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Magdalena Kwiatkowska
Wilfrid Laurier University

Effect of Ondansetron and Δ^9-Tetrahydrocannabinol on the Establishment of Lithium-Induced Conditioned Taste Avoidance in the House Musk Shrew (*Suncus murinus*)

by

Magdalena Kwiatkowska

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Abstract

Recent evidence suggests that toxin-induced taste avoidance in the non-emetic rat is not mediated by conditioned sickness. In contrast, it appears that toxin-induced taste avoidance in an emetic species is mediated by conditioned sickness. The present experiments evaluated the potential of the anti-emetic agents, ondansetron [OND; a serotonin receptor ($5\text{-}HT_3$) antagonist] and Δ^9-tetrahydrocannabinol [THC; a cannabinoid (CB_1) agonist] to interfere with lithium chloride (LiCl)-induced taste avoidance in the house musk shrew (*Suncus murinus).* In Experiment 1, shrews were pretreated with OND (1.5 mg/kg) or saline 30 min prior to drinking 0.1% saccharin solution then they were injected with LiCl (390 mg/kg) or saline. When assessed by a two-bottle test over a 12 hr period, but not a one-bottle test, the shrews displayed a LiCl-induced saccharin avoidance that was prevented by pretreatment with OND. The relatively weak effects may have been due to floor effects in consumption of saccharin solution; therefore a highly preferred 0.3 M sucrose solution was used in Experiment 2. In Experiment 2, shrews were pretreated with OND, THC (5 and 10 mg/kg) or Vehicle 30 min prior to sucrose solution exposure. With a more highly preferred sucrose solution, OND and THC interfered with the establishment of LiCl-induced taste avoidance detected with one-bottle test. These results suggest that taste avoidance in the shrew, unlike the rat, is motivated by conditioned sickness.

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Table of abbreviations

2-AG	2-arachidonoyl glycerol; endocannabinoid
AP	Area postrema
CB	Cannabinoid receptor
CB_1	Specific cannabinoid receptor found throughout Central Nervous System
CB_2	Specific cannabinoid receptor found in the periphery
CBD	Cannabidiol
CR	Conditioned Response
CS	Conditioned Stimulus
CTA	Conditioned taste avoidance
DMNX	dorsal motor nucleus
5-HT	5-hydroxytrypatamine, Serotonin receptor
$5\text{-}HT_3$	5-hydroxytrypatamine type 3 receptor
HU-210	Synthetic cannabinoid
i.p.	Intraperitoneal
i.v.	Intravenously
LiCl	Lithium Chloride
NA	Nucleus accumbens
NTS	Nucleus of the solitary tract
OND	Ondansetron

8-OH-DPAT	5-HT_{1A} serotonin autoreceptor agonist
p.o.	Oral
PR	Preference Ratio
THC	-9-Tetrahydrocannabinol
SR	SR-141716A, rimonabant
	selective CB_1 antagonist
UCS	Unconditioned stimulus

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Effect of Ondansetron and Δ-9-Tetrahydrocannabinol on the Establishment of Lithium-Induced Conditioned Taste Avoidance in the House Musk Shrew (*Suncus murinus*)

Survival depends on an organisms ability to acquire adequate food. In order to maintain optimum internal homeostasis, organisms have innate and learned behavioural mechanisms that allow for the recognition of food and regulation of its intake. Much of what we know about food selection comes from studying the non-emetic rat. Omnivores, such as rats, can take advantage of all possible food substances as nutrients to survive; however, this benefit also induces the risk of lethal poisoning. Through natural selection, rats have developed protective mechanisms as a means of reducing the likelihood of poisoning. The most primitive device is the unconditioned reflex, involving receptors of the oral and nasal cavities. These receptors respond to bitter tastes that normally accompany natural toxins and signal the animal not to ingest these substances (Bures, Buresova & Krivanek, 1988).

At higher levels of protective devices the rat uses the anticipated consequences of the ingested food to make a decision to eat the food or to not eat. This decision is based on previous experience with the food. When a substance does not match a memory of a previous encounter, neophobic behaviour is elicited (Barnett, 1958). In other words, the rat consumes small amounts of the food and waits for a long period of time to resume consumption in order to prevent risk of poisoning. Through this behaviour, rats are better able to evaluate the consequences of the food ingestion as well as to reduce the potential of poisoning. The sensory attributes of the novel substance are stored in memory and

compared with the gustatory properties to mark the experience with the food as neutral, pleasant or aversive. If the outcome of digestion and absorption is good (nutrient repletion), the taste will be remembered as acceptable and will be consumed in the future. Since the food is still desirable, external cues associated with this food will continue to be sought by the animal as it forages in the external environment.

If the food is poisoned, a different series of events take place. Many poisons produce gastric distress which are signals that are brought by the vagus nerve from the gut to the nucleus of the solitary tract (NTS) of the brainstem, which is also known as the "emetic center" (Borison & Wang, 1949). In emetic species, sufficient stimulation in the NTS results in vomiting which purges the poison. Poisons that do not irritate the gut may remain long enough to be absorbed into the blood. If this is the case, then vomiting occurs when the area postrema (AP) is stimulated by blood-born poisons. If the poison is removed by vomiting or in the non-emetic rat by the lengthier and usually more punishing process of detoxification then the animal may fully recover. The only lasting effect will be a "memory" for the taste of food eaten before the onset of the illness. Upon subsequent exposure to that food the animal will avoid the food. These events are classified as Conditioned Taste Avoidance (CTA). CTA is a robust defence device protecting organisms against repeated consumption of toxic food.

Conditioned Taste Avoidance Learning

CTA is an evolutionary conserved behaviour, with similar forms of food aversion learning being found in vertebrate and invertebrate species despite the divergence of ancestral lines more than 500 million years ago (Bures, Bermudez-Rattoni & Yamamoto,

1998). Berenbaum & Miliczy (1983) reported that the preying mantis learned to avoid eating milkweed bugs that were previously poisoned with cardenolide poison. Similarly, blue jays have been observed to avoid monarch butterflies containing cardiac glycosides (Brower & Fink, 1985). Garden slugs (*Limax maximus*) have been documented to learn to associate vegetable flavour and poisons (Sahley, Gelperin & Rudy, 1981). In fact, this unique form of learning has been observed in a wide range of organisms.

The house musk shrew (*Suncus murinus*) is an established model for emesis research as it reliably vomits when exposed to a variety of stimuli, such as chemotherapeutic agents (Matsuki, Ueno, Kaji, Ishihara, Wang, & Saito, 1988), motion (Ueno, Matsuki, & Saito, 1988), radiation (Torii, Shikita, Saito, & Matsuki, 1993), and ethanol (Chen, Saito, & Matsuki, 1997; Hori, Fujii, Hatanaka, Suwa, 2003). Since the shrew, unlike the rat, vomits when challenged with toxins, it is a potential model for CTA that may be more similar to humans in this respect. The present investigation will evaluate CTA learning in the shrew and the potential of anti-emetic agents to interfere with this type of learning. However, since the preponderance of research in CTA learning has been conducted using rats, the literature review will first evaluate what we know of CTA learning in rats.

CTA has a number of unusual properties, which contrast sharply with the basic assumptions of traditional learning theories. In CTA in rats, the interval between the taste conditioned stimulus and the toxin-induced unconditional stimulus may occur with delays of minutes to hours, rather than seconds, yet learning still occurs (Garcia, Hankins & Rusiniak, 1974). Only a single taste-illness pairing is often sufficient to produce CTA (Garcia, Hankins & Rusiniak, 1974). Finally, CTA is extremely resistant to forgetting or

extinction and does not require repeated illness for it to be remembered long after recovery from the initial illness (Garcia, Hankins & Rusiniak, 1974).

In humans, there is evidence that the development of CTA occurs at a non-conscious level. If a cancer patient receives chemotherapy or radiotherapy and then eats food before the onset of illness induced by the cancer treatment, the patient will likely avoid eating that food in the future (Bernstein, 1985). The patient can consciously know that the illness was due to the cancer treatment rather than to the food, but will still be unable to consume this food. Also, it has been observed that wolves and coyotes, that have consumed a mutton bait containing an illness-causing substance will, long after having recovered from the illness, avoid live, moving sheep as long as they taste or smell like the bait. This is so even though the bait was not living and moving when eaten (Gustavson, Garcia, Hankins, & Rusiniak, 1974; Gustavson, Kelly, Sweeney, & Thomas, 1976). Thus, the CTA paradigm is successfully employed in conservation wildlife management and livestock protection programs. However, there are many aspects of CTA that are still not well understood; this is an area of active investigation.

In the laboratory, CTA is studied under regulated standard conditions. Typically, rats are maintained on a drinking schedule during which they have access to water for a limited period. Once a drinking schedule is established the animals are exposed to a new taste (conditioned stimulus, CS) in a bottle through which they drink and the amount of solution consumed is measured. Following the consumption period, the animal is exposed to an illness-producing agent, such as lithium chloride (LiCl) (unconditional stimulus, UCS). As a result of this pairing, upon subsequent re-exposure to the flavour, the animal avoids it (conditioned response, CR), and CTA has been formed.

Rats avoid consumption of flavours previously paired with illness produced by LiCl (Garcia & Koelling, 1967). Garcia and Koelling (1967) suggested that CTA occurred in this case as a result of the association of the flavour and the illness, making the taste unpalatable. Surprisingly, rats also avoid consumption of flavours paired with rewarding drugs, for instance amphetamine (Berger, 1970). In fact, when rats are injected with a rewarding drug, such as amphetamine or morphine and are placed in a distinctive chamber CS with a distinctive taste CS, they learn to approach the chamber but avoid the taste (Reicher & Holman, 1977). Additionally, when rats drink a saccharin solution prior to self-administration with amphetamine, they maintain self-administration of the drug but later avoid the saccharin (Wise, Yokel, & De Wit, 1976). Therefore, the same drug appears to be rewarding and aversive at the same time. These paradoxical reports in the non-emetic rat, led Gamzu (1977) to suggest that any novel change in state produces avoidance of a flavour that precedes it. It has been suggested that rats learn to avoid a taste paired with any novel change in state as a result of their inability to vomit when exposed to toxins (Davis, Harding, Leslie & Andrews, 1986).

Conditioned Taste Aversion Measured by the Taste Reactivity Test in Rats

In rats the typical measure of flavour-drug associations is by the avoidance of consumption of a flavoured solution in a bottle test. There is also an alternative measure of flavour-drug association, the taste reactivity test (Grill & Norgren, 1978). The taste reactivity test measures the orofacial reactions elicited by an intraoral infusion of the taste directly into the rat's mouth. When infused with sweet sucrose, rats display ingestive reactions of tongue protrusions, mouth movements, and paw licks. When infused with

bitter quinine, rats display rejection reactions of gaping, chin rubs and paw treads. When sweet sucrose is paired with the emetic LiCl, rats subsequently display rejection reactions during infusion of the sucrose. The most reliable rejection reaction is gaping. Parker and colleagues (1995) have shown that, although rewarding drugs (such as morphine, amphetamine and cocaine) produce avoidance of a taste with which they were paired, these agents do not produce the conditioned gaping response when paired with a flavoured solution. Only drugs that produce vomiting in emetic species can establish conditioned gaping in rats (Parker, 1998; Parker, 2003). It has been suggested that since the rat gape is topographically similar to a retch in the emetic shrew, conditioned gaping may represent a vestigial vomiting response in the non-emetic rat (Parker, 2003).

There is considerable evidence that rats experience nausea even though they are not capable of the motor response of vomiting. Grundy and colleagues (Blackshaw & Grundy, 1993; Grundy 1998; Hillsley, Kirkup & Grundy, 1992) have demonstrated that in rats the vagal gastric afferents respond in the same manner to physical and chemical (copper sulfate and cisplatin) stimulation that precedes vomiting in ferrets (seemingly resulting in nausea that precedes vomiting). Furthermore, 5-HT_3 antagonists that block vomiting in ferrets also disrupt this preceding vagal afferent reaction in rats. That is, in the rat, the detection mechanism of nausea is present but the vomiting response is absent (Davis, Harding, Leslie, & Andrews, 1986). In a classic review paper, Borrison and Wang (1953) suggest that the rats' inability to vomit can be explained as a species-adaptive neurological deficit and that, in response to emetic stimuli, the rat displays autonomic and behavioural signs corresponding to the presence of nausea, called the prodromata (salivation, pupillary dilation, tachypnoea and tachycardia).

Suppression of toxin-induced gaping but not taste avoidance, by anti-emetic pre-treatment in rats

Parker (1998) suggested that conditioned gaping in the taste reactivity test is a rat model of nausea. If conditioned gaping is motivated by nausea in rats, then anti-nausea agents should interfere with both the establishment and the expression of gaping. The most effective anti-emetic agents used to prevent the side effects of nausea and vomiting in chemotherapy patients are drugs that antagonize serotonin receptors. Limebeer and Parker (2000) found that ondansetron (OND), a 5-HT_3 receptor antagonist, prevented both the establishment and the expression of LiCl-induced conditioned gaping. When administered prior to conditioning, OND interfered with LiCl-induced nausea and subsequently prevented the establishment of conditioned gaping in rats. Furthermore, when administered prior to testing, OND interfered with previously established conditioned nausea and therefore prevented the expression of LiCl-induced conditioned gaping in rats. On the other hand, OND did not interfere with either the establishment or the expression of LiCl-induced conditioned taste avoidance using both one-bottle and two-bottle tests (see also, Rudd, Ngan & Wai, 1998). Similarly, the 5-HT_{1A} serotonin autoreceptor agonist, 8-OH-DPAT, which decreases serotonin availability, also interfered with the establishment and expression of LiCl-induced conditioned gaping reactions, but not taste avoidance (Limebeer & Parker, 2003). On the basis of these dissociations, Limebeer and Parker (2000, 2003) suggest that conditioned gaping, but not avoidance, reflects nausea in rats, a species that does not vomit in response to toxins.

Considerable anecdotal and recent experimental evidence (eg. Kwiatkowska, Parker, Burton & Mechoulam, 2004; Darmani, 2001a) suggests that marijuana is an

effective anti-nausea drug. Although marijuana contains over 60 different cannabinoid compounds, only delta-9-tetrahydrocannabinol (THC) is intoxicating. In addition to THC, the non-intoxicating compound, Cannabidiol (CBD) is highly prevalent in marijuana. Recent evidence (Limebeer & Parker, 1999; Parker et al., 2002; Parker et al., 2003) revealed that THC and CBD suppress nausea in rats. THC was also found to interfere with cyclophosphamide (a chemotherapeutic agent)-induced (Limebeer & Parker, 1999) and LiCl-induced conditioned gaping (Parker et al., 2003), but not taste avoidance. Interestingly, CBD also interferes with LiCl-induced gaping, but not taste avoidance (Parker, Mechoulam & Schlievert, 2002). Furthermore, the potent synthetic cannabinoid, HU-210, interfered with toxin-induced conditioned gaping, and the CB_1 receptor antagonist SR 141716 blocked this effect. Most intriguingly, the antagonist actually potentiated LiCl-induced conditioned gaping, suggesting that endogenous cannabinoids may play a role in the regulation of nausea (Parker, Mechoulam, Schlievert, Abbott, Fudge, & Burton, 2003). These findings, that anti-emetic agents suppress conditioned gaping but not taste avoidance, indicate that toxin-induced conditioned gaping in rats is mediated by nausea.

Gamzu (1977) suggested that any novel change in state (unpleasant or pleasant) produces CTA in the rat, because the rat cannot vomit. Davis, Harding, Leslie, & Andrews (1986) suggest that rats have a tiered system of defence against toxins which protects the rat against increasing penetration by toxins. The first line of defence signals danger any time a new food is consumed and is followed by a change in physiological state. This results in avoidance of the food in the future. The first line of defence is mediated by smell and taste receptors which can produce effects of nausea and avoidance

of the toxin. The second line of defence is intragastric, at this point gastric chemoreceptors mediate the defence action. Within the vascular system lies the third defence level, which is mediated by chemoreceptors in the trigger zone within the central nervous system. The effects of nausea (i.e. decreased gastric motility, vomiting, and avoidance of a toxin) can result through activation of the second and third lines of defence. Rats have developed a highly sensitive first line of defence (taste and smell) which signals danger when a novel taste is followed by a change in physiological state. As a result, the subsequent levels of defence are non-functional. As mentioned earlier, rats have similar physiological mechanisms in response to toxins as do emetic species; however, in rats the emetic response is absent (Davis et al., 1986). Since anti-emetic treatments, do not affect the strength of CTA produced by emetogenic drugs, a process other than conditioned nausea must mediate CTA in rats. Since conditioned taste avoidance does not appear to be motivated by conditioned nausea, Parker (2003) suggested that it may be motivated by conditioned fear.

Rats vs. Shrews: Conditioned Taste Avoidance/Preference

Rats cannot vomit, but shrews vomit in response to toxin challenge. Both rats and shrews avoid a taste paired with the emetic drug LiCl (Smith, Friedman & Andrews, 2001). However, they differ in their response to a taste paired with the rewarding drugs morphine and amphetamine. Parker, Corrick, Limebeer, & Kwiatkowska (2002) evaluated the hedonic properties of amphetamine and morphine in the *Suncus murinus* (house musk shrew), an insectivore that is capable of vomiting. Unlike rats, shrews displayed amphetamine and morphine conditioned sucrose (0.3 M) and saccharin (0.1%)

preference, when measured by both a one and a two bottle test. Amphetamine and morphine were also observed to produce a place preference. The results suggest the potential of rewarding drugs to produce taste avoidance may vary on the basis of the ability of the species to vomit.

The avoidance of a taste paired with LiCl in the shrew may be the result of conditional sickness, because they vomit in response to toxins. Although anti-emetic drugs do not interfere with the establishment of toxin-induced taste avoidance in the non-emetic rat (Limebeer & Parker, 2000, 2003), it is not known whether this would interfere with the establishment of toxin-induced taste avoidance in the emetic shrew. The experiments in this thesis address this issue.

Effects of serotonin anti-emetics on vomiting

Considerable evidence indicates that serotonergic agonists induce emesis and antagonists act as anti-emetics. In fact, the discovery of the anti-emetic properties of 5-HT_3 antagonists, such as OND, has had a remarkable impact on reducing the incidence of chemotherapy-induced vomiting in cancer patients (Hesketh et al., 2001; Hickok et al., 2003; Schnell, 2003). In the past decade, numerous investigations evaluated the anti-emetic properties of 5-HT_3 antagonists in a variety of species, including cats (Rudd, Tse, & Wai, 2000), ferrets (Ozaki, & Sukamoto, 1999) and shrews (Darmani, 1998). There is a high concentration of 5-HT_3 receptors in the emetic areas of the brainstem, including the area postrema, vagus and solitary tract nuclei (Mitchelson, 1992). Since serotonin is involved in the emetic responses, blocking receptors in these areas results in the attenuation of emesis.

The house musk shrew (*Suncus murinus*) is an established model for emesis research as it reliably vomits when exposed to a variety of stimuli, such as chemotherapeutic agents (Matsuki, Ueno, Kaji, Ishihara, Wang, & Saito, 1988), motion (Ueno, Matsuki, & Saito, 1988), radiation (Torii, Shikita, Saito, & Matsuki, 1993), and ethanol (Chen, Saito, & Matsuki, 1997; Hori, Fujii, Hatanaka, Suwa, 2003). Considerable research has been conducted with the *Suncus murinus* investigating the anti-emetic efficacy of a number of 5-HT_3 receptor antagonists including: GK-128 (Ito et al., 1995), OND (Ito et al., 1995; Torii, Saito, & Matsuki, 1991), granisetron (Torri et al., 1991; Andrews, Torii, Saito, & Matsuki, 1996), and tropisetron (Matsuki et al., 1997; Andrews et al., 2000).

The 5-HT_3 antagonists have been shown to be highly effective in reducing acute vomiting produced by cisplatin in the shrew. For example, Ito et al. (1995) investigated the inhibitory effects of GK-128, OND and granisetron on cisplatin-induced emesis in *Suncus murinus*. Shrews were administered cisplatin (30 mg/kg) as an emetic agent, but between 30 and 45 min prior to the toxin they received i.p., i.v. or oral GK-128 as an anti-emetic. By all routes of administration, GK-128 prolonged the latency to the first emetic episode and decreased the number of episodes in a dose-dependent manner. The inhibitory effect of the 5-HT_3 antagonists GK-128 and OND (3 mg/kg, i.p.) on vomiting disappeared about 6 hr after the injection. This may have valuable implications for anti-emetic therapy since cisplatin-induced emesis includes not only acute phases (1-12 hr), but also a delayed phase that lasts for several days. If OND anti-emetic effects are observed to disappear within 6 hours, this may not be the drug of choice for patients receiving cisplatin chemotherapy. Most recently, Kwiatkowska et al., (2004) reported that

OND (0.02-6.0 mg/kg) effectively inhibited cisplatin induced retching and vomiting in the shrew in a dose-dependent manner.

Effect of Cannabinoid anti-emetics on emesis

Another system that plays a role in nausea and vomiting is the endocannabinoid system (e.g. Darmani, 2001). Cannabinoids (the principle chemical entities of cannabis) have anti-emetic properties in humans (Sallen, Zinberg, & Frei, 1975), cats (McCarthy & Borison, 1981), ferrets (Simoneau et al., 2001; Ferrari, Ottani & Giuliani, 1999), pigeons (Feigenbaum, Richmond, Weissman, & Mechoulam, 1989), and shrews (Darmani, 1998; Darmani, 2001a; Darmani, 2001b; Darmani, 2001c). Δ^9-Tetrahydrocannabinol (THC) is the psychoactive constituent of marijuana (*Cannabis sativa*) (Ganoi & Mechoulam, 1964). THC acts at the cannabinoid (CB) receptors, CB_1 and CB_2. Only CB_1 receptors are found in the central nervous system; this receptor mediates the psychotropic and other effects of cannabinoids (Iversen, 2003). Both CB_1 and CB_2 receptors are found peripherally, with the CB_2 receptors predominantly found on immune cells; cannabinoids may alter immune functions by binding to CB_2 receptors (Pertwee, 1997). In the shrew, it appears that anti-emetic properties of cannabinoids result centrally from the activation of the CB_1 receptor (Darmani, 2001a; Darmani 2001b; Darmani 2001c; Simoneau et al., 2001).

Clinical evidence suggests that THC and its synthetic analog (nabilone) can prevent emesis in cancer patients receiving chemotherapy (Marmor, 1998; Andrews, Naylor, & Joss, 1998). Existing animal studies, while limited, also support the anti-emetic potential of cannabinoids. In a dose related manner, THC reduced the frequency

of vomiting and the number of shrews that vomited following an injection of cisplatin (chemotherapeutic agent) in the least shrew (*Cryptotis parva*) (Darmani, 2001b). Darmani (2001c) reports that SR-141716A (SR; CB_1 receptor antagonist) blocks the anti-emetic activity of CB agonists at low doses and produced vomiting on its own at higher doses (Darmani, 2001a). Van Sickle et al. (2001) investigated the role of CB_1 receptors and endocannabinoids in the anti-emetic properties of cannabinoid agonists, THC, WIN 55,212-2, and methanandamide in ferrets. The cannabinoid agonists inhibited emesis while the CB_1 antagonists reversed the effect. When administered alone, the antagonists had no effect; however, when administered with a toxin, the antagonists potentiated the vomiting response.

The dorsal vagal complex is involved in nausea and/or vomiting reactions induced by either vagal gastrointestinal activation or the administration of several humoral cytotoxic agents (Davis et al., 1986). In the rat and ferret, the dorsal vagal complex includes the area postrema (AP), nucleus of the solitary tract (NTS) and the dorsal motor nucleus (DMNX) of the vagus in the brainstem. It is considered the starting point of a final common pathway for the induction of emesis in vomiting species (Van Sickle et al., 2003). In rats, this area is also densely populated with CB_1 and 5-HT_3 receptors (Himmi, 1996; Kimura et al., 1998). CB_1 receptors in the NTS are activated by THC and this activation is blocked by the selective CB_1 antagonists, SR141716 (Himmi et al., 1998) and AM 251 (Simoneau et al., 2001; Van Sickle et al., 2001). Immediate early genes (IEG), such as *c-fos*, act as transcription factors to couple short-term neuronal activity with changes in the level of gene transcription. As a result the *c-fos* gene is a possible molecular marker of cell activation, increase in IEG expression levels often occur as a

consequence of experience (e.g. Curran & Morgan, 1985). Evaluation of *c-fos* expression following exposure to an anti-emetic stimulus in ferrets, such as THC, verified that CB_1 receptors in the dorsal vagal complex of the brainstem are responsible for the anti-emetic effects of cannabinoids. Indeed, *c-Fos* expression induced by cisplatin in the DMNX, specific subnuclei of the nucleus of the solitary tract and AP is significantly reduced by THC (Van Sickle et al., 2001; Van Sickle et al., 2003). Endogenous cannabinoid ligands, such as anandamide, as well as synthetic cannabinoids, such as WIN 55, 212-2, also act on these receptors (Felder & Glass, 1998). In rats, Anandamide has also been reported to interact with serotonin (Kimura et al., 1998).

Parker, Kwiatkowska, Burton & Mechoulam (2004) observed a suppression of LiCl-induced vomiting in the *Suncus murinus* by cannabinoid pretreatment; THC produced suppression of vomiting in a concentration dependent manner, at doses greater than 2.5 mg/kg. It was also found that the primary non-psychoactive cannabinoid found in marijuana, cannabidiol (CBD), produced a biphasic effect in suppressing LiCl-induced vomiting. Lower doses (5 and 10 mg/kg) suppressed vomiting, while higher doses (25 and 40 mg/kg) potentiated LiCl-induced vomiting. The suppressant effect of THC, but not CBD, on vomiting was blocked by pretreatment with the CB_1 receptor antagonist SR 141716, suggesting that the anti-emetic effect of THC is mediated by CB_1 receptors but that the anti-emetic effect of CBD is mediated by some other mechanism. This is consistent with receptor binding studies that show that CBD does not bind to the CB_1 receptor (Mechoulam, Parker, & Gallily, 2002).

Interaction of Cannabinoid and Serotonin anti-emetics

The cannabinoid and serotonin systems interact and both systems are involved in the control of emesis. However, there has been little evaluation of their relative effectiveness in control of nausea and vomiting. There have been no human clinical trials with chemotherapy patients that compare 5-HT_3 antagonists and cannabinoids in the treatment of emetogenic side effects of chemotherapy in cancer patients. A single recent experiment with human subjects, compared the effectiveness of a single dose of OND (8 mg) with one of two doses of a single puff of smoked marijuana (8.4 mg and 16.9 mg) in attenuating nausea and vomiting produced by syrup of ipecac (Soderpalm, Schuster, & de Wit, 2001). Unlike cisplatin, ipecac produces short lasting nausea and vomiting with a rapid onset. Although both agents reduced emesis produced by ipecac, OND was considerably more effective than a single puff of smoked marijuana in attenuating both vomiting and nausea. However, the relatively short duration of action of ipecac (60 min) compared with the long lasting (several days) effect of cisplatin chemotherapy treatment limits the generalizability of this experimental finding to treatments of chemotherapy-induced nausea and vomiting. Indeed, OND has been reported to be ineffective in treating delayed phases of chemotherapy-induced vomiting and nausea in cancer patients (e.g. Morrow, 1995).

The relative anti-emetic effectiveness of OND and THC has been compared in the *Suncus murinus*. Kwiatkowska et al. (2004) found that intraperitoneal (i.p.) administration of either OND or THC dose-dependently suppressed cisplatin-induced vomiting and retching. However, the minimally effective dose of OND (0.2 mg/kg) was lower than the minimally effective dose of THC (2.5 mg/kg). To evaluate the potential of

combined low doses of OND and THC to suppress cisplatin-induced emesis, Kwiatkowska et al. (2004) administered subthreshold doses of both OND (0.02 mg/kg) and THC (1.25 mg/kg) prior to injecting cisplatin in the shrews. This combination of OND and THC completely suppressed vomiting and retching elicited by cisplatin. These results suggest that combinational use of low doses of OND and THC may be an effective alternative treatment for the acute phase of chemotherapy-induced vomiting; therapeutically there may be fewer side effects than the use of higher doses of each agent alone.

Present research

When rats serve as the experimental subjects the establishment or the expression of LiCl-induced taste avoidance is unaffected by pretreatment with anti-nausea agents that prevent LiCl conditioned gaping (Limebeer & Parker, 1999; Parker et al., 2002; Parker et al., 2003; Rudd, Ngan, & Wai, 1998). Since taste avoidance, but not conditioned gaping, can also be produced by non-emetic (and even rewarding) drugs, Parker (2003) has suggested that drug induced CTA may be motivated by conditioned fear in rats. On the other hand, the emetic species, *Suncus murinus*, develops a conditional preference for a taste paired with a rewarding drug (Parker et al., 2002) and avoids a taste paired with the emetic drug, LiCl (Smith et al., 2001).

While shrews, like rodents, avoid a taste paired with LiCl (Smith et al., 2001), it is not known whether anti-emetic treatments will interfere with the establishment of LiCl-induced taste avoidance in shrews. The following experiments will determine if shrews learn to avoid a taste paired with LiCl. As well, the potential of the anti-emetic agents,

OND and THC, to interfere with the establishment of LiCl-induced taste avoidance will be evaluated. If unlike the non-emetic rodents (Limebeer & Parker, 2000, 2003; Parker et al., 2002; Parker et al., 2003; Rudd, Ngan, & Wai, 1998) the anti-emetics interfere with LiCl-induced taste avoidance in the shrew, it is likely that the mechanisms responsible for taste avoidance in these two species differ.

Experiment 1

Experiment 1 evaluated the potential of OND to prevent the establishment of LiCl-induced saccharin avoidance in *Suncus murinus*. The dose of OND (1.5 mg/kg) used was previously found to inhibit cisplatin-induced vomiting and retching in shrews (Kwiatkowska et al., 2004). Non-nutritive 0.1% saccharin solution served as a conditioned stimulus flavour, because it is the most commonly used flavour in taste avoidance experiment with rats. Parker et al. (2002) found that water deprived shrews consume 0.1 % saccharin. The CTA was evaluated first using a one bottle test in which saccharin alone was available. The one bottle test has been reported to more sensitively detect between group differences in aversion strength (Batsel & Best, 1993). In addition the CTA was evaluated using a two bottle test in which saccharin and water were both available. The two bottle test is a more sensitive measure of CTA *per se*, but may obscure between group differences (Batsel & Best, 1993).

Method

Subjects

The subjects were 15 male (28-50 g) and 13 female (20-29 g) S*uncus murinus* bred and raised in the Wilfrid Laurier University colony (original stock donated by E. Rissman, Department of Biology, University of Virginia). All animals were weaned at postnatal day 21. The animals were housed individually in 25 x 16 x 12 cm polyethylene cages with aspen wood shavings and shredded paper towel. The holding room was maintained on a 14:00 hr light/dark cycle (lights on at 0700 hr) at 23 ± 1°C. The shrews received *ad libitum* access to cat chow and mink pellets. In order to reduce risk of gastrointestinal disease, normal HCl (1ml/L) was added to tap water to maintain a pH of 5.5 delivered in drinking water. The 0.1 % saccharin solution was dissolved in this HCl-water. All procedures were approved by the Wilfrid Laurier University Animal Care Committee in accordance with regulations of the Canadian Council on Animal Care.

Drugs

OND (1.5 mg/kg) was prepared as a 0.5 mg/ml solution in a vehicle of ethanol/emulsifier/saline (1/1/36). This dose of OND was found to be effective in preventing cisplatin induced vomiting in the shrews (Kwiatkowska et al., 2004). The vehicle was also administered at a volume of 3 ml/kg. The LiCl (390 mg/kg) was dissolved in sterile water (0.15 M solution) and administered at a volume of 60 ml/kg. This concentration of LiCl has previously been found to reliably induce vomiting in shrews (Parker et al, 2003). All agents were administered i.p.

Procedure

The shrews were trained to drink water for 15 min on Days 1-5 while in their home cage. Each day the water bottles were removed from the cage at 2100 hr. Since shrews have a rapid metabolic rate they cannot be deprived of water for more than 12 hr (Parker & Kwiatkowska, unpublished observations). Therefore the shrews were deprived of water for 12 hours instead of the typical procedure used with deprived rats (i.e. 23 hr). While 12 hr water deprived the shrews were presented water in graduated tubes for 15 min in their home cage beginning at 0900 hr. At 1100 hr water bottles were presented for a 10 hr period to allow the shrews to replenish their water deficit.

The conditioning trial occurred on Day 6. The groups are defined on the bases of preconditioning drug (OND or VEH) and conditioning drug (LiCl or SAL): OND-LiCl (n=7), OND-SAL (n= 7), VEH-LiCl (n=7) and VEH-SAL (n=7). Each group there were 3-4 males or females. Depending on the group, the shrews were injected i.p. with 3 ml/kg of OND (1.5 mg/kg) or VEH 30 min prior to being presented with 0.1% saccharin solution in graduated tubes for 15 min. Immediately following consumption they were injected with 60 ml/kg of LiCl or SAL. Shrews had access to water between 1100 hr and 2100 hr in their home cage. On Days 7-9 shrews were maintained on the water deprivation schedule as on Days 1-5.

Testing began on Day 10 with a 15 min one bottle test. The shrews were presented with saccharin in place of water for 15 min at 900 hr. At 1100 h, water bottles were returned to their home cage and then removed at 2100h. On Day 11, the shrews received a two bottle test during which they received saccharin in one graduated tube and water in the second graduated tube for 12 hr. Each spout was presented until the shrew

licked from it, then both tubes, were presented in the home cage with saccharin always on the left. A measure of saccharin and water consumption was taken at 15 min and at 12 hr.

Data analysis

The Day 5 water, saccharin conditioning trial (Day 6), the Day 9 water and the Day 10 one bottle saccharin test intake measure were each entered into a 2 by 2 ANOVA with between group factors of pretreatment condition (VEH, 1.5 mg/kg OND) and conditioning drug (LiCl or SAL). Additionally, to control for individual differences in drinking both the saccharin conditioning trial data and the saccharin one bottle test trial data were converted into saccharin consumption ratios relative to the previous day water intake measure [Saccharin Consumption Ratios = mean ml saccharin/ (mean ml saccharin + previous day mean ml water)].

The Day 11 two-bottle test intake scores were converted into saccharin preference ratios (PR) [Preference ratio = mean ml saccharin consumed/ (mean ml saccharin + mean ml water consumption)]. Both the conditioning trial consumption ratio and the test trial saccharin PR were entered into a 2 by 2 between groups ANOVA. Finally, the Day 11 two bottle test 15 min and 12 hr measures were converted into saccharin PRs [mean ml saccharin/ (mean ml saccharin intake + mean ml water intake)] and entered into 2 by 2 between groups ANOVA.

Results

Conditioning Trial

OND pre-treatment during conditioning did not effect saccharin consumption. Figure 1 presents the mean ml (± SEM) water consumed during the 15 min drinking period on Day 5 (A), the mean ml (± SEM) saccharin solution consumed by the various groups during the conditioning trial on Day 6 (B) and the saccharin Consumption Ratio (C) relative to the Day 5 water. None of the 2 by 2 between groups ANOVA for any set of data revealed significant effects.

Test Trials

The 15 min single bottle test on Day 10 did not show evidence of a CTA. Figure 2 presents the mean ml (± SEM) water consumed on Day 9 (A), saccharin solution consumed during the 15 min one bottle test on Day 10 (B) and the saccharin Consumptions Ratio relative to the Day 9 water (C). For none of the data sets did the 2 by 2 between groups ANOVAs reveal any significant effects.

At the 12 hr measure only, LiCl-conditioned shrews showed saccharin CTA that was prevented by OND pretreatment. Figure 3 presents the Day 11 mean (± SEM) saccharin preference ratio in the two-bottle test measured at 15 min (top) and at 12 hr (bottom). At 15 min, the 2 x 2 between groups ANOVA revealed no significant effects. However, at 12 hr, the 2 x 2 ANOVA revealed a significant pretreatment by conditioning drug interaction ($F(1, 24) = 4.32$; $p < .05$) as well as a significant main effect of conditioning drug ($F(1, 24) = 7.68$; $p < .01$). At the 12 hr measure, independent t-tests revealed that shrews, pretreated with vehicle had a lower saccharin preference ratio when

they were conditioned with LiCl than when they were conditioned with saline ($t(12) = 3.9$; $p < .01$); however, the LiCl and Saline conditioned groups pretreated with OND did not significantly differ.

Discussion

Only after 12 hr of drinking did the shrews show significant saccharin avoidance; but, if they were pretreated with the anti-emetic drug, OND, they did not avoid the LiCl-paired saccharin. These results are markedly different than those reported using the non-emetic rat; that is, OND does not block the establishment of LiCl-induced taste avoidance in the rat (Limebeer & Parker, 2000; Rudd, Ngan & Wai, 1998).

Shrews developed weak conditioned taste avoidance for a saccharin solution paired with LiCl. This may have been due to a low baseline intake of saccharin that was equivalent to unflavoured water during the conditioning trial. Baseline drinking of the CS flavour should be sufficiently high to be able to detect suppression due to conditioning with LiCl. Saccharin solution is most commonly used with rats in the taste avoidance literature, because it is non-nutritive. However, shrews have been reported to dislike saccharin within the range of concentrations used in Experiment 1 (Iwasaki & Sato, 1982). To increase baseline consumption of the novel flavour, Experiment 2 evaluated the potential of LiCl to produce conditioned avoidance of 0.3 M sucrose solution consumption. This concentration of sucrose has previously been reported to be highly consumed by shrews (Iwasaki & Sato, 1982; Parker et al., 2002).

Experiment 2

Experiment 2 evaluated the potential of THC (5 and 10 mg/kg) and OND (1.5 mg/kg) to prevent LiCl–induced sucrose avoidance. The THC (5 and 10 mg/kg) doses were selected based on previous research showing that these optimally prevent LiCl-induced emesis in shrews (Parker et al., 2004). Additionally, Experiment 1 did not include a non-associative control group, which is a standard control used in taste avoidance learning in rats (see Schafe, Thiele & Bernstein, 1998). Such a group ensures that avoidance of taste is the result of its associative pairing with LiCl, and not merely the result of enhanced neophobia from prior experience with sickness (Domjan, 2002). Therefore, in Experiment 2, all shrews received an injection of LiCl; the LiCl-conditioned groups, were administered LiCl immediately following sucrose consumption, while the saline-conditioned groups, were administered LiCl 24 hr following sucrose consumption (a standard procedure used in the CTA literature, see Schafe et al., 1998). To evaluate the taste avoidance, a one bottle test was administered because it has been reported that the two bottle test may obscure between group differences in aversion strength (Batsel & Best, 1993).

Method

Subjects

The subjects were 27 male (32 - 43g) and 29 female (23 - 27g) S*uncus murinus.* During the course of the experiment, 5 shrews were removed from the study due to health problems. Unless otherwise indicated, the animals were treated in a similar manner as described in Experiment 1.

Drugs

The dose of OND (1.5 mg/kg) and of THC (5 and 10 ml/kg) were selected on the basis of their ability to prevent cisplatin- or LiCl- induced vomiting in the shrew (Parker et al., 2003; Kwiatkowska et al., 2004). All solutions were prepared in a vehicle of ethanol/emulsifier/saline (1/1/36). OND was prepared as a 0.5 ml/kg solution in vehicle and administered at a volume of 3 ml/kg. THC was prepared as a 1.67 mg/kg solution and administered at a volume of 3 ml/kg (5 mg/kg) and 6 ml/kg (10 mg/kg). Vehicle was administered at 3 ml/kg. The conditioning drug 0.15 M solution of LiCl (390 mg/kg) was prepared using sterile water; LiCl and Saline were administered at a volume of 60 ml/kg. All agents were administered i.p.

Procedure

The shrews were trained to drink water for 15 min on Day 1- 4 while in their home cage. Each day the water bottles were removed from the cage at 2200 hr. While 12 hr water deprived, beginning at 0900 hr, the shrews were presented water in graduated tubes for 15 min in their home cage. At 1200 hr, water bottles were presented for a 10 hr period to allow the shrews to replenish their water deficit.

The conditioning trial occurred on Day 5. Depending on the group, the shrews were injected with 3 ml/kg of OND (1.5 mg/kg), 3 ml/kg of THC (5 mg/kg), 6 ml/kg of THC (10 mg/kg) or 3 ml/kg of VEH 30 min prior to being presented with 10 % sucrose solution in graduated tubes for 15 min. Immediately following consumption they were injected with 60 ml/kg of LiCl (390 mg/kg) or SAL. Shrews had access to water between 1100 hr and 2100 hr in their home cage. The final groups were as follows: OND-SAL (n=

7), OND-LiCl (n= 7), 10 THC-LiCl (n= 5), 10 THC-SAL (n= 7), 5 THC-LiCl (n= 7), 5 THC-SAL (n= 4), VEH-LiCl (n= 7), VEH-SAL (n= 7).

On Day 6, all animals received a noncontingent injection 24 hours following the conditioning trial; Groups OND- SAL, 10 THC-SAL, 5 THC-Sal, VEH-SAL were administered 60 mg/kg of .15 M LiCl while Groups OND-LiCl, 10 THC-LiCl, 5 THC-LiCl, VEH-LiCl were administered 60 mg/kg of saline. The injections were administered at 1100 hr, 2 hr following the normal 15 min water access in the morning and 1 hr before the normal 10 hr water access to the water bottles in the afternoon. These noncontingent injections ensured that all animals had equivalent drug exposure.

On Days 7and 8 shrews were presented with water for 15 min in the morning and 10 hr in the afternoon on the water schedule. On Day 9, a one-bottle sucrose test was administered where the shrews had access to the sucrose solution for a period of 15 min.

Data Analysis

The Day 4 water intake, conditioning trial sucrose intake (Day 5), Day 8 water intake and the test trial sucrose intake (Day 9) were entered into a 4 x 2 between groups ANOVA with the factors of preconditioning drug (Vehicle, OND, 5 THC or 10 THC) and conditioning drug (LiCl or saline). Additionally, the data for the conditioning trial and the test trial were converted into sucrose consumption ratios relative to the previous day's 15 min water intake [Consumption Ratio = mean ml sucrose/ (mean ml sucrose + mean ml water consumed on the previous day)]. These consumption ratios control for individual differences in baseline drinking among the groups.

Results

Conditioning Trial

The highest dose of THC clearly suppressed sucrose consumption during conditioning. Figure 4 presents the mean amount of water consumed (A) on Day 4 and the mean amount of sucrose consumed (B) on the conditioning trial (Day 5) by the rats in each preconditioning and conditioning treatment in Experiment 2. The 4 by 2 ANOVA of the Day 4 water intake revealed no significant effects. On the other hand, the 4 x 2 ANOVA of the sucrose intake on the conditioning day (Day 5) revealed a significant preconditioning drug effect ($F(3, 43) = 5.83$; $p < .01$). Subsequent Least Significant Difference (LSD) pairwise comparison tests (pooled across conditioning drug) revealed that Groups 5 THC and 10 THC drank significantly less sucrose solution during conditioning than Group Vehicle (p's $< .01$) and Group 10 THC drank significantly less sucrose solution during conditioning than Group OND ($p < .025$).

Figure 4 also presents the mean (± SEM) conditioning day sucrose consumption ratios (C) relative to the Day 4 water intake for each group in Experiment 2. The 4 x 2 ANOVA of the conditioning day sucrose consumption ratios revealed only a significant effect of preconditioning drug ($F(3, 43) = 7.49$; $p < .01$); Group 10 THC displayed significantly lower sucrose consumption ratios than any other group (p's $< .01$), but no other groups differed. Using the sucrose consumption ratio measure only the highest dose of THC interfered with drinking during conditioning.

Test Trial

Only the shrews that had been pretreated with vehicle displayed LiCl-induced taste avoidance. Figure 5 presents the mean (± SEM) water intake (A) on Day 8 and the mean (± SEM) sucrose intake (B) on the test trial (Day 9). The 4 by 2 ANOVA of the Day 8 water intake revealed a significant effect of preconditioning drug ($F(3, 43) = 3.10$; $p < .05$); subsequent LSD pairwise comparison tests (pooled across conditioning drug) revealed that Group 10 THC drank more water on the day prior to the test trial than Groups Vehicle or 5 THC (p's $< .05$). The 4 x 2 ANOVA of the test trial sucrose intake revealed a significant main effect of preconditioning drug ($F(3, 43) = 5.47$; $p < .01$) and a significant preconditioning drug by conditioning drug interaction ($F(3, 43) = 3.24$; $p < .05$). The preconditioning drug effect was evaluated by LSD pairwise comparison tests (pooled across conditioning drug) which revealed that overall, Groups 5 THC and 10 THC drank more sucrose than Group Vehicle (p's $< .025$) and that Group 10 THC drank more sucrose than Group OND (p's $< .025$). The most interesting effect was the preconditioning drug by conditioning drug interaction which was evaluated by independent t-tests for each preconditioning drug group. These analyses revealed that the LiCl conditioned group drank significantly less sucrose than did the saline conditioned group only among the shrews pretreated with vehicle during conditioning ($p < .01$).

Since the groups pretreated with 10 mg/kg of THC drank significantly more water on Day 8 than any of the other preconditioning groups, the sucrose consumption ratio data (which controls for differences in baseline drinking) is a better measure of CTA than the raw sucrose intake measure. This measure revealed the same pattern of results. The bottom of Figure 5 presents the mean (± SEM) test day sucrose consumption ratios (C)

relative to the previous day water for each group in Experiment 2. The 4 by 2 between groups ANOVA of the test day sucrose consumption ratios revealed significant effects of preconditioning drug ($F(3, 43) = 5.27$; $p < .01$), conditioning drug ($F(1, 43) = 6.08$; $p < .025$) and a preconditioning drug by conditioning drug interaction ($F(3, 43) = 2.94$; $p < .05$). Overall, lithium conditioned groups had lower sucrose consumption ratios than saline conditioned groups and Groups 5 THC and 10 THC had higher sucrose consumption ratios than Group Vehicle (p's $< .01$). Analysis of the interaction revealed that only among the Vehicle pretreated groups were sucrose consumption ratios significantly ($p < .01$) lower among the shrews conditioned with lithium than the shrews conditioned with saline. Furthermore, the interaction was analyzed as a single factor ANOVA for the preconditioning drug among the lithium and the saline conditioned groups. This analysis revealed that the preconditioning effect was significant only for the lithium conditioned groups ($F(3, 22) = 6.15$; $p < .01$); the Vehicle pretreated group displayed a lower saccharin consumption ratio than any other group (p's $< .05$), but no other groups differed significantly.

Discussion

Doses of OND and THC previously shown to prevent LiCl- or cisplatin-induced vomiting in the *Suncus murinus* prevented the establishment of LiCl-induced CTA in this species. These results suggest that toxin-induced CTA may be motivated by conditioned sickness in the shrew, unlike the non-emetic rat. One caveat must, however, be considered regarding the effectiveness of THC as an anti-emetic in this paradigm. During conditioning, pretreatment with THC unconditionally suppressed sucrose intake resulting

in reduced exposure to the LiCl-paired taste. Bond and Di Giusto (1975) reported that the strength of the LiCl-induced CTA in rats is a function of the amount of solution consumed prior to conditioning; that is the less solution consumed during conditioning the weaker the CTA This concern may be more important in considering the effectiveness of 10 mg/kg THC than 5 mg/kg THC on the establishment of LiCl-induced CTA. The group pretreated with 10 mg/kg of THC showed reduced sucrose intake as well as reduced sucrose consumption ratios when their previous day water intake was taken into account. On the other hand, only the raw sucrose intake measure was suppressed in the group pretreated with 5 mg/kg THC. When their conditioning trial sucrose intake measure were transformed into consumption ratios relative to the previous days water intake, the group pretreated with 5 mg/kg of THC did not show suppressed sucrose consumption ratios during conditioning and they did not show LiCl-induced CTA during testing.

The doses of THC and OND were selected on the basis of their potential to interfere with LiCl-induced vomiting in shrews (Parker et al., 2003). It is not clear if lower doses of THC, which are ineffective in reducing vomiting, might be effective in reducing LiCl-induced CTA without suppressing fluid intake unconditionally. Future studies will examine the possibility of lower doses of THC interfering with establishment of CTA.

General Discussion

The primary purpose of this study was to evaluate the potential of the anti-emetic agents, OND and THC, to interfere with LiCl-induced conditioned taste avoidance in the shrew (*Suncus murinus*), an animal capable of vomiting. The present study found *Suncus murinus* to form CTA to saccharin (Experiment 1) and sucrose (Experiment 2) paired with the administration of LiCl. Furthermore, both anti-emetics, OND and THC, prevented the formation of CTA in the shrews. Unlike the non-emetic rodents (Limebeer & Parker, 1999; Parker et al., 2002; Parker et al., 2003; Rudd, Ngan, & Wai, 1998), the anti-emetics interfered with LiCl-induced taste avoidance in the shrew. These results suggest that the mechanisms responsible for taste avoidance in these species differ.

In Experiment 1, shrews pretreated with OND (1.5 mg/kg) prior to drinking 0.1% saccharin solution paired with LiCl, only showed saccharin avoidance after 12 hr drinking in the two-bottle test. A two-bottle test was employed, because the first single-bottle test did not reveal the presence of saccharin avoidance. The two-bottle test is more sensitive to the presence or absence of avoidance (Batsell & Best, 1993) because animals have an alternative solution (water) to consume in order to replete their thirst. By this measure, shrews demonstrated the presence of taste avoidance, but only at the 12 hr measure of drinking. The taste avoidance displayed by the vehicle pretreated group was prevented by OND pretreatment.

The relatively weak display of LiCl-induced taste avoidance evident in Experiment 1 may have been the result of relatively low baseline consumption of saccharin solution among the shrews, masking the suppressed intake in the LiCl conditioned animals. Indeed, Iwasaki & Sato (1981) reported that this species is reluctant

to consume 0.1 % saccharin solution. Therefore, in Experiment 2, the CS flavour was changed to 0.3 M sucrose solution, a flavour that shrews readily consume (Iwasaki & Sato 1981; Parker et al., 2002). Additionally, given the expected higher level of consumption of sucrose solution among the control groups, a single-bottle test was used to reveal taste avoidance. A single-bottle test has been shown to be preferential to the more sensitive two-bottle test in detecting group differences between animals with CTAs (Batsell & Best, 1993).

Experiment 2 revealed that shrews avoided the LiCl-paired sucrose solution, but that this avoidance was prevented by prior treatment with doses of OND (1.5 mg/kg) and THC (5 mg/kg and 10 mg/kg); these doses have been shown to prevent LiCl-induced vomiting in shrews (Parker et al., 2004). Therefore, as in Experiment 1, the anti-emetic pretreatment prevented the establishment of LiCl-induced taste avoidance in the shrew.

The interference with establishment of LiCl-induced taste avoidance by THC must be interpreted cautiously in light of the suppression of sucrose intake by the sedating doses required to suppress vomiting (Parker et al., 2003; Kwiatkowska et al., 2004). The doses of THC required to suppress LiCl (Parker et al., 2003) or cisplatin (Kwiatkowska et al., 2004) induced vomiting are clearly within the range that produce motor suppression and within this range they also suppressed sucrose intake during the conditioning trial. The reduced exposure to sucrose CS may have reduced the strength of the LiCl-induced taste avoidance (Bond & Di Giusto, 1975) among the THC-pretreated groups. The present design cannot rule out this alternative explanation. The absolute intake of sucrose during conditioning was suppressed in both the 5 mg/kg THC and the 10 mg/kg THC pretreated groups. However, when the sucrose intake scores were

transformed into preference ratios relative to shrews previous day's water intake, only the group pretreated with 10 mg/kg of THC showed suppressed sucrose preference ratios during conditioning. By this measure, the dose of 5 mg/kg THC did not suppress relative sucrose intake, but did prevent the establishment of LiCl-induced CTA. Clearly, the dose of THC (0.5 mg/kg) that suppresses conditioned gaping reactions in rats is considerably lower than the doses required to suppress vomiting in shrews. In fact, lower doses (0.5-1 mg/kg ip) of THC produce hyperphagia rather than hypophagia, in rats (Williams & Kirkham, 2002). Future research will evaluate the potential of lower doses of THC that are ineffective in suppressing vomiting in shrews to interfere with LiCl-induced conditioned taste avoidance, since lower doses of THC may not produce the potential confound of suppressed consumption during conditioning.

The ability of OND and possibly of THC to prevent the establishment of LiCl-induced taste avoidance in the shrew are in sharp contrast to those apparent when rats serve as subjects (Limebeer & Parker, 2000; Limebeer & Parker, 2003; Parker et al., 2003). Although OND prevented both the establishment and the expression LiCl-induced conditioned gaping reactions in the Taste Reactivity test in the rat (Limebeer & Parker, 2000), OND did not reduce either the establishment or the expression of LiCl-induced CTA in either a one-bottle or a two-bottle test. Similarly, 8-OH-DPAT, which decreases serotonin availability, also interfered with the establishment and expression of LiCl-induced conditioned gaping reactions, but not taste avoidance (Limebeer & Parker, 2003). On the basis of these dissociations, Limebeer and Parker (2000, 2003) suggest that conditioned gaping, but not avoidance, reflects nausea in rats, a species that does not vomit in response to toxins.

Cannabinoids also selectively reduce conditioned gaping, but not CTA in rats. In the rat, THC interfered with cyclophosphamide (a chemotherapeutic agent)-induced (Limebeer & Parker, 1999) and LiCl-induced conditioned gaping (Parker et al., 2003), but not taste avoidance. Even the non-psychoactive cannabinoid CBD interfered with LiCl-induced gaping, but not taste avoidance (Parker, Mechoulam & Schlievert, 2002). Furthermore, the synthetic cannabinoid, HU-210, interfered with LiCl-induced conditioned gaping (Parker, Mechoulam, Schlievert, Abbott, Fudge, & Burton, 2003). Therefore, considerable evidence indicates that toxin-induced conditioned gaping in rats, but not taste avoidance, is mediated by nausea. It has been suggested that this paradoxical phenomenon may be due to the inability of rodents to vomit (Gamzu, 1977; Davis, Harding, Leslie, & Andrews, 1986).

The results of the present study revealed that LiCl-induced CTA in an animal capable of vomiting may be mediated by sickness since anti-emetic action of OND and THC prevented CTA learning. Yet for decades of research of taste avoidance learning, investigators have mainly employed the rat model, an animal that does not vomit. Only recently, has research investigated the capacity of species, which are more closely related to humans, to develop a CTA (Smith et al., 2001; Parker, 2002). Indeed, unlike the non-emetic rat, shrews develop a preference (rather than avoidance) for a flavour paired with the rewarding drugs, amphetamine or morphine (Parker et al., 2002). With the use of the shrew, it appears that CTA is mediated by different mechanisms in species with the capability to vomit than those that do not. The present data suggest that LiCl-induced CTA in the shrew- an animal capable of vomiting- is mediated by sickness since anti-emetics prevent the formation of CTA to a flavour paired with LiCl.

The findings of these studies have highlighted certain issues that need to be attended to in future research. First, it will be important to evaluate the potential of lower doses of THC, which do not unconditionally suppress fluid intake, to prevent LiCl-induced taste avoidance. Second, in order to evaluate the role of conditioned nausea as a motivation for the expression of taste avoidance in the shrew, it will be interesting to determine if anti-emetics interfere with the expression of previously established CTA. Third, it would be valuable to explore the hedonic changes of flavour palatability after pharmacological manipulation in a species capable of vomiting using the taste reactivity test.

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Figure Caption

Figure 1. Mean (± SEM) consumption (ml) of water during the 15 min drinking period on Day 4 (A) and mean (± SEM) consumption (ml) of saccharin during the conditioning trial (B) by various groups in Experiment 1. The mean (± SEM) saccharin consumption ratios (C) relative to previous day water intake (Consumption ratio = mean ml saccharin intake/ mean ml saccharin intake + mean ml Day 4 water intake).

Figure 2. Mean (± SEM) (ml) water intake on Day 9 (A) and mean (± SEM) saccharin (B) consumed on a 15 min one-bottle test (Day 10) for various groups in Experiment 1. Mean (± SEM) saccharin consumption ratios (C) for saccharin consumed during one-bottle 15 min test relative to previous day water intake.

Figure 3. Mean (± SEM) saccharin preference ratio during two-bottle test at 15 min (top) and 12 hr (bottom) by various groups in Experiment 1. (** = $p < 0.01$)

Figure 4. Mean ml (± SEM) (ml) water consumed during the 15 min drinking period on Day 4 (A) and mean (± SEM) (ml) sucrose solution consumed (B) on conditioning day (Day 5) by the various groups in Experiment 2.

Also, mean (± SEM) sucrose consumption ratios (C) for sucrose consumed during conditioning relative to previous day water intake.

Figure 5. Mean (± SEM) (ml) water intake on Day 8 (A), mean (± SEM) (ml) sucrose consumed during the one-bottle test on Day 9 (B), and sucrose consumption ratios (C) for sucrose consumed during test relative to previous day water intake for various groups in Experiment 2. (** = $p < 0.01$)

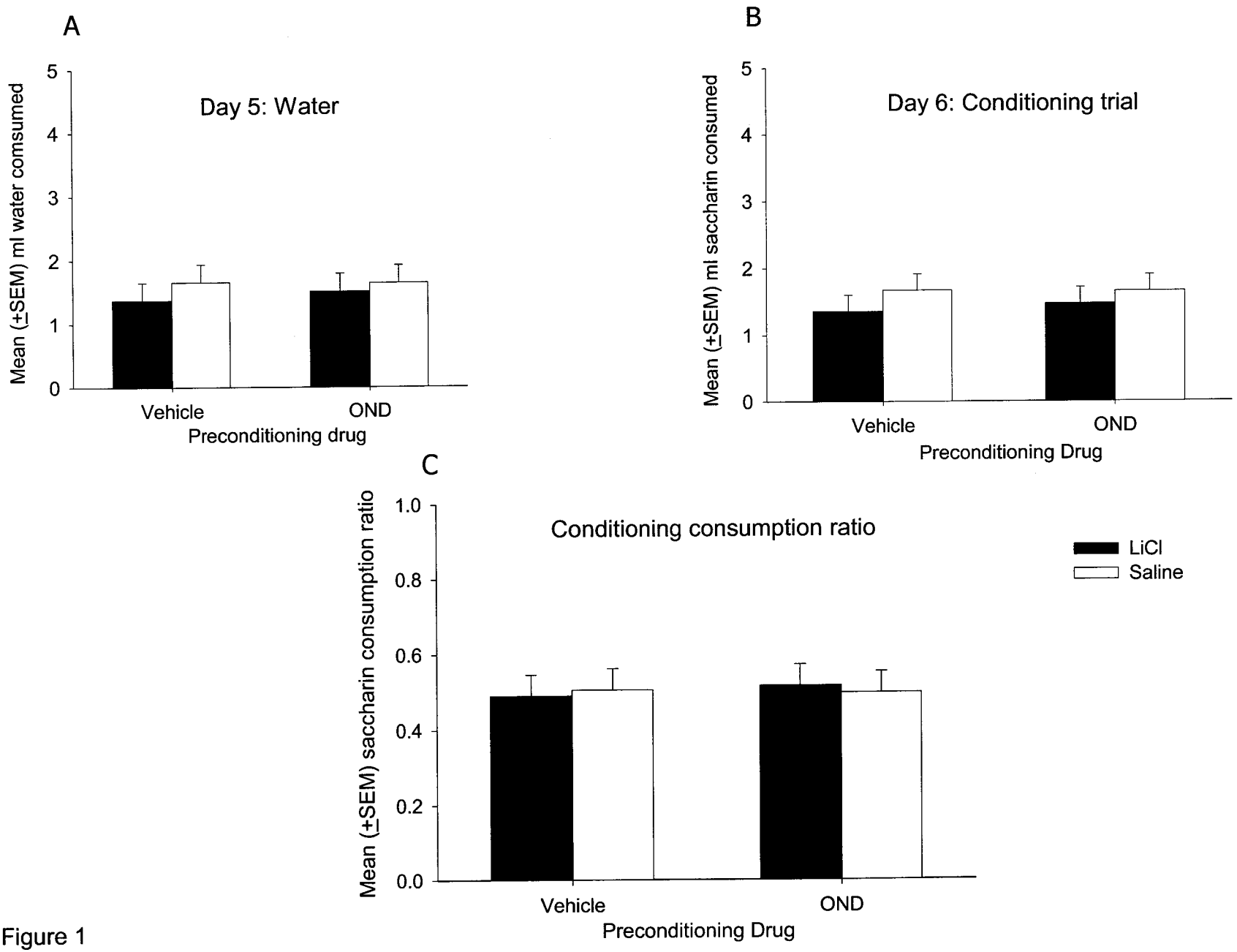

A
Day 5: Water
Mean (±SEM) ml water comsumed
5
4
3
2
1
0
Vehicle
OND
Preconditioning drug
B
Day 6: Conditioning trial
Mean (±SEM) ml saccharin consumed
5
4
3
2
1
0
Vehicle
OND
Preconditioning Drug
C
Conditioning consumption ratio
Mean (±SEM) saccharin consumption ratio
1.0
0.8
0.6
0.4
0.2
0.0
Vehicle
OND
Preconditioning Drug
LiCl
Saline

Figure 1

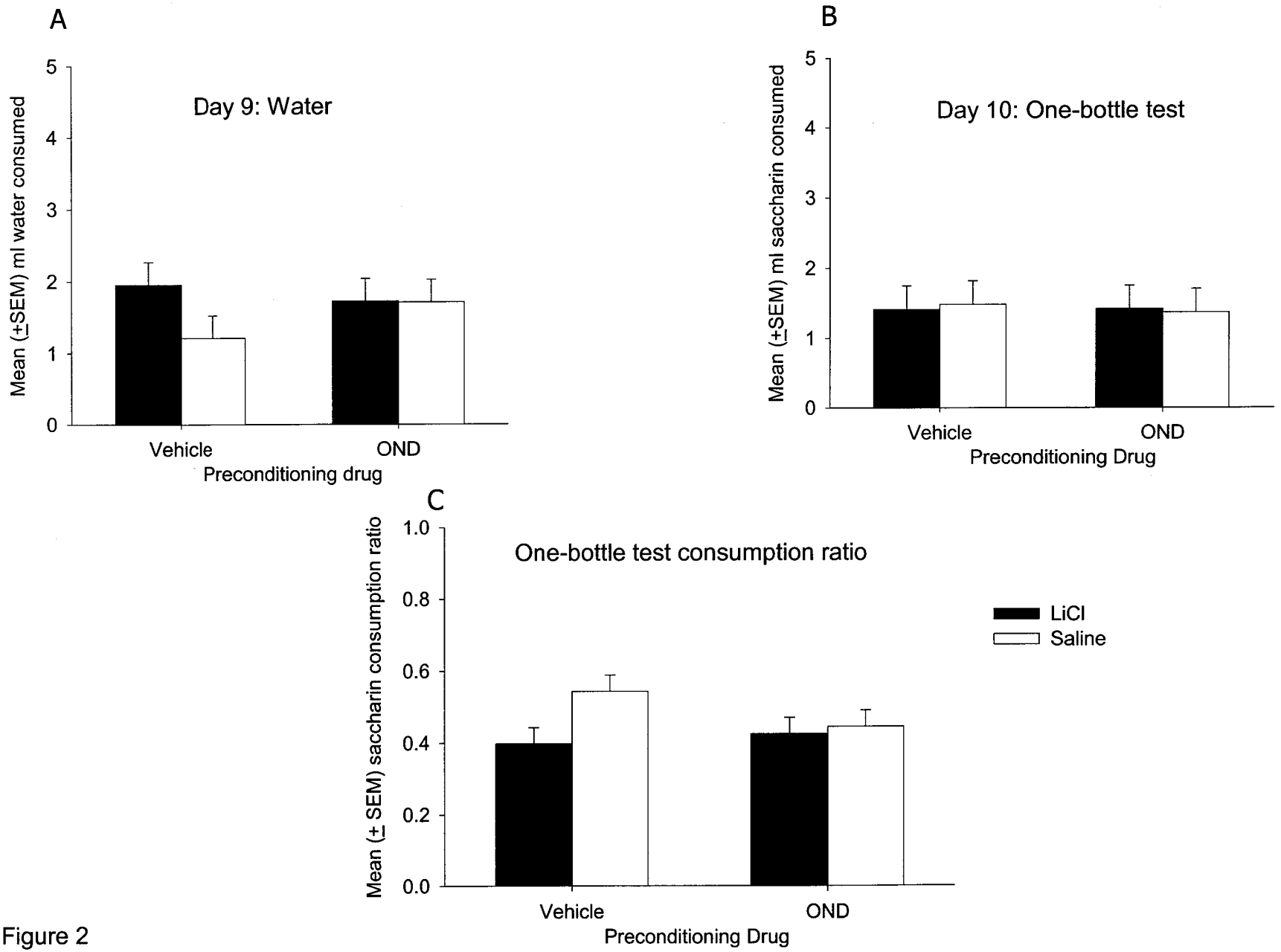
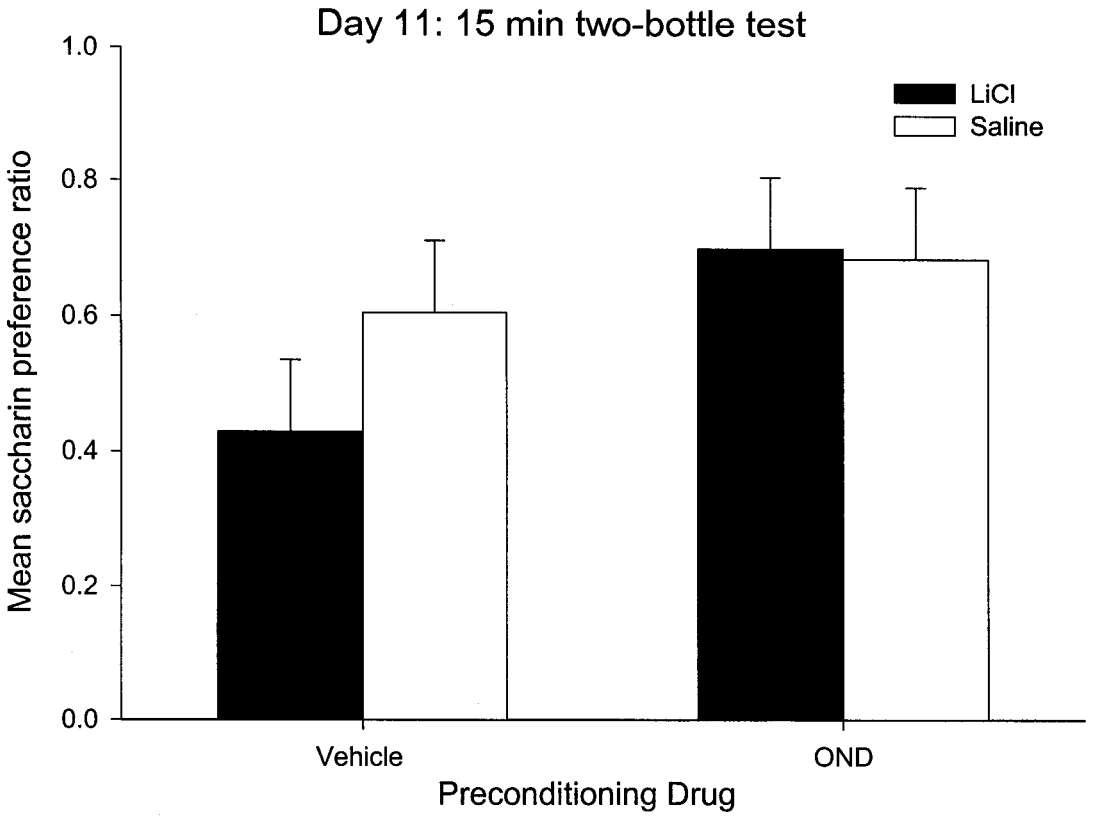

A
Day 9: Water
Mean (±SEM) ml water consumed
5
4
3
2
1
0
Vehicle
OND
Preconditioning drug
B
Day 10: One-bottle test
Mean (±SEM) ml saccharin consumed
5
4
3
2
1
0
Vehicle
OND
Preconditioning Drug
C
One-bottle test consumption ratio
Mean (± SEM) saccharin consumption ratio
1.0
0.8
0.6
0.4
0.2
0.0
LiCl
Saline
Vehicle
OND
Preconditioning Drug

Figure 2


Day 11: 15 min two-bottle test
LiCl
Saline
Mean saccharin preference ratio
1.0
0.8
0.6
0.4
0.2
0.0
Vehicle
OND
Preconditioning Drug

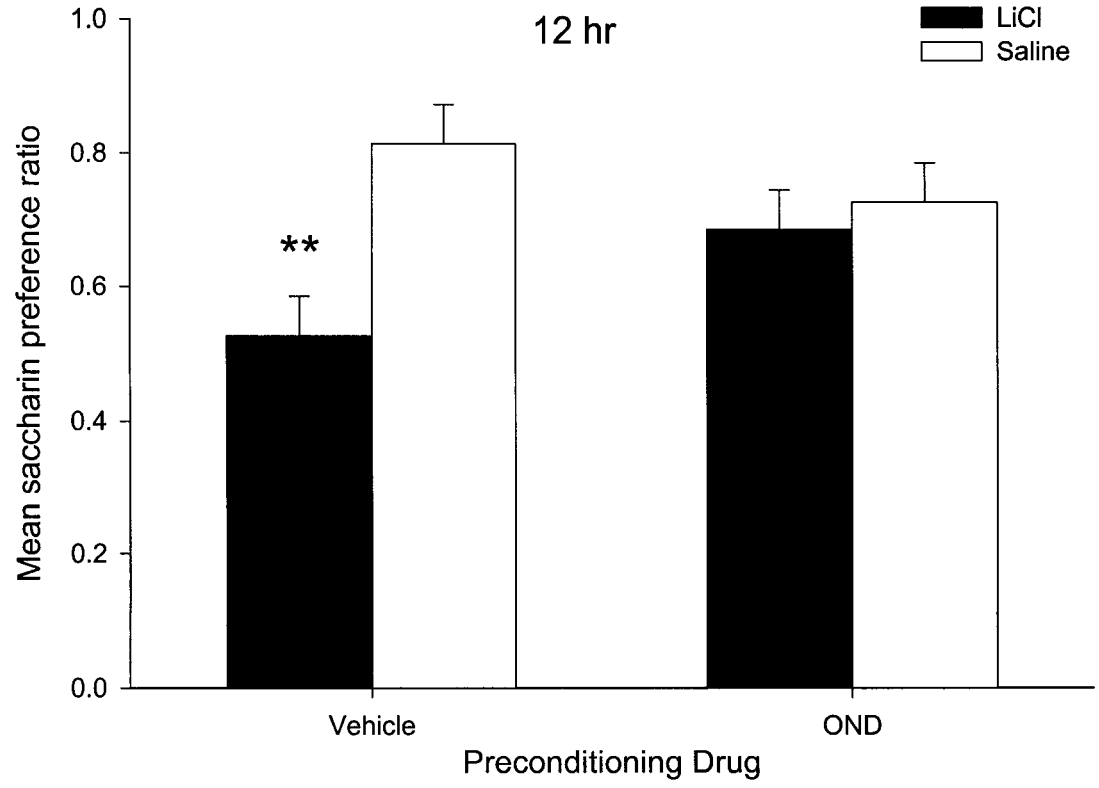

12 hr
LiCl
Saline
Mean saccharin preference ratio
1.0
0.8
0.6
0.4
0.2
0.0
**
Vehicle
OND
Preconditioning Drug

Figure 3

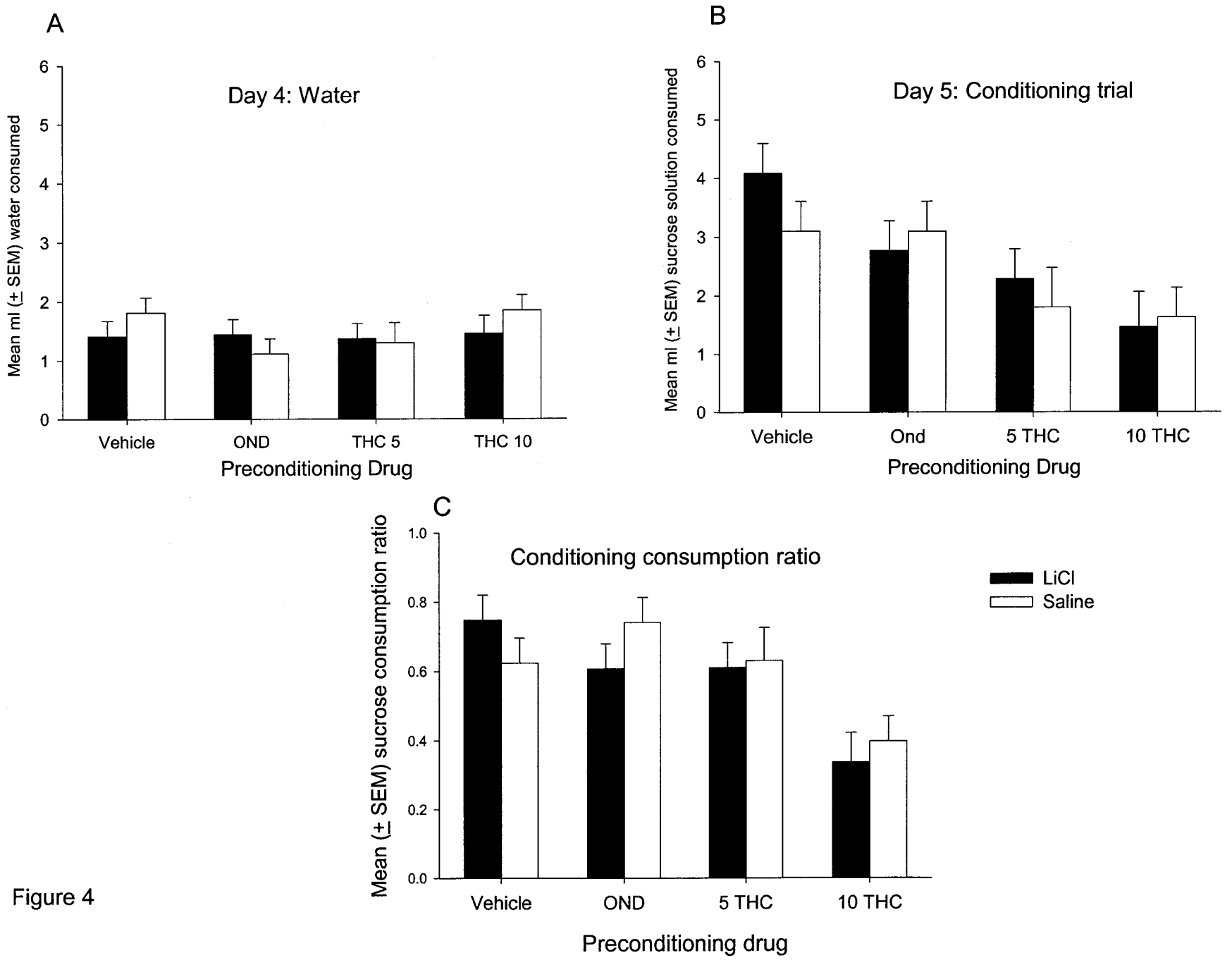

A
Day 4: Water
Mean ml (± SEM) water consumed
0
1
2
3
4
5
6
Vehicle
OND
THC 5
THC 10
Preconditioning Drug
B
Day 5: Conditioning trial
Mean ml (± SEM) sucrose solution consumed
0
1
2
3
4
5
6
Vehicle
Ond
5 THC
10 THC
Preconditioning Drug
C
Conditioning consumption ratio
Mean (± SEM) sucrose consumption ratio
0.0
0.2
0.4
0.6
0.8
1.0
Vehicle
OND
5 THC
10 THC
Preconditioning drug
LiCl
Saline

Figure 4

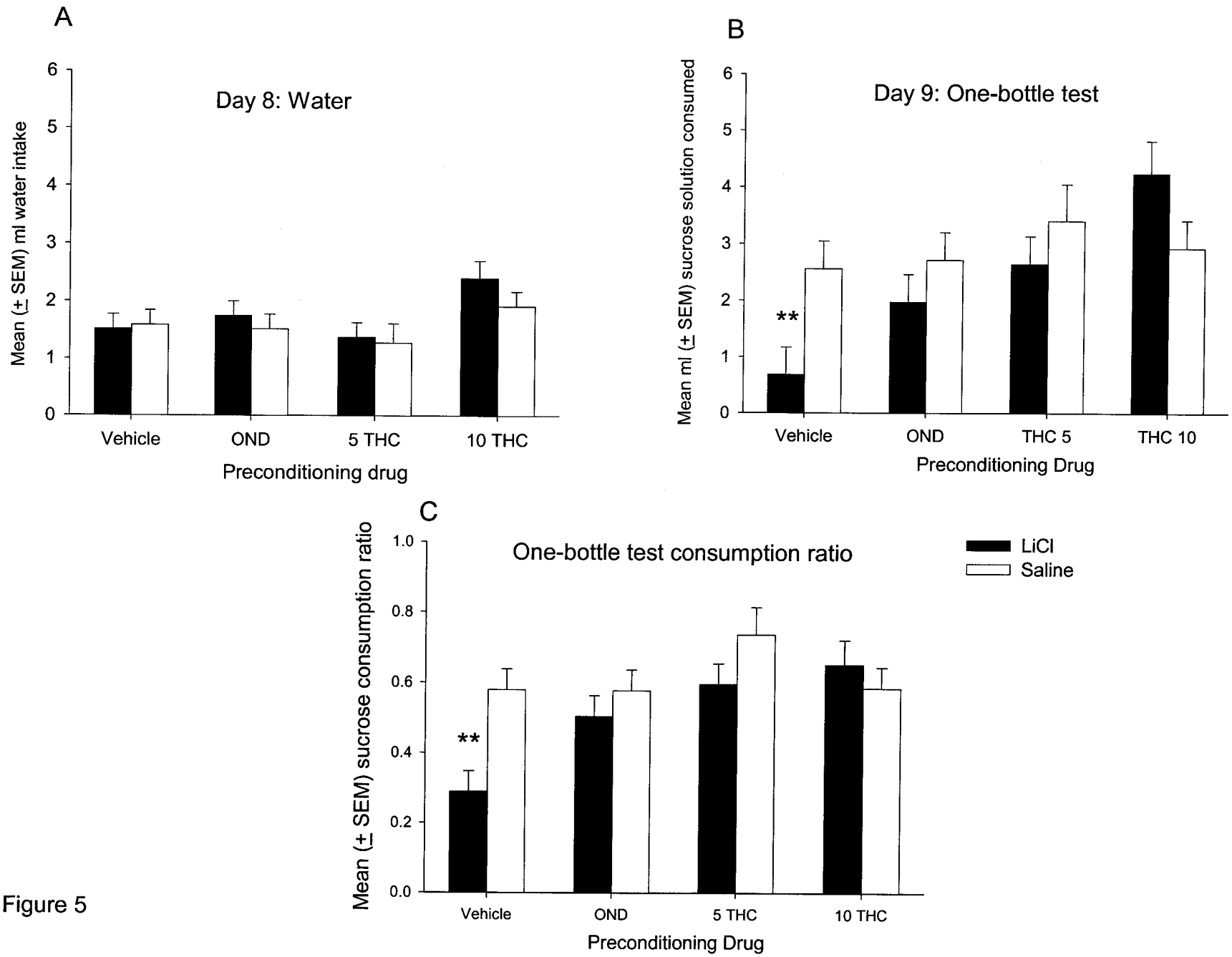

A
Day 8: Water
Mean (± SEM) ml water intake
0
1
2
3
4
5
6
Vehicle
OND
5 THC
10 THC
Preconditioning drug
B
Day 9: One-bottle test
Mean ml (± SEM) sucrose solution consumed
**
Vehicle
OND
THC 5
THC 10
Preconditioning Drug
C
One-bottle test consumption ratio
Mean (± SEM) sucrose consumption ratio
0.0
0.2
0.4
0.6
0.8
1.0
**
Vehicle
OND
5 THC
10 THC
Preconditioning Drug
LiCl
Saline

Figure 5