11-4-2016 12:00 AM

Oxytocin's Effects on Sickness Behaviours, Anxiety Responses, and Immune Function in Adult Male Mice

Julie Deleemans
The University of Western Ontario

Supervisor
Dr. Martin Kavaliers
The University of Western Ontario Joint Supervisor
Dr. Christine M. Tenk
The University of Western Ontario Joint Supervisor
Dr. Klaus-Peter Ossenkopp
The University of Western Ontario

Graduate Program in Psychology

A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

Abstract

The nonapeptide, oxytocin (OT), is implicated in a range of behavioural and physiological functions. However, OT's role in sickness behaviours remains unclear. This thesis examined effects of the OT agonist, carbetocin (CBT), and OT antagonist, L-368,899, on anxiety and locomotor sickness-related behaviours and pro-inflammatory cytokines, TNF-α and IL-6, in adult male CD-1 mice. Animals received 2 intraperitoneal treatment injections. The first treatment was carbetocin, L-368,899, or saline, while the second was lipopolysaccharide (LPS) or saline. Behaviours were evaluated via the light-dark test, and cytokines via immunoassay. OT antagonist treatment attenuated LPS induced perturbations in locomotor and anxiety-like behaviour, but produced no significant effects on cytokines. The 10mg/kg CBT-saline treatment suppressed locomotion, and augmented anxiogenic behaviour, while OT antagonist treatment augmented locomotor behaviour, and decreased anxiety-like behaviour. The present findings suggest that OT antagonist treatment has anxiolytic effects on basal anxiety-like behaviours, and attenuates the expression of sickness behaviour.

Keywords: oxytocin, agonist, carbetocin, antagonist, L-368,899, lipopolysaccharide, sickness behaviours, anxiety, cytokines, mice.

Acknowledgement of Co-Authorship

All experimental work was carried out by Julie Deleemans with the exception of assistance from Kai Wang who aided in the collection of the data presented in Chapter 3. Drs. Martin Kavaliers, Christine Tenk, and Klaus-Peter Ossenkopp contributed to the design of the experiments presented here and to the editing of the manuscripts.

Acknowledgements

I would first like to express my sincere gratitude and appreciation to my supervisors, Dr. Christine Tenk, Dr. Martin Kavaliers , and Dr. Klaus-Peter Ossenkopp, for their support, guidance, and encouragement throughout the years. Your understanding, patience and kindness have made this experience both a meaningful and enjoyable one. I am truly thankful for the many opportunities which you have given me to attend conferences and to share my work with the academic community. These opportunities, along with your guidance and support, have helped foster the development of my skills as a researcher, and have allowed me to gain invaluable experience in this field. Furthermore, I would like to thank Dr. Martin Kavaliers and the Psychology Department for the financial support that I received, as well as the Faculty of Graduate Studies for the scholarships that I have received.

I wish to next extend my deepest thanks to the other members of the lab. To Jacek for taking such excellent and knowledgeable care of the animals. To my lab mates Kai Wang, Caylen Cloutier, Natalie Boulet, and Jordan Ward – thank you. Kai, I greatly appreciate your mouse wrangling skills, and especially your assistance with the collection of serum samples, which I could not have done without you. Caylen, Natalie, and Jordan, thanks for your encouragement, insight, and much needed laughs over the years. From our lab lunch dates, to long chats and fun times out, I truly value each of you and I am so grateful that I have had the opportunity to get to know you all as colleagues and friends. I also want to thank Francis for patiently helping me with dose calculations, and Lisa for the enjoyable and thoughtful discussions we've had.

Finally, I would like to extend a massive thank you to my family and friends. To my parents, Maria and Dave, thank you for your support, patience, and encouragement over the years; I could not have done this without you. Robin, thank you for being such an amazing sister, my sounding board, and for helping me to gain perspective during challenging times. To all of my other dear friends, near and far, thank you for your encouragement and for believing in me, even when I did not believe in myself. Lastly, I would like to thank my dog, Oscar, and kitty, Calvin, for their unconditional love, extreme adorableness, and for bringing me joy, especially during my most difficult times.

Table of Contents

List of Tables

List of Figures

List of Appendices

CHAPTER 1

GENERAL INTRODUCTION

1.1. General Introduction

The nonapeptide, oxytocin (OT), has been implicated in a broad range of behavioural and physiological functions. For example, evidence from humans and non-human animals demonstrates that OT mediates various pro-social behaviours such as maternal nurturing, mother-infant bonding, pair-bonding, and alloparental care (Ross & Young, 2009). In humans, OT has also been shown to enhance interpersonal trust, affiliation, and sensitivity to inferring the emotions of others (Feldman et al., 2016; Ross & Young, 2009). There is, however, also evidence indicating that OT plays a role in other types of behaviour, including "antisocial" behaviour characterized by avoidance, aggression, and reduced social contact (Alcorn et al., 2015; Beery, 2015), as well as immune-related behaviours such as the recognition and mediation of avoidance response to infection and pathogen threat (Arakawa et al., 2015; Choleris et al., 2009; Kavaliers & Choleris, 2011). Such findings from previous studies are consistent with the idea that OT is involved in the mediation of responses to socially salient information, and that OT can either augment or attenuate social behaviour according to the social context (Feldman et al., 2016; Guzman et al., 2014; Guzman et al., 2013; Shamay-Tsoory & Abu-Akel, 2016). As previous studies have demonstrated, OT is associated with behavioural responses related to the detection of sickness and infection (eg. Arakawa et al., 2015). However, whether OT is also associated with the expression of sickness behaviours remains unclear.

1.1.1. Oxytocin, Stress, and Anxiety

OT is synthesized in 2 major sites within the hypothalamus: the paraventricular nucleus (PVN) and the supraoptic nucleus (SON); two areas which are also associated with hypothalamic-pituitary-adrenal (HPA) axis function (Insel, 1992; Love, 2014; Neumann & Landgraf, 2012; Ross & Young, 2009). After synthesis, OT is released into circulation via axonal projections from the posterior pituitary via the neurohypophyseal system (Insel, 1992; Neumann & Landgraf, 2012; Ross & Young, 2009). High densities of OT and its receptors are found in limbic regions including the nucleus accumbens, central amygdala, ventromedial hypothalamus, and as well as the hypothalamic endocannabinoid system (Choleris et al., 2013; De Laurentiis et al., 2010; Peters et al., 2014; Tops et al., 2004). These are neural regions which have been associated with

stress-related behaviours (eg. increased anxiety) and physiological cascades, as well as immune function (Duval et al., 2015; Viveros et al., 2005). Moreover, OT receptor expression is also influenced by genetic variation, age, sex, and early environmental experiences such as exposure to stress or trauma (Bakos et al., 2014; Buisman-Pijlman et al., 2014).

Increasing evidence suggests that OT plays an important role in modulating the stress response and potentially immune function as well (Wang et al., 2015). Importantly, there is substantial evidence that OT can have suppressive effects on the HPA-axis, helping to reduce circulating levels of cortisol/corticosterone and adrenocorticotropic hormone (ACTH) (Clodi et al., 2008; Brunton et al., 2012; Detillion et al., 2004; DeVries et al., 2007; Landgraf et al., 1995; Neumann et al., 2000). This impact of OT on the HPA-axis may mediate effects on immune function, decreasing pro-inflammatory cytokine activation (Wang et al., 2015). For example, in hamsters, treatment with OT or housing conditions allowing for social interaction led to reduced HPA-axis and pro-inflammatory cytokine activation, thus promoting more rapid wound healing (DeVries et al., 2007). Conversely, it was shown that hamsters who received treatment with an OT antagonist or who were individually housed displayed increased HPA-axis and pro-inflammatory cytokine activation, and inhibited wound healing (Detillion et al., 2004; DeVries et al., 2007; Neumann et al., 2000). Taken together, evidence from these studies clearly indicates a role for OT in mediating HPA-axis as well as immunological functions.

Accumulating evidence suggests that systemic levels of OT may also influence other behaviours associated with HPA activation, including locomotor and anxiety-like behaviour. Importantly, treatment with an OT agonist has been shown to reduce anxiety-like behaviour in a variety of animal models (Harari-Dahan & Bernstein, 2014; Neumann, 2008; Neumann & Slattery, 2015; Peters et al., 2014). Positive social interaction and social bonding experiences, such as being socially housed, mating, and parental care have been shown to increase endogenous levels of OT, and may also buffer against the deleterious effects of anxiety, and attenuate anxiety-like behaviour in rodents via OT mediated mechanisms (DeVries et al., 2007; Neumann, 2008; Tops et al., 2014). Conversely, studies have shown that when rodents receive treatment with an OT antagonist or are housed individually, anxiety-like behaviour increases (Neumann &

Slattery, 2015; Ring et al., 2006). However, there is also a small but growing body of data suggesting that OT may either have no effect, or may augment anxiety responses, which appears to be very much context and situation specific (Guzman et al., 2014; Guzman et al., 2013; Shamay-Tsoory & Abu-Akel, 2016).

Although augmented levels of OT tend to induce anxiolytic effects, and attenuated OT levels have been associated with increased anxiety, conflicting data suggests that these effects may be dose- and context-related (Guzman et al., 2014; Peters et al., 2014; Ring et al., 2006). For example, Ring et al. (2006) found dose-related effects of OT on anxiety-like behaviours in male mice as indexed by the four-plate test. Here, systemic OT (3.0 – 30 mg/kg i.p.) treatment resulted in anxiolytic effects on behaviour; the 10 mg/kg dose of OT produced the greatest number of increased punished crossings, indicating both dose-related, and anxiolytic effects of peripheral OT (Ring et al., 2006). Similarly, Peters et al. (2014) chronically administered high (10 ng/h) or low (1 ng/h) doses of OT intracerebroventricularly (ICV), and found that the high dose of OT increased anxiogenic behaviour as indexed by reduced time spent in the light chamber of the Light-Dark test, and less time spent in the open arms of an elevated plus maze (EPM), while the low dose of OT increased locomotor activity (Peters et al., 2014). Conversely, Guzman et al. (2014) showed that in male mice, ICV treatment to the lateral septum with an OT antagonist abolished social fear conditioning. Their results indicated a bidirectional role for OT in fear conditioning as OT led to decreased fear after positive, but increased fear after negative social encounters (Guzman et al., 2014). Evidently, the putative dose- and context-related nature of OT effects on anxiety-like behaviour remains poorly understood.

1.1.2. Oxytocin, Immune Function, and CD38

Results of studies examining CD38, a type II transmembrane glycoprotein, further support the link between OT, immune function, and behaviour. In humans and other animals CD38 plays a role in immune, social behaviour, and OT mediated functions (Algoe & Way, 2014; Feldman et al., 2016; Higashida et al., 2012; Jin et al., 2007; Lerer et al., 2010). For example, Partida-Sanchez et al. (2001) showed that trafficking of neutrophils to inflammatory and infected sites was dependent upon CD38 expression on neutrophils, providing evidence of a role for CD38 in immune function and inflammatory

mechanisms. Results of investigations with mice have also shown that CD38 regulates in vivo functions of immune-related cells, such as dendritic cells, monocytes, and neutrophils (Lund, 2006). Furthermore, Lerer et al. (2010) found that lymphocytes from Autism Spectrum Disorder (ASD) patients had significantly less CD38 expression, compared to the patients' unaffected parents. As patients diagnosed with ASD display hallmark dysfunction of social behaviour including the OT-related behaviours of social cognition, social approach, and affiliative behaviour (Lukas & Neumann, 2013), these data provide support for the integrated role of CD38 and OT in immune function and social behaviour.

1.1.3. Oxytocin, LPS, and Sickness Behaviour

Lipopolysaccharide (LPS) is an endotoxin from the outer cell wall of Gram-negative bacteria, which activates both immune and endocrine systems including the HPA-axis (Anisman & Merali, 2002; Anisman et al., 2003; Clodi et al., 2008; Ross et al., 2013). In both humans and non-human animals, LPS has reliably and consistently been shown to induce robust immune responses and sickness behaviours (Anisman & Merali, 2002). Sickness behaviours refer to a complex suite of coordinated behaviours that occur after the onset of infection, and are elicited by pro-inflammatory cytokines in both humans and other animals (Anisman et al., 2003; Aubert, 1999; Dantzer & Kelley, 2007; Kelley et al., 2003; Shattuck & Muehlenbein, 2015; York at el., 2012). Specific behaviours indicative of sickness include lethargy, anorexia, depression, cognitive changes, decreased sex drive, anhedonia, sleep disturbance, hyperalgesia, and social withdrawal and isolation (Kelley et al., 2003; Shattuck & Muehlenbein, 2015). For example, Banasikowski et al. (2015) showed dose- and context-related effects of LPS treatment on locomotor and anxiety-like behaviour in the light-dark test in male mice. LPS at 25μg/kg (i.p.), but not 1μg/kg or 5μg/kg, significantly enhanced anxiety-like behaviour on the first day of testing (Banasikowski et al., 2015). Similarly, Engeland et al. (2001) also found context-related effects on locomotor behaviour following LPS treatment as animals displayed significant reductions in locomotor activity, and environmental novelty was shown to mediate the locomotor reducing effects of LPS in male mice. Furthermore, in adult male rats, Reyes-Lagos et al. (2016) showed that LPS at 0.1mg/kg (i.p.) significantly increased mean heart rate and body temperature, and

attenuated locomotor behaviour in freely moving animals, indicating LPS-induced endotoxemia. These and other studies provide evidence for the role of LPS in affecting sickness behaviours, including anxiety, and immune responses.

Cytokines communicate with the central nervous system via the endocrine route or by direct neural transmission via the vagus nerve, alerting the brain that an infection has occurred (Anisman & Merali, 2002; Dantzer & Kelley, 2007; Kelley et al., 2003). Cytokines that are synthesized and released during the early stages of response to infection, and which have been strongly and consistently associated with sickness behaviour include interleukin 1 beta (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α) (Anisman & Merali, 2002; Anisman et al., 2003; Kelley at al., 2003; Shattuck & Muehlenbein, 2015).

OT has been shown to affect serum and brain levels of IL-1β, IL-6, and TNF-α levels (Wang et al., 2013). Results of studies have shown that pro-inflammatory cytokines, such as TNF-α and IL-1β, stimulate release of both OT and AVP both in vitro and in vivo (Anisman et al., 2003; Landgraf et al., 1995; Shattuck & Muehlenbein, 2015). For instance, Landgraf et al. (1995) found that central administration of IL-1β induced the release of OT and AVP within the supraoptic nucleus in rats. Moreover, LPS treatment, which stimulates the production of pro-inflammatory cytokines including TNF-α, similarly appears to cause increases in plasma OT (De Laurentiis et al., 2010). These studies further underscore the link between OT and immune function. There is, however, limited research on the effects of OT as it relates specifically to the expression of sickness behaviour. For example, only a recent study by Reyes-Lagos et al. (2016) investigated OT's role in mediating heart rate fluctuations, body temperature, and locomotor activity (ie. sickness behaviour) in adult male rats challenged with LPS. They found that in animals administered LPS plus OT, there was a decrease in mean heart rate, and OT appeared to moderate LPS-induced hyperthermia, while also increasing locomotor activity for up to 6 hours post LPS treatment (Reyes-Lagos et al., 2016), providing evidence that OT does influence these particular sickness behaviours. Based on previous limited research, it is clear that OT does play a role in mediating HPA-related physiological and behavioural mechanisms, immune function, and potentially sickness behaviour. However, more research is needed to clarify OT's role regarding specific

sickness behaviours, such as locomotor activity and anxiety-like behaviour, in the context of LPS-induced immune activation.

1.1.4. Present Study

Results of previous studies have produced mixed results regarding the effects of OT on both locomotor and anxiety-like behaviour, immune function, and that expressed during sickness following LPS treatment. In this study the effects of the OT agonist, carbetocin, and OT antagonist, L-368,899, on sickness and anxiety-like behaviour induced by LPS administration were examined, along with levels of pro-inflammatory cytokines TNF-α and IL-6. Locomotor and anxiety-like sickness-related behaviours were evaluated in the light-dark test, as the automated apparatus allows for examination of both anxiety and locomotor behavioural parameters (Arrant et al., 2013; Banasikowski et al., 2015; Bourin & Hascoet, 2003). Cytokine parameters were measured from trunk blood collection following LPS treatment and behavioural testing.

In the study of locomotor and anxiety-like behaviours affected by sickness, it was hypothesized that animals receiving treatment with the OT agonist, carbetocin (Engstrøm et al., 1998; Moertl et al., 2011), in combination with LPS would demonstrate attenuated anxiety responses and normalized locomotor activity. It was also hypothesized that animals treated with the OT antagonist, L-368,899 (Kuteykin-Teplyakov & Maldonado 2014; Olszewski et al., 2015; Olszewski et al., 2014), would show augmented anxiety-like behaviour and reduced locomotor activity. In the second study, which examined OT's role in pro-inflammatory responses, it was hypothesized that the OT agonist would result in reduced serum levels of pro-inflammatory cytokines TNF-α and IL-6, elicited by LPS treatment, while the OT antagonist was predicted to elevate these cytokine levels.

1.2 References

Alcorn, J. L., Green, C. E., Schmitz, J., & Lane, S. D. (2015). Effects of oxytocin on aggressive responding in healthy adult men. *Behavioural Pharmacology, 26*, 798-804. doi:10.1097/fbp.0000000000000173

Algoe, S. B., & Way, B. M. (2014). Evidence for a role of the oxytocin system, indexed by genetic variation inCD38, in the social bonding effects of expressed gratitude. *Social Cognitive and Affective Neuroscience, 9*(12), 1855-1861. doi:10.1093/scan/nst182

Anisman, H., & Merali, Z. (2002). Cytokines, stress, and depressive illness. *Brain, Behavior, and Immunity, 16*(5), 513-524. doi:10.1016/s0889-1591(02)00009-0

Anisman, H., Merali, Z., & Hayley, S. (2003). Sensitization associated with stressors and cytokine treatments. *Brain, Behavior, and Immunity, 17*(2), 86-93. doi:10.1016/s0889-1591(02)00100-9

Arakawa, H., Blanchard, D. C., & Blanchard, R. J. (2015). Central oxytocin regulates social familiarity and scent marking behavior that involves amicable odor signals between male mice. *Physiology & Behavior, 146*, 36-46. doi:10.1016/j.physbeh.2015.04.016

Arrant, A. E., Schramm-Sapyta, N. L., & Kuhn, C. M. (2013). Use of the light/dark test for anxiety in adult and adolescent male rats. *Behavioural Brain Research, 256*, 119-127. doi:10.1016/j.bbr.2013.05.035

Aubert, A. (1999). Sickness and behaviour in animals: A motivational perspective. *Neuroscience & Biobehavioral Reviews, 23*(7), 1029-1036. doi:10.1016/s0149-7634(99)00034-2

Bakos, J., Lestanova, Z., Strbak, V., Havranek, T., & Bacova, Z. (2014). Neonatal manipulation of oxytocin prevents lipopolysaccharide-induced decrease in gene expression of growth factors in two developmental stages of the female rat. *Neuropeptides, 48*(5), 281-286. doi:10.1016/j.npep.2014.06.004

Banasikowski, T. J., Cloutier, C. J., Ossenkopp, K., & Kavaliers, M. (2015). Repeated exposure of male mice to low doses of lipopolysaccharide: Dose and time dependent development of behavioral sensitization and tolerance in an automated light–dark anxiety test. *Behavioural Brain Research, 286*, 241-248. doi:10.1016/j.bbr.2015.03.004

Beery, A. K. (2015). Antisocial oxytocin: Complex effects on social behavior. *Current Opinion in Behavioral Sciences, 6*, 174-182. doi:10.1016/j.cobeha.2015.11.006

Bourin, M., & Hascoët, M. (2003). The mouse light/dark box test. *European Journal of Pharmacology, 463*(1-3), 55-65. doi:10.1016/s0014-2999(03)01274-3

Brunton, P. J., Bales, J., & Russell, J. A. (2012). Allopregnanolone and induction of endogenous opioid inhibition of oxytocin responses to immune stress in pregnant rats. *Journal of Neuroendocrinology, 24*(4), 690-700. doi:10.1111/j.1365-2826.2012.02295.x

Buisman-Pijlman, F. T., Sumracki, N. M., Gordon, J. J., Hull, P. R., Carter, C. S., & Tops, M. (2014). Individual differences underlying susceptibility to addiction: Role for the endogenous oxytocin system. *Pharmacology Biochemistry and Behavior, 119*, 22-38. doi:10.1016/j.pbb.2013.09.005

Choleris, E., Pfaff, D. W., & Kavaliers, M. (2013). *Oxytocin, vasopressin and related peptides in the regulation of behavior*. New York: Cambridge University Press.

Clodi, M., Vila, G., Geyeregger, R., Riedl, M., Stulnig, T. M., Struck, J., . . . Luger, A. (2008). Oxytocin alleviates the neuroendocrine and cytokine response to bacterial endotoxin in healthy men. *AJP: Endocrinology and Metabolism, 295*(3). doi:10.1152/ajpendo.90263.2008

Dantzer, R., & Kelley, K. W. (2007). Twenty years of research on cytokine-induced sickness behavior. *Brain, Behavior, and Immunity, 21*(2), 153-160. doi:10.1016/j.bbi.2006.09.006

De Laurentiis, A., Fernandez-Solari, J., Mohn, C., Burdet, B., Zubilete, M. A., & Rettori, V. (2010). The hypothalamic endocannabinoid system participates in the secretion of oxytocin and tumor necrosis factor-alpha induced by lipopolysaccharide. *Journal of Neuroimmunology, 221*(1-2), 32-41. doi:10.1016/j.jneuroim.2010.02.006

Detillion, C. E., Craft, T. K., Glasper, E. R., Prendergast, B. J., & Devries, A. (2004). Social facilitation of wound healing. *Psychoneuroendocrinology, 29*(8), 1004-1011. doi:10.1016/j.psyneuen.2003.10.003

DeVries, A. C., Craft, T. K., Glasper, E. R., Neigh, G. N., & Alexander, J. K. (2007). 2006 Curt P. Richter award winner*:* Social influences on stress responses and health*. Psychoneuroendocrinology, 32*(6), 587-603. doi:10.1016/j.psyneuen.2007.04.007

Duval, E., Javanbakht, A., & Liberzon, I., (2015). Neural circuits in anxiety and stress disorders: A focused review. *Therapeutics and Clinical Risk Management*, 115. doi:10.2147/tcrm.s48528

Feldman, R., Monakhov, M., Pratt, M., & Ebstein, R. P. (2016). Oxytocin pathway genes: evolutionary ancient system impacting on human affiliation, sociality, and psychopathology. *Biological Psychiatry, 79*(3), 174-184. doi:10.1016/j.biopsych.2015.08.008

Guzmán, Y. F., Tronson, N. C., Jovasevic, V., Sato, K., Guedea, A. L., Mizukami, H., . . . Radulovic, J. (2013). Fear-enhancing effects of septal oxytocin receptors. *Nature Neuroscience, 16*(9), 1185-1187. doi:10.1038/nn.3465

Guzmán, Y. F., Tronson, N. C., Sato, K., Mesic, I., Guedea, A. L., Nishimori, K., & Radulovic, J. (2014). Role of oxytocin receptors in modulation of fear by social memory. *Psychopharmacology, 231*(10), 2097-2105. doi:10.1007/s00213-013-3356-6

Harari-Dahan, O., & Bernstein, A. (2014). A general approach-avoidance hypothesis of oxytocin: Accounting for social and non-social effects of oxytocin. *Neuroscience and Biobehavioral Reviews, 47*, 506-519. doi:10.1016/j.neubiorev.2014.10.007

Higashida, H., Yokoyama, S., Kikuchi, M., & Munesue, T. (2012). CD38 and its role in oxytocin secretion and social behavior. *Hormones and Behavior, 61*(3), 351-358. doi:10.1016/j.yhbeh.2011.12.011

Insel, T. R. (1992). Oxytocin — A neuropeptide for affiliation: Evidence from behavioral, receptor autoradiographic, and comparative studies. *Psychoneuroendocrinology, 17*(1), 3-35. doi:10.1016/0306-4530(92)90073-g

Jin, D., Liu, H., Hirai, H., Torashima, T., Nagai, T., Lopatina, O., . . . Higashida, H. (2007). CD38 is critical for social behaviour by regulating oxytocin secretion. *Nature, 446*(7131), 41-45. doi:10.1038/nature05526

Kavaliers, M., & Choleris, E. (2011). Sociality, pathogen avoidance, and the neuropeptides oxytocin and arginine vasopressin. *Psychological Science, 22*(11), 1367-1374. doi:10.1177/0956797611420576

Kelley, K. W., Bluthé, R., Dantzer, R., Zhou, J., Shen, W., Johnson, R. W., & Broussard, S. R. (2003). Cytokine-induced sickness behavior. *Brain, Behavior, and Immunity, 17*(1), 112-118. doi:10.1016/s0889-1591(02)00077-6

Landgraf, R., Neumann, I., Holsboer, F., & Pittman, Q. J. (1995). Interleukin-1β stimulates both central and peripheral release of vasopressin and oxytocin in the rat. *European Journal of Neuroscience, 7*(4), 592-598. doi:10.1111/j.1460-9568.1995.tb00663.x

Lerer, E., Levi, S., Israel, S., Yaari, M., Nemanov, L., Mankuta, D., . . . Ebstein, R. P. (2010). Low CD38 expression in lymphoblastoid cells and haplotypes are both associated with autism in a family-based study. *Autism Research, 3*(6), 293-302. doi:10.1002/aur.156

Lukas, M., & Neumann, I. D. (2013). Oxytocin and vasopressin in rodent behaviors related to social dysfunctions in autism spectrum disorders. *Behavioural Brain Research, 251*, 85-94. doi:10.1016/j.bbr.2012.08.011

Lund, F. E. (2006). Signaling properties of CD38 in the mouse immune system: Enzyme-dependent and -independent roles in immunity. *Molecular Medicine, 12*(11), 238-333.

Love, T. M. (2014). Oxytocin, motivation and the role of dopamine. *Pharmacology Biochemistry and Behavior, 119*, 49-60. doi:10.1016/j.pbb.2013.06.011

Neumann, I. D., Wigger, A., Torner, L., Holsboer, F., & Landgraf, R. (2000). Brain oxytocin inhibits basal and stress-induced activity of the hypothalamo-pituitary-adrenal axis in male and female rats: partial action within the paraventricular nucleus. *Journal of Neuroendocrinology, 12*(3), 235-243. doi:10.1046/j.1365-2826.2000.00442.x

Neumann, I. D. (2008). Brain oxytocin: A key regulator of emotional and social behaviours in both females and males. *Journal of Neuroendocrinology, 20*(6), 858-865. doi:10.1111/j.1365-2826.2008.01726.x

Neumann, I. D., & Landgraf, R. (2012). Balance of brain oxytocin and vasopressin: Implications for anxiety, depression, and social behaviors. *Trends in Neurosciences, 35*(11), 649-659. doi:10.1016/j.tins.2012.08.004

Neumann, I. D., & Slattery, D. A. (2016). Oxytocin in general anxiety and social fear: A translational approach. *Biological Psychiatry, 79*(3), 213-221. doi:10.1016/j.biopsych.2015.06.004

Partida-Sanchez, S., Cockayne, D. A., Monard, S., Jacobson, E. L., Oppenheimer, N., Garvy, B., . . . Lund, F. E. (2001). Cyclic ADP-ribose production by CD38 regulates intracellular calcium release, extracellular calcium influx and chemotaxis in neutrophils and is required for bacterial clearance in vivo. *Nature Medicine, 7*(11), 1209-1216.

Peters, S., Slattery, D. A., Uschold-Schmidt, N., Reber, S. O., & Neumann, I. D. (2014). Dose-dependent effects of chronic central infusion of oxytocin on anxiety, oxytocin receptor binding and stress-related parameters in mice. *Psychoneuroendocrinology, 42*, 225-236. doi:10.1016/j.psyneuen.2014.01.021

Reyes-Lagos, J. J., Hadamitzky, M., Peña-Castillo, M. Á, Echeverría, J. C., Bösche, K., Lückemann, L., . . . Pacheco-López, G. (2016). Exogenous oxytocin reduces signs of sickness behavior and modifies heart rate fluctuations of endotoxemic rats. *Physiology & Behavior, 165*, 223-230. doi:10.1016/j.physbeh.2016.07.013

Ring, R. H., Malberg, J. E., Potestio, L., Ping, J., Boikess, S., Luo, B., . . . Rosenzweig-Lipson, S. (2006). Anxiolytic-like activity of oxytocin in male mice: Behavioral and autonomic evidence, therapeutic implications. *Psychopharmacology, 185*(2), 218-225. doi:10.1007/s00213-005-0293-z

Ross, K. M., Mcdonald-Jones, G., & Miller, G. E. (2013). Oxytocin does not attenuate the ex vivo production of inflammatory cytokines by lipopolysaccharide-activated monocytes and macrophages from healthy male and female donors. *Neuroimmunomodulation, 20*(5), 285-293. doi:10.1159/000351610

Ross, H. E., & Young, L. J. (2009). Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Frontiers in Neuroendocrinology, 30*(4), 534-547. doi:10.1016/j.yfrne.2009.05.004

Shamay-Tsoory, S. G., & Abu-Akel, A. (2016). The social salience hypothesis of oxytocin. *Biological Psychiatry, 79*(3), 194-202. doi:10.1016/j.biopsych.2015.07.020

Shattuck, E. C., & Muehlenbein, M. P. (2015). Human sickness behavior: Ultimate and proximate explanations. *American Journal of Physical Anthropology, 157*(1), 1-18. doi:10.1002/ajpa.22698

Tops, M., Koole, S. L., Ijzerman, H., & Buisman-Pijlman, F. T. (2014). Why social attachment and oxytocin protect against addiction and stress: Insights from the

dynamics between ventral and dorsal corticostriatal systems. *Pharmacology Biochemistry and Behavior, 119*, 39-48. doi:10.1016/j.pbb.2013.07.015

Viveros, M., Marco, E., & File, S. (2005). Endocannabinoid system and stress and anxiety responses. *Pharmacology Biochemistry and Behavior, 81*(2), 331-342. doi:10.1016/j.pbb.2005.01.029

Wang, P., Yang, H., Tian, S., Wang, L., Wang, S. C., Zhang, F., & Wang, Y. (2015). Oxytocin-secreting system: A major part of the neuroendocrine center regulating immunologic activity. *Journal of Neuroimmunology, 289*, 152-161. doi:10.1016/j.jneuroim.2015.11.001

York, J. M., Blevins, N. A., Baynard, T., & Freund, G. G. (2012). Mouse testing methods in psychoneuroimmunology: An overview of how to measure sickness, depressive/anxietal, cognitive, and physical activity behaviors. *Methods in Molecular Biology Psychoneuroimmunology*, 243-276. doi:10.1007/978-1-62703-071-7_13

CHAPTER 2

THE EFFECTS OF OXYTOCIN ON SICKNESS BEHAVIOURS AND ANXIETY RESPONSES IN ADULT MALE MICE

2.1 Introduction

There is accumulating evidence suggesting that the nonapeptide, oxytocin (OT), is involved in mediating various social behaviours. However, OT also appears to play a role in affecting behavioural and physiological mechanisms that underlie the experience and expression of stress and anxiety (Choleris et al., 2013; DeVries et al., 2007; Guzman et al., 2013; Neumann & Landgraf, 2012; Neumann & Slattery, 2015). "Sickness behaviours", which refer to a complex suite of coordinated behaviours that occur after the onset of infection, consist of numerous behavioural symptoms including anxiety, as well as lethargy, and social withdrawal and isolation, among others (Anisman et al., 2003; Aubert, 1999; Dantzer & Kelley, 2007; Kelley et al., 2003; Shattuck & Muehlenbein, 2015; York at el., 2012). Given the putative link between OT, stress, and anxiety (Neumann & Landgraf, 2012; Neumann & Slattery, 2015) and the relation between sickness behaviours and anxiety, it is plausible that OT may also participate in the regulation and expression of sickness behaviours. This is a topic which has received relatively minimal attention to date, and remains largely unclear.

2.1.1. Oxytocin, LPS, and Sickness Behaviour

Lipopolysaccharide (LPS) is an endotoxin from the outer cell wall of Gram-negative bacteria, which activates both immune and endocrine systems including the HPA-axis (Anisman & Merali, 2002; Anisman et al., 2003; Clodi et al., 2008; Ross et al., 2013). In both humans and non-human animals, previous studies have shown that LPS reliably and consistently induces robust immune responses and sickness behaviours (Anisman & Merali, 2002). For instance, Banasikowski et al. (2015) showed dose- and context-related effects of LPS treatment on locomotor and anxiety-like behaviour in the light-dark test in male mice. LPS at 25μg/kg (i.p.), but not 1μg/kg or 5μg/kg, significantly enhanced anxiety-like behaviour on the first day of testing (Banasikowski et al., 2015). Likewise, Reyes-Lagos et al. (2016) showed that LPS at 0.1mg/kg (i.p.) significantly increased mean heart rate and body temperature, and attenuated locomotor behaviour in freely moving adult male rats, indicating LPS-induced sickness via cytokine mediated mechanisms.

Cytokines communicate with the central nervous system via the endocrine route or by direct neural transmission via the vagus nerve, alerting the brain that infection has

occurred in the periphery (Anisman & Merali, 2002; Dantzer & Kelley, 2007; Kelley et al., 2003). Cytokines, including interleukin 1 beta (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α), are synthesized and released during the early stages of an acute phase response to infection and have consistently been associated with sickness behaviour (Anisman & Merali, 2002; Anisman et al., 2003; Dantzer & Kelly, 2007; Kelley at al., 2003; Shattuck & Muehlenbein, 2015). OT has been linked to alterations in IL-1β, IL-6, and TNF-α levels during the sickness response (Wang et al., 2013), and pro-inflammatory cytokines, such as TNF-α and IL-1β, have been shown to stimulate the release of both OT and AVP (Anisman et al., 2003; Landgraf et al., 1995; Shattuck & Muehlenbein, 2015).

Sickness behaviours refer to a constellation of coordinated behaviours that occur after the onset of infection, and are elicited by pro-inflammatory cytokines in both humans and non-human animals (Anisman et al., 2003; Aubert, 1999; Dantzer & Kelley, 2007; Kelley et al., 2003; Shattuck & Muehlenbein, 2015; York at el., 2012). Specific behaviours indicative of sickness include lethargy, depression, anxiety, anorexia, cognitive changes, decreased sex drive, anhedonia, sleep disturbance, hyperalgesia, and social withdrawal and isolation (Kelley et al., 2003; Shattuck & Muehlenbein, 2015). Sickness behaviours are an adaptive response to illness and infection, and motivate an organism in reorganize its priorities so as to promote recovery and survival (Aubert, 1999; Dantzer & Kelley, 2007; Shattuck & Muehlenbein, 2015). Previous findings illustrate a link between OT and immune function. However, research on the effects of OT specifically relating to sickness behaviours is quite sparse, thus the current study provides a novel examination of OT's role in specific sickness behaviours following an immune challenge.

2.1.2. Oxytocin and Anxiety

Studies have demonstrated that systemic levels of OT appear to influence behaviour associated with HPA activity, such as anxiety behaviour, as well as that related to immune activation-induced sickness behaviour (eg. locomotor activity). Previous studies have found both systemic and central treatment with various OT agonists and antagonists to be efficacious in examining OT's effects on anxiety behaviours. For instance, treatment with an OT agonist was shown to reduce anxiety-like behaviour in a

variety of rodent species, such as rats and mice, using a battery of anxiety-specific behavioural assays, including the light-dark test, elevated plus maze, four plate test, and open field test (Harari-Dahan & Bernstein, 2014; Neumann, 2008; Neumann & Slattery, 2015; Peters et al., 2014; Ring et al., 2006; York et al., 2012). Similarly, social bonding experiences such as being socially housed, mating, and parental care seem to buffer against the deleterious effects of anxiety (eg. immune suppression, adrenal fatigue, aberrant social behaviour, etc.), and attenuate anxiety-like behaviour presumably via OT mediated mechanisms (DeVries et al., 2007; Neumann, 2008; Tops et al., 2014). For example, in female Siberian hamsters treatment with OT, or housing conditions allowing for social interaction with a same-sex sibling, led to reduced activity within the HPA-axis, and pro-inflammatory cytokine activation, promoting more rapid wound healing (DeVries et al., 2007). Conversely, studies have shown that when animals receive OT antagonist treatment or are housed in isolation, anxiety-like behaviour (Neumann & Slattery, 2015; Ring et al., 2006), HPA activity, and pro-inflammatory cytokines increase, and wound healing is inhibited (Detillion et al., 2004; DeVries et al., 2007; Neumann et al., 2000).

Previous studies show inconsistent findings with respect to dose-related effects of OT on behavioural and physiological parameters. For example, to evaluate OT's putative dose-related effects on anxiety- and stress-related parameters in male mice, Peters et al. (2014) chronically administered high (10 ng/h) or low (1 ng/h) doses of OT intracerebroventricularly (ICV). High dose OT resulted in increased anxiogenic behaviour indexed by reduced time spent in the light chamber of the Light-Dark (LD) test, and less time spent in the open arms of an elevated plus maze (EPM), while low dose OT animals displayed increased locomotor activity in the LD test (Peters et al., 2014). Similarly, Ring et al. (2006) found dose-related effects of OT and the OT antagonist, WAY-162720, on anxiety-like behaviours in male mice as indexed by the four-plate test (FPT). Findings showed that systemic OT (3.0 – 30 mg/kg i.p.) treatment 30 minutes prior to testing resulted in anxiolytic effects on behaviour. Importantly, the 10 mg/kg dose of OT produced the greatest number of increased punished crossings, indicating both a dose-related, and anxiolytic effects of peripheral OT. Treatment with the OT antagonist, WAY-162720, reversed the anxiolytic effects of OT (Ring et al.,

2006). In evaluating the effects of 3 doses (2 mg/kg, 6.4 mg/kg, or 20 mg/kg. i.p.) of the OT agonist, carbetocin, on open field behaviour in male rats, Chaviaras et al. (2010) found that only the 20 mg/kg dose led to a significant decrease in the total distances animals travelled, providing further evidence of dose related effects of OT treatment on behaviour.

Within human research, results have also been variable regarding OT's role in anxiety. Consistent with much animal research, Scantamburlo et al. (2007) found that endogenous OT appears to have anxiolytic effects, as patients with major depression with higher plasma levels of OT show attenuated anxiety. Others have reported that in individuals with depressive symptoms, lower levels of OT tend to be associated with augmented anxiety levels (Harari-Dahan & Bernstein, 2014). OT may be related to depressive symptoms, and the often comorbid syndrome of anxiety, through the inflammatory hypothesis of depression. This hypothesis states that symptoms of depression, including depressive and anxiety behaviour, may result from augmented levels of pro-inflammatory cytokine activity (ie. inflammation) (Maes, 2011).

Although augmented levels of OT tend to induce anxiolytic effects, and attenuated OT levels are associated with increases in anxiety, other data suggests that these effects may be context dependent (Peters et al., 2014). Furthermore, there are a small but growing number of studies suggesting that OT may either have no effect, or cause a slight augmentation of anxiety responses following OT receptor activation (Choleris et al., 2007). For example, Donhoffner et al. (2016) showed that in female adult rats central (ICV) OT treatment had no effect on either locomotor or anxiety-like behaviour in the elevated plus maze. Inconsistent findings highlight the critical role of contextual cue salience within OT mediated behavioural responses, and whether such cues are social or non-social in nature (Choleris et al., 2013; Shamay-Tsoory & Abu-Akel, 2016). The present study provides necessary additional examination of the putative dose-related and context-dependent nature of OT's effects on anxiety-like behaviour within animal models.

2.1.3. Present Study

This study evaluated the effects of the OT agonist, carbetocin, and OT antagonist, L-368,899, on the expression of locomotor and anxiety-like sickness-related behaviours

affected by LPS treatment. Locomotor and anxiety-like behaviours were indexed via an automated light-dark test. This apparatus allowed for examination of both anxiety-like and locomotor behaviours simultaneously (Arrant et al., 2013; Banasikowski et al., 2015; Bourin & Hascoet, 2003). Importantly, this study provided a novel investigation into OT's role in the expression of specific sickness behaviours, along with anxiety-like behaviour.

Based on previous research, it was hypothesized that OT agonist treatment would attenuate the expression of sickness responses, thereby suppressing locomotor activity and anxiety-like behaviour among animals treated with the OT agonist in conjunction with LPS. That is, animals treated with the OT agonist plus LPS were expected to exhibit normal levels (ie. similar to that of controls) of locomotor activity, and to spend less time in the dark chamber of the LD test. Furthermore, animals treated with the OT agonist plus saline were hypothesized to show increased locomotor activity and anxiolytic behaviour. Conversely, animals treated with the OT antagonist, L-368,899, in combination with LPS were hypothesized to show reduced locomotor activity and augmented anxiety-like behaviour.

2.2 Methods

2.2.1. Animals

A total of 91 adult male CD-1 mice (30 to 35 grams) from Charles River, Quebec, were used. Animals were housed in a temperature controlled colony room at 21±1°C, with photoperiod set to a 12:12 Light:Dark cycle with lights on at 0700, and were provided food (ProLab rodent chow, RMH 3000) and water ad libitum. Animals were housed individually in polypropylene cages (29.5 x 18.8 x 13 cm). Effects of individual housing on male mice are strain and density dependent, with previous studies indicating negligible differences in CD-1 mice with regard to basal stress responses between individually and socially housed males (Bartolomucci et al., 2003; Bartolomucci, 2007). However, individual housing was used in our study to prevent effects of inter-male aggression and minimize dominance-subordinance related differences in individual stress and OT responses, as social contextual variables have been shown to affect these (Choleris et al., 2013; DeVries et al., 2007). Mice were left undisturbed for 10 days prior to testing to ensure adequate adjustment time after arrival. All procedures were approved

by the Western University Animal Care Committee and animals were handled and tested according to guidelines set out by the Canadian Council on Animal Care (CCAC).

2.2.2. Drug Treatment

Mice were randomly divided into 8 groups. Beginning between 12:00 and 13:00h, all animals received a series of 2 intraperitoneal (i.p.) injections, 15 minutes apart. Table 2.1 outlines the group treatments and group sizes. The first injection manipulated OT levels. Here, 2 groups received a 20mg/kg dose of OT agonist, carbetocin (CBT) (Carbetocin acetate SML0748, no. 073M4722V, Sigma-Aldrich, St. Louis, MO, USA) dissolved in 0.9% isotonic saline, while 2 other groups received a 10mg/kg dose. Two different groups received a 10mg/kg dose of the OT antagonist, L-368,899 (L-368,899 hydrochloride, no. 1A/82628, Tocris Bioscience, Minneapolis, MN, USA) dissolved in 0.9% isotonic saline for their first injection, while the remaining 2 groups were administered a control injection of an equivalent volume of isotonic saline (0.9%). CBT was chosen as the OT agonist for its relatively long half-life (85 – 100 mins) (Engstrøm et al., 1998; Moertl et al., 2011) and the doses were based on behaviourally active doses previously reported in mice (eg. Chaviaras et al., 2010). Similarly, results from previous studies have shown L-368,899, at varying dosages, to be an effective OT antagonist in mice with the dose used here being based on these studies (Kuteykin-Teplyakov & Maldonado 2014; Olszewski et al., 2015; Olszewski et al., 2014).

The second injection 15 mins later initiated an immune challenge. Animals received either 75μg/kg Lipopolysaccharide (LPS) (derived from Escherichia Coli serotype 0111:B4, no. L2630-028K4090, Sigma Chemical, St. Louis, MO, USA) dissolved in a 0.9% isotonic saline, or saline as a control. This dose was selected based on the results of previous studies with CD-1 mice (Engeland et al., 2001).

Table 2.1. *Group sizes and treatment injections for OT and LPS*

Group	*n*	*Injection 1*	*Injection 2*
20CBT-LPS	12	20 mg/kg carbetocin	75 μg/kg LPS
10CBT-LPS	12	10 mg/kg carbetocin	75 μg/kg LPS
20CBT-NaCl	12	20 mg/kg carbetocin	0.2 to 0.35 mL saline
10CBT-NaCl	12	10 mg/kg carbetocin	0.2 to 0.35 mL saline
OTant-NaCl	7	10 mg/kg L-368,899	0.2 to 0.35 mL saline
OTant-LPS	7	10 mg/kg L-368,899	75 μg/kg LPS
NaCl-LPS	15	0.2 to 0.35 mL saline	75 μg/kg LPS
NaCl-NaCl	14	0.2 to 0.35 mL saline	0.2 to 0.35 mL saline

2.2.3. Apparatus

The Light-Dark (LD) apparatus consisted of 8 Plexiglas VersaMax Animal Activity Monitors (40 cm x 40 cm x 30.5 cm) (Accuscan Model RXYZCM-16, Columbus, OH), divided into two chambers. Infrared sensor beams were located every 2.54 cm along the perimeter (16 along each side) and 2.5 cm above the floor. Additionally, 2 sets of 16 infrared beams were located 8.0 cm above the floor on opposite sides. Beam breaks generate measures of the animals' activity. Black Plexiglas boxes (40 cm x 20cm x 30 cm) with small holes in the sides to avoid obstructing the infrared beams were inserted into each Activity Monitor. Thus the open-field was divided into two equal sized “dark” and “light” chambers. Animals had unrestricted access to both chambers via a 13.0 x 5 cm doorway. Clear Plexiglas lids with air-holes were used to prevent escape. Three fluorescent lights were located above the activity boxes providing a light source of approximately 900 lux at the floor of each light chamber. Data were collected and analyzed by a VersaMax Analyzer (Accuscan Model CDA-8, Columbus, OH), which sent information to an IBM computer where it was recorded for future statistical analysis.

2.2.4. Procedure

Animals were habituated to the VersaMax LD test apparatus for 15 minutes on the day before drug administration and testing. On test day, animals received an injection of either CBT, L-368,899, or saline, followed 15 minutes later by an injection of either saline or LPS, and were then left undisturbed for 2 hours. This time frame was selected as

other studies have found that effects of the acute-phase sickness response, including autonomic, endocrine and behavioural events, appear to peak at approximately 2 hours following LPS administration (e.g. Engeland et al., 2001). Mice were gently placed into the light chamber of the apparatus after this 2 hour period and left undisturbed during testing. Data were collected for 15 minutes in the LD apparatus, as activity tends to markedly decline after 15 minutes (Bourin & Hascoet, 2003; Engeland et al., 2001).

The LD test allows for the collection of data on both locomotor and anxiety parameters (Arrant et al., 2013; Bourin & Hascoet, 2003). For each chamber, measures of locomotor activity and anxiety-like behaviour were obtained and then corrected for unequal amounts of time spent in the chambers by dividing the given variable by the duration of time spent in the corresponding light or dark chamber. Horizontal movement variables included Total Distance travelled (total distance travelled in cm in each chamber), Speed (distance in centimeters travelled per second) and Movement Time (amount of time animals spent moving in the horizontal plane per second). Vertical activity variables included the time animals spent Rearing (time moving vertically per second), and the Number of Rears (number of times animals made vertical movements per second). Measures of anxiety-like behaviour included the Duration of time spent in seconds in the light chamber, the number of Nose Pokes into the light chamber (single beam breaks through the doorway between chambers), and the number of Transitions into the light chamber (full body crossed the center line).

2.2.5. Data Analysis

The effects of the OT agonist, CBT, OT antagonist, L-368,899, and LPS on locomotor activity and anxiety-like behaviours were evaluated using a multivariate Analysis of Variance (MANOVA) with between subjects factors of OT (4 levels: 20mg/kg CBT, 10mg/kg CBT, 10mg/kg OT antagonist, saline (control)) and LPS (2 levels: 75μg/kg and saline (control). Post hoc pairwise comparisons were then completed where appropriate using the Least Significant Difference (LSD) to evaluate significant main effects and interactions. Behavioural data were analyzed using SPSS 23.0 for windows with $\alpha = 0.05$, and the Greenhouse-Geisser correction factor where appropriate.

2.3 Results

2.3.1. Effects of LPS on Locomotor Behaviour

In the dark chamber LPS treatment induced robust attenuations in both horizontal and vertical locomotor behaviour for all locomotor measures including Total Distances travelled, Speed, horizontal Movement Time, Time Rearing, and the Number of Rearing Movements, indicating sickness behaviour. In the light chamber LPS treatment suppressed locomotor behaviour for Total Distances travelled, Time Rearing, and the Number of Rearing Movements, demonstrating the occurrence of sickness behaviour in a context-dependent manner.

Total Distance travelled in the dark and light chambers is shown in Figures 2.1: A – D. There was a significant main effect of LPS in both the dark [$F(1, 85) = 74.68$, $p = .000$] and the light chambers [$F(1, 85) = 32.87$, $p = .000$], with LPS treated animals travelling a significantly shorter distance in both chambers relative to non-LPS treated mice.

Speed in the dark and light chambers is shown in Figure 2.2: A – D. A significant main effect of LPS in the dark chamber was found [$F(1, 85) = 66.48$, $p = .000$]. LPS treated animals travelled significantly slower than those treated with saline, suggesting that LPS treated animals experienced lethargy.

Movement Time in the horizontal plane in both the dark and light chambers is shown in Figure 2.3: A – D. ANOVA analysis revealed a significant main effect of LPS treatment in the dark chamber [$F(1, 85) = 34.76$, $p = .000$]. LPS treated animals spent significantly less time moving in the horizontal plane relative to non-LPS treated animals, suggesting lethargy.

Time Rearing in the dark and light chambers is shown in Figure 2.4: A – D. A significant main effect of LPS was observed in both the dark [$F(1, 85) = 70.30$, $p = .000$] and light [$F(1, 85) = 55.86$, $p = .000$] chambers. Here, LPS treated animals exhibited less rearing behaviour in both chambers relative to non-LPS treated mice, demonstrating LPS induced reductions in locomotor activity.

The Number of Rearing Movements in the dark and light chambers is shown in Figure 2.5: A – D. ANOVA analysis revealed a significant main effect of LPS treatment for both the dark [$F(1, 85) = 83.34$, $p = .000$] and light [$F(1, 85) = 22.58$, $p = .000$]

chambers. LPS treated animals made significantly fewer rearing movements in both chambers relative to non-LPS treated groups, further reflecting an attenuation of locomotor activity following LPS treatment.

2.3.2. OT Agonist and OT Antagonist Effects on Locomotor Behaviour

In the dark chamber the OT agonist, CBT, was shown to affect locomotor behaviour in a dose-related manner for Speed, Time Rearing, and the Number of Rearing Movements. In particular, the 10mg/kg CBT-saline treated group tended to show reduced locomotor activity. OT antagonist treatment was also shown to affect locomotor behaviour in the dark chamber for Speed, Time Rearing, and the Number of Rearing Movements. OT antagonist-saline treated animals tended to show increased locomotor behaviour. Importantly, animals spent significantly more Time Rearing, and made a greater Number of Rearing Movements, than all other LPS groups, suggesting that OT antagonist treatment may attenuate aspects of sickness induced lethargy.

In the light chamber the OT agonist, CBT, was shown to affect locomotor behaviour in a dose-related manner for the Total Distances animals' travelled, such that 10mg/kg CBT treated mice showed reductions in locomotor behaviour for this variable. However, no other significant main effects or interactions were found for locomotor behaviour in the light chamber, suggesting context-related effects of OT on locomotor behaviour.

Total Distance travelled in the dark and light chambers is shown in Figures 2.1: A – D. There was a significant main effect of OT manipulation in the light chamber [$F(3,85) = 3.43$, $p = .021$]. Analysis of this main effect revealed a dose-related effect of the OT agonist, CBT, with the 10mg/kg treated mice travelling a significantly shorter distance than the 20 mg/kg CBT treated group ($p = .034$) (Fig. 2.1 C). However, neither group differed significantly from the control group (p's > .05). Main effects analysis also revealed a significant effect of the OT antagonist in the light chamber as these animals travelled significantly further compared to the 10 mg/kg CBT treated mice ($p = .003$) (Fig. 2.1 D). A significant LPS x OT interaction was not observed for Total Distances travelled in either the dark chamber [$F(3, 85) = 2.51$, $p = .064$] nor the light chamber [$F(3, 85) = 2.16$, $p = .099$].

Speed in the dark and light chambers is shown in Figure 2.2: A – D. A significant main effect of OT manipulation on Speed was found in the dark chamber [$F(3, 85) = 3.89$, $p = .012$]. Analysis of this main effect indicated that animals treated with the OT agonist, 10mg/kg CBT, travelled more slowly than those treated with 20mg/kg CBT ($p = .029$), but not saline controls ($p = .119$) (Fig. 2.2 A). Furthermore, effects of OT antagonist treatment were also found, where this group travelled significantly faster relative to the 10mg/kg CBT ($p = .002$) and saline control ($p = .039$) groups in the dark chamber (Fig. 2.2 B). A significant LPS x OT interaction was not found for either the dark chamber [$F(3, 85) = 2.48$, $p = .066$] nor the light chamber [$F(3, 85) = .623$, $p = .602$]. Furthermore, these locomotor activity effects on Speed were specific to the dark chamber as no significant main effects of OT or LPS, nor any significant interactions were found in the light chamber.

Movement Time in the horizontal plane in both the dark and light chambers is shown in Figure 2.3: A – D. Significant main effects of OT were not observed. Moreover, a significant LPS x OT interaction was not found in either the dark [$F(3, 85) = 1.56$, $p = .206$] or light chambers [$F(3, 85) = .29$, $p = .833$].

Time Rearing in the dark and light chambers is shown in Figure 2.4: A – D. In the dark chamber, the ANOVA revealed a significant main effect of OT [$F(3, 85) = 2.84$, $p = .043$] on Time Rearing. Main effects analysis showed that mice treated with 10mg/kg CBT reared significantly less than animals treated with the OT antagonist ($p = .006$), but not saline controls ($p = .073$) (Fig. 2.4 A). This effect was specific to the dark chamber as no significant differences were observed in the light chamber. In the dark chamber a significant LPS x OT interaction was found [$F(3, 85) = 2.95$, $p = .038$] for Time Rearing (Fig. 2.4 B). Post hoc analyses revealed dose related effects of CBT in the dark chamber as the 10mg/kg CBT-saline animals spent significantly less time rearing than the 20mg/kg CBT-saline group ($p = .014$) and the saline-saline group ($p = .017$) . These data are again suggestive of suppressive effects of the 10mg/kg CBT dose on locomotor activity, though these effects appear to be context dependent as they were only observed in the dark chamber. Furthermore, OT antagonist-LPS treated animals spent significantly more time rearing than all other LPS groups (p's $\leq .011$) providing evidence that OT antagonist treatment may attenuate aspects of sickness induced lethargy.

The Number of Rearing Movements in the dark and light chambers is shown in Figure 2.5: A – D. In the dark chamber, the main effect of OT manipulation approached significance [$F(3, 85) = 2.65$, $p = .054$]. This trend was again driven by dose-specific effects of OT agonist, where the 10mg/kg CBT treated group made fewer rearing movements compared to the OT antagonist group ($p = .006$), but not saline controls ($p = .223$) (Fig. 2.5 A). A significant main effect of OT manipulation in the light chamber was not observed. In the dark chamber a significant LPS x OT interaction was also found [$F(3, 85) = 2.97$, $p = .036$] on the Number of Rearing Movements animals made (Fig. 2.5 B). Post hoc analyses indicated that dose-related effects of CBT treatment occurred as the 10 mg/kg CBT-saline treated animals made significantly fewer rearing movements compared to the 20 mg/kg CBT-saline group ($p = .024$), though not saline controls ($p = .084$). This effect further highlights the dose-related effects of CBT treatment, and in particular that the 10 mg/kg CBT dose may have suppressive effects on locomotor behaviour. Furthermore, in the dark chamber the OT antagonist-LPS treated animals made significantly more rearing movements relative to all other LPS groups (p's $\leq .008$), suggesting an attenuation of sickness induced lethargy. These effects appear to be context-dependent as effects of OT on locomotor behaviour only occurred in the dark chamber.

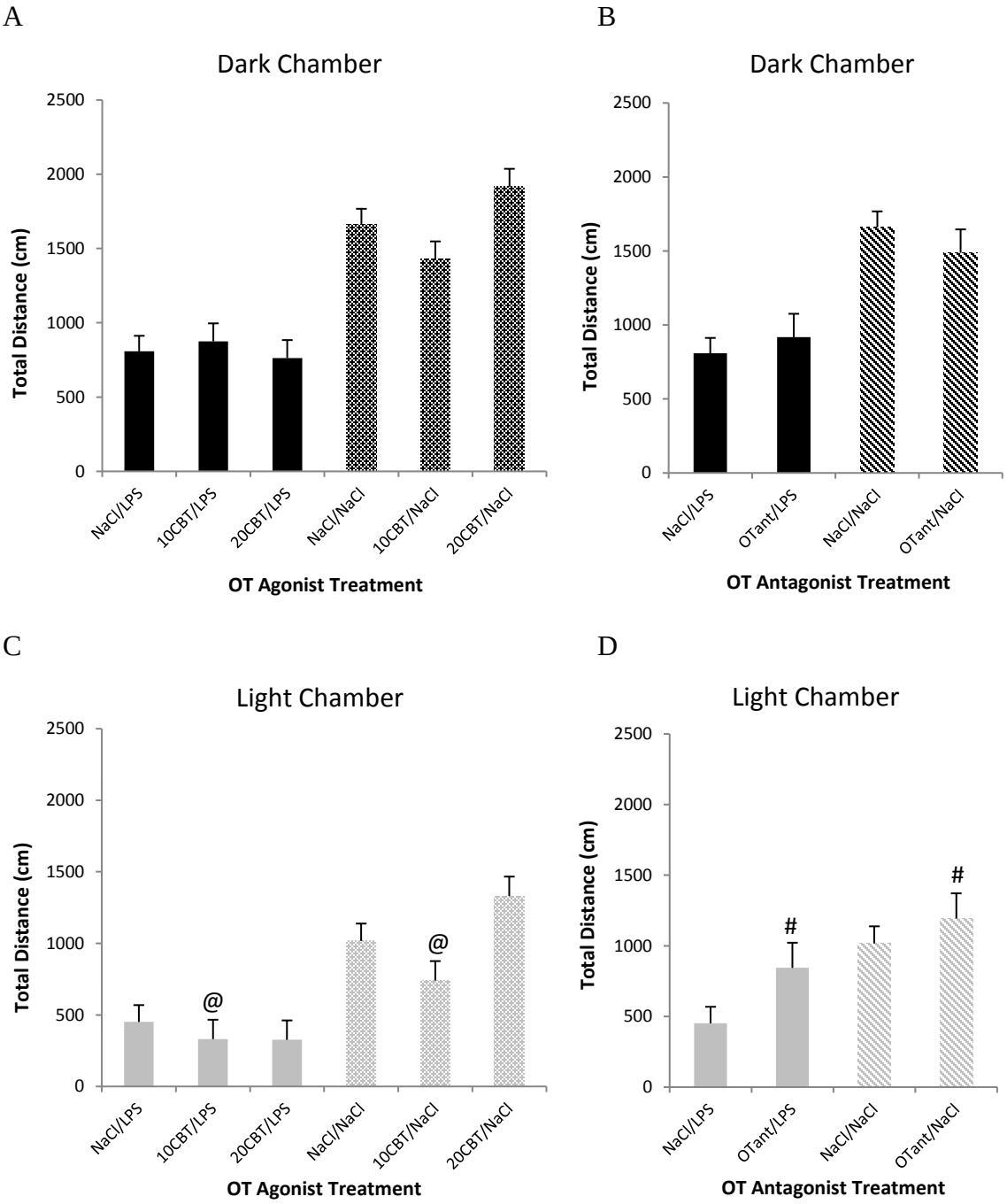


Fig. 2.1. (A – D) Mean Total Distance travelled in the dark (A – B) and the light (C – D) chamber during a 15 minute testing session for mice treated i.p. with an OT agonist (CBT), or OT antagonist, plus LPS or saline. Control groups are displayed twice for ease of data comparison. Vertical lines indicate standard error. LPS groups travelled significantly less in both light and dark chambers than non-LPS groups ($p \leq .001$; not marked on graph). In the light chamber, 10 mg/kg CBT treatment significantly suppressed distances travelled relative to 20 mg/kg CBT (@ $p = .034$). OT antagonist treated mice in the light chamber travelled significantly further compared to the 10 mg/kg CBT treated mice (# $p = .003$).

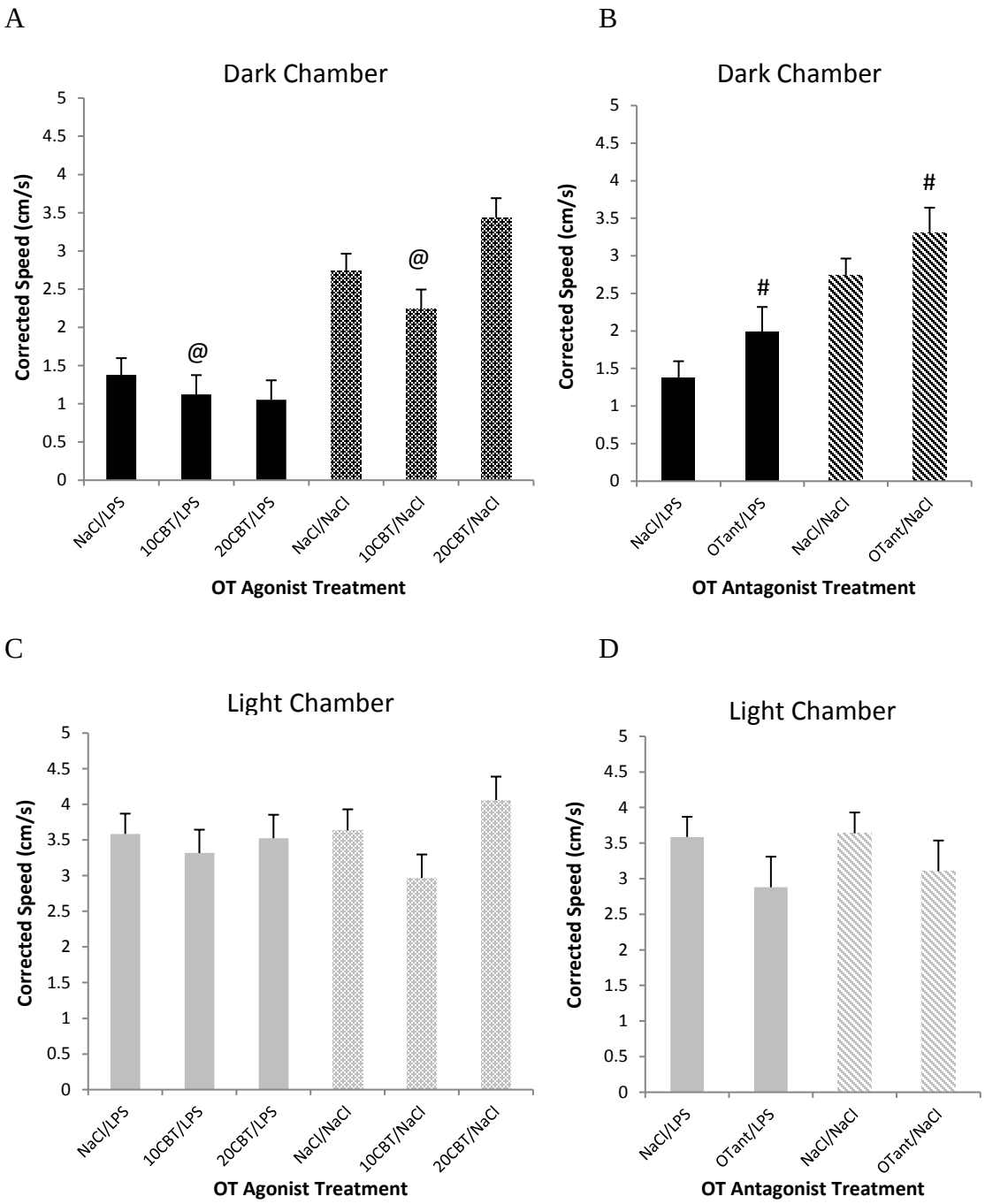


Fig. 2.2. (A – D) Mean Speed in the dark (A – B) and light (C – D) chamber during a 15 minute testing session for mice treated i.p. with an OT agonist (CBT), or OT antagonist, plus LPS or saline. Control groups are displayed twice for ease of data comparison. Vertical lines indicate standard error. LPS treated groups moved significantly slower in the dark chamber relative to non-LPS treated animals ($p \leq .001$; not marked on graph). In the dark chamber, 10 mg/kg CBT treatment significantly attenuated Speed relative to 20mg/kg CBT (@ *p*'s = .029). OT antagonist treatment significantly augmented Speed in the dark chamber relative to 10 mg/kg CBT and saline controls (# *p's* $\leq$ .039).

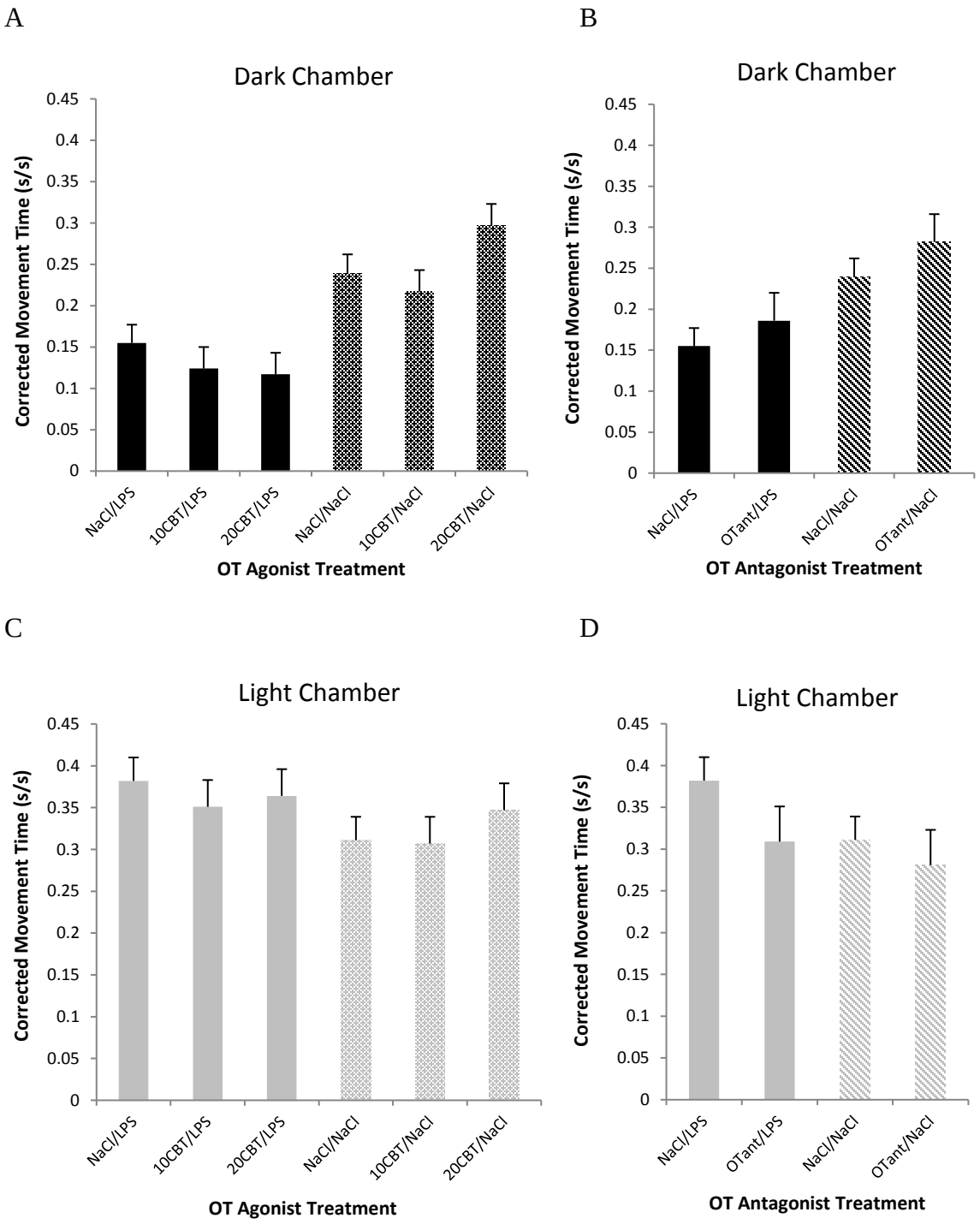


Fig. 2.3. (A – D) Mean horizontal Movement Time in the dark (A – B) and the light (C – D) chamber during a 15 minute testing session for mice treated i.p. with an OT agonist (CBT), or OT antagonist, plus LPS or saline. Control groups are displayed twice for ease of data comparison. Vertical lines indicate standard error. In the dark chamber, LPS groups travelled significantly less in the horizontal plane compared to saline groups ($p \leq .001$; not marked on graph).

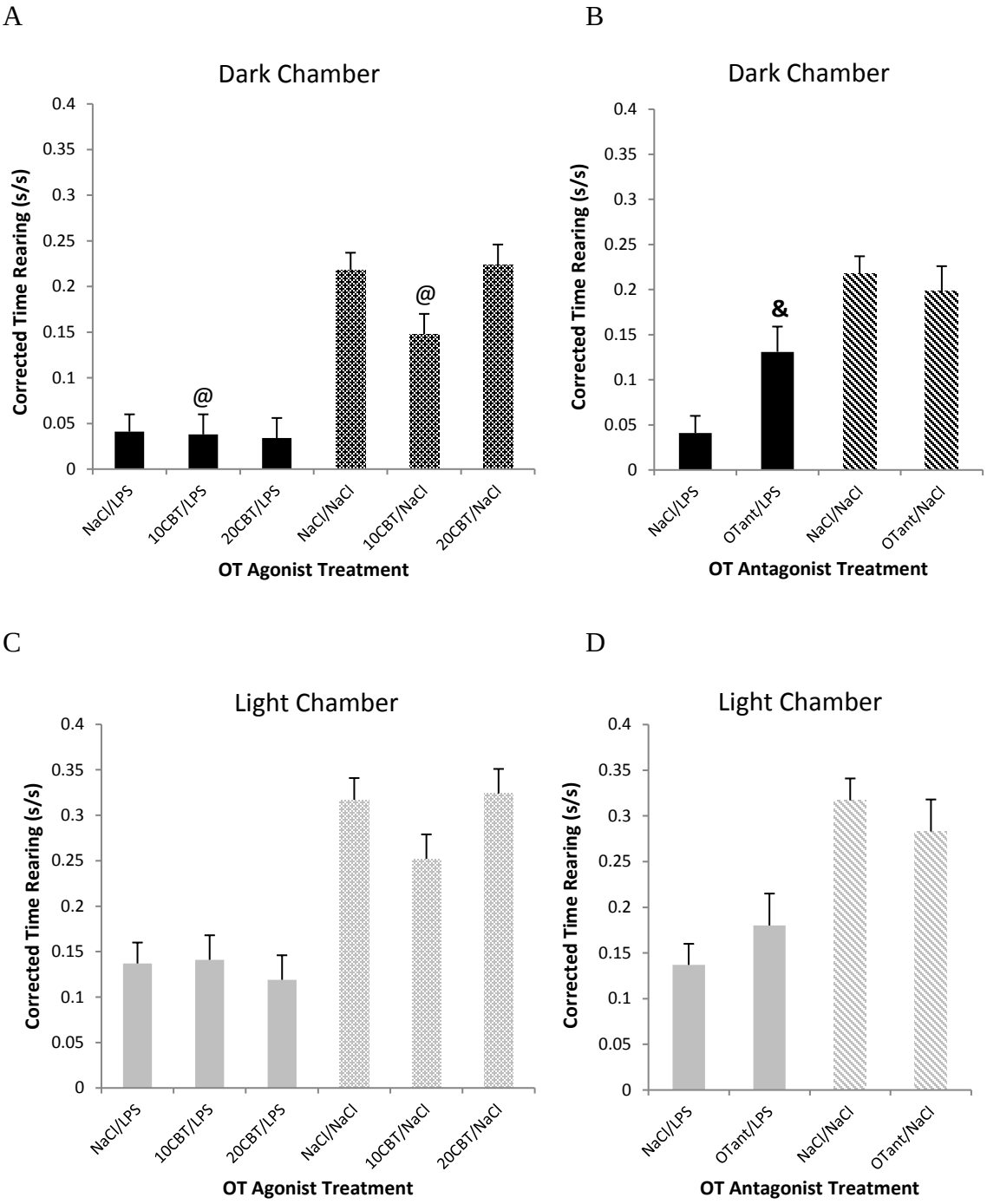


Fig. 2.4. (A – D) Mean Time Rearing in the dark (A – B) and light (C – D) chamber during a 15 minute testing session for mice treated i.p. with an OT agonist (CBT), or OT antagonist, plus LPS or saline. Control groups are displayed twice for ease of data comparison. Vertical lines indicate standard error. LPS groups spent significantly less time rearing in both chambers than non-LPS groups ($p \leq .001$; not marked on graph). In the dark chamber, OT antagonist-LPS mice reared significantly more relative to all other LPS groups (& p's $\leq .011$). The 10 mg/kg CBT-saline mice spent significantly less time rearing than 20 mg/kg CBT-saline and saline-saline control groups (@ p's $\leq .017$).

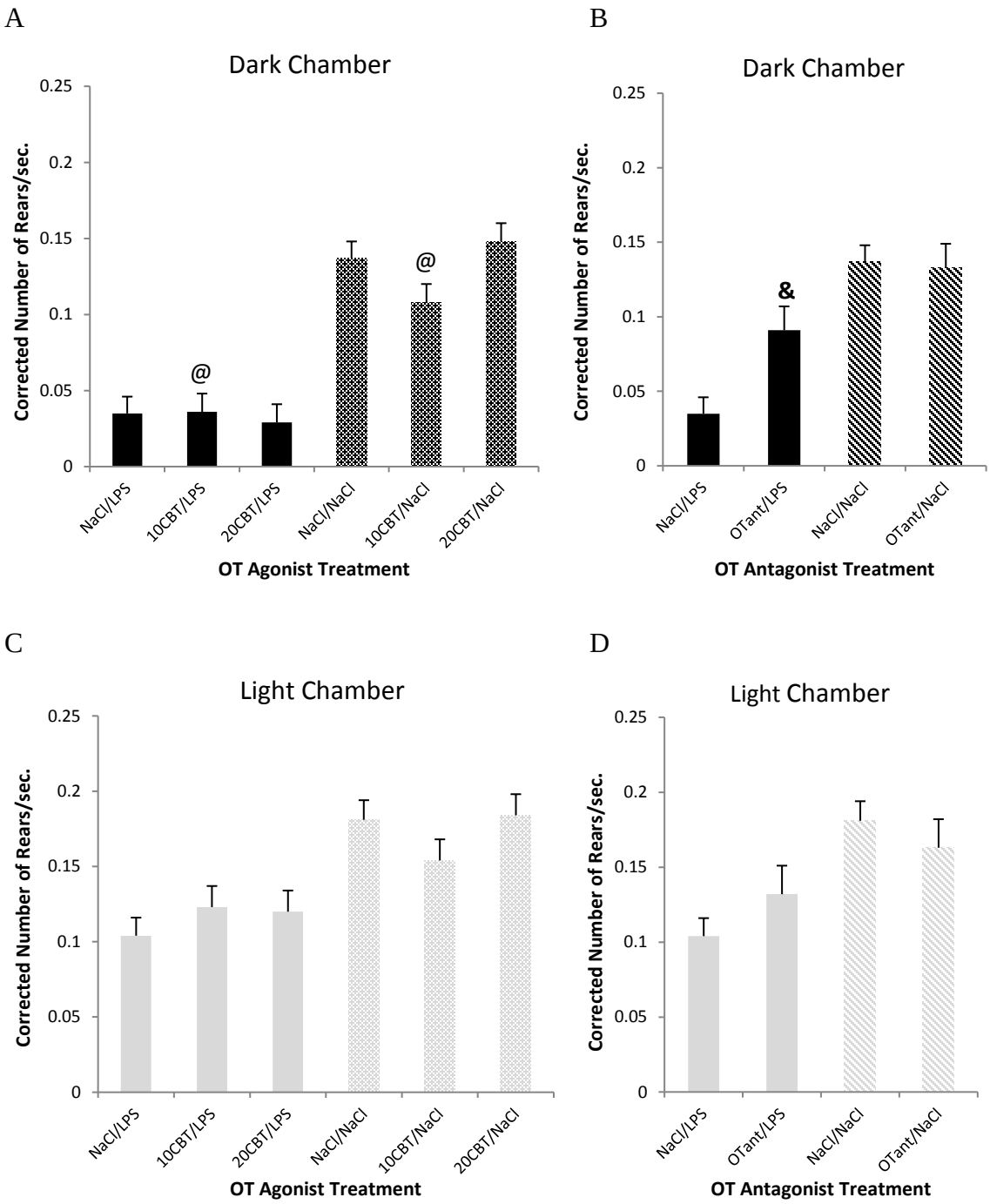


Fig. 2.5. (A – D) Mean Number of Rearing Movements in the dark (A – B) and light (C – D) chamber during a 15 minute testing session for mice treated i.p. with an OT agonist (CBT), or OT antagonist, plus LPS or saline. Control groups are displayed twice for ease of data comparison. Vertical lines indicate standard error. LPS groups spent significantly less time rearing in both chambers than non-LPS groups ($p \leq .001$; not marked on graph). In the dark chamber, OT antagonist-LPS treated mice made significantly more rearing movements compared to all other LPS treated groups (& p's $\leq .008$) and 10 mg/kg CBT-saline mice spending significantly less time rearing relative to the 20 mg/kg CBT-saline group (@ $p = .024$).

2.3.3. Effects of LPS on Anxiety-like Behaviour

In the light chamber LPS treatment increased anxiety-like behaviour as indicated by the reduced duration of Time animals spent in the light chamber, along with fewer Nose Pokes and Transitions into the light chamber, indicating sickness-induced anxiety-like behaviour.

Time in the light chamber is shown in Figure 2.6: A – B. Time in the dark chamber was not analyzed because the light-dark test was conducted in a forced-choice 2 chamber apparatus and thus analysis would have yielded identical statistics to those stemming from the light chamber. ANOVA analysis revealed a significant main effect of LPS treatment [$F(1, 85) = 21.71$, $p = .000$], such that LPS animals spent significantly less time in the light chamber ($p \leq .001$). This finding is indicative of the immune challenge augmenting anxiety-like behaviour as LPS animals spent less time in the light chamber relative to saline treated mice.

Number of Nose Pokes into the light chamber is illustrated in Figure 2.7: A – B. The ANOVA revealed a significant main effect of LPS for Nose Pokes [$F(1, 85) = 44.51$, $p = .000$], whereby LPS treated animals made significantly fewer nose poke investigations into the light chamber, suggesting increased anxiety.

The Number of Transitions into the light chamber is shown in figure 2.8: A – B. The ANOVA revealed a significant main effect of LPS treatment [$F(1, 85) = 45.49$, $p = .000$], as LPS groups made significantly fewer transitions into the light chamber than did saline groups (p's $< .001$), indicative of enhanced anxiety.

2.3.4. OT agonist and OT antagonist Effects on Anxiety-like Behaviour

In the light chamber the OT agonist, CBT, was shown to affect anxiety-like behaviour for the Number of Nose Pokes and also Transitions into the light chamber in a dose-related manner. Specifically, mice treated with the 20mg/kg CBT dose made more Nose Pokes overall, and the 20mg/kg-saline, but not the 10mg/kg CBT-saline, treated mice made significantly more nose pokes into the light chamber. Furthermore, the 10mg/kg CBT treated mice made fewer transitions into the light chamber overall.

Effects on anxiety-like behaviour in the light chamber for the OT antagonist, L-368,899, were also found for duration of Time spent in the light chamber, as well as for the Number of Nose Pokes and for Transitions into the light chamber. OT antagonist

treated mice spent more time in the light chamber relative to both OT agonist groups, and saline controls. As well, OT antagonist treated mice showed a greater number of Nose Pokes and Transitions into the light chamber, suggesting an attenuation of anxiety. Importantly, OT antagonist-LPS mice showed a greater number of Nose Pokes into the light chamber, demonstrating anxiolytic effects of the OT antagonist on sickness-induced anxiety-like behaviour.

Time in the light chamber is shown in Figure 2.6: A – B. Time in the dark chamber was not analyzed because the light-dark test was conducted in a forced-choice 2 chamber apparatus and thus analysis would have yielded identical statistics to those stemming from the light chamber. For duration of Time spent in the light chamber, a significant main effect of OT treatment was found [$F(3, 85) = 4.21$, $p = .008$]. Main effects analysis indicated that OT antagonist treated animals spent significantly more time in the light chamber relative to both the 10mg/kg ($p = .001$) and the 20mg/kg ($p = .013$) CBT treated groups, and saline control animals ($p = .008$) (Fig. 2.6 B). No significant LPS x OT interactions were observed [$F(3, 85) = .414$, $p = .744$].

Number of Nose Pokes into the light chamber is illustrated in Figure 2.7: A – B. The ANOVA revealed a significant main effect of OT on Number of Nose Pokes into the light chamber [$F(3, 85) = 2.95$, $p = .037$]. Animals treated with the 20mg/kg CBT dose or the OT antagonist made significantly more Nose Pokes into the light chamber compared to saline treated animals ($p's \leq .036$) (Fig. 2.7: A – B). A significant LPS x OT interaction was also identified for Nose Pokes into the light chamber [$F(3, 85) = 3.17$, $p = .028$] (Fig. 2.7 B). Dose-related effects of CBT treatment were again observed as the 20mg/kg-saline, but not the 10mg/kg CBT-saline, treated animals made significantly more nose pokes into the light chamber compared saline-saline controls ($p = .002$). Furthermore, post hoc analyses also revealed that the OT antagonist-LPS treated animals made significantly more nose pokes into the light chamber relative to all other LPS groups (p's $\leq .033$). This provides additional evidence that OT antagonist treatment moderates the effect of LPS, here reducing LPS induced anxiety-like behaviour.

The Number of Transitions into the light chamber is shown in figure 2.8: A – B. The ANOVA revealed a significant main effect of OT for transitions into the light chamber [$F(3, 85) = 2.88$, $p = .041$]. Analysis of main effects indicated that the 10mg/kg

CBT group made significantly fewer transitions into the light chamber compared to the OT antagonist group (p = .016), but not the 20 mg/kg group nor saline controls (Fig. 2.8: A – B). Furthermore, the OT antagonist animals transitioned more into the light chamber than saline controls (p = .031). A significant LPS x OT interaction was not observed for Transitions into the light chamber [$F(3, 85) = 2.22$, p = .091].

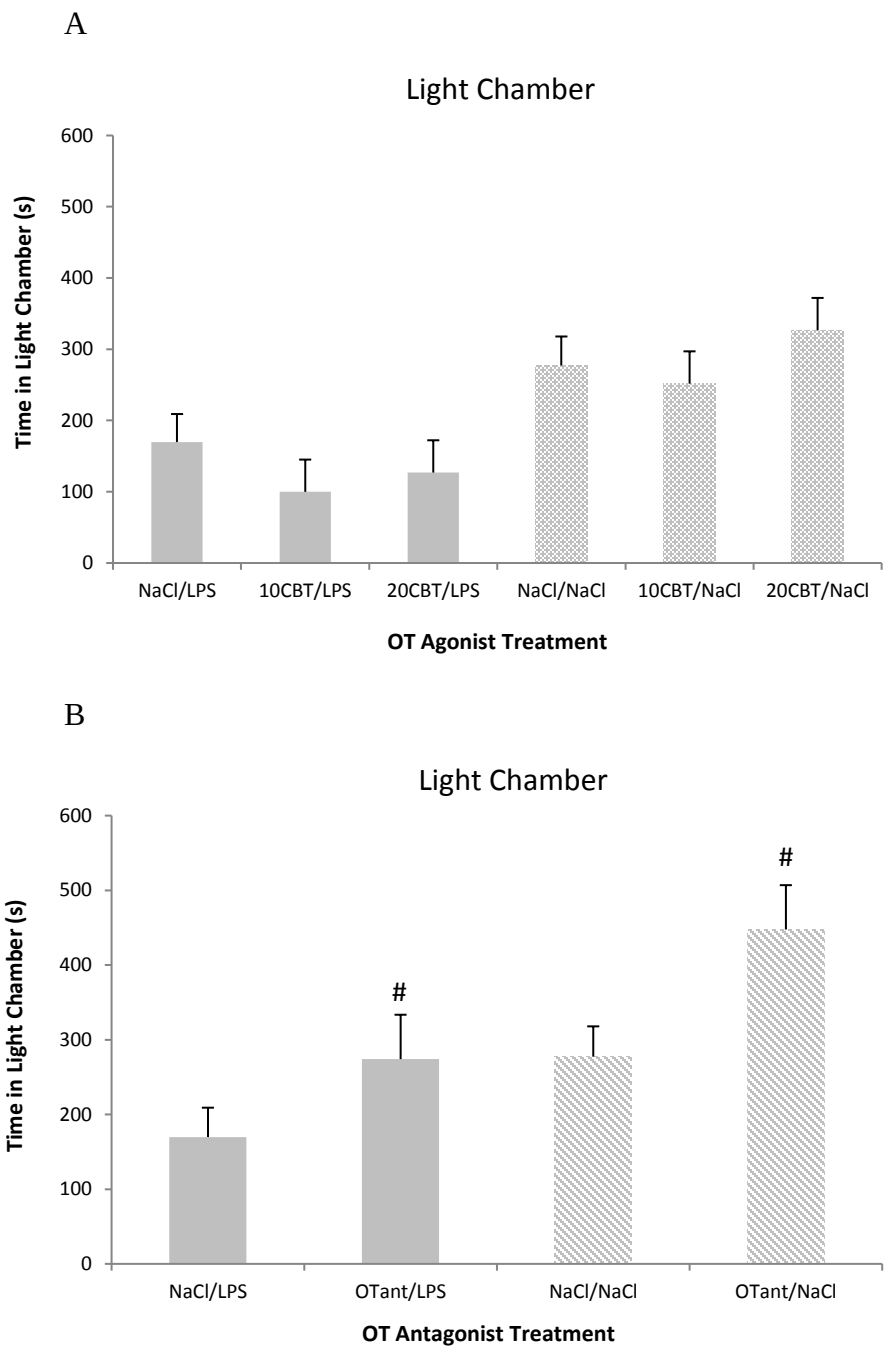
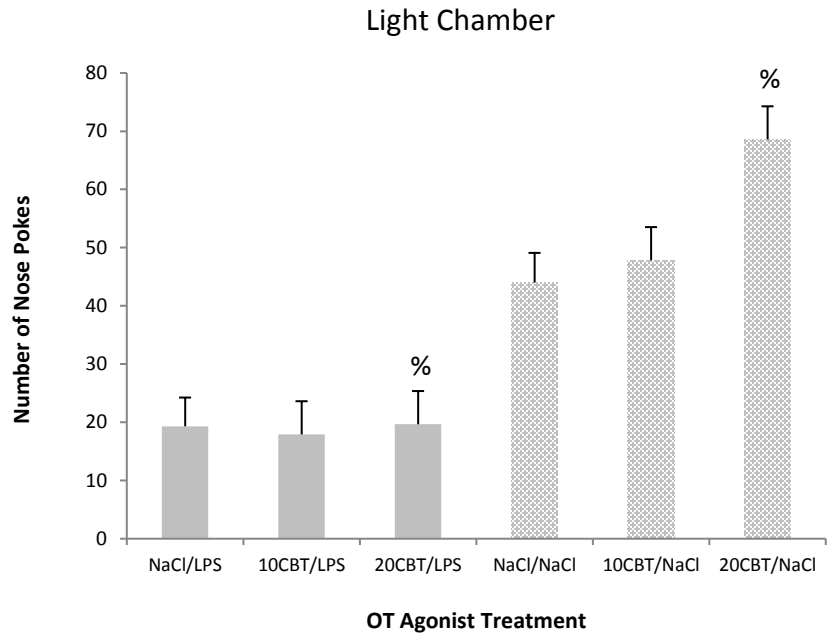


Fig. 2.6. (A – B) Mean Time spent in the light chamber (A – B) during a 15 minute testing session for mice treated i.p. with an OT agonist (CBT), or OT antagonist, plus LPS or saline. Control groups are displayed twice for ease of data comparison. Vertical lines indicate standard error. LPS treated animals spent significantly less time in the light chamber relative to non-LPS treated groups of animals ($p \leq .001$; not marked on graph). OT antagonist treated mice spent significantly more time in the light chamber compared to CBT treated groups and the saline control group (# p's $\leq .013$).

A



B

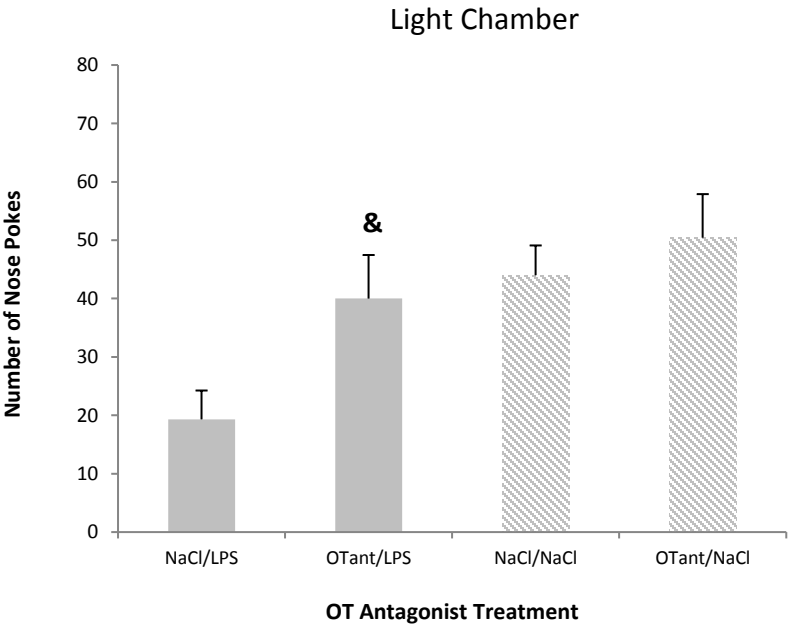
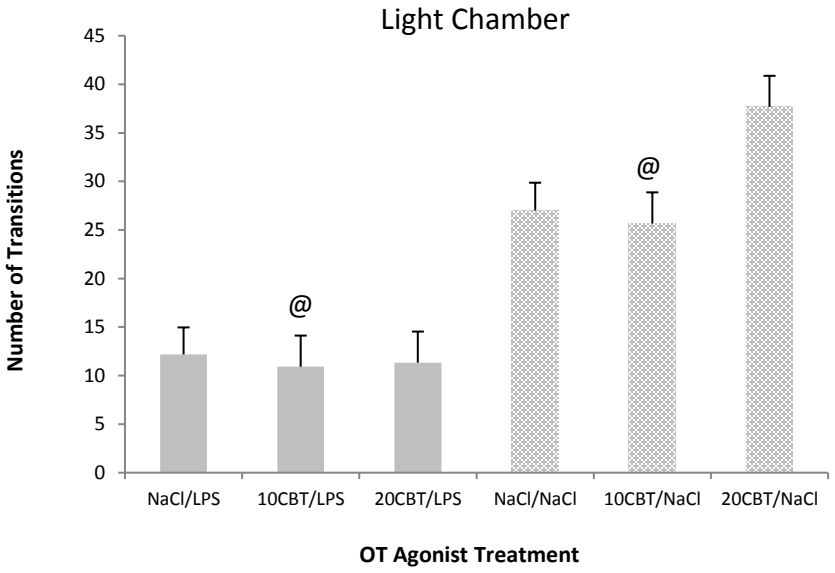


Fig. 2.7 (A – B) Mean number of Nose Pokes into the light chamber (A – B) during a 15 minute testing session for mice treated i.p. with an OT agonist (CBT), or OT antagonist, plus LPS or saline. Control groups are displayed twice for ease of data comparison. Vertical lines indicate standard error. LPS treated animals spent significantly less time in the light chamber relative to non-LPS treated groups of animals ($p \leq .001$; not marked on graph). OT antagonist-LPS animals made significantly more nose pokes compared to all other LPS treated groups (& p's $\leq .033$) and 20mg/kg CBT-saline made significantly more nose pokes than saline-saline control animals (% $p = .002$).

A



B

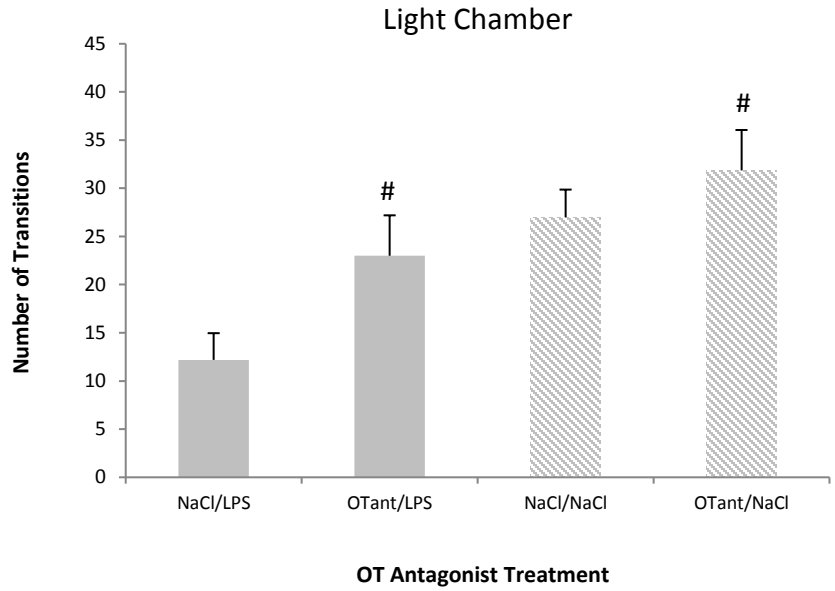


Fig. 2.8 (A – B) Mean number of Transitions into the light chamber (A – B) during a 15 minute testing session for mice treated i.p. with an OT agonist (CBT), or OT antagonist, plus LPS or saline. Control groups are displayed twice for ease of data comparison. Vertical lines indicate standard error. LPS treated animals spent significantly less time in the light chamber relative to non-LPS treated groups of animals ($p \leq .001$; not marked on graph). 10 mg/kg CBT mice made significantly fewer transitions into the light chamber compared to OT antagonist treated mice (@ $p = .016$). OT antagonist animals made significantly more transitions into the light chamber relative to saline control animals (# $p = .031$).

2.4 Discussion

To date few studies have examined the effects of OT on the expression of sickness behaviours, including locomotor and anxiety-like behaviour. Results of the present study demonstrate that treatment with the OT antagonist, L-368,899, in conjunction with 75 μg/kg of LPS attenuates locomotor and anxiety-like sickness-related behaviours. Furthermore, dose-related effects of the OT agonist, carbetocin (CBT), on basal locomotor activity and anxiety-like behaviour were also shown. In particular, in conjunction with saline treatment the 10 mg/kg dose of CBT suppressed both horizontal and vertical locomotor behaviours, and augmented anxiety-like responses.

2.4.1. Effects of OT on Sickness Behaviours

Consistent with previous research (Engeland et al., 2001; Tenk et al., 2008), 75μg/kg of LPS treatment elicited a robust attenuation of both horizontal and vertical locomotor activity indicating sickness behaviour, as male CD-1 mice showed significant increases in lethargy in both chambers of the LD test. LPS treatment also significantly augmented anxiety-like behaviour in the light-dark test as demonstrated by significant reductions in the duration of time that animals spent in the light chamber, as well as fewer Nose Pokes and Transitions into the light chamber. This finding agrees with previous accounts of sickness-induced increases in anxiety-like behaviour characterized by an avoidance of brightly lit open spaces and more threatening contexts (Banasikowski et al., 2015; Bourin & Hascoet, 2003).

The present study revealed that treatment with the OT antagonist, L-368,899, suppressed LPS-induced perturbations on locomotor and anxiety-like behavioural responses, while the OT agonist had no effect on sickness behaviour. Specifically, it was found that OT antagonist-LPS treated mice display increased time rearing, and an increased number of rearing movements in the dark chamber relative to all other LPS groups, illustrating the suppressive effect of the OT antagonist on LPS-induced lethargy. While this effect was not observed in the light chamber, previous research suggests that sickness behaviours are expressed in a context-dependent manner. In particular, sickness behaviours are more strongly expressed in the dark versus light chamber due to motivational reorganization since it is not adaptive for rodents to appear sick in threatening contexts, such as brightly lit open spaces (Arrant et al., 2013). As such, the

dark chamber may be a more accurate representation of the true level of sickness behaviour. OT antagonist-LPS mice also showed attenuation of sickness-induced anxiety-like behaviour as these animals made significantly more Nose Poke investigations into the light chamber (ie. the more anxiogenic context) relative to all other LPS treated groups. As Figures 2.6 (B), 2.7 (B) and 2.8 (B) illustrate, OT antagonist-LPS treated mice behaved comparably to that of saline control mice for all anxiety measures. These findings are suggestive of OT antagonist treatment attenuating sickness behaviour by normalizing animals' behaviour to levels similar to that of saline-control animals.

There have been limited investigations of the effects of OT on the expression of sickness behaviours. However, Reyes-Lagos et al. (2016) did investigate OT's role in mediating heart rate fluctuations, body temperature, and locomotor activity in adult male rats challenged with LPS. They found that in LPS treated animals OT decreased mean heart rate, moderated LPS-induced hyperthermia, and increased locomotor activity for up to 6 hours post LPS treatment (Reyes-Lagos et al., 2016). This finding is in contrast with that of the present study, which found that the OT agonist had no effect on sickness behaviours, while the OT antagonist suppressed sickness-induced locomotor and anxiety-like behavioural perturbations.

The difference in findings between the present study and that of Reyes-Lagos et al. (2016) could largely be due to methodological differences. Specifically, in their study locomotor activity was evaluated using telemetric electrocardiogram recording in freely moving rats in the home cage, whereas in the present study locomotor activity was evaluated in the more anxiogenic and stressful context of the light-dark test. Thus, in this study the more stressful context may have affected HPA-axis mediated OT mechanisms, causing differential effects on locomotor behaviour. Thus it is possible that the contextual differences between studies may, at least to some extent, account for the differential behavioural findings. Regardless, findings from the present study suggest that suppression of systemic OT may postpone the onset of sickness behaviour expression, or that it may block LPS induced pro-inflammatory mechanisms and suppress manifestation of sickness behaviours. However, the underlying physiological mechanisms responsible for these behavioural effects can only be speculated at this time. As such, more research

is needed to investigate OT's apparent role in sickness behaviours, and elucidate underlying physiological mechanism that affect the expression of such behaviours.

2.4.2. Effects of OT Agonist Treatment

Treatment with the OT agonist, CBT, yielded dose-related effects on locomotor and anxiety-like behaviour. Specifically, the 10 mg/kg CBT treatment resulted in depressed locomotor activity in both the dark and light chamber as animals travelled shorter distances, more slowly, spent less time rearing, and made fewer rearing movements. This effect was not observed among the 20 mg/kg CBT or saline-control groups. These findings suggest that a dose of 10 mg/kg OT agonist suppressed locomotor activity in non-novel contexts. Interestingly, the 10 mg/kg CBT group demonstrated a reduced number of transitions into the light chamber relative to the 20 mg/kg CBT group, indicative of the 10 mg/kg CBT treatment augmenting anxiety-like responses and reducing exploratory behaviour in this context. In addition, the 20 mg/kg CBT treated animals made significantly more Nose Pokes into the light chamber relative to saline-controls, suggestive of attenuated anxiety-like responses.

Consistent with previous studies, the results of this study demonstrate dose-related effects of OT on measures of anxiety in male rodents. For instance, Ring et al. (2006) observed dose related effects of OT as measured by the four-plate test (FPT) in adult male mice. However, in contrast with the present findings, Ring et al. (2006) found that administration of 10 mg/kg (i.p.) of OT 30 minutes prior to testing led to increased punished crossings, suggesting an anxiolytic effect of peripheral OT. Conversely, the present study showed that a dose of 10 mg/kg (i.p.) resulted in anxiogenic effects on behaviour. Chaviaras et al. (2010) found that in the open field test male rats treated with a high (ie. 20 mg/kg) but not low (ie. 2 mg/kg; 6.4 mg/kg) dose (i.p.) of OT agonist, carbetocin, 30 minutes prior to testing showed a significant decrease in total distances animals' travelled, suggesting suppressed locomotor activity and increased anxiety. Again these findings are in contrast to those of the present study, as here it was found that the 20 mg/kg CBT (i.p.) dose resulted in anxiolytic effects on behaviour.

The differences observed between the results of this and other studies could be attributed to the amount of time elapsed between OT administration and commencement of behavioural testing. In this study, behavioural testing did not occur until 2 hours after

OT agonist treatment, which is longer than the time elapsed between OT treatment and behavioural testing in other studies (Chaviaras et al., 2010; Ring et al., 2006). This differential finding may suggest that temporal variables play a role in mediating OT's effects on anxiety-like behaviour, with OT exerting anxiolytic effects shortly after treatment, but having anxiogenic effects in a dose-related manner, following longer time delays between administration and testing. Importantly, another consideration for the present findings is that different anxiety tests will often produce different outcomes on behaviour. For instance, Hascoet et al. (2000) showed differential drug effects in a dose-related manner between the light-dark and four plate tests in mice.

Taken together, the results of this study in conjunction with previous research illustrate the dose-related and potentially temporally-dependent nature of anxiolytic and anxiogenic effects of OT on behaviour. Specifically, the present study confirms dose-related effects of OT on behaviour as seen in previous studies. However, this study has also demonstrated that temporal and context-dependent effects exist. Compared to other studies that administered a 10mg/kg dose of OT but waited only 30 minutes between drug treatment and testing (Chaviaras et al., 2010; Ring et al., 2006), the 2 hour latency between OT administration and behavioural testing in the present study seemed to affect mice differently at this dose, increasing anxiety-like behaviour and suppressing locomotor activity. These findings also illustrate novel context-dependent effects of OT in relation to anxiety and locomotor behaviour, as previous studies examining OT have used other measures of anxiety, such as the elevated zero maze and four plate test (eg. Ring et al., 2006), finding differential effects on these behaviours compared to those of the current study.

2.4.3. Effects of OT Antagonist Treatment

Treatment with the OT antagonist, L-368-899, yielded intriguing results as animals showed increased locomotor activity in the dark chamber, travelled faster, and spent more time rearing relative to other groups. Furthermore, in the light chamber OT antagonist treated mice travelled a greater distance compared to other groups. These data suggest that a 10mg/kg (i.p.) dose of the OT antagonist may enhance locomotor and exploratory behaviour in male CD-1 mice. Behavioural anxiety indicators were also shown to be affected by treatment with the OT antagonist. In particular, OT antagonist

treated mice spent significantly more time in the light chamber, indicating anxiolytic-like behaviour. Furthermore, OT antagonist treated animals made significantly more Nose Pokes and Transitions into the light chamber, indicating an overall reduction in anxiety-like behaviour.

The present findings are in contrast with those of Ring et al. (2006) who found that the OT antagonist, WAY-162720, at 10 mg/kg (i.p.) 30 minutes prior to testing reversed the anxiolytic effects of OT in the four plate test, thereby demonstrating anxiogenic effects of peripheral OT antagonist treatment on behaviour. However, more recent studies have shown an effect consistent with that of the present study, as others have found that OT antagonist treatment has either no effect, or anxiolytic effects, in certain contexts. For example, Duque-Wilckens et al. (2016) showed that in female mice treated with L-368,899 (i.p.) 30 min before behavioural testing, the OT antagonist blocked the effects of stress in both the social interaction and odor preference tests, such that animals showed high levels of social interaction, and preferred the odor of an unfamiliar individual. Conversely, when stressed, saline treated mice showed reduced social interaction and a preference for the odor of a familiar cagemate, suggesting that OT antagonist treatment has anxiolytic effects on behaviour in social contexts (Duque-Wilckens et al., 2016). Similarly, Arakawa et al. (2015) showed that in male mice OT antagonist treatment had limited effects on social approach behaviour, although it did significantly decrease the amount of social engagement time. These conflicting findings again demonstrate the role of context, temporal, and test-related effects of OT on behaviour (Hascoet et al., 2000; Ring et al., 2006).

2.4.4. Limitations and Future Directions

Methodological limitations within the present study may account for the results observed. In particular, calculating the dose of OT drugs to be used in mice was challenging as there are relatively few existing studies with which to determine dosages for mice. Furthermore, time-related factors, such as the 2 hour latency between OT treatment and behavioural testing, likely also influenced behavioural findings. As most studies conduct behavioural testing 30 minutes after OT treatment due to the relatively short half-life of OT (eg. Chaviaras et al., 2010; Ring et al., 2006), in the present study behavioural effects of OT may have been affected differently due to lower systemic

levels of OT, thus reducing its effects on behaviour. Future studies could examine OT's effects on sickness and anxiety-like behaviour in mice at the same dosages used in this study, along with other doses, to elucidate dose-related effects of OT in mice. As well, examining OT's effects on sickness and anxiety-like behaviours at different time points after drug administration may help to clarify time-related effects of OT on behaviour.

Another methodological limitation of this study can be attributed to the nature of the behavioural task, and difficulty distinguishing locomotor behaviour exclusively from anxiety-like behaviour. It is possible that other tests of sickness and anxiety-like behaviour in rodents, such as those relating to social behaviour, may have yielded different results. As such, using other behavioural assays might be an effective way to resolve this limitation in the future. As OT has consistently been linked to mediation of social behaviours, such as social affiliation (Choleris et al., 2013; Ross & Young, 2009), and LPS induced sickness has been shown to affect social behaviour in rodents (Kavaliers & Choleris, 2011), it would be useful to employ social behavioural paradigms to examine OT's effects on sickness behaviour in social contexts where OT mediated behaviours are more likely to be affected by social-contextual cues (Shamay-Tsoory & Abu-Akel, 2016). An example of this could be the social preference test, which is a well-established model in animals for studying OT's effects on social behaviour (Toth & Neumann, 2013). Another potential route could be to apply the socially based odor preference test to examine how OT affects sickness behaviour and pathogen avoidance in rodents (Arakawa et al., 2015; Kavaliers & Choleris, 2011).

With respect to sex differences, OT may play a less important role in moderating behaviour in male animals, compared to females. In females, the estrous cycle has been shown to influence immune function and sickness behaviour in a manner that is dependent on estrous phase (Engeland et al., 2006). As such, future studies could examine effects of OT on sickness and anxiety-like behaviours more thoroughly in both males, and in females, by tracking estrous cycle to determine if and how OT differentially affects behavioural responses based on estrous hormone levels.

2.4.5. Conclusions

This study showed that treatment with the OT antagonist, L-368,899, attenuated sickness induced LPS perturbations in locomotor activity and anxiety-like behaviour,

effectively normalizing sickness behaviours. The exact mechanism by which this occurred remains to be elucidated. The present study demonstrates that OT antagonists do affect locomotor and anxiety-like behaviour related to sickness, and in particular that the absence of OT may lead to later onset of sickness behaviours, or perhaps suppress sickness behaviour altogether. It was also found that 10 mg/kg-saline treatment of the OT agonist, CBT, exerts suppressive effects on locomotor and anxiety-like behaviour in the LD test. Given that studies using OT antagonists tend to show increased anxiety-like behaviour in rodents, although a few studies have shown either no affect or anxiolytic effects, the finding that OT antagonist treatment augments locomotor activity and suppresses anxiety-like behaviour in male CD-1 mice is intriguing. Importantly, this finding illustrates that observed behavioural effects may be highly dose- and context-related. Evidently, further research is needed to explore OT's effects on behaviour, and in particular to distinguish any dose- and time-related effects of OT on sickness behaviour.

2.5 References

Anisman, H., & Merali, Z. (2002). Cytokines, stress, and depressive illness. *Brain, Behavior, and Immunity, 16*(5), 513-524. doi:10.1016/s0889-1591(02)00009-0

Anisman, H., Merali, Z., & Hayley, S. (2003). Sensitization associated with stressors and cytokine treatments. *Brain, Behavior, and Immunity, 17*(2), 86-93. doi:10.1016/s0889-1591(02)00100-9

Arakawa, H., Blanchard, D. C., & Blanchard, R. J. (2015). Central oxytocin regulates social familiarity and scent marking behavior that involves amicable odor signals between male mice. *Physiology & Behavior, 146*, 36-46. doi:10.1016/j.physbeh.2015.04.016

Arrant, A. E., Schramm-Sapyta, N. L., & Kuhn, C. M. (2013). Use of the light/dark test for anxiety in adult and adolescent male rats. *Behavioural Brain Research, 256*, 119-127. doi:10.1016/j.bbr.2013.05.035

Aubert, A. (1999). Sickness and behaviour in animals: A motivational perspective. *Neuroscience & Biobehavioral Reviews, 23*(7), 1029-1036. doi:10.1016/s0149-7634(99)00034-2

Banasikowski, T. J., Cloutier, C. J., Ossenkopp, K., & Kavaliers, M. (2015). Repeated exposure of male mice to low doses of lipopolysaccharide: Dose and time dependent development of behavioral sensitization and tolerance in an automated light–dark anxiety test. *Behavioural Brain Research, 286*, 241-248. doi:10.1016/j.bbr.2015.03.004

Bartolomucci, A. (2007). Social stress, immune functions and disease in rodents. *Frontiers in Neuroendocrinology, 28*(1), 28-49. doi:10.1016/j.yfrne.2007.02.001

Bartolomucci, A., Palanza, P., Sacerdote, P., Ceresini, G., Chirieleison, A., Panerai, A., & Parmigiani, S. (2003). Individual housing induces altered immuno-endocrine responses to psychological stress in male mice. *Psychoneuroendocrinology, 28*(4), 540-558. doi:10.1016/s0306-4530(02)00039-2

Bourin, M., & Hascoët, M. (2003). The mouse light/dark box test. *European Journal of Pharmacology, 463*(1-3), 55-65. doi:10.1016/s0014-2999(03)01274-3

Chaviaras, S., Mak, P., Ralph, D., Krishnan, L., & Broadbear, J. H. (2010). Assessing the antidepressant-like effects of carbetocin, an oxytocin agonist, using a modification of the forced swimming test. *Psychopharmacology, 210*(1), 35-43. doi:10.1007/s00213-010-1815-x

Choleris, E., Pfaff, D. W., & Kavaliers, M. (2013). *Oxytocin, vasopressin and related peptides in the regulation of behavior*. New York: Cambridge University Press.

Choleris, E., Little, S. R., Mong, J. A., Puram, S. V., Langer, R., & Pfaff, D. W. (2007). Microparticle-based delivery of oxytocin receptor antisense DNA in the medial amygdala blocks social recognition in female mice. *Proceedings of the National Academy of Sciences, 104*(11), 4670-4675. doi:10.1073/pnas.0700670104

Clodi, M., Vila, G., Geyeregger, R., Riedl, M., Stulnig, T. M., Struck, J., . . . Luger, A. (2008). Oxytocin alleviates the neuroendocrine and cytokine response to bacterial endotoxin in healthy men. *AJP: Endocrinology and Metabolism, 295*(3). doi:10.1152/ajpendo.90263.2008

Dantzer, R., & Kelley, K. W. (2007). Twenty years of research on cytokine-induced sickness behavior. *Brain, Behavior, and Immunity, 21*(2), 153-160. doi:10.1016/j.bbi.2006.09.006

Detillion, C. E., Craft, T. K., Glasper, E. R., Prendergast, B. J., & Devries, A. (2004). Social facilitation of wound healing. *Psychoneuroendocrinology, 29*(8), 1004-1011. doi:10.1016/j.psyneuen.2003.10.003

DeVries, A. C., Craft, T. K., Glasper, E. R., Neigh, G. N., & Alexander, J. K. (2007). 2006 Curt P. Richter award winner: Social influences on stress responses and health. *Psychoneuroendocrinology, 32*(6), 587-603. doi:10.1016/j.psyneuen.2007.04.007

Donhoffner, M. E., Goings, S. P., Atabaki, K., & Wood, R. I. (2016). Intracerebroventricular oxytocin self-administration in female rats. *Journal of Neuroendocrinology*. doi:10.1111/jne.12416

Duque-Wilckens, N., Steinman, M. Q., Tan, P. B., Hao, R., & Trainor, B. C. (2016). Systemic inhibition of oxytocin receptors blocks stress induced social anxiety in female California mice [Abstract]. *Society For Neuroscience*.

Engeland, C. G., Nielsen, D. V., Kavaliers, M., & Ossenkopp, K. (2001). Locomotor activity changes following lipopolysaccharide treatment in mice: A multivariate assessment of behavioral tolerance. *Physiology & Behavior, 72*(4), 481-491. doi:10.1016/s0031-9384(00)00436-4

Engeland, C. G., Kavaliers, M., & Ossenkopp, K. (2006). Influence of the estrous cycle on tolerance development to LPS-induced sickness behaviors in rats. *Psychoneuroendocrinology, 31*(4), 510-525. doi:10.1016/j.psyneuen.2005.11.007

Engstrøm, T., Barth, T., Melin, P., & Vilhardt, H. (1998). Oxytocin receptor binding and uterotonic activity of carbetocin and its metabolites following enzymatic degradation. *European Journal of Pharmacology, 355*(2-3), 203-210. doi:10.1016/s0014-2999(98)00513-5

Guzmán, Y. F., Tronson, N. C., Jovasevic, V., Sato, K., Guedea, A. L., Mizukami, H., . . . Radulovic, J. (2013). Fear-enhancing effects of septal oxytocin receptors. *Nature Neuroscience, 16*(9), 1185-1187. doi:10.1038/nn.3465

Hascoët, M., Bourin, M., & Dhonnchadha, B. A. (2000). The influence of buspirone, and its metabolite 1-PP, on the activity of paroxetine in the mouse light/dark paradigm and four plates test. *Pharmacology Biochemistry and Behavior, 67*(1), 45-53. doi:10.1016/s0091-3057(00)00293-8

Harari-Dahan, O., & Bernstein, A. (2014). A general approach-avoidance hypothesis of oxytocin: Accounting for social and non-social effects of oxytocin. *Neuroscience and Biobehavioral Reviews, 47*, 506-519. doi:10.1016/j.neubiorev.2014.10.007

Kavaliers, M., & Choleris, E. (2011). Sociality, pathogen avoidance, and the neuropeptides oxytocin and arginine vasopressin. *Psychological Science, 22*(11), 1367-1374. doi:10.1177/0956797611420576

Kelley, K. W., Bluthé, R., Dantzer, R., Zhou, J., Shen, W., Johnson, R. W., & Broussard, S. R. (2003). Cytokine-induced sickness behavior. *Brain, Behavior, and Immunity, 17*(1), 112-118. doi:10.1016/s0889-1591(02)00077-6

Kuteykin-Teplyakov, K., & Maldonado, R. (2014). Looking for prosocial genes: ITRAQ analysis of proteins involved in MDMA-induced sociability in mice. *European Neuropsychopharmacology, 24*(11), 1773-1783. doi:10.1016/j.euroneuro.2014.08.007

Maes, M. (2011). Depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 35*(3), 664-675. doi:10.1016/j.pnpbp.2010.06.014

Moertl, M., Friedrich, S., Kraschl, J., Wadsack, C., Lang, U., & Schlembach, D. (2011). Haemodynamic effects of carbetocin and oxytocin given as intravenous bolus on women undergoing caesarean delivery: A randomised trial. *BJOG: An International Journal of Obstetrics & Gynaecology, 118*(11), 1349-1356. doi:10.1111/j.1471-0528.2011.03022.x

Neumann, I. D., Wigger, A., Torner, L., Holsboer, F., & Landgraf, R. (2000). Brain oxytocin inhibits basal and stress-induced activity of the hypothalamo-pituitary-adrenal axis in male and female rats: partial action within the paraventricular nucleus. *Journal of Neuroendocrinology, 12*(3), 235-243. doi:10.1046/j.1365-2826.2000.00442.x

Neumann, I. D. (2008). Brain oxytocin: A key regulator of emotional and social behaviours in both females and Males. *Journal of Neuroendocrinology, 20*(6), 858-865. doi:10.1111/j.1365-2826.2008.01726.x

Neumann, I. D., & Landgraf, R. (2012). Balance of brain oxytocin and vasopressin: Implications for anxiety, depression, and social behaviors. *Trends in Neurosciences, 35*(11), 649-659. doi:10.1016/j.tins.2012.08.004

Neumann, I. D., & Slattery, D. A. (2016). Oxytocin in general anxiety and social fear: A translational approach. *Biological Psychiatry, 79*(3), 213-221. doi:10.1016/j.biopsych.2015.06.004

Olszewski, P. K., Allen, K., & Levine, A. S. (2015). Effect of oxytocin receptor blockade on appetite for sugar is modified by social context. *Appetite, 86*, 81-87. doi:10.1016/j.appet.2014.10.007

Olszewski, P. K., Ulrich, C., Ling, N., Allen, K., & Levine, A. S. (2014). A non-peptide oxytocin receptor agonist, WAY-267,464, alleviates novelty-induced hypophagia in mice: Insights into changes in c-Fos immunoreactivity. *Pharmacology Biochemistry and Behavior, 124*, 367-372. doi:10.1016/j.pbb.2014.07.007

Peters, S., Slattery, D. A., Uschold-Schmidt, N., Reber, S. O., & Neumann, I. D. (2014). Dose-dependent effects of chronic central infusion of oxytocin on anxiety, oxytocin receptor binding and stress-related parameters in mice. *Psychoneuroendocrinology, 42*, 225-236. doi:10.1016/j.psyneuen.2014.01.021

Reyes-Lagos, J. J., Hadamitzky, M., Peña-Castillo, M. Á, Echeverría, J. C., Bösche, K., Lückemann, L., . . . Pacheco-López, G. (2016). Exogenous oxytocin reduces signs of sickness behavior and modifies heart rate fluctuations of endotoxemic rats. *Physiology & Behavior, 165*, 223-230. doi:10.1016/j.physbeh.2016.07.013

Ring, R. H., Malberg, J. E., Potestio, L., Ping, J., Boikess, S., Luo, B., . . . Rosenzweig-Lipson, S. (2006). Anxiolytic-like activity of oxytocin in male mice: Behavioral and autonomic evidence, therapeutic implications. *Psychopharmacology, 185*(2), 218-225. doi:10.1007/s00213-005-0293-z

Ross, K. M., Mcdonald-Jones, G., & Miller, G. E. (2013). Oxytocin does not attenuate the ex vivo production of inflammatory cytokines by lipopolysaccharide-activated

monocytes and macrophages from healthy male and female donors. *Neuroimmunomodulation, 20*(5), 285-293. doi:10.1159/000351610

Ross, H. E., & Young, L. J. (2009). Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Frontiers in Neuroendocrinology, 30*(4), 534-547. doi:10.1016/j.yfrne.2009.05.004

Scantamburlo, G., Hansenne, M., Fuchs, S., Pitchot, W., Maréchal, P., Pequeux, C., . . . Legros, J. (2007). Plasma oxytocin levels and anxiety in patients with major depression. *Psychoneuroendocrinology, 32*(4), 407-410. doi:10.1016/j.psyneuen.2007.01.009

Shamay-Tsoory, S. G., & Abu-Akel, A. (2016). The social salience hypothesis of oxytocin. *Biological Psychiatry, 79*(3), 194-202. doi:10.1016/j.biopsych.2015.07.020

Shattuck, E. C., & Muehlenbein, M. P. (2015). Human sickness behavior: Ultimate and proximate explanations. *American Journal of Physical Anthropology, 157*(1), 1-18. doi:10.1002/ajpa.22698

Tenk, C. M., Kavaliers, M., & Ossenkopp, K. (2008). Sexually dimorphic effects of neonatal immune system activation with lipopolysaccharide on the behavioural response to a homotypic adult immune challenge. *International Journal of Developmental Neuroscience, 26*(3-4), 331-338. doi:10.1016/j.ijdevneu.2008.01.001

Tops, M., Koole, S. L., Ijzerman, H., & Buisman-Pijlman, F. T. (2014). Why social attachment and oxytocin protect against addiction and stress: Insights from the dynamics between ventral and dorsal corticostriatal systems. *Pharmacology Biochemistry and Behavior, 119*, 39-48. doi:10.1016/j.pbb.2013.07.015

Toth, I., & Neumann, I. D. (2013). Animal models of social avoidance and social fear. *Cell Tissue Res Cell and Tissue Research, 354*(1), 107-118. doi:10.1007/s00441-013-1636-4

Wang, P., Yang, H., Tian, S., Wang, L., Wang, S. C., Zhang, F., & Wang, Y. (2015). Oxytocin-secreting system: A major part of the neuroendocrine center regulating immunologic activity. *Journal of Neuroimmunology, 289*, 152-161. doi:10.1016/j.jneuroim.2015.11.001

York, J. M., Blevins, N. A., Baynard, T., & Freund, G. G. (2012). Mouse testing methods in psychoneuroimmunology: An overview of how to measure sickness, depressive/anxietal, cognitive, and physical activity behaviors. *Methods in Molecular Biology Psychoneuroimmunology*, 243-276. doi:10.1007/978-1-62703-071-7_13

CHAPTER 3

THE EFFECTS OF OXYTOCIN ON IMMUNE RESPONSES IN ADULT MALE MICE

3.1 Introduction

3.1.1. Oxytocin and Immune Function

There is accumulating evidence suggesting that the nonapeptide, oxytocin (OT), is involved in the regulation of immune activity (eg. Wang et al., 2015). However, results of research focusing on relations between OT and immune function have been limited. Poutahidis et al. (2013) found that both endogenous OT and exogenous OT augmented wound healing capacity in male and female mice, and that wound healing in OT gene knockout mice was significantly improved following at least 3 days of peripheral OT treatment. As well, daily oral treatment with *Lactobacillus reuteri*, which interacts with the gut microbiome to up-regulate OT, was shown to accelerate wound healing by compressing the wound repair cascade, increasing collagen deposition, and importantly, by down-regulating the pro-inflammatory response (Poutahidis et al., 2013).

Studies examining the type II transmembrane glycoprotein, CD38, further support the link between OT and immune function. CD38 plays a role in immune, social behavioural, and OT mediated functions (Algoe & Way, 2014; Feldman et al., 2016; Higashida et al., 2012; Jin et al., 2007; Lerer et al., 2010). In studies with mice, activities of immune-related cells, such as dendritic cells, monocytes, astrocytes, and neutrophils, are shown to be regulated by CD38 (Lund, 2006). In addition, neutrophil trafficking to sites of inflammation and infection is dependent upon CD38 expression on neutrophils (Partida-Sanchez et al. (2001). Among Autism Spectrum Disorder (ASD) patients, in which OT deficiencies have been extensively reported (Lukas & Neumann, 2013), abnormalities in CD38 expression may also point to a possible regulatory role of OT. Importantly, Lerer et al. (2010) found that lymphocytes from ASD patients had significantly less CD38 expression, compared to patients' unaffected parents, providing further support for the integrated role of CD38 and OT in immune function and social behaviour.

3.1.2. Oxytocin, Stress, and Immune Function

The interplay between OT and the immune system may be mediated by the stress axis. OT has been shown to have suppressive effects on the stress response, reducing circulating levels of cortisol/corticosterone and adrenocorticotropic hormone (ACTH) (Brunton et al., 2012; Clodi et al., 2008; Detillion et al., 2004; DeVries et al., 2007;

Landgraf et al., 1995; Neumann et al., 2000). Higher HPA activation, and subsequently higher cortisol and ACTH, lead to increased levels of pro-inflammatory cytokines such as Tumor Necrosis Factor Alpha (TNF-α) and Interleukin-6 (IL-6) (Anisman & Merali, 2002). As such, OT's potential to dampen immune function via HPA mediated actions is an important consideration.

In rodents, positive social interaction paradigms such as same-sex sibling pair housing, and pair bonding in monogamous prairie voles, were generally shown to augment endogenous OT levels and to reduce HPA activity and pro-inflammatory cytokines (DeVries et al., 2007). For instance, in extensive studies with adult female Siberian hamsters, Detillion et al., (2004) housed animals in pairs with a sibling, or individually, and the effects on wound healing were evaluated. They also manipulated OT levels by administering either OT (20 mg/kg i.p. for 5 days prior to wounding) or an OT antagonist (desGly-NH_2-d$(CH_2)_5$(D-Tyr^2,Thr^4]OVT^a at 3 μl, intracerebroventricular (ICV), 1 day before wounding). Their results indicate that both OT treatment and positive social interaction promote wound healing via OT mediated mechanisms that suppress HPA activity in animals subjected to restraint stress (Detillion et al., 2004). Conversely, it was shown that female hamsters who received treatment with an OT antagonist or who were individually housed displayed increased HPA activity, pro-inflammatory cytokine activation, and inhibited wound healing (Detillion et al., 2004; DeVries et al., 2007; Neumann et al., 2000). Similarly, Vitalo et al. (2009) proposed that OT may facilitate wound healing through attenuation of the peripheral stress response similar to that seen in environmental enrichment paradigms. In their study, male Sprague-Dawley rats were reared in isolation and treated chronically with injections of OT (10 mg/kg i.p.) 5 days a week for 6 weeks, and/or received additional bedding as a means of environmental enrichment. They found that both OT and environmental enrichment facilitated wound healing rates comparably, suggesting both OT and environmental enrichment act in a similar manner to enhance wound healing, likely through attenuation of the peripheral stress response (Vitalo et al., 2009).

3.1.3. OT, LPS, and Immune Function

Lipopolysaccharide (LPS) is an endotoxin from the outer cell wall of Gram-negative bacteria, which activates both the immune and endocrine systems, including the

HPA-axis, and increases pro-inflammatory cytokine levels in rodents (Anisman & Merali, 2002; Anisman et al., 2003) and humans (Clodi et al., 2008; Ross et al., 2013). A recent study by Yuan et al. (2016) found that in murine cells OT attenuated LPS induced microglial activation and pro-inflammatory cytokine levels. Furthermore, Reyes-Lagos et al. (2016) showed that in adult male rats treated with LPS, subcutaneous treatment with synthetic OT at 6μg/kg moderated LPS-induced cytokine mediated hyperthermia (ie. fever). However, in contrast Ross et al. (2013) showed that in ex vivo experiments on healthy human monocytes and macrophages, OT did not attenuate LPS-induced cytokine production.

While results of studies in non-human animals have established a link between LPS immune challenge and subsequent initiation of the stress response and immune activation (Anisman & Merali, 2002; Reyes-Lagos et al., 2016), relatively few studies have examined the effect of OT on immune function and its relation to the stress response in humans. Clodi et al., (2008) investigated the role of exogenous OT treatment on immune responses in men by administering an LPS immune challenge as an acute stressor. It was shown that among men treated intravenously with a dose of 1 pmol/kg/min of OT plus 2 ng/kg of LPS for 90 minutes, there was an overall attenuation of typical LPS-induced increases in markers of HPA activation (ie. ACTH and cortisol), as well as in markers of immune activation (ie. TNF-α, IL-1, IL-4, and IL-6). They concluded that OT was effective in attenuating neuroendocrine and pro-inflammatory cytokine activation caused by LPS treatment in men (Clodi et al., 2008). However, when Ross et al. (2013) examined OT's effects on LPS activated healthy human monocytes and macrophages they found that OT did not attenuate pro-inflammatory cytokines. This led them to conclude that the anti-inflammatory effects of OT observed in other studies may be dependent on other classes of immune cells or lymphoid organs. These contradictory findings thus indicate the need for further investigations of the relations between OT, stress, LPS, and immune function.

3.1.4. Present Study

In the present study, effects of the OT agonist, carbetocin, and OT antagonist, L-368,899, on pro-inflammatory cytokine levels of TNF-α and IL-6 following LPS administration were evaluated. On the basis of limited previous studies, it was

hypothesized that the OT agonist would result in reduced serum levels of pro-inflammatory cytokines TNF-α and IL-6 among animals treated with LPS, while the OT antagonist was anticipated to augment levels of these cytokines.

3.2 Methods

3.2.1. Animals

The same animals used in experiment 1 were also used in this study. A total of 41 adult male CD-1 mice (30 to 35 grams) from Charles River, Quebec, were used to study the effects of the OT agonist, CBT, while another 31 adult male CD-1 mice were used to evaluate effects of the OT antagonist, L-368,899. Animals were housed in a temperature controlled colony room at 21±1°C, with photoperiod set to a 12:12 Light:Dark cycle with lights on at 0700, and were provided food (ProLab rodent chow, RMH 3000) and water ad libitum. Animals were housed individually in polypropylene cages (29.5 x 18.8 x 13 cm). Effects of individual housing on male mice are strain and density dependent, with previous studies showing negligible differences in CD-1 mice with regard to basal stress responses between individually and socially housed males (Bartolomucci, 2007; Bartolomucci et al., 2003). However, individual housing was used in this study to prevent effects of inter-male aggression and minimize dominance-subordinance related differences in individual stress and OT responses, as social contextual variables have been shown to affect these (Choleris et al., 2013; DeVries et al., 2007). Mice were left undisturbed for 10 days prior to testing to ensure adequate adjustment time after arrival. All procedures were approved by the Western University Animal Care Committee and animals were handled and tested according to guidelines set out by the Canadian Council on Animal Care (CCAC).

3.2.2. Drug Treatment – OT Agonist: Carbetocin

Mice were randomly divided into 6 groups. Beginning between 12:00 and 13:00h, all animals received a series of 2 intraperitoneal (i.p.) injections, 15 minutes apart. Table 3.1 outlines the group treatments and group sizes. The first injection manipulated OT levels. Here, 2 groups received a 20mg/kg dose of OT agonist, carbetocin (CBT) (Carbetocin acetate SML0748, no. 073M4722V, Sigma-Aldrich, St. Louis, MO, USA) dissolved in 0.9% isotonic saline, while 2 other groups received a 10mg/kg dose, and the remaining 2 groups were administered a control injection of an equivalent volume of

isotonic saline (0.9%). CBT was chosen as the OT agonist for its relatively long half-life (85 – 100 mins) (Engstrøm et al., 1998; Moertl et al., 2011) and the doses were based on behaviourally active doses previously reported in mice (eg. Chaviaras et al., 2010). The second injection 15 mins later initiated an immune challenge. Animals received either 75 μg/kg Lipopolysaccharide (LPS) (derived from Escherichia Coli serotype 0111:B4, no. L2630-028K4090, Sigma Chemical, St. Louis, MO, USA) dissolved in a 0.9% isotonic saline, or an equivalent volume of saline as a control. This dose of LPS was selected based on previous work done in CD-1 mice (Engeland et al., 2001).

Table 3.1. *Group sizes and treatment injections for OT agonist and LPS*

Group	*Injection 1*	*Injection 2*	*n: TNF-α assay*	*n: IL-6 assay*
20CBT-LPS	20 mg/kg carbetocin	75 μg/kg LPS	5	5
10CBT-LPS	10 mg/kg carbetocin	75 μg/kg LPS	11	7
20CBT-NaCl	20 mg/kg carbetocin	0.2 to 0.35 mL saline	4	4
10CBT-NaCl	10 mg/kg carbetocin	0.2 to 0.35 mL saline	12	12
NaCl-LPS	0.2 to 0.35 mL saline	75 μg/kg LPS	5	3
NaCl-NaCl	0.2 to 0.35 mL saline	0.2 to 0.35 mL saline	4	4

3.2.3. Drug Treatment – OT Antagonist: L-368,899

Mice were randomly divided into 4 groups. Beginning between 12:00 and 13:00h, all animals received a series of 2 intraperitoneal (i.p.) injections, 15 minutes apart. Table 3.2 outlines the group treatments and group sizes. First, 2 groups received a 10mg/kg dose of the OT antagonist, L-368,899 (L-368,899 hydrochloride, no. 1A/82628, Tocris Bioscience, Minneapolis, MN, USA) dissolved in 0.9% isotonic saline. The other 2 groups were administered an equivalent volume of isotonic saline (0.9%) for their first injection. Previous studies have shown L-368,899, at varying dosages, to be an effective OT antagonist in mice and the dose used here was based on these studies (Kuteykin-Teplyakov & Maldonado 2014; Olszewski et al., 2015; Olszewski et al., 2014). The second injection 15 mins later initiated an immune challenge. Animals received either 75 μg/kg Lipopolysaccharide (LPS) (derived from Escherichia Coli serotype 0111:B4, no.

L2630-028K4090, Sigma Chemical, St. Louis, MO, USA) dissolved in a 0.9% isotonic saline, or saline as a control. This dose was selected based on previous work done in CD-1 mice (Engeland et al., 2001).

Table 3.2. *Group sizes and treatment injections for OT antagonist and LPS*

Group	*Injection 1*	*Injection 2*	*n: TNF-α assay*	*n: IL-6 assay*
OTant-NaCl	10 mg/kg L-368,899	0.2 to 0.35 mL saline	10	10
OTant-LPS	10 mg/kg L-368,899	75 μg/kg LPS	10	10
NaCl-LPS	0.2 to 0.35 mL saline	75 μg/kg LPS	6	4
NaCl-NaCl	0.2 to 0.35 mL saline	0.2 to 0.35 mL saline	5	5

3.2.4. Procedure

After behavioural testing (as described in experiment 1), approximately 4 hours after LPS treatment and within 20 minutes of removal from the VersaMax activity box, animals were killed via cervical dislocation followed by immediate decapitation. Trunk blood samples of approximately 0.5mL were collected, placed on ice, and then centrifuged at 3000 RPM for 10 minutes. Serum were separated from red blood cells and frozen at -80 degrees until needed for cytokine assays. TNF-α and IL-6 were analyzed in parallel using Luminex Mouse Cytokine /Chemokine Magnetic Bead kits manufactured by EMD Millipore Corporation (Billerica, MA 01821 USA) according to manufacturer instructions, as seen in Appendix A. IL-1β was also analyzed, however these values were out of range and thus were not included for further analysis. Assay sensitivity was determined using the Minimum Detectable Concentration (MinDC), and was calculated with MILLIPLEX® Analyst 5.1. For TNF-α the MinDC is 2.3 pg/mL, while the MinDC+2SD is 3.4 pg/mL. For IL-6 the MinDC is 1.1 pg/mL, while the Min DC+2SD is 2.0 pg/mL. Accuracy for the TNF-α and IL-6 assays were calculated at 94%, and 92.2%, respectively.

3.2.5. Data Analysis

The effects of the OT agonist, CBT, OT antagonist, L-368,899, and LPS on pro-inflammatory cytokines TNF-α and IL-6, were each evaluated using a univariate

Analysis of Variance (ANOVA) with between subjects factors of CBT (3 levels: 20mg/kg CBT, 10mg/kg CBT, saline (control)), or OT antagonist (2 levels: 10mg/kg OT antagonist, saline (control)) and LPS (2 levels: 75μg/kg and saline (control)). Post hoc pairwise comparisons were then completed, where appropriate, using the Least Significant Difference (LSD) to evaluate significant main effects and interactions. Tests of polynomial linear contrasts were also conducted to examine potential linear relationships within the data. Data were analyzed using SPSS 23.0 for windows with $\alpha = 0.05$, and the Greenhouse-Geisser correction factor where appropriate.

3.3 Results

3.3.1. LPS and OT Agonist Effects on Cytokines

Serum concentrations of TNF-α for animals treated with LPS and the OT agonist, CBT, are shown in Figure 3.1. ANOVA analysis revealed a significant main effect of LPS [$F(1, 35) = 47.54$, $p = .000$], such that LPS treated animals showed significantly higher serum concentrations of TNF-α relative to non-LPS treated groups (p's $\leq .001$). This is suggestive of pro-inflammatory cytokine induced inflammation.

No significant main effects of CBT treatment were found [$F(2, 35) = 1.67$, $p = .202$], indicating that OT agonist treatment did not significantly influence TNF-α serum concentration levels. Furthermore, linear contrasts were non-significant demonstrating a lack of a linear relationship between CBT dose and TNF-α serum concentrations ($p = .265$).

No significant interactions were observed [$F(2, 35) = .69$, $p = .510$]; However, planned comparisons were conducted to examine potential dose-related differences among individual groups. Post hoc analysis revealed dose-related effects of CBT as the 10mg/kg CBT-LPS, but not the 20mg/kg CBT-LPS group, had significantly greater serum concentration levels of TNF-α relative to saline-LPS treated mice ($p = .045$).

Serum concentrations of IL-6 for animals treated with LPS and the OT agonist, CBT, are shown in Figure 3.2.The ANOVA revealed a significant main effect of LPS [$F(1, 29) = 51.32$, $p = .000$] for serum concentrations of IL-6. LPS treated animals had significantly higher concentrations of pro-inflammatory cytokine IL-6 relative to non-LPS groups (p's $\leq .001$), indicating inflammation.

The analysis found no significant main effects of CBT treatment [$F(2, 29) = 1.71$, $p = .198$] on IL-6 serum concentrations. This shows that OT agonist treatment has no significant effect on IL-6 pro-inflammatory cytokine levels. Furthermore, non-significant linear contrasts indicated no significant linear relationship between CBT dose and IL-6 serum concentrations ($p = .375$).

Significant interactions for effects on IL-6 serum levels were not observed [$F(2, 29) = 1.93$, $p = .163$]; However, planned comparisons were nevertheless conducted to evaluate potential dose-related differences among groups. Post hoc analysis revealed effects of the OT agonist treatment as both the 10mg/kg CBT-LPS ($p = .034$) and the 20mg/kg CBT-LPS ($p = .023$) treated groups had significantly higher serum concentrations of IL-6 relative to saline-LPS treated mice.

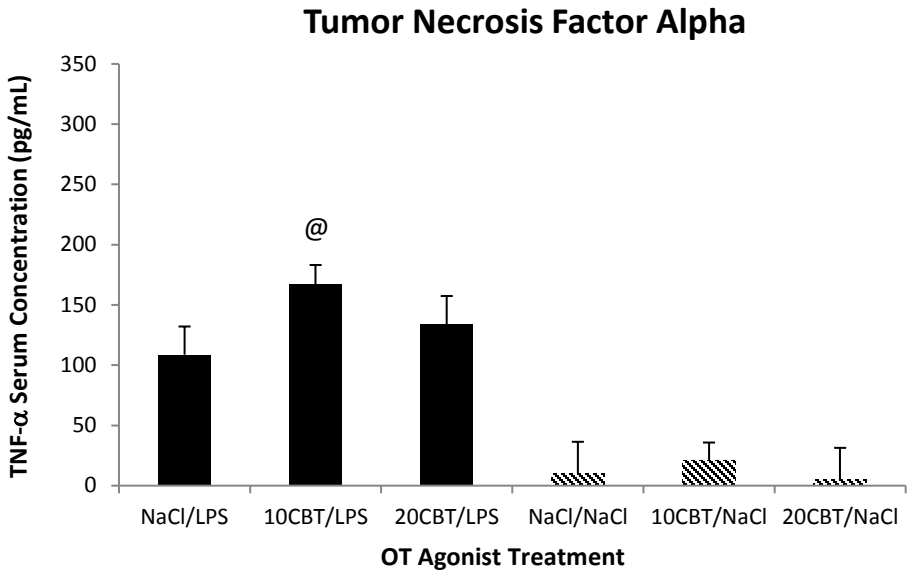


Fig. 3.1. Mean TNF-α serum concentrations (pg/mL) for mice treated i.p. with the OT agonist, CBT, plus LPS or saline. Vertical lines indicate standard error. LPS groups showed significantly higher serum levels of TNF-α relative to non-LPS groups ($p \leq .001$; not marked on graph), indicating LPS-induced inflammation. 10mg/kg CBT-LPS treated mice had significantly higher serum levels of TNF-α than saline-LPS treated mice (@ $p < .05$).

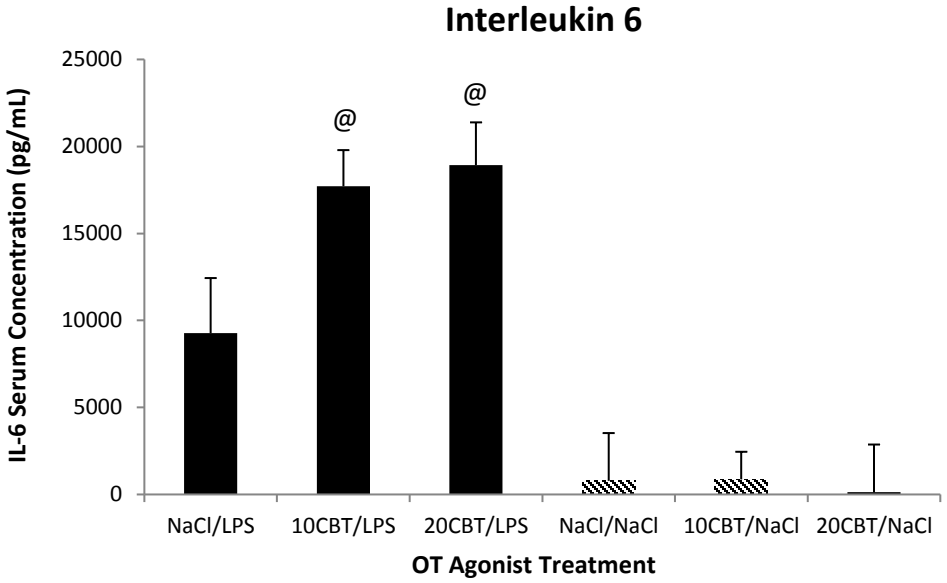


Fig. 3.2. Mean IL-6 serum concentrations (pg/mL) for mice treated i.p. with the OT agonist, CBT, plus LPS or saline. Vertical lines indicate standard error. LPS groups showed significantly higher serum levels of IL-6 relative to saline groups ($p \leq .001$; not marked on graph), indicative of LPS-induced inflammation. Both 10mg/kg CBT-LPS and 20mg/kg CBT-LPS (@ $p < .05$) treated groups had significantly higher serum concentrations of IL-6 relative to saline-LPS treated mice.

3.3.2. LPS and OT Antagonist Effects on Cytokines

Serum concentrations of TNF-α for animals treated with LPS and the OT antagonist, L-368,899, are shown in Figure 3.3. A significant main effect of LPS was found [$F(1, 27) = 38.16$, $p = .000$]. LPS treated mice had significantly higher serum concentrations of TNF-α relative to saline treated groups (p's $\leq .001$), suggesting LPS-induced inflammation.

Significant main effects of OT antagonist treatment were not found [$F(1, 27) = 1.16$, $p = .292$], indicating that OT antagonist treatment did not significantly affect TNF-α serum concentration levels. ANOVA analysis revealed no significant OT antagonist x LPS interactions [$F(1, 27) = 1.16$, $p = .291$].

IL-6 serum concentrations for animals treated with LPS and the OT antagonist, L-368,899, are shown in Figure 3.4. ANOVA analysis revealed a significant main effect of LPS treatment [$F(1, 25) = 36.25$, $p = .000$] for IL-6 serum concentrations. Relative to saline treated animals, LPS treated mice had significantly higher serum concentrations of pro-inflammatory cytokine IL-6 (p's $\leq .001$), indicative of LPS induced inflammation.

No significant main effects of OT antagonist treatment on IL-6 serum concentrations were found [$F(1, 25) = .14$, $p = .716$]; suggesting that OT antagonist treatment did not significantly affect IL-6 pro-inflammatory cytokines. Furthermore, significant OT antagonist x LPS interactions for effects on IL-6 serum levels were not observed [$F(1, 25) = .00$, $p = .987$].

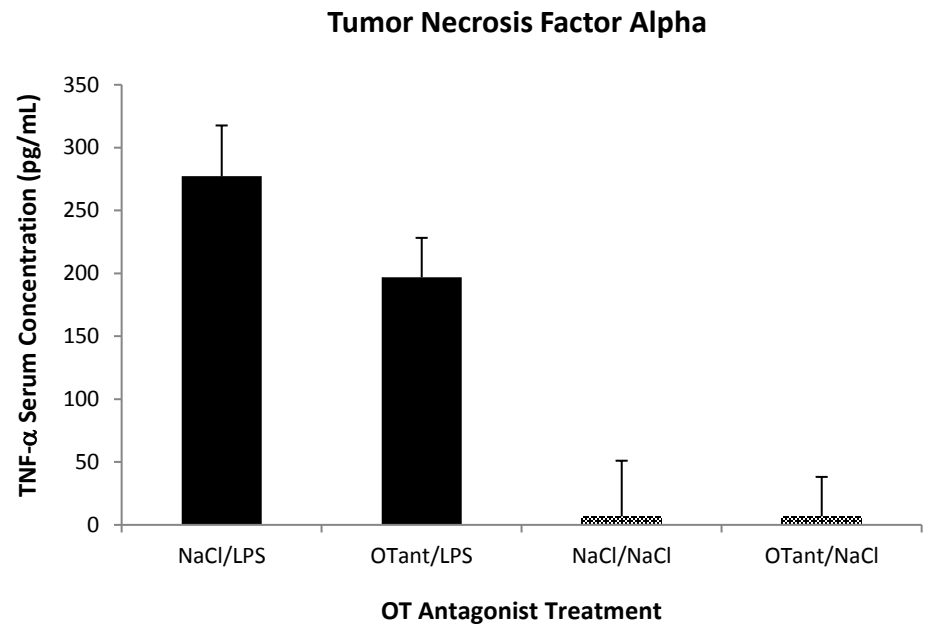


Fig. 3.3. Mean TNF-α serum concentrations (pg/mL) for mice treated i.p. with the OT antagonist, L-368,899, plus LPS or saline. Vertical lines indicate standard error. LPS groups showed significantly higher serum levels of TNF-α relative to non-LPS groups ($p \leq .001$; not marked on graph).

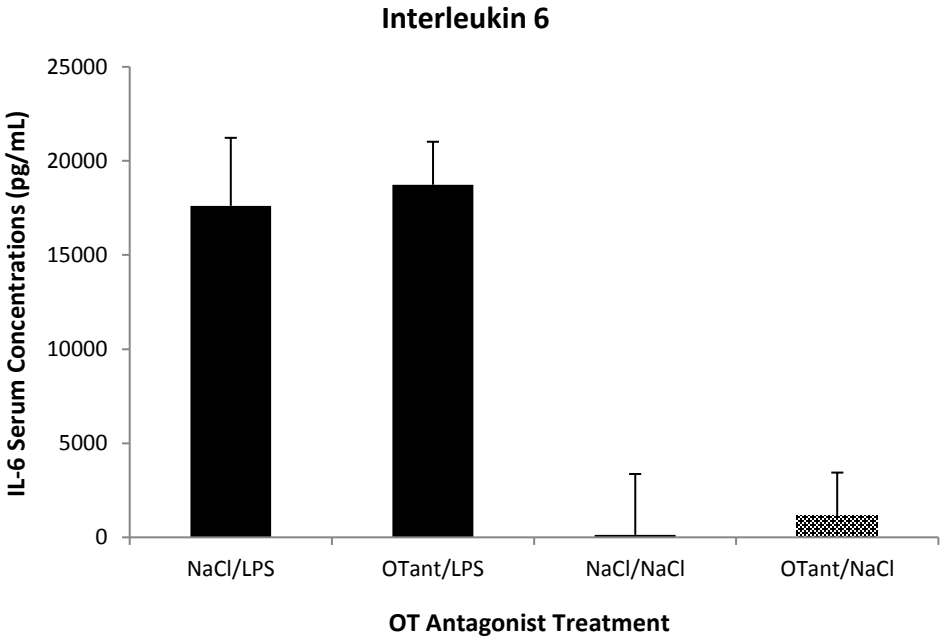


Fig. 3.4. Mean IL-6 serum concentrations (pg/mL) for mice treated i.p. with the OT antagonist, L-368,899, plus LPS or saline. Vertical lines indicate standard error. LPS groups showed significantly higher serum levels of IL-6 relative to saline groups (p's $\leq$ .001; not marked on graph).

3.4 Discussion

3.4.1. Effects of LPS on Cytokines

The current study examined the effects of the OT agonist, CBT, and the OT antagonist, L-368,899, on serum concentrations of TNF-α and IL-6 in male CD-1 mice following treatment with LPS (75 μg/kg i.p.). LPS treatment robustly and consistently augmented serum levels of both TNF-α and IL-6, thereby confirming and extending the results of previous research (Anisman & Merali, 2002; Anisman et al., 2003). All LPS treated groups showed significantly higher concentrations of both cytokines relative to non-LPS treated animals, thus indicating that the immunoassay was providing reliable measures that were consistent with cytokine values obtained from other studies (Hubner et al., 2008; Iseri et al., 2010; Starkhammer et al., 2012). For example, Hubner et al. (2008) found that in both male and female adult mice treated with LPS there was a significant increase in levels of pro-inflammatory cytokines, including IL-6 and TNF-α, which persisted for up to between 1 to 48 hours after LPS challenge.

3.4.2. Effects of OT on Cytokines

Neither the OT agonist, CBT, nor the OT antagonist, in conjunction with saline, had significant effects on basal levels of either TNF-α or IL-6. This finding suggests that OT does not affect these cytokines in the absence of LPS treatment. In the context of immune challenge via injury or infection (eg. LPS), results from the present study contrast with those from previous limited research. For example, Iseri et al. (2010) showed that OT treatment at 5 mg/kg (s.c.) twice a day for 5 days attenuated basal TNF-α at day 5 following burn injury. Furthermore, Clodi et al. (2008) found that in men intravenous LPS treatment in conjunction with OT attenuated serum levels of pro-inflammatory cytokines, including TNF-α and IL-6.

Despite the lack of significance found for OT's effects on cytokines in the current study, planned comparisons were conducted to explore potential dose-related effects of the OT agonist treatment when combined with LPS. Mice treated with 10 mg/kg CBT-LPS, but not 20 mg/kg CBT-LPS, showed significantly higher plasma concentrations of TNF-α relative to saline-LPS treated mice. This finding contrasts with those of previous studies which have shown that OT treatment tends to decrease serum levels of pro-inflammatory cytokines (Clodi et al., 2008; Wang et al., 2015; Yuan et al., 2016).

However, others indicate that in vivo, OT does not attenuate pro-inflammatory cytokines (Ross et al., 2013). It is not clear then, the impact that OT has on serum levels of TNF-α and further studies are needed to clarify these effects. Furthermore, the present data suggest that OT's effects on TNF-α may be dose-related. As previous studies have not examined dose-related effects of OT on serum TNF-α levels, additional studies are needed to clarify any dose-related effects of OT on TNF-α immune functions.

OT agonist treatment had no suppressive effects on serum levels of IL-6 in LPS treated mice. In both the 10 mg/kg CBT-LPS and the 20 mg/kg CBT-LPS treated groups there were significantly higher serum concentrations of IL-6 compared to saline-LPS treated mice. This finding is in contrast to previous studies which have shown that OT agonist treatment tends to decrease serum levels of pro-inflammatory cytokines, such as IL-6 (Clodi et al., 2008; Wang et al., 2015; Yuan et al., 2016). Other studies (eg. Ross et al., 2013), however, have shown that in vivo, OT does not attenuate pro-inflammatory cytokines. Together, these findings highlight that OT's effects on pro-inflammatory cytokines, such as IL-6, are still not clearly understood and that further research is needed to characterize the nature of these effects.

3.4.3. Limitations and Future Directions

In light of previous research, findings from the current study are intriguing. In particular, OT treatment has generally been associated with attenuations in pro-inflammatory cytokine levels, while OT antagonist treatment seems to augment levels of pro-inflammatory cytokine activation (Wang et al., 2015). However, previous studies did not specifically examine the effects of systemic OT on cytokines in the context of LPS treatment, but rather evaluated i.p. or ICV administered OT or OT antagonist treatment effects on subcutaneous wound healing as an index of immune function (Detillion et al., 2004; Iseri et al., 2010). As such, it is possible that OT differentially affects immune responses dependent on the type and magnitude of injury or infection, and route of OT administration. Future research should evaluate the role of OT on immune function using a variety of infection types and severities, as well as by comparing systemic versus central OT treatment routes.

Another potential confound that may have obscured OT's effects on TNF-α and IL-6 in the present study is the large degree of variability among group sizes coupled

with some rather small samples, which likely increased variability in the data. Future studies could resolve this issue by increasing and equalizing group sizes to produce more consistent data with less variability. The present study was also conducted following behavioural testing. Although attempts were made to ensure animals were as stress-free as possible, behavioural activity and subsequent arousal undoubtedly caused some degree of anxiety among mice, which would likely confound cytokine activity via HPA mediated mechanisms. Future research could circumvent this limitation by using separate animals for behavioural versus immunological testing.

3.4.4. Conclusions

The present study both confirmed and extended knowledge pertaining to OT's role in immune function by examining effects of the OT agonist, CBT, and the OT antagonist, L-368,899, on cytokines TNF-α and IL-6, in conjunction with LPS treatment. The LPS immune challenge predictably produced robust pro-inflammatory responses as indicated by increased levels of both TNF-α and IL-6. However, OT failed to produce any significant suppressive effects on levels of these cytokines, although dose-related effects of OT agonist treatment were observed among LPS treated mice. In light of previous research, which has investigated OT's role regarding pro-inflammatory cytokines in the context of subcutaneous wound healing (eg. Detillion et al., 2004; Iseri et al., 2010), in vivo experiments (Ross et al., 2013), and most recently in conjunction with LPS immune challenge (Yuan et al., 2016), the present results were curious. As neither the OT agonist nor the OT antagonist had any significant effect on mediating pro-inflammatory cytokines, the present study indicates that OT's role in immune function may be dependent on the type and severity of infection, administration route, and dose.

3.5 References

Algoe, S. B., & Way, B. M. (2014). Evidence for a role of the oxytocin system, indexed by genetic variation inCD38, in the social bonding effects of expressed gratitude. *Social Cognitive and Affective Neuroscience, 9*(12), 1855-1861. doi:10.1093/scan/nst182

Anisman, H., & Merali, Z. (2002). Cytokines, stress, and depressive illness. *Brain, Behavior, and Immunity, 16*(5), 513-524. doi:10.1016/s0889-1591(02)00009-0

Anisman, H., Merali, Z., & Hayley, S. (2003). Sensitization associated with stressors and cytokine treatments. *Brain, Behavior, and Immunity, 17*(2), 86-93. doi:10.1016/s0889-1591(02)00100-9

Bartolomucci, A. (2007). Social stress, immune functions and disease in rodents. *Frontiers in Neuroendocrinology, 28*(1), 28-49. doi:10.1016/j.yfrne.2007.02.001

Bartolomucci, A., Palanza, P., Sacerdote, P., Ceresini, G., Chirieleison, A., Panerai, A., & Parmigiani, S. (2003). Individual housing induces altered immuno-endocrine responses to psychological stress in male mice. *Psychoneuroendocrinology, 28*(4), 540-558. doi:10.1016/s0306-4530(02)00039-2

Brunton, P. J., Bales, J., & Russell, J. A. (2012). Allopregnanolone and induction of endogenous opioid inhibition of oxytocin responses to immune stress in pregnant rats. *Journal of Neuroendocrinology, 24*(4), 690-700. doi:10.1111/j.1365-2826.2012.02295.x

Chaviaras, S., Mak, P., Ralph, D., Krishnan, L., & Broadbear, J. H. (2010). Assessing the antidepressant-like effects of carbetocin, an oxytocin agonist, using a modification of the forced swimming test. *Psychopharmacology, 210*(1), 35-43. doi:10.1007/s00213-010-1815-x

Choleris, E., Pfaff, D. W., & Kavaliers, M. (2013). *Oxytocin, vasopressin and related peptides in the regulation of behavior*. New York: Cambridge University Press.

Clodi, M., Vila, G., Geyeregger, R., Riedl, M., Stulnig, T. M., Struck, J., . . . Luger, A. (2008). Oxytocin alleviates the neuroendocrine and cytokine response to bacterial endotoxin in healthy men. *AJP: Endocrinology and Metabolism*, *295*(3). doi:10.1152/ajpendo.90263.2008

Detillion, C. E., Craft, T. K., Glasper, E. R., Prendergast, B. J., & Devries, A. (2004). Social facilitation of wound healing. *Psychoneuroendocrinology, 29*(8), 1004-1011. doi:10.1016/j.psyneuen.2003.10.003

DeVries, A. C., Craft, T. K., Glasper, E. R., Neigh, G. N., & Alexander, J. K. (2007). 2006 Curt P. Richter award winner: Social influences on stress responses and health. *Psychoneuroendocrinology, 32*(6), 587-603. doi:10.1016/j.psyneuen.2007.04.007

Engeland, C. G., Nielsen, D. V., Kavaliers, M., & Ossenkopp, K. (2001). Locomotor activity changes following lipopolysaccharide treatment in mice: A multivariate assessment of behavioral tolerance. *Physiology & Behavior*, *72*(4), 481-491. doi:10.1016/s0031-9384(00)00436-4

Engstrøm, T., Barth, T., Melin, P., & Vilhardt, H. (1998). Oxytocin receptor binding and uterotonic activity of carbetocin and its metabolites following enzymatic degradation. *European Journal of Pharmacology, 355*(2-3), 203-210. doi:10.1016/s0014-2999(98)00513-5

Feldman, R., Monakhov, M., Pratt, M., & Ebstein, R. P. (2016). Oxytocin pathway genes: evolutionary ancient system impacting on human affiliation, sociality, and psychopathology. *Biological Psychiatry, 79*(3), 174-184. doi:10.1016/j.biopsych.2015.08.008

Higashida, H., Yokoyama, S., Kikuchi, M., & Munesue, T. (2012). CD38 and its role in oxytocin secretion and social behavior. *Hormones and Behavior, 61*(3), 351-358. doi:10.1016/j.yhbeh.2011.12.011

Hubner, M. P., Pasche, B., Kalaydjiev, S., Soboslay, P. T., Lengeling, A., Schulz-Key, H., . . . Hoffmann, W. H. (2008). Microfilariae of the filarial nematode

litomosoides sigmodontis exacerbate the course of lipopolysaccharide-induced sepsis in mice. *Infection and Immunity, 76*(4), 1668-1677. doi:10.1128/iai.01042-07

Işeri, S. Ö, Düşünceli, F., Erzik, C., Uslu, B., Arbak, S., & Yeğen, B. Ç. (2010). Oxytocin or social housing alleviates local burn injury in rats. *Journal of Surgical Research, 162*(1), 122-131. doi:10.1016/j.jss.2009.02.018

Jin, D., Liu, H., Hirai, H., Torashima, T., Nagai, T., Lopatina, O., . . . Higashida, H. (2007). CD38 is critical for social behaviour by regulating oxytocin secretion. *Nature, 446*(7131), 41-45. doi:10.1038/nature05526

Kelley, K. W., Bluthé, R., Dantzer, R., Zhou, J., Shen, W., Johnson, R. W., & Broussard, S. R. (2003). Cytokine-induced sickness behavior. *Brain, Behavior, and Immunity, 17*(1), 112-118. doi:10.1016/s0889-1591(02)00077-6

Kuteykin-Teplyakov, K., & Maldonado, R. (2014). Looking for prosocial genes: ITRAQ analysis of proteins involved in MDMA-induced sociability in mice. *European Neuropsychopharmacology, 24*(11), 1773-1783. doi:10.1016/j.euroneuro.2014.08.007

Landgraf, R., Neumann, I., Holsboer, F., & Pittman, Q. J. (1995). Interleukin-1β stimulates both central and peripheral release of vasopressin and oxytocin in the rat. *European Journal of Neuroscience, 7*(4), 592-598. doi:10.1111/j.1460-9568.1995.tb00663.x

Lerer, E., Levi, S., Israel, S., Yaari, M., Nemanov, L., Mankuta, D., . . . Ebstein, R. P. (2010). Low CD38 expression in lymphoblastoid cells and haplotypes are both associated with autism in a family-based study. *Autism Research, 3*(6), 293-302. doi:10.1002/aur.156

Lukas, M., & Neumann, I. D. (2013). Oxytocin and vasopressin in rodent behaviors related to social dysfunctions in autism spectrum disorders. *Behavioural Brain Research, 251,* 85-94. doi:10.1016/j.bbr.2012.08.011

Lund, F. E. (2006). Signaling properties of CD38 in the mouse immune system: Enzyme-dependent and -independent roles in immunity. *Molecular Medicine, 12*(11), 238-333.

Moertl, M., Friedrich, S., Kraschl, J., Wadsack, C., Lang, U., & Schlembach, D. (2011). Haemodynamic effects of carbetocin and oxytocin given as intravenous bolus on women undergoing caesarean delivery: A randomised trial. *BJOG: An International Journal of Obstetrics & Gynaecology, 118*(11), 1349-1356. doi:10.1111/j.1471-0528.2011.03022.x

Neumann, I. D., Wigger, A., Torner, L., Holsboer, F., & Landgraf, R. (2000). Brain oxytocin inhibits basal and stress-induced activity of the hypothalamo-pituitary-adrenal axis in male and female rats: Partial action within the paraventricular nucleus. *Journal of Neuroendocrinology, 12*(3), 235-243. doi:10.1046/j.1365-2826.2000.00442.x

Olszewski, P. K., Allen, K., & Levine, A. S. (2015). Effect of oxytocin receptor blockade on appetite for sugar is modified by social context. *Appetite, 86*, 81-87. doi:10.1016/j.appet.2014.10.007

Olszewski, P. K., Ulrich, C., Ling, N., Allen, K., & Levine, A. S. (2014). A non-peptide oxytocin receptor agonist, WAY-267,464, alleviates novelty-induced hypophagia in mice: Insights into changes in c-Fos immunoreactivity. *Pharmacology Biochemistry and Behavior, 124*, 367-372. doi:10.1016/j.pbb.2014.07.007

Partida-Sanchez, S., Cockayne, D. A., Monard, S., Jacobson, E. L., Oppenheimer, N., Garvy, B., . . . Lund, F. E. (2001). Cyclic ADP-ribose production by CD38 regulates intracellular calcium release, extracellular calcium influx and chemotaxis in neutrophils and is required for bacterial clearance in vivo. *Nature Medicine, 7*(11), 1209-1216.

Poutahidis, T., Kearney, S. M., Levkovich, T., Qi, P., Varian, B. J., Lakritz, J. R., . . . Erdman, S. E. (2013). Microbial symbionts accelerate wound healing via the neuropeptide hormone oxytocin. *PLoS ONE, 8*(10). doi:10.1371/journal.pone.0078898

Reyes-Lagos, J. J., Hadamitzky, M., Peña-Castillo, M. Á, Echeverría, J. C., Bösche, K., Lückemann, L., . . . Pacheco-López, G. (2016). Exogenous oxytocin reduces signs of sickness behavior and modifies heart rate fluctuations of endotoxemic rats. *Physiology & Behavior, 165*, 223-230. doi:10.1016/j.physbeh.2016.07.013

Ross, K. M., Mcdonald-Jones, G., & Miller, G. E. (2013). Oxytocin does not attenuate the ex vivo production of inflammatory cytokines by lipopolysaccharide-activated monocytes and macrophages from healthy male and female donors. *Neuroimmunomodulation, 20*(5), 285-293. doi:10.1159/000351610

Shattuck, E. C., & Muehlenbein, M. P. (2015). Human sickness behavior: Ultimate and proximate explanations. *American Journal of Physical Anthropology, 157*(1), 1-18. doi:10.1002/ajpa.22698

Starkhammar, M., Georén, S. K., Swedin, L., Dahlén, S., Adner, M., & Cardell, L. O. (2012). Intranasal administration of poly(I:C) and LPS in BALB/c mice induces airway hyperresponsiveness and inflammation via different pathways. *PLoS ONE, 7*(2). doi:10.1371/journal.pone.0032110

Vitalo, A., Fricchione, J., Casali, M., Berdichevsky, Y., Hoge, E. A., Rauch, S. L., . . . Levine, J. B. (2009). Nest making and oxytocin comparably promote wound healing in isolation reared rats. *PLoS ONE, 4*(5). doi:10.1371/journal.pone.0005523

Wang, P., Yang, H., Tian, S., Wang, L., Wang, S. C., Zhang, F., & Wang, Y. (2015). Oxytocin-secreting system: A major part of the neuroendocrine center regulating immunologic activity. *Journal of Neuroimmunology, 289*, 152-161. doi:10.1016/j.jneuroim.2015.11.001

Yuan, L., Liu, S., Bai, X., Gao, Y., Liu, G., Wang, X., . . . Wang, Z. (2016). Oxytocin inhibits lipopolysaccharide-induced inflammation in microglial cells and attenuates microglial activation in lipopolysaccharide-treated mice. *Journal of Neuroinflammation, 13*(1). doi:10.1186/s12974-016-0541-7

CHAPTER 4

GENERAL DISCUSSION

4.1 General Discussion

The present study examined the effects of OT manipulations on the expression of LPS-induced sickness behaviours, including anxiety and locomotor behaviour, and immune function. Adult male CD-1 mice were treated with the OT agonist, carbetocin, or the OT antagonist, L-368,899, in conjunction with LPS immune challenge, and the effects on locomotor and anxiety-like behaviour, as well as pro-inflammatory cytokines TNF-α and IL-6, were evaluated. It was found that treatment with the OT antagonist, L-368,899, attenuated LPS induced perturbations in locomotor and anxiety-like behaviour. In contrast, the OT agonist, carbetocin, had no effect on locomotor and anxiety-related sickness behaviours. However, effects on basal locomotor and anxiety-like behaviour were found for some measures for both the OT agonist and antagonist.

The current results were interesting in light of findings from previous research, which has investigated OT's role in immune activation in the context of subcutaneous wound healing (eg. Detillion et al., 2004), and most recently in conjunction with LPS immune challenge and behaviour (Reyes-Lagos et al., 2016; Yuan et al., 2016). Importantly, results of previous studies have generally shown that OT reduces cytokine activation and in the case of the Reyes-Lagos et al., (2016), rescues LPS induced perturbations in locomotor behaviour. In the present study, cytokine assays showed that LPS treatment produced a robust pro-inflammatory response as indicated by increased serum levels of both TNF-α and IL-6. Although dose-related effects of CBT were observed, neither the OT agonist, nor the OT antagonist, produced any significant effects on levels of these cytokines, which could be a function of the large degree of variability in that data, small sample sizes, or perhaps due to a floor effect. Regardless, behaviourally speaking the present study demonstrates that systemic treatment with an OT antagonist attenuates the expression of locomotor and anxiety-related sickness behaviour, perhaps via delaying the onset, or by suppressing behavioural expressions of sickness altogether.

4.1.1. Effects of OT Agonist Treatment

In the present study, treatment with 10 mg/kg of the OT agonist, CBT, plus saline, had suppressive effects on locomotor and anxiety-like behaviour in the LD test. The 10 mg/kg CBT treatment led to reduced locomotor activity overall. This effect was not

observed among the 20 mg/kg CBT or saline-control groups, suggesting that the 10 mg/kg CBT dose suppressed locomotor activity in a non-novel context. Interestingly, the 10 mg/kg CBT group also demonstrated a reduced number of transitions into the light chamber, which is a more anxiogenic context (Bourin & Hascoet, 2003), relative to the 20 mg/kg CBT group. This suggests that the 10 mg/kg dose of CBT augmented anxiety-like responses and reduced exploratory behaviour in this context. To some degree, the present findings contrast with those of previous studies. For instance, Ring et al. (2006) found that 10 mg/kg (i.p.) of OT 30 minutes prior to testing led to anxiolytic effects of peripheral OT on behaviour. Chaviaras et al. (2010) found that male rats treated with a high (ie. 20 mg/kg) but not low (ie. 2 mg/kg; 6.4 mg/kg) dose (i.p.) of OT agonist, carbetocin, 30 minutes prior to testing showed a significant decrease in total distances animals' travelled, suggesting suppressed locomotor activity and increased anxiety in the open field test.

The differences observed between the results of the present study and those of others could be attributed to the amount of time elapsed between OT administration and commencement of behavioural testing, as well as species and/or test-specific differences. Furthermore, these differential findings illustrate the dose-related differences in effects on anxiety-like behaviour in male rodents. In the present study, behavioural testing did not occur until 2 hours after OT agonist treatment. This may suggest that temporal variables play a role in mediating OT's effects on anxiety-like behaviour. Specifically, OT may exert anxiolytic effects shortly after treatment, as seen in other studies (eg. Ring et al. 2006), and anxiogenic effects in a dose-related manner following longer time delays between administration and testing, as seen in the present study. In the context of previous research, results of this study demonstrate the dose-related and potentially temporally-dependent nature of anxiolytic and anxiogenic effects of OT on behaviour. Furthermore, the existing ambiguity in findings illustrate the need for further research to explore OT agonist effects on behaviour, and to distinguish potential dose-and time-related effects of OT on sickness behaviour.

4.1.2. Effects of OT Antagonist Treatment

In the present study, treatment with the OT antagonist, L-368-899, at a 10mg/kg (i.p.) dose was found to augment locomotor and exploratory behaviour in male CD-1

mice. Furthermore, OT antagonist treatment resulted in mice spending significantly more time in the light chamber of the light-dark test, and making more Nose Pokes and Transitions into the light chamber, indicating an overall reduction in anxiety-like behaviour. These results are intriguing as previous research often attributes OT antagonist treatment with anxiogenic effects on behaviour (Neumann & Landgraf, 2012; Olszewski et al., 2014). For example, findings from a study by Ring et al. (2006) show that the OT antagonist, WAY-162720, at 10 mg/kg (i.p.) 30 minutes prior to testing lead to anxiogenic effects on behaviour in male mice. However, more recent studies have also shown effects consistent with that of the present study, as others have found that OT antagonist treatment has anxiolytic effects in certain contexts. For instance, Duque-Wilckens et al. (2016) showed that in female mice treated with L-368,899 (i.p.) 30 min before behavioural testing, the OT antagonist blocked the effects of stress in both the social interaction and odor preference tests, such that animals showed high levels of social interaction, and preferred the odor of an unfamiliar individual. Conversely, when stressed, saline treated mice showed reduced social interaction and a preference for the odor of a familiar cagemate, suggesting that OT antagonist treatment has anxiolytic effects on behaviour in social contexts (Duque-Wilckens et al., 2016). Guzman et al. (2014) showed that in male mice OT receptor function appears to exert bidirectional effects on anxiety-like behaviour related to social fear, as OT appears to decrease anxiety following positive, but increase anxiety after negative, social encounters. Importantly, it was shown that OT antagonist treatment to the lateral septum of mice abolished anxiogenic behaviour during fear conditioning, suggesting that OT antagonists have anxiolytic effects on anxiety-like behaviour in social contexts (Guzman et al., 2014). Therefore, it is possible that in the current study the OT antagonist reduced anxiety-like behaviour in male mice due to context-dependent effects, and that the 2 hour time delay between drug administration and behavioural testing also influenced effects on anxiety-like behaviour.

4.1.3. LPS and OT Effects on Cytokines

The present study showed that LPS treatment robustly increased serum levels of both TNF-α and IL-6, indicating inflammation, as all LPS treated groups showed significantly higher concentrations of both cytokines relative to non-LPS treated animals.

Neither the OT agonist, CBT, nor the OT antagonist, significantly affected serum levels of TNF-α or IL-6 in saline controls, suggesting that OT does not affect these cytokines in absence of LPS treatment. However, further analyses showed that animals treated with 10 mg/kg CBT-LPS, but not 20 mg/kg CBT-LPS, had significantly higher serum concentrations of TNF-α relative to saline-LPS treated mice. These results are suggestive of OT's effects on TNF-α being dose-related, though further studies are needed to clarify any dose-related effects. Effects of OT agonist treatment in conjunction with LPS were also observed for IL-6. However, dose-related effects were not observed in this case as both CBT-LPS treated groups showed significantly higher serum concentrations of IL-6 compared to saline-LPS treated mice. Previous research suggests that OT treatment tends to decrease serum levels of pro-inflammatory cytokines, such as TNF-α and IL-6 (Clodi et al., 2008; Wang et al., 2015; Yuan et al., 2016), while other studies such as that done by Ross et al. (2013) have shown that OT does not attenuate pro-inflammatory cytokines. Evidently, further research is needed to elucidate the effects and mechanisms by which OT influences inflammation, since considerable variability between studies remains regarding OT's effects on pro-inflammatory cytokines.

4.1.4. Limitations and Future Research

Methodological limitations in the present study may account for the results observed. In particular, since there are relatively few existing studies in mice with which to determine dosages, calculating the dose of OT drugs to be used here was challenging. As well, the 2 hour time delay between OT drug administration and testing may have differentially affected behavioural and cytokine outcomes. In the majority of previous studies, there is often only a 30 minute time delay between OT treatment and subsequent testing due to the relatively short half-life of OT (eg. Chaviaras et al., 2010; Ring et al., 2006). Therefore, in the present study behavioural and immune effects of OT may have been complicated due to lower systemic levels of OT, potentially reducing its effects on behaviour and cytokines. Future research could examine OT's effects on sickness and anxiety-like behaviour, as well as cytokines, at varying dosages to elucidate dose-related effects of OT in mice. As well, examining OT's effects on sickness and anxiety-like behaviours and cytokines at different time points after drug administration may be useful in clarifying time-related effects of OT.

The nature of the behavioural task used in this study is another potential methodological limitation that could be addressed in future studies. In particular, the light-dark test presents a challenge in that it can be difficult to distinguish locomotor behaviour exclusively from anxiety-like behaviour. As such, using other behavioural assays that can more clearly distinguish anxiety and sickness-related behaviours might be a way to resolve this potential limitation in the future. Since numerous studies have shown that OT is strongly associated with the mediation of social behaviours, such as social affiliation (Choleris et al., 2013; Ross & Young, 2009), and LPS induced sickness has been shown to affect social behaviour in rodents (Arakawa et al., 2015; Kavaliers & Choleris, 2011), future studies should apply social behavioural paradigms, such as the social interaction or odor preference tests, to investigate OT's effects on anxiety and sickness behaviour in social contexts where OT mediated behaviours are more likely to be affected (Guzman et al., 2013; Shamay-Tsoory & Abu-Akel, 2016).

Stress experienced during behavioural testing, as well as small and uneven treatment group sizes, may have adversely influenced results of the cytokine assays. While attempts were made to ensure that animals were comfortable, handling stress, isolation housing, and behavioural testing would undoubtedly have caused some degree of distress among mice. This stress may have confounded cytokine activity via HPA mediated mechanisms, as HPA activity has previously been shown to increase levels of pro-inflammatory cytokines (Anisman & Merali, 2002), potentially creating a ceiling effect. This limitation could be circumvented in future studies by using separate animals for behavioural versus immunological testing, as well as increasing and equalizing group size numbers.

In the present study only male animals were used due to time constraints and the potential confounding effects of the estrous cycle in females. Future research could more thoroughly examine OT's effects on anxiety and sickness-related behaviour in both males and females, by tracking the estrous cycle in females. Previous studies have shown that the estrous cycle influences immune function and sickness behaviour in a manner that is dependent on estrous phase (Engeland et al., 2006). As such, investigating the effects of OT on sickness behaviours in females at specific points in the estrous cycle may reveal important hormone-related effects of OT on behavioural responses and immune function.

Literature on the role of cytokines in relation to anxiety remains quite sparse. However, a limited number of studies have shown a link between levels of pro-inflammatory cytokines and anxiety-like behaviour in rats and mice. For example, Ramirez et al. (2016) found that in male mice treated daily with 15mg/kg (orally) of the anti-depressant, imipramine, there was an attenuation of social defeat stress-induced corticosterone and IL-6 levels in serum, with corresponding reductions in anxiety-like behaviour in the open field test. Furthermore, in another study it was shown that daily treatment with 20mg/kg (i.p.) of imipramine in male mice subjected to repeated social defeat stress, there was an increase in social interaction time indicating reduced social anxiety, with corresponding reductions brain levels of pro-inflammatory cytokine, IL-6 (Ramirez et al., 2015). Furthermore, in response to LPS immune challenge, Ramirez et al. (2015) also found that imipramine reduced levels of TNF-α, IL-6, and IL-1β in the brain microglia of mice subjected to social defeat stress. These studies clearly demonstrate a link between stress-induced pro-inflammatory cytokines and anxiety-like behaviour in social contexts, with a potential role for OT. As such, future research should be conducted to better understand the link between pro-inflammatory cytokines, anxiety, and sickness-related behaviour, along with the potential role for OT in mediating these behaviours and immune function.

4.1.5. Conclusions

The present study showed that treatment with the OT antagonist, L-368,899, attenuates the expression of LPS-induced perturbations in locomotor activity and anxiety-like sickness-related behaviour, effectively normalizing these particular sickness behaviours. However, neither the OT agonist, nor the OT antagonist, produced any significant effects on serum TNF-α and IL-6 levels. Together, these findings demonstrate that systemic treatment with an OT antagonist attenuates the expression of locomotor and anxiety-related sickness behaviours, perhaps by delaying the onset or suppressing sickness behaviour altogether, and that this effect may be independent of cytokine mediated mechanisms. As such, this study provides evidence that OT antagonists may be efficacious in treating some behavioural symptoms of sickness, such as lethargy and anxiety. Results of this study also suggest that OT antagonists may have potential as anxiolytic treatments. However, in consideration of the present findings along with that

of previous research, further research is justified as the precise effects of, and mechanisms by which, OT influences anxiety, sickness behaviour, and immune function remain to be elucidated.

4.2 References

Anisman, H., & Merali, Z. (2002). Cytokines, stress, and depressive illness. *Brain, Behavior, and Immunity, 16*(5), 513-524. doi:10.1016/s0889-1591(02)00009-0

Arakawa, H., Blanchard, D. C., & Blanchard, R. J. (2015). Central oxytocin regulates social familiarity and scent marking behavior that involves amicable odor signals between male mice. *Physiology & Behavior, 146*, 36-46. doi:10.1016/j.physbeh.2015.04.016

Bourin, M., & Hascoët, M. (2003). The mouse light/dark box test. *European Journal of Pharmacology, 463*(1-3), 55-65. doi:10.1016/s0014-2999(03)01274-3

Chaviaras, S., Mak, P., Ralph, D., Krishnan, L., & Broadbear, J. H. (2010). Assessing the antidepressant-like effects of carbetocin, an oxytocin agonist, using a modification of the forced swimming test. *Psychopharmacology, 210*(1), 35-43. doi:10.1007/s00213-010-1815-x

Choleris, E., Pfaff, D. W., & Kavaliers, M. (2013). *Oxytocin, vasopressin and related peptides in the regulation of behavior*. New York: Cambridge University Press.

Clodi, M., Vila, G., Geyeregger, R., Riedl, M., Stulnig, T. M., Struck, J., . . . Luger, A. (2008). Oxytocin alleviates the neuroendocrine and cytokine response to bacterial endotoxin in healthy men. *AJP: Endocrinology and Metabolism, 295*(3). doi:10.1152/ajpendo.90263.2008

Detillion, C. E., Craft, T. K., Glasper, E. R., Prendergast, B. J., & Devries, A. (2004). Social facilitation of wound healing. *Psychoneuroendocrinology, 29*(8), 1004-1011. doi:10.1016/j.psyneuen.2003.10.003

DeVries, A. C., Craft, T. K., Glasper, E. R., Neigh, G. N., & Alexander, J. K. (2007). 2006 Curt P. Richter award winner: Social influences on stress responses and health. *Psychoneuroendocrinology, 32*(6), 587-603. doi:10.1016/j.psyneuen.2007.04.007

Duque-Wilckens, N., Steinman, M. Q., Tan, P. B., Hao, R., & Trainor, B. C. (2016). Systemic inhibition of oxytocin receptors blocks stress induced social anxiety in female California mice [Abstract]. *Society For Neuroscience*.

Engeland, C. G., Kavaliers, M., & Ossenkopp, K. (2006). Influence of the estrous cycle on tolerance development to LPS-induced sickness behaviors in rats. *Psychoneuroendocrinology, 31*(4), 510-525. doi:10.1016/j.psyneuen.2005.11.007

Guzmán, Y. F., Tronson, N. C., Jovasevic, V., Sato, K., Guedea, A. L., Mizukami, H., . . . Radulovic, J. (2013). Fear-enhancing effects of septal oxytocin receptors. *Nature Neuroscience, 16*(9), 1185-1187. doi:10.1038/nn.3465

Guzmán, Y. F., Tronson, N. C., Sato, K., Mesic, I., Guedea, A. L., Nishimori, K., & Radulovic, J. (2014). Role of oxytocin receptors in modulation of fear by social memory. *Psychopharmacology, 231*(10), 2097-2105. doi:10.1007/s00213-013-3356-6

Kavaliers, M., & Choleris, E. (2011). Sociality, pathogen avoidance, and the neuropeptides oxytocin and arginine vasopressin. *Psychological Science, 22*(11), 1367-1374. doi:10.1177/0956797611420576

Neumann, I. D., & Landgraf, R. (2012). Balance of brain oxytocin and vasopressin: Implications for anxiety, depression, and social behaviors. *Trends in Neurosciences, 35*(11), 649-659. doi:10.1016/j.tins.2012.08.004

Olszewski, P. K., Ulrich, C., Ling, N., Allen, K., & Levine, A. S. (2014). A non-peptide oxytocin receptor agonist, WAY-267,464, alleviates novelty-induced hypophagia in mice: Insights into changes in c-Fos immunoreactivity. *Pharmacology Biochemistry and Behavior, 124*, 367-372. doi:10.1016/j.pbb.2014.07.007

Ramirez, K., Shea, D. T., Mckim, D. B., Reader, B. F., & Sheridan, J. F. (2015). Imipramine attenuates neuroinflammatory signaling and reverses stress-induced social avoidance. *Brain, Behavior, and Immunity, 46*, 212-220. doi:10.1016/j.bbi.2015.01.016

Ramirez, K., & Sheridan, J. F. (2016). Antidepressant imipramine diminishes stress-induced inflammation in the periphery and central nervous system and related anxiety- and depressive- like behaviors. *Brain, Behavior, and Immunity*, *57*, 293-303. doi:10.1016/j.bbi.2016.05.008

Reyes-Lagos, J. J., Hadamitzky, M., Peña-Castillo, M. Á, Echeverría, J. C., Bösche, K., Lückemann, L., . . . Pacheco-López, G. (2016). Exogenous oxytocin reduces signs of sickness behavior and modifies heart rate fluctuations of endotoxemic rats. *Physiology & Behavior, 165*, 223-230. doi:10.1016/j.physbeh.2016.07.013

Ring, R. H., Malberg, J. E., Potestio, L., Ping, J., Boikess, S., Luo, B., . . . Rosenzweig-Lipson, S. (2006). Anxiolytic-like activity of oxytocin in male mice: Behavioral and autonomic evidence, therapeutic implications. *Psychopharmacology, 185*(2), 218-225. doi:10.1007/s00213-005-0293-z

Ross, K. M., Mcdonald-Jones, G., & Miller, G. E. (2013). Oxytocin does not attenuate the ex vivo production of inflammatory cytokines by lipopolysaccharide-activated monocytes and macrophages from healthy male and female donors. *Neuroimmunomodulation, 20*(5), 285-293. doi:10.1159/000351610

Ross, H. E., & Young, L. J. (2009). Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Frontiers in Neuroendocrinology, 30*(4), 534-547. doi:10.1016/j.yfrne.2009.05.004

Wang, P., Yang, H., Tian, S., Wang, L., Wang, S. C., Zhang, F., & Wang, Y. (2015). Oxytocin-secreting system: A major part of the neuroendocrine center regulating immunologic activity. *Journal of Neuroimmunology, 289*, 152-161. doi:10.1016/j.jneuroim.2015.11.001

Yuan, L., Liu, S., Bai, X., Gao, Y., Liu, G., Wang, X., . . . Wang, Z. (2016). Oxytocin inhibits lipopolysaccharide-induced inflammation in microglial cells and attenuates microglial activation in lipopolysaccharide-treated mice. *Journal of Neuroinflammation, 13*(1). doi:10.1186/s12974-016-0541-7

Appendices

Appendix A – Luminex Mouse Cytokine/ Chemokine Magnetic Bead Panel Kit Manual

Julie M. Deleemans

Education

Master of Science in Psychology 2014 – 2016
Western University, London, ON.

- Specialized in behavioural and cognitive neuroscience
- *Thesis Title*: Oxytocin's effects on sickness behaviours, anxiety responses, and immune function in adult male mice
    - Supervisors: Dr. Martin Kavaliers, Dr. Christine Tenk, & Dr. Klaus-Peter Ossenkopp

Bachelor of Arts - Honors Specialization in Psychology 2011 – 2014
Brescia University College, London, ON

- Specialized in developmental and health psychology
- *Thesis Title*: The effects of neonatal LPS exposure on depressive-like behaviours in male and female adolescent rats
    - Supervisor: Dr. Christine Tenk

Certificate in Human Services 2007 – 2008
Fanshawe College, London, ON

- Deans honor roll (2008)

Academic & Teaching Experience

Teaching Assistant 2014 – 2016
Western University, London, ON

- Psychology 1000 – Introduction to Psychology

Teaching Assistant 2014 – 2016
Brescia University College, London, ON

- Psychology 2054 A/B – The Psychology of Eating

Undergraduate Honors Thesis Student Co-Supervisor 2014 – 2016
Western University, London, ON

- Provided guidance and assistance with experimental procedures, data collection, analysis, and writing of thesis

Publications

- **Deleemans, J. M.**, Wang, K., Ossenkopp, K. P., Kavaliers, M., & Tenk, C. M. (2016). *Effects of an oxytocin agonist and antagonist on lipopolysaccharide elicited locomotor, anxiety, and immune responses in male mice*. Manuscript in preparation, Western Ontario University.

- **Deleemans, J. M.**, Ossenkopp, K. P., Tenk, C. M., & Kavaliers, M. (2015). Effects of the oxytocin agonist, carbetocin, on sickness behaviours and immune responses in male mice. *Society For Social Neuroscience Abstracts*.

- **Deleemans, J. M.**, Ossenkopp, K. P., Tenk, C. M., & Kavaliers, M. (2015). Effects of the oxytocin agonist, carbetocin, on sickness behaviours and immune responses in male mice. *Society For Neuroscience Abstracts*. (No. 811.02)

- **Deleemans, J. M.**, Ossenkopp, K.P., Tenk, C. M., & Kavaliers, M. (2015). The effects of oxytocin on sickness behaviours and anxiety responses in male mice. *Southern Ontario Neuroscience Association Abstracts*.

Poster Presentations

- **Deleemans, J. M.**, Ossenkopp, K. P., Tenk, C. M., & Kavaliers, M. (2015). Effects of the oxytocin agonist, carbetocin, on sickness behaviours and immune responses in male mice. Presented at the *Society For Neuroscience Meeting* (Chicago, Illinois).

- **Deleemans, J. M.**, Ossenkopp, K. P., Tenk, C. M., & Kavaliers, M. (2015). Effects of the oxytocin agonist, carbetocin, on sickness behaviours and immune responses in male mice. Presented at the *Society For Social Neuroscience Meeting,* (Chicago, Illinois).

- **Deleemans, J. M.**, Ossenkopp, K.P., Tenk, C. M., & Kavaliers, M. (2015). The effects of oxytocin on sickness behaviours and anxiety responses in male mice. Presented at the *Southern Ontario Neuroscience Association Meeting* (Hamilton, Ontario).

- **Deleemans, J. M.**, & Tenk, C. M. (2014). The effects of neonatal LPS exposure on depressive-like behaviours in male and female adolescent rats. Presented at the *Brescia University College Psychology Honors Program Conference* (London, Ontario).

- K. Colasanti, **J.M. Deleemans**, K.P. Ossenkopp, & M. Kavaliers (2016). Effects of adolescent corticosterone treatment on anxiety-like behaviours in male rats. Presented at the *Western University Psychology Honors Program Conference* (London, Ontario).

- K.M. Kezele, **J.M. Deleemans**, K.P. Ossenkopp, & M. Kavaliers (2015). Sexually dimorphic effects of neonatal LPS on anxiety-like behaviour in the dark-light paradigm following administration of a second acute adult immune challenge. Presented at the *Western University Psychology Honors Programs* Conference (London, Ontario).

Related Experience

Graduate Course - Animal Models in Behavioural Neuroscience 2015
Western University, London, ON

- *Major Research Paper*: Novel approaches to behavioural testing and naturopathic pharmacological treatments for depression
- *Research Project*: Probiotic intervention in nutritionally deficient diet induced developmental models of mental illness
- Conducted literature reviews for research studies, subsequent writing of research proposals, and developed protocols for project implementation

Graduate Course - Research Design and Statistical Modeling 2014 – 2015
Western University, London, ON

- Univariate and multivariate statistical and modeling procedures
- I. Foundational Statistics: sampling distributions, inferential statistics, confidence intervals, effect size, and power
- II. ANOVA, ANCOVA and MANOVA: experimental and quasi experimental designs
- III. Multiple Regression and Extensions: mediation, moderation, multilevel modeling, and models for categorical outcomes such as logistic regression
- IV. Factor Analysis and Structural Equation Modeling

Summer Research Assistant 2014
Western University, Kavaliers/Ossenkopp Lab

- Conditioned place preference study with LPS and scopolamine – data collection, analysis, and report writing

Professional Certifications

- Western University Animal Care and Veterinary Services 2013
  - WebCT Animal Care and Use Course
  - Basic Rat and Basic Mouse Training
  - Injections and Blood Collection Techniques Training
  - Supported by the Canadian Council on Animal Care
- Western University Basic WHMIS Training 2014
- Western University Accessibility at Western (AODA) 2014
  - Accessibility in Teaching
- Western University General Laboratory Safety and Hazardous Waste Management Training 2014
- Western University Safe Campus Community 2014
  - Preventing Harassment, Violence and Domestic Violence
- Western University Worker Health and Safety Awareness 2014

Honors & Awards

- Brescia University College Entrance Scholarship 2011
- Brescia University College Deans honor roll 2012 – 2014
- Western Graduate Research Scholarship 2014 – 2016

Volunteer Experience

- Editor – Western Undergraduate Psychology Journal 2014 – 2016
- Nutrition/recipe contributor
  - InHealth Technologies Speakers Club newsletter 2016
- Big Brothers Big Sisters mentoring program 2015 – 2016
- Canadian Cancer Society 2009 – 2010

Affiliations

- Canadian Association of Psychosocial Oncology 2016
- Society for Neuroscience 2015 – 2016
- Society for Social Neuroscience 2015 – 2016