2011

SIMULTANEOUS CONDITIONING OF ANTICPATORY NAUSEA AND TASTE AVOIDANCE AND THE INFLUENCE OF IMMUNE SYSTEM STIMULATION

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SIMULTANEOUS CONDITIONING OF ANTICPATORY NAUSEA AND TASTE AVOIDANCE AND THE INFLUENCE OF IMMUNE SYSTEM STIMULATION

(Spine title: Endotoxin Effects on Simultaneous Aversion-related Conditioning)

(Thesis format: Integrated-Article)

by

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Graduate Program in Psychology

A thesis submitted in partial fulfillment of the

Requirements for the degree of Master of Science

School of Graduate and Postdoctoral Studies

The University of Western Ontario

London, Ontario, Canada

THE UNIVERSITY OF WESTERN ONTARIO
SCHOOL OF GRADUATE AND POSTDOCTORAL STUDIES

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entitled:

Simultaneous Conditioning of Anticipatory Nausea and Taste Avoidance and the Influence of Immune System Stimulation

is accepted in partial fulfillment of the
requirements for the degree of

Master of Science

Date_____

Chair of the Thesis Examination Board

ABSTRACT AND KEY WORDS

Immune system stimulation with lipopolysaccharide (LPS) elicits a specific set of physiological and behavioral responses termed "sickness behavior". LPS treatment has been found to impair learning and memory in a variety of learning paradigms, including those for anticipatory nausea and conditioned taste avoidance. Traditional conditioning paradigms typically employ a single conditioned stimulus (CS) and unconditioned stimulus (US). This thesis used an intravascular (intraperitoneal) saccharin "taste" cue, together with the toxin LiCl, given immediately prior to anticipatory nausea context conditioning, in order to simultaneously condition responses to both internal (taste) and external (context) conditioning stimuli. The effects of LPS on the simultaneous acquisition of anticipatory nausea and taste avoidance were then examined. In addition to the establishment of a concurrent conditioning model, the present findings suggest that LPS pre-treatment was effective in disrupting both conditioned nausea and taste avoidance.

Keywords: endotoxin, lipopolysaccharide, toxin effects, anticipatory nausea, conditioned taste avoidance, learning, memory, conditioning, rats.

ACKNOWLEDGEMENT OF CO-AUTHORSHIP

This is acknowledgement of the contributions of Drs. Klaus-Peter Ossenkopp and Martin Kavaliers for their input into the conception of the present experiments and valuable comments on the writing of the manuscripts.

ACKNOWLEDGEMENTS

I would first like to thank Drs. Klaus-Peter Ossenkopp and Martin Kavaliers for their continued support and valuable guidance throughout my Masters. I also want to thank Dr. Shelley Cross-Mellor for her influence on my interests in this area of research, and for her guidance which became an integral part of my learning process. To Jen and Kelly, my lab mates and dear friends- thank you for all of your help.

DEDICATION

I would like to dedicate this thesis to my parents. Your unwavering support and positive encouragement throughout my life has helped me to build confidence, and to believe in myself. Thank you for giving me so many opportunities to learn and to grow.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

The aversive side-effects of chemotherapy treatment have been well-documented, where it is common for patients to experience severe nausea and/or vomiting after treatment sessions (Molassiotis, 2005; Morrow et al., 1998; Morrow, Roscoe, Korshner, Hynes, & Rosenbluth, 1998). Current anti-emetic treatments will attenuate vomiting, yet patients will report sustained subjective feelings of nausea (Molassiotis, 2005). Approximately 30% of all cancer patients who undergo chemotherapy treatment will experience an aversive type of conditioned learning called anticipatory nausea (AN). Anticipatory nausea is acquired through classical conditioning, wherein the repeated pairing of a conditioned stimulus (CS) with an unconditioned stimulus (US) will come to elicit a conditioned response (CR) upon re-exposure to the CS in the absence of the US (Hickok et al., 2003). After as little as one pairing, an association can form between the contextual elements of the hospital environment (CS) and the noxious side-effects of chemotherapy (US). Thus, patients will display vomiting and/or nausea (CR) prior to subsequent treatment sessions when they are re-exposed to the context stimuli of the hospital environment. Anticipatory nausea is reported by patients to be the most aversive side effect of chemotherapy, often causing many to forego further treatment that could be life-saving (Molassiotis, 2005).

A rodent model of anticipatory nausea has been established. Exposure to a context previously associated with "nausea" elicits an aversion-related response in the rat termed "conditioned gaping" behavior (Limebeer & Parker, 2000; Limebeer, Hall, & Parker, 2006; Limebeer et al., 2008; Chan, Cross-Mellor, Kavaliers, & Ossenkopp, 2009) providing an

animal model that can serve as a valuable preclinical tool for examining anticipatory nausea in chemotherapy patients.

Vomiting is often co-morbid with feelings of nausea (Stockhorst, Enck, & Klosterhalfen, 2007). Rats, however, lack an emetic reflex which leaves them incapable of vomiting (Hatcher, 1924). This inability to vomit has been thought to be due to elongated esophageal structures that cannot physically produce an emetic response (Travers & Norgren, 1986). Although it is difficult to determine when a rodent is subjectively experiencing nausea, prior reports have shown that rats will exhibit a "conditioned gaping" behavior in response to a contextual environment that was previously paired with a nausea-inducing stimulus (e.g., lithium chloride (LiCl) and other toxins, and, provocative vestibular stimulation) (Limebeer et al., 2006; Limebeer, Litt, & Parker, 2009; Rock et al., 2009; Tuerke, Leri, & Parker, 2009). LiCl is an emetic toxin that has repeatedly served as an effective unconditioned stimulus capable of producing robust conditioned responses in a variety of learning paradigms (e.g., Riley & Freeman, 2004), including the anticipatory nausea paradigm. This conditioning model demonstrates that rats form an association between feelings of nausea and distinct contexts, and subsequently retrieve these associations to display aversion-related behaviors, such as gaping, upon re-exposure to the context (Chan et al., 2009; Limebeer et al., 2006; Limebeer et al., 2008; Parker & Limebeer, 2006; Ossenkopp et al., 2011).

"Conditioned gaping" behavior involves the repeated opening and closing of the lower mandible in rapid succession approximately 5-7 times per bout (Travers & Norgren, 1986), similar in topography to the retching behavior that precedes vomiting in

emetic species, such as, the house musk shrew, *Suncus murinus* (Andrews, Friedman, Liu, Smith, & Sims, 2005). Gaping behavior is a conditioned behavior, and it has to date not been observed as a reflexive response to emetic treatment. However, treatment with anti-emetic agents, such as, ondansetron (Limebeer & Parker, 2000) and the 5-HT_{1A} agonist 8-OH-DPAT (Limebeer & Parker, 2003), have been shown to attenuate the gaping response, thus providing evidence that gaping behavior is an index of a nauseous state. Thus, "conditioned gaping" has been accepted as the most quantifiable index of nausea in the rat.

Rats, like humans, also form strong associations between feelings of nausea and salient tastes. Conditioned taste aversion/avoidance is a behaviorally adaptive form of learning that enables animals to successfully reject or avoid consumption of potentially harmful food agents (Garcia, Lasiter, Bermudez-Rattoni, & Deems, 1985). Gustatory conditioning to solutions paired with (Eckel & Ossenkopp, 1996; Kent, Cross-Mellor, Kavaliers, & Ossenkopp, 2000; Ossenkopp & Eckel, 1995; Spector, Breslin, & Grill, 1988) or foods infused with (Cross-Mellor, Clarke, & Ossenkopp, 2004; Loy & Hall, 2002; Ossenkopp & Eckel, 1995; Ossenkopp, Ladowsky, & Eckel, 1997) an emetic toxin is acquired rapidly and can be very robust. It is important to note the distinction between taste aversion and taste avoidance. A conditioned taste *aversion* has been established when animals exhibit active aversive rejection responses (i.e., gapes, forelimb flails, head shakes, passive drip, and chin rubs) to an intraoral infused taste that was previously paired with a nausea-inducing US (i.e., LiCl). The taste reactivity test (TRT) is commonly employed to test for conditioned taste aversion. This test involves the involuntary

infusion (via intraoral cannula) of a salient taste that was previously paired with feelings of nausea during the conditioning phase (Berridge, Grill, & Norgren, 1981; Grill & Norgren, 1978). Upon infusion of the salient taste, animals will display aversion-related rejection responses to the taste, in the absence of any actual noxious treatment. A conditioned taste *avoidance* has been established when an animal refuses to voluntarily consume a salient taste that was previously paired with a nausea-inducing US (i.e., LiCl). In the classic two-bottle preference test for conditioned taste avoidance, animals previously infused with a palatable taste in conjunction with feelings of nausea will, in a drug-free state, prefer to drink a safe fluid, such as water, and avoid voluntary consumption of the taste originally associated with nausea during the conditioning phase (e.g., Rana & Parker, 2008).

The traditional oral presentation of taste cues in a taste avoidance paradigm is sufficient, but not necessary, for the acquisition of conditioned taste avoidance. Intravascular administration of a taste (e.g., saccharin) at high concentrations allows the taste to be transported through the blood, eventually stimulating taste receptors in the oral cavity (Fishberg, Hitzig, & King, 1933). This phenomenon was first noted when patients receiving intravenous (i.v.) drug treatment reported being able to taste their medication. This intravascular technique was later used to measure circulation time to and from various regions of the body (Fishberg et al., 1933). Intravenously administered saccharin sodium has been shown to produce conditioned taste avoidance in rats exposed to gamma radiation (illness-inducing agent) during conditioning (Bradley & Mistretta, 1971). Rapid extinction of gustatory conditioning has also been achieved through intraperitoneal

application of saccharin in the absence of the nausea-inducing unconditioned stimulus that was previously paired with the taste (Baum, Foidart, & Lapointe, 1974; Bellingham & Lloyd, 1987; Buresova & Bures, 1977).

The rodent models of anticipatory nausea and conditioned taste avoidance demonstrate the rat's ability to associate the aversive feelings of toxin-associated nausea with a distinct context or a salient taste, respectively. Until now, conditioning paradigms have focussed on the rodent's ability to condition to only one mode of the conditioned stimulus (i.e., either context or taste). Anticipatory nausea and conditioned taste avoidance represent robust forms of associative learning. Additionally, while it is important to examine the processes responsible for conditioned responses, it is also important to investigate ways in which this type of associative learning can be disrupted. For example, stimulation of the immune system by endotoxin treatment, such as, lipopolysaccharide (LPS) has been shown to affect the development of these conditioned responses in a deleterious manner.

Lipopolysaccharide is the smallest component of Gram-negative bacteria outer cell wall (Rietschel et al., 1994), and systemic treatment with this immunogen is widely used to mimic bacterial infection, and associated immune activity, in a variety of animal species. Bacteria-related immunogens, such as LPS, activate phagocytes, resulting in the release of pro-inflammatory cytokines, such as, interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), and interleukin-6 (IL-6), which in turn produce a specific set of behaviors collectively termed "sickness behavior" (Gatti & Bartfai, 1993; Laye, Parnet, Goujon, & Dantzer, 1994). The "sickness behavior" profile often includes, fever (Hart,

1988; O'Reilly, Vander, & Kluger, 1988; Roth, Aslan, Storr, & Zeisberger, 1997), decreased locomotor activity (Hart, 1988; Engeland, Kavaliers, & Ossenkopp, 2003; Franklin, Engeland, Kavaliers, & Ossenkopp, 2007; Yirmiya, Rosen, Donchin, & Ovadia, 1994), hypersomnia (Hart, 1988), decreased grooming (Hart, 1988), adipsia, and anorexia (Cross-Mellor, Kent, Kavaliers, & Ossenkopp,2000; Gayle, Ilyin, Flynn, Plata-Salaman, 1998; Langhans, 2000; Langhans, Harlacher, Balkowski, Scharrer, 1990), all of which are considered to be behaviorally adaptive and serve to help the organism counter bacterial infection (Hart, 1998).

In addition to producing sickness behavior, LPS treatment has been found to exert deleterious effects on learning and memory. For example, LPS administration has been shown to disrupt spatial learning in the Morris Water Maze and the Y-maze (Arai, Matsuki, Ikegaya, and Nishiyama, 2001; Min et al., 2009), and, inhibit context-dependent fear conditioning (Pugh et al., 1998). When the paradigm requires the animal to exert itself physically, whether it is swimming or avoiding one chamber to enter another, a potential confound may exist in that the learning deficits may be due to the decreased locomotor activity elicited by LPS. For example, Sparkman, Kohman, Scott, and Boehm (2005) were able to show that latency to find the hidden platform of the Morris Water Maze was due to decreased swimming speeds in LPS-treated animals, and thus could not conclude that any specific learning deficits were present.

The benefit of both the anticipatory nausea and taste avoidance paradigms is that they do not require the animal to exert significant motor output. Therefore, the performance deficits that have been observed following LPS treatment, such as, failing to

establish "conditioned gaping" in the anticipatory nausea paradigm (Chan et al., 2009), or failing to establish conditioned taste aversion or avoidance (Cross-Mellor, Foley, Parker, & Ossenkopp, 2009) can be more reliably concluded to be a consequence of cognitive impairment due to drug treatment, as opposed to reductions in locomotor behavior.

Treatment with LPS has been shown to produce an initial drop in voluntary saccharin (Yirmiya, 1996; Langhans, 1996) or sucrose (Cross-Mellor et al., 1999) consumption. However, it has been further demonstrated that this avoidance-related behavior following LPS treatment is transient and is only present during the acute-phase response to the drug, when the animals show a maximal aversive response (Cross-Mellor et al., 2009). The results of prior studies show that LPS by itself does not produce conditioned taste aversion, instead, it has been shown to block conditioned taste aversion that is typically produced through the pairing of an emetic treatment (i.e., LiCl) and a palatable sucrose solution (Cross-Mellor et al., 2009).

The first objective of this thesis was to examine the ability of rodents to simultaneously process and associate two different modes of stimulus presentation- an external mode consisting of a novel context and an internal mode involving an intravascular taste. In this first study, rats were tested in the anticipatory nausea (external) and conditioned taste avoidance (internal) paradigms concurrently by means of intraperitoneal/intravascular taste administered during the traditional anticipatory nausea conditioning phase. The use of intravascular taste allowed the rodents to be exposed to a salient taste and a distinct context simultaneously, while experiencing toxin action. After conditioning, animals were tested on two separate drug-free test days for evidence of

anticipatory nausea and conditioned taste avoidance.

The second aim of this thesis was to examine the effects of immune system stimulation on the concurrent acquisition of anticipatory nausea and taste avoidance. As indicated, there is a growing body of literature that strongly suggests that immune stimulation by LPS exerts deleterious effects on memory consolidation processes. Individually, taste avoidance and "conditioned gaping" behavior have been shown to be inhibited following LPS treatment, thus, it was hypothesized that LPS administration may disrupt learning processes in the formation of associations between a nausea-inducing LiCl US and both an external (context) and internal (taste) CS. To examine these effects, the same methodological design as for the first study was employed, but with a pre-injection of either LPS or saline (NaCl) 90 minutes prior to conditioning. After conditioning, animals were tested on two separate drug-free test days for anticipatory nausea and conditioned taste avoidance.

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CHAPTER 2

SIMULTANEOUS CONDITIONING OF "GAPING" RESPONSES AND TASTE AVOIDANCE IN RATS INJECTED WITH LICL AND SACCHARIN: EXAMINING THE ROLE OF CONTEXT AND TASTE CUES IN THE RODENT MODEL OF ANTICIPATORY NAUSEA

A version of this chapter has been accepted for publication (C.J. Cloutier, S.K. Cross-Mellor, M. Kavaliers, & K.-P. Ossenkopp. Simultaneous conditioning of "gaping" responses and taste avoidance in rats injected with LiCl and saccharin: Examining the role of context and taste cues in the rodent model of anticipatory nausea. Neurosci. Lett. (2011), D.O.I: 10.1016/j.neulet.2011.07.003).

2.1 Introduction

Exposure to a context previously associated with nausea elicits a conditioned "gaping" response in the rat (Limebeer & Parker, 2000; Limebeer, Hall, & Parker, 2006; Limebeer et al., 2008; Chan, Cross-Mellor, Kavaliers, & Ossenkopp, 2009) providing an animal model that can serve as a valuable preclinical tool for examining anticipatory nausea in chemotherapy patients (Molassiotis, 2005). Anticipatory nausea is reported by patients as being the most aversive side effect to chemotherapy, often causing many patients to forego further treatment (Molassiotis, 2005). Gaping behavior in the rat is suggested to be indicative of nausea, as evidenced by the prevention of lithium chloride (LiCl)-induced conditioned gaping when rats are administered anti-emetic treatment, such as Ondansetron, or the 5-HT_{1A} agonist 8-OH-DPAT (Limebeer & Parker, 2000; Limebeer & Parker, 2003). This conditioning model demonstrates that rats form an association between feelings of nausea and distinct contexts, and subsequently retrieve these associations to display aversion-related behaviors, such as gaping, upon re-exposure to the context (Limebeer et al., 2006; Limebeer et al., 2008; Parker & Limebeer, 2006; Chan et al., 2009; Ossenkopp, Biagi, Cloutier, Kavaliers, Cross-Mellor, 2011).

Conditioned taste aversion/avoidance is a behaviorally adaptive form of learning that enables animals to successfully reject or avoid consumption of potentially harmful food agents (Garcia, Lasiter, Bermudez-Rattoni, & Deems, 1985). Gustatory conditioning to solutions paired with (Eckel & Ossenkopp, 1996; Kent, Cross-Mellor, Kavaliers, & Ossenkopp, 2000; Ossenkopp & Eckel, 1995; Spector, Breslin, & Grill, 1988) or foods infused with (Cross-Mellor, Clarke, & Ossenkopp, 2004; Loy & Hall, 2002; Ossenkopp

& Eckel, 1995; Ossenkopp, Ladowsky, & Eckel, 1997) an emetic toxin is acquired rapidly and can be very robust. In a classic two-bottle preference test, animals previously infused with a palatable taste in conjunction with feelings of nausea will, in a drug-free state, prefer to drink water and avoid consumption of the taste originally presented with nausea during the conditioning phase (e.g., Rana & Parker, 2008).

The traditional oral presentation of taste cues in a taste avoidance paradigm is sufficient, but not necessary, for the acquisition of conditioned taste avoidance. Systemic administration of a taste (e.g., saccharin) at high concentrations allows the taste to be transported through the blood, eventually stimulating taste receptors in the oral cavity (Fishberg et al., 1933). Intravenously administered saccharin sodium has been shown to produce conditioned taste avoidance in rats exposed to gamma radiation (illness-inducing agent) during conditioning (Bradley & Mistretta, 1971). Rapid extinction of gustatory conditioning has also been achieved through intraperitoneal application of saccharin following repeated pairings of an orally presented saccharin taste with the effects of a toxin (Baum, Foidart, & Lapointe, 1974).

The rodent models of anticipatory nausea and conditioned taste avoidance demonstrate the rat's ability to associate the aversive feelings of toxin-associated nausea with a distinct context or a salient taste, respectively. In both paradigms, LiCl has repeatedly served as an effective unconditioned stimulus capable of producing robust conditioned responses (Riley & Freeman, 2004). Until now, conditioning paradigms have focussed on the rodent's ability to condition to only one mode of the conditioned stimulus (i.e., either context or taste).

This study examined the ability of rodents to simultaneously process and associate two different modes of stimulus presentation- an external mode consisting of a novel context and an internal mode involving an intravascular taste. We tested rats in the anticipatory nausea (external) and conditioned taste avoidance (internal) paradigms concurrently by means of intraperitoneal/intravascular taste administered during the traditional anticipatory nausea conditioning phase. The use of intravascular taste allowed the rodents to be exposed to a salient taste and a distinct context simultaneously, while experiencing toxin action.

2.2 Methods

2.2.1 Animals

Subjects were thirty-two naive adult male Long-Evans rats (Charles River, Quebec, Canada) weighing between 200-250 g at the start of the experiment. The rats were initially pair-housed in standard polypropylene cages in a colony room with a temperature of 21 ± 1 °C. The colony room was maintained on a 12-h light:12-h dark cycle with the lights on from 07:00 to 19:00 h. All rats had free access to food (ProLab rat chow) and tap water throughout the experiment. Four days prior to a 2-bottle preference test, rats were individually-housed under the identical conditions in order to familiarize each animal with the presence of two water bottles in its cage. The experimental methodology was carried out according to the Canadian Council on Animal Care guidelines and was approved by the Institutional Animal Care Committee.

2.2.2 Apparatus

The apparatus (used on all conditioning days and the test day) consisted of a white

Plexiglas box (29 cm × 25 cm × 29 cm) set atop a clear glass plate. A mirror was mounted at a 45° angle beneath the glass plate in order to view the rat's ventral surface. Two 40 W red lights were placed below the glass plate. Lighting cues were kept consistent with previous studies employing this rodent model of anticipatory nausea (e.g., Chan et al., 2009; Limebeer et al., 2006). Behavioral responses on the test day were videotaped with a video camera (Sony DCR-DVD201; London, Ontario) positioned approximately 1 m from the mirror.

2.2.3 Experimental procedure

The conditioning phase consisted of four days, each spaced 72 hours apart. There were four groups (n= 8/group). On each conditioning day, animals were injected intraperitoneally with NaCl (0.9%, 10 ml/kg), LiCl (0.15M; 127 mg/kg), NaCl plus saccharin (NaCl+Saccharin; 0.9% with 2% saccharin, 10 ml/kg), or LiCl plus saccharin (LiCl+Saccharin; 127 mg/kg with 2% saccharin). Immediately following drug administration, each animal was exposed to the novel context for 30 minutes and then returned to its home cage.

2.2.4 Testing days

Seventy-two hours following the final conditioning day, each rat was re-exposed to the specific context (conditioning apparatus) for ten minutes on a drug-free anticipatory nausea test day. Behaviors were recorded and scored using the Observer (Noldus Information Technology, Sterling, VA) event-recording program. Dependent behavioral variables analyzed consisted of gaping frequencies and the composite scores (Ossenkopp & Mazmanian, 1985) of aversive responses that did not include gaping (paw treads,

forelimb flails, head shakes, passive drip), and spontaneous orofacial behaviors (tongue protrusions, and mouth movements). Tongue protrusions were defined as both midline and lateral extensions of the tongue. Mouth movement consisted of lowering of the jawbone. Gaping was defined as lowering of the jawbone and the pushing or thrusting out of the lower teeth (e.g., Parker & Limebeer, 2006).

The following day, each animal received a 24-hour two-bottle preference test with a choice between water and a normally palatable saccharin solution (0.2% saccharin). The bottles were presented in the home cage to the animals at 09:30 h, with consumption (ml of fluid) measured after 6 and 24 h. Fluid consumption was then converted into a saccharin-preference ratio for each rat (saccharin solution consumption/ (water consumption + saccharin solution consumption)).

2.2.5 Data analysis

Spontaneous orofacial, gaping, and aversive (minus gaping) behavioral responses recorded on the conditioning test day were analyzed using a between-subjects analysis of variance (ANOVA), with two factors, Drug 1 (at two levels: LiCl or NaCl) and Drug 2 (at two levels: saccharin or no saccharin). Saccharin preference ratios were analyzed using a mixed factor design ANOVA. The between subjects factors were Drug 1 (at two levels: LiCl or NaCl) and Drug 2 (at two levels: saccharin or NaCl), and the within-subjects factor was Time (at two levels: 6 hours and 24 hours). Post hoc analyses were performed using Tukey's Honestly Significant Difference (HSD). All statistical tests used a significance criterion of $\alpha = 0.05$.

2.3 Results

2.3.1 *Spontaneous orofacial behaviors*

The conditioning effects of systemic LiCl and saccharin on spontaneous orofacial behaviors were examined and are shown in Figure 2.1 A. The ANOVA revealed no main effects of, or interactions between, Drug 1 (NaCl or LiCl) and Drug 2 (saccharin or no saccharin), suggesting that neither drug significantly influenced the frequency of spontaneous orofacial behavior.

2.3.2 *Gaping behavior*

Conditioned anticipatory nausea was indexed by the frequency of gaping responses during the drug-free text day. The ANOVA revealed a strong main effect of Drug 1 (NaCl or LiCl), $F(1,28)= 35.72$, $p< .001$, showing that animals treated with LiCl treatment produced significantly higher frequencies of conditioned gaping relative to animals treated with NaCl. There was no main effect for saccharin treatment, nor was there a significant interaction between Drug 1 (NaCl or LiCl) and Drug 2 (saccharin or no saccharin), $F<1$, demonstrating that saccharin administration did not influence gaping frequencies in any group. Post hoc analyses revealed that animals in Groups LiCl and LiCl+Saccharin displayed significantly higher gaping frequencies than animals in Groups NaCl and NaCl+Saccharin, $ps< .001$. Groups LiCl and LiCl+Saccharin did not differ significantly in gaping frequency from each other (Figure 2.1 B).

2.3.3 *Non-gaping Aversion-related Behaviors*

Aversive behaviors other than gaping (paw treads, head shakes, forelimb flails, passive drip) were also examined and are presented in Figure 2.1 C. The ANOVA revealed no significant main effects of, or interactions between, Drug 1 (NaCl or LiCl) and Drug 2

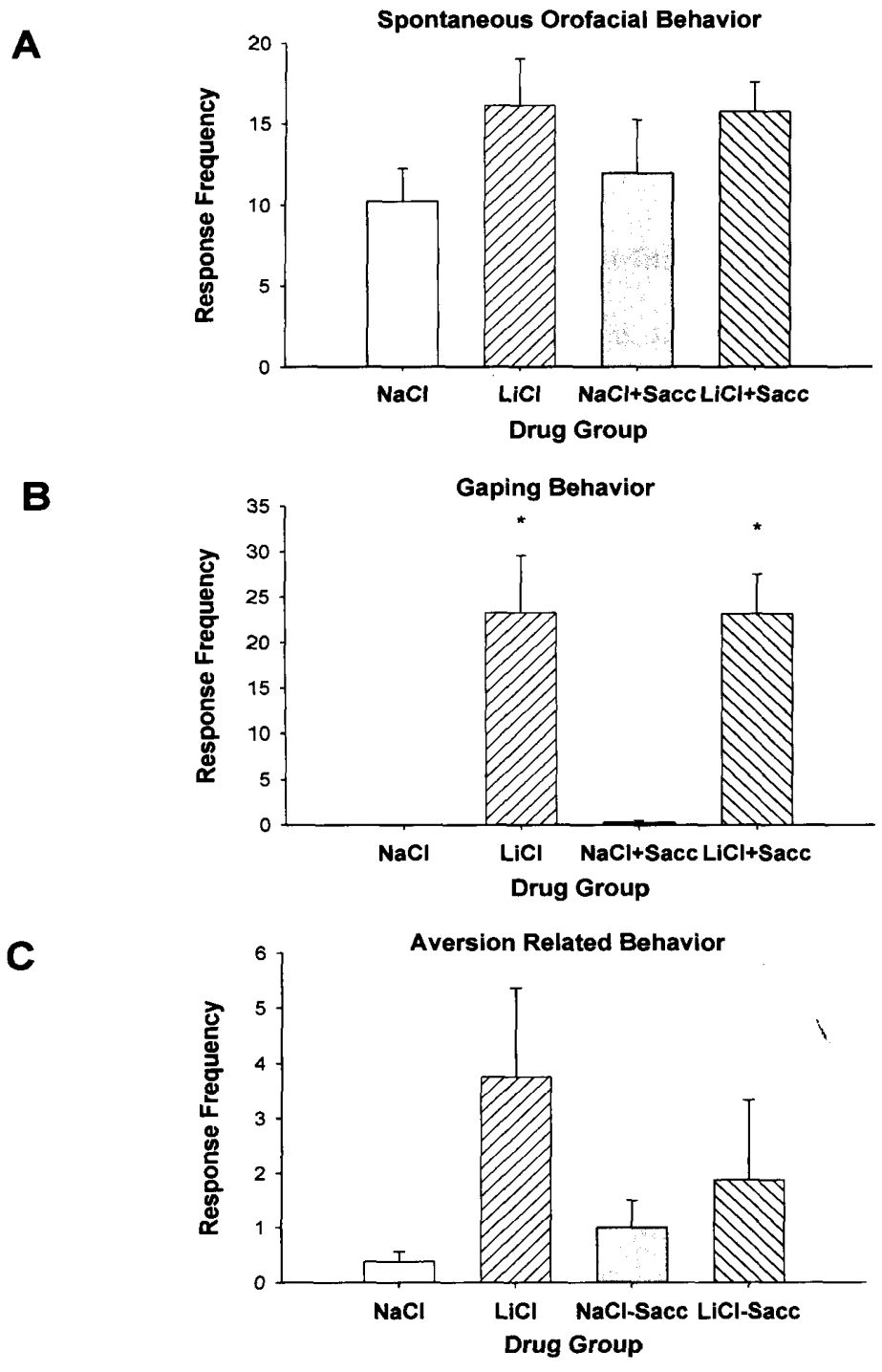

Figure 2.1 (A) Mean (+S.E.M.) frequency of total spontaneous orofacial behaviors expressed by all groups during the 30 min test in the distinctive context in the absence of drug treatment (n = 8/experimental group). Spontaneous orofacial behaviors consisted of the sum total of tongue protrusions, and mouth movements. **(B)** Mean (+S.E.M.) frequency of gaping responses expressed by groups during the 30 min test in the distinctive context in the absence of drug treatment. LiCl and LiCl+Saccharin treated animals showed significantly more gaping than all other groups ($*p < 0.01$), and did not significantly differ from each other. **(C)** Aversive Behaviors. Mean (+S.E.M.) frequency of aversive responses (minus gaping) expressed by groups during the 30 min test in the distinctive context in the absence of drug treatment.

(saccharin or no saccharin), indicating that neither drug significantly influenced the frequency of non-gaping-related aversive responding.

2.3.4 *Saccharin preference levels*

A split-plot ANOVA was performed for all conditioned taste avoidance analyses, where saccharin preference was the dependent measure. A significant main effect of Time, the within-subjects variable, was obtained, $F(1,28)= 44.47, p< .001$, but no interactive effects were yielded between Time (6 and 24 h) and Drug 1 (NaCl or LiCl), and/or Drug 2 (saccharin or no saccharin). Significant main effects were obtained for the between-subjects factors of Drug 1 (NaCl or LiCl), $F(1,28)= 5.254, p< .05$, as well as, Drug 2 (saccharin or no saccharin), $F(1,28)= 6.412, p< .05$. Most importantly, a significant interaction between Drug 1 (NaCl or LiCl) and Drug 2 (saccharin or no saccharin) was obtained, $F(1,28)= 17.86, p< .001$. Post hoc analyses revealed that animals in Group LiCl+Saccharin had significantly lower saccharin preferences relative to Groups NaCl, LiCl, and NaCl+Saccharin, $ps< .01$, indicating a significant conditioned taste avoidance (Figure 2.2 A-B). Groups NaCl, LiCl, and NaCl+Saccharin did not differ significantly from one another in terms of saccharin preference.

2.4 Discussion

The current study demonstrates that rats can simultaneously form an association between toxin-induced nausea and internal (taste) and external (context) presentation of conditioning stimuli. It was found that systemic (intraperitoneal/intravascular) administration of LiCl+Saccharin conditions both anticipatory nausea and taste avoidance. This finding was evidenced by significantly higher gaping frequencies in

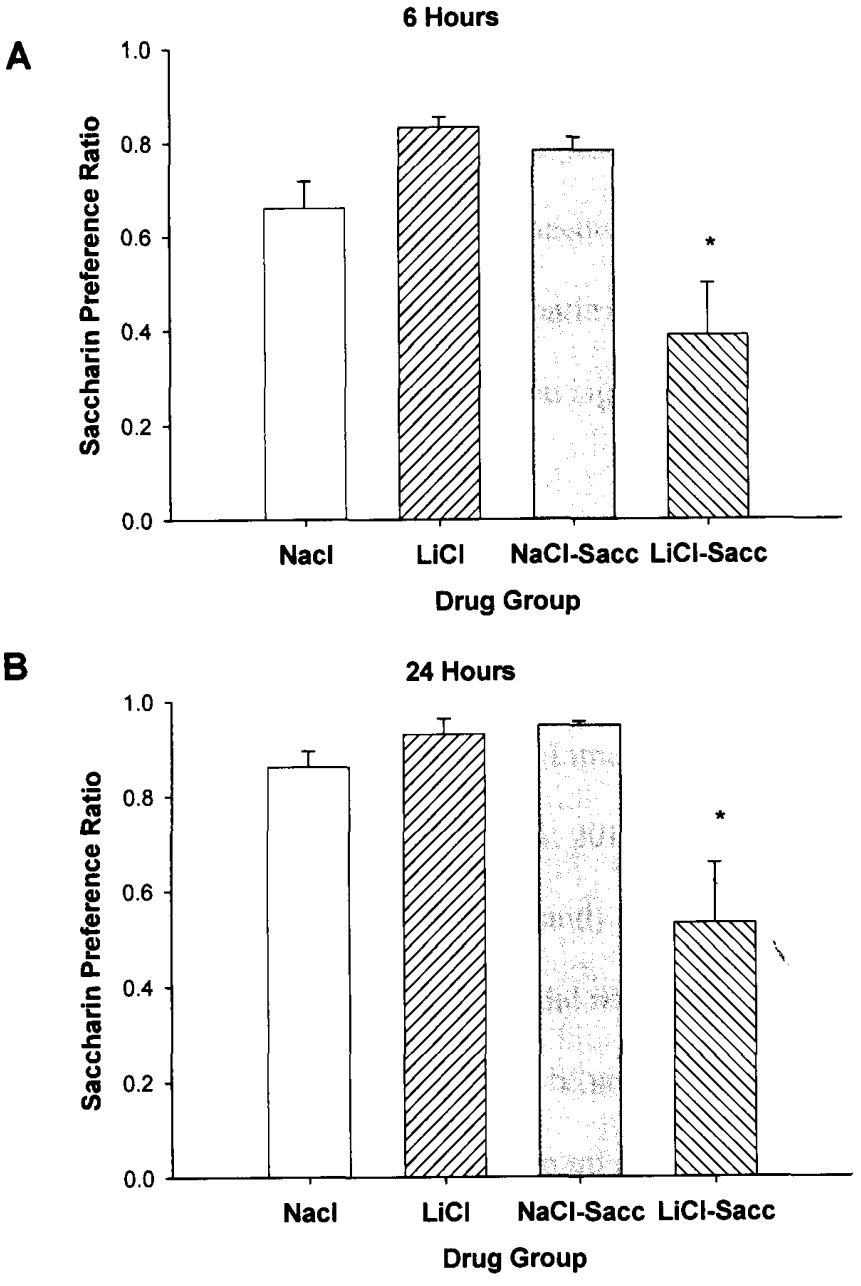

Figure 2.2 Saccharin Preference. Mean (+S.E.M.) saccharin preference ratio at 6h (A) and 24h (B) expressed by groups NaCl, LiCl, NaCl+Saccharin, and LiCl+Saccharin during the 24h 2-bottle intake test in the absence of drug treatment (n = 8/experimental group). Group LiCl+Saccharin displayed significantly lower saccharin preferences than all other groups (*p <.05) at both 6h and 24h.

Group LiCl+Saccharin relative to NaCl and NaCl+Saccharin controls in the context-conditioning test day, and a significantly lower saccharin preference ratio relative to all other groups in the two bottle taste choice test. In the anticipatory nausea test, drug influences were specific to gaping behavior and not a general behavioral effect. Groups did not differ significantly in the frequency of spontaneous orofacial behaviors, demonstrating that drug treatment (LiCl and/or saccharin) failed to alter the levels of tongue protrusions and mouth movements, despite having a significant effect on gaping frequency.

Animals treated with LiCl displayed significantly higher gaping frequencies relative to animals treated with NaCl only, consistent with previous demonstrations of anticipatory (conditioned) nausea (Chan et al., 2009; Limebeer et al., 2006; Limebeer et al., 2008; Limebeer & Parker, 2000; Ossenkopp et al., 2011). Animals treated with LiCl+Saccharin during conditioning showed significantly more conditioned gaping than animals treated with NaCl or NaCl+Saccharin, and did not differ significantly in gaping frequency from group LiCl. Although other aversive behaviors were observed on the drug-free test day, they failed to exhibit significant group differences. Thus, conditioned gaping presents as a robust outcome of anticipatory nausea conditioning, and is only present in animals treated with the toxin LiCl.

This study also replicated and extended previous studies showing that conditioned taste avoidance can be established with systemic presentation of a taste (Baum et al., 1974; Bellingham & Lloyd, 1987; Bradley & Mistretta, 1971; Buresova & Bures, 1977). Rats tested with saccharin and LiCl in the present study exhibited significantly lower

saccharin preference ratios relative to the other groups, showing evidence of a conditioned taste avoidance based on a learned association of the saccharin taste with the aversive (nausea) effects of LiCl.

Although the neural mechanisms underlying nausea conditioning need further clarification, it does appear that an intact area postrema is crucial for successful taste avoidance/aversion learning with LiCl (Ossenkopp & Eckel, 1995). The chemosensitive area postrema is a circumventricular medullary structure implicated in the detection of blood-borne toxins, such as LiCl (Borison, 1989). Animals with area postrema lesions will fail to acquire conditioned taste avoidances/aversions conditioned with toxins, such as LiCl (Eckel & Ossenkopp, 1996; Ossenkopp & Eckel, 1995). The role of area postrema in forming associations between feelings of nausea and specific contexts or environments, such as those in anticipatory nausea or conditioned place avoidance paradigms, has not been examined yet.

The simultaneous presentation of two distinctive conditioning stimuli in any learning paradigm introduces the possibility for overshadowing, wherein the saliency of one conditioned stimulus will be markedly stronger than that of the other conditioned stimulus, thus causing the less salient stimulus to form a weaker association with the unconditioned stimulus (Best & Meachum, 1986; Lindsay & Best, 1973). In the current study, it is difficult to determine whether overshadowing occurred, or to what extent. The presence of a saccharin taste cue during anticipatory nausea conditioning in group LiCl+Saccharin failed to interfere with the establishment of robust conditioned gaping that was not significantly different from gaping frequencies observed in LiCl-only

animals. However, it could not be determined whether exposure to a distinct context during taste avoidance conditioning altered the animals' abilities to form conditioned taste avoidances due to the absence of a control group that received taste conditioning only. Although saccharin avoidance for group LiCl+Saccharin did not appear to be as robust as taste avoidances obtained with oral intake of saccharin (e.g., Rana & Parker, 2008) this could be due to the route of administration of the saccharin as opposed to an overshadowing effect. Taste perception of intravascularly applied saccharin depends on transport through the blood and may not be perceived as strongly as orally administered saccharin. Despite this alternate route of administration, a significant conditioned taste avoidance was observed.

2.4.1 Conclusions

The present study demonstrates that the association of systemic treatment with saccharin plus lithium chloride with a novel context will condition both anticipatory nausea and taste avoidance. Thus, a robust rodent model of simultaneous aversive conditioning to both external and internal cues has been established. This model may prove instrumental in the elucidation of learning and memory processes involved with the conditioning of nausea responses to various modes of conditioned stimuli at both the behavioral and neurological levels.

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CHAPTER 3

THE EFFECTS OF IMMUNE SYSTEM STIMULATION WITH LIPOPOLYSACCHARIDE ON THE SIMULTANEOUS CONDITIONING OF ANTICIPATORY NAUSEA AND TASTE AVOIDANCE

3.1 Introduction

Lipopolysaccharide (LPS), the smallest component of Gram-negative bacteria outer cell wall (Rietschel et al., 1994), is used to mimic bacterial infections. Administration of LPS stimulates the immune system, thus activating phagocytes and resulting in the release of pro-inflammatory cytokines. Cytokines, such as, interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TFN-α), and interleukin-6 (IL-6), produce a specific set of behaviors collectively known as "sickness behavior". The "sickness behavior" profile often includes, fever (Hart, 1988; O'Reilly, Vander, & Kluger, 1988; Roth, Aslan, Storr, & Zeisberger, 1997), decreased locomotor activity (Hart, 1988; Engeland, Kavaliers, & Ossenkopp, 2003; Franklin, Engeland, Kavaliers, & Ossenkopp, 2007; Yirmiya, Rosen, Donchin, & Ovadia, 1994), hypersomnia (Hart, 1988), decreased grooming (Hart, 1988), adipsia, and anorexia (Cross-Mellor, Kent, Kavaliers, & Ossenkopp,2000; Gayle, Ilyin, Flynn, Plata-Salaman, 1998; Langhans, 2000; Langhans, Harlacher, Balkowski, Scharrer, 1990), all of which are considered to be behaviorally adaptive and serve to help the organism counter bacterial infection (Hart, 1998).

Bacteria-related immunogens have been shown to affect learning and memory in a variety of learning paradigms. Results from previous studies which have examined the effects of LPS administration on learning and memory are somewhat inconsistent. Arai, Matsuki, Ikegaya, and Nishiyama (2001) reported marked deficits in the Morris water maze and Y-maze tasks following acute LPS-induced immune stimulation of mice. Latency to reach the hidden platform of the water maze and a higher number of incorrect Y-maze arm choices suggested that administration of LPS adversely affects spatial

learning acquisition in mice (Arai et al., 2001). Deficits in Morris water maze performance after LPS administration have also observed in other studies (Sparkman, Kohman, Scott, & Boehm, 2005). They found decreases in swimming speed and suggested that the performance deficits were due to decreased locomotor activity, as opposed to spatial learning impairments. Similarly, it has been suggested that LPS may impair the ability to form representations of distinct contexts in contextual fear conditioning paradigms, as demonstrated by a reduction in freezing responses upon re-exposure to a context previously paired with an aversive foot shock in LPS-treated rats (Pugh et al., 1998). Learning paradigms that require the animal to produce significant motor output present potential confounds. As LPS is known to produce reductions in locomotor behavior (Hart, 1988; Engeland, Kavaliers, & Ossenkopp, 2003; Franklin, Engeland, Kavaliers, & Ossenkopp, 2007; Yirmiya, Rosen, Donchin, & Ovadia, 1994) during the acute-phase response to endotoxin treatment, it can be difficult to determine whether learning decrements are a product of disruptions in cognitive processes or simply due to reductions in locomotor behavior. In the current experiment, this confound was circumvented by employing two learning paradigms (anticipatory nausea and conditioned taste avoidance) that do not depend on significant motor output from the animals. Thus, any observed learning decrement could be attributed to the immune stimulation effects of LPS on learning or memory, as opposed to restrictions imposed by behavioral sickness behaviors.

The rodent models of anticipatory nausea and conditioned taste avoidance demonstrate the rat's ability to associate the aversive feelings of toxin-associated nausea

with a distinct context or a salient taste, respectively. In both paradigms, LiCl has repeatedly served as an efficacious unconditioned stimulus capable of producing robust conditioned responses (Riley & Freeman, 2004). Exposure to a context previously associated with feelings of nausea elicits a conditioned gaping response in the rat, providing an animal model that can serve as a valuable preclinical tool for examining anticipatory nausea treatments in chemotherapy patients (Limebeer et al., 2008; Molassiotis, 2005). Gaping behavior in the rat is suggested to be indicative of nausea, as evidenced by the prevention of LiCl-induced conditioned gaping when rats are administered an anti-emetic treatment, such as, ondansetron or the 5-HT_{1A} agonist 8-OH-DPAT, following conditioning in a lithium-induced taste avoidance (Limebeer et al., 2008; Limebeer et al., 2003). Thus, rats can learn and remember associations between distinctive environments and experienced nausea, and subsequently retrieve these associations to show aversion-related behaviors, such as gaping, upon re-entering the environment (Limebeer et al., 2008).

Anticipatory nausea is produced by classical conditioning, with the subject experiencing nausea or vomiting (conditioned response, CR) upon re-exposure to a context (conditioned stimulus, CS) previously paired with an emetic treatment (unconditioned stimulus, US) (Limebeer et al., 2008; Limebeer et al., 2006). A recent study investigated the effects of systemic LPS administration on the acquisition of anticipatory nausea in rats (Chan et al.,2009). Treatment with LPS followed by LiCl during a conditioning phase in a distinctive environment resulted in significantly reduced gaping responses on a drug-free test day, in comparison to NaCl-LiCl controls (Chan et

al., 2009). In addition, no significant differences in aversive behavioral responding were observed between the LPS-NaCl and NaCl-NaCl groups of animals, thus demonstrating that LPS was affecting learning and memory as opposed to decreasing locomotor behavior (Chan et al., 2009). These findings were consistent with previous literature indicating that administration of LPS can disrupt learning and memory (Sparkman et al., 2005; Min et al., 2009; Cross-Mellor, Foley, Parker, & Ossenkopp, 2009).

Conditioned taste aversion/avoidance is a behaviorally adaptive form of learning that enables animals to successfully reject or avoid consumption of potentially harmful food agents. Gustatory conditioning to solutions paired with (Ossenkop & Eckel, 1995; Eckel & Ossenkopp 1996; Kent, Cross-Mellor, Kavaliers, & Ossenkopp, 2000; Spector, Breslin, & Grill, 1988), or foods infused with (Cross-Mellor, Clarke, & Ossenkopp, 2004; Loy and Hall, 2000; Ossenkopp et al., 1997) an emetic toxin is acquired rapidly and can be very robust. In a classic two-bottle preference test, animals previously infused with a normally palatable taste in conjunction with feelings of nausea avoided consumption of the taste originally presented with nausea during the conditioning phase (Eg., Rana & Parker, 2008).

The traditional oral presentation of taste cues in a taste avoidance paradigm is sufficient, but not necessary, for the acquisition of conditioned taste avoidance. Systemic intravascular administration of a taste (e.g., saccharin) at high concentrations allows the taste to be transported through the blood, eventually stimulating taste receptors in the oral cavity (Fishberg, Hitzig, & King, 1933). Intravenously (i.v.) administered intravascular saccharin sodium has been shown to produce conditioned taste avoidance in rats exposed

to gamma radiation (illness-inducing agent) during conditioning (Bradley & Mistretta, 1971). Rapid extinction of gustatory conditioning has also been achieved through the intraperitoneal (i.p.) application of a saccharin taste substance following the traditional gustatory conditioning phase, wherein the saccharin taste was presented orally in conjunction with toxin action (Baum, Foidart, & Lapointe, 1974).

The effects of LPS on gustatory conditioning have been previously examined. Langhans (1996) demonstrated that the association of LPS effects and a novel saccharin taste results in a pronounced reduction in saccharin preference. Yirmiya (1996) also found significant reductions in saccharin preference in fluid-deprived rats, as well as, reductions in free consumption of saccharin relative to water in non-fluid-deprived animals. Similarly, it has been shown that systemic administration of cytokines IL-1 and TNF-α produce conditioned taste avoidance to a novel saccharin taste, or a novel diet, respectively (Goehler et al., 1995; Bernstein, Taylor, & Bentson, 1991).

Cross-Mellor, Kent, Ossenkopp, & Kavaliers (1999) demonstrated that although LPS treatment does significantly reduce sucrose intake initially, sucrose consumption increased in rats treated with LPS over several LPS treatment days, eventually leading to the absence of significant differences in sucrose consumption between LPS-treated and control animals. Furthermore, this study by Cross-Mellor et al., (2009) showed that LPS treatment does not produce active aversive responding in the taste reactivity test, where animals were involuntarily infused with sucrose, but it in fact increased ingestive responding to the taste.

LPS by itself fails to produce conditioned taste aversion, but pre-treatment with

LPS has been shown to block conditioned taste aversion that is typically produced through the pairing of an emetic treatment (i.e., LiCl) and a palatable sucrose solution (Cross-Mellor et al., 2009). Animals pre-treated with LPS prior to infusions of a LiCl-sucrose paired solution during the conditioning phase displayed increased ingestive responding and decreased aversive responding to an involuntarily infused LiCl-sucrose solution on a drug-free test day (LPS was not injected). Taken together, the anorectic effects of LPS treatment appear to be a part of the acute-phase response as opposed to the result of sucrose palatability shifts, conditioned avoidance/aversion, or enhanced satiety (Cross-Mellor et al., 1999; Cross-Mellor et al., 2009).

Previously, it was shown that intravascular/intraperitoneal administration of a LiCl-saccharin mixture was effective at concurrently establishing significant "conditioned gaping" behavior and taste avoidance (Cloutier et al., 2011). Independently, pre-treatment with LPS has been shown to reduce both "conditioned gaping" (Chan et al., 2009), and the rapid acquisition of conditioned taste aversion/avoidance (Cross-Mellor et al., 2009). In the present study, the effects of immune stimulation with LPS on the simultaneous acquisition of "conditioned gaping" and conditioned taste avoidance were examined. It was hypothesized that LPS would be effective in reducing the acquisition of anticipatory nausea, as it is an effect previously reported in the literature. It was also hypothesized that pre-treatment with LPS would reduce the acquisition of conditioned taste avoidance.

3.2 Methods

3.2.1 Animals

Subjects were 79 adult naive male Long-Evans rats (Chalers River, Quebec,

Canada) weighing between 200-250 g at the start of the experiment. The rats were initially pair-housed in standard polypropylene cages (45 x 22 x 20 cm) in a colony room with a temperature of 21 ± 1 °C. The colony room was maintained on a 12-h light: 12-h dark cycle with the lights on from 07:00 to 19:00 h. All rats had free access to food (ProLab rat chow) and tap water throughout the experiment. Four days prior to a 2-bottle preference test, rats were singly-housed under the identical conditions in order to acclimatize the animals to the presence of two water bottles in their cages. The experimental methodology was carried out according to the Canadian Council on Animal Care guidelines and was approved by the Institutional Animal Care Committee.

3.2.2 Apparatus

The apparatus (used on all conditioning days and the test day) consisted of a white Plexiglas box (29 cm × 25 cm × 29 cm) set atop a clear glass plate. A mirror was mounted at a 45° angle beneath the glass plate in order to view the rat's ventral surface. Two 40 W red lights were placed below the glass plate. Lighting cues were kept consistent with previous studies employing this rodent model of anticipatory nausea (e.g., Chan et al., 2009; Limebeer et al., 2006; Cloutier et al., 2011). Behavioral responses on the test day were videotaped with a video camera (Sony DCR-DVD201; London, Ontario) positioned approximately 1 m from the mirror.

3.2.3 Experimental procedure

An illustration of the testing injection schedule is provided in Figure 3.1. All conditioning and testing was performed during the light cycle. The conditioning phase consisted of four days, each spaced 72 hours apart. There were eight groups (n= 10/group

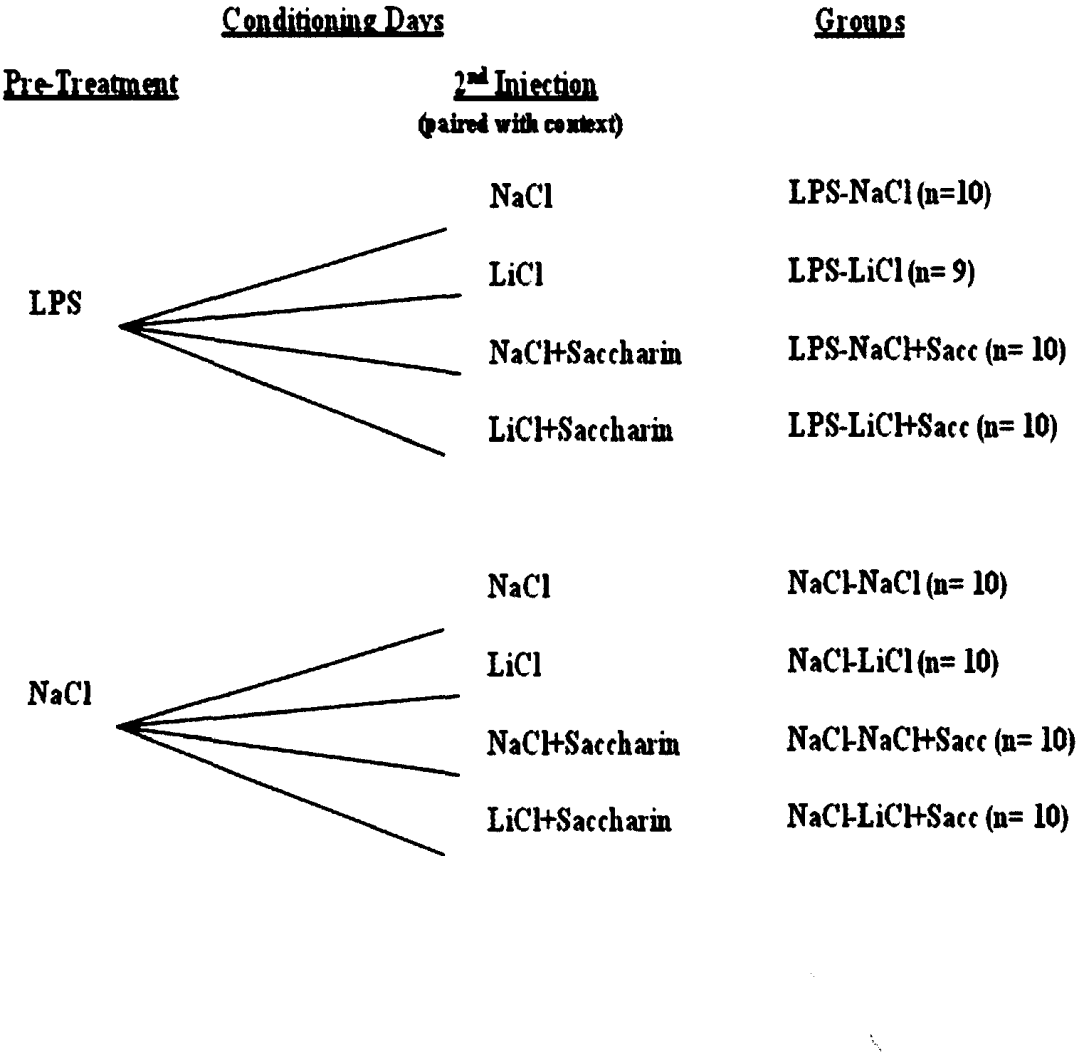

Figure 3.1 Illustration of experimental injection schedule and groups.

except Group LPS-LiCl, n= 9). On each conditioning day, animals were injected intraperitoneally with either LPS (200 μg/kg; derived from *E. coli* serotype 0111:B4, no. L-2630, Sigma, St. Louis, MO) or NaCl (1 mL/kg, 0.9%), followed 90 minutes later by an intraperitoneal injection of either NaCl (10 mL/kg, 0.9%), LiCl (0.15M; 127 mg/kg), NaCl plus saccharin (NaCl+Saccharin; 10 mL/Kg, 0.9%, with 2% saccharin), or LiCl plus saccharin (LiCl+Saccharin; 127 mg/kg with 2% saccharin). Immediately following the second injection, each animal was exposed to the novel context for 30 minutes and then returned to its home cage.

3.2.3.1 Body Weight Change

Body weight was measured prior to conditioning and 24 h following each of the four conditioning days. LPS induces an acute-phase response, wherein the initial immune system stimulation produces a specific set of sickness behaviors that includes anorexia and adipsia (Cross-Mellor et al., 2000; Fosset et al., 2003; Gayle et al., 1998). The anorectic and adipsic effects lead to significant weight loss following LPS treatment (Cross-Mellor et al., 2000; Fosset et al., 2003; Gayle et al., 1998) and are a reliable physiological measure of an effect of LPS.

3.2.4 Testing Days

Seventy-two hours following the final conditioning day, each rat was re-exposed to the conditioning context for 10 minutes on a drug-free test day. All behavioral responding to the context alone was video recorded for later scoring and analysis. Dependent behavioral variables analyzed consisted of gaping frequencies and the composite scores (Ossenkopp & Mazmanian, 1985) of aversive responses that did not

include gaping (paw treads, forelimb flails, head shakes, passive drip), and spontaneous orofacial behaviors (tongue protrusions, and mouth movements). Tongue protrusions were defined as both midline and lateral extensions of the tongue. Mouth movement consisted of lowering of the jawbone. Gaping was defined as lowering of the jawbone and the pushing or thrusting out of the lower teeth (e.g., Parker & Limebeer, 2006).

The following day, each animal received a 24-hour two-bottle preference test with water and a normally palatable saccharin solution (0.2% saccharin). The bottles were presented in the home cage to the animals at 09:30 h, with consumption (ml of fluid) measured after 6 and 24 h. Fluid consumption was then converted into a saccharin-preference ratio for each rat with the following equation: (saccharin solution consumption)/ (water consumption + saccharin solution consumption).

3.2.5 Data Analysis

Changes in body weight following drug treatment were analyzed using a mixed design repeated measures anlaysis of variance (ANOVA), with 3 between-subjects factors and one within-subjects factor. The between-subjects factors were Drug 1 (at two levels: LPS or NaCl) Drug 2 (at 2levels: NaCl or LiCl), and Drug 3 (at two levels: saccharin or no saccharin). The within-subjects factor was Conditioning Day (at four levels: Conditioning Day 1-4). Spontaneous orofacial, gaping, and aversive (minus gaping) behavioral responses recorded on the conditioning test day were analyzed using a between-subjects ANOVA, with 3 factors, Drug 1 (at two levels: LPS or NaCl), Drug 2 (at 2levels: NaCl or LiCl), and Drug 3 (at two levels: saccharin or no saccharin). Saccharin preference ratios were analyzed using a mixed factor design ANOVA. The

between subjects factors were Drug 1 (at two levels: LPS or NaCl), Drug 2 (at two levels: NaCl or LiCl), and Drug 3 (at two levels: saccharin or no saccharin).The within-subjects factor was Time (at two levels: 6 hours and 24 hours). Post hoc analyses were performed using Tukey's Honestly Significant Difference (HSD). All statistical tests used a significance criterion of $\alpha = 0.05$.

3.3 Results

3.3.1 Body weight change

The mixed design repeated measures ANOVA yielded a significant main effect of Conditioning Day, $F(2,109) = 30.37$, $p < .001$, and more importantly, a significant interaction between Conditioning Day (Days 1-4) and Drug 1 (LPS or NaCl), $F(2,109) = 47.56$, $p < .001$. Post hoc analyses revealed that following Conditioning Day 1, animals pre-treated with LPS (LPS-NaCl, LPS-LiCl, LPS-NaCl+Saccharin, and LPS-LiCl+Saccharin) lost significantly more weight relative to animals in NaCl pre-treated groups (NaCl-NaCl, NaCl-LiCl, NaCl-NaCl+Saccharin, and NaCl-LiCl+Saccharin), $ps < .05$. No other groups differed significantly following Conditioning Day 1.

Following Conditioning Day 2, animals in Group LPS-LiCl+Saccharin lost significantly more weight than Groups LPS-NaCl, NaCl-LiCl+Saccharin, and NaCl-NaCl+Saccharin, $ps < .05$. Likewise, animals in Groups LPS-LiCl and LPS-NaCl+Saccharin lost significantly more weight than Groups NaCl-LiCl+Saccharin and NaCl-NaCl+Saccharin, $ps < .01$. Following Conditioning Day 3, only animals in Group LPS-NaCl+Saccharin lost more weight than Groups LPS-NaCl and NaCl-LiCl+Saccharin, $ps < .05$. No significant differences were found among groups on

Conditioning Day 4.

Significant main effects were obtained for: Drug 1(LPS or NaCl), $F(1,71)= 171.223$, $p< .001$; Drug 2 (NaCl or LiCl), $F(1,71)= 5.103$, $p< .05$; and, Drug 3(saccharin or no saccharin), $F(1,71)= 5.146$, $p< .05$. In addition, a significant interaction was obtained between Drug 1 and Drug 3, $F(1,71)= 15.053$, $p< .001$, demonstrating increased weight gain in animals pre-treated with NaCl followed by saccharin (mixed with LiCl or NaCl), relative to NaCl pre-treated animals that were not administered saccharin.

Body weight was recorded on each conditioning day and 24 h following each conditioning day. Percentages of weight loss for each 24 h period following each conditioning day are depicted in Figure 3.1 A-B.

3.3.2 Gaping behavior

Conditioned anticipatory nausea was indexed by the frequency of gaping responses during the drug-free test day. The effects of systemic LPS, LiCl and saccharin treatment on gaping responses in the distinct context are depicted in Figure 3.3 A. The ANOVA revealed a main effect of Drug 1 (LPS or NaCl), $F(1,71)= 4.575$, $p< .05$, as well as, Drug 2 (NaCl or LiCl), $F(1,71)= 13.192$, $p< .001$. A significant interaction between Drug 1 and Drug 2 was also obtained, $F(1,71)= 6.340$, $p<.05$, with post hoc tests revealing that animals in Group NaCl-LiCl displayed significantly higher conditioned gaping frequencies than Groups LPS-LiCl, LPS-NaCl, LPS-LiCl+Saccharin, LPS-NaCl+Saccharin, NaCl-NaCl, and NaCl-NaCl+Saccharin, $ps< .01$, but did not differ significantly from Group NaCl-LiCl+Saccharin. Furthermore, gaping frequencies in Group NaCl-LiCl+Saccharin did not differ significantly from any other group.

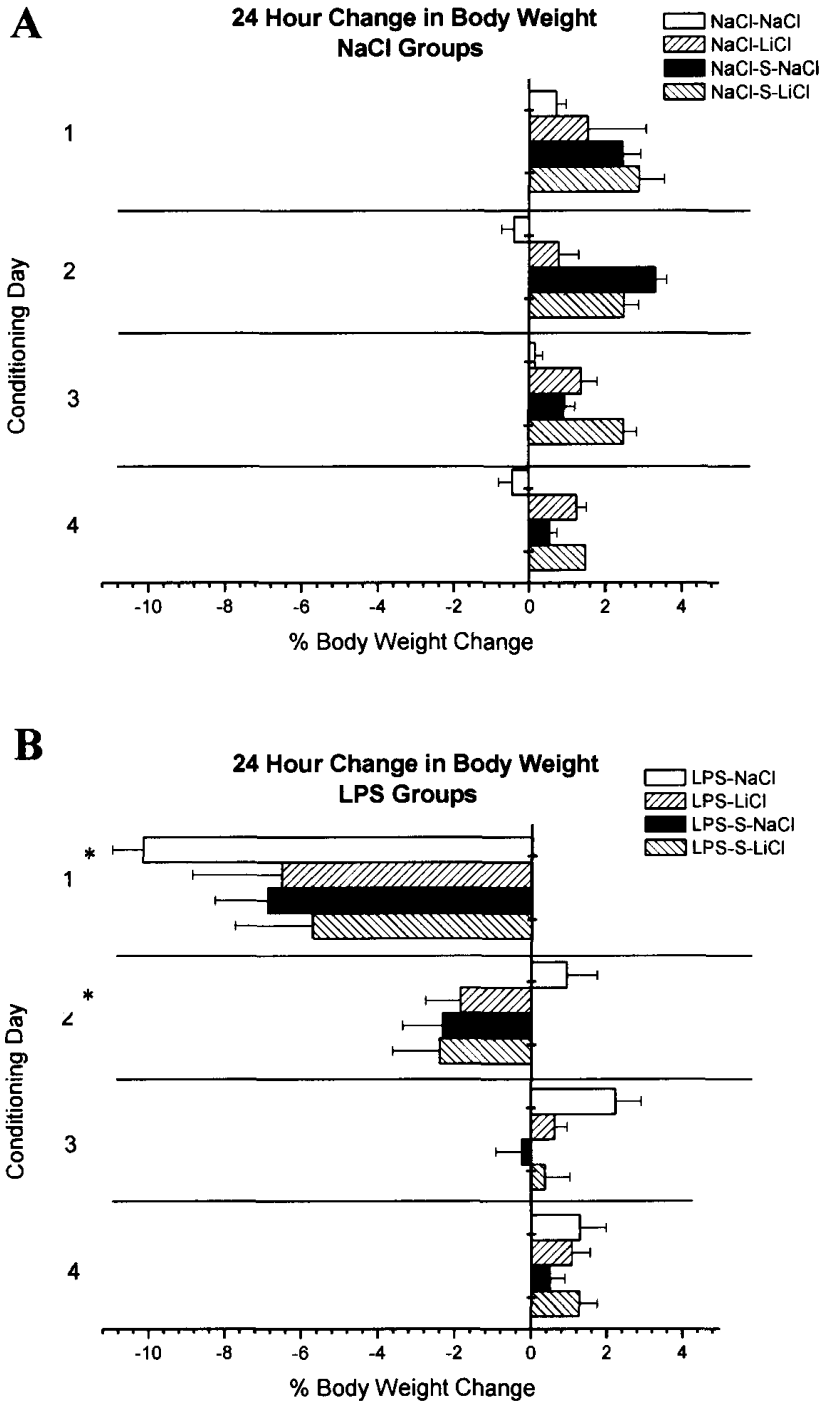

Figure 3.2 Group mean (+S.E.M.) 24 h change in body weight after systemic injection of (A) NaCl or (B) LPS (N= 79). Injection days were 72 h apart. Negative values represent a loss in weight and positive values represent a gain in weight. LPS-treated animals lost significantly more body weight than NaCl treated animals on injection days 1 and 2 (*$p< 0.05$).

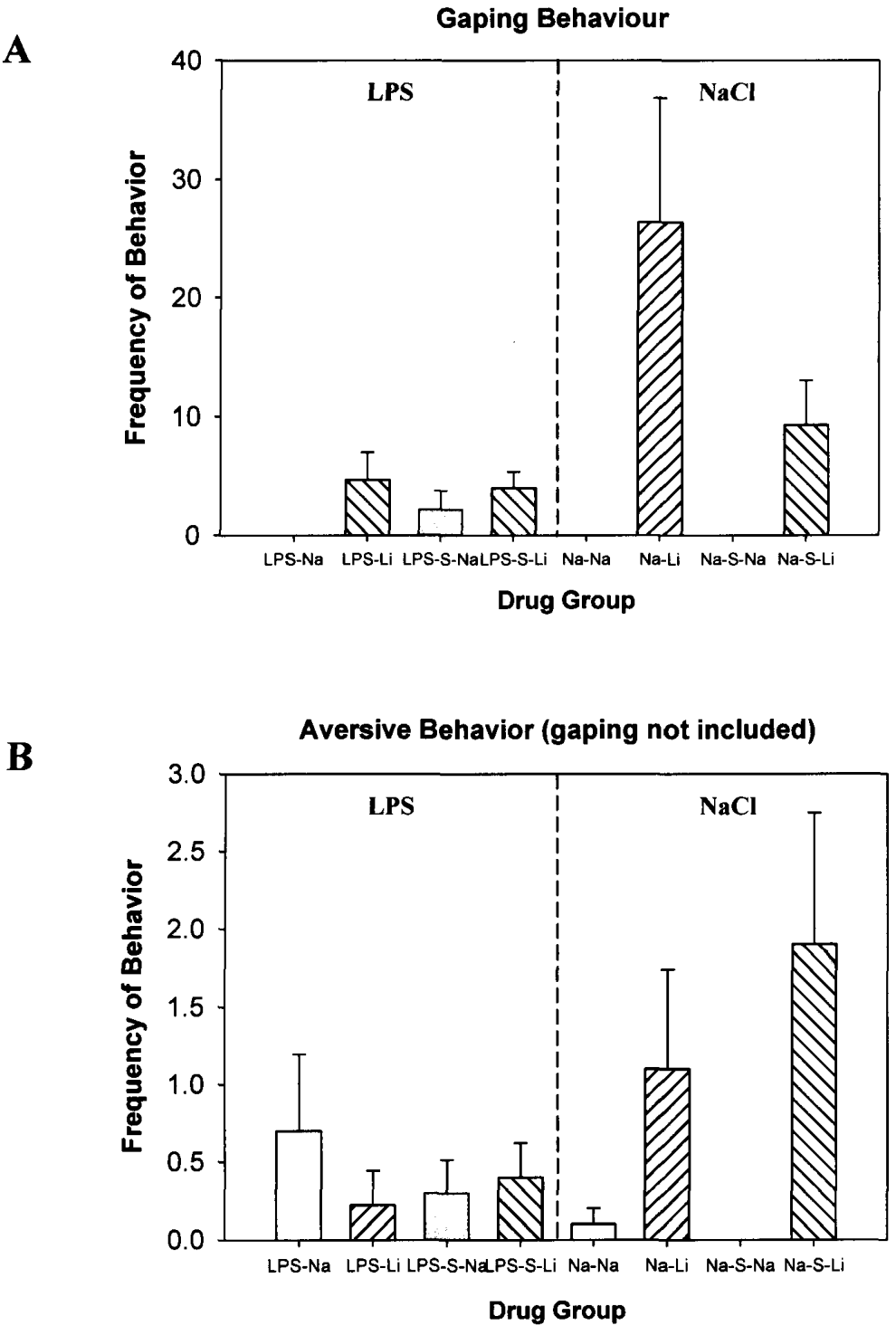

Figure 3.3 (A) Gaping Behavior. Mean (+S.E.M.) frequency of gaping responses expressed by groups during the 30 min test in the distinctive context in the absence of drug treatment. LiCl treated animals showed significantly more gaping than all other groups ($*p < 0.01$), except for Group NaCl-LiCl+Saccharin. **(B) Aversive Behaviors.** Mean (+S.E.M.) frequency of aversive responses (minus gaping) expressed by groups during the 30 min test in the distinctive context in the absence of drug treatment.

3.3.3 Non-gaping aversion-related behaviors

Aversive behaviors other than gaping (paw treads, head shakes, forelimb flails, and passive drip) were also examined and are presented in Figure 3.3 B. The ANOVA revealed a main effect of Drug 2 (NaCl or LiCl), $F(1,71)= 4.069$, $p< .05$. A significant interaction was also obtained between Drug 1 (LPS or NaCl) and Drug 2 (NaCl or LiCl), $F(1,71)= 6.872$, $p< .05$. Animals treated with LiCl displayed significantly higher frequencies of non-gaping aversion-related behaviors relative to animals treated with NaCl; however, this effect was stronger in animals pre-treated with NaCl as opposed to LPS prior to LiCl treatment.

3.3.4 Spontaneous orofacial Behaviors

The conditioning effects of systemic LiCl and saccharin on spontaneous orofacial behaviors were examined and are shown in Figure 3.4. The ANOVA revealed a main effect of Drug 2 (NaCl or LiCl), $F(1,71)= 4.58$, $p< .05$, showing that animals treated with LiCl displayed significant increases in the frequency of tongue protrusions, mouth movements, or paw licks, relative to animals who received NaCl.

3.3.5 Saccharin preference levels

A split-plot ANOVA was performed for all conditioned taste avoidance analyses, where saccharin preferences at 6 and 24 h were the dependent measure. Saccharin preference data are depicted in Figure 3.5 A-B. The analysis revealed a main effect of Time, $F(1,71)= 24.185$, $p< .001$, as well as, a four-way interaction between Time (6 or 24 hours), Drug 1 (LPS or NaCl), Drug 2 (NaCl or LiCl), and Drug 3 (saccharin or no saccharin), $F(1,71)= 4.829$, $p< .05$.

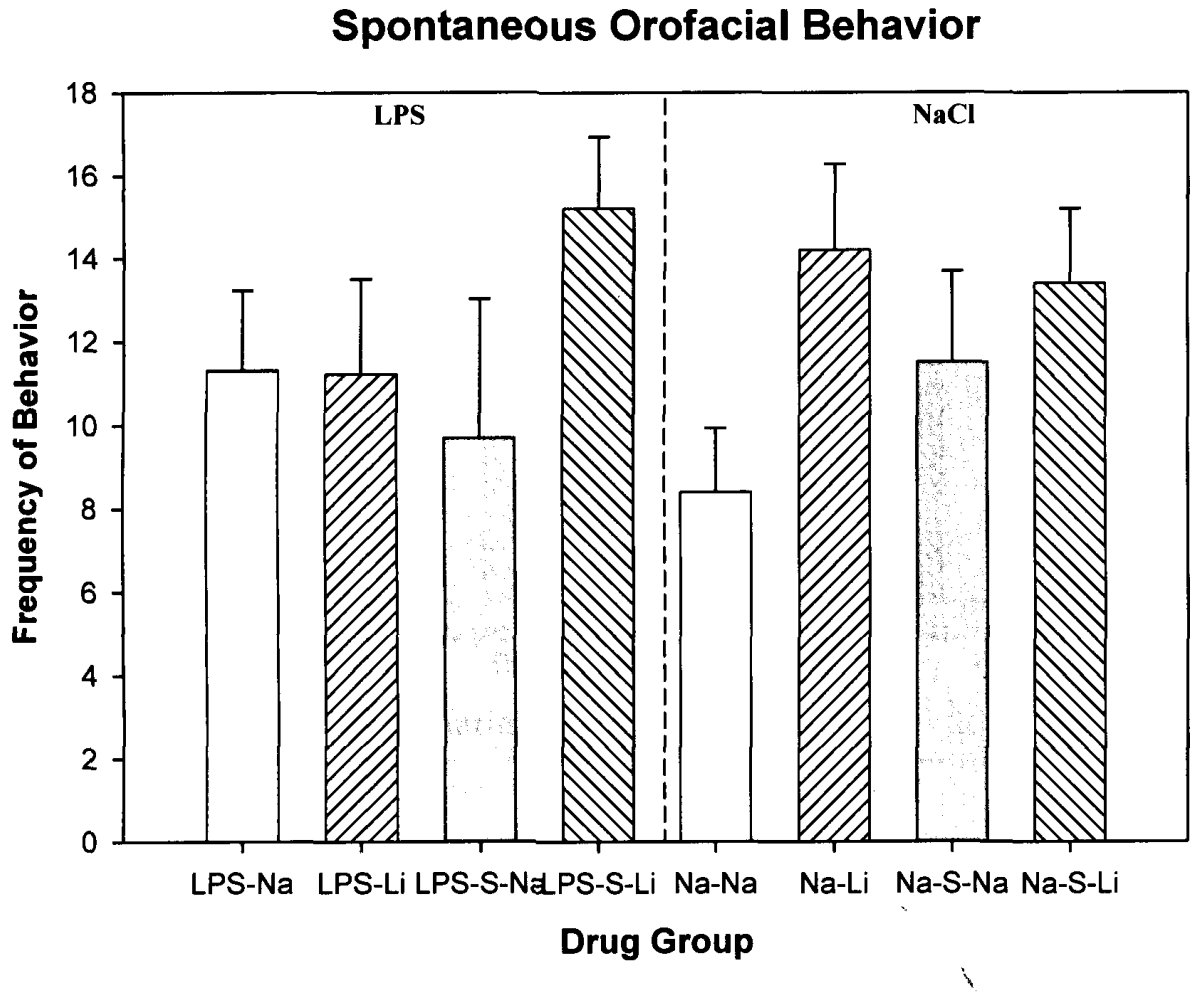

Figure 3.4 Spontaneous Orofacial Behaviors. Mean (+S.E.M.) frequency of total spontaneous orofacial behaviors expressed by all groups during the 30 min test in the distinctive context in the absence of drug treatment (n = 10/experimental group, Group NaCl-LiCl n=9). Spontaneous orofacial behaviors consisted of the sum total of tongue protrusions, and mouth movements.

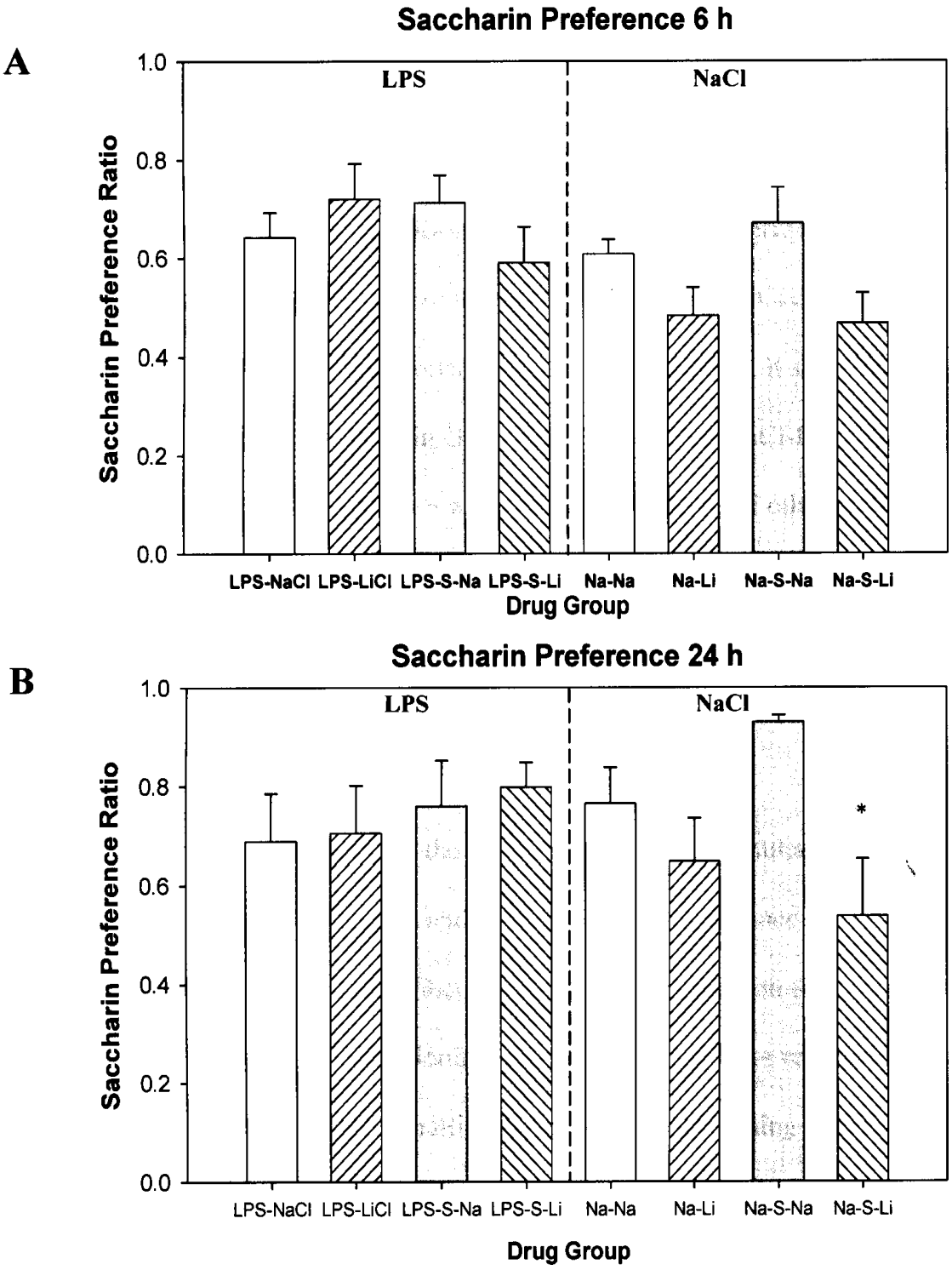

Figure 3.5 Saccharin Preference. Mean (+S.E.M.) saccharin preference ratio at 6h **(A)** and 24h **(B)** expressed by all gorups during the 24h 2-bottle intake test in the absence of drug treatment (n = 10/experimental group, Group NaCl-LiCl n=9). Group NaCl-LiCl+Saccharin displayed significantly lower saccharin preferences than Group NaCl-NaCl+Saccharin at 24h (*$p< 0.05$).

Post hoc analyses at the 6 h time point revealed that animals treated with LiCl plus saccharin as the second injection had significantly lower saccharin preferences relative animals who received NaCl mixed with saccharin. Post hoc analyses at the 24 h time point revealed a significant difference in saccharin preference between Groups Na-S-LiCl and Na-S-NaCl, where animals in Group Na-S-LiCl displayed a significantly lower saccharin preference relative to Group Na-S-NaCl. It should also be noted that at the 6 h time point, animals in Groups NaCl-NaCl and NaCl-LiCl had a significantly higher total fluid (saccharin + water) intake relative to all other groups of animals (p's< .001). However, by the 24 h time point after the typical nocturnal feeding period, no significant differences in total fluid consumption were observed. No further significant differences were observed among the groups.

3.4 Discussion

In the present study, the effects of LPS on the simultaneous acquisition of LiCl-induced anticipatory nausea and conditioned taste avoidance were examined. Thus, this experiment examined the effects of immune stimulation on the concurrent conditioning of external and internal conditioning cues, respectively. Prior reports have demonstrated the ability of LPS to disrupt conditioning in associative learning paradigms, such as contextual fear conditioning, anticipatory nausea, and conditioned taste aversion/avoidance (Pugh et al., 1998; Chan et al., 2009; Cross-Mellor et al., 2009). In this study, animals were pre-treated with LPS or saline (NaCl), followed 90 minutes later by an intraperitoneal injection of one of four drug treatments (NaCl, LiCl, NaCl plus 2% saccharin, or LiCl plus 2% saccharin), immediately prior to exposure to the novel context

of the anticipatory nausea conditioning paradigm. Systemic injection of LiCl plus 2% saccharin allowed for the simultaneous perception of the salient taste and the noxious effects of the LiCl during the time spent the novel context. Previous studies have shown that taste avoidance conditioning and rapid extinction of gustatory conditioning can be achieved through intraperitoneal (i.p.) application of a saccharin taste substance when paired with the effects of a toxin (Baum et al., 1974; Buresova & Bures, 1977; Bellingham & Lloyd, 1987). Following the conditioning phase, animals were tested for conditioned gaping and conditioned taste avoidance on two separate drug-free test days. To date, the effects of bacterial endotoxin treatment have been evaluated in paradigms that condition responses to one mode of conditioning stimulus (e.g., context, or, taste). It was hypothesized that LPS pre-treatment would attenuate the concurrent associations formed between the LiCl-induced nausea and two different modes of conditioning stimuli- an external cue consisting of a novel context, and an external cue consisting of a salient saccharin taste.

The current hypothesis was partially confirmed. Measures of body weight loss 24 h following each conditioning day provided an indirect measure of peripheral LPS tolerance development. LPS pre-treated animals lost significantly more weight than saline pre-treated animals following Conditiong Days 1-2, but did not differ significantly from saline pre-treated animals on Conditioning Days 3-4, indicating tolerance development to the peripheral effects of LPS treatment. Animals pre-treated with saline followed by LiCl treatment displayed significantly higher gaping frequencies upon re-exposure to the context in a drug-free state, relative to LPS pre-treated groups, and saline controls.

Animals in Groups NaCl-LiCl and NaCl-LiCl+Saccharin also displayed significantly higher frequencies of non-gaping, aversion-related responses to the context in a drug-free state. Furthermore, animals pre-treated with LPS followed by LiCl or LiCl+Saccharin failed to establish "conditioned gaping" frequencies that were significantly different from other LPS groups and saline controls. A significant taste avoidance was obtained in Group NaCl-LiCl+Saccharin, but only relative to Group NaCl-NaCl+Saccharin and not all other groups of animals. The results of the current study are discussed further in the following sub-sections.

In summary, the current results provide evidence for the deleterious effects of LPS on learning and memory in a simultaneous conditioning paradigm. Animals pre-treated with LPS failed to establish significant levels of "conditioned gaping" or saccharin taste avoidance that differed significantly from control animals, suggestive of a deleterious effect of LPS on associative learning and memory processes.

3.4.1 Body Weight Change

Consistent with prior reports, treatment with LPS produced significant decreases in body weight 24 h following Conditioning Day 1(Limebeer et al., 2006; Chan et al., 2009). Although significant reductions in body weight were observed 24 h following Conditioning Day 2 in Groups LPS-LiCl+Saccharin, LPS-NaCl+Saccharin, and LPS-LiCl; and, 24 h following Conditioning Day 3 in Group LPS-NaCl+Saccharin, these data remain consistent with the tolerance effects (significantly decreased physiological effects of LPS following repeated treatment) to LPS observed in previous studies (Cross-Mellor et al., 1999; Engeland et al., 2003; Dantzer, 2004). 24 h following Conditioning Day 3,

only the LPS-NaCl+Saccharin group exhibited significant body weight loss relative to Groups LPS-NaCl and NaCl-LiCl+Saccharin, but did not differ from any other group. Following Conditioning Day 4, LPS treated animals failed to show significant differences in body weight loss relative to NaCl pre-treated animals.

Interestingly, treatment with LPS seems to have an interactive effect with the saccharin treatment. In saline pre-treated animals, saccharin administration (with LiCl or NaCl) led to greater weight gains relative to saline pre-treated animals that were not treated with saccharin. However, this effect was not observed in LPS pre-treated animals treated with saccharin. It could be suggested that the sustained intravascular perception of the saccharin taste positively influenced appetite. However, the role of an intravascular saccharin cue on body weight, as well as how it interacts with LPS, has received little attention and requires further investigation.

3.4.2 Conditioned Gaping Behavior

It was shown that pre-treatment with saline (NaCl) followed by LiCl treatment (Group NaCl-LiCl) resulted in the establishment of robust "conditioned gaping" behavior when animals were re-exposed to the context on a drug-free test day. This finding was consistent with prior reports demonstrating that rats can associate feelings of nausea to salient contexts and will display aversion-related "conditioned gaping" behavior upon re-exposure to the context in a drug-free state (Limebeer et al.,2006; Chan et al., 2009; Ossenkopp et al., 2011). Furthermore, it was demonstrated that animals pre-treated with LPS prior to LiCl treatment exhibited significantly attenuated "conditioned gaping" responses relative to Group NaCl-LiCl, and were not significantly different from other

LPS pre-treated groups or saline controls. This finding was also consistent with the results of Chan et al., (2009), where it was found that LPS pre-treatment significantly attenuated "conditioned gaping" behavior in LiCl treated animals relative to saline pre-treated animals treated with LiCl. In the present study, it was also found that animals pre-treated with LPS followed by LiCl plus saccharin treatment displayed significantly lower "conditioned gaping" frequencies relative to Group NaCl-LiCl, but did not differ significantly from other LPS pre-treated animals or saline controls, suggestive of an attenuation of aversion-related behavior due to LPS treatment.

Conditioned gaping frequencies in saline pre-treated animals that were then treated with LiCl plus saccharin (Group NaCl-LiCl+Saccharin) did not differ significantly from animals pre-treated with saline followed by LiCl-only. This finding is partially consistent with the results in Chapter 2, where it was shown that conditioned gaping in animals treated with LiCl plus saccharin displayed comparable gaping frequencies to LiCl-treated animals. However, gaping frequencies in Group NaCl-LiCl+Saccharin also failed to differ significantly from LPS pre-treated animals and saline controls. Although "conditioned gaping" behavior did not present as a robust outcome of anticipatory nausea conditioning in Group NaCl-LiCl+Saccharin, an intermediate level of "conditioned gaping" was observed. Due to a lack of robust conditioned gaping in this group of animals, it is difficult to discern whether or not LPS was responsible for the absence of significant gaping behavior in Group LPS-LiCl+Saccharin. However, it should be noted that the relatively low gaping frequencies in this group were comparable to all other LPS pre-treated animals and saline controls. Gaping frequencies in Group LPS-

LiCl+Saccharin were also significantly reduced relative to Group NaCl-LiCl, a group that did display robust "conditioned gaping". Thus, perhaps LPS exerted a deleterious effect on the establishment of conditioned gaping in Group LPS-LiCl+Saccharin, something that requires further investigation.

It is unclear why Group NaCl-LiCl+Saccharin failed to establish "conditioned gaping" frequencies significantly different from LPS pre-treated animals and control animals. It is possible that overshadowing occurred in the current experiment. It may be that the injection stress caused by the extra pre-injection administered in this study altered the associative learning of the stimuli in this paradigm, but this requires further investigation. The current study was exploratory in nature; therefore, it cannot be conclusively determined whether this intermediate frequency of gaping behavior was due to random variation among animals, or, if the added pre-treatment in the current study caused additional injection stress that ultimately affected learning.

3.4.3 Non-gaping aversion-related behaviors

Gaping behavior consistently presents as a robust outcome of anticipatory nausea conditioning. Other aversion-related behaviors, however, can also be commonly observed, though they typically do not appear to be as strong (e.g. Chan et al., 2009). Aversion-related behaviors that did not include gaping behavior (an aggregated score of paw treads, forelimb flails, head shakes, and/or passive drip) were examined and compared. Animals treated with LiCl or LiCl+Saccharin displayed significantly higher frequencies of non-gaping, aversion-related behaviors relative to animals treated with saline (NaCl). This effect was attenuated, however, in LPS pre-treated animals receiving

LiCl or LiCl+Saccharin treatment, demonstrating once again the ability of LPS to disrupt classical aversion conditioning. Prior reports have shown that similar aversion-related behaviors are also attenuated in LPS pre-treated animals that are involuntarily infused with a salient taste that was previously associated with LiCl-induced nausea (Cross-Mellor et al., 2009). This finding provides support for the current hypothesis by demonstrating that LPS pre-treatment interfered with the establishment of conditioned aversive behavioral responding that was present in saline pre-treated LiCl and LiCl+Saccharin-treated groups of animals. Furthermore, this finding suggests that Group NaCl-LiCl+Saccharin did establish aversion learning, despite a lack of robust conditioned gaping in this group relative to controls.

3.4.4 Spontaneous orofacial behaviors

The frequency of spontaneous orofacial behaviors (tongue protrusions, mouth movements, and paw licks) were examined and compared. Animals treated with LiCl displayed significantly more spontaneous orofacial behaviors relative to controls. This finding is inconsistent with Chan et al. (2009), who did not find any significant differences among groups for tongue protrusions, mouth movements, and paw licks. However, this effect has been observed before (Chan, 2010), where animals pre-treated with saline followed by LiCl displayed significantly more spontaneous orofacial behaviors relative to LPS pre-treated animals and saline controls. Most importantly, these results support the proposal that LPS treatment specifically exerts a deleterious effect on the conditioning of aversive behaviors (including gaping), and does not simply depress all orofacial behavior.

3.4.5 Conditioned taste avoidance

Saccharin preferences were calculated at six hours and twenty-four hours following the start of the voluntary two-bottle choice test with tap water and a 0.2% palatable saccharin solution mixed with tap water. Firstly, it should be noted that a significant post hoc analysis at 6 h revealed that animals treated with LiCl plus saccharin exhibited significantly lower saccharin preferences than animals treated NaCl plus saccharin, thus replicating the findings in Chapter 2 (Cloutier et al., 2011). Furthermore, at the 24 h time point, it was found that animals in Group NaCl-LiCl+Saccharin exhibited a significant saccharin avoidance relative to animals in the NaCl-NaCl+Saccharin control group. In addition, animals in Group LPS-LiCl+Saccharin did not display saccharin avoidance, demonstrating a clear preference for the 0.2% saccharin solution as opposed to tap water. Although animals in Group LPS-LiCl+Saccharin were exposed to the saccharin taste in conjunction with LiCl-induced nausea during conditioning, a saccharin preference was observed during the voluntary two-bottle choice test, providing evidence for the negative effects of LPS on the acquisition of gustatory conditioning. This finding is consistent with prior reports by Cross-Mellor et al. (2009), where it was demonstrated that LPS pre-treatment during taste aversion/avoidance conditioning failed to produce aversion/avoidance-related conditioned responses upon re-exposure to a salient sucrose taste previously paired with LiCl-induced nausea.

Group NaCl-LiCl+Saccharin displayed a significant saccharin avoidance relative to Group NaCl-NaCl+Saccharin at the 24 h time point; however, this group of animals did not show significant saccharin avoidance when compared to any other group. Similar

to the prior discussion on "conditioned gaping" behavior, where animals in this group displayed an intermediate level of gaping as opposed to robust gaping when compared to controls, this group failed to show robust differences when compared to LPS pre-treated animals and saline control groups other than Group NaCl-NaCl+Saccharin. It is unclear why avoidance conditioning in this group did not result in significantly stronger effects than most other groups. Saccharin avoidance in group NaCl-LiCl+Saccharin did not appear to be as robust as taste avoidances obtained with the traditional oral intake of saccharin (e.g., Rana & Parker, 2008), though this could be due to the route of administration.

Taste perception of intravascularly applied saccharin depends on transport through the blood (Fishberg et al., 1933) and may not be perceived as strongly as orally administered saccharin. General saccharin preferences increased significantly between the 6 and 24 h time points across all groups, which may suggest that extinction processes occurred during this time interval. Since extinction occurs with repeated exposure to the CS (taste) in the absence of the US (LiCl-induced nausea), it is possible that initial exploratory ingestion of the saccharin solution during the first 6 hours of the two-bottle test, without the accompanying feelings of nausea, produced an extinguishing effect that ultimately increased saccharin preference. It can therefore be argued that systemically administered taste during gustatory conditioning may condition weaker taste avoidances that require more sensitive tests (e.g., Taste Reactivity Test for taste aversion).

3.4.6 Putative mechanisms

There is evidence suggesting that LPS treatment inhibits aversion conditioning by

disrupting memory consolidation processes. Examination of LPS-induced chronic neuroinflammation on the induction of NMDA-dependent, and NMDA-independent, long-term potentiation (LTP) showed that intracerebroventricular administration of LPS produced significant spatial memory impairment in the Morris water maze (Min et al., 2009). A prior report by Pugh et al. (1998) showed that LPS treatment impaired the ability to form representations of distinct contexts in contextual fear conditioning paradigms, as demonstrated by a reduction in freezing responses upon re-exposure to a context previously paired with an aversive foot shock in LPS-treated rats (Pugh et al., 1998). In both studies, it was suggested that LPS may affect the functioning of the hippocampus. Recordings of postsynaptic potentials showed that the induction of NMDA-dependent and NMDA-independent LTP were impaired in the Schaffer collateral-CA1 synapse of the hippocampus (Min et al., 2009). Contextual fear conditioning has been shown to be, at least in part, a hippocampal-dependent learning paradigm, as demonstrated by the elimination of contextual fear conditioned responses after hippocampal lesions one day following conditioning (Kim & Fanselow, 1992). Tanaka et al., (2006) reported that LPS administration to the CA1 region of the hippocampus activated microglial cells and resulted in an increased production of IL-1β and TNF-α in this region. After 5 d of injections, it was found that long-term activation of microglia induced by LPS resulted in a decrease of glutamatergic transmission and learning and memory impairments without neuronal cell death (Tanaka et al., 2006).

It has also been reported that peripheral inflammation by LPS causes a reduction of trophic supply in the brain (Schnydrig et al., 2007). Neurotrophins, such as, brain-

derived neurotrophic factor (BDNF) and nerve growth factor (NGF) are known to play an important role in synaptic plasticity and long-term potentiation (Schnydrig et al., 2007). An experiment by Hennigan, Trotter, & Kelly (2007) demonstrated that synaptic plasticity in the dentate gyrus of the hippocampal complex is related to neurotrophin signaling changes, and that the disruption of these changes in plasticity by LPS may be partially due to a strong effect on these signaling cascades. Guan and Fang (2006) found that LPS treatment decreased BDNF expression in not only the hippocampus, but also the frontal cortex, the parietal cortex, the temporal cortex, and the occipital cortex. LPS also exerts a depressive effect on the expression of other neurotrophins, such as, NGF and neurotrophic factor 3 (NT-3), where expression was significantly reduced in cortical regions, as well as, the hippocampus (Guan & Fang, 2006).

It is possible that LPS affects the saliency of the nausea induced by LiCl; however, this has not yet been investigated. The results of the studies discussed above strongly suggest that treatment with LPS, or specific cytokines, such as IL-1β, disrupts memory consolidation processes that are vital for associative learning in paradigms, such as those for anticipatory nausea and taste avoidance. Furthermore, these neurotrophin data also suggest that although tolerance develops to the peripheral effects of LPS treatment (i.e., reduction in behavioral sickness behaviors), it seems to have a longer lasting central effect. It has been demonstrated that the acquisition of "conditioned gaping" continues to be attenuated in peripherally LPS-tolerant animals (Chan, 2010), thus providing behavioral data to support this hypothesis.

3.4.7 Conclusions

It was hypothesized that LPS pre-treatment would disrupt the concurrent acquisition of anticipatory nausea and conditioned taste avoidance in a simultaneous conditioning model that employed intravascular/intraperitoneal administration of a toxin (LiCl) and a palatable saccharin taste. A number of findings in the current experiment partially support the current hypothesis. Firstly, measures of body weight loss were consistent with previous reports that indirectly measured the development of peripheral tolerance to LPS (Chan et al., 2009). Significant reductions in "conditioned gaping" responses and saccharin preference relative to Group NaCl-LiCl were observed in LPS pre-treated animals that received LiCl or LiCl+Saccharin- also consistent with the results of Chan et al. (2009). Furthermore, animals in these groups displayed significantly attenuated non-gaping, aversion-related behaviors in the novel context, relative to Group NaCl-LiCl and Group NaCl-LiCl+Saccharin. Conditioned gaping in Group NaCl-LiCl+Saccharin did not differ from LPS-treated animals or saline controls; however, this group displayed other aversion-related behaviors that were significantly higher in frequency relative to controls and comparable to aversion-related behaviors observed in Group NaCl-LiCl. Thus, there is evidence of aversion-related conditioning in Group NaCl-LiCl+Saccharin. In addition, saccharin avoidance in this group was significant when compared to the control group NaCl-NaCl+Saccharin. Although this avoidance was not robust enough to differ significantly from other groups, this may be due to a taste avoidance test that was not ideal for measuring weaker, intravascularly-derived, gustatory conditioning.

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CHAPTER 4

GENERAL DISCUSSION

4.1 General Discussion

The current thesis examined the roles of internal and external modes of conditioning stimuli (taste and context) in a rodent model designed to simultaneously condition anticipatory nausea and taste avoidance. Among cancer patients receiving chemotherapy treatment, anticipatory nausea and taste avoidance produce aversive conditioning consequences to the treatment (Molassiotis, 2005; Morrow et al., 1998; Morrow, Roscoe, Korshner, Hynes, & Rosenbluth, 1998). To expand upon the establishment of such a model, the effects of immune stimulation by LPS on the formation of these learned associations were examined. Though there are many studies that have reported robust learning and memory impairments following LPS treatment in the Morris Water Maze, taste avoidance/aversion paradigms, fear conditioning paradigms, and the anticipatory nausea paradigm, other studies have been shown to either improve or have no effect on learning (Sparkman, Kohman, Scott, & Boehm, 2005; Min et al.,2009; Arai, Matsuki, Ikegaya, & Mishiyama, 2001; Pugh et al., 1998; Chan, Cross-Mellor, Kavaliers, & Ossenkopp, 2009; Cloutier, Cross-Mellor, Kavaliers, & Ossenkopp, 2011; Ossenkopp, Biagi, Cloutier, Kavaliers, & Cross-Mellor, 2011).

In Chapter 2, a rodent model for the simultaneous conditioning of anticipatory nausea and taste avoidance was established using an internal taste stimulus. Systemic intraperitoneal injection of a solution containing a palatable saccharin taste (internal CS) mixed with nausea-inducing LiCl (US), administered immediately prior to exposure to the novel context (external CS) of the anticipatory nausea paradigm, produced robust "conditioned gaping" responses to the context, indicative of nausea. Significantly lower

saccharin preferences in a voluntary 2-bottle choice test between saccharin and water, relative to control animals were also obtained. This study demonstrated that rats are able to process and associate two different modes of conditioning stimuli (context and taste) with the same aversive unconditioned stimulus, LiCl. Since both anticipatory nausea and taste avoidance are observed among cancer patients undergoing chemotherapy, this model may prove important for elucidating the underlying mechanisms responsible for the formation of aversive conditioned associations between more than one conditioning stimulus (context or taste) and a nausea-inducing unconditioned stimulus (i.e., LiCl or chemotherapy). Although overshadowing effects cannot be ruled out, there was no evidence to suggest that the saliency of one stimulus (context or taste) was greater than the other when forming an association with the toxin effects of LiCl treatment.

In Chapter 3, the effects of the Gram-negative bacterial endotoxin, LPS, on the simultaneous establishment of anticipatory nausea and taste avoidance were examined. Since LPS has been shown to independently inhibit learning and memory in both the anticipatory nausea and taste avoidance paradigms, it was proposed that LPS would be effective in disrupting the learning processes involved in forming associations between the nausea induced by the LiCl and both the salient saccharin taste (internal CS) and the novel context (external CS).

The current hypothesis regarding the effects of LPS treatment on learning and memory was partially confirmed. The traditional anticipatory nausea paradigm was replicated, with LiCl-treated animals displaying significantly higher "conditioned gaping" frequencies relative to saline controls, consistent with prior reports (Chan et al., 2009;

Limebeer et al., 2006; Ossenkopp et al., 2011; Cloutier et al., 2011). The findings of Chan et al., (2009) were also replicated further, where animals pre-treated with LPS and given LiCl during conditioning did not show "conditioned gaping" frequencies that were significantly different from controls, but were significantly lower than LiCl, saline pre-treated, animals. It was further shown here that animals pre-treated with LPS followed by LiCl plus saccharin also displayed significantly attenuated "conditioned gaping" relative to group NaCl-LiCl.

Animals in Group NaCl-LiCl+Saccharin displayed an intermediate level of "conditioned gaping", where gaping frequency was not found to be significantly different from Group NaCl-LiCl; however, this group did exhibit significantly more non-gaping aversion-related behaviors (forelimb flails, paw treads, head shakes) relative to Groups LPS-LiCl, LPS-LiCl+Saccharin, LPS-NaCl, LPS-NaCl+Saccharin, NaCl-NaCl, and NaCl-NaCl+Saccharin, and did not differ significantly in frequency from Group NaCl-LiCl. This finding suggests that animals in Group NaCl-LiCl+Saccharin did condition aversive responding in the anticipatory nausea paradigm despite showing gaping frequencies that were not robust.

It was also shown that animals in Group NaCl-LiCl+Saccharin displayed a significant conditioned saccharin avoidance relative to Group NaCl-NaCl+Saccharin. Saccharin preferences in Group NaCl-LiCl+Saccharin did not differ significantly from any of the other groups; however, this may be due to saccharin intake measures that were not sensitive enough to capture a weaker taste avoidance that was produced through intravascular/intraperitoneal injection of LiCl plus saccharin, as opposed to orally

presented taste.

LPS-induced impairments are associated with reductions in neurotrophin expression, and the subsequent reductions in long-term potentiation observed in context-dependent memory areas of the brain, such as the CA1 region of the hippocampus (Tanaka et al., 2006). Many regions of the cortex, including the frontal, parietal, and temporal lobes, have also exhibited reduced neurotrophic expression, such as, reductions in nerve growth factor and brain-derived neurotrophic factor expression (Guan & Fang, 2006;). Repeated LPS administration has been shown to produce an accumulation of beta-amyloid peptide ($A\beta_{1-42}$) in both the hippocampus and cerebral cortex of mice (Lee et al., 2008). The neuroinflammation and intracellular protein accumulation observed following extended LPS treatment has been suggested to potentially be associated with the development of memory-related neurodegenerative disorders, such as Alzheimer's Disease, where neuroinflammation and amyloid plaques are commonly observed (Lee et al., 2008).

LPS-treated animals show significant reductions in body weight following initial LPS treatments, but these physiological effects are transient and animals treated with LPS will eventually show weight gains/losses comparable to control animals (tolerance development) (e.g., Chan et al., 2009; Ossenkopp et al., 2011; Cloutier et al., 2011; Chan, 2010). It has been shown that LPS-tolerant animals that no longer display any acute-phase response "sickness behaviors" (peripheral tolerance) fail to establish "conditioned gaping" in the anticipatory nausea paradigm (Chan, 2010), demonstrating that central tolerance to LPS does not develop in the same way as peripheral tolerance. One particular

finding of interest in the current study was the interactive effects observed between LPS pre-treatment and saccharin administration. NaCl pre-treated animals treated with saccharin mixed with LiCl or NaCl displayed larger gains in body weight relative to NaCl pre-treated animals that did not experience saccharin. It has not yet been examined how systemic saccharin interacts with LPS treatment. It could be suggested that an intravascular saccharin taste influenced appetite following conditioning, but this requires further investigation.

There is substantial evidence to support the hypothesis that non-pathological immune stimulation by LPS exerts a deleterious effect on learning- and memory-related cognitive processes. The deleterious effects of LPS treatment extend beyond vertebrates to honeybees and bumblebees, where it has been demonstrated that immune stimulation impairs performance in odor-sugar reward associations and free-flying learning paradigms, respectively (Mallon, Brockmann, & Schmid-Hempel, 2003; Alghamdi, Dalton, Rosato, & Mallon, 2008).

Although the neural mechanisms underlying nausea conditioning need further clarification, it does appear that an intact area postrema is crucial for successful taste avoidance/aversion learning with LiCl (Ossenkopp & Eckel, 1995). The chemosensitive area postrema is a circumventricular medullary structure implicated in the detection of blood-borne toxins, such as LiCl (Borison, 1989). Animals with area postrema lesions will fail to acquire conditioned taste avoidances/aversions conditioned with toxins, such as LiCl (Eckel & Ossenkopp, 1996; Ossenkopp & Eckel, 1995; Ossenkop, Ladowsky, & Eckel, 1997). The role of area postrema in forming associations between feelings of

nausea and specific contexts or environments, such as those in anticipatory nausea or conditioned place avoidance paradigms, has not been examined yet. Here, a robust rodent model that demonstrates the rat's ability to process and associate both an external (context) and an internal (taste) cue with the experience of LiCl-induced nausea has been established, and might help us to further elucidate the roles and functions of brain areas implicated in different types of learning and memory.

4.2 Conclusions

In the present thesis, a rodent model of simultaneous anticipatory nausea and taste avoidance conditioning was established through the use of intravascular/intraperitoneal LiCl mixed with saccharin that was administered prior to exposure to the novel context of the anticipatory nausea paradigm. Expanding on this finding, it was shown that animals pre-treated with LPS failed to show significant "conditioned gaping" or taste avoidance. Due to conditioning effects in Group NaCl-LiCl+Saccharin that were not robust, factors, such as, injection stress and the interactive role of saccharin should be examined further.

There is much left to be explored in this area of research. The presence of robust conditioned aversion learning in chemotherapy patients provides a modern day context in which processes that are generally considered to be adaptive and protective become maladaptive, dissuading individuals from continuing life-saving treatments. It is, therefore, important to study these associative processes on both behavioral and neurological levels, focusing on how these associations form, and how they can be disrupted. Examining the effects of immune stimulation on learning and memory will hopefully help to elucidate the cognitive consequences of neuroinflammation, with the

goal of contributing to the growing body of literature linking neuroimmunological processes with neurodegenerative disease and impairment.

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