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EFFECT OF CONDITIONED TASTE AVOIDANCE LEARNING ON ELEVATED PLUS MAZE PERFORMANCE AS A MEASURE OF POTENTIATED FEAR

by

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Abstract

The terms conditioned taste aversion and conditioned taste avoidance are often used interchangeably in the literature; however considerable evidence indicates that they may represent independent processes. Recent studies have demonstrated that conditioned taste aversion is mediated by conditioned sickness; however the mechanism(s) responsible for the establishment and expression of conditioned taste avoidance remains unknown. An early theory suggests that a taste that has been previously paired with a drug produces avoidance of that taste because it elicits some novel change in physiological state which signals danger to the rat. The emotional state produced may be one of conditioned fear. The present experiments evaluated the effect of exposure to a drug-paired flavor on avoidance of open arms in the elevated plus maze, an index of fear. If conditioned taste avoidance is mediated by conditioned fear, exposure to a drug-paired flavor should potentiate a rat's fear reaction to the open arms of an elevated plus maze.

In Experiment 1, rats were exposed to an amphetamine (3 mg/kg)- or lithium (25 mg/kg)- paired saccharin solution by bottle just prior to a test in a novel plus maze. Experiment 2 was conducted in a similar manner as Experiment 1, except rats were tested in a familiar plus maze. In Experiment 3, lithium-paired flavor was delivered by intraoral infusion prior to and during plus maze testing. Finally, in Experiment 4, rats were conditioned with 130 mg/kg of lithium and tested by intraoral infusion of saccharin solution 15 min prior to and during plus maze testing.

In Experiments 2 and 3, when the CS flavor was delivered by bottle or intraoral infusion immediately prior to plus maze testing, the rats in the CS^+ groups exhibited an

increase in open arm activity compared to CS^- groups. However, when the CS flavor was delivered during plus maze testing, as in Experiments 3 and 4, rats in the CS^+ groups exhibited increased avoidance of the open arms compared to rats in the CS^- groups. The opponent process model may account for the pattern of results found here. The presentation of the CS flavor may have produced a state of fear (the *a* process), however upon termination of the CS flavor, the opposite emotional state, relief may have been revealed (the *b* process).

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Table of Contents

List of Figures

Effect of Conditioned Taste Avoidance Learning on Elevated Plus Maze Performance as a Measure of Potentiated Fear

Conditioned taste avoidance learning is a form of associative learning that serves a protective function, because an animal learns to discriminate and to avoid consuming noxious substances by their flavor (Garcia & Koelling, 1966). In such learning, Garcia, Hankins and Rusiniak (1974) suggested that the flavored solution serves as the conditioned stimulus (CS) and gastrointestinal malaise serves as the unconditioned stimulus (US). Taste avoidance occurs when consumption of novel flavored food is followed by gastrointestinal malaise, and upon re-exposure to the flavored food, the animal avoids ingesting the food. An association between the flavor of the substance and the malaise is quickly established and persists over a long period of time.

Conditioned taste avoidance is not necessarily the same as conditioned taste aversion

Flavor-illness associations are most typically measured by consumption tests, which represent measures of conditioned taste avoidance. These tests include a one-bottle test in which a thirsty rat receives a single bottle of the flavored solution or a two-bottle test in which a thirsty rat has a choice between the flavored solution and water. In the avoidance test, the rat has control over the amount of exposure it receives to the flavored solution. Since the rat is required to approach the bottle in order to sample the flavored solution, the taste avoidance measure includes not only the consummatory phase of responding, but also the appetitive phase of responding (Konorski, 1967).

A more direct measure of taste aversion has been described, called the Taste Reactivity (TR) test (Grill & Norgren, 1978). The TR test measures the orofacial and somatic reactions elicited by a flavored substance infused directly into a rat's mouth

(Grill & Norgren, 1978). In the TR test, the experimenter controls exposure to the CS and the rat reacts with only the consummatory phase of responding. Two categories of reactions include ingestive reactions and rejection reactions. Ingestive reactions elicited by sweet sucrose solution include: tongue protrusions, mouth movements and paw licking. Rejection reactions elicited by bitter quinine solution include: gaping, chin rubbing and paw treading. Grill and Norgren (1978) reported that the ingestive pattern elicited by sucrose solution changes to a rejection pattern following pairings with the emetic drug lithium chloride; that is, rats displayed a conditioned aversion to the taste of sucrose.

It was once believed that taste avoidance was the result of a conditioned aversion to the taste; however, it is becoming increasingly clear that avoidance of a substance is not always motivated by taste aversion (Parker, 1982; 2003; Pelchat, Grill, Rozin & Jacobs, 1983). It has been found that rats not only avoid tastes paired with drugs having emetic properties (Garcia et al., 1974), but they also avoid tastes that have been paired with rewarding drugs that do not possess emetic properties (Hunt & Amit, 1987), for example cocaine. Yet, taste avoidance produced by rewarding drugs is not accompanied by conditioned rejection reactions in rats (Parker, 1995). It has been found that the failure of rewarding drugs to produce conditioned rejection reactions is not simply due to differential sensitivity of consumption tests and the TR test. Across nine conditioning/testing trials, Parker (1984) found that rats displayed equivalent avoidance of a lithium-paired taste and an amphetamine-paired taste, but only rejected the lithium-paired taste. Furthermore, Zalaquett and Parker (1989) demonstrated that when doses were adjusted to produce weaker taste avoidance with lithium than amphetamine, only

the lithium-paired flavor elicited rejection reactions. Since rejection reactions are only produced by drugs that induce vomiting in emetic species, Parker (1995) argues that taste avoidance produced by rewarding drugs is not motivated by conditioned sickness.

Not only can taste avoidance and taste aversion be dissociated on the basis of conditioning with emetic and rewarding drugs, but they can also be dissociated on the basis of conditioning with emetic drugs and shock (Pelchat et al., 1983). Although shock is less effective than emetic drugs in producing taste avoidance (e.g., Garcia & Koelling, 1966), the parameters of each aversive treatment may be adjusted to produce equivalent taste avoidance. Indeed, Pelchat and colleagues (1983) demonstrated that when paired with saccharin, footshock produced equivalent strength taste avoidance across multiple trials as did a low dose of lithium; however, only lithium produced conditioned rejection reactions.

Anti-nausea treatments interfere with taste aversion, but not taste avoidance

Recent evidence suggests that even taste avoidance produced by emetic drugs may not be the result of conditioned sickness. Limebeer and Parker (2000) demonstrated that pretreatment with the anti-emetic drug, ondansetron interfered with both the establishment and the expression of lithium-induced conditioned rejection reactions, but not lithium-induced taste avoidance. The rats learned the association between saccharin solution and lithium; however they did not reject the saccharin solution in the TR test. A similar effect has also been shown using cannabinoid agents that have been recently reported to have anti-emetic properties in animal models (Darmani, 2001a; 2001b; 2001c; 2002; Parker et al., 2003; Simoneau et al., 2001; Van Sickle et al., 2001). The cannabinoid agonists THC, HU-210, and cannabidiol, interfered with both the

establishment and the expression of lithium- and cyclophosphamide-induced conditioned rejection reactions, but not taste avoidance (Limebeer & Parker, 1999; Parker et al., 2003). Treatments that reduce vomiting in emetic species also reduce conditioned rejection reactions in rats, but do not reduce taste avoidance in rats.

Taste avoidance may be motivated by conditioned fear in the rat

If rats learn to avoid a flavor paired with lithium in the absence of nausea, then what might be responsible for avoidance of the taste? An early theory that attempted to explain paradoxical reports that reinforcing drugs produce taste avoidance suggested that any novel change in state signals danger to the rat, a species that cannot vomit (e.g., Gamzu, 1977; Hunt & Amit, 1987). A flavor paired with this change in state comes to signal danger (e.g., Pelchat et al., 1983), resulting in subsequent avoidance of that taste. Since anti-nausea treatments do not interfere with the establishment or the expression of conditioned taste avoidance produced by emetic drugs, it appears that such a mechanism may account not only for taste avoidance based on reinforcing drugs, but also for taste avoidance produced by emetic drugs. That is, even though nausea is prevented by pretreatment with the anti-nausea drug, the rat develops an association between a flavored solution and the disruption of the "milieu interne" produced by the emetic drug. The drug treatment is responsible for physiological changes which are novel in drug-naïve rats. Hence, nausea produces conditioned rejection of the taste, but it appears to be independent of avoidance of the taste.

Further evidence to suggest that taste avoidance in rats may result from any novel change in state, be it hedonic or aversive, is provided by a recent finding with an animal that vomits in response to toxin exposure. Parker, Corrick, Limebeer and Kwiatkowska

(2002) reported that the *Suncus murinus* (house musk shrew) develops a conditioned taste preference, rather than taste avoidance, when a novel saccharin flavor is paired with high doses of amphetamine or morphine, drugs which also produce a place preference in this species. Yet the shrew avoids saccharin that is paired with lithium chloride (Smith, Friedman & Andrews, 2001). Since rats, unlike shrews, are incapable of vomiting, it has been suggested that rats have evolved a highly sensitive first line of defense (Davis, Harding, Leslie & Andrews, 1986) that signals danger any time a novel food is tasted and followed by a change in physiological state. This first line of defense results in avoidance of the novel food in the future. The taste is avoided because it signals a physiological change, not because it elicits conditioned nausea. This change in state may serve as the US which when paired with the CS of a consumed solution or food produces the CR of fear.

Elevated plus maze as a measure of fear

If avoidance of a taste is mediated by conditioned fear, then exposure to the taste might be expected to potentiate a rat's reaction in a behavioral test of fear. One of the most widely used animal models for evaluation of fear and anxiety is the elevated plus maze (Pellow, Chopin, File & Briley, 1985). This model relies on the natural aversion of rodents to both heights and open spaces, and is sensitive to anxiolytic and anxiogenic agents (Rodgers & Cole, 1993). Rats normally avoid the two open arms of the elevated plus maze and exhibit a preference for the two enclosed arms of the maze. Avoidance of the open arms during plus maze testing is an index of fear (Pellow et al., 1985; Steenbergen, Heinsbroek, Van Hest & Van de Poll, 1990). When rats are forced to stay in the open arms, they exhibit fear reactions such as freezing, defecation and increased

plasma corticosterone. Anxiolytic compounds such as diazepam increase both the number of entries and percentage of time spent in the open arms (Pellow & File, 1986; Pellow et al., 1985). It has also been found that prior stressful/fearful situations can reduce open arm entries (Rodgers & Cole, 1993; Zangrossi & File, 1992).

The purpose of the present series of experiments was to determine whether prior exposure to a conditionally avoided flavor would reduce time spent in the open arms of the plus maze. In Experiment 1, rats were tested on a novel plus maze, and in Experiment 2, rats were tested on a plus maze to which they had received one pre-exposure. One might expect that fear of the open arms would habituate over trials; however, Treit, Menard and Royan (1993) found that open arm avoidance increased after a single 5 min exposure to the plus maze and showed no evidence of habituation or sensitization after 17 additional trials. Although anxiolytic drugs increase open arm entries and time spent in the open arms on trial 1, they are ineffective on trial 2 (Dawson, Crawford, Stanhope, Iverson & Tricklebank, 1994; File, 1990; 1993; File, Mabbutt & Hitchcott, 1990; Rodgers & Shepherd, 1993; Rodgers, Lee & Shepherd, 1992) regardless of whether animals received the drug on both trials or only on the second trial (Cruz-Morales, Santos & Brandao, 2002; File, 1993), or when trials 1 and 2 are separated by 24 hr or 2 weeks (File, 1993). These findings have led investigators (File & Zangrossi, 1993; Holmes & Rodgers, 1998) to suggest that trial 1 measures a different emotional process than trial 2 on the elevated plus maze.

The two emotional states elicited by plus maze exposure represent unlearned fear on trial 1 and learned fear on trial 2. Trial 1 is a test of anxiety in which the animal is placed in an approach-avoidance conflict situation with exploration of the novel open

arms in conflict with avoidance generated by unconditioned fear or anxiety (File & Zangrossi, 1993; Holmes & Rodgers, 1998; Treit et al., 1993). During trial 1, the rats rapidly learn to fear the open arms over the typical 5 min session, but not when the length of trial 1 is only 1 min (Dal-Col et al., 2003). The potentiated avoidance of the open arms on trial 2, therefore, reflects learned fear of the open arms. Because anxiolytics do not modify open arm activity on trial 2, File and colleagues (1996) suggest that the nature of the anxiety evoked on the second trial is similar to specific phobias which are also insensitive to benzodiazepine treatment (Marks, 1987). In support of such an analysis, File, Gonzalez and Galant (1998) demonstrated that rats that received reversible bilateral lesions of the basolateral amygdala immediately after trial 1 responded with an anxiolytic response to chlordiazepoxide when tested 48 hr later or 2 weeks later on trial 2. Since the basolateral amygdala has been shown to be critical for the consolidation of conditioned fear (Davis, 1998; Dunn & Everitt, 1988; McGaugh, Cahill & Roozendall, 1996; Killcross, Robbins & Everitt, 1997), these findings suggest that the shift from trial 1 to trial 2 reflects conditioned fear (Holmes & Rodgers, 1998).

It is not clear whether exposure to a drug paired flavor would be more effective in potentiating trial 1 or trial 2 plus maze performance, given that the trials appear to reflect different fear/anxiety processes. Therefore, in the first experiment rats were tested on a novel maze and in the second experiment rats were tested on a familiar maze. Experiment 1 evaluated the effect of prior exposure to a lithium- or amphetamine-paired flavor on open arm activity during trial 1 exposure to the elevated plus maze which should elicit unconditioned fear. Experiment 2 evaluated the effect of prior exposure to a lithium-or amphetamine-paired flavor on open arm activity during trial 2 exposure to the

elevated plus maze which should elicit learned fear. To the best of our knowledge, the effects of immediate prior exposure to a shock-paired cue on elevated plus maze performance has not been evaluated. In Experiment 3, the lithium-paired and saline-paired saccharin solution was delivered by intraoral infusion (rather than bottle) immediately prior to plus maze testing and during plus maze testing to determine whether delivery of the CS^+ flavor immediately prior to or during plus maze testing would affect plus maze performance. Finally, in Experiment 4, rats were conditioned with a higher dose of lithium and tested by intraoral delivery of saccharin solution 15 min prior to plus maze testing and during plus maze testing to maximize detection of a state of fear or a state of relief produced by CS exposure.

EXPERIMENT 1

Experiment 1 evaluated the effect of prior exposure (by bottle) to a lithium-paired or an amphetamine-paired saccharin solution on performance on a novel plus maze. The doses of lithium and amphetamine were equated to produce equivalent strength taste avoidance (Zalaquett & Parker, 1989). It was predicted that rats conditioned with lithium or amphetamine would exhibit potentiated open arm avoidance during a plus maze test compared to rats that received saline-paired saccharin.

Method

Subjects

Thirty-two male Sprague-Dawley rats, weighing between 260 and 290 g on the first conditioning trial served as subjects. The animals were obtained from Charles River Breeding Laboratory in St. Constant, Quebec. They were individually housed in stainless

steel hanging cages. The rats had ad-lib access to Purina rat chow and water. They were maintained under a 12:12 h illumination cycle (lights on at 0600h), at a room temperature of 20-22°C and humidity of 60%-70%. The rats arrived one week prior to the beginning of the experiment. All procedures employed in this series of experiments were approved by the WLU Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care.

Drugs

All drugs were injected intraperitoneally (ip). Lithium chloride was prepared in sterile water as a .15 M solution and was injected at a volume of 4 ml/kg (25 mg/kg). D-amphetamine sulfate was prepared in saline solution (.75 mg/ml) and was injected at a volume of 4 ml/kg (3 mg/kg). Physiological saline solution was injected at a volume of 4 ml/kg. The doses of lithium and amphetamine were selected to produce equivalent conditioned taste avoidance reactions (Zalaquett & Parker, 1989).

Apparatus

The plus maze apparatus was a wooden, plus-shaped maze, elevated to a height of 68 cm and painted black. It consisted of a center (10 x 10 cm), two open arms (50 x 10 cm) and two enclosed arms (50 x 10 x 45 cm) arranged such that the closed arms were opposite to each other and the open arms were opposite to each other. The removable floors of the plus maze were made of black rubber. On each trial, the rat was placed in the center of the plus-maze facing a closed arm. A video camera located in the ceiling transmitted the image of the rat to a computer using VideoPro software (Noldus, Inc). The software monitored the location of the largest image provided by the rat. The program provides a summary of the frequency of entries into each of the closed and open

arms, and the amount of time spent in each of the closed and open arms. The removable floors of the maze were cleaned with soapy water and dried after each rat was tested.

Procedure

The design of the experiment included the between groups factors of CS condition (paired [CS^+], unpaired [CS^-]) and US drug (lithium, amphetamine) with 8 rats per group. The rats were randomly assigned to treatment conditions.

On Days 1-4, the rats were placed on a water deprivation schedule in which they were presented water for 5 min at 0800 h and for 60 min at 1600 h and the amounts consumed were measured.

The conditioning trials occurred on Days 5 and 8. On each trial, the rats were presented with 0.1% saccharin solution in a graduated tube for 5 min beginning at 0800 h and the amount consumed was measured. Immediately following the saccharin presentation, the rats were injected with lithium (CS^+ lithium), amphetamine (CS^+ amphetamine) or saline (CS^- lithium and CS^- amphetamine) and were returned to their home cage, with 60 min access to water at 1600 h. Twenty-four hr after the saccharin exposure (Days 6 and 9), the rats were presented with water for 5 min in the morning and two hours later were injected with saline (CS^+ lithium and CS^+ amphetamine), lithium (CS^- lithium) or amphetamine (CS^- amphetamine). At 1600 h they all received access to water for 60 min. On Days 7, 10, and 11, the rats received access to water for 5 min at 0800 h and 60 min of water at 1600 h while in their home cage.

The test trial occurred on Day 12. Each rat was presented with 0.1% saccharin solution for 5 min beginning at 0800 h and the amount consumed was measured. Immediately following removal of the saccharin, the rat was placed in the plus maze and

its activity was recorded for 5 min. The proportion of entries into the open arms (proportion OE = open/open + closed) and the proportion of time spent in the open arms (proportion OT = open/open + closed) served as measures of anxiety/fear. The mean total arm entries (TE = open + closed) served as a measure of general activity.

Results

Consumption test

Figure 1 presents the mean amount (in milliliters) consumed by Groups CS^+ and CS^- during the 5-min one-bottle test prior to testing in the novel plus maze. The mean amount of saccharin consumed by the various groups was analyzed as a 2 (CS condition) by 2 (US condition) Analysis of Variance (ANOVA). The analysis revealed a significant effect of CS condition, $F(1, 28) = 135.70$, $p<0.01$. Consumption of saccharin was affected by drug contingency. Groups CS^+ lithium and CS^+ amphetamine consumed less saccharin than Groups CS^- lithium or CS^- amphetamine. No other effects were significant.

Plus maze test

Figure 2 presents the mean proportion OE (upper panel) and the mean proportion OT (lower panel) on the novel plus maze, by CS^+ and CS^- groups conditioned with lithium or amphetamine. Although the CS^+ groups appeared to spend a greater proportion of their time (OT) on the open arms than the CS^- groups, the 2 by 2 ANOVA revealed no significant effects of CS condition nor US drug. No other significant effects were observed. Contrary to predicted results, prior exposure to drug-paired saccharin did not affect performance on a novel plus maze. The CS^+ groups did not differ from CS^-

groups in proportion of entries into open arms (OE) or proportion of time spent in the open arms (OT) on a novel plus maze.

Figure 3 presents the mean total arm entries (TE) on the plus maze by CS^+ and CS^- groups. The 2 by 2 ANOVA revealed no significant effects, indicating that prior exposure to the CS flavor did not alter general activity level.

Discussion

It was predicted that prior exposure to a lithium- or amphetamine- paired saccharin solution would enhance fear of the open arms in the elevated plus maze; however, the results of Experiment 1 did not confirm this prediction. Considerable recent evidence (e.g., Dawson et al., 1994; Treit et al., 1993) indicates that a single 5-min exposure to the elevated plus maze sensitizes avoidance of the open arms and reduces exploration of the plus maze (Bertoglio & Carobrez, 2000; Dawson et al., 1994; Rodgers, Cole, Aboualfa & Stephenson, 1995). It is therefore possible that pre-exposure to the plus maze may also enhance rats sensitivity to the fear manipulation. In Experiment 2, rats were pre-exposed to the plus maze prior to conditioning and testing.

EXPERIMENT 2

Experiment 2 was conducted in the same manner as Experiment 1 except rats received a pre-exposure to the plus maze (pretest) prior to conditioning. Rats conditioned with lithium or amphetamine were expected to exhibit greater open arm avoidance than rats conditioned with saline.

Method

Subjects

Thirty male Sprague-Dawley rats weighing between 260 and 295 g on the first conditioning trial served as subjects. They were treated in a similar manner as in Experiment 1, except as indicated. There were 7-8 rats per group.

Procedure

On Days 1-4, the rats were placed on a water deprivation schedule, 5 min water exposure at 0800 h and 60 min at 1600 h, and the amounts consumed were measured. On Day 4, the rats were given a pretest on the plus maze immediately following their 5 min of water in the morning, they were placed on the plus maze and their activity was automatically recorded for 5 min. They were assigned to treatment conditions matched on the basis of proportion of time spent in the open arms.

Conditioning trials took place on Days 5 and 8 as described in Experiment 1. On each trial, rats were presented the CS flavor by bottle for 5 min at 0800 h and amount consumed was measured. Immediately following CS exposure, rats in the CS^+ groups were injected with either lithium (25 mg/kg) or amphetamine (3 mg/kg) and the rats in the CS^- groups were injected with saline and returned to their home cage, followed by 60 min access to water at 1600 h. Twenty-four hours following each conditioning trial, the rats were given 5 min water exposure in the morning followed by non-contingent injections two hours later. On Days 7, 10 and 11, all rats received their 5 min and 60 min water sessions in their home cage.

The test trial occurred on Day 12 in a similar manner as described in Experiment 1. The rats were presented with the 0.1% saccharin solution for 5 min followed by a plus maze test.

Results

Consumption Test

Figure 4 presents the mean amount (in milliliters) consumed by CS^+ and CS^- groups during the 5 min one-bottle test prior to plus maze testing. The mean amount of saccharin consumed by the various groups was analyzed as a 2 (CS condition) by 2 (US drug) ANOVA. The analysis revealed a significant effect of CS condition, $F(1, 26) = 196.45$, $p < 0.01$. Consumption of saccharin was affected by drug contingency. Groups CS^+ lithium and CS^+ amphetamine consumed less saccharin than Groups CS^- lithium and Groups CS^- amphetamine. No other effects were significant.

Plus Maze

Figure 5 presents the mean proportion OE (upper panel) and the mean proportion OT (lower panel), by the CS^+ and CS^- groups conditioned with lithium or amphetamine. Contrary to the expected results, the CS^+ groups spent a *greater* proportion of time in the open arms than the CS^- groups, although the groups did not significantly differ in the proportion of open arm entries. A 2 by 2 ANOVA of the proportion of time spent in open arms revealed only a significant effect of CS condition, $F(1, 26) = 6.36$, $p < .025$. The proportion of open arm entries revealed no significant effects, however the CS condition approached statistical significance, $F(1,26) = 3.52$, $p = .07$. Again, the CS^+ groups tended to display proportionately more open arm entries than the CS^- groups. Although it appears that the marginal CS effect was specific to the lithium groups, the 2

(CS condition) by 2 (US drug condition) ANOVA was not significant, $F(1, 26) = 2.67$, $p = 0.11$.

The literature (e.g., File, 1993) suggests that prior maze exposure sensitizes rats' avoidance of open arms. Indeed, the overall proportion of open arm entries ($t(60) = 2.01$, $p < .05$) and mean proportion of time spent in the open arms ($t(60) = 2.39$, $p < .025$) were greater in Experiment 1 (mean proportion OE = .47, sem = .02; mean proportion OT = .41, sem = .02) when the maze was novel than in Experiment 2 (mean proportion OE = .41, sem = .03; mean proportion OT = .24, sem = .03) when the maze was familiar.

Figure 6 presents the mean total arm entries (TE), by the CS^+ and CS^- groups conditioned with lithium or amphetamine. A 2 by 2 ANOVA of this data revealed no significant effects, indicating that the groups did not differ in overall activity. It is interesting to note, however, that the mean overall activity (mean = 18.5, sem = 1.2) across all groups in Experiment 2 when the maze was familiar was significantly lower than the mean overall activity (mean = 26.9, sem = 0.7) in Experiment 1 when the maze was novel ($t(60) = 6.15$, $p < .01$).

Discussion

Consistent with the literature (Dal-Col et al., 2003; Dawson et al., 1994; Rodgers & Shepherd, 1993), pre-exposure to the plus maze appeared to sensitize rats' avoidance of the open arms, as well as reduce their overall activity level in the maze. However, contrary to predicted results, prior exposure to a conditionally avoided taste did not potentiate open arm avoidance. In fact, rats in the CS^+ groups spent a *greater* proportion of time in the open arms than rats in the CS^- groups. Since proportion of time spent in the open arms of the elevated plus maze is a well-accepted measure of fear, these results

suggest that prior exposure to the CS^+ flavor may have attenuated fear, possibly the result of a compensatory (Siegel, 1978) or an opponent process (Soloman & Corbit, 1974) mechanism. However, before exploring such a mechanism, one potential alternative explanation must be discounted. During the 5 min consumption test that preceded exposure to the plus maze, the CS^+ groups drank significantly less saccharin than the CS^- groups. It is conceivable that the difference in proportion of time spent in the open arms is simply a function of the amount consumed during the consumption test. Therefore, in order to control amount of CS exposure prior to testing in the plus maze, in Experiment 3 all rats were given exposure to the CS flavor by intraoral infusion instead of by bottle.

EXPERIMENT 3

Surprisingly, prior exposure to a lithium- or amphetamine- paired taste resulted in an *increase* in the proportion of time spent in the open arms of the familiar plus maze relative to the unpaired control groups in Experiment 2. The saccharin solution was presented in a bottle during the 5-min consumption test just prior to placement in the plus maze. As expected, the CS^+ groups drank less saccharin than the CS^- groups. Therefore, the groups not only differed in the associative relation between the CS and US, but they also differed in the amount of solution that they consumed prior to testing in the plus maze. In Experiment 3, the CS^+ and CS^- groups were exposed to saccharin by intraoral infusion rather than by bottle during testing to control the amount of CS exposure. Additionally, only lithium (25 mg/kg) served as the US drug. Finally, the rats received two test trials: On the first trial, they were intraorally infused with saccharin immediately prior to placement on the plus maze and on the second trial, they were intraorally infused

with saccharin solution during plus maze testing in an attempt to enhance fear produced by CS exposure.

Method

Subjects

Twenty-one male Sprague-Dawley rats weighing between 260 and 320 g on the first conditioning trial were treated in a similar manner as in Experiment 2, except as indicated. During the course of the experiment, 3 rats lost their cannulae. The group assignments reflect the number of rats that completed the experimental procedures.

Surgery

The rats were surgically implanted with intraoral cannulae as in a modified version of the procedure described by Parker (1980). They were anaesthetized with isofluorane followed by subcutaneous (sc) injection of anafen (Ketoprofen, 10 mg/kg). A thin-walled 15-gauge stainless steel needle was inserted at the back of the neck and directed subcutaneously around the ear and brought out behind the first molar inside the mouth. Intramedic Polyethylene tubing with an inner diameter of 0.86 mm and an outer diameter of 1.27 mm was run through the needle and the needle was removed. A circular rubber adapter with a diameter of 3.00 mm was attached to the exposed tubing at the back of the neck. The tubing was held secure in the oral cavity by an O-ring, which was sealed behind the tubing. Post-operative treatment included intraoral infusions of Nolvadent oral cleansing solution and application of Savlon topically, for a period of three days.

Procedure

Following recovery from the surgery, the rats were placed on a water deprivation schedule, as described in Experiment 1, for each of three days. On Day 4, the rats

received an intraoral infusion of water in their home cage rather than by bottle at 0800 h. An infusion pump (Harvard Apparatus, South Natick, MA) delivered water at the rate of 1 ml/min over a 5 min period through a tube attached to the rats' cannula. Immediately following the infusion, the rats were given a pretest on the elevated plus maze and their activity was recorded. Following the pretest, the rats were returned to their home cage and given 60 min of water from a bottle at 1600 h. The rats were assigned to CS^+ (n= 8) and CS^- groups (n=10), matched on the basis of the proportion of time spent on the open arms during the pretest.

The conditioning trials took place on Days 5 and 8, exactly as in Experiment 2. On each trial, the rats were presented with 0.1% saccharin solution in a graduated tube for 5 min beginning at 0800 h and the amount consumed was measured. Immediately following the saccharin presentation, the rats in the CS^+ group were injected with lithium (25 mg/kg) and the rats in the CS^- group were injected with saline and returned to their home cage, with 60 min access to water at 1600 h. Twenty-four hours after each saccharin exposure (Days 6 and 9), the rats were presented with water for 5 min at 0800 h and two hours later were injected with saline (CS^+ group) or lithium (CS^- group). On Days 7, 10 and 11, they received 5 min of water at 0800 h and 60 min of water at 1600 h in their home cage.

The first test trial occurred on Day 12 in a similar manner as described in Experiment 1. The 0.1% saccharin solution was infused directly into the rat's mouth at a rate of 1 ml/min for 5 minutes while in its home cage. Immediately following infusion of the saccharin, the rats were placed in the plus maze and their activity was recorded for 5 minutes (CS Before test). A second test trial occurred on Day 13. During this trial, the

rats were intraorally infused with the saccharin solution at the rate of 1 ml/min for 5 min while being tested on the plus maze (CS During test).

Results

Consumption test (Conditioning trial 2)

Consumption of saccharin was affected by drug contingency. Group CS^+ lithium (Mean = 1.93, sem = 1.15) consumed less saccharin than the Group CS^- lithium (Mean = 8.05, sem = 1.30) on the second contingent conditioning trial ($t(16) = 3.54$, $p < .01$).

Plus maze tests

Figure 7 presents the mean proportion of open arm entries (OE) and the proportion of time spent in the open arms (OT) by the CS^+ and the CS^- groups during trial 1 (CS before test) and trial 2 (CS during test). The 2 by 2 mixed factor ANOVA for the proportion of open arm entries revealed a significant interaction of CS condition by infusion test ($F(1,16) = 7.01$, $p = .018$). When saccharin was presented immediately prior to plus maze testing (trial 1), the CS^+ group entered the open arms more frequently than the CS^- group ($t(16) = 2.03$, $p < .025$. Interestingly, when saccharin was presented during plus maze testing (trial 2), the CS^+ group entered the open arms proportionately fewer times than the CS^- group ($t(16) = 1.98$, $p < .05$). The 2 by 2 ANOVA for the proportion of time spent in the open arms revealed only a significant effect of infusion test ($F(1,16) = 12.68$, $p = .003$). The rats spent proportionately more time in the open arms when the CS^+ flavor was delivered prior to plus maze testing than when the CS^+ flavor was delivered during plus maze testing.

The total arm entries (TE) on each of the trials is presented in Figure 8. The 2 by 2 ANOVA revealed only a significant effect of infusion test ($F(1,16) = 37.72$, $p < .01$). The rats were more active in the plus maze when the CS^+ flavor was delivered immediately prior to plus maze testing than when the CS^+ flavor was delivered during plus maze testing. Although there appears to be a significant interaction of CS condition by infusion test, it only approached significance ($F(1,16) = 3.80$, $p = .069$).

Discussion

When the flavor was infused intraorally prior to placement in the plus maze, the CS^+ group displayed more open arm entries than the CS^- group, but the proportion of time spent in the open arms did not differ among the groups. In Experiment 2, the CS^+ groups spent proportionately more time in the open arms than the CS^- groups, and the proportion of open arm entries approached statistical significance. Both experiments suggest that exposure to the CS^+ prior to plus maze testing reduced fear of the open arms, when evaluated by at least one of the two behavioral measures typically employed in this paradigm.

When saccharin was infused during plus maze testing, the CS^+ group was less active than the CS^- group. Furthermore, the proportion of open arm entries was reduced and the proportion of time spent in the open arms tended to be reduced among the CS^+ group. Because the order of trials was not counterbalanced, this result should be interpreted cautiously; however, the pattern of findings across both tests is consistent with predictions of the opponent process theory of motivation (Solomon & Corbit, 1974).

The opponent process theory assumes a homeostatic mechanism controls emotional behavior; disturbances that move the system in one direction are met by an opposing force that counteracts the disturbance. When an emotion-arousing stimulus is presented, it elicits the *a* process that represents the emotion that occurs in the presence of the stimulus, for example fear. This *a* process is terminated upon removal of the stimulus which, in turn, reveals an opponent process or a *b* process that generates the opposite emotional reaction, for example, relief. The emotional changes that occur when a stimulus is presented and removed reflect the net result of the *a* and the *b* processes. The opponent process subtracts from the *a* process producing the net experienced emotional state. Figure 9 presents the standard pattern of affective dynamics predicted by the opponent process model of motivation. The standard pattern has five distinctive properties: (a) the 'peak' of the primary hedonic state, precipitated by stimulus onset; (b) a period of hedonic 'adaptation' during which the intensity of the hedonic state declines while stimulus intensity is maintained; (c) a 'steady level' of the hedonic process that remains active as long as the stimulus intensity is maintained; (d) a 'peak of affective after-reaction', which follows immediately after stimulus termination and is hedonically opposite in direction from that of the primary hedonic state, and (e) the afterstate decays and then disappears (Solomon & Corbit, 1974).

Solomon and Corbit (1974) interpret the results of a transfer of control experiment (Rescorla & LoLordo, 1965) as providing evidence for their model. Rescorla and Lolordo (1965) trained dogs to avoid shocks on a Sidman non-signaled avoidance schedule. The avoidance rate was used as a measure of fear intensity. A CS^{+} that had previously been paired with shock was presented during stable avoidance performance.

They reported that avoidance performance was greatly enhanced during the CS^+, but it was suppressed (below baseline) upon termination of the CS^+, and baseline responding slowly recovered over a 30-45 sec period. The results of Experiments 2 and 3 of the present study show a similar pattern as those of Rescorla and Lolordo (1965), using a different paradigm. In Experiments 2 and 3, rats exposed to the lithium-paired saccharin solution prior to placement on the elevated plus maze displayed reduced fear of the open arms which may represent the *b* process, that is, relief produced by the removal of the fear-producing stimulus. However, rats exposed to the lithium-paired saccharin solution during plus maze testing in trial 2 (CS During test) of Experiment 3 displayed enhanced fear of the open arms which may represent the *a* process, that is, fear produced during presentation of the fear-producing stimulus.

An opponent process mechanism has also been previously suggested to account for conditioned suppression/enhancement of consumption following intraoral exposure to a lithium-paired taste. When rats were intraorally infused with a lithium-paired taste during a consumption test with water or a different novel taste, they displayed conditioned suppression of drinking (Parker, 1980). On the other hand, when rats were intraorally infused with a lithium-paired taste 15 min prior to a consumption test, they displayed conditioned enhancement of drinking (Domjan & Gillan, 1977). The suppressed consumption might be interpreted to reflect the *a* process and the enhanced consumption the *b* process.

EXPERIMENT 4

Experiment 4 evaluated whether an opponent process mechanism might account for the findings of Experiments 2 and 3. In Experiment 4, the lithium-paired (CS^+) or saline-paired (CS^-) saccharin solution was infused intraorally during a plus maze test (to detect the *a* process) and 15 min prior to a plus maze test (to detect the *b* process). A 15 min post-infusion interval was selected because this was the interval that Domjan and Gillan (1977) found to be effective in detecting compensatory drinking following exposure to a lithium-paired taste. If exposure to a lithium-paired flavor elicits conditioned fear (*a* process) followed by an opposite state of relief (*b* process), then exposure to the saccharin solution during plus maze testing should elicit greater avoidance of the open arms in the CS^+ group than in the CS^- group, but exposure to the saccharin solution 15 min prior to plus maze testing should elicit less avoidance of the open arms in the CS^+ group than in the CS^- group. To maximize detection of conditioned fear and relief, a higher dose of lithium (130 mg/kg of .15 M) served as the US drug. Additionally, the rats were administered water baseline tests prior to the saccharin tests following conditioning.

Method

Subjects

Thirty-two male Sprague-Dawley rats weighing between 270 and 340 g on the first conditioning trial were treated in a similar manner as in Experiment 3, except as indicated. All rats received food and water *ad lib* for the duration of the experiment. During the course of the experiment, 8 rats lost their cannulae. The final group numbers are described below.

Procedure

Following recovery from the surgery, the rats received two pretest trials (one on Day 1 and the other on Day 2) separated by 24 hr in a counterbalanced order. On one trial, rats were infused intraorally with water (1 ml/min) for 5 min in their home cage and 15 min later tested in the plus maze; and on the other trial, rats were infused with water during the 5 min plus maze test. The chambers were cleaned with soapy water and dried following each test.

The conditioning trials occurred on Days 4 and 7. During each trial, the rats were infused intraorally with 0.1% saccharin solution for 5 min in their home cage. Immediately following the saccharin infusion, the rat was injected with either 130 mg/kg of lithium (CS^+ group, n=14) or saline (CS^- group, n = 10). Half the rats in each group received non-contingent injections 24 hr prior to each conditioning trial (Days 3 and 6) and half received non-contingent injections 24 hr after each conditioning trial (Days 5 and 8). On the non-contingent trials, the rats in the CS^+ group were injected with saline and the rats in the CS^- group were injected with the 130 mg/kg of lithium.

On Days 10 and 11, all rats were given baseline water posttests in which water was delivered intraorally 15 min prior to placement on the plus maze on one day, and while in the plus maze on the other day in the same manner as on Days 1 and 2. On Days 12 and 13, saccharin test trials occurred. The tests were conducted identically to the water posttests, except the rats were infused with 0.1% saccharin solution. The order of the delayed and simultaneous tests was counterbalanced among the groups for all tests.

Data Analysis

The proportion OE and the proportion OT following conditioning during the water tests and saccharin tests were entered into a 2 by 2 by 2 mixed factors ANOVA with the between-group factors of conditioning group (CS^+, CS^-) and non-contingent trial order (before, after) and the within-group factor of infusion test (Solution 15 min Before, Solution During).

Additionally, the water posttests were treated as baseline measures for the saccharin tests. The purpose of conducting water posttests prior to saccharin tests was to expose the CS^+ and CS^- groups to the two methods of testing, infusion 15 min before testing and infusion during testing, to eliminate the confound of novelty of testing procedure. The relative proportion OE and OT on the saccharin and water tests were determined (Saccharin/[Saccharin + Water]). For example, the relative proportion of OE was determined by taking the proportion of OE on the saccharin tests over the proportion of OE on the saccharin test plus the proportion of OE on the water tests. These relative proportion scores were also entered into a 2 by 2 by 2 ANOVA. Finally, as a measure of activity, TE on saccharin trials relative to water trials was determined (TE Saccharin/[TE Saccharin + TE Water]) and entered into a 2 by 2 by 2 ANOVA. Since the factor of non-contingent injection order was not significant in any analysis, all figures are prepared pooled across this factor.

Results

Water post-tests (Days 10-11)

The mean proportion OE and OT by the CS^+ and CS^- groups on the test that occurred 15 min after water exposure and on the test that occurred during water exposure

is presented in Figure 10. The 2 by 2 by 2 mixed factors ANOVAs revealed only a significant effect of infusion test for the proportion of time spent in the open arms ($F(1, 20) = 5.14$, $p < .05$); rats spent proportionately less time in the open arms during the Water During test than during the Water 15 min Before test. Neither the CS condition nor the order of non-contingent trials influenced plus maze performance during the water tests.

Saccharin post-tests (Days 12-13)

The mean proportion OE and OT by the CS^+ and CS^- groups on the test that occurred 15 min after saccharin exposure and on the test that occurred during saccharin exposure are presented in Figure 11. The proportion OE was suppressed following exposure to a lithium-paired saccharin solution. The 2 by 2 by 2 mixed factors ANOVAs revealed only a significant effect of conditioning group ($F(1, 20) = 5.64$, $p < .05$) for the proportion of open arm entries; the CS^+ group proportionately avoided the open arms more than the CS^- group. Neither the infusion tests nor the order of non-contingent injection was significant. The analysis of the proportion of OT revealed no significant effects.

Figure 12 presents the mean TE for the CS^+ and CS^- groups given saccharin 15 min prior to and during plus maze testing. Although rats were less active when infused during a plus maze test than before the plus maze test, the CS condition did not affect activity level. The 2 by 2 by 2 ANOVA revealed only a significant effect of infusion test, $F(1,20) = 17.44$, $p < .01$. The CS condition was not significant, $F(1,20) = 2.11$, $p = .16$.

Saccharin tests relative to Water tests

Figure 13 presents the mean relative proportion OE (upper panel) and relative proportion OT (lower panel) displayed on the saccharin tests relative to the baseline water tests. The relative proportion OE during infusion of the lithium-paired saccharin was less than during infusion of the saline-paired saccharin, but not when the infusion occurred 15 min before the test. The 2 by 2 by 2 ANOVA for the relative proportion OE revealed a significant effect of CS condition by infusion test, $F(1,20) = 4.36$, $p < .05$; when saccharin was presented during plus maze testing, but not when saccharin was presented 15 min prior to plus maze testing, the CS^+ group displayed relatively greater avoidance of the open arms than the CS^- group, $t(22) = 2.98$, $p < .01$. The 2 by 2 by 2 ANOVA for the relative proportion OT revealed no significant effects.

Figure 14 presents the mean relative TE on the saccharin tests relative to the baseline water tests. The 2 by 2 by 2 ANOVA revealed no significant activity effects. Therefore, the CS^+ induced suppression of open arm entries was not simply a function of suppressed activity.

Discussion

The opponent process theory predicts that an *a* process, an emotional state that is directly elicited by an emotionally arousing stimulus, is followed by a *b* process, which produces an opposite emotional state when that stimulus is terminated. Since the opponent process theory may account for the results found in Experiments 2 and 3, we predicted that presentation of the lithium-paired saccharin during testing would elicit conditioned fear that would exacerbate avoidance of the open arms in the elevated plus maze, but presentation of the lithium-paired saccharin 15 min prior to plus maze testing

would elicit an opponent process similar to what was observed in Experiments 2 and 3 when maze testing occurred immediately following CS exposure.

Only an *a* process was detected in Experiment 4. Analysis of the saccharin tests alone suggests that when conditioned with a much higher dose of lithium (130 mg/kg) than in Experiments 2 and 3 (25 mg/kg), rats displayed greater reluctance to enter the open arms of an elevated plus maze than those conditioned with saline regardless of whether the CS exposure was during the plus maze test (to intensify the *a* process) or 15 min prior to the plus maze test (presumably to intensify the *b* process). However, when the proportion of open arm entries and time on the open arms during the saccharin tests were converted to relative scores using water tests as the baseline, rats infused with lithium-paired saccharin 15 min before plus maze testing no longer differed from those infused with saline-paired saccharin. On the other hand, relative to their performance on water tests, those rats infused with lithium-paired saccharin during plus maze testing displayed enhanced avoidance of the open arms relative to those infused with saline-paired saccharin. Therefore, when baseline responding is taken into account, only on the test in which rats received the CS solution during testing did they display evidence of enhanced fear. This is what the opponent process theory would predict should happen. However, in contrast to the results of Experiments 2 and 3, there was no evidence of the presence of the opponent *b* process.

GENERAL DISCUSSION

The experiments reported here evaluated the role of fear in the motivation of conditioned taste avoidance. The rationale of the experimental design was that if

avoidance of a taste is mediated by conditioned fear, then exposure to that taste should potentiate a rat's avoidance of the open arms in an elevated plus maze, an index of fear.

In Experiment 1, rats were exposed to a drug-paired saccharin solution by bottle immediately prior to a test in a novel elevated plus maze. The CS^+ and CS^- groups did not differ, but suggested a pattern reflecting a paradoxical reduction of fear following CS exposure. Because the literature suggests that prior exposure to the plus maze sensitizes open arm avoidance (Dawson et al., 1994; Treit et al., 1993), Experiment 2 was a replication of Experiment 1, but the rats were tested in a plus maze to which they had been pre-exposed. Indeed, contrary to predicted results, rats that were given the drug-paired flavor (CS^+ groups) spent a greater proportion of time in the open arms than control rats (CS^- groups) even though the CS^+ groups drank less of the saccharin solution in the consumption test than the CS^- groups. To control for differential consumption of the CS flavor prior to plus maze testing, in Experiment 3, rats in the CS^+ and CS^- groups were exposed to an equivalent amount of saccharin solution via intraoral infusion, and a similar pattern of reduced fear of the open arms was once again evident. However, in a second plus maze test, rats were exposed to saccharin by intraoral infusion during the plus maze test. Simultaneous exposure resulted in greater avoidance of the open arms by the CS^+ group than by the CS^- group. An opponent process mechanism was proposed to account for these findings.

Solomon and Corbit (1974) argued that an emotionally arousing event elicits two opposing processes, an *a* process followed by a slave *b* process. The *a* process reflects the emotional reaction to the stimulus itself and the opponent *b* process reflects the emotional reaction to the termination of the stimulus. The emotional changes observed

when the emotion-arousing stimulus is presented and then removed reflect the net result of the *a* and *b* processes. Hence, upon presentation of an emotion-arousing stimulus, an *a* process is produced and during the duration of stimulus exposure, an opposing *b* process is produced, however the *b* process (the opposite emotional state) is not evident until after termination of the *a* process. If the opponent process model accounts for the pattern of results seen in Experiments 2 and 3, then the *a* process elicited by presentation of the CS flavor may reflect fear and the *b* process elicited by termination of the CS flavor may reflect relief. In Experiments 2 and 3, rats exposed to the drug-paired saccharin solution immediately *prior* to a plus maze test demonstrated attenuated fear of the open arms, but rats exposed to the drug-paired saccharin *during* the plus maze test (in the second test trial of Experiment 2), displayed enhanced fear of the open arms.

Experiment 4 was designed to determine whether an opponent process mechanism might account for the findings of Experiments 2 and 3. The CS flavor was infused intraorally during a plus maze test to detect the *a* process, and 15 min prior to a plus maze test to detect the *b* process. A 15 min delay was imposed, because prior research indicated that a delay of this duration produces compensatory drinking (the opposite effect of lithium) elicited by exposure to a lithium-paired flavor (Domjan & Gillan, 1977). Hence, if infusion of a lithium-paired flavor elicits conditioned fear followed by the opposite emotional state of relief, then exposure to the CS flavor during plus maze testing should potentiate open arm avoidance (fear) and exposure to the CS flavor 15 min prior to plus maze testing should reduce open arm avoidance (relief). Furthermore to maximize detection of both states, the dose of lithium was increased from 25 mg/kg in Experiments 1-3 to 130 mg/kg in Experiment 4. Indeed, infusion of the CS flavor during

plus maze testing resulted in fewer open arm entries when assessed as the proportion of open arm entries on saccharin tests relative to baseline water tests. However, infusion of the CS flavor 15 min prior to the test did not modify open arm entries relative to the same behavior following water tests. These results suggest that, under the conditions of Experiment 4, when a lithium-paired saccharin solution is delivered during plus maze testing, fear is enhanced. However, if the lithium-paired flavor is presented 15 min before plus maze testing, the emotional reaction does not differ from that produced by water.

Our results suggest that removal of a drug (lithium or amphetamine)-paired flavor immediately elicits a state of relief (*b* process) that is reflected by an enhanced exploration of open arms in the elevated plus maze (Experiments 2 and 3). On the other hand, presentation of the drug (lithium only)-paired flavor immediately elicits a state of fear (*a* process) that is reflected by enhanced avoidance of the open arms (Experiments 3 and 4). Further investigations are ongoing to confirm or deny this interpretation of our results.

Future Studies

Validity of Plus Maze performance as a measure of fear

The elevated plus maze is currently being used as an animal model of anxiety/fear by a number of investigators, it has been reported that over 100 laboratories use the apparatus and pharmaceutical companies continue to use it as a first screen for putative anxiolytics (Hogg, 1996). The plus maze continues to be one of the most popular animal models of anxiety based on the study of unconditioned behavior. Avoidance of the open arms is a well accepted index of fear. However, despite the successful application of this

animal model of anxiety in a number of pharmacological studies of anxiolytic effects (File, 1990; Lister, 1987) there remains considerable controversy over the validity of its ability to detect anxiety/fear especially when the manipulations are more complex and require more than one exposure to the plus maze. For example, certain anxiogenic compounds such as CCK-4 and isoproterenol fail to affect plus maze performance, which may support the idea that different animal models of anxiety measure different components of anxiety and the elevated plus maze may induce just one form of anxiety (Rodgers & Dalvi, 1997; Rodgers et al., 1995).

There is also evidence to suggest that plus maze performance is significantly affected by pre-exposure to the plus maze, so much so that performances during trial 1 and trial 2 reflect two different emotional states. A number of studies have shown potentiated open arm avoidance in rats with previous plus maze experience (Bertoglio & Carobrez, 2000). File (1990) demonstrated that during a plus maze test, rats treated with chlorodiazepoxide (CDP), a putative anxiolytic, spent substantially more time on the open arms of the plus maze compared to controls, however when the rats were given a second plus maze test, pretreatment with CDP had no effect on plus maze performance. Although File interpreted the findings as the development of tolerance to CDP, Dal-Col and colleagues (2003) found that when rats were pre-exposed to the plus maze for 5 min (trial 1 with no drug manipulation), and then were given plus maze exposure 24 hours later, the rats pretreated with the anxiolytic, midazolam, during trial 2 did not differ in plus maze performance from untreated controls. The findings suggest a shift in emotional states from an unconditioned anxious state due to the novelty of the apparatus (trial 1) to a learned fear response (trial 2).

It is possible that avoidance of open arms in the elevated plus maze may not be the best measure of conditioned fear. The amygdala is involved in emotional behavior and its role in conditioned fear is well-documented. Serial processing in regions of the amygdala has been implicated in the acquisition and expression of conditioned fear (Killcross et al., 1997). Several recent studies have shown that amygdaloid lesions do not interfere with open arm activity on a novel plus maze (Decker, Curzon & Brioni, 1995; Treit & Menard, 1997; Treit, Aujla & Menard, 1998; Treit, Pesold & Rotzinger, 1993). However, since the emotional processes represented by trial 1 open arm activity appears to be different from that of trial 2, it would be of interest to investigate the effect of amygdaloid lesions on open arm activity in rats that have received prior exposure on the plus maze.

On the other hand, the basolateral amygdala (BLA) appears to be necessary for the consolidation of emotional learning acquired during trial 1 in the plus maze (File et al., 1998). Rats were lesioned immediately after an elevated plus maze test (trial 1) and tested again 2 days later (trial 2). Prior to trial 2 some lesioned and sham rats received CDP while others received vehicle. It has previously been shown that anxiolytics are ineffective when administered on trial 2 (Dawson et al., 1994; File, 1990; 1993; File et al., 1990; Rodgers et al., 1992). However, this was not the case with the BLA lesioned rats. Among the lesioned rats, but not the sham rats, CDP effectively enhanced open arm activity on trial 2. The performance of CDP-pretreated lesioned rats on trial 2 was similar to performance of CDP-pretreated non-lesioned rats on trial 1 which demonstrated that the BLA may be affecting the consolidation of emotional memories acquired in trial 1.

Despite the fact that we found evidence to suggest that conditioned taste avoidance is mediated by conditioned fear using the elevated plus maze, further evidence to strengthen the argument might be provided using another behavioral paradigm, the fear-potentiated startle effect, as well as a neurobehavioral paradigm, i.e. effects of amygdala lesions on conditioned rejection reactions.

Startle Experiments

Another animal model of fear employs the fear-potentiated startle response, where the amplitude of the startle reflex in rats can be modified by a state of fear (Davis, 1998). In a typical experiment, rats are placed in a specialized chamber that measures the amplitude of the startle reflex when rats are presented with a set number of startle-eliciting stimuli (Davis, Walker & Lee, 1997). In contrast to open arm avoidance in the plus maze, studies have found that amygdala lesions completely block fear-potentiated startle (Davis, 1998; Davis et al., 1997). Specifically, localized destruction of cell bodies via NMDA (neurotoxin) infusion into the basolateral amygdala (BLA) blocked fear-potentiated startle when the lesions were made before or after conditioning (Davis, 1998).

If conditioned taste avoidance is motivated by conditioned fear, then exposure to a lithium-paired taste may potentiate the acoustic startle response. In a future experiment rats will be exposed to lithium-paired saccharin (CS^+ group) prior to placement in the startle apparatus and their acoustic startle response reported. If termination of CS exposure elicits a state of relief (as suggested by Experiments 2 and 3), then it may be necessary to devise a technique for delivery of the taste during startle testing. However, if the elevated plus maze is simply a less sensitive measure of conditioned fear, potentiated startle may be elevated by prior exposure to the lithium-paired taste.

Basolateral Amygdala Lesions

Considerable evidence has suggested lesions of the basolateral amygdala can interfere with taste avoidance learning (e.g., Dunn & Everitt, 1988). This structure is also known to be involved in both the acquisition and expression of conditioned fear responses to shock-paired stimuli (Killcross et al., 1997). However, it has not been determined what role the BLA plays in conditioned rejection reactions (conditioned taste aversion). Hence, if taste avoidance is mediated by conditioned fear of a tastant, then bilateral BLA lesions may interfere with taste avoidance produced by lithium paired with saccharin, but not necessarily modify taste aversion (conditioned rejection reactions). A future study will examine the role the BLA plays in conditioned taste aversion and conditioned taste avoidance using lesion studies.

Final Comments

The present study provides some support for the suggestion that conditioned fear motivates conditioned taste avoidance. When rats conditioned with lithium-paired saccharin were infused with the CS flavor in the plus maze, they consistently exhibited potentiated fear compared to control rats. However, when rats conditioned with lithium-paired saccharin were placed in the plus maze following saccharin exposure on the test day, under some conditions, but not others, they exhibited suppressed fear response compared to control rats. The findings might be explained in terms of an opponent process mechanism. Future research which includes startle experiments and amygdala lesion studies will further evaluate the hypothesis that conditioned fear mediates conditioned taste avoidance.

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Figure Captions

Figure 1. Mean (±sem) amount (ml) of 0.1 % saccharin solution consumed by CS^+ and CS^- groups conditioned with lithium or amphetamine and tested in a novel plus maze.

Figure 2. Mean (±sem) proportion of open arm entries and mean (±sem) proportion of time spent in the open arms in a novel plus maze during the 5-min test that immediately followed exposure to the CS^+ or the CS^- saccharin solution.

Figure 3. Mean (±sem) total arm entries in a novel plus maze during the 5-min test that immediately followed exposure to the CS flavor.

Figure 4. Mean (±sem) amount (ml) of 0.1 % saccharin solution consumed by CS^+ and CS^- groups conditioned with lithium or amphetamine and tested in a familiar plus maze.

Figure 5. Mean (±sem) proportion of open arm entries and mean (±sem) proportion of time spent in the open arms in a familiar plus maze during the 5-min test that immediately followed exposure to the CS^+ or the CS^- saccharin solution.

Figure 6. Mean (+sem) total arm entries in a familiar maze by CS^+ and CS^- groups following exposure to CS flavor

Figure 7. Mean (±sem) proportion of entries into the open arms and mean proportion of time spent in open arms during two 5-min tests by CS^+ lithium and CS^- lithium groups. Trial 1 occurred 1 min following intraoral infusion of the saccharin solution. Trial 2 occurred during intraoral infusion of the saccharin.

Figure 8. Mean (±sem) total arm entries during trials 1 and 2 by CS^+ lithium and CS^- lithium groups.

Figure 9. The five distinctive features of the standard pattern of affective dynamics. The bold black bar presents the time spent during which the emotion-arousing stimulus is present. The ordinate represents two hedonic scales, one for the primary affect, and the other for the affective after-reaction (Solomon & Corbit, 1974).

Figure 10. Mean (±sem) proportion of entries into open arms and mean (±sem) proportion of time spent in open arms while infused with water 15 min prior to placement on plus maze and while infused on plus maze, by CS^+ and CS^- groups, during water tests.

Figure 11. Mean (±sem) proportion of entries into the open arms and mean (±sem) proportion of time spent in open arms while infused with saccharin solution 15 min prior to plus maze test (CS 15 min before) and while infused with saccharin solution on the plus maze (CS during), by CS^+ and CS^- groups, during saccharin tests.

Figure 12. Mean (±sem) total arm entries while infused with CS solution 15 min prior to plus maze testing and while infused with CS solution during plus maze testing, by CS^+ and CS^- groups, during saccharin tests.

Figure 13. Mean (±sem) proportion of entries into the open arms and mean (+sem) proportion of time spent in open arms 15 min prior to plus maze testing and during plus maze testing on saccharin tests relative to baseline water tests, by CS^+ and CS^- groups.

Figure 14. Mean (±sem) total arm entries 15 min prior to plus maze testing and during plus maze testing on saccharin test relative to baseline water tests, by CS^+ and CS^- lithium groups.

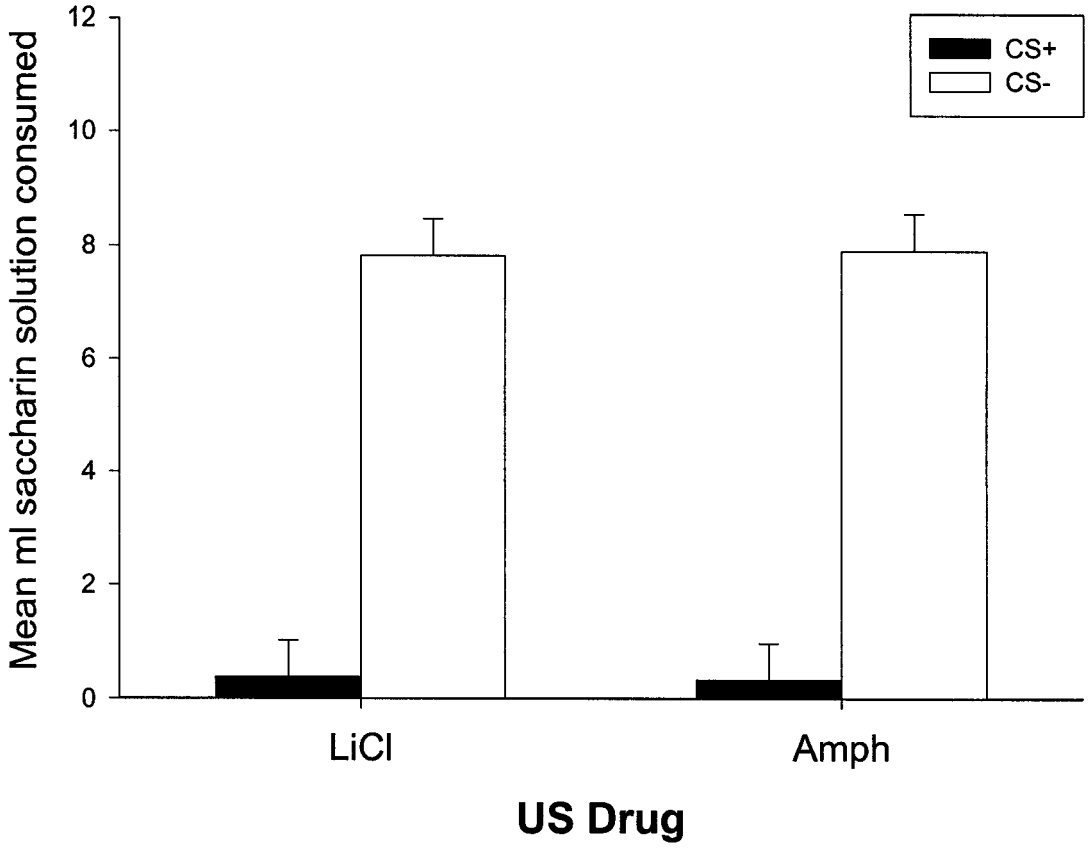
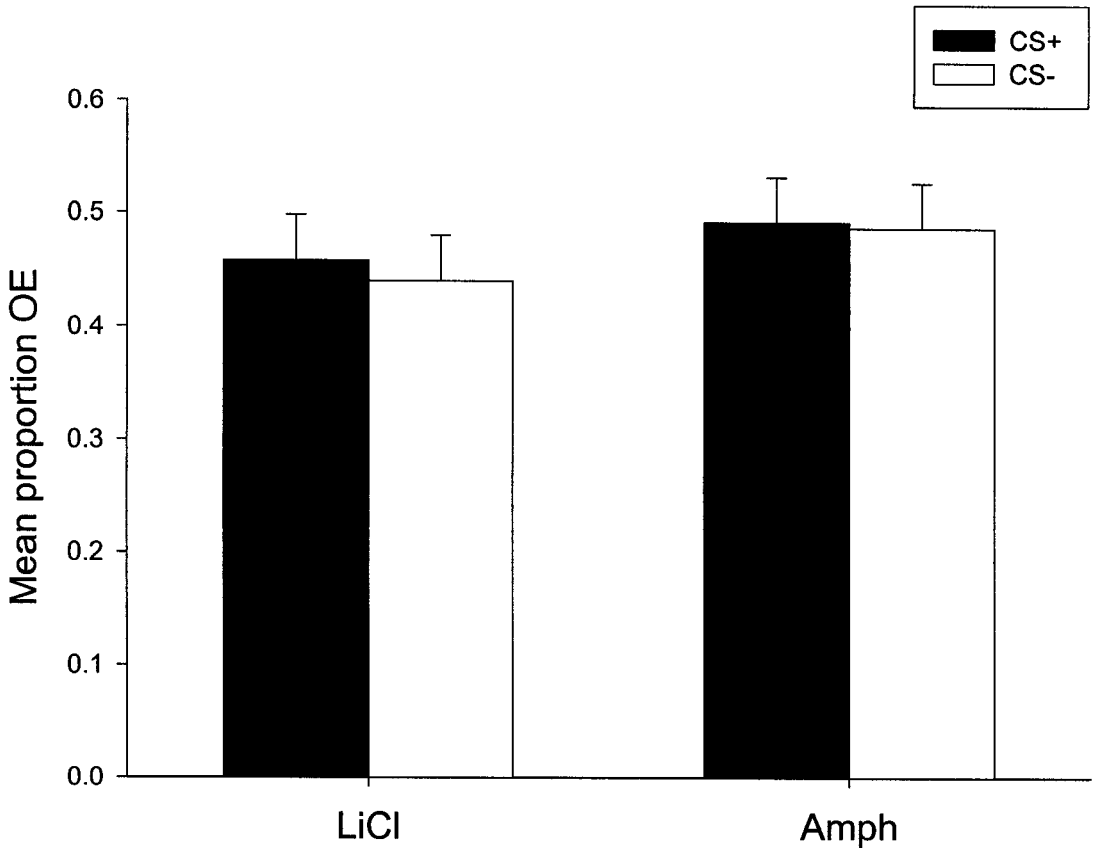

Mean ml saccharin solution consumed
12
10
8
6
4
2
0
CS+
CS-
LiCl
Amph
US Drug

Figure 1


CS+
CS-
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Mean proportion OE
LiCl
Amph

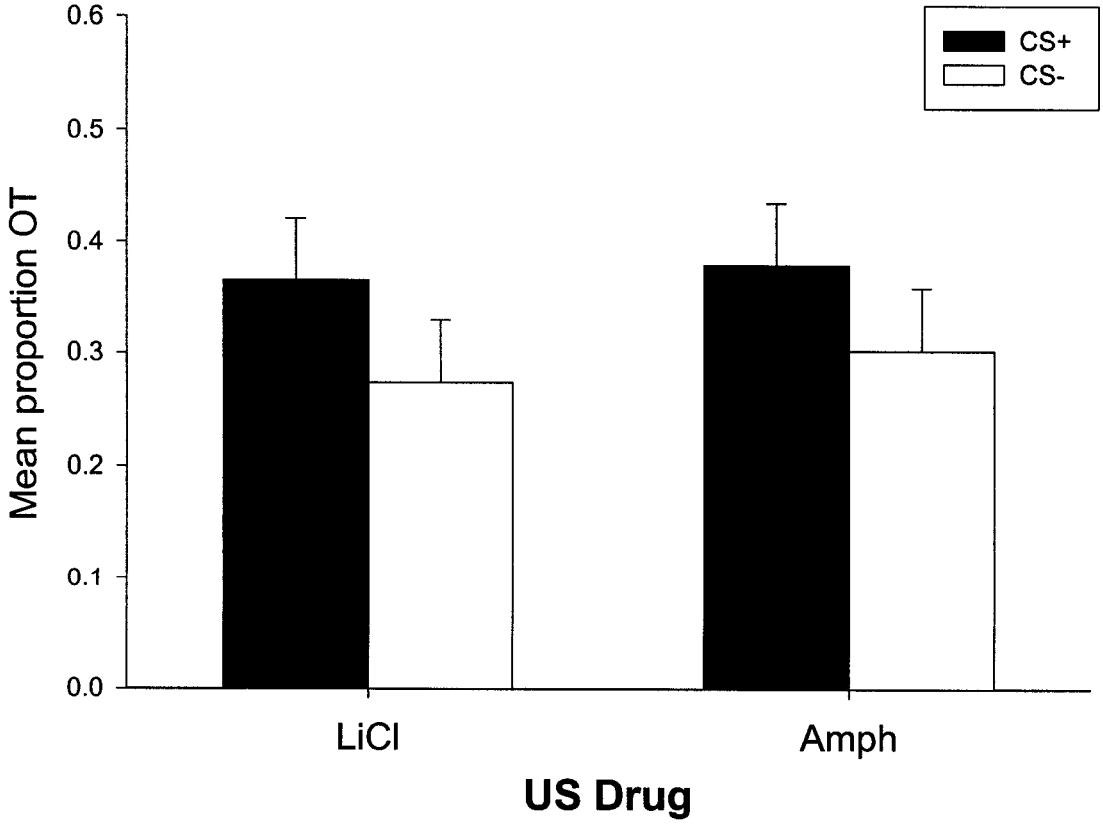

CS+
CS-
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Mean proportion OT
LiCl
Amph
US Drug

Figure 2

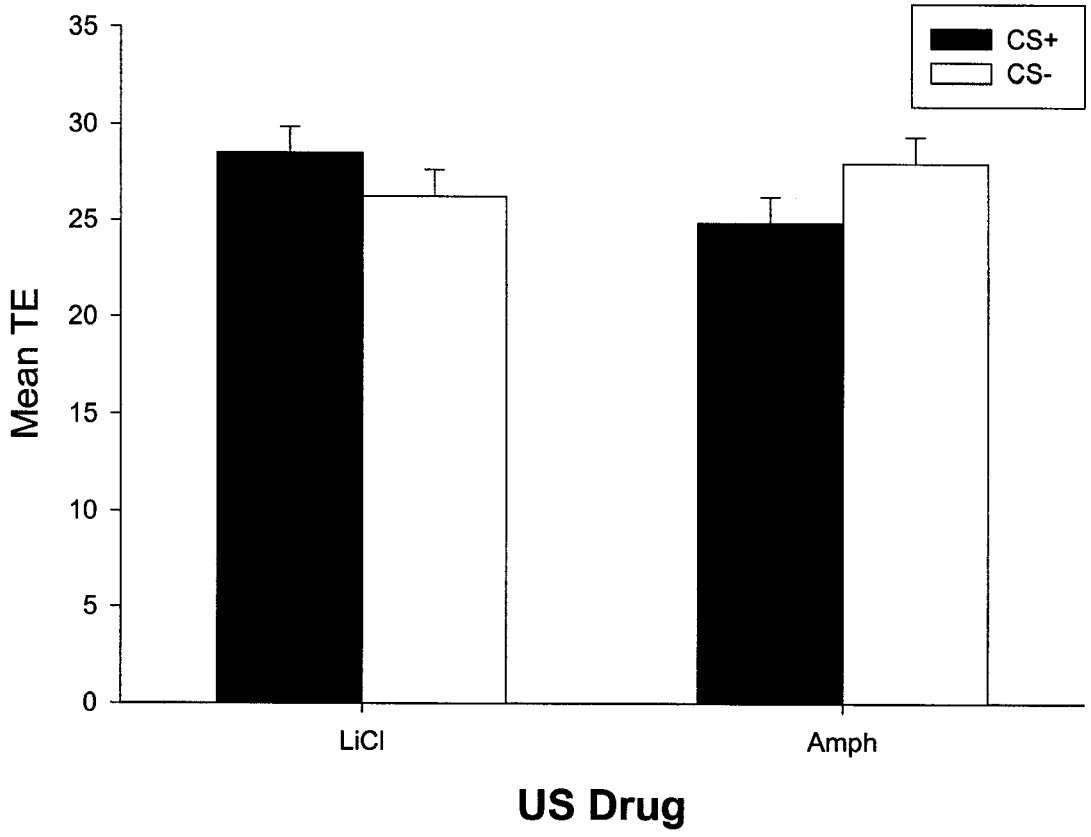


Figure 3

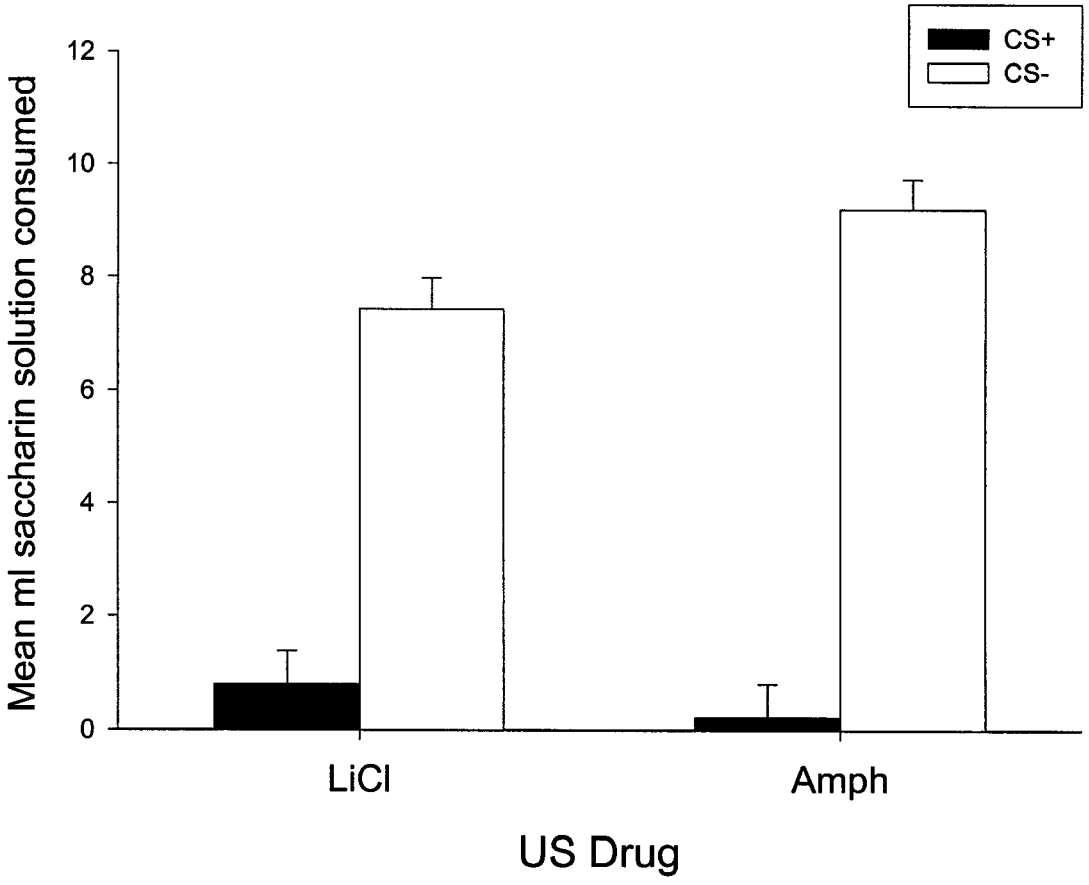
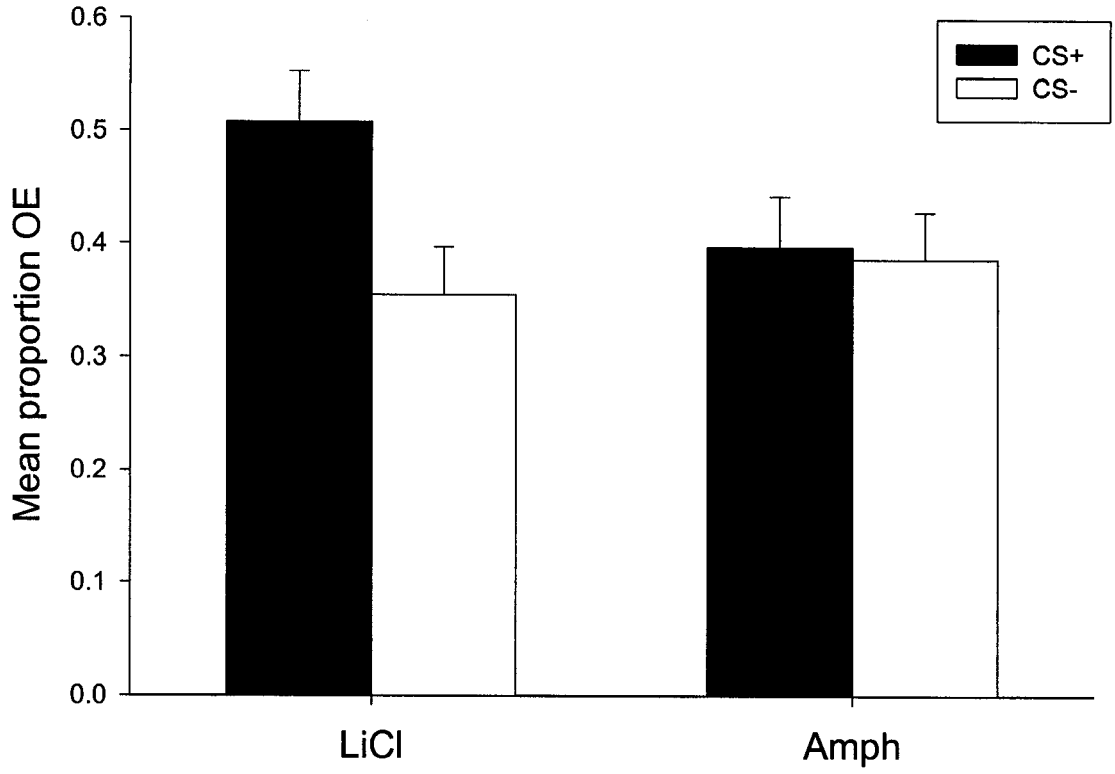

CS+
CS-
Mean ml saccharin solution consumed
12
10
8
6
4
2
0
LiCl
Amph
US Drug

Figure 4


0.6
0.5
0.4
0.3
0.2
0.1
0.0
Mean proportion OE
CS+
CS-
LiCl
Amph

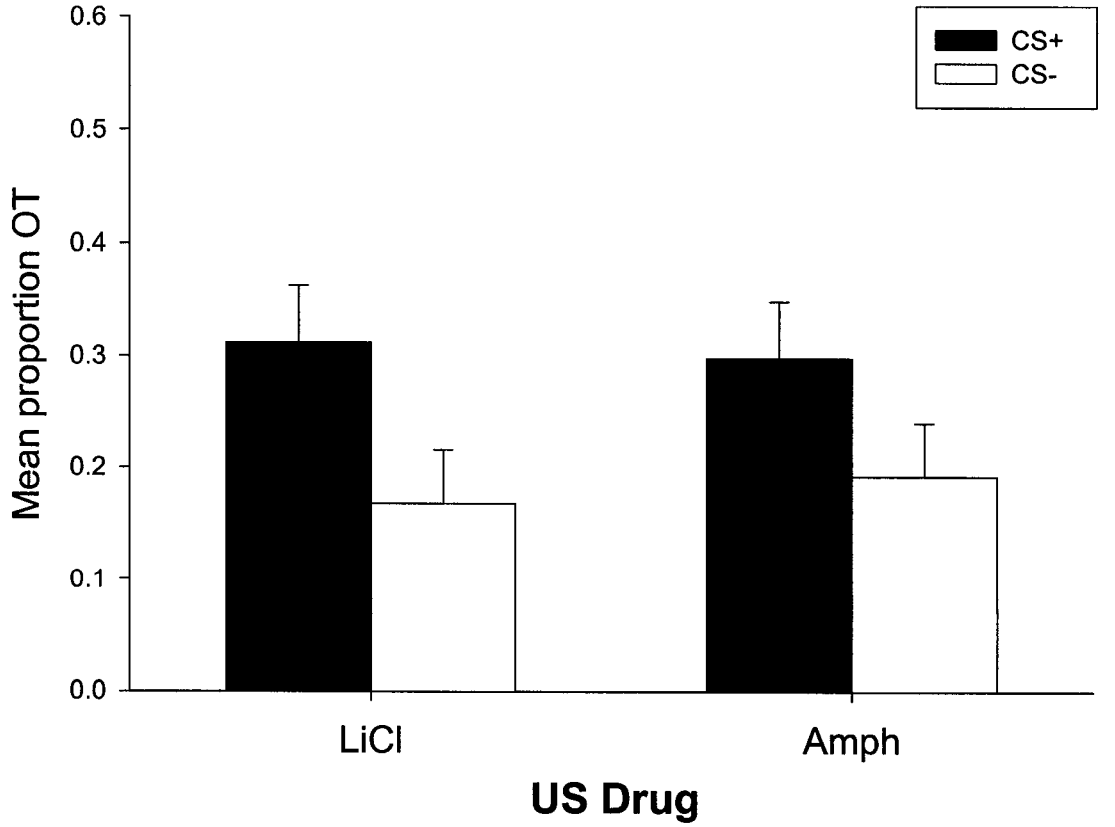

0.6
0.5
0.4
0.3
0.2
0.1
0.0
Mean proportion OT
CS+
CS-
LiCl
Amph
US Drug

Figure 5

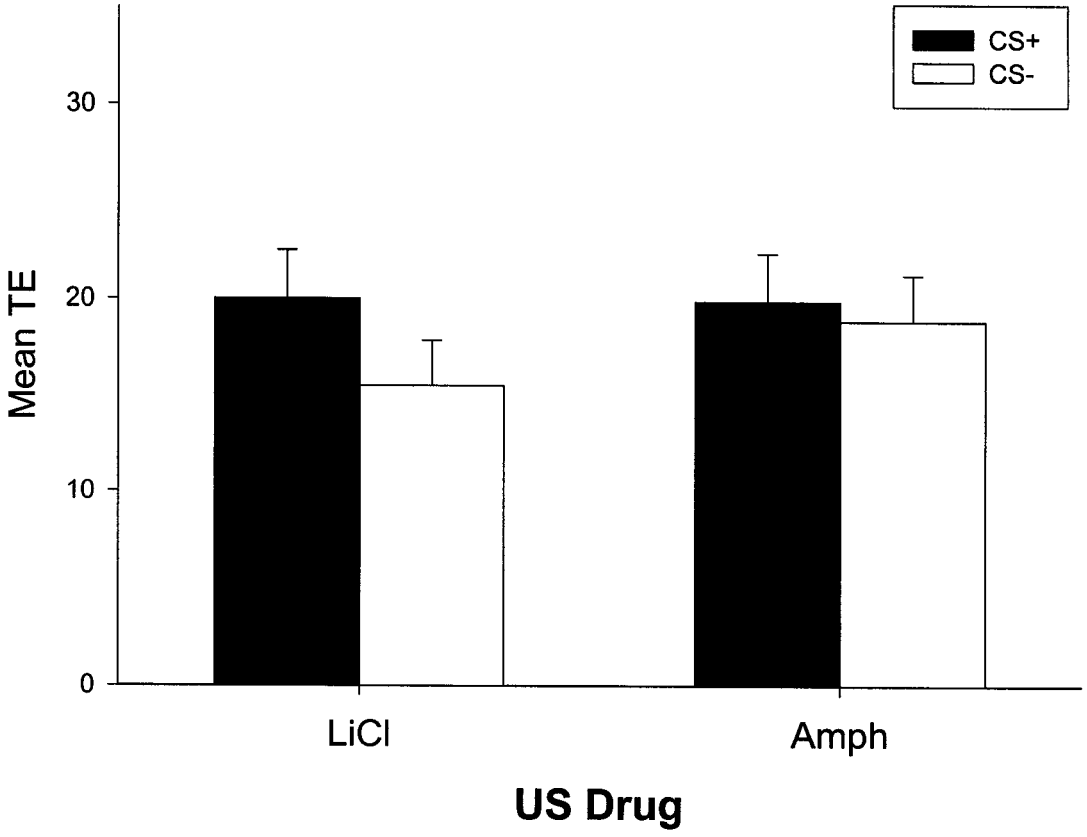
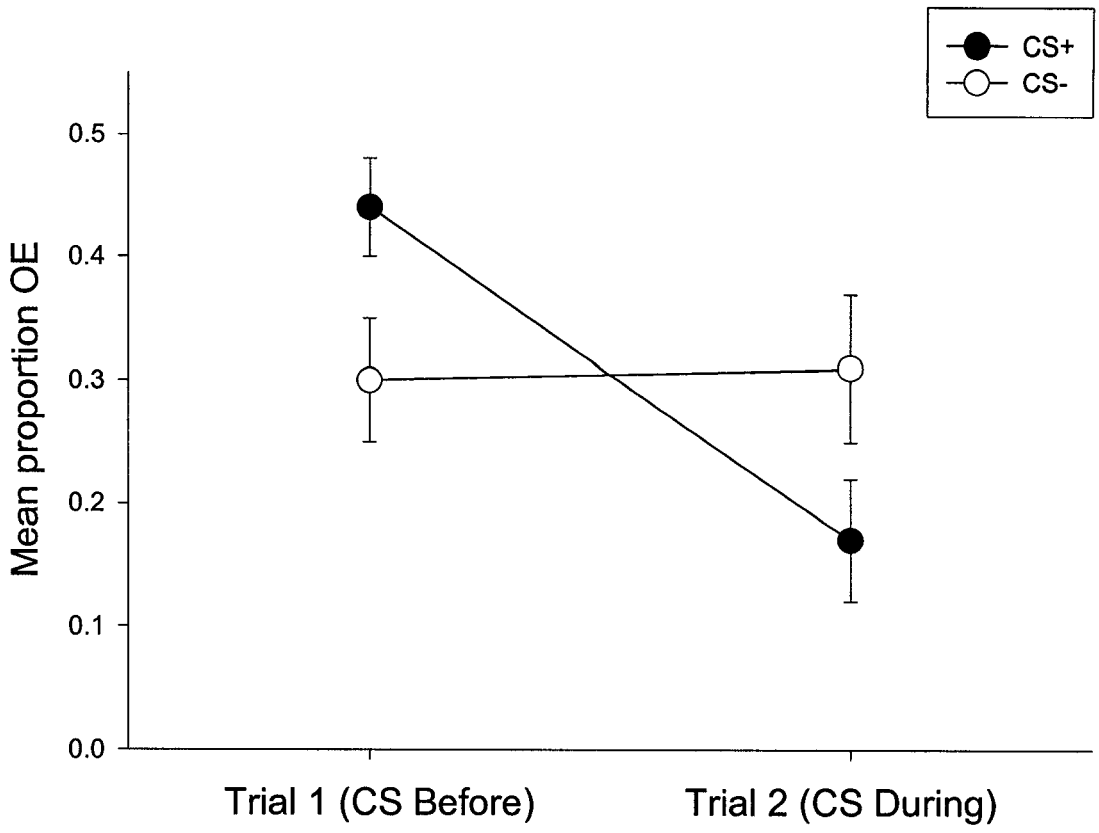


Figure 6


CS+
CS-
Mean proportion OE
0.5
0.4
0.3
0.2
0.1
0.0
Trial 1 (CS Before)
Trial 2 (CS During)

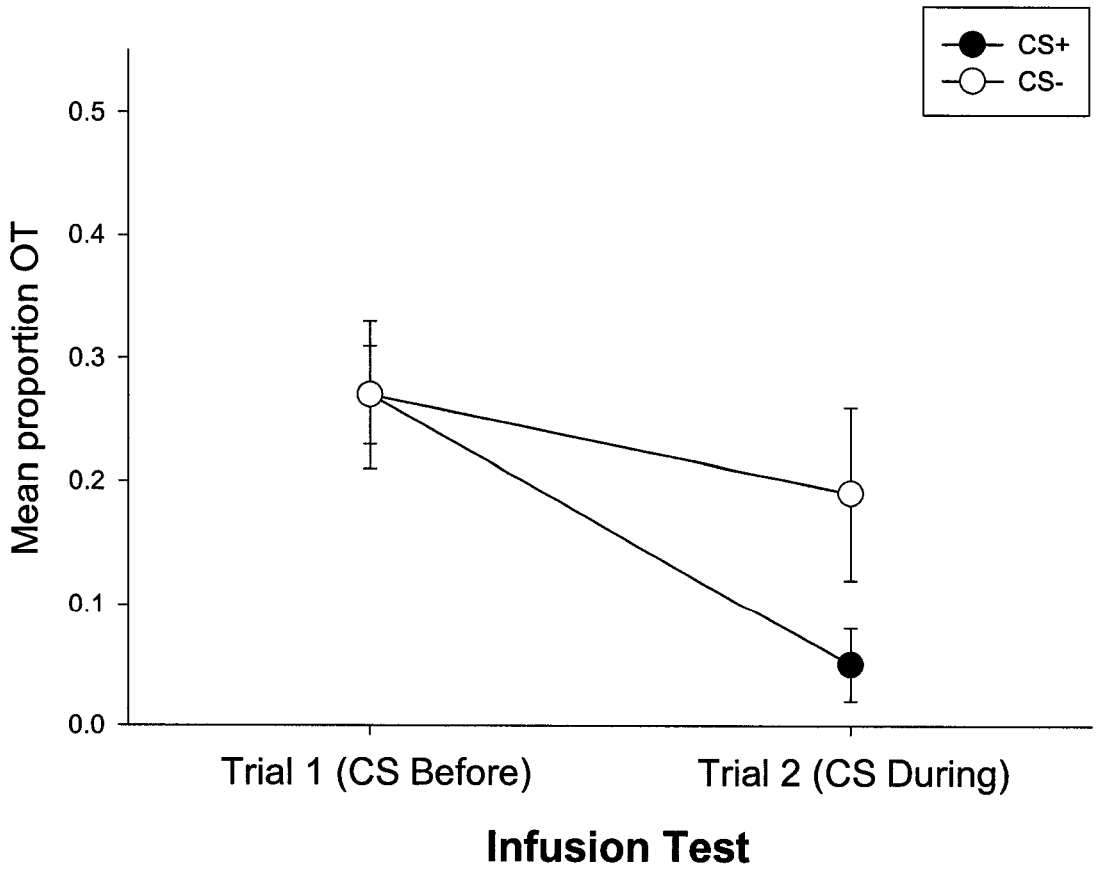

CS+
CS-
Mean proportion OT
0.5
0.4
0.3
0.2
0.1
0.0
Trial 1 (CS Before)
Trial 2 (CS During)
Infusion Test

Figure 7

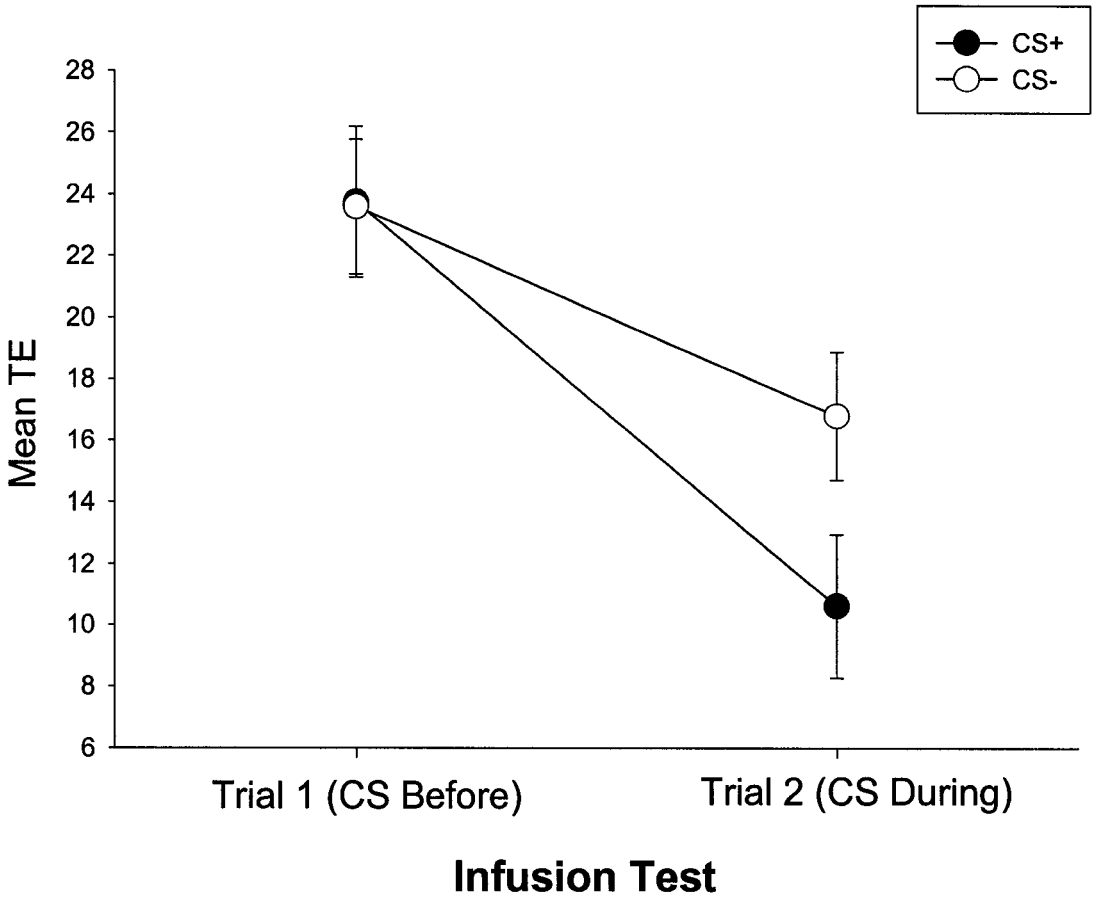

CS+
CS-
28
26
24
22
20
18
16
14
12
10
8
6
Mean TE
Trial 1 (CS Before)
Trial 2 (CS During)
Infusion Test

Figure 8

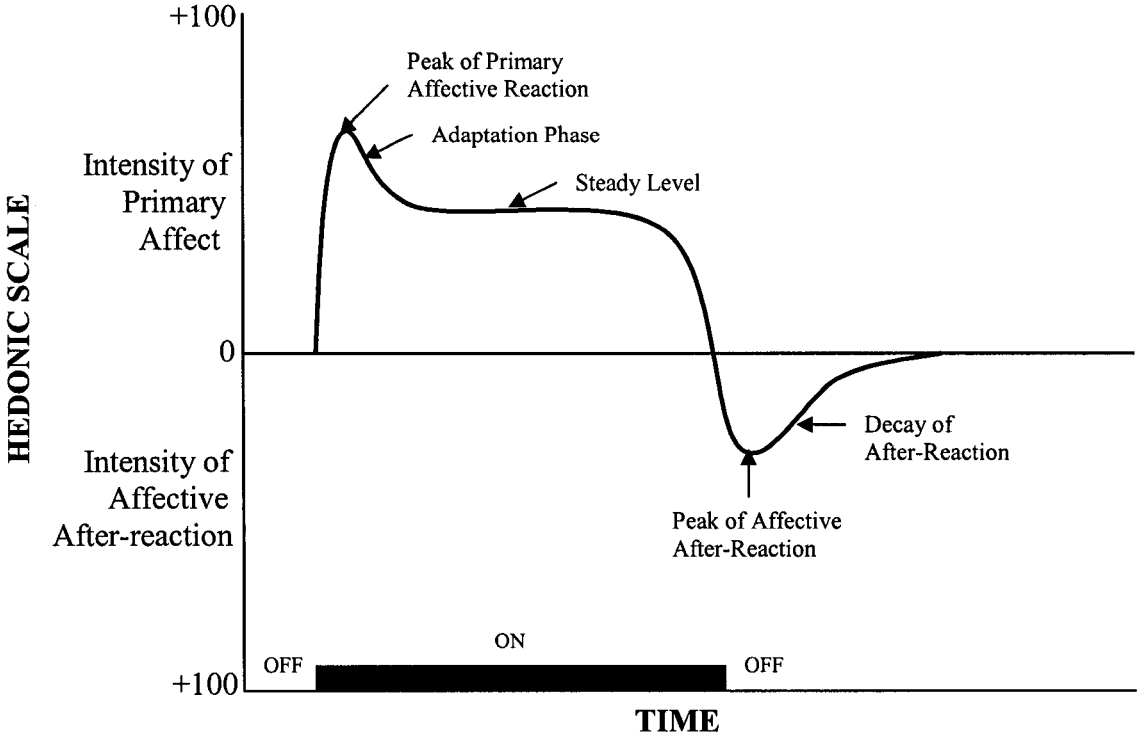

+100
Peak of Primary
Affective Reaction
Adaptation Phase
Intensity of
Primary
Affect
Steady Level
HEDONIC SCALE
0
Decay of
After-Reaction
Intensity of
Affective
After-reaction
Peak of Affective
After-Reaction
ON
OFF
OFF
+100
TIME

Figure 9

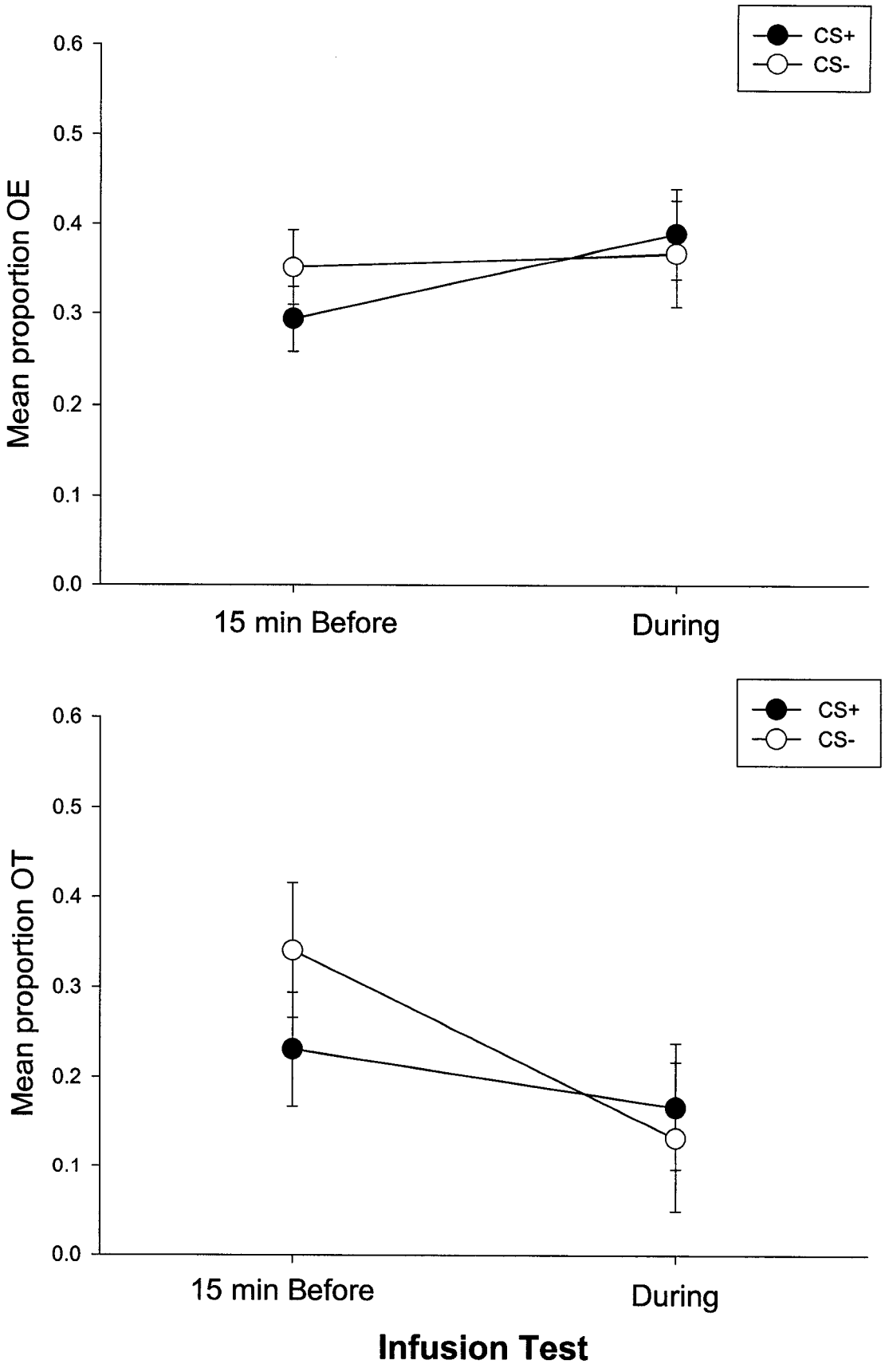
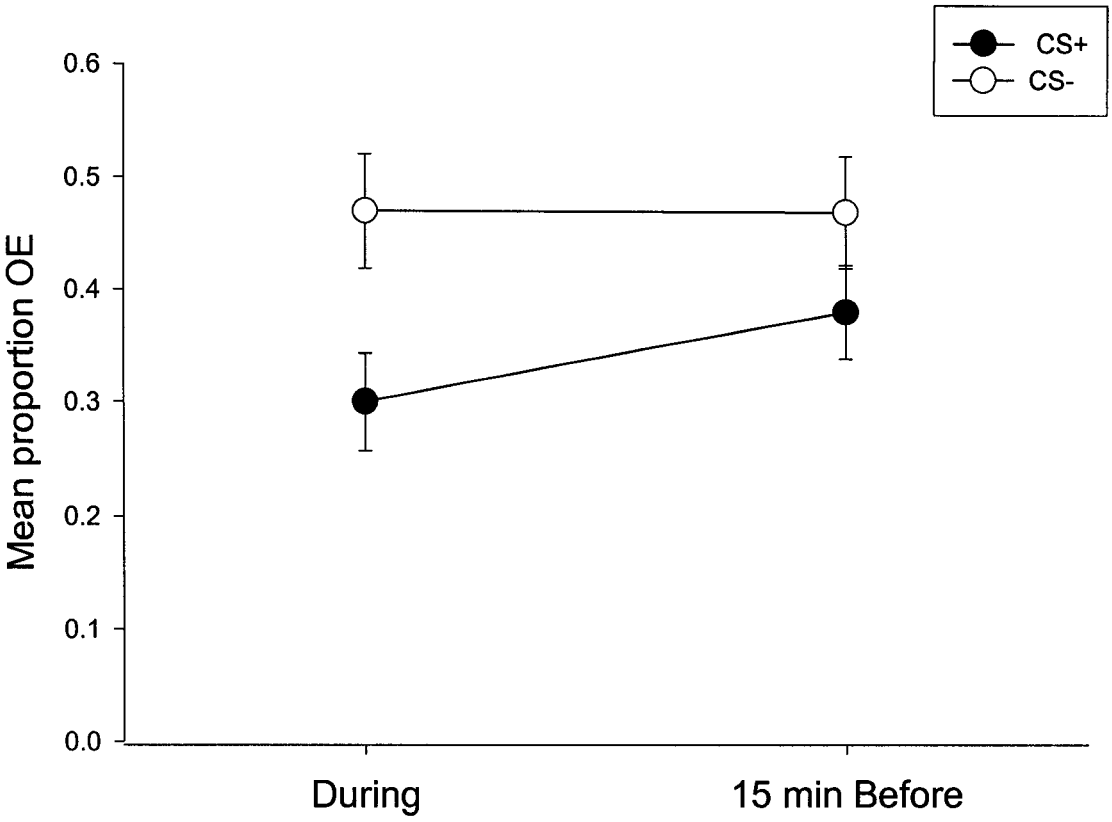

Water Tests
CS+
CS-
Mean proportion OE
0.6
0.5
0.4
0.3
0.2
0.1
0.0
15 min Before
During
CS+
CS-
Mean proportion OT
0.6
0.5
0.4
0.3
0.2
0.1
0.0
15 min Before
During
Infusion Test

Figure 10


CS+
CS-
Mean proportion OE
0.6
0.5
0.4
0.3
0.2
0.1
0.0
During
15 min Before

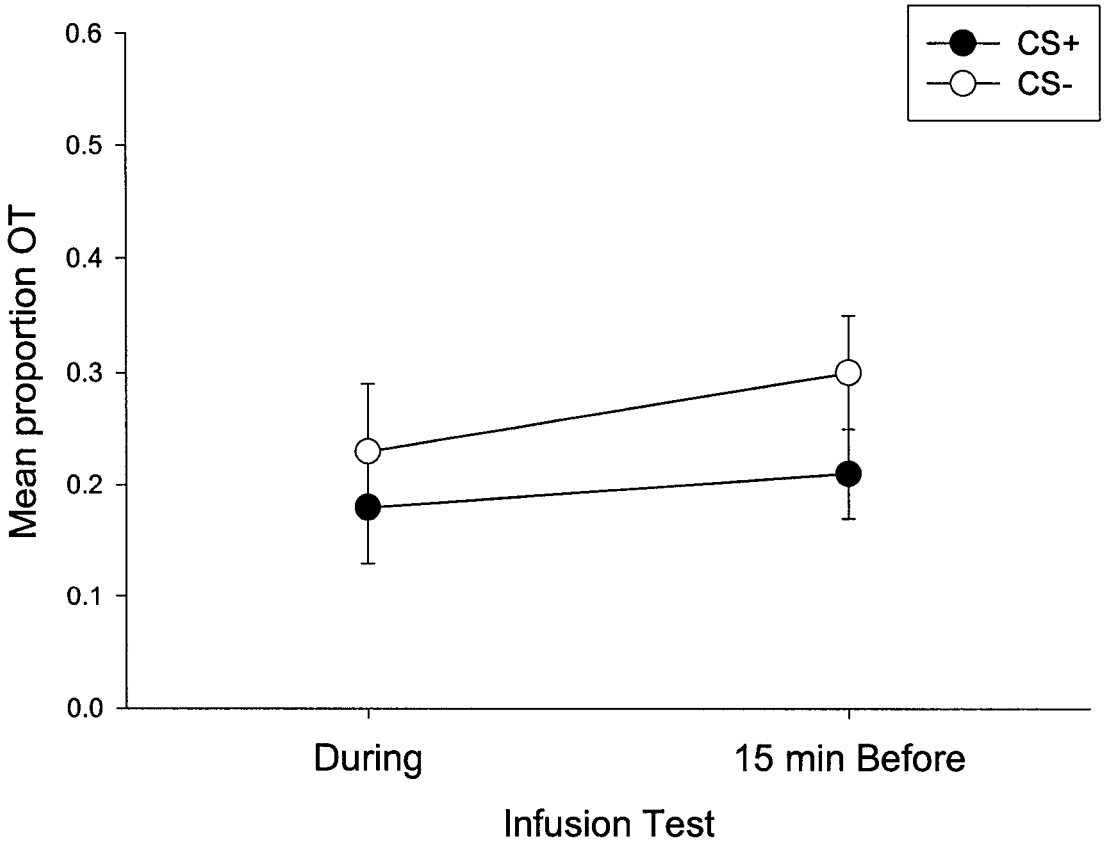

CS+
CS-
Mean proportion OT
0.6
0.5
0.4
0.3
0.2
0.1
0.0
During
15 min Before
Infusion Test

Figure 11

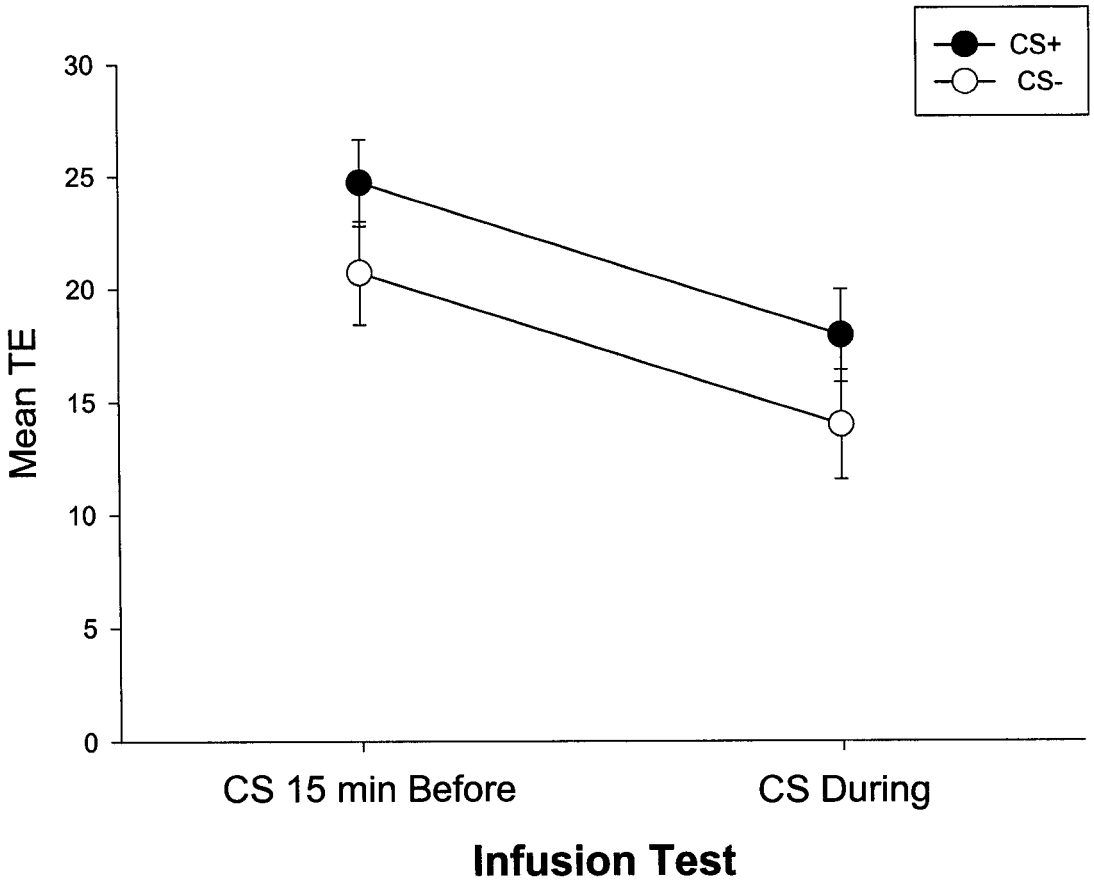

CS+
CS-
30
25
20
15
10
5
0
Mean TE
CS 15 min Before
CS During
Infusion Test

Figure 12

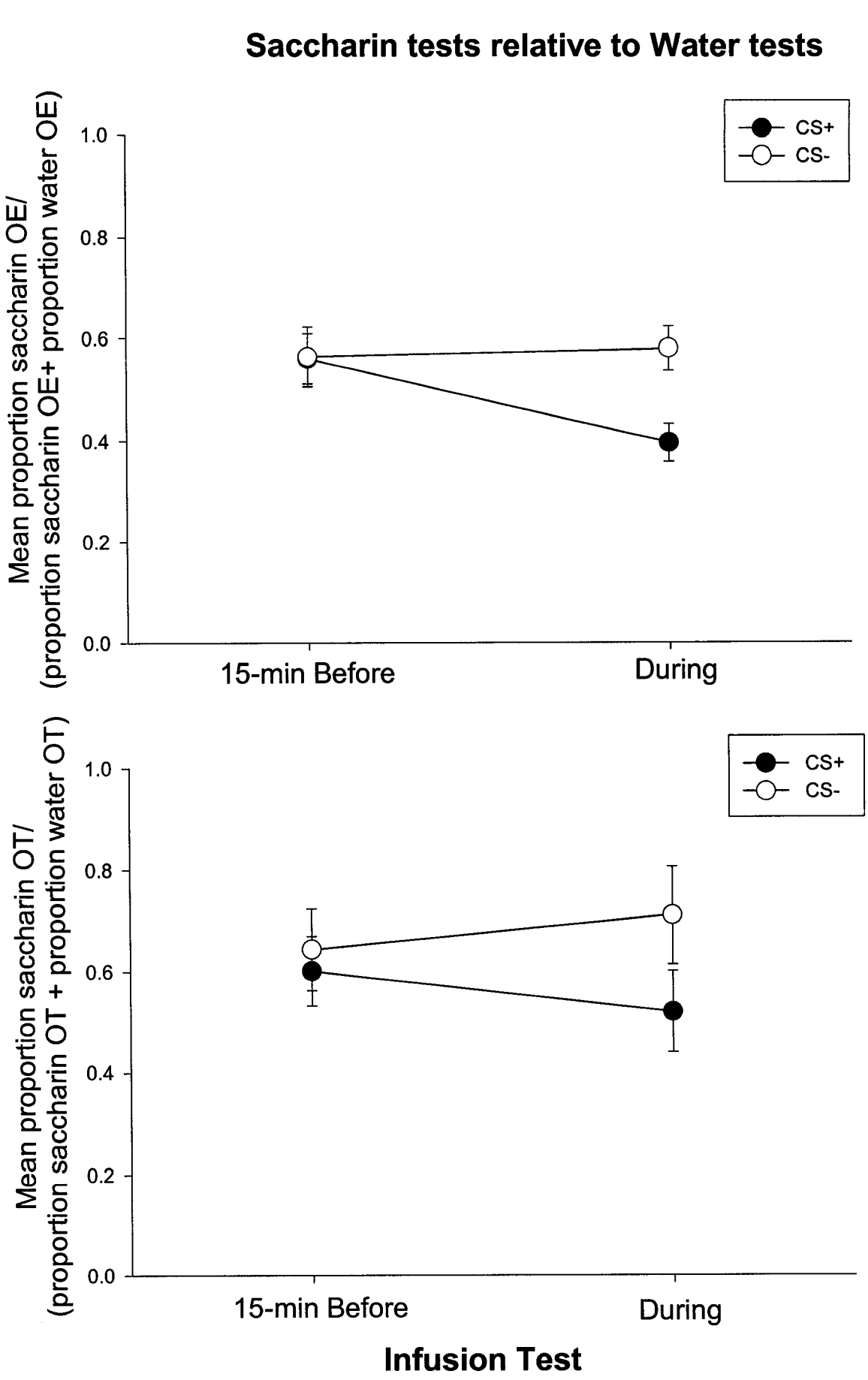

Saccharin tests relative to Water tests
CS+
CS-
Mean proportion saccharin OE/
(proportion saccharin OE+ proportion water OE)
1.0
0.8
0.6
0.4
0.2
0.0
15-min Before
During
CS+
CS-
Mean proportion saccharin OT/
(proportion saccharin OT + proportion water OT)
1.0
0.8
0.6
0.4
0.2
0.0
15-min Before
During
Infusion Test

Figure 13

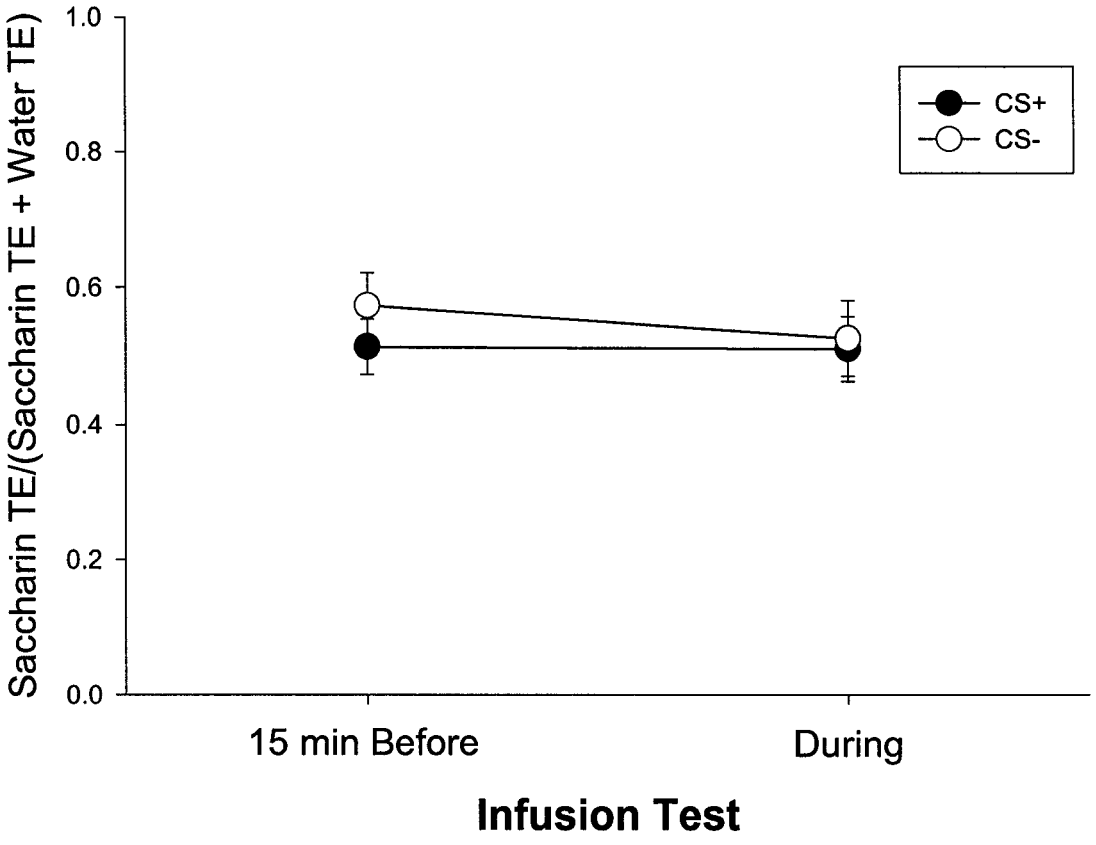

Saccharin TE/(Saccharin TE + Water TE)
1.0
0.8
0.6
0.4
0.2
0.0
CS+
CS-
15 min Before
During
Infusion Test

Figure 14