CONTEXTUAL CONTROL OVER FLAVOUR AVOIDANCE AND FLAVOUR AVERSION BY VISUAL CUES

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Contextual Control Over Flavour Avoidance and Flavour Aversion by Visual Cues

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Abstract

Eight rats were trained to consume (or withhold consumption of) a saccharine flavoured solution in a discrimination task. On Safe days, water deprived rats were placed in one context (either white or black box) for 20 min. During the first 10 min fluid was absent. During the second 10 min rats were given access to a saccharine solution through a hole in either the long or short wall of the test box. Immediately following this trial, rats were injected with saline and placed back into their home cage. Danger days consisted of the same rat being placed in the opposite colour context with the spout placed through the hole that was not used on the Safe day. Rats were injected with LiCl after the 20 min Danger trial. The location of the saccharine was fixed on Safe and Danger days. Both amount of saccharine, and taste reactivity responses were measured in parallel for each trial. Rats drank less saccharine on Danger days relative to Safe days and these changes in fluid consumption were correlated with aversive and appetitive behavioural changes. The aversive and appetitive behavioural changes occurred in anticipation of fluid delivery. Hole-poking behaviour revealed that animals anticipate fluid delivery on Safe days, but do not show anticipatory hole-poking on Danger days. A retention test 25 days later revealed that rats remembered the discrimination, with levels of fluid consumption and behavioural measures remaining intact. These findings indicate that conditional control of fluid consumption observed during discrimination training mirrors aversive and appetitive responses. These findings suggest that environmental cues can gain control over anticipatory nausea and may prove helpful in the control of nausea in clinical settings.

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1. Introduction

Nausea is a common side effect of cytotoxic chemotherapy drug treatment used to treat many forms of cancer. Many patients report feeling nauseated during or after chemotherapy treatment, and some of these patients report feeling ill prior to subsequent treatments when they enter the clinic (Andrykowski & Redd, 1987). This feeling of sickness prior to receiving an agent that has produced illness in the past can be referred to as anticipatory nausea. In the case of receiving chemotherapy treatment, anticipatory nausea occurs when cues from the treatment clinic, such as sights, sounds and smells, come to elicit the feeling of nausea that was experienced to previous treatment.

Anticipatory nausea is considered a product of Pavlovian conditioning (Stockhorst, Steingrueber, Enck & Klosterhalfen, 2006). In the clinical paradigm mentioned above, the unconditioned stimulus (US) is the chemotherapy drug, and the unconditioned response (UR) is nausea and\or vomiting. The conditioned stimulus (CS) is the treatment clinic (including sights, sounds and smells). With repeated pairings of the clinic and drug, the conditioned response (CR) of nausea is seen when patients enter the clinic. It has been demonstrated that if nausea and/or vomiting remains unmanaged during chemotherapy treatment, anticipatory nausea will likely follow (Tomoyasu, Bovbjerg & Jacobsen, 1996), and that once developed, anticipatory nausea is very resistant to anti-emetic drug treatment (Morrow, Roscoe, Hynes & Rosenbluth, 1998).

Anticipatory nausea has been studied using animal models (Limebeer, Hall & Parker, 2006; Limebeer, Krohn, Cross-Mellor, Litt, Osenkopp & Parker, 2008; Parker, 2003; Parker & Limebeer, 2006; Rodriguez, Lopez, Symonds & Hall, 2000). These animal models often use conditioned flavour avoidance or aversion learning as part of the

experimental protocol for examining anticipatory nausea. In both cases, a rat is usually given a novel flavour solution to consume which is then paired with a toxin. In most cases, the rat will withhold consumption of this flavour solution on subsequent pairings, indicating that the flavour has become associated with the effects of the toxin. Conditioned flavour avoidance is simply the learned response to avoid the flavour solution. Conditioned flavour aversion is an actual learned aversion to a target flavour solution, and this learning is often promoted by invoking a state of emesis. Parker (2003) stated that not all instances of conditioned flavour avoidance are accompanied by an aversion to the flavour solution, and that for a conditioned aversion to develop there must be some form of gastrointestinal distress projected on the rat. Conditioned flavour avoidance and conditioned flavour aversion are therefore considered to be distinct (Parker, 2003).

1.1 Conditioned flavour avoidance

Conditioned flavour avoidance is almost always measured using a consumption test. An animal is allowed to freely consume a target flavour solution to gain a baseline measure of consumption. This solution is then paired with an aversive stimulus, in many cases lithium chloride (LiCl), which produces a state of nausea. During the consumption test, the amount of flavour solution consumed after the aversive pairings is measured; if it is reduced or absent, conditioned flavour avoidance is said to have occurred. Many authors have demonstrated that conditioned flavour avoidance can be achieved when a flavour solution is paired with an emetic agent such as LiCl (Best, Batson, Meachum, Brown and Ringer, 1985; Best, Brown and Sowell, 1982; Symonds, Hall, Lopez, Loy, Ramos and Rodriguez, 1998). However, conditioned flavour avoidance has been

observed when a flavour solution is paired with wheel running (Lett and Grant, 1996), rewarding drugs (Berger, 1972) and aversive footshock (Pelchat, Grill, Rozin and Jacobs, 1983) in addition to toxins.

1.2 Conditioned flavour aversion

It has been suggested that the consumption test may not be adequate for assessing conditioned nausea in rats (Parker, 2003). In fact, she proposed that this type of test adequately measures only the avoidance of rather than the aversion to a flavour solution. Aversive reactions to a flavour are typically measured by assessing orofacial rejection responses (Grill & Norgren, 1978). With conditioned flavour avoidance, there is both an appetitive (approach the drinking spout) and a consummatory (consume the solution) response needed, whereas conditioned flavour aversion can develop without the appetitive response (flavour solution can be delivered via intra-oral cannula). Furthermore, conditioned flavour avoidance can be produced without the use of emetic agents (Berger, 1972; Lett and Grant, 1996; Parker, 1995; Pelchat, et al., 1983). Parker (1995) found that rewarding drugs could be used to create conditioned flavour avoidance, and that this conditioned avoidance is not accompanied by orofacial or somatic rejection reactions, suggesting that these drugs do not produce a conditioned flavour aversion. Also in line with this research, it has been found that conditioned flavour avoidance produced by rewarding drugs is not attenuated by anti-nausea treatments (Limebeer & Parker, 2000; Parker & Macleod, 1991), indicating that nausea may not be the underlying factor in promoting conditioned flavour avoidance.

In addition to using a measure of fluid consumption, conditioned flavour aversion is typically measured using the taste reactivity test (Grill & Norgren, 1978). With this

method, an animal's orofacial and somatic responses are recorded, usually to a flavour solution that has been previously paired with an emetic agent. The flavour solution is usually infused via an implanted intra-oral cannula, and orofacial and somatic indicators of either palatability or disgust are measured. Indicators of palatability include tongue protrusions (both lateral and rhythmic) and paw licking. In contrast, indicators of disgust include gaping, chin rubbing, headshaking, paw wiping and flailing of the forelimbs (Berridge, 2000; Grill & Norgren, 1978).

Parker (2003) suggested that the taste reactivity test may be a better measure of conditioned flavour aversion and nausea in the rat than the consumption test. Rats display conditioned rejection reactions (gaping, chin-rubbing, etc) to an otherwise palatable flavour solution after it has been paired with an emetic agent such as LiCl (Parker and Limebeer, 2006). LiCl has been shown to produce vomiting in species that can vomit, and it produces a gaping reaction in rats, a species that cannot vomit (Parker, 1998; Parker, 1991). Gaping can be described as rhythmic, large amplitude openings of the rat mandible, with the corners of the mouth drawn back. The rat gape essentially mimics the action of the vomiting response in animals that can vomit, and this reaction appears to accurately reflect conditioned nausea in this species (Parker and Limebeer, 2006). Furthermore, anti-emetic agents, such as ondansetron, lessen or eliminate conditioned rejection reactions, whereas these agents have no effect on conditioned flavour avoidance (Limebeer & Parker, 2000). Nausea seems to mediate the development of conditioned rejection reactions (which are usually evoked by an administered emetic agent) (Parker, 2003). In the case of conditioned flavour avoidance, nausea (whether induced by emetic drugs or in some other way) is not solely necessary,

but rather what is required is a change in physiological state (e.g., vestibular stimulation, wheel running, etc) that will cause the rat to avoid a palatable flavour solution. This conditioned avoidance due to a change in physiological state is a defence mechanism for the rat, as they cannot vomit (Davis, Harding, Leslie & Andrews, 1986).

1.3 Flavour Avoidance and Aversion Learning in a Distinct Context

It has been demonstrated that a context with aversive properties can suppress flavour solution consumption. This occurs when a reduction in consumption of an otherwise palatable solution is observed in a context that was paired with an emetic agent (Best, et al., 1982; Best, et al., 1985; Symonds, et al., 1998). Symonds, et al. (1998) provided rats with two distinct contexts, and all rats received LiCl paired with one of the contexts. Furthermore, half of these animals received water in the lithium-paired context, and half received nothing. During a test phase, all animals received access to a sucrose solution in both of the contexts at two different times. They found that animals that received water in the lithium-paired context had consumed less of the sucrose solution in that same context during the test phase, thus revealing conditioned context-flavour avoidance. The animals that did not receive water in the lithium-paired context did not have reduced levels of consumption. A blocking procedure was then employed in order to test the associative strength of the context as a conditioned stimulus (see also Symonds and Hall, 1997 for the original procedure). With this procedure, rats were trained in two phases; first, rats consumed water in a target context which was followed by an injection of LiCl. Second, rats were allowed to consume a novel flavour in their home cage before being placed in the target context, which was then followed by an injection of LiCl. In a test phase, it was noted that only a weak aversion to the flavour existed when it was

presented in the home cage. This was because of a context-illness association that formed in the first phase of training that served to block the aversion to the novel flavour. Using this blocking procedure, it was noted that the context alone had conditioned aversive properties, regardless of whether or not fluid was presented. Essentially, if a context is paired with LiCl, a context-illness association will likely form.

Rodriguez, et al. (2000) proposed that the suppressed consumption of a distinct palatable flavour solution while in a target context could be used as a model of anticipatory nausea. These researchers injected LiCl prior to the pairing of a target context with a sucrose flavour. A separate context was paired with saline. Reduced consumption of the sucrose flavour was evident in the lithium-paired context as compared to sucrose consumption in the saline-paired context during a consumption test. Also, by pairing a context with LiCl prior to flavour-LiCl pairings, the authors showed that the context cues could serve to block the acquisition of a flavour aversion to LiCl. From this, they concluded that context cues alone can acquire the power to elicit a conditioned nausea response, which can further be attributed to anticipatory nausea.

Symonds and Hall (2002) conducted a series of experiments that both extended and confirmed the findings of Rodriguez, et al. (2000) that contextual cues can come to elicit conditioned nausea. Although not a new finding, they first reiterated that consumption of a novel flavour solution could be reduced in response to an injection of LiCl. Second, the findings of Rodriguez, et al. (2000) were replicated, and further, the authors showed that the post-injection response to LiCl could be enhanced if it was measured in the same context where the lithium presentations/exposures took place. In conclusion, the results of the studies by Rodriguez, et al. (2000) and Symonds and Hall (2002) on conditioned

flavour avoidance point to two facts; first, that suppressed consumption of a novel solution while in a lithium-paired context possibly reflects conditioned nausea, and second, that following a number of pairings of the context with LiCl, the context itself could acquire the ability to elicit conditioned nausea in the absence of a flavour solution.

Although the issue of how fluid consumption actually maps onto measuring a state of conditioned nausea to a context is somewhat contentious, two things are clear – first, an injection of LiCl induces a state of nausea in rats (as evidenced by conditioned rejection reactions), and second, that rats decrease consumption of a palatable flavour solution when it is paired with LiCl in a target context more so than in a neutral context. Another way to demonstrate that the context has become associated with nausea is to measure the orofacial and somatic responses that are associated with the context using the taste reactivity test developed by Grill and Norgren (1978). Breslin, Spector and Grill (1992) intraorally infused rats with a sucrose flavour then later paired this infusion with LiCl. They demonstrated that as pairings with the toxin increased appetitive-type reactions decreased and aversive-type reactions increased, indicating that the sucrose flavour became less palatable when it was associated with illness.

Furthermore, when a rat is poisoned with LiCl in a target context that is paired with a flavour solution, the rat displays increased aversive-type behaviours to both the flavour solution that was presented in that context, and to the context itself (Limebeer, et al., 2006; Limebeer, et al., 2008). Limebeer et al. (2006) reported that rats gape when placed in a context that was previously associated with LiCl. In this study, the authors paired a target context with LiCl for four conditioning trials then infused the animal with saccharine solution via an implanted intraoral cannula on a test trial. A separate control

group received the context alone without an injection of LiCl. They found that rats that were conditioned in the lithium-paired context gaped more than the rats in the unpaired context when infused with saccharine. They also found that the gaping occurred at inter-infusion intervals, while the rat was still in the context, suggesting that the rats were gaping to the context alone when no fluid was present. This measure of gaping to the context alone could serve as a directly identifiable indicator of anticipatory nausea in the rat.

To further investigate conditioned gaping to a lithium-paired context, Limebeer, et al. (2008) gave rats an injection of LiCl prior to placement in an odour-permeated context, or the context alone, both in the absence of any flavour solution. They found that rats trained in a LiCl-paired context that was permeated with a distinct vanilla odour gaped when presented with the odour and context in the absence of LiCl. In the same study, the authors also paired the context alone with an injection of LiCl prior to being placed in the context. They discovered that the context alone could serve as the conditioned stimulus, evoking a gaping reaction when the rat was placed in the lithium-paired context in the absence of a LiCl injection. As mentioned earlier, this gaping reaction is solely produced by treatments that induce a state of nausea (Parker, 1995; Parker, 2003), and conditioned gaping can be prevented by administering anti-emetic agents beforehand (Limebeer and Parker, 2000; Limebeer and Parker, 2003).

1.4 Conditional control of fluid consumption

The above findings suggest that rats can associate a target context with nausea, and that these contexts can come to elicit conditioned rejection reactions. Conditional control of fluid consumption has also been demonstrated, and with this, one can further

investigate conditioned nausea (Jaegar and Mucha, 1990; Lopez and Cantora, 2003; Martin, Gans and van der Kooy, 1990; Mastropaolo, Moskowitz, Dacanay and Riley, 1989; Murphy and Skinner, 2005; Skinner, Martin, Pridger and van der Kooy, 1994). For example, conditional control of fluid consumption was initially demonstrated using drugs as the conditional cue, including phencyclidine (Mastropaolo, et al., 1989), morphine (Martin, et al., 1990) and pentobarbital or fentanyl (Jaeger & Mucha, 1990). With each of these drug experiments, it was shown that rats could be trained to discriminate when to consume a palatable solution based on the rat's assessment of the prior drug state. In sum, injections of the drug were paired with a flavour-illness contingency, such that the drug state could come to predict illness, and the rat would learn to withhold consumption of the flavour solution based on the drug state. Furthermore, injections of a vehicle only that did not predict illness enabled the animal to consume the flavour solution on subsequent trials. Also, in each of these studies it was shown that the drug and the vehicle could be reversed, so that when the vehicle injection predicted illness, the animal withheld consumption of the solution, whereas the drug injection enabled the animal to consume the flavour solution.

Subsequently, conditional control of fluid consumption was also demonstrated using context as the conditional cue (Lopez and Cantora, 2003; Murphy and Skinner, 2005; Rodriguez, et al., 2000; Skinner, et al., 1994; Symonds and Hall, 2002; Symonds, et al., 1998). Skinner et al. (1994) demonstrated that animals can learn to discriminate when to drink a saccharine flavour in two distinct contexts. Rats received pairings of saccharine and LiCl in one context, while saccharine was paired with saline in another context. Contextual control over fluid consumption was seen in the LiCl-paired context,

with markedly reduced fluid consumption in this context. Murphy and Skinner (2005), as well as others (Lopez and Cantora, 2003; Rodriguez, et al., 2000; Symonds and Hall, 2002; Symonds, et al., 1998), demonstrated this same contextual control of fluid consumption, which essentially is a type of discrimination learning.

There are differing explanations for how contexts control fluid consumption. Lopez and Cantora (2003) argue that the learned discrimination can be explained using Pavlovian conditioning terms, in that the context enters into an association with the unconditioned stimulus. The authors conclude that the

> "differential fluid consumption observed after discrimination learning can be explained in terms of summation between the Pavlovian properties of the fluid and those of the context in which it (*the fluid*) is ingested and (*the rat*) poisoned" (pp. 384; note that words in brackets were added by the present author for clarity).

Essentially, it is hypothesized that contextual control of fluid consumption is mediated by a simple association between the context and lithium (Lopez and Cantora, 2003; Rodriguez, et al., 2000; Symonds and Hall, 2002). In contrast, Skinner, et al. (1994) and later, Murphy and Skinner (2005), argued that the learned discrimination is explained in terms of occasion setting. Occasion setting is a learning phenomenon whereby the conditional cue tells an animal when to respond to an explicit conditioned stimulus. In the case of the previous studies, the context (or drug) would become the feature (occasion setter), telling the animal whether or not to consume the accompanying flavour solution in that context. Furthermore, Skinner et al. (1994) suggested that the context cues come to modulate the drinking response, rather than the physical or hedonic properties of the fluid. Regardless of the mechanism involved (Pavlovian conditioning or occasion setting) the context can evoke conditional control of fluid consumption. Conditional

control of the drinking response could be tested using an extinction procedure. Rats that are exposed to the context in the absence of drinking should not show extinction of the conditional control of the saccharine solution relative to those that are allowed to consume tap water during extinction.

The problems with the above studies on conditional control are twofold; first, the target contexts often differed on multiple combined dimensions, such as visual cues, odour, and texture. If the context did gain conditional control over fluid consumption, it is not possible to draw firm conclusions as to what aspect of the context actually served as the feature (e.g., odour, visual characteristics, or texture). Second, all of the studies on conditional control thus far measured conditioned flavour avoidance, in that a simple consumption test was used to assess the supposed dislike of the flavour solution. In order to assess the hedonic properties of the solution (or the aversion to it), orofacial and somatic behaviours should be measured to better assess nausea (Parker, 2003).

1.5 The current experiment

A context discrimination procedure was used to answer several questions. First, could conditional control over fluid consumption be obtained when only visual cues are used? Other studies that have focused on contextual control of fluid consumption have used multi-dimensional differences between the two contexts, such as colour, smell, and texture of the floor (Lopez and Cantora, 2003; Murphy and Skinner, 2005; Skinner et al., 1994). The current experiment used visual cues only to distinguish the Safe and Danger contexts, in that the contexts differed in the colour (white or black) that they were painted, and the hole in which the fluid (drinking tube) was presented. These training boxes were always located in the same place relative to the outside environment. It was

hypothesized that these two visual changes would be enough to exert conditional control over fluid consumption.

Second, it was investigated whether changes in fluid consumption were also reflected in the scored behavioural responses. Using elements of the taste reactivity test (Berridge, 2000; Grill and Norgren, 1978; Limebeer et al., 2006; Limebeer et al., 2008), both appetitive and aversive-type behaviours were scored for every trial of the experiment. If the context does come to exert conditional control over fluid consumption and the rat learns the discrimination, it was hypothesized that animals should display an increase in aversive-type behaviours (gaping, chin rubbing, paw wiping, headshaking and forelimb flails) on Danger days relative to Safe days. Correspondingly, appetitive-type behaviours (paw licking, tongue protrusions and grooming) should be suppressed on Danger days and more evident on Safe days. These behaviours were scored while the rat was allowed to freely consume a saccharine flavour, which is unlike the typical intraoral infusion of a flavour. The behavioural responses should enable us to draw conclusions with regards to conditioned nausea in the rat.

Third, it was investigated whether the context alone could come to elicit the appetitive or aversive behaviours associated with Safe and Danger days. During the first 10 min of each trial, the rat received context cues in the absence of fluid. During this time, both appetitive and aversive orofacial and somatic behaviours were scored. It has been demonstrated that rats show aversive gaping reactions to contexts that were previously paired with LiCl (Limebeer, et al., 2006; Limebeer, et al., 2008). To extend the finding of Limebeer, et al. (2008), other somatic and orofacial responses besides aversive gaping were scored, and a discrimination task was used by exposing rats in a

separate context that was not paired with LiCl. This was done to help achieve a complete picture of the taste reactivity responses to a presentation of the context alone, as well as to a separate context in which the rat was not poisoned. Another procedural difference to that of Limebeer, et al. (2008) was that animals were injected with LiCl after they had completed a trial (and prior to being placed back into their home cage), thus with this forward conditioning procedure, the rats in the present study were never actually sick while in the context. It was hypothesized that if rats learn to discriminate between the Safe and the Danger contexts, then the animals would show increased aversive-type behaviours and suppressed appetitive-type behaviours when in the Danger context during the first 10 min when fluid is not present. The opposite should hold true while the animal is placed in the Safe context. If rats gape and display more aversive-type behaviours to the context alone on Danger days than on Safe days, this can be interpreted as a display of anticipatory nausea because no fluid is present.

Fourth, the relationship between changes in fluid consumption and the behavioural measures that were taken was investigated. Essentially, there should be a significant negative relationship between the aversive measures and fluid consumption on Danger days. Likewise, on Danger days, appetitive behaviours should be positively related to fluid consumption, as they should both decrease. On Safe days, aversive behaviours should also be negatively related to consumption; as fluid consumption increases, aversive behaviours should decrease. In turn, appetitive behaviours should be positively related to consumption on Safe days, as they should both increase. These results should be consistent with a factor analysis performed by Parker (1995), where appetitive and aversive reactions were correlated with fluid intake.

Finally, whether the learned discrimination could be retained over an extended retention period was examined. Fluid consumption, as well as the taste reactivity responses on a single Safe-Danger-Safe cycle that began 25 days after the last trial of the experiment, was measured. It was hypothesized that rats would maintain the discrimination over the 25 day retention interval. It has been demonstrated that rats can maintain a learned avoidance to a flavour solution over an extended period (Biederman, Milgram, Heighington, Stockman and O'Neill, 1974; Dragoin, Hughes, Devine and Bentley, 1973) and a discrimination can be maintained over time when a drug acts as the conditional cue (Martin, et al., 1990). To my knowledge, maintenance of the taste reactivity responses has not been investigated. However, if the learned discrimination and the fluid consumption measure remains intact, then it is hypothesized that the taste reactivity measures should remain as well.

To answer these questions, two distinct contexts, differing only in colour and location of the hole in which the drinking tube is inserted to distinguish between Safe and Danger days, were used. On Safe days, water deprived rats were placed in one context (e.g., white plus drinking tube on long wall) for a total of 20 min. During the first 10 min of this trial, rats received only the context cues, while during the second 10 min they received this same context plus a saccharine flavour that was added through the appropriate spout hole. Immediately following this trial, rats were injected with saline and placed back into their home cage. On Danger days, the same rats received the same protocol in the opposite context (e.g., black plus drinking tube on short wall) to that received during Safe days however this was followed by an injection of LiCl before being placed back into their home cage. This is a standard forward conditioning procedure

known to reliably produce conditioned flavour avoidance. There were 49 trials in total, with 10 Danger days interspersed amongst 39 Safe days. Bottle weights were used to determine the amount of saccharine consumption. All trials were videotaped, and orofacial and somatic behaviours, both appetitive and aversive in nature, were scored for each 20-min trial. On the 25th day after the last trial, a retention test consisting of a single Safe-Danger-Safe cycle was completed.

2. Method

2.1 Subjects

Eight male Long-Evans rats, weighing between 330 g and 430 g, were used in this experiment. The rats had been used previously in an unrelated water maze experiment. The animals were housed individually in the colony room at Memorial University of Newfoundland. This room was held at a constant temperature of 20 ± 2 °C, as well as functioning on a 12 hr light/dark cycle, with lights on at 0700 hrs and off at 1900 hrs.

Rats were tested in two squads of four rats per squad. Experimentation with each squad took place on two separate occasions; that is, once squad one had finished all trials, squad two was then started.

2.2 Apparatus

The rats were housed in cages made of clear plastic. These cages measured 45 X 25 X 21 cm and were covered by a lid of metal bars. The two test boxes for this experiment were two rectangular wooden drinking chambers, each with a recessed wooden lid and containing no floor. The boxes and lids were constructed from 1.91 cm thick plywood, and the inner dimensions of each chamber measured 25.40 X 15.24 X 38.10 cm. The lid was recessed into the wooden chamber so that it measured 20.32 cm

from the bottom of the chamber walls (see Figure 1), and the lid was fitted with a handle so that it could be removed or added as necessary. One chamber and lid combination was painted black, and the other chamber and lid was painted white.

Two holes to allow the entry of a curved drinking tube (Model 5.5F, Girton Mfg. Co., Millville, PA) attached to a small drinking bottle (Model 8-38, Girton Mfg. Co., Millville, PA) were drilled in each chamber. One hole was drilled in the long side of the chamber approximately 7.62 cm from one side, and 7.62 cm from the bottom. The other hole was drilled in the middle of the short side opposite to that of the first hole, so that it was 7.62 cm from the bottom. (see Figure 2). The bottles were held in place by an elastic band that was stretched over two small brass hooks on either side of the drinking hole.

The test boxes were placed on top of a glass table to allow the animals to be videotaped from underneath. The table stood approximately 73.70 cm from the floor, and the glass top was square in shape (85.10 X 85.10 cm). Furthermore, this table and the corresponding drinking chambers were always positioned in the same place within the room. There were two desk-type, gooseneck lamps placed underneath the table that were pointed upwards to illuminate the underside of the test boxes. One lamp contained a frosted 60 W bulb, while the other contained a small, clear 40 W bulb. This was necessary to provide sufficient lighting for videotaping the animal.

During training, rats were given an intraperitoneal (ip) injection of either saline (0.9% NaCl; 3.0 ml/kg) or lithium chloride (LiCl) (0.47 M; 3.0 ml/kg), depending on the experimental protocol for that given trial. A 0.1% saccharine solution (1 g/1000 ml of water) stored at room temperature, was the novel flavour that the rats could consume during the conditioning phase

2.3 Procedure

Rats were maintained on ad lib food and water until the start of the deprivation regimen. Starting one week prior to the start of experimentation, and throughout the entirety of the experiment, rats were allowed to consume water during one 15 min drinking session per day for water repletion purposes. On experimental days, this drinking session occurred in the colony room immediately after all rats had finished the conditioning trial for the day.

Animals were transported daily from the colony room to the experimental room on the same rack that they were housed. This rack was then situated just outside of the experimental room. Rats remained on the rack in their home cage until their conditioning trial was about to begin that day. A single rat was carried in its home cage into the experimental room. The rat was weighed before being placed into the test box, which marked the start of a conditioning trial.

The conditioning trial was 20 min long. During the first 10 min of the trial, no fluid was present (i.e. no spout protruded from either hole). During the second 10 min, saccharine flavoured water was made available to the rat through a spout protruding through one of the holes. There were two different test boxes, one white and one black. The experiment was divided into Safe Days and Danger Days. A Safe Day consisted of a rat being placed in one context (for example, the white test box) for a 20 min trial, and immediately following this trial, removed from the test box and given an ip injection of saline. A Danger Day consisted of the same animal being placed in the opposite context to that of the Safe Day (to be consistent with the previous example, this would be the black test box), and immediately following this trial, removed and given an ip injection of

LiCl. Half of the animals (n=4) experienced the white test box on Safe Days, and half (n=4) received the black test box on Safe Days. The hole from which the spout of the drinking bottle entered the drinking chamber also differed on Danger Days from that of Safe Days, as half of the rats (n=4) received the saccharine solution through the hole on the long wall on Safe Days, while the other half (n=4) received the saccharine solution through the hole on the short wall on Safe Days. The opposite hole was used on Danger Days. There were two rats in each combination of box colour and drinking bottle entrance hole. Immediately following a trial, the rat was placed back into its home cage and returned to the metal holding rack located outside of the experimental room. The drinking bottles containing the saccharine solution were weighed before and after each trial so the amount of consumption could be calculated.

For this part of the experiment, the rats received a total of 49 trials. This consisted of 39 Safe Days interspersed with 10 Danger Days (See Table 1).

2.3.1 Procedure – Retention Test

The purpose of this probe was to test the rats' memory of the experimental procedures. On the 25th day after the cessation of the experiment, a cycle of three trials was conducted for each squad. This cycle consisted of a Safe Day, followed by a Danger Day, and ended with another Safe Day. These trials were administered in exactly the same fashion as the trials of the main experiment. The drinking chambers were held constant for all rats so that they matched with those experienced during the main experiment. As before, bottle weights were taken before and after to assess consumption rates. For the time between the end of the main experiment and the start of retention test,

the rats were housed in the colony room and were maintained on ad libitum food and a single 15 min daily watering session.

2.3.2 Scoring of Videotaped Behaviour

While in the test box, the rat's behaviour throughout the entire experiment and retention test was recorded with a Canon high definition (HD) video camcorder (Model HV-10) that was mounted on a small Canon tripod on the floor underneath the glass table. The camcorder was situated with the lens pointed upward to record the rats' behaviour in the test boxes. The video tapes used were Maxell MiniDV cassettes (Model DVM60SE). The recorded HD video was transferred to an Apple iMac computer (Model iMac 6.1) using a Firewire connection with iMovie HD software (Apple Inc, Version 6.0.3). The raw HD video was both compressed and de-interlaced using MPEG Streamclip software (Squared 5, Version 1.8) to have a final encoded video in H.264 format.

This video was later scored by the investigator for both aversive and appetitive rat behaviours, as well as other measures (see below) during the entire 20 min time period of test box filming. The video was played using Apple QuickTime Player software (Apple Inc., Version 7.4.5), while a keystroke program that functioned along with QuickTime was loaded at the same time. The keystroke program (written by Avery Earle, 2007) was able to accurately pair a keystroke from a computer keyboard to a timestamp from the QuickTime Player. Different behaviours were assigned different keys, thus a behaviour that was coded as happening at a certain time could be verified at a later date by going back to the corresponding timestamp in the video.

The behaviours of interest for this experiment were derived from the taste reactivity test developed by Grill and Norgren (1978), as well as from categorizations by Berridge (2000). Appetitive behaviours that were coded included paw licking (see Figure 3), and tongue protrusions (see Figure 4). Aversive behaviours that were coded included gaping (see Figure 5) and chin rubbing (see Figure 6). The aversive measures of headshaking, and flailing of the forelimbs were also scored, but not observed to occur very often. This observation is consistent with Parker (1995). In addition to these behaviours, fluid consumption, drinking (both in number of bouts and duration; see Figure 7), grooming (both in number of bouts and duration; see Figure 8), face-washing (see Figure 9), and hole-pokes (poking of the left and right drinking holes; see Figure 10) were also coded. Although face washing is part of the normal grooming regimen, it was coded separately in this study. Berridge (2000) stated that a face-wash could be considered an aversive event, however, more frequently it was seen to occur in conjunction with the normal grooming regimen in the present study. This finding is also consistent with that from Parker (1995).

An independent observer also scored some of the behaviours from the video tapes in order to test inter-rater reliability. A single day was chosen from the collection of videos in which a particular behaviour was known to have occurred. The independent observer then scored all eight rats for that given day for the particular measure in question. This process (pairing a particular day with a measure) was repeated until all of the behaviours were scored. The independent observer was blind to whether they were scoring behaviours occurring on a Safe or Danger day. All of the measures from the independent observer correlated significantly with the measures obtained by the

experimenter. These correlations are presented in Table 2. An analysis of these behaviours, as well as an analysis of drinking consumption follows.

2.4 Data Analysis

A 2-factor, 10 X 3 Cycle (Safe-Danger-Safe) X Day (Safe Day, Danger Day, Safe Day), repeated measures ANOVA was carried out on the measures of fluid consumption, drinking duration and drinking bouts. A 3-factor, 10X 3 X 2 Cycle X Day X Order (first 10 min vs. second 10 min), repeated measures ANOVA was carried out on all behavioural measures including the aversive measures of gaping, chin rubbing, head shaking, and forelimb flailing, the appetitive measures of paw licking and tongue protrusions, as well as face-washing and grooming (both in bouts and duration). A 3-factor, 10 X 3 X 2 Cycle X Day X Type (safe drinking hole vs. danger drinking hole) repeated measures ANOVA was carried out on the measure of hole-poking that occurred during the first 10 min of a trial. For this particular analysis, the safe drinking hole is the hole from which they receive the fluid on Safe days, while danger drinking hole is the hole from which they receive the fluid on Danger days.

The above analyses were carried out on the 10 Safe-Danger-Safe cycles, rather than over all 49 days of the experiment. A variable number of Safe days were interspersed between each Safe-Danger-Safe cycle. These additional Safe days were needed to get the rat's fluid consumption back to a suitable level after receiving a Danger day. This was necessary during the early part of the experiment before the discrimination was learned, as two or more Danger days in close proximity would likely have led to complete flavour avoidance, thus masking experimental effects.

All behaviours were analyzed during the retention test 25 days later using a 2-factor, 3 X 2 Day (Safe day, Danger day, Safe day) X Order (first 10 min vs. second 10 min) repeated measures ANOVA. Hole pokes were analyzed with a 2-factor, 3 X 2 Day X Type (safe drinking hole vs. danger drinking hole) repeated measures ANOVA.

All analyses were followed by planned comparisons where the rat's behaviour on the Danger day was compared to the behaviour on the following Safe day for the last four cycles of the experiment. Planned comparisons were also carried out on the Danger day and following Safe day for the retention test as well. This test provided a strong test of the learned discrimination. The comparisons were done between these two days because any differences in behaviour that occurred due to the learned discrimination were not confounded by the extinction trials that lead up to a Safe day that preceded a Danger day. Also, as evident by the measure of fluid consumption, and consistent with previous research (Murphy and Skinner, 2005), the discrimination began to appear at about cycle 7 for most rats, so only the last four cycles were relevant to the planned comparisons.

For all analyses and planned comparisons in this experiment, only statistically significant results are reported.

3. Results

3.1. Fluid Consumption

Analyses showed that animals acquired conditional control of fluid consumption. This was demonstrated through the measures of fluid intake, drinking duration, and drinking bouts (see below).

3.1.1. Fluid intake

A 10 X 3 repeated measures ANOVA revealed a significant Cycle X Day interaction, $F(18,126) = 9.537$, $p < .01$ (see Figure 11). Also significant was the Cycle main effect, $F(9,63) = 5.305$, $p < .01$, and the Day main effect $F(2,14) = 5.525$, $p < .05$. Follow up t-tests showed that rats drank significantly less on the Danger day relative to the subsequent Safe day on Cycle 7: $t(7) = 1.970$, $p < .05$, Cycle 8: $t(7) = 2.102$, $p < .05$, Cycle 9: $t(7) = 2.843$, $p < .05$, and Cycle 10: $t(7) = 3.218$, $p < .01$.

3.1.2. Fluid intake – retention test

A repeated measures ANOVA that was carried out on the retention test 25 days after the last trial (probe in Figure 11) revealed that rats maintained the discrimination, $F(2,14) = 9.180$, $p < .01$. A t-test showed that the rats drank significantly less on the Danger day relative to the subsequent Safe day, $t(7) = 3.149$, $p < .01$.

3.1.3. Drinking duration

A 10 X 3 repeated measures ANOVA revealed a significant Cycle X Day interaction, $F(18,126) = 10.072$, $p < .01$ (see Figure 12). Also significant was the Cycle main effect, $F(9,63) = 5.481$, $p < .01$, and the Day main effect $F(2,14) = 8.717$, $p < .01$. Follow up t-tests showed that rats spent significantly less time drinking on the Danger day relative to the subsequent Safe day on Cycle 7: $t(7) = 2.371$, $p < .05$, Cycle 8: $t(7) = 2.218$, $p < .05$, Cycle 9: $t(7) = 2.693$, $p < .05$, and Cycle 10: $t(7) = 3.571$, $p < .01$.

3.1.4. Drinking duration – retention test

A repeated measures ANOVA that was carried out on the retention test 25 days after the last trial (probe in Figure 12) revealed that rats maintained the discrimination, *F*

(2,14) = 9.099, $p < .01$. A t-test showed that the rats spent significantly less time drinking on the Danger day relative to the subsequent Safe day, t (7) = 3.259, $p < .01$.

3.1.5. Drinking bouts

A 10 X 3 repeated measures ANOVA revealed a significant Cycle X Day interaction, $F(18,126) = 1.774$, $p < .05$ (see Figure 13). Also significant was the Cycle main effect, $F(9,63) = 9.016$, $p < .01$. Follow up t-tests showed that rats had significantly fewer drinking bouts on the Danger day relative to the subsequent Safe day on Cycle 10: $t(7) = 3.571$, $p < .01$. These effects were not significant on the retention test 25 days later.

3.2. Aversive Measures

Analyses showed that animals demonstrated more gaping and chin rubbing on Danger days relative to Safe days once the discrimination was learned. The measures of headshaking and forelimb flailing did not change between Safe and Danger days. These two measures are presented in Figures 14 and 15.

3.2.1. Gaping

A 10 X 3 X 2 repeated measures ANOVA revealed a significant Cycle X Day X Order interaction, $F(18,126) = 3.358$, $p < .01$ (see Figure 16). Also significant was the Day X Order interaction, $F(2,14) = 7.651$, $p < .01$, and the Cycle X Day interaction, $F(18,126) = 6.602$, $p < .01$. All of the main effects were significant as follows; the Cycle main effect, $F(9,63) = 6.312$, $p < .01$, the Day main effect $F(2,14) = 14.704$, $p < .01$, and the Order main effect, $F(1,7) = 5.833$, $p < .05$.

Follow up t-tests were conducted on the last four cycles for both the first 10-min of the trial, and for the second 10-min of the trial. During the first 10 minutes, rats gaped

significantly more on the Danger day relative to the subsequent Safe day on Cycle 7: $t(7) = 2.547$, $p < .05$, Cycle 8: $t(7) = 3.066$, $p < .01$, Cycle 9: $t(7) = 4.034$, $p < .01$, and Cycle 10: $t(7) = 4.000$, $p < .01$. During the second 10 minutes, rats gaped significantly more on the Danger day relative to the subsequent Safe day on Cycle 7: $t(7) = 2.527$, $p < .05$, and Cycle 9: $t(7) = 2.149$, $p < .05$.

3.2.2. Gaping – retention test

A 3 X 2 repeated measures ANOVA was also carried out on the retention test 25 days after the last trial (probe in Figure 16). This test revealed that rats maintained this behaviour, as evident by a significant Day X Order interaction, $F(2,14) = 5.476$, $p < .05$. Also significant was the Day main effect, $F(2,14) = 8.010$, $p < .01$, and the Order main effect $F(1,7) = 7.760$, $p < .05$. T-tests showed that the rats gaped significantly more on the Danger day relative to the subsequent Safe day, during both the first 10 min, $t(7) = 2.945$, $p < .01$, and during the second 10 min, $t(7) = 2.511$, $p < .01$.

3.2.3. Chin rubbing

A 10 X 3 X 2 repeated measures ANOVA revealed a significant Cycle X Day X Order interaction, $F(18,126) = 2.240$, $p < .01$ (see Figure 17). Also significant was the Day X Order interaction, $F(2,14) = 4.270$, $p < .05$, the Cycle X Order interaction, $F(9,63) = 2.271$, $p < .05$, and the Cycle X Day interaction, $F(18,126) = 5.538$, $p < .01$. As for the main effects, the following were also significant; the Cycle main effect, $F(9,63) = 8.913$, $p < .01$, and the Day main effect $F(2,14) = 8.096$, $p < .01$.

Follow up t-tests were conducted on the last four cycles for both the first 10-min of the trial, and for the second 10-min of the trial. During the first 10 minutes, rats chin rubbed significantly more on the Danger day relative to the subsequent Safe day on Cycle

8: $t(7) = 2.374$, $p < .05$, Cycle 9: $t(7) = 3.220$, $p < .01$, and Cycle 10: $t(7) = 2.308$, $p < .05$. During the second 10 minutes, rats chin rubbed significantly more on the Danger day relative to the subsequent Safe day on Cycle 7: $t(7) = 1.994$, $p < .05$, Cycle 8: $t(7) = 2.560$, $p < .05$, Cycle 9: $t(7) = 2.952$, $p < .05$ and Cycle 10: $t(7) = 2.526$, $p < .05$.

3.2.4. Chin rubbing – retention test

A 3 X 2 repeated measures ANOVA was also carried out on the retention test 25 days after the last trial (probe in Figure 17). This test revealed that rats maintained this behaviour, as evident by a significant Day X Order interaction, $F(2,14) = 4.641$, $p < .05$. However, follow-up t-tests failed to distinguish between the Danger day and the subsequent Safe day.

3.3. Appetitive Measures

Analyses showed that animals demonstrated more tongue protrusions and paw licks on Safe days relative to Danger days once the discrimination was learned (see below).

3.3.1. Tongue protrusions

A 10 X 3 X 2 repeated measures ANOVA revealed a significant Cycle X Order interaction, $F(9,63) = 3.752$, $p < .01$, and a significant Cycle X Day interaction, $F(18,126) = 3.462$, $p < .01$ (see Figure 18). There was also a significant Cycle main effect, $F(9,63) = 6.370$, $p < .01$, a significant Day main effect $F(2,14) = 4.646$, $p < .05$, and a significant Order main effect, $F(1,7) = 14.460$, $p < .01$.

Follow up t-tests were conducted on the last four cycles for both the first 10-min of the trial, and for the second 10-min of the trial. During the first 10 minutes, rats demonstrated significantly more tongue protrusions on the Safe day relative to the

preceding Danger day on Cycle 8: $t(7) = 2.428$, $p < .05$. During the second 10 minutes, rats demonstrated significantly more tongue protrusions on the Safe day relative to the preceding Danger day on Cycle 7: $t(7) = 1.997$, $p < .05$, Cycle 9: $t(7) = 2.244$, $p < .05$ and Cycle 10: $t(7) = 2.630$, $p < .05$.

3.3.2. Tongue protrusions – retention test

A 3 X 2 repeated measures ANOVA was also carried out on the retention test 25 days after the last trial (probe in Figure 18). This test revealed that rats maintained differences in tongue protrusions, as evident by a significant Day X Order interaction, $F(2,14) = 4.369$, $p < .05$. The Day main effect was also significant in this analysis, $F(2,14) = 4.182$, $p < .05$. A t-test showed that rats demonstrated significantly more tongue protrusions on the Safe day relative to the preceding Danger day during the second 10 min, $t(7) = 4.536$, $p < .01$.

3.3.3. Paw licking

A 10 X 3 X 2 repeated measures ANOVA revealed a significant Day X Order interaction, $F(2,14) = 5.203$, $p < .05$, and a significant Cycle X Day interaction, $F(18,126) = 1.891$, $p < .05$ (see Figure 19). There was also a significant Cycle main effect, $F(9,63) = 2.247$, $p < .05$, a significant Day main effect $F(2,14) = 11.687$, $p < .01$, and a significant Order main effect, $F(1,7) = 9.074$, $p < .05$.

Follow up t-tests were conducted on the last four cycles for both the first 10-min of the trial, and for the second 10-min of the trial. During the first 10 minutes, rats demonstrated significantly more paw licking on the Safe day relative to the preceding Danger day on Cycle 10: $t(7) = 2.049$, $p < .05$. During the second 10 minutes, rats

demonstrated significantly more paw licking on the Safe day relative to the preceding Danger day on Cycle 7: $t(7) = 2.252$, $p < .05$, and Cycle 8: $t(7) = 2.671$, $p < .05$.

3.3.4. Paw licking – retention test

A 3 X 2 repeated measures ANOVA was also carried out on the retention test 25 days after the last trial (probe in Figure 19). Statistically, however, differences in paw-licking were not maintained over the 25 day retention interval.

3.4. Other Measures

Other behavioural measures were also recorded during the scoring procedure. Grooming, both in duration and bouts, was measured, as well as face-washing and hole-poking. Hole-poking was scored as the rat inserted its snout into one of the two drinking holes (see below).

3.4.1. Grooming bouts

A 10 X 3 X 2 repeated measures ANOVA revealed a significant Day X Order interaction, $F(2,14) = 4.208$, $p < .05$, Cycle X Order interaction, $F(9,63) = 2.818$, $p < .01$, and a significant Cycle X Day interaction, $F(18,126) = 2.130$, $p < .01$ (see Figure 20). There was also a significant Cycle main effect, $F(9,63) = 7.480$, $p < .01$, a significant Day main effect $F(2,14) = 6.493$, $p < .05$, and a significant Order main effect, $F(1,7) = 5.901$, $p < .05$.

Follow up t-tests were conducted on the last four cycles for both the first 10-min of the trial, and for the second 10-min of the trial. During the first 10 minutes, rats demonstrated significantly more grooming bouts on the Safe day relative to the preceding Danger day on Cycle 7: $t(7) = 2.118$, $p < .05$, Cycle 8: $t(7) = 3.000$, $p < .05$, and Cycle 9: $t(7) = 2.049$, $p < .05$. During the second 10 minutes, rats demonstrated significantly

more grooming bouts on the Safe day relative to the preceding Danger day on Cycle 8: $t(7) = 2.393$, $p < .05$, Cycle 9: $t(7) = 2.862$, $p < .05$ and Cycle 10: $t(7) = 2.619$, $p < .05$.

3.4.2. Grooming bouts – retention test

A 3 X 2 repeated measures ANOVA was also carried out on the retention test 25 days after the last trial (probe in Figure 20). This test revealed that differences in grooming bouts were maintained, as evident by a significant Day X Order interaction, $F(2,14) = 5.252$, $p < .05$. The Day main effect was also significant in this analysis, $F(2,14) = 4.609$, $p < .05$. A t-test showed that rats demonstrated significantly more grooming bouts on the Safe day relative to the preceding Danger day during both the first 10 min, $t(7) = 3.000$, $p < .05$, and during the second 10 min, $t(7) = 2.815$, $p < .01$.

3.4.3. Grooming duration

A 10 X 3 X 2 repeated measures ANOVA revealed a significant Cycle main effect, $F(9,63) = 3.204$, $p < .01$, and a significant Order main effect, $F(1,7) = 14.027$, $p < .01$ (see Figure 21).

Follow up t-tests were conducted on the last four cycles for both the first 10-min of the trial, and for the second 10-min of the trial. During the first 10 minutes, rats demonstrated significantly more grooming on the Safe day relative to the preceding Danger day on Cycle 7: $t(7) = 2.453$, $p < .05$, and Cycle 8: $t(7) = 2.270$, $p < .05$. During the second 10 minutes, rats demonstrated significantly more grooming on the Safe day relative to the preceding Danger day on Cycle 9: $t(7) = 2.118$, $p < .05$ and Cycle 10: $t(7) = 2.040$, $p < .05$.

3.4.4. Grooming duration – retention test

A 3 X 2 repeated measures ANOVA was also carried out on the retention test 25 days after the last trial (probe in Figure 21). This test revealed that differences in grooming duration were maintained, as evident by a significant Day X Order interaction, $F(2,14) = 5.088$, $p < .05$. The Day main effect was also significant in this analysis, $F(2,14) = 4.859$, $p < .05$. A t-test showed that rats demonstrated significantly more grooming on the Safe day relative to the preceding Danger day during both the first 10 min, $t(7) = 2.617$, $p < .05$, and during the second 10 min, $t(7) = 2.480$, $p < .01$.

3.4.5. Face-washing

A 10 X 3 X 2 repeated measures ANOVA revealed a significant Cycle X Order interaction, $F(9,63) = 2.781$, $p < .01$ (see Figure 22). There was also a significant Cycle main effect, $F(9,63) = 11.560$, $p < .01$, and a significant Day main effect $F(2,14) = 5.108$, $p < .05$.

Follow up t-tests were conducted on the last four cycles for both the first 10-min of the trial, and for the second 10-min of the trial. During the first 10 minutes, rats face-washed significantly less on the Danger day relative to the subsequent Safe day on Cycle 9: $t(7) = 2.376$, $p < .05$. During the second 10 minutes, rats face-washed significantly less on the Danger day relative to the subsequent Safe day on Cycle 9: $t(7) = 2.517$, $p < .05$ and Cycle 10: $t(7) = 2.250$, $p < .05$.

3.4.6. Face-washing – retention test

A 3 X 2 repeated measures ANOVA was also carried out on the retention test 25 days after the last trial (probe in Figure 22). This test revealed that rats maintained differences in this behaviour in the same direction as the main experiment, as evident by

a significant Day X Order interaction, F (2,14) = 10.208, $p < .01$. A t-test showed that the rats face-washed significantly less on the Danger day relative to the subsequent Safe day during the second 10 min, t (7) = 3.658, $p < .01$.

3.4.7. Hole-pokes

A 10 X 3 X 2 repeated measures ANOVA revealed a significant Cycle X Day X Type (Safe hole vs. Danger hole) interaction, F(18,126) = 2.710, $p < .01$ (see Figure 23). Also significant was the Day X Type interaction, F(2,14) = 11.794, $p < .01$, and the Cycle X Day interaction, F(18,126) = 3.936, $p < .01$. All of the main effects were also significant as follows; the Cycle main effect, F(9,63) = 2.265, $p < .05$, the Day main effect F(2,14) = 6.873, $p < .01$, and the Type main effect, F(1,7) = 21.374, $p < .01$.

Follow up t-tests were conducted on the last four cycles. These analyses revealed that rats poked the safe hole more on the Safe day after a Danger day on cycle 8 (t (7) = 2.953, $p < .05$), cycle 9 (t (7) = 2.650, $p < .05$), and cycle 10 (t (7) = 2.101, $p < .05$). Hole-pokes to the danger hole were less on the Safe day than on the preceding Danger day on cycle 7, t (7) = 4.490, $p < .01$. However, hole-pokes to the danger hole did not differ between the Danger day and following Safe day on cycles 8, 9 and 10 ($p > .05$).

Paired t-tests were used to compare safe versus danger hole-poking on each day (Safe day - Danger day - Safe day) of Cycles 7 to 10. On cycle 7, rats poked the safe hole more when compared to the danger hole on all three days as follows: first Safe day (t (7) = 5.427, $p < .01$), Danger day (t (7) = 2.368, $p < .05$), and second Safe day (t (7) = 3.158, $p < .01$). On cycle 8, rats poked the safe hole significantly more than the danger hole on both Safe days; first Safe day (t (7) = 3.747, $p < .01$), and second Safe day (t (7) = 1.909, $p < .05$). There were no differences in poking behaviour on the Danger day ($p >$

.05). On cycle 9, rats poked the safe hole significantly more than the danger hole on both Safe days; first Safe day (t (7) = 2.310, $p < .05$), and second Safe day (t (7) = 2.710, $p < .05$). There were no differences in poking behaviour on the Danger day ($p > .05$). On cycle 10, rats also poked the safe hole significantly more than the danger hole on both Safe days; first Safe day (t (7) = 2.334, $p < .05$), and second Safe day (t (7) = 2.979, $p < .05$). Again, there were no differences in poking behaviour on the Danger day ($p > .05$).

3.4.8. Hole-pokes – retention test

A 3 X 2 repeated measures ANOVA was also carried out on the retention test 25 days after the last trial. This test revealed that rats maintained differential hole-poking behaviour, as evident by a significant Day X Type interaction, F (2,14) = 4.329, $p < .05$. The Day main effect was also significant in this analysis, F (2,14) = 4.859, $p < .05$, as well as the Type main effect, F (1,7) = 10.806, $p < .05$.

Follow up t-tests revealed that rats poked the safe hole more on the Safe day after the Danger day, t (7) = 2.096, $p < .05$. Hole-pokes to the danger hole did not differ between the Danger day and following Safe day ($p > .05$). Consistent with the main experiment, rats poked the safe hole significantly more than the danger hole on both Safe days; first Safe day (t (7) = 2.862, $p < .05$), and second Safe day (t (7) = 3.124, $p < .01$). There were no differences in poking behaviour on the Danger day ($p > .05$).

3.5. Correlations of behavioural measures with fluid intake.

All of the significant behavioural measures, for the aversive, appetitive, and other measures, were correlated with fluid consumption. Drinking duration and drinking bouts were also correlated with fluid consumption. All measures were averaged across the last three cycles. Both the order of the day in the cycle, as well as the order of the first and

second 10 min was held constant, so that I was left with an averaged Safe-Danger-Safe cycle. All correlations calculated were 1-tailed, and are presented in Tables 3 and 4.

3.5.1. Fluid consumption

Overall, fluid consumption was positively correlated with both drinking duration ($p < .01$), and drinking bouts ($p < .01$) (see Table 3). This is the case on all averaged days with an exception on the Danger day, when fluid consumption was not significantly correlated with the number of drinking bouts ($p > .05$).

3.5.2. Aversive measures (Gaping and Chin rubbing)

The discriminating aversive measures, gaping and chin rubs were correlated with fluid consumption (see Table 4). Significant correlations were as follows: during the first 10 min of the averaged Danger day, gaping was negatively correlated with fluid consumption ($p < .01$). During the second 10 min of the averaged Danger day, both gaping and chin rubs were negatively correlated with fluid intake ($p < .05$).

During the first 10 min of the first averaged Safe day, chin rubs correlated negatively with fluid consumption ($p < .05$). During the second 10 min of the first averaged Safe day, chin rubs again was negatively correlated with fluid consumption ($p < .05$).

During both the first 10 min, and the second 10 min of the second averaged Safe day, neither of the aversive measures correlated significantly with fluid consumption ($p > .05$).

3.5.3. Appetitive measures (Tongue protrusions and Paw licking)

Like the aversive measures, both appetitive measures, tongue protrusions and paw licking, were also correlated with fluid consumption (see Table 4). During the first 10

min of the first averaged Safe day, neither tongue protrusions, nor paw licks correlated significantly with fluid consumption ($p > .05$). During the second 10 min of the averaged first Safe day, both of these measures were positively correlated with fluid consumption ($p < .01$).

During the first 10 min of the averaged Danger day, tongue protrusions correlated significantly with fluid consumption ($p < .05$). Likewise, during the second 10 min of the averaged Danger day, tongue protrusions correlated significantly with fluid intake ($p > .01$).

Finally, during the first 10 min of the second averaged Safe day, neither tongue protrusions, nor paw licks correlated significantly with fluid consumption ($p > .05$). During the second 10 min of the second averaged Safe day, both of these behavioural measures were again positively correlated with fluid consumption ($p < .05$).

3.5.4. Other measures

The other measures, grooming bouts and face-washing, were also both correlated with fluid consumption (see Table 4). During the first 10 min of the first averaged Safe day, face-washing was positively correlated with fluid intake ($p < .05$). During the second 10 min of the first averaged Safe day, both of these behavioural measures were positively correlated with fluid consumption ($p < .01$).

During the first 10 min of the averaged Danger day, grooming bouts correlated significantly with fluid consumption ($p < .01$). During the second 10 min of the averaged Danger day, neither grooming bouts, nor face-washing correlated significantly with fluid consumption ($p > .05$).

Finally, during the first 10 min of the second averaged Safe day, only grooming bouts correlated significantly with fluid consumption ($p < .05$). During the second 10 min of the second averaged Safe day, both grooming bouts ($p < .05$) and face-washing ($p < .01$) were again positively correlated with fluid consumption.

4. Discussion

4.1 Summary of Results

Conditional control of fluid consumption and appetitive and aversive behaviours was obtained when only visual cues distinguished the two contexts. Increases in fluid consumption on Safe days and decreases on Danger days were appropriately correlated with appetitive and aversive behavioural changes that were measured in parallel. The appetitive and aversive behavioural changes occurred not only during, but also in anticipation of fluid delivery. Hole-poking behaviour revealed that animals anticipate fluid delivery on Safe days, but do not show anticipatory hole-poking on Danger days. Finally, a retention test 25 days later revealed that rats remembered the discrimination, with levels of fluid consumption and behavioural measures remaining intact.

4.2 General Discussion – Comparison of Changes in Fluid Consumption with Orofacial and Somatic Responses.

The measures of consumption indicate that rats learned to avoid the saccharine flavour in the context where it had been paired with LiCl, indicated by their discrimination between Safe and Danger days based on visual cues from the context. The orofacial and somatic responses that are elements of the taste reactivity test were measured (Berridge, 2000; Grill and Norgen, 1978) and these behavioural changes were correlated with consumption. The usual taste reactivity test involves forced tasting of a

solution through an implanted intraoral cannula and subsequently assessing the animal's orofacial and somatic responses; however the same behavioural reactions are also present when a solution is freely available to the animal (Pelchat, et al., 1983). The latter method was used so that the relationship between fluid consumption and taste reactivity measures could be assessed.

Throughout the entire 20 min trial for each day of the experiment, the rat's behaviour was videotaped and later reviewed and scored by the experimenter. It was found that rats increased aversive-type behaviours (gaping and chin rubbing) and decreased appetitive-type behaviours (paw licking and tongue protrusions) on Danger days. This trend was reversed on Safe days, where aversive-type behaviours became less frequent, and appetitive-type behaviours became more frequent. These trends became apparent once the rats learned the discrimination as evident in the consumption measures, which began at approximately Cycle 7 of the experiment. The timing of this learned discrimination is consistent with a similar experiment from Murphy & Skinner (2005), where they found that a discrimination emerged at Cycle 7.

From these findings, one could interpret that appetitive responses are related to fluid consumption, while aversive responses are related to avoidance of the fluid. Pelchat, et al. (1983) noted that aversive taste reactivity responses were evoked by a lithium-paired sucrose solution that was freely available for consumption. Further to this, it been known for some time that rats will avoid a palatable flavour solution previously paired with lithium (Nachman, 1970), and also display taste reactivity responses to such a solution when infused intraorally (Grill and Norgren, 1978). The data confirmed that the observed changes in fluid consumption are related to both aversive and appetitive

behaviours that were measured in parallel in the ways hypothesized. To be more specific, both tongue protrusions and paw licking were positively related to fluid consumption after the fluid was delivered on Safe days, while there was either a negative relationship or none at all on Danger days. Gaping and chin rubbing were both negatively related to fluid consumption on Danger days, as fluid consumption decreased these behaviours increased. These findings support those obtained by Parker (1995). This author found that tongue protrusions correlated positively with drinking, and also correlated negatively with the aversion. Further, both gaping and chin rubbing were negatively correlated with drinking sucrose, and subsequently were positively correlated with the aversion.

Also evident in the current study, significant amounts of gaping were present in the period before fluid consumption, thus was a strong predictor of decreased fluid consumption. As for face-washing and grooming bouts, the general trend is that they both increase significantly after the fluid is presented on Safe days, which is not evident on Danger days. Although these behaviours are not considered to be strictly appetitive nor aversive, one could suggest that animals would have to be in a "safe" environment to perform these types of behaviours.

4.3 General Discussion – Contextual Control of Fluid Consumption

Contextual control of fluid consumption using only visual cues was demonstrated in this experiment. In earlier experiments, conditional control of fluid consumption was achieved using different drugs as the conditional cue (Jaeger & Mucha, 1990; Martin, et al., 1990; Mastropaolo, et al., 1989). In these studies, animals learned that preceding injections of either the drug or the vehicle could predict forthcoming illness, thus they learned to withhold consumption of an otherwise palatable solution. Subsequently,

conditional control of fluid consumption has also been demonstrated using the context as the conditional cue (Lopez and Cantora, 2003; Loy, Alvarez, Rey, and Lopez, 1993; Murphy and Skinner, 2005; Skinner, et al., 1994). In much the same manner as the earlier drug studies, these authors demonstrated that contextual cues can substitute as the conditional cue, in that animals can learn to discriminate when to consume a flavour solution in two distinct contexts. Rats received pairings of a flavour solution and an emetic agent in one distinct context, while the flavour solution was paired with a vehicle in another context. Like the drug studies, rats learned to withhold fluid consumption in the context that was paired with the emetic agent.

These results were replicated using the context as the conditional cue. The data indicated that rats learned to discriminate between Safe and Danger days of the experiment, in that rats learned to withhold consumption of a saccharine flavour that was succeeded by an injection of LiCl in one colour context (Danger context) and to consume the same flavour solution in the other that was paired with saline (Safe context). This was clearly evident in the measures of consumption. The amount of saccharine consumed as well as the drinking duration, both demonstrated that the rats had learned the discrimination by Cycle 7 of the experiment. This is an example of conditioned flavour avoidance, and supports the hypothesis that visual cues can exert conditional control over fluid consumption.

What differs in this study from those of others that have used the context as a conditional cue (Lopez and Cantora, 2003; Loy, et al., 1993; Murphy and Skinner, 2005; Skinner, et al., 1994), is that it was found that the consumption (or avoidance) of the saccharine flavour could be brought under conditional control using only visual cues

supplied by the context. Previous studies that have used the context as a conditional cue also contained other stimuli besides visual cues, such as olfactory cues and different floor textures. The present experiment used only visual cues (box colour and drinking spout orientation) in the absence of any olfactory or texture cues. Both of the conditioning chambers were constructed at the same time using the same building materials, and the odours from the different colour paint were minimal. The two boxes were constructed as equivalent as possible, and there are no differences (other than colour) known to exist. Using these visual cues alone, the consumption of saccharine was successfully brought under conditional control of the visual context. It should be noted that the drinking spout orientation, besides being obviously visual in nature, could also be classified as a spatial cue, as animals consumed the fluid in a different physical place within the larger space. In essence, my experiment likely did involve two cues, one visual (colour) and one spatial (spout orientation), however as shall be discussed later, animals still displayed evidence of conditional learning based solely on contextual visual cues, as was evident in the taste reactivity responses during the first 10 min of each trial.

4.4 General Discussion – Contextual Control of Orofacial and Somatic Responses

In order to assess the extent to which the context alone could come to elicit appetitive or aversive behaviours, the rat's behaviour was recorded in the absence of fluid during the first 10 min of each trial. It was found that in addition to fluid consumption, appetitive and aversive orofacial and somatic responses associated with the taste reactivity test could also come under contextual control. To summarize, rats displayed significantly more rejection reactions (gaping and chin rubbing) on Danger days than on Safe days in the 10 min prior to saccharine delivery. These aversive behaviours were

significantly less on Safe days. There was a trend for the appetitive measure of tongue protrusions to increase on Safe days relative to Danger days in the period prior to fluid presentation however these increased levels were not statistically significant. This trend was also evident for both grooming and face-washing. The other appetitive measure of paw licking was not evident during the first 10 min when no fluid was present. It should be noted that all of these behaviours (tongue protrusions, paw licking, grooming and face-washing) were all significantly higher on Safe days relative to Danger days once the fluid was presented. For all behaviours, the anticipatory responses were most evident when the discrimination was learned as evident in the fluid consumption measures, occurring at approximately Cycle 7. Taking these taste reactivity measures into account, it can be concluded that the context did come to elicit conditioned behaviours, however, this was true for only the aversive measures, as their presence during the first 10 min of each trial predicted a Danger trial.

Initially, flavour avoidance of a palatable solution was used to gauge the conditional properties of the context when trained in conjunction with an injection of an emetic agent (Lopez and Cantora, 2003; Loy, et al., 1993; Murphy and Skinner, 2005; Rodriguez, et al., 2000; Skinner, et al., 1994; Symonds and Hall, 2002). Animals withheld consumption of a palatable flavour solution in a context that was paired with an emetic agent, but consumed the flavour solution in an unpaired context. This demonstrates that fluid consumption can be put under contextual control. Furthermore, because the context was paired with an emetic agent, it is plausible that the context alone was endowed with the power to elicit nausea. This could serve as an explanation for the decreased consumption of the palatable flavour solution while in this context.

Recent studies have further evaluated this claim using the taste reactivity test, which is considered a more sensitive measure of conditioned nausea than a standard consumption test (Parker, 2003). By using the taste reactivity test, orofacial and somatic responses are measured in the absence of an appetitive approach-the-bottle response. Limebeer, et al. (2006) conducted a study to investigate taste reactivity responses in an experimental protocol very similar to the one by Rodriguez, et al. (2000). Rodriguez, et al. (2000) claimed that animals avoided sucrose in a lithium-paired context, while consuming this fluid in an unpaired context, due to the fact that the lithium-paired context had been endowed with the property to evoke nausea. They drew this conclusion based on the fact that lithium induces nausea, and that rats avoided the palatable flavour solution while in this context. In the comparable experiment by Limebeer, et al. (2006), taste reactivity responses confirmed the conclusions reached by Rodriguez et al. (2000); that suppressed consumption in a lithium-paired reflects conditioned nausea. In essence, animals gaped more while in the lithium-paired context when infused with a saccharine flavour, and gaping is considered the rats' display of emesis (Parker, 1991).

Also during the experiment by Limebeer, et al. (2006), it was discovered that rats continued to gape during the inter-infusion interval. The fact that rats continued to gape in the absence of fluid delivery suggests that rats gaped to the context alone, implying that the context was endowed with the property to induce a state of nausea. In a later study by Limebeer, et al. (2008), rats displayed a conditioned gaping reaction to a context that was paired with LiCl in the absence of a flavour solution. In this study, rats gaped to a distinctive context that was either permeated with a distinct odour (context + odour), or without the odour (context + no odour). This also held true with either high or low doses

of LiCl. With this, it can be concluded that a context can come to elicit nausea, even in the absence of a flavour solution or other gustatory stimuli.

In the present study, gaping was recorded to a lithium-paired context that differed only in visual cues in the absence of fluid delivery. The results are consistent with those obtained by Limebeer, et al. (2008); conditioned gaping to a context that only contained visual cues was achieved. The present study differed in the following ways; first, rats were injected with LiCl after they received a trial consistent with a forward conditioning procedure. Second, other appetitive and aversive behaviours were recorded to gain a more comprehensive picture of the taste reactivity responses. Third, rats were trained in a separate non-poisoned context in order to evaluate these behavioural changes in a discriminative learning task. Finally, a freely accessible saccharine flavour was provided in order to gauge the relationship between the behavioural responses and a measure of intake. This study suggests that the context can be conditioned to elicit nausea as evidenced by increased amount of gaping and chin rubbing (aversive responses) on Danger days prior to fluid presentation, even though the animal did not actually experienced sickness while in that context. Furthermore, this conditioned nausea can be put under conditional control of visual context cues alone, as these behaviours occur significantly more on Danger days relative to Safe days during the first 10 min of the trial, when both contexts only differ in colour (black or white).

It is also interesting to note that the animals in this study took slightly longer to learn the discrimination than other studies report (Limebeer, et al., 2006; Lopez and Cantora, 2003; Rodriguez, et al., 2000). A possible explanation for this could be the reduced number of contextual cues, in that only used visual context cues were used, with

the exception of the spatial cue (spout orientation) present only during fluid delivery. All of the previous studies training a contextual discrimination have used multiple cues from different modalities (e.g., visual, odour, and floor texture). Furthermore, Skinner, et al. (1994) also found that training the context as an occasion setter took longer than training drugs as a conditional cue.

4.5 General Discussion – Hole-poking Behaviour

Rats always had the saccharine flavour presented through a fixed hole on the Safe and Danger days (e.g., hole on long wall on Safe day and hole on short wall on Danger day, counterbalanced between subjects). Three findings came from analyses of the hole-poking behaviour. First, rats hole-poked more on Safe days than on Danger days. Second, rats poked the hole associated with "safe" saccharine insertion significantly more than the hole associated with "dangerous" saccharine insertion on Safe days. Finally, rats did not seem to have a preference for hole-poking either hole on Danger days. In addition to the aversive and appetitive taste reactivity behaviours, this is yet another behavioural difference between the Danger and the Safe days. The increased poking of the "safe" hole on Safe days indicated that rats were anticipating fluid delivery on Safe days, and possibly not anticipating delivery on Danger days. Furthermore, this same trend in hole-poking behaviour was also evident on the retention test 25 days later. This indicates that rats remembered the discrimination, and still anticipated fluid delivery on Safe days.

The findings obtained here are consistent with the observation that rodents can be trained to discriminate and nose-poke a target hole for food, where other holes are present in the operant chamber (Delcasso, Jeantet and Cho, 2006). In this study, mice were

trained to nose-poke a target hole for food reward, and this hole was remembered for a period of 24 hr. These authors also report that unpublished findings from their lab suggest that the memory for the target hole could last seven to eight weeks. This demonstrates that rodents will poke a hole for reward, and that memory for this target hole persists over long periods. The rats in the present study, although not poking for immediate reward, still nose-poked a hole associated with reward (in anticipation), and the memory for this "correct" hole persisted over a 25 day retention interval.

The pattern of nose-poking will require further analysis. Others have reported (e.g., Weingarten and Martin, 1989) that rats do not reduce overall nose-poking in anticipation of an aversive solution. These authors found that an aversion develops to food paired with LiCl, however, anticipatory head-poking in this group was not reduced and no different from a control group that received saline. This is contrary to the results obtained here, where anticipatory nose-poking was significantly reduced on lithium-paired Danger days. One possibility for the discrepancy is that the rats in the Weingarten and Martin (1989) study only had one food cup to head-poke. In the current study, there were two holes used throughout training and this may have encouraged discrimination between holes, and subsequently may have contributed to rats nose-poking less on Danger days than Safe days. A study where only one hole is used would allow a test for how important two holes during training are.

4.6 General Discussion – Retention Test

The rats in the current study received a retention test 25 days after the last trial of the experiment. This retention test was designed to determine if the learned discrimination would be remembered, and to what extent the behavioural measures would

persist. It was found that animals did retain the discrimination 25 days later, as fluid consumption and drinking duration were significantly higher on both Safe days compared to the Danger day. As for the aversive behavioural measures, both gaping and chin rubbing were present 25 days later in much the same capacity as they were on the last cycle of the experiment. The appetitive measure of tongue protrusions were also present 25 days later, whereas paw licking was not as evident at this time. Furthermore, both face-washing and grooming bouts persisted over the hiatus in much the same fashion as in the last cycle. This demonstrates that both learned avoidance and learned aversion can persist over a 25 day retention interval.

It has been demonstrated that rats can maintain learned flavour avoidance over a relatively long period of time. Biederman, et al. (1974) found that rats can maintain flavour avoidance to lithium-paired saccharine for at least 14 days (a longer period was not tested). Furthermore, Dragoin, et al. (1973) found that rats can maintain avoidance behaviour to a novel solution for at least 90 days (a longer period was not tested). Also, Martin, et al. (1990) found that conditional control of fluid consumption by a morphine or saline conditioned cue could be maintained over a 30 day interval. Similarly, the present experiment demonstrated that rats maintained the discrimination over a 25 day period, during which the rats were simply maintained in their home cages on a water-restricted diet.

To my knowledge, there is no published research on the retention of conditioned taste reactivity responses. The rats in the present study maintained many of the taste reactivity behaviours that were recorded throughout the experiment. The aversive measures of gaping and chin rubbing re-emerged after the 25 day retention interval in

much the same pattern as they appeared during the last cycle of the experiment, with these behaviours increasing on the Danger day. Appetitive tongue protrusions were also present during the retention test in much the same manner as the last cycle of the experiment, with this behaviour increasing on Safe days. Likewise, the other measures of face-washing and grooming also persisted, as they were both increased on Safe days. Also during the retention test, the fact that the two aversive behaviours (gaping and chin rubbing) were still evident in the 10 min prior to fluid presentation on the Danger day suggests that the context alone still has the capacity to elicit aversive responses. These results indicate that anticipatory nausea persists over a time period of at least 25 days. Further research could evaluate a longer time frame.

4.7 Conclusions

These findings reveal that conditional control of fluid consumption that was demonstrated with drugs and environmental cues can also be obtained when the discriminative cue is colour. These findings also show that the conditional control of fluid consumption is mirrored in aversive and appetitive behaviours both before and during the presence of a flavoured solution. Subsequent research will be required to elucidate the nature of this control.

Furthermore, this study has demonstrated that contextual control of anticipatory nausea can be attained, and that the memory persists over a delay. This research contributes to the body of literature designed to understand anticipatory nausea that affects patients undergoing chemotherapy treatment.

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Table 1

Timeline of Experimental Procedure.

Day	Type	Day	Type
1	Safe	26	Danger
2	Safe	27	Safe
3	Safe	28	Safe
4	Danger	29	Safe
5	Safe	30	Safe
6	Safe	31	Danger
7	Safe	32	Safe
8	Safe	33	Safe
9	Danger	34	Safe
10	Safe	35	Safe
11	Safe	36	Danger
12	Safe	37	Safe
13	Safe	38	Safe
14	Danger	39	Safe
15	Safe	40	Danger
16	Safe	41	Safe
17	Safe	42	Safe
18	Safe	43	Safe
19	Safe	44	Safe
20	Danger	45	Danger
21	Safe	46	Safe
22	Safe	47	Safe
23	Safe	48	Danger
24	Safe	49	Safe
25	Safe		

Note. This table represents the timeline for the main body of the experiment. A retention test consisted of a single Safe-Danger-Safe cycle and occurred on the 25th day after the end of the last trial of the experiment. The animals were maintained on ad lib food and one 15 min daily access to water.

Table 2

Inter-rater Correlations for Behavioural Measures

Behaviour	Correlation
Gapes	.922**
Chin rubs	.989**
Paw licks	.974**
Tongue protrusions	.800**
Left hole-poke	.975**
Right hole-poke	.981**
Grooming bouts	.986**
Grooming duration	.999**

** $p < .01$

Note. Data obtained from the chief investigator was correlated with data obtained from an independent observer. All significance levels are based on a non-directional 1-tailed test. Headshaking and forelimb flailing were not scored by the independent observer, as these measures only surfaced on rare occasions.

Table 3

Correlations Between the Two Measures of Drinking and Fluid Consumption.

Behaviour	Safe Day 1	Danger Day	Safe Day 2
Drinking duration	.959**	.797**	.941**
Drinking bouts	.911**	.578^{NS}	.830**

* $p < .05$
** $p < .01$
NS = Not significant

Note. Each of the above behaviours was correlated with fluid intake for that given day. The calculated correlations are at 1-tailed significance level.

Table 4

Correlations Between Each Behavioural Measure and Fluid Consumption.

	Safe Day 1		Danger Day		Safe Day 2	
Behaviour	1st 10	2nd 10	1st 10	2nd 10	1st 10	2nd 10
Gapes	.292^{NS}	.312^{NS}	-.813**	-.686*	-.034^{NS}	.553^{NS}
Chin rubs	-.744*	-.727*	-.530^{NS}	-.642*	-.606^{NS}	-.233^{NS}
Tongue protrusions	.367^{NS}	.845**	.693*	.827**	.372^{NS}	.695*
Paw licks	-.115^{NS}	.903**	-.149^{NS}	-	.097^{NS}	.721*
Face-washes	.667*	.847**	.476^{NS}	.421^{NS}	.201^{NS}	.809**
Grooming bouts	-.236^{NS}	.833**	.918**	.169^{NS}	-.683*	.775*

* $p < .05$
** $p < .01$
NS = Not significant

Note. All of the above behaviours were correlated with fluid intake for that given day. Even though fluid was not present during the first 10 min of a trial, the behaviours occurring during the first 10 min were correlated with fluid consumption on that day. The calculated correlations are at the 1-tailed significance level.

Figure Caption

Figure 1. Dimensions of the drinking chamber.

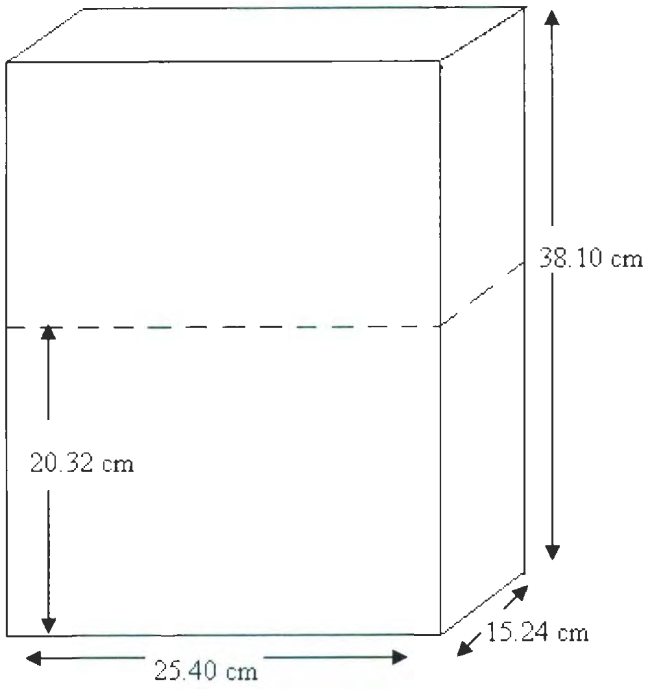

38.10 cm
20.32 cm
15.24 cm
25.40 cm

Figure Caption

Figure 2. Dimensions of the drinking spout holes in the drinking chamber.

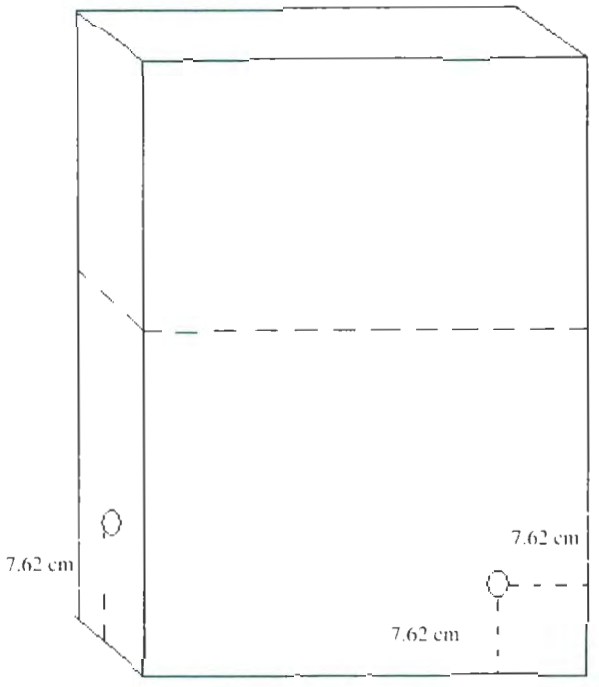

7.62 cm
7.62 cm
7.62 cm

Figure Caption

Figure 3. Still picture of paw-licking (an appetitive behaviour).

Figure Caption

Figure 4. Still picture of a tongue protrusion (an appetitive behaviour).

Figure Caption

Figure 5. Still picture of gaping (an aversive behaviour).

Figure Caption

Figure 6. Still picture of chin-rubbing (an aversive behaviour). Note that the rat is rubbing its chin on the glass floor.

Figure Caption

Figure 7. Still picture of drinking saccharine.

Figure Caption

Figure 8. Still picture of grooming behaviour.

Figure Caption

Figure 9. Still picture of face-washing.

Figure Caption

Figure 10. Still picture of nose-poking into a drinking hole.

Figure Caption

Figure 11. Mean (±SEM) amount of fluid consumed (g) on each Safe-Danger-Safe cycle (1 through 10) and on the probe trial.

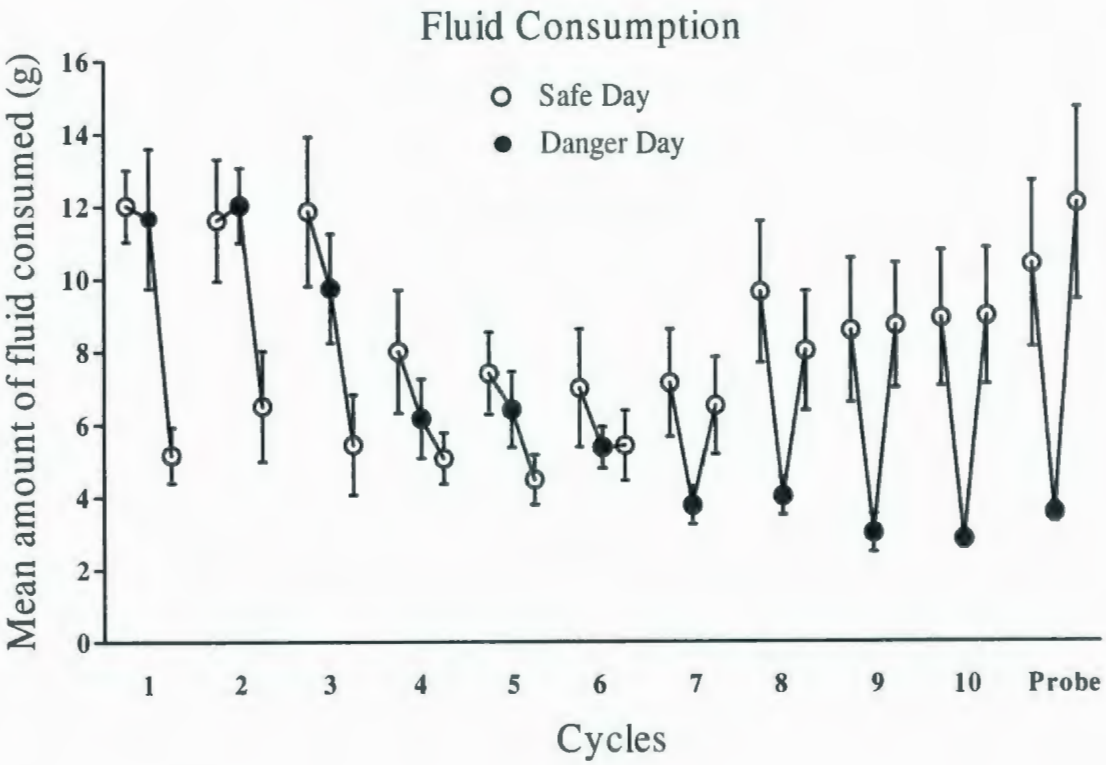

Fluid Consumption
Safe Day
Danger Day
Mean amount of fluid consumed (g)
0
2
4
6
8
10
12
14
16
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

Figure Caption

Figure 12. Mean (±SEM) drinking duration (sec) on each Safe-Danger-Safe cycle (1 through 10) and on the probe trial.

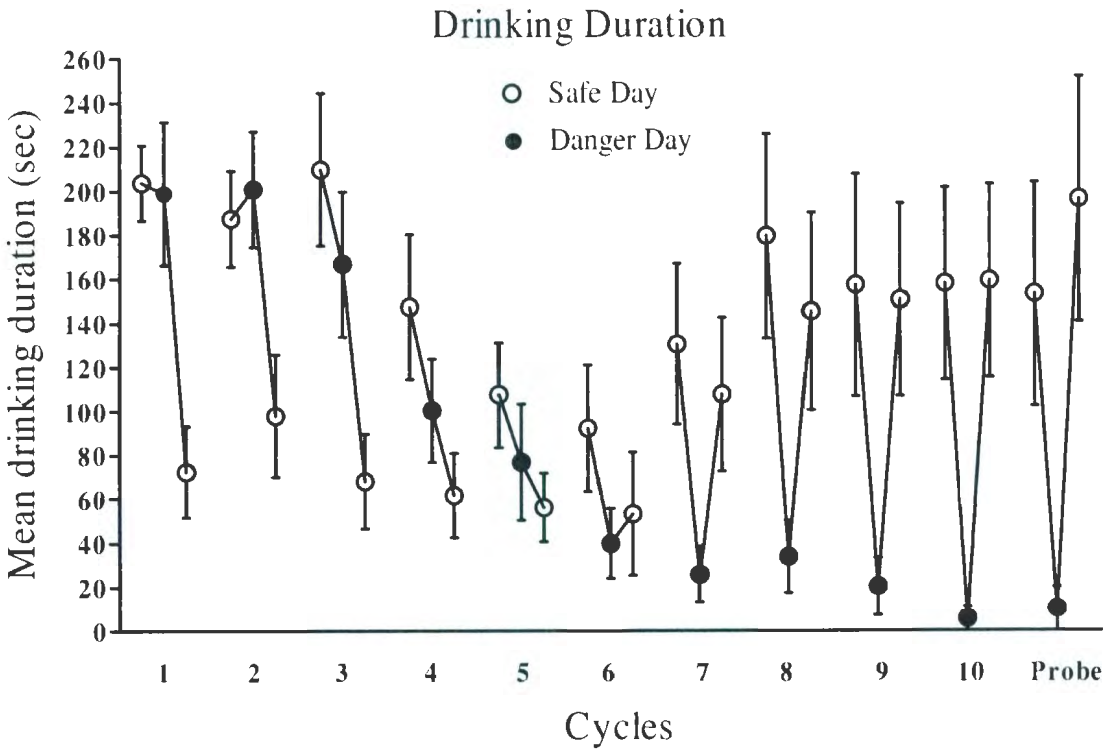

Drinking Duration
Safe Day
Danger Day
Mean drinking duration (sec)
260
240
220
200
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

Figure Caption

Figure 13. Mean (±SEM) number of drinking bouts on each Safe-Danger-Safe cycle (1 through 10) and on the probe trial.

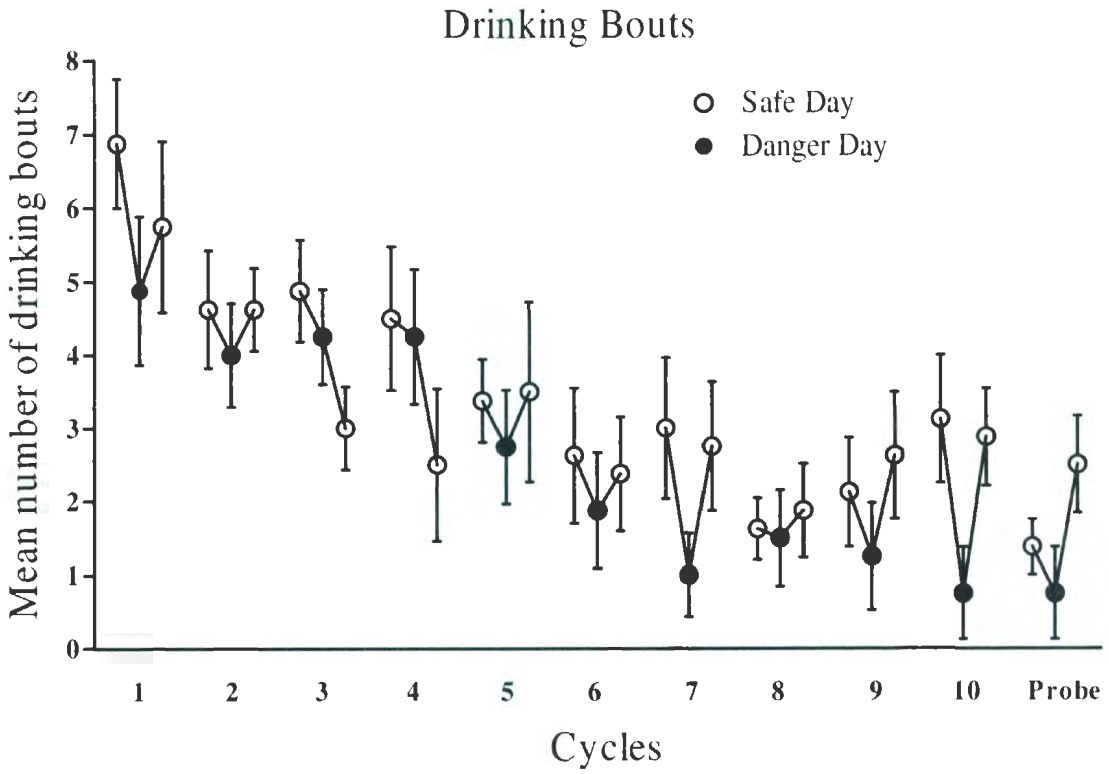
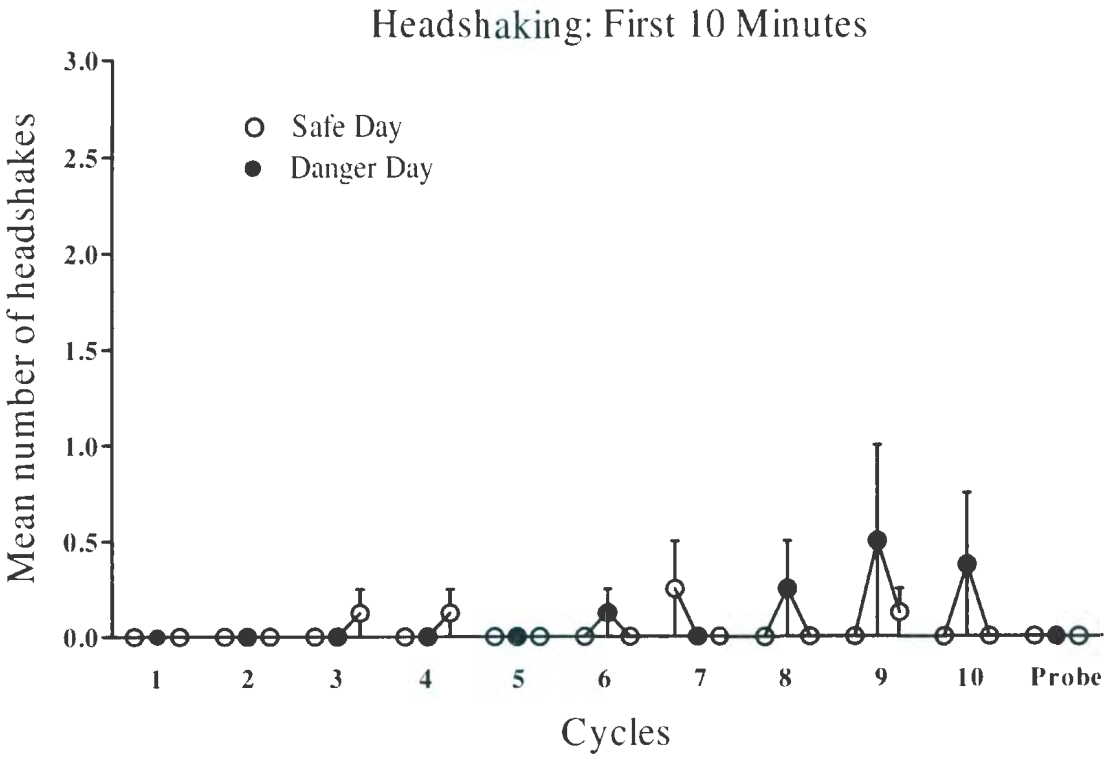

Drinking Bouts
Safe Day
Danger Day
Mean number of drinking bouts
0
1
2
3
4
5
6
7
8
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

Figure Caption

Figure 14. Mean (±SEM) number of headshakes on each Safe-Danger-Safe cycle (1 through 10) and on the probe trial for the first and second ten minute intervals.


Headshaking: First 10 Minutes
Safe Day
Danger Day
Mean number of headshakes
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

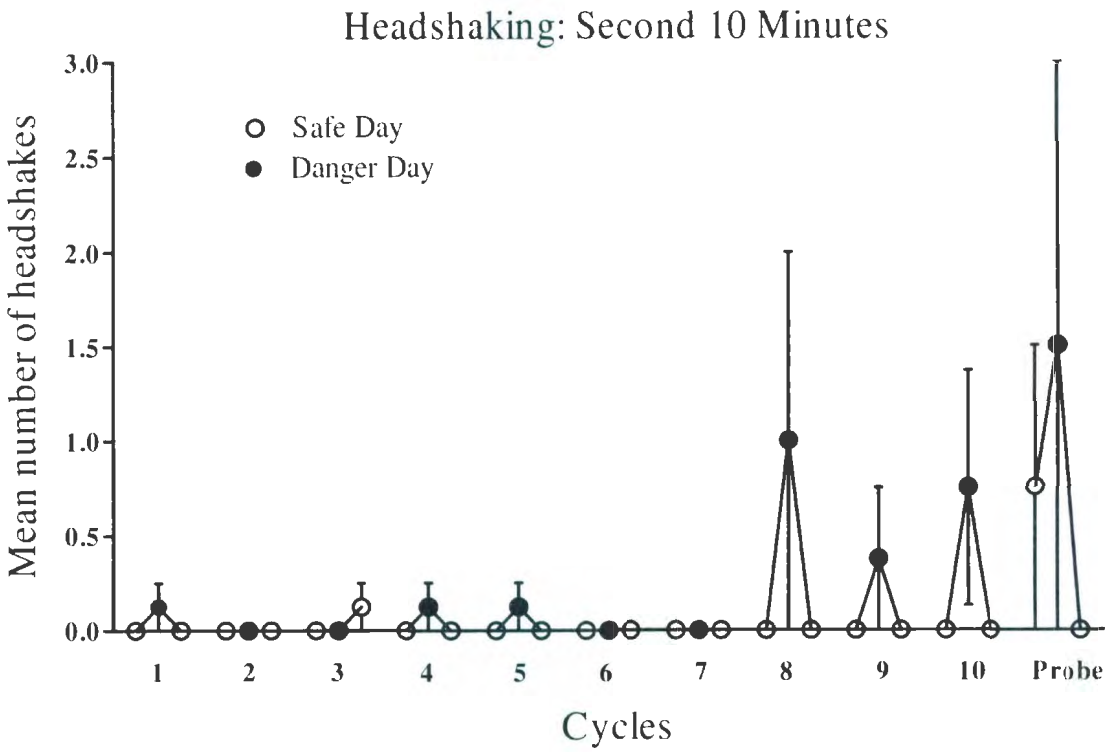
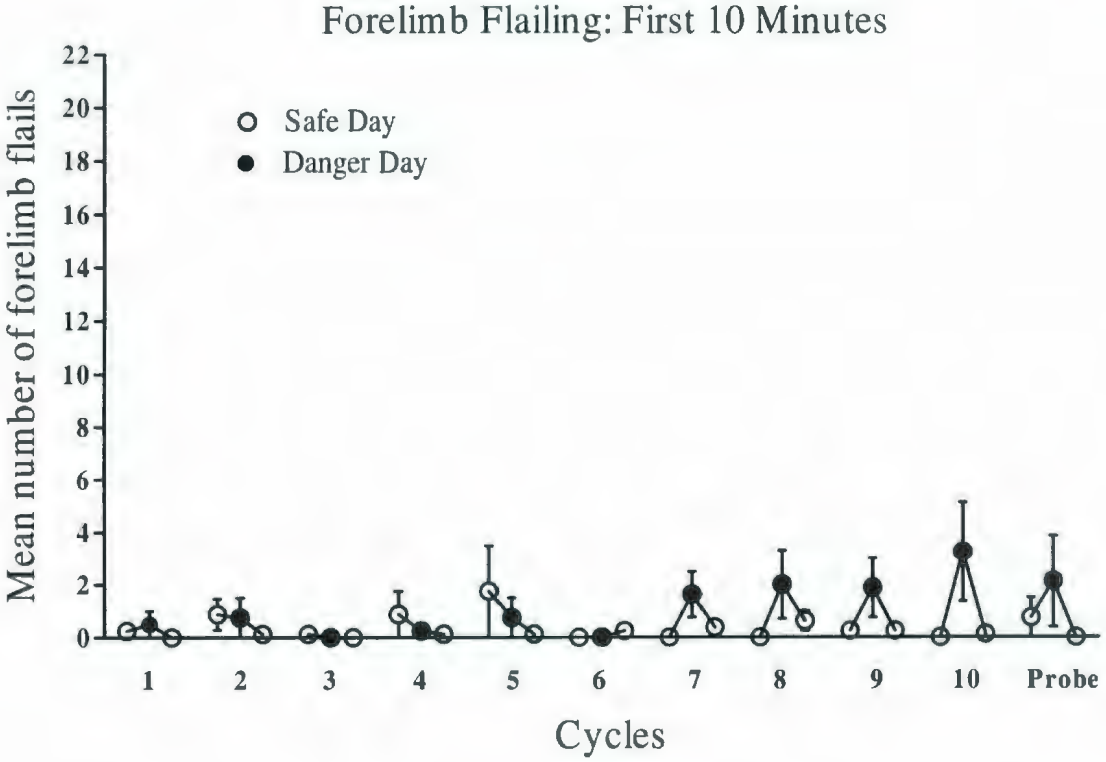

Headshaking: Second 10 Minutes
Safe Day
Danger Day
Mean number of headshakes
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

Figure Caption

Figure 15. Mean (±SEM) number of forelimb flails on each Safe-Danger-Safe cycle (1 through 10) and on the probe trial for the first and second ten minute intervals.


Forelimb Flailing: First 10 Minutes
Mean number of forelimb flails
Safe Day
Danger Day
0
2
4
6
8
10
12
14
16
18
20
22
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

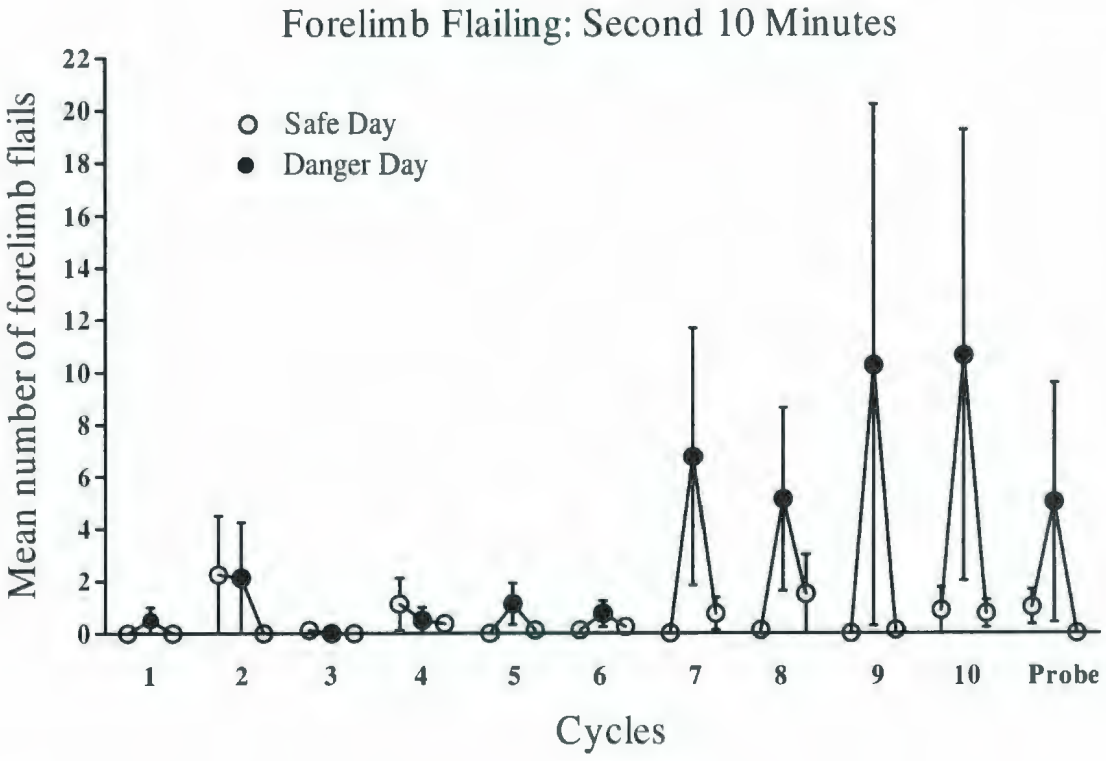
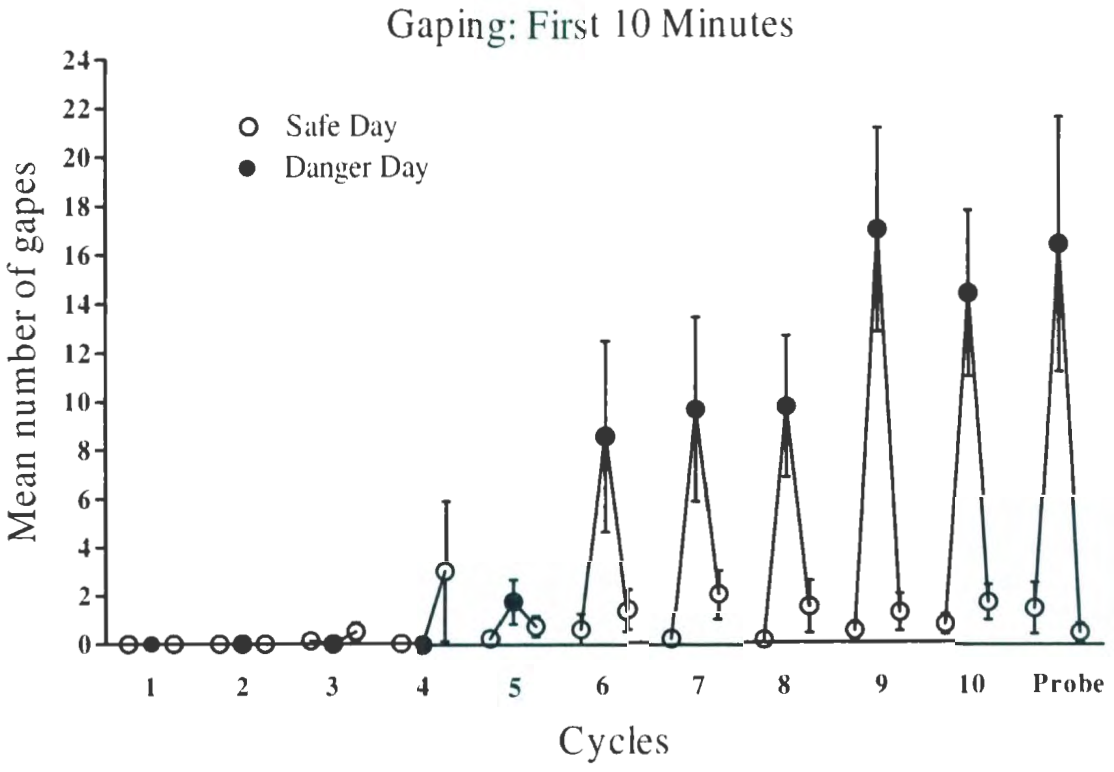

Forelimb Flailing: Second 10 Minutes
Mean number of forelimb flails
Safe Day
Danger Day
0
2
4
6
8
10
12
14
16
18
20
22
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

Figure Caption

Figure 16. Mean (±SEM) number of gapes on each Safe-Danger-Safe cycle (1 through 10) and on the probe trial for the first and second ten minute intervals.


Gaping: First 10 Minutes
Safe Day
Danger Day
Mean number of gapes
0
2
4
6
8
10
12
14
16
18
20
22
24
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

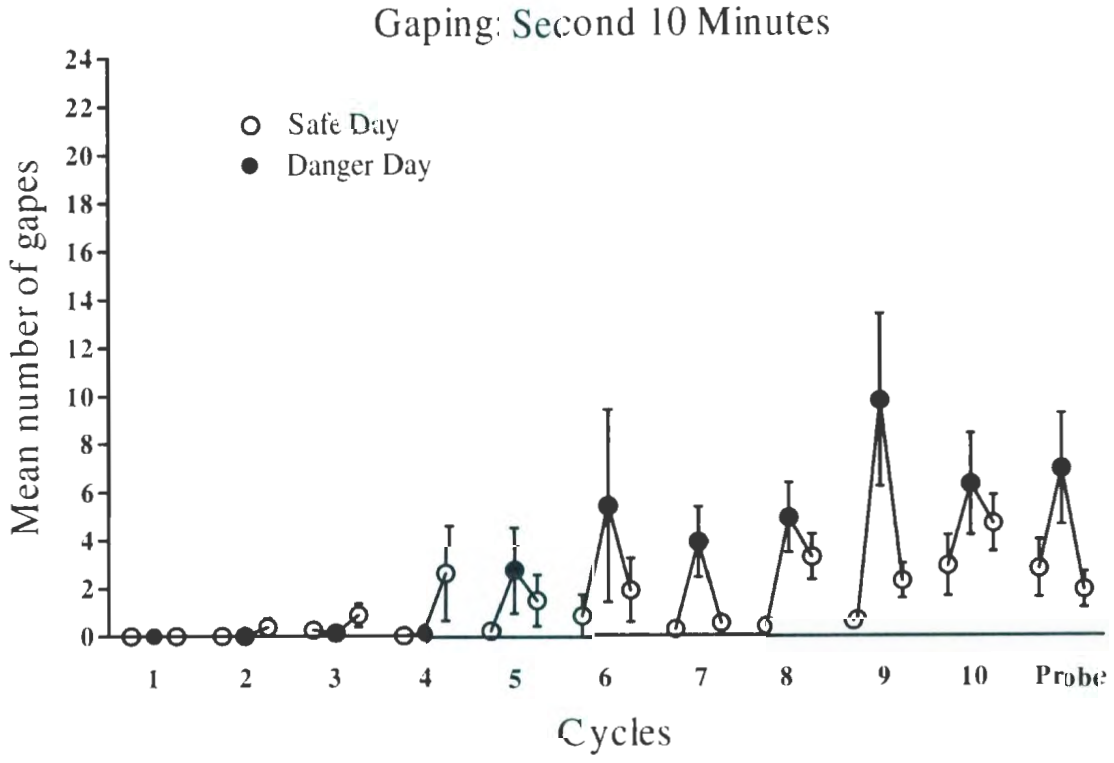
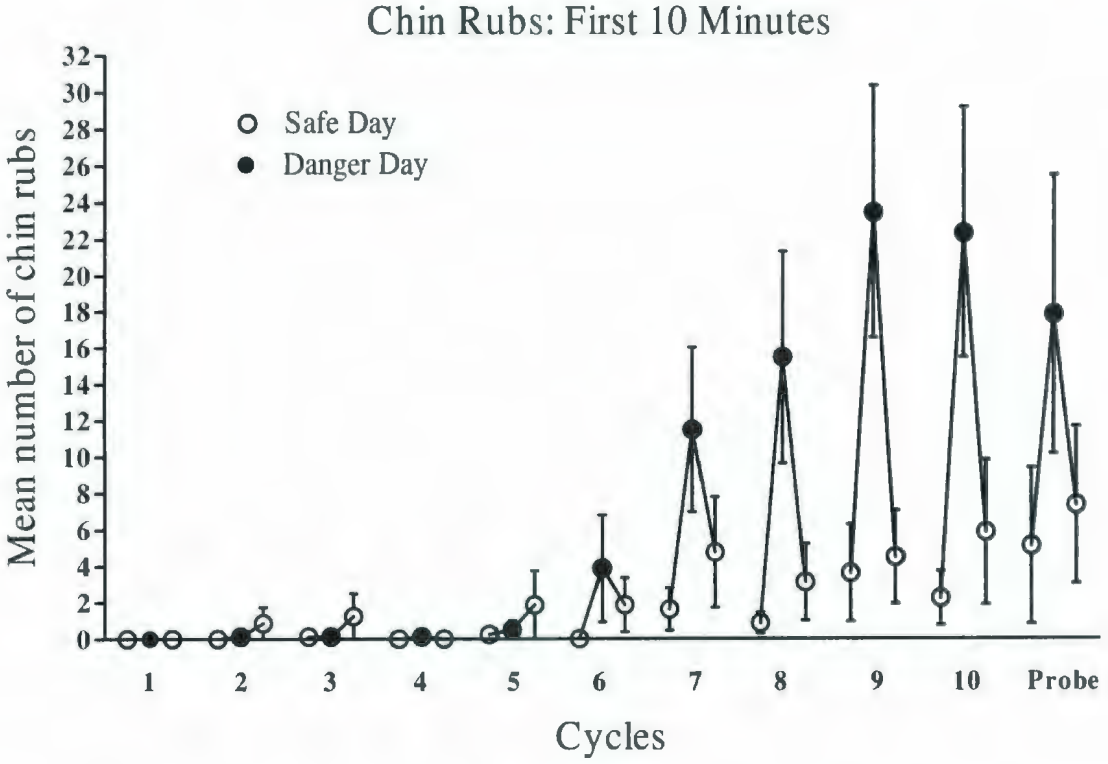

Gaping: Second 10 Minutes
Safe Day
Danger Day
Mean number of gapes
0
2
4
6
8
10
12
14
16
18
20
22
24
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

Figure Caption

Figure 17. Mean (±SEM) number of chin rubs on each Safe-Danger-Safe cycle (1 through 10) and on the probe trial for the first and second ten minute intervals.


Chin Rubs: First 10 Minutes
Mean number of chin rubs
Safe Day
Danger Day
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

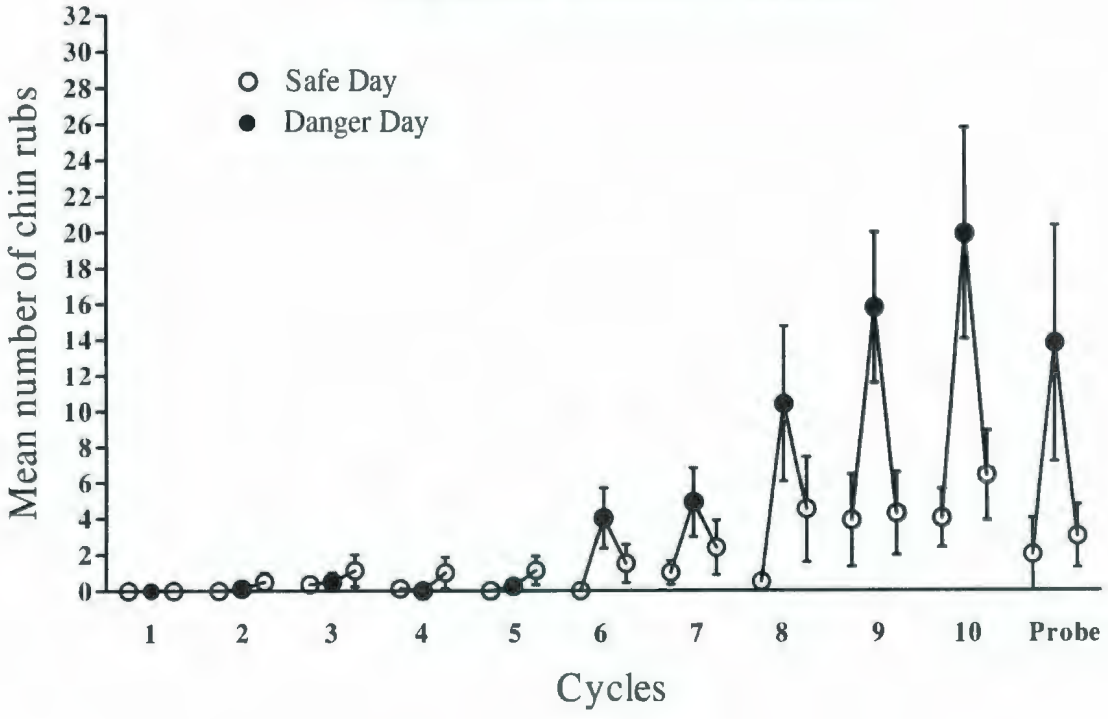
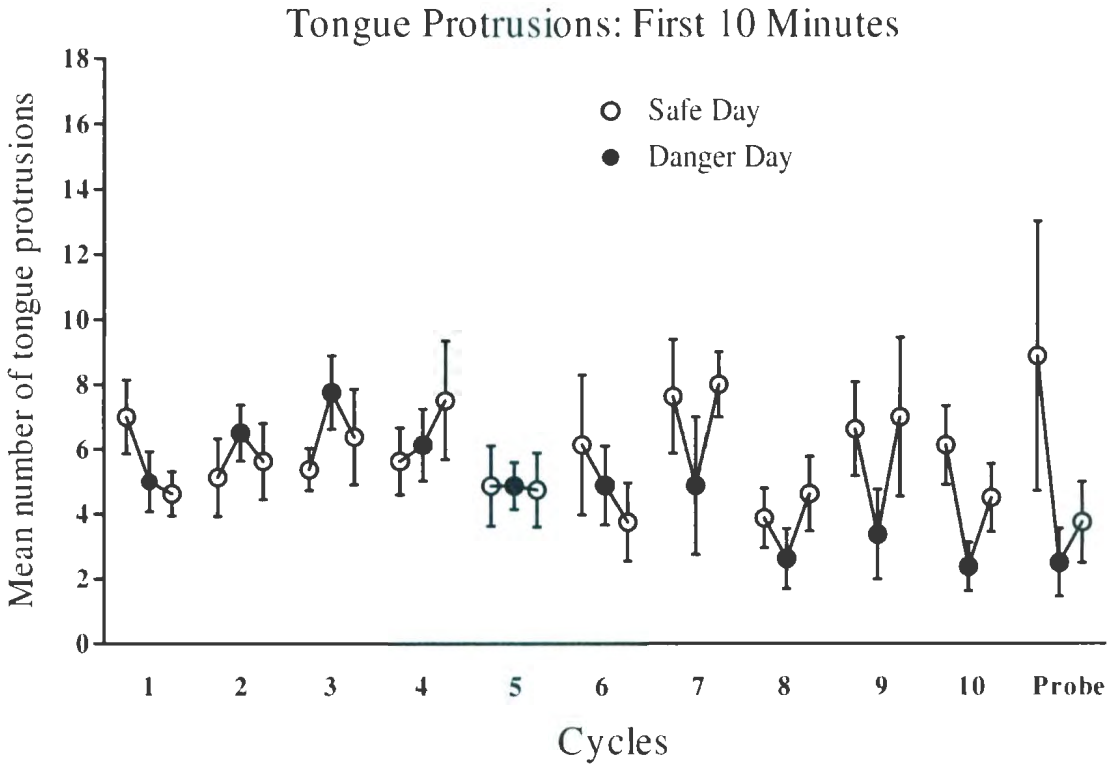

Chin Rubs: Second 10 Minutes
Mean number of chin rubs
Safe Day
Danger Day
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

Figure Caption

Figure 18. Mean (±SEM) number of tongue protrusions on each Safe-Danger-Safe cycle (1 through 10) and on the probe trial for the first and second ten minute intervals.


Tongue Protrusions: First 10 Minutes
Safe Day
Danger Day
Mean number of tongue protrusions
0
2
4
6
8
10
12
14
16
18
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

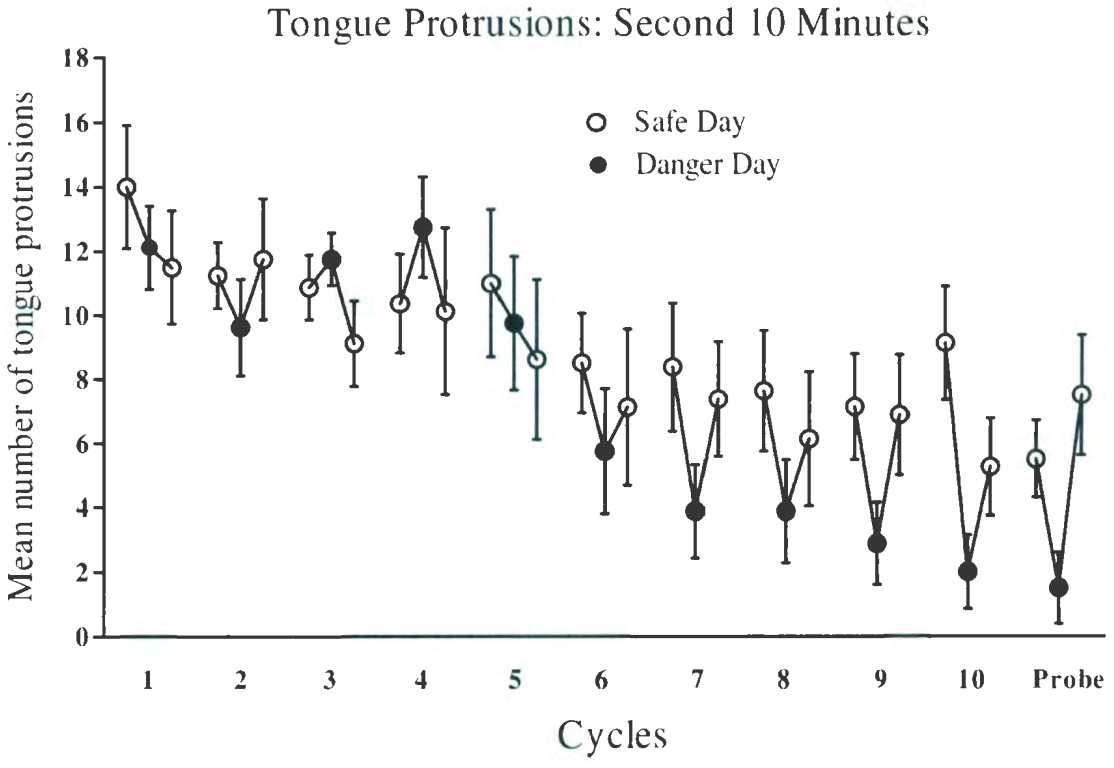
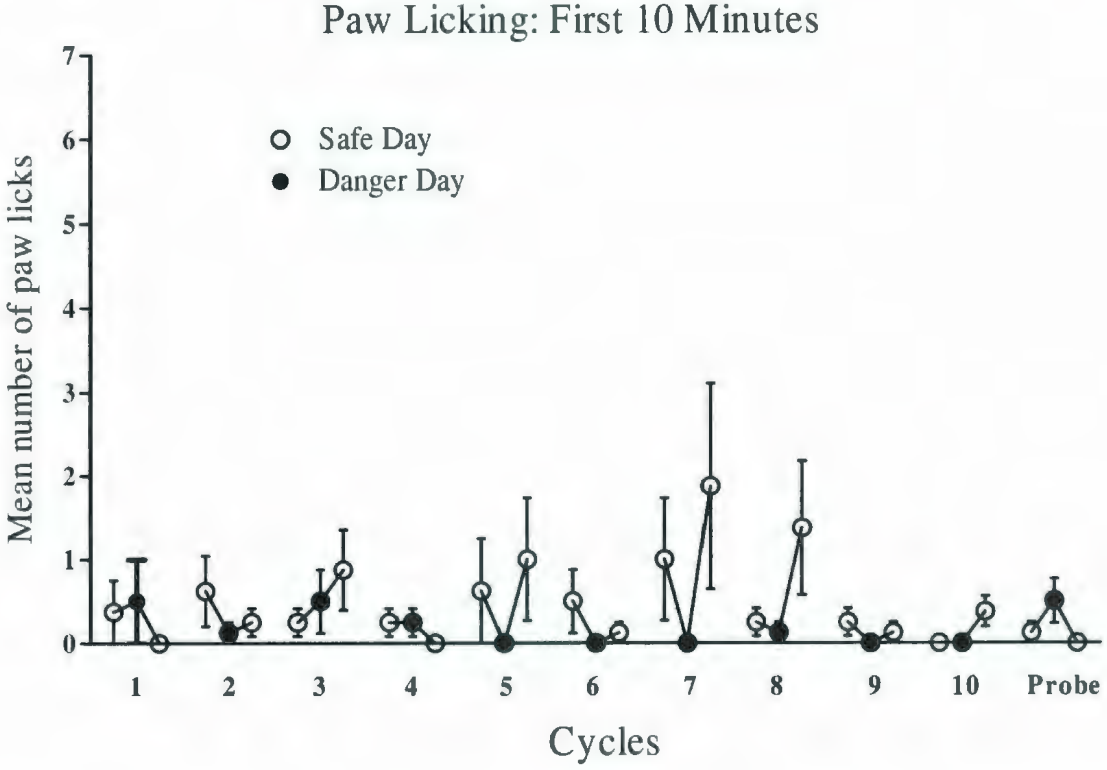

Tongue Protrusions: Second 10 Minutes
Safe Day
Danger Day
Mean number of tongue protrusions
0
2
4
6
8
10
12
14
16
18
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

Figure Caption

Figure 19. Mean (±SEM) number of paw licks on each Safe-Danger-Safe cycle (1 through 10) and on the probe trial for the first and second ten minute intervals.


Paw Licking: First 10 Minutes
Safe Day
Danger Day
Mean number of paw licks
0
1
2
3
4
5
6
7
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

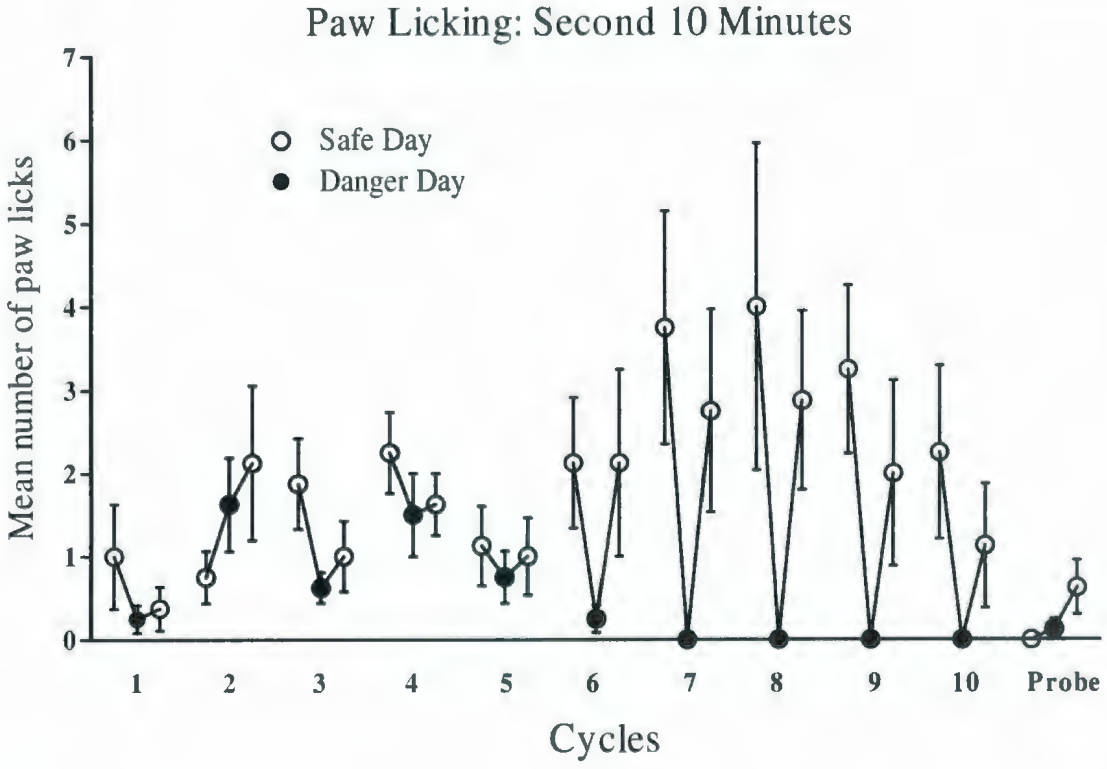
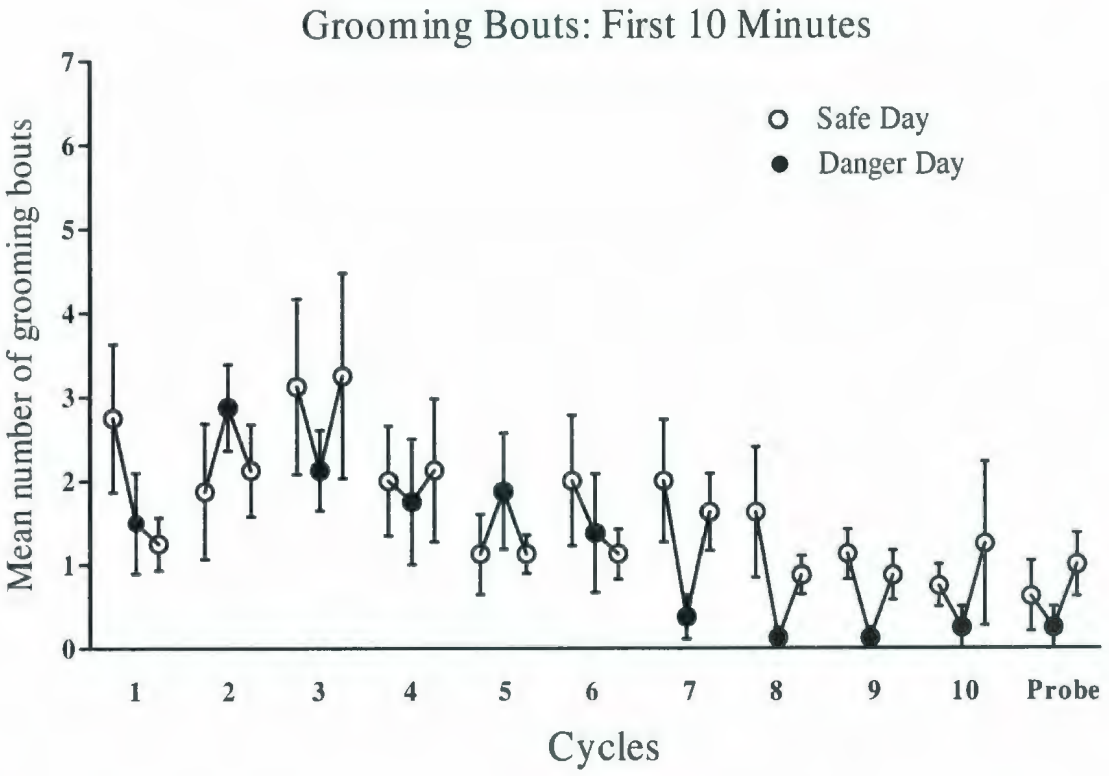

Paw Licking: Second 10 Minutes
Safe Day
Danger Day
Mean number of paw licks
0
1
2
3
4
5
6
7
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

Figure Caption

Figure 20. Mean (±SEM) number of grooming bouts on each Safe-Danger-Safe cycle (1 through 10) and on the probe trial for the first and second ten minute intervals.


Grooming Bouts: First 10 Minutes
Safe Day
Danger Day
Mean number of grooming bouts
0
1
2
3
4
5
6
7
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

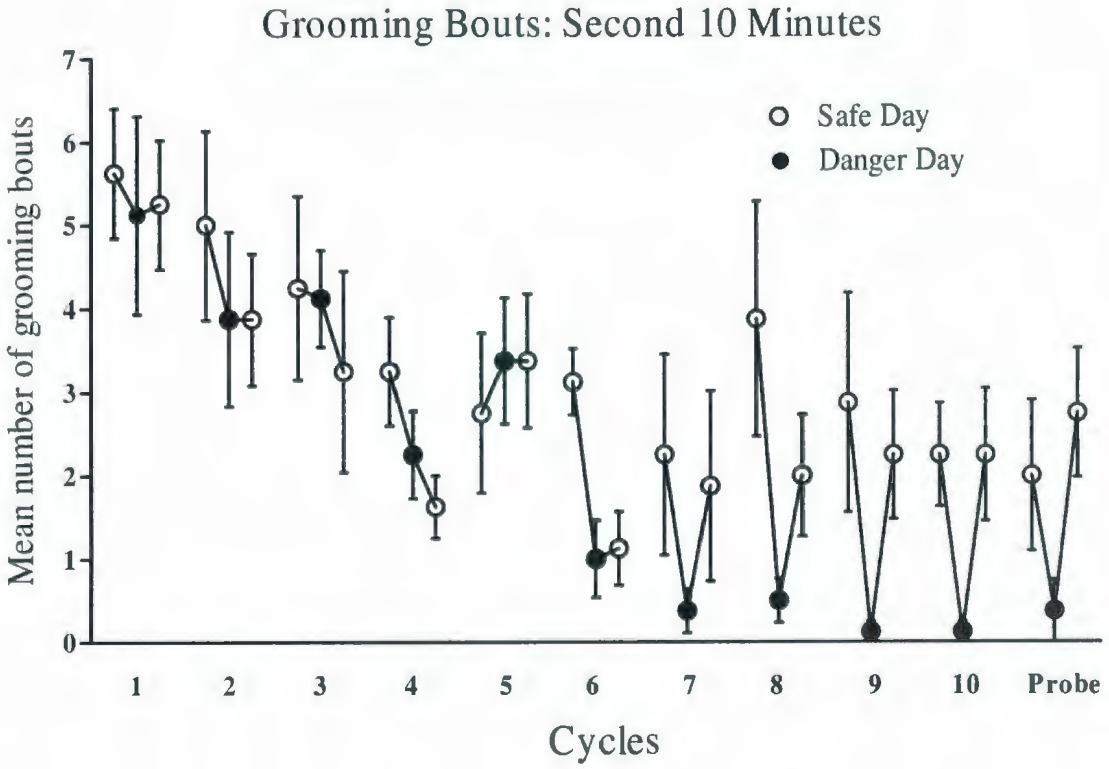
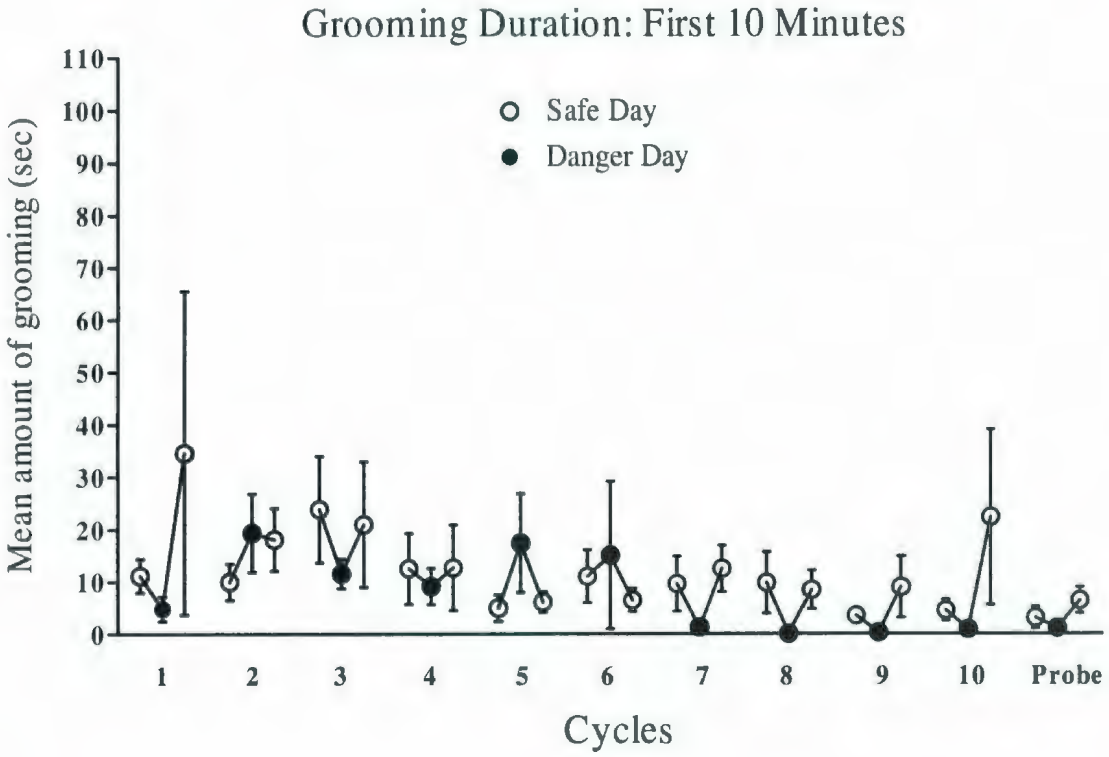

Grooming Bouts: Second 10 Minutes
Safe Day
Danger Day
Mean number of grooming bouts
0
1
2
3
4
5
6
7
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

Figure Caption

Figure 21. Mean (±SEM) amount of grooming (sec) on each Safe-Danger-Safe cycle (1 through 10) and on the probe trial for the first and second ten minute intervals.


Grooming Duration: First 10 Minutes
Safe Day
Danger Day
Mean amount of grooming (sec)
110
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

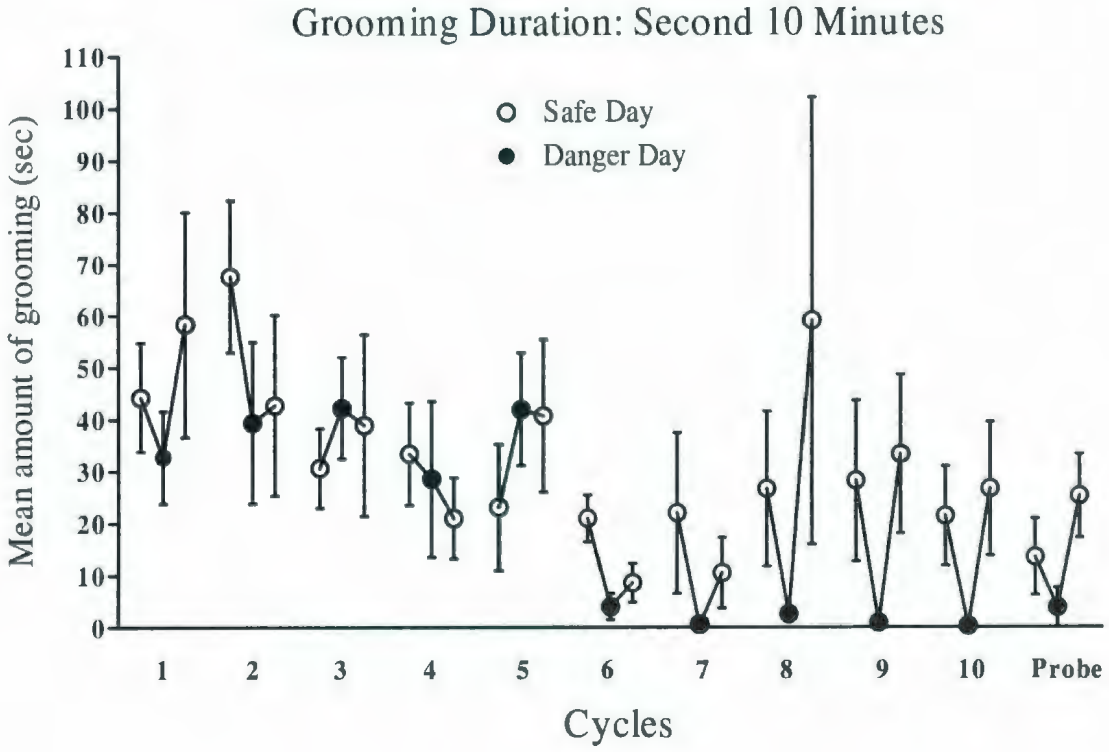
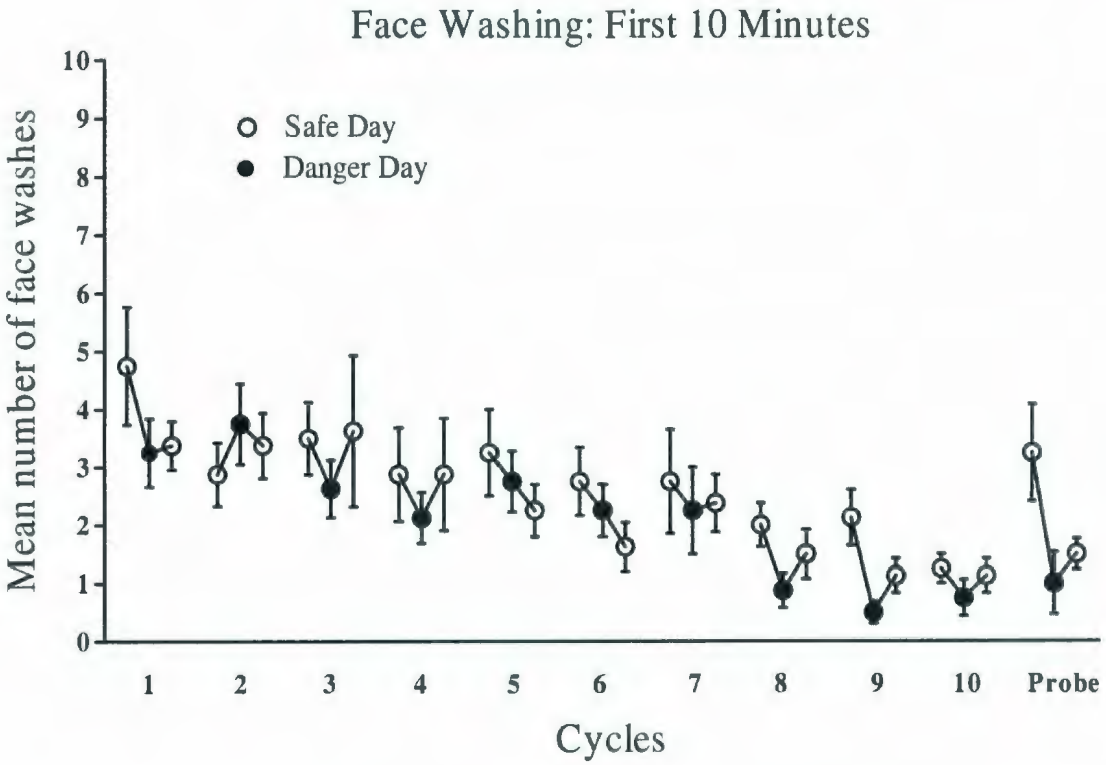

Grooming Duration: Second 10 Minutes
Safe Day
Danger Day
Mean amount of grooming (sec)
110
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

Figure Caption

Figure 22. Mean (±SEM) number of face-washes on each Safe-Danger-Safe cycle (1 through 10) and on the probe trial for the first and second ten minute intervals.


Face Washing: First 10 Minutes
Mean number of face washes
Safe Day
Danger Day
0
1
2
3
4
5
6
7
8
9
10
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

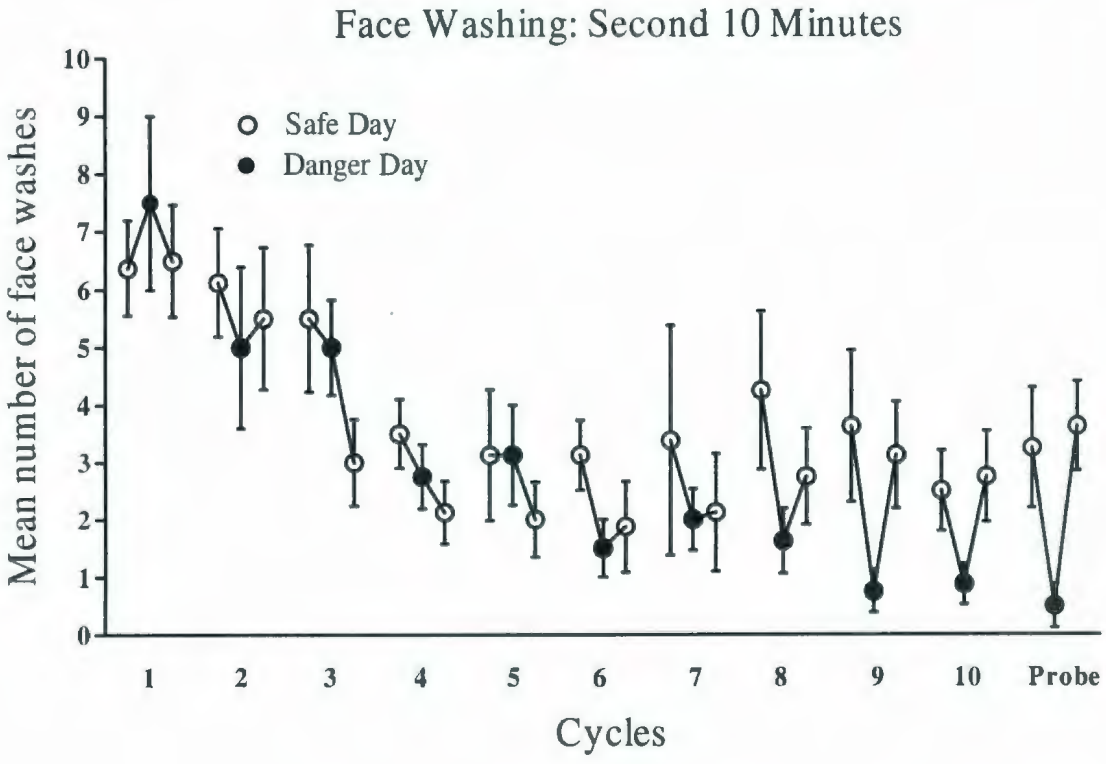
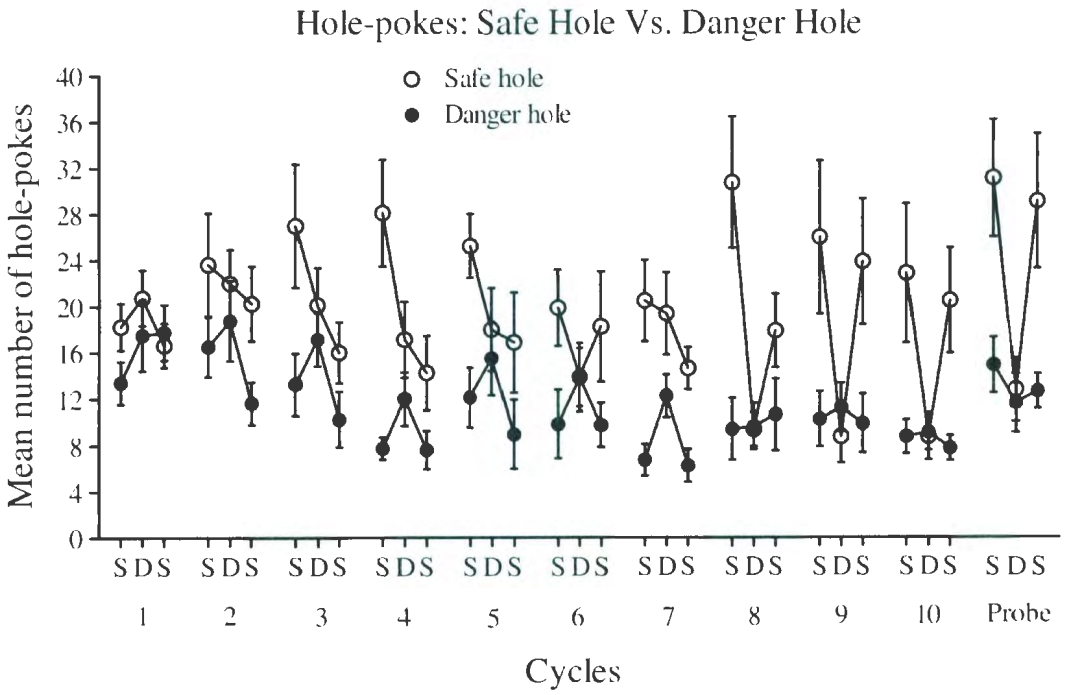

Face Washing: Second 10 Minutes
Mean number of face washes
Safe Day
Danger Day
0
1
2
3
4
5
6
7
8
9
10
1
2
3
4
5
6
7
8
9
10
Probe
Cycles

Figure Caption

Figure 23. Mean (±SEM) number of hole-pokes on each Safe-Danger-Safe cycle (1 through 10) and on the probe trial during the first ten minute interval.


Hole-pokes: Safe Hole Vs. Danger Hole
Safe hole
Danger hole
Mean number of hole-pokes
40
36
32
28
24
20
16
12
8
4
0
S D S
1
2
3
4
5
6
7
8
9
10
Probe
Cycles