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# The effect of Delta9-tetrahydrocannabinol (THC) on the establishment and the expression of place conditioning (Rattus norvegicus)

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# The Effect of Δ9-Tetrahydrocannabinol (THC) On the Establishment and the Expression of Place Conditioning

by

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## Abstract

The place conditioning paradigm has been used to assess the antinausea potential of drugs in non-emetic animal species. The present experiments were designed to determine the potential of Δ9-tetrahydrocannabinol (THC) to alleviate both conditioned and unconditioned sickness in rats (*Rattus norvegicus*). The results in the present study suggest that when the place aversion is strong (Experiment 1), THC may interfere with the establishment; however when the aversion is weak (Experiments 2 and 3), THC may interfere with the expression. Also, when administered during acquisition of a strong amphetamine-induced place preference, THC also seems to interfere with the establishment of the preference. Therefore it appears that when the place association is strong, THC may interfere with the associative process itself, but when the place association is weak THC may interfere with the conditioned sickness or the retrieval of the association.

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# The Effects of Δ9-Tetrahydrocannabinol (THC) as evaluated in the Place Conditioning Paradigm

For centuries marijuana, *Cannabis sativa*, has been used by many cultures as a herbal remedy and it has only been in the last few decades that the primary active component of marijuana's over 60 chemicals, Δ9- tetrahydrocannabinol (THC), has been identified. From early on people realized the antinausea effects of smoking marijuana and it has been these reports that have led scientists to test the various effects of the purified and unpurified drug (Iverson, 1999).

Nausea can be defined as a "feeling of sickness with the inclination to vomit" (Hawkins & Allen, 1991). Therefore, in order to properly treat the primarily subjective experience of nausea, behavioural measures must be utilized, especially when testing animals. Although nausea and vomiting can each occur independently of one another, it is often the case that vomiting, as caused through treatment with emetic agents, is preceded by nausea, thus nausea could be described as a lesser degree of emetic stimulation within the emetic system (Andrews, Rapeport & Sanger, 1988). Because nausea is a subjective experience, its mechanism is not clearly understood. However, generally therapy for vomiting has some effect on nausea (Andrews et al., 1988). With an awareness of these limitations, in the present thesis the terms "antiemetic" and "antinausea" will be used interchangeably. Following this logic, in nonemetic species antiemetic agents may also function as antinausea agents as well. In fact, many drugs that reduce vomiting caused by emetic agents also reduce the feeling of nausea and the behaviours associated with the nausea.

With the recent rise in the number of patients receiving chemotherapy for cancer-related illnesses, investigators have been searching for countermeasures to chemotherapy-induced nausea and vomiting. Anecdotal reports indicate that THC alleviates not only the drug-induced nausea or vomiting that immediately follows a chemotherapy treatment, but also the anticipatory classically conditioned nausea that occurs upon re-exposure to cues previously associated with the treatment. Reports of the effectiveness of marijuana in relief of nausea during chemotherapy have come from numerous patients including the Harvard biologist Stephen Jay Gould (Ottawa Citizen, 5 August 1998) who was quoted as saying "it is beyond comprehension that any humane person would withhold such a beneficial substance [marijuana] from people in such need simply because others use it for different purposes." The medical community, however, has not been as easy to persuade of the medical benefits of marijuana, partially due to the lack of experimental evidence for the antiemetic properties of THC. Indeed only a few oncologists have prescribed or recommended THC to their patients (Schwartz & Beveridge, 1994; Voth & Schwartz, 1997).

## History of the Discovery of the Cannabinoid System

Once the active component of marijuana, Δ9-THC, was isolated by Mechoulam and Gaoni (1967), pharmaceutical companies began the search for an analogue of Δ9-THC without psychoactive side effects. In 1967, Pfizer developed the first analogue called nantradol and marketed it for pain relief. Later, nabilone and dronabinol were developed, which had fewer side effects than nantradol. Nabilone and dronabinol were evaluated for their antiemetic properties in clinical trials. These early trials revealed that

THC was as effective as other available antiemetics, primarily the dopamine antagonists (eg. Sallan, Zinberg & Frei, 1975). As these analogues of THC were developed, concern for their abuse potential resulted in their classification as Schedule II drugs, limiting their accessibility and resulting in a decline in clinical research.

In the early 1990's, Mechoulam discovered that the brain contained specific cannabinoid (CB) receptors, CB1 and CB2, through which THC acts. Shortly thereafter the primary endogenous cannabinoid, anandamide, was discovered (Devane et al, 1992). Synthetic CB agonists (CP-55,940, WIN 55,212-2, HU 210) have subsequently been developed with the goal of developing drugs with fewer psychoactive properties than THC, but with similar antinausea properties. Like THC, these agents serve as nonspecific agonists of both CB1 and CB2 receptors. Most recently, specific CB1 (SR 141716A) and CB2 (SR 14428) receptor antagonists have been developed. Unlike the nonspecific agonists, these antagonists serve as tools required to determine the receptor type responsible for a given behavioural effect. In fact, it has also been recently discovered that the CB1 receptor is a G protein-coupled receptor that signals inhibition of adenylyl cyclase (Matsuda, Lolait, Brownstein, Young & Bonner, 1990).

CB1 receptors are found in both the central and peripheral nervous system, whereas CB2 receptors have been localized only in the peripheral nervous system. The greatest density of CB1 receptors in the brain can be found in the globus pallidus, substantia nigra pars reticulata, dentate gyrus of the hippocampus, nucleus accumbens and in the cerebral cortex in rats, monkeys and humans (Howlett et al., 1990). Through the use of other receptor ligands it has been possible to determine that there is little binding of the ligands in the lower brainstem (the site of breathing and pulmonary

regulation), which could somewhat explain why high doses of THC are not lethal (Herkenham et al., 1990). On the other hand, the CB2 receptors are located outside the blood-brain barrier and are primarily located in the immune cells in the body (macrophages, T-cells, B-cells and mast cells). This leads to an interesting question as to the role of the CB2 receptors in the adverse side effects of immunosuppression that may be produced by high doses of Δ9-THC.

By acting on central CB1 receptors, THC has been reported to negatively affect memory systems (Chaperon, Soubrie, Puech & Thiebot, 1998; Mallet & Beninger, 1998) and can play a role in stress recovery by relieving some stress-induced responses and leading to sedation (DiMarzo, Melck, Bisogno & De Petrocellis, 1998). THC has also been shown to influence dopamine (DA) transmission in the nucleus accumbens through an enhancement in dopamine release, which may be related to evidence suggesting that dependence may develop to THC (Tanda, Loddo & Di Chara, 1999). Indeed, rats and mice administered THC show withdrawal behaviour (e.g., paw tremors, head shakes) when administered a CB1 receptor antagonist (SR 141716) after only two days of chronic exposure (Cook, Lowe & Martin, 1998).

Most recently, CB1 receptors have been found in the nucleus of the solitary tract in rat brain slices (Himmi, Dallaporta, Perrin & Orsini, 1996; Himmi, Perrin, El Ouzzani & Orsini, 1998), a structure within the emetic system of the brain. The nucleus of the solitary tract is involved in nausea reactions induced by either vagal gastrointestinal activation or several humoral cytotoxic agents. It is considered the starting point of the final common pathway for the induction of emesis in vomiting species. This area is also highly populated with 5-HT3 (serotonin) receptors (Higgins, Kilpatrick, Bunce, Jones &

Tyers, 1989), potentially a site for the antiemetic effects of 5-HT3 antagonists. In fact, THC has recently been shown to interact with 5-HT3 receptors (Kimura, Ohta, Watanabe, Yoshimura & Yamamoto, 1998) by decreasing the ability of serotonin to bind to the 5-HT3 receptor. THC activates the CB1 receptors of the nucleus of the solitary tract and this activation is blocked by the selective CB1 antagonist SR 141716A (Himmi et al., 1998). Endogenous CB ligands, such as anandamide, as well as synthetic CB receptor agonists, such as WIN 55,212-2, also act on these receptors (Felder & Glass, 1998).

## CB Agonists as Antiemetic Agents: Experimental Evidence

The experimental evaluation of the antiemetic properties of THC and other CB agonists often employ animal models of emesis. Since rats and mice do not vomit, most work that evaluates the putative antiemetic agents involves dogs, cats, ferrets, *Suncus murinus* (house musk shrew), or pigeons, all of which vomit when injected with a toxin. Using these models, there is evidence that nonspecific CB agonists attenuate emesis. McCarthy and Borison (1981) showed that THC decreased cisplatin-induced vomiting in cats. Cisplatin is a drug used in chemotherapy that produces nausea in human patients. The non-psychotropic cannabinoid, HU-211, has subsequently been reported to elicit dose related inhibition of cisplatin-induced vomiting in the pigeon (Feigenbaum, Richmond, Weissman & Mechoulam, 1989). Most recently, Ferrari and colleagues (Ferrari, Ottani & Guilani, 1999) reported that the synthetic cannabinoid, HU-210, interfered with cisplatin-induced vomiting in pigeons, even at a dose (12.5 ug/kg, sc) that did not produce sedation. Finally, Darmani (2001) reports that THC interferes with

vomiting elicited by a CB1 receptor antagonist (SR 141716A) in the *Cryptotis parva* (least shrew).

Since rats and mice do not vomit, other behavioural measures of sickness must be used to evaluate the antiemetic potential of CB agonists in these species. Humans have historically used THC to reduce diarrhea, a symptom of gastrointestinal sickness (Iverson, 1999). Recently investigators have evaluated the ability of CB agonists to interfere with gastro-intestinal transit in humans and other animals. Cannabinoids have been shown to delay the emptying of gastric contents in humans (McCallum et al., 1999), and reduce the rate of intestinal propulsion/motility in rats (Crawley et al., 1993; Izzo, Mascolo, Pinto, Capasso & Capasso, 1999) and mice (Calignano et al., 1997; Colombo, Agabio, Lobina, Reali & Gessa, 1998). In fact, Izzo et al (1999) also found that the CB1 receptor antagonist SR 141716A increased fecal output suggesting that endogenous cannabinoids may naturally inhibit the system.

A putative measure of nausea in rats is that of conditioned rejection reactions (Parker, Limebeer & Simpson, 1998). Flavours paired with emetic agents produce conditioned rejection reactions in rats displayed as gapes, chin rubs and paw treading. Although drugs that are not emetic agents (e.g. cocaine, amphetamine) also produce taste avoidance in rats, these drugs do not produce conditioned rejection reactions (Parker & Gillies, 1995). Therefore, Parker and colleagues (1998) suggested that these rejection reactions reflect nausea in rats. If conditioned rejection reactions reflect conditioned nausea in rats, then pretreatment with an antiemetic drug should reduce conditioned rejection. Limebeer and Parker (1999) evaluated the potential of THC to interfere with the establishment and with the expression of cyclophosphamide-induced conditioned

rejection reactions. Cyclophosphamide is a drug used in chemotherapy with humans that produces severe nausea and vomiting during treatment. It also produces conditioned rejection reactions in rats (Parker et al., 1998). Limebeer and Parker (1999) gave rats THC or Vehicle prior to a pairing of saccharin solution with cyclophosphamide or saline during conditioning and/or prior to a test. They found that THC interfered with the *establishment* of cyclophosphamide-induced conditioned rejection when administered during the conditioning phase and with the *expression* of conditioned rejection when administered prior to the testing phase. These results suggest that THC alleviated the nausea produced by cyclophosphamide during the conditioning and also alleviated the conditioned nausea elicited by cyclophosphamide paired saccharin solution at test. Furthermore, in another test of the antiemetic potential of a drug in this paradigm, Limebeer and Parker (2000) demonstrated that the commonly employed antiemetic agent, ondansetron, interfered with both the establishment and the expression of lithium-induced conditioned rejection reactions.

Another paradigm used to measure sickness in rodents that has been described in the literature is that of conditioned place aversion learning (Frisch, Hasenohrl, Mattern, Hacker & Huston, 1995). Emetic drugs consistently produce a conditioned place aversion (CPA). In this conditioning paradigm the animal is given a drug, like lithium chloride (LiCl), and is then placed in a distinctive chamber for a time duration long enough for the animal to form an association between the cues of the chamber and the effects of the drug. On a different day the animal is given saline and is placed in another distinctive chamber. Following a number of such pairings the animal is placed in the apparatus with the barrier between the two distinct chambers removed and is allowed to

explore both chambers. In such a test, rats consistently avoid the lithium-paired chamber even after a single conditioning trial (Parker, 1992).

The place aversion paradigm has been used to evaluate the antiemetic potential of drugs. Frisch et al (1995) evaluated the potential of the antiemetic drug, metoclopramide, to interfere with the establishment of a lithium-induced place aversion. This drug that effectively attenuated nausea and vomiting in human chemotherapy patients also interfered with the establishment of a lithium-induced place aversion, presumably by reducing the nausea produced by lithium. Although not evaluated by Frisch et al (1995), the place aversion paradigm might also be useful to evaluate the potential of antiemetic treatments to reduce conditioned nausea elicited by cues previously paired with emetic drugs; such an evaluation would be done by administering the antiemetic treatment prior to a preference test. Interference with the expression of a conditioned place aversion might indicate that the agent interfered with conditioned nausea, a potential measure of anticipatory nausea that occurs in chemotherapy patients (Morrow, Hickok & Rosenthal, 1995).

## The Present Study

The present experiments employed the conditioned place aversion paradigm to evaluate the antinausea properties of THC. If THC interferes with lithium-induced nausea, then it was expected to attenuate the *establishment* of a place aversion when administered prior to lithium during conditioning. If THC interferes with the *expression* of previously established conditioned nausea, then it was expected to attenuate the expression of place aversion learning when administered prior to a place preference test.

The dose of THC (0.5 mg/kg, ip) that was employed in the following experiments was the same used by Limebeer and Parker (1999). This dose interfered with the establishment and expression of cyclophosphamide-induced conditioned rejection reactions, another putative measure of nausea in rats; however this dose did not affect general activity level in the rats. A suppression of motor activity could, potentially, result in reduced attentional processing of the contextual stimuli. Parker and Gillies (1995) reported that a dose of 1.5 mg/kg of THC or above produces a conditioned place aversion as well as conditioned saccharin rejection reactions. However, doses of 0.25–0.75 mg/kg produced neither preference nor aversion by either test. Therefore, a dose of 0.5 mg/kg was expected to have no hedonic or motoric effects on its own.

## Experiment 1

Experiment 1 evaluated the potential of THC to interfere with the establishment and/or with the expression of a lithium-induced conditioned place aversion. The design of the experiment is presented in Table 1. There were 4 groups, which differed on the basis of pretreatment drug (THC or VEH) and the conditioning drug (Lithium or Saline). On each of two treatment trials, rats were pretreated with THC or VEH 30 min prior to an injection of lithium or saline and placement in Chamber A. On each of two non-treatment trials, rats were injected with VEH 30 minutes prior to an injection of saline and placement in Chamber B. The chamber paired with the treatment was counterbalanced among the groups.

Using a repeated measures design, all rats were tested for their place preference 30 min following an injection of THC or 30 min following an injection of VEH, alternating every other day, with the order counterbalanced among the groups. All rats

received a total of 6 ten-minute test trials; on alternate days, rats received test trials 30 min following an injection of THC or Vehicle. The order of THC and Vehicle trials were counterbalanced among each pair of alternate days. Therefore, each rat received a total of 3 tests in a THC state and 3 tests in a Vehicle state. The design of Experiment 1 was a 2 by 2 by 2 by 3 mixed factors design with the between groups factors of Pretreatment Drug (THC, VEH) and Conditioning Drug (Lithium, Saline) and the within groups factors of Test Drug (THC, VEH) and Test Trial Cycle (1-3).

## Method

### Subjects

The subjects were 64 male Sprague-Dawley rats obtained from Charles River Laboratories, St. Constant, Quebec, weighing 225-300g at the beginning of the experiment. The rats were housed in pairs in plastic cages with woodchip bedding and were maintained on ad-lib food and water for the duration of the experiment. The colony room was kept on a 12L: 12D (lights on at 0700h with manipulations occurring at 0900h) schedule with relative humidity at 50-60%. The procedures were approved by the Wilfrid Laurier University Animal Care Committee according to the guidelines of the Canadian Council on Animal Care. All rats in the experiment were treated identically except where experimental procedure dictated otherwise.

### Drugs

The THC (1.0 mg/ml) was prepared in a mixture of polyvinylpyrrolidone (PVP) and saline according to the procedure described by Fenimore and Loy (1971). THC was administered intraperitoneally (ip) at a dose of 0.5 mg/kg (0.5 ml/kg) with controls

receiving an ip injection of 0.5 ml/kg of the PVP vehicle. Lithium chloride (LiCl) was also used (12 ml/kg of 0.15M LiCl mixed with distilled water – 75 mg/kg).

Apparatus

The place-conditioning apparatus used for the experiment was that described by Parker (1992) and included two boxes separated by a removable wooden partition. The walls and floor of the boxes were painted flat black and were of the following dimensions: 35 x 25 x 30 cm. There were distinct textural cues available in each of the two boxes consisting of a wire mesh grid (0.625 cm) and sandpaper strips (3 cm) located approximately 3 cm apart. The rats were conditioned in each box on successive days and the divider was removed during testing to allow the rat the opportunity to explore both sides of the apparatus. During testing, the activity of the rats was monitored via an overhead video camera (Videomex-V, Columbia Instruments, Columbus, OH), which was then fed to a computer allowing the experimenter to determine the amount of time spent on each side of the place-conditioning chamber. In addition, each chamber was divided into 7 zones and the number of crossings among the zones was recorded.

Procedure

The rats arrived in the laboratory one week prior to their use in the experiments and were handled daily by the experimenter. Each rat received a total of two conditioning trial cycles, (Table 1 presents the procedures for one conditioning trial cycle), within each cycle one trial was a Treatment trial and another trial was a Non-Treatment trial. There were four groups: THC – Lithium (TL, n=16), Vehicle – Lithium (VL, n=16), THC-Saline (TS, n=16) and Vehicle-Saline (VS, n=16). On the Treatment trial, depending upon the group, rats were injected ip with THC or Vehicle 25 min prior to another ip

injection of Lithium or Saline. Five min later, the rats were placed in the appropriate chamber (mesh or sandpaper, indicated by Chamber A) for 30 minutes. On the Non-Treatment trial of a cycle, all the rats were injected ip with Vehicle, 25 min prior to receiving another ip injection of Saline before being placed in the appropriate chamber as during the treatment trial (Chamber B), again for 30 minutes. The time spent between the pretreatment injection and the injection of the conditioning drug was spent in the home cage. The order of the treatment trial within a cycle and the chamber paired with the treatment were counterbalanced within each group. The amount of time between administration of THC and placement in the chamber was based on the procedure of Limebeer and Parker (2000).

Two days following the second conditioning cycle, testing began. For each test the wooden divider was removed allowing the rat to move freely between chambers. On each trial, the rats were injected with 0.5 mg/kg THC or Vehicle 30 min prior to being placed in the chamber for the 10 min test. The rats received a total of 3 cycles (6 test trials); in each cycle the rats were given one THC and one Vehicle test, separated by 24 hours. The order of THC and Vehicle tests were counterbalanced among the groups. The videotracking apparatus recorded the activity of the rat and the data was converted into the amount of time spent on each side of the chamber. Each chamber was divided into 7 equivalent zones (14 total during testing) so that the number of zone crossings could be calculated as a measure of the general activity level of the rat during the tests.

## Results

### Place Preference Test

Lithium produced a place aversion that extinguished more rapidly among the rats administered THC during conditioning than among the rats administered VEH during conditioning, although the THC-Lithium (TL) and VEH-Lithium (VL) groups did not differ on the first test trial cycle. On the other hand, THC administered during testing did not modulate the expression of place avoidance. Finally, there is no evidence that THC produced a hedonic effect on its own.

Figure 1 presents the mean number of seconds that the rats in each group spent on the treatment- minus the non-treatment-paired floor during the first cycle of testing with THC and VEH. A 2 by 2 by 2 mixed factors ANOVA with the between subjects factors of Pretreatment Drug (THC, VEH) and Conditioning Drug (LiCl, Saline) and the within subjects factor of Test Drug (THC, VEH) revealed only a significant effect of Conditioning Drug, $F(1,60)=21.10$; $p<.001$; those groups conditioned with lithium (Groups TL and VL) displayed a significantly stronger aversion than those groups conditioned with saline (Groups TS and VS). The Pretreatment Drug effect, $F(1,60)=2.64$, the Test Drug effect, $F(1,60)-1.41$, and all interactions were nonsignificant.

Figure 2 presents the place aversion test results for the three cycles of testing. The main effect of test drug was not significant, nor did it enter into any significant interaction with any other factor. Therefore, for clarity of presentation, the data presented in Figure 2 are pooled across the Test Drug factor. That is, the data for each cycle is the mean difference score pooled across the THC and the VEH test for that cycle.

The 2 by 2 by 2 by 3 mixed factors ANOVA revealed a significant main effect of Conditioning Drug, $F(1,60) = 10.4$; $p < .01$; the lithium conditioned groups displayed a significant place aversion. The analysis also revealed a significant Pretreatment by Conditioning Drug by Trial Cycle interaction, $F(2, 120) = 3.4$; $p < .05$. These were the only significant effects in this analysis, largest $F(2, 120)=2.8$; $p=.07$. The interaction was analyzed in two ways. First, separate 2 by 2 between groups ANOVAs were conducted on the difference scores pooled across Test Drug for each cycle of testing. These ANOVAs revealed only a significant effect of Conditioning Drug during cycle 1, $F(1, 60) = 21.1$; $p<.01$, and during cycle 2, $F(1, 60) = 7.26$; $p<.01$, but not during cycle 3, $F(1, 60) = 3.45$; $p=.068$. These ANOVAs revealed no significant Pretreatment by Conditioning Drug interaction during any cycle of testing. Secondly, the interaction was also analyzed by conducting separate 2 by 3 (Conditioning Drug by Test Trial Cycle) mixed factors ANOVAs of the difference scores pooled across Test Drug for each of the THC pretreated groups and the VEH pretreated groups. This analysis revealed a significant Conditioning Drug by Trial interaction for the THC pretreated groups, $F(2, 60) = 5.6$; $p<.01$, but not for the VEH pretreated groups, $F(2, 60) = .17$. Among the VEH pretreated groups, those rats conditioned with lithium significantly displayed lower difference scores than saline conditioned groups on each trial cycle, $F's(1,30) > 6.4$; $p's < .025$; however, among the THC pretreated groups, lithium conditioned groups displayed a significant place aversion during the *first* trial cycle, $F(1, 30) = 8.95$; $p < 0.01$, but *not* on trial cycle 2, $F(1,30) = 1.99$; $p = .17$; or cycle 3, $F(1, 30) = .068$; $p = .797$.

To better understand this effect seen in Group TL, separate 2 by 2 ANOVAs were performed with the within groups factors of Test Drug (THC, VEH) and Test Trial Cycle

(1-3). Only in Group TL was there a significant effect of Test Trial Cycle, $F(2,30)=5.58$; $p<.01$, although the interaction was nonsignificant. In no other group were any effects close to significance, largest $F(2,30)=1.45$. Therefore, in only Group TL did the strength of the conditioned place aversion change across extinction, thus THC modulated the establishment of the aversion.

In order to determine whether THC produced place conditioning on its own, the scores for Group TS were compared with those of Group VS. A 2 by 3 mixed factors ANOVA was conducted with the between groups factor of Pretreatment Drug (THC or VEH) and the within groups factor of Test Trial Cycle (1-3). This analysis revealed no significant effects [ largest $F(2, 60)=1.33$; $p=.27$], thus THC did not produce place conditioning on its own.

Activity Data

Figure 3 presents the mean ($\pm$ sem) number of zone crossings during testing for rats in each of the four groups by Test Drug. Rats were more active when tested under THC than when tested under VEH. A 2 by 2 by 2 by 3 mixed factors ANOVA with the between groups factors of Pretreatment Drug (THC, VEH) and Conditioning Drug (Lithium, Saline) and the within groups factors of Test Drug (THC, VEH) and Test Trial Cycle (1-3) was run on the activity data recorded during testing. The analysis revealed significant main effects of Test Drug, $F(1,60)=16.74$; $p<.001$, and Test Day, $F(2,120)=57.96$; $p<.001$, as well as a Test Drug by Pretreatment Drug interaction that was statistically significant, $F(1,60)=3.96$; $p=.05$. A simple main effects analysis revealed a significant Test Drug effect [$F(1,35)=17.5$; $p<.001$] for the groups pretreated with VEH during conditioning (Groups VL and VS) but the Test Drug effect only approached

significance for the groups pretreated with THC during conditioning (Groups TL and TS) [$F(1, 35)=4.0$; $p=.06$]. THC, when administered during testing *increased*, or enhanced, activity of the rats as measured by mean number of zone crossings, regardless of pretreatment condition. However, the fact that there was not a significant effect of Test Drug in the analysis of the place aversion suggests that, though THC may have enhanced activity, this enhancement did not alter the rat's ability to move about the chamber. Additionally, the results revealed a Conditioning Drug effect that approached statistical significance, $F(1, 60)=3.3$; $p=.07$; those groups conditioned with lithium tended to show less activity than those conditioned with saline

## Discussion

THC has a dose dependent biphasic effect on activity in rats; at high doses it suppresses activity and at low doses it increases activity (Chaperon et al., 1999). The present results show that THC administration led to an increase in activity as expressed by number of zone crossings during the test, suggesting that it had behavioural effects consistent with those produced by low doses. This shows that any effects of THC on lithium-induced place aversion are not due to the motor suppressive effects of THC possibly promoting attentional deficits.

Lithium produced a conditioned place aversion in rats pretreated with Vehicle that persisted for three extinction trials; however, the place aversion extinguished across test trials among the rats pretreated with THC during conditioning. One measure of the strength of avoidance conditioning is the number of trials required to reach a criterion of extinction (Domjan, 1998). By this criterion, THC appeared to interfere with the establishment of the lithium-induced place avoidance. Although THC attenuated the

establishment of a lithium induced place aversion during conditioning, THC did not affect the expression of a previously established place aversion. However, the strength of the place aversion displayed on the first test cycle by rats tested following THC injections did not differ from that displayed by rats tested following vehicle injections.

The attenuation of lithium place avoidance by THC cannot be explained as a summation of hedonic properties of THC and aversive properties of lithium, because Group VS and Group TS groups did not differ. Therefore, at a dose of 0.5 mg/kg, THC produced neither a place preference nor a place aversion.

## Experiment 2

In Experiment 1, after only 2 conditioning trials, lithium produced a strong conditioned place aversion in vehicle-pretreated rats. In fact, even after a total of 6 test trials, rats showed no evidence of extinction of this place aversion in the vehicle-pretreated group (Group VL). Such an effect is consistent with that previously reported in place avoidance paradigms (e.g., Parker & McDonald, 2000). THC pretreatment during conditioning, however, reduced resistance to extinction across the test trials. On the basis of this finding, it is assumed that THC pretreatment attenuated the strength of a lithium-induced place aversion (or sickness produced by lithium) in Experiment 1. Yet during the first cycle of testing in Experiment 1, there was no evidence that THC reduced the strength of the lithium-induced place aversion; such an effect was only apparent across extinction trials. It is possible that the potential of THC to interfere with lithium-induced place aversion learning would be evident if the strength of the aversion were reduced.

Therefore in Experiment 2, the potential of THC to interfere with the establishment of a weaker conditioned place aversion was evaluated using a lower dose of lithium (6 ml/kg as opposed to 12 ml/kg of 0.15 M LiCl). The rats in this experiment were given only a single test trial cycle with one day being a THC test and the other a VEH test, counterbalanced among the groups.

## Method

### Subjects

The subjects were 32 male Sprague-Dawley rats obtained from Charles River Labs, St. Constant, Quebec, weighing 220-250g at the beginning of the experiment. The rats were maintained as in Experiment 1.

### Procedure

The rats were conditioned identically to the procedure described in Experiment 1 except that they were given a single test trial cycle and there were only 2 groups; THC-Lithium (TL, n=16) and VEH-Lithium (VL, n=16). The rats were given two conditioning trial cycles, with lithium preceding placement in the chamber on one trial and saline preceding placement in the chamber on the other trial. The order of the trials and the chamber paired with the lithium were counterbalanced.

The rats were given a single test trial cycle following conditioning with half of the rats being injected with THC 30 minutes prior to placement in the chamber and half receiving injections of VEH prior to their placement in the chamber. On the following day the rats received the pretreatment drug they had not received on the previous day 30 minutes prior to placement in the test chamber. The order of the test drug administration was counterbalanced among the groups.

The data were analyzed as a 2 by 2 mixed factors ANOVA with the between groups factor of Pretreatment (THC, VEH) and the within groups factor of Test Drug (THC, VEH).

## Results

Figure 4 presents the mean number of seconds that the rats in groups THC-Lithium and VEH-Lithium spent on the Treatment-paired floor minus the Non-Treatment-paired floor during the THC and the VEH test trial. The analysis revealed only an effect of Test Drug that approached statistical significance, $F(1,30) = 3.9$; $p = .056$; the rats tended to display a weaker place aversion when tested in a THC state than when tested in a VEH state. One sample t-tests comparing the mean difference scores to a value of 0.0 of the pooled conditioning groups revealed a significant effect during the VEH test, $t(31)=4.0$; $p<.001$, but not during the THC test, $t(31)=1.65$; $p=.109$.

## Discussion

There were no significant effects of THC on the establishment or expression of a weaker lithium-induced conditioned place aversion as evaluated in Experiment 2; however there was a tendency for THC to interfere with the expression of the lithium-induced place aversion. Although marginal, these results suggest that THC interfered with the expression of a weak conditioned place aversion produced by a low dose of lithium. Consistent with these results are those of Parker and Kemp (2001) who demonstrated that THC reduced the expression of previously established lithium-induced conditioned retching in the *Suncus murinus* (house musk shrew). Following two prior pairings of a test chamber and lithium, shrews were injected with THC or VEH 30 min prior to being returned to the chamber. During these test trials, the VEH treated shrews

displayed conditioned retching but THC treated shrews did not retch. This was not the result of THC producing suppressed activity, because (consistent with Experiment 1 of the present study) it enhanced, rather than suppressed activity, which is seen at low doses (Chaperon et al., 1999). Parker and Kemp (2001) interpreted these findings to indicate that THC interfered with the conditioned emetic reactions, a potential model of anticipatory nausea and vomiting in human chemotherapy patients. The marginally significant finding of Experiment 2 confirms that, indeed, at low doses THC may interfere with conditioned nausea in rats, at least when the conditioned nausea is weak.

## Experiment 3

In Experiment 2, a lower dose of lithium chloride (6 ml/kg of 0.15M) was administered than in Experiment 1 (12 ml/kg of 0.15M) across two conditioning trials. Indeed a weaker place aversion was established in Experiment 2 than in Experiment 1. However, the pattern of results was not similar across the two experiments. As measured by resistance to extinction, the results of Experiment 1 suggest that THC interfered with the establishment of a stronger lithium-induced place aversion. However, the results of Experiment 2 suggest that when the place aversion is very weak, then THC may interfere with the expression of that aversion.

In Experiments 1 and 2, rats were administered two conditioning trials. Experiment 3 evaluated the potential of THC to interfere with either the establishment or the expression of a one-trial place aversion produced by 12 ml/kg of 0.15M LiCl. A single conditioning trial using the higher dose of lithium was expected to produce a weaker place aversion than that of Experiment 1.

## Method

### Subjects

The subjects were 24 male Sprague-Dawley rats obtained from Charles River Labs, St. Constant, Quebec, weighing 220-250g at the beginning of the experiment. The rats were maintained as in Experiment 1.

### Procedure

The rats were conditioned identically to the procedure described in Experiment 1 except that they were given a single conditioning trial cycle and there were two groups as in Experiment 2: Group THC-Lithium (TL, n=12) and Group VEH-Lithium (VL, n=12). The rats were given a single conditioning trial cycle, with lithium preceding placement in the chamber on one trial and with saline preceding placement in the chamber on the other trial. The order of the trials and the chamber paired with the lithium were counterbalanced. Half of the rats were injected with THC and half were injected with VEH 30 min prior to placement in the lithium-paired chamber.

The rats received one cycle of two test trials, one 30 min following an injection of THC and the other following an injection of VEH. The order of the trials was counterbalanced.

## Results

Figure 5 presents the mean number of seconds spent on the lithium-paired minus the saline-paired floor following a single conditioning cycle in Experiment 3. The 2 by 2 ANOVA with the between groups factor of Pretreatment (THC, VEH) and the within groups factor of Test Drug (THC, VEH) revealed no significant effects. Unlike Experiment 2, the main effect of Test Drug did not approach statistical significance, F(1,

22) = 1.89; p=.18. However, as in Experiment 2, single sample t-tests of the pooled conditioning group difference scores compared against a value of 0 revealed a significant place aversion during the VEH test, t(23)=2.26; p<.05, but not during the THC test, t(23)=1.02; p=.317.

## Discussion

As in Experiment 2, there were no significant effects of THC on the establishment or expression of the lithium-induced conditioned place aversion, but the results suggested THC-induced interference with expression of the lithium-induced place aversion. The effects of THC on place aversion in Experiment 3 were marginal at best. When the strength of the lithium-induced place aversion was weak, THC may have modulated the expression of the aversion. In Experiment 2 rats received two conditioning trials with a low dose of lithium (6 ml/kg of 0.15M) and in Experiment 3 rats received only one conditioning trial with a higher dose of lithium (12 ml/kg of 0.15M). In each of these experiments, rats displayed a place aversion (relative to the expected value of 0) during the VEH tests, but not during the THC test (although the difference scores did not significantly differ between the two tests). On the other hand, when the place aversion was stronger in Experiment 1 (two conditioning trials with the high dose of 12 ml/kg of 0.15M LiCl), THC did not modulate the expression or establishment of a lithium-induced place aversion when evaluated in the first test cycle, but showed evidence of modulating the strength of the established place aversion across 3 extinction cycles.

## Experiment 4

Experiment 1 suggested that THC interfered with the establishment of a lithium-induced conditioned place aversion. Indeed, if THC interfered with nausea it might be

expected to reduce the strength of the lithium-induced nausea during conditioning. By reducing the nausea, the THC conditioned group would be expected to form a weaker association than the VEH conditioned group. However, a similar effect would be evident if THC interfered with learning of the association itself.

In fact, recent work shows that THC interferes with learning and memory processes, but at higher doses than 0.5 mg/kg, the dose used in Experiments 1 through 3. THC has been shown to interfere with learning of both a radial arm maze and T-maze tasks (Lichtman & Martin, 1996; Molina-Holgado, Gonzalez & Leret, 1995; Nakamura, da Silva, Concilio, Wolkinson & Masur, 1991; Nava, Carta, Battasi & Gessa, 2000); however, the doses required to interfere with learning in these tasks range from 1.5 mg/kg – 5 mg/kg. To our knowledge a dose of 0.5 mg/kg (that used in Experiments 1-3) has never been reported to interfere with learning. Experiment 4 attempted to determine whether this dose indeed interfered with place preference learning.

In Experiment 1, if THC interfered with lithium-induced nausea, and only the nausea, then it should *not* interfere with an amphetamine-induced conditioned place preference. Experiment 4 evaluated the potential of THC to interfere with the establishment and the expression of a place preference produced by a dose of 3.0 and 9.0 mg/kg of amphetamine. It should be noted that Erb and Parker (1994) reported that 10.0 mg/kg of amphetamine produced a stronger place preference than 3.0 mg/kg of amphetamine.

## Method

### Subjects

The subjects were 48 male Sprague-Dawley rats obtained from Charles River Labs, St. Constant, Quebec, weighing 250-350g at the beginning of the experiment. (One rat was improperly administered amphetamine on a Non-Treatment day and was not included in the analysis). The rats were maintained as in Experiment 1.

### Procedure

The rats received a total of 2 cycles of conditioning. During each cycle they received a Treatment and a Non-Treatment Trial (see Table 2). On the Treatment Trial the rats were injected with THC or VEH 25 minutes prior to an injection of d-amphetamine (3.0 or 9.0 mg/kg, ip) and 5 minutes prior to placement in Chamber A (mesh or sandpaper). On the Non-Treatment Trial, the rats were injected with VEH 25 minutes prior to an injection of Saline and 5 minutes before placement in Chamber B (sandpaper or mesh). Within the cycle, the order of the trials and the chamber associated with amphetamine was counterbalanced among the groups. The rats were given a total of 6 test trials separated by 24 hours, consisting of 3 THC trials and 3 VEH trials. The conditioning groups were as follows: (1) THC-Amph (Group TA3, 3.0 mg/kg, n=11), (2) THC-Amph (Group TA9, 9.0 mg/kg, n=12), (3) VEH-Amph (Group VA3, 3.0 mg/kg, n=12), and (4) VEH Amph (Group VA9, 9.0mg/kg, n=12).

The experiment was a 2 by 2 by 2 by 3 mixed factors design with the between groups factors of Pretreatment Drug (THC, VEH) and Dose (3.0 or 9.0 mg/kg) and the within groups factors of Test Drug (THC, VEH) and Test Day (1-3).

## Results

Figure 6 presents the mean number of seconds that the rats spent on the treatment-paired minus saline-paired floor during the first place preference test cycle when the test drug was THC or VEH. A 2 by 2 by 2 mixed factors ANOVA with the between groups factors of Pretreatment Drug (THC, VEH) and Dose (3.0 or 9.0 mg/kg) and the within groups factor of Test Drug (THC, VEH) revealed a significant effect of Pretreatment Drug, $F(1, 45)=3.90$; $p=.05$. No other effects were significant. Those rats pretreated with VEH during conditioning displayed a place preference that was abolished by THC pretreatment. Because Dose was not significant and did not enter into any significant interactions, the data shown in Figure 7 are pooled across Dose for clarity of presentation.

Figure 8 presents the results of the place preference tests across the three cycles of testing in Experiment 4. In the overall analysis, the main effects of Test Drug and Amphetamine Dose were not significant, nor did they enter into any significant interaction with any other factor. Therefore the data presented in Figure 8 are pooled across the Test Drug factor and the data in Figure 9 are pooled across the Test Drug and Dose factors and the Groups are represented as Group TA (consisting of Group TA3 and TA9) and Group VA (consisting of Groups VA3 and VA9).

A 2 by 2 by 2 by 3 mixed factors ANOVA revealed a significant main effect of Test Day, $F(2, 86)= 4.4$; $p< .05$, and a significant Test Day by Pretreatment interaction, $F(2, 86) = 5.2$; $p<.01$. A simple main effects analysis of the difference scores pooled across Test Drug and Dose revealed a significant effect of Test Day for the VEH pretreated group, $F(2, 46) = 10.5$; $p<.001$, but not for the THC pretreated group, $F(2, 45) = .367$; $p =.695$. The VEH pretreated group displayed a stronger preference on Day 1

than on Days 2 or 3 (p's <.05). This shows that the rats pretreated with THC did not show a preference during any of the tests, whereas the rats pretreated with VEH did. Furthermore, on test trial cycle 1, Group VA displayed a stronger place preference than Group TA [$F(1,45)=4.01$, $p=.05$] but not on test trial cycle 2 [$F(1,45)=.05$] or 3 [$F(1,45)=1.1$]. Among the VEH pretreated rats, single sample t-tests show that the rats displayed a significant preference (taken against a value of 0.0 meaning no aversion or preference) on the first day, $t(23) = 2.72$; $p<.01$, but not on the second, $t(23)=.51$; $p=.62$, or third, $t(23) = .93$;$p=.37$ test trial cycle.

## Discussion

THC interfered with the establishment of both a lithium-induced place aversion (in Experiment 1) and an amphetamine-induced conditioned place preference, suggesting that THC interfered with learning.

## General Discussion

The pattern of results from the present study suggests that THC may interfere with the establishment of strong place conditioning (Experiments 1 and 4). However, when the place conditioning is weak (Experiments 2 and 3) the results suggest a possibility that THC may interfere with the expression of place conditioning. Parker and Kemp (2001) demonstrated that THC could reduce conditioned retching in house musk shrews; a measure of anticipatory nausea and vomiting. Limebeer and Parker (1999) have also shown that THC interferes with the establishment and expression of conditioned rejection reactions in rats as a result of pairing cyclophosphamide with saccharin solution. These results, taken together, provide evidence that THC may have the potential to attenuate conditioned nausea in both emetic and non-emetic species; however the

results could also be interpreted as retrieval failure. When the conditioned place aversion is stronger (as that in Experiment 1), THC does not interfere with the expression of the conditioned aversion, but rather the establishment of the association.

Taken across test trials, those rats pretreated with THC (Group TL) during conditioning displayed a weaker aversion than those pretreated with VEH (Group VL) during conditioning. Indeed, rats in Group TL displayed a place aversion much less resistant to extinction. This is evidence that THC had an effect on the establishment of the lithium-induced conditioned place aversion. While THC may be reducing the nausea elicited by treatment with lithium chloride during conditioning, it may also be interfering with the rats' ability to form the association between the sickness and the chamber in which it experienced the sickness – i.e. it's ability to learn. To test this hypothesis rats were conditioned with two doses of amphetamine (3.0 and 9.0 mg/kg, ip) and pretreated with THC or VEH and tested for their preference. During the first cycle of testing, those rats pretreated with THC did *not* show a significant preference for the amphetamine-paired floor, while those rats pretreated with VEH *did* display a preference. Presumably, since there was no nausea experienced by the animals during testing, THC did not have any antiemetic effects and did not modulate the expression of the conditioned place preference. The fact that the THC pretreated rats did not display a preference, whereas VEH pretreated rats did, gives clear evidence that the rats did not learn the association when in a THC state, thus THC must be interfering with learning.

There is evidence that THC interferes with learning in animals. Before reviewing this literature the distinction between working memory and reference memory will be described. Working memory, or short-term memory as it is often referred to, is thought of

as the thoughts in conscious awareness or the ideas and memories that are currently being processed. Traditionally experiments designed to evaluate effects on working memory are done with pigeons using a delayed match to sample task. In these tasks pigeons are trained to peck a sample stimulus and, after a delay, are rewarded for pecking the correct comparison stimulus. Evidence has shown that by varying the time between presentation of the sample and the comparison stimulus (the delay interval) pigeons' performance can be altered. It is thought that information in working memory is only accessible for a short period and degrades over time. Memory for the sample stimulus can also be altered by use of proactive interference techniques (Grant & Roberts, 1973). In these experiments pigeons are given the incorrect sample preceding the correct sample and must, then make the correct comparison choice. Again, as the delay between the samples and the comparison increases, the performance declines.

In rats, the tasks designed to evaluate working memory are generally the 8-arm radial maze or T-maze tasks. These paradigms involve the rat being allowed to explore the various arms of a maze and being rewarded for avoiding arms of the maze that it had previously visited on successive trials. Research has ruled out the possibility of mediation by odor cues, because investigators have replaced food in visited arms, (Roberts, 1979) or disrupting response algorithms by using forced choice (Roberts & Dale, 1981). It is therefore thought that performance is determined by recalling correctly the previously visited arms and visiting only those arms thought to contain food reward. Again, when the delay between exposure to the maze is increased between the visit to the fourth and fifth arm, performance declines showing an impairment of working memory. Another method for testing effects on working memory in rats is the use of delayed non-match-to-

position tasks utilizing left and right levers. In these tasks rats are presented with either lever and must depress it to move into the delay interval. After the delay, the rat is presented with a choice between two levers and must depress the lever opposite to that with which it was previously presented. With an increase in the delay interval, performance declines as expected. Similarly, rats have been tested in the delayed match-to-sample procedure. In this procedure the rat is presented with one of two levers and must depress the lever to move into the delay interval. During the delay the lever is retracted and the rat must perform a task designed to interfere with orientation behaviours such as performing a nosepoke on the opposite side of the test chamber. After the delay the left and right lever are presented together and the rat must depress the previously depressed lever to be reinforced.

In the radial arm maze tasks, THC has been shown to interfere with working memory processes when administered prior to testing (Lichtman & Martin, 1996; Molina-Holgado et al., 1995; Nakamura et al., 1991) using a 5 second delay between the fourth and fifth arm choice. The rat incorrectly entering a particular arm, which it had previously visited before the delay, illustrates this interference. In each case, however the effective dose was 1.25, 3.0, and 5.0 mg/kg of THC, which is considerably higher than the dose used in the present experiments (0.5 mg/kg). Also using a T-maze, THC was shown to lead to memory impairments (indicated by incorrect arm entries) at doses of 2.5 and 7.5 mg/kg, but not at a dose of 1.0 mg/kg (Nava et al., 2000). This experiment required the rats to run down the maze and enter the opposite arm to that which was previously correct, thus the rat had to learn to alternate its choice of arms by remembering which arm it had most recently been rewarded for visiting. The researchers

found that administration of THC before placement in the T-maze affected the animal's ability to alternate between arms maximally at 60 minutes following injection. This impairment is believed to be the result of the rat's inability to recall which arm was visited before the delay. Heyser and colleagues (Heyser, Hampson & Deadwyler; 1993) investigated the effects of THC in the delayed match-to-sample (DMTS) paradigm and found that performance was impaired following administration of THC at delays above 5 seconds. The experimenters used doses above 1.0 mg/kg and found that the behavioural effects were very similar to rats with hippocampal lesions (a discussion of this relation will be addressed later). In investigations determining the effect of THC on delayed non-match to position (DNMP) tasks, it has been demonstrated that THC interferes with the performance on these tasks as well, although, again the doses are considerably higher (2.0 and 4.0 mg/kg) than the dose used in Experiments 1 through 4 in the present study. Mallet and Beninger (1996) attribute this disruption, caused by THC, to its effects on working memory processes involved with correct performance on the tasks utilized in their design. They found that rats could perform the conditional discrimination task involving reference memory under the influence of THC, but that they showed a decrement when performing the non-match-to-position task, thought to involve primarily working memory.

Although THC has been shown to interfere with working memory at relatively high doses, there is little evidence that it interferes with the establishment of the reference memory. Reference memory, as opposed to working memory, generally involves learning a behaviour (associative learning) or outcome over repeated trials. Also, these memories persist for longer periods of time than those of working memory. In each of the above

cases demonstrating working memory deficits, reference memory played an integral role. While THC may have disrupted the rats' ability to recall what action it had recently performed, the rat was able to recall the rules necessary to complete the task – hence reference memory was intact. Only in cases where a drug was administered during training can the effect on the establishment of reference memory be evaluated, and in only one case in the studies reported above (Molina-Holgado et al., 1995) did THC (5.0 mg/kg) impair reference memory. THC pretreated animals showed significantly more incorrect choices before the delay, although the difference in the number of errors committed before and after the delay did not differ in the THC treated rats. Furthermore, Brodkin and Moerschbaecher (1997) demonstrated that THC (5.0-18.0 mg/kg) impaired learning in a task that required rats to correctly acquire a sequence of responses.

The fact that Heyser and colleagues (1993) noticed that rats administered THC behaved similarly to those rats with hippocampal lesions is not merely coincidental. The hippocampus is an area in the brain that is very densely populated with cannabinoid receptors (Herkenham et al., 1990). THC is a non-selective cannabinoid agonist that acts on both the CB1 and CB2 receptors. Since CB1 receptors are found centrally, recent research suggests that the CB1 receptor is the receptor responsible for this disruption and interference with memory. Indeed, Brodkin and Moerschbaecher (1997) demonstrated that THC (3.2-18.0 mg/kg) impaired learning, but more specifically, that the CB1 receptor antagonist SR 141716 attenuated this impairment. Those rats treated with THC at a dose higher than 5.0 mg/kg showed a marked increase in percent errors, but this was reduced when SR 141716 was administered paired with the THC. THC also was found to decrease response rate to the stimuli, which could have been evidence of motoric

suppression caused by a high dose of THC. Following up on their previous work, Mallet and Beninger (1998) administered SR 141716 along with THC during a working memory task (DNMP). Their earlier research (Mallet & Beninger, 1996) had shown that THC at a dose of 2.0 and 4.0 mg/kg led to a deficit in working memory. When THC was paired with SR 141716 in their DNMP task, the disruption was attenuated, while SR 141716 had no effect on performance at any of the doses tested when administered alone. These results suggest that preventing the agonist properties of THC, by blocking the CB1 receptors, prevents the learning and memory disruptive effects of THC. If CB1 receptor activation disrupts learning and memory processes, it is possible that CB1 receptor antagonists may enhance these processes. Indeed it has been reported that treatment with SR 141716, can facilitate the establishment of conditioned freezing (Guarraci, Frohardt, Falls & Musty, 2000) and the establishment of social recognition learning (Terranova et al, 1996) in rats when administered during training. This research suggests that the cannabinoid system is involved in learning and memory processes.

Future research should address whether SR 141716 can also attenuate the interference caused by THC on the establishment of lithium-induced conditioned place aversions. It might be expected that SR 141716 would enhance the weak place aversion shown in Experiment 2 and 3. Of course, the first step in this research would have to investigate whether SR 141716 possessed any hedonic or aversive properties when administered in the absence of lithium in the place conditioning paradigm. To dissociate the role of nausea and learning, the future direction would be to determine if the CB1 receptor antagonist SR 141716 had the ability to attenuate the interference of the amphetamine-induced conditioned place preference evidenced in Experiment 4. If SR

141716 interfered with both processes, then the disruption caused by THC on the establishment of the place preference was due to THC's actions on the CB1 receptor and this effect would reflect its effect on learning.

Recently there has been much research investigating the cannabinoid receptors and their role in brain, and specifically memory, functions. It appears that natural cannabinoids (anandamide) have a role in "turning down," or regulating, neurons in the hippocampus that are firing. Barinaga (2001) suggested that this may be a way of priming the neurons in the hippocampus for long-term potentiation (LTP) – a process that strengthens neuronal association. If this is the case, then high doses of THC may simply overload the system and disrupt the neuronal regulatory mechanisms. Hampson and Deadwyler (1999) argue that the cannabinoid receptors in the hippocampus may play a role in regulation of information encoding allowing the system to prevent overwriting of stored information. This would effectively allow the cannabinoid system to filter the information that gets routed to the hippocampus for storage. Indeed, Nava et al. (2000) showed that THC (at doses of 2.5 and 7.5 mg/kg) decreased the concentration of acetylcholine in the hippocampus and that the selective CB1 receptor antagonist SR 141716 attenuated this reduction of acetylcholine. Miller and Branconnier (1983) hypothesized that the principal action of cannabinoids in the brain was to reduce synthesis of acetylcholine in the limbic pathways. Acetylcholine is an important neurotransmitter at many synapses in the peripheral and central nervous system. Although the actions of cannabinoids on acetylcholine uptake and synthesis are not clear, cannabinoids do influence hippocampal functioning.

Current evidence has indicated that the CB1 receptor is a G protein-coupled receptor that inhibits adenylyl cyclase in a dose-dependent manner and that the receptor is more responsive to psychoactive cannabinoids, possibly leading to the aforementioned disruptions (Matsuda et al., 1990). Kim and Thayer (2001) demonstrated that CB1 receptor agonists (THC and anandamide), in fact, inhibit adenylyl cyclase (part of the cAMP pathway involved in ATP production) in rat hippocampal cells in culture. This leads to an inhibition of new synapse formation in the hippocampus, possibly the cause of memory disruptive effects of THC and an inability to form new associations. Campbell (2001) has also shown that THC increases cortical degeneration, or cell death, although this research has only demonstrated this in neonatal cell tissues. This process leading to cell death is thought to occur due to CB1 activation leading to generation of ceramide, which has a role in control of cell death as it influences neural growth and development (for a more complete description of the processes and mechanisms involved see Guzman, Galve-Roperh & Sanchez, 2001).

The pattern of results in the present study suggests that when the association is strong (Experiments 1 and 4), THC may interfere with establishment; however when the association is weak (Experiments 2 and 3), THC may interfere with expression. The effect of THC on the establishment of a strong association is not specific to the hedonic valence of the drug, because it interfered with lithium-induced place aversion learning as well as amphetamine-induced place preference learning, suggesting that it may be interfering with the associative process itself. On the other hand, the effect of THC on the expression of a weak lithium-induced place aversion suggests that it interfered with (1) the memory of the association or (2) the conditioned response (conditioned nausea) itself.

A future experiment that would evaluate the effect of THC on the expression of a weaker amphetamine-induced association (place preference) may shed some light on the mechanism.

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Table 1: Procedure of Conditioning Cycles and Testing Cycles in Experiment 1

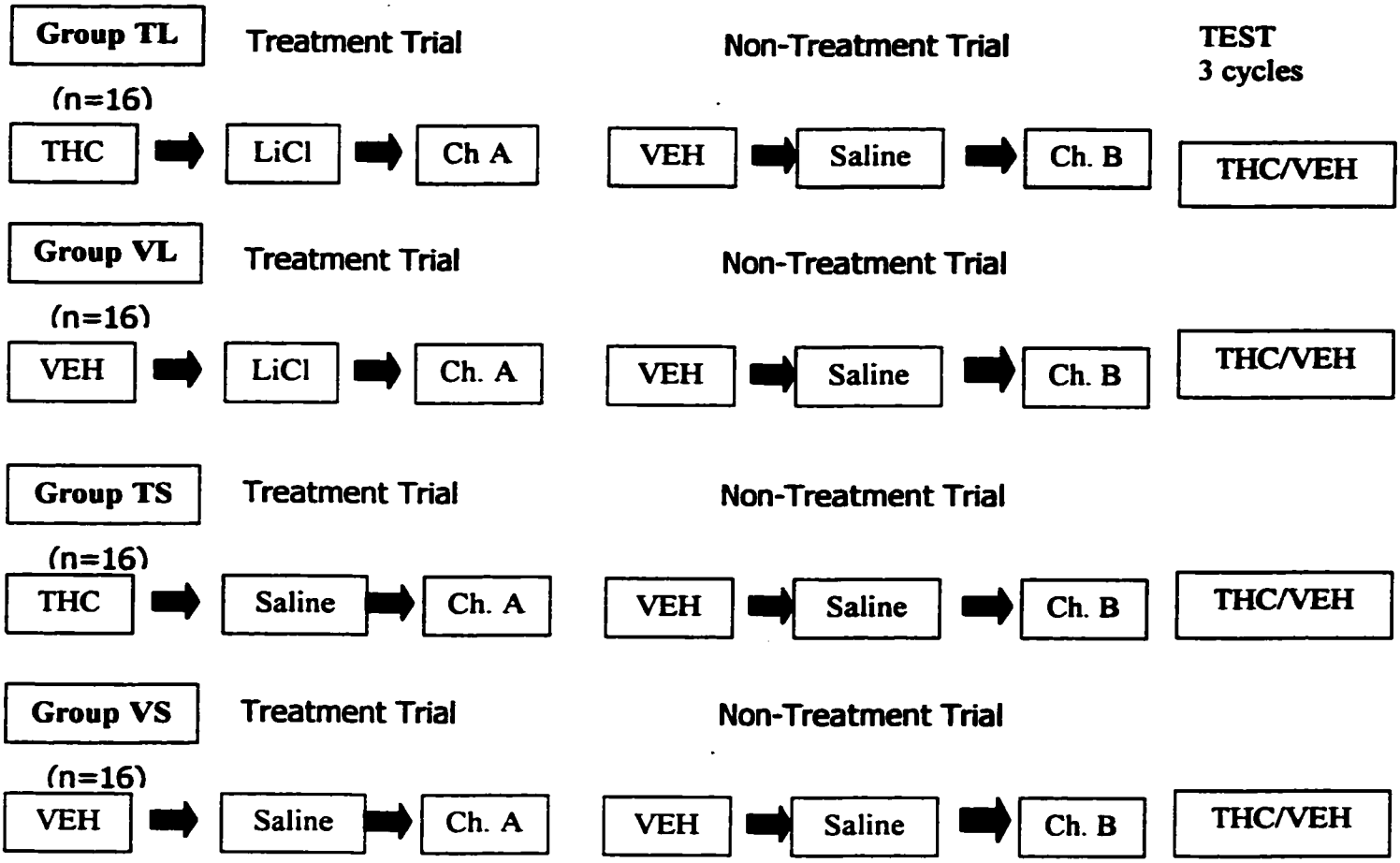
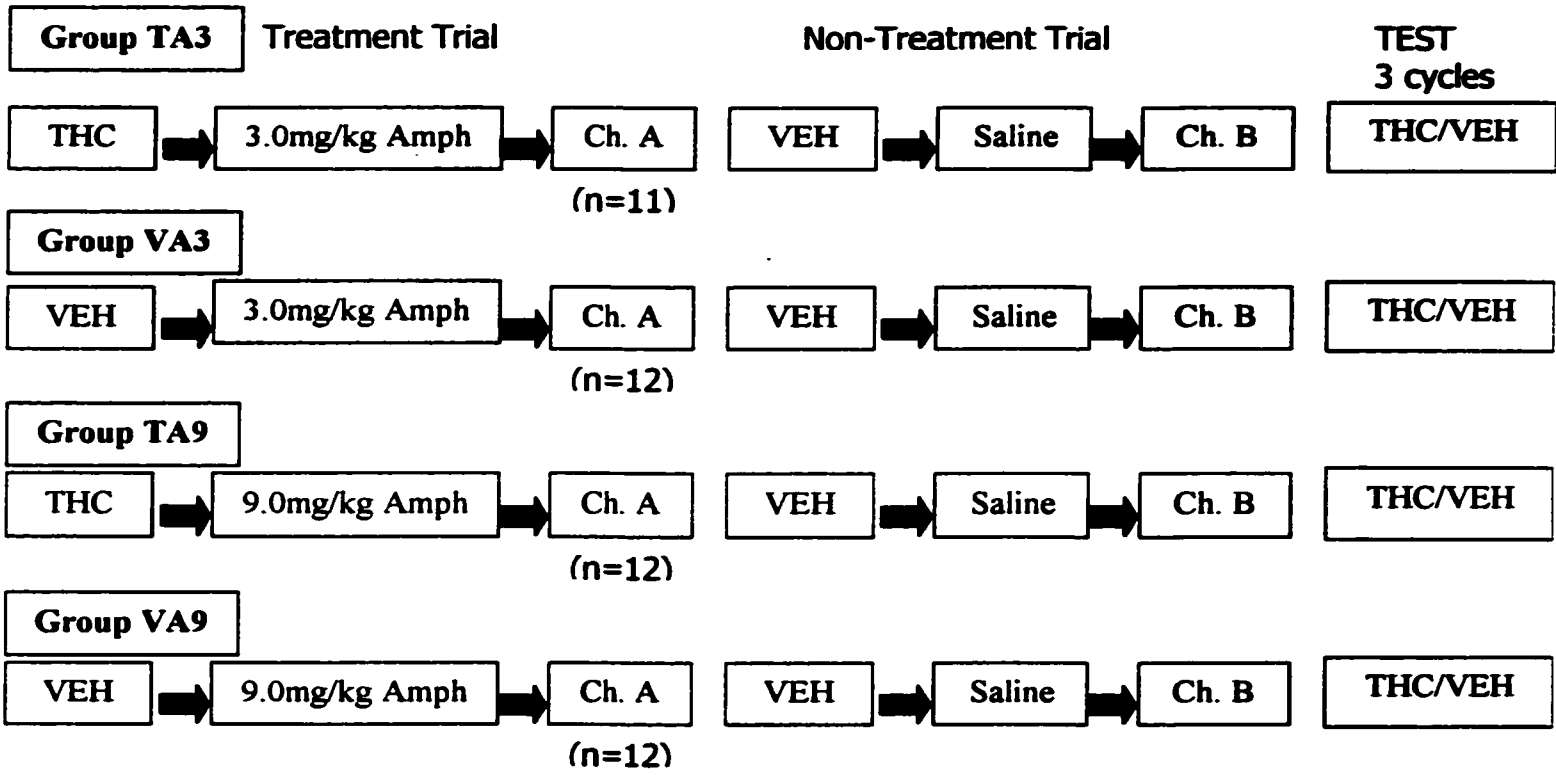


Table 2: Conditioning and Testing Procedure for Experiment 4



## Figure Captions

Figure 1: Mean (± sem) seconds spent of treatment minus non-treatment-paired floor during the first cycle of testing in Experiment 1.

Figure 2: Mean seconds (± sem) spent on the treatment minus the non-treatment-paired floor during test trial cycles pooled across Test Drug factor for Experiment 1.

Figure 3: Mean number (± sem) of zone crossings for the four groups during testing in Experiment 1.

Figure 4: Mean seconds (± sem) spent on the treatment minus the non-treatment-paired floor during each test trial in Experiment 2.

Figure 5: Mean seconds (± sem) spent on the treatment minus the non-treatment-paired floor during testing after a single conditioning cycle in Experiment 3.

Figure 6: Mean (± sem) seconds spent on treatment minus non-treatment-paired floor during the first cycle of testing in Experiment 4.

Figure 7: Mean (± sem) seconds spent on treatment minus non-treatment-paired floor during the first cycle of testing pooled across Dose of amphetamine in Experiment 4.

Figure 8: Mean seconds (± sem) spent on the treatment minus the non-treatment-paired floor during testing pooled across Test Drug in Experiment 4.

Figure 9: Mean seconds (± sem) spent on the treatment minus the non-treatment-paired floor during testing pooled across Test Drug and Dose of Amphetamine in Experiment 4.

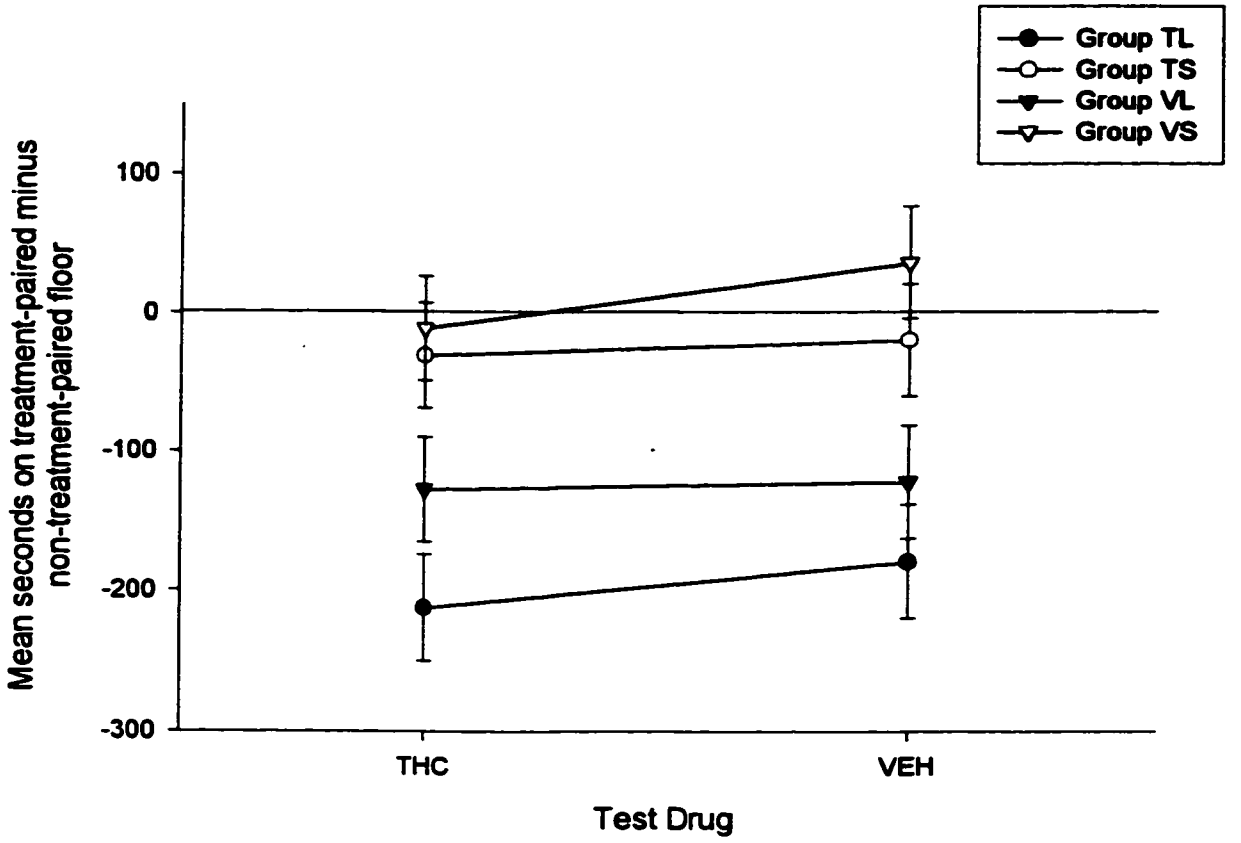

Group TL
Group TS
Group VL
Group VS
Mean seconds on treatment-paired minus non-treatment-paired floor
100
0
-100
-200
-300
THC
VEH
Test Drug

Figure 1

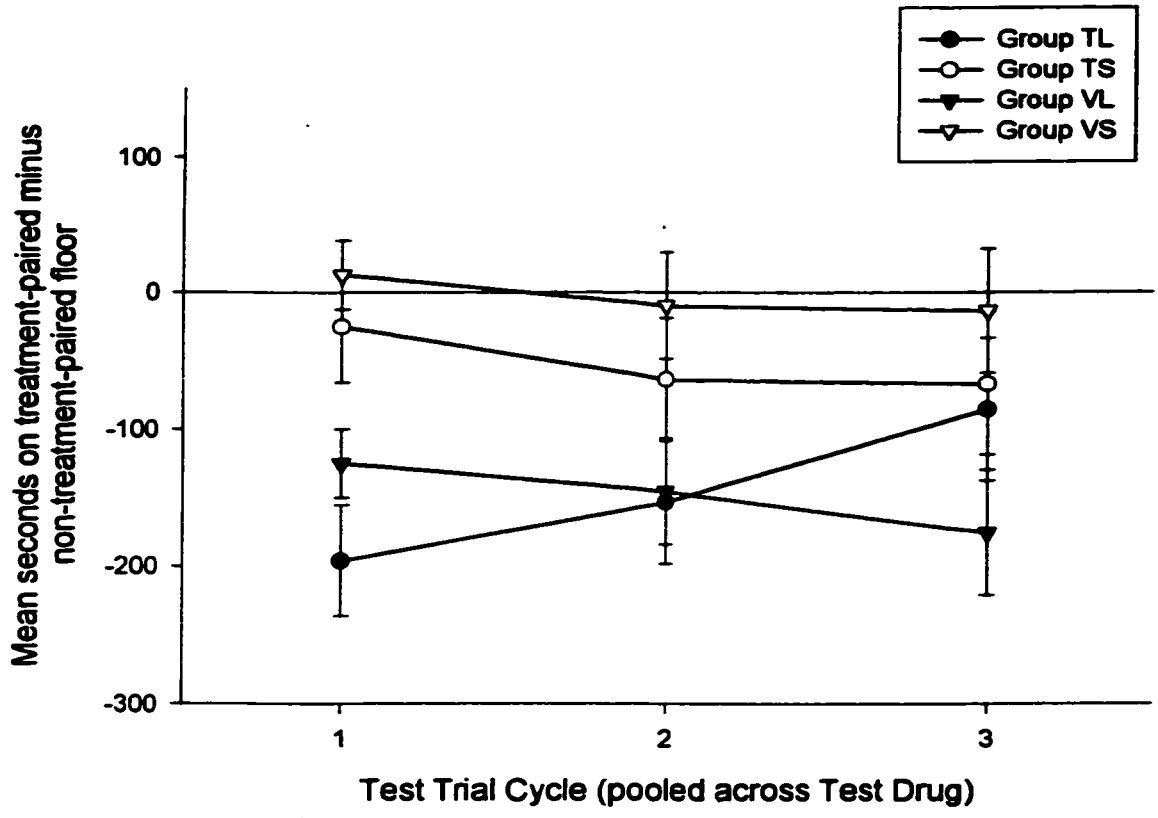

Group TL
Group TS
Group VL
Group VS
Mean seconds on treatment-paired minus non-treatment-paired floor
100
0
-100
-200
-300
1
2
3
Test Trial Cycle (pooled across Test Drug)

Figure 2

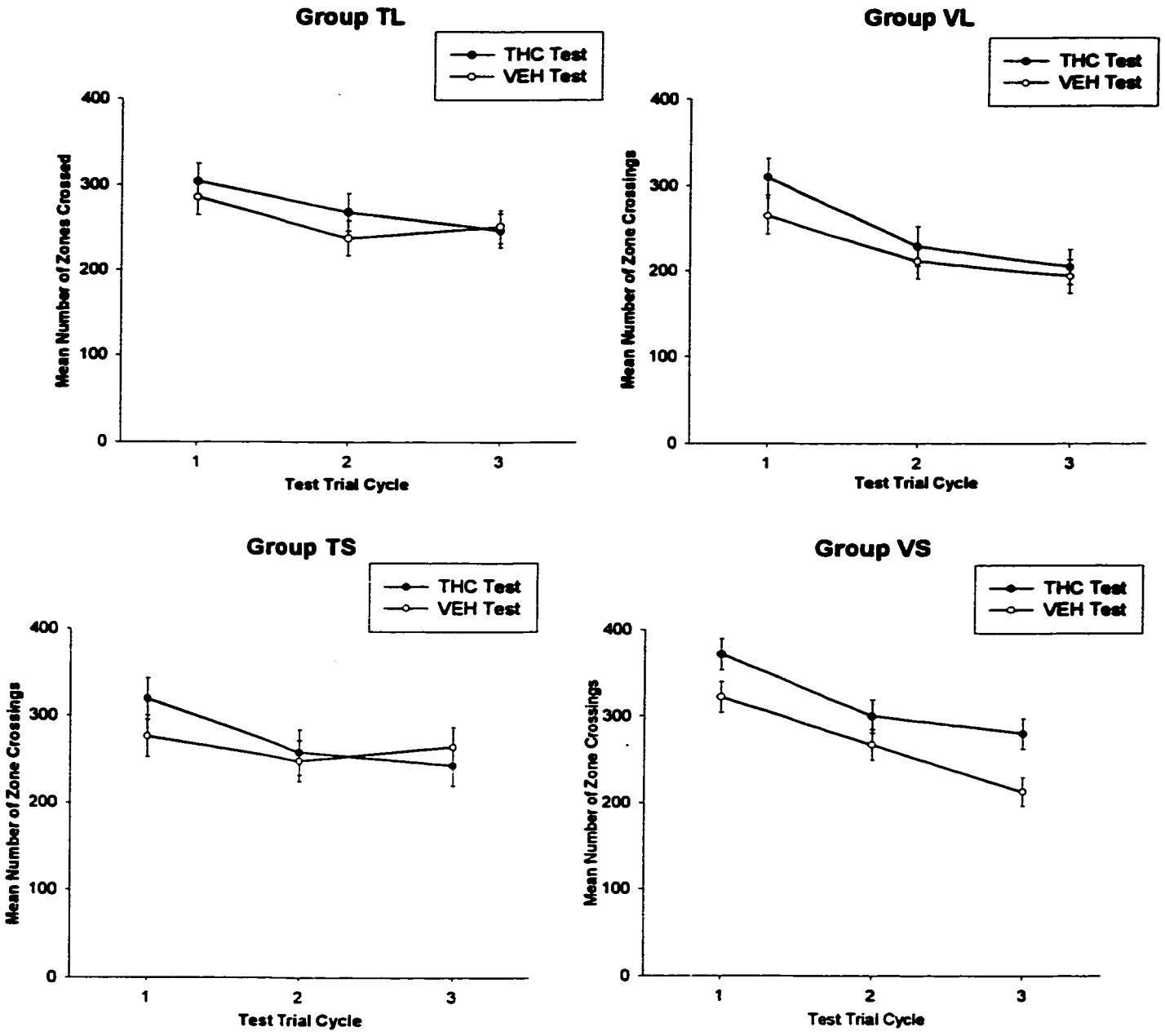

Group TL
THC Test
VEH Test
Mean Number of Zones Crossed
400
300
200
100
0
1
2
3
Test Trial Cycle
Group VL
THC Test
VEH Test
Mean Number of Zone Crossings
400
300
200
100
0
1
2
3
Test Trial Cycle
Group TS
THC Test
VEH Test
Mean Number of Zone Crossings
400
300
200
100
0
1
2
3
Test Trial Cycle
Group VS
THC Test
VEH Test
Mean Number of Zone Crossings
400
300
200
100
0
1
2
3
Test Trial Cycle

Figure 3

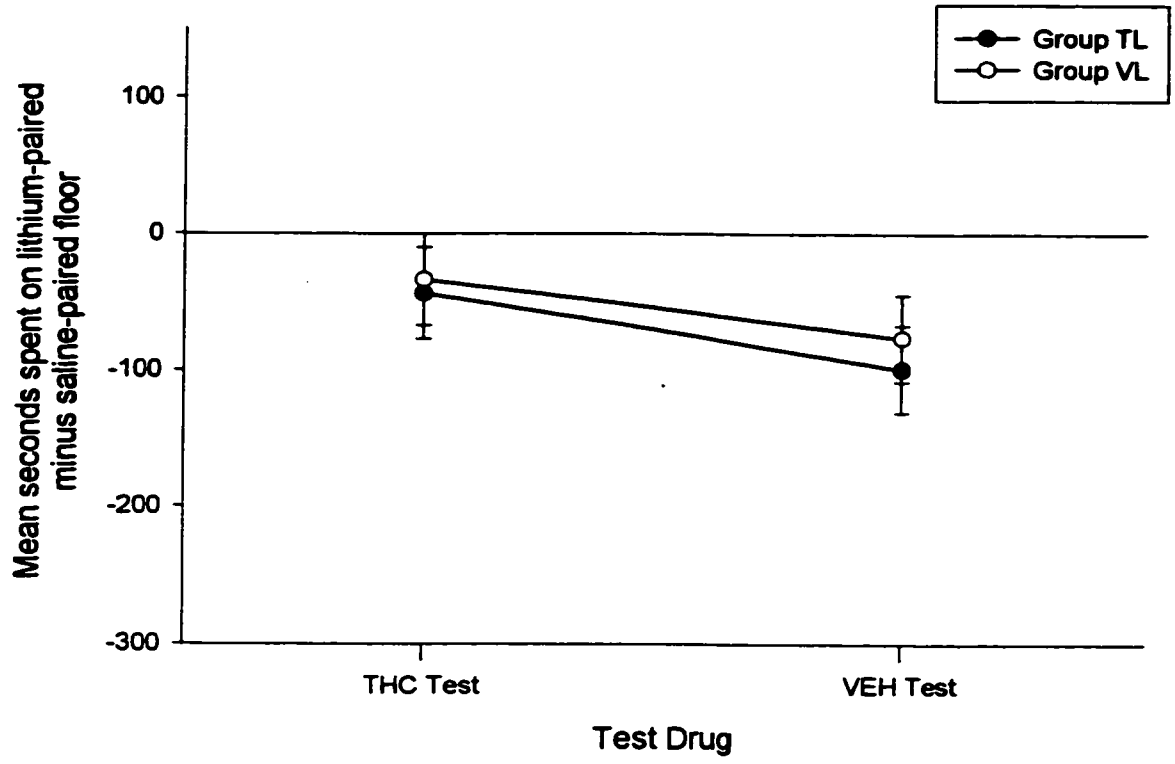

Group TL
Group VL
Mean seconds spent on lithium-paired minus saline-paired floor
100
0
-100
-200
-300
THC Test
VEH Test
Test Drug

Figure 4

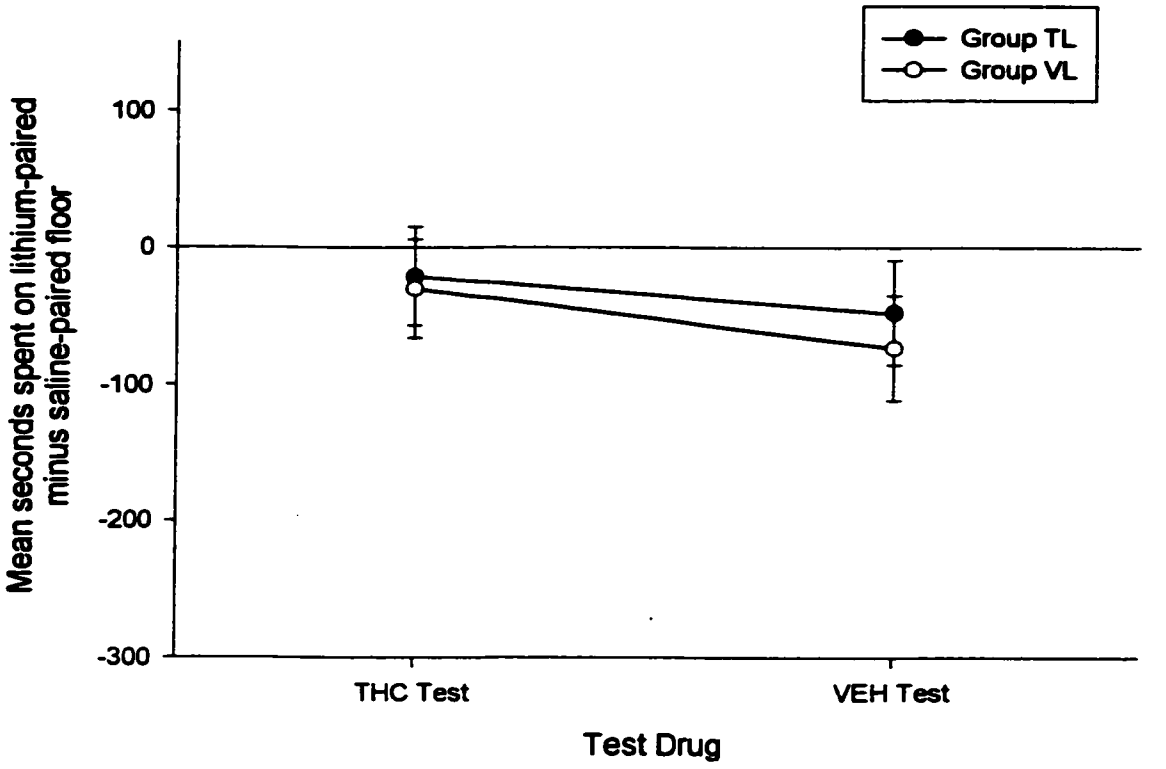

Group TL
Group VL
Mean seconds spent on lithium-paired minus saline-paired floor
100
0
-100
-200
-300
THC Test
VEH Test
Test Drug

Figure 5

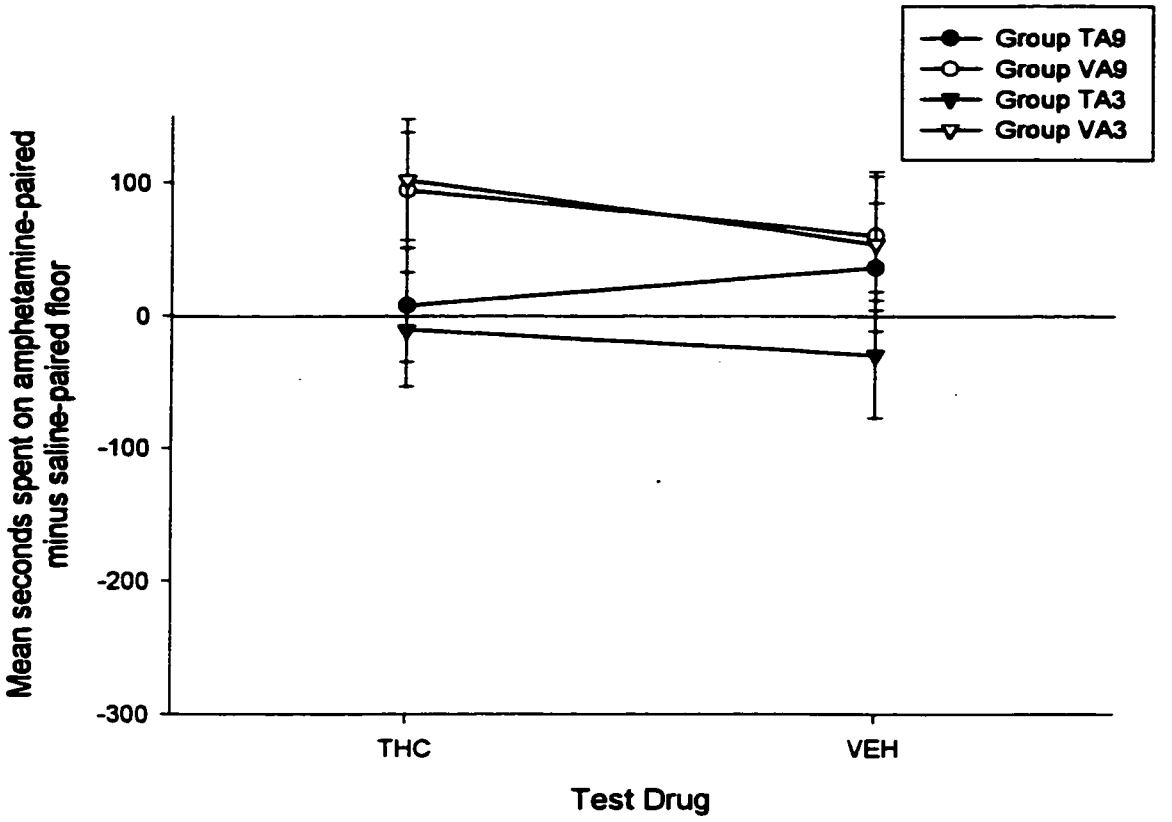

Group TA9
Group VA9
Group TA3
Group VA3
Mean seconds spent on amphetamine-paired minus saline-paired floor
100
0
-100
-200
-300
THC
VEH
Test Drug

Figure 6

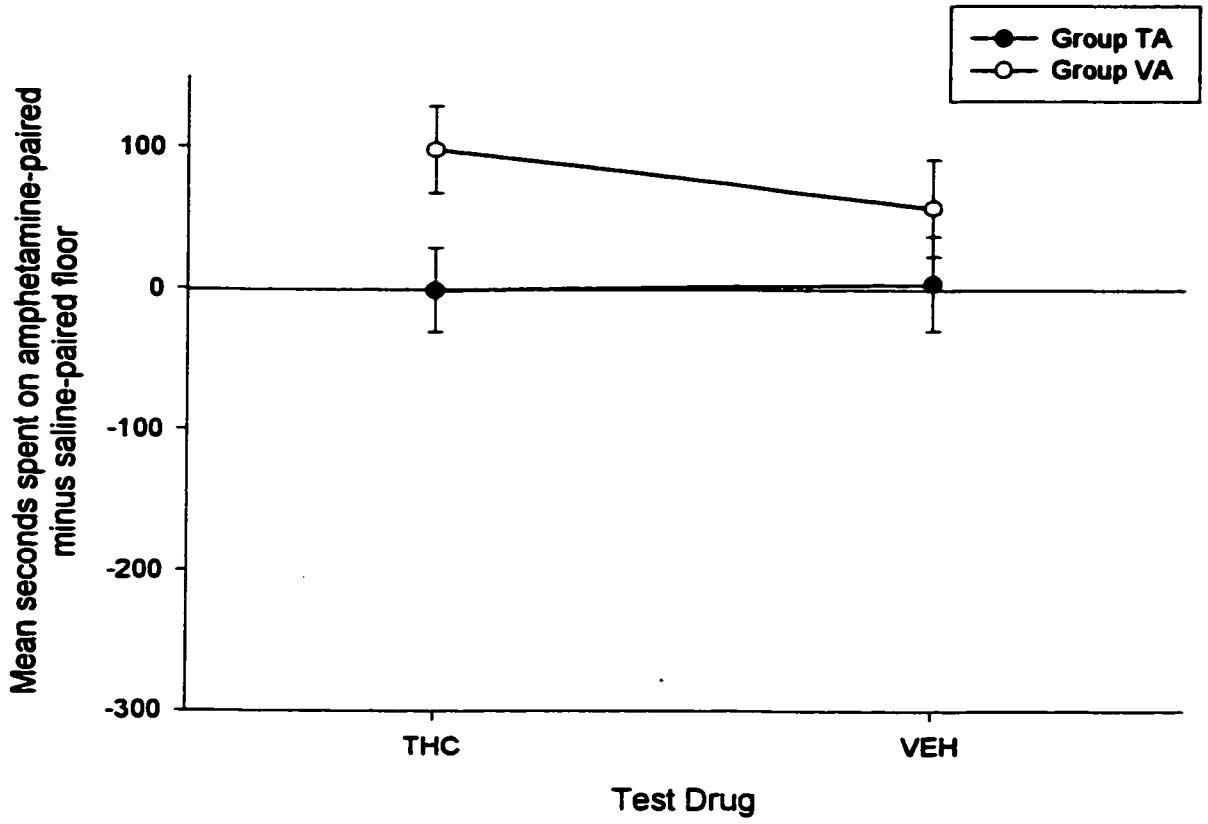

Group TA
Group VA
Mean seconds spent on amphetamine-paired minus saline-paired floor
100
0
-100
-200
-300
THC
VEH
Test Drug

Figure 7

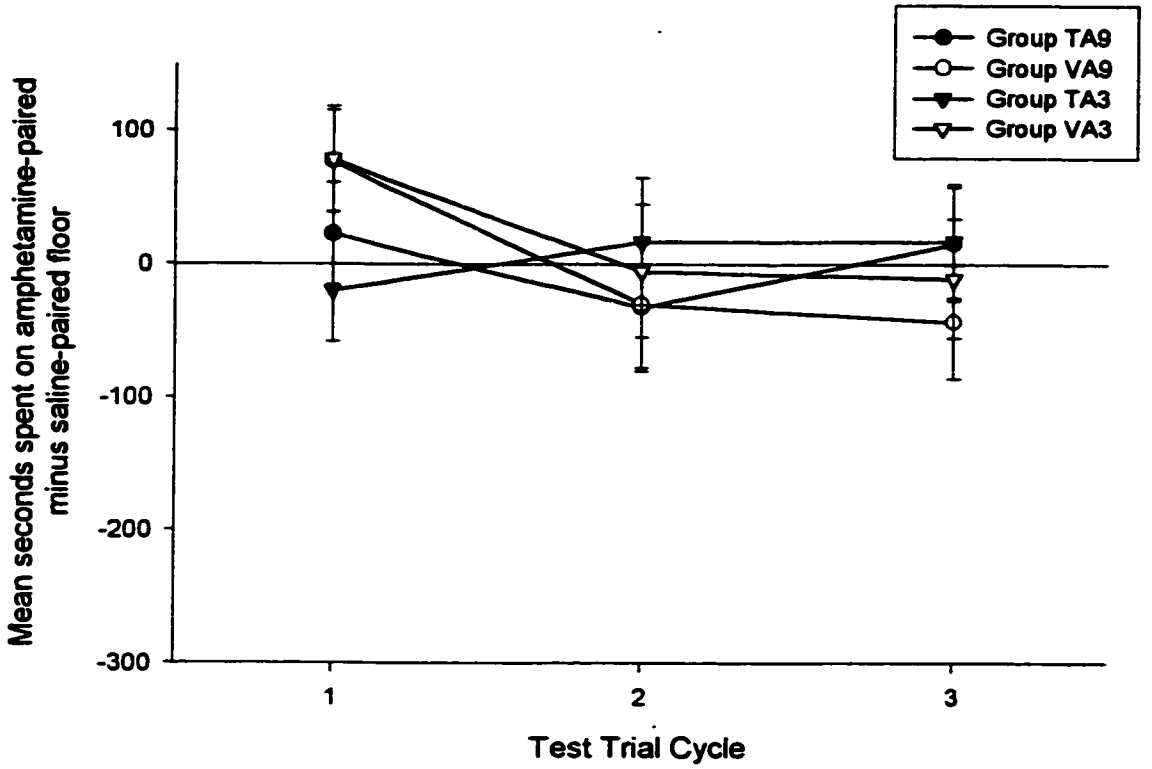

Group TA9
Group VA9
Group TA3
Group VA3
Mean seconds spent on amphetamine-paired minus saline-paired floor
100
0
-100
-200
-300
1
2
3
Test Trial Cycle

Figure 8

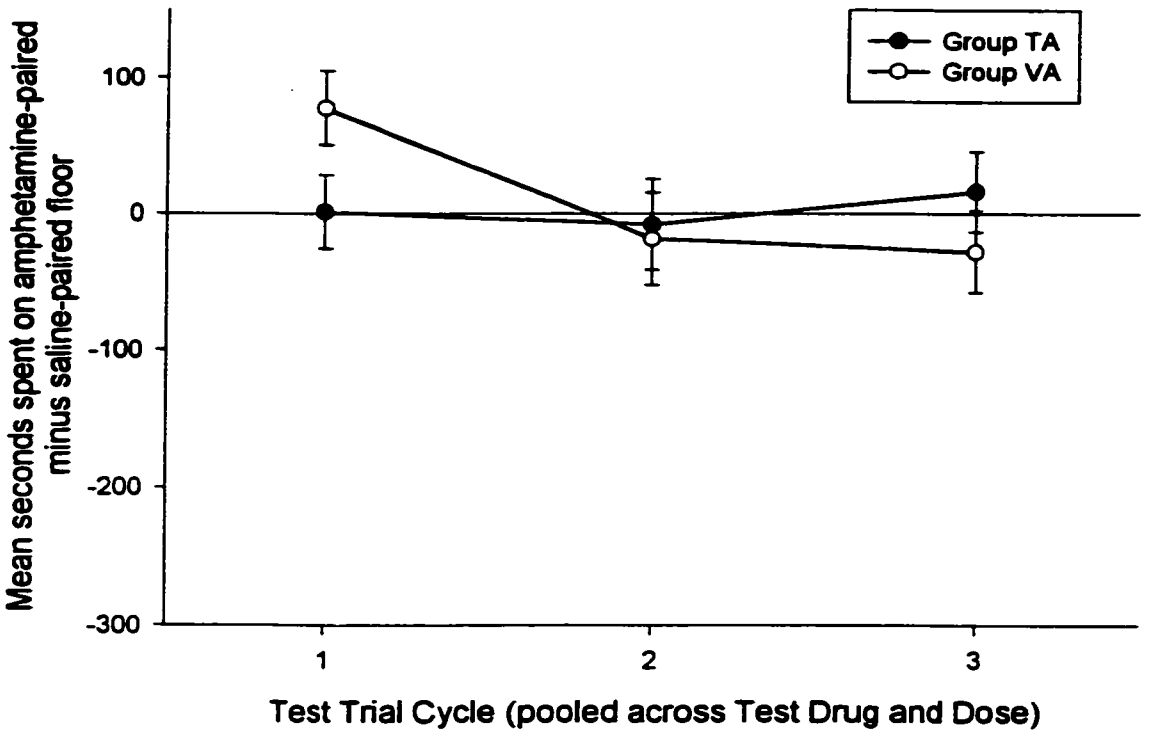

Group TA
Group VA
Mean seconds spent on amphetamine-paired minus saline-paired floor
100
0
-100
-200
-300
1
2
3
Test Trial Cycle (pooled across Test Drug and Dose)

Figure 9