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Ondansetron interferes with the establishment and expression of lithium-induced conditioned rejection reactions

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Ondansetron Interferes with the Establishment and Expression of Lithium-Induced Conditioned Rejection Reactions

By

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Abstract

Conditioned rejection reactions displayed in the taste reactivity test may reflect conditioned nausea in rats because they are exclusively produced by emetic agents. The present experiments demonstrated that pretreatment with the anti-nausea agent, ondansetron, interfered with the establishment of conditioned rejection reactions (Experiment 1) and interfered with the expression of previously established conditioned rejection reactions (Experiment 2). Ondansetron selectively interfered with conditioned nausea as reflected by conditioned rejection reactions because it did not modify the unconditioned rejection reactions elicited by unpalatable quinine solution (Experiment 2). Although ondansetron blocked the expression of the selective taste reactivity reaction of conditioned rejection, it did not modify the nonselective reactions of conditioned taste or place avoidance (Experiment 3). The results suggest that conditioned rejection reactions reflect conditioned nausea in rats that is attenuated by pretreatment with the anti-nausea agent, ondansetron.

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To laugh often and much; to win respect of intelligent people and the affection of children; to earn the appreciation of honest critics and endure the betrayal of false friends; to appreciate beauty; to find the best in others; to leave the world a bit better; whether by a healthy child, a garden patch or a redeemed social condition; to know even one life has breathed easier because you lived. This is to have succeeded.

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Rats avoid drinking or eating fluids or food that previously made them sick. This flavour-illness association is typically measured by a consumption test, the taste avoidance test. Garcia, Hankins, & Rusiniak (1974) suggest that conditioned taste avoidance is motivated by conditioned sickness. Paradoxically, non-emetic drugs which are self-administered and produce a preference for a place in rats, such as amphetamine and cocaine, also produce taste avoidance in the consumption test (Wise, Yokel, & DeWit, 1976; Reicher & Holman, 1977). Since drugs that do not produce illness also produce taste avoidance, taste avoidance does not necessarily reflect conditioned sickness.

The sensitivity of the consumption test is a disadvantage when using it as a measure of conditioned sickness. It has been demonstrated that a single drug-paired saccharin conditioning trial can result in minimal intake of saccharin during test (Parker, 1988). This floor effect may mask group differences. Another measure of flavour-drug associations is the taste reactivity (TR) test, a direct measure of palatability. The measure of palatability is defined by the orofacial and somatic responses of the animal to infusion of a flavoured solution that has either a positive or negative hedonic value (Grill & Norgren, 1978). In the TR test, the orofacial and somatic reactions elicited by an intraorally infused flavoured solution are measured. During an intraoral infusion of bitter quinine, rats display rejection reactions of gaping, chin rubbing, and paw treading. On the other hand, during an intraoral infusion of sweet sucrose solution, they display

ingestion reactions of mouth movements, tongue protrusions, and paw licking. Interestingly, during an intraoral infusion of a sweet sucrose solution that has previously been paired with an emetic drug such as lithium chloride (LiCl), rats display rejection reactions as if they were infused with quinine (Parker, 1988). An increase in the expression of rejection reactions to LiCl-paired saccharin also occurs within a test session (Spector, Breslin & Grill, 1988). This finding has led to the proposition that *conditioned* rejection reactions may reflect the state of conditioned nausea.

Flavour-illness associations are accompanied by rejection reactions

Recent literature, testing different doses of drugs across studies, has provided evidence that rats display conditioned rejection reactions when exposed to a sucrose solution that has been paired with LiCl (Berridge, Grill and Norgren, 1981; Grill and Norgren, 1978; Parker, 1982; Parker, 1984; Parker, 1993), high doses of nicotine (Parker, 1993), apomorphine (Parker & Brosseau, 1990), novel ethanol (Parker, 1982), fenfluramine (Parker, 1982) and novel Δ^9-tetrahydrocannabinol (THC) (Parker & Gillies, 1995). None of these agents has been shown to be self-administered by rats or to produce a place preference at equivalent doses. In fact, each of these agents has been shown to be capable of producing a conditioned place aversion at the dose that produces rejection reactions in the TR test (Best, Best & Mickley, 1973; Jorenbv, Steinpreis, Sherman & Baker, 1990; Davies & Parker, 1993; Cunningham, 1979; Mucha & Iverson, 1985; Parker & Gillies, 1995). It is thus likely that at doses that produce rejection TR reactions, each of these agents produces an aversive state such as nausea which becomes associated with the taste.

Although rats avoid consumption of a flavoured solution previously paired with a rewarding drug such as amphetamine, they do not exhibit conditioned rejection reactions during an intraoral infusion of that solution in the TR test (Zalaquett & Parker, 1989; Parker, 1998). Since flavours paired with an emetic drug, such as LiCl elicit both taste avoidance and conditioned rejection reactions in the TR test, conditioned rejection reactions may reflect conditioned sickness.

Anatomy of Emesis

Emetic reflexes in dogs and cats are integrated in a brainstem "emetic center" of the lateral reticular formation (Borison & Wang, 1953) which overlaps the *nucleus of the tractus solitarius* (NTS), the site to which primary taste afferents project to the brainstem via cranial nerves VII and IX (Norgren & Pfaffman, 1975). Two distinct afferent routes are known to project to the emetic area: 1) The vagal afferent route (cranial nerve X) carries the stimulus effects of local stomach irritation, such as that caused by intragastric copper sulfate, directly to the NTS (Wang & Borison, 1951). Agents that elicit emesis by this route are detected by their failure to elicit emesis after vagotomy. 2) A circulatory route monitors bloodborne toxins, such as intravenously administered apomorphine, via chemoreceptors in the area postrema which then project to the emetic area (Wang & Borison, 1952). A weak blood-brain-barrier at this site allows it to detect toxins in the blood. Toxins that elicit emesis solely by this route no longer do so after destruction of the area postrema (Bernstein et al., 1992; Eckel & Ossenkopp, 1996). Both forms of stimulation induce vomiting in cats and dogs (Borison & Wang, 1953), however rats cannot vomit due to the anatomy of their peripheral musculature. Since rats cannot

vomit, treatments for emesis are typically evaluated in dogs, cats or ferrets. A rat model of nausea would be cost effective for medical research. This model could be used to test the efficacy of established and newly developed anti-emetic therapies used to control the debilitating nausea that is the result of most chemotherapy treatments.

Cyclophosphamide- and LiCl-induced Emesis

A complication of the use of high doses of chemotherapeutic agents is nausea and vomiting (Mitchelson, 1992). In fact about 80% of patients receiving cyclophosphamide, a cytotoxic agent, experience vomiting (Morran, Smith, Anderson & McArdle, 1979). Furthermore, anticipatory vomiting may develop in humans after one or more treatments (Morrow & Morrell, 1982; Weddington, Miller & Sweet, 1982; Morrow & Dobkin, 1988; Carey & Burish, 1988). Morrow and Morrell (1982) report that approximately 20% of patients report nausea or vomiting before receiving treatment. There is evidence that chemotherapeutic drug-induced emesis produces taste avoidance in humans; children and adults avoid consumption of a novel flavoured ice cream previously paired with an exposure to a chemotherapeutic drug (Bernstein, 1978; Bernstein & Webster, 1980). Revusky & Martin (1988) report that cyclophosphamide produces conditioned taste avoidance in rats. Cyclophosphamide-induced sickness appears to be vagally mediated, because cyclophosphamide-induced vomiting is reduced by section of the abdominal vagus and sympathetic nerves (Hawthorn et al, 1988), but is not affected by area postrema ablation (McCarthy & Borison, 1984). Administration of 5-HT_3 antagonists (Cubeddu, et al, 1990) reduce cyclophosphamide-induced emesis in ferrets. It is speculated that activation of peripheral serotonin 5-HT_3 receptors in vagal and sympathetic afferents relay

information to the area postrema. Furthermore, 5-HT_3 receptors in the area postrema are involved in the transmission of that activation to the vomiting center (Mitchelson, 1992).

Parker (1998) explored the ability of cyclophosphamide to produce conditioned rejection reactions in the taste reactivity test. Cyclophosphamide, like LiCl, produced dose-dependent rejection taste reactions when paired with sucrose solution. Cyclophosphamide and LiCl are agents that notably produce emesis and also produce rejection of a flavour with which they are paired. These data provide additional support for the hypothesis that the taste aversion produced by emetic drugs is accompanied by a conditioned palatability shift.

Conditioned rejection reactions may reflect conditioned sickness

If rejection reactions in the TR test are elicited exclusively by flavours paired with emetic agents, then these reactions may reflect conditioned sickness (or conditioned nausea) reactions. Garcia and his colleagues (Garcia, Hankins, & Rusiniak, 1974) argue that conditioned taste avoidance produced by emetic agents is mediated by a conditioned sickness reaction. That is, the conditioned stimulus (CS; the taste) having been paired with the unconditioned stimulus (US; the emetic agent, such as LiCl) which produces the unconditioned response (UR; sickness) gains the associative capacity to elicit the conditioned response (CR; sickness). They argue that since the rat cannot vomit, the CR elicited by the CS flavour reflects a hedonic shift displayed as rejection reactions which include gaping and chin rubbing. These responses are elicited by unconditionally or conditionally aversive tasting solutions.

Parker's (1995a) findings that rewarding drugs do not elicit conditioned rejection

taste reactions suggest that taste avoidance produced by rewarding drugs is not motivated by conditioned sickness, but taste avoidance produced by an emetic drug, like LiCl, may be motivated by conditioned sickness. Indeed, if LiCl-based taste avoidance and the display of rejection reactions in the TR test are both motivated by conditioned sickness, then pretreatment with an anti-emetic drug prior to a test for the expression of learning should reduce the strength of that conditioned sickness. Coil, Hankins, Jenden, and Garcia (1978) report that pretreatment with a number of anti-emetic agents attenuates the expression of previously established LiCl-based taste avoidance. More recently, Balleine, Garner, and Dickinson (1995) found that ondansetron (OND), a 5-HT_3 antagonist anti-emetic agent, interfered with the ability of LiCl to devalue a reinforcer in an operant paradigm. Yet, others have reported that anti-emetic pretreatment does not interfere with a LiCl- (Goudie, Stolerman, Demellweek & D'Mello, 1982; Mele, McDonough, McLean & O'Halloran, 1992) or cisplatin- (Mele, et al, 1992) induced taste avoidance. These contradictory reports are based on experiments that employed the standard consummatory taste avoidance test which only indirectly measures CRs elicited by the flavoured solution. A more direct measure of the aversive CRs elicited by a tastant is the TR test. If rejection taste reactions reflect conditioned sickness (nausea) reactions, then pretreatment with an anti-emetic agent may attenuate the strength of these responses. In fact, Parker and MacLeod (1991) report that this is exactly what happens.

Following a single pairing of sucrose with LiCl, amphetamine or saline, rats were administered a taste reactivity test to determine the palatability of sucrose. Twenty min prior to the TR test, rats were injected either with trimethobenzamide, an anti-emetic

agent, or physiological saline. During the TR test, only the LiCl-conditioned group displayed rejection reactions that differed from saline controls during the sucrose infusion, however, those reactions were reduced in the trimethobenzamide pretreated group. On the other hand, trimethobenzamide did not modify the strength of rejection reactions displayed during an intraoral infusion of bitter quinine solution. These results suggest that the rejection reactions displayed to a LiCl-paired flavour may reflect conditioned nausea.

A Rat Model of Nausea

The present study evaluates the role of conditioned nausea in the establishment and expression of LiCl-induced conditioned rejection reactions. The emetic effects of LiCl are believed to be related to enhancement of serotonin availability in the gut and in the brainstem (Wegener, 1997), likely the result of LiCl-induced antagonism of serotonin autoreceptors which normally regulate the release of serotonin. OND, a serotonin post-synaptic receptor antagonist, is an anti-emetic drug that has been very effective for use with cancer patients who experience nausea and vomiting following toxic chemotherapy treatments (Fox, Einhorn, Cox, Powell, & Abdy, 1993; Beck, 1995).

The affective reaction to a LiCl-paired flavour is reflected in a sequence of rejection reactions in the TR test. The experiments that follow examine the ability of OND to interfere with the establishment and the expression of LiCl-induced saccharin rejection reactions.

Experiment 1

Experiment 1 examined the ability of OND to interfere with the establishment of

LiCl-induced conditioned rejection reactions. Rats were pretreated with OND or saline 30 min prior to saccharin presentation, allowing 50 min (30 min + 20 min saccharin) for the drug to act before the illness inducing treatment. The anti-emetic efficacy of OND using this interval has been demonstrated in previous studies (Beck, 1995; Fox, et al, 1993). Immediately following 20 min saccharin consumption the animals were injected with LiCl or saline. Saccharin was used as the novel flavour stimulus as it is consistent with other experiments conducted in our lab.

Method

Subjects. Twenty-four male Sprague-Dawley rats (Charles River Labs, St. Constant, Quebec) weighing 320-450 g at the beginning of the experiment served as subjects. They were individually housed in stainless steel hanging cages in a colony room kept at 21^{0}C on a 12:12 light/dark schedule with the lights on at 7:00 AM. Half of the rats had received a single exposure to morphine (20 mg/kg) one month prior to the beginning of the experiment. They were assigned to groups matched by prior experience and were habituated to the colony room for five days prior to surgical implantation of intraoral cannulae. During habituation, surgery, and recovery, the animals were maintained on ad-lib water after which they were placed on the water deprivation schedule. During the course of the entire experiment they were maintained on ad-lib rat chow.

Drugs. Fifteen minutes prior to administration of the anesthetic the animals were given an intraperitoneal (IP) injection of 1 ml Atropine. The anesthetic was prepared using 75 mg/kg of Ketamine combined with a dose of 10 mg/kg of Xylazine mixed WFI

(Water for Injection) and injected IP at a volume of 1 mg/kg. On the conditioning day the animals were given an IP injection of 0.15 M Lithium Chloride (LiCl) prepared in distilled water and delivered at a volume of 20 ml/kg. The anti-emetic OND (0.1 mg/kg) was obtained from Sigma Chemical Co. (St. Louis, MO) and prepared in a saline solution (0.1 mg/ml) and injected IP at a volume of 1 ml/kg.

Surgery. On the sixth day, the animals were surgically implanted with intraoral cannulae. A thin walled 15 gauge stainless steel needle was inserted at the back of the neck and directed subcutaneously around the ear and brought out behind the first molar inside the mouth. Intramedic Polyethylene tubing with an inner diameter of 0.86 mm and an outer diameter of 1.27 mm was then run through the needle and the needle was removed. An Intramedic Luer Stub Adapter (20 gauge) was attached to the exposed tubing at the back of the neck. The tubing was held secure in the oral cavity by an o-ring which was sealed behind the tubing. The rats were then allowed to recover from surgery for three days before the experiment began.

Procedure. Table 1 presents the procedure implemented for this experiment. On each of 4 days, the rats were trained to drink their daily water supply in 20 min while 24 hour water deprived. During this 20 min period the amount of water consumed was not restricted. On the fifth day (conditioning day), the rats were injected with OND (n=13) or saline (n=11) 30 min before being presented 0.1% saccharin in place of water for 20 minutes. Immediately following saccharin consumption the amount consumed was measured and the animals were injected with LiCl (n=12) or saline (n=12). All animals were returned to their home cages after the conditioning drug injection. Water bottles

were returned 6 hours later.

On the day after the conditioning trial the rats received a one minute adaptation trial in the Plexiglas Taste Reactivity (TR) chamber (22.5 x 26 x 20 cm). This trial served to adapt the animals both to the TR chamber and the duration of infusion that would be used for the TR test. During this time the animals' cannulae were attached to the infusion pump (Harvard Apparatus) using a 30 cm infusion hose. They were infused with water at a rate of 1 ml/min to adapt them to the TR procedure. On the next day, the rats were given the TR test. The animals were placed in the TR chamber where they were infused for one minute with a 0.1% saccharin solution while their orofacial and somatic responses were videotaped. A videocamera was focused on a mirror mounted at a 45^0 angle beneath the test chamber. This provided a clear view of the ventral surface of the rat facilitating recording of their orofacial and somatic responses. Immediately following the TR test the rats were returned to their home cages.

Taste Reactivity Measures. An experienced rater, blind to the experimental conditions, scored the videotapes using "The Observer" (Noldus, NL) event recording program. The frequency of the rejection reactions of gaping (rapid large amplitude opening of the mandible with retraction of corners of mouth), chin rubbing (mouth or chin in direct contact with the floor or wall of the chamber and body projected forward) and paw pushing (sequential extension of one forelimb against the floor or wall of the chamber while the other forepaw is being retracted) were summated to provide a rejection reaction score. The frequency of the ingestion reactions of mouth movements (movement of the lower mandible without opening the mouth), tongue protrusions (extensions of the

tongue out of the mouth), and paw licks (licking the flavoured solution from the forepaws) were summated to provide an ingestion reaction score. Finally, the frequency of instances of forward locomotion (movement of the rat's forepaws along the floor of the chamber) and rearing (both front forepaws lifted off the floor, whether placed against wall or not) were summated to provide an activity score.

The relationships between behaviours scored in the TR test have been previously defined by Parker (1995a). Correlational analysis indicates that the defined rejection reactions of gapes, chin rubbing, and paw pushing are positively correlated with one another. The ingestion reactions of mouth movements, tongue protrusions, and paw licks are positively correlated with each other and negatively correlated with the rejection reactions previously mentioned.

Results

Conditioning Trial. OND pretreatment did not modify saccharin consumption during the conditioning trial (Figure 1). The mean amount of saccharin consumed was analyzed using a 2 by 2 Analysis of Variance (ANOVA). There were no significant effects of pretreatment drug or conditioning drug.

TR Test. Figure 2 presents the mean frequency of rejection reactions, ingestion reactions, and activity elicited by LiCl or saline-paired saccharin solution during the TR test of Experiment 1. As is apparent in the top section of Figure 2, LiCl-paired saccharin elicited more rejection reactions than did saline-paired saccharin [$F(1, 20) = 5.249$; $p = .033$] and overall, the animals that received OND pretreatment expressed significantly fewer rejection reactions than those that received saline pretreatment [$F(1,20) = 4.596$; p

= .045]. The 2 by 2 factorial analysis of variance (ANOVA) revealed a pretreatment by conditioning drug interaction that approached significance [F(1,20) = 4.1; p = .056] for the rejection reaction category. A subsequent simple main effects analysis revealed that OND significantly reduced the frequency of rejection reactions for the rats conditioned with LiCl [F(1, 20) = 8.568; p< .01], but not for the rats conditioned with saline.

The middle section of Figure 2 presents the mean frequency of ingestion reactions displayed during the TR test of Experiment 1. Rats conditioned with LiCl displayed significantly fewer ingestion reactions overall than did rats conditioned with saline [F(1,20) = 8.398; p = .009]. Additionally, rats pretreated with OND displayed significantly more ingestion reactions overall than rats pretreated with saline [F (1, 20) = 4.341; p = .05]. There were no other significant effects.

For activity measures, the bottom section of Figure 2 demonstrates there were no significant effects of conditioning drug or pretreatment for the animals.

Discussion

Experiment 1 demonstrated that the anti-emetic OND interfered with the establishment of conditioned rejection reactions as expressed in the TR test. Administration of LiCl following novel saccharin presentation shifted the palatability of saccharin, evidenced by the increased expression of rejection reactions for animals in the LiCl-paired saccharin group compared to the saline-paired saccharin group. For those animals conditioned with LiCl, pretreatment with OND significantly attenuated the expression of conditioned rejection reactions. The ability of OND to interfere with establishment of conditioned rejection reactions does not appear to be achieved by

aversive effects of the drug itself (US pre-exposure effect) as OND pretreatment: 1) did not conditionally modify saccharin consumption during conditioning; 2) did not result in increased expression of conditioned rejection reactions for the OND-saline animals; 3) did not suppress expression of LiCl-induced rejection reactions by impacting on the general activity of the animals. In fact, OND alone conditionally enhanced the palatability of saccharin as displayed by the increased frequency of ingestion reactions. This suggests that OND may have produced a preference for the saccharin solution.

Experiment 2

Experiment 2 examined the ability of OND to interfere with the expression of previously established conditioned rejection reactions. Following a single saccharin-LiCl pairing, rats were injected (in a within-subject design) with saline, 0.01 mg/kg OND, or 0.1 mg/kg OND 30 min before a TR test. Additionally, in order to determine whether OND selectively interferes with rejection reactions produced by conditioned nausea, the rats were given a subsequent TR test with unconditionally aversive quinine solution 30 min after an injection of OND or saline.

Method

Subjects. Thirty two male Sprague-Dawley rats weighing 210-250 g (Charles River Labs, St. Constant, Quebec) at the beginning of the experiment served as subjects. They were treated in a manner similar to Experiment 1, except as indicated.

Procedure. Table 2 presents the procedure implemented for this experiment. Following recovery from the cannulation procedure, the rats were placed on a 24 hr water deprivation schedule and were trained to drink their daily water in 20 min on each of

three days. On the next day, they were presented with 0.1% saccharin solution for 20 min and the amount consumed was measured. Immediately following removal of saccharin the rats were injected IP with LiCl (n=16) or saline (n=15). Following the appropriate injection, the animals were placed back in their home cages and ad-lib water was returned 6 hours later. On the following day, the animals were given a 1 min TR adaptation trial as described in Experiment 1.

On each of three TR test days the rats received a 1 min intraoral infusion of 0.1% saccharin solution 30 minutes after receiving an injection of saline, 0.01 mg/kg OND, or 0.1 mg/kg OND. The order of drug treatments across days was counterbalanced among the groups.

In order to evaluate the effect of OND on unconditioned rejection reactions elicited by quinine solution, the rats were given a 1 min TR test (1 ml/min) with 0.05% quinine solution on the day following the third test trial. Thirty min prior to the quinine TR test, the rats were injected with saline (n=10), 0.01mg/kg OND (n=11), or 0.1 mg/kg OND (n=11).

The videotapes were scored by the same rater as in Experiment 1, who was again blind to the experimental conditions. The frequency of rejection reactions, ingestion reactions, and activity were scored as described in Experiment 1.

Results

Saccharin TR Test. OND interfered with the expression of previously established LiCl-induced conditioned rejection reactions in the saccharin TR test. The top section of Figure 3 presents the mean frequency of rejection reactions elicited by LiCl or saline-

paired saccharin solution in the TR test subsequent to administration of saline, 0.01 mg/kg OND, and 0.1 mg/kg OND. The 2 by 3 mixed ANOVA revealed a significant conditioning drug by test drug interaction [$F(2,58) = 7.497$; $p = .001$]. Subsequent repeated measures ANOVAs revealed a significant effect of test drug for animals conditioned with LiCl [$F(2,30) = 12.995$; $p < .001$], but not for animals conditioned with saline. For the LiCl conditioned group, Bonferroni pairwise comparison tests revealed that animals in the LiCl-paired saccharin group expressed significantly fewer rejection reactions when administered 0.01 mg/kg OND or 0.1 mg/kg OND than when administered saline (p's $< .05$), 30 minutes prior to the TR test.

As indicated in the middle section of Figure 3, LiCl-paired saccharin elicited fewer ingestion reactions than did saline-paired saccharin [$F(1,29) = 5.266$; $p = .029$]. Additionally, there was an overall significant effect of test drug [$F(2,58) = 3.925$; $p = .025$]. A Bonferroni pairwise comparison revealed that rats displayed significantly more ingestion reactions overall following exposure to 0.01 mg/kg OND than 0.1 mg/kg OND ($p < .05$). No other groups significantly differed on this measure. As in Experiment 1 and demonstrated in the bottom section of Figure 3, the analysis of activity scores revealed no significant effects.

Quinine TR Test. OND did not modify the display of rejection reactions elicited by quinine solution. As evidenced in Figure 4, a single factor ANOVA revealed no significant test drug effect. Therefore, OND selectively interferes with the display of rejection reactions, but does not modify unconditioned rejection reactions elicited by naturally unpalatable flavoured solutions. This suggests that OND specifically interferes

with nausea.

Discussion

Experiment 2 demonstrated that LiCl-paired saccharin animals expressed significantly more rejection reactions in the TR test. This is consistent with the results of Experiment 1 where LiCl conditioned animals displayed significantly more rejection reactions than saline conditioned animals. While Experiment 1 demonstrated that OND had the ability to interfere with the establishment of conditioned rejection reactions, Experiment 2 now demonstrates that once conditioned rejection reactions are established, OND has the ability to attenuate expression of these conditioned rejection reactions when administered 30 min prior to the TR test. ONDs' interference in the expression of rejection reactions appears to be related to conditioned nausea as OND does not interfere with the expression of rejection reactions elicited by infusion of unconditionally unpalatable quinine solution.

Experiment 3

The previous two experiments demonstrated that rats express significantly more rejection reactions to infusion of a saccharin solution following a single LiCl-paired saccharin experience compared to saline-paired saccharin. This is consistent with the findings of Parker (1995a) which demonstrated that these conditioned rejection reactions are expressed to infusion of a flavour previously paired with an emetic drug such as LiCl and not to a flavour paired with a rewarding drug such as amphetamine. The argument we have presented is that conditioned rejection reactions reflect a state of conditioned nausea in the rat that has resulted in a palatability shift of the tastant.

Based on this argument, we proposed that the known anti-emetic OND would have the ability to interfere with both the establishment and expression of conditioned rejection reactions displayed in the TR test. This is exactly what we found. In Experiment 1, animals administered OND prior to LiCl-paired saccharin expressed significantly fewer rejection reactions when subsequently infused with the saccharin solution in the TR test. This demonstrated the ability of OND to interfere with the establishment of conditioned rejection reactions. Furthermore, when animals with established conditioned rejection reactions were given an injection of OND 30 min prior to the TR test in Experiment 2, they expressed significantly fewer rejection reactions to infusion of the saccharin solution. This demonstrated the ability of OND to interfere with the expression of established conditioned rejection reactions. The interference of OND on conditioned rejection reactions was not due to any non-specific effects of OND on the tastant, as OND did not interfere with the expression of rejection reactions to infusion of a naturally unpalatable quinine solution.

Although OND interfered with the expression of LiCl-induced conditioned rejection reactions in Experiment 2, it is not known whether it would also interfere with the more traditional LiCl-induced taste avoidance. Using a standard taste avoidance paradigm, Coil et al. (1978) found that pretreatment with a number of anti-emetic agents (not including OND) attenuated previously established LiCl-induced taste avoidance, but others (Goudie et al., 1982; Mele et al., 1992; Rabin & Hunt, 1983) failed to replicate this effect. More recently, Balleine et al. (1995) report that OND interfered with both the establishment and expression of LiCl-induced taste avoidance. However, their procedure

differed considerably from former studies.

Balleine et al. (1995) used a discrimination procedure to produce LiCl-induced conditioned taste avoidance. Over two days, each of two flavours was paired with LiCl; on one day, OND preceded LiCl, and on the other day, saline preceded LiCl. In a subsequent two choice test, rats preferred the LiCl-paired flavour preceded by OND to that preceded by saline. During the preference test between the two LiCl-paired flavours, Balleine et al. (1995) also evaluated the ability of OND to interfere with the expression of previously learned avoidance; half of the rats were tested in an OND state and half were tested in a saline state. Those rats tested in an OND state displayed a weaker avoidance than those tested in the saline state, regardless of the pretreatment during conditioning. OND interfered with both the establishment and the expression of LiCl-induced taste avoidance. Furthermore, the interference with LiCl-induced taste avoidance by OND was not a state-dependent effect, because although OND attenuated the avoidance of both LiCl-paired flavours, it most effectively attenuated the avoidance of the flavour conditioned following saline pretreatment than the flavour conditioned following OND pretreatment.

The procedure used by Balleine et al. (1995) may have been a more sensitive assessment of the ability of an anti-emetic drug to interfere with LiCl-induced taste avoidance than is the more traditional two-choice test, in which rats are provided with a choice between the LiCl-paired flavour and water. Since the ability of OND to interfere with the expression of LiCl-induced taste avoidance has not been evaluated in the more traditional two choice test, it is possible that OND is more effective than other anti-emetic

agents tested in earlier studies (Goudie, et al, 1982; Rabin & Hunt, 1983).

Another well established paradigm used to demonstrate the aversive effects of drug administration is place conditioning (van der Kooy, 1987). Parker (1982) has demonstrated that LiCl administration paired with a distinctive chamber resulted in establishment of a place aversion. More recently, Frisch, Hasenohrl, Mattern, Hacker, and Huston (1995) have found that the known anti-emetic metoclopramide attenuates the development of a LiCl-induced place aversion.

Experiment 3 examined the ability of OND to interfere with the expression of a conditioned taste avoidance as well as a conditioned place aversion, both induced by LiCl administration. In Experiment 3a, rats experienced LiCl administration paired with novel saccharin. The resulting taste avoidance was assessed for animals receiving 0.1 mg/kg OND or saline 30 minutes prior to a two bottle choice test. In Experiment 3b, the animals were administered LiCl in one distinctive environment and saline in another. The amount of time that the animal spent in the LiCl-paired chamber compared to the saline-paired chamber, after administration of either 0.1 mg/kg OND or saline, was assessed.

Method

Experiment 3a: *(Taste Avoidance Conditioning)*

Subjects. Thirty two male Sprague-Dawley rats weighing between 196 and 237 gm on the first conditioning day were housed as in Experiment 1.

Procedure. Table 3 outlines the procedure for Experiment 3a. Three days after arriving in the laboratory, the rats were deprived of water. On each of three days, they were presented water for 20 min per day to train the rats to consume their daily water in

20 min. On the fourth day of water deprivation, the rats received the conditioning trial.

On the conditioning trial, the rats were presented with 0.1% saccharin solution for 20 min in graduated tubes and were immediately injected with LiCl (n=16) or saline (n=16) upon removal of the saccharin. The amount of saccharin solution consumed was measured. The dose and volume of LiCl and saline were the same as in Experiment 1. On the following day, all rats received 20 min of water during the designated drinking period.

Forty-eight hr after the conditioning trial, the rats received a two-bottle taste avoidance test. Thirty min prior to presentation of a bottle of water and a bottle of saccharin solution, half of the LiCl conditioned animals (n=8) and half of the saline conditioned animals (n=8) received an injection of 0.1 mg/kg OND and the other half received an injection of saline. In the two-bottle test, each rat was first presented the spout of a graduated tube containing saccharin on the right-hand side until it drank, and was then presented with the spout of a graduated tube containing water on the left-hand side until it drank. Both graduated tubes were then placed on the cage for 20 min and the amounts consumed were measured. Water bottles were returned six hours subsequent to testing.

Analysis. The amount of saccharin and water consumed was converted to a saccharin preference ratio [saccharin consumed/ (saccharin + water consumed)] for the purposes of analysis. A low saccharin preference score would indicate that consumption of water was preferred over saccharin where a higher saccharin preference score would indicate that consumption of saccharin was preferred over water.

Experiment 3b: *(Place Conditioning)*

Subjects. The saline conditioned animals (n=16) from Experiment 3a were used for the place conditioning phase of Experiment 3 as it was expected that LiCl preexposure would attenuate the strength of a LiCl-induced place aversion.

Apparatus. The place conditioning apparatus consists of two chambers (35 x 25 x 30 cm) painted flat black, each with a distinctively different floor, separated by a black wooden divider used during conditioning. One floor consists of sandpaper strips (5 cm wide) with a 5 cm space between strips. The other floor consists of a wire mesh with 2 cm spaces. The wooden divider is removed for testing allowing free access by the rat to either chamber. The activity of the rats during testing was recorded by a videocamera mounted in the ceiling above the place conditioning chambers and monitored by a videotracking apparatus (Videomex-V, Columbus Instruments, Columbus, OH). This equipment records the amount of time the rat spends in each chamber.

Procedure. Table 4 outlines the procedure for Experiment 3b. One week following the taste avoidance experiment, the rats received a single discrimination conditioning cycle consisting of one chamber-LiCl pairing and one chamber-vehicle pairing. On Day 1 of the conditioning trial, rats were injected with saline 5 min prior to placement in the appropriate chamber for 30 min. On Day 2, rats were injected with 20 ml/kg of 0.15 M LiCl and placed in the alternate chamber. In a discriminative conditioning paradigm, half of the rats (n=8) had the chamber with the meshfloor (Group Mesh+) paired with LiCl, and the other half of the rats (n=8) had the chamber with the mesh floor paired with saline (Group Mesh-). The order of the LiCl trial within the

conditioning cycle was counterbalanced across both groups. Following the time spent in the distinctive chamber the animals were returned to their home cages.

The test trial occurred 48 hr after the conditioning cycle. On the test trial, rats were placed at the intersection of the two chambers with the divider removed, and the amount of time spent in each chamber was recorded by the videotracking apparatus over a period of 15 min. Thirty min prior to the test trial, half the rats in group Mesh+ and half the rats in group Mesh- were injected IP with 0.1 mg/kg of OND, while the remaining rats in each group were injected with saline.

Analysis. The mean number of sec that Group Mesh+ and Group Mesh− spent in the mesh chamber served as a measure of place aversion. Rats in Group Mesh+ were expected to spend less time in the mesh chamber than rats in Group Mesh−. However, if OND interferes with the expression of a LiCl-induced place aversion, then the OND pretreated rats in Group Mesh+ should spend more time in the mesh chamber than the saline pretreated rats in Group Mesh+.

Results

Experiment 3a (Taste Avoidance). OND did not attenuate the expression of previously established LiCl-induced taste avoidance. As is evident in Figure 5, rats preferred LiCl-paired saccharin less than saline-paired saccharin [$F(1,28)=25.458$; $p<.001$], but OND did not modify the strength of that avoidance.

Experiment 3b (Place Avoidance). OND also did not modify the expression of previously established LiCl-induced place avoidance. Figure 6 shows that rats spent less time on the mesh floor if that floor had been paired with LiCl (Mesh+) than if it had been

paired with saline (Mesh-), indicating that LiCl indeed produced a place aversion in a single conditioning trial [$F(1,12)=7.812$; $p=.016$]. However, OND pretreatment did not modify the strength of the LiCl-induced place avoidance.

Discussion

OND did not modify the expression of a previously established taste or place avoidance. The results of Experiment 3a are consistent with those of Goudie et al (1982) and Rabin & Hunt (1983) who demonstrated that anti-emetic pretreatment during testing did not modify the strength of a LiCl-induced taste avoidance.

In a discrimination conditioning paradigm, Balleine et al (1995) found that OND pretreatment does modify, both the establishment and expression of LiCl-induced taste avoidance, as well as the ability of LiCl to devalue an instrumental outcome. In Experiments 1 and 2, we also demonstrate that OND interferes with the establishment and the expression of LiCl-induced conditioned rejection reactions. Therefore, it is most likely that the failure of OND to interfere with the expression of LiCl-induced taste avoidance or place avoidance is a function of test sensitivity.

General Discussion

Although rats are incapable of vomiting, they do display conditioned rejection reactions upon re-exposure to an illness-paired flavour. These conditioned rejection reactions are produced exclusively by treatments which produce emesis in other species. Neither non-emetic drugs (Parker, 1982; 1995a) nor shock (Pelchat et al, 1983) produce these conditioned rejection reactions when paired with a flavoured solution, even at doses that are titrated to produce equivalent taste avoidance as that produced by LiCl.

Since they are exclusively elicited by emetic treatments, these conditioned rejection reactions may reflect conditioned nausea in rats. Experiments 1 and 2 demonstrate that one of the most effective treatments for reducing nausea in humans (e.g., Fox et al, 1993; Beck, 1995), also reduces conditioned rejection reactions in rats. OND interfered with both the establishment of conditioned rejection reactions (Experiment 1) and with the expression of previously established conditioned rejection reactions (Experiment 2). However, OND did not modify unconditioned rejection reactions elicited by unpalatable quinine solution, suggesting that it selectively interferes with conditioned rejection reactions, which may reflect conditioned nausea.

Surprisingly, in Experiment 1 OND conditionally enhanced the palatability of saccharin solution. This effect was nonspecific, because it was evident whether saccharin had been paired with LiCl or saline. Another drug that produces this nonspecific enhancement of palatability is chlordiazepoxide (Berridge & Treit, 1986; Parker, 1995b; Berridge & Pecina, 1995). OND, like chlordiazepoxide, has been reported to produce anti-anxiety effects (Costall & Naylor, 1991), suggesting that its ability to enhance saccharin palatability may be a function of the anxiolytic effects, rather than the anti-nausea effect. However, the effect of OND on ingestion reactions is most likely independent of its effect on rejection reactions, because, in Experiment 2 a dose of 0.1 mg/kg reduced the expression of conditioned rejection reactions, but did not modify saccharin-elicited ingestion reactions.

Role of State Dependent Learning

In Experiment 1, rats pretreated with OND during conditioning, but tested drug

free, displayed suppressed conditioned rejection reactions and in Experiment 2, rats conditioned in the absence of OND displayed suppressed conditioned rejection reactions when pretreated with OND. These results could be interpreted as a state dependent learning decrement.

Although we did not specifically evaluate the role of state dependent learning, a number of findings discount this possibility:

1. In Experiment 3, OND pretreatment did not modify the expression of LiCl-induced conditioned taste avoidance or LiCl-induced conditioned place avoidance. If state dependent learning accounts for the suppression of the establishment of conditioned rejection reactions (Experiment 1) or the expression of previously established conditioned rejection reactions (Experiment 2), then it would also predict a suppression of conditioned taste avoidance and place avoidance (Experiment 3).
2. In order for a drug cue to serve as a discriminative stimulus for state dependent learning, it must be easily discriminable (Overton, 1974). Yet, rats displayed neither a change in intake of saccharin solution in Experiment 1 while in the OND state during conditioning nor a change in activity in Experiment 2 when in the OND state during testing, suggesting that it produced little state changes.
3. At the doses employed in the present experiments, OND fails to interfere with either the establishment or expression of conditioned reinforcement (Fletcher & Higgins, 1997) or passive avoidance conditioning (Roychoudhury & Kulkarni, 1997). In fact, OND has been reported to improve basal performance in rodent

and primate tests of cognition as well as reducing the impairments in performance caused by cholinergic deficits (Barnes et al, 1990).

4. Finally, Balleine et al (1995) specifically demonstrated that the interference with discriminative LiCl-induced taste avoidance by OND was not the result of state dependent learning. Rats that were trained and tested in an OND state displayed a *weaker* taste avoidance than rats trained in a saline state and tested in an OND state. If the attenuation of LiCl-induced taste avoidance was due to state dependency, rats should show a *stronger* avoidance of a flavour tested in the same state in which the rats were conditioned.

A more plausible explanation of our findings is that OND interfered with LiCl-induced nausea in Experiment 1 and with conditioned nausea in Experiment 2 resulting in complete suppression of conditioned rejection reactions.

Conditioned rejection reactions: A rat model of nausea

Reliable animal models of nausea are necessary to better understand the neurobiology of nausea and to assess treatment effectiveness. However, since rats do not vomit, such models rely on species more costly to maintain such as dogs, cats and ferrets. Although rats are a non-emetic species and ferrets are an emetic species, Grundy and his colleagues (Blackshaw & Grundy, 1993a; Blackshaw & Grundy, 1993b; Grundy, 1998) report that the vagal response to electrical stimulation and chemical stimulation by 5-hydroxytryptamine and cytotoxic drugs in these two species is similar. Furthermore, 5-HT_3 antagonists disrupt this neural afferent reaction similarly in both ferrets and rats. This suggests that the gastrointestinal signals that precede emesis in ferrets are similar to

those in non-emetic rat species, suggesting that both species may experience nausea.

We propose that conditioned rejection reactions reflect conditioned nausea. Unlike the conditioned taste avoidance consumption test, these conditioned rejection reactions predict the likelihood that the drug produces sickness. It is interesting to note that hedonic ratings of liking are also more predictive of nausea than consumption of the flavoured solution in human chemotherapy patients (Schwartz et al, 1996). Furthermore, humans who report nausea as a symptom of food allergies not only avoid the food, but also report a conditional distaste for the food (Pelchat & Rozin, 1982). On the other hand, when the allergic symptoms are mouth sores or hives, the subjects avoid the food, but do not dislike its taste. Nausea may be a necessary condition for the establishment of conditioned dislike for a taste.

If the display of conditioned rejection reactions in the taste reactivity test reflects conditioned nausea, then pretreatment with an anti-nausea drug either during conditioning or at test should reduce the strength of those reactions. Indeed, the present results demonstrate that pretreatment with OND, a commonly employed anti-nausea agent used in conjunction with chemotherapy treatment of cancer in humans, interferes with both the establishment and the expression of LiCl-induced conditioned rejection reactions in rats. Conditioned rejection reactions reflect conditioned nausea in rats.

References

Balleine, B., Garner, C., & Dickinson, A. (1995). Instrumental outcome devaluation is attenuated by the anti-emetic ondansetron. Quarterly Journal of Experimental Psychology. B, 48, 235-251.

Barnes, J. M., Costall, B., Coughlan, J., Domeney, A. M., Gerrard, P. A., Kelly, M. E., Naylor, R. J., Onaivi, E. S., Tomkins, D. M., & Tyers, M. B. (1990). The effects of ondansetron, a 5-HT3 receptor antagonist, on cognition in rodents and primates. Pharmacology, Biochemistry and Behavior, 35, 955-962.

Beck, T. M. (1995). The pattern of emesis following high-dose cyclophosphamide and the anti-emetic efficacy of ondansetron. Anticancer Drugs, 6, 237-242.

Bernstein, I. L. (1978). Learned taste aversions in children receiving chemotherapy. Science, 200, 1302-1303.

Bernstein, I. L., Chavez, M., Allen, D., & Taylor, E. M. (1992). Area postrema mediation of physiological and behavioral effects of lithium chloride in the rat. Brain Research, 575, 132-137.

Bernstein, I. L., & Webster, M. M. (1980). Learned taste aversions in humans. Physiology and Behavior, 25, 363-366.

Berridge, K. C., Grill, H. J., & Norgren, R. (1981). Relation of consummatory responses and preabsorptive insulin release to palatability and learned taste aversions. Journal of Comparative and Physiological Psychology, 95, 363-382.

Berridge, K. C., & Pecina, S. (1995). Bendzodiazepines, appetite, and taste palatability. Neuroscience and Biobehavioral Reviews, 19, 121-131.

Berridge, K. C., & Treit, D. (1986). Chlordiazepoxide directly enhances positive ingestive reactions in rats. Pharmacology, Biochemistry and Behavior, 24, 217-221.

Best, M., Best, P., & Mickley, G. (1973). Conditioned aversion to distinct environmental stimuli resulting from gastrointestinal distress. Journal of Comparative and Physiological Psychology, 85, 250-257.

Blackshaw, L. A., & Grundy, D. (1993a). Effects of 5-hydroxytryptamine on discharge of vagal mucosal afferent fibres from the upper gastrointestinal tract of the ferret. Journal of the Autonomic Nervous System, 45, 41-50.

Blackshaw, L. A., & Grundy, D. (1993b). Effects of 5-hydroxytryptamine (5-HT) on the discharge of vagal mechanoreceptors and motility in the upper gastrointestinal tract of the ferret. Journal of the Autonomic Nervous System, 45, 51-59.

Borison, H. L., & Wang, S. C. (1953). Physiology and pharmacology of vomiting. Pharmacology Review, 5, 193-230.

Carey, M. P., & Burish, T. G. (1988). Etiology and treatment of the psychological side effects associated with cancer chemotherapy: A critical review and discussion. Psychological Bulletin, 104, 307-325.

Coil, J. D., Hankins, W. G., Jenden, D. J., & Garcia, J. (1978). The attenuation of a specific cue-to-consequence association by antiemetic agents. Psychopharmacology, 56, 21-25.

Costall, B., & Naylor, R. J. (1991). Anxiolytic effects of 5-HT_3 antagonists in animals. In: R. J. Rodgers and S. J. Cooper (Eds.), 5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines: their comparative behavioral pharmacology (pp. 133-159). Chichester: Wiley.

Cubeddu, L. X., Hoffmann, I. S., Fuenmayor, N. T., & Finn, A. L. (1990). Efficacy of ondansetron (GR38032F) and the role of serotonin in cisplatin-induced nausea and vomiting. New England Journal of Medicine, 322, 810-816.

Cunningham, C. L. (1979). Flavor and location aversions produced by ethanol. Behavioral and Neural Biology, 27, 362-367.

Davies, A. M., & Parker, L. A. (1993). Fenfluramine-induced place aversion in a three-choice apparatus. Pharmacology, Biochemistry and Behavior, 44, 595-600.

Eckel, L. A., & Ossenkopp, K. P. (1996). Area postrema mediates the formation of rapid, conditioned palatability shifts in lithium-treated rats. Behavioral Neuroscience, 110, 202-212.

Fletcher, P. J., & Higgins, G. A. (1997). Differential effects of ondansetron and alpha-flupenthixol on responding for conditioned reward. Psychopharmacology, 134, 64-72.

Fox, S. M., Einhorn, L. H., Cox, E., Powell, N., & Abdy, A. (1993). Ondansetron versus ondansetron, dexamthasone, and chlorpromazine in the prevention of nausea and vomiting associated with multiple-day cisplatin chemotherapy. Journal of Clinical Oncology, 11, 2391-2395.

Frisch, C., Hasenohrl, R. U., Mattern, C. M., Hacker, R., & Huston J. P. (1995). Blockade of lithium chloride-induced conditioned place aversion as a test for antiemetic agents: Comparison of metoclopramide with combined extracts of *zingiber officinale* and *ginkgo biloba*. Pharmacology Biochemistry and Behavior, 52, 321-327.

Garcia, J., Hankins, W. G., & Rusiniak, K. W. (1974). Regulation of the milieu interne in man and rat. Science, 185, 823-831.

Goudie, A. J., Stolerman, I. P., Demellweek, C., & D'Mello, G. D. (1982). Does conditioned nausea mediate drug-induced conditioned taste aversion? Psychopharmacology, 78, 277-281.

Grill, H. J., & Norgren, R. (1978). The taste reactivity test. I: Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Research, 143, 263-279.

Grundy, D. (1998) Visceral afferents and their modulation in animal models of nausea and vomiting. Paper presented at the International Symposium on Nausea and Vomiting - A Multidisciplinary Approach. Tutzing, Germany, June, 1998.

Hawthorn, J., Ostler, K. J., & Andrews, P. L. (1988). The role of the abdominal visceral innervation and 5-hydroxytryptamine M-receptors in vomiting induced by the cytotoxic drugs cyclophosphamide and cis-platin in the ferret. Quarterly Journal of Experimental Physiology, 73, 7-21.

Jorenby, D. E., Steinpreis, R. E., Sherman, J. E., & Baker, T. B. (1990). Aversion instead of preference learning indicated by nicotine place conditioning in rats. Psychopharmacology, 82, 241-247.

McCarthy, L. E., & Borison, H. L. (1984). Animal models for predicting antiemetic drug activity. In: Laszlo, J. (Ed.) Antiemetics and cancer chemotherapy (pp. 21-33). Baltimore, MD: Williams and Wilkins.

Mele, P. C., McDonough, J. R., McLean, D. B., & O'Halloran, K. P. (1992). Cisplatin-induced conditioned taste aversion: Attenuation by dexamthasone but not zacopride or GR38032F. European Journal of Pharmacology, 218, 229-236.

Mitchelson, F. (1992). Pharmacological agents affecting emesis. A review (Part II). Drugs, 43, 443-463.

Morran, C., Smith, D. C., Anderson, D. A., & McArdle, C. S. (1979). Incidence of nausea and vomiting with cytotoxic chemotherapy: A prospective randomized trial of antiemetics. British Medical Journal, 1, 1323-1324.

Morrow, G. R., & Dobkin, P. L. (1988). Behavioral approaches for the management of adversive side effects of cancer treatment. Psychiatric Medicine, 5, 299-314.

Morrow, G. R., & Morrell, C. (1982). Behavioral treatment for the anticipatory nausea and vomiting induced by cancer chemotherapy. New England Journal of Medicine, 307, 1476-1480.

Mucha, R. F., & Iverson, S. (1985). Reinforcing properties of morphine and naloxone revealed by conditioned place preferences: A procedural examination. Psychopharmacology, 82, 241-247.

Norgren, R., & Pfaffman, C. (1975). Pontine taste area in the rat. Brain Research, 91, 99-117.

Overton, D. A. (1974). Experimental methods for the study of state-dependent learning. Federation Proceedings, 33, 1800-1813.

Parker, L. A. (1982). Nonconsummatory and consummatory behavioral CRs elicited by lithium- and amphetamine-paired flavors. Learning and Motivation, 13, 281-303.

Parker, L. A. (1984). Behavioral conditioned responses across multiple conditioning/testing trials elicited by lithium- and amphetamine-paired flavors. Behavioral and Neural Biology, 41, 190-199.

Parker, L. A. (1988). A comparison of avoidance and rejection responses elicited by conditionally and unconditionally aversive tasting solutions. Learning and Motivation, 19, 1-12.

Parker, L. A. (1993). Taste reactivity responses elicited by cocaine-, phencyclidine-, and methamphetamine-paired sucrose solutions. Behavioral Neuroscience, 107, 118-129.

Parker, L. A. (1995a). Rewarding drugs produce taste avoidance, but not taste aversion. Neuroscience and Biobehavioral Reviews, 19, 143-151.

Parker, L. A. (1995b). Chlordiazepoxide enhances the palatability of lithium-, amphetamine-, and saline-paired saccharin solution. Pharmacology, Biochemistry and Behavior, 50, 395-400.

Parker, L. A. (1998). Emetic drugs produce conditioned rejection reactions in the taste reactivity test. Journal of Psychophysiology, 12, 3-13.

Parker, L. A., & Brosseau, L. (1990). Apomorphine-induced flavor-drug associations: A dose-response analysis by the taste reactivity test and the conditioned taste avoidance test. Pharmacology, Biochemistry, and Behavior, 35, 583-587.

Parker, L. A., & Gillies, T. (1995). THC-induced place and taste aversions in Lewis and Sprague-Dawley rats. Behavioral Neuroscience, 109, 71-78.

Parker, L. A., & McLeod, K. B. (1991). Chin rub CRs may reflect conditioned sickness elicited by a lithium-paired sucrose solution. Pharmacology, Biochemistry and Behavior, 40, 983-986.

Pelchat, M. L., Grill, H. J., Rozin, P., & Jacobs, J. (1983). Quality of acquired responses to tastes by *Rattus norvegicus* depends on type of associated discomfort. Journal of Comparative Psychology, 97, 140-153.

Pelchat, M. L., & Rozin, P. (1982). The special role of nausea in the acquisition of food dislikes by humans. Appetite, 3, 341-355.

Rabin, B. M., & Hunt, W. A. (1983). Effects of antiemetics on the acquisition and recall of radiation and lithium chloride induced conditioned taste aversions. Pharmacology, Biochemistry and Behavior, 18, 629-636.

Reicher, M. A., & Holman, E. W. (1977). Location preference and flavor aversion reinforced by amphetamine in rats. Animal Learning and Behavior, 5, 343-346.

Revusky, S., & Martin, G. M. (1988). Glucocorticoids attenuate taste aversions produced by toxins in rats. Psychopharmacology, 96, 400-407.

Roychoudhury, M., & Kulkarni, S. K. (1997). Effects of ondansetron on short-term memory retrieval in mice. Methods Find Exp Clin Pharmacol, 19, 43-46.

Schwartz, M. D., Jacobsen, P. B., & Bovbjerg, D. H. (1996). Role of nausea in the development of aversions to a beverage paired with chemotherapy treatment in cancer patients. Physiology & Behavior, 59, 659-653.

Spector, A. C., Breslin, P., & Grill, H. J. (1988). Taste reactivity as a dependent measure of the rapid formation of conditioned taste aversion: A tool for the neural analysis of taste-visceral associations. Behavioral Neuroscience, 102, 942-952.

van der Kooy, D. (1987). Place conditioning: A simple and effective method for assessing the motivational properties of drugs. In M. A. Bozarth (Ed.), Methods of assessing the reinforcing properties of abused drugs (pp. 229-240). New York: Springer-Verlag.

Wang, S. C., & Borison, H. L. (1952). A new concept of organization of the central emetic mechanism: Recent studies on the sites of action of apomorphine, copper sulfate and cardiac glycosides. Gastroenterology, 22, 1-12.

Weddington, W. W., Miller, N. J., & Sweet, D. L. (1982). Anticipatory nausea and vomiting associated with cancer chemotherapy. New England Journal of Medicine, 307, 825-826.

Wegener, G., Smith, D. F., & Rosenberg, R. (1997). 5-HT1A receptors in lithium-induced conditioned taste aversion. Psychopharmacology, 133, 51-54.

Wise, R. A., Yokel, R. A., & DeWit, H. (1976). Both positive reinforcement and conditioned aversion from amphetamine and from apomorphine in rats. Science, 191, 1273-1275.

Zalaquett, C., & Parker, L. A. (1989). Further evidence that CTAs produced by lithium and amphetamine are qualitatively different. Learning and Motivation, 20, 413-427.

Table 1

Procedure for Experiment 1

Surgical Implantation of Intraoral Cannulae

Water Deprivation Schedule (4 days)

Conditioning Trial (1 trial)

OND → 0.1% Saccharin →LiCl

Saline → 0.1% Saccharin →LiCl

OND → 0.1% Saccharin →Saline

Saline → 0.1% Saccharin →Saline

Taste Reactivity (TR) Adaptation Trial (1 trial)

1 min intraoral infusion of water

Taste Reactivity (TR) Test

1 min intraoral infusion of 0.1% saccharin

Table 2

Procedure for Experiment 2

Surgical Implantation of Intraoral Cannulae

Water Deprivation Schedule (3 days)

Conditioning Trial (1 trial) (group assignment)

0.1% Saccharin → LiCl

0.1% Saccharin → Saline

Taste Reactivity (TR) Adaptation Trial (1 trial)

1 min intraoral infusion of water

Taste Reactivity (TR) Test

(within groups design counterbalancing across 3 test days)

0.01, 0.1 mg/kg OND, or saline → 1 min intraoral infusion of 0.1% saccharin

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Table 3

Procedure for Experiment 3a

Water Deprivation Schedule (4 days)
Conditioning Trial (1 trial)
0.1% Saccharin → LiCl
0.1% Saccharin → Saline
Taste Avoidance Test (48 hours after conditioning)
(LiCl conditioned group): OND → Saccharin & Water
Saline → Saccharin & Water
(Saline conditioned group): OND → Saccharin & Water
Saline → Saccharin & Water

Table 4

Procedure for Experiment 3b

	Conditioning Trial (1 cycle)		
	Day 1	Day 2	Test Trial (48 hr after conditioning)
[Group MESH +]	Saline + Sand	LiCl + Mesh	OND → Test
			Saline → Test
[Group MESH -]	Saline + Mesh	LiCl + Sand	OND → Test
			Saline → Test

Figure Captions

Figure 1. Mean (± sem) ml saccharin solution consumed during conditioning trial of Experiment 1 in rats pretreated with saline or 0.1 mg/kg ondansetron.

Figure 2. Mean (± sem) frequency of rejection reactions, ingestion reactions, and activity elicited by LiCl- or saline-paired saccharin solution during the TR test of Experiment 1. These animals received ondansetron or saline 30 min prior to conditioning.

Figure 3. Mean (± sem) frequency of rejection reactions, ingestion reactions, and activity elicited by LiCl- or saline-paired saccharin solution following exposure to 0.0, 0.01, or 0.1 mg/kg of ondansetron 30 min prior to the TR tests of Experiment 2.

Figure 4. Mean (± sem) frequency of rejection reactions elicited by unconditionally unpalatable quinine solution displayed following injection of 0.0, 0.01, or 0.1 mg/kg of ondansetron (Experiment 2).

Figure 5. Mean (± sem) preference ratio for LiCl- and saline-paired saccharin solution following pretreatment with saline or ondansetron in the taste avoidance test.

Figure 6. Mean (± sem) sec on mesh side for groups Mesh+ and Mesh- following pretreatment with saline or ondansetron in the place preference test.

Figure 1

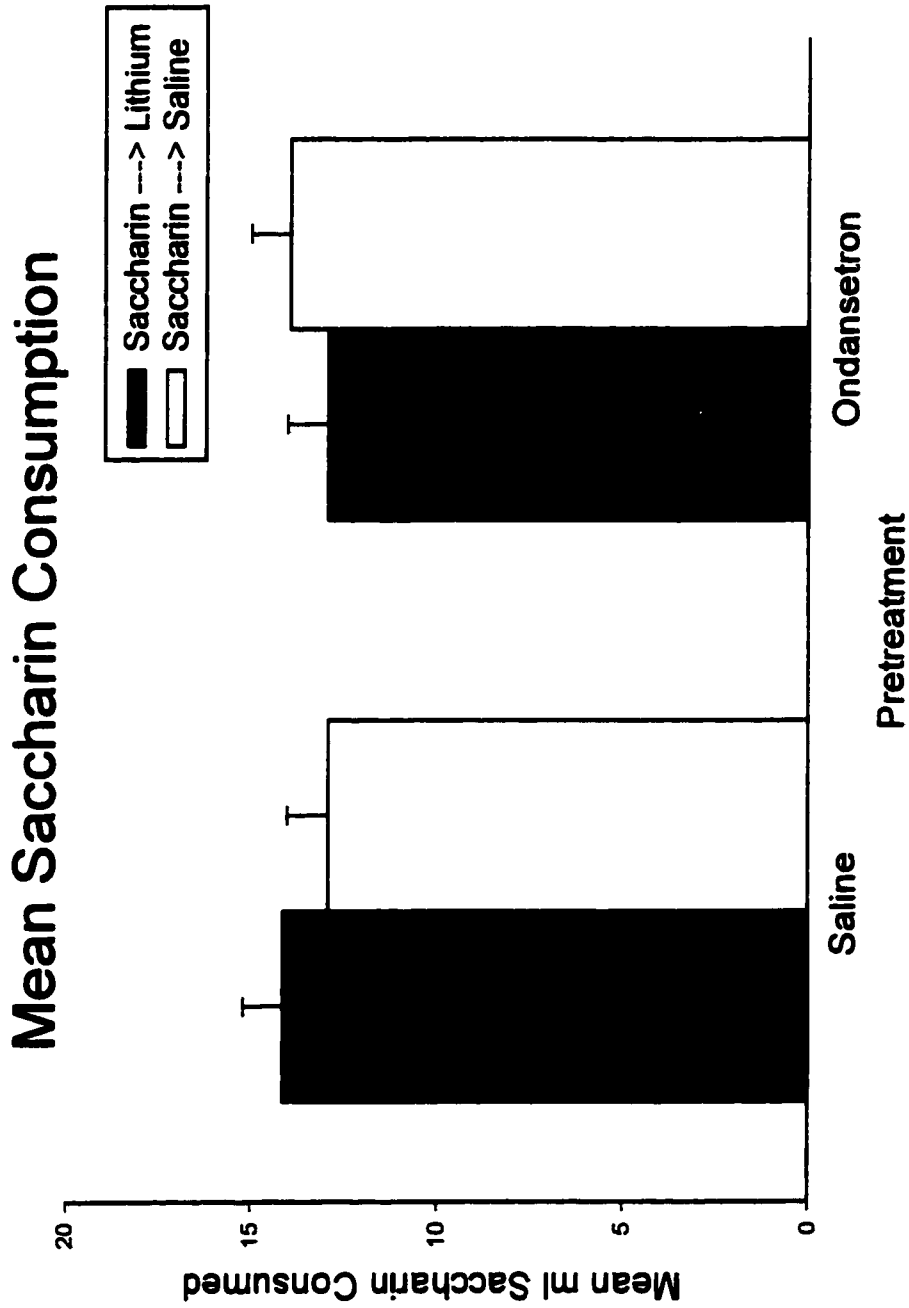
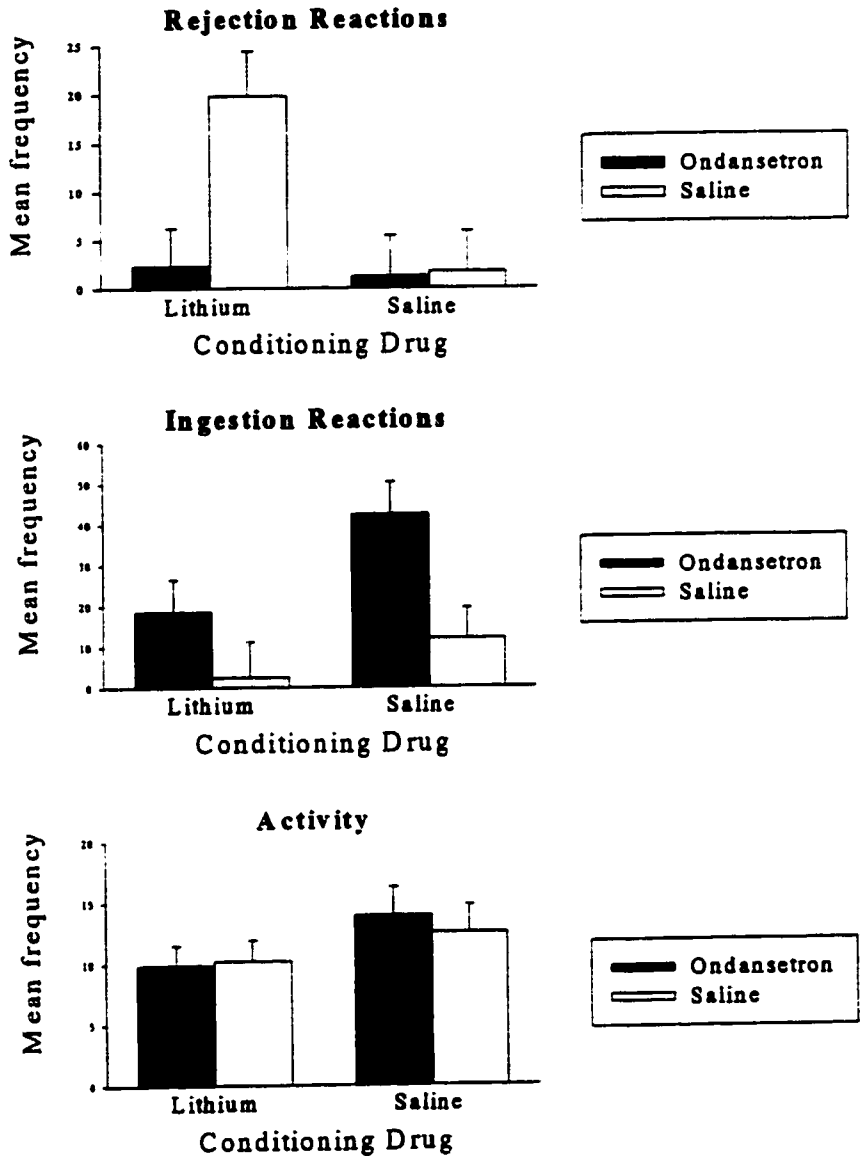

Mean Saccharin Consumption
Saccharin ---> Lithium
Saccharin ---> Saline
Mean ml Saccharin Consumed
0
5
10
15
20
Saline
Ondansetron
Pretreatment

Figure 2

Taste Reactivity Measures

Establishment



Taste Reactivity Measures

Figure 3

Expression

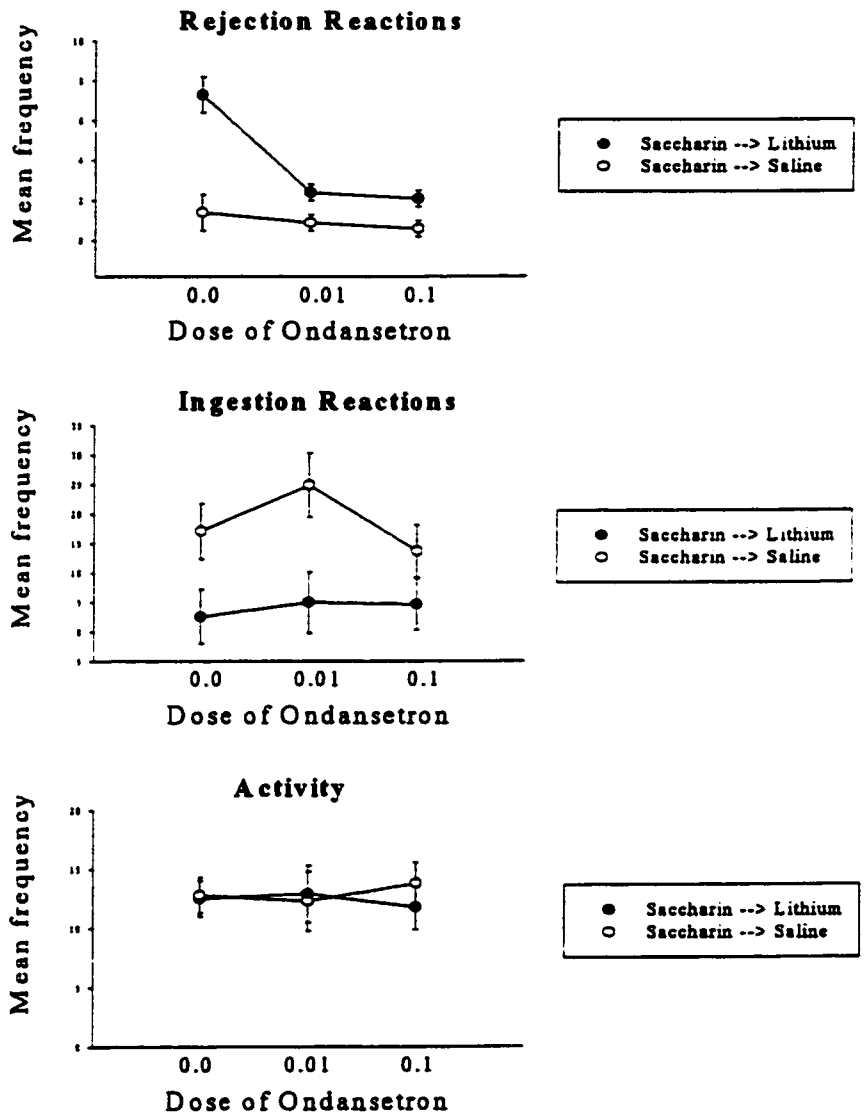


Figure 4

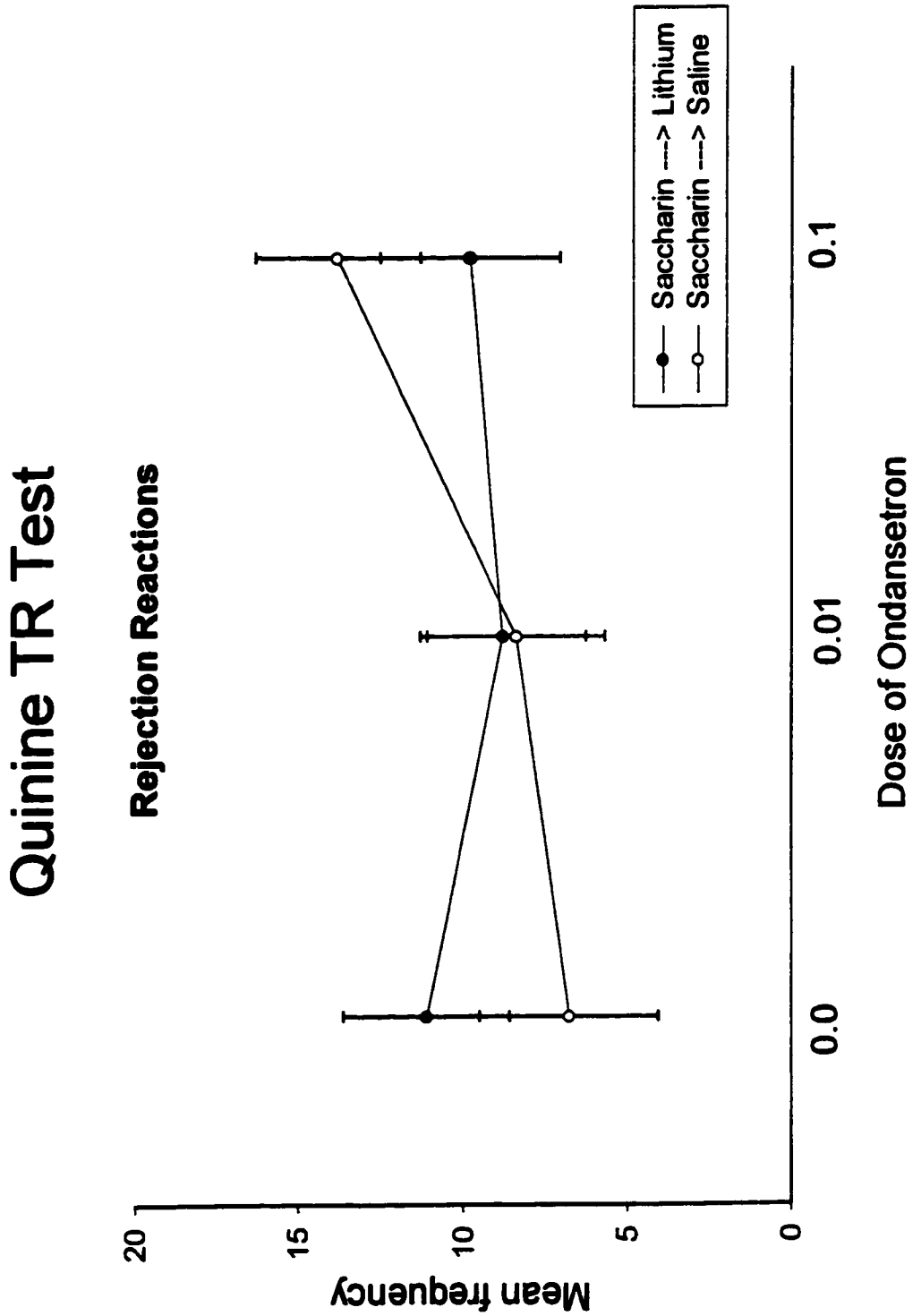

Quinine TR Test
Rejection Reactions
Mean frequency
20
15
10
5
0
0.0
0.01
0.1
Dose of Ondansetron
Saccharin ---> Lithium
Saccharin ---> Saline

Figure 5

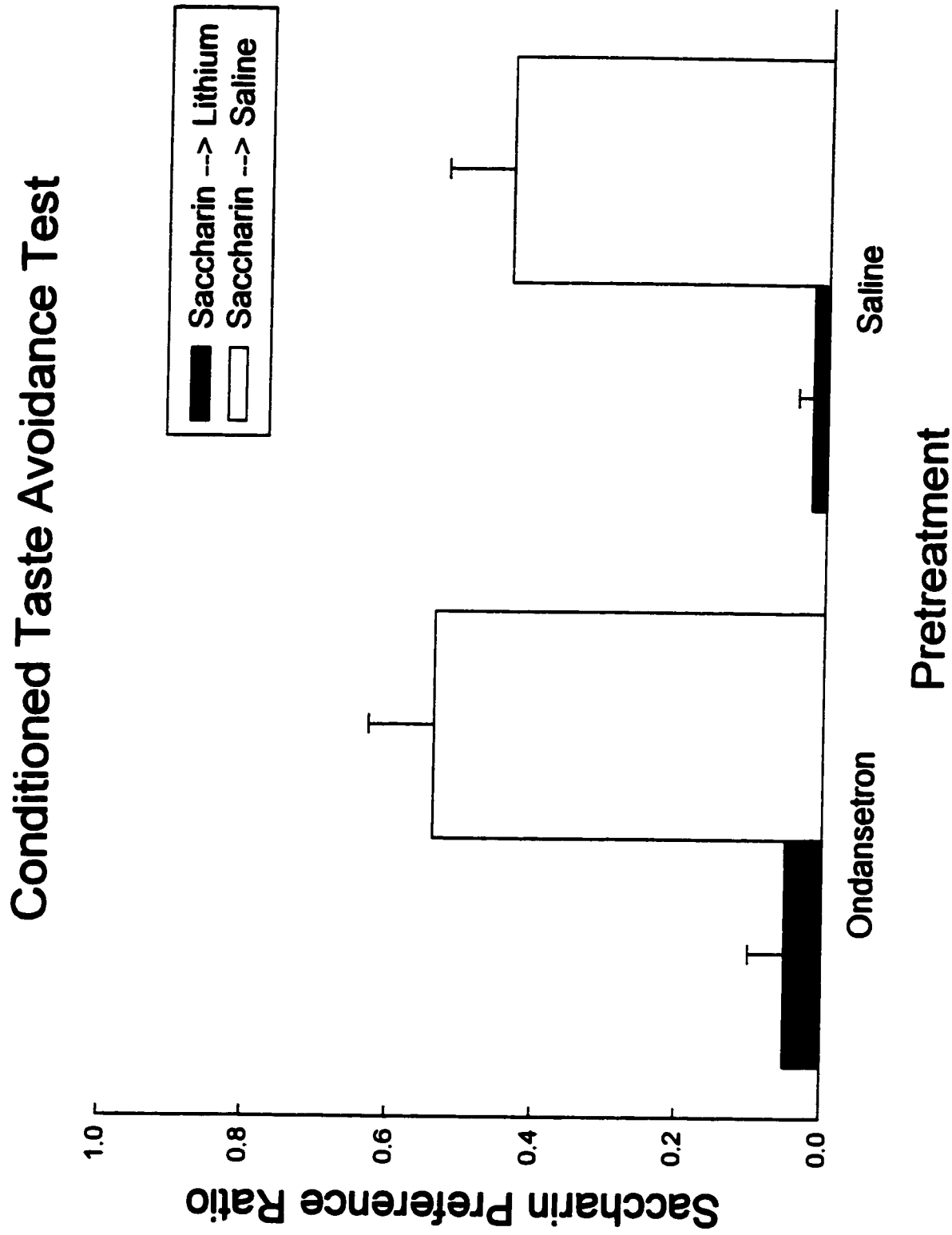
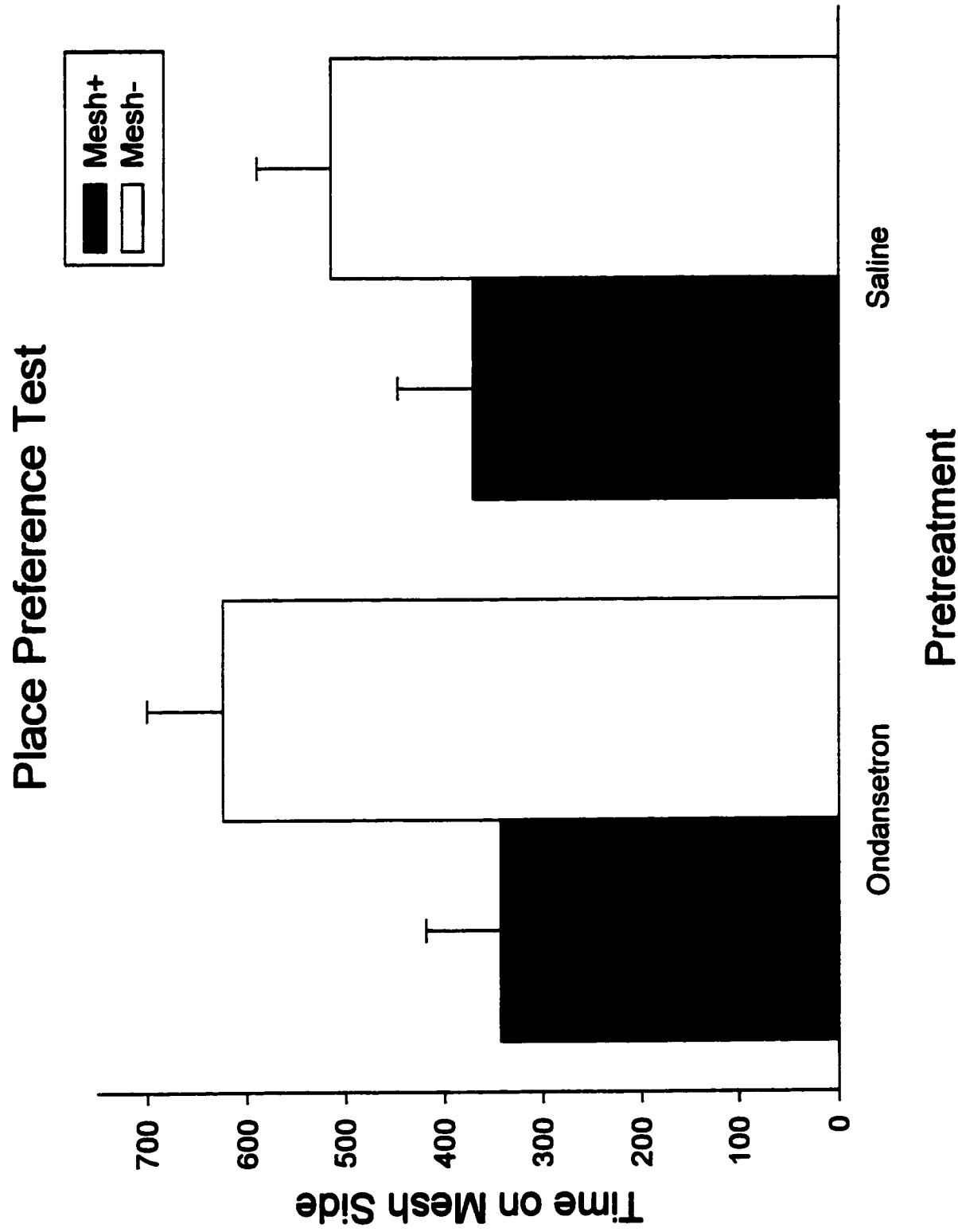

Conditioned Taste Avoidance Test
Saccharin --> Lithium
Saccharin --> Saline
Saccharin Preference Ratio
1.0
0.8
0.6
0.4
0.2
0.0
Ondansetron
Saline
Pretreatment

Figure 6


Place Preference Test
Mesh+
Mesh-
Time on Mesh Side
0
100
200
300
400
500
600
700
Ondansetron
Saline
Pretreatment