INTERSPECIFIC ATTACHMENT: SOCIAL BONDS BETWEEN HUMANS AND THEIR 'BEST FRIENDS'

by

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A thesis submitted to the School of Graduate Studies

in partial fulfilment of the requirements for the degree of

Master of Science

Cognitive and Behavioural Ecology Programme/Department of Psychology

Memorial University of Newfoundland

October 2015

St. John's Newfoundland and Labrador

ABSTRACT

Attachment refers to an individual seeking and maintaining close proximity to another individual. Although, relatively few studies have examined attachment in an interspecific context the human-dog bond has recently gained a great deal of attention, as this relationship has been subjected to thousands of years of co-evolutionary history. I examined the nature of the human-dog bond in the context of an amended Ainsworth's Strange Situation procedure, in which dogs experience a series of separation and reuniting events from their owners and are introduced to a stranger. Several facets of attachment were tested, predominantly preference (physical proximity and contact) and separation-induced stress. Dogs and owners also provided saliva samples to obtain physiological indicators of stress: cortisol (CORT) and chromogranin A (CgA). Owners completed a series of questionnaires including: human personality (NEO-FFI-3), dog personality (MCPQ-R), attachment (DAQ) and supplemental questions regarding health and about the dyad's relationship (e.g., duration of cohabitation). Overall, dogs demonstrated behavioural manifestations of attachment, as they spent more time in close proximity and in physical contact with owners compared to strangers. Neither dogs nor owners showed elevated CgA levels at the throughout the procedure. Owners experienced a decrease in CORT throughout the procedure, whereas CORT levels in some dogs increased and some dogs decreased. CORT was related to dog behaviour, e.g., dogs with higher CORT scratched the door more frequently and engaged in more contact bouts with owners. Owners and dogs did not 'match' on analogous personality factors, but they did complement each other in interesting ways (e.g., owners scoring high on Conscientiousness had dogs that scored high on Training-focus).

ACKNOWLEDGEMENTS

Allow me to begin by thanking my supervisors Dr. Carolyn Walsh and Dr. Anne Storey for their guidance and support. This co-supervision has provided me with the resources from two brilliant researchers, resulting in a thorough analysis (or as thorough as a Master's thesis can be) of the human-dog relationship. Carolyn passed on her wisdom of compartmentalization—something I needed desperate help in. She was always there with an open door and a way to make sense of the data. As this was my second time working with Anne, it was nice to have the perspective of someone I have modelled my research skills after. Like Carolyn, her door was open and she always had interesting suggestions for the overall 'story' or 'take home message' of each chapter. In combination, I could not have asked for a better supervision arrangement and I am truly grateful for them both. It does, however, take the hard work of many to produce and execute a research project and I have been blessed with an excellent team of collaborators, lab-mates and family to top it off!

The individuals on my committee were deliberate and obvious choices. At the side of my main supervisors, I had two additional committee members, Dr. Rita Anderson (cognitive psychologist) and Dr. Hélène Volkoff (endocrine biologist), who have been extremely helpful in the development of my research design, but also in problem solving and data analysis. Having a team with a variety of specializations demonstrated the importance of collaboration and that seeing the many facets of my topic is better than viewing it as a piece separated from an elaborate puzzle. As with many theses, my topic grew and evolved past what I originally imagined it would be. Some setbacks did occur, which led to the topic and original design changing quite drastically in some areas, while still addressing my main question—what is the nature of the human-dog relationship? Therefore, I will mention individuals who have helped me

along the way rather than simply addressing those who helped with aspects directly relevant to this thesis.

I would like to thank Dr. Donald McKay for the use of his lab and for teaching me the necessary techniques to carry out the oxytocin ELISA protocol. This protocol also would not have been possible without my collaboration with a summer student, Kylie Goodyear, who helped me implement pilot analyses and shared her insight on biochemical analytes as well as her connections within the relevant university departments.

The behavioural scenario, the Strange Situation, used in my study would not have been possible without my 'strangers' who were individuals not known to the dog being tested. These individuals included Mariana Castro, Lydia Ottenheimer Carrier, Julie Posluns and Ashley Quirke. The role of 'stranger' was no easy task as these women had to make themselves available to me for testing periods, they had to interact with the dog and owner, and they often had to hide near the study room so as to not meet the dog prematurely.

Having two supervisors also led to my participation in two research labs—one predominantly seabird and human researchers, and one predominantly dog and pig researchers. In total my lab-mates are: Sarah Adcock, Hayley Alloway, Marianna Castro, Michelle Fitzsimmons, Amy-Lee Kouwenberg, Kalita McDowell, Lydia Ottenheimer Carrier, Julie Posluns and Kathy Unger—thank you all for your constant input of ideas and suggestions. The aforementioned individuals were permanent fixtures in the lab if you will; however, we were also fortunate to have individuals who have worked with us for brief periods of time through MUCEP, WISE or other student employment opportunities. Therefore, I would like to highlight the contribution of Juanita Bartlett, Christina Hamlyn, Melissa Lye, Kimberly Williams and

Brittany White, thank you all for helping me achieve my deadlines (pre-ABS conference) and to complete the work with professionalism and accuracy.

To videotape my procedure (Strange Situation) I used a series of security cameras attached to a hard drive, which led to some technical problems. Fortunately, I had the help of a willing and interested technician, Avery Earle, who I affectionately call the 'magic man'—behind his back, obviously. Thank you for your technical support and love of 'impossible' things! Though I will never understand how Python Code works or how my Logger output gets turned into frequencies and durations, I am grateful that you do and that I did not have to do my calculations by hand.

To the BGSA (Biology Department) and Dr. Gerard Martin (Psychology Department), thank you for providing me with a space to perform the "Strange Situation". Space is extremely hard to find on campus, and it was looking grim for a while, but then you both stepped in giving me the room I needed!

Last but not least, to my family and friends, thank you for your continued support throughout my education. Whether you expressed an interest in my research or helped me take a step back, I am immensely appreciative of your contribution. To my mother, you have always been my greatest supporter in my educational endeavours. You always showed an interest in my field—even to the extent of conducting your own animal behaviour experiments! Dad, thank you for your handiwork. You were always there to humour me in my research, from creating a box to 'candle' and carry seabird eggs to helping me set-up and take-down my study rooms. Justin Foley, thank you for listening to and reading whatever I put in front of you. To the Entomology lab (Agriculture Canada), thank you for your encouragement and advice throughout this process

and for giving me time to work on my own project; namely, Jordan Bell, Jeff Bonazza, Ryan Oram, Dr. Peggy Dixon, Dr. Carolyn Parsons and Todd Power. Last but not least I would like to thank my church family for their prayers and support, with special mention to Daniel Bentum, Eni-Oto Familusi and Sarah McMeekin.

Thank you to all who have made this project possible. Aside from the important individuals, I would also like to make mention of the funding sources that fuelled this research, namely in the grants obtained by my supervisors from NSERC (NSERC Discovery Grants) as well as my personal funding sources: NSERC's Alexander Graham Canadian Graduate Scholarship, Memorial University's School of Graduate Studies Fellowship, Faculty of Science (Dean's Excellence Award) and the Dr. Anna C. Templeton Memorial Graduate Scholarship.

This degree has been an excellent experience and has motivated me to pursue future research when I complete my M.D. at Memorial University of Newfoundland. I can only hope that the next step brings as many wonderful people as this degree has afforded me with.

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CHAPTER 1: INTRODUCTION AND CO-AUTHORSHIP STATEMENT

1.1 INTRODUCTION

The human-dog relationship is an enduring interspecific bond, originating at least 10 000 years ago according to archaeological studies, although certain genetic studies suggest that domesticated dogs (*Canis familiaris*) were living with humans as early as 100 000 years ago (Axelsson et al., 2013; Germonpré et al., 2009; Hare, Wobber & Wrangham, 2012; Miklósi, 2007; Nagasawa, Kikusui, Onaka, & Ohta, 2009; Vilá et al., 1997). Cave drawings produced approximately 5000 years ago in Africa (Fenton, 1992) and the discovery of dog remains in ancient Natufian burial grounds (~10 000 years ago, Israel; Tchernov, 1997), depict our close association with dogs and illustrates, historically, the respect and honour humans had for them.

The mechanism of origin for this relationship is not clearly defined, namely, in whether dogs or humans initiated social contact or if the relationship stemmed from mutual social tolerance leading to cooperation (Range & Virányi, 2015; Trut, Oskina, & Kharlamova, 2009). Some theories suggest that early humans kept dogs for symbolic and/or utilitarian purposes, while others implicate the dog-like ancestor in 'self' domestication for personal gain (Germonpré et al., 2015; King, Marston & Bennett, 2012; Waller et al., 2013). The human-dog relationship was likely forged, however, from the advantages of communal living, which led to intense mutualism as dogs reaped the benefits of scraps and detritus to feed on and humans gained added security and hunting prowess from their dog companions (Axelsson et al., 2013; Coppinger & Coppinger, 2001).

One aspect that clouds dog domestication research is the discrepancy in the geographic location of where domestication originated. Some sources suggest several geographic regions of

origin, while others predominantly implicate the Middle East, Europe or East Asia (Dayan, 1994; Larson et al., 2012; Lupo & Janetski, 1994; Ovodov et al., 2011; Pang et al., 2009; Pennisi, 2013; Savolainen, Zhang, Luo, Lundeberg & Leitner, 2002; Wayne, 1993). Further, these geographic discrepancies impact how and when the wolf-like ancestor diverged to produce wolves and domesticated dogs, respectively. The history remains unclear predominantly because of the conflicting evidence available; that is most archaeological samples have been found in Europe, but dogs are genetically more similar to wolves from the Middle East than they are to Asian and European wolves.

Most researchers do agree, however, that domestication started with a wolf-like ancestor that possessed the necessary social structure required for approaching and maintaining close interactions with humans (Miklósi, 2007; Coppinger & Coppinger, 2001; Koler-Matznick, 2002). Many behavioural attributes of the domestic dog seem to be unlike those of other canids (*Canis*), a genus that recognizes eight species (seven wild dogs and the domestic dog; Miklósi, 2007; Fahey & Myers, 2000) that differ substantially in behaviour and morphology.

Dogs are thought to be unique in performing many problem-solving tasks that require aid from human gestures (e.g., distal pointing task to locate hidden food; Hare, Call & Tomasello, 1998; Kundey et al., 2010; Lakatos, Gácsi, Topál & Miklósi, 2012; Passalacqua et al., 2011; Scheider, Grassmann, Kaminsk & Tomasello, 2011; Topál, Kis & Oláh, 2014), in developing attachments (preferences) towards human caregivers (e.g., Gácsi et al., 2005; Mariti et al., 2013; Palestrini, Prato-Previde, Spiezo & Verga, 2005; Palmer & Custance, 2008; Prato-Previde, Custance, Spiezo & Sabatini, 2003; Rehn, McGowan & Keeling, 2013; Topál, Miklósi, Csányi & Dóka, 1998; Topál et al., 2005) and in overall eye contact and gaze durations towards humans (Call, Bräuer, Kaminski & Tomasello, 2003; Miklósi et al., 2003). In contrast, other canids such

as wolves (*C. lupus*), dingoes (*C. dingo*) and foxes (e.g., *Vulpes vulpes*) tend to do poorly in tasks relying on human help, but equal and often surpass domesticated dogs in novel, independent tasks, e.g., rope pulling to obtain a food reward (Gácsi et al., 2005; Hiestand, 2011; Miklósi et al., 2003; Smith & Litchfield, 2010a; Smith & Litchfield, 2010b; Smith & Litchfield, 2013; Trut, 2001). Recent literature has brought to light, however, that for other canids (e.g., wolves) to perform on par with domestic dogs, constant human interaction (captivity) and the right environment is required (Udell, Spencer, Dorey & Wynne, 2008; Udell, Spencer, Dorey & Wynne, 2012). These findings highlight the influence of domestication on dog behaviour as compared to other canid relatives, suggesting that during the early stages of domestication, the wolf-like ancestor likely possessed a distinctive set of personality and behavioural characteristics required to initiate close, interspecific associations. For example, dogs have the ability to maintain and use eye contact to communicate and the boldness to initiate social interactions (e.g., Miklósi et al., 2003).

Substantial evidence suggests that dogs have been selected for personality characteristics, attentional focus and attachment behaviours required for domestic life with humans (Hare, Call & Tomasello, 1998; Miklósi, Topál & Csányi, 2002; Miklósi, 2007; Mongillo, Bono, Regolin, & Marinelli, 2010). These abilities include perception of human behaviour and the ability to adapt to quick, random changes occurring in any given social context (Nagasawa et al., 2009; Pongracz, Miklósi, Vida & Csányi, 2005; Range, Aust, Steuer & Huber, 2008). Indeed, domestic dogs seem to be unique in their 'social competence' (Topál, Kis & Oláh, 2014), as they have developed communicative sensitivity towards humans, which is required to interact and extract information, such as signalling *wants* (e.g., obtaining food) and perceiving human vocal and visual cues (Call et al., 2003; Mongillo et al., 2010; Range et al., 2008).

Despite our long history with dogs, we currently know very little about the ontogeny and function of many dog behaviours (Scott & Fuller, 1998). This gap is partially due to the fact that ethologists originally believed that the artificial selection involved in domestication prevented conclusions about the 'wild' or 'natural' behaviours typically studied in undomesticated animals, such as mating systems, sexual selection and foraging for food in nature (Miklósi, 2007). Additionally, domestic dogs were regarded as being very clever and perceptive of human movements, vocalizations and gestures, leading researchers to assume that dogs may learn tasks too quickly or respond too much to human cues, thus tainting behavioural or invasive experimentation (Griffin, 1984; Miklósi, 2007). Therefore, due to these preconceptions, dogs were simply not studied in these contexts. However, the last 20 years have marked the advent of dog research that goes beyond experiments involving conditioning (e.g., Pavlov, 1927) or invasive physiological procedures (e.g., Banting, Best, Collip, Campbell & Fletcher, 1921). Over this time, ethologists acknowledged that domestic dogs' natural environment was in human society and that understanding and documenting dog behaviour could not only aid in developing methods to effectively study them, but it could also shed light on human evolutionary history (Miklósi, 2007; Topál et al., 2014).

Dog research may help us to uncover certain mysteries regarding human evolutionary history, namely social behaviour and early human movements (i.e., biogeography). Consider social bonding, for example, even though many authors accept the human-dog relationship as an attachment bond, little is known about the neurobiological and physiological systems underlying the social aspect of this interspecific bond. Results of certain studies suggest that the human-dog relationship taps into similar hormonal pathways as those seen in parent-child interactions (e.g., oxytocin increases in response to physical contact in both owners and dogs, Handlin et al., 2011).

Therefore, it is important to understand the mechanisms leading to human attachments to non-human animals and the ways in which this bond mirrors other affiliative, intraspecific social interactions (Hare, Brown, Williamson & Tomasello, 2002; Horn, Huber & Range, 2013; Mariti et al., 2013; Miklósi et al., 2003; Palestrini et al., 2005; Palmer & Custance, 2008; Prato-Previde et al., 2003; Rehn, McGowan & Keeling, 2013; Topál et al., 1998; Topál et al., 2005). It is also important to evaluate whether the formation and maintenance of individual social bonds occurs in predictable ways and whether this information could shed light on how these relationships evolved.

The human-dog relationship, based on mutual needs, also presents an interesting model to examine the neurobiology of attachment-based relationships. A recent study by Stoeckel, Palley, Gollub, Niemi and Evins (2014) revealed some neurobiological similarities and differences between the human-dog and mother-child bond. Mothers were asked to view photographs of familiar and unfamiliar children and dogs, and rate these photographs, while an fMRI (functional magnetic resonance imaging) recorded activation in brain regions. It was evident that while both images of familiar dogs and children elicited pleasant emotions, only familiar (own) children produced activation in the nucleus accumbens, substantia nigra and the ventral tegmental area, all of which are crucial brain regions involved in the formation of pair bonds. Therefore, despite the vast similarities between the human-dog and the parent-child bond, there are some unique differences that may be a part of our natural instincts to reproduce and pass along our genetic information to subsequent generations (Stoeckel et al., 2014). Further research is required to understand how an interspecific relationship can form and progress. Specifically, what ingredients are needed to form and maintain these affiliations and to what extent do they mimic an ‘intraspecific parental system’?

This thesis aims to address factors (e.g., personality) that contribute to interspecific attachment between owners and their dogs, as well as to determine the relationships between the physiological and behavioural manifestations of attachment and separation-induced stress in dogs and their caregivers. I will also assess whether owner-perceived attachment can predict physiological and behavioural responses of their dog companions. In order to address these questions, owners and their dogs participated in a variation of the Ainsworth's Strange Situation test (Ainsworth, 1969), which was originally designed to gauge attachment styles (i.e., secure, avoidant, ambivalent, disorganized) of young children towards their mothers. This procedure involved the dependant (dog) experiencing a series of separation and reuniting events from the caregiver as well as the introduction of a complete stranger, which was used to elicit attachment responses and separation-induced stress in dogs and owners. The procedure was videotaped, which allowed for the examination of whether dogs would use owners as a secure base (an element of attachment theory) by initiating and maintaining close proximity and contact, and whether stress behaviours would occur during the owner's absence. Saliva samples were also collected from both the owner and dog to establish a physiological indicator of stress, namely cortisol (CORT; a steroid stress hormone) and chromogranin A (CgA; an acidic protein that indicates sympathetic nervous system activity) concentrations.

Human participants were also required to complete a series of questionnaires including: a personality inventory for dogs (Monash Canine Personality Questionnaire—Revised, MCPQ-R, Ley et al., 2009), a personality inventory for humans (NEO-Five Factor Inventory-3, NEO-FFI-3, Costa & McCrae, 1992), an attachment questionnaire for owner-dog relationships (Dog Attachment Questionnaire, DAQ; Archer & Ireland, 2011) and a series of demographic, health and lifestyle based questions regarding the owner and the dog. Personality questionnaires were

used to examine whether personality matching or complementing occurred in owner-dog pairs, if certain personality factors contributed to attachment bonds, and if personality was linked to physiological and behavioural responses. The DAQ was used to investigate whether owner attachment predicted behavioural and physiological responses in the Strange Situation, i.e., whether the report was indicative of the bond demonstrated. Finally, supplemental questions were asked to ensure that the chemical concentrations in saliva samples were valid (e.g., caffeine intake influences salivary results), to gauge the amount of time owners spent with their dogs and to understand the dog's history (e.g., whether they were obtained from shelter). This thesis provides a comprehensive investigation of the proximate mechanisms contributing to the human-dog relationship (e.g., personality, stress analytes and duration of cohabitation). These aspects may shed light on ultimate levels of causation, regarding the adaptive nature of affiliative relationships and why or how domesticated dogs and humans began their close association.

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1.3 CO-AUTHORSHIP STATEMENT

This research project was implemented under the supervision of Dr. Carolyn Walsh and Dr. Anne Storey where, under their guidance, I developed the research questions and procedural design described within this manuscript. Procedural designs were also presented to my committee members, Dr. Rita Anderson and Dr. Hélène Volkoff, who gave feedback and suggestions. All data, including saliva samples, questionnaires and video footage were collected (2011-2013) by myself or under my supervision. Salimetrics (State College, PA, U.S.A), an external analyst company, completed the chemical assays (cortisol and chromogranin A), however. I recruited all participants in collaboration with the Canine Research Unit at Memorial University of Newfoundland, but all questions or concerns regarding this research project were directed to me.

Each chapter contains data that I exclusively analyzed and reports that I have written, with edits and suggestions provided by my supervisors and committee members. Dr. Carolyn Walsh and Dr. Anne Storey have earned co-authorship on all publications stemming from these chapters as they contributed directly to both the intellectual property of this document and provided financial support for this project (NSERC Discovery Grants).

CHAPTER 2: INTERSPECIFIC ATTACHMENT IN THE STRANGE SITUATION: BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES OF THE DOMESTIC DOG (*CANIS FAMILIARIS)* AND ATTACHMENT FIGURE

2.1 ABSTRACT

Behaviourally, attachment is demonstrated when one individual seeks and maintains close proximity to another individual (Bowlby, 1958; 1972). To examine attachment in an interspecific relationship, 29 human-dog dyads participated in a variation of Ainsworth's Strange Situation test (Ainsworth, 1969). Dogs experienced a series of separation and reuniting events from their owners and were introduced to a stranger. Saliva samples from owners and dogs were collected before and after the procedure to measure stress chemicals, namely cortisol (CORT) and chromogranin A (CgA), in response to this behavioural challenge. Stress in dogs was also evaluated through two behavioural responses: door scratching and body shaking. Additionally, proximity to focal objects/individuals and contact maintenance/seeking was recorded relative to the dog's movements within the room. Dogs had relatively high CORT levels (on par with previously reported levels in arousing contexts) and their CgA concentrations decreased during the procedure. Owners, however, experienced a decrease in CORT and had relatively low CgA levels across the procedure. Dogs displaying the highest CORT levels scratched the door more frequently and used owners as a secure base; i.e., spent more time in close proximity and instigated more contact with the owner compared to strangers. Overall, dogs exhibited a preference towards owners, compared to strangers, during all episodic comparisons. There were no significant relationships between dog CORT and body shaking, or between CgA and any of the dog behaviours analyzed.

2.2 INTRODUCTION

Behaviourally, attachment refers to one individual seeking and maintaining close proximity to another individual (Bowlby, 1958; 1972; Klagsbrun & Bowlby, 1976). The 'attachment figure' is often used as a 'secure base' for exploration, providing social and emotional support that is important for handling stressful situations and new environments (Ainsworth, 1979, 1989; Mikulincer, Shaver, Bar-On & Sahdra, 2014; Waters & Cummings, 2000). Consequently, individuals show a distinct preference for their attachment figure(s) and typically experience a stress response when separated from them (Insel & Young, 2001; Milkósi, 2007; Mongillo, Bono, Regolin & Marinelli, 2010).

Attachment has been extensively studied in conspecific parental interactions with offspring and pair bonds between mates in primates (e.g., Ainsworth, 1979, 1989; Harlow, Harlow & Suomi, 1971; Hertenstein, 2002; Insel & Young, 2001; Mendoza & Mason, 1989; Rawashdeh & Dubocovich, 2014) and various other species (e.g., Barrett et al., 2013; Mimura, Nakamura & Koshiba, 2013; Rehn, McGowan & Keeling, 2013; Remage-Healey, Adkins-Regan & Romero, 2003). However, very few studies have investigated attachment bonds between two different species, such as that seen among humans and domesticated animals, with the notable exception of the dog-human bond (e.g., Gácsi, Topál, Miklósi, Dóka & Csányi, 2001; Mariti et al., 2013; Palmer & Custance, 2008; Paul et al., 2014). The relationship between humans and domesticated dogs (*Canis familiaris*) represents an ideal model for interspecific attachment, as this relationship has been subjected to thousands of years of evolutionary history, producing a pseudo-parental social structure (Axelsson et al., 2013; Miklósi, 2007; Topál, Kiss & Oláh, 2014).

Like infants and parents, dogs rely on owners for basic needs. Dogs bred strictly for companionship often have friendly, affectionate temperaments and many have been created through artificial selection to retain infantile features (e.g., large eyes, bulging cheeks) throughout adulthood (Archer & Monton, 2011; Coppinger et al., 1987; Parslow & Jorm, 2003; Wayne, 1993). Therefore, it is not surprising that domesticated dogs appear able to tap into human care-giving mechanisms.

The strength of the human-dog relationship has resulted in many individuals, who lack offspring, to opt for a dog companion that they often consider akin to children (Nagasawa, Kikisui, Onaka & Ohta, 2009; Serpell, 2003). Dog ownership has been linked to lower blood pressure and heart rate, increased physical activity and higher survival rates, demonstrating that social support provided by dog companions aids in buffering against negative stressors (Bushman, 2014; Friedmann, Katcher, Thomas, Lynch & Messent, 1983; Garrity, Stallones, Marx & Johnson, 1989; Krause-Parello, Wesley & Campbell, 2014; Kurdek, 2009; Marcus, 2013; McNicholas et al., 2005; Parslow & Jorm, 2003; Serpell, 1991).

Furthermore, the effect of social support from dogs is reflected at a short-term physiological level, as dog owners usually experience decreases in blood cortisol (CORT, a stress hormone) levels when making physical contact with their dogs (Handlin et al., 2011). The human-dog bond also shares certain neurobiological mechanisms with intraspecific parental interactions. For example, the hormone oxytocin, involved in lactation and bond formation in mammals, increases when mothers hold and breastfeed their infants (Feldman, Gordon, Schneiderman, Weisman & Zagoory-Sharon, 2010; White-Traut et al., 2009), as well as when owners pet and interact with their dogs (Beetz, Uvnäs-Moberg, Julius & Kotrschal, 2012; Handlin et al., 2011; Odendaal & Meintjes, 2003).

Dogs also exhibit behavioural manifestations of attachment by showing a preference for owners (versus strangers) by spending more time in close proximity and by paying more attention (i.e., longer gaze durations) to owners compared to other individuals (e.g., Horn, Range & Huber, 2013; Kerepesi, Dóka & Miklósi, 2014; Mongillo et al., 2010), and in reacting to the absence of the owner (e.g., Konok, Dóka & Miklósi, 2011; Tuber, Sanders, Hennessy, & Miller, 1996; Mariti et al., 2013). Reaction to separation has been extensively investigated, particularly from a veterinary (behavioural and health management) perspective, as owner absence can elicit a number of moderately stressed to neurotic behaviours including: waiting by the door, refusal to interact with a caregiver 'substitute', excessive self-licking, defecation/urination, pacing or destruction of property (Flannigan, 2001; King et al., 2000; Overall, 2001; Scaglia et al., 2013; Schwartz, 2003; Sherman, 2008;Takeuchi, 2000).

Researchers have recently begun to investigate the behavioural components of human-dog attachment using Ainsworth's Strange Situation (1969), a procedure originally developed to address attachment styles of young children towards their mothers (i.e., secure or insecure; Ainsworth & Bell, 1970). This behavioural protocol subjects a dependant to a series of separation and reuniting events from her/his attachment figure and introduces dependants to a stranger. Recently, this protocol has been amended for use with dogs and has been employed to investigate preference (caregiver vs. stranger), reaction to separation, whether the caregiver is used as a secure base and if subjective reports of 'closeness' can predict behavioural responses of dogs during the procedure (Gácsi et al., 2001; Fallani et al., 2007; Mariti et al., 2013; Palestrini, Prato-Previde, Spiezio & Verga, 2005; Palmer & Custance, 2008; Prato-Previde, Custance, Spiezio & Sabatini, 2003; Rehn, Lindholm, Keeling & Forkman, 2014; Rehn et al., 2013;

Schöberl, Wedl, Beetz & Kotrschal, in press; Topál et al., 2005; Topál, Miklósi, Csányi & Dóka, 1998).

Although it is well established (through behavioural assessments) that some dogs experience stress upon separation from their owners, only a few studies (Palestrini et al., 2005; Rehn et al., 2013; Schöberl et al., in press) have examined physiological manifestations of stress (i.e., heart rate and CORT levels) during the Strange Situation procedure with dogs. This lack of research is largely because behavioural assessments typically produce context-dependent reactions (e.g., dog park versus a research facility), which makes finding a link between physiological measurements and associated 'stress' behaviours in dogs difficult (Beerda, Schilder, van Hooff, de Vries & Mol, 1998, Beerda, Schilder, van Hooff, de Vries & Mol , 1999; Beerda, Schilder, van Hooff, de Vries & Mol, 2000; Hennessy et al., 2001; Ottenheimer Carrier, Cyr, Anderson & Walsh, 2013; Rooney, Gaines & Bradshaw, 2007). However, using physiological measures during the Strange Situation, in particular, would aid in ascertaining whether behavioural reactions in dogs during this test could be attributed to separation anxiety. Additionally, using physiological measures would enhance our ability to predict how dogs respond in scenarios they experience on a daily basis, namely being separated from and reunited with owners (e.g., during the workweek).

One physiological measure of stress is CORT, which has been validated as a measure of stress for decades. CORT is a steroid hormone produced and released by the adrenal cortex, and its secretion is governed by the hypothalamic-pituitary-adrenal (HPA) axis. CORT increases in response to psychosomatic and physical stress; therefore, it is not as fast as the alternate, sympathetic pathway involved in our quick evaluations of perceived threats (Cannon, 1932; de Veld, Riksen-Walraven & de Weerth, 2014; Frodi & O'Keane, 2013; Harrison, Ratcliffe,

Mitchell & Smith, 2014; Kudielka, Hellhammer & Wüst, 2009; Sapolsky, 2003; van Eck, Berkhof, Nicolson & Sulon, 1996). However, CORT levels also experience slight fluctuations in accordance to circadian rhythms (Blagrove et al., 2012; Chan & Debono, 2010; Yehuda, Golier, & Kaufman, 2005). CORT has been successfully measured in saliva in both dogs (e.g., Beerda et al., 1998; Berganasco et al., 2010; Fallani, Prato-Previde, & Valsecchi, 2007; Ottenheimer Carrier et al., 2013; Schöberl et al., 2012) and humans (e.g., Adam & Kumari, 2009; Hellhammer, Wüst & Kudielka, 2009; Richardson, Rice & Devine, 2014). Salivary CORT levels have also been found to correlate strongly with levels found in plasma, albeit at lower concentrations (e.g., Calixto, Martinez, Jorge, Moreira & Martinelli, 2002; Lebelt, Schonreiter & Zanella, 1996).

Another physiological measure of stress is an acidic protein called chromogranin A (CgA). CgA is a stress marker that has been recently linked to the fast, sympathetic adrenomedullary system activity (SAM), as it is co-released with catecholamines (epinephrine and norepinephrine) from the adrenal medulla and sympathetic axons (Kanno et al., 1999; Stefanescu, Schipor, Paun, Dumitrache, & Badiu, 2011; van Kammen et al., 1992). The SAM system is responsible for our fight-or-flight response, which describes a collection of almost instantaneous physiological responses that occur when potentially stressful stimuli are perceived (Cannon, 1932; Sapolsky, 2003). CgA is an excellent tool for measuring SAM activity because it is more stable than catecholamines in the circulatory system as it lasts longer and is consequently easier to measure, especially in saliva (Kanno et al., 1999). Like CORT, CgA concentrations in the saliva have been measured in both dogs and humans and a strong correlation is present between salivary and plasma levels (Akiyoshi et al., 2005; Den, Toda, Nagasawa, Kitamura &

Morimoto, 2007; Kanai et al., 2008; Nakane et al., 1998; Nakane, Asami, Yamada, & Ohira, 2002; O'Connor, Frigon, Sokoloff, 1984; Stefanescu et al., 2011).

The time course for increases or decreases in CORT or CgA in response to a behavioural challenge is not well established, as is the case with most salivary analytes to date (e.g., oxytocin; Minton, 1994; Nakane et al., 2002). For this reason, it was important to incorporate both CORT and CgA together to best capture changes that might occur during the protocol (i.e., slow and fast system) for this current investigation. In addition, saliva sampling in general also helped to best capture physiological changes because it minimizes stress that might be caused by alternate, invasive procedures (e.g., blood collection; Granger et al., 2007; Obayashi, 2013).

Due to the nature of the protocol, it was predicted that several separation and reuniting events would elicit HPA and/or SAM activity, resulting in CgA and CORT increases (final greater than baseline levels) or in greater overall concentration changes (i.e., stress reactivity). It was also expected that dogs would display more frequent stress-related behaviours (e.g., body shaking and door scratching) when in the presence of the stranger exclusively and when alone, compared to episodes when the owner was present (see Palestrini et al., 2005). Individual differences in stress reactivity were also anticipated as not all individuals present physiological and behavioural markers of stress in the same way. For example, certain dogs may present exaggerated responses because of possible past history of separation or abuse, for example, which could contribute heavily to those responses.

As mentioned, the human-dog bond has been described as a strong social relationship, 'mutually' shared by both parties, and physiological responses in owners and dogs have been found to mirror one another (e.g., simultaneous oxytocin increases in owners and dogs; Handlin

et al., 2011). With this in mind, it was predicted that separation-induced stress would be observed in owners as well as in dogs. This will be the first study to examine separation-induced stress in the caregiver (owner) and the first to test directly whether body shaking in dogs, a behaviour often observed by dog trainers, serves as a coping mechanism, i.e., an action that buffers against an internal evaluation of 'emotional' tension and arousal during stressful situations (Beerda et al., 1998; Beerda et al., 1999; Beerda et al., 2000; Glenk et al., 2013; Koolhaas et al., 1999).

Overall, this study aimed to replicate past Strange Situation results, i.e., that dogs will show a distinct preference for owners compared to strangers as seen through increased contact and physical proximity maintenance and initiation. It also aimed to shed light on the nature of stress observed in the Strange Situation test by incorporating physiological measures of stress.

2.3 METHOD AND MATERIALS

2.3.1 Participants

This protocol was completed by 29 volunteer owner-dog dyads. They were gifted with a complimentary poop bag dispenser at the end of the study, but were unaware of this prior to participation. In an attempt to obtain a wide-ranging sample of Newfoundland dog owners, participants were recruited through a variety of social media (e.g., public posters, booths at dog shows and at a local Pet Expo, departmental e-mails and local classified ads such as www.kijiji.ca). Owners consisted of six males and 23 females, ranging from 20 to 71 years old ($X \pm$ SD, 40 ± 14.8 years). Eight (27.6%) owners had children either living with them or living outside the household as independent adults and seven women (30%) reported using oral

contraceptives. There were 13 male and 16 female dogs, ranging from eight months to 14 years old ($X \pm SD$, 6 ± 3.9 years).

Of the 29 dogs tested, five were sexually intact; one female (not in estrus at the time of the study, according to owner's report) and four males, while the remaining 24 dogs were neutered/spayed. No specific dog breed was targeted; rather, a variety of medium to large (greater than or equal to 8 kg; see Table 2.1) dogs were used, with the exception of one Yorkshire Terrier, to ensure the success of saliva sampling. All dogs were kept strictly for companionship or recreation purposes, i.e., there were no working or service dogs in this study. The majority of households (N =19, 66.0%) had one dog at the time of the study; the remainder of households owned multiple dogs (maximum of four dogs).

Prior to participation, dogs and owners were screened to ensure that they were free from endocrine disorders or pathologies and that the dog and owner had cohabitated for at least six months. None of the dogs had aggressive tendencies (according to owner reports) and all were familiar with travelling outside of their homes. A few individuals reported that their dogs were afraid of men, and they suggested that this fear was possibly attributed to abusive backgrounds. If a dog seemed abnormally apprehensive in the experimental situation (e.g., panting excessively, trembling, etc.), the owner was encouraged to terminate her/his participation; this occurred three times during the study (final N= 29).

Recruitment occurred between August 9th, 2012 and February 25th, 2013. Owners and dogs arrived at Memorial University of Newfoundland between 1300 and 1900h to ensure consistent windows of time, to minimize natural analyte fluctuations (e.g., CORT is highest in the morning; Rosmond, Dallman & Björntorp, 1998; Wüst, Federenko, Hellhammer, &

Kirschbaum, 2000). Participants were asked to refrain from eating (especially dairy products) one hour before arrival, to refrain from drinking caffeine two hours before arrival and not to excessively pet their dogs on route to the study location, as these factors may influence salivary results (Handlin et al., 2011**;** Hofman, 2001; Kaufman & Lamster, 2002; Schultheiss, Schiepe & Rawolle, 2012).

2.3.2 Study Location

Upon arrival, owners were seated while providing signed consent to the researcher. Two different study rooms were used because of the availability and seasonal use of office space on campus. Due to the layout of the first room (i.e., 3 m x 4.4 m, multiple desks, poor camera mounting locations), certain behaviours could not be coded with accuracy (N = 3), therefore, all behaviours analyzed are from dogs and owners tested in the second or "main" room (N = 26 of the 29 participants).

The main study location was in a 2.7m x 5.3m office, which consisted of a desk, two chairs, a speakerphone, a basket of toys, a water bowl and a series of storage units (filing cabinets and book shelves). Additionally, to prevent damage and to make the room easier to clean between participants, a thin rubber mat was secured on the floor. Four synchronous security cameras (LH114000 series, Lorex, Plainsfield, IL, USA) were set-up in the room at a variety of angles, so that most of the room was captured (see Figure 2.1). The cameras were connected to a hard drive (where the video recording was stored) and a monitor. As the video format produced by this system was .264, a series of file conversions were performed using Wondershare™ (Surrey, BC, Canada) before the final .mp4 files were created. Cooling fans were

551 also placed in the room to minimize external ambient noise, and to prevent dogs and owners
552 from overheating.

553 *2.3.3 Strange Situation Procedure*

554 A variation of a well-known behavioural protocol, "Ainsworth's Situation", was
555 performed using dogs and their owners (Ainsworth, 1969). This test is typically used to assess
556 attachment styles in infants towards their mothers; however, the dog-amended version has been
557 used by other investigators to evaluate attachment in owner-dog dyads (e.g., Palestrini et al.,
558 2005; Palmer & Custance, 2008; Prato-Previde et al., 2003, Topál et al., 1998). Additional
559 modifications were made to the dog-amended protocol typically used, namely in the durations of
560 the episodes and the incorporation of saliva sampling. The basic protocol involves a dog
561 experiencing a series of separation and reuniting events from her/his owner and exposure to a
562 stranger (Table 2.2). The stranger was chosen for each individual to ensure that the dog had no
563 previous interactions with that person and all four strangers used were females.

564 The procedure consisted of a brief (30 sec) introductory period and seven 'episodes',
565 each lasting approximately 3min (27.5 min total; Table 2.2). All episode changes and
566 instructions were administered over a speakerphone and saliva sampling occurred at specific
567 intervals throughout the procedure (Table 2.2). Ten minutes after dyads arrived, baseline saliva
568 samples were taken from both owners and their dogs. This timing was used to ensure that the
569 most accurate baseline was obtained as it allowed the dyad some time to adjust to the new
570 environment. Owners and dogs were then introduced to the room, the dog was unleashed and
571 they were shown the speakerphone, toys and water they could avail of. Episode 1 began when
572 the researcher left and owners were instructed to interact/play with their dog. During Episode 2,

a stranger entered the room, and they sat while engaging in conversation with the owner. Near the end of Episode 2 the owner left, while the stranger attempted to interact/play with the dog. Episode 3 was the first episode that the dog and stranger were alone. Strangers were instructed to attempt interactions/play with the dog and near the end of the episode they took the dog's second saliva sample (8 min into the procedure).

In Episode 4, the owner was instructed to enter the room and the stranger was asked to exit, while the owner interacted with their dog. For Episode 5, the dog was completely alone. During Episode 6, the stranger entered the room and took the dog's third saliva sample (15 min into the procedure), while the owner provided their second saliva sample outside of the room. Following the sample, strangers attempted interactions/play with the dog. Episode 7 was the final episode; the stranger exited the room and the owner returned and was instructed to interact/play with their dog. At the end of Episode 7 both the dog and the owner had their last saliva sample (22 min into the procedure).

2.3.4 Saliva Sampling

Two sampling techniques were used to collect saliva: a swab technique for dogs and the passive drool technique for humans. For dogs, the individual taking the sample held an 8 mm x 125 mm swab (Salimetrics Children's Swab, © Salimetrics, PA, USA) made from a durable, inert polymer in the dog's mouth (typically near the cheek) for approximately 1-2 min or until the swab was saturated. The swab was then placed within a 17 mm x 100 mm polypropylene, barcoded tube (Swab Storage Tube, © Salimetrics, PA, USA) and laid on ice. Humans were asked to lean their head forward, allow the saliva to pool in their mouth and then to guide that saliva into a 10 mm x 46 mm polypropylene tube (Passive Drool Cryovial, © Salimetrics, PA,

USA) using a collection device (Saliva Collection Aid, © Salimetrics, PA, USA) similar to a straw, and then the tube was placed inside the ice chest. Collection supplies were chilled on ice prior to use. It is important to note that due to the expense and nature of the collection methods, only the baseline and final saliva sample for dogs and humans was analyzed. The most crucial factor in this decision, however, was the relative 'quality' of sample obtained at intermediate times, as they tended to have less saliva than baseline and final samples.

2.3.5 Salivary Analytes

Once the procedure was complete, samples were placed in storage containers in a -20°C freezer until they were shipped, immersed in dry ice, to Salimetrics LLC. (State College, PA, USA) for analysis. Each sample with adequate volume was analyzed for two stress markers: CORT and CgA. Both analytes were measured using Enzyme Immunoassay (EIA): Cortisol, 1-3002 (Salimetrics, State College, PA, USA) and Chromogranin A, YII-YK070-EX (Cosmo Bio CO., LTD., Japan), respectively. Concentration values were expressed as μg/dL for CORT and pmol/mL for CgA. These values were also used to obtain a measure of individual stress reactivity, calculated as a percent change score ($\frac{baseline-final}{baseline}$ *100%).

2.3.6 Behaviour

Videos converted to .mp4 files were watched using QuickTime Media Player 7 (Apple, Toronto, ON, Canada), synchronized with a behavioural coding program logger.app (http://play.psych.mun.ca/_apps/log/; ©Avery Earle, Memorial University of Newfoundland). This coding program synchronized with the video's time signature and allowed a one-letter code to be assigned to each behaviour, providing a time stamp for when the behaviour occurred. The

resulting .txt data files were processed using a unique Python code (programming language) on an Apple interface to extract durations and frequencies of the behaviours analyzed.

The behaviours coded included physical proximity, physical contact, body shaking and door scratching (Table 2.3). Proximity was assessed using the dog's travel pattern such that one state could change into another depending on the dog's position in the room (e.g., "near owner" could change to "near door"). Dogs were considered to be in close proximity to a person or object if they were within one distance of their own body length (snout to rear) from a person(s) or object(s). This method was preferred to choosing an arbitrary numerical value (e.g., 1 m), as the latter would have resulted in some dogs being very close to the focal object while others would be father away (depending on body size) to be considered in close proximity.

Both the duration and frequency of the state changes were predominantly acquired from the main camera (Channel 1 of 4), which gave the most complete view of the room (though other channels were used as a reference when dogs were not visible from that source). Proximity to the door, however, was analyzed using the camera that exclusively monitored activity near the door. Physical proximity was coded from the beginning of Episode 1 until the end of the final episode (Episode 7) and was analyzed both as a comprehensive measure across all episodes and for each episode separately.

The duration of physical contact between dogs and humans was determined by output produced from the Python code calculations. During coding, notes were made for each bout regarding who initiated contact or whether the contact seemed intentional, i.e., clear indication of movement goal (forward gaze, dog often coming to retrieve a toy) instead of unintentionally brushing-off of that individual (e.g., sniffing a toy on the ground as her/his tail brushes off

someone's leg). Therefore, while these bouts were considered 'close proximity' they did not count for the contact bouts observed. Contact behaviour was coded from the beginning of Episode 1 to the end of the last episode. Both frequencies and durations were measured on a per episode basis and as a total measurement.

Body shaking and door scratching were noticed in several of the videos while the other measures were being coded. Body shaking was defined as any one continuous bout of side-to-side movement starting at the head and extending down the body (as if the dog was drying off). Door scratching was counted each time the dog made contact using their paw with the door. A new bout was counted when contact was broken (i.e., all paws on the floor) and then resumed.

All duration values recorded for each attachment-related behaviour expressed as a proportion of time; that is, the duration spent performing the given behaviour was divided by the total time the focal individual had available to interact with the dog. Therefore, for episodes that involved the stranger taking a saliva sample from the dog, the time required for saliva sample was subtracted from the total duration of the episode. The duration of the behaviour was then divided by the 'total interaction time', thereby producing a fair and accurate picture of the attachment behaviours.

2.3.7 Statistical Analyses

All statistical analyses were carried out using IBM SPSS Statistics 20 (IBM, Armonk, NY, USA). A series of normality tests (binomial and Kolmogorov-Smirnov tests) were performed to ensure that the data were normally distributed. Consequently, several variables required transformations in order to use parametric tests; specifically, a square root transformation was performed for door scratching frequency and a $\log_{10}$ transformation was

performed for CORT and CgA concentrations resulting from a positive skew, which is typical for hormonal data (Dreschel & Granger, 2009).

Due to the novel nature of this research, many analyses were exploratory; however, there were planned comparisons analyzing preference (proximity and contact) and hormonal changes. All analyses involving comparisons between how the dog spent time with the owner versus the stranger, and chemical changes within individuals involved Paired t-tests. Other analyses comparing individuals (e.g., sex comparisons) were performed using Independent Samples t-tests. Given the exploratory nature of certain correlational relationships present in this thesis, Bonferroni corrections for multiple comparisons were not utilized as they were thought to be too restrictive (see Jaeger & Halliday 1998; Ottenheimer Carrier et al., 2013). Correlations reported are Pearson r bivariate tests.

All significance probabilities reported in this manuscript are two-tailed with, $\alpha = 0.05$, unless otherwise stated. Episode 1 was excluded from certain comparisons, as it was an introductory period. Even though dogs were solely with owners in Ep1, dog movements appeared to reflect exploration rather than preference (e.g., 17.7% of time near the door compared to 5.6% and 5.0% in later episodes; Ep1 versus Ep4, Paired t-test: $t_{25} = 6.58$, $p < 0.001$; Ep1 versus Ep7, Paired t-test: $t_{25} = 6.14$, $p < 0.001$). However, when evaluating possible correlates with physiological stress, data obtained during Episode 1 were relevant. The sample size quite often deviates from the total number of participants collected ($N = 29$). This is due to the fact that only 26 participants qualified for behavioural measurements due to the layout of the first room and because not all salivary measures yielded enough saliva to quantify the chemical analytes.

2.4 RESULTS

BEHAVIOURAL MEASURES

2.4.1 Physical Proximity

Overall, a given dog's movements depended on which individuals were present (or absent) in the room (Figures 2.2a and 2.2b). For all physical proximity comparisons, the maximum sample size was $N = 26$. When the owner was alone with her/his dog for the entire episode (Episodes 4 and 7), dogs spent proportionally more time in close proximity to the owner compared to other focal objects (e.g., door) or exploring the room (Figure 2.2a). The remaining time was spent in areas not seen by the cameras ('other'), when the dog was not close to any focal objects/individuals or was near multiple focal objects simultaneously (Figure 2.2a).

When the dog was exclusively with the stranger (Episodes 3 and 6), a large portion of time was devoted to the saliva sample ('sample'; Figure 2.2b). Most of the dog's remaining time, during stranger exclusive episodes, was spent near the door, which comprised a significantly larger proportion of time than in episodes with the owner (i.e., time near door; Episode 3 vs. 4: $t_{25} = -14.83$, $p < 0.001$; Episode 3 vs. 7: $t_{25} = -14.83$, $p < 0.001$; Episode 6 vs. 4: $t_{25} = 11.95$, $p < 0.001$; Episode 6 vs. 7: $t_{25} = -11.96$, $p < 0.001$). Dogs in Episodes 3 and 6 also spent time with the stranger or exploring the room (Figure 2.2b). In the second episode with the stranger (Episode 6), dogs spent more time near the door than they did in the first episode with the stranger (Episode 3; $t_{25} = 2.70$, $p = 0.012$), but there was not a difference between the amount of time dogs spent near the door for the episodes spent exclusively with the owner (Episodes 4 and 7)

Time spent in 'other' locations was in areas not seen by the cameras, when the dog was not close to any focal objects/individuals, or when the dog was near multiple focal object(s)/individual(s) simultaneously (Figure 2.2b). An example of the occurrence of proximity to multiple focal objects/individuals was when dogs spent time between the door and the stranger who was attempting contact. When dogs were completely alone (Episode 5), they spent their time in close proximity to the door, by the desk (where owner and stranger sat in prior episodes) or exploring the room. The remaining time was spent in areas not seen by the cameras or when the dog was not by any focal objects/individuals or near multiple simultaneously.

Physical proximity was more finely examined by comparing the duration of time spent near the owner versus the stranger during specific episodes, expressed as a proportion of time, in which the total time the dog spent in close proximity to the owner was divided by the total time in the room (i.e., the duration of the episode). For physical proximity to the stranger, the total time in the room was expressed as the duration of the episode minus the duration of the dog's saliva sample, as the sampling time does not reflect the dog's 'choice' or 'preference' to be near the stranger. In a series of comparisons between episodes when the dog was exclusively with the stranger (Episodes 3 and 6) or the owner (Episodes 4 and 7), a strong preference was shown for the owner, as dogs spent more time in close proximity to owners compared to strangers (Episode 3 vs. 4: $t_{25} = -14.91$, $p < 0.001$; Episode 3 vs. 7: $t_{25} = -15.45$, $p < 0.001$; Episode 6 vs. 4: $t_{25} = 14.34$, $p < 0.001$; Episode 6 vs. 7: $t_{25} = -15.47$, $p < 0.001$; Figure 2.3). When the owner and the stranger were in the room together (Episode 2), dogs showed a preference to stay in close proximity to the owner compared to the stranger ($t_{25} = 2.60$, $p = 0.015$). Physical proximity to the stranger during the first episode alone with the stranger (Episode 3) was negatively correlated

with dog age (r = -0.405, p = 0.040, N = 26, Table 2.4). Therefore, younger dogs spent more time in close proximity to strangers than did older dogs.

2.4.2 Physical Contact

Physical contact was recorded as durations and frequencies of contact between the dog and focal individuals present within the room over the entire Strange Situation procedure. For all physical contact comparisons, the maximum sample size was N = 26. Frequency of physical contact bouts were examined more closely in episodes with the stranger (3 and 6) and episodes with the owner (4 and 7) to determine which individual initiated the contact bout, typically measured as a moving individual approaching the stationary individual prior to contact. For contact bouts and contact initiated by the dog, frequency of contact was converted to a rate within each episode (scaled by the amount of time the individual had to interact with the dog). As with physical proximity, dogs engaged in more physical contact bouts overall (e.g., petting, rough-housing) with their owner (X ± SE = 53.65 ± 3.79, N = 26) compared to the stranger (X ± SE = 22.92 ± 2.29, N = 26, t_{25} = 8.32, p < 0.001), and spent more time with the owners (X ± SE = 220.95 ± 17.58 sec, N = 26) during respective bouts compared to strangers (X ± SE = 97.28 ± 10.18 sec, N = 26, t_{25} = 4.42, p < 0.001).

Overall, dogs initiated more bouts of contact with their owners than with strangers (Episode 3 vs. 4: t_{25} = -3.62, p = 0.001; Episode 3 vs. 7: t_{25} = -4.16, p < 0.001; Episode 6 vs. 4: t_{25} = 4.76, p < 0.001; Episode 6 vs. 7: t_{25} = -4.42, p < 0.001; Figure 2.4). Also, contact initiation with strangers did not change as a result of short-term familiarity with the strangers, as there was no significant difference between Episode 3 and Episode 6 (t_{25} = 1.622, p = 0.117). Frequency of contact initiated by the dog towards the stranger during the second episode with the stranger

(Episode 6) was lower for older dogs (r = -0.394, p = 0.046, N = 26), but not so for the first episode in which the stranger and dog were alone (i.e., Episode 3).

2.4.3 Body Shaking and Door Scratching

Of the 26 dogs recorded, 80.8% (N = 21) displayed body shaking behaviour. Body shaking typically occurred when the dog reunited with their owner, immediately after the dog had been with the stranger (55.8%), or when the dog was alone with the stranger (34.6%). This behaviour rarely occurred if the dog was alone (7.7%) or when both the owner and stranger were present simultaneously (1.9%). Door scratching occurred in 50% (N = 13) of dogs recorded and was almost exclusively performed when the dog was alone in the room (72.5%), or when the dog was alone with the stranger (20%). This behaviour rarely occurred when the dog was with their owner (3.8%) or when both the owner and the stranger were present simultaneously (3.8%).

PHYSIOLOGICAL MEASURES

2.4.4 Cortisol

Dog CORT changes did not show an overall consistent pattern throughout the procedure (t_{22} = 0.771, p = 0.481, Figure 2.5). This result can be largely attributed to the fact that there were large individual differences in reactivity among dogs as 48% (N = 11) experienced an increase in CORT and 52% (N = 12) experienced a decrease. There were no significant differences in CORT levels between either sexually intact (N = 5) and altered dogs (N = 24), or male (N = 13) and female (N = 16) dogs. Humans differed from dogs in that their CORT levels decreased across the procedure, as baseline concentrations exceeded final concentrations (t_{28} = 4.850, p = 0.014, Figure 2.5). There were no significant differences between human male (N = 6) and female (N = 23) CORT concentrations.

2.4.5 Chromogranin A

Dogs experienced a decrease in CgA during the test, as the initial baseline concentrations were significantly higher than final concentrations ($t_{15} = 6.69$, $p < 0.001$; Figure 2.6). CgA concentrations were independent of whether dogs were intact or neutered/spayed; however, males ($X \pm SE = 148.20 \pm 20.56$ pmol/mL, $N = 7$) had significantly higher baseline CgA concentrations than females ($X \pm SE = 71.51 \pm 15.01$ pmol/mL, $N = 10$, $t_{15} = 2.18$, $p = 0.042$). Unlike dogs, there were no significant differences between human baseline and final CgA concentrations ($t_{23} = 0.837$, $p = 0.411$; Figure 2.6).

2.4.6 Dog and Human Physiological Stress

Individual baseline and final CORT levels were strongly and positively correlated for dogs ($r = 0.789$, $p < 0.001$, $N = 23$), and for humans ($r = 0.836$, $p < 0.001$, $N = 29$). CgA concentrations were also positively correlated between baseline and final concentrations for dogs ($r = 0.570$, $p = 0.021$, $N = 16$) and for humans ($r = 0.810$, $p < 0.001$, $N = 24$). Final CORT levels for dogs, but not baseline levels, were positively correlated with both their owners' baseline ($r = 0.512$, $p = 0.012$, $N = 23$) and final ($r = 0.606$, $p = 0.002$, $N = 23$) CORT levels. For humans, baseline CgA levels positively correlated with their final CORT levels, $r = 0.404$, $p = 0.037$, $N = 28$.

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2.4.7 Relationships Between Physiological and Behavioural Stress

Of the 29 dyads tested, 27.6 % ($N = 8$) of owners reported that their dogs had separation anxiety or that their dogs demonstrated behaviours associated with separation-induced anxiety

(e.g., excessive barking, destruction of property; Wren, 2000). The presence or absence of owner-reported separation anxiety did not seem to be related to the behaviours or physiological changes within individual dogs (e.g., dogs with reported anxiety did not have higher CORT levels). Dogs that had higher baseline and/or final CORT levels scratched the door more frequently than dogs with lower CORT levels (baseline: $r = 0.494$, $p = 0.023$, $N = 21$; final: $r = 0.510$, $p = 0.018$, $N = 21$; Figure 2.7; Table 2.5). Dog CORT levels were not linked to the presence or frequency of body shaking in dogs and neither door scratching nor body shaking were related to dog CgA (baseline and final levels).

CgA reactivity in dogs, as calculated as a percent change score ($\frac{baseline-final}{baseline}$ *100%), increased as the duration of time owners and dogs had been living together increased ($r = 0.550$, $p = 0.027$, $N = 16$, Table 2.5). Thus, dogs that lived with their owner for a longer period of time experienced larger changes between baseline and final CgA, possibly indicating dogs were more stress-reactive. CORT reactivity for dogs, also expressed as a percent change score, increased as the overall frequency of door scratching increased ($r = 0.481$, $p = 0.027$, $N = 21$, Table 2.5). Therefore, more stress-reactive dogs (i.e., those showing the largest differences between baseline and final CORT) scratched the door more frequently.

2.4.8 Relationships Between Stress and Attachment-Related Behaviours

Dogs with higher baseline CORT concentration values had more overall contact bouts (summation of Episodes 1, 2, 4 and 7) with owners than dogs with lower concentrations ($r = 0.461$, $p = 0.036$, $N = 21$, Table 2.5). Dogs with higher final CORT concentration values spent more time in close proximity to the owner during Episode 7 (final episode; owner and dog alone

in the room; $r = 0.499$, $p = 0.021$, $N = 21$, Table 2.5). Dogs with higher final CORT levels also had more frequent contact bouts ($r = 0.427$, $p = 0.031$, $N = 21$, Table 2.5) with the owner.

Humans with higher final CORT levels also spent more time in close proximity to their dog during Episode 7 (final episode; $r = 0.437$, $p = 0.025$, $N = 26$, Table 2.6). Humans with greater CORT reactivity also had dogs that frequently initiated contact bouts with strangers ($r = 0.591$, $p = 0.001$, $N = 26$, Table 2.6).

Dogs with higher initial baseline CgA spent less time with the stranger in Episode 3 ($r = -0.672$, $p = 0.008$, $N = 14$, Table 2.5), while dogs with higher baseline CORT spent more time with strangers during Episode 6 ($r = 0.524$, $p = 0.015$, $N = 21$, Table 2.5). Dogs that spent more time near the door during Episode 5 (dog alone) had higher CgA baseline ($r = 0.695$, $p = 0.006$, $N = 14$. Table 2.5). Both baseline and final CgA concentrations were higher for humans who had dogs that initiated more contact with them during Episode 4 (baseline: $r = 0.534$, $p = 0.006$, $N = 25$; final: $r = 0.672$, $p = 0.000$, $N = 25$, and Episode 7, with owner, final: $r = 0.416$, $p = 0.048$, $N = 23$; Table 2.6). Humans with greater CgA percent change had dogs that spent less time in close proximity to them in Episode 4 ($r = -0.691$, $p < 0.000$, $N = 22$, Table 2.6) and in Episode 7 ($r = -0.614$, $p = 0.004$, $N = 22$, Table 2.6). Interestingly, humans with higher baseline and final CgA concentrations had dogs that spent spent more time in close proximity with them in Episode 7 (Baseline: $r = 0.472$, $p = 0.017$, $N = 25$; Final: $r = 0.437$, $p = 0.025$, $N = 26$, Table 2.6), however.

Dogs that scratched the door more frequently engaged in shorter overall physical contact bouts with the stranger ($r = -0.429$, $p = 0.029$, $N = 26$). Also, there was a positive correlation between frequency of door scratching and frequency of owner contact ($r = 0.389$, $p = 0.050$, $N =$

26). Dog CgA expressed as a percent change was also negatively related to overall frequency of physical contact with the stranger (r = -0.620, p = 0.018, N = 14, Table 2.5).

2.5 DISCUSSION

2.5.1 Preference

In this Strange Situation test, dogs showed a distinct preference for their owners compared to the stranger as seen through the greater proportions of time spent in close proximity and in physical contact with owners and by the greater frequency of contact initiated by the dogs towards owners, compared to strangers. Owner preference has also been a common finding in the dog-amended Strange Situation literature (Gácsi et al., 2001; Fallani et al., 2007; Mariti et al., 2013; Palestrini et al., 2005; Palmer & Custance, 2008; Prato-Previde et al., 2003; Rehn et al., 2014; Rehn et al., 2013; Schöberl et al., in press; Topál et al., 2005; Topál et al., 1998). Preference is usually defined as the degree of contact seeking and maintenance, gaze orientation, searching behaviours (e.g., waiting by the door after the owner exited) and the relative occurrence of passive (e.g., laying down) and play behaviours in the presence of the owner versus the stranger. These findings are consistent with other dog-amended Strange Situation studies as well (e.g., Palestrini et al., 2005).

2.5.2 Behavioural Stress

In this study, preference was also demonstrated through behavioural 'stress' documented specifically by the frequency of door scratching. Door scratching was linked to stress, as dogs with higher baseline and final CORT concentrations scratched the door more frequently than those with lower CORT concentrations. Door scratching occurred almost exclusively when the dog was alone or when the dog was with the stranger, therefore, it appears as though removing

854 the secure base (owner) elicited a stress response and the substitute (stranger) did not minimize
855 the effect (e.g., Palestrini et al., 2005).

856 Body shaking occurred in approximately 80% of dogs, usually just after the dog was with
857 the stranger or just after the stranger episode ended and the owner was reunited with their dog.
858 Therefore, body shaking may also be used as a way to communicate arousal or alleviate
859 emotional tension. As shown by Glenk et al. (2013), body shaking may serve as a coping
860 mechanism to manage stress, rather than serving as a manifestation of stress. In this study, body
861 shaking was not linked to either physiological stress measurement or door scratching, and it was
862 rarely performed when the dog was alone (arguably the most stressful episode). Relationships to
863 other stress measurements would likely be present if body shaking exclusively signified arousal
864 or stress.

865 *2.5.3 Physiological Stress*

866 It is important to take into consideration the nature of the Strange Situation and that the
867 focal individual is the dependant, which in this case was the dog. The procedure is designed to
868 evoke a stress response within the dog, which in turn, will cause the dog to display attachment
869 behaviours. With this in mind, it is evident that the dog would be subjected to a larger degree of
870 stress than their human counterpart as they enter a novel environment (university campus) and
871 interact with a new individual. Additionally, this current investigation required dogs to provide a
872 series of saliva samples, and, despite being a relatively 'non-invasive' procedure; it is still a very
873 novel experience for most dogs. Therefore, it is not surprising to find that dogs experienced
874 changes in CgA concentrations (faster SAM system), and CORT levels (slower HPA system)
875 that were on par with studies investigating hormones in arousing contexts such as a dog park or

dog daycare (e.g., this investigation, baseline CORT: 0.259 μg/dL, final CORT: 0.250 μg/dL; Ottenheimer Carrier et al., 2013, baseline CORT: 0.14 μg/dL, final CORT: 0.20 μg/dL; similar to Dreschel & Granger, 2009; Posluns, Anderson & Walsh, 2014).

In this study, dog baseline and final salivary CORT concentrations did not change in a consistent pattern throughout the entire procedure (some increased and some decreased), while CgA decreased significantly. These findings can likely be explained by the biological stress systems themselves. The process of coming to campus itself may have actually been more 'stressful' or 'arousing' than the Strange Situation, as shown through the higher baseline CgA levels (compared to final levels) for dogs suggesting that the sympathetic adrenomedullary system (SAM), the faster stress system (compared to hypothalamic-pituitary-adrenal, HPA) was activated. A decrease in CgA was observed during the test probably as a result of the protocol (e.g., owner returns during subsequent episodes), the speed at which the SAM system changes when stress is increased and reduced, and the deleterious effects of prolonged SAM activity (Esler & Kaye, 2000; Glaser & Kiecolt-Glaser, 2005; Schommer, Hellhammer & Kirschbaum, 2003). This finding is in contrast to the slower HPA stress system for which no CORT decrease was observed for dogs. Indeed, a change in CORT may not have been observed because it simply was not captured in our sampling intervals (~30 min span). However, this does appear to be the sampling period chosen by many current researchers (e.g., Koda, Wantanabe, Miyaji, Ishida & Miyaji, 2015; Sandri, Colussi, Perrotta & Stefanson, 2015).

The salivary CgA values reported in this study were considerably lower than the concentrations reported in Kanai et al. (2008) for dogs; i.e., 3.28 ± 0.22 pmol/mg = 3280 pmol/mL, who passively monitored salivary CgA over a 24 hour period, as my values were: 148.20 ± 20.56 pmol/mL (baseline) and 71.51 ± 15.01 pmol/mL, respectively. Although this

finding seems counter intuitive, as one would expect a behavioural challenge to elicit a greater response than passive sampling (reflected in higher concentrations), it is important to note that Kanai et al. (2008) used a cotton substrate to obtain their samples, whereas this study used an inert polymer swab. In past studies, sampling materials have been shown to influence salivary results; therefore, these concentrations may not be comparable for that reason (Granger et al., 2007; Granger, Shirtcliff, Booth, Kivlighan, & Schwartz, 2004). Another reason that could be contributing to this finding is the nature of behavioural challenge (Strange Situation).

Dog CgA concentrations reported in this manuscript were not directly compared to human CgA because a human antibody was used to assay both dog and human CgA. Reports on salivary CgA have almost exclusively been performed on human subjects (Den, Toda, Ohira & Morimoto, 2011; Kanamaru, Kikukawa & Shimamura, 2006; Koh & Koh, 2007; Takatsuji, Sugimoto, Ishizaki, Ozaki, Matsuyama, & Yamaguchi, 2008), except for Kanai et al. (2008). It is possible that the amino acid sequences for CgA in humans may not be entirely structurally conserved in dogs as amino acid signature human-dog differences have been identified (i.e., human sequence has 457 amino acids, while dogs have 425, www.ncbi.nlm.nih.gov). Therefore, dog CgA values may not represent concentrations in their truest form and may not be directly comparable with human samples due to possible interfering differences in molecular structure. However, the CgA assay has been validated as recent literature has confirmed the relative cross reactivity (with human assays) and success in measuring of dog chromogranins (Stridsberg, Pettersson, Hagman, Westin & Höglund, 2014).

The human hormonal results contradicted our original predictions that dog and human hormonal levels would mirror one another. Unlike dogs, human CORT decreased, which might be attributed to owner-perceived participation expectations and the actual process of being

'tested' or events prior to testing (e.g., getting the dog into the car, running late or in heavy traffic; Storey, Walsh, Quinton & Wynne-Edwards, 2000; Takatsuji, Sugimoto, Ishizaki, Ozaki, Matsuyama, & Yamaguchi, 2008). Thus, the decrease for participants may have occurred because they became more comfortable over time. Also, because owners understood the Strange Situation requirements, they were probably relatively non-responsive to the effects of separation-induced stress.

Dogs with relatively low initial baseline CgA concentrations spent more time in close physical proximity to strangers (in Episode 3), and spent less time close to the door (Episode 5) than dogs with higher levels. Further, dogs that scratched the door more frequently interacted (physical contact) with the stranger for shorter durations of time. Taken together, these findings suggest that stress may have mediated their responses in the Strange Situation as higher chemical and behavioural measures of stress tended to be associated with more antisocial behaviours in dogs, i.e., spending time alone, avoiding interactions from the stranger.

Although changes in CORT concentrations were not mirrored in dogs and humans, final CORT levels in dogs were positively correlated with both baseline and final CORT levels for humans. It is possible that dogs are seeking information from their owners to better understand their environment so that they can respond accordingly, depending on the context, which may be reflected in this hormonal relationship (Buttner, Thompson, Strasser & Santo, 2015; Sümegi, Oláh, & Topál, 2014). Human baseline CORT was also positively correlated with human final CgA, which may indicate the relative speed in which these stress systems operate. Dogs with relatively high final CORT levels spent more time with their owner during the final episode (Episode 7) and across the entire procedure, compared to dogs with lower concentrations. Additionally, dogs that frequently scratched the door initiated frequent contact with their owner

and seldom engaged in contact with the stranger. This combination of results suggests that dogs under more stress solicited more contact from the owner, potentially utilizing them as a secure base from which to explore.

Furthermore, dogs with greater SAM stress reactivity (measured as CgA percent change, i.e., larger average decreases in CgA levels), sought less contact with strangers across the entire procedure, but, during the first episode that the dog was left alone with the stranger (Episode 3), dogs with higher final CORT (reflecting HPA axis) concentrations initiated fewer contact bouts with the stranger. Therefore, the faster SAM system was activated in response to a perceived threat. However, it is acknowledged that alternate methods of calculating stress reactivity should be explored to best represent changes over time.

Although not analyzed in this manuscript, there was one additional sample taken for owners and two additional saliva samples taken from dogs during the procedure. The goal had been to produce a salivary profile for both CORT and CgA, neither of which has been addressed within the literature. These samples (particularly for the dog) had to be taken during the episodes, thus limiting the natural interaction shared between the dog and stranger. While this was taken into account for duration percentages, saliva sampling may have influenced the ways in which the dog interacted with the stranger during the remainder of the respective episode. One interesting finding, which certainly deserves further exploration, is the relationship between human CgA concentrations and dog attachment-related behaviours (e.g., correlation between human CgA reactivity and physical proximity). It may be possible that dogs can 'detect' human stress and thereby adjust their own behaviours accordingly. Perhaps dogs can look to comfort their owners in stressful contexts? This result complements what was found in Buttner et al.

(2015) as they too found hormonal synchronization between dogs and their handlers in the context of a competitive agility trial.

Future research should work on obtaining ideal baseline concentrations for physiological stress indicators. Currently, samples taken as baseline measurements are most often taken when the subject is in a novel environment and/or in the presence of unfamiliar people. For example, in this study, the baseline measure was assessed 10 min after the participants arrived. On route to the study, subjects were asked to abstain from giving treats or petting their dog. This may have disrupted an individual dyad's travel routine and therefore been stressful to the dog in addition to entering an unfamiliar environment not to mention the excitement of traveling. Research should also aim to examine more closely the communicative and stress-coping functions of the body shaking behaviour to determine whether it is used exclusively in arousing contexts. Procedures, ideally, should be developed to achieve more close-scale measurements to collect unobtrusive saliva samples during a behavioural challenge.

Summary

Using an interspecific model for attachment helps to define the underlying motivation to develop 'attachment' systems as an adaptation for survival, dictated by stress in the environment. These findings suggest that dogs perceive owners as a 'secure base' from which to explore their environment and seek comfort, consistent with the results of other Strange Situation studies (e.g., Mariti et al., 2013; Palestrini et al., 2005; Palmer & Custance, 2007; Prato-Previde et al., 2003). Physiological stress responses appear to be mediating or working reciprocally with appraisals of stress (e.g., door scratching) to produce the behaviours of dogs in the Strange Situation, such as contact initiation and proximity to their owner. The Strange Situation also seems to be tapping

989 into the faster, SAM stress system. Further research is required to truly capture the profile of
990 these stress measures in response to a behavioural challenge.

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Table 2.1: Approximate sizes of dog breeds that participated in this study (numbers in parentheses represent *N*).

Breed	Mass (kg)	Height (cm)	
Beagle (5)	10-11	33-38	
Cavalier King Charles Spaniel (1)	5.5-8	30-33	
Collie (1)	22.5-34	56-66	
Eurasier (1)	31-32	60	
German Sheppard (1)	34-36	64	
Golden Doodle (1)	22-40	50-60	
Labrador Husky (1)	27-45	53-60	
Labrador Retriever (3)	27-34	57-62	
Miniature Golden Doodle (1)	7-8	28-38	
Newfoundland (1)	54-67.5	66-71	
Old English Bull Dog (1)	25-36	40-50	
Pit Bull Terrier (1)	10-35	35-60	
Samoyed (1)	16-30	48-60	
Yorkshire Terrier (1)	1-3	20-23	
Mixed Breed (9)	11-36	30-76	No distinct appearance.

Note: All source material for measurements are from the Canadian Kennel Club breed (2015) standard guidelines or from other internet sources (dogtime.com and dogbreedinfo.com).

Table 2.2: Summary of the Strange Situation procedure. Each episode is outlined with respective durations and events that occurred in the room.

Event	Saliva Sample	Time	Description
Introduction		30 sec	Owner + dog + researcher enter room
Episode 1		3 min	Owner + dog
Episode 2		3 min	Owner + dog greeted by stranger, owner exits
Episode 3	8.5 min (dog)	3 min	Stranger + dog
Episode 4		3 min	Owner + dog
Episode 5		3 min	Dog alone
Episode 6	15.5 min (dog + owner)	3 min	Stranger + dog
Episode 7	21.5 min (dog + owner)	3 min	Owner + dog

Table 2.3: Ethogram of dog behaviours analyzed.

Behaviour	Definition
Physical proximity to: Owner Stranger Desk Door Two focal items simultaneously Other	Physical closeness, excluding actual contact, to any focal object/individual or combination of focal objects/individuals in space, while within the distance of the dog's own body length (snout to rear). Both frequencies and durations were measured. Each interval was based on the dog's position and could be ended by any state change. For example, the dog might be close to the owner and then the stranger enters and approaches the dyad. This would subsequently transition proximity to *owner* to proximity to *two focal items simultaneously* and the duration would be marked within this interval.
Physical contact Owner Stranger Researcher Two focal people simultaneously Cannot see	Contact occurring between a person and the dog, including petting (stroking), patting (hit lightly), jumping up on, sitting on, kissing, pawing, and extended touch (making physical contact using a toy or touching/pulling the dog's collar). Physical contact within the context of the saliva sample was not considered contact with stranger or researcher and extended touch by lifting a bowl for the dog to drink was excluded. The individual initiating the contact was recorded and frequency and duration were measured.
Body shaking	A side-to-side motion that begins at the head and extends down the body. This behaviour mimics a typical wet dog dry-off routine, without the context of being wet.
Door scratching	A bout of physical contact made with the door such that continual touching was considered a single bout and if contact was broken (neither paw touching the door) the bout was ended. Under some circumstances when one paw fell and at the exact same time the other paw resumed position on the door, contact was said to be unbroken.

Table 2.4: Correlations between physical proximity durations (expressed as proportions of available interaction time between the dog and focal individuals) in Episodes 1, 2, 3, 4, 5, 6 and 7, and dog age, duration of cohabitation and body shaking (max $N = 26$).

Episode	Proximity to	Age (yrs)	Cohabitation duration (yrs)	Body shaking
Episode 1	Owner	-0.054	-0.084	-0.284
	Door	-0.138	-0.322	0.307
Episode 2	Owner	0.345	0.320	-0.253
	Stranger	-0.041	-0.210	-0.085
	Door	-0.188	-0.202	0.036
Episode 3	Stranger	**-0.406***	-0.297	0.096
	Door	0.070	0.116	0.301
Episode 4	Owner	0.088	0.141	0.052
	Door	-0.308	-0.266	-0.040
Episode 5	Door	0.142	0.067	-0.034
Episode 6	Stranger	0.151	0.201	0.179
	Door	-0.183	-0.125	0.011
Episode 7				
	Owner	0.112	0.045	0.172
	Door	-0.081	-0.209	0.228

* Indicates a significant result at $p < 0.05$; all values were generated from Pearson R correlations.

Table 2.5: Correlations between physiological measures of stress (CORT and CgA) in dogs, and contact durations (D) and frequencies (F) that dogs spent with the owner and stranger (overall and specifically in Episodes 3, 4, 6, and 7), door scratching, body shaking, duration of cohabitation, and durations (D) that dogs spent in close proximity to owners and strangers.

	Dog CORT			Dog CgA		
	Baseline	Final	%	Baseline	Final	%
Stranger contact (Ep3, F)	-0.044	-0.404	–	0.138	-0.175	–
Owner contact (Ep4, F)	0.235	0.074	–	-0.378	-0.320	–
Stranger contact (Ep6, F)	-0.020	-0.075	–	-0.061	-0.099	–
Owner contact (Ep7, F)	-0.051	-0.292	–	-0.495	-0.330	–
Door scratching (F)	**0.494***	**0.510***	**0.481***	-0.260	0.242	0.295
Body Shaking (F)	-0.092	-0.069	-0.161	0.140	0.081	0.146
Cohabitation (years)	0.111	0.119	-0.051	0.113	0.363	**0.550***
Owner contact (D)	0.164	**0.427***	-0.005	0.020	0.074	0.130
Stranger contact (D)	-0.273	-0.292	-0.227	0.352	-0.102	-0.057
Owner contact (F)	**0.461***	**0.442***	0.281	0.064	-0.014	-0.145
Stranger contact (F)	0.253	-0.105	0.392	0.095	-0.379	**-0.620**
Proximity Ep1 (O, D)	0.382	0.180	0.269	-0.421	-0.257	-0.162
Proximity Ep3 (S, D)	0.065	0.196	-0.143	**-0.672***	-0.240	-0.038
Proximity Ep4 (O, D)	0.047	0.043	-0.243	-0.314	-0.072	0.293
Proximity Ep5 (door, D)	-0.009	0.028	0.116	**0.695***	**0.503***	0.199
Proximity Ep6 (S, D)	**0.524***	0.491	0.130	0.355	0.187	0.260
Proximity Ep7 (O, D)	0.360	**0.499***	-0.092	0.166	0.027	0.055

* Indicates a significant result at $p < 0.05$; all values were generated from Pearson R correlations. CORT and CgA reactivity (%) was calculated using: $\frac{baseline-final}{baseline}$ *100%). "O" represents "owner", "S" represents "stranger".

Table 2.6: Correlations between physiological measures of stress (CORT and CgA) in humans, and contact durations (D) and frequencies (F) that dogs spent with the owner and stranger (overall and specifically in Episodes 3, 4, 6, and 7), door scratching, body shaking, duration of cohabitation, and durations (D) that dogs spent in close proximity to owners and strangers.

	Human CORT			Human CgA		
	Baseline	Final	%	Baseline	Final	%
Stranger contact (Ep3, F)	-0.308	**-0.516***	–	-0.121	-0.252	–
Owner contact (Ep4, F)	0.046	0.085	–	**0.534***	**0.670***	–
Stranger contact (Ep6, F)	-0.233	-0.282	–	-0.172	-0.259	–
Owner contact (Ep7, F)	-0.237	-0.301	–	0.287	**0.432***	–
Door scratching (F)	-0.195	-0.047	-0.254	0.080	0.154	0.191
Body Shaking (F)	0.112	0.086	-0.045	0.248	0.183	-0.227
Cohabitation (years)	0.134	0.238	-0.172	-0.051	0.020	0.168
Owner contact (D)	0.285	0.355	0.087	-0.119	-0.082	0.037
Stranger contact (D)	0.297	0.099	0.354	-0.028	-0.211	-0.068
Owner contact (F)	0.118	-0.089	0.333	0.064	-0.014	-0.145
Stranger contact (F)	0.050	-0.252	**0.591***	0.095	-0.379	0.223
Proximity Ep1 (O, D)	-0.031	-0.117	0.217	-0.169	-0.187	-0.113
Proximity Ep3 (S, D)	0.077	0.185	-0.178	0.002	-0.129	-0.116
Proximity Ep4 (O, D)	-0.107	0.138	-0.050	0.042	-0.090	**-0.691***
Proximity Ep5 (door, D)	-0.080	-0.049	-0.101	-0.200	-0.239	0.197
Proximity Ep6 (S, D)	0.241	0.270	-0.065	0.002	-0.041	0.061
Proximity Ep7 (O, D)	0.392	**0.437***	-0.053	**0.472***	0.162	**-0.614***

* Indicates a significant result at $p < 0.05$; all values were generated from Pearson R correlations. CORT and CgA reactivity (%) was calculated using: $\frac{baseline-final}{baseline}$ *100%). "O" represents "owner", "S" represents "stranger".

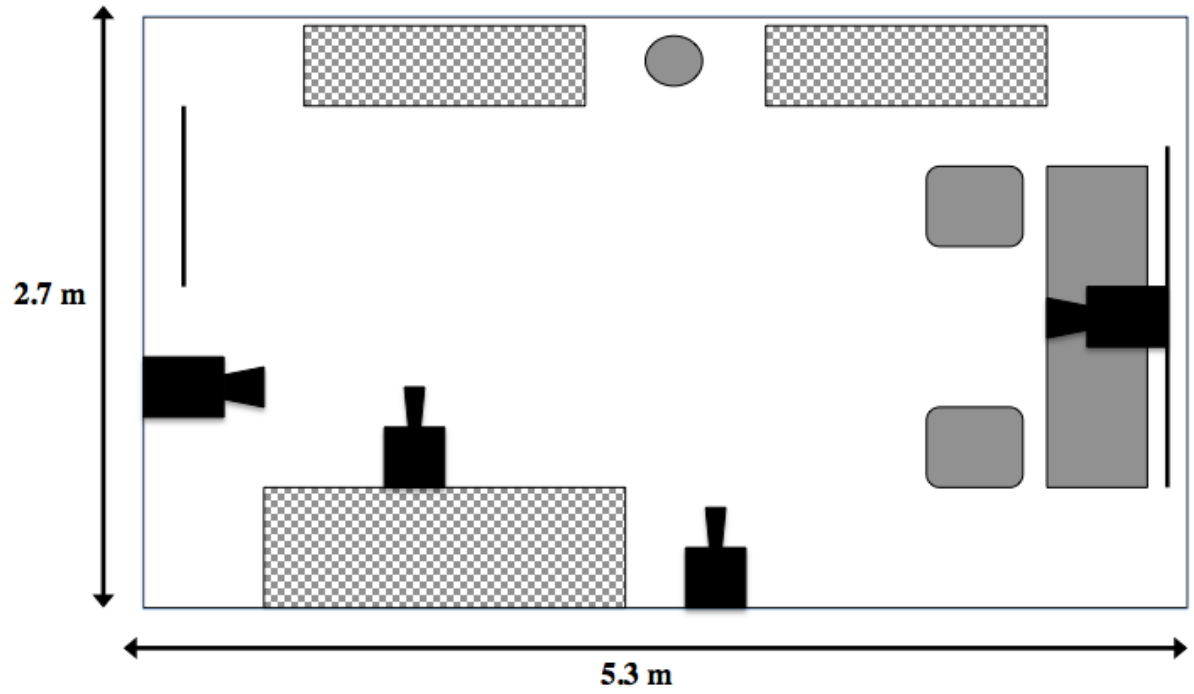

Figure 2.1: Layout of the Strange Situation room. Solid black objects represent camera placements in the room, the circle represents the water dish available to the dog, the grey and white checker rectangles represent storage units, and the solid grey shapes represents the desk area where the sample supplies, speakerphone and basket of toys were stored. Solid black lines represent a window (immediate right) and door (immediate left).

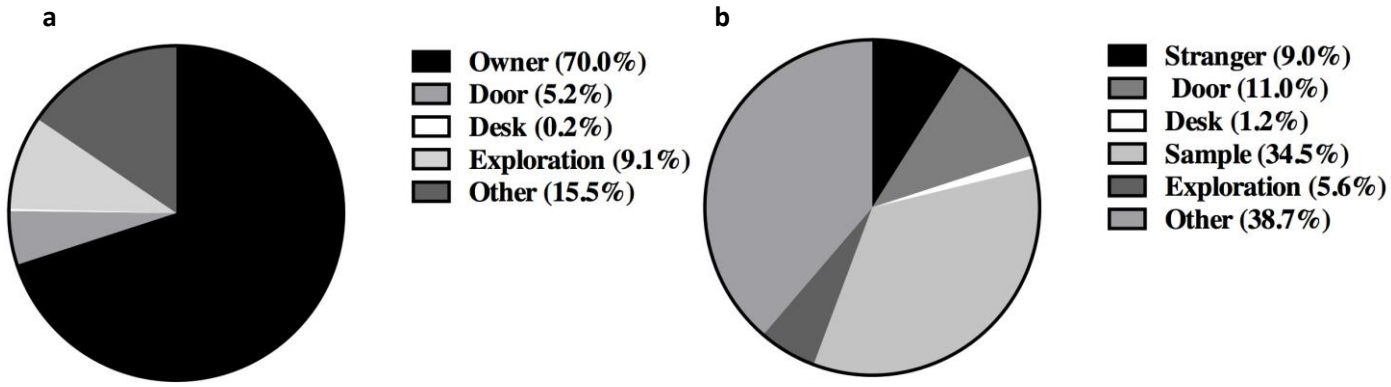

Figure 2.2: Proportion of time spent by the dogs in close proximity to focal objects and individuals within the room (*N* = 26). Figure 2.2a represents an average of Episodes 4 and 7, which were episodes in which the owner was with the dog exclusively and Figure 2.2b represents an average of Episodes 3 and 6, which were episodes in which the stranger was with the dog exclusively. Proportions are based on the total time spent in close proximity to the focal object/individual within a given episode divided by the total (average) duration of the episode. Note: Figure 2.2a is divided into 5 sections; however, only 4 are visible as the proportion of time spent near the desk is almost negligible.

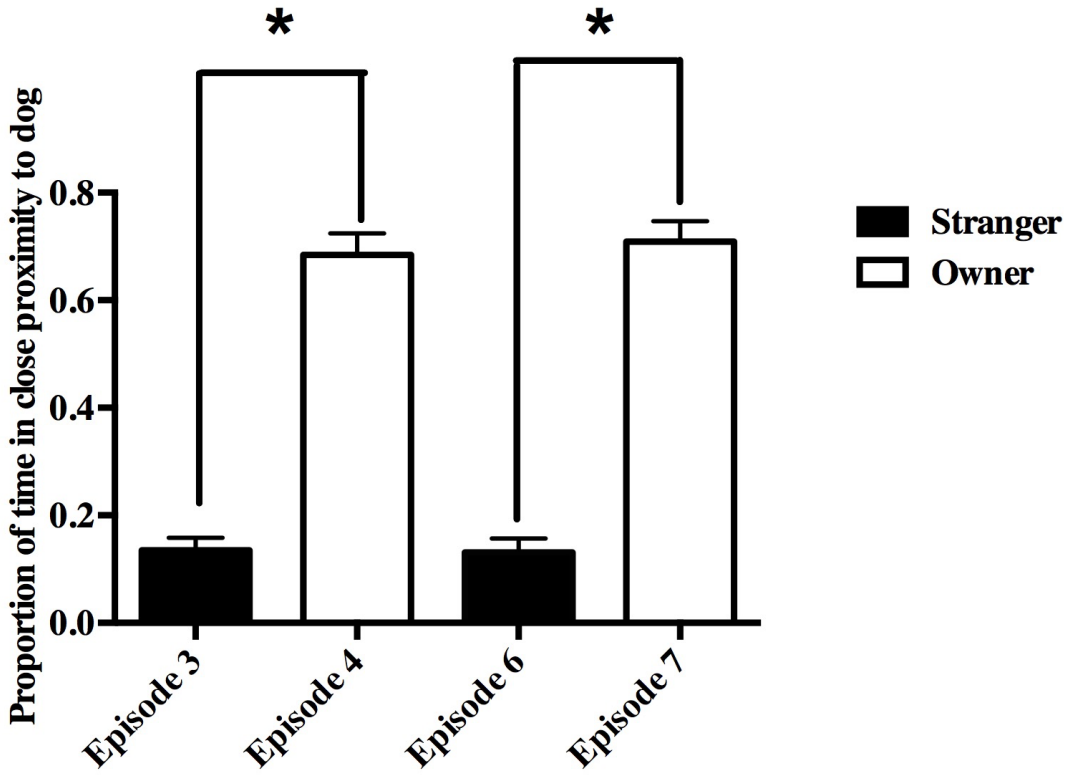

Figure 2.3: Overall duration that dogs spent in close proximity to strangers (Episode 3 and 6) and to owners (Episode 4 and 7). Proportions indicate the total duration dogs spent in close proximity to the focal individual (stranger or owner) over the total duration of the episode or the total time the individual had available to interact with the dog for Episodes 3, 4, 6 and 7. Significant differences are indicated by asterisks and error bars indicate SEM ($p < 0.001$; $N = 26$).

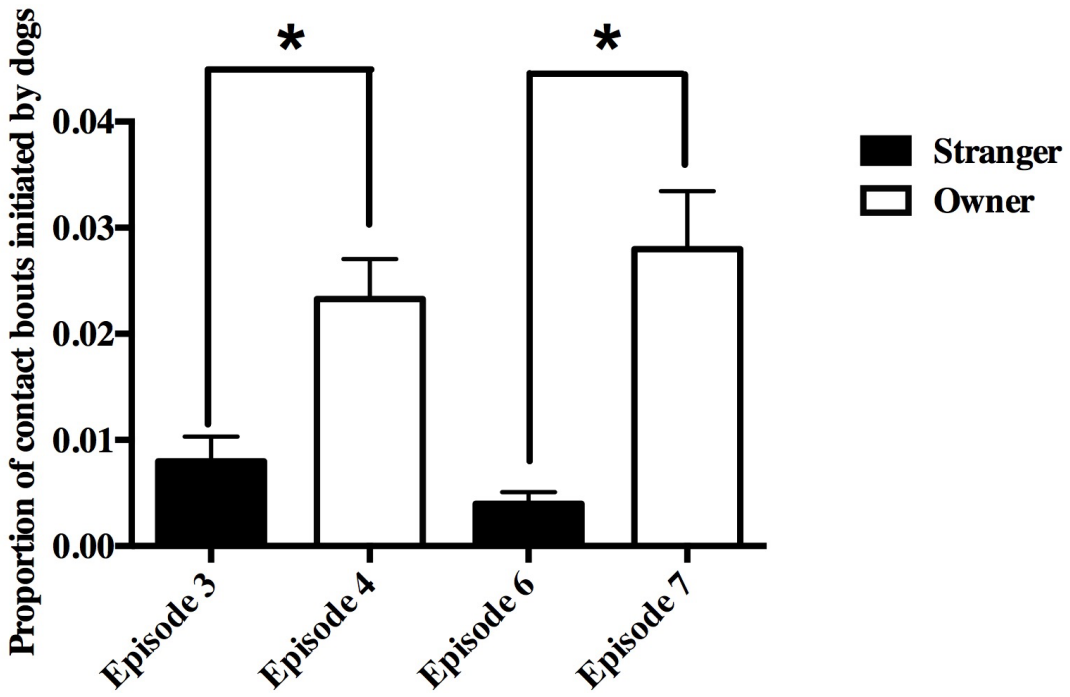

Figure 2.4: Proportion of physical contact bouts initiated by the dog towards the stranger (Episode 3 and 6) and the owner (Episode 4 and 7). Proportions represent total frequencies with each respective episode divided by the total time available for interaction with the dog. Significant differences are indicated by asterisks and error bars indicate SEM ($p < 0.001$; $N = 26$).

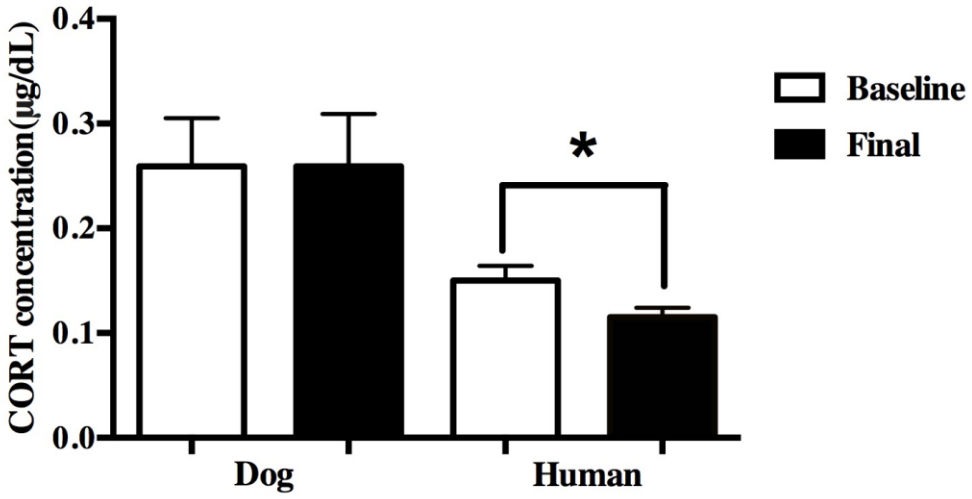

Figure 2.5: Baseline and final CORT concentrations in saliva of dogs and humans. No changes were observed in dogs for CORT concentrations. Human CORT concentrations decreased over the course of the Strange Situation. Significant differences are indicated by asterisks and error bars indicate SEM ($p < 0.001$; Dog: baseline $N = 24$, final $N = 23$; Human: baseline/final $N = 29$).

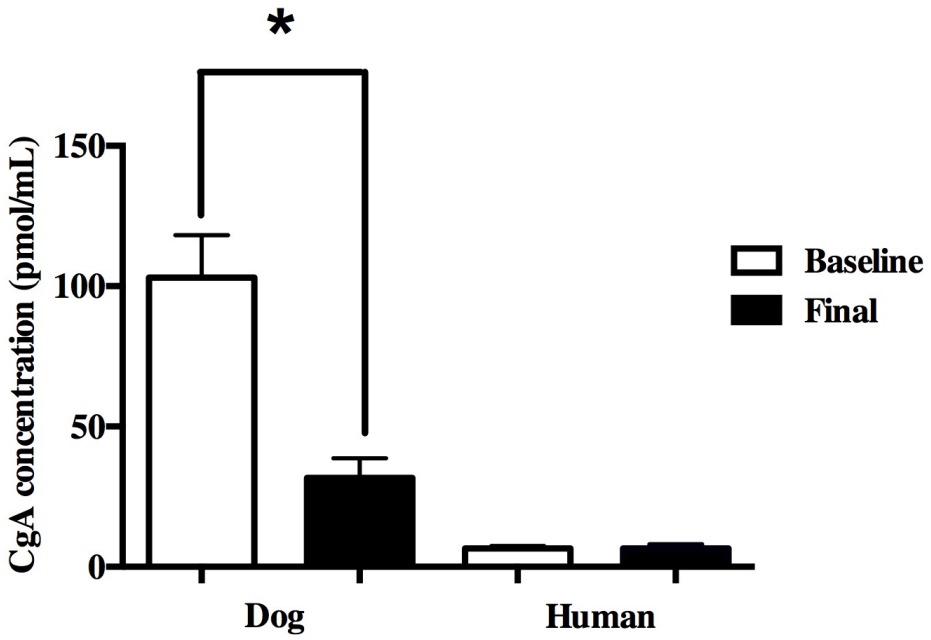

Figure 2.6: Baseline and final CgA concentrations for dogs and humans. Dog CgA concentrations decreased over the course of the Strange Situation. Human CgA concentrations did not change. Significant differences are indicated by asterisks and error bars indicate SEM ($p < 0.001$; Dog: baseline $N = 17$, final $N = 20$; Human: baseline $N = 28$, final $N = 25$).

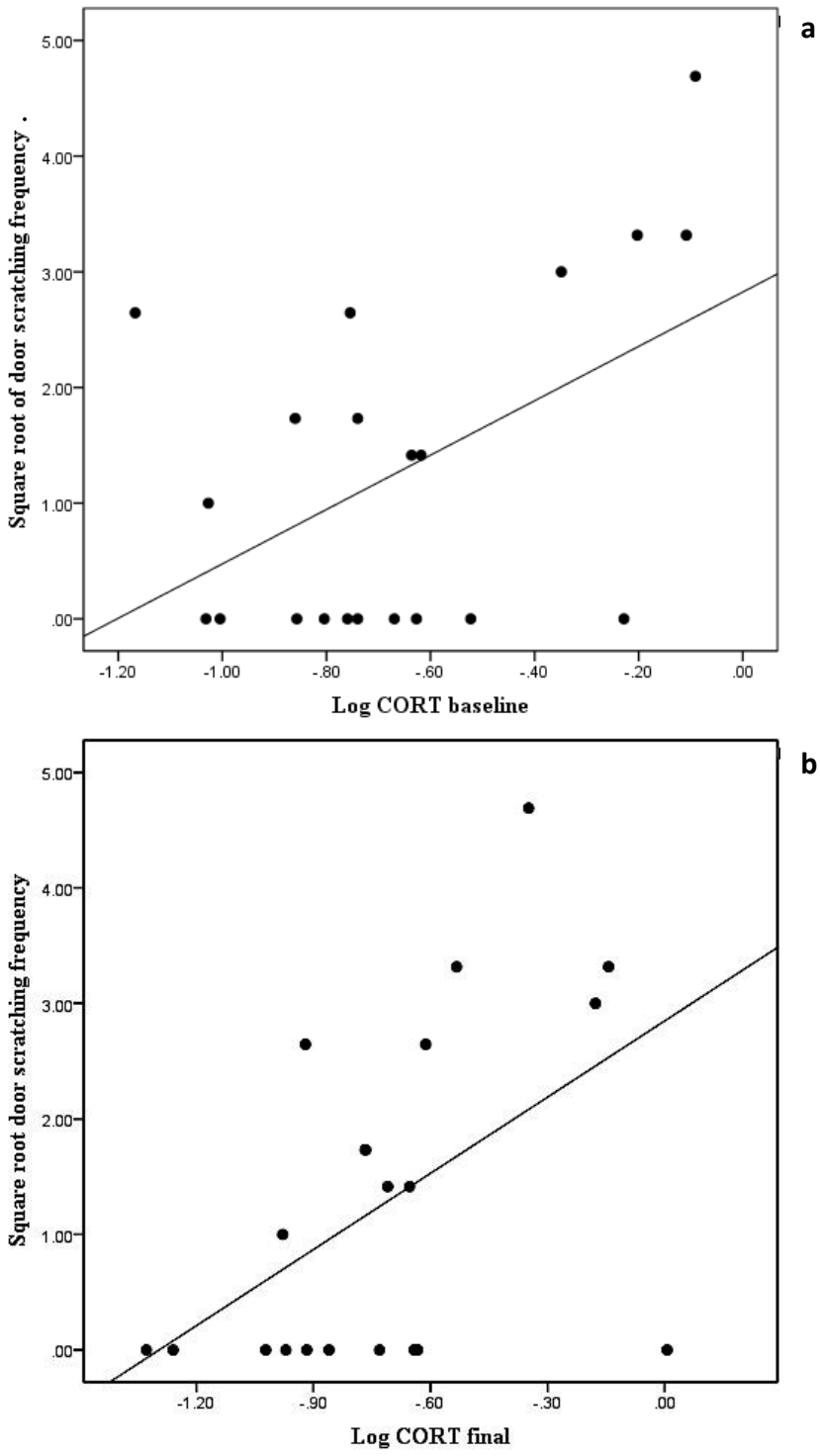

Figure 2.7: Correlation between the square root transformation of door scratching frequency and baseline (2.7 a; $r = 0.494$, $p = 0.023$, $N = 23$) and final (2.7 b; $r = 0.510$, p = 0.018, $N = 21$) CORT for dog.

CHAPTER 3: EXAMINING THE RELATIONSHIP BETWEEN PERSONALITY AND ATTACHMENT IN THE HUMAN-DOG RELATIONSHIP

3.1 ABSTRACT

Personality refers to enduring patterns of behaviours, attitudes and thoughts throughout an individual's lifespan, which are influenced by environmental and genetic factors. Personality has been linked to attachment *styles* acquired during early development, which tremendously impact social relationships and coping mechanisms adopted in adulthood. Since the human-dog bond has been described as a pseudo-parental relationship, this current investigation aimed to explore the possible link between personality and attachment in this interspecific affiliation. Here, attachment-related dog behaviours (proximity and contact) were recorded in the context of a dog-amended Ainsworth's Strange Situation test. Additionally, a series of questionnaires were used to measure human personality factors (NEO-FFI-3), dog personality dimensions (MCPQ-R) and self-reported owner attachment to the dog (DAQ). Overall, human and dog personality were not linked in predictable ways. However, certain logical associations were found, e.g., owners scoring high in Conscientiousness (ambitious) and low in Neuroticism (low anxious) had dogs scoring high in Training-focus (trainable). Human personality was also linked to attachment, as owners scoring high on Extraversion (outgoing) had higher attachment (DAQ) scores and they initiated significantly more contact with dogs than less extraverted owners. Dogs rated by owners as high in Amicability and low in Neuroticism engaged in more physical contact bouts with strangers in the Strange Situation test. Overall, distinct relationships were present between owner and dog personality and between personality and attachment-related behaviours.

3.2 INTRODUCTION

Personality refers to an individual's enduring pattern of behaviours, attitudes and thoughts, which are stable throughout their lifespan (Carere & Locurto, 2011; Cloninger, 2008; Fratkin, Sinn, Patall & Gosling, 2013; Ley, Bennett & Coleman, 2008; Lofgren et al., 2014). To describe personality, human-based approaches obtain scores across a series of overarching 'factors'. These identified characteristics (factors) have been shown to predict coping strategies, mental health outcomes, relationship satisfaction/success and academic performance (e.g., Holland & Roisman, 2008; Körner et al., 2015; O'Connor & Paunonen, 2007). Currently, the predominant, unifying theory for human personality recognizes five main factors, referred to as the 'big five': Openness, Conscientiousness, Extraversion, Agreeableness and Neuroticism (see Table 3.1; Costa & McCrae, 1985; Wiggins, 1996). These five factors are often measured using comprehensive questionnaires like the *Neuroticism Extraversion Openness- Five Factor Inventory-3* (NEO-FFI-3; Costa & McCrae, 1985), which ask behaviour-based statements and scores on the *big five* reflect the level of agreement on each associated statement.

Unlike humans, non-human animal personality studies lack uniformity, as there is not one specific or species-specific approach adopted, such as with the 'big five' in human research (Gosling, 2001). Instead, behavioural observations of non-humans typically describe personality in terms of relative exploration, coping styles, boldness and aggression (Carere & Locurto, 2011; Gosling, 2001; Mehta & Gosling, 2008). Identifying personality *traits* in non-humans may be limited to observed behaviours, which might not fall in the same 'factor' categories as seen in human personality inventories. Of course, the current human labels may restrict the true, underlying traits in non-human animals (Ley et al., 2008; Mehta & Gosling, 2008). It is irrefutable, however, that non-human animals possess their own collection of unique behavioural

attributes, which contribute to producing individual differences (Mehta & Gosling, 2008; Sinn & Moltschaniwskyj, 2005).

Considering that humans and dogs share extensive evolutionary history, it is not surprising to learn that attempts have been made to use human-analogous traits to describe dog personality (Draper, 1995; Germonpré et al., 2013; Pang et al., 2009). Most dog personality evaluations to date collect information through descriptive or observational inventories on a variety of different facets such as stress reactivity, trainability and sociability (e.g., Fratkin et al., 2013; Hsu & Serpell, 2003; Svartberg & Forkman, 2002; Svartberg, Tapper, Temrin, Radesäter & Thorman, 2005; van den Berg, Heuven, van den Berg, Duffy & Serpell, 2010). They also assume that traits remain stable over time, which has been recently supported; Fratkin et al., 2013; Mirkó, Kubinyi, Gácsi & Miklósi, 2012).

Ley and her colleagues (2008) created the *Monash Canine Personality Questionnaire-Revised* (MCPQ-R), which records owner's scores for dogs on five personality *dimensions*: Training-focus, Motivation, Extraversion, Amicability and Neuroticism, using a series of adjectives (see Table 3.2; Ley et al., 2008; Ley, McGreevy & Bennett, 2009). This questionnaire produces generalizations about dog behaviour and temperament, which produces normative data. Another well-known personality inventory for dogs is the Canine Behavioural Assessment and Research Questionnaire (C-BARQ), which assess dogs on a series of 100 online questions that produce 11 factors (van den Berg et al., 2010). Despite its popularity, the C-BARQ is not quite as short and user friendly as the MCPQ-R and arguably more of an inventory of 'problem behaviours' rather than an assessment of global personality (e.g., Marshall-Pescini, Valsecchi, Petak, Accorsi & Previde, 2008; Nagasawa et al., 2011; Ottenheimer Carrier et al., 2013; Walker, 2014).

Human personality research can greatly benefit from studying comparative, non-human animal species (Dingemmanse & Wolf, 2010; Gosling, 2001; Mehta & Gosling, 2008). Various animal models share many of the same physiological and behavioural attributes with humans; therefore, certain personality commonalties are likely found (Gosling, Kwan & John, 2003; Schöberl et al., 2012). Using comparative models allows for more experimental control and manipulation, which extends theories in an evolutionary and ecological context (Gosling, 2001; Mehta & Gosling, 2008). Examining dog personality, specifically, affords many advantages considering that they are the most popular pet in the Western world as 83.3 million people in the US alone own dogs (Bhattacharyya & Mukhopadhyay, 2014; Curb, Abramson, Grice & Kennison, 2013; Hart, 1995; Kis, Turcsán, Miklósi & Gácsi, 2012). Choosing appropriate personality characteristics in dogs can be crucial in certain contexts such as in the selection of working dogs (e.g., guide dogs, herding dogs). It may also be useful in making compatible 'matches' for dogs and owners, which could lower relinquishment rates in shelters and aid in managing problem behaviours (e.g., separation anxiety; Curb et al., 2013). Pet matching programs are being implemented and work is being done to find out more information regarding the factors that best predict relationship satisfaction (Mondelli et al., 2004; Mornement, Coleman, Toukhsati & Bennett, 2010). For example, Curb et al. (2013) found that personality matching, enjoyment of shared activities and absence of destructive behaviours influenced owners' perceived dog satisfaction with their dogs.

Owners may originally choose dogs that they share personality characteristics with or ones that complement their desired lifestyle (Hoffman, Chen, Serpell & Jacobson, 2013; Kwan, Gosling & John, 2008). However, it is also possible that owners influence their dogs' personality over time. The human-dog bond has been investigated at length and research suggests that this

unique relationship is analogous to that of a parent and child (e.g., Rehn, Lindholm, Keeling & Forkman, 2014; Sable, 2013; Topál, Miklósi, Csányi, & Dóka, 1998). Dogs also demonstrate emotional connectivity to their owners as they have the ability to 'empathize' with their owner (Bennett & Rohlf, 2007; Buttner & Strasser, 2014; Custance & Mayer, 2012; Hilby, Rooney & Bradshaw, 2004; Romero, Konno & Hasegawa, 2013; Schöberl et al., 2012; Silva & Sousa, 2011; Yong & Ruffman, 2015). Obedience corrections and positive training exercises (e.g., agility exercises) are the most well accepted examples of how owners influence their dogs' behaviour (Horowitz, 2009; Kis et al., 2012; Ostojić, Tkalčić, & Clayton, 2015; Schöberl, Wedl & Kotrschal, 2013). Although it is important to consider that not all influences are of a positive nature, e.g., abuse towards dogs can produce aggressive temperaments.

Owner personality has also been linked to dog behaviour, owner-dog performance on practical tests, and on their dogs' physiological stress response (Deldalle & Gaunet, 2014; Horváth, Dóka, & Miklósi, 2008; Payne, Bennett & McGreevy, 2015; Schöberl et al., 2012; Topal, Miklosi & Csanyi, 1997). Owners that score high on Neuroticism (nervousness) and Openness (creativity) tend to use more physical and verbal commands when asking their dogs to sit, which appeared to cause dogs to obey for longer periods of time (i.e., continue to sit for longer durations; Kis et al., 2012). Additionally, Turcsàn, Range, Viranyi, Miklósi & Kubinyi (2012) found that scores of owner-dog dyads were similar on four of the five main personality factors: Neuroticism, Extraversion, Conscientiousness and Agreeableness by using the Big Five Inventory (BFI) for owners and an amended BFI for dogs (they did find similarities between dogs and owners on Openness, however; Gosling et al., 2003; Kis et al., 2012; Turcsàn, Kubinyi, Virányi, & Range, 2011; Wedl, Schöberl, Bauer, Day & Kotrschal, 2010.)

Furthermore, the human-dog bond appears to be influenced by personality as owners scoring high on Neuroticism view their dogs as a social support system (Kotrschal, Schöberl, Bauer, Thibeaut, & Wedl, 2009). Additionally, owners scoring high on Extraversion were more likely to report that they enjoyed activities with their dogs (Kis et al., 2012; Kotrschal et al., 2009). In addition to the behavioural effects, owners scoring high on Neuroticism and low on Conscientiousness (NEO-FFI) had dogs with higher morning cortisol (a glucocorticoid hormone related to stress and arousal; Schöberl et al., 2012). This result was in the context of performing several 'experimental challenges' (i.e., playing with their owners or being taught a novel task) and it reflects the owner's ability to modulate coping strategies in their dog companions (Schöberl et al., 2012). Therefore, it is reasonable to suggest that owners select dogs based on a series of predetermined criteria (i.e., visible behavioural traits); however, this literature also suggests that owners have the ability to impact their dogs' behavioural and physiological responses.

It is important to consider that persistent individual differences are moulded by environmental factors, genetic predispositions, and physiological states, which are influenced by context dependent interactions (Haworth, Davis, & Plomin, 2013; Johnson, Carver, Joormann & Cuccaro, 2014; Knutson et al., 1998; Lewis, Haworth & Plomin, 2014; Southard, Zeigler-Hill & Shackelford, 2014; Tackett, Herzhoff, Harden, Page-Gould & Josephs, 2014). One environmental factor that reciprocally interacts with personality is the attachment *style* adopted by an individual during early developmental stages. At times, it is difficult to distinguish between the origin of certain behaviours and whether they could be attributed to the effects of attachment styles or the effects of personality in humans (Sibley & Overall, 2008). For example, Neurotic personalities can produce anxious styles of attachment, and insecure attachments in

early-development can also elevate the predisposition towards Neuroticism, especially in terms of how insecurely attached adults cope in intimate relationships (Heaven, Da Silva, Carey & Holen, 2004; Shaver & Brennan, 1992; Shiota, Keltner & John, 2006). As well, personality may provide a "genetic effect" on attachment; thus, those possessing a genetic predisposition to respond to differences in the quality of attachment figures may be more likely to develop a particular attachment style (Donnellan, Burt, Levendosky & Klump, 2008).

In humans, at least, attachment styles are most likely produced by a parent's relative attentiveness to their offspring's needs and they have been described as being 'secure' or 'insecure' (Ainsworth, Blehar, Waters & Wall, 1978). Secure offspring are those that exhibit uninhibited exploration in novel contexts when in close proximity to their attachment figure, but they experience distress upon the departure of this caregiver, refusing to interact with a substitute, and they are delighted upon the return of the caregiver. Conversely, insecure offspring are either 'resistant' or 'avoidant'. Children in both categories of insecure attachment have difficulties adjusting to novel environments or individuals as well as in exploring away from the attachment figure. 'Resistant' children are distressed by their caregiver's departure, with no change in distress upon their return, while 'avoidant' children are not distressed by their caregiver's departure or return (Ainsworth et al., 1978; Donnellan et al., 2008). Therefore, attached individuals have a greater ability to cope in novel environments and they are able to adapt to brief separation periods from their attachment figure, as they are able to resume to normal (relaxed) behaviour when their caregiver returns.

While the dog-attachment literature has not addressed attachment styles directly, it has suggested that dogs can have insecure attachments to their owners or develop *hyper* attachments to them, which often leads to separation anxiety (Konok et al., 2015; Sherman, 2008). Owners'

attachment styles have also influenced whether their dogs develop/present separation anxiety, as owners scoring high on attachment avoidance have dogs with higher Neuroticism scores and higher rates of reported separation anxiety than securely attached owners (Konok, Dóka & Miklósi, 2011). Within the context of a separation and greeting test (analogous, but not identical to Ainsworth's Strange Situation test), dogs whose owners reported past separation anxiety issues also tended to use owners as a secure base less frequently than dogs without such issues (Konok et al., 2011). Progress has also been made in the scoring of owner-perceived attachment, through the Dog Attachment Questionnaire (DAQ; Archer & Ireland, 2011), which reflects the extent to which the owner feels bonded towards his/her dog. This questionnaire has only been used a handful of times in the recent literature, namely in evaluating the attractiveness in infant and pet facial features and in the context of behavioural and hormonal states during a dog agility competition (Archer & Monton, 2011; Buttner, Thompson, Strasser & Santo, 2015). This test has never been used in conjunction with a personality evaluation or analyzed with direct measures of behavioural attachment such as the Strange Situation test.

This current investigation examines whether owner personality may influence traits observed in dogs and whether personality matching in owner-dog dyads may be related to owner-perceived relationship strength (DAQ). Additionally, this study tests the effects of personality on behavioural manifestations of attachment and it is the first to integrate the Strange Situation with measures of both human and dog personality. I predicted that there would be sex differences in owner personalities as past reports typically find that women score higher than men on Agreeableness and Neuroticism, and sometimes on Conscientiousness and Extraversion (e.g., Cavallera, Passerini & Pepe, 2013; Chapman, Duberstein, Sörensen & Lyness, 2007; Costa, Terracciano & McCrae, 2001). Furthermore, I thought that similar sex differences might

be found in analogous personality traits for dogs, though no literature to date has reported significant sex differences. Sex differences were also expected for owner-perceived attachment (DAQ) as past findings showed that females tend to score higher than males (Archer & Ireland, 2011). Personality matching was also predicted to occur as previous reports concluded that certain personality attributes (e.g., Extraversion) are positively correlated for owner-dog dyads (Curb et al., 2013; Kis et al., 2012; Turscán et al., 2011; Turscán et al., 2012). Finally, personality variables were expected to influence both perceived attachment (DAQ) and attachment-related behaviours, such that owners with higher DAQ scores would have dogs with more agreeable qualities, such as high Amicability and Training-focus, and display a stronger preference for owners during the procedure.

3.3 METHOD AND MATERIALS

3.3.1 Participants

This protocol was completed by 29 volunteer owner-dog dyads. They were given a complimentary poop bag dispenser at the end of the study, but were unaware of this prior to participation. In an attempt to obtain a wide-ranging sample of Newfoundland dog owners, participants were recruited through a variety of social media (e.g., public posters, booths at dog shows and at a local Pet Expo, departmental e-mails and local classified ads such as www.kijiji.ca). Owners consisted of six males and 23 females, ranging from 20 to 71 years old ($X \pm SD$, 40 ± 14.8 years). Eight (27.6%) owners had children either living with them or living outside the household as independent adults.

There were 13 male and 16 female dogs, ranging from eight months to 14 years old ($X \pm SD$, 6.0 ± 3.9 years). Of the 29 dogs tested, five were sexually intact; one female (not in estrus at

the time of the study, according to owner's report) and four males, while the remaining 24 dogs were neutered/spayed. No specific dog breed was targeted. See Chapter 2 of this thesis for details regarding participation requirements and for all other methods not directly related to the questionnaires discussed in the current chapter. In particular, the methods used during the Strange Situation test (order of episodes) and the ethogram outlining the behaviours measured (e.g., Table 2.3, Chapter 2). It is important to note here, however, that the Strange Situation uses a series of separation and reuniting events from a caregiver (owner) to elicit attachment behaviours in the dependant (dog). For this protocol, in Episodes 1, 4 and 7 the dog was exclusively with the owner, in Episode 2 the dog was with the owner and a stranger, and during Episodes 3 and 6 the dog was exclusively with the stranger.

3.3.2 Questionnaires

Supplemental questions and standardized questionnaires were given to participants in the context of a study examining the behavioural and physiological manifestations of attachment in owner-dog dyads (see Appendix A). Supplemental questions required owners to report basic information pertaining to their own health, their dogs' health and general activities the dog and owner engaged in together. The majority of supplemental questions were not used for analysis, save for owner reported separation anxiety in dogs, dog age and how long the dog and owner had lived together. Supplemental questions were designed as a means of explaining possible outlying chemical concentrations or scores on the standardized questionnaires. The standardized questionnaires consisted of: the Neuroticism Extraversion Openness-Five-Factor-Inventory-3 (NEO-FFI-3; Costa & McCrae, 1986), the Monash Canine Personality Questionnaire Revised (MCPQ-R; Ley et al., 2009) and the Dog Attachment Questionnaire (DAQ; Archer & Ireland, 2011). All participants that came to Memorial University of Newfoundland to participate in the

study completed the MCPQ-R (N = 29) and the DAQ (N = 29), but not all participants completed the NEO-FFI-3 (N = 25). Participants were approached after the study to fill out the NEO-FFI-3 on a computer and submit their results electronically, as it was not a component of the original study.

3.3.3 NEO-FFI-3

The NEO-FFI-3 (Costa & McCrae, 1986) is a standardized questionnaire designed for adolescents and adults (12 to 99 years old) that uses a series of 60 statements, rated on a 5-point Likert scale. Each statement relates to one of the big five personality factors: Openness, Conscientiousness, Extraversion, Agreeableness and Neuroticism, and the inventory yields an overall and adjusted (for age and sex) score for each factor (Table 3.1). Statements within the inventory include, “I try to be courteous to everyone I meet”, “I like to have a lot of people around me” and “At times I have felt bitter and resentful”.

This questionnaire was completed after the original Strange Situation procedure at the participant’s convenience using an online platform (recovery rate: 25/29, 86.2%; PAR iConnect; Psychological Assessment Resources, Inc., Lutz, FL). As this was not an original participation requirement and fell outside of the original consent form, a response to the e-mail sent and subsequent completion of the inventory was taken as the participant’s method of informed consent, which was approved by the ethics committee.

3.3.4 MCPQ-R

Ley and colleagues (2009) developed the MCPQ-R to identify adjectives, and consequently, condense these adjectives into related super categories for the purpose of describing individual differences in dogs (Ley et al., 2008). The validated MCPQ-R asks owners

to rate how well each of a series of 26 adjectives describes their dog on a 6-point Likert scale (Table 3.1). Based on factor analyses (Ley et al., 2009), each adjective belongs to one of five dimensions: Training-focus, Amicability, Neuroticism, Extraversion and Motivation (Table 3.2). Each dimension score is based on the rating given to the adjectives belonging to that category divided by the number of adjectives for that category. It is important to note that the MCPQ-R dimensions are not directly comparable to the big five personality traits observed in humans. Some factors do, however, share common elements with the MCPQ-R dimensions, e.g., Amicability and Agreeableness.

3.3.5 DAQ

The DAQ (Archer & Ireland, 2011) requires owners to rate how much they agree with each of a series of 35 statements on a 5-point Likert scale. Statements were designed to gain information regarding the depth of the human-dog relationship. Some statements were positively scored such that strong agreement with those items conveyed a strong bond, whereas, other statements were negatively weighted such that strong agreement dismissed or scorned the importance of the relationship. For example, "My dog is an important part of my life" versus "Having a dog means that you cannot do what you want to". Upon completion, each response is taken into account, added (or subtracted) together and averaged across all responses (DAQ score = total score/35). A score of three or greater was considered to suggest moderate to high levels of attachment.

3.3.6 Statistical Analyses

All statistical analyses were carried out using IBM SPSS Statistics 20 (IBM, Armonk, NY, USA). A series of normality tests (binomial and Kolmogorov-Smirnov tests) were

performed to ensure that the data were normally distributed. Due to the novel nature of this research, many analyses were exploratory. Analyses comparing individuals (e.g., sex comparisons) were performed using Independent Samples t-tests. Given the exploratory nature of certain correlational relationships present in this thesis, Bonferroni corrections were not utilized as they were thought to be too restrictive (see Jaeger & Halliday 1998; Ottenheimer Carrier et al., 2013). Correlations reported indicate Pearson r bivariate tests. All significance probabilities reported in this manuscript are two-tailed, p = 0.05. The sample size quite often deviates from the total number of participants collected (*N* = 29), as only 26 participants qualified for behavioural measurements due to the layout of the first room and because not all participants completed the NEO-FFI-3 (*N* = 25).

3.4 RESULTS

3.4.1 NEO-FFI-3

There were no sex differences present for any of the NEO-FFI-3 factors; average raw scores are presented in Table 3.3 for men (*N* = 4), women (*N* = 21) and their combined average, respectively (*N* = 25).

3.4.2 MCPQ-R

Extraversion was the only dimension to show a sex difference, as female dogs scored significantly higher than male dogs (t_{27} = -2.49, *p* = 0.019; Table 3.4). No personality dimension differed as a result of whether females or males were sexually intact.

3.4.3 DAQ: Owner Perceived Attachment

Participants of both sexes met the attachment criteria, as all individuals obtained a score higher than 3.A sex difference was found in DAQ scores as women (X± SE: 3.71± 0.054) scored significantly higher than men (X± SE: 3.30± 0.115; t_{27} = -3.35, p = 0.002, N = 29). The overall mean (males + females) was also nearly identical to that reported in one of the earlier papers (this study: 3.62 ± 0.057 versus Archer & Monton, 2011: 3.61 ± 0.049), which had 163 participants.

3.4.4 Human and Dog Personality

Human and dog personality scores lacked any predicted links, e.g., human Agreeableness (NEO-FFI-3) did not correlate with dog Amicability (MCPQ-R), nor did human Neuroticism and dog Neuroticism (see Table 3.5; N = 25). However, owners scoring higher on Openness (i.e., creative) had dogs that scored lower on Amicability (i.e., friendly; r =-0.508, p = 0.010) and higher on Extraversion (i.e., active; r = 0.421, p = 0.036). Additionally, dogs scoring higher in Training-focus had more Conscientious (r = 0.399, p = 0.048), less Neurotic (r = -0.528, p = 0.009) and less Open (r = -0.509, p = 0.009) owners than dogs that scored lower on this dimension.

3.4.5 DAQ Scores and Personality

Human and dog personalities did not appear to affect owner-perceived attachment (DAQ scores), with the exception of human and dog Extraversion. Humans with higher DAQ scores had higher Extraversion scores (NEO-FFI-3; r = 0.443, p = 0.026, N = 25, Table 3.6) and had dogs that *tended* to have higher Extraversion scores (MCPQ-R; r = 0.366, p = 0.051, N = 29, Table 3.6) than owners with lower DAQ scores.

3.4.6 Human Attachment-related Behaviours and the NEO-FFI-3

The only human-initiated attachment behaviour that was related to the big five personality factors was contact initiated by the owner in Episode 4 (second episode dogs spent with the owner exclusively) and human Extraversion. Owners scoring high on Extraversion initiated more contact in this episode with their dog ($r = 0.433$, $p = 0.044$).

3.4.7 Dog Attachment-related Behaviours and the NEO-FFI-3

Physical proximity of the dog to the owner was related to several personality factors. Dogs that spent more time in close proximity to the stranger during Episode 6 (second episode dogs spent with the stranger) had owners that scored lower on Openness ($r = -0.479$, $p = 0.024$, $N = 22$). Owners scoring high on Openness also spent more time in close proximity to their dogs in Episode 1 ($r = 0.430$, $p = 0.046$, $N = 22$). Owners that scored low on Agreeableness and Conscientiousness had dogs that spent more time in close proximity to the door during Episode 3 (first episode dogs spent with the stranger exclusively; $r = -0.477$, $p = 0.025$, $N = 22$; $r = -0.533$, $p = 0.011$, $N = 22$, respectively). Compared to owners with low Extraversion scores, owners with high Extraversion scores had dogs that spent less time in close proximity to them in Episode 1 (first episode the dogs spent with their owners; $r = -0.522$, $p = 0.013$, $N = 22$). Physical contact initiated by the dog was not related to any of the human personality factor (see Table 3.7 for all significant relationships).

3.4.8 Dog Attachment-Related Behaviours and the MCPQ-R

Dog personality dimensions were related to both physical contact and physical proximity behaviours (see Table 3.8 for all significant relationships, with the exception of two correlations

between personality dimensions and contact, no other correlations were found with this behaviour). Motivation and Training-focus were not related to any measured behaviour. Dogs with higher Amicability scores spent less time with their owners during Episode1 (first episode the dogs spent with their owners*;* $r = -0.479$, $p = 0.013$, $N = 26$), more time near the door during Episode1($r = 0.587$, p = 0.002, $N = 26$) and they initiated more contact overall with strangers ($r = 0.500$, $p = 0.009$, $N = 26$). Dogs scoring high on Neuroticism initiated less overall contact with strangers ($r = -0.409$, $p = 0.038$, $N = 26$), less overall contact with owners ($r = -0.433$, $p = 0.027$, $N = 26$), and they spent a lower proportion of time near the door during Episode 3 (second episode dogs spent with the stranger*;* $r = -0.497$, $p = 0.010$, $N = 26$). Dogs with higher Extraversion scores spent less time near the stranger during Episode 2 (dog with owner and stranger, $r = -0.414$, $p = 0.036$, $N = 26$).

3.4.9 Attachment-Related Behaviours and Owner-Perceived Attachment

DAQ scores did not predict how dogs or owners behaved during the Strange Situation test, and they were not related to physical contact or physical proximity measurements.

3.5 DISCUSSION

Collectively these findings suggest that links are present between owner and dog personality as well as between attachment and personality for human-dog dyads. However, no previously reported personality matches between human-dog dyads were replicated (e.g., Kis et al., 2012; Turscán et al., 2012). Even though 'direct' matching was not present, some interesting associations were found. For example, owners scoring high in Openness, which refers to adventurous and creative individuals, had less Amicable (i.e., friendly, relaxed) and more Extraverted (i.e., energetic, active) dogs. It may be that owners have the ability to contribute to

their dogs' collection of personality traits, that they choose particular *types* of dogs, or that certain types of owners place their dogs in the appropriate contexts to view or elicit specific traits. As in the case of 'high Openness' owners, they are more likely to be adventurous, therefore, their dogs may be more active (Extraverted). Similarly, the association for highly Conscientious (ambitious) and low Neurotic (anxious) owners to have dogs with higher Training-focus (obedient) makes sense as ambitious, non-anxious individuals are more likely to value training their dogs.

Unlike past research, sex differences were not found in the *big five* personality factors for humans in this small sample. For example, women typically score higher than men on Agreeableness and Neuroticism (e.g., Cavallera et al., 2013). While this was not true here, it is not surprising to learn as this study used a fairly homogenous population (others have expanded to multicultural/multi-geographical cohorts) with a very small sample size for male participants ($N = 6$). Variation may have been further reduced by the fact that certain personality types may be more likely to participate in research and to own dogs (Covell, Frisman & Essock, 2003; Perrine & Osbourne, 1998; Westgarth et al., 2007).

Female dogs in this study scored higher on Extraversion than male dogs (MCPQ-R). There is no literature, however, that reports sex differences in the MCPQ-R directly. Studies have revealed that male and female dogs can show behavioural differences, such as male dogs possessing higher prey drives than female dogs, therefore, it is reasonable that personality differences could also be present (e.g., Wilsson & Sundgren, 1997). Furthermore, it is important to consider that Extraversion in the context of dog personality speaks more about activity level, rather than how 'outgoing' or 'sociable'. Therefore, this difference may be attributed to owners feeling more comfortable taking female dogs for activities as male dogs often have a reputation

for being rambunctious (e.g., pulling on the leash) and aggressive (Borchelt, 1983; Roth & Jensen, 2015).

The only sex difference present for owners was that women scored higher than men in the DAQ, which mirrors past findings (Archer & Ireland, 2011). Additionally, it is not surprising as women tend to be more empathetic than men and they tend to express more caring attitudes (e.g., Hojat et al., 2014; Prato-Previde, Fallani & Valsecchi, 2006; Schöberl et al., 2012). DAQ scores also seemed to be influenced by dog and human personality, since high DAQ scores were related to high Extraversion scores for humans and dogs. Even though duration of cohabitation did not influence attachment in any respect, it is possible that because Extraversion refers to activity level for dogs, outgoing owners are engaging in more shared activities. This increase in activities may, in turn, alter or enhance the perceived strength/satisfaction of the owner-dog bond, as reflected in DAQ scores (Curb et al., 2013).

Attachment-related behaviours were also linked to personality as more extraverted owners initiated more contact during specific episodes and more amicable (friendly), less neurotic (nervous) dogs initiated more contact with strangers. It may be that outgoing owners may feel comfortable expressing affection towards their dogs, especially when being filmed than individuals scoring lower on Extraversion. Furthermore, it makes intuitive sense that less inhibited dogs would be more likely to approach a complete stranger. Moreover, dogs with high Amicability scores spent less time with their owners during Episode 1, suggesting uninhibited exploration, a hallmark of ‘secure’ attachment. Taken together it appears as though dogs may in fact be behaving similarly to securely attached children during this protocol. Alternate evaluations of dog attachment would be beneficial for future research such as examining the physiological responses or genetic predisposition for bonding hormones (i.e., oxytocin; Johnson

& Young, 2015; Kis, Hernádi, Kanizsár, Gácsi & Topál, 2015). It is particularly important to incorporate other evaluations of attachment because it is difficult to differentiate between seeking physical proximity due to attachment and seeking proximity due to the positive reinforcement given (i.e., petting, food reward; Payne et al., 2015). It would also be beneficial to find a way to accurately categorize the behaviour patterns shown by dogs during tests such as the Strange Situation. Currently, attempts have only been made to create associations between attachment and behaviours that may convey how the dog perceives the relationship (e.g., proximity seeking), and not to describe the type of relationship (e.g., 'secure' and 'insecure'). This study does provide some evidence that dog attachments may be able to be placed in similar categories as human secure and insecure attachments. To move in this direction, future research would require a more fine examination of individual behaviours within each given episode, unobstructed by saliva sampling (see Chapter 2). Particularly, specific behaviours would need to be examined, such as a measurement for the 'type' (i.e., level of enthusiasm/indifference) of greeting during a reuniting episode or how averse they are to engaging in interactions with the stranger in the absence of the owner.

It is important to consider, however, that the current methods for evaluating dog personality are mostly limited to adjective-based approaches. Many participants, while completing the MCPQ-R, for example, commented that their dog is *sometimes* 'energetic' or 'obedient' in particular environments, but not in others and perhaps it is not sufficient to measure a dog's personality based on their average behaviour. This current investigation could have greatly benefitted from behavioural assessments of dog personality from independent observers as well as a written questionnaire that provides 'context' for each given adjective (e.g., Dog Personality Questionnaire, Jones, 2008). Nonetheless, recent literature has suggested that

behavioural observations of personality do coincide nicely with that achieved by written inventories (e.g., Kubinyi, Gosling & Miklósi, 2015). It would have also been interesting to examine whether these personality dimensions differed as a result of breed differences (Duffy, Hsu & Serpell, 2008; Hart, 1995; Lofgren et al., 2014). Unfortunately, this current study did not include enough dogs in any particular breed category to make this comparison feasible.

This investigation suggests that there is a unique relationship between attachment and personality for human-dog dyads. Research like this provides broader applications to understanding personality, its origins and evolutionary underpinning. Knowing more about the personality of non-human animals, in particular, creates an interdisciplinary approach that integrates proximate mechanisms, evolution and ecology (Carere & Locurto, 2011). Understanding personality in domesticated animals may help to combat behavioural problems, reduce relinquishment statistics and decrease separation anxiety. Research regarding attachment and personality may help to uncover ways to circumvent these problems through early interventions and better matching of owner-dog pairs.

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Table 3.1: Adjectives to describe the *big five* personality traits used in the NEO-FFI-3. This table is from Cloninger (2008), Table 8.3, pg 237.

The Big Five Factors of Personality

Factor	Description of a High Scorer	Description of a Low Scorer
Openness	Creative	Uncreative
	Imaginative	Down-to-earth
	Prefers variety	Prefers routine
Conscientiousness	Conscientious	Negligent
	Hardworking	Lazy
	Ambitious	Aimless
	Responsible	Irresponsible
Extraversion	Talkative	Quiet
	Passionate	Unfeeling
	Active	Passive
	Dominant	
	Sociable	
Agreeableness	Good-natured	Irritable
	Soft-hearted	Ruthless
	Trusting	Suspicious
Neuroticism	Worrying	Calm
	Emotional	Unemotional
	Vulnerable	Hardy
	Anxious	Self-controlled
		Sense of well-being

Table 3.2: Adjectives used in the MCPQ-R to describe dog personality dimensions (Ley et al., 2009).

Dimension	Adjectives
Training-focus	Attentive
	Biddable
	Intelligent
	Obedient
	Reliable
	Trainable
Motivation	Assertive
	Determined
	Independent
	Persevering
	Tenacious
Extraversion	Active
	Energetic
	Excitable
	Hyperactive
	Lively
	Restless
Amicability	Easy-going
	Friendly
	Non-aggressive
	Relaxed
	Sociable
Neuroticism	Fearful
	Nervous
	Submissive
	Timid

Table 3.3: Average (± standard error of the mean) human (female: $N = 21$, male: $N = 4$, combined: $N = 25$) scores for the NEO-FFI-3 factors: Openness, Conscientiousness, Extraversion, Agreeableness and Neuroticism.

	Openness	Conscientiousness	Extraversion	Agreeableness	Neuroticism
Female	32.3 ± 1.24	33.2 ± 1.60	29.5 ± 1.49	35.1 ± 1.43	20.4 ± 2.03
Male	31.5 ± 0.87	31.0 ± 2.19	27.0 ± 2.34	34.0 ± 3.42	24.0 ± 4.06
Combined	32.2 ± 1.05	32.8 ± 1.39	29.1 ± 1.30	34.9 ± 1.29	21.0 ± 1.81

Table 3.4: Average (± standard error of the mean) dog scores (female: *N* = 16, male: *N* = 13, combined: *N* = 29) for MCPQ-R dimensions: Motivation, Training-focus, Extraversion. Amicability and Neuroticism.

	Motivation	Training-focus	Extraversion	Amicability	Neuroticism
Female	70.5 ± 0.032	73.8 ± 0.036	77.1 ± 0.033	79.8 ± 0.034	51.0 ± 0.044
Male	61.8 ± 0.041	70.1 ± 0.028	62.2 ± 0.053	81.5 ± 0.041	52.9 ± 0.045
Combined	66.6 ± 0.026	72.1 ± 0.023	70.4 ± 0.033	80.6 ± 0.026	51.9 ± 0.031

Table 3.5: Correlations between dog (MCPQ-R) and human (NEO-FFI-3) personality traits. Namely, Amicability, Extraversion, Motivation, Neuroticism and Training-focus for dogs (MCPQ-R) and Agreeableness, Conscientiousness, Extraversion, Neuroticism and Openness for humans (NEO-FFI-3).

	Amicability	Extraversion	Motivation	Neuroticism	Training-focus
Agreeableness	0.033	-0.010	0.090	0.137	0.139
Conscientiousness	-0.079	0.141	-0.002	0.139	**0.399***
Extraversion	0.245	-0.175	-0.068	-0.142	0.393
Neuroticism	-0.114	0.013	0.001	-0.082	**-0.528***
Openness	**-0.508***	**0.421***	0.301	-0.189	**-0.509***

*Significant at $p < 0.05$; N=25.

Table 3.6: Correlations between dog (MCPQ-R) and human (NEO-FFI-3) personality scores and Dog Attachment Questionnaire scores (DAQ).

		DAQ scores
MCPQ-R	Amicability (dog)	-0.163
	Extraversion (dog)	*0.366*~
	Motivation (dog)	0.264
	Neuroticism (dog)	-0.098
	Training-focus (dog)	0.150
NEO-FFI-3	Agreeableness (human)	0.258
	Conscientiousness (human)	0.192
	Extraversion (human)	**0.443***
	Neuroticism (human)	-0.084
	Openness (human)	0.012

* Significant at $p < 0.05$; $N = 29$ for dog correlations and $N = 25$ for human correlations.

~ represents a marginally significant result ($p = 0.051$).

Table 3.7: Correlations between physical proximity durations (expressed as proportions of available interaction time between the dog and focal individuals) in Episodes 1-7, and owner personality factors: Openness, Conscientiousness, Extraversion, Agreeableness and Neuroticism (NEO-FFI-3).

		Openness	Conscientiousness	Extraversion	Agreeableness	Neuroticism
Episode 1	Owner	**0.430***	-0.315	**-0.522 ***	-0.278	0.388
	Door	-0.230	-0.080	0.150	0.100	-0.132
Episode 2	Owner	-0.135	-0.306	-0.291	-0.148	0.127
	Stranger	-0.032	-0.054	0.379	0.093	0.095
	Door	0.298	-0.242	-0.157	-0.027	0.224
Episode 3	Stranger	0.122	0.288	0.255	0.177	-0.088
	Door	-0.087	**-0.533***	-0.172	**-0.477***	0.166
Episode 4	Owner	0.247	-0.060	-0.140	-0.085	-0.051
	Door	-0.228	-0.262	0.251	-0.097	0.075
Episode 5	Door	-0.197	0.004	0.221	-0.098	-0.288
Episode 6	Stranger	**-0.479***	-0.044	0.098	0.025	-0.318
	Door	0.129	0.118	-0.014	-0.171	-0.182
Episode 7	Owner	-0.193	-0.016	-0.141	-0.054	0.041
	Door	0.205	0.093	0.322	0.052	-0.146

1998 * Significant at $p < 0.05$; $N = 22$.

Table 3.8: Correlations between physical proximity durations (expressed as proportions of available interaction time between the dog and focal individuals) in Episodes 1-7, and dog personality factors: Openness, Conscientiousness, Extraversion, Agreeableness and Neuroticism (MCPQ-R).

		Motivation	Training-focus	Extraversion	Amicability	Neuroticism
Episode 1	Owner	0.306	-0.113	0.298	**-0.479***	-0.025
	Door	-0.325	-0.233	-0.243	**0.587***	-0.258
Episode 2	Owner	0.127	0.000	-0.047	-0.039	0.252
	Stranger	-0.278	-0.165	**-0.414***	0.214	0.072
	Door	0.095	-0.047	0.155	-0.159	0.081
Episode 3	Stranger	-0.189	0.155	-0.132	-0.143	-0.091
	Door	-0.112	0.107	-0.056	0.372	**-0.497***
Episode 4	Owner	0.104	0.129	-0.121	0.043	-0.111
	Door	-0.002	-0.045	0.137	0.273	-0.255
Episode 5	Door	0.028	-0.150	0.065	0.162	-0.001
Episode 6	Stranger	-0.088	0.355	-0.233	0.267	-0.071
	Door	0.129	-0.021	0.215	-0.262	-0.190
Episode 7	Owner	-0.165	0.230	-0.243	0.212	-0.100
	Door	0.003	-0.063	-0.111	0.232	-0.275

1999 * Significant at $p < 0.05$; $N = 26$.

CHAPTER 4: GENERAL DISCUSSION AND CONCLUDING REMARKS

4.1 INTRODUCTION

Within the past decade, advancements have been made in understanding dog behaviour and physiological responses to behavioural challenges, though certain areas in this field remain unclear and/or not well researched (Miklósi, 2014). Our desire to learn more about dogs likely stems from the thousands of years of evolutionary history we share with them (Germonpré et al., 2009). Due to the pervasive and close bond between humans and dogs, it is not surprising that the term *attachm*ent has been used to describe this relationship. This thesis aimed to address personality and other factors contributing to interspecific attachment in owners-dog dyads, and whether dogs would demonstrate secure-base effects (e.g., Waters & Cummings, 2000) in the context of a dog-amended Ainsworth's Strange Situation test (such as that seen in: e.g., Gácsi, Topál, Miklósi, Dóka & Csányi, 2001; Mariti et al., 2013; Palmer & Custance, 2008; Rehn, Lindholm, Keeling & Forkman, 2014;Topál, Miklósi, Csányi, & Dóka, 1998; Topál et al., 2009). The presence of attachment-related behaviours (proximity and contact) were examined in relation to the physiological (cortisol and chromogranin A; de Veld, Riksen-Walraven & de Weerth, 2014; Harrison, Ratcliffe, Mitchell & Smith, 2014; Kanno et al., 1999; Kudielka, Hellhammer & Wüst, 2009; Stefanescu, Schipor, Paun, Dumitrache, & Badiu, 2011; van Kammen et al., 1992) and behavioural (e.g., door scratching) manifestations of separation-induced stress. Here, I will highlight the main results in the preceding chapters and make suggestions regarding the significance of these findings.

4.2 DOGS IN THE CONTEXT OF THE STRANGE SITUATION TEST

4.2.1 Attachment in Owner-Dog Dyads

The current results were consistent with those in past dog-amended Strange Situation tests in that dogs demonstrated a distinct preference (i.e., greater durations and frequencies of physical proximity and contact for their owners compared to strangers) and they performed some separation-induced stress behaviours (e.g., door scratching) during the procedure. Dogs spent more time near the door and scratched the door more frequently either when they were with the stranger exclusively or when they were alone. Therefore, it is likely that dogs are utilizing owners as a 'secure base' for exploring new environments, despite the presence of a potential substitute (stranger; e.g., Topál et al., 1998).

Additionally, owners reported attachment relationships with their dogs; all owners scored within the 'attached' range (greater or equal to 3) on the Dog Attachment Questionnaire (DAQ; Archer & Ireland, 2011), with women scoring higher than men. This scale would benefit from further research, as the current questionnaire does not leave any room for the complete absence of the 'attachment scenarios' provided without impacting the achieved score. For example, when asked whether their dog is 'encouraged' to sleep in the owner's bed, some participants reported that the dog had a bed in their room and they were uncertain as to how to answer the question. The option to omit the behaviour may have impacted scores substantially, which may have contributed to the lack of significant correlations between the DAQ and attachment-related behaviours. It would also be interesting to see whether people with low (less than 3) scores on this questionnaire perform fewer attachment-related behaviours. It would also be beneficial to analyze whether there are any differences between non-attached owners (scores less than 3) and attached owners (scores greater than or equal to 3) in terms of alternate dog relationship inventories or behavioural evaluations of attachment.

4.2.2 Stress

Overall, neither humans nor dogs experienced increases in cortisol (CORT) or chromogranin A (CgA) levels during the Strange Situation procedure, but human CORT and dog CgA levels decreased over time. It is probable that owners and dogs became more comfortable during the procedure as the setting became less 'novel'. Dogs, in particular, may be performing certain behaviours to serve as coping mechanisms like the body shaking behaviour observed in this study. Other authors have suggested that dogs body shake to relieve stress (Beerda, Schilder, van Hoff, de Vries & Mol, 1998; Beerda, Schilder, van Hoff, de Vries & Mol, 1999; Beerda, Schilder, van Hoff, de Vries & Mol, 2000; Glenk et al., 2013; Kogan, Schoenfeld-Tacher & Simon, 2012; De Palma et al., 2005; Rehn & Keeling, 2011). Furthermore, it is important to note that CORT levels for dogs in this study were comparable to those found in arousing contexts (e.g., Dreschel & Granger, 2009; Ottenheimer Carrier, Cyr, Anderson & Walsh, 2013). Therefore, even though a decrease in 'stress' occurred, dogs likely did experience a stress response to the protocol.

Dog CORT was also linked to door scratching, which occurred almost exclusively when the dog was in the presence of the stranger or when the dog was alone, as dogs with higher CORT (baseline and final) and those with an increase over the testing period scratched the door more frequently. It was also interesting to see that most significant behavioural correlations occurred during episodes when the dog was with the stranger (Episodes 2, 3 and 6) or when the dog was first introduced to the room (Episode 1). Moreover, dogs with lower baseline CgA were less inhibited in interacting with strangers than dogs with higher CgA levels (specifically in Episode 3, which is the first episode when the dog was with the stranger exclusively). Stress relationships were also present in what appeared to be a synchronization effect as final CORT was highest for dogs that had owners with highest baseline and final CORT. Therefore, owners

and dogs may be in tune with each other or dogs may be seeking information from owners, thus detecting and matching their stress levels (Buttner, Thompson, Strasser & Santo, 2015).

Preliminary exploratory results did not indicate any significant relationships between personality and the physiological measures examined, therefore, connections between these measures were not discussed in this thesis.

4.2.3 Human and Dog Personality

Substantial evidence suggests that dogs have been selected for personality characteristics and behaviours required for domestic life with humans (e.g., Hare, Call & Tomasello, 1998; Miklósi, 2014; Mongillo, Bono, Regolin, & Marinelli, 2010). This current investigation did not uncover any natural links between human and dog personality using the Neuroticism-Extraversion-Openness Five Factor Inventory (NEO-FFI-3) for humans and the Monash Canine Personality Questionnaire Revised (MCPQ-R). Past studies using the Big Five Inventory (BFI) and a dog amended version of this questionnaire found (similar to NEO-FFI-3 and MCPQ-R), positive correlations between the major personality factors, namely in: Conscientiousness, Extraversion, Agreeableness and Neuroticism (Kis, Turcsán, & Gácsi, 2012; Turcsán, Range, Virányi, Miklósi, & Kubinyi, 2012). Despite not finding similar results using different instruments, some interesting connections were found, for example, owners that scored higher on Conscientiousness (responsible), lower on Neuroticism (relaxed) and lower on Openness (regimented) had dogs with high Training-focus (intelligent, trainable). Such associations are reasonable and may suggest that responsible, non-anxious dog owners are probably more likely to engage in and have success with training regimes for their dogs and may choose dogs with

high trainability, although other underlying factors which may influence these relationships cannot be ruled out.

Dog personality was found to predict dog behaviour during the Strange Situation test as dogs scoring higher on Neuroticism (anxiety) initiated less contact with strangers. Dogs also seemed to be impacted by owner personality as owners scoring lower on Agreeableness (unfriendly) and Conscientiousness (irresponsible) had dogs that spent more time by the door in Episode 3 (first time alone with stranger). Therefore, owners may influence their dogs' behaviour, which produces a consistent pattern of behaviours that can be detected by canine personality inventories such as the MCPQ-R.

4.3 IMPLICATIONS

Researching dogs affords many immediate benefits to current society. We can use our knowledge of dog behaviour to implement efficient training regimes such as achieving optimal performance of working dogs (e.g., search and rescue dogs) or in finding ways to best 'match' owner-dog pairs to lower relinquishment rates to shelters. The relationship between owners and their dogs demonstrates the main elements of attachment, namely in dogs seeking and maintaining contact with owners and in reacting to separation from the owner, as seen through many studies (e.g., Gácsi et al., 2001; Fallani, Prato-Previde, & Valsecchi, 2007; Palmer & Custance, 2008; Prato-Previde, Custance, Spiezio & Sabatini, 2003; Rehn et al., 2014; Topál et al., 1998).

This study only scratches the surface of the ingredients needed to form and maintain interspecific affiliations. For example, is it important that dogs and owners match on major personality factors? Even though the results of this current study lend no support for previously

reported personality similarities between dogs and their owners, it does seem in part seem that dispositional characteristics are at least *complimentary* or somewhat *intuitive*. I am not able to ascertain from this study how these personality associations were produced as owners may have 'parental' influence on their dogs, they may simply prefer to select dogs based on predetermined criteria, or some combination of the two. Regardless of the origin, it appears as though these personality combinations are satisfactory for each dyad as every owner expressed attachment (i.e., high DAQ scores). It would be beneficial, however, to adopt a better, more exhaustive questionnaire to record owner-reported attachment or have a better list of dog-directed attachment performed by owners. The measurement of dog personality, specifically is something that would benefit from more uniformity and consensus in terms and this study would have greatly benefitted by the introduction of an independent observer to assess dog personality dimensions (Gosling, 2001). Recent literature, however, has suggested that behavioural observations of personality do coincide with assessments made by written inventories (e.g., Kubinyi, Gosling & Miklósi, 2015).

Another area for improvement would be in finding the best possible method to achieve an accurate baseline measurement for salivary analytes and to choose the most appropriate sampling intervals. The short length of the episodes in the Strange Situation limited the time allocated to saliva sampling. While only two measurements were actually quantified (baseline and final), two additional samples were collected mid-procedure, which limited the natural interaction between the stranger and the dog. Therefore, eliminating the *within-procedure* samples and simply measuring a baseline (pre-procedure) and final (post-procedure) saliva sample would have been favourable. Alternatively, the Strange Situation procedure for dogs could be better adjusted to accommodate for these samples by increasing the length of each episode and setting aside

specific times where neither the owner nor the stranger could interact with the dog save for taking the sample. Further, it is still not well established whether behaviours and hormones measured in saliva synchronize or at least the mechanism of how this might occur.

Taken together, it is undeniable that humans and dogs share a unique relationship and these results suggest that this interspecific relationship does classify as an attachment bond. Moving in this direction may even allow for dogs to be classified in terms of human-analogous attachment systems, i.e., *secure* and *insecure* attachment, which may be useful in correcting behavioural problems caused by separation-anxiety. Making these specific extensions, however, would require a more detailed history from owner-dog pairs as well as a closer examination of certain behaviours, such as the nature of the greeting events upon the owners return or the degree of avoidance in interacting with a stranger. That being said, it does appear that the dogs in this current investigation displayed a stereotyped *secure* attachment style as seen by the large proportion of time dogs spent near the door in the absences of their owner (decreased exploration) and their unwillingness to interact with the stranger.

This study was the first to combine the dog-amended Strange Situation test with behavioural measures of attachment, an attachment inventory (DAQ), physiological measures of stress (CORT and CgA) and personality questionnaires (NEO-FFI-3: humans, MCPQ-R: dogs). In combination, this experimental design allowed for an evaluation of separation-induced stress from a physiological and behavioural perspective during the protocol. Additionally, it demonstrated that owners may be influencing their relationship with their dogs through their own unique personalities, and that dog personality contributes to how attachment is presented during the Strange Situation.

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Appendix A

Hello (*Participant's Name*),

My name is Morag Ryan and I am a member of the Canine Research Unity (CRU) at Memorial University of Newfoundland. I am E-mailing in response to your expressed interest in my project. First of all, I would like to thank you for your interest in our research! As a Masters student, I am studying the human-dog bond and the hormones that may be involved in this relationship. If you decide to participate in my study, you would be required to:

1) Come to Memorial University to perform our behavioural protocol with your dog (i.e., the 'strange situation')
2) Take your own saliva samples (with our instruction) and allow our researchers to take saliva samples from your dog
3) Fill out a questionnaire regarding your relationship with your dog, your dog's personality, and certain health questions to aid us in understanding the hormonal results we obtain

Total participation should take no more than 40min. If you think that you might be interested in participating in my study please respond to this E-mail or call me at (709) 764-7681 and I will send you a copy of the consent form, which will provide you with a more detailed description of my study. If you have any questions or concerns please do not hesitate to contact me.

For more information about the research being carried out at the Canine Research Unit in the Department of Psychology at Memorial University can be found here: http://dogsbody.psych.mun.ca

Thank you again!

Sincerely,

Morag Ryan, M.Sc. Candidate
Canine Research Unit
Cognitive and Behavioural Ecology
Memorial University of Newfoundland

Questionnaire

Interspecific attachment: Social bonds between humans and their 'best friends'

Dyad #: *(filled in by researcher)* Date of completion: (*filled in by the participant*)

Dog name: *(filled in by participant)*

NOTE: This questionnaire should be completed by the **primary caregiver** of the dog. We define "primary caregiver" as **the person who typically feeds and walks the dog**. If you have any questions or concerns about the content of this questionnaire, please do not hesitate to ask the supervising researcher. If at any time you feel uncomfortable responding to a question, please skip that question and move on to the next.

On behalf of the Canine Research Unit, thank you for participating!

Principal investigator: Morag Ryan, M.Sc. Candidate, Cognitive and Behavioural Ecology Program

Supervisors: Dr. Carolyn Walsh and Dr. Anne Storey, Department of Psychology

Human participant information

Please note that all personal questions regarding general health and life choices are important to this study. These questions will enable the researcher to better understand the hormonal levels we obtain as certain substances or health conditions may impact the hormones we measure.

Pet ownership and care giving experience

1. How many pets do you own?
   _____
   a. How many of your pets are dogs?
      _____
   b. Of the following, what kinds of pets do you own (please circle all that apply)?
      i. Cats
      ii. Small rodents (hamsters, rats, mice, etc.)
      iii. Reptiles (lizards, snakes, turtles etc.)
      iv. Fish
      v. Other (please specify):_____
2. Did you have a pet during your childhood? YES or NO
   a. How many of your pets during your childhood were dogs?
      _____
   b. Of the following, what kinds of other pets did you own (please circle all that apply)?
      i. Cats
      ii. Small rodents (hamsters, rats, mice, etc.)
      iii. Reptiles (lizards, snakes, turtles etc.)
      iv. Fish
      v. Other (please specify):_____
3. Do you have any children? YES or NO
   If yes:
   a. How many children do you have?
      _____
   b. How many of them are living with you?
      _____
   c. How old are your children?
      _____

4. As an adult, did you have a pet (of which you were the primary care giver) or children first? Please describe.

_____

_____

_____

Health and life choices

5. Your date of birth (day/month/year):

_____

6. Your approximate height (feet) and weight(pounds)

_____

7. Approximately how many hours per week (on average) do you engage in physical activity (i.e., gym, hiking, swimming, organized sports, etc.)?

_____

8. Do you currently smoke? If so, when was the last time you had a cigarette?

_____

9. Approximately how many hours of sleep did you have the night before participating in this study? _____
10. Have you consumed an alcoholic beverage in the last 12 hours? YES or NO
11. Have you consumed a caffeinated beverage in the last 2 hours (e.g., coffee, tea, soda pop, etc.)? YES or NO
12. Have you consumed any dairy products (e.g., milk, yogurt, cheese, etc.) in the last 20min? YES or NO
13. Have you eaten a major meal within the last 60 min? If so, what was it?

_____

14. Are you prone to or currently have an oral diseases (i.e., gingivitis) or lacerations?

_____

15. Are you currently taking or have you taken any hormonal supplements or medications that contain steroids? **NOTE:** Prescription medications containing some steroids (cortisol, hydrocortisone, prednisone, and prednisolene) interfere with the way we measure hormones in your saliva sample. These include inhalers containing steroids as well as some prescription skin ointments, and eye/ear/nasal suspensions. This does **NOT** refer to the use of over-the-counter antibiotic ointments such as Neosporin, Polysporin, Polydem, etc. (If you are uncertain about a drug you are taking, please consult one of the investigators). Please check off one of the following responses below:

    No I have not taken medication containing hormones and/or steroids _____
    Yes I have taken medication containing hormones and/or steroids_____
    If yes, how recently did you take this medication (e.g., today, yesterday, past few days)? Please describe.

_____

_____

16. Please indicate, if you feel comfortable, whether you have an endocrine disorder, and the name of your condition. This may include: hypothyroidism, hyperthyroidism, Cushing's syndrome, diabetes 1 or 2, etc. This question is asked because certain endocrine issues can affect the hormonal analyses performed.

_____

_____

17. Sex: Male or Female (please circle)

    For females, the following factors are known to influence the hormones that we are measuring.

    If you are FEMALE circle all that apply:

    a. I am pregnant
    b. I have been pregnant within the last year
    c. I take birth control (e.g., the pill, Norplant, Depo-Provera)
    d. I am going through menopause
    e. I am currently menstruating

Dog participant information

1. Your dog's date of birth (day/month/year):_____

NOTE: If the birth date of your dog is unknown, write the approximate age of your dog (e.g., years, months)

2. Sex: Male or Female (Please circle one)
3. Breed (if unknown, please write unknown or mixed-breed)_____
4. Approximate height and weight:

   _____
5. Has your dog been neutered/spayed? YES or NO
6. How is your dog typically fed (please circle):

   a) Free fed (dish with food is left so that your dog can eat at any time)
   b) Fed on a routine schedule (once or twice a day)
   c) Fed using a combination of a routine and free fed, please describe:

   _____

   _____

7. Where did you get your dog? (Shelter, rescue group, from another owner, from a breeder, etc.)

   _____
8. Approximately how long has your dog lived with you?

   _____

9. How old was your dog when you got her/him?

_____

10. What are your reasons for having a dog (select all that apply)?
    a. Companionship
    b. Working (e.g., hunting dog)
    c. Service dog (for any special needs, e.g., deafness, epilepsy, blindness, etc.)
    d. For recreation
    e. Guarding property
    f. Breeding
    g. Other (please specify)

       _____

11. Approximately how many waking hours do you spend with your dog per day?

_____

12. Many individuals do not walk their dog, as they prefer to engage in other physical activity with them (e.g., fetch, off leash runs, etc.). If you do walk your dog, approximately how often do engage in this activity per week (e.g., average number of hours)?

_____

_____

13. Has your dog had any health issues or currently have a health condition? If so, please describe.

_____

_____

_____

14. Is your dog currently taking ANY medication (particularly any medication containing steroids or hormonal supplements)? If so, please list the name of the medication(s).

_____

_____

_____

15. Has your dog completed any kind of training (obedience or other)? If so, please describe.

_____

_____

16. Do you participate in any regular activities with your dog (e.g., walks, dog sports such as agility, showing, etc.)?

_____

_____

17. Where does your dog typically sleep?_____
18. Does your dog show any of the following behaviours? Circle all that apply:
    a. Chewing on furniture, shoes, or other personal belongings
    b. Whining
    c. Barking excessively
    d. Pacing
    e. Excessive licking
    f. Waiting for you by the door
19. Do you think your dog has separation anxiety? Yes or No (please circle one)
20. Has anyone ever suspected that your dog has separation anxiety? If so, please describe.

    _____

    _____

Monash Canine Personality Questionnaire— Revised (MCPQ-R)

Please rate your dog's personality using the MCPQ-R by recording how well each word describes your dog's personality by marking the appropriate box.

1 = really does not describe my dog, 6 = really describes my dog

	Really does **NOT** describe my dog					Really describes my dog
Friendly	1	2	3	4	5	6
Persevering	1	2	3	4	5	6
Nervous	1	2	3	4	5	6
Energetic	1	2	3	4	5	6
Attentive	1	2	3	4	5	6
Easy going	1	2	3	4	5	6
Independent	1	2	3	4	5	6
Trainable	1	2	3	4	5	6
Non-aggressive	1	2	3	4	5	6
Hyperactive	1	2	3	4	5	6
Submissive	1	2	3	4	5	6
Determined	1	2	3	4	5	6
Relaxed	1	2	3	4	5	6
Tenacious	1	2	3	4	5	6
Timid	1	2	3	4	5	6
Biddable*	1	2	3	4	5	6
Active	1	2	3	4	5	6
Intelligent	1	2	3	4	5	6
Sociable	1	2	3	4	5	6
Restless	1	2	3	4	5	6
Fearful	1	2	3	4	5	6
Obedient	1	2	3	4	5	6
Lively	1	2	3	4	5	6
Reliable	1	2	3	4	5	6
Assertive	1	2	3	4	5	6
Excitable	1	2	3	4	5	6

*biddable: your dog's willingness to follow directions/obey commands

Dog Attachment Questionnaire (DAQ)

Please complete the following questions regarding your relationship with your dog using the DAQ. Please rate how well each word describes your dog's personality by marking the appropriate box.

1 = I strongly disagree, 5 = I strongly agree

	I strongly DISAGREE				I strongly AGREE
1. Life without my dog would be unbearable as though a vital part were missing.	1	2	3	4	5
2. My dog is treated like a family member.	1	2	3	4	5
3. The loss of my dog would mean as much to me as the loss of a family member or friend.	1	2	3	4	5
4. There was an increase in happiness after getting my dog.	1	2	3	4	5
5. Having to deal with the death of my dog would be very hard.	1	2	3	4	5
6. My dog is an important part of my life.	1	2	3	4	5
7. When I think of losing my dog I become very upset.	1	2	3	4	5
8. It's hard to express to others what the loss of my dog would mean to me.	1	2	3	4	5
9. What I like about my dog is its acceptance, love and loyalty.	1	2	3	4	5
10. When upset or anxious I turn to my dog for comfort.	1	2	3	4	5
11. I spend a lot of time talking to my dog.	1	2	3	4	5
12. I/we do not celebrate my dog's birthday.	1	2	3	4	5

13. I feel a strong companionship with my dog.	1	2	3	4	5
14. If my dog became lost I would not give up until I found him or her.	1	2	3	4	5
15. A reward would be offered for their return.	1	2	3	4	5
16. Having a dog is a source of contact and comfort.	1	2	3	4	5
17. I feel very close to my dog.	1	2	3	4	5
18. Extra care is taken to ensure my dog is well taken care of while on holiday.	1	2	3	4	5
19. I enjoy feeling my dog sitting close to me.	1	2	3	4	5
20. Extra care is taken to ensure my dog does not escape or get lost.	1	2	3	4	5
21. I often find myself talking about my dog when in company.	1	2	3	4	5
22. Having a dog increased my self-esteem and self-worth.	1	2	3	4	5
23. When I'm alone, I often think about my dog.	1	2	3	4	5
24. I feel more relaxed in company when my dog is present.	1	2	3	4	5
25. He/she is encouraged to sleep on my bed at night.	1	2	3	4	5
26. I hate going home when my dog is not there to greet me.	1	2	3	4	5

27. I never go away on holiday where my dog cannot accompany me.	1	2	3	4	5
28. When talking to my dog I often use endearing terms or baby talk.	1	2	3	4	5
29. Having a dog means that you cannot do what you want to.	1	2	3	4	5
30. If I am on holiday without my dog I hardly even think about him or her.	1	2	3	4	5
31. People are more important to me than my dog is.	1	2	3	4	5
32. When people let me down I don't find that I rely more upon my dog for companionship and solace.	1	2	3	4	5
33. I find it easier to talk to my dog than to people.	1	2	3	4	5
34. I receive more companionship from friends or family than from my dog.	1	2	3	4	5
35. I spend a lot of the time stroking and petting my dog.	1	2	3	4	5

This is the last page of the questionnaire.

Thank you for your time and effort! ☺

Sincerely,

Morag Ryan, M.Sc. candidate, Memorial University of Newfoundland