Intra- and inter-specific cortisol coregulation

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Summary

Coregulation is the bidirectional modulation of social partners' physiology, including hypothalamic-pituitary-adrenal (HPA) axis activity, and it is key for forming and maintaining attachment bonds between individuals. By investigating coregulation of acute and chronic cortisol in dog-human, dog-dog, and human-human dyads, this thesis aims to further our understanding of social and temporal drivers of coregulation and their potential links to attachment. Chapter 1 outlines how coregulation regulates attachment bonds and reviews existing cortisol coregulation literature in parent-infant and spousal dyads, non-human dyads, and human-non-human dyads. In Chapter 2, I describe the methodology used across Chapters 3-6. Chapter 3 explores whether factors commonly associated with stronger coregulation in parent-infant dyads also predict dog-owner coregulation strength. Chapter 4 tests for coregulation in cohabiting dogs, and demonstrates that the absence of the owner (i.e. an external social influence) may strengthen dog-dog cortisol coregulation. Chapter 5 explores how chronic cortisol concentrations (derived from hair) may enhance the study of coregulation, and presents preliminary evidence suggesting that the predictors of coregulation strength depend on the temporal nature of the cortisol measure utilised (i.e. acute or chronic cortisol concentrations). Chapter 6 highlights how greater dyad similarity in Big-5 personality traits may strengthen romantic couples' coregulation – an area that has received minimal attention in the literature. Lastly, in the general discussion (Chapter 7), I place the key findings in a wider context and outline potential implications for future studies. Overall, results from Chapters 3-6 further our knowledge of cortisol coregulation and indicate that the physiological mechanism has shared characteristics across types of social relationships and species. Additionally, I show how external social influences and inter-individual differences in personality have the potential to be key predictors of coregulation strength, which could have considerable implications for the methodologies of future coregulation studies (provided these findings persist in a larger sample).

Contents

Declarations

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed ..[redacted]....... (candidate)

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This thesis is the result of my own investigations, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked in a footnote(s).

Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

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The University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.

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Date: 29/01/2023

Acknowledgments

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Statement of Contributions

Table 1 Summary of the contributions made by myself (CS), Dr Ines Fürtbauer (IF), and Dr Andrew King (AJK) towards the completion of this thesis.

Contributor Role	**Persons Involved**
Conceptualisation	CS, IF
Ethical approval	IF, CS
Participant recruitment and data collection	CS
Biological sample analysis	CS
Supervision	IF, AJK
Data analysis and visualisation	CS
Writing – original draft preparation	CS
Writing – review and editing	CS, IF, AJK

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Chapter 1 - General Introduction

Sociality has evolved and persisted across many branches of the animal kingdom – from colonies of bees and ants, shoals of fish and marine animals, to flocks of birds and herds of mammals (Krause and Ruxton, 2002; Silk, 2007; Ward and Webster, 2016). For some species, social aggregation – where individuals group in the same place at the same time – provides benefits to individuals such as protection from predators (reviewed by Lehtonen and Jaatinen, 2016), and more effective use of food resources (e.g. King et al., 2011; Snijders et al., 2021). However, in other species, individuals interact with each other repeatedly, developing and maintaining meaningful interactions across their lifetime or the duration of time that both individuals remain in the same social group (Krause et al., 2009). For these individuals, repeated interactions and shared experiences result in the formation of differentiated social relationships within groups – meaning some individuals interact with each other more than with others based on non-random characteristics (Busia and Griggio, 2020; Krause et al., 2009). For example, stronger relationships are observed between individuals who are more closely related, or share other similar heritable characteristics, such as body size (e.g. Croft et al., 2005) and appearance (e.g. Dugatkin and Godin, 1998; Killen et al., 2017; Romano and Stefanini, 2022). Individuals can also assort by similarity or differences in behavioural traits, such as dominance rank (Silk et al., 2010; Sueur et al., 2011) or personality (Croft et al., 2005; Pike et al., 2008).

Like non-human animals, humans rely on sociality for survival and reproductive success (Boyd, 2006). However, compared to other animals, the importance and range of social interaction exhibited by humans is huge, and is linked with the evolution of a large brain size and a complex psychology (Boyd and Richerson, 2006). Indeed, for humans, sociality has benefits ranging from social learning of complex socio-cognitive processes early in ontogeny to information transmission within and between cultural groups (Herrmann et al., 2007), and can improve individuals' long-term physical and mental health (reviewed by Zihlman and Bolter, 2017). Throughout the human lifespan individuals form close and enduring bonds with a variety of individuals, not least with social (mating) partners (Luo, 2017; McPherson et al., 2001) and these regular interactions between two individuals have been studied as attachment bonds (Bowlby, 1970; reviewed by Carter, 2005).

Attachment Theory

Early work in ethology formed the theoretical basis of **attachment theory** (see glossary in Table 1.1 for definitions of all terminology in bold script throughout Chapter 1); particularly **imprinting** studies in geese which demonstrated social bonds formed during immediate postnatal development without the need for affiliative or assortative interactions (Ainsworth and Bowlby, 1991; Lorenz, 1937). Attachment theory was proposed as a mechanism of facilitating the formation of strong social bonds between infants and parents (Ainsworth and Bowlby, 1991; Bowlby, 1970), with stronger attachment bonds linked to increased likelihood of offspring surviving to reproductive age (Ainsworth and Bowlby, 1991; Bowlby, 1970; reviewed by Fitton, 2012). The basis of attachment theory is that an infant is entirely dependent on their caregiver (also referred to as 'primary attachment figure') for survival, and a strong attachment bond will therefore be characterised by four main features: i) a sense of security (creating a 'safe haven'), ii) proximity seeking, iii) distress upon separation, and iv) creating a reliable, 'secure base' from which to explore (Bowlby, 1970; reviewed by Fitton, 2012). The theory was later extended to the relationship that forms between adults, particularly romantic partners (Pietromonaco and Beck, 2015; Zeifman, 2019). In this case, however, attachment is not formed on the basis of either individual being dependent on their partner for survival (Pietromonaco and Beck, 2015; Zeifman, 2019). Instead, attachment between adults functions to regulate negative affect and prevent distress in response to trauma (reviewed by Pietromonaco and Beck, 2015). Attachment theory has developed and advanced considerably in the six decades since its conception (reviewed by Fitton, 2012), with the theory now being applied to a broad range of research areas, including the development of mental health services for patients (reviewed by Bucci et al., 2015), establishing and maintaining functional employee-employer relationships (reviewed by Yip et al., 2018), and understanding how customers develop brand loyalty (e.g. Boateng et al., 2020).

Despite finding its origins in ethology, the study of attachment theory in non-human animals is a relatively recent advancement (e.g. Beck and Madresh, 2008; Prato-Previde et al., 2003; reviews by Prato-Previde and Valsecchi, 2014; Rockett and Carr, 2014). Studies across many animal species, for example mice and rats, birds, and non-human primates have demonstrated attachment-related behaviours towards a social partner, such as the secure-base effect and distress upon separation (Colonnello et al., 2011; Livia Terranova et al., 1999; Munteanu et al., 2017; Savidge & Bales, 2020). Attachment research in non-human animal species has, however, primarily focussed on the bonds formed between domesticated animals, such as dogs and cats, and their owners (Payne et al., 2015; Rockett and Carr, 2014; Vitale et al., 2019). The dog-owner bond, in particular, meets all four key attachment features set out by Bowlby and Ainsworth (see above; reviews by Fitton, 2012 & Payne et al., 2016). Dogs experience diminished behavioural and physiological responses to threatening situations when in the 'safe haven' of their owner (i; Gácsi et al., 2013), regularly seek close physical proximity with their owner (ii; Schöberl et al., 2012; reviewed by Payne et al., 2016), exhibit distress behaviours such as barking, whining, and shaking upon separation from the owner (iii; e.g. Rehn and Keeling, 2011; Topál et al., 1998), and demonstrate more inquisitive behaviours when the presence of their owner acts as a 'secure base' (iv; e.g. Horn et al., 2013). Notably, dog-owner attachment is bidirectional, with owners often placing considerable importance on the relationship with their dog (reviews by Amiot et al., 2016 & Rockett and Carr, 2014). Dog ownership has been associated with a more positive psychological outlook and being more inclined to socialise with others (reviewed by Amiot et al., 2016). Recent work further suggests that companion animal owners can form stronger attachment bonds with their pet compared to their romantic partner (Beck and Madresh, 2008; Rockett and Carr, 2014).

Table 1.1 Glossary of terms.

Term	Definition
Attachment theory	Psychological theory developed by John Bowlby and Mary Ainsworth which outlined the key characteristics of parent-infant relationships (Bowlby, 1970), and has since been applied to understanding adult social relationships and non-human animal interactions.
Coregulation	A physiological mechanism in which social dyads bidirectionally modulate each other's physiological stress levels (i.e. both individuals up/down-regulate each other's physiological state) and is believed to underpin *attachment* bonds (Sbarra and Hazan, 2008). The mechanism can also be referred to as synchrony, attunement, and linkage (reviewed by Timmons et al., 2015).

Cortisol	A steroid hormone, specifically a glucocorticoid, released from the adrenal cortex of the adrenal glands. It is key to the distribution of energy in the body and is also released as part of the *hypothalamic-pituitary-adrenal (HPA) axis* in response to a *stressor* (Nelson, 2011).
Hypothalamic-pituitary-adrenal (HPA) axis	A series of hormone secretions from the hypothalamus, pituitary gland, and adrenal glands that is triggered by exposure to a stressor, and is key to regulating the body's physiological response (Nelson, 2011).
Imprinting	The psychobiological process in which an animal forms an attachment bond to the first moving object they see upon hatching (Lorenz, 1937).
Social buffering	A mechanism in which the presence of a social partner reduces the stress response of the focal individual (reviewed by Hennessy et al., 2009).
Stressor	An internal (e.g. disease) or external (e.g. environmental conditions) stimulus that disrupts the homeostasis of an individual (Chrousos and Gold, 1992).
Trier Social Stress Test (TSST)	A laboratory-based stress protocol designed to elicit psychological stress under experimental conditions (e.g. mock job interview, mental arithmetic), and used to understand how individual differences predict the stress response to controlled stimuli (Kirschbaum et al., 1993).

Physiological underpinnings of attachment bonds

For attachment bonds to persist between social partners, the associated sense of safety and security must be reinforced through proximate mechanisms on a neurophysiological level (reviewed by Carter, 2005; Figure 1.1). When a positive interaction between two individuals triggers the reward system of the brain through the release of neurochemicals such as oxytocin (peptide hormone produced in the hypothalamus) and dopamine (organic chemical that acts as a neurotransmitter), individuals will be motivated to seek the same reward again in the future, thus promoting further interactions between the two individuals (Carter, 2005; Johnson and Young, 2015). In the same way, interactions between individuals that lead to an attenuated physiological response to a threatening/stressful situation will also initiate further social interactions through positive feedback (Johnson and Young, 2015; Sbarra and Hazan, 2008). One such mechanism of an individual mediating the physiology of a social partner is known as '**social buffering**' (reviewed by Hostinar et al., 2014).

Social buffering

Social buffering refers to a unidirectional reduction in physiological stress levels in the presence of a familiar social partner when exposed to sub-optimal or aversive conditions (Hennessy et al., 2009; Hostinar et al., 2014; Figure 1.1). In this instance, 'unidirectional' refers to the fact that the social interaction buffers the physiological stress of one individual in the dyad – the physiological state of the other individual remains unchanged. This biological mechanism has been identified across many different animal species and social contexts (reviewed by Hostinar et al., 2014), as outlined in the below examples. Comparisons of endocrinological and behavioural stress responses in the presence of familiar and unfamiliar conspecifics in guinea pigs (Hennessy et al., 2008) and rats (Kiyokawa et al., 2014) have shown that stress buffering effects are greater when housed with a familiar individual. Meanwhile, pair-housing in captive rhesus macaques also promotes social buffering between individuals when exposed to a visual **stressor** of a third conspecific being restrained and anesthetised (Gilbert and Baker, 2011). In humans, social buffering has been identified as an important relief for individuals experiencing stressful conditions (Gunnar et al., 2015; Gunnar and Hostinar, 2015; Hostinar et al., 2014); for example, women who had the opportunity to socially interact with their male spouse prior to a **Trier Social Stress Test** exhibited significantly reduced stress responses in cortisol concentrations and heart rate compared to women who had no social interaction or were restricted to verbal interaction (Ditzen et al., 2007).

Over time and following repeated instances of social buffering in response to acute stressors, social partners will start to associate each other with the sense of safety and security across other interactions, not just those that take place during a stressful event (Sbarra and Hazan, 2008). As each individual comes to rely on their social partner as their primary attachment figure, this bond begins to manifest in the form of covarying physiology. The context of the relationship is determined by the shared physical and emotional environment of the individuals, and the synchronisation of physiological traits comes as a result of both individuals maintaining homeostasis within this same environment – termed ‘**coregulation**’ (Sbarra and Hazan, 2008; Figure 1.1).

Coregulation

Whilst social buffering is an important mechanism that contributes to the formation and maintenance of attachment bonds, it is not a defining characteristic that is exclusive to attachment (Sbarra and Hazan, 2008). Coregulation, on the other hand, consists of the synchronised physiological state of social partners as both individuals repeatedly up/down-regulate the stress responses of their partner in the long-term – i.e. individuals’ physiological state will continuously fluctuate to maintain a dyadic homeostasis (Sbarra and Hazan, 2008). This synchrony is generally considered as representing a functioning attachment bond between two individuals and demonstrates, on a physiological level, the long-term safe haven and secure base effects outlined in Bowlby and Ainsworth’s attachment theory (Ainsworth and Bowlby, 1991; Sbarra and Hazan, 2008; Figure 1.1). In line with social baseline theory, coregulation is believed to function as an energy-saving mechanism (Coan and Sbarra, 2015; Sbarra and Hazan, 2008). By forming an attachment with another individual, the energetic costs and negative affect associated with daily tasks can be subconsciously perceived as less costly when that individual is present, as well as simply sharing the energetic costs through completing tasks together (Coan and Sbarra, 2015). This minimisation of physical and psychological stress reduces the likelihood of energy being diverted away from important systems such as the immune system and cardiovascular system (Coan and Sbarra, 2015; Harris, 2015; McEwen, 2017). In an evolutionary context, attachment bonds – and the underlying mechanism of coregulation – therefore constitute a social resource which an individual can rely upon to improve the likelihood of survival and increase individual fitness (Coan, 2008; Coan and Sbarra, 2015).

Various physiological measures have been used to identify coregulation within social dyads (reviews by Davis et al., 2018; Mayo et al., 2021; Palumbo et al., 2017; Timmons et al., 2015). Recent studies have shown that, under certain contexts, both mother-infant dyads and spousal dyads coregulate heart rate interval (Corner et al., 2019; Creaven et al., 2014; Wilson et al., 2018), respiratory sinus arrhythmia (the change in cardiac rhythm across the respiratory cycle; Amole et al., 2017; Gates et al., 2015; McKillop and Connell, 2018), and electrodermal activity (a measure of skin conductivity, which is then used to calculate sympathetic nervous system (SNS) responsiveness; Baker et al., 2015; Corner et al., 2018; Coutinho et al., 2018; Karvonen, 2017). Synchronised prefrontal cortical activity in response to controlled stimuli was also observed in romantic couples (Azhari et al., 2020). Additionally, coregulation has been identified in the endocrine system, particularly in partner's levels of oxytocin (Feldman et al., 2011; Schneiderman et al., 2014) and **cortisol** (reviews by Davis et al., 2018 & Timmons et al., 2015). Of all the measures used to investigate physiological coregulation, cortisol is most commonly used (reviewed by Davis et al., 2018), and is the focus of this thesis.

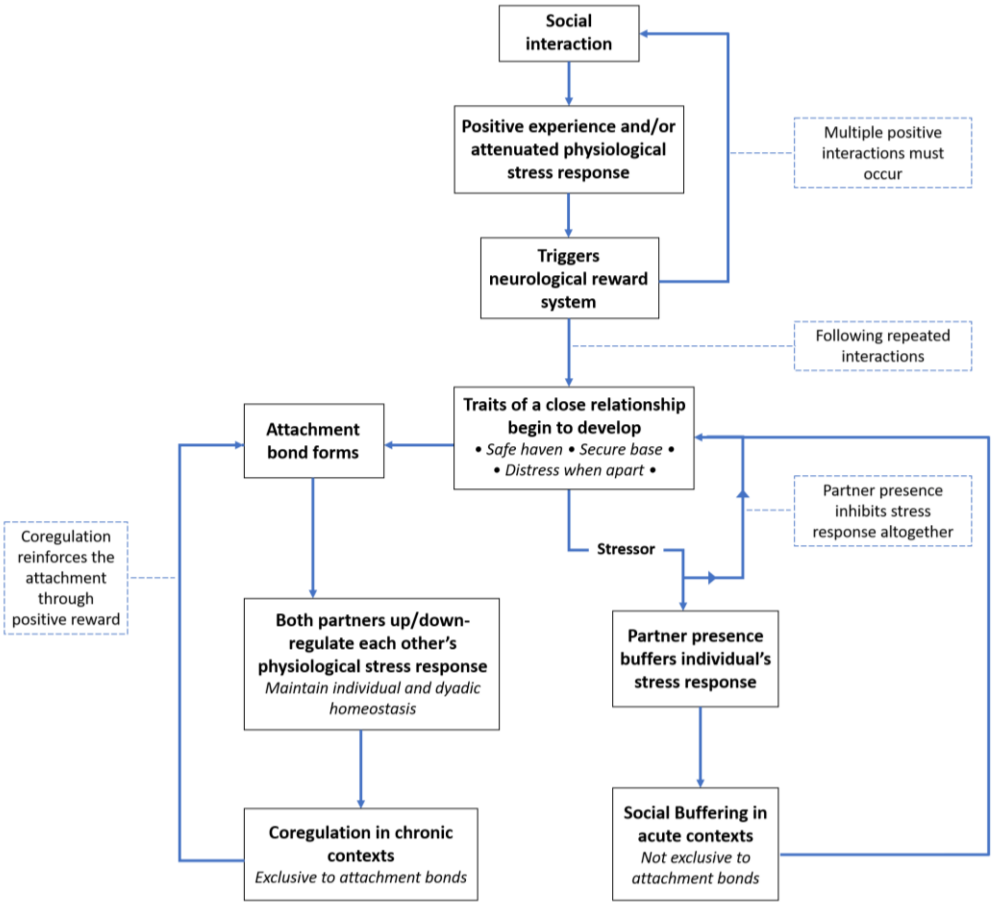


Figure 1.1 Process of forming an attachment bond with another individual, and the physiological mechanisms that underpin this relationship. The information in the dotted boxes describes the processes involved in the labelled steps within the diagram. Diagram created by myself based on the theory outlined by Sbarra and Hazan (2008).

Cortisol coregulation

Cortisol is a steroid hormone released from the adrenal cortex as part of the stress response, regulated by the **hypothalamic-pituitary-adrenal (HPA) axis** (Nelson, 2011). The perception of a stressor triggers the cascade of hormone secretions which constitute the HPA axis, starting with an increase in the release of corticoptropin-releasing-hormone (CRH) from the hypothalamus. Increased CRH concentrations stimulate the release of adrenocorticotropic hormone (ACTH), which subsequently signals the release of the glucocorticoid cortisol (or corticosterone, depending on the species; Chrousos and Gold, 1992; Nelson, 2011). The increased concentration of circulating cortisol diverts energy away from processes not necessary for immediate survival, such as growth, digestion, reproduction, and immune response (Nelson, 2011; Smith and Vale, 2006), with the ultimate function of the HPA axis being to overcome the stressor and return the body to homeostasis (Nelson, 2011; Sapolsky et al., 2000). The stress response is an energetically expensive biological process, and chronic activation of the HPA axis can have adverse effects on health and wellbeing, such as increased susceptibility to disease, fatigue, hypertension, and depression (Nelson, 2011; Raber, 1998). Therefore, mechanisms, such as cortisol coregulation, that either limit the frequency with which the stress response is activated in the first place, or reduce the duration of HPA axis stimulation are crucial (reviewed by Coan and Sbarra, 2015; Hennessy et al., 2009; Sbarra and Hazan, 2008).

The majority of cortisol coregulation research to date has focussed on human dyads, i.e. parents and infants (Table 1.2) or romantic couples (Table 1.3) (reviews by Davis et al., 2018; DePasquale, 2020; Meyer and Sledge, 2020). In parent-infant and romantic dyads, attachment is an adaptive process which is dependent upon the social and environmental context of the dyad; therefore, the physiological mechanism underlying attachment should also vary according to context (Sbarra and Hazan, 2008). For this reason, cortisol coregulation has been studied in a number of different environmental, temporal, and social contexts (reviews by Davis et al., 2018 & Timmons et al., 2015). Parent-infant and spousal cortisol coregulation have been measured across various time scales, ranging from a single laboratory visit lasting a number of hours (e.g. Ha et al., 2016; Saxbe et al., 2014), to daily sample collection at the participants' homes (e.g. Hibel et al., 2014; Liu et al., 2013), to sampling/home visits across multiple years (e.g. Ouellette et al., 2015; Saxbe et al., 2015). With this temporal variation, we also see variation in the environmental context of the studies –

many studies measure cortisol coregulation in the dyads' familiar, naturalistic home surroundings (e.g. Hibel et al., 2014; Saxbe and Repetti, 2010), whilst others measure coregulation in an unfamiliar, controlled environment with some form of social interaction test (i.e. strange situation test, conflict discussions, and performance-focused tasks; e.g. Borelli et al., 2019; Ha et al., 2016; Laurent et al., 2012; Saxbe et al., 2014). Whilst a small number of studies find no coregulation in parent-infant and spousal dyads (Bader et al., 2021; Hall, 2013; Neu et al., 2014), the general consistency over time and context shown in the majority of the cortisol coregulation literature (see Table 1.2 and Table 1.3) highlights the regularity with which this biological phenomenon maintains attachment relationships (Sbarra and Hazan, 2008).

Table 1.2 Studies investigating cortisol coregulation and predictors of coregulation strength in parent-infant dyads.

Study dyad	**Study Context**	**Dyads (n)**	**Cortisol Measure**	**Predictors of Coregulation Strength**	**Reference**
Mothers and infants aged 2-5 years	Mother observed child on gymnastics beam	64	Saliva	Maternal sensitivity to child's behavioural cues	Sethre-Hofstad et al. (2002)
Mother, father, child (and sibling), and families with twins	3-day study period under naturalistic home conditions split across 2 studies (S1 and S2)	S1: 321 S2: 233	Saliva	Shared environmental factors better explained variation in cortisol similarity than shared genetic factors	Schreiber et al. (2006)
Mothers and pre-term babies	Comparison of holding techniques across 2-hour period	20	Saliva	Coregulation stronger following holding, but no effect of holding technique	Neu et al. (2009)
Mothers and infants aged 7-months	Infant stress test during a home visit	702	Saliva	Stronger in dyads where mother is exposed to intimate partner violence	Hibel et al. (2009)
Mothers and adolescents	2-day study period under naturalistic home conditions	45	Saliva	More time together and more shared activities predicted stronger coregulation	Papp et al. (2009)
Mothers and pre-school children	IQ tests and free-play sessions during two home visits	75	Saliva	Coregulation only present during periods of increased challenge, and with greater maternal sensitivity.	Ruttle et al. (2011)
Mothers and infants aged 18-months	Strange situation test with repeated separation	86	Saliva	Dependent on the stress context rather than dyad characteristics	Laurent et al. (2012)
Mothers and infants aged 4-10 months	4-day infant sleep training programme	25	Saliva	Coregulation ceased following removal of behavioural cue (infant crying)	Middlemiss et al. (2012)

Table 1.2 cont.

Mothers and children aged 7-12 years	2-day study period under naturalistic home conditions	27	Saliva	Maternal anxiety and family environment	Williams et al. (2013)
Working mothers and infants aged 2-4 years	4-day study period under naturalistic home conditions	47	Saliva	Stronger on non-workdays compared to workdays	Hibel et al. (2014)
Mothers, fathers, and adolescents	Conflict discussion during a single lab session	103	Saliva	Strongest between same-sex dyads (i.e. mother-daughter, father-son)	Saxbe et al. (2014)
Mothers and infants	Home visit involving an infant stress task, observed by the mother	1292	Saliva	Similarity decreased from pre- to post task. Decline was steeper in dyads with less maternal sensitivity	Hibel et al. (2015)
Mothers and adolescent daughters	2-day study period under naturalistic home conditions	112	Saliva	Not influenced by history of maternal depression	LeMoult et al. (2015)
Mothers and pre-term babies	Comparison of holding techniques during first 4 months since birth	42	Saliva	Coregulation only present when utilising skin-to-skin holding technique (compared to standard care)	Mörelius et al. (2015)
Mothers and infants aged 0-12 months	Home visit and a 1-day study period under naturalistic home conditions	54	Saliva	Coregulation only at bedtime, and was stronger in breastfeeding dyads	Neelon et al. (2015)
Mothers and 3-year-old daughters	Home visit aged 3, phone interview aged 5, and a second home visit aged 7	28	Hair	Only groups classified as 'high-stress' coregulated	Ouellette et al. (2015)
Mothers and 12-month-old infants	90-minute lab session involving mother-infant free play	111	Hair	None indicated	Flom et al. (2017)

Table 1.2 cont.

Mothers and infants aged 5-8 months	Single home visit involving mother-infant free play	121	Maternal hair and infant saliva	Correlation in maternal HCC and infant salivary cortisol found in groups with more intrusive mothers	Tarullo et al. (2017)
Mothers and children aged 3 years	2-day study period under naturalistic home conditions	82	Saliva	Coregulation only present in groups where the mother had experienced abuse during childhood	Fuchs et al. (2017)
Mothers and infants aged 4 years	2-day study period under naturalistic home conditions	139	Saliva	Parental lifetime depression and +ve/-ve infant emotionality predicted coregulation strength	Merwin et al. (2017)
Mothers and children aged 6 years	2-day study period under naturalistic home conditions	97	Saliva	Maternal depressive symptoms where unrelated to coregulation	Pratt et al. (2017)
Mothers and 12-month-old infants	Single home visit for sample collection	129	Hair	Coregulation observed in HCC representing 9-12 months postpartum. Suggestion that it is stronger in high-risk contexts (e.g. inhabiting Brazilian slums)	Liu et al. (2017)
Parents and infants with autism spectrum disorder	Infant interaction tests during two home visits in a single month	115	Saliva	Father-infant coregulation was stronger than mother-infant. Autism only influenced maternal coregulation	Saxbe et al. (2017)
Mothers and infants aged 6-12 months	Two 2-day study periods at 6 and 12 months postpartum	93	Saliva	Coregulation in breastfeeding dyads, and non-breastfeeding dyads displayed inverse coregulation	Jonas et al. (2018)
Mothers and 2-year-old infants	90-minute lab session involving infant strange situation tests	70	Saliva	Stronger coregulation linked to lower risk of infant internalising symptoms	Kalomiris and Kiel (2018)

Table 1.2 cont.

Mothers and 3-month-old infants, either full-term or very pre-term	Face-to-face still face paradigm test during a single lab visit	82	Saliva	Pattern of coregulation differs significantly between the full-term and pre-term groups	Provenzi et al. (2019)
Mothers of children aged 4-5 years with ADHD	Home visit (interaction observations and child intelligence test), playroom session, and telephone interview over 6-week period	111	Hair	Stronger with high maternal sensitivity and fewer ADHD symptoms in the child	Schloß et al. (2019)
Mothers and children aged 9-12 years	Child performance challenge task during a single lab visit	99	Saliva	Maternal overcontrol and child age predicted a greater change in strength from pre- to post-task	Borelli et al. (2019)
Mothers and 17-month-old infants	Home visit involving a strange situation test	256	Saliva	Inverse coregulation associated with a disorganised (asynchronous) attachment bond	Nofech-Mozes et al. (2020)
Low-income mothers and adolescent daughters	Disagreement discussion during a single lab visit	118	Saliva	Maternal cortisol has the greater influence on coregulation	Byrd-Craven et al. (2020)
Parents and adolescents	8-day study period involving daily diary phone calls with researchers	318	Saliva	Adolescents’ cortisol awakening response and bedtime cortisol predicted that of parents the following day	Lippold et al. (2020)
Women in third trimester of pregnancy	Comparison of perinatal coregulation in mothers with and without severe psychiatric disorders	45	Hair	Coregulation only observed in mothers without psychiatric disorders	Broeks et al. (2021)

Whilst the ultimate function of cortisol coregulation is to increase individual fitness and survival (Coan and Sbarra, 2015), its day-to-day role in social interactions is somewhat unclear (Danyluck and Page-Gould, 2019; Timmons et al., 2015). When a relationship is functioning well and both individuals positively benefit from their social interactions, cortisol coregulation is predicted by indices that tend to reflect this positive social environment – cortisol coregulation is stronger when in closer physical proximity and spending more time together doing shared activities (Table 1.2 and 1.3; Hibel et al., 2014; Laws et al., 2015; Mörelius et al., 2015; Neu et al., 2009; Papp et al., 2013, 2009; Pauly et al., 2020; Saxbe and Repetti, 2010) and in dyads where there is greater emotional depth and sensitivity to behavioural cues (Table 1.2 and 1.3; Engert et al., 2018; Hibel et al., 2015; Ruttle et al., 2011; Sethre-Hofstad et al., 2002). However, in contexts where a relationship becomes dysfunctional and the attachment bond is tested, stronger cortisol coregulation tends to be linked to factors such as low relationship satisfaction, high strain (Table 1.2 and 1.3; Borelli et al., 2019; Laws et al., 2015; Liu et al., 2013; Ruttle et al., 2011; Saxbe and Repetti, 2010), and high-stress situations, such as exposure to domestic abuse (Table 1.2 and 1.3; Fuchs et al., 2017; Hibel et al., 2009; Timmons et al., 2015). A history of depression and anxiety can also strengthen cortisol coregulation (Table 1.2 and 1.3; Braren et al., 2020; Broeks et al., 2021; Merwin et al., 2017; Ouellette et al., 2015; Williams et al., 2013; but see LeMoult et al., 2015 & Pratt et al., 2017).

Variation in the types of social and environmental factors that predict the strength of coregulation therefore demonstrates the complexity of the mechanism (Danyluck and Page-Gould, 2019). Furthermore, the inclusion of hair cortisol analysis as a chronic measure of HPA axis activity (reviewed by Burnard et al., 2017; see section 2.2.2 in Chapter 2) introduces further complexity as studies begin to consider variation in coregulation across short- and long-term contexts (Broeks et al., 2021; Ouellette et al., 2015; see Tables 1.2 and 1.3). As a result, it is uncertain whether it is truly beneficial to coregulate with a social partner, or if the mechanism can be disadvantageous under certain social and temporal contexts (reviewed by Timmons et al., 2015). For example, coregulation during a shared acute stressor may be more effective in limiting activation of the HPA axis in functioning relationships, whilst sustained coregulation may be more heavily influenced by factors more commonly associated with non-functioning relationships (Timmons et al., 2015). One way in which to better understand the day-to-day functional role of coregulation is to broaden the scope of the research and consider attachment bonds beyond those of human parent-infant dyads and romantic couples (Sbarra and Hazan, 2008; Timmons et al., 2015).

Table 1.3 Studies investigating predictors of cortisol coregulation in romantic couples.

Study dyad	**Study Context**	**Dyads (n)**	**Cortisol Measure**	**Predictors of Coregulation Strength**	**Reference**
New and expectant mothers and fathers	Situational reactivity test (auditory, visual, and olfactory cues of newborns)	31	Blood	Mother-father coregulation strength linked to the time remaining before the expected birth date	Storey et al. (2000)
Expectant mothers and fathers	Weekly sampling under naturalistic conditions for 6 months (surrounding the birth)	9	Saliva	None indicated	Berg and Wynne-Edwards (2002)
Married couples, both working full-time	3-day study period under naturalistic home conditions	32	Saliva	Stronger with close physical proximity and low marital satisfaction	Saxbe and Repetti (2010)
Romantic/married couples	4-day study period under naturalistic home conditions	19	Saliva	Stronger in groups where relationship was perceived as highly strained	Liu et al. (2013)
Married couples	2-day study period under naturalistic home conditions	47	Saliva	Moderated by time spent together. Coregulation not maintained when apart	Papp et al. (2013)
Newly-married couples	Two lab sessions 18 months apart	183	Saliva	Low relationship satisfaction increased strength. Cohabitation also influenced coregulation	Laws et al. (2015)
Couples new to parenthood	Repeated sampling over 2-year study period following childbirth	122	Saliva	Stronger with higher levels of mother-reported partner aggression	Saxbe et al. (2015)
Adolescent romantic couples	Conflict discussions and interaction task during single lab session	91	Saliva	Degree of perceived support in the relationship	Ha et al. (2016)

Table 1.3 cont.

Romantic couples	2-day study period under naturalistic home conditions and single lab visit involving a mock job interview	44	Saliva	Dyads with higher female stress resonance and emotionally deeper relationships coregulated more strongly	Engert et al. (2018)
Romantic couples	5-day study period under naturalistic home conditions	40	Saliva	Weaker following interaction between the two individuals	Doerr et al. (2018)
Married/cohabiting couples for ≥ 2 years	Single study day consisting of a conflict (and resolution) discussion	62	Saliva	Negative marital conflict disrupts coregulation	Seiter et al. (2019)
Expectant mothers and fathers	2-day study period under naturalistic home conditions	445	Saliva	Stronger with greater maternal psychological stress levels	Braren et al. (2020)
Older romantic couples	Two interview sessions, one either side of a 7-day study period under naturalistic home conditions	S1: 85 S2: 77	Saliva	Positive socioemotional interactions prior to sample collection increased coregulation strength	Pauly et al. (2020)
Older romantic couples	7-day study period under naturalistic home conditions	160	Saliva	Dependent on the political context of the area the couple reside. Openness personality trait linked to weaker coregulation	Pauly et al. (2021)
Pairs made up of a romantic couple, friends, or strangers	Single lab visit consisting of three naturalistic interaction tasks	82	Saliva	Cortisol concentrations of male romantic partners had greater effect on coregulation than female partner cortisol	Djalovski et al. (2021)

As outlined previously, attachment bonds are also studied within non-human animal species (Meehan et al., 2017; Payne et al., 2016; Rockett and Carr, 2014), and the apparent similarities in the defining characteristics of human and non-human attachment bonds (e.g. seeking proximity, separation anxiety, etc.) suggest a common underlying physiological mechanism to maintain these bonds, i.e. cortisol coregulation (see Figure 1.1; Sbarra and Hazan, 2008). Studying coregulation within non-human and human-non-human (i.e. interspecific) dyads allows comparisons to the human literature, for example with regards to potential moderators of coregulation. Any similarities and differences between study systems could help to better understand the functional role of coregulation within the context of the different attachment bonds. Non-human animal species currently represented in the coregulation literature include domestic dogs (Buttner et al., 2015; Cunningham, 2017; Sundman et al., 2019) and horses (Strzelec et al., 2013) as part of interspecific dyads with their owner/rider; and intraspecific dyads of prairie voles (Burkett et al., 2016) and three-spined stickleback fish (Fürtbauer and Heistermann, 2016; Table 1.4). Whilst limited to a small number, these studies demonstrate that cortisol coregulation is not exclusive to human social relationships. Furthermore, preliminary evidence suggests that the predictors of coregulation strength in non-human and human-non-human dyads are analogous to those identified in human dyads (Table 1.4) – stronger coregulation has been observed in stressful experimental conditions (Burkett et al., 2016; Fürtbauer and Heistermann, 2016; Ryan et al., 2019) and high-pressure contexts such as dog agility competitions (Buttner et al., 2015). Coregulation strength is also influenced by the sex of the non-human (dog-owner dyad: Cunningham, 2017; Sundman et al., 2019) and human (horse-rider dyad: Strzelec et al., 2013) individuals within the dyads.

The dog-human relationship has received the most attention in the non-human and human-non-human coregulation literature (see Table 1.4), likely due to dogs' advanced cognitive perception of human cues providing a strong basis for synchronisation (Duranton and Gaunet, 2015), as well as the wealth of research into the attachment bond that forms between dogs and owners (see above; reviewed by Payne et al., 2015). Dogs are the oldest domesticated species, with dogs and humans having coevolved for at least 30,000 years (Galibert et al., 2011; Pionnier-Capitan et al., 2011) and, in this time, dogs' role in human society has majoritively shifted from utilitarian to social companion (Marshall-Pescini and Kaminski, 2014; Prato-Previde and Valsecchi, 2014). In the same way that infants are reliant on their parent/caregiver for survival, dogs also develop a strong reliance on their owner for day-to-day care, which is why dog-owner attachment bonds are often compared to those that form between infants and parents (reviewed by Meehan et al., 2017; Prato-Previde and Valsecchi, 2014). With this key similarity in dog-owner and parent-infant attachment bonds, we should expect to observe similarities in factors that moderate cortisol coregulation. Equally, identifying differences between the dyads' coregulatory mechanisms could help to discern how coregulation functions differently within the context of the relationships.

Table 1.4 Studies investigating predictors of cortisol coregulation in non-human animal dyads or human-non-human dyads.

Dyad Species	**Study Context**	**Dyads (n)**	**Cortisol Measure**	**Predictors of Coregulation Strength**	**Reference**
Horse (*Equus ferus caballus*) – Human	Equestrian competition	36	Saliva	Dependent on context and rider sex	Strzelec et al. (2013)
Prairie voles (*Microtus ochrogaster*)	Separation of co-housed rodents. One exposed to separation or stressor	20	Blood (cortico-sterone)	Coregulation only in stressor condition	Burkett et al. (2016)
Three-spined stickleback fish (*Gasterosteus aculeatus*)	Comparison between cohabitation, separation, and shared stressor	11	Water-borne	Coregulation only in stressor condition	Fürtbauer and Heistermann (2016)
Dog (*Canis lupus familiaris*) – Human	Dog agility competition	58	Saliva	Not influenced by handler behaviour or assessment of dog performance	Buttner et al. (2015)

	Dog agility competition and home environment	49	Saliva	Dependent on the sex and neuter status of the dog	Cunningham (2017)
	Naturalistic home conditions	58	Hair	Influenced by season, sex of dog, and dog lifestyle (companion vs competition)	Sundman et al. (2019)
	Single lab visit consisting of a strange situation test	29	Saliva	Coregulation only after the strange situation test	Ryan et al. (2019)
	Single home visit including a 15 min walk	68	Saliva	Inverse coregulation – dog cortisol lower with greater owner cortisol	Harvie et al. (2021)

Thesis objectives and overview

The aim of this thesis is to develop our understanding of the individual and social factors that influence cortisol coregulation across short- and long-term naturalistic contexts in interspecific dog-human dyads and intraspecific dog-dog and human-human dyads and explore how this physiological mechanism may contribute towards a functional attachment bond between social partners. In particular, I will be studying the effect of factors that have received little attention in the coregulation literature to date – the presence/absence of external (to the dyad) social influences and personality similarity of social partners (but see Pauly et al., 2021; Saxbe and Repetti, 2010). Understanding how such factors affect cortisol coregulation strength, along with further investigation of acute and chronic measures, could have important methodological implications for the way coregulation research moves forward and is used to better understand the daily role of coregulation across a vast range of social relationships (see reviews by Davis et al., 2018; Timmons et al., 2015). Additionally, given the important role of coregulation in regulating social bonds, continuing to develop our knowledge of the mechanism will enable a better understanding of how sociality aids survival and improves mental health and welfare for human and non-human animal species.

Given the different time-scales represented, a combination of saliva (acute) and hair (chronic) cortisol analysis will be utilised in this thesis to study variation in coregulation across short-and long-term naturalistic contexts. To my knowledge, this is the first research to concurrently measure saliva and hair cortisol concentrations to address research questions across varying temporal contexts (see Tables 1.2, 1.3, and 1.4). Chapter 3 uses saliva sampling to investigate whether proximity to a social partner, a commonly identified predictor of cortisol coregulation in human dyads (e.g. Hibel et al., 2014; Saxbe and Repetti, 2010), moderates the strength of dog-owner cortisol coregulation. Here, I consider both the owners' physical proximity and emotional closeness with their dog (determined using a dog-owner relationship questionnaire). Given the many similarities in the behavioural characteristics of dog-owner and parent-infant attachment bonds (see above; Meehan et al., 2017; Prato-Previde and Valsecchi, 2014), it is reasonable to expect these similarities to extend to the physiological mechanism that maintains this attachment (Sbarra and Hazan, 2008). Based on this hypothesis, we should observe stronger dog-owner cortisol coregulation with greater physical and emotional closeness, as per the parent-infant literature (reviewed by Davis et al., 2018).

Chapter 4 is the first study to investigate whether the attachment between cohabiting dogs (Sipple et al., 2021) is underpinned by cortisol coregulation, and whether the owner's presence influences the strength of dog-dog coregulation. To date, most research has considered how dyadic characteristics or features of the surrounding environment influence coregulation strength (see Tables 1.2-1.4). However, in addition to their interactions with a cohabiting conspecific, dogs also have interactions with the owner, who holds an important role as caregiver and key attachment figure (e.g. Payne et al., 2015). This presents a context for both dogs in which each dog can choose between intra- and inter-specific interactions, and the associated benefits of each attachment bond (Sipple et al., 2021), meaning that dog-dog coregulation strength could vary accordingly.

Chapter 5 focusses on long-term cortisol coregulation in both dog-owner and dog-dog dyads using hair cortisol concentrations (HCCs). Given that coregulation mediates attachment bonds in the long-term, not only during acute stress events (Sbarra and Hazan 2008), hair cortisol – a measure of chronic HPA axis activity – is well suited (see Burnard et al., 2017) to measuring long-term cortisol coregulation (e.g. Broeks et al., 2021; Sundman et al., 2019). By providing an average cortisol concentration that is unaffected by acute daily cortisol rhythms, HCCs are beneficial for investigating the effects of standard, long-term social conditions within the individuals' surrounding environment on cortisol coregulation. In addition to measuring dog-owner and dog-dog HCC coregulation, I investigate how the presence of a second dog influences dog-owner coregulation strength compared to a one-dog household, which could have important implications for our perception of dog sociality in multi-dog households.

In Chapter 6, I investigate the effect of personality similarity on coregulation strength in romantic couples. Despite couple similarity commonly forming the foundation of romantic relationships (Luo, 2017), it has, to date, rarely been considered as a predictor of cortisol coregulation strength (but see Saxbe and Repetti, 2010).

Chapter 2 - General Methodology

To address the aims and objectives of this thesis (see Chapter 1), saliva and hair cortisol concentrations and behavioural questionnaire data were collected from groups of companion dogs and owners. At a minimum, each study group must have contained at least one dog and one owner (i.e. one unique dog-owner dyad) in order to be included in the sample to measure salivary cortisol coregulation (Chapter 3) and/or hair cortisol coregulation (Chapter 5). Groups with multiple dogs and two owners in a romantic relationship were also recruited to investigate dog-dog (Chapter 4) and human-human (Chapter 6) cortisol coregulation respectively. These multi-dog and multi-owner groups could also be included in Chapters 3 and 5 if they met the inclusion criteria (see Table 2.1 and Figure 2.1).

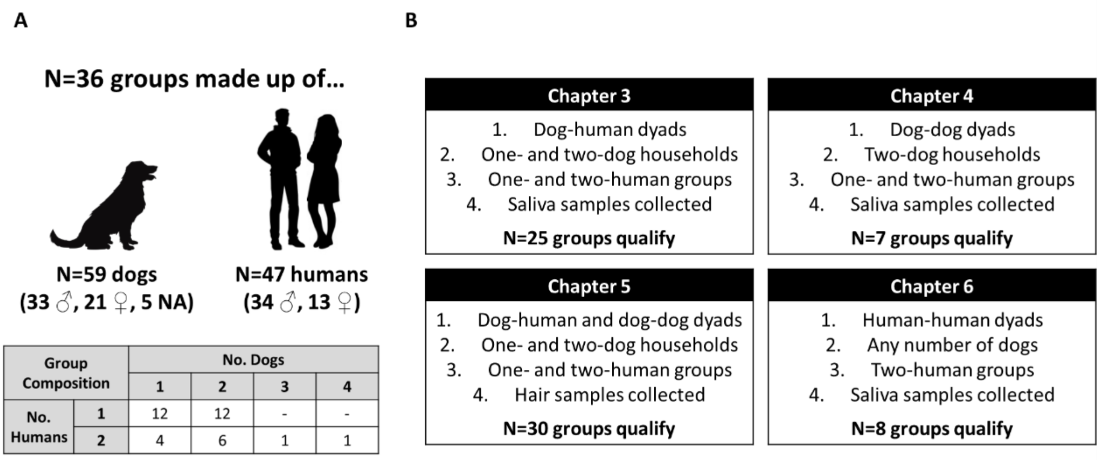
The study was advertised using emails and posters (Appendix 2) sent to Swansea University staff and postgraduate students, as well as recruitment via word-of-mouth. One limitation of this strategy is that the majority of those receiving the recruitment materials belong to WEIRD societies – populations made up of Western, Educated, Industrialised, Rich and Democratic individuals (Henrich et al., 2010; see section 7.4 for further discussion). Groups were recruited opportunistically in the order that they responded to the research call. Participants were recruited on a voluntary basis with no financial reward for completing the study. As the absence of a reward may have deterred potential participants, there were limited criteria for participation in order to maximise the available population from which groups could be sampled. All dogs and owners in each study group must have been cohabiting in the same household. Additionally, all owners participating in the study needed to be aged 18 years or over. Whilst there were no restrictions on the breed, sex, or age of the dog, the owner needed to confirm that their dog would, to the best of their knowledge, cope with the sampling procedures (see sections 2.1.3 and 2.1.4), and owners were encouraged to withdraw their dogs from the study if their dog exhibited any signs of distress as a result of the study. Dogs may have been excluded if they suffered from health conditions that could affect cortisol production and regulation, but there were no instances where exclusion was required.

2.1 Study subjects and procedure

2.1.1 Study subjects

All procedures in the following methodology were approved by Swansea University's Animal Welfare and Ethical Review Body (AWERB; IP-1819-02 and CSH-001-2015; Appendix 1). When explaining what participation in the study would involve, the purpose of the research and the specific research questions were withheld from the participants so as not to influence the way they took part in the study and how they responded to the questionnaires. Consent forms were signed by all participants before data collection, in which it was made clear that participation in the study was entirely optional and that they were free to withdraw at any point during the study (Appendix 3).

Between December 2018 and February 2020 a total of n=36 groups were recruited, of which n=8 groups withdrew from the study prior to completion (either for personal reasons or for health and safety reasons following the Covid-19 outbreak) but provided hair samples in the initial study meeting. Group size varied according to the number of dogs and owners (Figure 2.1), with a maximum of six different dyads per group (based on a 2-dog, 2-owner group). In this thesis, 'group' is the overarching identifier, and 'dyad' is used to refer to a specific relationship within the group. N=16 groups included one dog, n=18 groups included two dogs, n=1 group included 3 dogs, and n=1 group included four dogs (Table 2.1). I recruited n=25 groups with one owner and n=11 groups with two owners (Table 2.1) In total, the study included n=47 humans (n=34 females, n=13 males; age 26-70 years) and n=59 dogs (n=33 females, n=21 males, n=5 not recorded prior to withdrawal from study; age 1-12 years; Figure 2.1, Table 2.1). The sample size for the four data chapters differed according to the specific objectives of the chapter, but each chapters' subset of data was derived from the n=36 groups outlined above. All groups (except G35 and G36) contained at least one dog-owner dyad which was included in the analyses for Chapter 3 and/or Chapter 5. Groups with two dogs were included in Chapter 4 to measure dog-dog cortisol coregulation, and human-human cortisol coregulation between romantic couples in Chapter 6 was measured using the groups containing two owners.



Group Composition		No. Dogs			
		1	2	3	4
No. Humans	1	12	12	-	-
	2	4	6	1	1

Chapter 3

1. Dog-human dyads
2. One- and two-dog households
3. One- and two-human groups
4. Saliva samples collected

N=25 groups qualify

Chapter 4

1. Dog-dog dyads
2. Two-dog households
3. One- and two-human groups
4. Saliva samples collected

N=7 groups qualify

Chapter 5

1. Dog-human and dog-dog dyads
2. One- and two-dog households
3. One- and two-human groups
4. Hair samples collected

N=30 groups qualify

Chapter 6

1. Human-human dyads
2. Any number of dogs
3. Two-human groups
4. Saliva samples collected

N=8 groups qualify

Figure 2.1 A) Summary of the number of dogs and humans making up the n=36 participating groups, including the number of males (♀) and females (♂) (or NA if unknown), and B) the inclusion criteria that each group must meet to be included in the four data chapters. The number of applicable groups is provided for each chapter, and a more detailed breakdown of the groups included in each chapter can be found in Table 2.1.

2.1.2 Study schedule and sample collection

The study consisted of two face-to-face meetings with myself at Swansea University Park Campus and n=8 study days across two weeks in the owners’ homes according to their natural daily routines (Figure 2.2). The average time between the meetings at the start and end of the study was n=35 ± 28 days. Participation in the study involved the collection of saliva and hair samples from both the dogs and owners, and the completion of five questionnaires (see section 2.2).

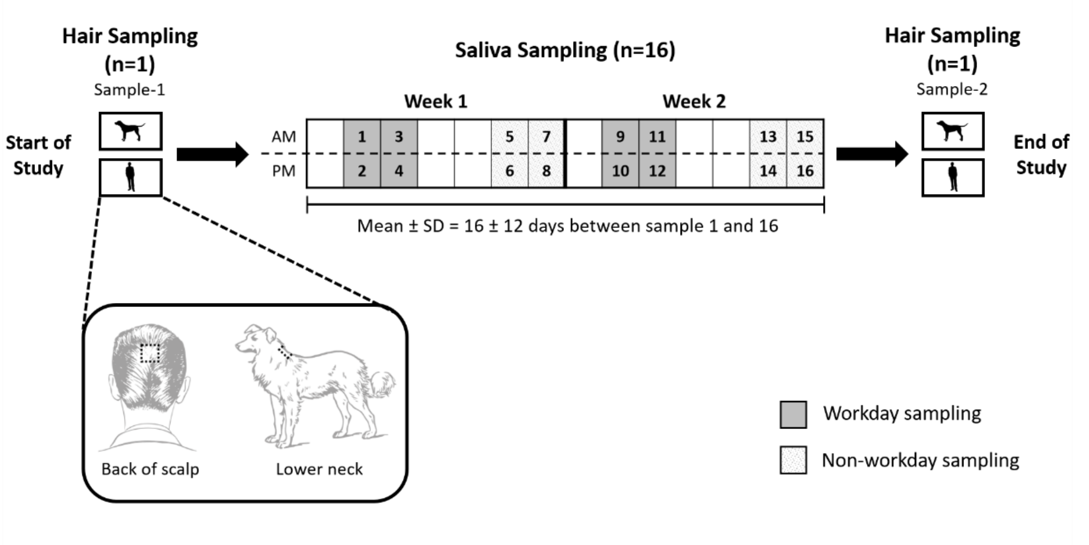


Figure 2.2 Sampling schedule for hair and saliva collection. Owners collected n=2 saliva samples per day (AM and PM) from themselves and their dog(s) across n=8 study days (totalling n=16 saliva samples per individual). Study days were split equally between the owners’ workdays and non-workdays. Hair sampling took place before (sample-1) and after (sample-2) the saliva sampling period, with dog and owner hair samples being collected from the lower neck region and posterior vertex of the scalp respectively (denoted by the dotted line on the images above).

Table 2.1 Composition of the n=36 groups that participated in the study, categorised by the number of dogs living in the household. ‘Collected saliva samples’ represents the number of samples collected by the participants for each individual in the group, whist the ‘analysed saliva samples’ column details the number of samples that were of sufficient volume for cortisol analysis. The number of hair samples analysed per individual is also provided. Inclusion of the group in the datasets for each of the four data chapters is indicated in the last four columns. Owners were asked to indicate their sex, but it is not known whether they reported their biological sex at birth or the gender that they identify with now.

Group	**ID**	**Subject**	**Sex**	**Age (years)**	**Collected Saliva Samples (n)**	**Analysed Saliva Samples (n)**	**Hair Samples (n)**	**Chapter 3**	**Chapter 4**	**Chapter 5**	**Chapter 6**
One-Dog Households											
1	D1	Dog	Female	2	16	10	1	✓	✗	✓	✓
	P1A	Owner	Male	60	16	10	0				
	P1B	Owner	Female	46	16	10	1				
2	D2	Dog	Male	2	16	15	2	✓	✗	✓	✓
	P2A	Owner	Female	37	16	15	2				
	P2B	Owner	Male	37	16	15	2				
3	D4	Dog	Female	-	0	0	1	✗	✗	✓	✗
	P4	Owner	Male	48	0	0	1				
4	D6	Dog	Female	8	16	12	2	✓	✗	✓	✗
	P6	Owner	Female	36	16	14	2				
5	D7	Dog	Male	5	16	0	1	✗	✗	✓	✗
	P7	Owner	Female	67	16	0	1				

Table 2.1 cont.

6	D11	Dog	Female	4	16	6	2	✓	✗	✓	✗
	P11	Owner	Female	65	16	6	2				
7	D12	Dog	Female	2	14	13	2	✓	✗	✓	✗
	P12	Owner	Female	31	14	13	2				
8	D13	Dog	Male	4	16	16	2	✓	✗	✓	✗
	P13	Owner	Female	49	16	16	2				
9	D17	Dog	Female	3	16	10	0	✓	✗	✗*	✗
	P17A	Owner	Female	30	16	11	0				
	P17B**	Owner	Male	30	0	0	0				
10	D19	Dog	Female	1	16	6	2	✓	✗	✓	✗
	P19	Owner	Female	43	16	6	2				
11	D20	Dog	Female	6	17	9	2	✓	✗	✓	✗
	P20	Owner	Female	55	17	9	2				
12	D30	Dog	Female	-	0	0	1	✗	✗	✓	✗
	P30	Owner	Female	42	0	0	1				
13	D31	Dog	Male	1	16	16	2	✓	✗	✓	✓
	P31A	Owner	Male	32	16	16	2				
	P31B	Owner	Female	30	16	16	2				
14	D33	Dog	Female	3	0	0	1	✗	✗	✓	✗
	P33	Owner	Female	52	0	0	1				

Table 2.1 cont.

15	D35	Dog	Male	9	14	14	2	✓	✗	✓	✗
	P35	Owner	Female	52	15	14	2				
16	D36	Dog	Female	5	15	13	2	✓	✗	✓	✗
	P36	Owner	Female	32	15	13	2				

Two-Dog Households

17	D3A	Dog	Male	7	22	19	0	✓	✓	✗*	✓
	D3B	Dog	Female	3	22	3	0				
	P3A	Owner	Female	33	21	19	0				
	P3B	Owner	Male	32	18	18	0				
18	D5A	Dog	Male	7	17	17	2	✓	✓	✓	✗
	D5B	Dog	Female	6	17	5	2				
	P5	Owner	Female	32	17	17	2				
19	D8A	Dog	Female	11	16	3	2	✓	✗	✓	✗
	D8B	Dog	Male	11	16	2	2				
	P8	Owner	Female	32	16	6	2				
20	D9A	Dog	-	-	0	0	1	✗	✗	✓	✗
	D9B	Dog	-	-	0	0	1				
	P9	Owner	Female	43	0	0	1				

Table 2.1 cont.

21	D10A	Dog	Male	6	16	5	0	✓	✗	✗*	✗
	D10B	Dog	Female	2	16	3	0				
	P10	Owner	Female	31	16	9	0				
22	D14A	Dog	Male	11	16	12	2	✓	✓	✓	✗
	D14B	Dog	Male	11	16	13	2				
	P14	Owner	Female	39	16	15	2				
23	D15A	Dog	Female	6	16	16	2	✓	✓	✓	✓
	D15B	Dog	Female	4	16	16	2				
	P15A	Owner	Male	67	16	16	2				
	P15B	Owner	Female	70	16	15	2				
24	D16A	Dog	Female	6	14	12	2	✓	✓	✓	✗
	D16B	Dog	Female	2	14	12	2				
	P16	Owner	Female	64	14	14	2				
25	D18A	Dog	Female	9	16	0	2	✗	✗	✓	✗
	D18B	Dog	Female	10	16	0	2				
	P18A	Owner	Male	65	14	0	0				
	P18B	Owner	Female	61	16	0	2				
26	D21A	Dog	Female	5	16	16	2	✓	✓	✓	✓
	D21B	Dog	Female	3	16	16	2				
	P21A	Owner	Male	33	16	16	2				
	P21B	Owner	Female	30	16	16	2				

Table 2.1 cont.

27	D22A	Dog	Female	11	16	3	0	✓	✗	✗*	✗
	D22B	Dog	Female	11	16	2	0				
	P22	Owner	Female	36	16	6	0				
28	D23A	Dog	Female	6	16	8	2	✓	✗	✓	✗
	D23B	Dog	Male	7	16	4	2				
	P23	Owner	Female	31	16	9	2				
29	D24A	Dog	Male	2	16	16	2	✓	✓	✓	✓
	D24B	Dog	Male	10	16	15	2				
	P24A	Owner	Male	27	16	16	2				
	P24B	Owner	Female	26	16	16	1				
30	D26A	Dog	Female	-	0	0	1	✗	✗	✓	✗
	D26B	Dog	Female	-	0	0	1				
	P26	Owner	Female	46	0	0	1				
31	D27A	Dog	Male	3	16	15	2	✓	✗	✓	✗
	D27B	Dog	Female	9	0	0	2				
	P27	Owner	Female	51	16	15	2				
32	D32A	Dog	Male	12	8	3	2	✓	✗	✓	✗
	D32B	Dog	Male	3	8	6	2				
	P32	Owner	Female	56	8	5	2				

Table 2.1 cont.

33	D34A	Dog	Female	-	0	0	1	✗	✗	✓	✗
	D34B	Dog	Female	-	0	0	1				
	P34A	Owner	Male	62	0	0	0				
	P34B	Owner	Female	61	0	0	1				
34	D38A	Dog	Male	-	0	0	1	✗	✗	✓	✗
	D38B	Dog	Male	-	0	0	1				
	P38	Owner	Male	51	0	0	0				
Three-Dog Households											
35	D28A	Dog	Female	6	16	16	2	✗	✗	✗	✓
	D28B	Dog	Male	4	16	16	2				
	D28C	Dog	Female	1	16	16	2				
	P28A	Owner	Female	56	16	16	2				
	P28B	Owner	Male	60	16	16	2				

Table 2.1 cont.

Four-Dog Households											
36	D29A	Dog	-	-	0	0	1	✕	✕	✕	✕
	D29B	Dog	-	-	0	0	1				
	D29C	Dog	-	-	0	0	1				
	D29D	Dog	-	-	0	0	1				
	P29A	Owner	Female	41	0	0	1				
	P29B	Owner	Male	47	0	0	1				

* groups who did not provide hair samples but were included for the questionnaire data analysis

** participant completed the questionnaires but did not partake in sample collection

N.B. Age and sex is not reported for all dogs as this information was not provided prior to the group withdrawing from the study.

2.1.3 Saliva sampling procedure

Owners from n=28 groups collected saliva samples from themselves and their dog(s) on n=8 separate study days (spread equally across two different weeks) between the two face-to-face meetings (Figure 2.2). Samples were collected twice per day – once in the morning (AM) and once in the afternoon (PM) – resulting in n=16 saliva samples per individual in the group (Table 2.1). Additionally, sample collection was spread evenly across owners' workdays and non-workdays with the view to understanding how the absence of the owner (e.g. Hibel et al., 2014) influences inter-specific dog-owner coregulation.

During the first meeting, owners were provided with all sampling materials (including gloves to prevent contamination of the dog saliva samples), and I instructed them on how to collect the samples. Written instructions for the saliva sample collection process were given to all owners for them to follow whilst they collected the samples, and the participants were encouraged to contact myself if they had any questions. Owner samples were collected by passive-drooling down a Saliva Collection Aid (Salimetrics, Stratech Scientific Limited, Ely, UK) into a 1 mL microcentrifuge tube, with the aim of providing at least 0.5 mL of saliva for each sample. Owners were asked not to eat, smoke, or drink alcohol in the 30 minutes immediately preceding sample collection. Dog saliva samples were collected with synthetic Salivettes (Sarstedt, Leicester, UK). Synthetic swabs were used as opposed to cotton swabs because they absorb more saliva (Lensen et al., 2015) and return more of the absorbed saliva on centrifugation (Hansen et al., 2008). Compared to the passive-drooling technique for the human samples where the participants can see whether enough saliva has been collected in the microcentrifuge tube, for the dog saliva samples it is difficult to judge how much saliva has been absorbed by the synthetic swab, so the owners were advised to maximise saliva uptake by moving the synthetic swab around the cheeks and under the tongue for at least 30 seconds (e.g. Bennett and Hayssen, 2010; Glenk et al., 2014). Food treats could also be used as an incentive to stimulate the dogs' saliva production, but these were not to be fed to the dog until after the sample collection process was complete (see review by Chmelíková et al., 2020). N=1 dog exhibited signs of distress during attempts to collect a saliva sample and was subsequently withdrawn from this part of the study (see G31 in Table 2.1). Immediately after sample collection, samples were placed in a zip-lock plastic bag and stored in the owner's freezer (exact freezer temperature for each group was unknown, but it is typically around -18 °C). Following collection of all saliva samples (Figure 2.2), owners returned the samples in the second meeting, and all human cellular material was removed from the owner saliva samples by centrifugation and disposed of in accordance with the Human Tissue Act (2004), and subsequently stored along with the dog samples at -20 °C in the endocrinology laboratory at Swansea University until analysis.

2.1.4 Hair sampling collection and processing

Dog and owner hair samples were collected from n=32 groups – the remaining n=4 groups were unable to bring their dogs to Singleton Campus for the hair sampling. Hair samples were collected by myself during the two study meetings either side of the saliva sampling period, hereafter referred to as ‘sample-1’ and ‘sample-2’ (Figure 2.2), resulting in n=2 hair samples per individual in the group (Table 2.1). N=10 groups withdrew from the study before sample-2 could be collected (Table 2.1). The time between the collection of sample-1 and sample-2 varied per group due to the owners’ schedule (mean ± SD = 35 ± 28 days).

Dog hair samples were collected using clippers (BaoRun P6 Pro Clippers) from the lower dorsal neck region (Rosén, 2016; Roth et al., 2016; Veronesi et al., 2015; Figure 2.2) – this region was chosen given it is a difficult area for the dog to groom and so should have limited contamination from cortisol-containing saliva (Meyer and Novak, 2012). Dogs were given time to familiarise themselves with myself and the sound of the clippers, and hair samples would only be collected if the dog was settled and showed no signs of distress – no dogs had to be excluded from the hair sampling for this reason. Owner hair samples were collected using scissors from the posterior vertex region of the scalp (reviewed by Greff et al., 2019; Figure 2.2). Both dog and owner hair samples were clipped/cut as close to the skin as possible in order to include the most recently grown hair, and the sample location remained the same across all samples to eliminate any variance in cortisol caused by body region (see Burnard et al., 2017; Fürtbauer et al., 2019). The clippers/scissors were cleaned between samples using disinfectant for groups with multiple dogs and/or owners (Table 2.1). Once collected, all hair samples were stored in aluminium foil within paper envelopes at room temperature until preparation for analysis (Davenport et al., 2006; Wennig, 2000).

In total, n=87 dog hair samples and n=65 owner hair samples were collected. The full length of the dog hair samples was used to provide an average measure of cortisol due to the varying hair growth rates across dog breeds (reviewed by Mesarcova et al., 2017). For long human hair samples, only the most proximal (closest to the scalp) 6 cm of the hair sample was used to avoid any influence of the ‘wash-out effect’ (the leaching of cortisol from the hair shaft due to general wear-and-tear and damage from washing with water and shampoo; Dettenborn et al., 2012; Hamel et al., 2011). All samples were washed twice with 3 mL of isopropanol to remove any surface cortisol or dirt, and then left to air-dry under a fume hood at room temperature for 7 days (Davenport et al., 2006).

Using a ball mill (BeadBug^{TM} 3 Microtube Homogenizer; Benchmark Scientific), samples were ground into a fine powder and weighed to approximately 0.02 g (mean ± SD = 0.021 ± 0.005 g, n=152) with a fine scale balance. Cortisol was extracted by adding 1 mL of methanol to each sample, vortexing for 10 min at 1500 rpm, and then leaving to incubate for 24 hours. The samples were vortexed again for 10 min at 1500 rpm following incubation and centrifuged for 5 min at 13,000 rpm. Using a stream of nitrogen gas at 37 °C, 700 µL of the cortisol-containing supernatant was evaporated (e.g. D’Anna-Hernandez et al., 2011; Fürtbauer et al., 2019; Ghassemi Nejad et al., 2014; Ouschan et al., 2013).

2.1.5 Sample analysis

All saliva and hair samples were analysed using a commercially available enzyme immunoassay (EIA) kit with a sensitivity of 0.007 µg/dL (1-3002, Salimetrics LLC, State College, PA). This EIA kit has previously been used to measure saliva and hair cortisol concentrations in dogs (e.g. Colussi et al., 2018; Packer et al., 2019) and humans (e.g. D’Anna-Hernandez et al., 2011; Ha et al., 2016). Salivettes were centrifuged at 3000 rpm for 10 minutes. The centrifuged Salivettes needed to contain at least 25 µL of saliva for analysis – n=430 dog samples contained enough saliva for analysis and the remaining n=248 were discarded, equating to a 63 % success rate for dog saliva sample collection. Human saliva samples that could be time-matched to dog saliva samples of sufficient volume were defrosted ready for analysis (n=444 out of n=585 samples; the remaining n=141 samples were not analysed). Evaporated hair samples were reconstituted with 150 µL of assay buffer. Sample analysis was completed in accordance with the protocol provided with the EIA kit. All n=152 hair samples were analysed in duplicate, whilst n=130 saliva samples (out of n=874) were run in singlet as their volume was below the 50 µL required for duplicate analysis. Any samples run in duplicate with a coefficient of variance (CV) above 10 % were re-analysed. Intra-assay coefficients of variation (CVs) were calculated using the duplicate high and low controls, which were 5.57 % (high) and 11.10 % (low) for the saliva analysis (n=27 plates), and 7.21 % (high) and 17.84 % (low) for the hair analysis (n=5 plates). The inter-assay CVs for the n=27 saliva plates and n=5 hair plates were 6.53 % and 7.39 % respectively.

2.2 Assessment of dyad characteristics and psychometrics

In addition to providing saliva and hair samples, owners were asked to complete five different questionnaires about i) basic group information (section *2.2.1*), ii) their own personality (section *2.2.2*), iii) their dog's behaviour (section *2.2.3*), and iv) the relationship they have with their dog (sections *2.2.4* and *2.2.5*; Table 2.2). Sections *2.2.1* through to *2.2.5* provide specific details on each of the five questionnaires. All questionnaires were completed in the owner's home at any point during the study period and did not need to be completed in any particular order. It was the choice of the participant as to whether they completed all questionnaires in one go or split it across multiple days – this approach was used to prevent the possibility of respondent fatigue (e.g. see Ben-Nun, 2008). Additionally, participants were not made fully aware of the focus and specific details of the research (known as demand characteristics) until after completion of the study to avoid the participants completing the questionnaires having formed a perception of "correct" responses that support the hypotheses (McCambridge et al., 2012; Robb and Shellenbarger, 2020). Where two owners took part together, each owner completed their own set of questionnaires and were specifically asked not to share their responses with each other. In groups with multiple dogs, the owner(s) completed each questionnaire separately for each dog (except for the owner personality questionnaire outlined in section *2.2.2*).

Table 2.2 Questionnaires completed by participants during the study. The final column. 'Chapter', indicates the data chapter(s) in which each questionnaire was used.

Topic	**Questionnaire**	**Description**	**Reference**	**Chapter**
Basic group information	Study specific questionnaire	Used to obtain basic information about the dog (e.g. sex, age, length of ownership), the owners' daily routine, and dog-owner shared activities	-	3, 4, 5
Owner personality	Big-5 personality test	Used to assign each owner a score for the following personality traits: openness, conscientiousness, extroversion, agreeableness, and neuroticism.	Goldberg (1999, 1990)	6
Dog behaviour traits	Canine Behavioural Assessment and Research Questionnaire	Used to obtain an understanding of the dog's behaviour, with particular focus on aggression, fear, attachment, and separation-related behaviours	Hsu and Serpell (2003)	3, 5
Dog-owner relationship	Monash Dog-Owner Relationship Scale	Owners rate how they perceive the relationship they have with their dog in terms of dog-owner interactions, emotional closeness, and perceived costs of dog ownership	Dwyer et al. (2006)	3
	Pet Attachment Questionnaire	Used to assess the attachment style of the owner towards their dog. Considers attachment anxiety and attachment avoidance	Zilcha-Mano et al. (2011)	3, 5

2.2.1 Study-specific questionnaire

General information about all individuals within the group (dogs and humans) was obtained using a bespoke questionnaire designed by myself (Appendix 4). The questionnaire assessed individual characteristics (age, sex, etc.), daily routine and living arrangements, time spent together/apart, shared activities, and any health conditions in the dogs. The 26-item study-specific questionnaire was made up of a combination of open questions with free-text response fields (e.g. *'In years, how old is your dog?'*) and closed questions with a set of fixed responses for the participant to choose from (e.g. *'What sex is your dog?'*: *'male'* or *'female'*). These closed questions instructed the participants to 'select one' response or 'select all that apply'.

2.2.2 Big-5 personality test

Owner personality was assessed using the 50-item self-report questionnaire known as the 'Big-5' personality test (Goldberg, 1999, 1990; Appendix 5). Using a 5-level Likert scale ranging from *1 = very inaccurate* to *5 = very accurate*, participants were asked to rate how accurately the 50 statements describe themselves and their personality. Each statement relates to one of the five personality dimensions outlined in the five-factor model (McCrae and John, 1992): openness, conscientiousness, extroversion, agreeableness, and neuroticism (Table 2.3). High scores relate to the participants identifying more closely with that personality trait for all dimensions except neuroticism, where a low score indicates a more neurotic personality. Standardised scores were calculated for each personality dimension, which included the reversal of scores for particular items on the questionnaire (e.g. items 2, 4, and 6; Appendix 5). The minimum and maximum possible scores for each personality dimension, and thus the range of recorded responses, is 10 and 50 respectively.

Table 2.3 Overview of the five personality traits assessed using the Big-5 personality test and the types of behaviours associated with each trait (McCrae and John, 1992; Novikova and Vorobyeva, 2019), as well as an indication of what a high score means for each dimension.

Personality Dimension	**Definition**	
Openness (to experience)	Reflects a preference for variety, new experiences, and imagination, high levels of (intellectual) curiosity, and attentiveness to the inner emotions of themselves and others.	High score = more open
Conscientiousness	Reflects goal-oriented, driven individuals who are highly organised, responsible/reliable, and strive to achieve and maintain high standards.	High score = more conscientious
Extroversion	Reflects a preference for stimulating environments and the presence of many other individuals. Generally energetic, talkative, and tend to be more assertive and expressive in social situations.	High score = more extrovert
Agreeableness	Reflects a cooperative rather than competitive nature, and individuals are generally kind, trusting, compassionate, and forgiving.	High score = more agreeable
Neuroticism	Reflects emotional sensitivity and instability. Individuals regularly experience negative emotions such as worry and anxiety, fear, anger, and self-consciousness, and can regularly act impulsively.	High score = less neurotic

2.2.3 Canine Behavioural Assessment and Research Questionnaire (C-BARQ)

Information about the dogs' general day-to-day behaviour was gathered using the Canine Behavioural Assessment and Research Questionnaire (C-BARQ; Hsu and Serpell, 2003; Appendix 6). Owners completed the 101-item questionnaire, scoring their dogs' behaviour on a Likert scale from *0 = never/no observed behaviours* to *4 = always/extreme behaviours*. C-BARQ considers 14 subscales of dog behaviour and temperament, covering areas such as aggression, separation-related behaviour, and attachment (Appendix 6). The sections on fear and anxiety account for any variation that exists in stranger-, owner-, and dog-directed behaviours. Scores were reversed for items 5, 6, and 7 (as instructed), and a standardised scoring method was followed to generate an average score for each subscale. Average subscale scores range from 0 to 4. The C-BARQ survey was selected for this thesis because it has become an increasingly relied-upon tool for assessing dog behaviour and temperament, and it is commonly applied to the study of human-dog interactions and bonds (e.g. Canejo-Teixeira et al., 2020; González-Ramírez, 2019; Hoffman et al., 2013).

2.2.4 Monash Dog-Owner Relationship Scale (MDORS)

The strength/quality of the relationship between the dogs and owners was assessed using the Monash Dog-Owner Relationship Scale, a 28-item psychometric, self-report questionnaire (MDORS; Dwyer et al., 2006; Appendix 7). Owners responded on a 5-point Likert scale (1 to 5) to a series of questions and statements relating to their i) dog-owner interactions, ii) perceived emotional closeness, and iii) perceived costs of owning a dog. Where instructed, individual item scores were reversed, and the sum was calculated for each of the three subscales. Subscale scores ranged from 9 to 45 for dog-owner interactions and perceived costs, and from 10 to 50 for the perceived emotional closeness. Note, a higher score for perceived costs means the owner believes there is a low level of cost involved with dog ownership.

The MDORS questionnaire has sound theoretical grounding in relation to the benefits and costs of social relationships, as outlined in social exchange theory (Dwyer et al., 2006; reviewed by Rodriguez et al., 2018) and is therefore widely used and considered the most reliable measure of dog-human relationships (Payne et al., 2015). It is important, however, to consider potential biases towards the humans' perspectives of the relationship given the self-report nature of the questionnaire (reviewed by Payne et al., 2015).

2.2.5 Pet Attachment Questionnaire (PAQ)

Owners' attachment to their dog(s) was assessed using the Pet Attachment Questionnaire (PAQ; Zilcha-Mano et al., 2011; Appendix 8). Attachment theory forms a key part of our current understanding of cortisol coregulation (reviewed by Gross and Medina-DeVilliers, 2020; Sbarra and Hazan, 2008), making it important to understand how predictors of dog-owner coregulation strength also influence markers of attachment. Owners were asked to rate how much they agree or disagree with each of the 26 statements listed in the questionnaire. Owner responses were recorded on a Likert scale ranging from *1 = disagree strongly* to *7 = agree strongly*. Half of the questionnaire items relate to the 'avoidant' dimension (defined as the 'maintenance of emotional and cognitive distance' and 'denial of attachment tendencies'), and the other half relate to the 'anxiety' dimension (defined as 'hyperactivating strategies to attain greater proximity and support' and 'worry that this support will not be attained'; Zilcha-Mano et al., 2011). As instructed, the scores for the first item were reversed, and the sums of the scores relating to each dimension were calculated. For both dimensions, the possible range of scores was 13 to 91, with higher scores representing greater avoidance or anxiety.

Chapter 3 - Physical but not emotional closeness predicts cortisol coregulation strength between dogs and owners

Abstract

Coregulation is a biological mechanism in which emotionally attached individuals bidirectionally modulate each other's physiological stress levels. Many similarities exist between dog-owner and parent-child attachment bonds, including the overall dependence of the dog/child on the caregiver (owner/parent) and the coregulation that underpins attachment in both relationships. Currently, predictors of dog-owner cortisol coregulation strength are not well known, but similarities to parent-child coregulation are likely. In parent-child dyads, physical and emotional closeness predict cortisol coregulation strength, and the present study aimed to identify whether markers of physical proximity (owner time away, co-sleeping) and emotional closeness predict cortisol coregulation strength (i.e. degree of correlation in cortisol concentrations) in dog-owner dyads. Questionnaires that assess dog attachment and separation behaviours and owner attachment anxiety were also used to understand how these same measures of physical and emotional closeness influence dog-owner attachment on a behavioural level. As expected, cortisol coregulation was stronger in groups where the owner reported being away less, whilst co-sleeping had no effect. Furthermore, dogs and owners from these groups scored significantly higher for separation-related behaviours and attachment anxiety, indicating that dogs and owners in dyads where the owner is away less may exhibit more of the behavioural characteristics typically associated with attachment, which could explain the stronger cortisol coregulation. Emotional closeness had no effect on dog-owner coregulation. Overall, the findings presented here suggest some similarities in the factors that moderate parent-child and dog-owner cortisol coregulation. Importantly, this study highlights that physical proximity alone (i.e. co-sleeping) may not be sufficient for the maintenance of cortisol coregulation, and that an element of interaction between social partners is required (such as affiliative tactile interactions – cuddling, stroking, grooming). This finding should be investigated further across inter- and intra-specific social dyads as it could have considerable implications for the way we understand the role of cortisol coregulation within social dyads.

3.1 Introduction

The attachment bond is an affectional tie between two individuals and forms as a result of continued stable companionship in which the regularity of positive affiliative behaviours (e.g. grooming, infant care, huddling/cuddling) outweigh negative interactions (Carter, 2005; Fitton, 2012). On a physiological level, these positive interactions can inhibit/buffer the stress response to threats or challenges, and positive feedback of these physical benefits promotes further social interactions between the two individuals (see Chapter 1; Sbarra and Hazan, 2008). Two such physiological mechanisms include i) social buffering, where the presence of one social partner reduces the stress response of the other partner (see Chapter 1; reviewed by Hennessy et al., 2009), and ii) cortisol coregulation in which both individuals bidirectionally modulate each other's hypothalamic-pituitary-adrenal (HPA) axis activity (Davis et al., 2018; Timmons et al., 2015). Understanding social bonds at a physiological level (in particular coregulation) enables us to better understand the mutual, bidirectional affiliations often observed in nature, and allow us to explore their functional and adaptive benefits within an evolutionary context (Carter, 2005).

Attachment bonds are common in parent-infant relationships and, as a result, cortisol coregulation has consistently been observed within the dyad (see Table 1.2 in Chapter 1). Another relationship that bears considerable similarities with parent-infant attachment is that of dogs and owners (reviewed by Payne et al., 2015). Despite being a relationship between individuals of two different species, dogs exhibit the same dependence on their caregiver as infants do, and both dog and owner seek physical proximity and benefit from the characteristic 'safe haven' and 'secure base' effects associated with attachment (reviewed by Payne et al., 2015; Rockett and Carr, 2014). Furthermore, initial evidence suggests that the mechanism of cortisol coregulation is present in dog-owner dyads (see Table 1.4, reviewed by Kikusui et al., 2019), and coregulation in both dog-owner and parent-infant dyads is stronger in periods of high stress or challenge (e.g. *dog agility competitions*, Buttner et al., 2015; *mother exposed to abuse from partner*, Hibel et al., 2009). Given these similarities in the behavioural and physiological properties of attachment in dog-owner and parent-infant dyads, we can expect that further predictors of dog-owner cortisol coregulation strength will be closely aligned to those previously observed in the parent-infant coregulation literature.

One of the earliest identified predictors of cortisol coregulation strength within parent-child dyads is physical proximity (e.g. Neu et al., 2009; Papp et al., 2009). Cortisol coregulation was shown to be stronger when mothers practiced more regular skin-to-skin holding routines of their new-born babies (Mörelius et al., 2015; Neu et al., 2009), as well as spending more time doing shared activities with their adolescent child (Papp et al., 2009). Further to this, separation of mother-child dyads due to the mother's work routine was found to significantly impact cortisol coregulation, with a stronger correlation between mother and child cortisol concentrations on non-workdays, when the individuals are not physically separated from each other (Hibel et al., 2014). Close physical proximity also affects coregulation strength in romantic couples (Laws et al., 2015; Papp et al., 2013; Saxbe and Repetti, 2010).

In addition to physical closeness, the emotional closeness of the relationship between two social partners also predicts the strength of coregulation (e.g. Hibel et al., 2015; Saxbe and Repetti, 2010), whereby coregulation is believed to be a physiological indicator of a dyad's shared emotional responses and experiences (Feldman, 2007; Hibel et al., 2015). Emotional closeness relates to a dyad's ability to recognise, understand, and react to each other's emotional state, and the human literature has demonstrated that cortisol coregulation is stronger in parent-child dyads with more emotionally-sensitive parents (Hibel et al., 2015; Ruttle et al., 2011) and in emotionally-deeper romantic couples (Engert et al., 2018). Individuals' perception of their romantic relationship, in particular how satisfied they are with the relationship, has also been shown to influence the strength of cortisol coregulation (Ha et al., 2016; Laws et al., 2015; Liu et al., 2013; Saxbe and Repetti, 2010). With this in mind, emotional closeness too, in addition to physical closeness, should also influence the strength of dog-owner cortisol coregulation.

The present study investigates how both physical and emotional closeness between dogs and owners affects the strength of cortisol coregulation, using salivary cortisol measurements and questionnaires. Given that cortisol coregulation is stronger in human dyads with closer physical proximity (e.g. Hibel et al., 2014; Laws et al., 2015; see above), I predict that dog-owner cortisol concentrations will be more strongly correlated in dyads where the owner is away less, maintains more physical contact with their dog, and rate the relationship with their dog highly in terms of emotional closeness. Furthermore, given that cortisol coregulation is the mechanism that underpins social attachment bonds (Sbarra and Hazan, 2008), the factors that influence coregulation strength should also moderate the types of attachment behaviours exhibited by the social partners and thus the security and quality of the attachment bond. Physical closeness is one of the four defining features of an attachment bond (Bowlby, 1970) and emotional closeness between two individuals contributes to the safe haven and secure base effect of the relationship (e.g. Carter, 2005), therefore I expect the markers of physical and emotional closeness identified as predicting stronger cortisol coregulation in the first part of this study will also predict increased questionnaire-based scores for i) attachment and separation anxiety in dogs and ii) attachment anxiety in the owners.

3.2 Methodology

3.2.1 Participants

N=25 groups of dogs and owners were recruited (see Table 2.1 in Chapter 2) without specific requirements for participation. N=12 groups contained one dog and n=13 had two dogs. N=18 groups included one owner and n=7 groups included two owners (Table 2.1). In total, data were collected from n=38 dogs (n=16 male, n=22 female) and n=32 owners (n=7 male, n=25 female; Table 2.1). Ethical approval was granted by Swansea University's Animal Welfare and Ethical Review Body (AWERB; Reference IP-1819-02 and CSH-001-2015; Appendix 1) prior to commencing the study.

3.2.2 Saliva sampling schedule, collection, and analysis

Dog and owner saliva samples were collected as per the schedule and methodology outlined in Chapter 2. The total number of days between the collection of the first and last saliva samples was 16 ± 13 days (mean ± SD) on average (Figure 3.1). N=376 of the swabs contained a minimum of 25 μL of saliva for analysis – the other n=225 samples were discarded. Salivary cortisol concentrations were measured using a commercial enzyme immune-assay (EIA) kit (Salimetrics LLC, State College, PA; Item no. 1-3002; sensitive to 0.007 ug/dL), in accordance with the provided protocol (see Chapter 2 for methodology). N=256 of the dog samples contained the 50 μL required for duplicate-well analysis, whilst the remaining n=120 were analysed as singlets. N=6 owner samples (out of n=399) were run as singlets. For plates relevant to the present chapter, the inter-assay coefficients of variance (CV), calculated using duplicate high and low controls, were 5.21 % for the high control and 9.35 % for the low control (n=26 plates). Intra-assay CVs for all n=26 plates were below 12.09 %.

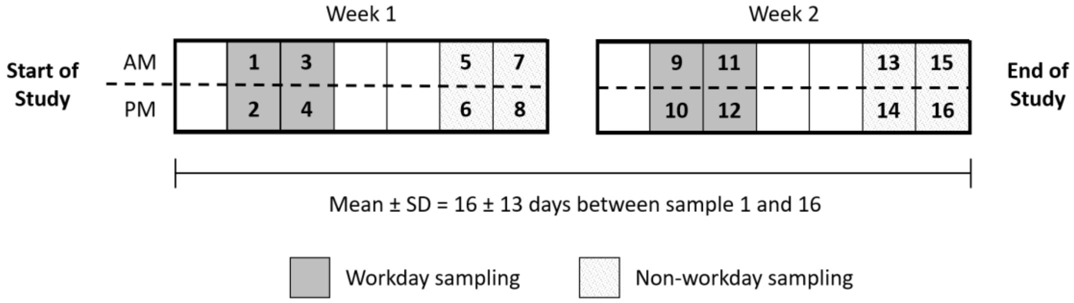


Figure 3.1 Saliva sample collection schedule. Owners collected samples from both themselves and their dog(s). Sampling took place over two weeks (not always consecutive weeks) – a morning (AM) and afternoon/evening (PM) sample was collected from each individual on all n=8 study days, totalling n=16 saliva samples per individual. Sample collection days were spread across workdays and non-workdays (n=4 days each).

3.2.3 Questionnaire data

A study-specific questionnaire was developed to obtain basic information about the dogs and owners (see section 2.2.1), as well as data regarding daily routine and two proxies of physical proximity. First, the owners indicated (chose from one of 7 categories; see Table 3.1 and questionnaire in Appendix 4) how much time they generally spend away from their dog(s) on weekdays (Q15) and weekends (Q16). An average of the upper and lower time estimates for each of the seven categories was calculated. Saliva samples collected on weekdays were matched with the owners' responses to Q15, and weekend samples matched with Q16. Second, owners indicated the regularity with which their dog(s) sleep(s) on the owners' bed (hereafter referred to as 'co-sleeping') using the following categories: 'every night', 'once every few nights', 'once a week', 'once a month', or 'never'. Prior to statistical analysis, the 5 categories were condensed into a 3-level categorical variable to avoid overfitting the linear mixed models (LMMs). The categories 'once every few nights', 'once a week', and 'once a month' were combined into a single level, represented as 'sometimes', making the final three levels: 'every night', 'sometimes', and 'never'.

Table 3.1 Categories used to measure owner time away (hours) in a normal week, and the band average used for statistical analyses.

	Owner Time Away (hours)						
Q15 Weekdays (Mon-Fri)	0	≤ 5	6-15	16-25	26-35	36-45	46+
Average	0	2.5	10.5	20.5	30.5	40.5	NA*
Q16 Weekend (Sat-Sun)	0	≤ 2	3-6	7-10	11-14	15-18	19+
Average	0	1	4.5	8.5	12.5	16.5	19

* no responses in this category, therefore an average was not calculated.

The relationship between dog and owner was assessed using the perceived emotional closeness subscale from the 28-item Monash Dog-Owner Relationship Scale (MDORS; Dwyer et al., 2006; Appendix 7). Ten items within the questionnaire directly assess emotional closeness, which are scored on a 1-5 Likert scale. The sum of the ten item scores is calculated, ranging from 10 to 50, with a high score representing an emotionally closer dog-owner relationship as perceived by the owner (Dwyer et al., 2006).

To understand how physical and emotional closeness influence behavioural markers of attachment in both dogs and owners, subscale scores from the Canine Behavioural Assessment and Research Questionnaire (C-BARQ; Hsu and Serpell, 2003; Appendix 6) and Pet Attachment Questionnaire (PAQ; Zilcha-Mano et al., 2011; Appendix 8) were used. The 'separation-related behaviour' section of the C-BARQ assesses dogs' abnormal behaviours (e.g. shaking, barking/howling, damaging household items, and loss of appetite) *"when left, or about to be left, on its own"* (items 55-62), and the 'attachment and attention-seeking' section reflects dog sociability with and protectiveness of a familiar individual (items 69-74; Hsu and Serpell, 2003). Owners rated how regularly they observe each of the behaviours on a 0-4 Likert scale (0 = never observed, 4 = always observed). Overall scores were calculated as the average of all item scores for each subscale. Owner attachment towards the dog(s) was assessed using the PAQ – specifically the anxiety dimension, which reflects the owners' worries regarding the unavailability of emotional support from their dog (Zilcha-Mano et al., 2011). The sum of the 13 item scores was calculated, and high scores (range from 13 to 91) represent a high degree of owner anxiety.

The study questionnaire, MDORS, and C-BARQ were completed by all n=32 owners, whilst the PAQ was completed by n=28 owners. In two-dog households the owner(s) completed the questionnaire separately for each dog. In groups with two owners, the participants were requested to complete the questionnaires independently from each other. See section 2.2 for further details about each of the questionnaires and the data collection methodology.

3.2.4 Statistical analysis

All statistical analyses were conducted using linear mixed models (LMMs), using the 'lme4' and 'lmertest' packages (Bates et al., 2015; Kuznetsova et al., 2017) in R version 4.1.1 (R Core Team, 2021). All cortisol data were log-transformed, and Q-Q plots and residuals vs. fitted values were used to assess model diagnostics. Additionally, all LMMs were checked for influential data points ('performance' package; Lüdecke et al., 2021) and groups ('influence.ME' package; Nieuwenhuis et al., 2012).

The correlation between dog and owner cortisol concentrations was assessed (LMM1; Table 3.2). Only samples collected in the afternoon (see Figure 3.1) were used for the dog-owner coregulation analysis because of the considerable individual variation in the human cortisol awakening response (CAR; reviewed by Law et al., 2013), and limited evidence of a similar awakening cortisol rhythm in dogs (reviewed by Cobb et al., 2016). With a sample size of n=265 observations, dog saliva cortisol was fitted as the response variable and interactions were fitted between i) time away from dog (continuous variable), ii) regularity of co-sleeping (categorical variable: every night/sometimes/never), and iii) emotional closeness (MDORS subscale score – continuous variable) and owner cortisol concentrations. An interaction between owner cortisol and the number of dogs in the household (categorical variable; one- vs. two-dog household) was also included. Sample analysis method (singlet/duplicate) was controlled for as a fixed effect. Owner cortisol concentration was also included as a random slope term with dyad ID, and group ID was included as a random effect. Non-significant interactions were removed from the final model.

The effect of physical and emotional closeness on dog and owner attachment-related behaviours was assessed using the following response variables: C-BARQ separation (LMM2); C-BARQ attachment and attention-seeking (LMM3); and PAQ attachment anxiety (LMM4; Table 3.2). Owner time away (continuous), regularity of co-sleeping (categorical: every night/sometimes/never), and emotional closeness (continuous) were included as fixed effects, and dog ID and group ID included as random effects.

Table 3.2 Summary of the linear mixed models (LMM) used to assess the effect of physical and emotional closeness on dog-owner cortisol coregulation in PM saliva samples (LMM1) and attachment-related behaviours in dogs and owners (LMM2-4).

Model	**Description**	**Response Variable**	**Fixed Effects**	**Random Effects**
LMM1	Dog-owner cortisol coregulation	Dog cortisol	Owner cortisol*owner time away; owner cortisol*co-sleep; owner cortisol*MDORS score; owner cortisol*household dogs; analysis method	(Owner Cortisol |Dyad ID); Group ID
LMM2	Dog separation behaviours	C-BARQ separation score	Owner time away; co-sleep; emotional closeness	Dog ID; Group ID
LMM3	Dog attachment and attention-seeking behaviours	C-BARQ attachment score	Owner time away; co-sleep; emotional closeness	Dog ID; Group ID
LMM4	Owner attachment anxiety	PAQ anxiety score	Owner time away; co-sleep; emotional closeness	Dog ID; Group ID

3.3 Results

3.3.1 Dog-owner cortisol coregulation

Dog PM salivary cortisol concentrations ranged from 0.015-0.916 μg/dL with a median of 0.113 μg/dL, and owner cortisol concentrations ranged from 0.012-1.540 μg/dL with a median of 0.108 μg/dL. Average owner time away was 20.4 hours (min = 2.5 hours, max = 40.5 hours) for the weekday period (Mon-Fri) and 3.5 hours (min = 0 hours, max = 19 hours) for the weekend (Sat-Sun). The interaction between owner cortisol and owner time away was significant (LMM1: estimate ± se = -0.01 ± 0.003, t = -2.70, p = 0.008; Table 3.3; Figure 3.2), indicating that the positive relationship between dog and owner salivary cortisol concentrations is stronger in groups where the owner reports being away less (Figure 3.2).

Table 3.3 Summary of LMM1 (n=265 from n=25 groups) following the removal of the non-significant interactions. All statistically significant results are shown in bold, and p-values between 0.05 and 0.1 are italicised.

Model	**Predictor Variable**	**Estimate ± se**	**p-value**
LMM1 Dog salivary cortisol			
Fixed Effects	Intercept	-1.42 ± 0.48	
	log(Owner Cortisol)	0.21 ± 0.06	**0.002**
	Owner time away	-0.02 ± 0.01	**0.015**
	Emotional closeness	-0.01 ± 0.01	0.238
	Co-sleep *sometimes*	0.32 ± 0.21	0.142
	Co-sleep *every night*	0.41 ± 0.22	*0.080*
	Household dogs *one-dog*	0.01 ± 0.19	0.960
	Analysis *singlet*	-0.08 ± 0.07	0.267
	log(Owner Cortisol):Owner Time Away	-0.01 ± 0.003	**0.008**
Random Effects		χ^2	**p-value**
	log(Owner Cortisol)|Dyad ID	38.40	< **0.001**
	Group ID	41.63	< **0.001**

One influential observation was identified by the ‘performance’ package in LMM1 (from group 32; see Table 2.1); removal of the influential data point did not affect the results in LMM1 (estimate ± se = -0.01 ± 0.003, t = -2.80, p = 0.006). The 'influential.ME' package found no influential groups within LMM1.

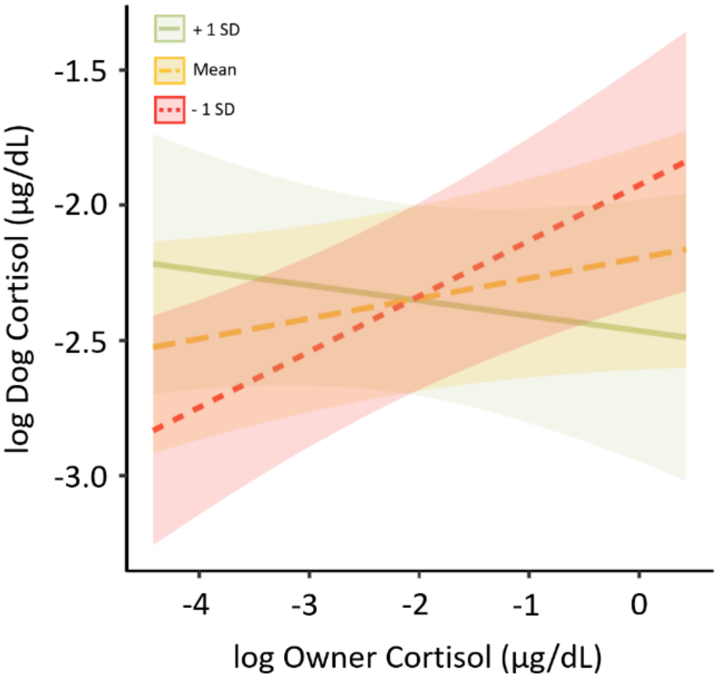


Figure 3.2 Predictive interaction plot for the effect of owner time away on the correlation between dog and owner cortisol concentrations (LMM1; plotted using 'interactions' R package; Long, 2019). Shown are three levels of the moderator: mean, one standard deviation below the mean, and one standard deviation above the mean, indicating stronger dog-owner cortisol coregulation in dyads where owners are away less.

3.3.2 Physical and emotional closeness linked to attachment-related behaviours

Dog separation-related behaviours (e.g. shaking, barking/howling, loss of appetite) were significantly lower in groups where the owner reports being away for more hours across an average week (LMM2: estimate ± se = -0.01 ± 0.01, t = -2.188, p = 0.035; Figure 3.3A) and having an emotionally closer relationship with their dog (LMM2: estimate ± se = -0.04 ± 0.01, t = -3.03, p = 0.005; n=48; Figure 3.3B). Regularity of co-sleeping did not predict dog separation-related behaviours. Dogs showed more attachment and attention-seeking behaviours (e.g. seeking and maintaining physical proximity) where owners spent more time away in an average week (LMM3: estimate ± se = 0.02 ± 0.01, t = 2.13, p = 0.042; Figure 3.3C), but no effect on attachment and attention-seeking was found for emotional closeness (LMM3: estimate ± se = -0.01 ± 0.02, t = -0.77, p = 0.450) or co-sleeping regularity (LMM3:

estimate ± se = 0.32 ± 0.37, t = 0.86, p = 0.398; n=48). Owners' attachment anxiety towards their dog(s) (i.e. worries regarding the emotional availability of their dog) was greater with greater time away from the dog(s), but this trend was not statistically significant (LMM4: estimate ± se = 0.16 ± 0.08, t = 1.91, p = 0.064; n=43; Figure 3.3D).

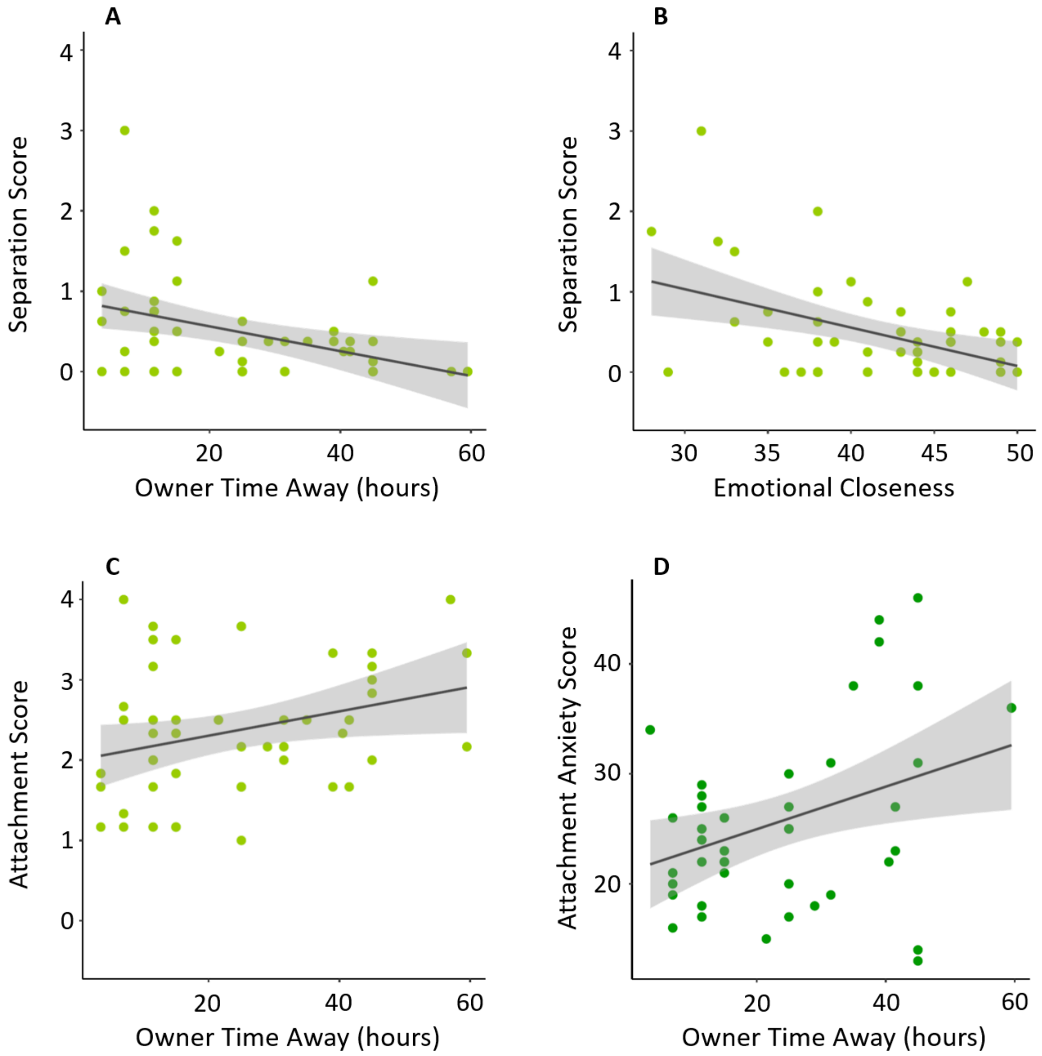


Figure 3.3 Relationship between dog separation-related behaviours (C-BARQ separation scores) and A) owner time away (hours), and B) MDORS emotional closeness (n=48 from n=25 groups), and C) the relationship between dog attachment and attention seeking behaviours (C-BARQ attachment scores) and owner time away (n=48 from n=25 groups). Sub-scale scores range from 0 to 4. D) Relationship between owners' time away (hours) in an average week and their PAQ attachment anxiety score (ranges from 13 to 91; n=43 from n=21 groups). Light-green data points represent dog attachment markers (C-BARQ separation and attachment) and dark-green points represent owner attachment (PAQ attachment anxiety). The black line represents the predicted effect of owner time away/emotional closeness, and the 95 % confidence intervals are shown by the shaded area.

3.4 Discussion

Cortisol coregulation is crucial for the maintenance of human attachment bonds (Davis et al., 2018; Sbarra and Hazan, 2008; Timmons et al., 2015), and preliminary studies of coregulation in non-human animals (e.g. Burkett et al., 2016; Buttner et al., 2015; Fürtbauer and Heistermann, 2016) have demonstrated that the predictors of coregulation (e.g. periods of high stress/challenge) closely align with the human literature (reviewed by Davis et al., 2018; Timmons et al., 2015). Akin to the human parent-infant relationship, dog-owner dyads represent a good model for cortisol coregulation research because of their strong attachment (Meehan et al., 2017; Payne et al., 2015), in addition to the complex human-directed sociocognitive capabilities of companion dogs (e.g. Cordoni and Palagi, 2019; Lazzaroni et al., 2020). Therefore, research into coregulation between dogs and their owners could improve our understanding of this interspecific attachment bond, as well as contribute to our wider understanding of cortisol coregulation. As dogs and children are both highly reliant on their caregiver (Payne et al., 2015; Prato-Previde and Valsecchi, 2014), factors that influence the strength of parent-child coregulation, such as physical proximity (e.g. Hibel et al., 2014; Neu et al., 2009) and emotional closeness (e.g. Hibel et al., 2015; Saxbe and Repetti, 2010), should also influence dog-owner coregulation. The present study investigated the effects of physical and emotional closeness on dog-owner cortisol coregulation strength and attachment-related behaviours in dogs and owners.

3.4.1 Physical closeness

In the present study, greater dog-owner cortisol synchrony was observed in groups where the owner is away for fewer hours on average. This result is comparable to the stronger mother-infant coregulation observed on non-workdays when mothers and infants spend more time together (Hibel et al., 2014), and further demonstrates how predictors of dog-owner cortisol coregulation closely align with those identified in the human literature (e.g. Davis et al., 2018; Hibel et al., 2014; Papp et al., 2009). Closer physical proximity has repeatedly been reported as a predictor of stronger cortisol coregulation, whether it be in the form of postpartum holding of new-borns (Mörelius et al., 2015; Neu et al., 2009), spending more time together and doing activities together (Hibel et al., 2014; Papp et al., 2009; Saxbe and Repetti, 2010), or cohabitation with a spouse (Laws et al., 2015; Papp et al., 2013). Greater physical proximity as a result of less owner time away likely increases dog-owner coregulation strength as a result of individuals' HPA axes being exposed to the same cues and stimuli within a shared environment, whilst weaker/absent coregulation in more physically distant dyads results from experiencing unrelated stimuli in different environments (Papp et al., 2013, 2009; Saxbe and Repetti, 2010). However, the regularity with which the dog sleeps on the owner's bed – another measure of physical proximity – did not affect coregulation strength.

Taken together, the differing effects of owner time away and co-sleeping on dog-owner cortisol coregulation suggest that physical proximity alone is not enough to facilitate stronger coregulation, and an element of social interaction is required. Having likely evolved through a mutual need for protection, dog-owner co-sleeping fulfils the 'safe haven' criteria of attachment theory and inhibits separation-related behaviours for both individuals (Smith et al., 2017). However, whilst regular co-sleeping is widely considered as being a marker of an especially close dog-owner relationship (Blouin, 2012; Voith et al., 1992), the evidence directly linking this behaviour to measures of attachment is mixed. Martens et al. (2016) identified higher attachment scores in owners whos' dog slept in their bedroom, but Harter (2018) found no differences in attachment. Additionally, no effect on dog separation-related behaviours has been observed (Flannigan and Dodman, 2001; McCrave, 1991). This absence of a clear link between co-sleeping and attachment further suggests that the maintenance of attachment bonds requires more than physical proximity. To develop a better understanding of the role social interaction plays in cortisol coregulation, future studies should measure dyads' coregulation strength across varying experimental conditions (e.g. i) individuals in separate rooms, ii) together but not interacting, iii) together with interaction).

The presence of a second cohabiting dog did not affect dog-owner coregulation, unlike results obtained from hair cortisol coregulation analysis (see Chapter 5). This difference in the findings is likely caused by the varying time-scales represented in saliva (acute) and hair (chronic) cortisol concentrations (Burnard et al., 2017; see Chapter 2). Therefore, the long-term cortisol measure obtained from hair samples is more suited to studying stable factors within the dyads' social environment, such as cohabitation with a second dog (see Chapter 5).

As well as affecting physiological coregulation, owner time away was found to influence behavioural markers of dog-owner attachment, which is to be expected given that cortisol coregulation underpins attachment bonds (see Chapter 1; Sbarra and

Hazan, 2008). Separation anxiety in response to owner absence is a commonly observed attachment-related behaviour in companion dogs (Buckland et al., 2014; Payne et al., 2015; Prato-Previde and Valsecchi, 2014; Verga and Michelazzi, 2009) however, in contrast to my prediction and previous studies (e.g. see Rehn and Keeling, 2011), separation scores were higher for dogs whos' owner was away less. It is possible that, as the measure of time away represents a normal weekly average, dogs with owners who are away for longer periods of time become habituated to this and develop a coping mechanism whereby separation behaviours are exhibited less frequently/not at all. In turn, this could weaken the dog-owner attachment bond as greater owner absence limits the opportunities for the dog to be reliant on the owner (Payne et al., 2015; Topál et al., 1998). When the owner is away for fewer hours, on the other hand, the dog may not develop this coping mechanism because they are more familiar with the owner being present. By being away less, the owner has the opportunity to interact more with the dog, thereby forming a more secure, dependable attachment bond, which is then accompanied by more severe separation behaviours (Schöberl et al., 2016). Alternatively, with n=15 out of the n=25 groups having at least one other human in the household who did not take part in the study, it is not known whether the participating owners' time away estimate represents a period in which the dogs were home alone or in the presence of another cohabiting owner. Future research should record the presence of all human cohabitants so as to understand how they may impact dog-owner coregulation when the primary owner is absent.

In owners, more time away from the dog(s) was associated with higher attachment anxiety scores, suggesting that more physically absent owners have more worries about the emotional support they receive from their dog(s) (i.e. attachment anxiety). Leaving dogs for a prolonged period of time (whether home alone or supervised by a different owner) has been shown to create feelings of regret and guilt in owners, which can lead to considerable anxiety (Norling and Keeling, 2010). In line with attachment theory (Ainsworth and Bowlby, 1991; Bowlby, 1970), it is likely that this anxiety will impact on the dog-owner attachment bond as owners may perceive their absence as a barrier preventing them from forming an emotionally secure attachment with their dog, and could subsequently manifest as separation anxiety disorder in the owner (Dowsett et al., 2020). Given that separation anxiety contributes towards the formation and functioning of attachment bonds (Ainsworth and Bowlby, 1991; Bowlby, 1970), it follows that factors affecting the degree of separation anxiety should affect the underlying mechanism of attachment – cortisol coregulation – although contextual factors would be key to understanding the direction of this effect (Sbarra and Hazan, 2008).

Overall, based on the findings discussed above, owners' time away from their dog(s) persistently seems to influence both the behavioural and physiological markers of dog-owner attachment. This suggests that, as expected, physical proximity is as important in dog-owner dyads as it is in human parent-infant and spousal dyads (reviews by Davis et al., 2018; Timmons et al., 2015). Importantly, however, these results are correlational and therefore do not imply causation, and further research is required to directly measure how physical proximity, particularly owner time away, influences cortisol coregulation and attachment. A study design such as that used by Rehn and Keeling (2011) – whereby dogs and owners are separated for set durations of time (e.g. 0.5 h, 2 h, 4 h) and saliva samples are collected immediately prior to separation and upon reuniting – would assist in further understanding how the mechanism of coregulation functions before, during, and after these periods of separation.

3.4.2 Emotional closeness

The owners' perceived emotional closeness of the relationship with their dog(s) did not predict the degree of synchrony in dog and owner cortisol concentrations. This is surprising given the well-reported influence that relationship satisfaction and emotional depth/closeness has on human dyad physiological coregulation (e.g. Engert et al., 2018; Laws et al., 2015; Saxbe and Repetti, 2010; see Introduction). Additionally, dogs in the present study tended to score higher for separation-related behaviours when emotional closeness within the dog-owner dyad was lower, indicating a link between emotional closeness scale and dog-owner attachment on a behavioural level.

One possible explanation for this absence of an effect in the cortisol coregulation analysis is the self-report style of the MDORS questionnaire, whereby all responses are solely from the owner's perspective of the relationship (Rodriguez et al., 2018). The questionnaire score does not account for how the dogs experience the relationship. This potential bias could be removed by introducing objective, independent observations of dyadic interactions across a select number of controlled, experimental situations to accompany the scores from the questionnaire (Rodriguez et al., 2018). Alternatively, the recruitment process for the study may also introduce bias – individuals who are emotionally closer with their dog and who place more importance on the relationship they have with their dog may be more likely to volunteer for a study in which they will learn more about this relationship (e.g. Dwyer et al., 2006; van Lange et al., 2011). For example, whilst the full range of scores for the MDORS emotional closeness scale is 10 to 50, participant responses in this study range from 28 to 50, and only n=31 observations (out of the n=265 observations in the coregulation analysis) ranked on the lower end of this scale with a score below 35. To counter this bias, a reward/compensation could be offered so as to incentivise participation across a broader variety of individuals (e.g. see Flom et al., 2017).

3.5 Conclusion

Previous research has demonstrated the negative effect that long periods of separation from the owner can have on dog physical and psychological wellbeing (e.g. Rehn and Keeling, 2011), and the present findings demonstrate that certain markers of physical proximity also affects physiological linkage and attachment-related behaviours in dog-owner dyads. As in the human coregulation literature (Davis et al., 2018; Timmons et al., 2015), greater owner time away (i.e. less physical proximity) predicts weaker coregulation in dog and owner salivary cortisol concentrations. However, to fully understand whether stronger coregulation with greater physical proximity positively or negatively influences the relationship, more must be understood about the dyad’s environmental and social context (Sbarra and Hazan, 2008). For example, whilst more time together may lead to a more secure attachment bond in which the individuals are better able to load-share the energetic costs of daily living (Coan, 2008; Coan and Sbarra, 2015), it could also represent a stressful context in which the owner is hyper-attached to their dog and does not provide the dog with sufficient space (e.g. Hill et al., 2020; Mota-Rojas et al., 2021; equivalent to coregulation between romantic couples under high emotional strain; Saxbe and Repetti, 2010; Timmons et al., 2015). Once we have a better understanding of the function of cortisol coregulation, being aware of the factors that predict the strength of coregulation in dog-owner dyads, such as owner time away, could then be applied to improving companion dog welfare and understanding how dog and owner behaviours interact to influence overall dyad wellbeing (e.g. Payne et al., 2015).

It is not, however, just the dog-owner bond that closely mirrors the human parent-infant attachment bond – close social relationships between parents and infants exist across the animal kingdom (see review by Bales, 2017), and we can therefore expect the requirements for attachment (and, thus, cortisol coregulation) to be met in many non-human species. This study builds on previous non-human animal cortisol coregulation research (see Burkett et al., 2016; Buttner et al., 2015; Cunningham, 2017; Fürtbauer and Heistermann, 2016) and further highlights the similarities that exist in human and non-human coregulation. It is possible that research across many different non-human study systems (e.g. alloparenting and maternal/paternal/biparental care) could be invaluable in developing a more holistic understanding of the mechanism of cortisol coregulation across social vertebrates.

Chapter 4 - Salivary cortisol coregulation between cohabiting companion dogs is moderated by owner time away

Abstract

Recent evidence of cortisol coregulation between dogs and owners demonstrates that dogs can experience a shared physiological state with another individual. Further to this, attachment bonds have been shown to form in dog-dog dyads. Taken together, this presents the possibility for dog-dog cortisol coregulation, given that coregulation is the physiological mechanism that underpins attachment bonds. The present study investigated salivary cortisol coregulation in dog-dog dyads (n=7) and tested for a potential moderating effect of owner absence. Results indicate that cortisol coregulation is present in dog-dog dyads, and greater synchrony in the dogs' cortisol concentrations was found when owners spent more time away. During an owner's absence, the cohabiting dogs may interact more or maintain closer physical proximity to each other. Alternatively, increased strength of cortisol coregulation could be a result of the owner's absence creating a stress-inducing context, under which the mechanism of cortisol coregulation is used as a coping mechanism by the dogs – however no elevation in cortisol was observed with greater time away. Overall, despite the small sample size, this study provides further evidence that cortisol coregulation is a biological mechanism that not only exists in humans but across social vertebrates more generally. Furthermore, the moderating effect of owner absence on dog-dog coregulation highlights the potential importance of external social influences on the functioning of cortisol coregulation, which has rarely been incorporated into previous coregulation studies. Individuals regularly maintain multiple attachment bonds with different social partners simultaneously, therefore, understanding the interplay between these different bonds on a physiological level will be key to developing a more realistic representation of attachment and coregulation in dyads' naturalistic home environments.

4.1 Introduction

Over the last 30,000 years, dogs have evolved closely alongside humans, and artificial selection for traits such as tameness, docility, and sociality has resulted in the companion dogs we are familiar with today (Cordoni and Palagi, 2019; Driscoll et al., 2009). The process of domestication is believed to have enhanced dogs' receptiveness to social cues, particularly those of humans, which in turn contributes to the formation of dog-human attachment bonds (Payne et al., 2015; Udell et al., 2010). As in human parent-infant dyads and romantic couples (reviews by Davis et al., 2018; Timmons et al., 2015), the attachment between dog and owner is underpinned by cortisol coregulation via a positive feedback loop (see Chapter 1; Sbarra and Hazan, 2008). Dog-owner cortisol coregulation has been measured consistently across a number of studies (Chapter 3; Buttner et al., 2015; Cunningham, 2017; Ryan et al., 2019; Sundman et al., 2019). This mechanism of synchronised physiology is thought to be a product of a mutation in the dog's hypothalamic-pituitary-adrenal (HPA) axis caused by the domestication process (Kikusui et al., 2019), suggesting that dogs may only coregulate with humans.

However, in recent years, the first examples of cortisol coregulation in non-human dyads have been presented (*Microtus ochrogaster*, Burkett et al., 2016; *Gasterosteus aculeatus*, Fürtbauer and Heistermann, 2016), indicating that cortisol coregulation may be a mechanism that is shared across social vertebrates and may have already existed in wolves (*Canis lupus*) prior to domestication. Despite being studied as part of inter-specific dyads with their owners, there is, to my knowledge, no study measuring intra-specific cortisol coregulation in dog-dog dyads. This is surprising given that dogs are a species descended from the wolf, in which strong familial and pack bonds form regularly, monogamy is the predominant mating system, and biparental care of offspring is common (Feddersen-Petersen, 2007). The social network that exists within multi-dog households is also known to be highly complex, with dominance hierarchies often established (Dantas de Castro, 2017; Mariti et al., 2017; Trisko et al., 2016; Wallis et al., 2020), as well as the occurrence of behaviours such as play and jealousy (Mehrkam and Wynne, 2021; Prato-Previde et al., 2018). Additionally, dogs have been shown to exhibit separation behaviours, such as increased vocalisation, maintaining proximity to the exit door, and stereotypic behaviours (e.g. pacing, repetitive grooming), when separated from a familiar conspecific (Mariti et al., 2018; Walker et al., 2014). Together, there is growing evidence for characteristics of attachment in cohabiting dog dyads (Cimarelli et al., 2019; Mariti et al., 2017, 2014; Sipple et al., 2021). Added to our increasing understanding of the physiological underpinnings of attachment relationships (Carter, 2005; Sbarra and Hazan, 2008), this suggests that cortisol coregulation is likely to occur between cohabiting dogs.

In this study, I test for evidence of salivary cortisol coregulation within cohabiting dog dyads and test for a potential moderating effect of owner time away from the dogs. Whilst physical proximity has regularly been shown to predict the strength of cortisol coregulation – with stronger coregulation being observed when individuals spend more time together (Chapter 3; Hibel et al., 2014; Laws et al., 2015; Papp et al., 2013, 2009; Saxbe and Repetti, 2010) – this factor is unlikely to influence dog-dog coregulation given that cohabiting dogs are rarely separated and have the same daily routine as each other (e.g. Sipple et al., 2021). It is possible, however, that the amount of time that the owner is away from their dogs does influence the strength of dog-dog coregulation. In addition to the complex intra-specific social interactions between dogs in multi-dog households (see above; Mariti et al., 2017), the dogs also form attachment bonds with their owner(s) and will benefit from different, but comparable, inter-specific dog-owner social interactions (Cimarelli et al., 2019; Sipple et al., 2021). Therefore, in households where the owner spends a longer duration of time away from their dogs, the absence of dog-owner interactions and the potential increase in dog-dog interactions may strengthen the attachment bond that forms between the two dogs (Sipple et al., 2021). This is especially true if the absence of the owner presents a social context in which separation behaviours are triggered and the dog subsequently seeks companionship from their cohabiting conspecific (Cimarelli et al., 2019). The findings could have important implications for how we approach companion dog care and ensure that the living environment is as suitable and stress-relieving as possible (see Sonntag and Overall, 2014).

Whilst separation behaviours can be stress-inducing for the individuals (reviewed by Sherman and Mills, 2008), some dog-dog relationships have been found to be stress-alleviating (Cimarelli et al., 2019), so cohabiting dogs may be more likely to form a stress-alleviating bond in households where the owner is away more often compared to dogs in households where the owner is away less. With this in mind, I predict that the cortisol concentrations of dogs in groups where the owner is away more will be more stable and may decrease across the day, as measured using the dogs' percentage change in cortisol across the study day.

4.2 Methodology

4.2.1 Study animals

All dogs (*Canis lupus familiaris*) included in this study were privately owned, and consent for their participation was obtained from the owner prior to commencing sample collection. Ethical approval was granted by Swansea University's Animal Welfare and Ethical Review Group (Reference IP-1819-02 and CSH-001-2015). Only dogs that lived in a household with a second dog were considered, and households with more than two dogs were discounted given the complex interactions and hierarchies that can develop (Dantas de Castro, 2017; see Table 2.1 in Chapter 2). In total, n=7 dog dyads were included in the study. Of these n=14 dogs, n=7 were male and n=7 were female, and ages ranged from 2 to 11 years. The sample contained a variety of dog breeds.

4.2.2 Sample schedule, collection, and analysis

Owners collected dog saliva samples using Salivettes (Sarstedt, Leicester, UK) twice daily across eight study days (split equally between two weeks) under naturalistic home conditions (Figure 4.1; for more details on the study schedule and sample collection process, see Chapter 2). N=150 samples received from the owners contained the minimum 25 μL of saliva required for analysis – all other samples were discarded (n=84). Sample analysis was conducted as described in Chapter 2 using a commercially available enzyme immune-assay (EIA) kit (Salimetrics LLC, State College, PA; Item no. 1-3002). N=110 samples were analysed in duplicate, whilst n=40 were run as singlets using the 25 μL that was retrieved from the Salivette swab. Inter-assay coefficients of variation (CV) were calculated using duplicate high and low controls, and were 6.3 % and 9.5 % respectively for the n=12 plates used for these samples. The intra-assay CV for all 12 plates was under 11.8 %.

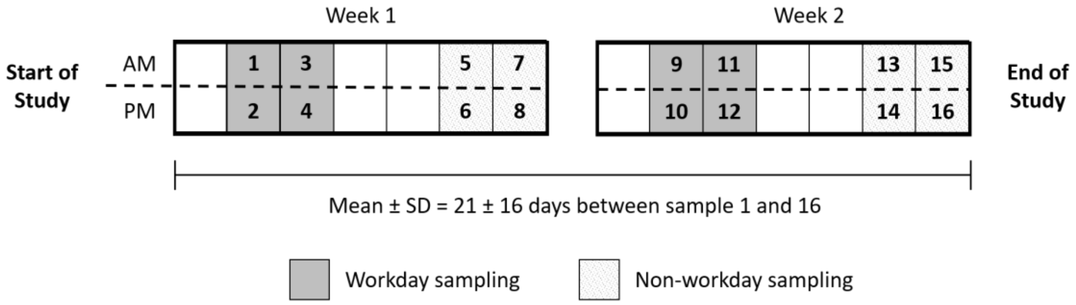


Figure 4.1 Saliva sampling schedule. Owners collected n=16 samples from each dog, resulting in n=32 samples per dyad. Samples were collected twice per study day (AM and PM). Study days were evenly distributed between owner workdays and non-workdays.

4.2.3 Owner time away

The owners of the dog dyads completed a 30-item questionnaire designed specifically for the present study (Appendix 3). Questions 15 and 16 asked the owner to estimate how many hours they are away from their dogs during a normal week (Mon-Fri) and weekend (Sat-Sun) respectively, choosing one of seven provided time bands. Owner time away was converted into a continuous variable by taking the average of the upper and lower time estimates for each time band (see Table 3.1 in Chapter 3). Where two-owners participated in the study, an average of their time estimates was calculated for both Q15 and Q16.

4.2.4 Statistical analysis

Using the ‘lme4’ and ‘lmertest’ (Bates et al., 2015; Kuznetsova et al., 2017) packages in R version 4.1.1 (R Core Team, 2021), linear mixed models (LMMs) were used to determine the effect of owner time away on dog-dog coregulation and dog daily cortisol rhythms. Log-transformations accounted for the non-normality of the cortisol data, and model diagnostics were performed using Q-Q plots and standardised residuals vs. fitted values. The presence of possible influential observations and dyads was checked using the ‘performance’ (Lüdecke et al., 2021) and ‘influence.ME’ (identified using Cook’s distance; Nieuwenhuis et al., 2012) packages respectively.

LMM1 assessed the effect of owner time away on dog-dog coregulation in time-matched samples using an interaction between partner dog cortisol concentration and owner time away (continuous variable ranging from 1 to 40.5 hours), with dyad ID included as a random slope term with partner dog cortisol. Dog sex and age had no effect on LMM1 and were subsequently removed to improve the fit of the model according to Akaike Information Criterion (AIC) (Bozdogan, 1987). The analysis method for the saliva sample (duplicate vs. singlet; see Chapter 2) was controlled for as a categorical fixed effect. For the time away data, the day of the week that the samples were collected was matched to the owners’ estimation of time away specific to the weekdays and weekend (see section 4.2.3).

Where there were matching morning and afternoon samples for a single study day, percentage change in cortisol from AM to PM was calculated to assess how owner time away influenced dog daily cortisol rhythms (n=60 data points). LMM2 included percentage change in cortisol for both the focal and partner dogs as the response variable and owner time away as a continuous fixed effect (ranging from 1 to 40.5

hours). Sample analysis method was included as a categorical variable (any percentage change measures made up of at least one singlet cortisol measure were labelled as ‘singlet’). Individual ID and group ID were included as random effects.

4.3 Results

Salivary cortisol concentrations ranged from 0.030 µg/dL to 1.365 µg/dL, with a median concentration of 0.101 µg/dL (n=150 samples, n=14 dogs). A non-significant trend was found for a small effect of the interaction between owner time away and partner dog cortisol (LMM1: estimate ± se = 0.01 ± 0.01, t = 1.84, p = 0.071; Table 4.1; Figure 4.2), suggesting stronger dog-dog coregulation when owners spend more time away.

Table 4.1 Summary of the output from LMM1 for the effect of partner dog cortisol and owner time away on focal dog cortisol concentrations (n=75 observations from n=7 groups). Significant p-values are in bold, and those between 0.05 and 0.1 are italicised.

Model	**Predictor Variable**	**Estimate ± se**	**p-value**
LMM1 Focal dog cortisol			
Fixed Effects	Intercept	-1.71 ± 0.20	
	log(Partner Dog Cortisol)	0.21 ± 0.11	0.142
	Owner Average Time Away	0.03 ± 0.02	**0.047**
	Analysis *singlet*	-0.05 ± 0.12	0.662
	log(Partner Dog Cortisol):Owner Average Time Away	0.01 ± 0.01	*0.071*
Random Effects		χ^2	**p-value**
	log(Partner Dog Cortisol)|Dyad ID	49.72	< **0.001**

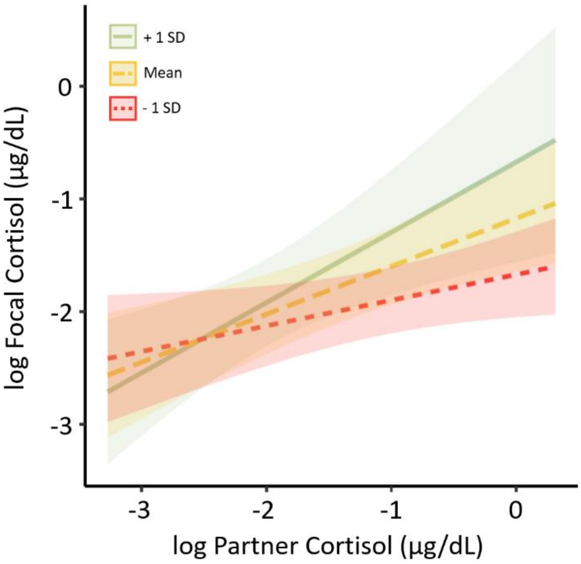


Figure 4.2 Interaction plot for the effect of partner dog cortisol on focal dog cortisol, moderated by owner time away at three levels: mean, one standard deviation below the mean, and one standard deviation above the mean (plotted using 'interactions' R package; Long, 2019).

Owner time away did not predict the dogs' change in cortisol from morning to afternoon (LMM2: estimate ± se = 0.45 ± 0.48, t = 0.93, p = 0.357, n=60 observations; Figure 4.3).

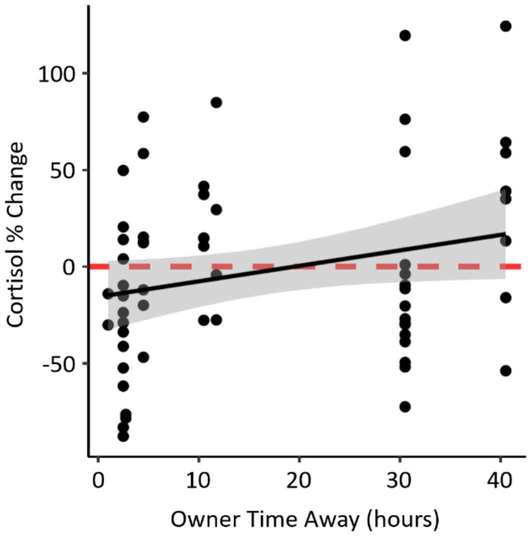


Figure 4.3 Correlation between the daily percentage in dogs' cortisol concentrations and owner time away (hours). The solid black line and the surrounding shaded area represents the predicted effect of owner time away on dog cortisol % change and the 95 % confidence intervals. The dashed red line indicates no change in cortisol – points above this line show an increase in cortisol from AM to PM, whilst points below represent a decrease in cortisol across the day.

4.4 Discussion

Despite the growing number of dog-owner coregulation studies (Buttner et al., 2015; Cunningham, 2017; Sundman et al., 2019) and the wealth of literature exploring dog sociality and attachment with a conspecific (e.g. Mariti et al., 2017; Sipple et al., 2021; Trisko et al., 2016), there are, to my knowledge, no studies measuring cortisol coregulation in dog-dog dyads. The aim of the present study was to test for cortisol coregulation between cohabiting companion dogs and to test for an effect of owner presence on the strength of dog-dog cortisol coregulation. I predicted stronger coregulation when owners were away more, given that prolonged absence of the owner could promote more interactions and a more secure attachment between cohabiting dogs (Cimarelli et al., 2019). Despite the small sample size, the present study provides evidence for cortisol coregulation between cohabiting companion dogs. This, together with the two other studies investigating cortisol coregulation in non-human animal dyads (three-spined sticklebacks: Fürtbauer and Heistermann, 2016; prairie voles: Burkett et al., 2016) suggests that cortisol coregulation is more widespread across vertebrates.

As predicted, cortisol coregulation tended to be stronger in dog dyads where owners spent more time away from the dogs, suggesting that owner routine may affect physiological linkage between dogs. To date, most coregulation research has focused on dyadic characteristics as predictors of coregulation strength, and do not consider the potential impact of other individuals in the surrounding environment (reviews by Davis et al., 2018; Meyer and Sledge, 2020; Timmons et al., 2015; for an exception see Pauly et al. (2021), showing that the wider political context of the area that a romantic couple live in can influence the extent of cortisol synchrony).

The stronger coregulation between cohabiting dogs when the owner is away more is most likely caused by the dogs having longer periods of time together uninterrupted by interactions with the owner. These periods of owner absence could represent greater physical proximity between the two dogs, which is a key predictor of cortisol coregulation (Chapter 3; Hibel et al., 2014; Papp et al., 2009); but they may also influence the strength of dog-dog coregulation through a change in the type of social interactions available to the dogs. When both the owner and cohabiting conspecific are present, the dog has a choice of social partners and will choose which individual to interact with based on the immediate surrounding context (Cimarelli et al., 2019; Sipple et al., 2021). Whilst the attachment that forms between dogs and owners is often compared to that of parents and children (Meehan et al., 2017; Payne et al., 2015; Sipple et al., 2021), adaptation of a human sibling behavioural ethogram for dog-dog interactions suggests that cohabiting companion dogs form a bond more attuned to sibling attachment (Sipple et al., 2021). As a result, dogs experience different benefits from interactions with their owner and conspecific, such as more reference and information-seeking benefits and greater attachment security in dog-owner interactions, and it is these differences that form the basis for the choice of who to interact with at a given time (Cimarelli et al., 2019; Sipple et al., 2021). However, when the owner is absent, the dog no longer has a choice between a parent-like or sibling-like attachment. Therefore, perhaps in an attempt to limit boredom and loneliness (Burn, 2017), the dog may shift its focus to interactions with their conspecific, which develops into a more secure, functional dog-dog attachment bond and stronger cortisol coregulation (Sbarra and Hazan, 2008).

Alternatively, cohabiting dogs may rely on one another as a coping mechanism during periods of owner absence, which could also explain the effect of owner time away on dog-dog coregulation. Dog owners' role as a caregiver means that they represent a key figure in their dog's life (Payne et al., 2015), and the absence of the owner can be a significant stressor for dogs (Buckland et al., 2013). Separation anxiety in response to owner absence is a common problem for companion dogs and can manifest in many forms of stress-related behaviours (e.g. barking/howling, shaking, whining; Ogata, 2016; Scaglia et al., 2013). Therefore, the stronger dog-dog cortisol coregulation in groups where the owner is away more could be a result of the owners' absence being a stressful event for the dogs, which leads the dogs to seek the 'safe-haven' and potentially stress-alleviating-effects (Cimarelli et al., 2019) of their cohabiting conspecific under such contexts to minimise the negative impacts associated with separation anxiety. Previous coregulation literature demonstrates that cortisol concentrations of human and non-human social partners tend to be more synchronised in a stressful context (Burkett et al., 2016; Fürtbauer and Heistermann, 2016; Ouellette et al., 2015; Saxbe et al., 2015). Based on the evidence available in this study, there is no link between owner time away and the dogs' percentage change in cortisol, which could indicate that the dogs have become habituated to owner absences (e.g. Müller et al., 2012) given the regularity and predictability surrounding the owners' work schedules (most owners in the study held full-time employment). However, a more specific study design (e.g. collecting dog saliva samples during periods of separation and reunion; similar to Rehn and Keeling, 2011) is required to test the effect of owner time away on dog cortisol concentrations, such as those employed by Ryan et al. (2019) and Shin and Shin (2016) who do observe a link between dog cortisol and dog-owner separation.

Before we can confidently interpret the possible stress-alleviating role of dog-dog cortisol coregulation, we must first develop a sound understanding around the role of a second dog during owner absences (e.g. are they a support or stressor?; Stephan et al., 2021). Whilst the company of a second dog is widely believed to ameliorate the negative impacts associated with dogs being left alone (see Coren, 2021; Dreschel and Granger, 2005; Mariti et al., 2014; Tiira and Lohi, 2015), the first empirical study to directly compare singly- and co-housed dogs' behavioural responses to being home alone observed more signs of anxiety in multi-dog households (e.g. vocalisations and less resting; Stephan et al., 2021), and higher dog hair cortisol concentrations were observed in two-dog households in Chapter 5. Aggression between cohabiting dogs is also relatively common (Casey et al., 2013). Following on from this, future cortisol coregulation studies should focus on directly measuring the link between dog-dog coregulation and owner time away. This could be achieved by having more precise measures of owner time away specific to each study day, making comparisons with days when the owner does not leave the dogs (see Hibel et al., 2014), and incorporating video-based behavioural analysis to identify and quantify separation-related behaviours and/or dog-dog interactions when home alone (see Stephan et al., 2021). This is a key avenue of research that should continue to be pursued given that it could have considerable ramifications for how we approach the housing and living arrangements of dogs and other companion animals, and therefore work to improve companion animal welfare (see Sonntag and Overall, 2014).

The results presented in this study demonstrate how factors external to the dyad, here the presence of the owner (i.e. caregiver), could influence the strength of cortisol coregulation – an area which has received relatively little attention in the existing coregulation literature (but see Pauly et al., 2021). Additionally, given the analogous nature of dog-dog attachment bonds to human sibling bonds, we could also test whether these findings extend to human dyad coregulation by considering whether sibling cortisol coregulation is affected by the presence/absence of a parent/caregiver (for a review of sibling attachment, see Whiteman et al., 2011). For both cohabiting dogs and human siblings, the attachment bonds formed within these dyads have a key bearing on the way the individuals socialise and form bonds outside of their immediate household/family (e.g. Brumbaugh, 2017; Sipple et al., 2021; Tiira and Lohi, 2015), and the application of coregulation analysis to these “sibling” bonds can help us further understand how individuals rely on different social partners to share the energetic costs of daily life (Coan and Sbarra, 2015).

4.5 Conclusion

By providing the third example of intra-specific cortisol coregulation in a non-human animal species, this study adds to the growing body of literature indicating that coregulation is a physiological mechanism shared among social vertebrates (Burkett et al., 2016; Fürtbauer and Heistermann, 2016). Expanding the scope of coregulation research beyond humans presents the opportunity to study the mechanism across a variety of different social systems and environmental contexts, and potentially develop a clearer understanding of the functional role of coregulation (see review by Timmons et al., 2015). Further to this, future coregulation studies should also begin to consider the importance of external social influences on cortisol coregulation strength (but see Pauly et al., 2021) given the potential moderating effect of owner absence on dog-dog coregulation shown here. Social networks in human and non-human animals are highly complex (Krause et al., 2015), and individuals maintain many attachment bonds simultaneously with multiple social partners (e.g. Lewis, 2005; Saxbe et al., 2014; Sipple et al., 2021). Therefore, as coregulation underpins these attachment bonds (Sbarra and Hazan, 2008), it is key that we consider how external influences, such as the presence of another social partner, affect the strength and role of coregulation within social dyads.

Chapter 5 - Hair cortisol synchrony in dog-owner and dog-dog dyads

Abstract

Cortisol coregulation has been studied extensively across a variety of species, but mostly using acute salivary cortisol concentrations. Integration of hair cortisol analysis (a measure of chronic HPA-axis activity) into coregulation research, however, presents an opportunity to measure how the long-term social context influences the strength of cortisol coregulation. The relationship between dogs and their owners where more than one dog is present in the household provides opportunity to investigate inter- (dog-owner) and intra-specific (dog-dog) attachment bonds simultaneously using physiological samples. Here, I used hair samples and attachment questionnaire data (from the Canine Behavioural Assessment & Research Questionnaire; C-BARQ) collected from dogs (n=45) and owners (n=38) in one-dog (n=15) and two-dog households (n=15). In two-dog households, dog-dog coregulation was also investigated. Although based on a small sample, the results suggest that cortisol coregulation occurred in one- but not in two-dog households, and provided some evidence to suggest coregulation occurred between cohabiting dogs. Additionally, dogs in one-dog households scored higher on the C-BARQ attachment scale compared to dogs in two-dog households, indicating dogs from one-dog households exhibit more attachment-related behaviours. The absence of dog-owner coregulation in two-dog households is unexpected (see Chapter 3). It is possible this result is due to a combination of the complex multi-species social network in two-dog households and the limited number of hair samples per group make it difficult to distinguish dog-owner coregulation using a measure of chronic cortisol. Whilst based on non-significant trends, these findings contribute to existing literature and provide further support for the use of hair cortisol analysis as an important tool in the study of coregulation. Additionally, taken together with the findings from Chapter 3, the results provide preliminary evidence indicating the varying temporal nature (i.e. acute vs. chronic) of the predictors of coregulation strength.

5.1 Introduction

When social partners regularly interact and form a close attachment bond, regulation of this attachment occurs on a physiological level through coregulation (Sbarra and Hazan, 2008). Research over the last two decades has demonstrated that cortisol coregulation is a mechanism present across a variety of species, such as humans (*Homo sapiens*; e.g. Chapter 6; Davis et al., 2018; Saxbe and Repetti, 2010; Timmons et al., 2015), prairie voles (*Microtus ochrogaster*; Burkett et al., 2016), three-spined stickleback fish (*Gasterosteus aculeatus*; Fürtbauer and Heistermann, 2016), and dogs (*Canis lupus familiaris*; Chapter 4); and interspecific dog-owner (Chapter 3; Buttner et al., 2015; Cunningham, 2017; Ryan et al., 2019; Sundman et al., 2019) and horse-rider dyads (*Equus caballus*; Strzelec et al., 2013). Comparatively less research has gone into understanding how different endocrinological techniques and biological sample types could be used to enhance our understanding of cortisol coregulation.

The majority of cortisol coregulation studies have used short-term measures of cortisol concentrations (obtained from saliva, blood, and water samples; see Tables 1.1-1.3 in Chapter 1), however, very few studies have addressed long-term aspects of cortisol coregulation (e.g. Broeks et al., 2021; Ouellette et al., 2015; see Tables 1.1-1.3). Long-term cortisol concentrations can be obtained from hair samples, as cortisol produced during the growth phase accumulates in the hair shaft (Sheriff et al., 2011; Stalder and Kirschbaum, 2012). Depending on the hair growth rate and species, hair cortisol concentrations (HCCs) can cover a period of months, which is considerably longer than the single time-point measures (at point of collection) provided by saliva and blood samples (Russell et al., 2012; Sheriff et al., 2011; Stalder and Kirschbaum, 2012). Whilst mostly associated with the stress response, cortisol continuously circulates in the bloodstream due to its function in metabolism and energy distribution (reviewed by Lee et al., 2015). As a result, the acute measure of cortisol obtained from saliva and blood samples can vary considerably due to natural daily patterns in cortisol production and metabolism, as well as in response to acute stress events (reviewed by Burnard et al., 2017). The key benefit of hair cortisol analysis is that the resultant measure of hypothalamic-pituitary-adrenal (HPA) axis activity is unaffected by acute daily fluctuations in cortisol concentrations, therefore making it a more reliable measure of chronic cortisol concentrations (reviewed by Burnard et al., 2017).

Given that cortisol coregulation research generally concerns the study of well-established, long-term social relationships (e.g. Papp et al., 2013, 2009; Pratt et al., 2017; Saxbe and Repetti, 2010; but see Fürtbauer and Heistermann, 2016), incorporating a chronic measure of cortisol introduces the possibility of studying and understanding coregulation across a longer period of time. Recent studies have used hair cortisol concentrations to consider predictors of coregulation strength that are more stable (chronic) factors within the study dyads (e.g. Broeks et al., 2021; Ouellette et al., 2015). For example, hair cortisol has been used to understand the role coregulation plays in pre- and post-natal child development (Broeks et al., 2021; Flom et al., 2017), how a child's attention-deficit/hyperactivity disorder (ADHD) symptoms can influence coregulation strength with their mother (Schloß et al., 2019), as well as the effects of long-term (post-natal) maternal stress/depression, psychological disorders, and parenting styles (Broeks et al., 2021; Liu et al., 2017; Ouellette et al., 2015; Tarullo et al., 2017).

Hair cortisol synchrony has also been studied in dog-owner dyads, revealing mixed results (Höglin et al., 2021; Sundman et al., 2019). In a sample of Shetland sheepdogs and border collies, Sundman et al. (2019) demonstrated that dog-owner HCC coregulation was stronger in the summer; when the dyad included a female dog; and in competition dogs compared to pet dogs. Social factors such as the presence of other dogs and the owners' work status did not have any effect (Sundman et al., 2019). Höglin et al. (2021), in contrast, found no such association in HCCs for a sample of ancient and hunting dog breeds. These studies present interesting insight into HCC coregulation in dog-owner dyads, but further research is required to validate the presence of HCC coregulation in dog-owner dyads and subsequently build upon these initial findings to better understand the role that hair cortisol analysis can play in advancing coregulation research.

Chronic cortisol measures from hair samples have the potential to be particularly helpful in understanding how the social environment on a dyad influences the strength of coregulation. The social behaviour of dogs is highly complex, and there is added complexity in multi-dog households through the combination of, and interchange between, dog-owner and dog-dog interactions (Cimarelli et al., 2019; Sipple et al., 2021). Dogs in one-dog households, on the other hand, socialise predominantly with their owner(s) (with potential for short-term interactions with conspecifics when outside of the house, e.g. Ottenheimer Carrier et al., 2013), resulting in an overall reliance of singly-housed dogs on their owner to meet their social needs (Marinelli et al., 2007). Based on attachment theory, this has the potential to increase the regularity of support-seeking behaviours from dogs in one-dog households, thus promoting the formation of a more secure dog-owner bond and stronger physiological coregulation (Sbarra and Hazan, 2008). Differences in social behaviour have been observed in dogs from single- and multi-dog households; singly-housed dogs tend to show more signs of fear and anxiety (Savalli et al., 2019), can exhibit more behaviours associated with separation anxiety (Harvey et al., 2016; but see Stephan et al., 2021), and attachment behaviours have been found to be stronger due to the increased care and attention from the owner (Marinelli et al., 2007). Additionally, lower HCCs previously observed in dogs from one-dog households (Bennett and Hayssen, 2010) introduce the possibility that these differences in attachment may be reflected on a physiological level and could further influence the mechanism that regulates attachment – cortisol coregulation.

The present study investigates the effect of social environment, here the presence of a second cohabiting dog, on dog and owner HCCs and the level of HCC synchrony within the dog-owner dyads. Despite the mixed results in the existing literature (Höglin et al., 2021; Sundman et al., 2019), I expect the positive correlation in dog and owner salivary cortisol observed in Chapter 3 to be reflected also in the chronic measure of HPA axis activity. Whilst Chapter 3 found no effect of a second cohabiting dog on dog-owner coregulation, here I predict stronger dog-owner cortisol coregulation in one-dog households compared to two-dog households. This is because acute salivary cortisol concentrations may be less suited to investigating the effect of a long-term factor such as living with another dog. For example, Christensen et al. (2022) demonstrated in chacma baboons (*Papio ursinus*) that urinary glucocorticoid metabolite concentrations (represent HPA axis activity for a time-window of up to a few hours) were predicted by short-term circadian factors such as weather, whilst faecal glucocorticoid metabolite concentrations (time window of up to a few days) were affected by long-term factors such as reproductive state and changes in day length. Similar to this comparison of urinary and faecal glucocorticoid patterns (Christensen et al., 2022), the different time-scales of saliva (acute) and hair (chronic) cortisol concentrations mean that individual, environmental, and social characteristics are likely to influence the two cortisol measures differently depending on whether the characteristics trigger short- or long-term activation of the HPA axis (reviewed by Burnard et al., 2017; Russell et al., 2012; Sheriff et al., 2011). Using questionnaire data, I further test the prediction that dog-owner attachment is stronger in one- compared to two-dog households (e.g. Marinelli et al., 2007). In addition, building on the identification of dog-dog salivary cortisol coregulation in Chapter 4, I also expect that the regular social interactions and close relationship formed between cohabiting dogs (Cimarelli et al., 2019; Sipple et al., 2021) will be reflected in synchrony of chronic HPA axis activity in the dog-dog dyads of two-dog households.

5.2 Methodology

5.2.1 Participants

Hair cortisol data were collected from n=38 owners (n=10 male, n=28 female) and n=45 dogs (n=19 male, n= 26 female), from one-dog (n=15) or two-dog households (n=15) (see Table 2.1 in Chapter 2), on two separate occasions, resulting in n=137 hair samples. N=4 additional groups did not provide hair samples, but completed the relevant questionnaires for the questionnaire-based analysis of the present study (n=1 one-dog and n=3 two-dog households; Table 2.1).

5.2.2 Hair sample collection and analysis

Hair was collected twice per individual, and the time between sample-1 and sample-2 varied per group depending on the schedule of the owner (mean ± SD = 36 ± 29 days, ranged from 14 days to 121 days; Figure 5.1).

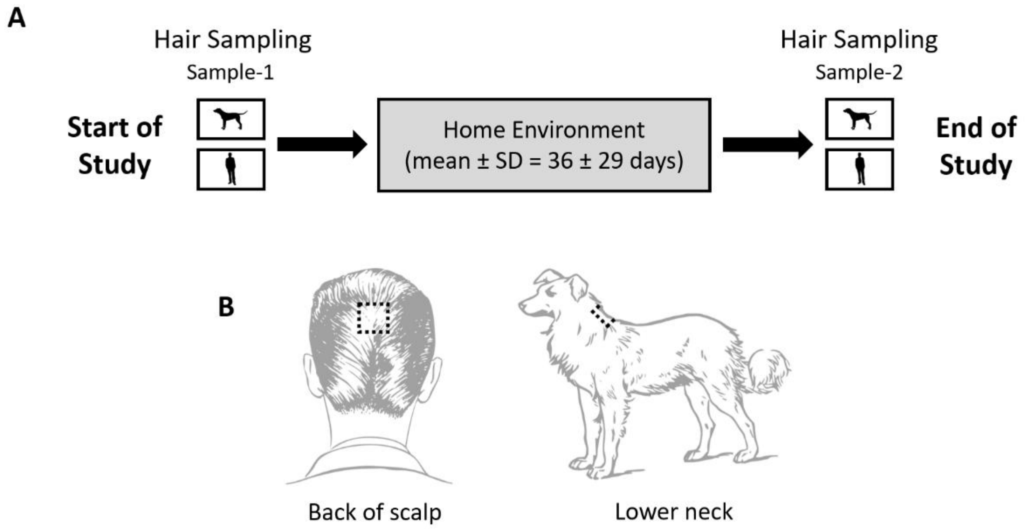


Figure 5.1 (A) Hair sample collection schedule. Dog and human hair samples were collected on two occasions (Sample-1 and Sample-2). (B) Body location of hair sample collection for owners and dogs (denoted by the dotted line).

The method for preparing the hair samples for analysis is outlined in Chapter 2. The average weight of powdered hair sample used for incubation was 0.021 ± 0.005 g (mean ± SD, n = 137). Samples were analysed by enzyme immunoassay (EIA; Salimetrics LLC, State College, PA). High and low quality controls were included in duplicate on each plate and used to calculate the inter-assay CVs which were 7.2% and

17.8%, respectively for the n=5 plates used for hair sample analysis. The intra-assay CV was 7.4 ± 3.7 % (mean ± SD; n=5 plates). The cortisol concentrations of n=2 samples were below the limit of detection, leading to a final sample size of n=135.

5.2.3 Questionnaire data

The dog-owner attachment bond was assessed using two self-report questionnaires. Owner attachment was assessed using the Pet Attachment Questionnaire (PAQ; Appendix 8; Zilcha-Mano et al., 2011) which is scored on two dimensions – i) 'attachment avoidance'; defined as the maintenance of cognitive and emotional distance from your pet, and ii) 'attachment anxiety'; defined as seeking greater proximity and support from your pet, and worry that this type of relationship may not be attained. Each dimension has 13 items and are scored on a 1 ('*disagree strongly*') to 7 ('*agree strongly*') Likert scale, with possible scores ranging from 13 to 91 (high scores represent greater avoidance/anxiety).

The dogs' attachment behaviours were assessed using the 'Attachment and Attention-Seeking' section of the Canine Behavioural Assessment and Research Questionnaire (C-BARQ; Hsu and Serpell, 2003 ; https://vetapps.vet.upenn.edu/cbarq/). Owners were asked to rate how often their dog(s) exhibited the attachment and attention-seeking behaviours outlined in the six items (e.g. following owner, seeking proximity, agitation when not receiving attention) in the recent past. The Likert scale ranged from 0 ('*never*') to 4 ('*always*'). Total scores were calculated as the average score for all six items, with higher scores indicating greater need for physical proximity and more attention-seeking behaviours.

The study-specific questionnaire (see Appendix 4) was used to determine owner time away. Scores for Q15 and Q16 were averaged as per Table 3.1 (see Chapter 3 methodology), and weekly time away was obtained by calculating the sum of Q15 (Mon-Fri time away) and Q16 (Sat-Sun time away).

Questionnaires could be completed at any point during the study. Owners with two dogs were asked to complete a separate questionnaire for each dog. Where two owners from the same household participated in the study, both owners were asked to complete the questionnaires and to not share their responses with each other.

5.2.4 Statistical analysis

Data was analysed using linear mixed models (LMMs) in R version 4.1.1 (R Core Team, 2021; package 'lme4', Bates et al., 2015; package 'lmerTest', Kuznetsova et al., 2017). Where necessary, HCC were log-transformed to achieve normality, and Q-Q plots and standardised residuals vs. fitted values were used for model diagnostics. The 'performance' package identified influential data points within the LMMs (Lüdecke et al., 2021), and influential groups were detected by Cook's distance using the 'influence.ME' package (Nieuwenhuis et al., 2012). Where influential data points/groups were detected, the analysis was re-run following their removal. This process was repeated until no further influential data points/groups were identified and, in instances where results changed, new model outputs are reported alongside the original results.

First, I investigated basic predictors of variation in HCC for dogs (LMM1; Table 5.1) and humans (LMM2; Table 5.1) to understand which variables should be controlled for in the coregulation models. Both models included individual ID as a random effect, and LMM1 also included group ID as a random effect (inclusion of group ID in LMM2 caused singularity issues, and was subsequently removed from the final model). Fixed effects were sex (male/female; categorical variable), age (continuous variable), social context (one-dog/two-dog household; categorical variable), number of cohabiting humans (continuous variable; 0-3 humans), and the presence of children under 18 years old (yes/no; categorical variable). As coat colour has been shown to affect dog HCC (Bennett and Hayssen, 2010), the dog model (LMM1) also included coat colour (light/mixed/dark; categorical variable). The effect of dog breed was non-significant and Akaike Information Criterion (AIC) (Bozdogan, 1987) estimated a better fit of LMM1 with breed excluded from the model. The presence of hair dye in the human samples was initially included as a categorical variable in LMM2 (see Camille Hoffman et al., 2014), but was non-significant in the model and reduced the overall fit of the model based on AIC, so was subsequently removed.

Second, to test for cortisol coregulation between dogs and owners, I used two LMMs including HCC (LMM3; Table 5.1) and percentage change in HCC between the two sampling points (LMM4; Table 5.1) as response variables. Collecting samples at two separate time points allowed me to investigate changes in HCC across the study and test for a correlation in dog and owner HCC % changes. This trend in HCC from sample-1 to sample-2 allows me to consider whether the directionality and steepness of dog and owner HCC change are correlated, given the seasonal effects observed by Sundman et al. (2019). To meet LMM normality assumptions, a constant was added to the percent change data and then log-transformed. Dyad ID (LMM3) and group ID (LMM3 and LMM4) were included as random effects. An interaction between social context (one- versus two-dog household) and owner HCC was included to test for the possible moderating effect of a second dog on dog-owner coregulation. Owner time away was initially included as an interaction with owner HCC (given the effect it has on dog-owner and dog-dog coregulation in Chapters 3 and 4), but was subsequently removed as it had no effect on dog HCC and decreased the fit of the model (measured using AIC). Where appropriate, post-hoc analyses were run separately for one- and two-dog households.

Third, to test for HCC coregulation in dog-dog dyads, a linear mixed model was used (LMM5; Table 5.1). Spearman's rank correlation tested for a correlation between dogs' percentage change in HCC between the two sampling points.

Lastly the questionnaire data was analysed to determine whether dog and owner attachment scores differed between one- and two-dog households. Questionnaire data from n=4 groups who could not provide hair samples, but took part in the wider study, was also included (n=6 individuals; see Table 2.1 in Chapter 2) Separate linear mixed models were run with PAQ attachment anxiety (LMM6; Table 5.1; Zilcha-Mano et al., 2011) and log-transformed PAQ attachment avoidance (LMM7; Table 5.1; Zilcha-Mano et al., 2011) as the response variable and household social context as the only fixed effect. Owner ID and group ID were included as random effects. For the C-BARQ 'attachment and attention-seeking' scores, a Mann-Whitney U test was used to determine a difference across the two social contexts. In groups with two participating owners, the C-BARQ attachment scores of both owners were correlated (Spearman's rho = 0.51, p = 0.065, n = 14), so an average of the two owners was used for the analysis.

Table 5.1 Summary of the linear mixed models (LMMs) used to analyse the predictors of hair cortisol and the presence of cortisol coregulation in dog-owner and dog-dog dyads, along with the effect of social context on questionnaire attachment scores.

Model	Description	Response Variable	Fixed Effects	Random Effects
LMM1	Predictors of dog hair cortisol	Dog HCC	Dog sex; dog age; household social context; cohabiting humans; children; coat colour	Dog ID; Group ID
LMM2	Predictors of owner hair cortisol	Owner HCC	Owner sex; owner age, household social context; cohabiting humans, children	Owner ID
LMM3	Dog-owner HCC coregulation	Dog HCC	Owner HCC*household social context	Dyad ID; Group ID
LMM4	Dog-owner HCC % change coregulation	Dog HCC % change	Owner HCC % change*household social context	Group ID
LMM5	Dog-dog HCC coregulation	Focal dog HCC	Partner dog HCC	Group ID
LMM7	Attachment and social context	PAQ anxiety score	Household social context	Owner ID; Group ID
LMM6	Attachment and social context	PAQ avoidance score	Household social context	Owner ID; Group ID

5.3 Results

5.3.1 Predictors of dog and owner HCC

Dog HCC ranged from 4.33 pg/mg to 32.98 pg/mg, with a median of 11.99 pg/mg (n=77 samples). Dogs in one-dog households tended to have lower HCCs than dogs from two-dog households, but this was not statistically significant (LMM1:

estimate ± se = -0.22 ± 0.11, t = -2.00, p = 0.057; Table 5.2; Figure 5.2). Sex, age, coat colour, the number of humans living in the household, and presence of children did not predict dog HCC (see 'LMM1' in Table 5.2). Significant differences in HCCs were identified at an individual-level (LMM1: p = 0.002, n=67 samples; Table 5.2), and were close to significant at the group-level (LMM1: p = 0.078, n=67 samples; Table 5.2).

Table 5.2 Factors affecting the hair cortisol concentrations (pg/mg) of dogs (LMM1; n=67 samples, n=35 individuals) and owners (LMM2; n=53 samples, n=29 individuals). Dog and owner HCCs were log-transformed to meet the normality assumptions of the LMM. Significant results are shown in bold, and near-significant results are italicised.

Model	**Predictor Variable**	**Estimate ± se**	**p-value**
LMM1 Dog HCC			
Fixed Effects	Intercept	2.48 ± 0.18	
	Sex *male*	0.04 ± 0.09	0.698
	Age	-0.02 ± 0.02	0.320
	Coat *light*	-0.06 ± 0.11	0.545
	Coat *mixed*	0.10 ± 0.18	0.596
	Social Context *one-dog*	-0.22 ± 0.19	*0.057*
	Cohabiting Humans	0.07 ± 0.09	0.440
	Children *yes*	0.08 ± 0.14	0.606
Random Effects		χ^2	**p-value**
	Dog ID	9.90	**0.002**
	Group ID	3.10	*0.078*
LMM2 Owner HCC			
Fixed Effects	Intercept	1.95 ± 0.45	
	Sex *male*	0.21 ± 0.29	0.480
	Age	0.003 ± 0.01	0.662
	Social Context *one-dog*	-0.41 ± 0.21	*0.061*
	Cohabiting Humans	-0.06 ± 0.18	0.736
	Children *yes*	0.26 ± 0.33	0.439
Random Effects		χ^2	**p-value**
	Owner ID	17.57	**< 0.001**

Owner HCCs ranged from 1.64 pg/mg to 80.04 pg/mg and the median was 6.20 pg/mg (n=58 samples). Owner HCCs tended to be lower for owners living in a one-dog household, but this effect was not significant (LMM2: estimate ± se = -0.41 ± 0.21, t = -1.97, p = 0.061; Table 5.2; Figure 5.2). Age and sex of the owner, the number of cohabiting humans, and the presence of children did not affect owner cortisol concentrations (see 'LMM2' in Table 5.2). Individual differences in owner HCC were found (LMM2: p < 0.001, n=53 samples; Table 5.2).

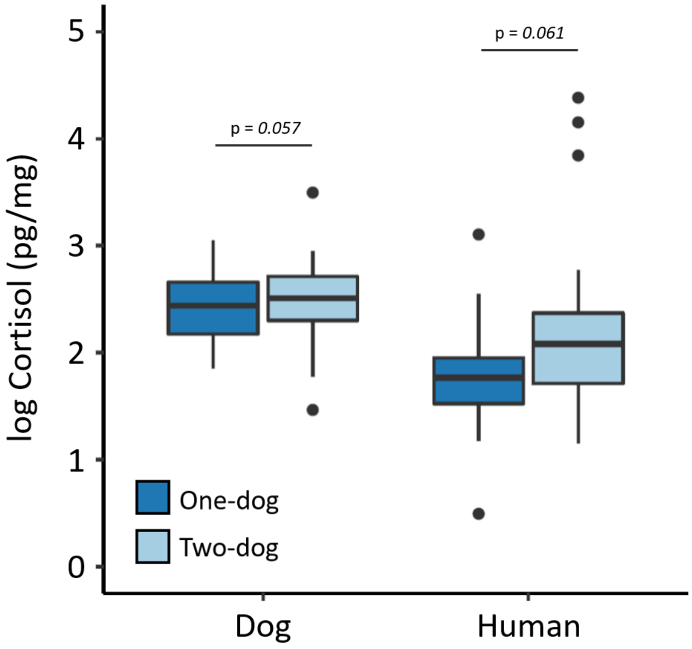


Figure 5.2 Effect of social context (one- vs. two-dog household) on dog and human HCCs (pg/mg). The bold line represents the median, and the box and whiskers show the interquartile range and the minimum and maximum cortisol concentrations. The statistical difference in cortisol concentrations between one- and two-dog households is indicated by the p-values above the dog (LMM1) and human (LMM2) boxplots.

5.3.2 Dog-owner cortisol coregulation

The interaction term testing the effect of social context (one- vs. two-dog household) on the correlation between dog and owner cortisol concentrations was non-significant (LMM3: estimate ± se = 0.18 ± 0.12, t = 1.48, p = 0.143; Table 5.3). Removal of the non-significant interaction revealed no correlation between dog and owner HCCs (LMM3: estimate ± se = 0.04 ± 0.05, t = 0.84, p = 0.404).

Table 5.3 Summary of LMM3, which tests for an interaction between owner HCC and the social context of the household (one- vs. two-dog). Significant p-values are shown in bold.

Model	**Predictor Variable**	**Estimate ± se**	**p-value**
LMM3 Dog HCC			
Fixed Effects	Intercept	2.49 ± 0.14	
	log(Owner HCC)	0.002 ± 0.06	0.973
	Social Context *one-dog*	-0.43 ± 0.25	0.090
	log(Owner HCC):Social Context	0.18 ± 0.12	0.143
Random Effects		χ^2	**p-value**
	Dyad ID	14.70	**< 0.001**
	Group ID	6.55	**0.011**

Following visual inspection of the plotted slopes for dog-owner cortisol correlation in one- and two-dog households (Figure 5.3A), separate post-hoc LMMs were run for each household type, as detailed in the methodology (section 5.2.4). A non-significant trend for a positive correlation between dog and owner HCCs was found in one-dog households ($\text{LMM3}_{\text{one-dog}}$: estimate ± se = 0.21 ± 0.11, t = 1.93, p = 0.065; Figure 5.3A), but not in two-dog households ($\text{LMM3}_{\text{two-dog}}$: estimate ± se = 0.003 ± 0.06, t = 0.05, p = 0.958; Figure 5.3A). No influential data points were detected by the performance package, however, the influence.ME package identified two influential groups in $\text{LMM3}_{\text{one-dog}}$ (groups 7 and 12; Table 2.1), and once these were removed the correlation between dog and owner cortisol concentrations was not significant (estimate ± se = 0.18 ± 0.12, t = 1.49, p = 0.152).

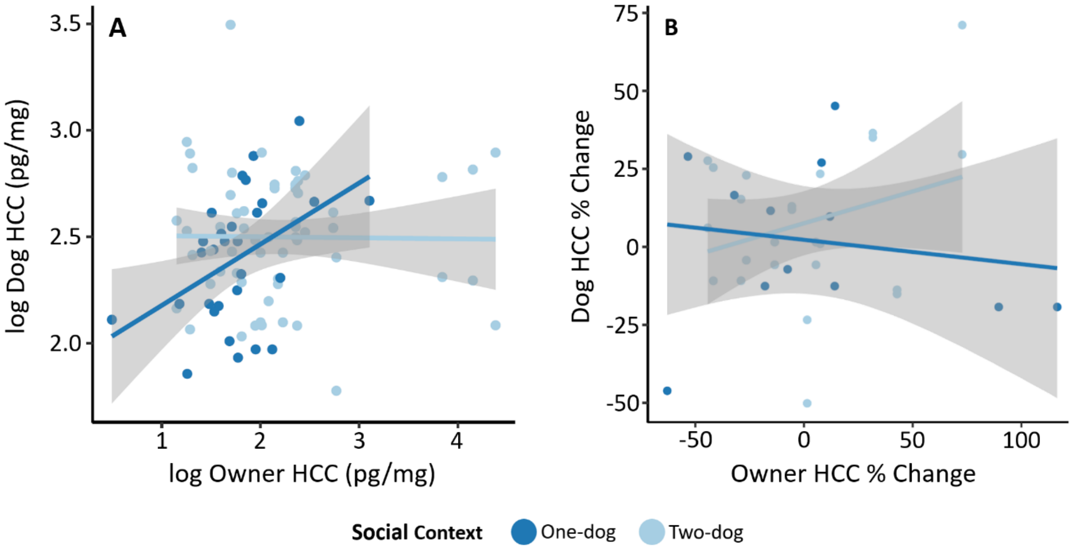


Figure 5.3 The effect of household type (one- versus two-dog) on the relationship between dog and owner (A) time-matched HCC and (B) percentage change in HCC between the two sampling points. The lines represent the predicted effect of owner HCC on dog HCC, and the shaded area demonstrates the 95 % confidence intervals.

Dog and owner percentage change in cortisol showed no interaction with social context (LMM4: estimate ± se = 1.13 ± 13.96, t = 0.08, p = 0.937; Figure 5.3B). Groups 7 and 12 were again highlighted as influential within LMM4, but removal of the groups did not change the results. Following removal of the interaction, there was no correlation between dog and owner % change in HCCs (LMM4.2: estimate ± se = 7.15 ± 5.83, t = 1.23, p = 0.234).

5.3.3 Dog-dog cortisol coregulation

Time-matched HCC of the two dogs in two-dog households were not correlated (LMM5: estimate ± se = 3.46 ± 2.04, t = 1.70, p = 0.105; Figure 5.4A). A non-significant trend for a positive correlation between the dogs' HCC % change from sample-1 to sample-2 was found (Spearman's rho = 0.60, p = 0.056, n = 11, Figure 5.4B).

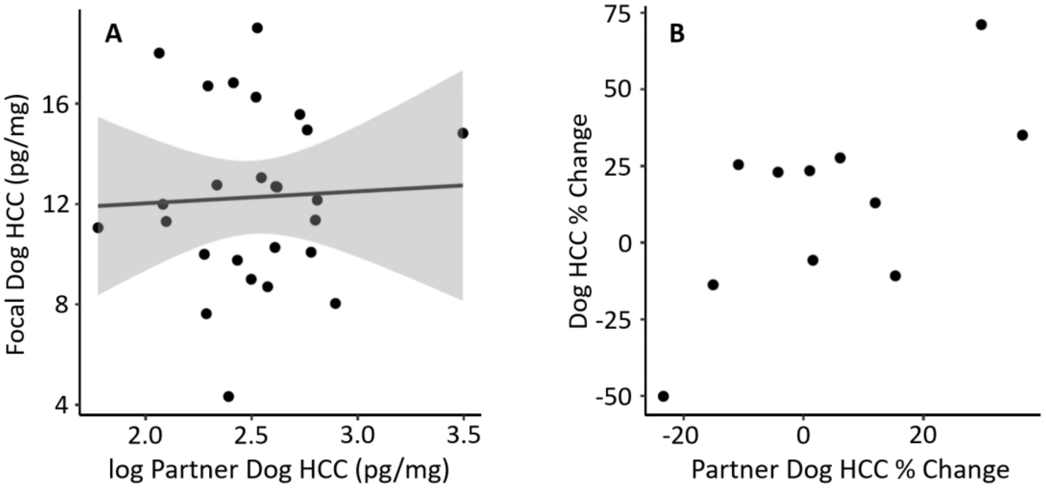


Figure 5.4 Relationship between focal and partner dog (A) time-matched HCC (n=25), and (B) the percentage change in HCC between the sampling points (n=11). The line in (A) represents the predicted effect of partner dog HCC on focal dog HCC, and the shaded area shows the 95 % confidence intervals.

5.3.4 Dog-owner attachment

Neither the PAQ attachment anxiety (LMM6: estimate ± se = -3.59 ± 2.66, t = -1.35, p = 0.184, n=49) or attachment avoidance (LMM7: estimate ± se = -0.07 ± 0.12, t = -0.55, p = 0.585, n=49) dimensions differed significantly between one- and two-dog households. C-BARQ attachment scores tended to be higher in one-dog households than in two-dog households, but this difference was non-significant (W = 125.5, p = 0.061, n=42, Figure 5.5).

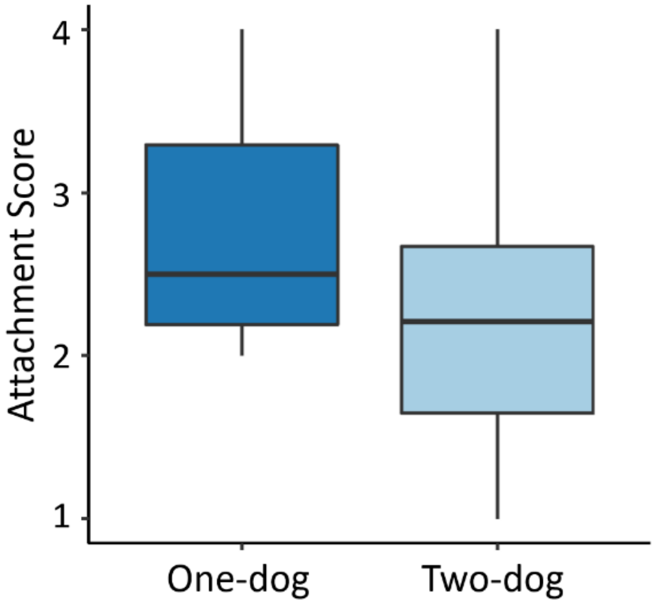


Figure 5.5 Difference in C-BARQ attachment scores between one- and two-dog households. The bold line represents the median, and the box and whiskers show the interquartile range and the minimum and maximum reported scores.

5.4 Discussion

Cortisol coregulation is a biological mechanism involved in the formation and maintenance of attachment bonds between social partners (Sbarra and Hazan, 2008), and being able to accurately measure coregulation helps us to understand how social relationships are regulated on a physiological level. Importantly, given the considerable variation in the patterns and daily rhythms of HPA axis activity (Nelson, 2011), it is key that the study design matches a suitable measure of cortisol to the temporal and contextual setting of the dyad (reviewed by Burnard et al., 2017). The majority of previous coregulation studies have focussed on salivary cortisol concentrations (see Tables 1.1-1.3; Davis et al., 2018; Timmons et al., 2015), but recent developments have seen the inclusion of hair cortisol analysis to measure coregulation using a measure of chronic HPA axis activity (Broeks et al., 2021; Ouellette et al., 2015; Sundman et al., 2019). Saliva samples represent a single time-point measure of circulating cortisol concentrations, whilst hair cortisol provides a measure of accumulated cortisol that has been secreted across a period that can span a number of months (depending on species, hair growth rate, and hair sample length;

reviewed by Burnard et al., 2017). Therefore, hair cortisol analysis lends itself towards understanding the stable, long-term predictors of coregulation as opposed to measuring daily fluctuations in coregulation strength in response to acute conditions/events (e.g. physical proximity). In this study, I use hair cortisol analysis to i) understand the predictors of dog and owner cortisol concentrations, and ii) identify whether the coregulation in short-term salivary cortisol concentrations from dog-owner (Chapter 3) and dog-dog (Chapter 4) dyads is also reflected in chronic hair cortisol concentrations. Further, for dog-owner dyads I test how the presence of a second dog living in the household (i.e. a consistent, stable factor of the social environment) influences the strength of dog-owner HCC coregulation. Below, I discuss the findings in detail and outline how hair cortisol analysis can be best utilised going forward in the study of cortisol coregulation more generally.

The present findings provide limited evidence for cortisol coregulation in dog and owner hair cortisol concentrations – dog-owner dyads from one-dog households tended to have stronger cortisol synchrony and higher attachment scores. However, the complete absence of a correlation in two-dog households is unexpected given that coregulation in salivary cortisol concentrations was identified in both household types in Chapter 3 (which largely includes the same groups of participants; see Table 2.1). In one-dog households, although a direct association has not been investigated, the combination of the possible presence of cortisol coregulation and more attachment-related behaviours in the dogs (e.g. following the owner, nudging/nuzzling the owner, and agitation from lack of attention) could be indicative of a stronger dog-owner attachment bond, as outlined by Sbarra and Hazan (2008). The C-BARQ measure of attachment is, however, primarily focused on the dogs' physical proximity to the owner and has limited consideration for the three other key aspects of attachment: 'safe-haven' and 'secure-base' effects, and anxiety upon separation (see Chapter 1; Bowlby, 1970). Therefore, this could be more of an indication towards the importance of physical proximity for cortisol coregulation, as shown in Chapter 3 and previous studies in the human literature (e.g. Hibel et al., 2014; Papp et al., 2009; Saxbe and Repetti, 2010).

In addition to the evidence of coregulation and higher attachment scores in one-dog households, both dogs and owners from one-dog households tended to have lower HCCs than those in two-dog households. Higher hair cortisol concentrations indicate prolonged activation of the HPA axis as a result of adverse environmental conditions that continually trigger a stress response (reviewed by Burnard et al., 2017). Therefore, it is possible that the lower concentrations in one-dog households results from the potentially more secure dog-owner attachment – the dyad is functioning well and providing each other with the emotional support they require, which leads to reduced activation of the stress response (Payne et al., 2015; Sbarra and Hazan, 2008). However, the relationship between attachment security and coregulation strength is unclear, as demonstrated by studies identifying stronger coregulation with negative relationship markers (e.g. low satisfaction, high strain; Laws et al., 2015; Saxbe and Repetti, 2010), meaning a better understanding of this is required before confidently linking cortisol coregulation to individuals' HPA axis activity.

A possible explanation for the absence of dog-owner HCC coregulation in two-dog households (both time-matched cortisol and % change in cortisol) could be that the combination of intra- and inter-specific social interactions for dogs in two-dog households may impact the way attachment bonds are established and maintained on a physiological level. Dogs derive different benefits from social interactions with their owner and cohabiting conspecific, and this is also reflected in the dog-owner and dog-dog attachment bonds that form (Cimarelli et al., 2019; Sipple et al., 2021) and the types of attachment behaviours exhibited by co-housed dogs (e.g. Stephan et al., 2021). Whilst the literature demonstrates that there is a difference in dog attachment behaviour between single- and multi-dog households, the direction of this trend is not consistent, with some studies reporting greater attachment from dogs in single-dog households (Harvey et al., 2016; Marinelli et al., 2007; Savalli et al., 2019) and vice versa (Meyer and Forkman, 2014; Stephan et al., 2021). Despite this inconsistency, the fact that the owner has to share their care and attention between two dogs may be perceived by the dogs as the owner being less emotionally available for their individual needs (Marinelli et al., 2007; for a review of dogs' capability to understand human expressions and emotions, see review by Benz-Schwarzburg et al., 2020). Even though there is no difference in owner attachment anxiety and avoidance in the present study, a 'sharing of property' mentality has commonly been reported among dogs and this can lead to signs of jealousy in an attempt to regain the attention of their owner (Abdai et al., 2018; Cook et al., 2018; Harris and Prouvost, 2014). The outcome of jealous behaviours can often be detrimental to a relationship (Chung and Harris, 2018), and could impact on the security and functionality of the dog-owner attachment bond. Dogs in one-dog households, on the contrary, are less likely to experience this mentality and more likely to receive individualised care and greater physical proximity with their owner, therefore reinforcing the attachment bonds and underlying physiological mechanisms (Marinelli et al., 2007; Sbarra and Hazan, 2008).

This explanation around dog jealousy, however, does not account for the presence of salivary cortisol coregulation in dog-owner dyads (Chapter 3). Instead, the option of intra- and inter-specific social interactions for a dog in a two-dog household introduces considerable plasticity depending on the immediate environmental context of the owner and two-dogs (Cimarelli et al., 2019; Sipple et al., 2021). Under certain contexts, interactions with the owner will be more beneficial, particularly when the dog is seeking information or reference from another individual; and dog-dog interactions are instead more associated with affiliative benefits (Cimarelli et al., 2019). Naturally, the dog will interchange between the inter- and intra-specific relationships, and attachment bonds can be simultaneously maintained with the owner and cohabiting conspecific (Cimarelli et al., 2019; Sipple et al., 2021). Additionally, the human coregulation literature has demonstrated that children, for example, coregulate simultaneously with their mother and father (Saxbe et al., 2017, 2014) Therefore, as a result of the complex social structure that exists in multi-dog households (Dantas de Castro, 2017), and the potentially frequent interchanges in which individual is the primary attachment figure at a given time, a chronic measure of HPA axis activity is perhaps not suitable to measure cortisol coregulation in multi-dog households.

One of the challenges of comparing dog and owner HCCs is the varying rate of hair growth between dogs and humans. Whilst human hair generally grows 1 cm per month (reviewed by Burnard et al., 2017), the growth rate of dog hair is highly variable and is dependent on the season, the body region sampled, and the breed of the dog (reviewed by Mesarcova et al., 2017). Therefore, it is highly possible that there was a mismatch in the hair growth rates of the dogs and owners participating in this study, meaning that the cortisol concentrations obtained from the samples may not be representative of the same period/duration of time. Alternatively, as a result of grinding the 6cm-long hair samples into a powder, the HCCs presented in this study represent an ‘average’ of the cortisol that has been stored in the hair shaft over the corresponding growth period which, for many of the samples would have been a number of months. Whilst this removes the influence of daily variation in HPA axis activity and acute stressors (Burnard et al., 2017), it also introduces the possibility of a very coarse measure of HPA axis activity, depending on the growth rate. Another approach would be to utilise segmental hair analysis, whereby the hair shaft is divided into equal segments, usually relative to 1-month's growth, and analysed separately so as to get a more accurate measure of long-term cortisol (known as a retrospective cortisol calendar; see Carlitz et al., 2014; Fürtbauer et al., 2019). Alternatively, acute cortisol concentrations from saliva samples may be the more appropriate measure to use in this context, as it should detect the short-term fluctuations necessary to identify dog-owner and/or dog-dog coregulation.

Whilst these findings support, in part, the positive correlation in dog and owner HCCs observed by Sundman et al. (2019), the present study indicates an effect of the presence of other household dogs, which is not found by Sundman et al. (2019). The main difference between the two studies is the inclusion of only border collies and Shetland sheepdogs (working dog breeds) by Sundman et al. (2019) and no breed restrictions in the present study (six out of the 7 recognised breed groups are represented, as well as mixed breed individuals). Domestication and strong selective breeding pressures (Lord et al., 2016) have made working breeds highly trainable (Serpell and Duffy, 2014; Turcsán et al., 2011) and reliant on contextual information obtained from human handlers (e.g. Barnard et al., 2019; Passalacqua et al., 2011). Visual and behavioural cues are believed to be key to facilitating cortisol coregulation (Timmons et al., 2015), so more perceptive breeds may be more likely to coregulate with humans, regardless of the surrounding social context (i.e. presence of another dog), as shown by Sundman et al. (2019). This possible breed-effect should be investigated further, because if consistent breed differences in coregulation strength exist (as with separation and attachment behaviours; e.g. Asp et al., 2015; Pongrácz et al., 2020), this could allow us to use breed as a proxy for comparing behaviour types and personalities in coregulation studies – something that has received little attention in the coregulation literature (but see Chapter 6 and Pauly et al., 2021).

The percentage change coregulation analysis showed no dog-owner coregulation, but provided evidence of coregulation in the dog-dog dyads from two-dog households. Evidence of dog-dog coregulation in the present chapter and Chapter 4 demonstrates that cortisol coregulation can be measured on both a short- and long-term time scale, and therefore highlights the importance of directly comparing how acute and chronic contextual factors in the surrounding environment differently influence coregulation strength (e.g. Burnard et al., 2017; Christensen et al., 2022). It is possible that the high variability in time between hair sample-1 and sample-2 (14 days to 121 days, median = 28 days) across the participating groups could have affected the percentage change coregulation analysis given that the resulting cortisol percentage change measures will represent different durations and could therefore be subject to the seasonal effects reported by Sundman et al. (2019). Due to the small sample size, it was not possible to determine predictors of dog-dog coregulation strength, however, it would be interesting to investigate whether the moderating effect of owner physical proximity on dog-dog salivary cortisol coregulation strength (Chapter 4) persists in coregulation of dogs' hair cortisol concentrations.

5.5 Conclusion

The presence of a second cohabiting dog introduces considerable complexity to the social network of the household (Cimarelli et al., 2019; Sipple et al., 2021; Trisko et al., 2016), and here I show that it may also impact processes involved in dog-owner cortisol coregulation. Overall, these results provide some evidence for the contribution that hair cortisol analysis can make towards coregulation research (Broeks et al., 2021; Ouellette et al., 2015; Sundman et al., 2019) and, taken together with the findings from Chapter 3, indicate that the factors affecting coregulation strength may be dependent on whether coregulation is being measured using acute (e.g. saliva) or chronic (e.g. hair) cortisol concentrations (see review by Burnard et al., 2017). Importantly, however, this study is based on a limited sample size and the results presented are non-significant trends in the data. Replicating this study across a larger sample with more frequent hair sampling will be key. In particular, the technique of segmental hair analysis could be well suited to studying cortisol coregulation, given that the resultant retrospective cortisol calendar (Carlitz et al., 2014) can be used to obtain monthly HCCs for a period that could easily span a year (depending on the number and length of hair samples) – this type of long-term data would be invaluable for enhancing our understanding of coregulation.

Chapter 6 - Effects of personality similarity on cortisol coregulation in cohabiting heterosexual couples

Abstract

Bidirectional modulation of partners’ cortisol concentrations, known as cortisol coregulation, has commonly been measured in human adult dyads, predominantly romantic couples. Depending on the context, predictors of coregulation strength can vary between physical proximity, relationship satisfaction, emotional depth, time spent together, and high-stress conditions. Despite the innate human tendency to associate with individuals similar in physical, behavioural, and demographic characteristics, little research has tried to understand how social partner similarity influences the strength of coregulation. The ‘dyadic coping model’, in particular, outlines how close social dyads generally exhibit similarities in the way that they approach and cope with stressful events, and thus experience a greater emotional depth within the relationship. Using saliva sampling and Big-5 personality questionnaires, I tested whether personality similarity within cohabiting couples (n=8) affected cortisol coregulation strength. Although based on a small sample size, the results show relatively strong (but non-significant) correlations between couple cortisol coregulation strength and couple similarity in both extroversion and neuroticism, whereby coregulation tended to be stronger in couples with greater personality similarity. Extroversion is important for communication skills, so increased coregulation strength with more similarity in extroversion may be a result of more effective dyadic communication associated with matching extroversion types (i.e. extrovert-extrovert, introvert-introvert). Additionally, greater similarity in neuroticism predicted stronger coregulation, which may be linked to the individuals having a more similar approach to coping with stress-inducing events. Overall, this study complements existing studies examining the modulating effects on cortisol coregulation and suggests that personality similarity within dyad partners should be considered and explored further in future studies.

6.1 Introduction

Married/romantic couples have been a key study dyad in the existing cortisol coregulation research due to the attachment bonds that form between pair-bonded individuals (Meyer and Sledge, 2020); a secure attachment promotes a social context under which coregulation can occur (Coan and Sbarra, 2015; Sbarra and Hazan, 2008). The majority of couple cortisol coregulation literature demonstrates that perceived relationship satisfaction is a key predictor of coregulation strength, with coregulation being stronger in couples experiencing low levels of relationship satisfaction (Ha et al., 2016; Laws et al., 2015; Liu et al., 2013; Saxbe and Repetti, 2010). Female-directed domestic abuse also increases coregulation strength (Hibel et al., 2009; Saxbe et al., 2015). At the same time, however, positive interactions between partners, such as spending more time in physical proximity and being part of an emotionally deeper relationship, are linked to stronger cortisol coregulation (Engert et al., 2018; Laws et al., 2015; Papp et al., 2013). Recent attention has shifted towards understanding how individual views and personality traits influence dyadic coregulation (Braren et al., 2020; Pauly et al., 2021). For instance, the strength of couple coregulation has been linked to markers of female psychological stress (Braren et al., 2020; Engert et al., 2018) and levels of negative mood (Saxbe and Repetti, 2010), as well as the political context (i.e. situation along the left-right political spectrum) of the area that the couple live in (Pauly et al., 2021). Additionally, openness – one of the 'Big-5' personality traits (Goldberg, 1999, 1990) and a trait associated with an individual's situation on the left-right political spectrum – has been linked to coregulation strength, with more open individuals experiencing weaker coregulation with their partner (Pauly et al., 2021). Whilst these studies have identified a number of individual traits that can affect the strength of cortisol coregulation, only one study, to my knowledge, has considered potential effects of partner similarity, showing that couples more similar in negative mood levels exhibit stronger association in cortisol concentrations (Saxbe and Repetti, 2010).

Humans have an innate tendency to associate with individuals who are similar physically, behaviourally, and demographically to oneself – known as assortative mating in romantic couples (Luo, 2017) and homophily in friendships (McPherson et al., 2001). Once relationships have become established, it is possible that partners/friends will converge to become even more similar in at least some traits as they begin to spend more time together and understand each other (Lenhausen et al., 2021; Luo, 2017). Benefits of assortative mating/homophily include improved perceptions of dyad relationship quality and functioning in both romantic/married couples (Böhm et al., 2010; Brandstätter et al., 2018; Decuyper et al., 2012; Gaunt, 2006; Gonzaga et al., 2007) and platonic friendships (Harris and Vazire, 2016; Linden-Andersen et al., 2009; Wrzus et al., 2017). Couple similarity has been shown in various traits, including age; ethnicity; level of education; political views and personal values; height, weight, and perceptions of attractiveness; lifestyle and hobbies; personality traits and mental wellbeing (Luo, 2017; McPherson et al., 2001). For example, individuals who perceived their partner as being more similar to themselves in optimism and personality rated their relationship as being of a higher quality (Böhm et al., 2010; Decuyper et al., 2012), and similarities in Big-5 personality traits and communication skills have been shown to increase friendship intensity and improve emotional expression between friends respectively (Burleson and Samter, 1996; Selfhout et al., 2009). Additionally, similarity in the way that human social partners cope with stressful situations is another key predictor of relationship quality and satisfaction (Badr, 2004; Chow et al., 2014).

Two personality traits associated with the way individuals cope with stress are neuroticism and conscientiousness (Afshar et al., 2015; Carver and Connor-Smith, 2010; Childs et al., 2014). Highly neurotic individuals are more likely to perceive a stressor as threatening and feel less able to cope in the situation, whilst highly conscientiousness individuals generally avoid impulsive actions that could lead to unexpected stressors, thereby making them better able to cope when they encounter predictable stressors (Afshar et al., 2015; Carver and Connor-Smith, 2010). Therefore, similarity in these two personality traits among romantic partners should indicate a more synchronised coping strategy – this is the basis of the dyadic coping model proposed by Bodenmann (2005). The theoretical basis of the dyadic coping model closely aligns with that of cortisol coregulation – the key characteristic of both mechanisms is that they are bidirectional; both individuals are reliant on their partners' regulation of coping behaviours/cortisol concentrations to function on a day-to-day basis (Bodenmann, 2005; Sbarra and Hazan, 2008). Additionally, both mechanisms can have positive and negative effects depending on the amount of strain upon the relationship (Falconier and Kuhn, 2019; Timmons et al., 2015). With this in mind, it is possible that couple similarity in personality traits that are associated with stress coping (i.e. neuroticism and conscientiousness; see above), could indicate a more congruent coping style within the dyad (Bodenmann, 2005). This more aligned dyadic coping style may result in a better functioning relationship which, in turn, may be reflected in stronger cortisol coregulation (Saxbe and Repetti, 2010). Alternatively, coregulation may be stronger when coping styles are misaligned, as this could reflect a relationship under high strain (see examples above; Saxbe et al., 2015; Timmons et al., 2015).

In this study, I investigate how similarity in personality traits (using Big-5 questionnaires; Goldberg, 1999, 1990) influences the strength of salivary cortisol coregulation in cohabiting couples. Whilst I test for effects of similarity in all Big-5 personality traits, I expect neuroticism and conscientiousness, in particular, to be linked to the strength of cortisol coregulation, with greater similarity resulting in stronger cortisol coregulation within couples. This is because they are more likely to have a functioning dyadic coping style that promotes an emotionally-deeper relationship (Bodenmann, 2005; Falconier et al., 2015), which has previously been shown to strengthen cortisol coregulation (Engert et al., 2018).

6.2 Methodology

6.2.1 Participants

N=8 heterosexual cohabiting adult couples were recruited following advertisement of the study across Swansea University (see Table 2.1 in Chapter 2). All dyads were in a romantic relationship, but the marital status and the duration of the romantic relationship was unknown. Except for one group (group 17; Table 2.1) who had one child under 18 years of age living in the household, all couples cohabited together without any other persons present.

6.2.2 Saliva sampling and analysis

Participants collected saliva samples twice daily across eight days (Figure 6.1), as per the methodology in Chapter 2. Once returned to the Swansea University Behavioural Endocrinology Laboratory, a total of n=242 saliva samples were analysed using a commercial enzyme immunoassay (EIA) kit (Salimetrics LLC, State College, PA; Item no. 1-3002). Where a participant did not provide sufficient saliva for analysis, the matching sample from their partner was also not analysed (n=7 sampling points from groups 1, 2, and 23; Table 2.1). The n=15 EIA plates used for analysis had inter-assay coefficients of variation (CV) of 6.0 % (high quality control) and 13.0 % (low quality control), and intra-assay CVs were under 11.76 %.

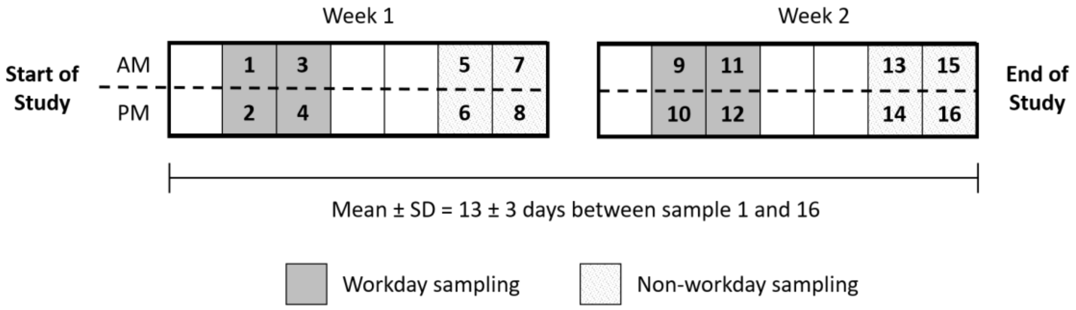


Figure 6.1 Saliva sampling schedule. Partners collected saliva samples in the morning (AM) and afternoon (PM) on n=4 workdays and n=4 non-workdays across n=2 weeks, resulting in n=16 sampling points per individual per dyad.

6.2.3 Personality measures

Participants' personality was assessed using the 50-item 'Big-5' questionnaire (Appendix 5; Goldberg, 1999, 1990). Participants were provided with a paper copy and were asked to complete the questionnaire at home at any point during the two-week study period (for further details, please see section 2.2). Each item, scored on a 5-level Likert scale, relates to one of five personality traits: openness, conscientiousness, extroversion, agreeableness, and neuroticism. With 10 items per personality trait, an overall trait score was calculated as the sum of the 10 item scores (totals range from 10 to 50). High overall scores represent the individual showing more behaviours attributed to the personality trait, except for neuroticism, where a high score is equivalent to low neuroticism. Using the overall scores for each trait, the similarity in male and female personality scores in each dyad was calculated by subtracting the male score from the female score, and absolute differences in scores were used in subsequent analyses.

6.2.4 Statistical analysis

Using R version 4.1.1, data was analysed using linear mixed models (Bates et al., 2015; Kuznetsova et al., 2017; R Core Team, 2019). All measures of male and female cortisol concentrations fell within the reported range (Ozgocer et al., 2017; Zhang et al., 2018), and were log-transformed to achieve a normal distribution. Model diagnostics were assessed using Q-Q plots and standardised residuals vs. fitted values.

A LMM was used to assess cortisol coregulation. As previous studies have demonstrated coregulation in couples' cortisol awakening response (CAR; e.g. Liu et al., 2013), both morning and afternoon samples were included (unlike in Chapter 3).

Female cortisol was fitted as the response variable and male cortisol as a fixed effect. Time of day (AM/PM) and work context (work-/non-work) were controlled for (Fries et al., 2009; Wendsche et al., 2021) and included as fixed effects. Individual ID was included as a random effect as well as random slopes for male cortisol. To test for the effect of personality similarity on the strength of coregulation, the slope coefficients for each group were extracted and used as a measure of coregulation strength. To test for potential effects of personality similarity on cortisol coregulation strength, I back-transformed the slope coefficients and used Spearman's rank correlations to test for a correlation between coregulation strength and each of the similarity scores for the five personality traits.

6.3 Results

6.3.1 Saliva cortisol coregulation

Salivary cortisol concentrations ranged from 0.018-1.728 µg/dL (median = 0.182 ug/dL) for men and 0.012-1.540 µg/dL (median = 0.186 ug/dL) for women. A significant positive correlation between male and female cortisol concentrations was found (estimate ± se = 0.35 ± 0.14, t = 2.47, p = 0.029; Table 6.1; Figure 6.2). The random slope for male cortisol was significant (Table 6.1; Figure 6.2), indicating dyad differences in coregulation strength.

Table 6.1 Summary of the linear mixed model measuring the correlation between male and female cortisol concentrations. All statistically significant results are in bold.

Model	**Predictor Variable**	**Estimate ± se**	**p-value**
LMM1 Female Cortisol			
Fixed Effects	Intercept	-0.66 ± 0.28	
	log(Male Cortisol)	0.35 ± 0.14	**0.029**
	Time *pm*	-0.93 ± 0.17	< **0.001**
	Work Context *workday*	0.06 ± 0.13	0.648
Random Slope		χ^2	**p-value**
	log(Male Cortisol)|Female ID	14.96	**0.002**

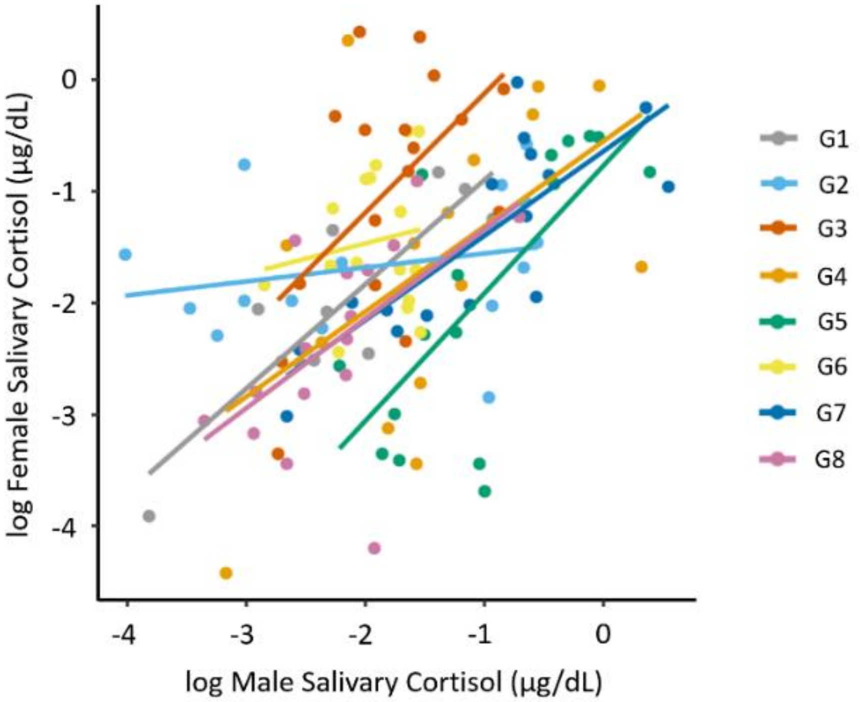


Figure 6.2 Relationship between male and female salivary cortisol concentrations (µg/dL), with individual regression lines and data points for each group (n=8).

6.3.2 Personality similarity and cortisol coregulation strength

Across all n=16 participants, average (±SD) scores for the Big-5 personality traits were 37.9±5.2 for agreeableness, 35.7±7.4 for conscientiousness, 26.4±5.9 for extroversion, 31.4±9.5 for neuroticism, and 34.4±5.1 for openness. Individuals' trait scores and the absolute difference between couples' scores are shown in Figure 6.3.

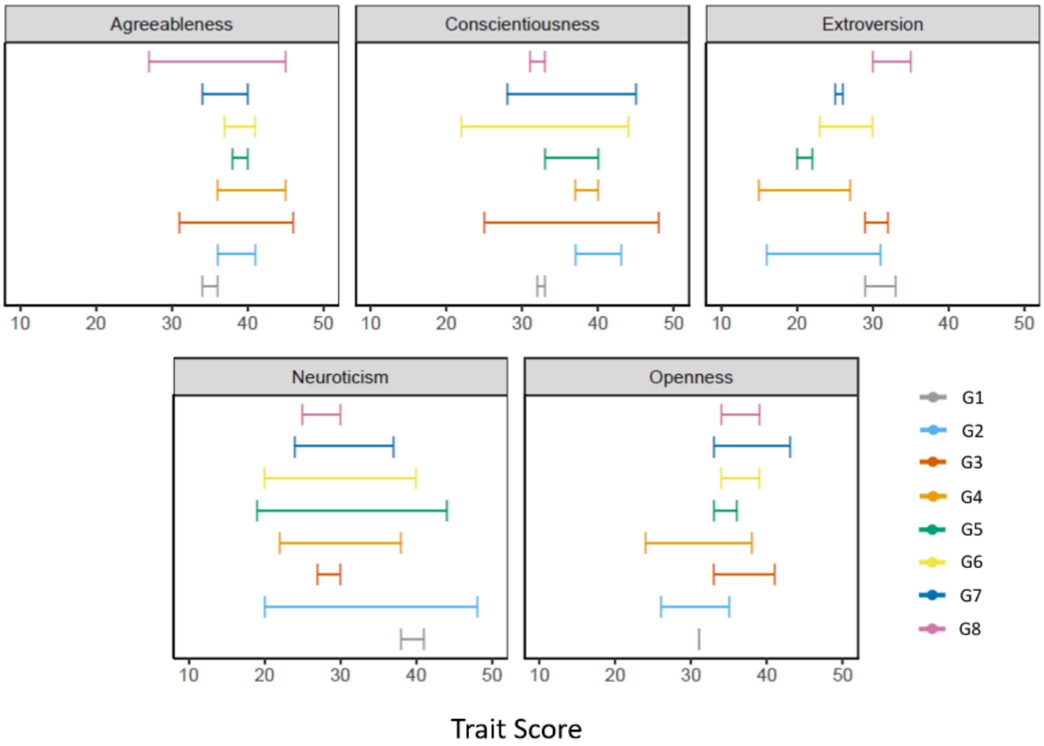


Figure 6.3 Range of Big-5 questionnaire scores for each personality trait for n=8 cohabiting, heterosexual couples. The horizontal line for each couple provides a visual representation of the difference between the two individuals' scores (represented by the vertical lines at either end). Thus, shorter lines are equivalent to greater similarity. Note, the scale for neuroticism is reversed, meaning that individuals with a higher score are considered to be less neurotic.

The strength of cortisol coregulation (random slopes extracted from LMM) was strongly but non-significantly negatively correlated with similarity in extroversion (Spearman's rho = -0.647, p = 0.083, n=8: Table 6.2; Figure 6.4C), neuroticism (Spearman's rho = -0.614, p = 0.105, n=8, Table 6.2; Figure 6.4D), and openness (Spearman's rho = -0.458, p = 0.254, n=8, Table 6.2; Figure 6.4E), indicating stronger cortisol coregulation in couples with more similarity in these three traits. Similarity in conscientiousness and agreeableness did not correlate with the strength of coregulation (Table 6.2; Figure 6.4A and B).

Table 6.2 Summary of the Spearman's correlation coefficients for the similarity measures of each of the Big-5 personality traits when correlated against couple coregulation strength (represented as each couple's back-transformed random slope estimate from the LMM; n=8 couples). Any p-values between 0.05 and 0.1 are shown in italics.

Personality Trait	**Spearman's rho**	**p-value**	
Agreeableness	0.048	0.910	Figure 6.4A
Conscientiousness	0.060	0.888	Figure 6.4B
Extroversion	-0.647	*0.083*	Figure 6.4C
Neuroticism	-0.614	0.105	Figure 6.4D
Openness	-0.458	0.254	Figure 6.4E

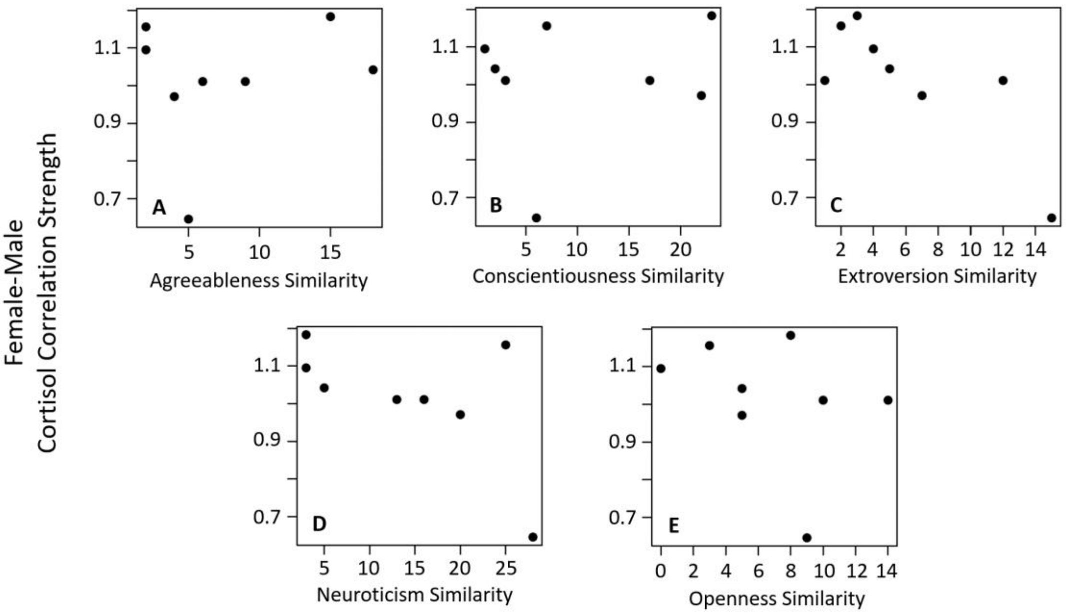


Figure 6.4 Correlation between cortisol coregulation strength (random slope estimates from LMM) and dyad similarity scores across the Big-5 personality traits: A) agreeableness, B) conscientiousness, C) extroversion, D) neuroticism, and E) openness – lower scores for each personality trait represent greater similarity within couples.

6.4 Discussion

Investigations into the role of cortisol coregulation in human adult relationships have been extensive in recent years (Cook, 2020; Meyer and Sledge, 2020; Timmons et al., 2015). Results demonstrate the importance of relationship satisfaction (Laws et al., 2015; Saxbe and Repetti, 2010), spending time together (Laws et al., 2015), personality and political views (Pauly et al., 2021), and emotional depth (Engert et al., 2018) in predicting the strength of cortisol coregulation between partners. Very little, however, has been studied in relation to how partner similarity influences dyadic coregulation (Saxbe and Repetti, 2010), despite the large body of research linking couple similarity and homophily with improved relationship satisfaction (Luo, 2017). In particular, similarity in coping styles is common within social relationships, and can be an indicator of greater emotional depth (Chow et al., 2014). Thus, markers of congruence in coping styles (such as similarity in neuroticism and conscientiousness; Carver and Connor-Smith, 2010) could also predict the strength of cortisol coregulation within the dyad. To test this hypothesis, I investigated the relationship between partners' similarity in the Big-5 personality traits (Goldberg, 1999) and the strength of cortisol coregulation. I predicted stronger cortisol coregulation in more similar couples, in particular similarity in neuroticism and conscientiousness.

Despite the small sample size, the present study provides preliminary evidence of a link between couple personality similarity and cortisol coregulation strength. In particular, I found a strong, but non-significant, negative correlation between neuroticism similarity and coregulation strength, indicating that coregulation is stronger in couples more similar in neuroticism. Similarity in conscientiousness, in contrast, had no effect. As outlined in the introduction (see section 6.1), the effect of neuroticism similarity on couple coregulation strength is likely due to its well-documented links to stress coping behaviours (Afshar et al., 2015; Bouchard, 2003; Carver and Connor-Smith, 2010), and the important biological role HPA-axis activity plays in modulating such coping styles and behaviours of an individual (Hori et al., 2010; Koolhaas et al., 1999). The non-significant effect of neuroticism similarity is likely due to the small sample size; however, another contributing factor could be the context under which the study was conducted. Given that the present study was conducted in the couples' home environment, each of the dyads will have had different life events and experiences occurring during the study period, and it is unknown how often the couples encountered challenging situations that tested the dyads' coping styles during this time. For example, similarity in neuroticism may determine a couple's response to a stressful event, but if there is no stressful event present, similarity in neuroticism may not be relevant for the dyad in that social context, and therefore has a reduced effect on cortisol coregulation. Instead, the influence of dyad similarity in coping styles (e.g. neuroticism and conscientiousness) on coregulation strength could be specifically assessed through the addition of a stress-inducing experimental task (e.g. conflict discussion/public speaking/mock job interview; Engert et al., 2018; Ha et al., 2016; Hall, 2013). Another option would be to measure stress related to the individuals' working environment and investigate whether similarity in neuroticism predicts coregulation strength in couples where one or both of the individuals are experiencing elevated work stress levels.

Similarity in extroversion had the strongest correlation with couple cortisol coregulation strength, with more similar couples having greater cortisol synchrony, and a relatively strong negative trend was also shown for openness similarity. Extroversion relates to the fulfilment that individuals experience when socialising with others; extroverts tend to be highly gregarious and energetic in social contexts, whilst introverts gain more fulfilment whilst alone (Goldberg, 1999). It is possible then that less similarity in extroversion scores within social dyads could create a barrier for establishing and maintaining the attachment bond that underpins coregulation (Roisman et al., 2007; Sbarra and Hazan, 2008; Shaver and Brennan, 1992).

The key components of an attachment bond are high dependence on the social partner, feeling safe and secure when in their presence, and experiencing anxiety on separation from this partner (Fitton, 2012; Zeifman, 2019). Extroversion plays an important role in the way that individuals communicate with each other – extroverts have been shown to be more emotionally expressive (Wu et al., 2018), use more abstract and subjective language (Beukeboom et al., 2013), maintain closer physical proximity and eye contact (Jensen, 2016), and use more positive emotion words (Chen et al., 2020). Therefore, in instances where there is a larger difference in male and female extroversion scores, and the dyads likely consist of a more extroverted individual and a more introverted individual, there may be a mismatch in the communication preferences of the individuals, whilst the complementary communication skills of more similar individuals (potentially extrovert-extrovert (E-E) and introvert-introvert (I-I) dyads) mean the social partners are better able to communicate with each other. This was demonstrated among a sample of newly acquainted social partnerships in an unstructured, experimental interaction scenario (Cuperman and Ickes, 2009). Communication skills are key to how satisfied individuals are with their romantic relationship (Johnson et al., 2021), and relationship satisfaction is a factor which often predicts couple coregulation strength (Timmons et al., 2015). The negative trend between openness similarity and coregulation strength could also be as a result of relationship satisfaction, as couple similarity in openness has recently been linked to increased relationship satisfaction (Liu et al., 2022). Furthermore, communication skills have long been linked to individuals' attachment styles (Anders and Tucker, 2000; Guerrero and Jones, 2005; Sessa et al., 2020). Therefore, a mismatch in communication skills could affect the type of attachment that forms between two individuals (e.g. secure/insecure, anxious, avoidant), and hence interfere with the establishment and functioning of the physiological regulatory mechanisms that result from these attachments (i.e. cortisol coregulation). The effect this has on the strength of coregulation would likely depend on the degree of mismatch – a small mismatch may result in weaker coregulation (as seen in this study), whilst a large discrepancy may create a high-stress environment in which coregulation strengthens (e.g. Hibel et al., 2009; Saxbe et al., 2015).

Additionally, extroversion is a personality trait that has been found to strongly predict the extent to which individuals experience and exhibit attachment avoidance and anxiety behaviours in their social relationships (Roisman et al., 2007). Therefore, in E-I dyads, one individual may be trying to maintain emotional and physical distance whilst the other is persistently seeking closeness, which can result in a demand-withdraw interaction (Conradi et al., 2021; Kilmann et al., 2013; Millwood and Waltz, 2008). Couples less similar in extroversion may experience less security within their attachment (Lewis and Yoneda, 2021), meaning the stable base from which cortisol coregulation develops is missing (Sbarra and Hazan, 2008), which may explain why coregulation was weaker with less similarity in extroversion in this study. However, reduced security could reduce relationship satisfaction in the dyad, which is commonly linked to stronger coregulation (e.g. Laws et al., 2015).

The present analysis is, however, based on a small sample size with limited variation in extroversion similarity scores. Whilst there is some variation in these similarity scores, most couples are situated in the middle of the introvert-extrovert spectrum, with no clear E-E or I-I couples (see Figure 6.3). The effect of extroversion similarity is also not clear cut, with existing studies having shown that greater differences in extroversion within romantic couples can actually improve marital quality and satisfaction (Barelds, 2005; Shiota and Levenson, 2007). Therefore, future research should determine whether this finding persists within a larger sample, and with greater variation across the full range of extroversion scores. This would also allow for a more robust analysis of how the dyads' position along the introvert-extrovert scale influences the mediating effect of extroversion similarity on cortisol coregulation (e.g. is the mediating effect of extroversion similarity only apparent when both individuals are highly extrovert?). These types of research questions should also be asked more broadly for the range of Big-5 personality traits, given the preliminary evidence presented in this study.

Another area to consider is the context under which cortisol coregulation is being measured (Danyluck and Page-Gould, 2019). Previous research in parent-child dyads demonstrated how cortisol coregulation itself may only be present under high-stress conditions (Hibel et al., 2009; Ouellette et al., 2015; Ruttle et al., 2011), and stronger cortisol coregulation between romantic couples has been consistently observed in more stressful contexts (Engert et al., 2018; Liu et al., 2013; Saxbe et al., 2015). With this is mind, the factors that predict the strength of cortisol coregulation could also be dependent on the stress context that the social dyad finds themselves in. For example, dyads more similar in neuroticism or conscientiousness may exhibit stronger coregulation under stress-inducing conditions but, outside of this context, neuroticism/conscientiousness similarity may not mediate coregulation strength. Going forward, research should continue to study the potential effect of personality similarity on the strength of coregulation. Such work should use a combination of naturalistic and experimental conditions (e.g. conflict discussions, Ha et al., 2016; mock job interview, Engert et al., 2018) to draw comparisons across varying contexts, as well as make the study more comparable across all participating groups.

6.5 Conclusion

Whilst based on a small sample size, the present study suggests that personality similarity – particularly extroversion, neuroticism, and openness – may have an important impact on cortisol coregulation in romantic couples. The overall findings suggest that coregulation is stronger with greater personality similarity, perhaps as a result of being able to form a more secure attachment bond (Shaver and Brennan, 1992). Future research should attempt to replicate the present findings with a larger sample size, in order to better understand how personality similarity between partners links into the functioning and maintenance of cortisol coregulation. This may highlight the importance of coregulation in regulating romantic relationships and could be applied to recognising triggers and/or markers of a poor-functioning relationship, which could help to improve the mental health of individuals in such a relationship (e.g. Kiecolt-Glaser et al., 2003).

Additionally, these preliminary findings highlight the importance of accounting for inter-individual behavioural differences (e.g. Kanai and Rees, 2011; Roche et al., 2016). Given the influence that individual personality has on the way we socialise and form attachment bonds with others (reviewed by Wolf and Krause, 2014), considering how individuals differ in terms of personality could have a considerable impact on the way we interpret existing and future coregulation research.

Chapter 7 - General Discussion

Regular meaningful social interactions between two individuals often develop into an attachment bond (Bowlby, 1970) which, in order to persist, must be regulated by an underlying physiological mechanism (Carter, 2005; Sbarra and Hazan, 2008). The bidirectional up-/down-regulation of social partners' hypothalamic-pituitary-adrenal (HPA) axis activity – known as cortisol coregulation – is a defining characteristic of attachment bonds (Sbarra and Hazan, 2008; but see Fürtbauer and Heistermann, 2016). Whilst our understanding of this biological phenomenon has developed considerably (reviewed by Davis et al., 2018; Meyer and Sledge, 2020), there remain many unanswered questions. In particular, with the inclusion of non-human animals into coregulation studies (intra-specific: Burkett et al., 2016; Fürtbauer and Heistermann, 2016); inter-specific with humans: Buttner et al., 2015; Ryan et al., 2019; Strzelec et al., 2013; Sundman et al., 2019), it is important to determine whether similarities in coregulatory patterns exist between human and non-human animals, given that highlighting potential differences in the mechanism across the wide variety of social systems in vertebrate species may help us develop a more specific understanding of the day-to-day function of the mechanism. Additionally, little research has tried to understand how coregulation and the predictors of coregulation strength differ across short- and long-term time scales (but see Broeks et al., 2021; Ouellette et al., 2015; Sundman et al., 2019), despite our ability to measure acute and chronic HPA axis activity through different sample media (reviewed by Burnard et al., 2017).

This thesis has focused on both inter- (dog-human) and intra- (dog-dog, human-human) specific dyads combined with saliva and hair cortisol measures to enhance our understanding of cortisol coregulation across human and non-human animals. Below, I summarise my key findings (Figure 7.1) and discuss their implications for future cortisol coregulation research.

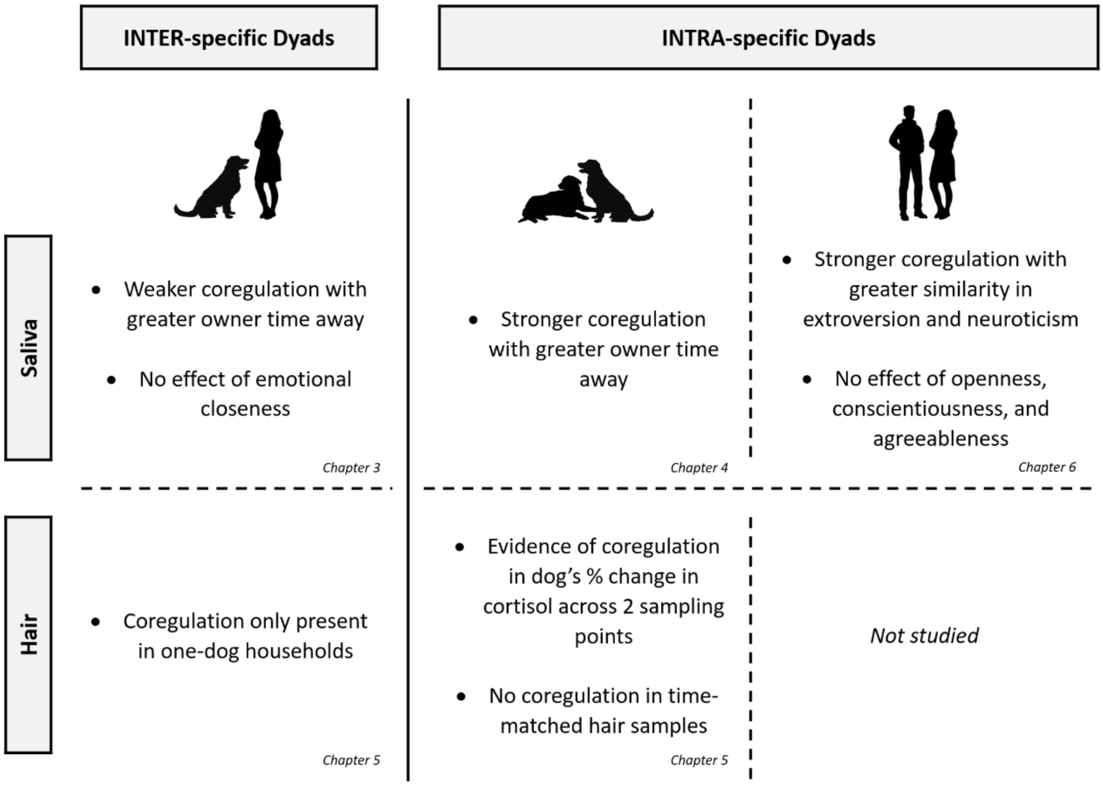


Figure 7.1 Summary of the main findings regarding cortisol coregulation presented in Chapters 3-6. Results are arranged as per the study dyad (*inter-specific*: dog-human; *intra-specific*: dog-dog, human-human) and the sample media (saliva/hair) used to quantify cortisol concentrations.

7.1 Moderators of cortisol coregulation strength

A key part of this thesis was to determine whether the predictors of coregulation strength identified in human dyads exist in dog-owner dyads. Previous research had demonstrated how stressful contexts, such as participation in a dog agility competition (Buttner et al., 2015), strengthens dog-owner cortisol coregulation, similar to parent-child dyads and romantic couples who experience low relationship satisfaction and domestic abuse (e.g. Hibel et al., 2009; Liu et al., 2013). Chapter 3 demonstrated stronger coregulation with increased physical proximity (Figure 7.1) similar to human-human dyads (see Hibel et al., 2014 & Papp et al., 2013 from the human literature). Further to this, the finding that dog-owner cortisol coregulation is dependent on the presence of a second cohabiting dog (Chapter 5; Figure 7.1) bears some similarities to studies of human mother-father-child triads whereby the strength of the child's coregulation depends on the sex of the parent they are interacting with

(Saxbe et al., 2017, 2014), and therefore could vary according to mother and father presence/absence (though, to my knowledge, this has not been directly tested). The congruence between the coregulation predictors presented here and the predictors commonly observed in the human literature increases confidence in the findings of this thesis.

In addition to demonstrating similarities with previously identified predictors of cortisol coregulation strength, Chapters 4 and 6 provide preliminary evidence for the effect of two factors that have received little attention to date: personality similarity (Chapter 6) and 'external social influences' (Chapter 4). Building upon the theoretical basis of assortative mating (attraction to individuals more similar to oneself; Luo, 2017), Chapter 6 indicates that cortisol coregulation is stronger in romantic couples who are more similar in extroversion, neuroticism, and openness (Figure 7.1). Personality traits and couple similarity are two aspects that are rarely considered in the coregulation literature (but see Pauly et al., 2021; Saxbe and Repetti, 2010) despite them each having considerable influence on the way that individuals select and interact with their social partners (e.g. Webster and Ward, 2011). 'External social influences' are defined here as conditions/contexts of the surrounding physical and/or social environment that are not an attribute of either one of the (coregulating) dyad partners, such as the moderating effect of owner absence on dog-dog cortisol coregulation in Chapter 4 (Figure 7.1). To date, only Pauly et al. (2021) considered how non-dyadic characteristics, i.e. the wider political context of the region the dyad inhabited, affected cortisol coregulation. Stronger coregulation was observed in couples living in federal states situated further right on the political spectrum (Pauly et al., 2021). Taken together, the effects of personality similarity and external social influences highlight the breadth of factors that can moderate coregulation and further demonstrate the complexities of the mechanism (Sbarra and Hazan, 2008). Whilst identifying predictors of coregulation can shed light on how dyads react to certain contexts and direct us in terms of the factors that should be controlled for in study designs, they are limited in helping us understand the function that coregulation plays within these contexts or the biological cues that facilitate synchronised physiology between social partners (see review by Timmons et al., 2015).

7.2 Using acute and chronic cortisol concentrations to measure coregulation

By combining short- and long-term measures of cortisol concentrations, this thesis is, to my knowledge, unique to the pre-existing cortisol coregulation literature in that it uses two different sample types (saliva and hair; see Tables 1.2-1.4 in Chapter 1) to measure coregulation in acute and chronic HPA axis activity across the same dog-owner and dog-dog dyads (see review by Burnard et al., 2017). Reviews of coregulation research have identified the importance of concurrently measuring multiple physiological indictors (Davis et al., 2018) and developing more longitudinal study designs to measure coregulation over a period of months or years (Timmons et al., 2015). The introduction of hair cortisol analysis developed our understanding of how chronic physiological state can be synchronised in social partners (Broeks et al., 2021; Flom et al., 2017; Liu et al., 2017; Ouellette et al., 2015; Schloß et al., 2019; Sundman et al., 2019; Tarullo et al., 2017; Chapter 5; but see Höglin et al., 2021), and can be used to understand the continual mediating role of synchronised physiology underlying social attachment bonds across long-term contexts.

Comparisons of cortisol coregulation across different sample types (i.e. saliva and hair) in the same dyads suggested that the potential effect of a second cohabiting dog on dog-owner cortisol coregulation differed according to the sample type the cortisol was derived from. Dog-owner coregulation in salivary cortisol concentrations was observed in one- and two-dog households (Chapter 3), whilst hair cortisol coregulation was observed exclusively in one-dog households (Chapter 5; but see Sundman et al., 2019). Although this observation is based on a relatively small sample size (see section 7.4) and indirectly compares the effect of one predictor variable (i.e. presence of second dog), it could indicate that the complex physiological mechanism of cortisol coregulation is mediated by different predictors according to the varying social and temporal contexts (e.g. Christensen et al., 2022), which could prove to be an important factor to consider and control for in future coregulation research. In this instance, the presence of a second dog may only influence coregulation of chronic cortisol concentrations because this is a factor that does not change on a daily basis (i.e. it is not acute, and therefore would not influence acute measures of cortisol).

Before studying how social and environmental factors affect acute and chronic measures of cortisol coregulation, it is important to first validate that synchrony in salivary cortisol and synchrony in hair cortisol are representative of the same physiological mechanism, i.e. coregulation (see review by Burnard et al., 2017). If this is the case, I would expect to observe a positive correlation between dyads' salivary and hair cortisol coregulation strengths, whereby a dyad more strongly synchronised in hair cortisol concentrations will also show strong synchrony in salivary cortisol. Unfortunately, due to considerable between-group variability in study duration (i.e. from first hair sample collection to second; see section 7.4), it was not possible to directly link the hair and saliva cortisol concentrations in this way. This would require a stricter study schedule consistent across all groups in which the participants must commit to specified dates for sample collection to ensure that salivary and hair cortisol concentrations are time-matched and comparable across all groups.

Additionally, linking chronic HPA axis activity to acute cortisol responses could improve our understanding of the role coregulation plays in social relationships. The ultimate function of cortisol coregulation is generally well understood – it is the load-sharing of energy expenditure with a social partner, which results in increased likelihood of survival and individual fitness (Coan, 2008; Coan and Sbarra, 2015). The daily function of coregulation, however, remains unclear, with markers of 'good' (e.g. *physical proximity*: Hibel et al., 2014; Saxbe and Repetti, 2010; *emotional depth*: Engert et al., 2018; *positive interactions*: Pauly et al., 2020) and 'bad' (e.g. *relationship strain and dissatisfaction*: Liu et al., 2013; Saxbe and Repetti, 2010; *high stress and anxiety*: Laurent et al., 2012; Ouellette et al., 2015; *subject to partner aggression*: Hibel et al., 2009; Saxbe et al., 2015) relationships predicting stronger coregulation. In the same way that Sandner et al. (2020) demonstrated how chronic cortisol concentrations influenced individuals' physiological response to an acute stressor, future coregulation research could investigate how hair cortisol synchrony with a primary attachment figure affects individual physiological response to an acute stressful experimental condition. This avenue of research could help to identify physiological benefits/costs of cortisol coregulation and, therefore, develop a clearer understanding of the day-to-day function of the mechanism (see review by Timmons et al., 2015).

Concurrently investigating acute and chronic measures of cortisol coregulation presents the opportunity to determine how individuals can maintain multiple attachment bonds simultaneously – known as polytropic attachment (see Lewis, 2005). Polytropic attachment is observed across human relationships (Lewis, 2005) and in human-dog households (Cimarelli et al., 2019; Sipple et al., 2021); and my findings of dog-owner (Chapter 3) and dog-dog (Chapter 4) coregulation, along with variation in predictors of dog-owner coregulation (Chapters 3 and 5), could be indicative of multiple attachment bonds influencing individuals' HPA axis activity. Whilst based on limited evidence, this perspective on attachment and coregulation should be investigated further so as to provide a more realistic representation of social relationships (Lewis, 2005) and how they are regulated on a physiological level. To date, most coregulation research has focused on coregulation on a dyadic level (e.g. parent-infant, romantic couple; see Tables 1.2-1.4 in Chapter 1; but see Saxbe et al., 2017, 2014) rather than how an individual may simultaneously modulate bonds with multiple attachment figures through physiological synchrony. However, through the use of chronic and acute cortisol concentrations and changing the focus of coregulation research to the individual (rather than a specific dyad), it could be possible, for example, to discern an individual's 'primary' attachment figure with whom chronic cortisol concentrations are consistently coregulated to a high degree and 'secondary' attachment figures with whom the level of synchrony is more variable and is perhaps more correlated under certain social contexts, depending on the relationship.

7.3 Cortisol coregulation across vertebrates

With the majority of early coregulation research focusing on human dyadic relationships, such as parent-child dyads and romantic couples (Davis et al., 2018; Meyer and Sledge, 2020), and the general belief that only humans would form attachment bonds with social partners (Fine and Beck, 2015), cortisol coregulation could easily be perceived as a biological mechanism that was exclusive to humans. However, as researchers expanded on Bowlby's theory of attachment (Bowlby, 1970), it became clear that non-human animals too show the types of complex behaviours that constitute attachment (reviews by Kenkel et al., 2017; Lim and Young, 2006; Payne et al., 2015), therefore indicating that coregulation could be a more widespread biological phenomenon. Studies of dog-owner and horse-rider dyads have indeed demonstrated that animals also experience covarying HPA axis activity with a social partner (e.g. Buttner et al., 2015; Strzelec et al., 2013; Sundman et al., 2019) and, given that these studies focussed on highly domesticated animal species, it was suggested that the ability of animals to coregulate was a product of domestication as opposed to a mechanism they inherently possessed prior to domestication (reviewed by Kikusui et al., 2019). However, studies in prairie voles (*Microtus ochrogaster*; Burkett et al.,

2016) and stickleback fish (*Gasterosteus aculeatus;* Fürtbauer and Heistermann, 2016), along with the evidence of correlated salivary and hair cortisol concentrations in cohabiting dog dyads in Chapters 4 and 5 respectively, demonstrate that coregulation is a more widespread mechanism throughout the animal kingdom than the initial evidence suggests.

The identification of intraspecific coregulation in a third non-human animal species (Chapter 4) highlights the important role that non-human vertebrates may have in coregulation research. The sheer number of different social systems in vertebrates (Kutsukake, 2009) presents an invaluable opportunity to study coregulation within different contexts and perhaps obtain a clearer picture of the day-to-day function of coregulation within social dyads (see review by Timmons et al., 2015). This is especially true given that vertebrate social behaviour often has an endocrine basis and this association between glucocorticoids and sociality, in many cases, leads to an overall down-regulation of stress levels (reviewed by Raulo and Dantzer, 2018).

In terms of these findings in an evolutionary context, the identification of intraspecific coregulation across a wide range of vertebrates (i.e. fish, rodents, canids, and humans) suggests that the mechanism likely originates from a common ancestor of vertebrates. Whilst it could be argued that dog-dog cortisol coregulation (Chapters 4 and 5) is a product of dogs' domestication by humans (evidenced by increased sensitivity to social cues (Udell et al., 2010) and occurrence of epigenetic mutations of the HPA axis (Kikusui et al., 2019; Pörtl and Jung, 2017)), and did not originate from a common ancestor, I believe that the coregulation in canids pre-dates domestication and was already a key aspect of the socioecology of the undomesticated ancestor of modern-day dogs, the wolf (*Canis lupus*). Wolves have been shown to be as sensitive to human cues as domesticated dogs (Lampe et al., 2017; Range et al., 2019; Wheat and Temrin, 2020) and can outperform dogs in conspecific cooperation tasks (Bräuer et al., 2019; Marshall-Pescini et al., 2017). It is believed to be these cooperative abilities, accompanied by selection (self and/or artificial) for behaviours that increase the propensity for wolves to be in close proximity to humans (Lazzaroni et al., 2020; Pendleton et al., 2018; e.g. reduction of flight initiation distance, Pörtl and Jung, 2017), that underpinned the shift from cooperating with conspecifics to dog-human cooperation, hence instigating the process of domestication (Cordoni and Palagi, 2019; Range and Virányi, 2015). Taken together, it seems unlikely that a mechanism to modulate conspecifics' HPA axis activity would evolve as dogs are moving away from the cooperative pack socioecology and living in closer proximity to humans. Instead, an adaptation in the pre-existing conspecific coregulation mechanism likely resulted in interspecific modulation of physiological state (e.g. see Pörtl and Jung, 2017). This is in line with the ‘canine cooperation hypothesis’, which proposes that dogs’ interspecific social interactions with humans stem from cooperation and sociality between their wolf ancestors (Benz-Schwarzburg et al., 2020; Range and Virányi, 2015). Wolves are believed to possess a high level of awareness to the social cues of conspecifics – a prerequisite to sociality and coordinated behaviours – and it is likely that the domestication process saw a shift to being aware of interspecific human cues instead of those of their conspecifics, rather than an improvement in their ability to perceive social cues (Range and Virányi, 2015, 2014).

Future studies should aim to measure cortisol coregulation in wild wolves or wolves unsocialised to humans to give a better indication of whether dogs inherited the trait for coregulation from their wolf ancestors. Broadening the study of coregulation to other animal families containing domesticated and undomesticated species, such as Felidae and Equidae (see Strzelec et al., 2013), could further contribute to our understanding of the origins of coregulation and the role it could have played in the domestication of these species. Studying cortisol coregulation in wild non-human animals also presents the opportunity to understand how different mating systems and social hierarchies/networks influence the strength and functioning of the mechanism.

7.4 Limitations of a “real-life” study design

A naturalistic study design was utilised in this thesis because there are many benefits associated with designing a “real-life” study that aims to keep interruptions to the participants’ normal daily routines to a minimum (reviewed by McLeod, 2012). Firstly, it is well documented that laboratory settings used in experimental study designs can elicit a stress response in humans (reviewed by Bali and Jaggi, 2015) and dogs (Bodnariu, 2008; Diederich and Giffroy, 2006), and the aim of this thesis was not to measure coregulation in activated HPA axis activity. Secondly, I aimed to keep the participants’ routine as realistic as possible so that the results could be a true reflection of the dyads’ relationship and shared physiological state. Thirdly, the naturalistic design also limited any interference by myself. Dogs' responses to a stranger can be highly variable – including fear, excitement, or no discernible reaction (e.g. Barrera et al., 2010; Feuerbacher and Wynne, 2017) – and these responses will last for different durations of time depending on the dog. Lastly, many coregulation studies in the human literature have utilised a naturalistic study design and demonstrated the contribution these types of studies can make towards developing our understanding of cortisol coregulation (e.g. Liu et al., 2013; Papp et al., 2013, 2009).

However, given the scope and resources available for my thesis, the naturalistic study design introduced a number of confounding variables that were difficult to control for with the small sample size and likely contributed to the limited statistical power of the analyses. Given the voluntary nature of participation, I tried to keep the study design as convenient as possible for the participants, however this then limited the comparability between groups. For example, the time between the first and second study meeting (see Figure 2.2 in Chapter 2) ranged from 14 days to 121 days (median = 28 days), and similarly for the saliva sampling the range between collection of the first and last samples was 9 to 51 days (median = 13 days). Another confounding factor was the variability in the human participants' daily routine. Whilst I had originally planned to test for the effect of the owners' work schedule on dog-owner coregulation, in the same way that Hibel et al. (2014) compared mother-infant coregulation on workdays and non-workdays, the variation in part-/full-time work routines and the possibility of working from home meant that a categorical classification of whether a study day was a workday or not did not contain sufficient detail for accurate analyses. Consideration of the type of work and how stress-inducing it may be should also be factored into future studies. In future work, a daily log that the participants complete on each study day (e.g. Papp et al., 2009) should be used to collect data about the work routine, time away from the dog(s), and the presence of other owners during the participants' absence, along with a daily perceived stress questionnaire to assess owner psychological stress levels specifically for each study day. Additionally, variables such as dog and owner age and sex, and the duration of ownership should be controlled for in the statistical analyses – the small sample size in this thesis meant that these non-significant factors in the models were removed so as to maintain as high a level of statistical power as possible.

Collection of the saliva samples by the participants in their familiar home environment was implemented so as to reduce the stress associated with the study procedures (particularly for the dogs) that may affect the cortisol concentrations obtained (e.g. Burnard et al., 2017). However, with this set-up, it was not possible to collect saliva samples from the dogs whilst the owner was away (i.e. during periods of dog-owner separation), meaning direct measures of the effect of owner absence on dog cortisol and dog-dog coregulation could not be made. Incorporating a small number of laboratory-based separation scenarios (e.g. Mariti et al., 2018; Schöberl et al., 2016) would allow for dog saliva collection by another individual, and would help to better understand dogs' physiological response and coping mechanism to owner absence.

A common limitation of scientific research involving human participants is that the sample population constitutes mostly Western, Educated, Industrialised, Rich and Democratic (WEIRD) individuals (Henrich et al., 2010). This, too, was the case for the sample population of this thesis. Henrich et al. (2010) demonstrate that WEIRD individuals often test as outliers in studies measuring markers of human behaviour and psychology and, therefore, findings based on WEIRD sample populations are less representative when generalising about the species as a whole. It is highly likely that this could apply to the findings of this thesis given that attitudes towards dogs vary greatly across the world depending on factors such as culture and wealth (Serpell, 2017). Replicating the studies presented in this thesis across different sample populations worldwide will be key to validating the results (Henrich et al., 2010) as well as understanding how human perspectives of dogs' role in society affect the formation of attachment bonds.

7.5 Wider applications of the findings

The focus of this thesis has been around advancing our understanding of the biological phenomenon of cortisol coregulation, with particular focus on dog-human and dog-dog social dyads; however, the presented findings, whilst preliminary, could have far-reaching applications to a wide variety of areas. Companion animal welfare is a crucial and fast-growing area of research which aims to ensure that the physical and emotional wellbeing of companion animals is prioritised and that their needs are compatible with the human lifestyle (Buckland et al., 2014; Sonntag and Overall, 2014). By considering dog-owner and dog-dog social relationships on a physiological level, the study of coregulation could have useful applications in understanding how the physiological effects of separation anxiety can be better minimised in domesticated animals, developing a clearer distinction on the benefits and disadvantages of social relationships with conspecific and interspecific social partners, and creating less stress-inducing home environments for singly- and group-housed pets (Sipple et al., 2021; Sonntag and Overall, 2014). This, in turn, could aid domestic animal rehoming centres in understanding the key drivers of pet relinquishment and reduce the regularity with which relinquishment occurs (e.g. see Cardoso et al., 2022).

Beyond humans and domesticated animals, there is a vast variety of social systems in wild non-human animal populations (Krause and Ruxton, 2002), for which we are continually learning more about social network dynamics, communication, and the ultimate benefits and costs of participating in social behaviours (Busia and Griggio, 2020). Measuring cortisol coregulation in wild animal populations could be a key step in understanding the proximate and ultimate function of the mechanism across a broad array of social systems, which, with the development and fine-tuning of hormone profiling from hair, faeces, and urine samples, has become easier to obtain such chronic hormone concentrations non-invasively (Burnard et al., 2017).

7.6 Conclusion

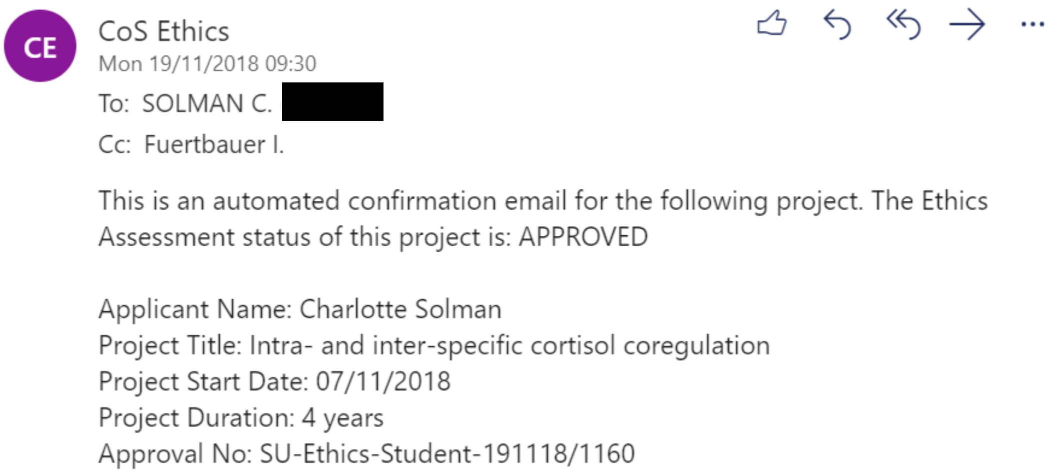
Using a combination of inter- and intra-specific social dyads, this thesis aimed to build upon the existing knowledge of coregulation (see Sbarra and Hazan, 2008), highlight the potential role that non-human animal species can have in coregulation research, and explore possible methodological considerations for future studies in the field. In particular, by identifying intraspecific cortisol coregulation in another non-human animal species (dog) and measuring an effect of two predictors of coregulation strength not previously studied (personality similarity and external social influences; but see Pauly et al., 2021; Saxbe and Repetti, 2010), this thesis expands our understanding of coregulation whilst also demonstrating that there is still a lot to learn about this physiological mechanism that underpins our social attachment bonds (Sbarra and Hazan, 2008; but see Fürtbauer and Heistermann, 2016).

The findings presented here suggest that there may be many important environmental, behavioural, and temporal factors of social interactions and relationships that could be key to coregulation, but have received little attention thus far in the literature (see Tables 1.2-1.4 in Chapter 1; reviews by Davis et al., 2018; Timmons et al., 2015). Developing a study design which incorporates factors such as individual differences/dyadic similarity in personality traits (Chapter 6), the presence/absence of social partners beyond the primary attachment figure (Chapter 4), and short- and long-term cortisol concentrations (Chapters 3 and 5) measured across varying naturalistic and experimental contexts could enable a more realistic representation of coregulation within the complex social networks formed by humans and non-human animals (Krause et al., 2015). A longitudinal study with a large sample size will be key, however, to ensure the influence of these many potentially interacting factors is considered.

Whilst the daily function of cortisol coregulation remains unclear, providing further evidence of cortisol coregulation being a widespread mechanism in social vertebrates (Chapter 4; Burkett et al., 2016; Fürtbauer and Heistermann, 2016) presents the opportunity to test for coregulatory mechanisms across a variety of social and environmental contexts, which would not be possible to achieve looking solely at human social dyads. In turn, being able to make comparisons between these study contexts could help to discern the daily function of the mechanism, given that it is highly context-specific (see Timmons et al., 2015). Understanding both the evolutionary and day-to-day functions of cortisol coregulation will help us to determine the role of coregulation within different types of social relationships, and subsequently apply this to areas such as health and welfare, group cohesion and performance (e.g. Gordon et al., 2020), and the role of physiology on animal hierarchies and societies.

Appendix 1: Ethical Approval

Appendix 1.1 Project approval confirmation email from the College of Science (CoS) Ethics Committee.



CE

CoS Ethics

Mon 19/11/2018 09:30

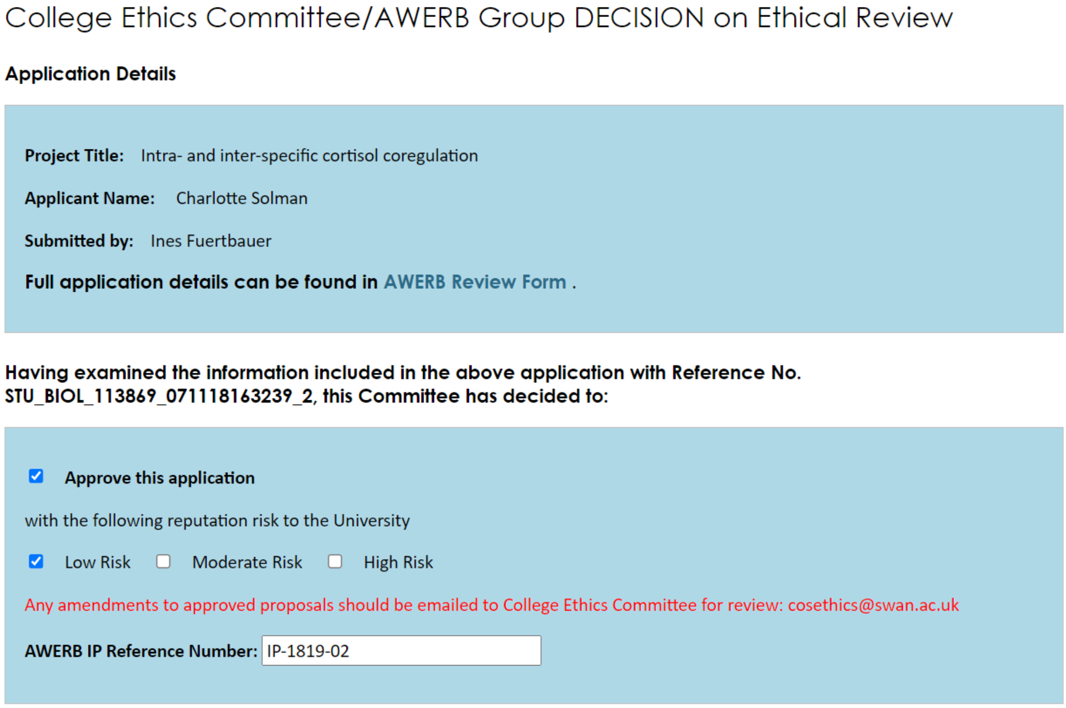
To: SOLMAN C. [redacted]

Cc: Fuertbauer I.

This is an automated confirmation email for the following project. The Ethics Assessment status of this project is: APPROVED

Applicant Name: Charlotte Solman
Project Title: Intra- and inter-specific cortisol coregulation
Project Start Date: 07/11/2018
Project Duration: 4 years
Approval No: SU-Ethics-Student-191118/1160

Appendix 1.2 Summary of project approval decision by CoS Ethics Committee and Animal Welfare and Ethical Review Body (AWERB).



College Ethics Committee/AWERB Group DECISION on Ethical Review

Application Details

Project Title: Intra- and inter-specific cortisol coregulation

Applicant Name: Charlotte Solman

Submitted by: Ines Fuertbauer

Full application details can be found in AWERB Review Form .

Having examined the information included in the above application with Reference No. STU_BIOL_113869_071118163239_2, this Committee has decided to:

☑ **Approve this application**

with the following reputation risk to the University

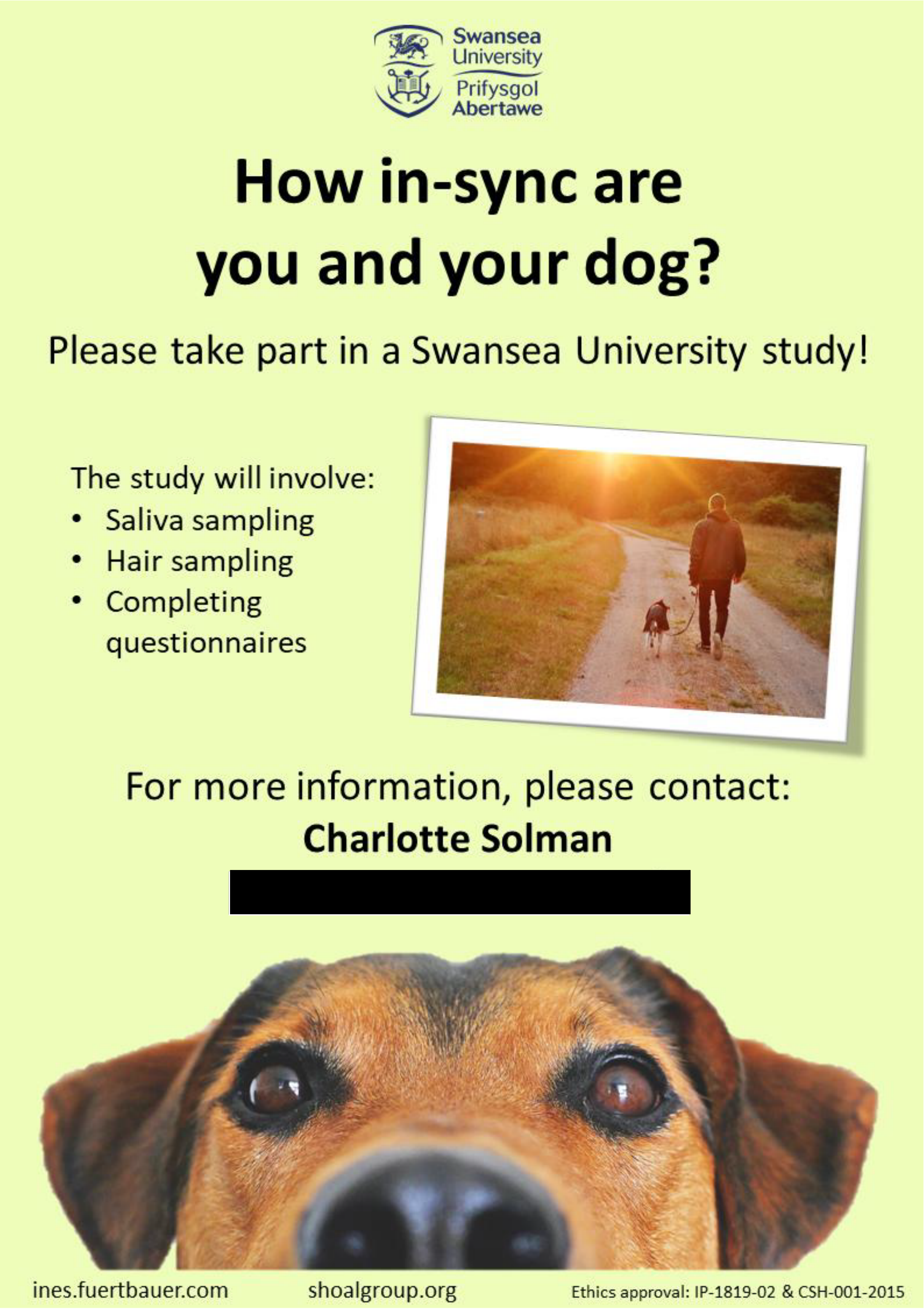
☑ Low Risk ☐ Moderate Risk ☐ High Risk

Any amendments to approved proposals should be emailed to College Ethics Committee for review: cosethics@swan.ac.uk

AWERB IP Reference Number: IP-1819-02

Appendix 2: Study Recruitment Poster

Appendix 2 The poster that was distributed across Swansea University Singleton Campus and sent via email to recruit participants for the study.



Swansea University
Prifysgol Abertawe

How in-sync are you and your dog?

Please take part in a Swansea University study!

The study will involve:

- Saliva sampling
- Hair sampling
- Completing questionnaires

For more information, please contact:

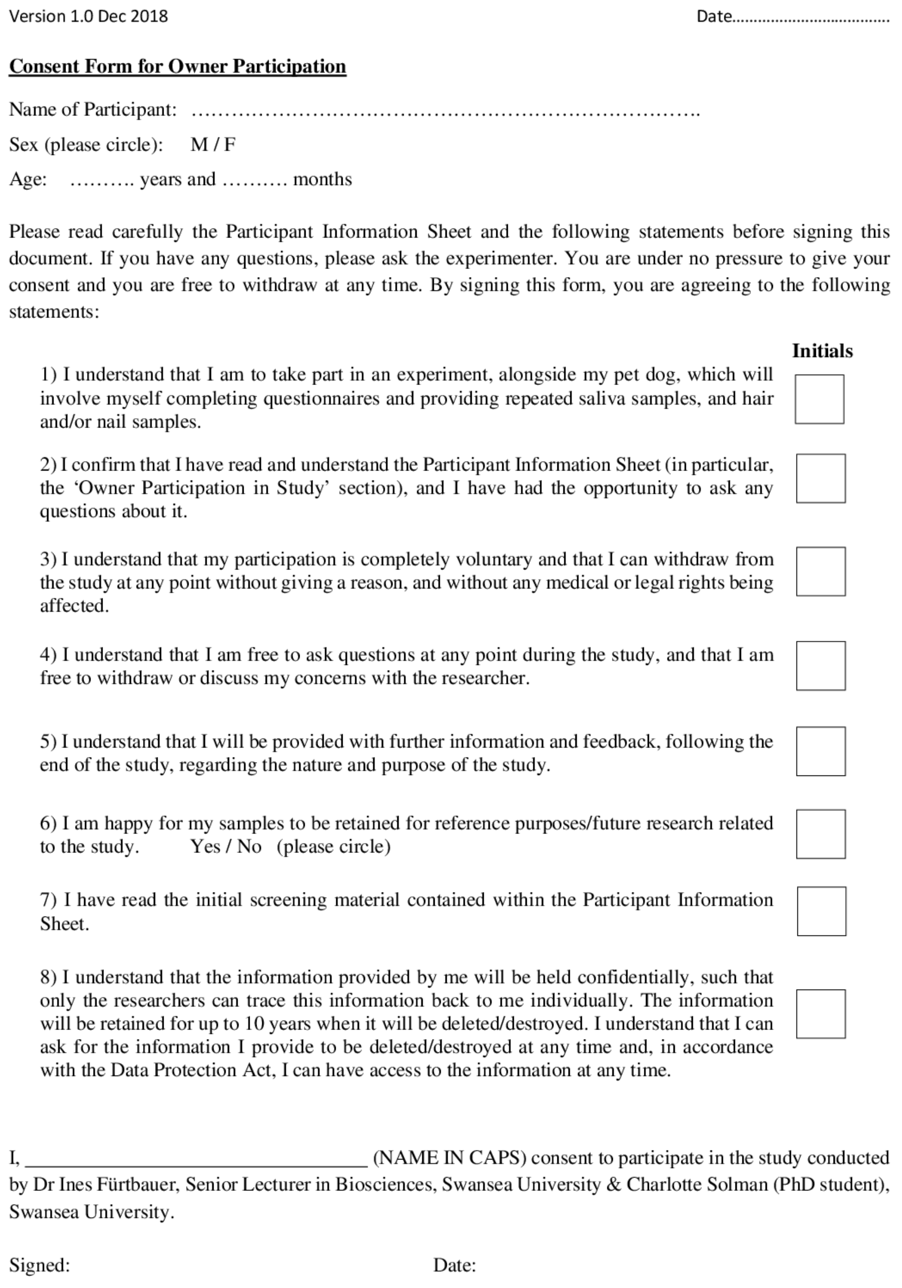
Charlotte Solman

[redacted]

ines.fuertbauer.com shoalgroup.org Ethics approval: IP-1819-02 & CSH-001-2015

Appendix 3: Study Consent Forms

Appendix 3.1 The consent form completed by all participants prior to commencing sample collection



Version 1.0 Dec 2018 Date………………………………

Consent Form for Owner Participation

Name of Participant: ……………………………………………………………………….

Sex (please circle): M / F

Age: ………. years and ………. months

Please read carefully the Participant Information Sheet and the following statements before signing this document. If you have any questions, please ask the experimenter. You are under no pressure to give your consent and you are free to withdraw at any time. By signing this form, you are agreeing to the following statements:

Initials

1) I understand that I am to take part in an experiment, alongside my pet dog, which will involve myself completing questionnaires and providing repeated saliva samples, and hair and/or nail samples. ☐

2) I confirm that I have read and understand the Participant Information Sheet (in particular, the 'Owner Participation in Study' section), and I have had the opportunity to ask any questions about it. ☐

3) I understand that my participation is completely voluntary and that I can withdraw from the study at any point without giving a reason, and without any medical or legal rights being affected. ☐

4) I understand that I am free to ask questions at any point during the study, and that I am free to withdraw or discuss my concerns with the researcher. ☐

5) I understand that I will be provided with further information and feedback, following the end of the study, regarding the nature and purpose of the study. ☐

6) I am happy for my samples to be retained for reference purposes/future research related to the study. Yes / No (please circle) ☐

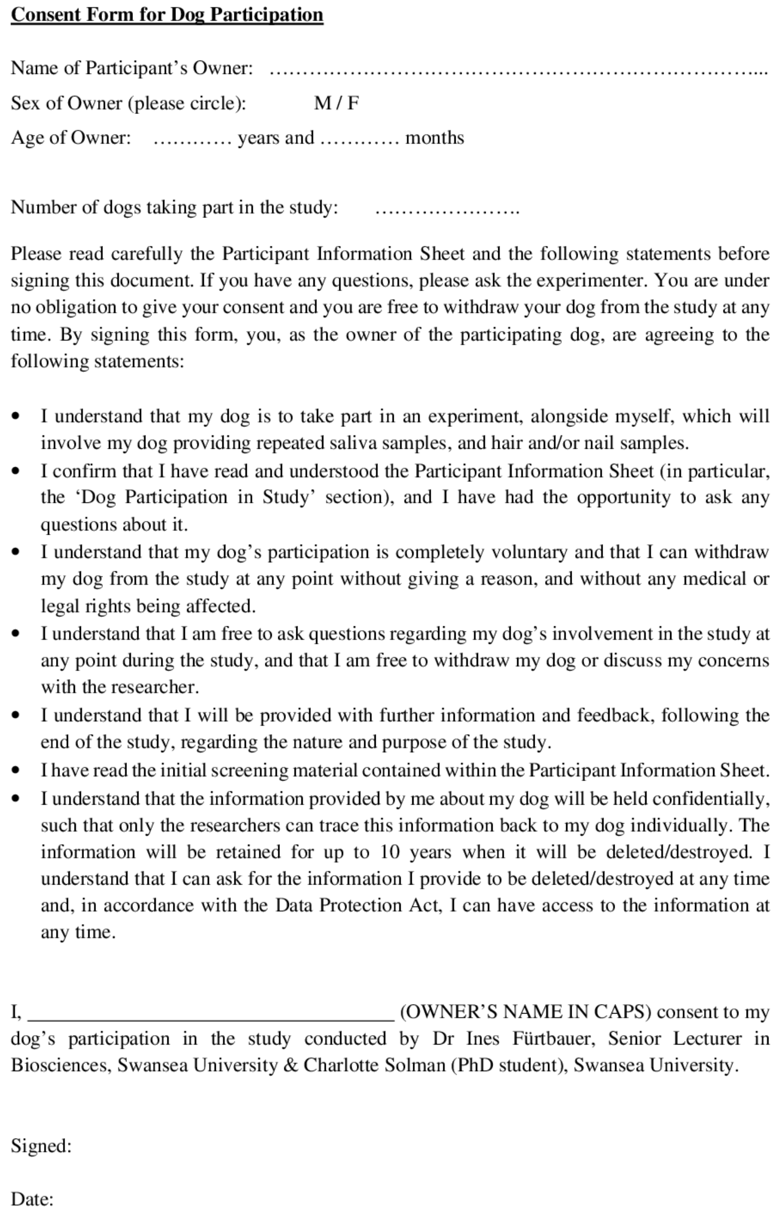
7) I have read the initial screening material contained within the Participant Information Sheet. ☐

8) I understand that the information provided by me will be held confidentially, such that only the researchers can trace this information back to me individually. The information will be retained for up to 10 years when it will be deleted/destroyed. I understand that I can ask for the information I provide to be deleted/destroyed at any time and, in accordance with the Data Protection Act, I can have access to the information at any time. ☐

I, ________________________________ (NAME IN CAPS) consent to participate in the study conducted by Dr Ines Fürtbauer, Senior Lecturer in Biosciences, Swansea University & Charlotte Solman (PhD student), Swansea University.

Signed: Date:

Appendix 3.2 A second consent form for participants to complete, specifically addressing consent for their dogs' participation in the research study.



Consent Form for Dog Participation

Name of Participant's Owner: ……………………………………………………………...
Sex of Owner (please circle): M / F
Age of Owner: ………… years and ………… months

Number of dogs taking part in the study: ………………….

Please read carefully the Participant Information Sheet and the following statements before signing this document. If you have any questions, please ask the experimenter. You are under no obligation to give your consent and you are free to withdraw your dog from the study at any time. By signing this form, you, as the owner of the participating dog, are agreeing to the following statements:

- I understand that my dog is to take part in an experiment, alongside myself, which will involve my dog providing repeated saliva samples, and hair and/or nail samples.
- I confirm that I have read and understood the Participant Information Sheet (in particular, the 'Dog Participation in Study' section), and I have had the opportunity to ask any questions about it.
- I understand that my dog's participation is completely voluntary and that I can withdraw my dog from the study at any point without giving a reason, and without any medical or legal rights being affected.
- I understand that I am free to ask questions regarding my dog's involvement in the study at any point during the study, and that I am free to withdraw my dog or discuss my concerns with the researcher.
- I understand that I will be provided with further information and feedback, following the end of the study, regarding the nature and purpose of the study.
- I have read the initial screening material contained within the Participant Information Sheet.
- I understand that the information provided by me about my dog will be held confidentially, such that only the researchers can trace this information back to my dog individually. The information will be retained for up to 10 years when it will be deleted/destroyed. I understand that I can ask for the information I provide to be deleted/destroyed at any time and, in accordance with the Data Protection Act, I can have access to the information at any time.

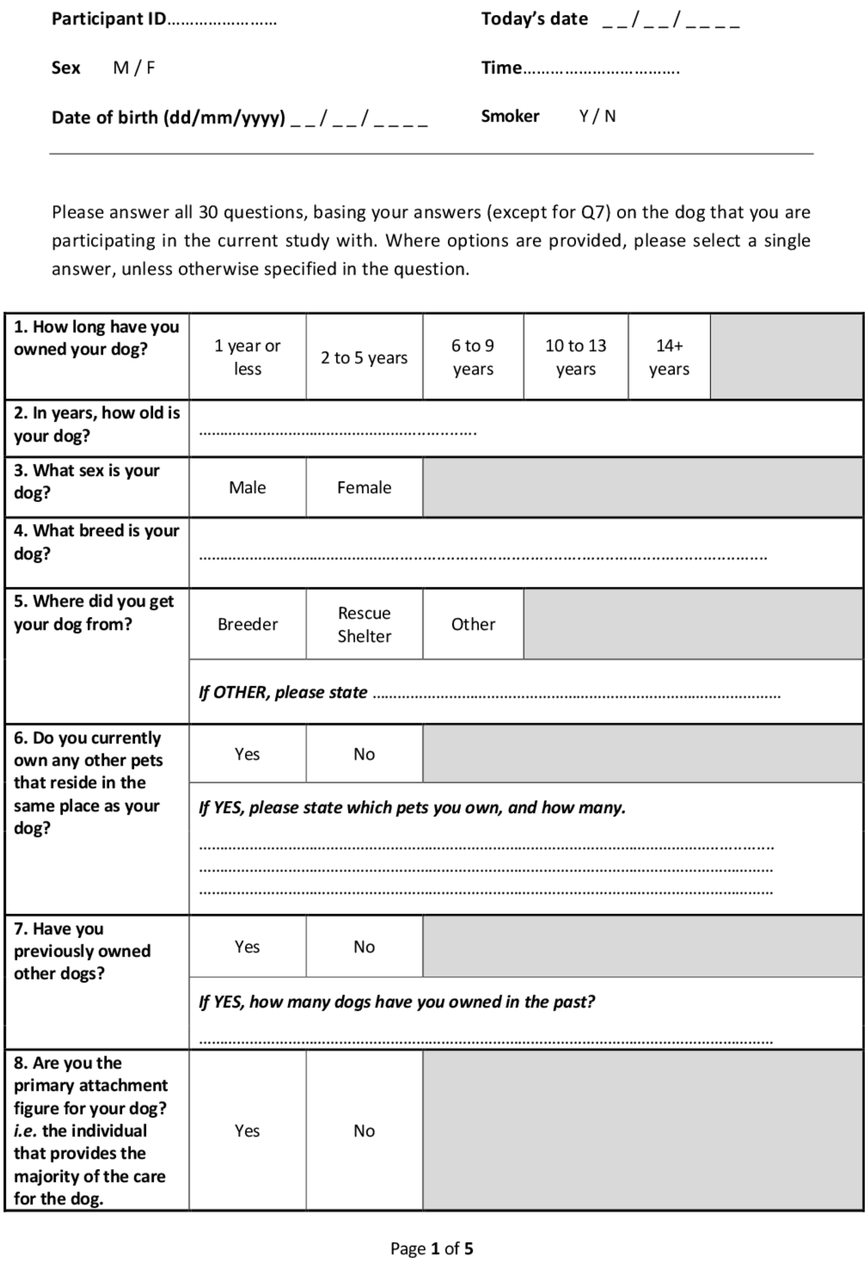
I, ___________________________________ (OWNER'S NAME IN CAPS) consent to my dog's participation in the study conducted by Dr Ines Fürtbauer, Senior Lecturer in Biosciences, Swansea University & Charlotte Solman (PhD student), Swansea University.

Signed:

Date:

Appendix 4: Study-Specific Questionnaire

Appendix 4 Questionnaire designed specifically for the study to collect basic information about the dogs and owners, as well as information about their daily routines and interactions between dog and owner (e.g. shared activities).



Participant ID……………………

Today's date _ _ / _ _ / _ _ _ _

Sex M / F

Time……………………………

Date of birth (dd/mm/yyyy) _ _ / _ _ / _ _ _ _

Smoker Y / N

Please answer all 30 questions, basing your answers (except for Q7) on the dog that you are participating in the current study with. Where options are provided, please select a single answer, unless otherwise specified in the question.

1. How long have you owned your dog?	1 year or less	2 to 5 years	6 to 9 years	10 to 13 years	14+ years	
2. In years, how old is your dog?	………………………………………………					
3. What sex is your dog?	Male	Female				
4. What breed is your dog?	………………………………………………………………………………					
5. Where did you get your dog from?	Breeder	Rescue Shelter	Other			
	If OTHER, please state ……………………………………………………………					
6. Do you currently own any other pets that reside in the same place as your dog?	Yes	No				
	If YES, please state which pets you own, and how many. …………………………………………………………………………… …………………………………………………………………………… ……………………………………………………………………………					
7. Have you previously owned other dogs?	Yes	No				
	If YES, how many dogs have you owned in the past? ……………………………………………………………………………					
8. Are you the primary attachment figure for your dog? *i.e.* the individual that provides the majority of the care for the dog.	Yes	No				

Page **1** of **5**

Appendix 4 cont.

9. How many people live with your dog on a regular basis (including children under 18)?	Just me	Me plus 1 other	Me plus 2 others	Me plus 3 others	Me plus 4 others		
	If MORE THAN 4, please state how many people, in addition to yourself, live with your dog. ………………………………………………………						
10. How many children under the age of 18 live with your dog?	………………………………………………………						
11. Where does your dog sleep at night? If your dog sleeps in different places, please select the place where your dog sleeps most often.	My bed	In my bedroom, but not in my bed	Relative's bedroom	Other room in house	Garage	Outside	Other
12. How often does your dog sleep in your bed?	Every night	Once every few nights	Once a week	Once a month	Never		
13. Do you take your dog with you to work?	Yes	No					
	If YES, how often do you take your dog to work?						
	Daily	Once every few days	Once a week	Monthly	A few times a year		
14. What is your annual household income?	Less than £25,000	Between £25,000 and £40,000	More than £40,000	Prefer not to say			
15. During a normal week (Mon-Fri), how many hours are you away from your dog? Please provide the sum of hours for all five days of the week.	0 hours	5 hours or less	6 to 15 hours	16 to 25 hours	26 to 35 hours	36 to 45 hours	46+ hours

Appendix 4 cont.

16. On a normal weekend (Sat-Sun), how many hours are you away from your dog? Please provide the sum of hours for both days of the weekend.	0 hours	2 hours or less	3 to 6 hours	7 to 10 hours	11 to 14 hours	15 to 18 hours	19+ hours
17. What time do you normally wake up on a workday?	……………………………………………						
18. What time do you normally wake up on a non-workday?	……………………………………………						
19. Is your dog ever in the house on its own for a prolonged period of time (i.e. more than 4 hours)?	Yes	No					
	If YES, how often does this occur?						
	Daily	Once every few days	Once a week	Once every couple of weeks	Monthly		
20. What arrangements do you make if you have to leave your dog alone? Please tick one or more of the following boxes.	€ I am never away from my dog. ☐ My dog is home alone. ☐ I leave my dog alone, but not for more than 4 hours at a time before I return home. ☐ My dog is home alone, but there are other pets also in the house. ☐ Family/friends look after my dog if I am out of the house. ☐ I hire a dog walker to visit my dog. ☐ I take my dog to dog day care ☐ Other… **If OTHER, please state.** …… …… ……						
21. When you return home after a period away from your dog, does your dog get excited and seek physical contact with you?	Yes	No					

Page **3** of **5**

Appendix 4 cont.

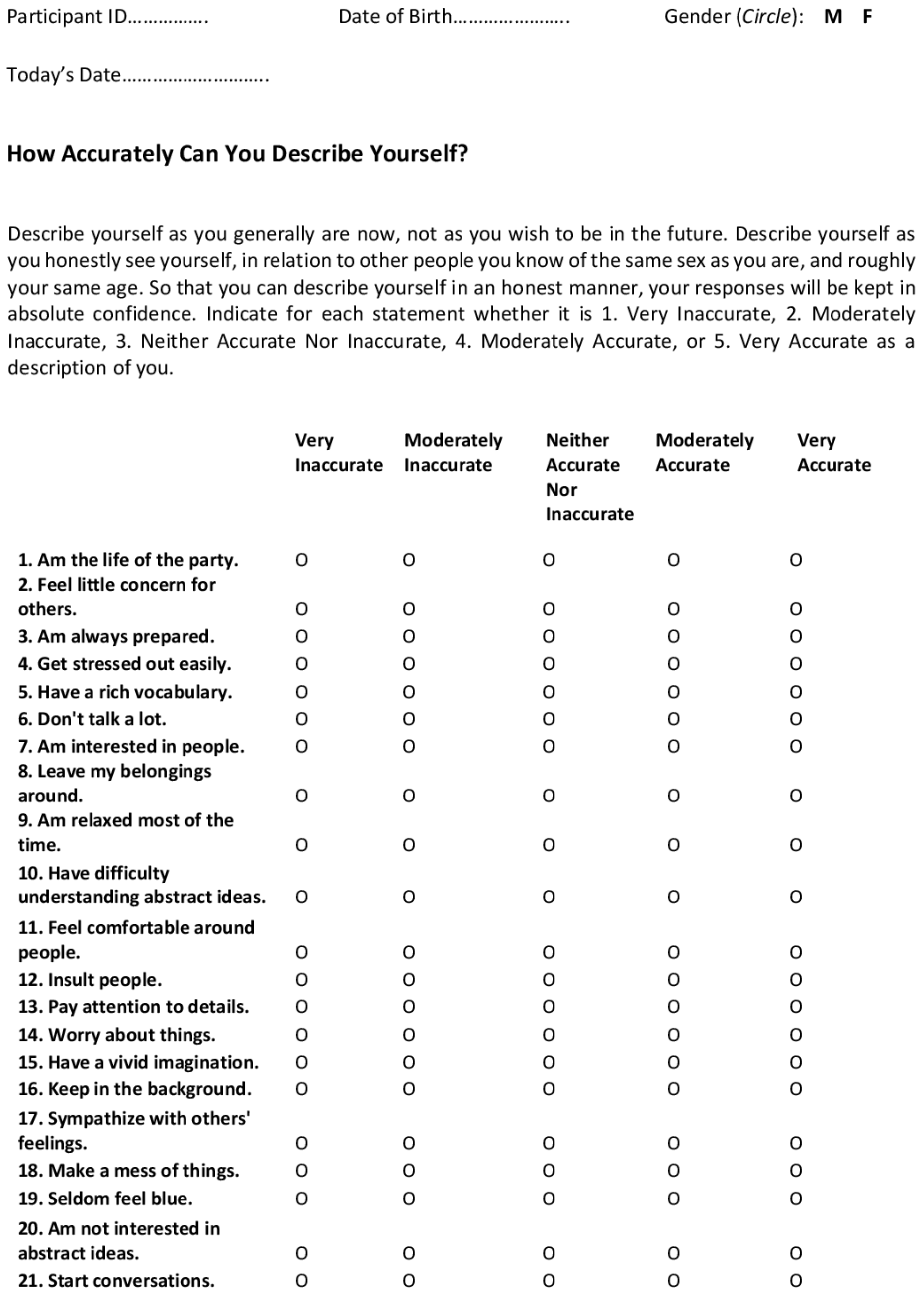
22. Does your dog tend to follow you around the house?	Yes	No				
23. Whilst at home together, roughly what proportion of time is your dog in the same room as you?	0%	25%	50%	75%	100%	
24. How often do you walk your dog during a normal week?	Twice a day	Once a day	Once every few days	Once a week	Never	Other
	If OTHER, please state. ………					
25. Did you own your dog from when it was a puppy?	Yes	No				
	If YES, did you attend a puppy training course(s)?					
	Yes	No				
26. Have you ever attended training or agility sessions with your dog (excluding puppy training sessions)?	Yes	No				
	If YES, how often do you attend training or agility sessions with your dog? *'Continuously' refers to training that does not finish after a set number of weeks like a training course would.*					
	Continuously each week	Continuously each month	One-off course	One course a year	Multiple courses in a year	Other
	If OTHER, please state. ………					

Appendix 4 cont.

27. What activities do you do with your dog? Tick all that apply.	☐ Running ☐ Play games (e.g. fetch) ☐ Swimming ☐ Grooming ☐ Relaxing and/or cuddling ☐ Hiking ☐ Obedience training ☐ Holidays ☐ Tracking (hiding items for dog to find)	☐ Trick training (teach your dog tricks) ☐ Yoga ☐ Dress in dog clothes ☐ Cycling (with dog running alongside) ☐ Agility training ☐ Agility competitions ☐ Other	
	If you selected OTHER, please state. …………………………………………………………………… …………………………………………………………………… …………………………………………………………………… ……………………………………………………………………		
28. To your knowledge, has your dog experienced any stressful events in the last month? This can include things such as visits to vets, or aggressive encounters with other dogs.	Yes	No	
	If YES, please describe the event, including when the event occurred. …………………………………………………………………… …………………………………………………………………… …………………………………………………………………… ……………………………………………………………………		
29. Does your dog suffer from any illnesses or diseases?	Yes	No	
	If YES, please state which illnesses/diseases. …………………………………………………………………… …………………………………………………………………… …………………………………………………………………… ……………………………………………………………………		
30. Does your dog require any medication?	Yes	No	
	If YES, please state which medications your dog requires. …………………………………………………………………… …………………………………………………………………… …………………………………………………………………… ……………………………………………………………………		

Appendix 5: ‘Big-5’ Personality Test

Appendix 5 Copy of the Big-5 personality questionnaire used to assess the owners’ personality in terms of agreeableness, conscientiousness, extroversion, neuroticism, and openness (Goldberg, 1990).



Participant ID……………. Date of Birth………………….. Gender (*Circle*): **M F**

Today’s Date………………………..

How Accurately Can You Describe Yourself?

Describe yourself as you generally are now, not as you wish to be in the future. Describe yourself as you honestly see yourself, in relation to other people you know of the same sex as you are, and roughly your same age. So that you can describe yourself in an honest manner, your responses will be kept in absolute confidence. Indicate for each statement whether it is 1. Very Inaccurate, 2. Moderately Inaccurate, 3. Neither Accurate Nor Inaccurate, 4. Moderately Accurate, or 5. Very Accurate as a description of you.

	Very Inaccurate	**Moderately Inaccurate**	**Neither Accurate Nor Inaccurate**	**Moderately Accurate**	**Very Accurate**
1. Am the life of the party.	○	○	○	○	○
2. Feel little concern for others.	○	○	○	○	○
3. Am always prepared.	○	○	○	○	○
4. Get stressed out easily.	○	○	○	○	○
5. Have a rich vocabulary.	○	○	○	○	○
6. Don't talk a lot.	○	○	○	○	○
7. Am interested in people.	○	○	○	○	○
8. Leave my belongings around.	○	○	○	○	○
9. Am relaxed most of the time.	○	○	○	○	○
10. Have difficulty understanding abstract ideas.	○	○	○	○	○
11. Feel comfortable around people.	○	○	○	○	○
12. Insult people.	○	○	○	○	○
13. Pay attention to details.	○	○	○	○	○
14. Worry about things.	○	○	○	○	○
15. Have a vivid imagination.	○	○	○	○	○
16. Keep in the background.	○	○	○	○	○
17. Sympathize with others' feelings.	○	○	○	○	○
18. Make a mess of things.	○	○	○	○	○
19. Seldom feel blue.	○	○	○	○	○
20. Am not interested in abstract ideas.	○	○	○	○	○
21. Start conversations.	○	○	○	○	○

Appendix 5 cont.

	Very Inaccurate	Moderately Inaccurate	Neither Accurate Nor Inaccurate	Moderately Accurate	Very Accurate
22. Am not interested in other people's problems.	○	○	○	○	○
23. Get chores done right away.	○	○	○	○	○
24. Am easily disturbed.	○	○	○	○	○
25. Have excellent ideas.	○	○	○	○	○
26. Have little to say.	○	○	○	○	○
27. Have a soft heart.	○	○	○	○	○
28. Often forget to put things back in their proper place.	○	○	○	○	○
29. Get upset easily.	○	○	○	○	○
30. Do not have a good imagination.	○	○	○	○	○
31. Talk to a lot of different people at parties.	○	○	○	○	○
32. Am not really interested in others.	○	○	○	○	○
33. Like order.	○	○	○	○	○
34. Change my mood a lot.	○	○	○	○	○
35. Am quick to understand things.	○	○	○	○	○
36. Don't like to draw attention to myself.	○	○	○	○	○
37. Take time out for others.	○	○	○	○	○
38. Shirk my duties.	○	○	○	○	○
39. Have frequent mood swings.	○	○	○	○	○
40. Use difficult words.	○	○	○	○	○
41. Don't mind being the centre of attention.	○	○	○	○	○
42. Feel others' emotions.	○	○	○	○	○
43. Follow a schedule.	○	○	○	○	○
44. Get irritated easily.	○	○	○	○	○
45. Spend time reflecting on things.	○	○	○	○	○
46. Am quiet around strangers.	○	○	○	○	○
47. Make people feel at ease.	○	○	○	○	○
48. Am exacting in my work.	○	○	○	○	○
49. Often feel blue.	○	○	○	○	○
50. Am full of ideas.	○	○	○	○	○

Appendix 6: Canine Behavioural Assessment and Research Questionnaire (C-BARQ)

Appendix 6 Copy of the Canine Behavioural Assessment and Research Questionnaire (C-BARQ) used to assess behavioural traits in the dogs, such as attachment, fear, separation, and aggression (Hsu and Serpell, 2003).

The C-BARQ questionnaire

The questions on the following pages are designed to allow you to describe how your dog has been behaving in the recent past. Studies have shown that most of these questions can be grouped or condensed into a set of thirteen major behavioral traits or factors that describe most of the variation in canine temperament, and which are relatively consistent across dogs of different sexes, breeds, and ages:

1. Stranger-directed aggression
2. Owner-directed aggression
3. Dog-directed aggression/fear
4. Trainability
5. Chasing
6. Stranger-directed fear
7. Nonsocial fear
8. Dog-directed fear
9. Separation-related behavior
10. Touch sensitivity
11. Excitability
12. Attachment or attention-seeking
13. Energy

Please try to answer all of the questions. Only use the "not observed/not applicable" option if you have never observed the dog in the situation described.

Section 1: Training and obedience

Some dogs are more trainable than others. By writing in the appropriate number from the scale, please indicate how trainable or obedient your dog has been in each of the following situations in the recent past.

Not observed/
Not applicable
N/A

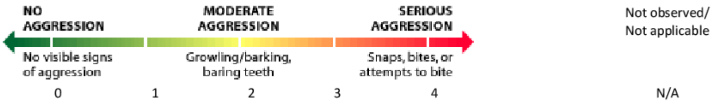
1. When off the leash, returns immediately when called.	
2. Obeys the "sit" command immediately	
3. Obeys the "stay" command immediately..	
4. Seems to attend/listen closely to everything you say or do .	
5. Slow to respond to correction or punishment; "thick-skinned".	
6. Slow to learn new tricks or tasks.	
7. Easily distracted by interesting sights, sounds, or smells.	
8. Will "fetch" or attempt to fetch sticks, balls, or objects.	

Appendix 6 cont.

C-BARQ

Section 2: Aggression

Some dogs display aggressive behavior from time to time. Typical signs of moderate aggression in dogs include barking, growling and baring teeth. More serious aggression generally includes snapping, lunging, biting, or attempting to bite. By writing in the appropriate number from the scale, please indicate your own dog's recent tendency to display aggressive behavior in each of the following contexts:



9. When verbally corrected or punished (scolded, shouted at, etc.) by you or a household member.	
10. When approached directly by an unfamiliar adult while being walked/exercised on a leash.	
11. When approached directly by an unfamiliar child while being walked/exercised on a leash.	
12. Toward unfamiliar persons approaching the dog while s/he is in your car (at the gas station, for example).	
13. When toys, bones or other objects are taken away by a household member.	
14. When bathed or groomed by a household member.	
15. When an unfamiliar person approaches you or another member of your family at home.	
16. When unfamiliar persons approach you or another member of your family away from your home.	
17. When approached directly by a household member while s/he is eating.	
18. When mailmen or other delivery workers approach your home.	
19. When his/her food is taken away by a household member.	
20. When strangers walk past your home while your dog is outside or in the yard.	
21. When an unfamiliar person tries to touch or pet the dog.	
22. When joggers, cyclists, rollerbladers or skateboarders pass your home while your dog is outside or in the yard.	
23. When approached directly by an unfamiliar male dog while being walked/exercised on a leash.	
24. When approached directly by an unfamiliar female dog while being walked/exercised on a leash.	
25. When stared at directly by a member of the household.	
26. Toward unfamiliar dogs visiting your home.	
27. Toward cats, squirrels or other animals entering your yard.	
28. Toward unfamiliar persons visiting your home.	

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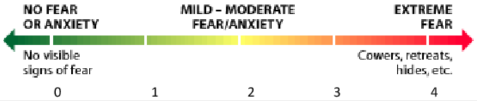
29. When barked, growled, or lunged at by another (unfamiliar) dog.	
30. When stepped over by a member of the household.	
31. When you or a household member retrieves food or objects stolen by the dog.	
32. Towards another (familiar) dog in your household.	
33. When approached at a favorite resting/sleeping place by another (familiar) household dog.	
34. When approached while eating by another (familiar) household dog.	
35. When approached while playing with/chewing a favorite toy, bone, object, etc., by another (familiar) household dog.	

Are there any other situations in which your dog is sometimes aggressive? If so, please describe briefly:

Section 3: Fear and anxiety

Dogs sometimes show signs of anxiety or fear when exposed to particular sounds, objects, persons or situations. Typical signs of mild to moderate fear include: avoiding eye contact, avoidance of the feared object, crouching or cringing with tail lowered or tucked between the legs, whimpering and whining, freezing, and shaking and trembling. Extreme fear is characterized by exaggerated cowering, and/or vigorous attempts to escape, retreat or hide from the feared object, person or situation. By writing in the appropriate number from the scale, please indicate your own dog's recent tendency to display fearful behavior in each of the following contexts:



Not observed/
Not applicable

N/A

36. When approached directly by an unfamiliar adult while away from your home.	
37. When approached directly by an unfamiliar child while away from your home.	
38. In response to sudden or loud noises (e.g. vacuum cleaner, car backfire, road drills, objects being dropped, etc.).	
39. When unfamiliar persons visit your home.	
40. When an unfamiliar person tries to touch or pet the dog.	
41. In heavy traffic.	
42. In response to strange or unfamiliar objects on or near the sidewalk (e.g. plastic trash bags, leaves, litter, flags flapping, etc.).	
43. When examined/treated by a veterinarian.	
44. During thunderstorms, firework displays, or similar events.	

45. When approached directly by an unfamiliar dog of the same or larger size.	
46. When approached directly by an unfamiliar dog of smaller size.	
47. When first exposed to unfamiliar situations (e.g. first car trip, first time in elevator, first visit to veterinarian, etc.).	
48. In response to wind or wind-blown objects.	
49. When having nails clipped by a household member.	
50. When groomed or bathed by a household member.	
51. When stepped over by a member of the household.	
52. When having his/her feet toweled by a member of the household.	
53. When unfamiliar dogs visit your home.	
54. When barked, growled, or lunged at by an unfamiliar dog.	

Section 4: Separation-related behavior

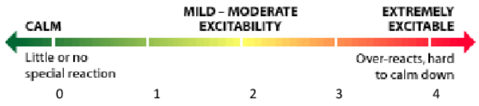
Some dogs show signs of anxiety or abnormal behavior when left alone, even for relatively short periods of time. Thinking back over the recent past, how often has your dog shown each of the following signs of separation-related behavior when left, or about to be left, on its own:

	Not observed/ Not applicable N/A
55. Shaking, shivering, or trembling.	
56. Excessive salivation.	
57. Restlessness, agitation, or pacing.	
58. Whining.	
59. Barking.	
60. Howling.	
61. Chewing or scratching at doors, floor, windows, curtains, etc.	
62. Loss of appetite.	

Are there any other situations in which your dog is fearful or anxious? If so, please describe briefly:

Section 5: Excitability

Some dogs show relatively little reaction to sudden or potentially exciting events and disturbances in their environment, while others become highly excited at the slightest novelty. Signs of mild to moderate excitability include increased alertness, movement toward the source of novelty, and brief episodes of barking. Extreme excitability is characterized by a general tendency to over-react. The excitable dog barks or yelps hysterically at the slightest disturbance, rushes toward and around any source of excitement, and is difficult to calm down. By writing in the appropriate number from the scale, please indicate your own dog's recent tendency to become excitable in each of the following contexts:



Not observed/
Not applicable
N/A

63. When you or other members of the household come home after a brief absence.	
64. When playing with you or other members of your household.	
65. When doorbell rings.	
66. Just before being taken for a walk.	
67. Just before being taken on a car trip.	
68. When visitors arrive at your home.	

Are there any other situations in which your dog sometimes becomes over-excited? If so, please describe briefly:

Section 6: Attachment and attention-seeking

Most dogs are strongly attached to their people, and some demand a great deal of attention and affection from them. Thinking back over the recent past, how often has your dog shown each of the following signs of attachment or attention-seeking:

Not observed/
Not applicable
N/A

69. Displays a strong attachment for one particular member of the household.	
70. Tends to follow you (or other members of the household) about the house, from room to room.	
71. Tends to sit close to, or in contact with, you (or others) when you are sitting down.	
72. Tends to nudge, nuzzle or paw you (or others) for attention when you are sitting down.	
73. Becomes agitated (whines, jumps up, tries to intervene) when you (or others) show affection for another person.	
74. Becomes agitated (whines, jumps up, tries to intervene) when you (or others) show affection for another dog or animal.	

Section 7: Miscellaneous

Dogs display a wide range of miscellaneous behavior problems in addition to those already covered by this questionnaire. Thinking back over the recent past, please indicate how often your dog has shown any of the following behaviors:

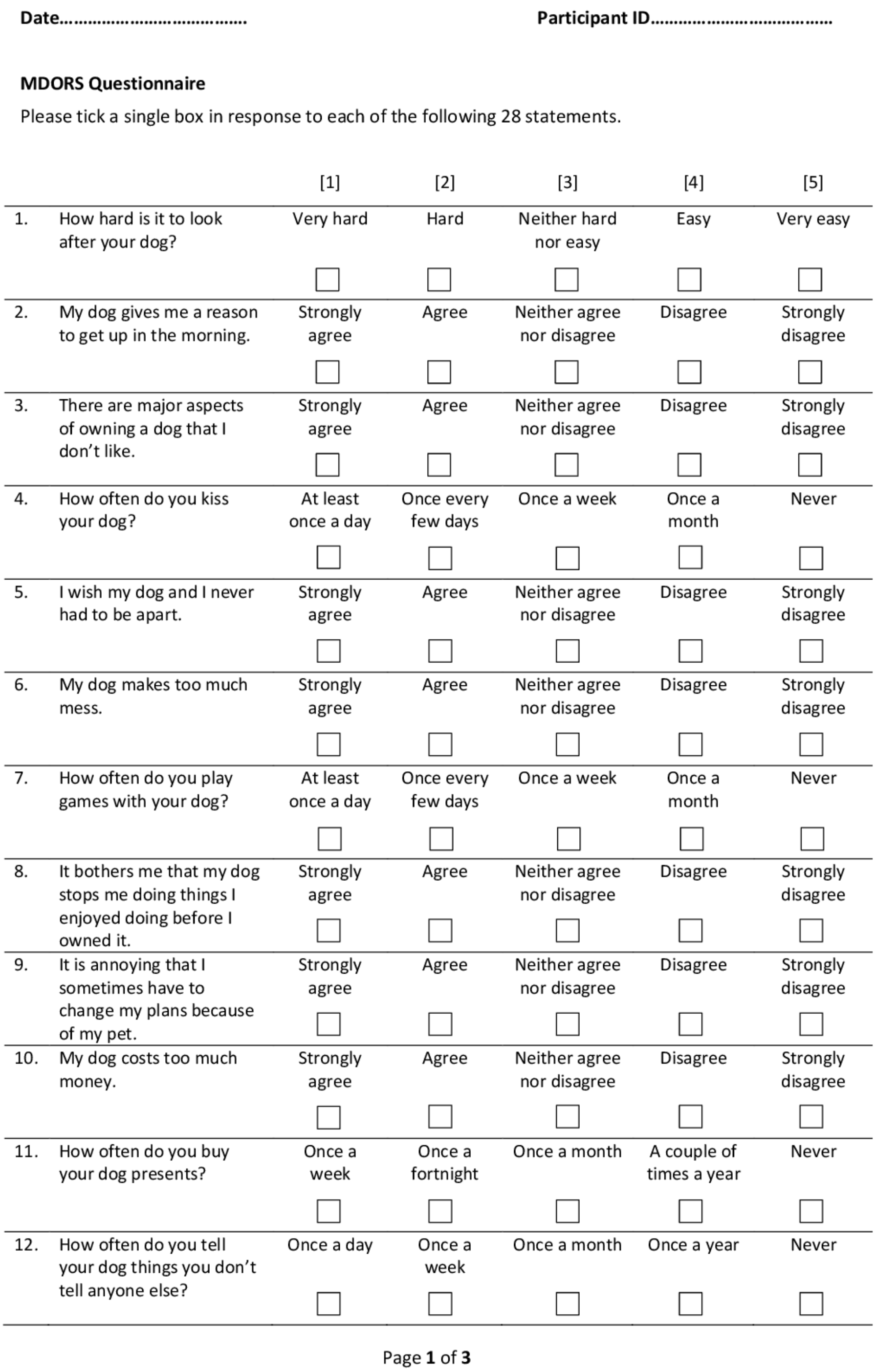
NEVER	SELDOM	SOMETIMES	USUALLY	ALWAYS	Not observed/ Not applicable
0	1	2	3	4	N/A

75. Chases or would chase cats given the opportunity.	
76. Chases or would chase birds given the opportunity.	
77. Chases or would chase squirrels, rabbits and other small animals given the opportunity.	
78. Escapes or would escape from home or yard given the chance.	
79. Rolls in animal droppings or other "smelly" substances.	
80. Eats own or other animals' droppings or feces.	
81. Chews inappropriate objects.	
82. "Mounts" objects, furniture, or people.	
83. Begs persistently for food when people are eating.	
84. Steals food.	
85. Nervous or frightened on stairs.	
86. Pulls excessively hard when on the leash.	
87. Urinates against objects/furnishings in your home.	
88. Urinates when approached, petted, handled or picked up.	
89. Urinates when left alone at night, or during the daytime.	
90. Defecates when left alone at night, or during the daytime.	
91. Hyperactive, restless, has trouble settling down.	
92. Playful, puppyish, boisterous.	
93. Active, energetic, always on the go.	
94. Stares intently at nothing visible.	
95. Snaps at (invisible) flies.	
96. Chases own tail/hind end.	
97. Chases/follows shadows, light spots, etc.	
98. Barks persistently when alarmed or excited.	
99. Licks him/herself excessively.	
100. Licks people or objects excessively.	
101. Displays other bizarre, strange, or repetitive behavior(s).*	

*Describe briefly:

Appendix 7: Monash Dog-Owner Relationship Scale (MDORS)

Appendix 7 Copy of the Monash Dog-Owner Relationship Scale (MDORS) questionnaire used to assess dog and owner interactions, emotional closeness, and perceived costs of dog ownership (Dwyer et al., 2006).



Date…………………………………. **Participant ID………………………………….**

MDORS Questionnaire

Please tick a single box in response to each of the following 28 statements.

		[1]	[2]	[3]	[4]	[5]
1.	How hard is it to look after your dog?	Very hard ☐	Hard ☐	Neither hard nor easy ☐	Easy ☐	Very easy ☐
2.	My dog gives me a reason to get up in the morning.	Strongly agree ☐	Agree ☐	Neither agree nor disagree ☐	Disagree ☐	Strongly disagree ☐
3.	There are major aspects of owning a dog that I don't like.	Strongly agree ☐	Agree ☐	Neither agree nor disagree ☐	Disagree ☐	Strongly disagree ☐
4.	How often do you kiss your dog?	At least once a day ☐	Once every few days ☐	Once a week ☐	Once a month ☐	Never ☐
5.	I wish my dog and I never had to be apart.	Strongly agree ☐	Agree ☐	Neither agree nor disagree ☐	Disagree ☐	Strongly disagree ☐
6.	My dog makes too much mess.	Strongly agree ☐	Agree ☐	Neither agree nor disagree ☐	Disagree ☐	Strongly disagree ☐
7.	How often do you play games with your dog?	At least once a day ☐	Once every few days ☐	Once a week ☐	Once a month ☐	Never ☐
8.	It bothers me that my dog stops me doing things I enjoyed doing before I owned it.	Strongly agree ☐	Agree ☐	Neither agree nor disagree ☐	Disagree ☐	Strongly disagree ☐
9.	It is annoying that I sometimes have to change my plans because of my pet.	Strongly agree ☐	Agree ☐	Neither agree nor disagree ☐	Disagree ☐	Strongly disagree ☐
10.	My dog costs too much money.	Strongly agree ☐	Agree ☐	Neither agree nor disagree ☐	Disagree ☐	Strongly disagree ☐
11.	How often do you buy your dog presents?	Once a week ☐	Once a fortnight ☐	Once a month ☐	A couple of times a year ☐	Never ☐
12.	How often do you tell your dog things you don't tell anyone else?	Once a day ☐	Once a week ☐	Once a month ☐	Once a year ☐	Never ☐

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Appendix 7 cont.

13.	How often do you feel that looking after you dog is a chore?	Once a day ☐	Once a week ☐	Once a month ☐	Once a year ☐	Never ☐
14.	How often does your dog stop you doing things you want to do?	Once a day ☐	Once a week ☐	Once a month ☐	Once a year ☐	Never ☐
15.	I would like to have my dog near me all the time.	Strongly agree ☐	Agree ☐	Neither agree nor disagree ☐	Disagree ☐	Strongly disagree ☐
16.	If everyone else left me, my dog would still be there for me.	Strongly agree ☐	Agree ☐	Neither agree nor disagree ☐	Disagree ☐	Strongly disagree ☐
17.	How often do you feel that having a pet is more trouble than it is worth?	Once a day ☐	Once a week ☐	Once a month ☐	Once a year ☐	Never ☐
18.	My dog helps me get through tough times.	Strongly agree ☐	Agree ☐	Neither agree nor disagree ☐	Disagree ☐	Strongly disagree ☐
19.	How often do you hug your dog?	At least once a day ☐	Once every few days ☐	Once a week ☐	Once a month ☐	Never ☐
20.	My dog provides me with constant companionship.	Strongly agree ☐	Agree ☐	Neither agree nor disagree ☐	Disagree ☐	Strongly disagree ☐
21.	How often do you have your dog with you while relaxing, *i.e.* watching TV?	At least once a day ☐	Once every few days ☐	Once a week ☐	Once a month ☐	Never ☐
22.	My dog is there whenever I need to be comforted.	Strongly agree ☐	Agree ☐	Neither agree nor disagree ☐	Disagree ☐	Strongly disagree ☐
23.	How traumatic do you think it will be for you when your dog dies?	Very traumatic ☐	Traumatic ☐	Neither traumatic nor untraumatic ☐	Untraumatic ☐	Very untraumatic ☐
24.	How often do you take your dog to visit people?	Once a week ☐	Once a fortnight ☐	Once a month ☐	A couple of times a year ☐	Never ☐
25.	How often do you give your dog food treats?	At least once a day ☐	Once every few days ☐	Once a week ☐	Once a month ☐	Never ☐

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Appendix 7 cont.

26.	How often do you take your dog in the car?	At least once a day ☐	Once every few days ☐	Once a week ☐	Once a month ☐	Never ☐
27.	How often do you groom your dog?	At least once a day ☐	Once every few days ☐	Once a week ☐	Once a month ☐	Never ☐
28.	My pet is constantly attentive to me.	Strongly agree ☐	Agree ☐	Neither agree nor disagree ☐	Disagree ☐	Strongly disagree ☐

End of questionnaire.

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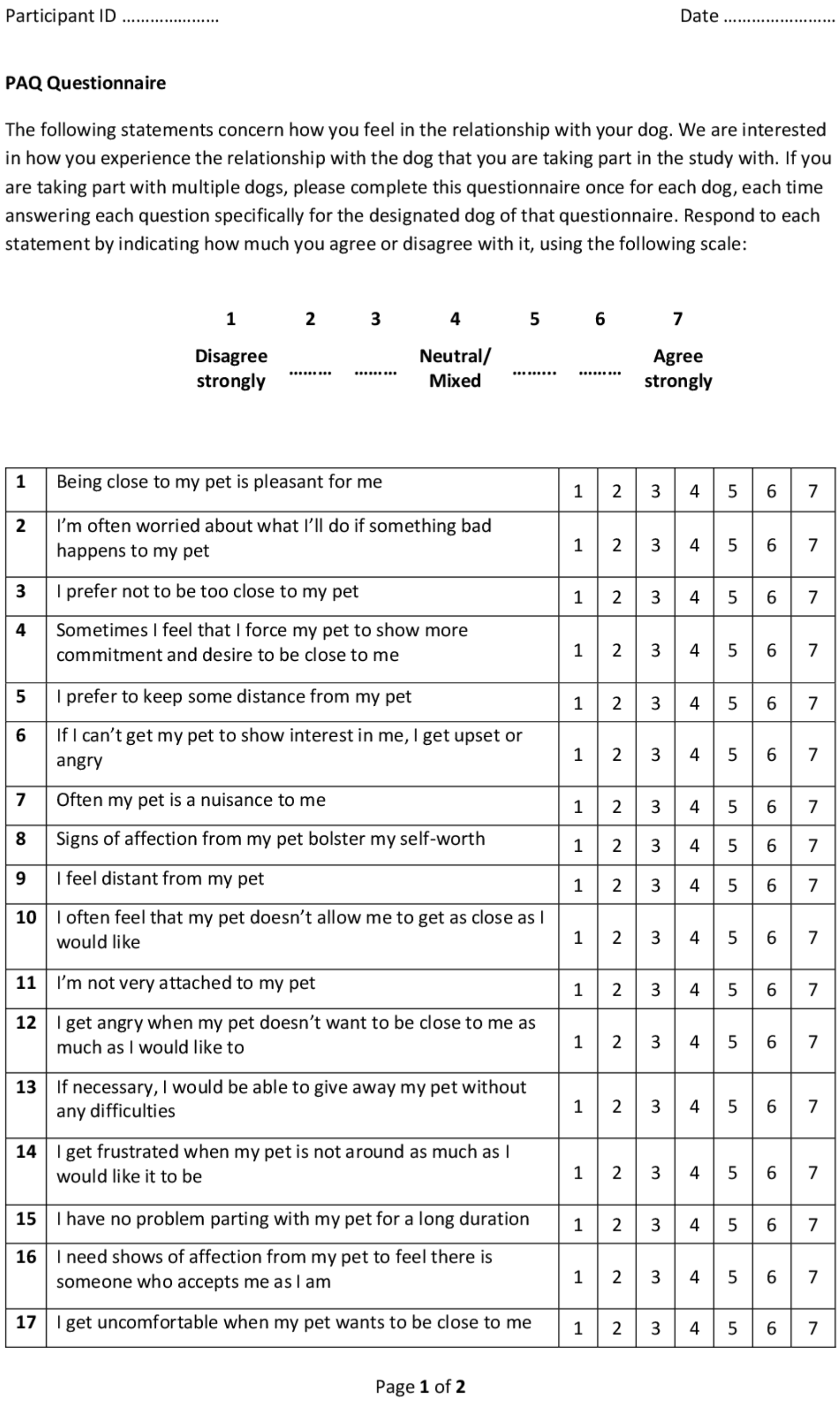
Appendix 8: Pet Attachment Questionnaire (PAQ)

Appendix 8 Copy of the Pet Attachment Questionnaire (PAQ) used to assess owner attachment avoidance and anxiety in the relationship with their dog (Zilcha-Mano et al., 2011)



Participant ID ………………… Date ……………………

PAQ Questionnaire

The following statements concern how you feel in the relationship with your dog. We are interested in how you experience the relationship with the dog that you are taking part in the study with. If you are taking part with multiple dogs, please complete this questionnaire once for each dog, each time answering each question specifically for the designated dog of that questionnaire. Respond to each statement by indicating how much you agree or disagree with it, using the following scale:

1	**2**	**3**	**4**	**5**	**6**	**7**
Disagree strongly	………	………	**Neutral/ Mixed**	………	………	**Agree strongly**

1	Being close to my pet is pleasant for me	1	2	3	4	5	6	7
2	I'm often worried about what I'll do if something bad happens to my pet	1	2	3	4	5	6	7
3	I prefer not to be too close to my pet	1	2	3	4	5	6	7
4	Sometimes I feel that I force my pet to show more commitment and desire to be close to me	1	2	3	4	5	6	7
5	I prefer to keep some distance from my pet	1	2	3	4	5	6	7
6	If I can't get my pet to show interest in me, I get upset or angry	1	2	3	4	5	6	7
7	Often my pet is a nuisance to me	1	2	3	4	5	6	7
8	Signs of affection from my pet bolster my self-worth	1	2	3	4	5	6	7
9	I feel distant from my pet	1	2	3	4	5	6	7
10	I often feel that my pet doesn't allow me to get as close as I would like	1	2	3	4	5	6	7
11	I'm not very attached to my pet	1	2	3	4	5	6	7
12	I get angry when my pet doesn't want to be close to me as much as I would like to	1	2	3	4	5	6	7
13	If necessary, I would be able to give away my pet without any difficulties	1	2	3	4	5	6	7
14	I get frustrated when my pet is not around as much as I would like it to be	1	2	3	4	5	6	7
15	I have no problem parting with my pet for a long duration	1	2	3	4	5	6	7
16	I need shows of affection from my pet to feel there is someone who accepts me as I am	1	2	3	4	5	6	7
17	I get uncomfortable when my pet wants to be close to me	1	2	3	4	5	6	7

Page **1** of **2**

Appendix 8 cont.

Participant ID ………………… Date ……………………

18	I feel frustrated if my pet doesn't seem to be available for me when I need it	1	2	3	4	5	6	7
19	I get nervous when my pet gets too close to me	1	2	3	4	5	6	7
20	Without acts of affection from my pet I feel worthless	1	2	3	4	5	6	7
21	I want to get close to my pet, but I keep pulling away	1	2	3	4	5	6	7
22	I am worried about being left alone without my pet	1	2	3	4	5	6	7
23	I try to avoid getting too close to my pet	1	2	3	4	5	6	7
24	I need expressions of love from my pet to feel valuable	1	2	3	4	5	6	7
25	When I'm away from my pet for a long period of time, I hardly think about it	1	2	3	4	5	6	7
26	I need a lot of reassurance from my pet that it loves me	1	2	3	4	5	6	7

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