rodriguez M, et al. Lithium-induced context aversion in rats as a model of anticipatory nausea in humans. *Physiology and Behavior* 2000;71:571–579. [PubMed: 11239677]
24. Wilensky AE, Schafe GE, LeDoux JE. The amygdala modulates memory consolidation of fear-motivated inhibitory avoidance learning but not classical fear conditioning. *The Journal of Neuroscience* 2000;20(18):7059–7066. [PubMed: 10995852]
25. Holahan MR, White NM. Intra-amygdala muscimol injections impair freezing and place avoidance in aversive contextual conditioning. *Learning and Memory* 2004;11:436–446. [PubMed: 15254220]
26. Dielenberg RA, Arnold JC, McGregor IS. Low-dose midazolam attenuates predatory odor avoidance in rats. *Pharmacology, Biochemistry and Behavior* 1999;62:197–201.
27. Holahan MR, White NM. Amygdala inactivation blocks expression of conditioned memory modulation and the promotion of avoidance and freezing. *Behavioral Neuroscience* 2004;118(1):24–35. [PubMed: 14979780]

28. Higgins GA, et al. Effects of 5-HT₃ receptor antagonists on behavioural measures of naloxone-precipitated opioid withdrawal. *Psychopharmacology* 1991;105(3):322–328. [PubMed: 1839177]
29. Suzuki T, et al. Attenuation of mecamylamine-precipitated nicotine-withdrawal aversion by the 5-HT₃ receptor antagonist ondansetron. *Life Sciences* 1997;61(16):PL249–PL254. [PubMed: 9353175]

Figure 1. LiCl Supports CPA, and Ondansetron Blocks This Effect

CPA data from rats pretreated with saline vehicle (left set of bars) or with OND (right set of bars). Vehicle-pretreated rats significantly reduced time spent within the LiCl-paired chamber during the 5 min post-conditioning CPA test, and increased time spent within the NaCl-paired chamber. OND pre-treatment before LiCl conditioning blocked the ability of LiCl to promote CPA of the LiCl-paired chamber or to increase time spent within the NaCl-paired chamber ($*P < 0.001$, difference scores in vehicle-pretreated vs. OND-pretreated groups). As reported in the *Results*, there was no significant effect of i.p. saccharin pairing condition on experimental outcomes, and so the CPA data were combined within each pre-treatment group for this figure.

Figure 2. Ondansetron Does Not Attenuate Conditioned Avoidance of LiCl-paired Saccharin
 Volume of 0.2% saccharin consumed by rats in each of the 4 experimental groups (n=6/group) during single-bottle 60 min access. Rats that were previously injected i.p. with LiCl+sac consumed less than half as much saccharin solution as rats that were previously injected i.p. with NaCl+sac, and there was no significant effect of pretreatment condition (OND vs. vehicle). Differences between bars with different letters are significant, $P < 0.001$.

Figure 3. Ondansetron Does Not Attenuate LiCl-Induced conditioned Flavor Avoidance

Average group preference ratios (mean $\pm$ SE) in two-bottle choice tests between flavors that were previously paired with i.p. injection of saline (0.15M NaCl) or with i.p. injection of LiCl. The central line indicates the expected intake preference ratio of 1.0 (i.e., 50%:50%) if there was no conditioning effect. Results were similar between rats that were pre-treated with NaCl vehicle before flavor exposure and conditioning (n=7, left set of bars) and rats that were pre-treated with OND (n=8, right set of bars). Both groups show equivalent and significantly lower preferences for the LiCl-paired flavor (dark bars) vs. the saline-paired flavor (open bars). Thus, OND pretreatment before LiCl conditioning does not attenuate subsequent avoidance of LiCl-

paired flavors. The values within each bar indicate volumes consumed during the 2-bottle choice test (group mean $\pm$ SE).

Table 1

Experimental design for simultaneous LiCl-induced conditioning of place and taste avoidance (Experiment 1).

	<i>Conditioning day 1</i>		<i>Conditioning day 2</i>	
	pre-treatment	treatment	pre-treatment	treatment
Group 1 (n=6)	OND	LiCl+sac	OND	NaCl
Group 2 (n=6)	vehicle	LiCl+sac	vehicle	NaCl
Group 3 (n=6)	OND	NaCl+sac	OND	LiCl
Group 4 (n=6)	vehicle	NaCl+sac	vehicle	LiCl