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Social Factors Modulate Toxin (LICL)-Induced Conditioned Disgust Responses in Male Rats

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ABSTRACT AND KEYWORDS

Rats, which are a non-emetic species, display conditioned disgust responses when re-exposed to a context previously associated with sickness. These conditioned disgust responses can be used to model anticipatory nausea in humans, a growing problem faced by numerous chemotherapy patients. This thesis found that social factors, in addition to contextual factors, can play a role in the expression of toxin (LiCl)-induced conditioned disgust in rats. The results show that a familiar, but not unfamiliar, social partner can serve as a cue for the display of conditioned gaping. Further, a variety of sensory cues may play a role in the development of socially-mediated conditioned disgust, as an odour cue (urine) alone was incapable of causing significant conditioned disgust. It was also found that socially-mediated conditioned disgust can be modulated by oxytocin, as an oxytocin receptor antagonist, L-368,899, significantly decreased the display of conditioned gaping. Therefore, these findings suggest that social factors can lead to the development and expression of toxin-elicited conditioned disgust responses in rats. This has implications for chemotherapy patients, as the development and expression of anticipatory nausea may also be impacted by social factors.

Keywords: toxin, malaise, sickness, anticipatory nausea, oxytocin, social recognition

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DEDICATION

I would like to dedicate this thesis to my parents. Your unwavering support and positive encouragement throughout my life has helped me build confidence and believe I am truly capable of anything I set my mind to. Thank you for giving me so many opportunities to learn and grow.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Disgust is an emotional response of revulsion characterized by a distinct facial expression, withdrawal response, and the possibility of an emetic reaction (Rozin and Fallon, 1987; Rozin, Haidt and McCauley, 2008). Disgust has been proposed to have evolved from an internal toxin and pathogen based food rejection system, to an external pathogen, toxin and infectious disease avoidance system (Curtis, de Barra & Aunger, 2011; Chapman & Anderson, 2012). Therefore, disgust is usually paired with an experience of nausea and revulsion, and sometimes it is accompanied by vomiting. This response can be observed in a variety of different species, including rats. Rats, however, are incapable of expelling harmful pathogens and toxins due to the lack of proper musculature and brainstem pathways (Horn et al., 2013). Although rodents are non-emetic species, they still display disgust through distinctive conditioned disgust reactions. Of these disgust reactions, the gaping response has been well documented as the most reliable indicator of disgust in rats (Parker, Rana & Limebeer, 2008). The gaping response is characterized by a large opening of the mouth, revealing the bottom incisors. This movement involves the repeated opening and closing of the lower mandible in rapid succession (approximately 5-7 times per bout) (Travers & Norgren, 1986). This mouth movement closely resembles the shrew retch, which is a facial movement made by the shrew, *Suncus murinus*, just before it vomits (Andrews et al., 2005; Horn et al., 2013). Studies have also shown that both the shrew retch and the rat gape require similar orofacial musculature (Travers & Norgren, 1986).

Gaping behavior is a conditioned behavior and has not been observed as a reflexive response to emetic treatments. Conditioned gaping responses can be seen when

rats are re-introduced into a context that has been previously associated with illness. Specifically, rats treated with a toxin (e.g. lithium chloride (LiCl) and other toxins) and placed in a context over a few conditioning trials, will show conditioned disgust responses, i.e. gaping, upon re-exposure to the context in a drug-free state (Limebeer, Hall and Parker, 2006; Limebeer et al., 2008; Rock et al., 2009; Tuerke, Leri & Parker, 2009; Ossenkopp et al., 2011). Although gaping is a conditioned response, treatment with anti-emetic agents, such as ondansetron (Limebeer & Parker, 2000) and the 5-HT1A agonist 8-OH-DPAT (Limebeer & Parker, 2003), have been shown to attenuate the gaping response, thus providing evidence that gaping behaviour is an index of a nauseous state. Therefore, "conditioned gaping" has been accepted as the most quantifiable and reliable indicator of nausea in rats.

Conditioned disgust responses exhibited by rats following toxin-induced sickness can be used to model anticipatory nausea (AN) in humans. Anticipatory nausea is a learned response following chemotherapy treatment which occurs in over a quarter of patients by the fourth treatment (Morrow & Roscoe, 1997). This learned response has been explained as a classically conditioned response (Matteson et al., 2002; Neese et al., 1980; Tomoyasu, Bovbjerg & Jacobsen, 1996). The sight of the hospital or nurse acts as a conditioned stimulus (CS). When the CS is paired with an unconditioned stimulus (US) (e.g. chemotherapy) it results in an unconditioned response (UR) (e.g. nausea). After as little as one chemotherapy treatment, the CS alone is able to elicit a UR; which is similar to the response produced by the chemotherapy drug itself. Although drug treatments exist to help manage acute vomiting (e.g. the 5- hydroxytryptamine 3 (5-HT3) receptor

antagonist ondansetron; Navari, 2009), nausea is still a growing problem faced by many chemotherapy patients today.

Many chemotherapy patients report that simply the sight of the hospital context is able to trigger feelings of nausea prior to chemotherapy (Roscoe et al., 2011). However, some patients also report that even the sight of the nurse or oncologist alone is able to trigger feelings of nausea and/or vomiting prior to the chemotherapy administration. In fact, one oncologist anecdotally reported that when his patient witnessed him out of the hospital context, the patient experienced nausea and vomiting (Divgi, 1989). Therefore, social factors, in addition to contextual factors, may play a role in the development and expression of conditioned disgust in rats and ultimately AN in humans.

To date, research using the rat model of AN has primarily focused on the ability of a rat to associate either a context or taste with sickness. However, there is reason to believe that social factors may also play a role in the modulation and expression of conditioned disgust and AN. Social factors have a large role in toxin avoidance and aversion, as well as toxin-elicited and interpersonal disgust in humans (Tybur et al., 2013). Rodents also display innate, and acquired, avoidant responses to an actual or potential infection threat from a conspecific, or cues associated with the conspecific. (Akawara, Cruz & Deak, 2011; Kavaliers et al., 2004). Early research using Mongolian gerbils found that animals treated with lithium chloride, immediately after a brief encounter with a conspecific, showed decreased approach to, and investigation of, the conspecific 48 hours later (Pettijohn, 1981). More recently a study investigated the role that social interactions have in the retrieval conditioned taste avoidance. They exposed a mouse to a novel saccharin solution, injected it with LiCl, and exposed it to a conspecific.

They found that the mice who received social interactions following, though not during, sickness significantly increased their consumption of saccharin throughout the test days, suggesting an attenuation of the taste avoidance (Hishimura, 2015).

A typical response for most species during sickness is to withdraw from social interaction. The withdrawal from social interaction has been proposed as a way for the animal to conserve energy and resources to help fight the infection and increase the animals' chances of survival (Hart, 1988). This is consistent with a recent study by Guitton, Klin and Dudai in 2008. Using a combination of conditioned taste avoidance and social interaction measures, they showed a decrease in social interactions and an increase in social withdrawal behaviours in rats following re-exposure to the conditioned taste. However, there is also evidence suggesting that animals seek social interaction during sickness to decrease the negative side-effects associated with malaise. One study found that male zebra finches displayed decreased sickness behaviours in a colony setting compared to in isolation (Lopes et al., 2012). They also displayed increased social initiations and interactions towards conspecifics. This is also, in part, consistent with studies showing ambivalent social responses by mice and rats towards either an infected, or potentially infected, individual, as well as the hesitant responses of humans towards unfamiliar individuals or endotoxins (Kavaliers et al., 2004; Parkinson et al., 2012; Lopes et al., 2012). Therefore, it appears that the presence of a conspecific, and social interactions, can modulate conditioned taste avoidance, and potentially the expression of disgust.

Olfactory cues have an important role during social interactions in many species, including humans. The social behaviours of many mammals relies on chemical signals

from conspecifics (Brennan & Kendrick, 2006). Rodents can distinguish and display aversive responses to infected individuals on the basis of odour (Kavaliers et al., 2004). Olfactory cues, therefore, help animals carefully navigate social interactions to avoid disgust associated social cues (Kavaliers et al., 2004). Odour cues are also involved in the mediation of various aspects of human behaviour, including the disgust response (Moshkin et al., 2012, Olsson, 2014). Odour cues, therefore, seems to play an essential role in social interactions, as well as social recognition, and could possibly modulate the expression of disgust.

Social recognition and the processing of other social information is primarily mediated by the nonapeptides oxytocin (OT) and arginine-vasopressin (AVP). OT, as well as AVP, play important roles in the mediation of social avoidance and social recognition in a variety of different species (e.g. Popik & van Ree, 1991; Donaldson & Young, 2008; Choleris et al., 2009; Lukas et al., 2011). Both rats and mice given OT antagonists showed significantly reduced naturally occurring social preference towards an unfamiliar conspecific (Lukas et al., 2011). Oxytocin has also been found to be involved in the mediation of olfactory-based social recognition in both male and female rodents (Kavaliers et al., 2004; Choleris et al., 2009). Human studies have shown that intranasal OT administration facilitates social encounters (Bartz & Hollander, 2006), as well as decreases social anxiety and fear responses (Petrovic et al., 2008; Kirsch et al., 2005). However, recent research has found that intranasal administration of OT led to ambivalent approach and avoidance motor responses to emotional stimuli (Theodoridou, Penton-Voak & Rowe, 2013). OT has also been shown to be involved in the expression of pathogen-related disgust in both humans (Theodoridou, Penton-Voak & Rowe, 2013)

and non-human rodents (Kavaliers et al., 2004). Therefore, OT could play a role in the expression of conditioned disgust, including that which is socially-mediated.

Early research by O'Connor, Cheng and North (1987) found that administering LiCl intraperitoneally resulted in increased plasma levels of OT/AVP. Later studies have shown that LiCl-induced conditioned taste avoidance (CTA) was associated with increased activation of OT/AVP neurons (Olszewski et al., 2013). The administration of an OT receptor antagonist, L-368,899, prior to the two-bottle test (retrieval of CTA) did not cause avoidance of the saccharin solution (Olszewski et al., 2013), whereas administration of the receptor antagonist during the CTA acquisition phase significantly impaired acquisition. Although, whether or not OT is associated with conditioned disgust and AN is not known.

The present study examined whether social factors and cues can have an impact on the development and expression of conditioned disgust in rats. Specifically, the study sought to determine how the presence of: (i) a familiar social partner, (ii) an unfamiliar social partner, (iii) an odour cue from a familiar social partner, and (iv) an oxytocin receptor antagonist, L-368,899, affect the acquisition and/or expression of conditioned disgust responses in rats. It was hypothesized that the animals would associate a familiar, but not an unfamiliar, social partner with sickness, and display the conditioned disgust responses. Further, it was hypothesized that a familiar social odour (urine) would lead to the display of conditioned disgust responses. Finally, it was hypothesized that an oxytocin receptor antagonist would diminish the gaping responses in LiCl-treated rats conditioned with a familiar social partner.

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CHAPTER 2

SOCIAL FACTORS MODULATE CONDITIONED DISGUST IN MALE RATS

2.1 Introduction

Disgust has long been recognized as a basic and universal human emotion that is consistent across cultures (Darwin, 1872). It has been proposed that disgust evolved to rid the body and mouth of noxious substances and toxins, as well as to motivate and facilitate avoidance of contact with disease-causing organisms and infectious materials (Curtis & de Barra, 2011). Disgust encompasses a typical facial expression, as well as a withdrawal response and experience of revulsion, which may be associated with vomiting (emesis) (Rozin & Fallon, 1987; Rozin, Haidt, & McCauley, 2008). These distinct responses can be observed in human adults and neonates (Greimel et al., 2006; Steiner, 1973) as well as in a variety of non-human animals including rodents (Grill & Norgren, 1987), apes and monkeys (Berridge, 2000). Non-emetic species, such as the rat, lack the musculature and brainstem pathway needed to expel harmful toxins (Horn et al., 2013). Therefore, disgust is inferred from facial movements such as gaping; a large opening of the mouth, revealing the bottom incisors. The gaping response is proposed to be a reliable indicator of disgust, with results of comparative, evolutionary and neurobiological investigations supporting the gape as an indicator of disgust and nausea in rats (Parker, Rana, & Limebeer, 2008). Comparative studies have revealed that the rodent gape involves similar orofacial musculature as vomiting in emetic species (Travers & Norgren, 1986), and is topographically similar to the orofacial components of retching in the shrew; a distinct facial expression made immediately before an emetic response (Horn et al., 2013). At an evolutionary level, disgust is proposed to have expanded from an internal toxin and pathogen based food rejection system, to an external pathogen and infectious disease avoidance system (Curtis, 2011). Early work by Garcia and colleagues (1985) showed

that the association between taste, sucrose, and malaise, elicited by a toxin (LiCl), resulted in conditioned taste aversions and conditioned disgust reactions in rats upon re-exposure to the taste. Results of neurobiological investigations have revealed similar neural systems in the regulation of disgust across species, with evidence that the insular cortex and its sub regions are involved in the expression of both gaping in rats and disgust responses in humans (Panksepp, 2007; Harrison et al., 2010; Chapman & Anderson, 2012; Tuerke et al., 2012). Humans and non-humans also can display disgust responses upon re-exposure to a context that has been previously associated with a toxin (Parker, 2003).

Anticipatory nausea (AN), a conditioned form of nausea occurring before administration of a chemotherapy drug (Roscoe et al., 2011), can be modeled by conditioned gaping in rats. Just as rats display nausea (conditioned disgust responses) following re-exposure to a context previously associated with sickness, chemotherapy patients experience a similar phenomenon before a chemotherapy session. In rats, Ossenkopp et al. (2011) demonstrated that dose related conditioned gaping occurs when the animal is placed in a context that has been previously paired with an emetic agent, such as lithium chloride (LiCl). This phenomenon can be explained using Pavlovian conditioning. When a conditioned stimulus (CS) (e.g. the sight of the hospital or nurse), is paired with an unconditioned stimulus (US) (e.g. chemotherapy), it ultimately produces an unconditioned response (UR) (e.g. nausea). After a few chemotherapy treatments, the CS alone is able to elicit a UR, which is similar to that produced by the chemotherapy drug itself.

There is increasing interest in the role that social cues have in mediation of disgust responses. Social factors have a key role in toxin detection and avoidance, as well as toxin-elicited and interpersonal disgust in humans (Tybur et al., 2013). Similarly, rodents display innate and acquired aversive, and avoidant, responses to potential, as well as actual, infection threats from conspecifics, or from cues associated with them (Arakawa, Cruz & Deak, 2011; Kavaliers et al., 2004). Many chemotherapy patients report that simply the sight of the nurse or oncologist alone is able to trigger feelings of disgust and nausea (Parkinson et al., 2012). In fact, one oncologist reported that seeing a patient in the mall triggered vomiting and nausea in the patient (Divgi, 1989). This raises the possibility that social factors may also play a role in the modulation of anticipatory nausea.

An early study by Pettijohn (1981) demonstrated that Mongolian gerbils treated with lithium chloride, immediately following a brief encounter with a conspecific, 48 hours later showed decreased approach to, and investigation of, the conspecific. More recently Hishimura (2015) investigated the role that social interactions may play in the expression of conditioned taste avoidance. They exposed a mouse to a novel saccharin solution, injected it with LiCl, and exposed it to a conspecific. They found that the mice who received social interactions following, though not during, sickness significantly increased their consumption of saccharin throughout the test days, suggesting an attenuation of the taste avoidance. Normally, animals experiencing sickness withdraw from social interactions, presumably to conserve energy and resources to help fight the infection and increase the animals' chances of survival (Hart, 1988). Consistent with this, a study by Guitton, Klin and Dudai (2008), using a combination of conditioned taste

avoidance and social interaction measures, showed a decrease in social interactions and an increase in social withdrawal behaviours in rats following re-exposure to the conditioned taste. Therefore, it appears that social interactions, or the presence of a conspecific, can have an impact on conditioned taste avoidance, and potentially the expression of disgust.

In rodents, olfactory cues have an important role in modulation of social interactions, as well as mediating disgust associated social cues (Kavaliers et al., 2004). The social behaviours of many mammals relies on chemical signals from conspecifics (Brennan & Kendrick, 2006). In rodents, odour cues play a major role in determining social interactions and mediating disgust associated aversive responses (Kavaliers et al., 2004, Choleris et al., 2009). Rodents can distinguish and display aversive responses to infected individuals on the basis of odour (Kavaliers et al., 2004). Odour cues are also involved in the mediation of various aspects of human behaviour, including that of disgust responses (Moshkin et al., 2012; Olsson et al., 2014). Odour, therefore, seems to play a vital role in social recognition, as well as acting as a modulator of potential positive or negative social interactions and potentially the expression of disgust.

The present study examined the roles of social factors in the expression of toxin (LiCl) elicited conditioned disgust (gaping and associated behaviours) in male rats by examining how the presence of: (i) a familiar social partner, (ii) an unfamiliar social partner and (iii) an odour cue from a familiar social partner, affects the acquisition and/or expression of conditioned disgust responses in rats.

2.2 Experiment 1: Effect of a familiar social partner on conditioned disgust

2.3 Methods

2.3.1 Animals

Subjects were forty-four naïve adult male Long-Evans rats (Charles River, Quebec, Canada) weighing between 250- 350g at the start of the experiment. Rats were pair-housed in translucent polypropylene cages (45 x 22 x 20cm) in a colony room maintained at 21 + 1 °C and under a 12 L: 12 D cycle (light 0700 – 1900h). Rats had *ad libitum* access to both food (ProLab Rat Chow RMH 3000) and water. Animals were tested during the light phase of the light:dark cycle between 0800 and 1500 h. All procedures were carried out in accordance to the Canadian Council of Animal Care guidelines and were approved by the Institutional (University of Western Ontario) Animal Care Committee.

2.3.2 Drugs

Lithium chloride was dissolved in distilled water to a molarity of 0.15M and given at a dose of 128 mg/kg (20 ml/kg). Isotonic saline (NaCl, 0.9%; 0.15M), at the same dose as the LiCl, was employed as the control vehicle injection (20 ml/kg). LiCl at 128 mg/kg has been previously shown to produce robust conditioned aversive responses in rats (Limebeer, Hall, & Parker, 2006; Limebeer et al., 2008; Cloutier et al., 2011). All injections were administered intraperitoneally immediately before conditioning.

2.3.3 Apparatus

The conditioning chamber (used on all conditioning days and test day 2) consisted of a white Plexiglas box (29 cm x 25cm x 29 cm) with two ventilation holes on opposite sides of the box. The box was set atop a clear glass plate with a mirror mounted at a 45-degree angle beneath the glass plate to view the ventral surface of the animal. A distinct context (used on test day 1) was provided by a transparent black and white striped box

(29 cm x 25 cm x 29 cm) with two ventilation holes (on opposite sides of the box), set atop the clear glass plate. A mirror was again mounted at a 45-degree angle beneath the glass plate. Two 40 W red lights positioned under the striped chamber provided additional distinctive lighting cues. Although rats are not considered to perceive the colour red (Jacobs, Penwick, & Williams, 2001), these lights provided illumination distinct from that on the conditioning day. Behavioural responses on the test days were videotaped with a video camera (Sony DCR-DVD201, London, Ontario) positioned approximately 1 m from the mirror. The camera was attached directly to a computer (LG, London, Ontario).

2.4 Procedure

The experimental procedures are summarized in Figure 2.1.

2.4.1 Social and non-social conditioning

Rats were individually housed for one week. Prior to conditioning trials, rats were habituated for one 10-minute session in the conditioning context located in a room different than the colony room, followed 24 hours later by habituation to the distinct striped context for 10 minutes, located in a different room than conditioning. Twenty-four hours after the second habituation, the conditioning phase commenced. The conditioning phase consisted of four days, each separated by 72 hours. On each conditioning day, each rat was intraperitoneally (i.p.) injected with either LiCl (0.15 M, 20 ml/kg) or saline vehicle (0.9 % NaCl, 20 ml/Kg) and immediately placed in the conditioning apparatus for 30 minutes. Half of the animals from each group were placed in the apparatus in the presence of an uninjected male social partner [Groups: LiCl-Social (n = 10) and Na-Social (n = 13),

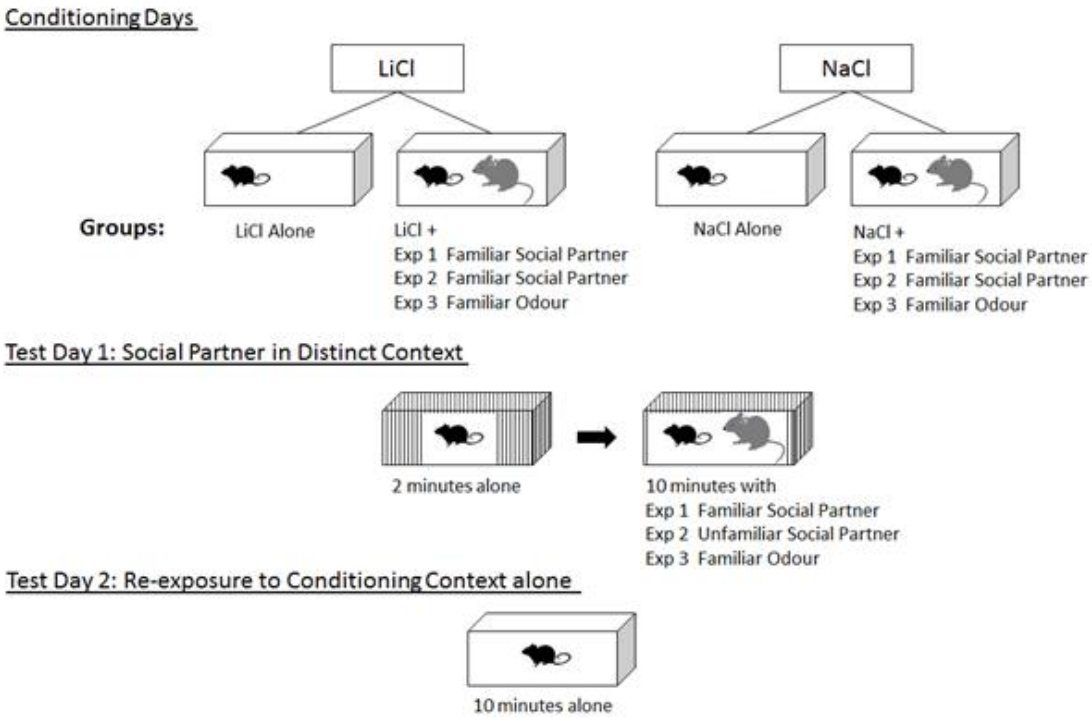

Figure 2.1. Outline of procedures used for experiments 1-3. Details of procedures are given in the text.

with the same social partner used on each conditioning day (familiar)], while the other half were conditioned alone [Groups: LiCl-Alone (n = 10) and Na-Alone (n = 11)]. Social partners were randomly selected and were animals different from the initial pair housed mates.

2.4.2 Social partner in a distinct context (Test Day 1)

Seventy-two hours following the fourth conditioning day each animal that had received either LiCl [LiCl-Social; LiCl-Alone] or NaCl [Na-Social; Na-Alone] was exposed to the distinctive striped chamber alone for two minutes, while in a drug free state, prior to the introduction of the social partner. They were then left undisturbed for 10 minutes while their interactions were recorded. Those animals that previously had a social partner during their conditioning [LiCl-Social; Na-Social] were exposed to the same familiar social partner, whereas those that had no social partner [LiCl-Alone; Na-Alone] now received a social partner. Conditioned disgust and
social behaviours displayed over the 10-minute period were recorded and scored using the Observer (Noldus Information Technology, Sterling Va) event –recording software.

Dependent disgust related behavioural variables analyzed included gaping frequencies and the composite scores of aversive responses that did not include gaping (paw treads, forelimb flails, chin rubs and head shakes (Cloutier et al., 2011, Cloutier, Kavaliers, & Ossenkopp, 2012), as well as spontaneous orofacial behaviours (tongue protrusions and mouth movements). Gaping was defined as lowering of the jawbone and the pushing or thrusting out of the lower teeth (Cloutier et al., 2011). Assessments of these distinct behaviours have been previously shown to have a very high inter-observer reliability (Cloutier et al., 2011, Cloutier, Kavaliers, & Ossenkopp, 2012).

Dependent social behaviours of the conditioned social partner were manually scored according to previously described criteria (Pellis et al., 1997). These behaviours included: 1. Number of social initiations: number of snout to nape contacts. 2. The number of facing defenses (withdrawal of the nape from the partner's snout by turning to face the partner) 3. The number of evasive defenses (withdrawal of the nape from the partner's snout by either running or turning away from the partner).

2.4.3 Re-exposure to original conditioning context

Twenty-four hours following Test Day 1, each experimental animal was exposed alone to the original white conditioning context (conditioning apparatus), for a 10-minute period, while in a drug free state. During this 10-minute period the rats' orofacial and aversive behaviours were again recorded.

2.5 Statistical analyses

The dependent conditioned disgust variables – gaping behaviour and composite aversive behaviours were each analyzed with separate 2X2 analysis of variance (ANOVA) for drug treatment and social condition. Gaping behaviour for the two-minute pre-exposure on Test Day 1 was also analyzed with a separate 2X2 ANOVA for drug treatment and social condition. Further, a split-plot ANOVA was employed to determine differences in spontaneous orofacial behaviours. These tests were repeated for Test Day 2. A repeated measures test was employed to measure differences in Test Day 1 and Test Day 2 for gaping and other aversive behaviours.

Social variables – social initiations, evasive defense, and facing defense were analyzed with separate split-plot ANOVAs for drug treatment and social condition. They were also analyzed with separate one way ANOVAs, with one between subject factor of

group (at 4 levels: Na-Alone; LiCl-Alone; Na-Social; and LiCl-Social). Least significant difference (LSD) post-hoc pair-wise comparisons were used following significant interactions and/or main effects to determine differences among the groups. LSD post-hoc test was chosen as this is an exploratory study. All hypothesis tests used an alpha of .05, and all data were analyzed using SPSS 18.0 for Windows.

2.6 Results

2.6.1 Distinct context (Test Day 1)

A 2X2 ANOVA for the two minute pre-exposure to the distinct context alone revealed a significant main effect of drug treatment on gaping behavior, $F(1, 40) = 5.867$, $p = 0.020$ (Figure 2.2A). Animals treated with LiCl gaped significantly more than animals treated with NaCl. A significant main effect of prior social condition (social versus alone) was also found, with rats conditioned with a social partner gaping significantly more than rats conditioned alone. $F(1, 40) = 4.075$, $p < 0.050$. Finally, a significant group X drug interaction effect was discovered, $F(1, 40) = 4.075$, $p < 0.050$, in that animals treated with LiCl and conditioned with a social partner gaped significantly more than animals treated with LiCl and conditioned alone.

2.6.2 Social partner in distinct context (Test Day 1)

Following this initial two minute exposure, social partners were introduced into the distinct chamber. The 2X2 ANOVA revealed a significant main effect of drug on gaping behavior, $F(1, 40) = 27.259$, $p < 0.01$, with LiCl treated rats gaping significantly more than NaCl treated rats. A significant main effect of social condition on gaping was also discovered, $F(1, 40) = 5.594$, $p = 0.023$. Rats who were conditioned with a social partner gaped significantly more than rats conditioned alone. Rats treated with LiCl and

conditioned with a social partner gaped significantly more than rats treated with LiCl and conditioned without a social partner (Figure 2.2B). These results show that a familiar social partner can serve as a cue for the expression of conditioned (anticipatory) disgust.

A 2X2 ANOVA for total aversive behaviors revealed a significant main effect of drug on aversive behaviors, $F(1, 40) = 6.489$, $p = 0.015$. LiCl treated rats showed significantly more aversive behaviors compared to the NaCl treated rats. No significant main effect of social condition or interaction effects were found.

A split-plot ANOVA of the composite score of regularly occurring spontaneous orofacial behaviors (mouth movements and tongue protrusions) revealed no significant main effect of drug, $F(1, 40) = 0.450$, $p = 0.506$, or significant main effect of social condition, $F(1, 40) = 0.756$, $p = 0.390$. This indicates that increased frequency of gaping and disgust responses in the LiCl treated groups is not associated with a higher frequency of spontaneous orofacial responses.

2.6.3 Social interactions with conspecific (Test Day 1)

A 2X2 ANOVA was used to determine the effect of drug and social condition on social initiations. Tests of between-subjects effects revealed a significant main effect of drug on social initiations, $F(1, 40) = 11.05$, $p = 0.002$. Animals treated with LiCl made significantly more social initiations towards their social partners compared to animals treated with NaCl. The analysis did not reveal a significant main effect of social condition on social initiations. Further, no significant interaction between drug and social condition on social initiations was found.

A one-way ANOVA was used to determine the effect of group on social initiations. Tests of between-subjects effects revealed a significant main effect of group

on social initiations, F (1, 40) = 4.43, p = 0.009. LSD post-hoc comparisons revealed that the LiCl-Social group made significantly more social initiations towards their partner (p = 0.002), compared to the Na-Social group. Further, the LiCl-Social group made significantly more social initiations compared to the Na-Alone group (p = 0.007) (Figure 2.3).

2.6.4 Re-exposure to original conditioning context (Test Day 2)

A 2X2 ANOVA revealed a significant main effect of drug on conditioned gaping frequency on Test Day 2. LiCl treated rats displayed significantly more gaping responses, F (1, 40) = 11.679, $p < 0.001$, compared to NaCl treated rats. However, no significant main effect of social condition on aversive behavior or interaction effect was determined (p = 0.296) (Figure 2.2C).

A split-plot ANOVA for drug and social condition on aversive behaviors revealed a significant main effect of drug, F (1, 40) = 6.559, p = 0.014. LiCl-treated rats displayed significantly more gaping behavior on Test Day 2 compared to NaCl-treated rats. No significant main effect of social condition or interaction effect was discovered.

A split-plot ANOVA was employed to uncover the effects of drug and social condition on facing defense behavior. A significant main effect of drug on facing defense was discovered, F (1, 40) = 4.37, p = 0.043, in that the LiCl treated animals displayed more facing defenses towards their social partner compared to the NaCl treated animals. The analysis did not reveal a significant main effect of social condition or a significant interaction effect of drug and social condition on facing defense behavior.

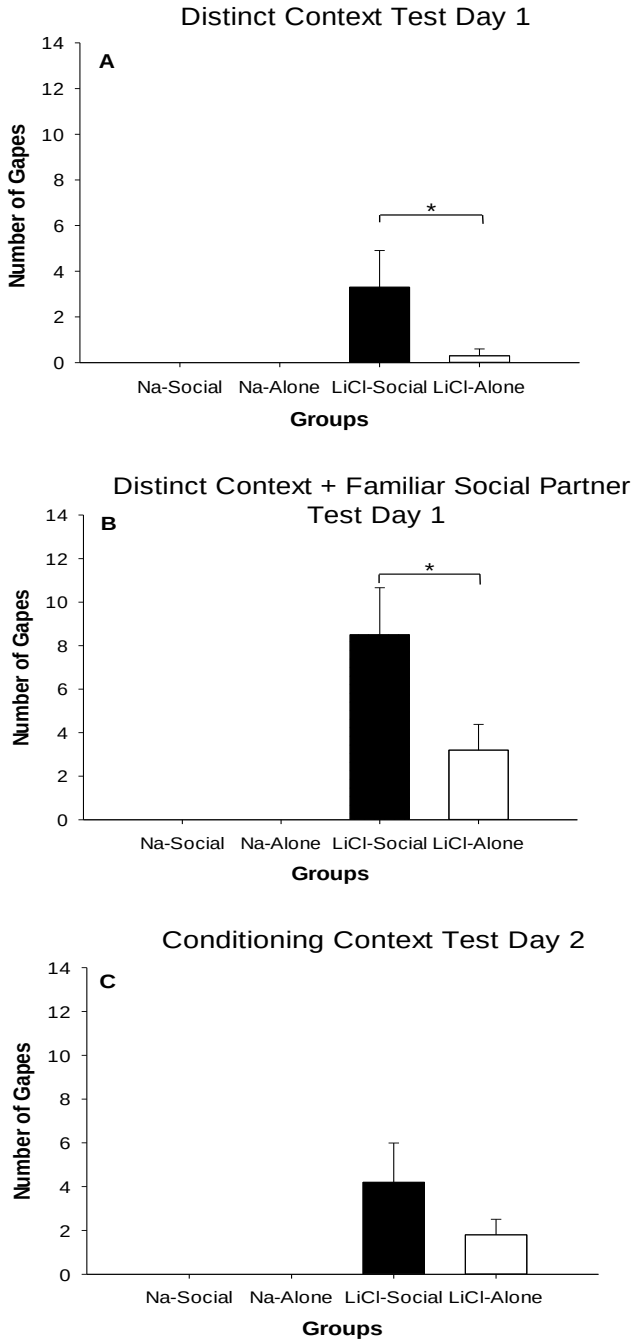

Figure 2.2. **(A)** Mean number of gapes displayed for each of the four treatment groups on Test Day 1 during the 2 minute period in the absence of a social partner in the distinct context. The LiCl-Social group gaped significantly more than the LiCl-Alone group (**p* = 0.009). **(B)** Mean number of gapes displayed by four treatment groups on Test Day 1 during the 10 minute exposure to a social partner in distinct context. The LiCl-Social group gaped significantly more than the LiCl-Alone group (**p* = 0.003). **(C)** Mean number of gapes displayed by four treatment groups on Test Day 2 while alone in original conditioning context. Error bars represent mean +S.E.M.

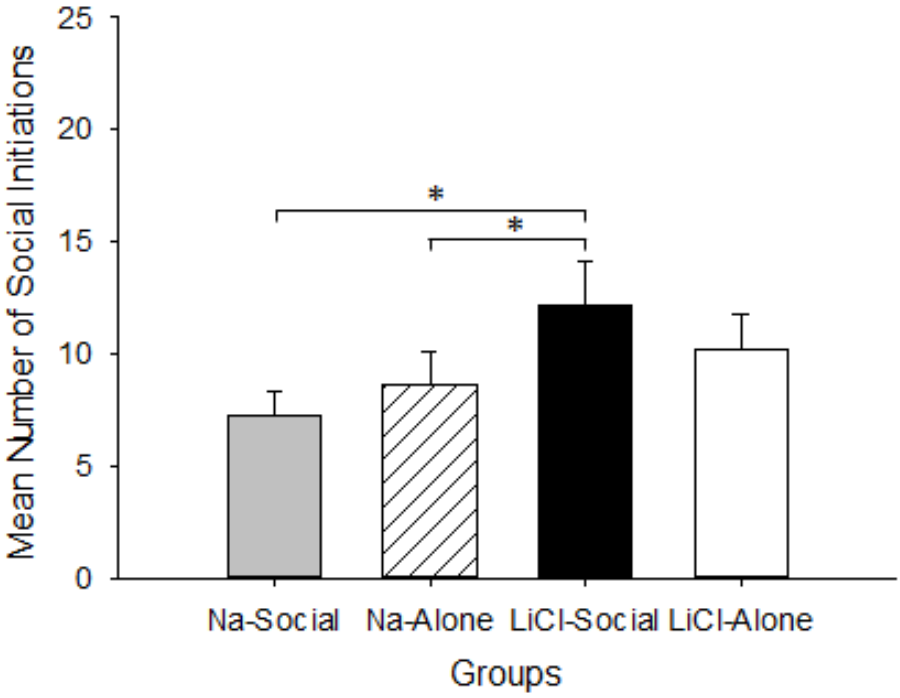

Figure 2.3. Mean number of social initiations of LiCl/NaCl treated animals towards social partner during Test Day 1. The LiCl-Social group displayed significantly more social initiations towards their social partner compared to Na-Social (**p* = 0.002) and Na-Alone (**p* = 0.007) groups. Error bars represent mean +S.E.M.

Finally, a 2X2 factorial ANOVA was utilized to determine the effects of drug and social condition on evasive defense behavior. No significant main effects or interactions were uncovered for evasive defense behavior.

2.6.5 Test Day 1 versus Test Day 2

A repeated measures ANOVA revealed that the LiCl-Social group displayed significantly more conditioned gaping behavior on Test Day 1 (distinct context with social partner) ($p < 0.001$) compared to Test Day 2 (conditioning context alone). There was no significant difference found between Test Day 1 and Test Day 2 for the LiCl-Alone group.

A repeated measures ANOVA revealed that the LiCl-Alone group displayed significantly more aversive behaviors on Test Day 2 (while in the conditioning chamber alone) compared to Test Day 1 ($p = 0.007$). No significant difference was found between Test Day 1 and Test Day 2 for LiCl-Social group for aversive behaviors.

2.7 Summary of results

On drug-free Test Day 1 (distinct context), rats that were treated with LiCl and conditioned in the presence of a social partner displayed significantly more gaping than animals treated with LiCl and conditioned without a social partner. Further, rats treated with LiCl, and specifically those conditioned with a social partner, displayed significantly higher numbers of social initiations towards their social partner compared to both of the NaCl [Alone and Social] treated groups. No significant differences in gaping frequencies were determined between LiCl treated groups on Test Day 2. However, the LiCl-Social group displayed significantly more conditioned gaping behavior on Test Day 1 (distinct context with social partner) compared to Test Day 2 (conditioning context alone).

3.1 Experiment 2: Effect of unfamiliar social partner on conditioned disgust

3.2 Methods

3.2.1 Animals

Subjects were thirty-two naïve adult male Long-Evans rats (Charles River, Quebec, Canada) weighing between 250- 350g at the start of the experiment. Rats were pair-housed in translucent polypropylene cages (45 x 22 x 20cm) in a colony room maintained at 21 + 1 °C and under a 12 L: 12 D cycle (light 0700 – 1900h). Rats had *ad libitum* access to both food (ProLab Rat Chow) and water. Animals were tested during the light phase of the light:dark cycle between the hours of 0800 and 1500 h. All procedures were carried out in accordance to the Canadian Council of Animal Care guidelines and were approved by the Institutional (University of Western Ontario) Animal Care Committee.

3.2.2 Drugs

Same drugs and dosages as Experiment 1.

3.2.3 Apparatus

The apparatus was as described in Experiment 1.

3.3 Procedure

The experimental procedures are summarized in Figure 2.1.

3.3.1 Social and non-social conditioning

Rats were individually housed for one week before the start of the experiment. Prior to conditioning trials, rats were habituated for one 10 min session in the conditioning context, followed by habituation to the distinct striped context for 10 min, 24 hours later (different room). Twenty-four hours after the second habituation, the

conditioning phase commenced. The conditioning phase consisted of four days, each separated by 72 hours. On each conditioning day, each rat was intraperitoneally (ip) injected with either LiCl (0.15 M, 128 mg/kg, 20 ml/kg) or saline vehicle (0.9 % NaCl, 20 ml/Kg) and immediately placed in the conditioning apparatus for 30 min. Half of the animals from each drug group were placed in the apparatus in the presence of an uninjected male social partner [Groups: Li-Unfam (n = 8); Na-Unfam (n = 8), with the same social partner used on each conditioning day (familiar)], while the other half were conditioned alone [Groups: Li-Alone (n = 8); Na-Alone, (n = 8)]. Social partners were animals different from experiment 1 and were selected at random. They are also different from the initial pair-housed mates.

3.3.2 Unfamiliar social partner in a distinct context (Test Day 1)

Test Day 1 took place 72 hours following the fourth conditioning day. Each animal that had received either LiCl [Li-Unfam; Li-Alone] or NaCl [Na-Unfam; Na-Alone] was exposed to the distinctive striped chamber alone for two minutes, while in a drug free state, prior to the introduction of the distinct (unfamiliar) social partner. They were then left undisturbed for 10 minutes while their interaction was recorded. Those animals that previously had a social partner (familiar) during their conditioning [Li-Unfam; Na-Unfam] were exposed to a distinct (unfamiliar) social stimulus, whereas those that had no social partner [Li-Alone; Na-Alone] now also received a distinct (unfamiliar) social stimulus. Conditioned disgust responses and social behaviours were recorded and scored using the Observer [Noldus Information Technology, Sterling Va] event – recording software. Dependent behavioural variables analyzed included gaping frequencies and the composite scores of aversive responses that did not include gaping

(paw treads, forelimb flails, chin rubs and head shakes, as well as spontaneous orofacial behaviours (tongue protrusions and mouth movements).

3.3.3 Re-exposure to original conditioning context (Test Day 2)

Twenty-four hours following Test Day 1 each experimental animal was exposed for a 10 minute period alone to the original white conditioning context (conditioning apparatus), while in a drug free state. During these tests the rats' orofacial and aversive behaviours were again recorded (See Figure 2.1).

3.4 Statistical analyses

The dependent conditioned disgust variables – gaping behaviour and composite aversive behaviours were each analyzed with separate 2X2 analysis of variance (ANOVA) for drug treatment and social condition. The same analyses were used for Test Day 2. Social behaviors during Test Day 1 (initiations, facing defense and evasive defense) were also analyzed with separate 2X2 ANOVA's for drug treatment and social condition. The gaping behavior (on Test Day 1) for the two minute period alone was analyzed with a 2X2 ANOVA for drug and social condition. A repeated measures design was used to compare Test Day 1 to Test Day 2 for differences in gaping behaviors and aversive behaviors. Least significant difference (LSD) post-hoc pair-wise comparisons were used following significant interactions and/or main effects to determine differences among the groups.

3.5 Results

3.5.1 Unfamiliar social partner in a distinct context (Test Day 1)

An analysis for the gaping behavior during the initial two minute period without the social partner, revealed no significant differences between groups (Figure 2.4A).

Following the two minute period alone, the unfamiliar social partner was placed in the distinct context. The split-plot ANOVA for drug and social condition on gaping behavior revealed a main effect of drug, with LiCl-treated rats gaping significantly more, $F(1, 32) = 6.713$, $p = 0.015$, than NaCl treated rats. No significant differences were discovered between social conditions for gaping behavior, and no social condition by drug interaction was uncovered (Figure 2.4B).

A 2X2 ANOVA for aversive behaviors did not reveal a significant main effect of treatment on aversive behaviors, $F(1, 32) = 3.316$, $p = 0.079$. Therefore, LiCl treated rats did not show more aversive behaviors than NaCl treated rats on test day 1 (distinct context).

A one-way ANOVA for group on the composite score of regularly occurring spontaneous orofacial behaviors (mouth movements and tongue protrusions) revealed no significant differences between groups, $F(1, 32) = 1.687$, $p = 0.193$.

3.5.2 Social interactions with conspecific (Test Day 1)

A 2X2 ANOVA was used to determine the effect of drug and social condition on social initiations. Tests of between-subjects effects revealed a significant main effect of condition on initiations, $F(1, 28) = 35.221$, $p < 0.001$. Animals conditioned alone displayed significantly more social initiations towards the social partner (distinct) compared to animals that were conditioned with an unfamiliar social partner. The analysis did not reveal a significant main effect of drug on social initiations. Finally, a significant interaction between drug and social condition on social initiations was found, $F(1, 28) = 7.800$, $p = 0.009$. Animals treated with LiCl and conditioned without a social partner (Li-Alone) displayed significantly more social initiations towards that partner

compared to the Na-Alone group ($p = 0.02$), the Na-Unfam group ($p < 0.001$) and the Li-Unfam group ($p < 0.001$) (Figure 2.5).

3.5.3 Re-exposure to original conditioning context (Test Day 2)

A main effect of drug was found in that LiCl treated rats gaped significantly more, $F(1, 32) = 4.671$, $p = 0.039$, than NaCl treated rats. A one-way ANOVA for group on conditioned gaping behavior revealed a significant effect of group on gaping behavior, $F(1, 32) = 3.758$, $p = 0.022$. Post hoc comparisons revealed that subjects who were treated with LiCl and conditioned without a social partner gaped significantly more than animals who were treated with LiCl and conditioned with a social partner (Figure 2.4C).

The split-plot ANOVA for social condition and drug on aversive behaviors revealed a significant main effect of drug on aversive behavior, $F(1, 32) = 6.577$, $p = 0.016$. LiCl-treated rats displayed significantly more aversive behaviors than NaCl treated rats. No other significant differences were discovered.

An ANOVA of the composite score of regularly occurring spontaneous orofacial behaviors (mouth movements and tongue protrusions) revealed no significant differences between groups, $F(1, 32) = 0.131$, $p = 0.941$.

A split-plot ANOVA was employed to uncover the effects of drug and social condition on facing defense behavior. A significant main effect of drug on facing defense was discovered, $F(1, 28) = 6.921$, $p = 0.014$, in that the LiCl treated animals displayed more facing defenses towards their social partner compared to the NaCl treated animals. The analysis did not reveal a significant main effect of social condition or a significant interaction effect of drug and social condition on facing defense behavior.

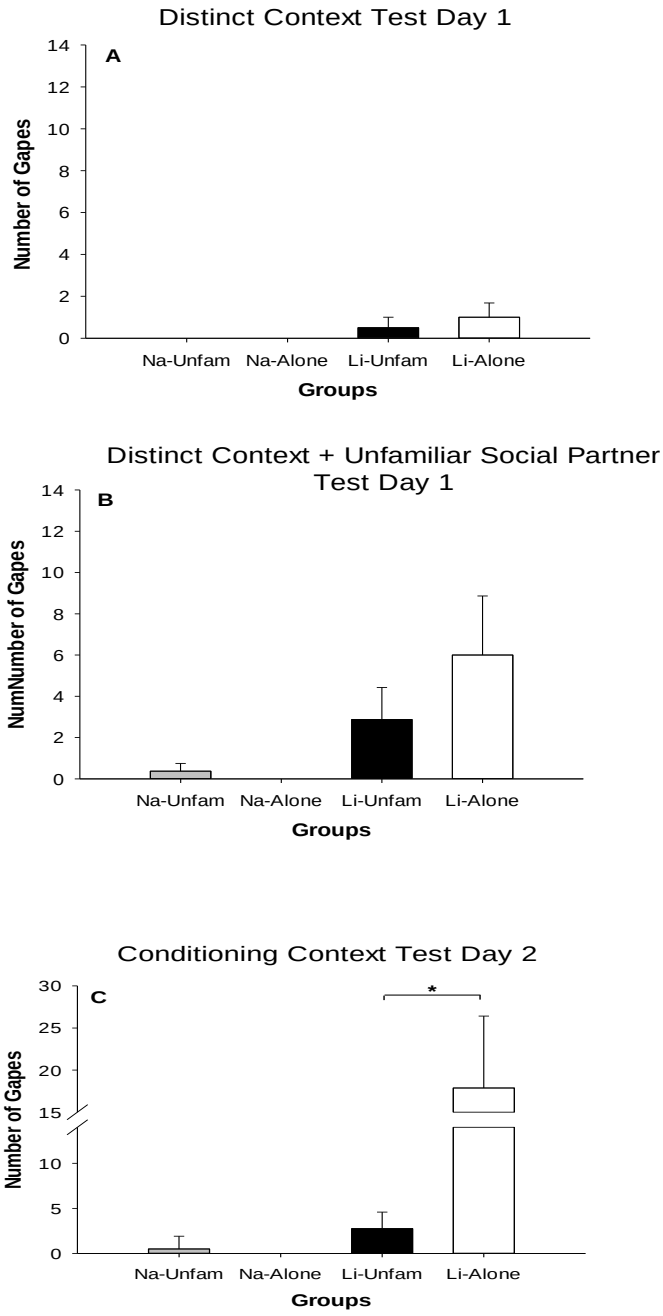

Figure 2.4. **(A)** Mean number of gapes displayed for each of the four treatment groups on Test Day 1 during the 2 minute period in the absence of a social partner in the distinct context. No significant differences were found between groups. **(B)** Mean number of gapes displayed by four treatment groups on Test Day 1 during the 10 minute exposure to an unfamiliar social partner in distinct context. No significant differences were discovered between the Li-Unfam and the Li-Alone groups. **(C)** Mean number of gapes displayed by four treatment groups on Test Day 2 while alone in original conditioning context (note change of the scale). The Li-Alone group gaped significantly more than the Li-Unfam group on test day 2 (*p = 0.021). Error bars represent mean +S.E.M.

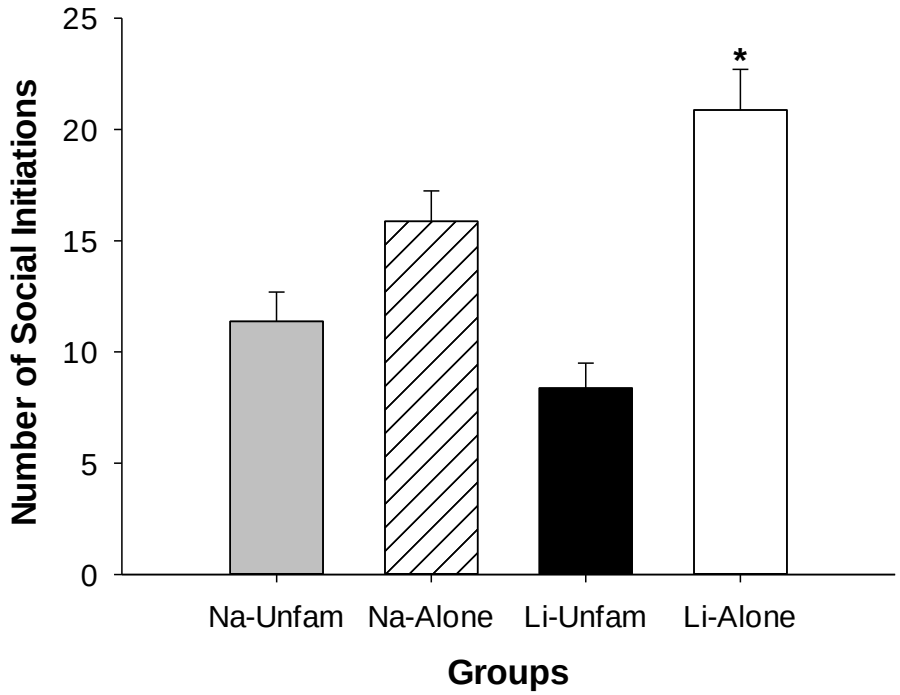

Figure 2.5. Mean number of social initiations of LiCl/NaCl treated animals towards social partner during Test Day 1. The Li-Alone group displayed significantly more social initiations towards their social partner compared to Li-Unfam (*$p < 0.001$), Na-Alone (*$p = 0.02$) and Na-Unfam (*$p < 0.001$) groups. Error bars represent mean +S.E.M.

Finally, a 2X2 factorial ANOVA was utilized to determine the effects of drug and social condition on evasive defense behavior. A significant main effect of condition on evasive behavior was determined, F (1, 28) = 4.673, p = 0.039. Animals conditioned with a social partner and then tested with a different partner (Unfam) showed more evasive behaviors than animals conditioned alone and tested with a social partner (Alone). No significant drug by condition interaction was discovered.

3.5.4 Test Day 1 versus Test Day 2

A repeated measures design revealed a significant effect of group on gaping behavior. Rats conditioned alone and treated with LiCl gaped significantly more on Test Day 2 than Test Day 1 (p = 0.006). No significant differences were found between Test Day 1 and 2 for rats treated with LiCl and conditioned with a social partner.

3.5.5 Familiar social partner versus unfamiliar social partner

A one-way ANOVA comparing differences in gaping frequency for LiCl-treated rats with a familiar or an unfamiliar social partner did not reveal any significant differences. The differences did however approach significance F (1, 17) = 4.051, p = 0.06, in that LiCl-treated rats conditioned and tested with a familiar social partner gaped more than LiCl-treated rats conditioned with a familiar social partner, but tested with an unfamiliar social partner.

3.6 Summary of results

Test Day 1 revealed no significant differences between LiCl-treated groups. However, despite the insignificance, the Li-Unfam group displayed less gaping behavior on Test Day 1 compared to the Li-Alone group. The Li-Unfam group also displayed low gaping frequencies on Test Day 2, whereas the Li-Alone group displayed significantly

more gapes on Test Day 2 (conditioning context alone) compared to Test Day 1. When comparing testing with an unfamiliar rat to testing with a familiar rat, the results suggest that rats gape more in the presence of their familiar conditioning partner rather than an unfamiliar individual.

4.1 Experiment 3: Effect of familiar social odour on conditioned disgust responses

4.2 Methods

4.2.1 Animals

Subjects were thirty-two naïve adult male Long-Evans rats (Charles River, Quebec, Canada) weighing between 250- 350g at the start of the experiment. Rats were pair-housed in translucent polypropylene cages (45 x 22 x 20cm) in a colony room maintained at 21 + 1 °C and under a 12 L: 12 D cycle (light 0700 – 1900h). Rats had *ad libitum* access to both food (ProLab Rat Chow) and water. Animals were tested during the light phase of the light:dark cycle between the hours of 0800 and 1500 h. All procedures were carried out in accordance to the Canadian Council of Animal Care guidelines and were approved by the Institutional (University of Western Ontario) Animal Care Committee.

4.2.2 Drugs

Same drugs and dosages as Experiment 1.

4.2.3 Apparatus

The apparatus was as described in Experiment 1. Q-tips® were used to collect urine from a conspecific other than the subject's cage mate. The urine soaked Q-tips® were then tapped to the outside of one of the air holes in the side of the conditioning

chamber. The Q-tips® were also tapped to the outside of one of the ventilation holes in the distinct chamber for Test Day 1.

4.3 Procedure

4.3.1 Social and non-social conditioning

Rats were individually housed for one week prior to initiation of the experiment. Prior to conditioning trials, rats were habituated for one 10 minute session in the conditioning context, followed by habituation to the distinct stripped context for 10 minutes, 24 hours later. Twenty-four hours after the second habituation, the conditioning phase commenced. The conditioning phase consisted of four days, each separated by 72 hours. Immediately before conditioning began, fresh urine was collected from a conspecific by means of a Q-tip®. These animals were different than the original pair-housed mates. The conspecifics were placed in an empty cage for a 30 min period prior to conditioning. The cages were then swabbed for urine and the Q-tip® was adhered to the outside of one of the ventilation holes on the conditioning chamber.

Each rat was intraperitoneally (ip) injected with either LiCl (0.15 M, 20 ml/kg) or saline vehicle (0.9 % NaCl, 20 ml/Kg) and immediately placed in the conditioning apparatus for 30 minutes. Half of the animals from each drug group were placed in the apparatus in the presence of an odour (urine) of a conspecific [Groups: Li-Odour (n = 8), Na-Odour (n = 8), with the same urine odour used on each conditioning day] while the other half were conditioned alone [Groups: Li-Alone (n = 8), Na-Alone (n = 8)].

4.3.2 Social odour in distinct context (Test Day 1)

Test Day 1 took place 72 hours following the fourth conditioning day. Each animal that had received either LiCl [Li-Odour; Li-Alone] or NaCl [Na-Odour; Na-

Alone] was exposed to the distinctive striped chamber alone for 10 minutes while their interaction was recorded. Those animals that were conditioned without an odour now received an odour, and those animals conditioned with an odour received the same urine odour as conditioning. Conditioned disgust responses were recorded and scored using the Observer [Noldus Information Technology, Sterling Va] event –recording software. Dependent behavioural variables analyzed included gaping frequencies and the composite scores of aversive responses that did not include gaping (paw treads, forelimb flails, chin rubs and head shakes), as well as spontaneous orofacial behaviours (tongue protrusions and mouth movements).

4.3.3 Re-exposure to original conditioning context (Test Day 2)

Twenty-four hours following Test Day 1 each experimental animal was exposed for a 10 minute period alone, without any odour, to the original white conditioning context (conditioning apparatus), while in a drug free state. During these tests the rats' orofacial and aversive behaviours were again recorded.

4.4 Statistical analyses

The dependent conditioned disgust variables – gaping behaviour and composite aversive behaviours were each analyzed with separate two-way analysis of variance (ANOVA) for drug treatment, social condition and test day. A split-plot ANOVA was employed to determine differences in spontaneous orofacial behaviors. These analyses were also employed for Test Day 2. A repeated measures design was used to compare Test Day 1 to Test Day 2 for differences in gaping behaviors. Least significant difference (LSD) post-hoc pair-wise comparisons were used following significant interactions and/or main effects to determine differences among the groups.

4.5 Results

4.5.1 Familiar social odour in distinct context (Test Day 2)

A 2X2 ANOVA revealed a significant main effect of drug on gaping behavior, $F(1, 28) = 9.366$, $p = 0.005$. Animals treated with LiCl gaped significantly more than animals treated with NaCl. No significant differences were found across conditions, $F(1, 28) = 0.212$, $p = 0.649$, in that animals conditioned with an odour displayed similar gaping frequencies to animals conditioned alone. Further, no significant interaction effect was determined between drug and social condition (Figure 2.6A).

A 2X2 ANOVA revealed a significant main effect of drug on aversive behaviors, $F(1, 28) = 5.119$, $p = 0.032$. Animals treated with LiCl showed significantly more aversive behaviors on Test Day 1 compared to animals treated alone. No significant differences were found across conditions, in that animals conditioned with an odour displayed a similar amount of aversive behaviors compared to animals conditioned alone. Further, no significant interaction effect was determined between drug and social condition.

4.5.2 Re-exposure to original conditioning context (Test Day 2)

A 2X2 ANOVA revealed a significant main effect of drug, $F(1, 28) = 8.085$, $p = 0.008$, in that rats treated with LiCl gaped significantly more and rats treated with NaCl. However no significant differences were discovered between conditions, as well as no significant drug by condition interaction (Figure 2.6B).

A 2X2 ANOVA for aversive behaviours revealed a significant main effect of drug $F(1, 28) = 12.064$, $p = 0.002$, in that rats treated with LiCl displayed significantly more

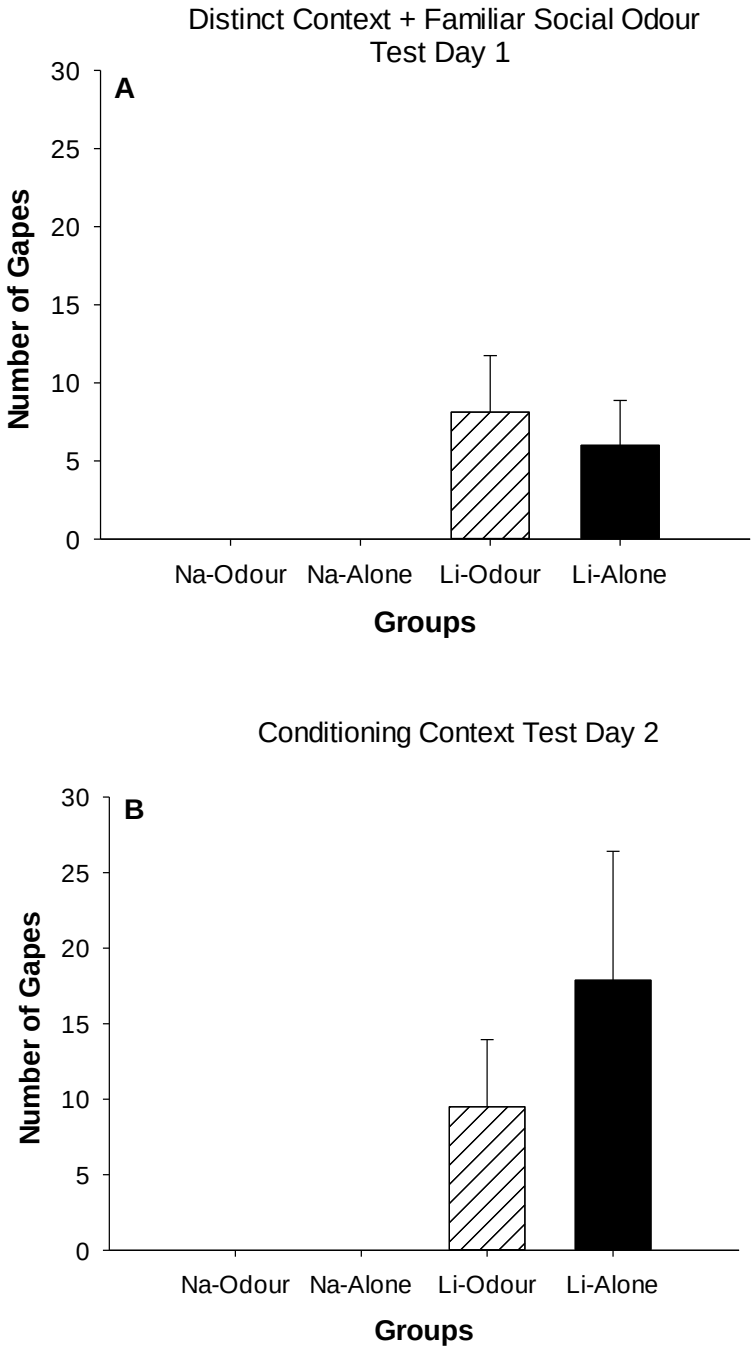

Figure 2.6. **(A)** Mean number of gapes displayed by four treatment groups on Test Day 1 during the 10 minute exposure to the familiar social odour in the distinct context. No significant differences were found between the LiCl-Odour group and the LiCl-Alone group. **(B)** Mean number of gapes displayed by four treatment groups on Test Day 2 while alone in original conditioning context. No significant differences were found between the LiCl-Odour group and the LiCl-Alone group. Error bars represent mean +S.E.M.

aversive behaviors than rats treated with NaCl. However, no main effect of condition or interaction effect was discovered for aversive behaviors.

4.5.3 Test Day 1 versus Test Day 2

No significant differences were discovered for the LiCl-Odour group between Test Day 1 and Test Day 2. However, the LiCl-Social group gaped significantly more on Test Day 1 compared to Test Day 2 ($p = 0.009$). Further, the LiCl-Alone group gaped significantly more on Test Day 2 compared to Test Day 1 ($p = 0.006$).

4.6 Summary of results

Test Day 1 (distinct context with familiar odour) revealed no significant differences between LiCl-treated groups. Rats treated with LiCl and conditioned with an odour gaped comparably to rats treated with LiCl and conditioned alone. Further, no significant differences were found between LiCl-treated rats for Test Day 2 (conditioning context alone).

5.1 Discussion

The results of the present study demonstrate that social factors are involved in the development and expression of conditioned disgust in male rats. It was found that; (i) a social partner can serve as a cue for eliciting anticipatory nausea (disgust/ gaping), (ii) this conditioned disgust is specific to a familiar individual, as an unfamiliar individual failed to elicit significant disgust responses, (iii) these responses likely involve a variety of sensory cues, as social odours (urine) alone failed to elicit significant conditioned disgust responses. As there is accumulating evidence for evolutionary and neural consistencies between gaping in rats and human disgust (Curtis, 2011; Garcia et al., 1985; Panksepp, 2007; Harrison et al., 2010; Chapman & Anderson, 2012; Tuerke et al., 2012),

the conditioned gaping seen here supports the presence of socially mediated conditioned disgust.

The presence of a familiar social partner during conditioning resulted in drug-free conditioned gaping and other aversive responses when the experimental rat was in the presence of the familiar social stimulus (Test Day 1). Compared to Test Day 1 (social partner in distinct context) LiCl-Social rats also had a lower gaping frequency on Test Day 2 (alone in conditioning context). If there is minimal context carry-over between the conditioning context and the distinct context, the gaping exhibited by the experimental rats can be attributed to the presence of their social partner, rather than the context itself. Minimal context carry-over is shown by the LiCl-Alone group gaping significantly less than the LiCl-Social group during the pre-social two-minute exposure in the distinct context. This confirms that the context had little carry-over from the original conditioning context, and was therefore not as aversive. However, in experiment 1, LiCl-Social rats gaped in the distinct context even in the absence of their social partner. This may have been due to the rats anticipating the arrival of their social stimulus, or simply that pairing a social stimulus with an illness inducing agent results in an amplified expression of disgust responses in these rats. The simultaneous presentation of two distinctive conditioned cues (social and non-social context) also introduces the possibility of overshadowing, wherein the saliency of one cue is greater than that of the other (Lindsey & Best, 1973, Best & Meachum, 1986). However, in experiment 2, no differences in gaping behaviour were found during the two-minute pre-exposure to the distinct context alone. Therefore, although the results of the present study suggest that social cues are more salient than non-social cues, further research is needed.

The increased gaping frequency seen in the LiCl-Social group in experiment 1 conflicts with the results of Hishimura (2015). They found that interactions with a conspecific decreased, and even attenuated, conditioned taste avoidance in mice. The mice that were exposed to a social stimulus following a taste (saccharin) paired with toxin-induced sickness consumed more saccharin compared to the controls, which received no social stimulus. However, in their experiment the social stimulus is not used as the cue for sickness, but rather is being presented after the conditioned taste avoidance is already established. Further, the experiment utilized a two-bottle test for conditioned taste avoidance which requires the animal to physically approach the bottles, as well as display both appetitive and consummatory responses (Best & Meachum, 1986). Parker, Rana and Limebeer (2008) have argued that this measures conditioned taste avoidance, and does not accurately measure disgust. Therefore, the present study utilizing a social stimulus as a conditioning cue may result in a more accurate depiction of socially mediated conditioned disgust.

Despite the fact that the presence of a familiar social partner elicited gaping and disgust responses, it did not lead to noticeable social avoidances. Rather, the LiCl-treated rats conditioned with a social partner displayed ambivalent social responses as seen by their propensity to engage in social contact, mixed with defensive and avoidant behaviours. This is similar to the mixed social responses seen in mice and rats towards either an infected or potentially infected individual, as well as the hesitant responses of humans towards unfamiliar individuals or endotoxins (Kavaliers et al., 2004, Parkinson et al., 2012, Lopes et al., 2012). The results of the current study show that rats treated with LiCl and conditioned with a social partner display significantly more initiations towards

their partner than animals treated with NaCl and conditioned with a social partner. These findings are, in part, consistent with apparent conditioned social aversions reported with Mongolian gerbils, where the animals show reduced, although not eliminated, social approach to, and interactions with, familiar animals that had been previously paired with LiCl (Pettijohn, 1981). The increased social initiations seen in the current study are also consistent with research by Lopes et al. (2012), who showed that animals can overcome the behavioural symptoms associated with sickness and display increased social interactions when in a social context. Specially, they showed that male zebra finches displayed decreased sickness behaviours in a colony setting compared to in isolation, as well as increased social initiations and interactions. Therefore, the increase in social initiations seen by the LiCl-Social group in experiment 1 may be due to an attempt to overcome the negative symptoms of sickness and benefit from the positive effects of social interaction. However, as seen in experiment 2, animals treated with LiCl and conditioned with a familiar social partner (Li-Unfam) showed decreased social initiations towards an unfamiliar social partner during Test Day 1. Interestingly however, the Li-Alone group displayed a relatively high number of social initiations when in the presence of an unfamiliar partner during Test Day 1. This may be due to the lack of social interaction during conditioning, leading to increased social initiations while in the presence of a social stimulus to ameliorate the negative symptoms of sickness (social buffering effect). These findings suggest animals may seek social interaction with familiar conspecifics rather than unfamiliar conspecifics, unless they have experienced no social contact during sickness.

Although LiCl-treated animals displayed significantly enhanced social initiations and gaping responses in the presence of their social partner, they failed to demonstrate increased aversive responses. In fact, these rats displayed minimal, if any, aversive responses while in the presence of their social partner. These results may be related to a phenomenon called social buffering; where animals show a better recovery from a distressful situation when they are in the presence of another conspecific. Davitz & Mason (1955) showed that rats displayed a decrease in fearful withdrawal in an open field apparatus when in the presence of another non-fearful rat. They also found that these rats displayed increased locomotor activity as well as increased affiliative behaviour towards the other rat. Further, Taylor (1981) found that rats who were stressed were more attracted to other non-stressed rats. Davitz & Mason (1955) hypothesized that these rats were actively seeking out interactions with conspecifics to potentially ameliorate their negative internal state. This may explain why in the present study the rats display increased social initiations towards the conspecific, as well as decreased aversive responses. The presence of these mixed social interactions further suggests that the rats are displaying a conditioned social disgust rather than social fear conditioning per se. Social fear conditioning has been shown to lead to marked social avoidance and social anxiety (Toth, Neumann, & Slattery, 2012). These differences in social responses are also consistent with the distinctions between fear and disgust reported in humans (Curtis, 2011; Toth, Neumann, & Slattery, 2012).

Social information and its processing is necessary for social and individual recognition, as well as the facilitation of social interactions (Choleris et al., 2009). In rats, social information is encoded via olfactory or pheromonal signals, as well as auditory and

visual signals (Popik & Vetulani, 1991). In the current study, rats are able to distinguish between the familiar conditioning partner and the unfamiliar testing partner, as demonstrated by a decreased gaping frequency in the presence of an unfamiliar social partner. True individual recognition can be operationally defined as unique modifications in the way an animal behaves towards another animal based on previous experiences with that specific individual (Gheusi et al., 1994). As such, whether or not they can distinguish between different familiar individuals remains to be determined.

Rodents utilize a variety of sensory cues to distinguish between conspecifics. The most prominent are olfactory cues. However, in the present study urine odours alone failed to elicit significant conditioned disgust. In experiment 3, animals conditioned with a urine odour cue displayed very similar gaping patterns to the animals conditioned alone. Upon re-exposure to the conditioning context alone (without the odour), animals conditioned with an odour displayed a similar number of gapes as Test Day 1 (distinct context). However, the Li-Alone group significantly increased their gaping on Test Day 2 in the original conditioning context. This further demonstrates that the distinct context is different than the original conditioning context which acts as a cue for the sickness behaviours for animals conditioned alone. Utilizing just urine odour for conditioning either may not be a strong enough cue and/or might be overshadowed by the context. As well, the degree of exposure to volatile and non-volatile odour cues and the role of the odours in addition to that of urine needs to be addressed. As suggested, further studies are needed to examine the roles that familiar conspecific olfactory cues have in the development of conditioned disgust.

The involvement of social stimuli in the mediation of anticipatory disgust in rats is also supported by associations between conditioned taste avoidance and elevations in the nonapeptides, oxytocin and arginine vasopressin (O'Connor, Cheng, & North, 1987; Verbalis et al., 1986). Results from human studies suggest that oxytocin can enhance the salience of disgust, leading to approach-avoidance of the disgust cues (Theodoridou, Penton-Voak, & Rowe, 2013). Further, findings from rats and suggestive human studies indicate that oxytocin and likely vasopressin are involved in the detection and modulation of socially related pathogen and infection threat disgust cues, as well as suppression of food intake (Kavaliers et al., 2004, Kavaliers & Choleris, 2011). As well, elevations in OT have been associated with social buffering (Smith & Wang, 2014). Therefore, elevations in oxytocin during LiCl conditioning may in part explain the increased social initiations followed by avoidance behaviours seen in experiment 1. However, further studies are needed to address the role elevations in oxytocin play in the modulation of socially mediated anticipatory nausea/ disgust.

This study clearly demonstrates that a social stimulus can act as a cue for the expression of anticipatory nausea. This may explain why some chemotherapy patients report seeing the nurse is enough to cause feelings of nausea prior to chemotherapy treatment. The findings from these experiments demonstrate the need to further explore the role that social factors play in the development and modulation of anticipatory nausea. Although these experiments suggest that social factors play a role in the expression of disgust there are a number of limitations. For example, the social behaviours of the untreated social partners were not quantified. The behaviours exhibited by the social partner in particular could help clarify the behaviours of the conditioned

rats. In addition, rates of extinction from Test Day 1 to Test Day 2 and roles of social buffering need to be considered more fully. As well, the exact nature of the social cues used needs to be addressed further. However, despite these limitations, the present findings do support a role for social factors in the development and expression of conditioned disgust.

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CHAPTER 3

OXYTOCIN RECEPTOR ANTAGONIST DECREASES SOCIALLY-MEDIATED GAPING RESPONSES IN MALE RATS

6.1 Introduction

Disgust responses play a pivotal role in mediating the avoidance of toxins and pathogens in both humans and non-human animals. These responses can be seen in both adult and neonate humans (Greimel et al., 2006; Steiner, 1973), rodents (Grill & Norgren, 1987), apes and monkeys (Berridge, 2000). The disgust response is characterized by a distinct facial expression and withdrawal response, with the possibility of an emetic episode (Rozin & Fallon, 1987; Rozin, Haidt & McCauley, 2008). In non-emetic species, such as the rat, that lack the musculature needed to expel harmful substances, disgust can be observed through typical facial movements, including the gaping movement (Horn et al., 2013). The gaping response is characterized by a large opening of the mouth, revealing the bottom incisors (Parker, Rana & Limebeer, 2008). Studies comparing non-emetic and emetic species have shown that the rodent gape utilizes similar musculature as vomiting does in emetic species (Travers & Norgren, 1986), and is topographically similar to the orofacial components of retching in the shrew; a distinct facial expression made immediately before an emetic response (Horn et al., 2013). Further, research has shown that rats are capable of associating taste, sucrose and malaise with toxin, lithium chloride (LiCl), induced sickness. This association results in conditioned taste avoidance and conditioned disgust reactions in rats upon re-exposure to the taste or context, respectively (Ossenkopp & Eckel, 1995; Limebeer et al., 2008).

Rats display a gaping response when re-exposed to a context that has been previously associated with illness. Specifically, rats that are conditioned with the toxin, LiCl, and placed in a specific environmental context, will display a dose related increase in gaping responses upon re-exposure to that specific context, while in a drug free state

(Parker, 2003; Ossenkopp et al., 2011). This established animal model of conditioned disgust closely parallels the anticipatory nausea (AN) experienced by many chemotherapy patients. Specifically, AN is a learned response following chemotherapy treatment which occurs in over 25% of patients by the fourth treatment (Morrow & Roscoe, 1997). This learned response has been interpreted as a classically conditioned response (Matteson et al., 2002; Neese et al., 1980; Tomoyasu, Bovbjerg & Jacobsen, 1996). When a conditioned stimulus (CS) (e.g. the sight of the hospital or nurse), is paired with an unconditioned stimulus (US) (e.g. chemotherapy), it ultimately produces an unconditioned response (UR) (e.g. nausea). After one or more chemotherapy treatments, the CS alone is able to elicit a UR; which is similar to the response produced by the chemotherapy drug itself. Although there are treatments available to help with the unpleasant chemotherapy side effect of acute vomiting (e.g. the 5- hydroxytryptamine 3 (5-HT3) receptor antagonist ondansetron; Navari, 2009), AN still a highly unmanageable symptom experienced by many patients.

Research on anticipatory nausea has been predominately focused on the association between the hospital context and nausea. However, results of recent studies have suggested that social factors may also have an impact on the development and modulation of AN. In fact, one patient has reported experiencing nausea and vomiting when they saw their oncologist in a mall setting (Divgi, 1989). In humans, social factors play an essential role in toxin detection and avoidance, as well as toxin elicited and interpersonal disgust (e.g. Tybur et al., 2013). Consistent with this, rodents also display innate and acquired aversive, and avoidant, responses to a potential, as well as an actual, infection threat from a conspecific, or from cues associated with them (Arakawa, Cruz &

Deak, 2011; Kavaliers et al., 2004). Recently, it was demonstrated that rats can associate a social cue, in addition to contextual cues, with LiCl-induced sickness. This is similar to the sight of the nurse or oncologist triggering nausea and/ or vomiting in humans. It was found that male rats given LiCl and conditioned with a social partner displayed significantly more gaping responses while in the presence of that individual in a distinct context, compared to controls (Boulet et al., 2016; submitted for publication). Further, it was found that partners used during distinct context testing must be the same partners used during LiCl conditioning (familiar social partner) to elicit conditioned disgust responses. Therefore, it appears that social interactions and the presence of a familiar conspecific can have an impact on the development and expression of conditioned disgust responses in rats.

Social learning and social recognition both play fundamental roles in guiding appropriate behavioural responses displayed during social interactions. In a variety of species, the processing of social information and the mediation of social recognition and avoidance is regulated by the nonapeptides oxytocin (OT) and arginine vasopressin (Popik & van Ree, 1991; Donaldson & Young, 2008; Lukas et al., 2011). OT, as well as AVP, play essential roles in the regulation of social behaviour. In rodents, OT is critical for the full expression of naturally-occurring social investigations (Ferguson, Young & Insel, 2002; Choleris et al., 2009; Lukas et al., 2011). Both rats and mice either given oxytocin antagonists, having genetic modifications/ deletions of OT or OT receptor activity, display impaired social recognition and reduction in responses to an unfamiliar individual (Choleris et al., 2009; Lukas et al., 2011). In terms of social recognition, OT has been found to be particularly involved in the mediation of olfactory-based social

recognition in both male and female rodents (Kavaliers et al., 2004; Choleris et al., 2009). OT-mediated responses to positive social cues, as well as familiar individuals, have been shown to lead to a positive affective state and an increase in social interaction and social approach (Choleris et al., 2009). Results of human studies have also shown that intranasal OT administration facilitates social encounters (Bartz & Hollander, 2006) and decreases social anxiety and fear responses to familiar individuals (Petrovic et al., 2008; Kirsch et al., 2005). It has been proposed that OT mediates responses to socially salient stimuli, leading to approach to positive stimuli and avoidance of negative stimuli (Shamay-Tsoory & Abu-Akel, 2015). Likewise, OT was found to be associated with the expression of pathogen-related disgust-like responses and avoidance in rodents (Kavaliers et al., 2004; Kavaliers & Choleris, 2011).

There is now accumulating evidence suggesting that OT may be associated with the expression of conditioned taste avoidance (CTA) and/or AN. Early work by O'Connor, Cheng and North (1987) found that intraperitoneal administration of LiCl increased plasma levels of OT and AVP. Consistent with this, Verbalis et al. (1986) found administration of other nausea associated agents also increased plasma levels of OT, and to a lesser extent AVP. Later Olzewski et al. (2013) showed that LiCl-induced conditioned taste avoidance was associated with increased activation of OT/AVP neurons in the hypothalamic paraventricular and supraoptic nuclei. They further found that the administration of an OT receptor antagonist, L-368,899, prior to the two-bottle test (retrieval of CTA) did not cause avoidance of the saccharin solution (Olszewski et al., 2013). However, administration of the OT receptor antagonist during the CTA acquisition phase significantly impaired acquisition of a LiCl-induced CTA to saccharin. This study

suggests that activation of the oxytocin receptor during CTA acquisition may be crucial for the formation of CTA. Whether or not OT is associated with AN and conditioned disgust is not known. Results of human imaging studies suggests that OT at the level of the insula is correlated with the effect of social factors and aversive (including disgust) responses to social stimuli (Striepens et al., 2012). Interestingly the anterior insula is also involved in the expression of conditioned disgust in rodents (Tuerke et al., 2012). This raises the possibility that OT may be associated in the expression of socially mediated conditioned disgust. As indicated, although originally conceived as pro-social, more recent work has shown that OT is responsive to the salience of social stimuli, leading to enhanced responses and approach to positive social cues and decreased responses and avoidance of negative social factors (Domes et al., 2007; Kemp & Guastella, 2011). Further, the results of recent work have suggested that intranasal OT can lead to increased expression of disgust responses in humans (Theodoridou, Penton-Voak & Rowe, 2013). As there is accumulating evidence that OT is also associated with the expression of conditioned taste avoidance, this leads to the possibility that it is also associated with conditioned disgust in rats.

The present study examined the effect of a specific OT receptor antagonist, L-368,899 (Pettibone & Freidinger, 1997), on the expression of conditioned disgust responses to a familiar social partner. It was hypothesized that administration of the OT receptor antagonist would block/alter the expression of socially-mediated anticipatory nausea (disgust) in male rats.

6.2 Methods

6.2.1 Animals

Subjects were thirty-two naïve adult male Long-Evans rats (Charles River, Quebec, Canada) weighing between 250- 350g at the start of the experiment. Rats were pair-housed in translucent polypropylene cages (45 x 22 x 20cm) in a colony room maintained at 21 + 1 °C and under a 12 L: 12 D cycle (light 0700 – 1900h). Rats had *ad libitum* access to both food (ProLab Rat Chow) and water. Animals were tested during the light phase of the light:dark cycle between 0800 and 1500 h. All procedures were carried out in accordance to the Canadian Council of Animal Care guidelines and were approved by the Institutional (University of Western Ontario) Animal Care Committee.

6.2.2 Drugs

Lithium chloride (LiCl) was dissolved in distilled water to a molarity of 0.15M and given at a dose of 128 mg/kg (20 ml/kg). Isotonic saline (NaCl, 0.9%; 0.15M), at the same dose as the LiCl, was used as the control injection. During Test Day 1, an oxytocin receptor antagonist, L-368,899 (Tocris) was employed at a dose of 5 mg/kg (10 ml/kg) (Olszewski et al., 2013; Herisson et al., 2014) 10 minutes before testing. All injections were administered intraperitoneally immediately before conditioning. For Test Day 1, either L-368,899 or NaCl was administered 10 minutes prior to testing.

6.2.3 Apparatus

The conditioning chamber (used on all conditioning days and Test Day 2) consisted of a white, Plexiglas box (29 cm x 25cm x 29 cm) with two ventilation holes (on two opposing sides of the box). The box was set atop a clear glass plate with a mirror mounted at a 45 degree angle beneath the glass plate to view the ventral surface of the

animal. Lights were kept on during conditioning days and Test Day 2. Conditioning was done in a room different than Test Day 1. A distinct context (used on Test Day 1) was provided by a transparent black and white striped box (29 cm x 25 cm x 29 cm) with two ventilation holes, set atop the clear glass plate. A mirror was again mounted at a 45 degree angle beneath the glass plate. Two 40 W red lights positioned under the striped chamber provided additional distinctive lighting cues. Although rats do not perceive the colour red (Jacobs et al. 2001), these lights provided lighting different from that to which they were previously accustomed. Lights were kept off in the room during Test Day 1. Behavioural responses on the test days were videotaped with a video camera (Sony DCR-DVD201, London, Ontario) positioned approximately 1 m from the mirror. The camera was attached directly to the computer.

6.3 Procedure

The experimental procedures are summarized in Figure 2.1.

6.3.1 Social conditioning

Rats were acclimatized to their new home cages for one week and were then handled on three separate days. Prior to conditioning trials, rats were habituated for one 10 minute session in the conditioning context, followed by habituation to the distinct stripped context for 10 minutes, 24 hours later. Twenty-four hours after the second habituation, the conditioning phase commenced. The conditioning phase consisted of four days, each separated by 72 hours. On each conditioning day, each rat was intraperitoneally (i.p.) injected with either LiCl (0.15 M, 20 ml/kg) or saline vehicle (0.9 % NaCl, 20 ml/Kg) and immediately placed in the conditioning apparatus for 30 minutes. All animals received an uninjected male rat as a social partner during conditioning. Social

partners were randomly selected and were animals different from the initial pair housed mates.

6.3.2 Social partner in distinct context (Test Day 1)

Seventy-two hours following the fourth conditioning day each animal that had received either LiCl or NaCl during conditioning, was administered either the OT receptor antagonist or saline, 10 minutes prior to placement in the distinct context. Each animal was then exposed to the distinctive striped chamber alone for two minutes prior to the introduction of the social partner. They were then left undisturbed for 10 minutes while their interactions were recorded. Conditioned disgust and social behaviours were recorded and scored using the Observer (Noldus Information Technology, Sterling Va) event –recording software.

Dependent disgust related behavioural variables analyzed included gaping frequencies and the composite scores (Ossenkopp & Mazmanian, 1985) of aversive responses that did not include gaping (paw treads, forelimb flails, chin rubs and head shakes (Cloutier et al., 2011 and Cloutier et al., 2012), as well as spontaneous orofacial behaviours (tongue protrusions and mouth movements). Gaping was defined as lowering of the jawbone and the pushing or thrusting out of the lower teeth (Limebeer et al., 2008; Cloutier et al., 2011). Assessments of these distinct behaviours have been previously shown to have a very high inter-observer reliability (Cloutier et al., 2011 and Cloutier et al., 2012).

Dependent social behaviours displayed by conditioned animals were manually scored according to previously described criteria (Pellis et al., 1997). These behaviours included: 1. Frequency of social initiations: number of snout to nape contacts. 2. The

number of facing defenses (withdrawal of the nape from the partner's snout by turning to face the partner) 3. The number of evasive defenses (withdrawal of the nape from the partner's snout by either running or turning away from the partner).

6.3.3 Re-exposure to original conditioning context (Test Day 2)

Twenty-four hours following Test Day 1, each experimental animal was exposed alone to the original white conditioning context (conditioning apparatus), for a 10 minute period, while in a drug free state. During these tests the rats' orofacial and aversive behaviours were again recorded. Figure 3.1.

6.4 Statistical analyses

The dependent conditioned disgust variables – gaping behaviour and composite aversive behaviours were each analyzed with separate 2X2 analysis of variance (ANOVA) for conditioning drug treatment and test day drug treatment. Gaping behavior was also analyzed with a one-way ANOVA with one between subject factor of group (at 4 levels: Na-NaCl; Li-NaCl; Na-OTX; and Li-OTX). Gaping behaviour for the two-minute pre-exposure was also analyzed with a separate 2X2 ANOVA for conditioning drug treatment and test day drug treatment. A split-plot ANOVA was employed to determine differences in spontaneous orofacial behaviours. These tests were repeated for Test Day 2. A repeated measures test was employed to measure differences in Test Day 1 and Test Day 2 for gaping and other aversive behaviours.

Social variables – social initiations, evasive defense, and facing defense were analyzed with separate split-plot ANOVAs for conditioning drug treatment and test day drug treatment. They were also analyzed with separate one way ANOVAs, with one between subject factor of group (at 4 levels: Na-NaCl; Li-NaCl; Na-OTX; and Li-OTX).

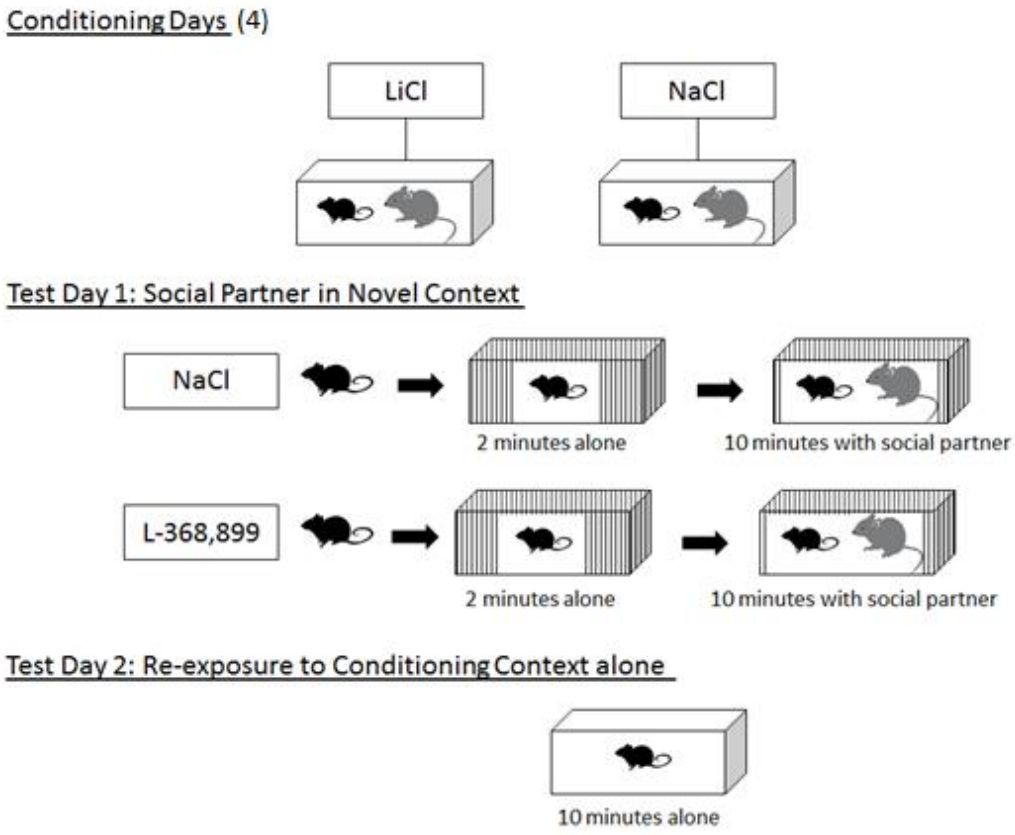

Figure 3.1. Illustration of experimental procedures used on conditioning and test days

Least significant difference (LSD) post-hoc pair-wise comparisons were used following significant interactions and/or main effects to determine differences among the groups. LSD post-hoc test was chosen as this is an exploratory study. All hypothesis tests used an alpha of .05, and all data were analyzed using SPSS 18.0 for Windows.

6.5 Results

6.5.1 Oxytocin receptor antagonist and social partner in distinct context (Test Day 1)

A 2X2 ANOVA for the two minute pre-exposure to the distinct context alone revealed no significant interaction between conditioning drug and test drug. Further, no main effects for either of these drugs on gaping behaviour was discovered (Figure 3.2A). Following this initial two minute exposure, social partners were introduced into the distinct chamber and a 2X2 ANOVA for gaping behaviour revealed the following differences. A significant main effect of conditioning drug on gaping behaviour was determined, $F(1, 31) = 4.82$, $p = .037$, in that LiCl treated rats gaped significantly more than NaCl treated rats. No significant main effect of test drug on gaping behaviour was discovered. Further, no conditioning drug by test drug interaction was found. However, it should be noted that the LiCl-OTX group showed no gaping while the LiCl-Na group did show some.

A one-way ANOVA for group on gaping behaviour revealed a significant main effect, $F(1, 31) = 4.15$, $p = 0.015$. Post-hoc analyses revealed that the LiCl-Na group gaped significantly more than the LiCl-OTX group, $p = 0.013$. The LiCl-Na group was also significantly different from the NaCl-Na ($p = 0.006$) and the NaCl-OTX groups ($p = 0.006$) (Figure 3.2B).

A 2X2 ANOVA for total aversive behaviours revealed no significant main effects of conditioning drug or test day drug. No significant interaction effect was discovered.

A split-plot ANOVA of the composite score of regularly occurring spontaneous orofacial behaviours (mouth movements and tongue protrusions) revealed no significant main effect of conditioning drug, $F(1, 31) = .116$, $p = .736$, or significant main effect of test drug, $F(1, 31) = .236$, $p = .631$. Further no significant interaction between conditioning drug and test drug was discovered.

6.5.2 Social interactions with conspecific (Test Day 1)

A 2X2 ANOVA was used to determine the effect of conditioning drug and testing drug on social initiations. Tests of between-subjects effects revealed a significant main effect of conditioning drug on social initiations, $F (1, 27) = 5.46$, $p = 0.027$. Animals conditioned with LiCl made significantly more social initiations towards their social partners compared to animals conditioned with NaCl. The analysis also revealed a significant main effect of test drug on social initiations, $F (1, 27) = 4.07$, $p < 0.05$. Animals pre-treated with NaCl on Test Day 1 showed more social initiations towards their partner compared to animals pre-treated with the OT receptor antagonist. Further, a significant interaction between conditioning drug and test drug was found, $F (1, 27) = 7.23$, $p = 0.012$. Animals treated with LiCl during conditioning and then given NaCl during test day 1 displayed significantly more initiations towards their social partner compared to all other groups (Figure 3.3).

A one-way ANOVA for group on social initiations revealed a significant effect, $F (1, 27) = 5.726$, $p = 0.004$. The LiCl-Na group displayed significantly more social

initiations towards their partner compared to the LiCl-OxAnt ($p = 0.003$), NaCl-OxAnt ($p = 0.004$) and NaCl-Na ($p < 0.001$).

A split-plot ANOVA was employed to uncover the effects of drug and social condition on facing defense behaviour. The analysis did not reveal any significant main effects or interaction effect. Finally, a 2X2 factorial ANOVA was utilized to determine the effects of drug and social condition on evasive defense behaviour. No significant main effects or interactions were uncovered for evasive defense behaviour.

6.5.3 Re-exposure to original conditioning context (Test Day 2)

A 2X2 ANOVA revealed a significant main effect of conditioning drug on conditioned gaping frequency. LiCl treated rats displayed significantly more gaping responses, $F(1, 27) = 6.13$, $p = 0.02$, compared to NaCl treated rats. However, no significant main effect of test drug on gaping behaviour or interaction effect was found (Figure 3.2C).

A one-way ANOVA for group on gaping behaviour revealed no significant effect between groups.

A 2X2 ANOVA for total aversive behaviours revealed a significant main effect of conditioning drug on aversive behaviour, $F(1, 27) = 8.31$, $p = 0.008$. Animals conditioned with LiCl showed significantly more aversive behaviours compared to animals conditioned with NaCl. However, no significant main effect of test day drug or interaction effect was discovered.

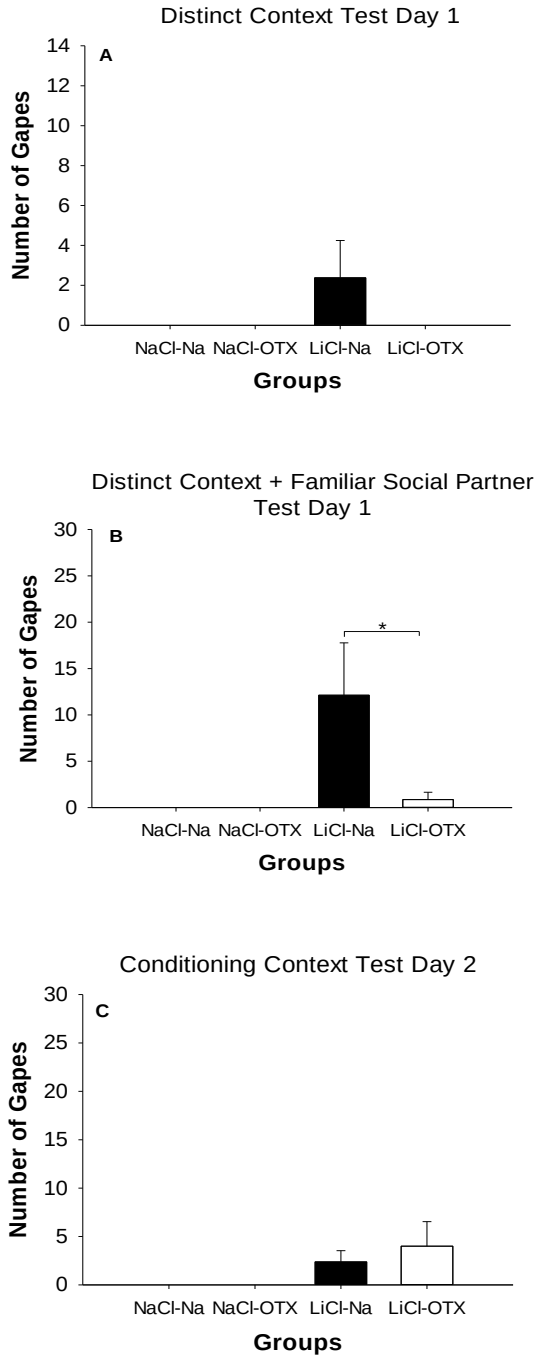

Figure 3.2. **(A)** Mean (+S.E.M.) number of gaping behaviours on Test Day 1 for the four treatment groups [NaCl-Na (n = 8), NaCl-OTX (n = 8), LiCl-Na (n = 8) and LiCl-OTX (n = 7)] for the 2-minute period in the absence of a social familiar partner. No significant differences were discovered between groups. **(B)** Mean (+S.E.M.) number of gaping behaviours on Test Day 1, in the presence of a familiar social partner for 10 min. The LiCl-Na group gaped significantly more than the LiCl-OTX group (**p* = 0.013). **(C)** Mean (+S.E.M.) frequency of gaping behaviour on Test Day 2. No significant differences in gaping frequencies were found between the two LiCl treated groups

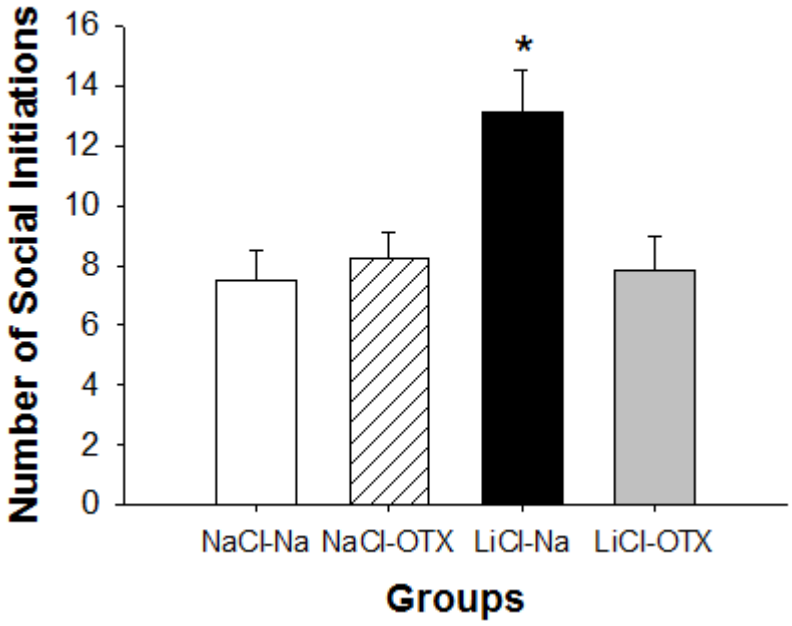

Figure 3.3. Mean (+S.E.M.) number of social initiations of experimental animals towards social partner during Test Day 1. The LiCl-Na group displayed significantly more social initiations towards their social partner compared to the LiCl-OTX (*p = 0.003), NaCl-OTX (*p = 0.004) and NaCl-Na (*p < 0.001).

6.5.4 Test Day 1 versus Test Day 2

A repeated measures ANOVA revealed a significant difference between groups across test days for gaping behaviour. LiCl-Na rats gaped significantly more on Test Day 1 (distinct context with social partner) compared to Test Day 2 (original conditioning context alone), $p = 0.002$.

A repeated measures ANOVA for aversive behaviours across test days revealed a significant difference for the LiCl-Na group. This groups displayed significantly more aversive behaviours on Test Day 2 compared to Test Day 1, $p < 0.001$.

6.6 Summary of results

On Test Day 1 (distinct context) during the initial 2 minute pre-social exposure, rats that were conditioned with LiCl and pre-treated with L-368,899 (LiCl-OTX) showed lower levels of conditioned disgust compared to animals conditioned with LiCl and pre-treated with NaCl before testing (LiCl-Na). Upon introduction of the social partner, the LiCl-Na group displayed significantly more gaping reactions compared to the LiCl-OTX group. The LiCl-OTX group also displayed decreased social initiations towards their partner compared to the LiCl-Na group, with no effect on social avoidance. No significant differences in gaping frequencies were determined between LiCl treated groups on Test Day 2. However, the LiCl-Na group gaped significantly more on Test Day 1 compared to Test Day 2. Although it was not significant, the LiCl-OTX group gaped more on drug-free Test Day 2 compared to Test Day 1. Further, the LiCl-Na group displayed significantly more aversive behaviours on Test Day 2 (conditioning context alone) compared to Test Day 1 (distinct context with social partner).

6.7 Discussion

The results of the present study demonstrate that oxytocin (OT) is involved in the expression of socially-mediated conditioned disgust in male rats. It was found that rats given an OT receptor antagonist, L-368,899, 10 minutes prior to testing, gaped significantly less in the distinct context in the presence of their social partner compared to controls. Rats conditioned with LiCl and pre-treated with the OT receptor antagonist also displayed more ambivalent social interactions with their social partner compared to the LiCl, NaCl-treated, control animals. These findings are consistent with, and extend, prior findings of OT involvement in the mediation of CTA in rats (Olzewski et al., 2013), and the expression of socially induced unconditioned disgust in humans (Theodoridou, Penton-Voak & Rowe, 2013), as well as pathogen and toxin-induced disgust in rodents (Kavaliers et al., 2004).

The present results demonstrate that social factors can function as cues for the expression of conditioned disgust. The presence of a familiar social partner during LiCl toxin conditioning resulted in drug-free conditioned gaping by LiCl-Na rats on Test Day 1. This is consistent with, and extends, prior findings showing that rats can associate a social partner with sickness, as evidenced by increased gaping in the presence of the partner in a distinct context, compared to alone in the original conditioning context (Boulet et al., 2016 submitted for publication). Interestingly, the increased gaping seen in the LiCl-Na animals did not correspond with decreased social initiations. This is again consistent with prior studies showing LiCl treated animals conditioned with a social partner showed more social initiations towards their partner compared to LiCl treated animals conditioned alone. However, although these animals displayed increased social

initiations, they also displayed hesitant and ambivalent aversive and avoidant social responses towards their partner, as seen by their propensity to engage in social contact, mixed with defensive and avoidant behaviours. This is in agreement with studies with Mongolian gerbils showing that the animals display hesitant social interactions with, and ambivalent aversive behaviours towards, animals that have been previously associated with LiCl (Pettijohn, 1981). The increased social initiations seen in the current study are also consistent, in part, with research by Lopes et al. (2012). They showed that male zebra finches displayed decreased sickness behaviours in a colony setting compared to in isolation, as well as increased social initiations and interactions. Therefore, the increase in social initiations seen by the LiCl-Na group may be due to an attempt to overcome the negative symptoms of sickness and benefit from the positive effects of social interaction. This is also similar to the mixed social responses seen in mice and rats towards either an infected, or potentially infected, individual, as well as the hesitant responses of humans towards unfamiliar individuals or endotoxins (Kavaliers et al., 2004, Parkinson et al., 2012, Lopes et al., 2012).

Animals conditioned with LiCl, and then pre-treated with the OT receptor antagonist on Test Day 1, showed decreased gaping responses compared to the other LiCl toxin conditioned rats treated with NaCl prior to testing. It was also found that compared to Test Day 1 (social partner in distinct context), LiCl-Na rats displayed a lower number of gapes on Test Day 2 (alone in conditioning context); whereas LiCl-OTX rats did not differ between test days. Therefore, the OT receptor antagonist attenuated, but did not fully eliminate, the expression of socially mediated conditioned disgust. Further, during the two minute pre- social stimulus exposure, rats treated with L-368,899 showed

completely eliminated gaping responses, whereas animals treated with NaCl still showed gaping prior to the introduction of their social partner. This raises the possibility that OT may play a role in the expression of both environmentally conditioned disgust and socially mediated conditioned disgust. However, further work is needed to determine OT involvement in the mediation of context mediated conditioned disgust.

In view of the data showing that LiCl results in an increase in the number and activity of vasopressin-neurons and oxytocin-neurons (Verbalis et al., 1986; O'Connor, Cheng & North, 1987), it is possible that OT may have a role in the establishment/ expression of sickness-related behaviors following LiCl toxin conditioning. This is in part consistent with the findings that the OT receptor antagonist decreased the expression of conditioned disgust in rats. However, this conflicts with research by Olszewski et al. (2013) who showed that oxytocin receptor blockade during acquisition, but not retrieval, of conditioned taste avoidance reduced aversion. In the current study, administration of L-368,899 10 minutes prior to drug-free testing resulted in a decrease in gaping behaviour, suggesting that the receptor antagonist blocked the retrieval of conditioned disgust. However, the study by Olszewski et al. (2013) utilized a two-bottle test for conditioned taste avoidance which required the animal physically approach the bottles, as well as display both appetitive and consummatory responses (Best & Mechoulam, 1986). Parker, Rana and Limebeer (2008) have argued that this measure of conditioned taste avoidance and does not accurately assess disgust. The current study did not, however, consider whether administration of an OT receptor antagonist plays a role in the acquisition of conditioned disgust. Despite these limitations, the present study may more

accurately depict the role oxytocin plays in socially mediated conditioned disgust, rather than conditioned taste avoidance, in rats.

Administration of an OT receptor antagonist prior to testing also led to decreased social initiations in the LiCl conditioned animals. This is in part consistent with the involvement of OT in the mediation of social investigations and social recognition in rodents (Dluzen et al., 2000; Lukas et al., 2011; Kavaliers & Choleris, 2011; Oettl et al., 2016). Therefore, it could be that the animals given the OT receptor antagonist were no longer able to recognize their familiar social partner. Results of prior investigations showed that an unfamiliar social partner elicited less gaping than a familiar social partner (re: Boulet et al., 2016). The OT receptor antagonist treated animals in the current study may be acting as if this is an unfamiliar individual, different from the one they were conditioned with. This is consistent with research showing that high levels of peripheral oxytocin antagonist administration decreases social memory (Popik & Vetulani, 1991; Popik, Vetulani & van Ree, 1992; Benelli et al., 1995). In addition, the decreased social initiations could be due to increased social fear and altered social salience (i.e. more negative) of the social partner. Rats given a foot shock during investigation of a conspecific showed decreased investigation of an unfamiliar conspecific compared to a familiar conspecific (Toth, Neumann & Slattery, 2012). Further, rats centrally infused with oxytocin prior to social fear extinction training showed completely eliminated social fear expression (Zoicas, Slattery & Neumann, 2014). Therefore, blockade of oxytocin in the current study may also be causing decreased social initiations in these animals due to increased social fear.

If the OT receptor antagonist leads to decreased social recognition, this would account for the decreased gaping, as the animals are no longer able to recognize the partner as a familiar social stimulus. As indicated, this is in part consistent with previous work demonstrating that LiCl-treated animals conditioned and tested with the same familiar social partner display significantly more gaping reactions compared to LiCl-treated animals conditioned with a familiar partner, but then tested with an unfamiliar social partner. However, oxytocin receptor blockade may have an actual effect on the expression of socially mediated conditioned disgust. This is suggested by findings that animals pre-treated with L-368,899 prior to testing showed completely eliminated gaping reactions during the initial two minute exposure in the absence of their familiar social partner. However, the gaping levels increased upon introduction of the social partner, indicating that the animals may recognize the partner but that L-368,899 is having an effect on the conditioned gaping per se. This is consistent with human research showing OT in humans has been associated with the expression of disgust, including that which is socially mediated (Theodoridou, Penton-Voak & Rowe, 2013; Striepens et al., 2012). Moreover, OT in rodents is associated with the expression of pathogen/ infection related disgust reactions and responses independent of effects on social recognition (Kavaliers et al., 2004; Kavaliers & Choleris, 2011).

In humans, the anterior insula (AI) is associated with the expression of disgust (e.g. Wicker et al., 2003; Chapman & Anderson, 2012). Specifically, it has been shown that elevated levels of OT in the AI are associated with the display of disgust. In rodents, the AI is also associated with the expression of anticipatory nausea and conditioned disgust (Striepens et al., 2012). Tuerke et al. (2012) showed that interrupting AI activity

blocked expression of conditioned disgust responses in rats. They showed that a 5-HT3 receptor in the insula was involved in the mediation of anticipatory nausea. Interestingly, and of relevance, OT has been shown to modulate 5-HT3 receptor activity (Mottolese et al., 2014). This further supports the possible involvement of OT in the mediation of socially conditioned disgust.

There are a number of limitations to the present study. The possible involvement of AVP in the expression of conditioned disgust was not considered. However, a previous study using vasopressin-deficient rats showed normal establishment of CTA (Yirmiya, Holder & Garcia, 1987). Further, recent research with adult Syrian hamsters has shown that both OT and AVP act on OT receptors, and not AVP V1a receptors, to enhance social recognition (Song et al., 2016). This suggests lack of AVP likely does not lead to decreased CTA, whereas lack of oxytocin does seem to lead to decreased acquisition of CTA (Olszewski et al., 2013). As indicated, it is also possible that the OT receptor antagonist is primarily affecting social recognition rather than the expression of the anticipatory nausea and conditioned disgust. Further studies are needed, using centrally and peripherally acting antagonists of various dosages and toxins other than LiCl, to tease apart the social recognition component from the conditioned disgust component. As well both the development, as well as its acquisition, of conditioned disgust needs to be assessed. Finally, considering female mammals tend to contain more oxytocin-producing neurons than males (Del Cerro, 1998; Nelson & Panksepp, 1998), and that the prevalence of anticipatory nausea in females is higher than males (Boakes et al., 1993; Cloutier, Kavaliers & Ossenkopp, 2016), research should be conducted to determine if sex differences exist in the involvement of OT in the expression of socially conditioned

disgust. The current study, however, does demonstrate that oxytocin has a role in the modulation and expression of socially-mediated conditioned disgust in rats.

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CHAPTER 4

GENERAL DISCUSSION

4.1 Discussion

There is accumulating evidence for the expression of disgust in both humans and non-human animals. The results of a variety of studies have suggested that rodents display conditioned disgust as evidenced by a gaping response to various contextual cues previously associated with sickness (Limebeer, Hall and Parker, 2006; Limebeer et al., 2008; Rock et al., 2009; Tuerke, Leri & Parker, 2009; Ossenkopp et al., 2011). The current thesis examined the involvement of social factors in the development, modulation and expression of conditioned disgust responses in male rats. It was shown that social factors have a role in the development and expression of conditioned disgust in rats, with a familiar, though not an unfamiliar partner, serving as a cue for the expression of anticipatory nausea (anticipatory disgust/ gaping). Moreover, these responses likely involve a variety of sensory cues, as a familiar social odour (urine) by itself failed to elicit significant conditioned disgust. Further, this socially-mediated conditioned disgust may be, in part, regulated by the nonapeptide, oxytocin (OT), with an OT receptor antagonist significantly decreasing the expression of socially-mediated conditioned disgust in rats.

In chapter 2 it was demonstrated that the presence of a familiar social partner during toxin (lithium chloride – LiCl) conditioning resulted in the display of conditioned gaping responses on Test Day 1 (distinct context with social partner). Further, these rats displayed decreased gaping, relative to a non-socially conditioned individual, while in the original conditioning context without a social partner (Test Day 2). This indicates that the distinct context in the presence of the social partner is more aversive than the original conditioning context without the social partner. Moreover, animals conditioned with a social partner also displayed a high number of social initiations and ambivalent social

approach-avoidance responses towards their partner, again suggestive of disgust associated with the social partner. In experiment 2, rats conditioned with a familiar social partner then tested with an unfamiliar social partner displayed significantly decreased gaping levels, as well as a reduced number of social initiations towards their partner. This indicates that a familiar social partner is necessary for the establishment of full conditioned disgust. In experiment 3, animals conditioned and tested with a familiar social odour (urine) (Li-Odour) showed gaping levels similar to that of animals conditioned alone. This suggests that the actual physical presence of the familiar social partner and the various sensory cues associated with that individual are required for the full establishment of socially-mediated conditioned disgust.

The involvement of social factors in the mediation of the expression of conditioned disgust was replicated in chapter 3. It was further found that rats conditioned with LiCl and pre-treated with an OT receptor antagonist prior to testing showed decreased gaping responses while in the distinct context in the presence of their familiar partner. These rats also showed a lower number of social initiations towards their partner. This indicates that OT is involved in the modulation of the expression of conditioned disgust in rats, and potentially anticipatory nausea in humans.

All of the groups of rats treated with LiCl and conditioned, and tested, with a familiar social partner (i.e. LiCl-Social and LiCl-Na) showed decreased gaping levels when re-exposed to the original conditioning context alone on Test Day 2. Decreased gaping displayed by the LiCl-Social and LiCl-Na groups on Test Day 2 could be due to extinction of the behavior. However, as shown in chapter 2 experiment 2, animals treated with LiCl and conditioned alone (LiCl-Alone) gaped significantly more on Test Day 2

compared to Test Day 1. Therefore, it is likely that the social partner is serving as a cue for the LiCl-Social and LiCl-Na animals, rather than the original conditioning context alone; and that the decreased gaping displayed by these groups on Test Day 2 is simply not due to extinction. It could be that on Test Day 1 the animals that were conditioned alone displayed a decreased gaping when exposed to another individual due to a "social buffering" effect, whereby the presence of another individual attenuates aversive/ stress responses (Davitz & Mason, 1955; Lopes et al., 2012). However, since the gaping response is a variable response, further research needs to be conducted.

Another possible explanation for the decreased gaping responses seen by the LiCl-Social and LiCl-Na groups on Test Day 2 is that the utilization of two distinct yet similar contexts (i.e. Plexiglas boxes on glass surface), leads to the possibility of context carry-over (generalization). Rats could be associating the distinct context itself with sickness as it is similar to the original conditioning context. However, the finding that the LiCl-Alone group gaping significantly less than the LiCl-Social group during the pre-social two-minute exposure in the distinct context suggests minimal context carry-over. Since the LiCl-Alone group hardly gaped during the 2 minutes in the distinct context, we can assume that the context was sufficiently different from the original conditioning context and was therefore not as aversive to this group. However, in experiment 1, LiCl-Social rats gaped in the distinct context even in the absence of their social partner. This may have been due to the rats anticipating the arrival of their social stimulus, or simply that pairing a social stimulus with an illness inducing agent results in an amplified expression of disgust responses in these rats. Presenting two distinct conditioned cues (social and non-social context) can lead to overshadowing, wherein the saliency of one

cue is greater than that of the other (Lindsey & Best, 1973; Best & Meachum, 1986). However, in chapter 2 experiment 2, during the initial pre-social 2 minute exposure, no differences were found between groups for gaping behavior. Therefore, it is likely that the social cue is more salient than the non-social cue. However, further research is necessary to clarify this.

Although animals treated with LiCl and conditioned with a social partner showed increased gaping compared to LiCl-treated controls, they did not display noticeable social avoidances. Instead, all of the rats treated with LiCl and conditioned with a social partner showed hesitant social initiations paired with ambivalent social withdrawals. These animals could be seeking social interaction to decrease the negative symptoms associated with sickness. This is consistent with research by Lopes et al. (2012), showing that male zebra finches displayed decreased sickness behaviours in a colony setting compared to in isolation, as well as increased social initiations and interactions. This is also, in part, consistent with studies showing ambivalent social responses by mice and rats towards either an infected, or potentially infected, individual, as well as the hesitant responses of humans towards unfamiliar individuals or endotoxins (Kavaliers et al., 2004; Parkinson et al., 2012; Lopes et al., 2012). As mentioned, the social initiations shown by the rats that were previously conditioned with a social partner could be seeking a “social buffering” effect and a reduction in malaise associated responses.

Animals conditioned with a familiar social partner and then tested with an unfamiliar social partner (experiment 2 chapter 2) show decreased gaping in the presence of this unfamiliar social partner. Therefore, it is likely that the animal associated a specific individual (e.g. the animal it was conditioned with) with sickness, and were able

to recognize and distinguish the conditioning social partner from the testing social partner. A variety of sensory processes are involved in social recognition. In rodents, social information is encoded via olfactory or pheromonal signals, as well as auditory and visual signals (Toth, Neumann, & Slattery, 2012). Rodents also have the ability to differentiate specific individuals on the basis of odour (Kavaliers et al., 2004). Further, odour cues are involved in the mediation of various aspects of human behaviour, including disgust (Moshkin et al., 2012; Olsson, 2014). Therefore, the socially-mediated conditioned disgust seen in chapter 2 experiment 1 may, in part, be due to odour cues. In chapter 2 experiment 3, it was found that urine odours alone failed to elicit significant conditioned disgust. Rats conditioned with a urine odour cue displayed very similar gaping patterns to the animals conditioned alone. Upon re-exposure to the conditioning context alone (without the odour), animals conditioned with an odour displayed a similar number of gapes as Test Day 1 (distinct context). Conditioning with urine odour alone may not be a salient cue and/or might be overshadowed by the context. In addition, the degree of exposure to both volatile and non-volatile urine odour cues and the role of odours in addition to that of urine needs to be addressed in future studies.

There is evidence suggesting the nonapeptides oxytocin (OT) and arginine-vasopressin (AVP) may be associated with the expression of conditioned taste avoidance (CTA) and/or AN. Prior research has shown that intraperitoneally administering LiCl leads to increased plasma levels of OT and AVP. Further, oxytocin has a major role in the determination of social interaction, specifically social recognition and social avoidance (Dluzen et al., 2000; Lukas et al., 2011; Kavaliers & Choleris, 2011; Oettl et al., 2016). This raised the possibility that oxytocin may play a role in the development and/or

expression of socially mediated toxin (LiCl) conditioned disgust. In chapter 3, animals conditioned with LiCl and a social partner, then pre-treated with an OT receptor antagonist, showed significantly decreased gaping responses on Test Day 1. This is consistent with studies showing associations between conditioned taste avoidance and elevations in the nonapeptides, OT and AVP (O'Connor, Cheng & North, 1987; Verbalis et al., 1986). Specifically, Verbalis et al. (1986) found administration of nausea associated agents, and other stimuli producing learned conditioned avoidance, increased plasma levels of OT and AVP. Further, intraperitoneal administration of LiCl leads to increase number of OT and AVP neurons in the hypothalamic paraventricular and supraoptic nuclei (O'Connor, Cheng, & North, 1987). Therefore, it is possible that OT release following LiCl administration leads to the establishment of conditioned disgust, and potentially socially-mediated conditioned disgust.

As oxytocin plays a role in the mediation of social recognition, the animals given the OT receptor antagonist may no longer recognize their social partner as familiar. The LiCl-OTX group may therefore be acting as if this is an unfamiliar individual. This is consistent with research showing that high levels of peripheral OT antagonist administration decreases social memory in rats (Popik & Vetulani, 1991; Popik, Vetulani & van Ree, 1992; Benelli et al., 1995), as well as impairments in social recognition seen with genetic ablations of OT in mice (Choleris et al. 2003). This finding of is also consistent with the findings from chapter 2 showing decreased gaping towards an unfamiliar social partner compared to a familiar social partner. However, it is likely that the OT receptor antagonist is playing a role in decreasing the socially-mediated disgust, as the LiCl-OTX rats showed no gaping during the initial 2 minute pre-social stimulus

exposure, whereas the LiCl-Na animals did. This suggests that the OT receptor antagonist is diminishing the gaping behavior in these animals even in the absence of their partner. Further, results of studies with humans have shown that OT is associated with the expression of disgust, including that which is socially mediated (Theodoridou, Penton-Voak & Rowe, 2013; Striepens et al., 2012). Likewise, in rodents, OT is associated with the expression of pathogen/ infection related disgust reactions and responses independent of effects on social recognition (Kavaliers et al., 2004; Kavaliers & Choleris, 2011).

There is suggestive evidence for the involvement of the anterior insula (AI) in the expression of conditioned disgust in both humans and non-human animals. Results of imaging studies have indicated that augmented activity of the AI is associated with the expression of disgust in humans (e.g. Wicker et al., 2003; Chapman & Anderson, 2012). Interestingly, elevated levels of OT in the anterior insula were shown to be associated with the display of disgust responses to social stimuli (Striepens et al., 2012). Furthermore, in rodents, the anterior insula has also been implicated in the expression of anticipatory nausea and conditioned disgust. Tuerke et al. (2012) showed that interrupting anterior insula activity blocked expression of conditioned disgust responses in rats. Additionally, they showed that a 5-HT3 receptor in the insula was involved in the mediation of anticipatory nausea. Interestingly, and of relevance, 5-HT3 receptor activity is modulated by oxytocin (Mottolese et al., 2014), further supporting the possible involvement of OT in the mediation of socially conditioned disgust.

Rats pre-treated with an OT receptor antagonist also showed decreased, although not completely eliminated, social initiations towards their partner compared to the LiCl-Na group. This is consistent with studies showing that both rats and mice given OT

antagonists, as well as deletions of OT receptor activity, show decreased social preference towards an unfamiliar individual (Choleris et al., 2009; Lukas et al., 2011). However, the decreased social initiations could be due to increased social fear and altered social salience (i.e. more negative) of the social partner. This is consistent with results from human studies suggesting that oxytocin can enhance the salience of disgust, leading to approach-avoidance of the disgust-related cues (Theodoridou, Penton-Voak & Rowe, 2013). It is also in agreement with the findings of Toth, Neumann and Slattery (2012) that rats given a foot shock during investigation of a conspecific displayed decreased investigation of an unfamiliar conspecific compared to a familiar conspecific. Likewise, central infusion into the dorsolateral septum with OT prior to social fear extinction training completely eliminated social fear expression (Zoicas, Slattery & Neumann, 2014). Specifically, animals who underwent social fear conditioning (i.e. given a foot shock every time they approached an unfamiliar social stimulus), showed diminished social fear expression during extinction training when intracerebroventricularly infused with oxytocin, compared to animals who did not receive oxytocin. Therefore, blockade of oxytocin in the current study may also be causing decreased social initiations in these animals due to increased social fear.

Although the results of the present study demonstrated that a familiar social stimulus can play a role in the development and expression of conditioned disgust, there are a number of limitations. For example, the social behaviours of the untreated social partners were not quantified. In addition, rates of extinction from Test Day 1 to Test Day 2 and roles of social buffering (i.e. seeking interaction to diminish sickness-associated behaviors) need to be considered more fully. Further, the possible involvement of AVP in

the expression of conditioned disgust was not considered here. However, a previous study using vasopressin-deficient (but not OT deficient) Battleboro rats showed normal patterns of CTA development and expression (Yirmiya, Holder & Garcia, 1987). As indicated, it is also possible that the OT antagonist is primarily affecting social recognition rather than the expression of the anticipatory nausea and conditioned disgust. Further studies are needed using centrally and peripherally acting OT antagonists of various dosages, and toxins other than LiCl. Further, both the development, as well as its acquisition, of conditioned disgust needs to be assessed. Finally, considering females have higher levels of OT as well as more oxytocin neurons than males (Del Cerro, 1998; Nelson & Panksepp, 1998), and that the prevalence of anticipatory nausea is higher in females than males (Boakes et al., 1993; Cloutier, Kavaliers & Ossenkopp, 2016), possible sex differences in the socially-mediated conditioned disgust need to be examined. Despite these limitations, the current thesis, provides evidence that conditioned disgust can be socially-mediated, with the expression, in part, being regulated by oxytocin.

The socially-mediated conditioned gaping seen in this thesis has major implications for patients experiencing anticipatory nausea (AN). The development of anticipatory nausea may be due to a variety of sensory cues, including social factors. This is consistent with the anecdotal report by one oncologist stating one of his patients vomited when they saw him in a setting other than the hospital setting (Divgi, 1989). It also suggests that a familiar, the same nurse always administering the drug, compared to an unfamiliar, different nurses administering the drugs, may have an impact on the severity of the anticipatory nausea.

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