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The Effect of Delta-9-Tetrahydrocannabinol (THC) on Lithium Induced Sickness Reactions in both Rats (*Rattus norvegicus*) and the House Musk Shrew (*Suncus murinus*)

by

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Abstract

The following experiments examined the ability of delta-9-tetrahydrocannabinol (THC) to attenuate lithium induced sickness in both the nonemetic rat (*Rattus norvegicus*) and the emetic house musk shrew (*Suncus murinus*). The ability of THC to attenuate the expression of previously established lithium induced conditioned sickness behavior in *Suncus* was also examined. Although unconditioned sickness behavior was displayed by both rats and shrews, THC did not attenuate this behavior in either species. However, THC did attenuate conditioned retching in the *Suncus murinus*. These results are the first to show the attenuation of conditioned sickness in *Suncus* by THC. They also experimentally verify anecdotal reports from chemotherapy patients that THC attenuates conditioned or "anticipatory" nausea and/or vomiting (ANV). The present findings suggest that *Suncus murinus* may serve as a reliable animal model to evaluate both pharmacological and/or behavioral interventions for conditioned emetic responses.

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Table of Contents

Introduction

Testimony of numerous cancer patients indicates that marijuana reduces nausea and vomiting associated with chemotherapy. These anecdotal reports have been encouraged by the results of clinical trials demonstrating decreased nausea and emesis in patients who smoke marijuana during a chemotherapy regime (Chang, Shilling, & Stillman, 1979). In a randomized, double-blind cross-over study, Chang et al (1979) found that 14 of 15 patients had decreased nausea and vomiting when smoking marijuana (17 mg THC/cigarette) than when a placebo was taken. Similar results have been shown when THC (psychoactive ingredient in marijuana) is administered orally in the synthetic pill form (nabilone; a synthetic cannabinoid derivative), rather than inhaled (Lucas & Laszlo, 1980; Sallan, Zinberg, & Frei, 1975). When administered at a dose of 15 mg/m^2 PO (oral), Lucas and Laszlo (1980) showed that THC was more effective than standard antiemetic regimes and placebo in 72% of patients tested. These antiemetic effects were also seen when THC was administered at a lower dose of 10 mg/m^2 PO (Sallan et al., 1975). Although patient self reports concerning the efficacy of THC and nabilone in attenuating chemotherapy induced nausea and vomiting are encouraging, there is an increasing need for experimental support of both patient anecdotal evidence and early clinical trials.

Experimental evidence of the antiemetic potential of cannabinoids

Experimental evidence of the antiemetic potential of THC is quite limited. Cannabinoids have been shown to interfere with cisplatin-induced vomiting in cats (McCarthy & Borison, 1984; McCarthy, Flora, & Vishnuvajjala, 1984) and

pigeons (Ferrari,Ottanik, & Giuliani, 1999). They have also been shown to attenuate vomiting induced by the cannabinoid CB_1 receptor antagonist/inverse agonist SR 141716A in the least shrew, *cryptotis parva* (Darmani, 2001). Furthermore, THC suppresses toxin-induced conditioned rejection reactions in rats (Limebeer & Parker, 1999), which is believed to be a model of nausea in these animals (Parker, 1995, 1998). THC has historically been used for the treatment of diarrhea (Chaperon & Thiebot, 1999). Not surprisingly, Coull, Johnston, Pertwee, & Davies (1997) report THC inhibits gastrointestinal motility in rats. This is similar to the delay of gastric emptying of solid food in response to THC administration in humans (McCallum, Soykan, Sridhar, Ricci, et al., 1999).

There is recent evidence that cannabinoid receptors are found within the emetic system of the rat's brain, in the nucleus of the solitary tract (Himmi, Dallaporte, Perrin, & Orsini, 1996; Himmi, Perrin, El Ouazzani, & Orsini, 1998). The nucleus of the solitary tract is involved in the nausea reactions induced by either vagal gastrointestinal activation or several humoral cytotoxic agents. It is considered the starting point of a final common pathway for the induction of emesis in vomiting species. The CB_1 receptors in the nucleus of the solitary tract are activated by THC and this activation is blocked by the selective CB_1 antagonist SR 141716A (Himmi et al., 1998). Endogenous cannabinoid ligands such as anandamide, as well as synthetic CB receptor agonists, such as WIN 55,212-2 and CP 55, 940 also act on these receptors (Darmani, 2001; Felder & Glass, 1998; Mallet & Beninger, 1998).

Effect of THC on lithium-induced nausea and/or vomiting

The potential of THC to interfere with lithium-induced nausea and/or vomiting has not been previously evaluated in animal models. Lithium is an emetic drug capable of producing sickness reactions (i.e., nausea and/or vomiting) similar to that seen with chemotherapeutic administration (i.e., cisplatin, cyclophosphamide, veratrine) in emetic species such as the ferret (Davey & Biederman, 1998) and is a commonly employed sickness-inducing agent in behavioral paradigms such as flavor aversion learning in rats (e.g., Gustavson, Garcia, Hankins, & Rusiniak, 1974).

Lithium induces a specific pattern of behavioral responses in rats which includes: suppressed activity, suppressed grooming, suppressed scratching, body dragging (stomach in constant contact with floor, with animal using its paws to drag itself in a forward motion) and "lying on belly" (LOB) in a flat position (Parker, Hills, & Jensen, 1984). If decreased activity and LOB represent sickness behavior in rats, then physiological manipulations that interfere with sickness should attenuate these behaviors. Bernstein, Chavez, Allen, and Taylor (1992) and Ladowsky and Ossenkopp (1986) reported that rats with lesions to the area postrema (emetic center of the brain) failed to show the behavioral suppression of activity after treatment with lithium chloride. Bernstein et al (1992) also showed that LOB was significantly attenuated in animals with lesions to the area postrema. These results suggest that both behavioral suppression and LOB represent unconditioned sickness in rats following lithium administration. There

have been no reports on the effects of antiemetic treatments on lithium-induced unconditioned sickness behavior in rats.

Another unconditioned sickness behavior displayed by rats is pica, the eating of nonnutritive substances such as kaolin (Mitchell, Krusemark, & Hafner, 1976a; Mitchell, Wells, Hoch, Lind, et al., 1976b). Mitchell and his colleagues demonstrated that rats engage in pica when subjected to rotation or the administration of emetic drugs. Subsequently, Morita, Takeda, Kubo, Yamatodani, et al (1988) found that pretreatment with a variety of antiemetic agents suppressed post-rotation consumption of kaolin when compared with saline controls.

The first two experiments below evaluated the potential of THC to interfere with lithium induced unconditioned sickness behavior in rats.

EXPERIMENT 1

Lithium chloride produces the following behavioral profile in rats: decreased activity, decreased grooming activity, body dragging, and LOB (Meachum & Bernstein, 1990, 1992; Parker et al., 1984). However, these studies only examined the unconditioned behavioral effects of lithium when administered at a high dose (129 mg/kg LiCl). There have been no reports of unconditioned behavioral effects over a range of lithium doses, and therefore it is unknown whether this behavioral profile will occur at low doses of lithium chloride. Subsequently, if THC alleviates lithium induced sickness in rats, then it should change the lithium-induced behavioral profile. The following experiment

examined the ability of THC to interfere with lithium induced sickness reactions in the rat.

Method

Subjects

The animals were 24 male Sprague-Dawley rats (*Rattus norvegicus*) weighing 300-350 g. Animals were pair-housed with *ad-lib* access to food (Lab Diet, PMI Foods) and water. Experiments were conducted during the light phase of 12:12 h light:dark cycle, with lights on at 7:00 AM.

Drugs

Delta-9-tetrahydracannabinol (THC) was prepared in a mixture of 1 mg THC per ml polyvinylpyrrolidone (PVP) and saline according to the procedure described by Fenimore and Loy (1971). THC and vehicle control injections were administered in a volume of 0.5 ml/kg (dose of 0.5 mg/kg). The dose of THC was selected on the basis of a previous report that 0.5 mg/kg interferes with the establishment of cyclophosphamide-induced conditioned rejection reactions in rats (Limebeer & Parker, 1999), which is presumably a measure of sickness in these animals. Parker and Gillies (1995) reported that doses of THC as low as 1.5 mg/kg produce both taste and place aversions in rats. Therefore, the dose of THC used in the first two experiments reported was 0.5 mg/kg. Lithium was prepared in a 0.15 M solution of LiCl in distilled water. Lithium injections were administered in a volume of 2 ml/kg (12.9 mg/kg), 8 ml/kg (51.6 mg/kg), and 20 ml/kg (129 mg/kg). Saline injections were administered in a volume of 8 ml/kg. All injections were administered intraperitoneally (i.p.).

Procedure

Animals were randomly assigned according to drug group with n=6/group. Each group was given two test trials, separated by one week. One test trial followed THC pretreatment and the other followed vehicle pretreatment, in a counterbalanced order.

On each trial, animals were administered THC or vehicle and were then immediately returned to their home cages. Thirty min later, animals were administered the appropriate volume of saline or lithium (12.9, 51.6, or 129 mg/kg) and placed in a test chamber (22.5 x 26 x 20 cm). The test chamber consisted of 4 plexiglass sides, a clear plexiglass bottom, and a clear plexiglass lid was placed over the chamber during testing. The room was illuminated by four 25-W light bulbs located 30 cm from either side of the test chamber. A glass mirror was positioned below the chamber at an angle which made viewing of orofacial and somatic responses possible. The behaviors of the rats were videotaped from the reflection of the mirror during the 30 min test using a Panasonic AG-5710p SVHS cassette recorder.

The behavioral measures included: frequency of grooming bouts (paws in contact with face for more than 1 sec or licking of body for more than 1 sec), active locomotion bouts (movement of both forepaws in a forward direction), rearing bouts (animal maintaining balance on hindlimbs exclusively for more than 1 sec), genital licks (licking of genital region for more than 1 sec), scratching bouts (scratching body with either hind or forelimb for more than 1 sec), and body dragging (stomach in constant contact with test chamber floor, with animal using

its paws to drag itself along the floor in a forward motion). In addition, the mean number of seconds were measured for LOB (belly in constant contact with chamber surface with no discernible body movement, with head and nose in contact with floor of the chamber).

Results

Body dragging was not displayed by animals during either the THC or vehicle test. Figure 1 presents the mean frequency of the behaviors displayed by rats injected with various doses of LiCl during a trial on which they were pretreated with THC (Solid lines) and on a trial on which they were pretreated with the Vehicle (Dotted lines). The general trend for each behavior is to decrease as the dose of LiCl increased, but THC did not consistently affect any behavior. A 4 by 2 mixed factors ANOVA for each of the behaviors displayed in Figure 1 revealed only significant effects of the Dose of Lithium: Rearing, $F(3, 20)=61.4$; $p<.001$, Active Locomotion Bouts, $F(3, 20)=13.1$; $p<.001$, Grooming Bouts, $F(3, 20)=18.7$; $p<.001$, Genital Licks, $F(3,20)=11.4$; $p<.001$, and Scratching, $F(3, 20)=4.4$; $p<.025$. In no case was there a significant effect of pretreatment drug or a pretreatment drug by dose of lithium interaction.

Clearly, the differences among the dose conditions were the result of a suppression of each of these behaviors as the dose of LiCl increased. Subsequent LSD pair-wise comparison tests were used to evaluate differences among dose conditions for each behavior. For the behavior of Rearing, as the dose of lithium increased, each dose produced significantly less rearing than the previous dose (p's<.01). For Active Locomotion, a dose of 51.6 or 129 mg/kg of

LiCl produced less activity than saline (p's<.05) and a dose of 129 mg/kg of LiCl produced less activity than either 12.9 or 51.6 mg/kg LiCl. For the behaviors of both Grooming and Genital Licks, a dose of 51.6 or 129 mg/kg of LiCl produced fewer grooming bouts and genital licks than a dose of 12.9 mg/kg of LiCl or saline (p's<.05). For the behavior of Scratching, a dose of 51.6 or 129 mg/kg of LiCl produced fewer scratching bouts than saline (p's<.05) and a dose of 129 mg/kg produced fewer bouts than 12.9 mg/kg of LiCl (p's<.05).

Figure 2 presents the mean number of seconds that the rats injected with each dose of LiCl spent LOB during the trial on which the rats were pretreated with THC and on the trial on which they were pretreated with Vehicle. A 4 by 2 mixed factors ANOVA revealed only a significant effect of LiCl dose, $F(3, 20)=4.4$; $p=.015$. LSD pair-wise comparison tests revealed that a dose of 130 mg/kg of LiCl produced significantly more LOB than any other dose (p's<.01). THC, however, did not modify the potential of LiCl to produce this effect.

Discussion

When administered lithium chloride, rats display the behavioral response of decreased locomotion, decreased grooming behavior, increased body dragging, and increased LOB (Meachum & Bernstein, 1990, 1992; Parker et al., 1984). In the preceding experiment, lithium elicited suppression of locomotor activity, rearing, grooming, genital licks, and scratching. This suppression was enhanced as the dose of LiCl increased, with the greatest suppression of behavior occurring in animals administered 129 mg/kg LiCl. Although body dragging was not seen in lithium treated animals, the mean duration of LOB

increased with higher doses of lithium, with animals administered 129 mg/kg LiCl spending the greatest time LOB.

Although the behavioral profile displayed by animals administered lithium is consistent with previous research, THC did not attenuate any behavior. There is no previous research evaluating the effect of THC or other antiemetic drugs on the lithium induced unconditioned sickness behavioral profile in rats. Therefore, it is impossible to compare these results with those of 5-HT_3 antagonists, NK_1 antagonists, or 5-HT_{1A} agonists, all of which are commonly employed in antiemetic research (Gardner, Twissell, Gale, Kilpatrick, et al., 1995; Gregory & Ettinger, 1998; Okada, Torii, Saito, & Matsuki, 1994).

Experiment 2

Mitchell et al., (1976b) demonstrated that rats increase soil consumption (a measure of gastrointestinal distress) after injection of lithium chloride. Antiemetic drugs have been successful in attenuating both motion induced increases in kaolin consumption (Morita et al., 1988; Takeda, Hasegawa, Morita, Horii, et al., 1995a), and increased kaolin consumption induced through administration of emetic agents (Takeda, Hasegawa, Morita, and Matsunaga, 1993; Takeda et al., 1995a; Takeda, Hasegawa, Morita, Horii, et al., 1995b). However, there has been no previous research evaluating the effect of antiemetic treatment on lithium induced pica (sickness induced kaolin consumption). If THC attenuates lithium induced sickness in rats, then it should interfere with pica.

Method

Subjects

The animals were 24 male Sprague Dawley rats, weighing 300-350 g. Animals were individually housed in wire mesh cages with *ad lib* access to food (Lab Diet, PMI Foods) and water throughout the course of the experiment. Experiments were conducted during the light phase of a 12:12 h light:dark cycle, with lights on at 7:00 AM.

Drugs

The drugs used and doses administered were identical to those used in Experiment 1.

Kaolin Preparation

The method of kaolin preparation was similar to that used by Mitchell et al., (1976a, b). A non-nutritive mixture of kaolin and acacia (Gum Arabic) was prepared by mixing pharmaceutical grade kaolin with 1% acacia (w/w) in distilled water to form a thick paste. This paste was then extruded through a syringe onto stainless steel trays and dried at room temperature. After drying overnight, the spaghetti-like strands were broken into aggregates averaging 1.0 x 0.5 cm (approximately 45 mg). Kaolin prepared in this manner was provided to all animals in metal containers attached to the right front corner of each cage.

Procedure

All animals were habituated to the presence of food, water, and kaolin for 10 days before the beginning of the experiment. During this period, baseline kaolin measures were taken every 24 h. This consisted of collecting any spilled

kaolin and weighing the entire container. Following the habituation period, animals were randomly assigned according to drug group with n=6/group.

Each group was given two test trials, separated by one week. One test trial followed THC pretreatment and the other followed vehicle pretreatment, in a counterbalanced order. Animals were administered THC or vehicle and were then immediately returned to their home cages. Thirty min later, animals were administered the appropriate volume of saline or lithium and returned to their home cages. During this 30 min interval, animals had *ad lib* access to food, water, and kaolin. Kaolin consumption levels were recorded for the first hour and a half during both test trials.

Results

Figure 3 presents the mean (± sem) grams of kaolin consumed by rats during the first hour and a half after LiCl administration. The Group by Test Drug mixed factors ANOVA for hour 1 revealed only a significant Group effect, $F(3, 20)=3.28$; $p=.042$. An LSD pair-wise comparison test (pooled across the nonsignificant factor of test drug) revealed that only Group 51.6 mg/kg LiCl consumed more kaolin during the first hour of testing than did group saline, but the remaining groups did not significantly differ from one another.

Discussion

When administered lithium chloride, rats will engage in pica, the eating of non-nutritive substances such as kaolin (Davey & Biederman, 1998; Mitchell et al., 1976b). In the preceding experiment, lithium increased kaolin consumption across the first hour of test, although only rats administered 51.6 mg/kg differed

significantly from saline control animals. Therefore, the insignificant difference seen between the high dose lithium group (129 mg/kg) and saline group is inconsistent with previous research (Mitchell et al., 1976b). Although animals administered 51.6 mg/kg LiCl displayed enhanced kaolin consumption when compared to saline animals, THC did not attenuate or enhance this lithium induced pica.

In both Experiment 1 and Experiment 2, a dose of 0.5 mg/kg THC did not attenuate lithium induced sickness behaviors in rats. Because a range of THC doses was not tested, it is impossible to determine whether the dose of THC was simply too low to interfere with these sickness reactions. Limebeer and Parker (1999) found that the same dose of THC used in Experiments 1 and 2 interfered with both the establishment and the expression of cyclophosphamide induced conditioned rejection reactions in rats. Therefore, conditioned rejection reactions displayed by rats may be more easily disrupted by the effects of THC than unconditioned behavioral sickness reactions or pica. However, an alternative explanation is that THC may be more effective at attenuating the emetic properties of cyclophosphamide than lithium.

Experiment 3

Since the rat is incapable of vomiting, animals models of emesis have typically relied on larger animals capable of vomiting such as dogs, cats, and ferrets (Andrews & Davis, 1993; King, 1990). A more recent and economical model of emesis has been developed using the mouse-sized house musk shrew,

Suncus murinus (Matsuki, Ueno, Kaji, Ishihara et al., 1988). *Suncus murinus* vomits in response to a variety of sickness inducing drugs such as cisplatin, cyclophosphamide, nicotine, and veratrine (Gardner & Perren, 1998; Matsuki, Torri, Ueono, & Saito, 1992; Matsuki, Wang, Okada, Tamura, et al., 1997; Selve, Friderichs, Reimann, & Reinartz, 1994; Ueno, Matsuki, & Saito, 1987). The potential of lithium to induce retching and vomiting in the *Suncus murinus* has not been previously reported. The present experiment evaluated the potential of THC to interfere with lithium induced retching and vomiting in the *Suncus*.

Method

Subjects

Animals were 15 male *Suncus murinus*, weighing 30-50 g. All animals were born and raised in the Wilfrid Laurier breeding colony (stock descends from animals raised at the University of Virgina). Animals were individually housed in 28 x 17 x 12 cm solid bottomed plastic cages with pine wood shavings and shredded paper towel for bedding. Animals were maintained on a 14:10 h light:dark cycle with lights on at 7:00 AM. The colony room was maintained at a constant 23^0C and animals had *ad-lib* access to food (10:1 ratio of Purina Cat Chow and mink pellets) and water throughout the course of the experiment. All experiments were conducted during the light phase of the cycle.

Drugs

Pilot testing determined a 129 mg/kg, ip, dose of lithium most commonly used as a high dose in rats was not sufficient to produce vomiting in the *Suncus murinus*. In fact, it was necessary to increase the dose by a factor of three to 390

mg/kg ip, to elicit vomiting in the shrew. Lithium was prepared in a 0.15 M solution LiCl in distilled water. Lithium and saline injections were administered in a volume of 60 ml/kg. All injections were administered intraperitoneally (i.p.).

Because of the failure of THC to attenuate unconditioned behavioral sickness reactions and pica in Experiments 1 and 2, a higher dose of THC was selected. Also, since the dose of lithium used was dramatically increased from that used with the rats, we reasoned that the dose of THC should be increased six-fold. Therefore, the dose of THC selected for shrews was 3.0 mg/kg, ip. As is evident in the results, this dose produced enhanced rather than suppressed general activity levels, an effect that is typically seen at the low end of the dose-response range for THC. THC produces a biphasic effect on activity in rats and mice, at low doses it produces hyperactivity and at high doses it produces suppressed activity and eventually catalepsy (Chaperon & Thiebot, 1999). Delta-9- tetrahydrocannabinol (THC) was prepared in a mixture of polyvinylpyrrolidone (PVP) and saline at a concentration of 1 mg/ml according to the procedure used in Fenimore and Loy (1971). THC and vehicle control injections were administered in a volume of 3.0 ml/kg; therefore, the dose of THC used was 3 mg/kg.

Procedure

Animals were assigned to groups on the basis of preconditioning drug (THC or vehicle), and the conditioning drug (lithium or saline) as follows: THC-lithium (n=6), THC-saline (n=2), vehicle-lithium (n=5), vehicle-saline (n=2). Because of the small number of subjects in the saline groups, they were

combined for the purpose of data analysis. The data were combined into a single factor design as follows: THC-lithium (n=6), Vehicle-lithium (n=5), saline (n=4). The design, therefore, did not evaluate the behavioral effects of THC alone.

Animals were administered THC or vehicle and were then returned immediately to their home cage for 30 min. After the 30 min interval, animals were administered either lithium or saline and placed immediately into the test chamber (22.5 x 26 x 20 cm) for a period of 30 min. The test chamber and videorecording procedure used were identical to those of Experiment 1.

Behavioral Measures

The frequency and latency to the first bout of each of the behaviors described below was measured using the Observer event recording program (Noldus, Inc.) The behaviors included: Vomiting, Retching (marked abdominal contractions and wide opening of the mouth appearing like a yawn), Gaping (smaller opening of the mouth exposing the bottom teeth), Chin Rubbing (chin rubbing directly against the chamber wall or floor), Backward Walking (sequential extension of one forelimb while simultaneously retracting the other forelimb), Forward Locomotion (forward movement along the surface of the floor), and Face Washing (forelimbs in contact with facial area for more than 1 sec). The behaviors were scored by two raters. The interrater reliability scores (Pearson Product Moment Correlation) for each behavior were: Vomiting (frequency, r=.99; latency, r=.99), Retching (frequency, r=.97; latency, r=.99), Gaping (frequency, r=.90; latency, r=.88), Chin Rubbing (frequency, r=.68; latency, r=.22), Backward Walking (frequency, r=.57; latency, r=.83), Forward Locomotion (frequency,

r=.91; latency, r=.30), and Face Washing (frequency, r=.97; latency, r=.97). For purposes of data analysis, the mean score of the two raters was entered into the analysis.

Results

Table 1 presents the mean (± sem) scores for each behavior recorded during Experiment 3. A single factor ANOVA for each behavior revealed a significant effect for the behaviors of Chin Rub Frequency, $F(2, 12)=4.8$; $p<.05$, Retch Latency, $F(2, 12)=4.9$; $p<.05$, Vomit Latency, $F(2, 12)=5.0$; $p<.05$, and Face Wash Latency, $F(2, 12)=7.9$; $p<.01$. Pairwise comparison tests (LSD) revealed that Group Vehicle-Lithium displayed more chin rubbing than either group THC-Lithium or Saline (p's<.05). Group THC-Lithium displayed a shorter latency to both retch and vomit than saline control animals (p's<.05). However, Group Vehicle-Lithium did not differ from either Group THC-Lithium or Saline in terms of both retch and vomit latency. Group THC-Lithium displayed a shorter latency to face wash than group Vehicle-Lithium ($p<.05$), but did not differ significantly from group saline. There were no differences between the groups in terms of gaping, forward locomotion, or backward walking.

Although the Groups did not differ significantly on the basis of frequency of retching or vomiting, examination of the mean scores revealed that Group Saline did not display these behaviors. A comparison of the lithium treated shrews and the saline treated shrews by an independent groups t-test revealed that lithium elicited significantly more vomiting [$t(13)=2.3$;$p<.05$], retching [$t(13)=2.3$; $p<.05$],

and gaping [$t(13)=2.4$; $p<.05$] than did saline, regardless of pretreatment condition.

Discussion

Lithium chloride induced both retching and vomiting in the *Suncus murinus*. In addition, shrews administered lithium displayed increased chin rubbing and gaping. Although both of these behaviors have not been previously measured in *Suncus*, a similar behavioral profile is displayed by rats administered lithium as conditioned rejection reactions. Parker (1995, 1998) argued that these types of conditioned rejection reactions may indicate nausea in rats. The act of gaping itself, which consists of a triangular opening of the mouth exposing the bottom teeth, may actually be a precursor to retching and subsequent vomiting in emetic species. The fact that the nonemetic rat displays this behavior in response to sickness producing and not to rewarding drugs supports this hypothesis.

In Experiment 3, THC did not attenuate the frequency of lithium induced gaping, retching, or vomiting. However, animals administered THC-lithium displayed a shorter latency to both retch and vomit than saline controls, although they did not differ from animals treated with vehicle-lithium. Animals administered vehicle-lithium did not differ from saline controls in terms of latency to both retch and vomit, however, animals administered saline did not display these behaviors. Although THC did not attenuate the latency to first retch and vomit, it did appear to suppress lithium-induced chin rubbing, which is thought to be a sickness behavior displayed by rats when administered an emetic agent (Parker, 1995,

1998). Therefore, if chin rubbing is a measure of sickness in *Suncus*, it may be more easily disrupted by the effects of THC than either retching or vomiting, both of which are direct measures of the emetic response.

Experiment 4

Anticipatory nausea and/or vomiting is highly prevalent among chemotherapy patients (Morrow, Burish, & Bellig, 1992). In this situation nausea and/or vomiting results from the pairing of a conditioned stimulus (sight, smells, or sounds associated with treatment environment) with administration of a sickness producing agent (Neese, Carli, & Curtis, 1980). These symptoms are not usually responsive to traditional antiemetic treatment (Morrow, Roscoe, Kirshner, Hynes, et al., 1998). However, anecdotal evidence indicates that THC alleviates anticipatory nausea and vomiting in chemotherapy patients (Cohen, Blanchard, Ruckdeschel, & Smolen, 1986; Iverson, 2000; Pratt, Lazar, Penman, & Holland, 1984). Experiment 4 evaluated the potential of THC to interfere with anticipatory nausea and vomiting using the *Suncus murinus* (house musk shrew).

Conditioned emetic reactions have been reported to cues previously paired with lithium chloride. For instance, Gustavson, Garcia, Hankins, & Rusiniak (1974) reported that coyotes display conditioned retching upon re-exposure to sheep meat that was previously paired with lithium induced vomiting. Although conditioned emetic reactions have been reported in a variety of species (Brett, Hankins, & Garcia, 1976; Davey & Biederman, 1998; Gustavson et al, 1974; Rusiniak, Gustavson, Hankins, & Garcia, 1976), there have been no experimental

demonstrations of interference with these conditioned emetic reactions by antiemetic agents.

Rats display a characteristic behavioral pattern of conditioned rejection reactions (i.e., gaping, paw pushing, and chin rubbing) when exposed to a flavoured solution that was previously paired with administration of an emetic drug (Parker, 1998; Parker & MacLeod, 1991). In fact, these conditioned rejection reactions are elicited exclusively by emetic agents and are not displayed when administered a rewarding drug like cocaine or amphetamine (Parker, 1995). Since these reactions are only displayed when administered emetic drugs, Parker (1998) has argued that these reactions may constitute symptoms of both unconditioned and conditioned nausea in the nonemetic rat. If the display of these reactions do indeed reflect conditioned nausea, then we would expect conditioned rejection reactions to be attenuated when treated with an antiemetic agent at test. The attenuation of conditioned rejection reactions in rats has been reported when animals were pretreated with trimethobenzamide (Parker & MacLeod, 1991), and THC (Limebeer & Parker, 1999). Since administration of antiemetics like THC has been successful in attenuating conditioned rejection reactions in rats (which may reflect nausea), it is therefore reasonable to assume that THC will attenuate conditioned sickness reactions in emetic species (i.e., conditioned gaping, retching, and vomiting). The following experiment examined the ability of THC to interfere with the expression of previously established conditioned emetic reactions when administered prior to a test.

Method

Subjects

The subjects were 14 male *Suncus murinus*, weighing 30-50 g. All shrews were born and raised in the Wilfrid Laurier breeding colony. The animals were individually housed in 28 x 17 x 12 cm solid bottomed plastic cages with pine wood shavings and shredded paper towels for bedding. Animals were maintained on a 14:10 h light:dark cycle with lights on at 7:00 AM. The colony room was maintained at a constant 23^0C and animals had ad-lib access to food and water. All animals were weaned at 20 days of age.

Drugs

The drugs used and doses administered were identical to those used in Experiment 3.

Procedure

Animals were randomly assigned on the basis of conditioning drug, lithium (n=7) or saline (n=7). The shrews received two conditioning trials, separated by 72 hr, during which the contextual chamber was paired with either lithium chloride or with saline. On each conditioning trial, each animal was injected with the appropriate solution and immediately placed in the test chamber (22.5 x 26 x 20 cm) for a period of 30 min. The test chamber used was identical to the one used in Experiments 1 and 3. The chamber was thoroughly washed with lemon scented soap between each animal's trial.

Test trials started six days after the final conditioning trial. The shrews received two test trials, separated by 72 hr, one following an injection of THC (3

mg/kg) and one following an injection of vehicle (4 animals administered THC during the first week, 3 during the second week). On each trial, the shrews were injected with THC or vehicle and returned to their home cage. Thirty min later, all animals were administered a second injection of physiological saline. Animals were then immediately placed in the test chamber and observed for a 30 min period. During the 30 min test, each animal's behavior was video recorded (Panasonic AG-5710p SVHS) from a mirror located below the chamber at an angle able to view orofacial and somatic responses of the shrew. After the 30 min interval, animals were immediately placed back in their home cages. The test chamber was cleaned with lemon scented soapy water following each test.

Behavioral Measures

The behavioral measures recorded were identical to those of Experiment 3 and were scored by a rater blind to the experimental conditions.

Results

All shrews conditioned with lithium vomited during the first 30 min conditioning trial and no shrews conditioned with saline vomited during conditioning. However, no shrew displayed vomiting during testing.

Figure 4 presents the mean frequency of each behavior displayed by the groups previously conditioned with lithium and saline when tested following an injection of THC or Vehicle. The data in the upper left-hand section represents conditioned retching behavior during exposure to the test chamber. A 2 by 2 mixed factors ANOVA of the frequency of retching revealed a significant conditioning drug by test drug interaction, $F(1, 12)=5.5$; $p<.05$. Subsequent LSD

pair-wise comparison tests for each test drug revealed that the lithium conditioned group displayed significantly more conditioned retching when tested under the vehicle condition than any other group (p's<.05) and no other groups differed significantly. Although one saline animal displayed conditioned retching upon re-exposure to the test chamber, this was probably due to experimental error since this behavior was not displayed by other saline animals. The upper right-hand section represents conditioned gaping reactions during exposure to the test chamber. The 2 by 2 ANOVA revealed a significant effect of conditioning drug, $F(1, 12)=5.2$, $p<.042$, and a significant effect of test drug, $F(1, 12)=5.6$; $p<.05$, but no significant interaction, $F(1, 12)=2.3$; $p=.16$. Shrews conditioned with lithium gaped more than those conditioned with saline and those tested with THC gaped less than those tested with Vehicle. Finally, analysis of the locomotion data revealed only a significant effect of test drug, $F(1, 12)=8.3$; $p<.025$; shrews were more active when tested under the influence of THC than when tested under the influence of Vehicle.

Discussion

During conditioning, all animals administered lithium displayed both retching and vomiting, while those administered saline did not. At test, shrews who were previously administered lithium chloride displayed both conditioned gaping and retching upon re-exposure to an environmental cue (test chamber) associated with lithium induced vomiting. Although gaping is defined as a smaller opening of the mouth than a retch, it may be that gaping naturally precedes retching in an emetic species. This is consistent with the fact that most animals

display gaping before they display retching when administered lithium chloride (Experiment 3). In the present experiment, THC completely interfered with the expression of conditioned retching without interfering with general activity levels of the shrew. In fact, THC enhanced locomotor activity in these animals, suggesting that the attenuation of conditioned retching was not due to a general disruption of responding. The effect of THC on behavior is biphasic; it produces an increase in activity levels at low doses and a suppression of activity at high doses (Chaperon & Thiebot, 1999). These results suggest that conditioned retching may be an effective measure of a conditioned emetic response. Furthermore, THC interferes with this conditioned emetic response in *Suncus murinus*. This may, therefore, serve as an effective animal model to evaluate anticipatory nausea and vomiting reported by chemotherapy patients.

General Discussion

Upon re-exposure to an environmental chamber associated with lithium induced sickness, *Suncus murinus* displays both conditioned gaping and conditioned retching. Similar results have been reported in coyotes, who display conditioned retching upon re-exposure to sheep meat that was previously paired with lithium induced vomiting (Gustavson et al., 1974), and in ferrets, who display this conditioned behavior when re-exposed to mice who were previously paired with lithium induced sickness. When administered lithium chloride, retching always precedes vomiting in the *Suncus*. The fact that these animals display this behavior as a conditioned response in the absence of lithium at test suggests that

conditioned retching may be an effective measure of a conditioned emetic response in the house musk shrew.

THC has been previously shown to interfere with the expression of conditioned rejection reactions in rats, which may reflect the attenuation of nausea in these animals (Limebeer & Parker, 1999). In Experiment 4, THC eliminated conditioned retching at a dose which did not impair general locomotor activity. In fact, when tested under the influence of THC, animals showed increased activity levels than when tested under the vehicle. This enhanced locomotor activity suggests that the dose of THC used falls within the low range of the dose-response curve (Chaperon & Thiebot, 1999). These results are the first to experimentally verify patient anecdotal reports that THC attenuates conditioned sickness behavior when presented with cues previously associated with toxin induced emesis. Therefore, it appears that conditioned retching in the *Suncus murinus* may serve as an effective animal model to evaluate both pharmacological and/or behavioral interventions for anticipatory nausea and/or vomiting in human patients.

Administration of lithium chloride in rats induced a repertoire of unconditioned behavioral responses including a suppression of locomotor activity, grooming, rearing, genital licks, and scratching. The mean duration spent lying on belly also increased for lithium treated animals. This suppression of behavior and increased lying on belly was enhanced as the dose of LiCl was increased, with the greatest suppression of behavior and LOB occurring in animals administered the high dose of lithium (129 mg/kg). Lithium also increased kaolin

consumption in rats across the first hour and a half of test, although this was seen only in animals administered 51.6 mg/kg. Although these behaviors were displayed in rats following lithium administration in Experiments 1 and 2, a dose of 0.5 mg/kg THC neither modified unconditioned behavioral sickness reactions elicited by lithium chloride nor lithium induced kaolin consumption (pica). However, it is impossible to determine whether the dose of THC was simply too low because a wide range of doses of THC was not tested. Limebeer and Parker (1999) showed that 0.5 mg/kg THC attenuates both the establishment and expression of cyclophosphamide induced conditioned rejection reactions in rats. If conditioned rejection reactions displayed by rats are more easily disrupted by the effects of THC, then future investigations concerning unconditioned sickness reactions might examine the effect of increasing doses of THC.

Future research evaluating the efficacy of THC at attenuating lithium induced unconditioned sickness behavior must be careful in the selection of THC doses to be used. In the place preference paradigm, a place aversion has been shown in rats administered cannabinoids such as THC (Mallet & Beninger, 1998; Parker & Gillies, 1995; Sanudo-Pena, Tsou, Delay, Hohman, et al., 1997), CP 55, 940 (McGregor, Issakidis, & Prior, 1996), and HU 210 (Cheer, Kendall, & Marsden, 2000). Place aversions to these cannabinoids occur at relatively low doses of drug administered. For instance, Parker and Gillies (1995) reported that a dose of 1.5 mg/kg THC produces both a place and taste aversion in both Lewis and Sprague-Dawley rats. These results are consistent with studies showing that

animals will not self administer cannabinoids (Corcoran & Amit, 1974; Harris, Waters, & McLendon, 1974).

Although place aversions to low doses of cannabinoids have been reported, place preference to THC has also been found in both rats (Lepore, Vorel, Lowinson, & Gardner, 1995), and mice (Valjent & Maldonado, 2000) under particular experimental conditions. Lepore et al., (1995) found a place preference for THC at doses of 2 and 4 mg/kg when THC and vehicle were administered on consecutive days during conditioning. In addition, a THC induced place preference was found at a dose of 1 mg/kg when the conditioning procedure was modified, so that each drug or vehicle pairing was followed by a 24 h washout period (e.g., drug – day off – vehicle – day off). In addition, Valjent and Maldonado (2000) found a THC place preference in mice when animals received a previous priming THC injection. These studies suggest that it may be worthwhile to examine a range of THC doses on lithium induced unconditioned sickness behavior, with doses as high as 4 mg/kg being tested.

Antiemetic drugs have been shown to be successful in attenuating both retching and vomiting in *Suncus murinus* elicited by radiation (Gardner et al., 1995), anesthesia (Gardner & Perren, 1998), and toxin induced sickness (Matsuki et al., 1997; Ueno et al., 1987). However, the results of Experiment 3 show that at a dose of 3 mg/kg, THC did not interfere with lithium induced gaping, retching, or vomiting displayed by the shrews. In fact, animals in Group THC-lithium displayed a shorter latency to both retch and vomit than saline controls, although they did not differ from Group Vehicle-Lithium. Athough saline animals did not

display retching or vomiting, animals in Group Vehicle-Lithium did not differ from them in terms of latency to first retch or vomit. THC did, however, suppress chin rubbing elicited by lithium in shrews, which has been previously argued to be a measure of nausea in rats (Parker, 1995, 1998). If this behavior is a measure of sickness in *Suncus*, it may be that it is more easily disrupted by the effects of THC than either gaping, retching, or vomiting.

Although THC attenuated conditioned retching in *Suncus murinus*, it did not attenuate unconditioned sickness behavior in either the nonemetic rat or the emetic shrew. Future research may use a shorter time interval between administration of THC and delivery of lithium chloride. Studies with human subjects suggest that when smoked, THC can start to have a maximum effect as early as 5-10 min after administration (Iverson, 2000). If this is indeed the case, then the efficacy of THC as an antiemetic would diminish after a 30 min period. The effect of testing a range of THC doses should also be further examined. However, a maximum dose must be obtained which does not impair normal locomotor or behavioral functioning, and is not aversive to the animal.

Future antiemetic research may also investigate the effect of THC in combination with 5-HT_3 (serotonin) antagonists, which possess antiemetic properties themselves (Gregory & Ettinger, 1998). The highest density of 5-HT_3 receptors in the central nervous system is located in a sub-region of the nucleus of the solitary tract , which has been named the area sub-postrema (Pratt, Bowery, Kilpatrick , Leslie, et al., 1990). Because cannabinoid receptors are found in the nucleus of the solitary tract, Himmi and colleagues have argued that

5-HT_3 receptors located in the hindbrain may be involved in the nausea-reducing effects of cannabinoids (Himmi et al., 1996, 1998). This hypothesis is supported by the fact that delta-9-tetrahydrocannabinol sensitive neurons in the solitary tract respond to the 5-HT_3 agonist 1-phenylbiguanide (which induces vomiting) in a direction opposite to that of THC itself (Himmi et al., 1996). In addition, Fan (1995) showed through patch-clamp studies that cannabinoid agonists inhibit the activation of 5-HT_3 receptors in rat nodose ganglion neurons. These studies suggest that 5-HT_3 receptors are acted upon by cannabinoids in the central nervous system, and that this may be a possible mechanism of the antiemetic effects of cannabinoids themselves.

These results suggest that THC may be more effective in attenuating conditioned, rather than unconditioned sickness. In addition, they show that conditioned retching in *Suncus murinus* may be an effective animal model to further evaluate pharmacological and/or behavioral interventions for conditioned or "anticipatory" nausea and/or vomiting in humans.

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Table 1. Mean (±sem) frequency and latency of unconditioned reactions displayed by animals in Experiment 3.

BEHAVIOR	Veh-Lithium	THC-Lithium	Saline
Gape			
Frequency	102.7(±44.6)	111.2(±30.5)	7.5(±2.8)
Latency	124.1(±23.8)	167.9(±49.3)	573.0(±358.0)
Chin Rub			
Frequency *	122.4(±28.9)	55.7(±16.9)	29.0(±11.7)
Latency	82.6(±14.5)	355.9(±150.1)	468.9(±176.9)
Backward Walk			
Frequency	56.0(±17.5)	27.5(±8.7)	16.9(±8.8)
Latency	304.2(±149.0)	519.1(±275.4)	720.0(±365.4)
Locomotion			
Frequency	204.9(±9.9)	119.2(±30.2)	133.3(±60.9)
Latency	5.8(±1.3)	7.7(±2.6)	233.4(±224.4)
Face Wash			
Frequency	1.8(±1.7)	10.9(±3.3)	8.0(±6.7)
Latency *	154.3(±61.9)	1477.1(±258)	1158.4(±379)
Retch			
Frequency	22.1(±11.0)	29.1(±8.8)	0.0
Latency *	1221.4(±286.0)	807.2(±202)	1800[1]
Vomit			
Frequency	5.8(±3.0)	5.8(±1.4)	0.0
Latency *	1224.5(±285)	809.6(±202)	1800[1]

[1] duration of test
* indicates significance at $p<.05$

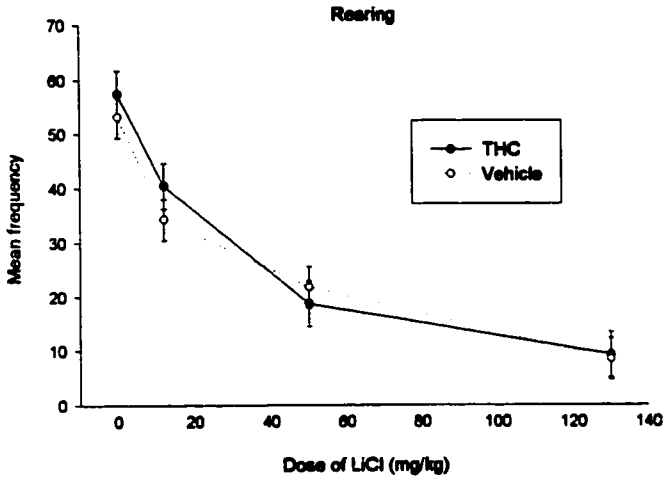
Figures

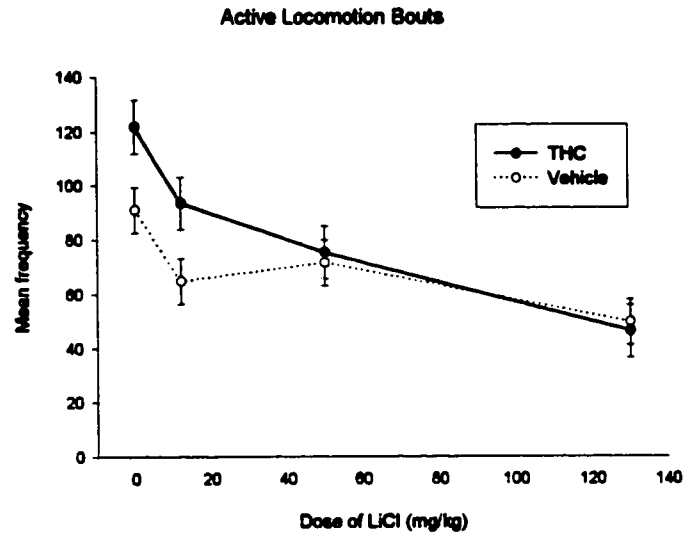
Figure 1. Mean (± sem) frequency of rearing, active locomotion, grooming, genital licks and scratching displayed by rats injected with various doses of LiCl during a trial on which they were pretreated with THC and on a trial on which they were pretreated with vehicle.

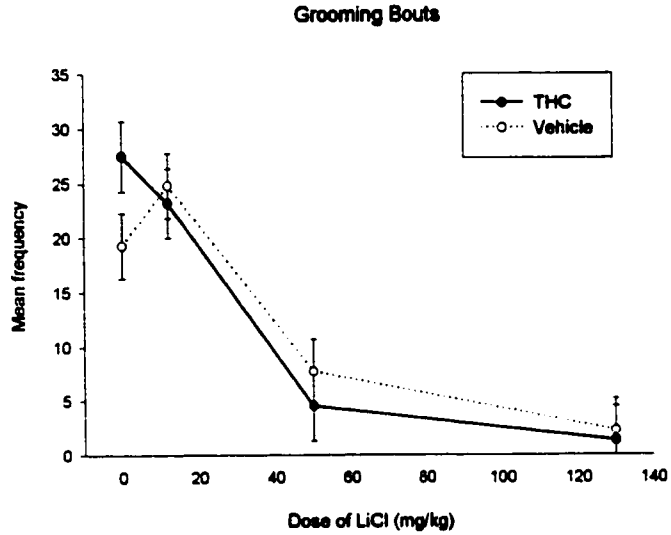
Figure 2. Mean (± sem) number of seconds that rats injected with each dose of LiCl spent lying on belly during the trial on which the rats were pretreated with THC and on the trial on which they were pretreated with vehicle.

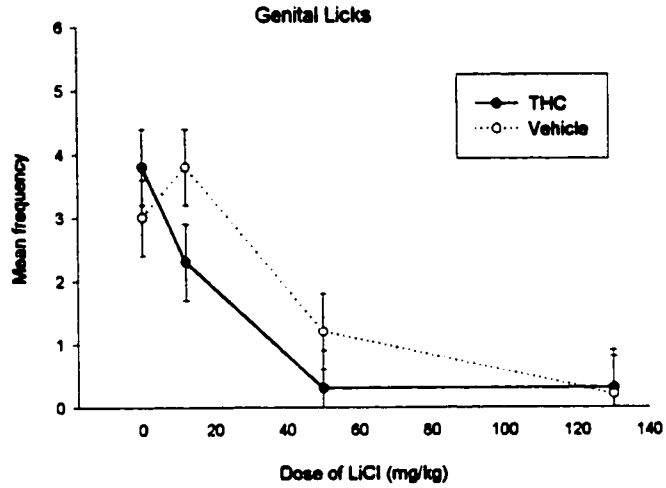
Figure 3. Mean (± sem) grams of kaolin consumed by rats conditioned with various doses of LiCl across 1 ½ h of testing.

Figure 4. Mean (± sem) frequency of retching, gaping, and bouts of forward locomotion displayed during a test for the expression of conditioned emetic activity. Shrews were administered 3 mg/kg of THC on one test day and the vehicle on another test day (counterbalanced), 30 min prior to placement in the conditioning chamber.


Rearing
Mean frequency
Dose of LiCl (mg/kg)
THC
Vehicle


Active Locomotion Bouts
Mean frequency
Dose of LiCl (mg/kg)
THC
Vehicle


Grooming Bouts
Mean frequency
Dose of LiCl (mg/kg)
THC
Vehicle


Genital Licks
Mean frequency
Dose of LiCl (mg/kg)
THC
Vehicle

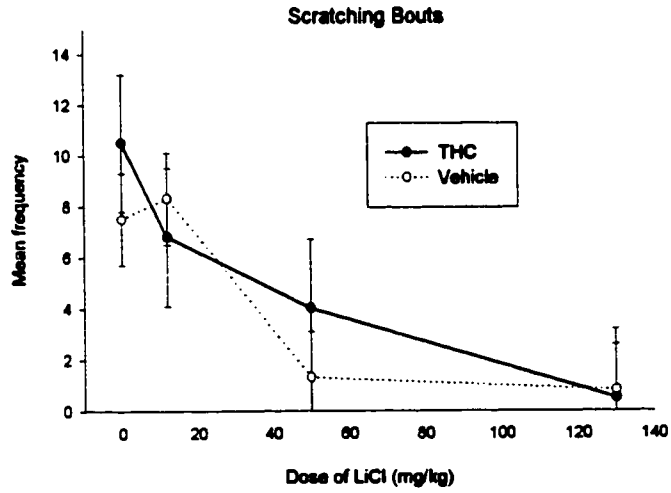

Scratching Bouts
Mean frequency
Dose of LiCl (mg/kg)
THC
Vehicle

Figure One

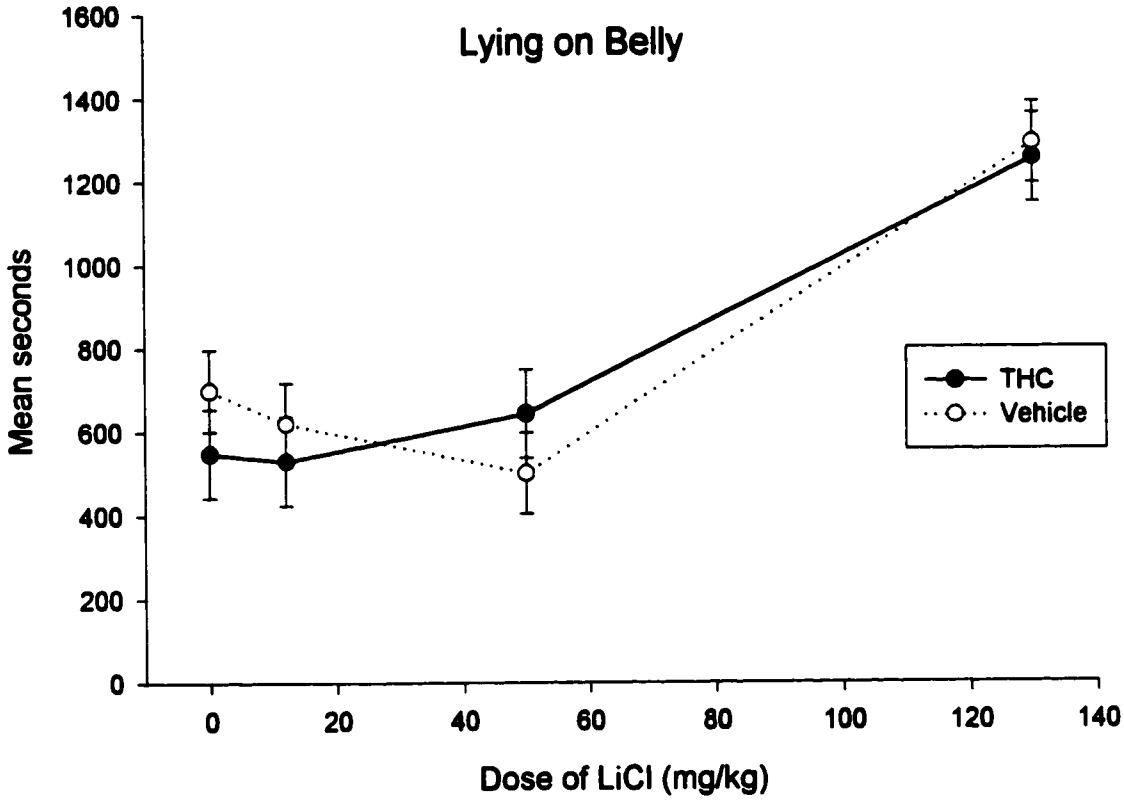

Lying on Belly
Mean seconds
1600
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
140
Dose of LiCl (mg/kg)
THC
Vehicle

Figure Two

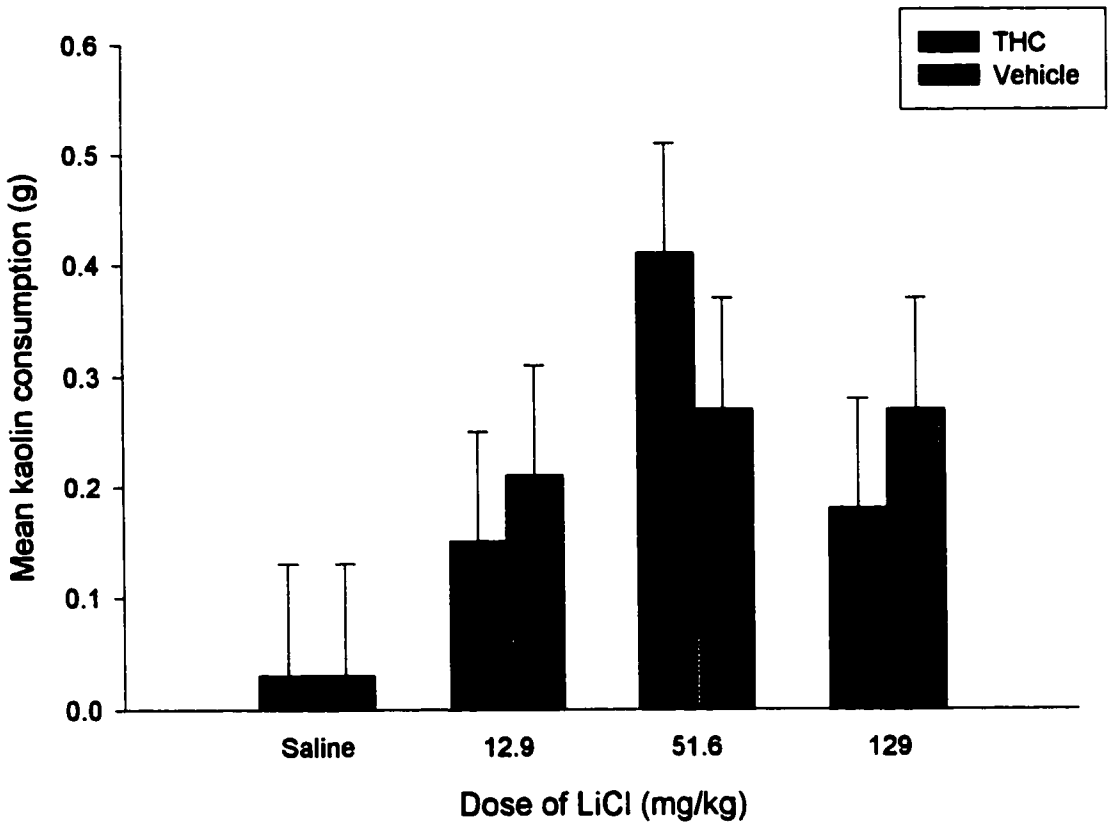


Figure Three

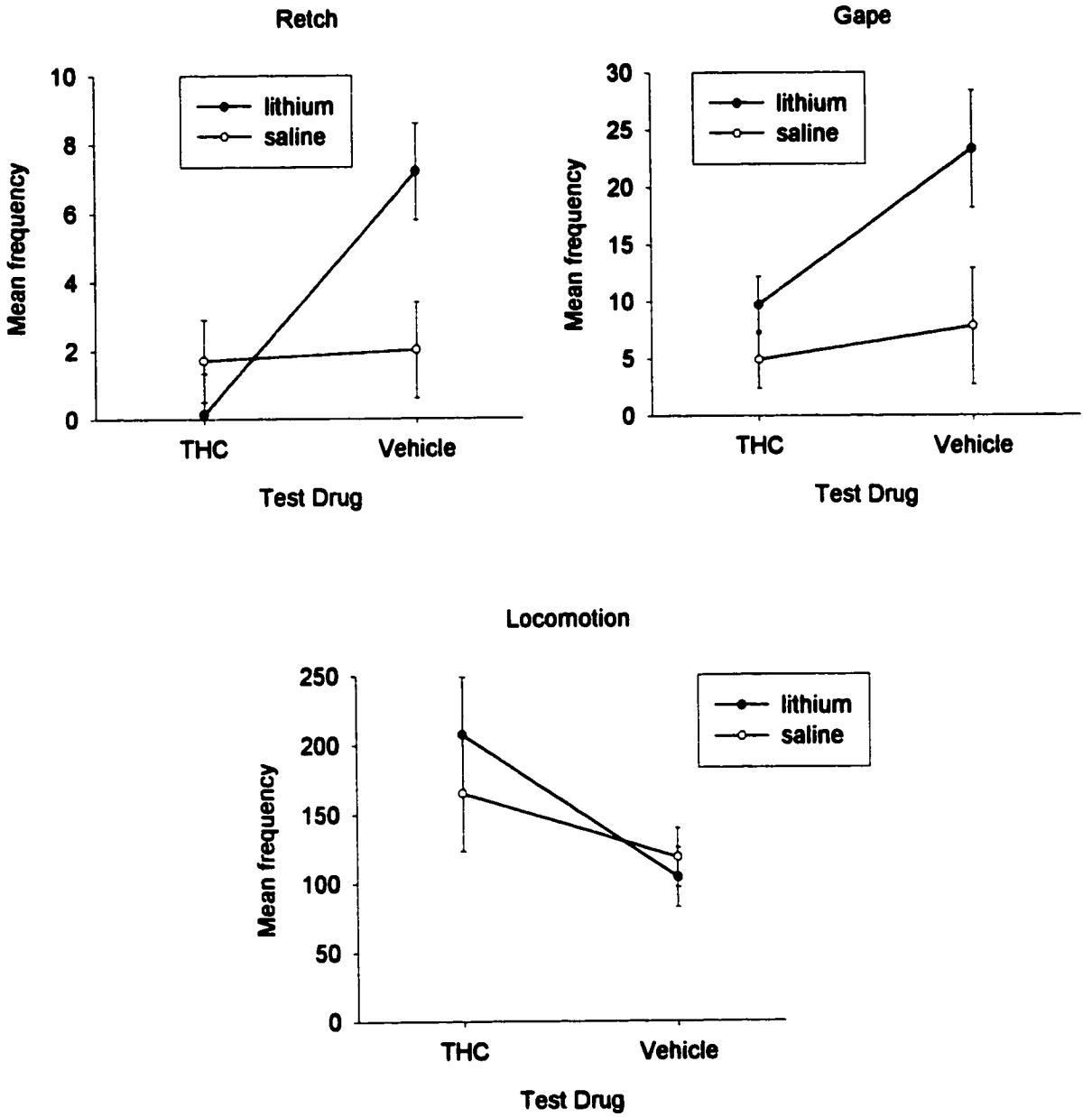

Retch
Mean frequency
10
8
6
4
2
0
lithium
saline
THC
Vehicle
Test Drug
Gape
Mean frequency
30
25
20
15
10
5
0
lithium
saline
THC
Vehicle
Test Drug
Locomotion
Mean frequency
250
200
150
100
50
0
lithium
saline
THC
Vehicle
Test Drug

Figure Four