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The Influence of Estrogen on Sex Differences in Chemotherapy-Induced Nausea and Vomiting (CINV)

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Abstract

Chemotherapy-induced nausea and vomiting (CINV) is one of the most distressing events that affects the quality of life of cancer patients. Evidence suggests that females are more susceptible to CINV than males, but the mechanism remains unknown. The current thesis examined whether higher levels of circulating estrogens in females contributes to this sex difference. CINV was analyzed in a pediatric oncology population, where it was revealed female patients demonstrate increased delayed CINV relative to male patients, in the post-pubertal age group. CINV was also studied by examining the influence of the estrous cycle on anticipatory nausea (AN) in rats. This study showed that rats in proestrus demonstrate increased AN relative to rats in diestrus. These results imply that females' greater likelihood to experience CINV may be partly due to their higher levels of circulating estrogens. Uncovering this mechanism will ultimately help to alleviate the burden of CINV on cancer patients.

Keywords

Chemotherapy-Induced Nausea and Vomiting (CINV); Anticipatory Nausea (AN); Estrogens; Estrous Cycle; Estradiol; Conditioned Disgust; Rat; Sex Differences; Nausea; Learning; Memory; Oncology; Pediatrics; Puberty

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Chapter 1

1 General introduction

The prevalence, course, and severity of many medical and behavioural conditions differ between males and females (Manton, 1999; Regitz-Zagrosek & Seeland, 2013). These sex differences arise from a combination of genetic, hormonal, and social factors that interact to produce widespread differences in the brain and behaviour of the human population. Despite knowledge of these sex differences, there is little sex-specific healthcare; sex and gender are often not taken into account when considering the prevention, treatment, and management of disease (Regitz-Zagrosek, 2012). Moreover, many animal studies are carried out with male subjects only, thus largely ignoring crucial sex differences. It is critical that researchers and clinicians take sex into account, as there are many questions concerning sex differences that have yet to be answered. Though sex differences in various diseases and conditions have been discovered, the mechanisms behind many of these sex differences remain largely unclear. Knowledge of these mechanisms is essential for the employment of more effective and personalized treatment strategies for many disorders that differentially affect males and females.

One area that has garnered recent interest is sex differences in the experience of nausea and nausea-related phenomena (Cloutier, Kavaliers, & Ossenkopp, 2017; Hilarius et al., 2011; Paillard et al., 2013). In a study identifying risk factors for post-operative nausea and vomiting (PONV), female sex was identified as the strongest patient-related predictor (Fujii, 2009; Gan, 2006). Female sex is also a known risk factor for motion sickness susceptibility (Paillard et al., 2013). Moreover, motion sickness has been shown to fluctuate throughout the menstrual cycle, in accordance with fluctuating estrogen levels (Golding, Kadzere, & Gresty, 2005). Women also report different factors which contribute to chronic migraine compared to men, with one of the more prevalent factors in women being nausea (Özge & Yalın, 2016). There is also a notable sex difference in the symptomatology reported during acute coronary syndromes. Females are more likely than males to report nausea, vomiting, and abdominal pain (Arslanian-Engoren, &

Engoren, 2010; Shin, Martin, & Suls, 2010). Finally, and critical to the present thesis, female sex is a known risk factor for chemotherapy-induced nausea and vomiting (CINV) (Molassiotis, Stamataki, & Kontopantelis, 2013; Sekine, Segawa, Kubota, & Saeki, 2013; Warr, 2014). Female patients experience CINV at higher frequencies than male patients in the acute, delayed, and anticipatory phases (Gonella & Di Giulio, 2015). In the delayed phase specifically, female sex is the only patient-related risk factor (Sekine et al., 2013).

Though the mechanism responsible for the sex difference in nausea-related phenomena has yet to be discovered, there is some evidence that implicates estrogens. The present thesis explored the possible influence of estrogens on CINV, and on anticipatory nausea (AN), which is a specific syndrome related to CINV. Two diverse study methodologies were used. The first consisted of a *post hoc* data analysis of pediatric oncology patients, examining sex differences in CINV in these patients at various developmental stages. The second study employed an animal model of AN which examined differences in conditioned disgust behaviours in female rats between two of the four phases of the estrous cycle.

There is no shortage of research in this area using solely male subjects. Unfortunately, inferences are often drawn from these studies that are applied to both males and females, which largely ignores the influence of female physiology and biology. The present thesis focused on female subjects, and the putative contribution of estrogens to CINV.

1.1 Chemotherapy-induced nausea and vomiting (CINV)

Cancer patients undergoing chemotherapy list nausea and vomiting as among the most debilitating and distressing treatment-related adverse effects (Aapro, Molassiotis, & Olver, 2005; Molassiotis, 2005). Chemotherapy-induced nausea and vomiting (CINV) is associated with significant deterioration of quality of life for these patients, and remains a severe problem in the oncology community (Feinberg, Gilmore, Haislip, Wentworth, & Burke, 2010). CINV can be acute (24 hours following chemotherapy administration), delayed (more than 24 hours after and within 7 days of chemotherapy administration), or anticipatory (within 24 hours prior to chemotherapy administration).

For adults undergoing chemotherapy, there are a number of known risk factors associated with CINV. These include: age below 55 years, female sex, non-habitual alcohol use, and history of nausea including pregnancy-related morning sickness and motion-sickness (Molassiotis et al 2013; Sekine et al., 2013; Warr, 2014). Taking these risk factors into account when selecting CINV prophylaxis for individual patients may improve complete CINV control rates. Unfortunately, in children, risk factors for the development of CINV are currently unknown. Although there are several possible risk factors, the present thesis focused on one potential risk factor in children: female sex. Knowledge of patient-related risk factors for CINV would permit tailoring of standard CINV prophylaxis to meet the needs of individual children and would likely result in complete CINV control in a higher proportion of children. In the present thesis, patients were split into three groups: pre-, mid-, and post-pubertal, in order to pinpoint exactly when the sex difference in CINV may arise.

One stage of CINV that is particularly challenging to study is anticipatory CINV. Anticipatory CINV is the experience of nausea and vomiting in the 24 hours leading up to chemotherapy administration, in anticipation of the treatment itself. Anticipatory CINV is difficult to control once it develops, and it does not respond well to pharmacological or non-pharmacological treatment strategies (Tyc, Mulhern, Barclay, Smith, & Bieberich, 1997). Currently, the best treatment of anticipatory CINV is to prevent it from occurring in the first place, by controlling acute and delayed CINV (Chan et al., 2015). The prevalence of anticipatory CINV reported in the literature ranges from 8%–30% of patients (Molassiotis et al., 2016; Morrow et al., 1998). It is posited that anticipatory CINV develops through classical conditioning (Bovbjerg, 2006; Stockhorst, Steingrueber, Enck, & Klosterhalfen, 2006). The chemotherapy acts as the unconditioned stimulus, which elicits nausea and vomiting, the unconditioned response. The physical hospital or room acts as the conditioned stimulus and after repeated pairings, these environmental stimuli alone can elicit nausea and vomiting (Tyc et al., 1997). The prevalence and severity of anticipatory CINV are the basis for the development of an animal model (Limebeer et al., 2006; 2008).

1.2 Animal model

A rat model of anticipatory CINV has been well established and is used as a preclinical tool to study possible treatments (Limebeer et al., 2006; 2008). This model refers to the condition as anticipatory nausea (AN; Limebeer et al., 2006; 2008). The AN model uses the toxin, lithium chloride (LiCl), a nausea-inducing drug, as the unconditioned stimulus, and a distinctive context as the conditioned stimulus. This paradigm demonstrates rats' ability to associate toxin-induced nausea with the context in which they experienced it. Rats are injected with LiCl and placed in a distinctive context immediately after injection. After numerous pairings, rodents are capable of associating this distinctive context with "feelings" of nausea. When rodents are re-exposed to the context drug-free, they will demonstrate a conditioned-gaping response, which is a learned response. This well-established animal model is a valuable pre-clinical tool for examining possible risk factors and treatments for AN (Cloutier et al., 2017; Limebeer et al., 2006; 2008).

Sex differences have been observed in this animal model. On drug-free test day, female rats demonstrate significantly more conditioned gaping responses than male rats (Cloutier et al., 2017). This is consistent with other animal studies; sex differences in favour of females have been seen in the development of motion-sickness in the musk shrew (Javid & Naylor, 1999), as well as in many clinical studies that point to female sex as a risk factor for a number of nausea-related phenomena (Arslanian-Engoren, & Engoren, 2010; Fujii, 2009; Gan, 2006), including CINV (Molassiotis et al., 2013; 2016).

1.3 Estrogens

Although this sex difference is well established, further exploration is necessary to examine the mechanism underlying this sex difference. It has been suggested that gonadal hormones, specifically estrogens, play a role in this etiology. Estradiol has been shown to have some natural illness-inducing properties (Ganesan & Simpkins, 1991; Goodman & Gilman, 1975). When paired with a novel sucrose taste, estradiol produces a conditioned taste avoidance and disgust response to the novel taste (Ossenkopp, Rabi, &

Eckel, 1996). Estradiol can also have an additive effect on the LiCl-induced conditioned disgust in castrated male rats (Lin, Tsai, Tai, & Yeh, 2015).

Estrogens also have stimulatory effects on the serotonergic system. Acute estradiol administration to ovariectomized rats increases serotonin levels in a number of brain regions, including the hippocampus (Johnson & Crowley, 1983). Evidence suggests that a large number of estrogen receptors are expressed in the dorsal raphe nuclei, where most serotonin neurons are located (Merchenthaler, Lane, Numan, & Dellovade, 2004). Consequently, estrogen replacement increases neuronal activity in this this region (Dalmasso, Amigone, & Vivas, 2011). Serotonin (specifically 5-HT_3) antagonism has well-established anti-emetic effects, thus implicating the serotonergic system in the production of nausea and vomiting (Hesketh, 2000). Therefore, estrogens may increase nausea and vomiting through a downstream serotonergic process.

There is some indication of an evolutionary explanation for estrogen's enhancing effect on nausea. It has been proposed that nausea and vomiting during pregnancy evolved as an evolutionarily adaptive mechanism to avoid the ingestion of harmful toxins, thus potentially harming the fetus (Buss, 1999; Profet, 1995). The vomiting and disgust reflexes have long been regarded as evolutionarily adaptive, as they protect the individual from ingesting potentially poisonous or noxious substances. It is possible that estrogen causes this sense to be particularly heightened in women, who have the added responsibility of protecting their developing offspring from harm (Sherman & Flaxman, 2002).

Estrogens are a class of hormones that naturally fluctuate through the course of a woman's lifetime. Estrogens begin to rise during puberty, fluctuate throughout the menstrual cycles, and begin to decline after menopause. Thus, it is possible to observe estrogen's effects on nausea by examining the course of nausea-related phenomena in different stages of the lifespan of a woman. Motion sickness has been shown to fluctuate throughout the menstrual cycle, in accordance with fluctuating estrogen levels (Golding, Kadzere, & Gresty, 2005). Additionally, motion sickness susceptibility and chronic migraine demonstrate age-dependent decreases in the experience of nausea in women but

not men. After menopause, motion sickness susceptibility decreases and nausea no longer predicts chronic migraine (Özge et al, 2014; Paillard et al., 2013).

1.4 Objectives of the current thesis

The first objective of this thesis was to explore the association between CINV control as assessed by pediatric oncology patients and/or their parents and the patient-related factors of sex and pubertal status. The study involved a post hoc analysis using integrated data from three prospective, observational studies which examined acute, delayed, and anticipatory CINV in pediatric oncology patients at The Hospital for Sick Children (SickKids) in Toronto. The study explored sex differences in pre-, mid-, and post-pubertal children in order to elucidate the possibility that sex differences in CINV only arise post pubertally.

The second objective of this thesis was to examine the ability of female rodents to process and associate a distinctive context with an internal sickness cue (i.e., LiCl-induced nausea) in the rodent model of AN, and to test whether estrous cycle phase influences the formation of AN. Specifically, using a conditioned disgust paradigm, female rats conditioned and tested during proestrus (high estrogen levels) were compared to those conditioned and tested during diestrus (low estrogen levels) to determine if estrogen is modulating the observed sex difference in the literature.

The current study examined sex differences in CINV by considering two very different subject groups. In Chapter 2, pediatric patients were examined, while in Chapter 3, adult female rats were the subjects. Much of the sex differences in CINV literature focuses on adult patients, while the pediatric population has been less investigated. The present study focused on this less examined patient age group, in order to shed some light on the possible sex differences that may or may not be present in this population.

Although it has been well established that females are more likely to develop CINV (including but not limited to the anticipatory phase), further research is necessary to determine the mechanism contributing to this sex difference. It was hypothesized that estrogen is the contributing factor to females' greater propensity to experience CINV than

their male counterparts. This hypothesis was explored through diverse studies, uniquely combining human and rodent subjects, whose results will strengthen the estrogen hypothesis with converging evidence.

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Chapter 2

2 Examining the Association Between Sex, Pubertal Status, and the Development of Chemotherapy-Induced Nausea and Vomiting (CINV) in Pediatric Oncology Patients

2.1 Introduction

Cancer patients undergoing chemotherapy list nausea and vomiting as among the most debilitating and distressing treatment-related adverse effects (Aapro, Molassiotis, & Olver, 2005; Molassiotis, 2005). Chemotherapy-induced nausea and vomiting (CINV) is associated with significant deterioration of quality of life for these patients, and remains a severe problem in the pediatric oncology community (Feinberg, Gilmore, Haislip, Wentworth, & Burke, 2010). CINV can be acute (24 hours following chemotherapy administration), delayed (more than 24 hours after and within 7 days of chemotherapy administration), or anticipatory (within 24 hours prior to chemotherapy administration). Despite the routine administration of modern CINV prophylaxis based on the emetic risk carried by the planned chemotherapy regimen, up to 55% of children receiving moderately or highly emetogenic chemotherapy experience CINV (Kang et al., 2015, Kovacs et al., 2016). Chemotherapy-induced nausea (CIN) and chemotherapy-induced vomiting (CIV) are considered as separate entities and are often analyzed separately. While modern antiemetics have vastly improved CIV control rates over the past decade, CIN remains more difficult to measure, as well as to control (Rapoport, Molasiotis, Raftopoulos, & Roila, 2015).

For adults undergoing chemotherapy, there are a number of known risk factors associated with CINV. Taking these risk factors into account when selecting CINV prophylaxis for individual patients may improve complete CINV control rates. These risk factors include: age (below 55 years), non-habitual alcohol use, past history of nausea including pregnancy-related morning sickness and motion-sickness, and female sex (Molassiotis,

Stamataki, & Kontopantelis, 2013; Sekine, Segawa, Kubota, & Saeki, 2013; Warr, 2014). Female patients experience CINV at higher frequencies than male patients in both the acute and delayed phases (Gonella & Di Giulio, 2015). In the delayed phase specifically, female sex is the only patient-related risk factor (Sekine et al., 2013). Although clinicians are aware of this, the mechanism by which this sex difference operates remains unclear. Sex differences in favour of females have also been seen in post-operative nausea and vomiting as well as susceptibility to motion sickness (Fujii, 2009; Paillard et al., 2013).

It has been suggested that sex hormones, specifically estrogens, may play a role in CINV. Several animal studies have shown that estrogens enhance conditioned aversion behaviour and may increase nausea and malaise (Fudge, Kavaliers, Baird, & Ossenkopp, 2009; Ganesan, 1994). Estradiol itself has been found to produce both conditioned taste avoidance when paired with a novel sucrose taste, as well as a strong shift in palatability of the sucrose taste (Ossenkopp, Rabi, & Eckel, 1996). This may occur because of estradiol's illness-inducing properties, which has been seen in animal studies (Ganesan & Simpkins, 1991) as well as in humans (Goodman & Gilman, 1975).

Although investigators have observed signals that CINV is more problematic in adult females compared to adult males, a sex difference in pediatric populations is less evident (Freedman et al., 2014). As of now, chemotherapy emetogenicity is the strongest known determinant of CINV in children. While recent pediatric CINV studies have not found female sex to be a significant predictor, these studies have not taken pubertal status into consideration when examining sex differences (Aseeri, Mukhtar, Khansa, Elimam, & Jastaniah, 2013; Dupuis et al., 2017; Holdsworth, Raisch & Frost, 2006; Vol et al., 2016). If in fact gonadal hormones such as estrogens contribute to the sex difference in adults, it is possible that a sex difference has not been seen in the pediatric population due to the relatively low levels of circulating gonadal hormones in children. The present study specifically differentiated pubertal status to examine the possibility that sex differences in CINV only arise post puberty. It was hypothesized that sex differences would only appear in patients in the post pubescent group.

The present study involved a post hoc analysis using integrated data from three prospective, observational studies which examined acute, delayed, and anticipatory CINV in pediatric oncology patients at The Hospital for Sick Children (SickKids) in Toronto. The primary objective of this study was to explore the association between CINV control as assessed by patients and/or their parents and the patient-related factors of sex and pubertal status. Any sex differences found (or lack thereof) may then be used by clinicians to personalize CINV prophylaxis provided to children receiving chemotherapy. Finding a post pubertal CINV sex difference would also point to a role for gonadal hormones in the observed sex difference in CINV seen in adults. Finally, knowledge of patient-related risk factors for CINV would permit tailoring of standard CINV prophylaxis to meet the needs of individual children and would likely result in more complete CINV control in a higher proportion of children.

2.2 Method

Data for the current analysis were pooled from three prospective, observational studies completed at SickKids. The three studies included in this post hoc analysis are outlined in Table 2.1. All studies collected information on the prevalence of acute, delayed, and anticipatory CINV in children receiving chemotherapy. All studies were approved by the SickKids Research Ethics Board. The following is a brief overview of the study methodology. For more detailed descriptions, see the methodologies from the original studies (Flank et al., 2017a; Flank et al., 2017b; Vol et al., 2016).

2.2.1 Patients

Data were collected from 160 patients (105 male and 55 female, ages 4 to 18, M= 9.56 years, SD= 3.90 years). Common eligibility criteria in all three studies were as follows: patients had to have been English speaking, and have the cognitive ability believed to be at least at a 4-year-old level according to a parent or health care professional. Informed consent was obtained from eligible patients aged 16 years or older. For eligible patients younger than 16 years, informed consent was obtained from the parent or guardian and,

Table 2.1

Summary of Studies Used in Post Hoc Analysis

Study	*N* (m:f)	Additional Eligibility Criteria
[a]*Chemotherapy-induced nausea and vomiting in children receiving ifosfamide plus etoposide, dactinomycin plus vincristine, high dose methotrexate, or high dose methotrexate plus vincristine*	36 (23:13)	- Receiving ifosfamide 1800mg/m^2/day plus etoposide 100mg/m^2/day, dactinomycin 45µg/kg/dose plus vincristine 1.5mg/m^2/dose, methotrexate >1g/m^2, methotrexate >1g/m^2 plus vincristine 1.5mg/m^2/dose or equivalent in cases of dose reduction in response to renal or hepatic impairment
[b]*Prevalence of chemotherapy-induced nausea and vomiting in children receiving hematopoietic stem cell transplantation conditioning (HSCT)*	54 (31:23)	- Receiving HSCT conditioning for their first HSCT for any indication other than immunodeficiency - HSCT conditioning regimen started at The Hospital for Sick Children
[c]*Chemotherapy-induced nausea and vomiting in children receiving intrathecal methotrexate with/without vincristine*	70 (51:19)	- Receiving intrathecal methotrexate in the context of maintenance therapy for acute lymphoblastic leukemia and as dosed by age per protocol. Patients also received their protocol driven maintenance chemotherapy which includes oral 6 mercaptopurine and may include IV vincristine and an oral steroid pulse (prednisone or dexamethasone). - Patients were past their first cycle of maintenance therapy to avoid interactions with the intensive chemotherapy phase - Not receiving chemotherapy other than dictated by protocol for maintenance therapy within 24 hours prior to or 24 hours following receipt of intrathecal methotrexate

[a] Vol, H., Flank, J., Lavoratore, S. R., Nathan, P. C., Taylor, T., Zelunka, E., . . . Lee Dupuis, L. (2016). Poor chemotherapy-induced nausea and vomiting control in children receiving intermediate or high dose methotrexate. *Supportive Care in Cancer, 24*(3), 1365-1371.

[b] Flank, J., Sparavalo, J., Vol, H., Hagen, L., Stuhler, R., Chong, D,, Courtney, S., Doyle, J. J., Gassas, A., Schechter, T., Dupuis, L. L. (2017b). The burden of chemotherapy-induced nausea and vomiting in children receiving hematopoietic stem cell transplantation conditioning: a prospective study. *submitted for publication*

[c] Flank, J., Nadeem, K., Moledina, S., Khanna, M., Schindera, C., Punnett, A. S., Dupuis, L. L. (2017a). Nausea and vomiting in children and adolescents receiving intrathecal methotrexate: a prospective, observational study**.** *submitted for publication*

in the case of children aged 7-15 years, assent was obtained from the child as well. See Table 2.1 for additional eligibility criteria for each study.

2.2.1.1 Pubertal status designation

Pubertal status of each participant was defined using sex-specific age cut-offs, based on norms for precocious and late onset puberty in the literature (Carel, Lahlou, Roger, & Chaussain, 2004; Sedlmeyer & Palmart, 2002). Males between the ages of 1 to 9 years were defined as "pre-pubertal", between 9.1 to 14 years as "mid-pubertal", and between 14.1 to 18 years as "post-pubertal". Females between the ages of 1 to 8 years were defined as "pre-pubertal", between 8.1 to 13 years as "mid-pubertal", and between 13.1 to 18 years as "post-pubertal". See Table 2.2 for sample size of each of these pubertal groups.

2.2.2 Data collection

Structured diaries were used to collect CINV data. A validated nausea assessment instrument, the pediatric nausea assessment tool (PeNAT; Dupuis, Taddio, Kerr, Kelly, & MacKeigan, 2006), was used to measure nausea. The PeNAT, which has been validated for children aged 4 to 18 years, incorporates a facial expression scale with a standard script. Through the use of the facial scale and script, children can focus on the expression being asked of them and select the facial expression that best describes their feelings. Nausea is then translated onto a Likert scale from 1 to 4 (1 being no nausea to 4 being worst nausea) (Dupuis et al., 2006). Emetic episodes as well as antiemetic administration were also recorded in the diaries. Vomiting was defined as the expulsion of any stomach contents by the mouth. Retching was defined as an attempt to vomit that is not productive of any stomach contents. An emetic episode was defined as a vomit or retch separated from another vomit or retch by at least 1 minute. A study investigator explained study procedures to each patient and/or parent or guardian and administered the PeNAT to the child at least once in the presence of the parent or guardian. The number of emetic episodes recorded by the child/parent/guardian was compared against the nursing flow

Table 2.2

Sample Size By Pubertal Status and Sex

	Pre Pubertal	Mid Pubertal	Post Pubertal	Totals
Males	52	43	10	105
Females	26	13	16	55

sheets for inpatients, and in the case of discrepancy, the higher number was used. All diary pages completed during the inpatient period were collected prior to discharge. The diary pages completed during the outpatient period were returned to the investigator via mail or in person at a subsequent hospital visit.

2.2.3 Outcomes

2.2.3.1 Anticipatory CINV

Anticipatory CINV was defined as nausea, vomiting, or retching in the 24 hours prior to chemotherapy administration in patients that have received chemotherapy previously. The child was asked if he/she felt nauseated or vomited in the last 24 hours before his/her first chemotherapy dose in the study course. The parent(s)/guardian(s) were asked separately if their child vomited or felt nauseated within the last 24 hours before his/her first chemotherapy dose in the course to confirm the information provided by the child. Anticipatory CINV was quantified by presence or absence.

2.2.3.2 Acute and delayed phase CINV

Acute CINV was defined as nausea, vomiting, or retching occurring within 24 hours following chemotherapy administration. Delayed CINV was defined as nausea, vomiting, or retching which occurs between 25 hours up to 7 days following chemotherapy administration. The time that each emetic episode occurred was recorded by the child or parent in the diary. Children were able to rank nausea as none, mild, moderate, or severe using the PeNAT. Both acute and delayed CINV were quantified with a score of "level of control over CINV" (Likert scale of 1-3, 1 being complete control, 2 being partially controlled, 3 being uncontrolled). The primary study endpoint was complete control of CINV which was defined as no nausea (maximum PeNAT score of 1) and no emetic episodes. Partial CINV control was defined as a maximum PeNAT score of 2 during the phase of interest, or 1 or 2 emetic episodes in any 24-hour period. Uncontrolled CINV was defined as a maximum PeNAT score of 3 or 4 during the phase of interest, or more than 2 emetic episodes in any 24-hour period.

2.2.4Data analysis

Dependent variables quantified were the proportion of participants that developed anticipatory CIN, CIV, and CINV, the overall level of control of acute CIN, CIV, and CINV, and the overall level of control of delayed CIN, CIV, and CINV. Independent variables examined were sex (male and female) and pubertal status (pre-pubertal, mid-pubertal, and post-pubertal). All statistical tests used α=0.05 as a significance criterion. Statistical analysis were performed using IBM SPSS Statistics 23 for Windows.

2.2.4.1 Anticipatory CINV

Data was analyzed using three separate chi-square tests to explore the association between sex and proportion of patients who develop anticipatory CIN, CIV, and CINV, for each of the three pubertal groups of interest.

2.2.4.2 Acute and delayed phase CINV

Data was analyzed using 2 X 3 between subjects analysis of variances (ANOVA) with two between subject factors of Sex (at two levels: male and female) and Pubertal Status (at three levels: pre-pubertal, mid-pubertal, and post-pubertal) for CIN, CIV, and CINV each separately. This analysis compared mean CINV control scores (higher indicating increased CINV) for each of the groups of interest. A priori planned comparisons were performed on each pubertal group separately, as this was the specific focus of the study. This was done using three separate one-way ANOVAs on each pubertal group, with one between subject factor of sex (at two levels: male and female).

The number of individuals experiencing either completely controlled, partially controlled, or uncontrolled CINV were also calculated as a proportion of total in each group of interest (pre-pubertal males, pre-pubertal females, mid-pubertal males, mid-pubertal females, post-pubertal males, and post-pubertal females). This was done separately for anticipatory, acute, and delayed CINV. Subsequently, chi square analyses were performed to compare these frequencies, to further ascertain if sex differences exist in level of control of CINV in each pubertal group.

2.3 Results

2.3.1 Anticipatory CIN, CIV, and CINV

Three separate chi-square analyses revealed no significant differences between males and females for any of the pubertal groups. The proportion of children experiencing anticipatory CINV in each of the pubertal groups is shown in Table 2.3. For all pubertal groups, no relationship was found between sex and development of CIN, CIV, or CINV.

2.3.2 Acute CIN, CIV, and CINV control

The proportion of children who experienced complete, partial, or no control of CIN, CIV, and CINV in the acute phase is summarized in Table 2.4. The 2 X 3 between-subjects ANOVA revealed no significant Sex or Pubertal Status effects for acute CIN, CIV, or CINV. Since pubertal status was of particular interest in this study, a priori planned comparisons were done in order to explore possible Sex effects in each of the pubertal status groups. The one-way ANOVA revealed no significant Sex effect in any of the pubertal status groups. The chi square analyses also revealed no significant differences in any of pubertal groups for acute CIN, CIV, and CINV control.

2.3.3 Delayed CIN, CIV, and CINV control

The proportion of children who experienced complete, partial, or no control of CIN, CIV, and CINV in the delayed phase is summarized in Table 2.5. For CIV, the 2 X 3 between subjects ANOVA revealed a significant main effect of Sex, $F(1,154)= 7.76$, $p < .01$. For CIN and CINV, the 2 X 3 between-subjects ANOVA revealed no significant Sex or Pubertal Status effects. Since pubertal status was of particular interest in this study, a priori planned comparisons were done in order to explore possible Sex effects in each of the pubertal status groups. For both CIN and CINV, the one-way ANOVA revealed a significant main effect of Sex in the post pubertal group, $F(1,24)= 5.01$, $p < .05$. As shown in Figure 2.1, post pubertal females demonstrated a significantly higher mean "level of control of delayed CINV" score, which translates to higher levels of

Table 2.3

Prevalence of Anticipatory CINV by Sex and Pubertal Status

	Number of patients experiencing anticipatory chemotherapy-induced nausea and vomiting (%)					
	CINV		CIV		CIN	
	Male	Female	Male	Female	Male	Female
Pre Pubertal	8 (15)	5 (19)	4 (8)	2 (8)	8 (15)	5 (19)
Mid Pubertal	9 (21)	2 (15)	5 (12)	2 (15)	7 (16)	2 (15)
Post Pubertal	1 (10)	5 (31)	0 (0)	2 (13)	1 (10)	4 (25)

CINV chemotherapy-induced nausea and vomiting, CIV chemotherapy-induced vomiting, CIN chemotherapy-induced nausea.

Table 2.4

Prevalence of Acute CINV by Sex and Pubertal Status

	Acute Phase					
	CINV		CIV		CIN	
	Male	Female	Male	Female	Male	Female
Number of patients experiencing complete control[a] (%)						
Pre Pubertal	14 (27)	10 (38)	38 (73)	17 (65)	14 (27)	11 (42)
Mid Pubertal	14 (33)	2 (15)	26 (60)	5 (38)	14 (33)	2 (15)
Post Pubertal	1 (10)	3 (19)	7 (70)	9 (56)	1 (20)	3 (19)
Number of patients experiencing partial control[b] (%)						
Pre Pubertal	16 (31)	4 (15)	7 (13)	3 (12)	17 (33)	5 (19)
Mid Pubertal	11 (26)	4 (31)	9 (21)	6 (46)	11 (26)	4 (31)
Post Pubertal	4 (40)	4 (25)	2 (20)	3 (19)	4 (40)	4 (25)
Number of patients experiencing no control[c] (i.e. uncontrolled) (%)						
Pre Pubertal	22 (42)	12 (46)	7 (13)	6 (23)	21 (40)	10 (38)
Mid Pubertal	18 (42)	7 (54)	8 (19)	2 (15)	18 (42)	7 (54)
Post Pubertal	5 (50)	9 (56)	1 (10)	4 (25)	5 (50)	9 (56)

CINV chemotherapy-induced nausea and vomiting, CIV chemotherapy-induced vomiting, CIN chemotherapy-induced nausea.

[a] Complete control is defined as a max PeNAT score of 1 and/or 0 vomits, retches, or gags.

[b] Partial control is defined as a max PeNAT score of 2 and/or 1-2 vomits, retches, or gags.

[c] Uncontrolled is defined as a max PeNAT score of 3-4 and/or > 2 vomits, retches, or gags.

Table 2.5

Prevalence of Delayed CINV by Sex and Pubertal Status

	Delayed Phase					
	CINV		CIV		CIN	
	Male	Female	Male	Female	Male	Female
Number of patients experiencing complete control (%)						
Pre Pubertal	18 (35)	8 (31)	39 (75)	12 (46)	18 (35)	9 (35)
Mid Pubertal	15 (35)	5 (38)	24 (56)	6 (46)	15 (35)	5 (38)
Post Pubertal	5 (50)	3 (19)	8 (80)	7 (44)	5 (50)	3 (19)
Number of patients experiencing partial control (%)						
Pre Pubertal	5 (10)	5 (19)	7 (13)	11 (42)	5 (10)	4 (15)
Mid Pubertal	5 (12)	3 (23)	15 (35)	2 (15)	5 (12)	3 (23)
Post Pubertal	3 (30)	3 (19)	1 (10)	6 (38)	3 (30)	3 (19)
Number of patients experiencing no control (i.e. uncontrolled) (%)						
Pre Pubertal	29 (56)	13 (50)	6 (12)	3 (12)	29 (56)	13 (50)
Mid Pubertal	23 (53)	5 (38)	4 (9)	5 (38)	23 (53)	5 (38)
Post Pubertal	**2 (20)**	**10 (63)**	1 (10)	3 (19)	**2 (20)**	**10 (63)**

CINV chemotherapy-induced nausea and vomiting, CIV chemotherapy-induced vomiting, CIN chemotherapy-induced nausea.

[a] Complete control is defined as a max PeNAT score of 1 and/or 0 vomits, retches, or gags.

[b] Partial control is defined as a max PeNAT score of 2 and/or 1-2 vomits, retches, or gags.

[c] Uncontrolled is defined as a max PeNAT score of 3-4 and/or > 2 vomits, retches, or gags.

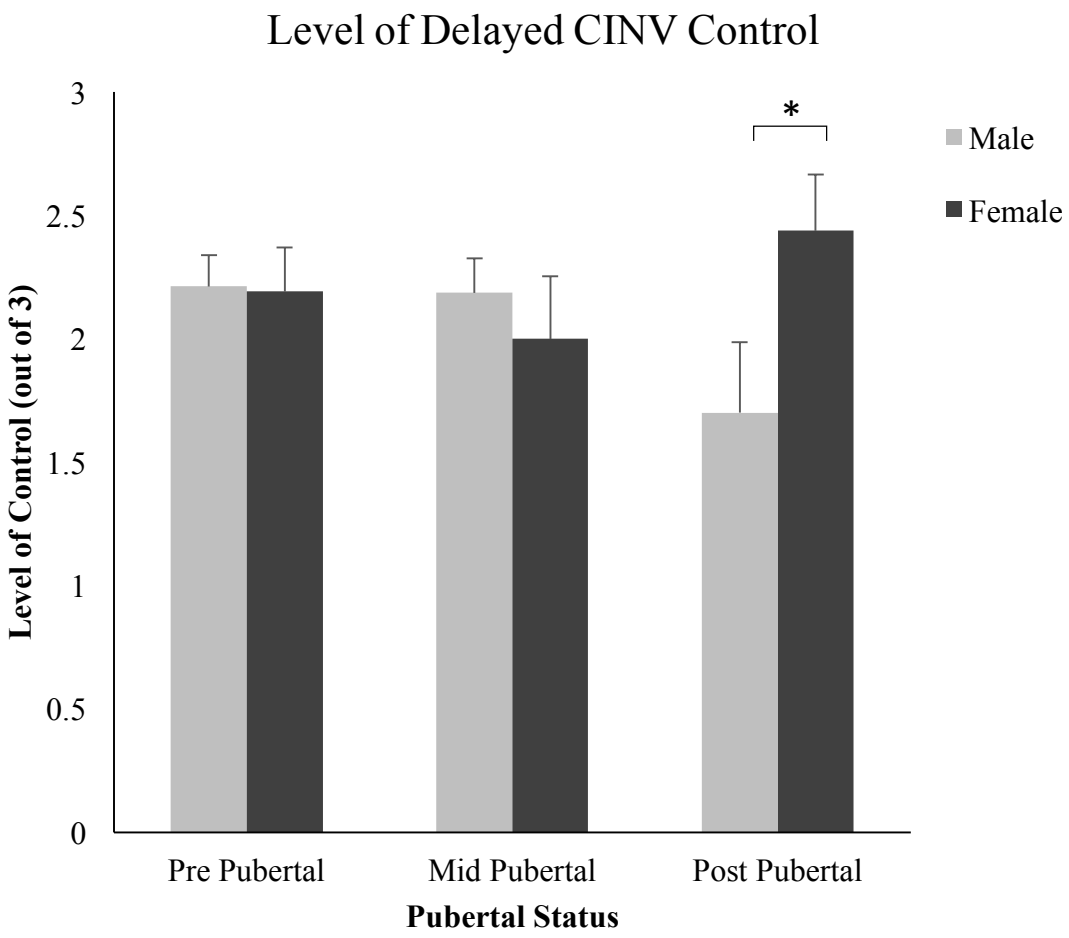

Figure 2.1. **Level of Delayed CINV Control.** Mean (+ sem) delayed CINV scores (out of 3, 1= complete control, 2= partial control, 3=uncontrolled) for pre, mid and post pubertal male and female participants who developed delayed CINV. In the post pubertal group only, there was a significant sex difference; post pubertal females had less control of delayed nausea (i.e. experienced more nausea) than post pubertal males *p < .05.

uncontrolled delayed CINV. No main effect of Sex was seen in any of the other pubertal groups. The chi square analysis also revealed an association between sex and proportion experiencing uncontrolled CIN and CINV, for the post pubertal group, X^2 (1, N = 26) = 4.301, $p < .05$. As shown in Figure 2.2, Post pubertal females demonstrated significantly higher proportions of uncontrolled CIN and CINV compared to post pubertal males. All other chi square analyses revealed no significant associations.

2.4 Discussion

The present study examined putative sex differences in the development of anticipatory, acute, and delayed CINV in pediatric oncology patients. Patients were split into three groups based on pubertal status: pre-, mid-, and post-pubertal. It was hypothesized that sex differences in the development of CINV would only be seen in the post-pubertal age group. This post-pubertal sex difference was in fact observed in delayed CINV.

Notably, in both the pre-, and mid-pubertal patients, no sex differences were seen in acute or delayed CINV control. This agrees with multiple studies which found that female sex is not a significant predictor of CINV in pediatric oncology patients (Aseeri et al., 2013; Dupuis et al., 2017; Holdsworth, Raisch & Frost, 2006; Vol et al., 2016). On the other hand, female sex is a well-established risk factor for CINV in adults (Hesketh, 2008; Sekine et al., 2013). This seemingly age-dependent sex difference points to some process occurring in females during adolescence, making them more susceptible to developing CINV. The results from the present study suggest that this said process may in fact be puberty. Puberty begins in females when gonadotropin-releasing hormone (GnRH) is secreted by the hypothalamus, which signals the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). This triggers ovarian production of estrogens, which are responsible for the behavioral and physiological changes that accompany puberty (Dietrich, 2014). Serum estradiol levels are significantly higher in post pubertal girls than post pubertal boys, as well as pre-pubertal children of both sexes (Courant et al., 2010). This marked rise in estradiol, beginning during puberty, fluctuating throughout the menstrual cycle, and lasting until menopause, may be connected to the increase in nausea-related phenomena seen in females. In a study identifying risk factors for post-

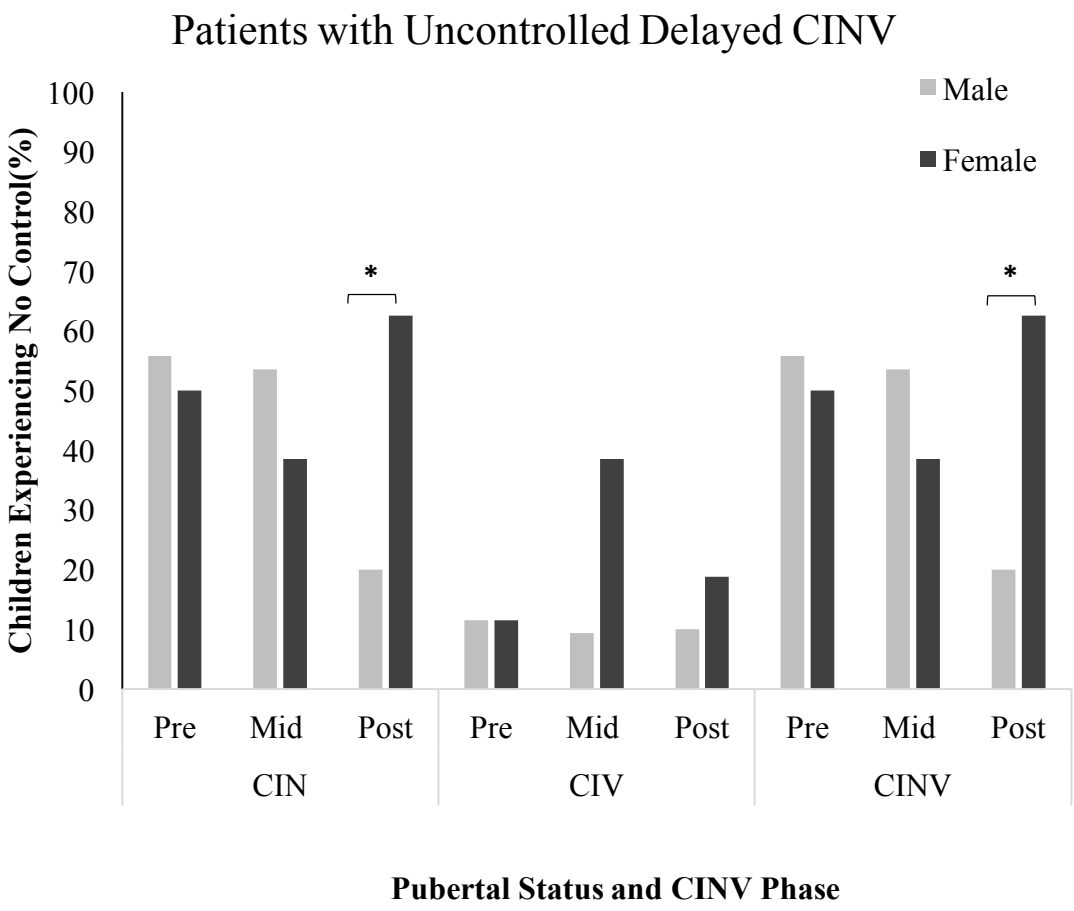

Figure 2.2. Uncontrolled Delayed CINV. Proportions of children experiencing uncontrolled CIN, CIV, and CINV in the delayed phase, grouped by sex and pubertal status. Post pubertal females demonstrated significantly higher proportions of uncontrolled delayed CIN and CINV than post pubertal males,* $p < .05$.

operative nausea and vomiting (PONV), female sex was identified as the strongest patient-related predictor (Fujii, 2009; Gan, 2006). Female sex is also a known risk factor for motion sickness susceptibility (Paillard et al., 2013). Moreover, motion sickness has been shown to fluctuate throughout the menstrual cycle, in accordance with fluctuating estrogen levels (Golding, Kadzere, & Gresty, 2005). Women also report different factors than men which contribute to chronic migraine, one of the more prevalent factors being nausea (Özge et al, 2014).

Motion sickness susceptibility and chronic migraine demonstrate additional age-dependent decreases in the experience of nausea in women but not men. After age 50 – 60 years, motion sickness susceptibility decreases and nausea no longer predicts chronic migraine (Özge et al, 2014; Paillard et al., 2013). Interestingly, this age range corresponds to menopause, a time period associated with fluctuations in hormone concentrations, particularly the gradual decline of estrogens, which may influence nausea responses in women.

Taken together, the lack of a sex difference in CINV in children, the emergence of a sex difference in post-pubertal patients in the present study, a well-established sex difference in the adult population, and finally, the menstrual cycle and menopause related changes in other nausea related phenomena, all point to some gonadal hormone process operating, likely estrogens.

The finding of a post-pubertal sex difference in delayed CINV in the present study provides some evidence that pubertal hormones contribute to the overall sex difference seen in the adult population. Though no post-pubertal sex differences were found in anticipatory CINV in the present study, we observed a signal that there may be an increased chance of anticipatory CINV in post-pubertal females. The lack of significance may be due to the particularly small sample size in this group. Notably, the most commonly diagnosed cancer in children is leukemia, which is most prevalent in children one to four years of age (Greenberg et al., 2015; Canadian Cancer Society, 2016). As such, the sample size for the post-pubertal age group was the smallest, and should be re-examined with a larger sample size in future studies.

Some limitations of the present analysis should be mentioned. This is a post hoc analysis of data, as such these conclusions can only be considered as hypothesis generation. It is also worth discussing the uneven sample sizes in the present study. Due to the large proportion of leukemia patients, which is seen predominantly in young children, the sample sizes in each pubertal group are substantially varied, with the post pubertal groups being the smallest (10 males and 16 females). Further, the original studies were not primarily designed to investigate sex differences within each pubertal group. Therefore, cut-offs for each pubertal group could only be determined retrospectively, using established norms for precocious and late-onset puberty. Future studies should examine this research question with a focus on puberty so that clinician-defined pubertal status determination is part of the study's methodology, as well as with larger and more evenly divided sample sizes. Limitations of the original studies include their observational nature as well as the possibility of selection bias. It is possible that patients who experienced more severe CINV were more likely to participate in the studies. Additionally, the studies are limited by the subjective nature of self-report by patients or their parents. However, the PeNAT has been validated and has shown to be a reliable and accurate indicator of nausea in pediatric patients (Dupuis et al., 2006).

2.4.1 Conclusions

It was hypothesized that post pubertal female patients would show less CINV control than post pubertal males, in the anticipatory, acute, and delayed phases. It was found that post pubertal female patients exhibited significantly less CINV control than post pubertal male patients. This sex difference was particular to the delayed phase, and was not seen in the anticipatory or acute phases. The present findings provide support for a post pubertal CINV sex difference, and provide some indication that gonadal hormones may be the underlying factor for the observed sex difference in CINV in adults. This study also indicates female sex as a possible risk factor for CINV in the post-pubertal pediatric population.

The effects of CINV on individuals experiencing chemotherapy can be debilitating and extremely discouraging. Although there are a number of known risk factors for

developing CINV in the adult population, research on risk factors in children up until this point has been scarce. Developing a comprehensive understanding of the risk factors for CINV in children will provide a stronger foundation for targeted CINV prophylaxis and, ultimately, will alleviate some of the stress and discomfort that accompany chemotherapy.

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Chapter 3

3 The Effect of the Estrous Cycle on the Development of Anticipatory Nausea (AN) in Female Rats

3.1 Introduction

3.1.1 Anticipatory nausea

Chemotherapy treatment largely contributes to survival rates in cancer patients, due to its cytotoxic and antineoplastic effects. Unfortunately, chemotherapy also has several undesirable side effects, including nausea and vomiting. Chemotherapy-induced nausea and vomiting (CINV) are reported as among the most debilitating and distressing treatment-related effects (Aapro, Molassiotis, & Olver, 2005; Molassiotis, 2005). As a result, many patients develop anticipatory CINV whereby they are conditioned to experience nausea and vomiting in anticipation of the chemotherapy administration. The prevalence of anticipatory CINV reported in the literature ranges from 8%–30% of patients (Molassiotis et al., 2016; Morrow et al., 1998). It is posited that anticipatory CINV develops through classical conditioning (Bovbjerg, 2006; Stockhorst, Steingrueber, Enck, & Klosterhalfen, 2006). The chemotherapy acts as the unconditioned stimulus, which elicits nausea and vomiting, the unconditioned response. The physical hospital or room acts as the conditioned stimulus and after repeated pairings, these environmental stimuli alone can elicit nausea and vomiting (Tyc, Mulhern, Barclay, Smith, & Bieberich, 1997). The prevalence and severity of this problem are the basis for the development of an animal model of anticipatory CINV (Limebeer, et al., 2006; 2008; Rodriguez, Lopez, Symonds, & Hall, 2000).

3.1.2 Animal model

A rat model of anticipatory CINV, coined anticipatory nausea (AN) for the purpose of this model, has been well established and is used as a preclinical tool to study possible treatments (Limebeer et al., 2006; 2008). The model uses the toxin lithium chloride

(LiCl), a nausea-inducing drug as the unconditioned stimulus, and a distinctive context as the conditioned stimulus. This paradigm demonstrates rats' ability to associate toxin-induced nausea with the context in which they experienced it. Rats are injected with LiCl and placed in a distinctive context immediately after injection. On the test day, rats are placed in the distinctive context drug free, and observed for the disgust behaviours of gaping, chin rubbing, paw treading, and forelimb flails. Rats are non-emetic species, so gaping has been well established as a reliable and selective indicator of nausea in rats (Parker & Limebeer, 2006). Gaping is seen when rats are exposed to drugs known to have emetic properties, i.e, cause vomiting in species that have this reflex (Parker & Limebeer, 2006; Travers and Norgren, 1983). A number of studies have shown that a distinctive context comes to elicit gaping responses in rats after it is paired with injections of LiCl (Cloutier, Cross-Mellor, Kavaliers, & Ossenkopp, 2011; Limebeer et al., 2006; 2008; Rodriguez et al., 2000). Disgust behaviours are also elicited by aversive tastes such as bitter quinine (Grill & Berridge, 1985; Grill & Norgren, 1978). This well-established animal model is a valuable pre-clinical tool for examining possible risk factors and treatments for AN.

3.1.3Sex differences and estrogens

Female sex is a known risk factor for AN in adult cancer patients undergoing chemotherapy. Women are significantly more likely to develop symptoms and less likely to achieve control (Fetting et al., 1983; Hilarius et al., 2012; LeBaron, Zeltzer, LeBaron, Scott, & Zeltzer, 1988). Women under the age of 50 are at particular risk for developing AN. Animal studies, using the rat model of AN, have demonstrated this sex difference as well. Female rats demonstrated significantly more conditioned gaping behaviour than male rats at three different doses of LiCl (64, 96, or 128 mg/kg) (Cloutier, Kavaliers, & Ossenkopp, 2017).

Although this sex difference is well established, further exploration is necessary to examine the mechanism(s) underlying this difference. It has been suggested that gonadal hormones, specifically estrogens, play a role in this etiology. Estrogens may be implicated in the formation of conditioned disgust through the attenuation of conditioned

learning, or by facilitating nausea itself. Estradiol has been found to produce both conditioned taste avoidance when paired with a novel sucrose taste, as well as conditioned disgust responses to the sucrose taste (Ossenkopp, Rabi, & Eckel, 1996). Estradiol has also been shown to enhance the acquisition of LiCl-induced conditioned taste aversion (CTA) in castrated male rats (Lin, Tsai, Tai, & Yeh, 2015). This may occur because of estradiol's illness-inducing properties, which have been seen in humans (Goodman & Gilman, 1975) as well as animal studies (Ganesan & Simpkins, 1991). Estrogens also have stimulatory effects on the serotonergic system. Acute estradiol administration to ovariectomized rats increases serotonin levels in a number of brain regions, including the hippocampus (Johnson & Crowley, 1983). Serotonin (specifically 5-HT_3) antagonism has well-established anti-emetic effects, thus implicating the serotonergic system in the production of nausea and vomiting (Hesketh, 2000). Therefore, estrogens may increase nausea and vomiting through a downstream serotonergic process.

Interestingly, estrogens—and consequently—serotonin, have stimulatory effects on hippocampal neurogenesis (Mahmoud, Wainwright, & Galea, 2016). There is no shortage of research on the involvement of the hippocampus in learning and memory (Josselyn, Kohler, & Frankland, 2015; Rosenbaum, Winocur, & Moscovitch, 2001; Winocur, Moscovitch, & Sekeres, 2013). While not the sole structure responsible for memory, the hippocampus has strong connections to spatial memory (Moser, Rowland, & Moser, 2015). Circulating estrogen levels are positively correlated with cell proliferation and hippocampal neurogenesis in the female rat. Adult female rats have significantly more newly proliferating cells in the dentate gyrus during proestrus in comparison to diestrus (Pawluski, Brummelte, Barha, Crozier, & Galea, 2009). Recent evidence has suggested that this estrogenic effect on the hippocampus may be mediated by serotonin (Mahmoud, Wainwright, & Galea, 2016). Thus, connecting estrogens to serotonin, which may have some involvement in enhancing both nausea and vomiting, and learning and memory, through different pathways.

There is some evidence that suggests estrogens are involved in learning and memory through a rapid mechanism that is controlled by intracellular signaling (Frick et al.,

2004). These effects have been seen in rodents specifically when estradiol is administered shortly after the acquisition phase of a learning task, implicating the early phase of memory formation. Additionally, these results have been extended to human research; estradiol levels are positively correlated to working memory performance in women of reproductive age (Hampson & Morley, 2013).

Studies have also shown that estradiol improves memory by potentiating glutamatergic and cholinergic activity. Administration of both 17 β-estradiol and estrone into the hippocampus of female ovariectomized mice improved retention of T-maze footshock avoidance (Farr, Banks & Morley, 2000). As well, normally subthreshold levels of estradiol become effective at improving memory retention when co-administered with either cholinergic or glutamatergic agnoists. (Farr, Banks, & Morley, 2000). Estradiol treatment in ovariectomized female rats also leads to increased hippocampal dendritic spine densities, further supporting the link between estrogens and memory (Woolley & McEwen, 1993).

3.1.4 Estrous cycle

The reproductive cycle of female rats is known as the estrous cycle. The typical rat estrous cycle lasts four days and consists of estrus, metestrus, diestrus, and proestrus (Long & Evans, 1922). Estrogen reaches peak levels at proestrus, while diestrus consists of baseline estrogen levels. The estrous cycle can be tracked by collecting samples of vaginal secretion and examining cell type and quantity in these samples. Thus, the effects of estrogens on various behaviours can be determined by tracking the rats' estrous cycle, and quantifying behaviours on specific days of the cycle. Rats in the current study were conditioned and tested during proestrus, a period of high estrogen circulation, or diestrus, a period of low estrogen circulation.

3.1.5 Current study

While sex differences in AN and conditioned disgust have been established in the human as well as animal population, further research is required to determine how estrogens modulate this phenomenon. The current study investigated possible explanations as to

why females are more prone to AN than males. Understanding the mechanisms underlying the sex difference in AN may provide differential prophylactic treatment strategies to the male and female populations undergoing chemotherapy.

The current study examined the effect of the estrous cycle on the formation of AN in a rat conditioned disgust model, using an intermediate dose (96 mg/kg) of the nausea-inducing toxin, LiCl. Based on reported effects of estrogens in the literature, it was hypothesized that rats in proestrus (i.e. high estrogen levels) would demonstrate stronger conditioned nausea responses than rats in diestrus (i.e. low estrogen levels).

3.2 Method

3.2.1 Animals

Subjects were 31 naïve female Long-Evans rats (Charles River, Quebec, Canada) weighing between 125-150g at the start of the experiment. Rats were pair-housed in standard polypropylene cages (45 cm x 22 cm x 20 cm) in a temperature-controlled colony room (20 ± 1°C) maintained on a 12:12-h light-dark cycle with lights on from 07:00 to 19:00 h. All testing took place during the light portion of the light-dark cycle. Rats had *ad libitum* access to ProLab Rat Chow RMH 3000 and water, except while in the testing apparatus. All procedures used were carried out in compliance with the Canadian Council of Animal Care and approved by the Institutional Animal Care Committee of Western University.

3.2.2 Drugs

Rats (n=15-16/group) were injected intraperitoneally with 96 mg/kg 0.15M LiCl, or 10 ml/kg 0.9% NaCl. All conditioning and testing took place immediately after drug administration. Doses were based on those employed in previous studies examining the effect of the drug (Cloutier et al., 2017; Ossenkopp, Biagi, Cloutier, Kavaliers, & Cross-Mellor, 2011).

3.2.3Materials and apparatus

The context-conditioning apparatus was an opaque Plexiglas chamber (22.5 cm x 26 cm x 20 cm) with a gray lid that rested on a transparent glass plate. Below the chamber, a mirror was mounted at a 45° angle to allow for viewing of the ventral surface of the rats. Rats were tested in a darkened room with two 40W red lights placed below the glass plate (see Figure 3.1). This lighting set up was to ensure testing environment was distinctive. Behavioral responses during the conditioning days and test day were videotaped with a video camera (Sony DCR-DVD201; London, Ontario) located 1 m from the mirror.

3.2.4Estrous cycle determination

Reference images for determination of estrous cycle phase can be seen in Figure 3.2. Rats were acclimatized to their home cages for one week and then handled on three separate occasions. Following handling, the estrous cycle was tracked daily at the same time each day for approximately 14 days. The estrous cycles were tracked by the vaginal smear technique. This involved sampling the cells of the vaginal canal with sterile saline using a pipette. The recovered solution (approximately 20 μl) containing cells was then placed on microscope slides for later determination of estrous cycle phase. This was done using a phase-contrast microscope with 10X magnification. Proestrus was determined by scattered distribution of predominantly cornified cells and the presence of nucleated cells. Diestrus was identified by the reduction in the number of cells and the presence of leukocytes (Marcondes, Bianchi, & Tanno, 2002). Vaginal smears were obtained on a daily basis for a period of three full estrus cycles prior to testing, after which rats were divided in proestrus or diestrus groups. All rats used in this study exhibited a regular 4-day cycle.

3.2.5Experimental procedure

The experimental procedure is summarized in Figure 3.3. The experiment consisted of two phases: a conditioning phase consisting of four days spaced 96 hours apart, and a drug-free test day, 96 hours following the final conditioning day. There were four

Figure 3.1. Distinctive Context. On conditioning days, rats are injected with either 96 mg/kg LiCl or 0.9% NaCl and immediately placed in this distinctive context for 30 minutes. On test day, rats are placed in this distinctive context drug-free.

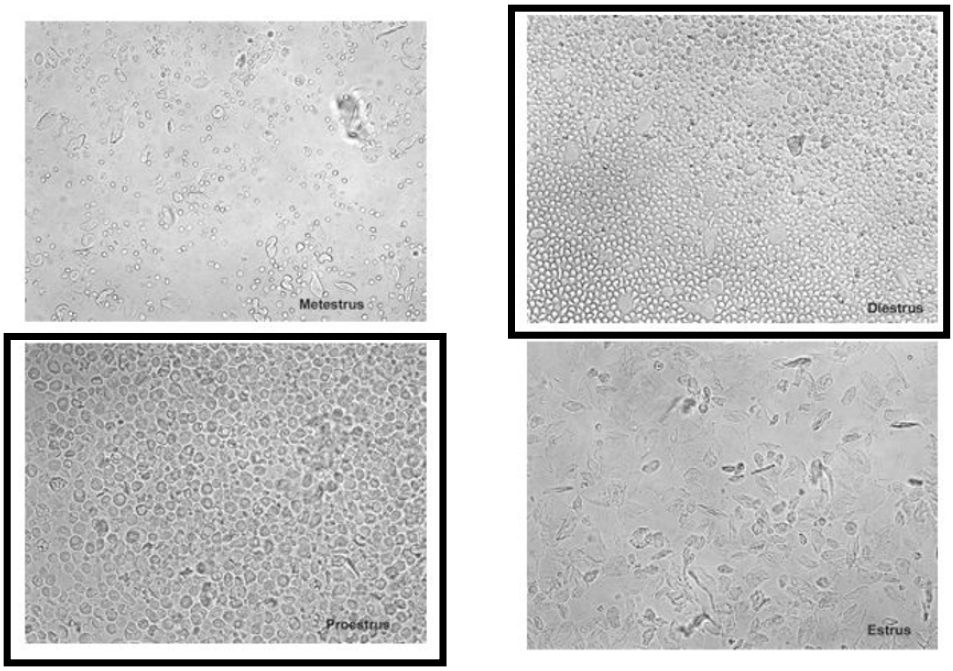

Figure 3.2. Estrous Cycle Phases. Sample slides for each estrous cycle phase. Metestrus consists of approximately equal proportions of cell types, many leukocytes, some cornified cells, and some nucleated cells. Diestrus consists of many small leukocytes, which often branch near edges of slides. Proestrus consists of large clumps of round, nucleated cells. Estrus consists of sheets of cornified cells and few to no nuclei. Phases of interest for this study (Proestrus and Diestrus) are shown in boxes.

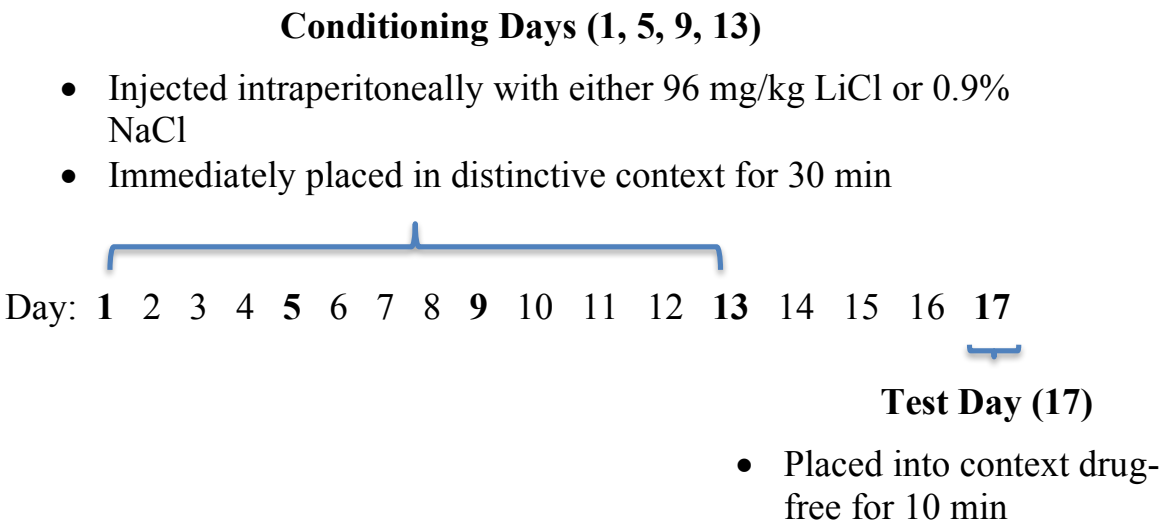

Figure 3.3. Experimental Procedure. On each conditioning day, rats are injected with 96 mg/kg LiCl or 0.9% NaCl and immediately placed in the distinctive context. Conditioning days are spaced 96 hours apart to ensure rats are consistently conditioned during the phase of each rats' respective group (proestrus or diestrus). Test day occurs 96 hours following the final conditioning day, where rats are placed into the distinctive context drug-free.

experimental groups (*n*= 7-8/group). Half of the rats were conditioned and tested during diestrus, and the other half during proestrus. Of those two groups, half were given intraperitoneal injections of 96 mg/kg 0.15 M LiCl, and half were given control injections of 0.9% 10 ml/kg NaCl. As shown in Figure 3.4, the four experimental groups were: Proestrus+LiCl, Proestrus+NaCl, Diestrus+LiCl, and Diestrus+NaCl.

3.2.4.1 Conditioning phase

On each of the four conditioning days (spaced 96 h apart), animals were injected intraperitoneally with NaCl (0.9%, 10 ml/kg), or LiCl (0.15 M; 96 mg/kg) depending on experimental group. Immediately following drug administration, each animal was placed in the distinctive context for 30 minutes. Behaviors were recorded for later scoring using the Observer (Noldus Information Technology, Sterling, VA) Event-Recording Program. Dependent behavioural variables analyzed consisted of gaping frequency and other aversive responses (forelimb flails, paw treads, and chin rubs). Following each exposure to the distinctive context, a vaginal smear was obtained from each animal to confirm stage of estrous cycle, and the rat was then returned to its home cage.

3.2.4.2 Drug-free test day

Ninety-six hours following the final conditioning day, each rat was re-exposed to the distinctive context for 10 min on a drug-free test day. Behaviors were video recorded for later scoring using the Observer (Noldus Information Technology, Sterling, VA) Event-Recording Program. Dependent behavioural variables analyzed consisted of gaping frequency and the other aversive responses (forelimb flails, paw treads, and chin rubs). Immediately following 10-minute exposure to distinctive context, a vaginal smear was again obtained to confirm stage of estrous cycle.

3.2.5Data analysis

On the four 30 minute conditioning days and on the 10 minute drug-free Test Day, dependent variables included conditioned gaping behaviour, and other aversion-related

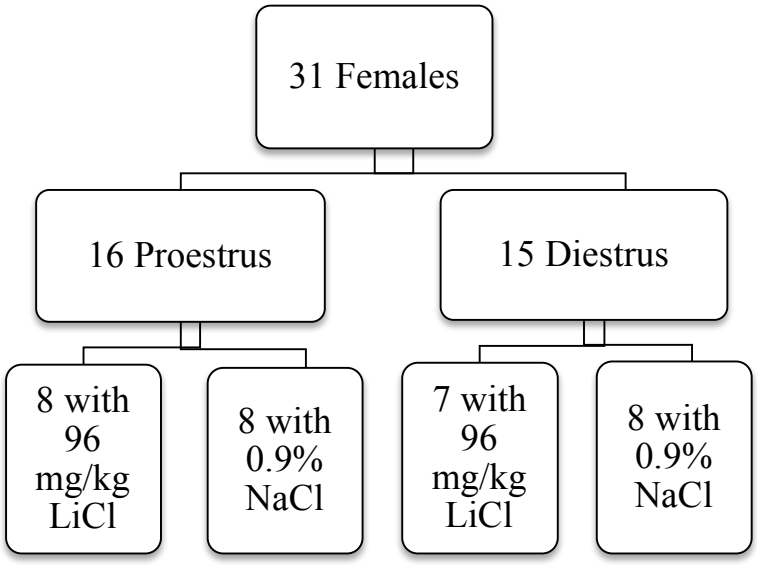

Figure 3.4. Group Designation.

behaviours, such as, forelimb flails, paw treading, and chin rubs, and finally, a composite score of these aversion-related behaviours (excluding gaping) (Ossenkopp & Mazmanian, 1985). Gaping was defined as lowering of the jawbone, opening of the mouth, and the protrusion of the lower teeth (Limebeer et al., 2006; 2008).

Data from conditioning days and test day were analyzed separately. Conditioned gaping responses and aversion-related behaviours (excluding gaping) on the conditioning days were analyzed using separate 2 x 2 x 4 mixed design analysis of variances (ANOVA) with two between-subject factors of Drug (at two levels: 96 mg/kg LiCl and 0.9% NaCl) and Estrous Cycle Phase (at two levels: proestrus and diestrus), and one within-subject factor of Conditioning Day (at four levels: conditioning days 1 to 4). Conditioned gaping responses and aversion-related behaviours (excluding gaping) from the drug-free test day were analyzed using separate, 2 x 2 ANOVAs. The between-subjects factors were Drug (at two levels: 96 mg/kg LiCl and 0.9% NaCl) and Estrous Cycle Phase (at two levels: proestrus and diestrus). As this study was exploratory, Fisher's least significant difference (LSD) test was used for post hoc pairwise comparisons. All statistical tests used α=0.05 as a significance criterion. Statistical analyses were performed using IBM SPSS Statistics 23 for Windows.

3.3 Results

3.3.1 Conditioning days

The frequency of gaping and aversion-related behaviour during the four conditioning days were analyzed in order to evaluate the differences in acquisition rate of conditioned disgust responses among the experimental groups.

3.3.1.1 Conditioned gaping behaviour

The 2 x 2 x 4 mixed design ANOVA revealed a significant effect of Conditioning Day (1 to 4), $F(3, 81) = 5.85$, $p < .01$, and a significant main effect of Drug (NaCl or LiCl), $F(1, 27) = 21.89$, $p < .001$. A significant Conditioning Day x Drug interaction was also found, $F(3, 81) = 5.85$, $p < .01$. As shown in Figure 3.5, both LiCl groups showed significantly

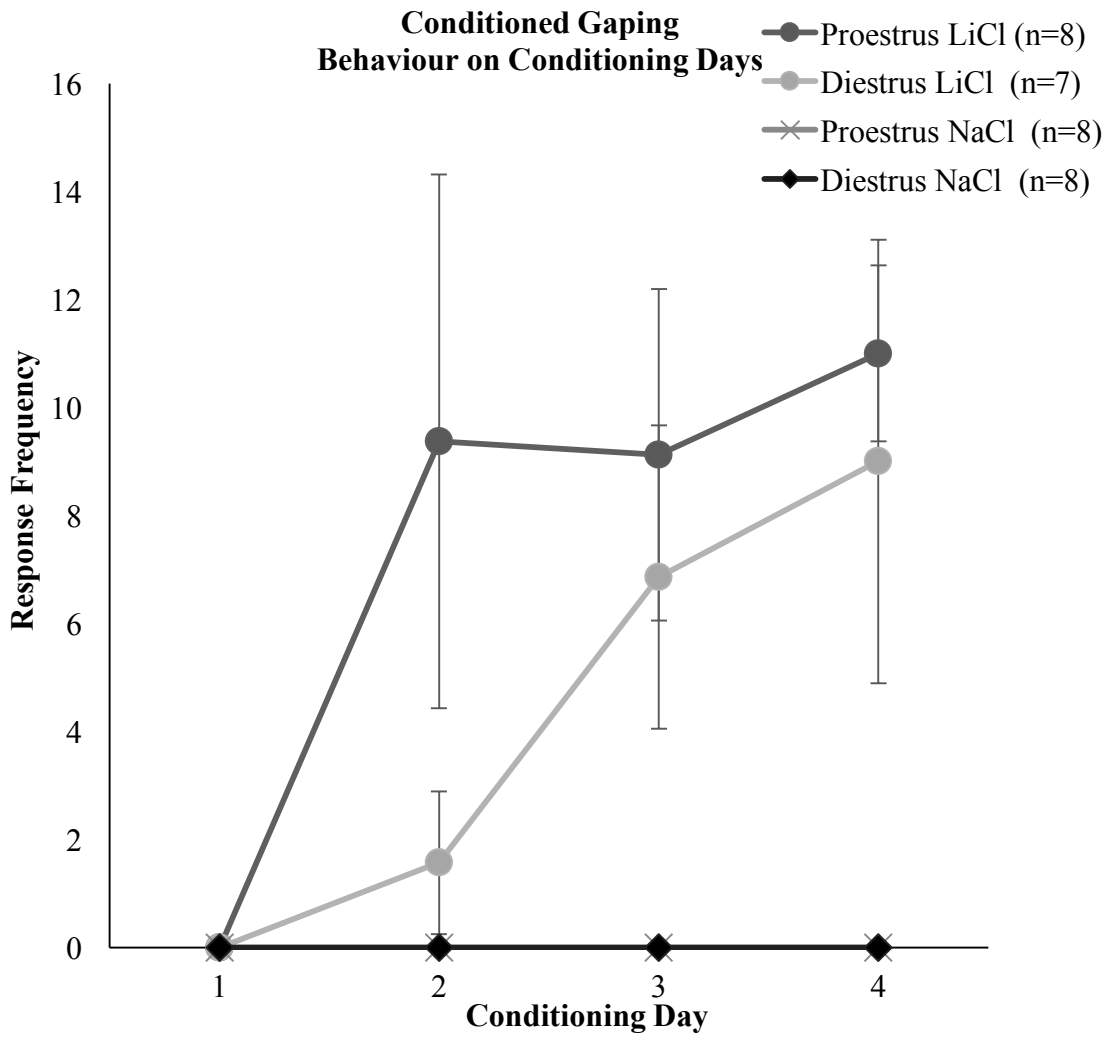

Figure 3.5. Conditioned Gaping Behaviour on Conditioning Days. Mean (± sem) frequency of conditioned gaping responses expressed by the 4 groups (Proestrus+LiCl, Diestrus+LiCl, Proestrus+NaCl, & Diestrus+NaCl), during the 30-minute conditioning in the distinctive context across four conditioning days (96h apart). Both LiCl groups showed significantly more gaping behaviour than both NaCl groups ($p < .001$). Both Proestrus+LiCl and Diestrus+LiCl showed increased gaping behaviour across conditioning days ($p < .01$), while Proestrus+NaCl and Diestrus+NaCl did not.

higher gaping responses than NaCl groups. Furthermore, there was a monotonic increase in gaping frequency for both Proestrus+LiCl and Diestrus+LiCl groups over the four conditioning days.

3.3.1.2 Conditioned aversion related behaviour

Conditioned aversion-related behaviours not including gaping (forelimb flails, paw treads, and chin rubs) were analyzed as a composite score. The 2 x 2 x 4 mixed design ANOVA revealed a significant main effect of Drug, $F(1, 27) = 5.88$, $p < .05$. As shown in Figure 3.6, both LiCl groups showed significantly more conditioned aversion-related behaviours than NaCl groups. No other significant main effects or interactions were found.

3.3.2 Drug-free test day

3.3.2.1 Conditioned gaping behaviour

The 2 x 2 between-subjects ANOVA revealed a significant main effect of Drug, $F(1,27) = 23.48$, $p < .001$. A significant main effect of Phase, $F(1,27) = 11.50$, $p < .01$, as well as a significant Drug x Phase interaction, $F(1,27) = 11.50$, $p < .01$ were found. As shown in Figure 3.7, post-hoc pair-wise comparisons (Fisher's LSD) for phase differences revealed that rats in Group Proestrus+LiCl displayed significantly higher frequencies of conditioned gaping responses, relative to Diestrus+LiCl, Proestrus+NaCl, and Diestrus+NaCl groups ($p < .001$).

3.3.2.2 Conditioned aversion related behaviour

Conditioned aversion-related behaviours not including gaping (forelimb flails, paw treads, and chin rubs) were analyzed as a composite score. The 2 x 2 between-subjects ANOVA revealed a significant main effect of Drug, $F(1, 27) = 19.82$, $p < .001$, and significant main effect of Phase $F(1, 27) = 4.50$, $p < .05$. No significant interaction was found. As demonstrated in Figure 3.8, post hoc analyses (Fisher's LSD) for phase differences revealed that rats in Group Proestrus+LiCl displayed significantly higher

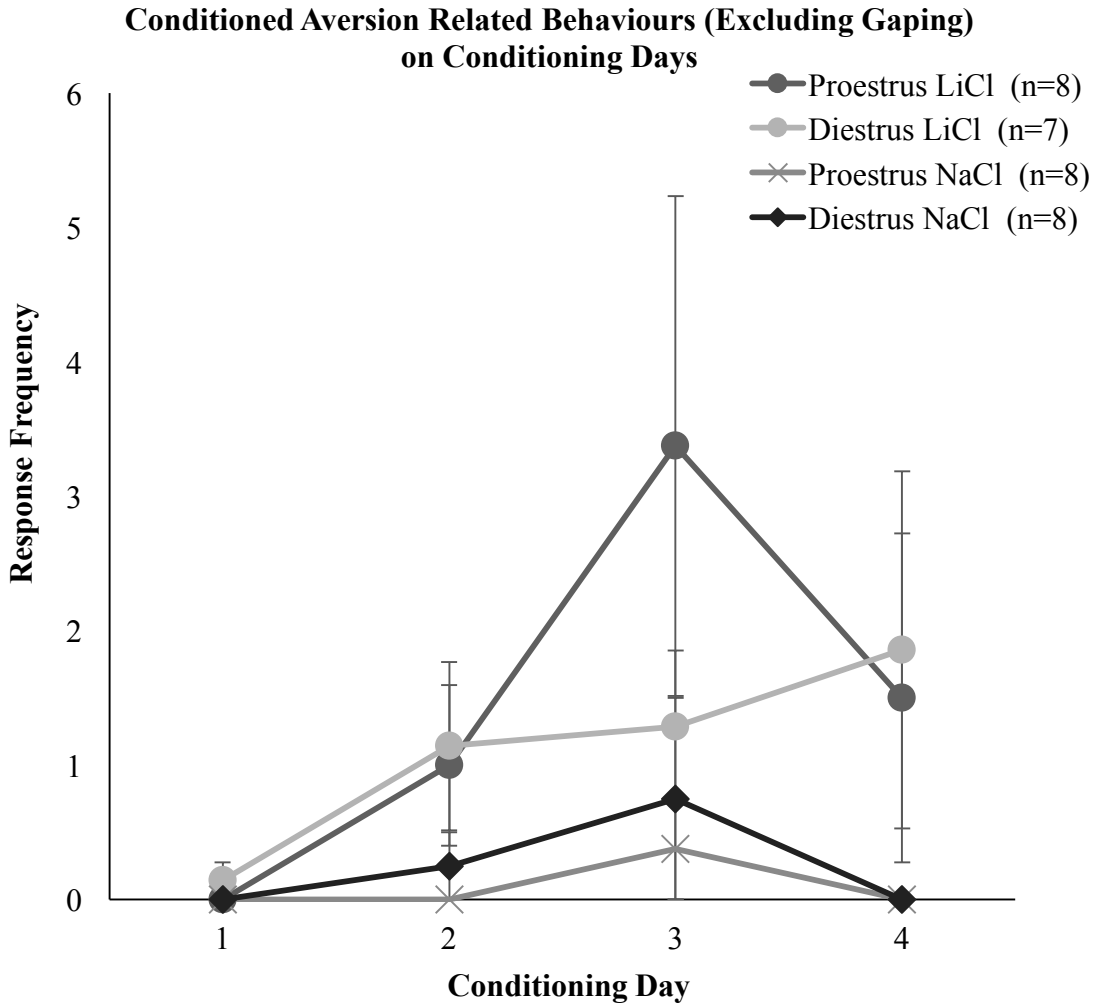

Figure 3.6. Conditioned Aversion Related Behaviours on Conditioning Days. Mean (± sem) frequency of conditioned aversion related behaviours expressed by the 4 groups (Proestrus+LiCl, Diestrus+LiCl, Proestrus+NaCl, & Diestrus+NaCl), during the 30-minute conditioning in the distinctive context across four conditioning days (96h apart). Both LiCl groups demonstrated significantly more aversion related behaviours than both NaCl groups ($p < .05$). No significant conditioning day effects were seen.

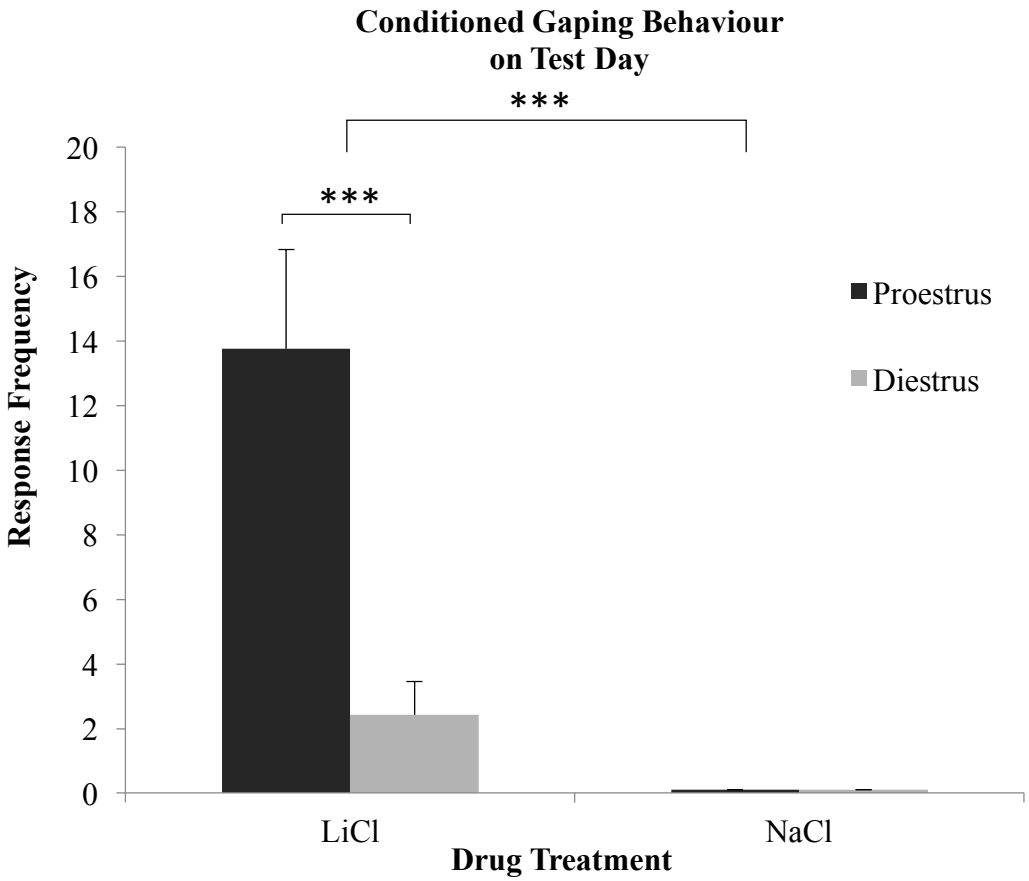

Figure 3.7. Conditioned Gaping Behaviour on Test Day. Group mean (+ sem) frequency of total gaping responses expressed by groups LiCl+Proestrus, LiCl+Diestrus, NaCl+Proestrus, and NaCl+Diestrus during the 10 min test in the distinctive context in the absence of drug treatment (*n*= 7-8/experimental group). LiCl treated animals showed significantly more gaping than NaCl treated animals ***($p < .001$), and LiCl+Proestrus showed significantly more gaping than LiCl+Diestrus ***($p < .001$).

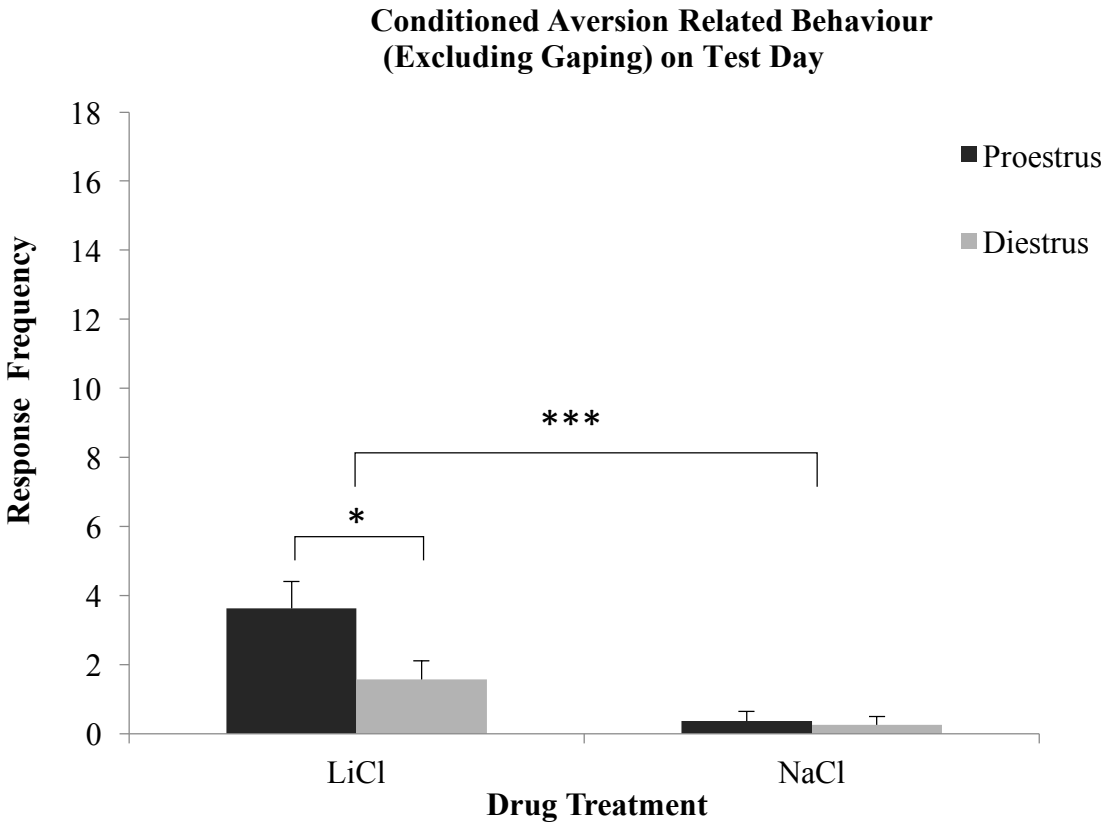

Figure 3.8. Conditioned Aversion Related Behaviour on Test Day. Group mean (+ sem) frequency of total aversion related responses (excluding gaping) expressed by groups LiCl+Proestrus, LiCl+Diestrus, NaCl+Proestrus, and NaCl+Diestrus during the 10 min test in the distinctive context in the absence of drug treatment (n= 7-8/ experimental group). LiCl treated animals showed significantly more aversion related behaviour than NaCl treated animals ***($p < .001$), and LiCl+Proestrus showed significantly more aversion related behaviour than LiCl+Diestrus *($p < .05$).

frequencies of conditioned aversion-related behaviours than Diestrus+LiCl ($p < .05$), Proestrus+NaCl ($p < .001$), and Diestrus+NaCl groups ($p < .001$).

3.3.3Estrous cycle determination

Following coding of the lavage samples under 10x magnification under the microscope, it was confirmed that each rat assigned to the diestrus or proestrus group was in fact conditioned and tested during diestrus or proestrus, respectively. No disruptions to estrous cycle phases were observed in the rats following drug treatment.

3.4 Discussion

The current study investigated the relationship of the estrous cycle with the formation of conditioned disgust behaviours in the rodent model of AN, using 96 mg/kg of nausea-inducing LiCl and a saline control. It was hypothesized that proestrus rats would show greater levels of conditioned disgust responses relative to diestrus rats and this hypothesis was supported. Results showed that on the drug free test day, rats conditioned and tested during proestrus demonstrated greater levels of conditioned gaping and other aversion-related behaviours (i.e., forelimb flails paw treads, and chin rubs) relative to rats conditioned and tested during diestrus. This finding is consistent with previously observed effects of estrogens on other conditioning paradigms (Farr, Banks, & Morley, 2000; Lin et al., 2015; Ossenkopp et al., 1996).

Although increased levels of estrogens were associated with increased conditioned disgust responses on test day, no such effects were seen on the conditioning days. That is, proestrus and diestrus rats learned the conditioned disgust behaviours at the same rate. During the conditioning trials, rats were injected with LiCl and immediately placed into the distinctive context. This allowed the association between feelings of nausea and the distinctive context to form, which then manifested as conditioned gaping. When the rats were placed into the distinctive context drug-free on test day, the presence of conditioned gaping behaviour indexed this learned association. In the current study, estrous cycle phase effects were only seen on drug-free test day. This suggests that estrogen enhances the memory component of the AN model, rather than augmenting learning during

conditioning trials. This inference is supported by numerous studies that have demonstrated estradiol's beneficial effects on hippocampal-dependent spatial memory (Al Abed et al., 2016; Barker & Galea, 2010; Frye, Duffy, & Walf, 2007; Sandstrom & Williams, 2004; Tuscher, Fortress, Kim & Frick, 2015). Estradiol likely exerts its effect on memory by increasing dendritic spine density on CA1 pyramidal neurons, as well as increasing proliferation of new neurons in the hippocampus. These effects of estradiol on the hippocampus were first discovered by Woolley and McEwen (1993) and have since been replicated by numerous labs (Barha, Dalton & Galea, 2010; Frankfurt & Luine, 2015: Frick et al., 2004). 17β-estradiol specifically dose-dependently upregulates cell proliferation in the dentate gyrus of adult female rats (Barha et al., 2009). Both ER-α and ER- β have been shown to be involved in estradiol-increased cell proliferation in the dentate gyrus of adult female rats (Mazzucco et al., 2006). With regards to non-spatial working memory, the effects of estradiol are more complex. Studies have shown that injections of low-dose estradiol facilitates non-spatial working memory, while high-dose estradiol impairs it (Barha et al., 2010; Wide, Hanratty, Ting, & Galea, 2004). The effects of estrogens on the brain are both region and memory task specific. Estrogens seem to facilitate hippocampal-dependent learning, but attenuate striatal-dependent learning (Davis, Jacobson, Aliakbari, & Mizumori, 2005). This is reiterated in the study by Fader, Johnson, and Dohanich (199), that demonstrated that estrogen improves spatial working memory, but not reference memory. Thus, though the enhancing effects of estrogen on hippocampal-dependent memory specifically are well documented (Joesselyn et al., 2015; Rosenbaum et al., 2001; Winocur et al., 2013), estrogen's effects on other types of memory are less clear.

The present study demonstrated a positive relationship between estrogen levels and presumed memory for learned disgust, but perhaps this result would be better explained by the increase in nausea/disgust itself during conditioned trials. As demonstrated in Figure 3.5, Proestrus+LiCl rats do have a higher gaping response frequency than Diestrus+LiCl rats, albeit this was not significant. It is possible that with a larger sample size and thus more power, a significant effect would emerge. In this case, estrogens would be implicated not only in the memory of the association between the context and disgust, but also in the acquisition of this association. This explanation is plausible as

there is substantial research connecting estrogens to nausea and malaise in general, such as estradiol-related increases in nausea during pregnancy (with contributions from progesterone as well), or in women taking oral contraceptives (Ganesan, 1994; Lagiou et al., 2003; Matchock, Levine, Gianaros, & Stern, 2008). Since estrogens have been linked to nausea in other studies, it is possible that in the current study, the increased levels of estrogen in proestrus rats is causing greater disgust-related behaviours during conditioning days. Future studies should examine this possibility by using larger sample sizes.

Past studies measuring exact hormone levels in rats have shown that during proestrus, estradiol levels in serum are approximately 90 pg/mL, while during diestrus estradiol levels are about 30 pg/mL (Faccio et al., 2013). This represents a marked difference in estradiol levels between the two phases. Progesterone levels in serum however, average at about 35 ng/mL during proestrus, and 20 ng/mL during diestrus (Faccio et al., 2013). Although the present study did not measure hormone levels in the rats, this evidence suggests that the change in behaviour seen in proestrus rats is likely due to the large difference in estradiol levels compared to diestrus rats. The difference in progesterone levels from proestrus to diestrus is not as pronounced, and likely not causing the increased AN behaviours seen in proestrus rats.

Tracking the estrous cycle is a clever way of using female rats' natural ebb and flow of hormones to examine the putative effects of hormones on certain behaviours. This method has the advantage of being relatively non-invasive and doesn't put the animal through the stress of a surgery. The caveat is, however, that the phases of the estrous cycle are a relative measure of estrogen and progesterone, and do not provide an exact measure of the hormones. Thus, although it is likely estrogens causing the increased AN, the present study does not definitively prove this. The current methodology was used due to the exploratory nature of the study. Now that additional support has been provided for estrogen's role in AN, a follow up study should explore this further by means of ovariectomy and estrogen and/or progesterone replacement.

AN is not the only syndrome in which women exhibit higher incidences of nausea than

men. In a study identifying risk factors for post-operative nausea and vomiting (PONV), female sex was identified as the strongest patient-related predictor (Fujii, 2009; Gan, 2006). Female sex is also a known risk factor for motion sickness susceptibility (Paillard et al., 2013), which is highly relevant to the current study given that provocative vestibular stimulation has been used as the unconditioned stimulus in similar conditioned disgust paradigms (Cordick, Parker, & Ossenkopp, 1999; Limebeer et al., 2008; Ossenkopp et al., 2003). Moreover, motion sickness has been shown to fluctuate throughout the menstrual cycle, in accordance with fluctuating estrogen levels (Golding, Kadzere, & Gresty, 2005).

Most studies employing animal models of AN have used male rats, with some recent studies examining putative sex differences (Cloutier et al., 2017). The existence of sex differences in multiple types of nausea highlights the importance of the use of female rats, and taking the estrous cycle into account, in the animal model for AN. In addition to context, taste cue is another stimulus that can elicit a conditioned disgust response when paired with a toxin. Taste aversion/avoidance paradigms, where rats are injected with a salient taste cue combined with a LiCl to induce nausea, produce a similar conditioned disgust effect to context-conditioning (Parker et al., 2006; Cloutier, Cross-Mellor, Kavaliers, & Ossenkopp, 2011). The effect of the estrous cycle has yet to be explored in taste aversion/avoidance paradigms. Future studies should examine a putative role of estrogen in the development of conditioned taste aversion/avoidance.

Developing a comprehensive understanding of AN and its relation to the estrous cycle will provide a strong foundation for which treatment and prevention efforts can be directed. At present, the best-known strategy to defend against AN is to prevent it from developing in the first place, by controlling acute and delayed CINV immediately using 5-hydroxytryptamine3 (5-HT_3) and neurokinin1 receptor (NK1) antagonists. These medications are well-documented to alleviate vomiting, but their effects on nausea are more complicated (Miner & Sanger, 1986; Costall, Domeney, Naylor, & Tattersall, 1986; Sanger & Andrews, 2006). And further, once it does develop, AN is refractory to anti-emetic medication for the most part. This study lends support to the hypothesis that estrogen augments the learning of this conditioned nausea. If this idea is further

supported, it is possible estrogen antagonism could become a new avenue to explore in terms of pharmacological treatment options. Additionally, women may be able to prevent the severity of AN by adjusting their chemotherapy schedule around their menstrual cycle, such that the treatments never coincide with the days where estrogen levels are the highest. Due to the clinical relevance of AN, further human studies are necessary in order to explore this phenomenon.

3.4.1 Conclusions

It was hypothesized that proestrus rats would condition higher levels of disgust responses relative to diestrus rats in the rodent model of AN. It was found that proestrus rats exhibited significantly stronger conditioned disgust responding via gaping and other aversion-related behaviours (i.e., chin rubs, paw treads, and forelimb flails), when re-exposed to a distinctive context previously paired with the toxin, LiCl. This estrous cycle phase effect was not seen during conditioning days, which suggests estrogens may be impacting memory for the conditioned association, rather than the learning process itself. The present findings provide support that estrogens may contribute to the higher incidence of AN in females, a finding which should be further examined.

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Chapter 4

4 General Discussion

Cancer patients list chemotherapy-induced nausea and vomiting (CINV) as one of the most distressing and bothersome events that negatively affects quality of life. While both sexes do experience this distressing side-effect, evidence suggests that there is a sex difference in the prevalence and severity of CINV, where females have been shown to be more susceptible relative to males (Molassiotis, Stamataki, & Kontopantelis, 2013; Sekine, Segawa, Kubota, & Saeki, 2013; Warr, 2014;). This sex difference is seen in all phases of CINV, which includes acute, delayed, and anticipatory. In the animal model of anticipatory nausea (AN), which was developed to study the anticipatory phase of CINV, a sex difference in favour of females has also been seen (Cloutier, Kavaliers, & Ossenkopp, 2017). Although this sex difference is well established, the reason behind the increase in CINV seen in females remains unknown. The current thesis explored the mechanism behind the known sex difference in CINV using two diverse approaches.

In Chapter 2, sex differences in the development of anticipatory, acute, and delayed CINV in pediatric oncology patients were examined. Patients were split into three groups based on pubertal status: pre-, mid-, and post-pubertal. It was hypothesized that sex differences in the development of CINV would only be seen in the post-pubertal age group, due to the higher levels of estrogen in this group of females. This post-pubertal sex difference was in fact observed in delayed CINV. Notably, in both the pre-, and mid-pubertal patients, no sex differences were seen in acute or delayed CINV control. This agrees with multiple studies which found that female sex is not a significant predictor of CINV in pediatric oncology patients (Aseeri, Mukhtar, Khansa, Elimam, Jastaniah, 2013; Dupuis et al., 2017; Holdsworth, Raisch & Frost, 2006; Vol et al., 2016). As previously established, female sex is a risk factor for the development of CINV in the adult oncology population. The present study demonstrated that the observed sex difference in CINV may in fact emerge around adolescence, which indicates the involvement of sex steroids. In terms of anticipatory CINV, no significant sex differences were seen in the pre-, mid-, or post-pubertal groups. However, although this difference was not significant, post-

pubertal females did show substantially higher proportions of anticipatory CINV than males. Since uncontrolled acute or delayed CINV is a risk factor for the development of anticipatory CINV (Lohr, 2008), and post-pubertal females did demonstrate significantly less delayed CINV control than males, it is possible that this post-pubertal sex difference in anticipatory CINV would prove to be significant with a larger sample size.

In order to further investigate estrogen's effects, in Chapter 3, the rodent model of anticipatory nausea (AN) was employed to test whether the estrous cycle influences the ability of female rats to form associations between a distinct context and feelings of disgust (i.e., AN). The estrous cycle is the reproductive cycle of the female rat, and consists of a cyclical rise and fall of estrogen and progesterone. The effects of estrogens on various behaviours can be determined by tracking the rats' estrous cycle, and quantifying behaviours on specific days of the cycle. As predicted, on the drug free test day, rats conditioned and tested during proestrus (high estrogen levels) demonstrated greater levels of aversion-related disgust behaviour relative to rats conditioned and tested during diestrus (low estrogen levels). And further, this effect was not seen on conditioning days, suggesting estrogen is acting on the memory component of conditioned disgust, rather than on the learning of the toxin-induced nausea itself. To the best of our knowledge, this was the first study to demonstrate a significant estrous cycle difference in conditioned disgust using the rodent model of AN. The results of this study strengthen the notion that estrogens are involved in nausea in general, and also suggest estrogens plays a role in CINV. This implies that females' greater likelihood to experience CINV may be in part due to their higher levels of circulating estrogens, in comparison to males.

Taken together with the findings of Chapter 2, a strong indication for the involvement of gonadal hormones has been established. The results of this thesis imply that estrogens affect the development of CINV in the delayed phase, through increasing nausea itself, and in the anticipatory phase, through increasing both nausea, and memory of the conditioned nausea.

One caveat of this thesis is that it does not definitively prove that estrogen is in fact the gonadal hormone contributing to the effects shown. It may be possible that another prominent female hormone, progesterone, is having an effect. While this possibility cannot be eliminated without conducting a study involving direct hormone manipulation, a number of findings in the literature suggest that progesterone has either minimal or unpredictable effects on learning and memory or nausea. In a study examining the effects of estradiol and progesterone on the acquisition of conditioned taste aversion (CTA), it was found that estradiol, but not progesterone affected the acquisition of CTA learning. In fact, some studies have shown that progesterone actually impairs performance of spatial and working memory, and avoidance tasks in female rats (Braden et al., 2015; Fry & Sturgis, 1995; Johansson et al., 2002). There is also evidence that women taking progesterone as part of hormone therapy for menopause have an increased risk of dementia as well as other cognitive impairments (Coker et al., 2010; Shumaker et al., 2003). With regards to nausea, the research on progesterone's connection has yielded mixed results. In some studies, progesterone has been shown to have minimal connections with the its development. In a study assessing pregnancy hormone levels in relation to nausea, it was found that estradiol was positively associated with nausea with or without vomiting, while no evidence was found for progesterone's connection to nausea during pregnancy (Lagiou et al., 2003). Conversely, there has been some research which has shown that the elevation of the combination of progesterone and estrogen are associated with the gastric dysrhythmias that correlate to pregnancy-related nausea (Walsh, Hasler, Nugent, & Owyang, 1996). In a literature review on Hyperemesis Gravidarum (HG), a condition which causes severe nausea and vomiting in early pregnancy, it was concluded that progesterone's connection to this condition is weak to inconclusive, while estrogens are likely causally related to HG (Verberg, Gillott, Al-Fardan, & Grudzinskas, 2005).

These studies strongly suggest that elevated progesterone is not the reason for the increase in CINV in females in comparison to males. While estrogen's involvement cannot be definitively proven without performing hormone manipulation experiments, much of the literature points to it being a key contributing factor. Estrogen can increase the number of dopamine receptors in the brain, while certain antiemetic drugs such as

droperidol are designed to inhibit these dopamine receptors (Hruska & Sibergeld, 1980; Tornetta, 1977). This suggests that estrogen may influence nausea through a dopamine-mediated pathway. It has also been postulated that estrogen sensitizes vomiting centers such as the area postrema and triggers nausea. The area postrema is a circumventricular medullary structure implicated in the detection of toxins, such as LiCl, and acts as a vomit-inducing center. Following exogenous administration of estradiol benzoate, markers for neuronal activity are activated in the area postrema. In addition, area postrema lesions eliminate the hypophagia expressed by male rats exposed to chronic estradiol treatment (Bernstein, Courtney, & Braget, 1986). An intact area postrema is critical for taste avoidance and aversion learning with LiCl (Eckel and Ossenkopp, 1996; Ossenkopp & Eckel, 1994;1995). Estrogen can also act as the unconditioned stimulus in a CTA learning paradigm (Hintiryan, Foster, Chambers, 2009; Ossenkopp et al., 1996), and it can enhance LiCl-induced CTA (Ganesan & Simpkins, 1991).

While further research is required to determine estrogen's specific involvement in CINV, there is no shortage of research on the connection between estrogens and nausea and vomiting in general. The results of the present thesis, along with the number of studies that have drawn connections between estrogens and nausea, point to estrogens being a key contributor to the sex difference seen in CINV in cancer patients.

4.1 Conclusions

In the present thesis, the possible mechanism behind the sex difference seen in CINV was explored. This was done using two diverse approaches: a post hoc data analysis of CINV in a pediatric oncology patient population, and a rodent model of AN to examine the influence of the estrous cycle on the establishment of context-based disgust conditioning.

The human post hoc analysis revealed female pediatric patients demonstrate less delayed CINV control than male patients, specifically in the post-pubertal age group. Both pre- and mid-pubertal patients did not show a sex difference in either anticipatory, acute, or delayed CINV. The rodent study showed that rats conditioned and tested during a period of high estrogen circulation (i.e., proestrus) demonstrate increased conditioned-disgust

behaviours relative to rats conditioned and tested during a period of low estrogen circulation (i.e., diestrus).

Future studies might extend these animal results by examining AN in rats in varying pubertal stages, in both females and males. Given the results from the human study which suggest a sex difference only appears once patients reach puberty, studying this theory in rodents would further strengthen the animal model of AN as a useful preclinical tool. Furthermore, the effects of ovariectomy and replacement of estrogen and/or progesterone should be evaluated in female rats, as this would provide a clearer indication of which gonadal hormone causes increased conditioned nausea-related behaviour. Future clinical studies should examine this research question with a focus on puberty so that clinician-defined pubertal status determination is part of the study's methodology, as well as with larger and more evenly divided sample sizes.

Until the mechanism behind the sex difference in CINV is completely understood, treatment of and prophylaxis for CINV will be suboptimal, and will continue to have a profound impact on cancer patients' lives. The current thesis provides substantial support to suggest that increased levels of estrogens in female patients may be the underlying cause for the observed sex difference. These results provide a basis for future studies to explore both direct hormone manipulation in the animal model, and hormone assays in the clinical population, in order to further elucidate estrogen's possible effects. Uncovering this mechanism will ultimately help to alleviate the burden of CINV on cancer patients, and the present thesis lays the groundwork that future studies can build on.

4.2 References

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women's health initiative memory study: A randomized controlled trial. *Jama, 289*(20), 2651-2662.

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Warr, D. (2014). Prognostic factors for chemotherapy induced nausea and vomiting. *European Journal of Pharmacology*, *722*, 192-196.

Danna Zevy

Curriculum Vitae

EDUCATION

Western University, London, ON **2015- 2017**
Master of Science, Neuroscience- MSc
Thesis Title: The Influence of Estrogen on Sex Differences in Chemotherapy-Induced Nausea and Vomiting
Supervisors: Dr. Klaus-Peter Ossenkopp & Dr. Martin Kavaliers

Western University, London, ON **2011- 2015**
Bachelor of Science (Honours), Psychology- BSc
Thesis Title: Hemispheric Lateralization of Theory of Mind
Supervisor: Dr. Adam Cohen

RESEARCH EXPERIENCE

WESTERN UNIVERSITY, London, ON **2014-2017**
Masters Thesis Project- *Supervisors: Dr. Klaus-Peter Ossenkopp and Dr. Martin Kavaliers*

Project: The influence of estrogen on sex differences in chemotherapy-induced nausea and vomiting
Roles: Full project responsibility including study design, animal care and handling, manuscript preparation, presenting results in seminar course. Involves intraperitoneal injections, vaginal lavage technique, scoring behavioural responses using Observer Event-Recording Program, and analysis using SPSS

HOSPITAL FOR SICK CHILDREN, Toronto, ON **2016- 2017**
Division of Haematology/Oncology *- Supervisor: Dr. Lee Dupuis*

Project: Risk factors in the development of chemotherapy-induced nausea and vomiting in pediatric cancer patients
Roles: Developed and implemented clinical research study, created the protocol, submitted to SRB and REB, presented to oncology departments (Leukemia/Lymphoma, Solid Tumour, and Neurooncology) and collected data

INTERNATIONAL PEDIATRIC ONCOLOGY GUIDELINES IN SUPPORTIVE CARE NETWORK, Toronto, ON **2016**
Systematic Review- *Supervisor: Dr. Paula Robinson*

Project: Dexrazoxane for prevention of cardiotoxicity due to anthracyclines in pediatric cancer patients
Role: Data transcription

PEDIATRIC ONCOLOGY GROUP OF ONTARIO, Toronto, ON **2016**
Clinical Practice Guidelines*- Supervisor: Dr. Paula Robinson*

Project: Clinical Practice Guideline-consistent care and patient outcomes
Role: Title and abstract screening

THE BRAIN AND MIND INSTITUTE, London, ON **2014-2015**
Undergraduate Thesis Project- *Supervisor: Dr. Adam Cohen*

Project: Hemispheric lateralization of Theory of Mind
Role: Full project responsibility including study design, participant recruitment, data collection, and data analysis

PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS

Zevy, D., Kavaliers, M., & Ossenkopp, K. (2016). Development of anticipatory nausea in female rats varies across the estrous cycle. *Canadian Journal of Experimental Psychology-Revue Canadienne De Psychologie Experimentale, 70*(4), 427-428. (abstract) **2016**

Zevy, D., Cohen, A. (April, 2016). Hemispheric lateralization of Theory of Mind. *Western Undergraduate Psychology Journal*. **2016**

PRESENTATIONS

Zevy, D. L., Kavaliers, M., Ossenkopp, K. P., (2017, February). *Exploring Sex Differences in Anticipatory Nausea: The Effect of the Estrous Cycle*. Poster for Steroids and Nervous System, Torino, Italy. **2017**

Zevy, D. L., Kavaliers, M., Ossenkopp, K. P., (2016, June). *Development of anticipatory nausea in female rats varies across the estrous cycle*. Poster for Canadian Society for Brain, Behaviour, and Cognitive Science, Ottawa, Canada. **2016**

Zevy, D. L., Kavaliers, M., Ossenkopp, K. P., (2016, May). *Development of anticipatory nausea in female rats varies across the* **2016**

estrous cycle. Poster for Southern Ontario Neuroscience Association, Waterloo, Canada.

Zevy, D. (2016, March). *Hemispheric lateralization of Theory of Mind*. Poster for Western Research Forum, London, Canada. **2016**

Zevy, D. (2015, March). *Hemispheric lateralization of Theory of Mind*. Poster for Western Student Research Conference, London, Canada. **2015**

ACADEMIC/TEACHING EXPERIENCE

WESTERN UNIVERSITY, London, ON **2017**
Teaching Assistant- *Course Instructor: Dr. Elizabeth Hampson*

Course: Psychology 3225A Sex Differences in Human Brain and Behaviour
Roles: Marked exams and quizzes, held office hours, assisted students with course materials, proctored exams and quizzes

WESTERN UNIVERSITY, London, ON **2016-2017**
Undergraduate Honours Student Thesis Supervision

Roles: Aided in the development of research project, gave instruction and hands-on aid in the experiment, provided feedback on thesis writing

WESTERN UNIVERSITY, London, ON **2016**
Invited Guest Lecturer- *Course Instructor: Dr. Klaus-Peter Ossenkopp*

Course: Psychology 3225A Sex Differences in Human Brain and Behaviour
Roles: preparing slides, teaching 3-hour lecture

WESTERN UNIVERSITY, London, ON **2016**
Teaching Assistant- *Course Instructor: Dr. Scott MacDougall-Shackleton*

Course: Psychology 3228A Evolution and Psychology
Roles: Marking assignments, proctoring exams, holding office hours, assisting students with course materials

WESTERN UNIVERSITY, London, ON **2015-2016**
Teaching Assistant- *Course Instructor: Dr. Klaus-Peter Ossenkopp*

Course: Psychology 3225A Sex Differences in Human Brain and Behaviour

Roles: Marked exams and quizzes, held office hours, assisted students with course materials, proctored exams and quizzes

HONOURS, SCHOLARSHIPS, AND AWARDS

Western Graduate Research Scholarship **2016- 2017**
Neuroscience Department, Western University, London, ON
Value: $12, 200

Western Graduate Research Scholarship **2015-2016**
Neuroscience Department, Western University, London, ON
Value: $12, 200

Faculty of Social Science Dean's Honour List **2014- 2015**
Faculty of Psychology, Western University, London, ON

Western Scholarship of Excellence **2011**
Western University, London, ON
Value: $2000

TECHNICAL SKILLS/PROFESSIONAL CERTIFICATES

Graduate Level Course in Statistics Using "R" **2017**
Multiple comparisons, ANOVA, ANCOVA, multiple regression, bootstrapping, maximum likelihood estimation, bayesian approaches

Experienced with Statistical Analysis Program, SPSS **2014-2017**

Western University Animal Care and Veterinary Services **2014**
Certification- Supported by the Canadian Council on Animal Care