Assessing the social organization of multi-dog households: Dog behaviour, hormones, personality, and demographics

by

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ABSTRACT

Domestic dogs and grey wolves, although related species, display differences in social organization. As has been shown in captive vs. free-living wolves, context differences affecting social organization are likely to exist in domestic dogs. Social status has been investigated in free-ranging domestic dogs, among dogs living in kennel environments, and among pet dogs at a day-care facility, but not among cohabiting pet dogs. This study examines whether three methods (a toy possession test (TPT), behavioural characteristics, and owner-reported dominance) for evaluating dominance among dogs living in multi-dog homes yield convergent results. There were no significant relationships among the outcomes of the three methods, indicating they are likely measuring different constructs. Higher TPT scores were related to higher baseline testosterone, an interaction between baseline cortisol and testosterone characterized by low cortisol/high testosterone levels, relative age, Extraversion and Motivation. Behaviours previously associated with dominance were not consistent in their relationships with dog demographics (sex, age), hormones, and personality traits, as measured by the Monash Canine Personality Questionnaire-Revised (MCPQ-R). Owner-reported dominant dogs had higher relative Motivation and Training Focus and lower Amicability. Cohabiting pet dog social organization is probably different from that found in other dog groups, perhaps due to owner behaviour. Assumptions derived from wolf and feral dog studies about the concept of dominance must be evaluated carefully before being accepted as valid for pet dogs.

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1. Social structure of cohabiting domestic dogs

1.1. Introduction

Domestic dogs (*Canis lupus familiaris*) are commonly kept as household pets throughout the world, but particularly in Westernized countries, where they frequently live with two or more humans as well as other dogs. The social organization of pet dogs in multi-dog households is not well understood. Popular culture has been invaded by notions of the presence of a "top dog" in a household dog "pack" (e.g., https://www.cesarsway.com/dog-psychology/pack/Dog-pack-hierarchy), which, until fairly recently, appeared to be the predominant view of dog social organization. More recent research, described below, has challenged these popular notions of dog social structure; however, while social hierarchies have been examined in feral dog groups (e.g., Cafazzo, Valsecchi, Bonanni, & Natoli, 2010), and kennelled dogs (e.g., Bradshaw, Blackwell, & Casey, 2009; Schilder, Vinke, & van der Borg, 2014), there has been no empirical work to date on pet dogs living in multi-dog households. The objective of this study is to examine the relationship between different methods for assessing social status in domestic dogs living in human households, and relate them to demographic, hormonal and personality characteristics. In this chapter, the social organization of domestic dogs is discussed in the context of the evolutionary connection between dogs and wolves, domestication, and previous studies on dog social structure.

Domestic dogs and modern grey wolves (*Canis lupus*) have evolved from a common wolf ancestor, but domestic dogs have been living in closer association with humans for many thousands of years (Frantz et al., 2016; Larson et al., 2012; Wayne &

Vilá, 2002). Questions such as when, where or how many times domestication took place are still debated (Frantz et al., 2016; Larson et al., 2012; Pang et al., 2009; Pennisi, 2013). Dog remains have been found in different archaeological sites, but some of these dog lineages likely did not contribute genetically to the current domestic dog (e.g., the 33,000-year-old remains found in the Altai Mountains in southern Siberia; Ovodov et al., 2011). Genetic and archaeological data have indicated that dogs originated from Europe and South Asia (Ding et al., 2012). It is assumed that wolves were attracted to permanent and semi-permanent human settlements and that persistent dog lineages arose in Europe, the Middle East and China by the end of the Last Glacial Maximum (approximately 26,500 to 19,000 years ago; Ovodov et al., 2011). This suggestion agrees with the idea that multiple dog-wolf interbreeding events lead to mixed results and that domestication probably happened through multiple events in those regions (Pennisi, 2013).

Archaeological evidence shows that dogs already differed in appearance from wolves 10,000 years ago, and mitochondrial DNA evidence suggests that dogs might have branched off from the wolf lineage earlier than that by as much as 30,000 years (Axelsson et al., 2013; Bradshaw, 2006; Germonpré et al., 2009). Because dogs and modern wolves share a recent common ancestor, it is sometimes assumed that their abilities and tendencies in the formation of social relationships are the same (discussed by Bradshaw et al., 2009). For example, in some studies, dog social organization is assumed to always include social status relationships, and these assumed relationships are evaluated with respect to other characteristics such as leader-follower relationships during walks (Akos, Beck, Nagy, Vicsek, & Kubinyi, 2014), urine investigation duration

(Lisberg & Snowdon, 2009), and social learning performance (Pongrácz, Vida, Bánhegyi, & Miklósi, 2008). Although studies of grey wolf social organization can offer valuable information about the evolution of dog behaviour, caution is warranted regarding assumptions that dogs and wolves share the same pattern of social organization.

There are two main problems with applying principles of wolf social behaviour to dogs. First, dogs and wolves display many physical differences. For example, cranial morphology including the shape and size of mandibles, teeth, and braincase is different between the two species, with dogs showing a larger orbital region, a smaller rostrum, and modifications in the shape and orientation of the skull (Schmitt & Wallace, 2014). Genetic studies have shown that dogs can have more copies of a gene for amylase (with high inter-individual and inter-breed variability), indicating that dogs might have been selected for a diet composed of a higher proportion of starch compared to wolves (Arendt, Fall, Lindblad-Toh, & Axelsson, 2014; Axelsson et al., 2013; Reiter, Jagoda, & Capellini, 2016). Dogs also show behavioural differences which include an increased responsiveness to their owner compared to unfamiliar humans (Topál et al., 2005) and a decreased likelihood of copying conspecifics (Range & Viranyi, 2014) in comparison to hand-reared captive wolves. It has also been shown that wolves, regardless of their social rank, monopolize food and exhibit agonistic behaviour, while in shelter dogs these behaviours are performed more often by individuals characterized as high-ranking (Range, Ritter, & Viranyi, 2015). Because of these and other differences, it is important to study domestic dogs separately from wolves to clarify how domestic dogs organize themselves socially.

The second issue with applying concepts of wolf social behaviour to dogs is that the early study on grey wolves (Schenkel, 1947), from which the conventional ideas of dominance and hierarchy in dogs derive, was performed using artificially formed groups of up to ten captive wolves housed in an approximately 10 x 20 m enclosure. Behavioural observations were made in the Basle Zoological Garden between 1934 and 1942, and wolf groups were comprised of unrelated individuals. At the time, the influence of kinship and limited space on wolf pack structure was not known. Wolves were said to organize themselves into two linear hierarchies, one for each sex, based on the exchange of agonistic behaviours (Schenkel, 1947). However, the grouping of unrelated wolves in an above-normal density and the artificial living conditions in which the animals were kept could have led to a more frequent exchange of agonistic behaviours because of exacerbated competition for space and other resources. Thus, there is some current consensus that Schenkel's (1947) results should be interpreted with caution when applied to free-living wolves and, in particular, dogs (e.g., Bradshaw et al., 2009; Mech, 1999).

Bradshaw et al. (2009) discuss that more recent work on wild grey wolves (i.e., Mech, 1999) indicates that, when free to associate as they please, wolves organize themselves into packs composed of one to three family groups, each including a breeding pair, juvenile offspring who have yet to disperse, and pups. There is a division-of-labour system in which the breeding female focuses on pup care and defence and the male focuses on foraging, food provisioning and travel. These family groups show a more fluid organization than the linear hierarchy found in some captive packs, with the breeding pair as the centre of the group and little to no aggression between group mates.

Domestic dogs living in multi-dog households may share similarities and differences with captive wolves. Both are kept by humans, rarely have control over group composition, and some share their living space with non-related individuals. Differences include the fact that, in some parts of the world, pet dogs are often gonadectomised, which leads to changes in hormone levels and behaviour, although the effects of gonadectomy on dog social behaviour are not well-studied (*cf.* socio-sexual behaviour, e.g., Beach, 1970). Additionally, the natural environment of wolves is the wild, while dogs have arguably been selected for traits that make them compatible with life in human households. Because of these differences, it is important to consider that pet domestic dog social behaviour might be quite different from that of captive wolves. Dog social behaviour and structure in multi-dog households requires further investigation, including consideration of how differences in the human-dog relationship across the world might impact dog social behaviour.

A particularly troubling example of the inappropriate application of the out-dated strict hierarchy concept derived from the wolf literature to multi-dog households is one of the suggested treatments for aggression problems between cohabiting dogs. In this view, the concept of dominance is posited to be useful in explaining and preventing undesired aggressive behaviour (Schilder, et al., 2014), i.e., the owner enforces a set hierarchy of access to resources for dogs under his or her care (reviewed by van Kerkhove, 2004; Wrubel, Moon-Fanelli, Maranda, & Dodman, 2011). This treatment is based on the idea that inter-dog household aggression is a result of dogs contesting a dominant-subordinate relationship (Wrubel et al., 2011). However, the American Veterinary Society of Animal

Behaviour (2008) advised that enforcing a set hierarchical structure of dominance on a group of dogs may be risky and should be avoided, since it might lead to increased fear and anxiety in some of the individuals involved and, as a consequence, an unwanted increase in aggressive behaviour (see also Schilder, et al., 2014). Only if the strategies used for treating aggressive behaviour increase consistency and predictability of social interaction outcomes might there consequently be a decrease in aggressive behaviour (Wrubel et al., 2011). Indeed, a better understanding of social structure in domestic dogs becomes important in the development and promotion of welfare-friendly training methods (Westgarth, 2015).

Some feral domestic dogs form consistent groups, and dominant-subordinate relationships leading to a linear hierarchy have been shown through the analysis of formal submission signals between group members (Bonanni & Cafazzo, 2014; Cafazzo et al., 2010). In contrast, pet domestic dogs live under increased human influence in an environment with quite different constraints. There are studies that presume the existence of a linear hierarchy in pet domestic dog groups (Akos et al., 2014; Lisberg & Snowdon, 2009; Pongrácz et al., 2008; van der Borg, Schilder, Vinke, & de Vries, 2015), each using a different methodology for assessing social status. Some evidence for the formation of linear hierarchies in a group of pet domestic dogs at a dog day care facility has been found, with submission displays being the most useful behaviour for building these hierarchies (Trisko & Smuts, 2015). However, both formal submission displays and aggressive behaviours were performed by a low proportion of animals, with clear one-way relationships appearing in less than one-third of dyads. Support for the formation of linear hierarchies in domestic dogs was also found by van der Borg et al. (2015) when using lowered posture during dyadic interactions as a formal submission display; however, this group of domestic dogs consisted of a high proportion of juveniles living in a kennel environment, so its broad applicability to pet dogs living in homes is uncertain.

As stated previously, the objective of this project is to investigate the social organization of domestic dogs living in multi-dog households. Because an individual's social status within a group can depend on many characteristics, different methods of characterizing a dog's relative status within its home group that have been used in previous studies, as well as owner-reported dominance, were compared and evaluated using demographic, behavioural, hormonal, and personality information. In dogs and other species, factors such as age (Bauer & Smuts, 2007; Cafazzo et al., 2010; Trisko & Smuts, 2015), sex (Mehta & Josephs, 2010), gonadectomy status, and size (Stulp, Buunk, Verhulst, & Pollet, 2015) often relate to an individual's status within a group. In domestic dogs specifically, aggressive behaviours, formal dominance, and formal submission displays have been used to investigate social status (Akos et al., 2014; Bauer & Smuts, 2007; Bonanni & Cafazzo, 2014; Bonanni et al., 2010; Bradshaw et al., 2009; Cafazzo et al., 2010; Pongrácz et al., 2008; Trisko & Smuts, 2015; van der Borg et al., 2015). As well, the toy possession test has been used to identify dogs of higher status within a group of cohabiting dogs (Lisberg & Snowdon, 2009), and most owners of multiple dogs have an opinion about relative social status of the dogs in their care. Testosterone and cortisol levels have been shown to jointly regulate dominance in humans (Casto & Edwards, 2016; 2010; Mehta & Prasad, 2015; Ponzi, Zilioli, Mehta, Maslov, & Watson,

2016) and these two hormones could also be related to the formation of dominance relationships in dogs. Personality characteristics have been shown to influence dominance relationships in several species such as brown trout (Adriaenssens & Johnsson, 2011), starlings (Boogert, Reader, & Laland, 2006), zebra finches (David, Auclair, & Cézily, 2011), great tits (Dingemanse & de Goede, 2004), mountain chickadees (Fox, Ladage, Roth, & Pravosudov, 2009), and barnacle geese (Kurvers et al., 2009). In domestic dogs, asymmetries in personality differences have been proposed to be responsible for the formation of apparent dominance relationships (Bradshaw, Blackwell, & Casey, 2016). Thus, it is important to examine which factors might reveal social status differences in dogs living in multi-dog households and how context might influence such relationships.

1.2. Co-authorship statement

This research project, including its research questions and procedural design described within this manuscript, was developed by me under the supervision of Dr. Carolyn Walsh. Dr. Rita Anderson and Dr. Anne Storey, as committee members, also helped shape the final procedural design through important suggestions and feedback. All data, including saliva samples, questionnaires, and video footage were collected by myself or under my supervision, but the results for cortisol and testosterone levels in saliva samples were obtained by hire of an external analyst, Salimetrics LLC. (Pennsylvania, USA). All participants were recruited by myself, my supervisor, and committee members through the Canine Research Unit in the Department of Psychology at Memorial University of Newfoundland, and all questions or concerns regarding this research project were directed to me.

I analysed and reported results under guidance from my supervisor and committee members. Dr. Carolyn Walsh and Dr. Rita Anderson are co-authors of all chapters presented as they contributed directly to the intellectual property of this document. Financial support for this project was provided by Natural Sciences and Engineering Council of Canada (NSERC) via a Discovery Grant to Dr. Carolyn Walsh. Support was also received from the School of Graduate Studies at Memorial University of Newfoundland.

2. Relating social status in domestic dogs to hormonal, personality, and behavioural characteristics

2.1. Introduction

2.1.1. Dominance in domestic dogs

In gregarious species, individuals are part of a group of conspecifics and operate within a social context. Associating with others can bring important advantages such as increasing access to and choice of mating partners, facilitating cooperative behaviour in resource management such as foraging and territory maintenance, and protecting against predatory behaviour (Croft, James, & Krause, 2010). However, in social groups, conspecifics are also often competitors for access to limited resources, such as food and mates. In many species, group social structures or hierarchies emerge and appear to function to reduce overt competitive conflict among group members, as well as to provide priority access to resources for some individuals (Clutton-Brock & Huchard, 2013), although these functions have long been debated (e.g., Rowell, 1974). Such social hierarchies involve dominance, a concept that can be difficult to define and measure (Drews, 1993). The concept of dominance generally refers to an inequality in the social relationship between two individuals (a dyad) within the group, such that one individual has a higher social rank or status than the other (e.g., Drews, 1993; Hinde, 1978). How dominance relationships are defined or measured can be based on agonistic or non-agonistic behaviours, with the association between dominance and aggression varying widely.

Dominance is used in at least four ways in the ethological literature, none of which are mutually-exclusive (reviewed in Bradshaw et al., 2009): 1) defining dominant individuals as having preferential access to key resources (e.g., food, mates) ; 2) defining relationships in which one individual repeatedly profits from interactions (Clutton-Brock, Albon, Gibson, & Guinness, 1979); 3) describing a pecking order in which lower ranking individuals inhibit their aggressive behaviours to avoid conflict with higher ranking individuals (e.g., Pagel & Dawkins, 1997); and 4) describing structures in which dominance is determined by formal agonistic displays, i.e., submission, and not aggression (Drews, 1993). There can be a temporal component to each of these descriptions of dominance. A dominance relationship might be temporary, which is the case when animals do not live in permanent groups and only meet and compete for resources occasionally. When animals do live in permanent groups, dominance might be contextual and depend on the quality and quantity of resources that are being contested, as well as the relative value of the resource to each individual (Bradshaw et al., 2009). Additionally, over time, dominance relationships between individuals can be altered (e.g., as the individuals age).

In permanent groups with more than two individuals, consistent social relationships between members of the group might develop into a social hierarchy, such as a transitive linear hierarchy, in which dominance relationships between pairs of individuals can be translated into individual ranks, with higher-ranking individuals always dominant over lower-ranking ones (De Vries, 1998). It is assumed that individuals compete for status that, if achieved, guarantees them preferential access to resources for an extended period. Non-transitive circular hierarchies might also appear if, for example, physical differences between individuals are not salient and memory of outcomes from previous encounters (e.g., wins and losses) persists (Bradshaw et al., 2009; van Doorn, Hengeveld, & Weissing, 2003). It should be noted that dominance relationships between individuals can exist in the absence of any social hierarchical structure within a group (e.g., Rowell, 1974; van Doorn, et al. 2003).

In linear hierarchies, individuals can be assigned a rank that determines the order of access to resources (Bradshaw et al., 2009). These dominance hierarchies can be found in many animals, including wasps (Tibbetts, 2002), fish (Fausch, 1984), and apes (Wittig & Boesch, 2003), and can lead to inequality in resource access and individuals' health outcomes (Sapolsky, 2005). Such inequality can create psychosocial stressors for both high and low-ranking individuals, wherein any animal anticipating a threat to homeostasis may suffer deleterious consequences from the stress of such anticipation (Creel, Dantzer, Goymann & Rubenstein, 2013). In some species, actual physiological stressors involving the prolonged release of glucocorticoids (the so-called "stress hormones") may be experienced by lower ranking individuals who are more drastically affected by the lack of access to resources (e.g., male olive baboons, Sapolsky, 2005; additional species reviewed in Creel et al., 2013). However, in other species, higher ranking individuals appear to experience an increased physiological stress response, as measured by glucocorticoid production (e.g., grey wolves, Sands & Creel, 2004; although see Molnar, et al. 2015). In either case, sustained activation of the stress response can lead to increased risk of cardiovascular problems, immune suppression, reproductive impairment, and affective disorders (Sapolsky, 2005), although it is recognized that other factors, such as group stability, breeding system type, and the behaviours by which dominance is obtained have significant impacts on the relationships between dominance and the effects of glucocorticoids (Creel et al. 2013).

Domestic dogs and wolves are popularly said to form dominance relationships, as exemplified by the expressions 'alpha dog' and 'top dog' used in colloquial language. As two closely related species, it is often falsely assumed that the formation and maintenance of social relationships happens similarly in both species, and the idea that there is a 'top dog' in every canid group derives from Schenkel's (1947) observations of captive groups of unrelated wolves in a small enclosure.

The differences between the social structure of wolves portrayed by the classic study by Schenkel (1947) on captive groups of unrelated wolves in a zoo and the more recent work on naturally occurring wolf packs (Mech, 1999; Mech & Cluff, 2010) show that social organization of wolves might be dependent on context. In the Schenkel (1947) study, the unrelated wolves were said to form two linear hierarchies, one for each sex, based on the exchange of agonistic behaviours. In wild wolf packs, however, the social group is organized around the breeding pair, while other group members are typically offspring that have yet to disperse from one or both breeding individuals (Mech & Cluff, 2010). Females have one reproductive period per year, usually mating with one male, and other pack members help care for pups. There is a division-of-labour system in which the breeding female focuses on pup care and defense and the male on foraging, food provisioning and travels (Mech, 1999). While the Schenkel (1947) study provides information about the behaviour of unrelated wolves kept in small enclosures, its results seem not to generalize to wild wolves or wolves kept in facilities more similar to their natural environment. However, in a recent study of an extended family group of captive Arctic wolves, Caffazo, Lazzaroni, and Marshall-Pescini (2016) found support for the existence of a social hierarchy with the group, based on age, but not sex. Most importantly, it is unlikely that the social organization structures of either extant wild or captive wolf species can be directly applied to domestic dogs, particularly those living with human owners in multi-dog homes, as wolves and dogs have been evolving under different constraints for thousands of years.

Feral dog (i.e., free-ranging domestic dog) studies provide an interesting opportunity for comparison with wild wolves. The investigation of agonistic behaviour of free-ranging dogs showed that, like wolves, they also associate with close kin, share territory with them, and are aggressive towards neighbouring groups (e.g., in Indian village dogs, Pal, Ghosh, & Roy, 1998; reviewed in Bradshaw et al., 2009). But unlike wolves, dog reproductive behaviour includes females copulating with multiple males in one reproductive period, an increase in the frequency of reproductive cycles to approximately twice per year, little reproductive suppression over other members of the group, little infanticide, and limited paternal care (Pal, Ghosh, & Roy, 1999). There are also fewer ritualized displays in feral dog interactions when compared to wild wolves, indicating that communication between dog pack members is subtle and may be based on mutual recognition (Bradshaw et al., 2009).

A significant linear hierarchy with a low level of linearity, indicating that some instances of relationships leading to a non-transitive hierarchy appear in the sample, was found in a group of free ranging dogs near Rome, Italy (Cafazzo et al., 2010). To create this hierarchy, outcomes of agonistic interactions at the dyadic level were used. In the same study, formal displays of submission such as avoiding eye contact, exposing the ventral side, flattening the ears, tucking the tail or lowering posture were unidirectional and appeared in all contexts analyzed, although they were not displayed by all dogs. These behaviours were displayed when dogs characterized as submissive approached dominant ones, while aggressive displays were mostly present during competition for resources. In another study, individuals in this pack were classified into different social statuses based on formal and agonistic dominance (Bonanni, Cafazzo, Valsecchi, & Natoli, 2010). Dominant dogs that received formal submission displays from other pack members were shown to be leaders of pack movement more often than dominant dogs that achieved that status through agonistic dominance (Bonanni et al., 2010).

However, companion domestic dogs live in contexts unlike those of feral dogs, as the degree of human influence on their behaviour is quite different. Of course, there is variation in the housing of companion dogs, ranging from relatively large permanent groups living together in no-kill shelters or kennels (e.g., research facility dogs), to smaller numbers living with their owners in the owner's home. Thus, even within non-feral domestic dogs, their housing and social group contexts may impact the type of social relationships or structures formed among conspecifics. For example, a group of neutered male domestic dogs living in a 0.28 ha enclosure of a rehoming charity did not seem to organize themselves into a linear hierarchy (Bradshaw et al., 2009). This could indicate that the social behaviour of gonadectomized dogs is different from the social behaviour of intact animals, possibly because of the removal of the primary sex organs and subsequent effects on hormone levels and endocrine system regulation. Bradshaw et al. (2009) suggest that these dogs could be classified according to their pattern of interaction with other group members, using “confident” and “submissive” agonistic behaviours as reference; some animals showed dominant relationships over other group members, some showed no dominant relationships over other group members, and some interacted so infrequently with other group members that it was difficult to define their relationships.

This issue of “low coverage” of the behaviours used to indicate a dominance relationship among dyads, i.e., the behaviours not only appear infrequently but are not shown by all dogs, is a common finding of most studies of canid social hierarchy (e.g., dogs, Trisko & Smuts, 2015; grey wolves, Cafazzo, et al. 2016). Trisko and Smuts (2015) investigated the formation of linear hierarchies in a group of pet domestic dogs at a dog day care facility based on formal submission displays such as muzzle licking, low posture, and retreat. The behaviours could be used to form linear hierarchies in the group, although the behaviours examined had low coverage (29% coverage for one-way relationships). While subjects in this study were pet domestic dogs, the dominance relationships were formed between dogs from different homes occasionally kept together at the day care facility, not cohabiting dogs of a multi-dog home. Additionally, there were probably few resources that dogs could contest at the day care facility, and the day care workers actively prevented conflicts between dogs.

Van der Borg et al. (2015) studied a mixed-breed group of 16 dogs (with a high proportion of pups, juveniles and sub-adults) at the dog kennel of a veterinary school. They examined different postures and behaviours exhibited by the dogs for their suitability as formal status indicators. They identified high posture and muzzle bite as formal dominance indicators, and body tail wag, mouth lick and pass under head as formal sumission indicators. Lowering of posture was deteremined to be the most reliable indictor of social status between dogs, as it had high coverage (unlike many other behaviours), was mostly unidirectional when observed between indivdiuals, and indicated a nearly linear hierarchy within the group.

Pongrácz et al. (2008) investigated whether a dog's relative dominance rank within a group of cohabiting dogs had an effect on social learning performance and measured the latency of dogs to detour around a V-shaped fence following no demonstration, demonstration by an unfamiliar dog, or demostration by an unfamiliar human. Relative dominance within the home group was obtained for each dog from owners' answers to a questionnaire with four questions about barking ("When a stranger comes to the house, which dog starts to bark first, or if they start to bark together, which dog barks more or longer?"), licking ("Which dog licks more often the other dog's mouth?"), eating ("If the dogs get food at the same time and at the same spot, which dog starts to eat first or eats the other dog's food?") and fighting ("If the dogs start to fight, which dog usually wins?"). Dogs were then categorized as either dominant or subordinate: if their owner answered "the present dog" to at least three questions and did not answer "some of my other dogs" to the question about fighting, the dog was said to be dominant. Submissive dogs were the ones for whom the owner answered “some of my other dogs” to at least three questions, provided that the answer to the question about fighting was not “the present dog”. Thus, owner-reported behavioural characteristics were used to qualify dogs as dominant or submissive. There were no significant differences in the performance of dogs characterized as dominant and those characterized as subordinate in the conditions without demonstration and with a human demonstrator. However, subordinate dogs had significantly shorter detour times than dominant dogs when a dog demonstrator was used. The authors suggested that perceived dominance rank within the group has a strong effect on a dog’s social learning ability that depended on the demonstrator species (Pongrácz et al., 2008). A shortcoming of this study is that it is not clear whether the behaviours assessed by the questionnaire are indeed related to dominance in cohabiting pet domestic dogs. As well, Bradshaw et al. (2016) note that these behavioural questions are no longer used to assign dominance to dogs by the research group with which Pongrácz is associated.

Using the Pongrácz et al. (2008) questions for evaluating the dominance relationships between six cohabiting dogs, Akos et al. (2014) investigated the influence of dominance or submissiveness on leadership and path characteristics during walking, such as speed, distance covered, and distance from owner. Leader-follower relationships were used to construct a linear hierarchy; a dog’s position in this hierarchy was strongly correlated with dominance as measured by the behavioural questions.

Lisberg and Snowdon (2009) examined the relationship between a dog’s social status within its home group and its investigation pattern of unfamiliar conspecific urine.

Dogs were allowed to walk through a course of wooden stakes containg either dog urine or water, and investigation times at each stake were measured. Each subject's relative dominance rank within its home group was subsequently determined by 1) the results of a toy possession test, in which a toy is tossed to the group of cohabiting dogs over three trials, and the score is defined by how many first possessions a dog gets, and 2) a mean tail-base position score obtained as the dogs approached each of the stakes in the urine course. For each approach to a stake, dogs received a score for tail-base position (1-low, 2-medium and 3-high), and mean tail-base position score was calculated. There was strong agreement between mean tail-base position scores and toy possession test scores, suggesting that mean tail-base position could be used as an indicator of dog social status. Using this metric, they found that dogs of lower status (lower tail base position) investigated unfamiliar urine longer than did dogs of higher status. The authors suggested that lower ranking dogs might investigate the cues associated with potential competitiors longer because they have a greater chance of losing a competitive interaction with a conspecific (Lisberg & Snowdon, 2009).

The above studies that have evaluated social status in cohabiting pet domestic dogs used different methods for determining status in the group, making it difficult to directly compare studies. Equally problematic is that which behaviours validly and reliably indicate dominance relationships in permanent social groups of dogs living together is not yet clear. Further investigation is required to determine exactly how and under which circumstances dominance relationships are formed between cohabiting pet domestic dogs, and whether reported methods for identifying a dog's social rank are robust.

For the present work, dominance is defined as a relationship between two individuals (dyad) in which one of the individuals is repeatedly favoured by outcomes of competitive interactions and achieves preferential access to a contested resource. The main objective of this study was to compare the different methods for assessing domestic dog social status in multi-dog households: the toy possession test, owner-reported behaviours based on a questionnaire, and overall owner impressions of dominance among their dogs. Hormonal (cortisol and testosterone), personality, and demographic information for these dogs was also examined to determine which factors are related to dominance relationships among the dogs, should evidence for such relationships exist.

2.1.2. Dominance and the dual hormone hypothesis

Testosterone, an androgen, is a steroid hormone produced in large quantities by the testes, and in smaller quantities by the adrenal cortex and ovaries (Smith, Mitchell, & McEwan, 2013). It is primarily involved in sex differentiation *in utero*, facilitating the formation of male reproductive organs, the development of male secondary sexual characteristics during puberty, the regulation of male gamete production and increased protein synthesis in skeletal muscle (Smith et al., 2013). It also seems to influence behavioural characteristics such as social status, with increased testosterone levels related to higher social status in some species (e.g., chimpanzees: Anestis, 2006; humans: Archer, 2006, Terburg et al., 2016; baboons: Beehner, Bergman, Cheney, Seyfarth, &

Whitten, 2006; lemurs: Cavigelli & Pereira, 2000; rock hyrax: Koren, Mokady, & Geffen, 2006; cichlid fish: Ramallo et al., 2015; canaries: Vergauwen, Groothuis, Eens, & Müller, 2014).

While there is a large body of work supporting the relationship between testosterone and social status behaviours, some studies have found weak or no effects (e.g., lemurs: Cavigelli & Pereira, 2000; baboons: Kalbitzer, Heistermann, Cheney, Seyfarth, & Fischer, 2015; rock hyrax: Koren et al., 2006; humans: see Mehta & Josephs, 2010; mole-rats: Oosthuizen, Viljoen, & Bennett, 2010; deer: Taillon & Côté, 2008; sparrow-weaver: York, Radford, de Vries, Groothuis, & Young, 2016). One possible explanation for this inconsistency is the dual-hormone hypothesis, in which the relationship between testosterone and dominance behaviours is different depending on whether cortisol levels of an individual are high or low; this has recently been studied in humans (e.g., Casto & Edwards, 2016; Ponzi et al., 2016; reviews in Knight & Mehta, 2014, Mehta & Josephs, 2010, Mehta & Prasad, 2015). Cortisol, also a steroid hormone, is a glucocorticoid produced by the adrenal cortex. It causes mobilization of energy reserves and suppression of the immune system (Costanzo, 2014; Yates et al., 2013), mediating physiological changes related to stress and arousal. It affects mood, appetite, libido, and sleep patterns, among other behavioural characteristics (Gardner & Shoback, 2011).

The dual-hormone hypothesis proposes that testosterone will be positively related to behaviours associated to increased social status only when cortisol levels are low. When cortisol levels are high, this relationship between testosterone and status-seeking behaviours should change (Knight & Mehta, 2014, Mehta & Prasad, 2015). The influence of the possible interaction between testosterone and cortisol has been related to dominance and competitive ability in humans. Mehta and Josephs (2010) related two different measurements of dominance to a possible testosterone and cortisol interaction. In their first study, dominance as measured by observer ratings during a leadership task was related to the interaction between testosterone and cortisol, as was dominance measured by willingness to compete again after defeat in a second study. There has been little investigation of the dual-hormone hypothesis in non-human animals, and none in domestic dogs.

Both cortisol and testosterone secretions are controlled by the hypothalamus and the anterior pituitary in the central nervous system. During periods of stress or arousal, the hypothalamus stimulates the anterior pituitary to secrete adrenocorticotropic hormone (ACTH), which stimulates the adrenal cortex to secrete glucocorticoids. This is the hypothalamic-pituitary-adrenal (HPA) axis of the endocrine system (Gardner & Shoback, 2011). Although androgen secretion by the adrenal cortex is in part controlled by ACTH, there can be a dissociation between adrenal androgen and cortisol secretion (Kelly, McKenna, & Young, 2004). Sex-steroid secretion, including androgens, estrogens and progesterone, is controlled by the hypothalamic-pituitary-gonadal (HPG) axes of males (hypothalamic-pituitary-testicular axis) and females (hypothalamic-pituitary-ovarian axis) (Gardner & Shoback, 2011). In these axes, the hypothalamus secretes gonadotropin releasing hormone (GnRH), which stimulates the anterior pituitary to release gonadotropins: follicle stimulating hormone (FSH) and luteinizing hormone (LH). These gonadotropins will stimulate production and secretion of sex steroids by either ovaries or testes (Gardner & Shoback, 2011).

The HPA and HPG axes interact with each other, in that HPA axis activation has a negative feedback effect on the HPG axis, decreasing sex steroid secretion in both males and females (Gardner & Shoback, 2011). However, male and female HPG axes seem to have different effects on the HPA axis: androgens increase, while estrogens decrease HPA activation (Goel, Workman, Lee, Innala, & Viau, 2014).

Thus, the investigation of HPA and HPG axes interactions and their influence on the social status behaviour of domestic dogs needs to take possible sex differences into consideration. Differences that may appear because of gonadectomy also must be considered, since the removal of testes and ovaries affects sex steroid levels and, thus, likely influences the regulation of HPA and HPG axes interactions. As gonadectomized male dogs secrete less testosterone than intact males upon GnRH stimulation (de Gier et al., 2012), there is support for the idea that gonadectomy could affect HPA and HPG regulation.

2.1.3. Personality

An individual's personality is the consistent pattern of behaviours it exhibits across situations and time (Ley, Bennett, & Coleman, 2008). Personality can be used to explain individual differences in response to a changing environment, and is usually distributed across different continua called personality dimensions (Ley et al., 2008).

Personality can be investigated from a variety of angles, including biological, cognitive, learning, and humanistic perspectives (Cloninger, 2009). While most of the work on personality has historically been focused on humans, there is a growing interest in examining personality in dogs (Jones & Gosling, 2005; Ley et al., 2008; Ottenheimer Carrier, Cyr, Anderson, & Walsh, 2013; Posluns, Anderson, & Walsh, 2017) and other animal species, including bees (Walton & Toth, 2016), squid (Sinn, Moltschaniwskyj, Wapstra, & Dall, 2010), reptiles (Siviter et al., 2016), and non-human primates (Kohn et al., 2016).

Five personality dimensions, referred to as the 'Big Five', have been identified in humans: Openness, Conscientiousness, Extraversion, Agreeableness and Neuroticism (Goldberg, 1993; Gosling & John, 1999); they have been related to many different behavioural characteristics. However, in the study of non-human animal personality, there is no consensus on which approach to use for identifying and measuring personality dimensions across different species (Gosling & John, 1999; Posluns et al., 2017). Studies examining personality in non-human animals have used exploration of novel objects or environments, behavioural flexibility, activity level, risk-taking and aggression as indicators of personality differences (e.g., trout: Adriaenssens & Johnsson, 2011; starlings: Boogert et al., 2006; zebra finches: David et al., 2011; great tits: Dingemanse & de Goede, 2004; mountain chickadees: Fox et al., 2009; barnacle geese: Kurvers et al., 2009).

The evaluation of dog personality can be done in two different ways: 1) behavioural coding, which measures frequency and/or duration of operationally-defined behaviours from direct observations, or 2) behavioural ratings, which are broader assessments of behavioural characteristics made by people familiar with the dog (Fratkin, Sinn, Patall, & Gosling, 2013). Behavioural ratings also have been used successfully to characterize personality in a study of personality characteristics of spotted hyenas (Gosling, 1998). While each of these methods has advantages regarding data collection, a recent meta-analysis performed for dogs found that there was no difference in consistency estimates for personality evaluation based on expert ratings compared to observed coded behaviours (Fratkin et al., 2013). Similarly, Barnard et al. (2016) demonstrated that subjective ratings of 2-month-old Border collie puppies strongly reflected objective behavioural coding.

The Monash Canine Personality Questionnaire – Revised (MCPQ-R) is a valid and reliable questionnaire containing a list of common adjectives owners use to describe their dogs' behaviour, each adjective related to one of five personality dimensions identified in this group: Extraversion, Motivation, Training Focus, Amicability and Neuroticism (Ley, Bennett, & Coleman, 2009; Ley, McGreevy, & Bennett, 2009). For each adjective in the list, the respondent, who is highly familiar with the dog in question (typically the owner), uses a five-point scale to rate how well the adjectives describe his or her dog. A percentage score is created for each personality dimension. Personality dimension scores based on the MCPQ-R have been used in studies to examine the relationships between canine personality and owner personality traits (Schöberl, Wedl, Beetz, & Kotrschal, 2017), dog response to separation from owner (Schöberl et al., 2016), and social behaviour and cortisol levels of dogs in a dog park (Ottenheimer Carrier et al., 2013).

The relationship between personality characteristics and dominance has been investigated in several species, but not often in domestic dogs. A higher social status has been related to the personality variables of increased proactivity, which reflects increased productivity and is associated with a higher metabolic rate and preferential access to resources in female zebra finches (David et al., 2011), lower exploratory behaviour in mountain chickadees (Fox et al., 2009), and fast exploration of a novel environment in adult wild great tits (Dingemanse & de Goede, 2004). Curiously, Dingemanse and de Goede (2004) found that this relationship was inverted in young birds, so there seems to be an interaction between age and exploratory behaviour in the determination of dominance status in that species. Adriaenssens and Johnsson (2011) showed that aggression is a poor indicator of dominance in brown trout, and Kurvers et al. (2009) related leadership, but not dominance, to exploratory behaviour in barnacle geese.

Thus, it is possible that dominance, or relatively higher social status, is related to individual personality characteristics, but the nature of the relationship and which personality characteristics are involved appear to vary among species. Bradshaw et al. (2016) suggested that dominance and submission are not personality characteristics of individual dogs, but rather a quality of pairwise relationships. According to Bradshaw et al. (2016), asymmetries in personality characteristics such as Motivation, among other factors, may influence formation of dominance-like relationships between dogs in specific contexts, without the construct of dominance being a particularly meaningful way to describe the overall dyadic relationship. Interestingly, genetic variation may be related to social dominance in some species; one proposed mechanism by which this may occur is via the effects of genes on individual differences in personality traits (van der Kooij & Sandi, 2015). One objective of the present study is to examine how personality dimensions as assessed by the MCPQ-Revised relate to potential dominance relationships in domestic dogs.

2.1.4. Owner-reported Behaviours

As outlined above, several studies have used specific behaviours to assess the social status of individuals within a group of domestic dogs (Akos et al., 2014; Bauer & Smuts, 2007; Bonanni & Cafazzo, 2014; Bonanni et al., 2010; Cafazzo et al., 2010; Lisberg & Snowdon, 2009; Pongrácz et al., 2008; Trisko & Smuts, 2015). However, none of these studies have examined permanent social groups of pet dogs living with owners using behavioural assessments in the dogs' homes. It is still not clear how some of the behaviours used to define a dog's social status relate to dominance. For example, rolling over during play had been used as an indicator of submission (e.g., Bauer & Smuts, 2007), but recent studies suggest that this behaviour is part of ongoing play sequences, unrelated to dominance status (Norman, Pellis, Barrett, & Henzi, 2015). Other interpretations for the same rollover behaviour also exist (Smuts, Bauer, & Ward, 2015), showing that interpreting the role behaviours play in dominant-subordinate relationships is not straightforward.

Another example of disagreement between different interpretations of the same behaviour can be found in the questionnaire created by Pongrácz et al. (2008). While the four behaviours (barking, muzzle licking, eating other dogs' food, and winning fights) in the questionnaire were used as indicators of dominance, the behaviour of licking muzzle has been considered an indicator of submissiveness by several other researchers (Akos et al., 2014; Bauer & Smuts, 2007; Bonanni et al., 2010; Cafazzo et al., 2010; Trisko & Smuts, 2015). Other behaviours have also been associated to social status: Cafazzo et al. (2010) identify laying a paw on a conspecific's back as a sign of dominance, and Bauer and Smuts (2007) suggest that biting another dog's muzzle and mounting another dog indicate higher social status.

Therefore, it is important to evaluate which behaviours exhibited by cohabiting pet domestic dogs are indeed related to social status differences between individuals.

2.1.5. Research questions

Examining the relationship between specific behaviours potentially associated with higher social status, toy possession test results, and owner impressions of dominance can help clarify whether these methods measure the same characteristic (e.g., dominance within a dyad or group) and how such methods might be related to demographic characteristics, such as age and size, cortisol and testosterone levels, and personality variables. If the different methods for determining a dog's dominance compared in this study assess the same characteristics, it is expected that they will be positively related to each other and to the same demographic, hormone, and personality variables.

The research questions of this thesis include:

- Are the three methods for assessing social status of domestic dogs (specifically, the toy possession test, owner-reported behaviours potentially related to social status, and owner-reported dominance) positively correlated for groups of cohabiting pet dogs?
- Are these three methods for assessing dog dominance in multi-dog homes related to the same demographic, hormonal, and personality factors?
- Does the interaction between testosterone and cortisol levels predict any difference in social status between domestic dogs, as measured by a competitive task (the toy possession test), owner-reported dog behaviours, and/or owner-reported dominance status?

Additionally, the relationships between the measured demographic, hormonal and personality variables were investigated. Given the exploratory nature of this aspect of the study, many of the possible relationships between the variables measured were examined, and no predictions will be presented here. Such results should be considered preliminary, and used as the basis to generate further testable hypotheses.

2.2. Methods

All procedures involving dog owners were approved by the Interdisciplinary Committee on Ethics in Human Research (ICEHR) at Memorial University of Newfoundland, protocol number 2012-182-SC. All procedures involving dogs were approved by Animal Care Services at Memorial University of Newfoundland, protocol number 11-01-CW.

2.2.1. Recruitment and subjects

Owners of multi-dog households and their dogs were recruited from the community. To qualify for participation, households had to be located within a 100-km radius from the city of St. John's, NL, Canada and have two or more cohabiting dogs older than six months of age. Dogs had to be living in the same home for at least three months so that they were familiar to each other.

Recruitment efforts were focused on four approaches: (1) network sampling, (2) presence at relevant events, (3) posters in relevant locations and (4) an advertisement posted on a website for free classified ads. (1) Network sampling was conducted by contacting acquaintances who fulfilled the criteria for participation as well as by asking participants to spread the word about the project to their personal networks. (2) Recruitment also took place at Canine Research Unit booths set up at the Newfoundland and Labrador Pet Expo fairs in May 2011 and May 2012 and at the Newfoundland All Breed Kennel Club Dog Show in April 2012. During these events, people who demonstrated interest in finding out more about the Canine Research Unit and its projects were asked to share their contact information so they could be later asked whether they were interested in participating in this study. (3) Posters (Appendix I) were distributed to pet stores and veterinary clinics in the St. John's metropolitan area, bulletin boards at Memorial University of Newfoundland and at the Quidi Vidi dog park in St. John’s, NL. (4) Lastly, an advertisement (Appendix II) was posted on a free classified advertisement website and re-posted whenever it expired to continue recruitment.

Potential participants who responded positively to any of the abovementioned methods of recruitment were contacted either by email or telephone depending on the type of contact information provided and their preferences for method of communication. They received the appropriate consent form and, once they agreed to participate, questionnaires and material for saliva collection were sent via regular mail.

A total of 23 multi-dog households (two-dog, N = 10; three-dog, N = 8; four-dog, N = 3; five-dog, N = 2) were recruited to participate in this study. The primary caregiver was female in 21 households. There were 66 participating dogs of various breeds, including mixed-breeds (Appendix III). For some analyses involving hormones, the dogs were assigned to one of four “sex/gonadectomy group”, describing their sex (male vs. female) and gonadectomy status (spayed/neutered vs. intact) as follows: spayed females, N = 34; intact females, N = 12; neutered males, N = 13; and intact males, N = 7. Dogs came from three different origins: breeders (N = 40), rescue organizations (N = 16) and other homes (N = 10). Age ranged from 7 to 170 months (75.45 ± 45.16, mean ± SD), weight from 1.6 to 86.2 kg (17.65 ±14.03), height at withers from 18 to 84 cm (42.95 ±15.62), age when acquired from birth to 84 months (10.01 ± 17.40), and age when gonadectomised from 5 to 120 months (22.75 ± 28.0) for 44 gonadectomised dogs (age at gonadectomy was not known for two dogs).

2.2.2. Data collection

Data collection took place between September 2011 and February 2013.

2.2.2.1. The data collection kit

Participants received a kit in the mail containing the material necessary for data collection. The kit contained:

(1) The instruction sheet (Appendix IV) containing a greeting, a list of kit components and instructions on how to proceed with data collection including detailed procedures and a list of necessary materials for each step.

(2) The consent form (Appendix V), a legal document that outlined the possible benefits and risks of participation, as well as the legal responsibilities of researchers and participants.

(3) The questionnaire for each dog (Appendix VI) of participating households requesting general information about the dog (dog's name, birth date, sex, breed, weight, height, neuter-status, origin, order of arrival compared to other dogs in the same home, health issues, medications being taken, training and regular activities), behavioural questions (owners were asked to report whether their dogs had performed them in the last six months) and the Monash Canine Personality Questionnaire - Revised (MCPQ-R) (Ley et al., 2009).

(4) A sheet describing the standard operating procedure for saliva samples (Appendix VII) that included the link to an online video showing how the procedure should be performed.

(5) One saliva sample record sheet (Appendix VIII) for each dog to record important information about the sample including the time and order of collection, vial number, blood contamination (which could affect the final hormone levels obtained) and any problems experienced during the collection.

(6) Five Salimetrics children's swabs (Salimetrics LLC, State College, Pennsylvania), an inert polymer cylindrical swab (8mm x 125mm), for each dog and three Salimetrics swab storage tubes for each dog.

2.2.2.2. Practice saliva sample

After receiving the kit, owners were asked to take one practice saliva sample to familiarize themselves with the technique and check that their dogs would not resist the procedure. This helped decrease the chances of problems while taking the first study sample before the visit. They were instructed to use the material and follow the instructions provided in the kit, as well as to discard the swab used since this sample would not be analyzed.

2.2.2.3. Visit, saliva samples and toy possession test

After owners had collected the practice saliva sample, a visit to their homes was scheduled. All visits took place between 1:00 and 9:00 pm to minimize the influence of circadian patterns of hormonal fluctuations on the values obtained from saliva samples (Giannetto et al., 2014).

For homes with two or three dogs (N = 18), visits followed the format illustrated in Figure 2.1. Owners were asked to collect a baseline saliva sample from each of their dogs approximately 30 minutes before researchers arrived and to store them in the freezer. Upon arrival, the research assistant began filming the dogs, with the restriction that the camera operator would always remain within the owner's field of view. The first part of the visit lasted approximately 25 minutes. During this period, the main researcher (MKC) asked the owners if they had any questions about the consent form or if there had been any problems with taking the first saliva sample. Then, the researcher and owner went through the questionnaires to ensure that all information had been filled in.

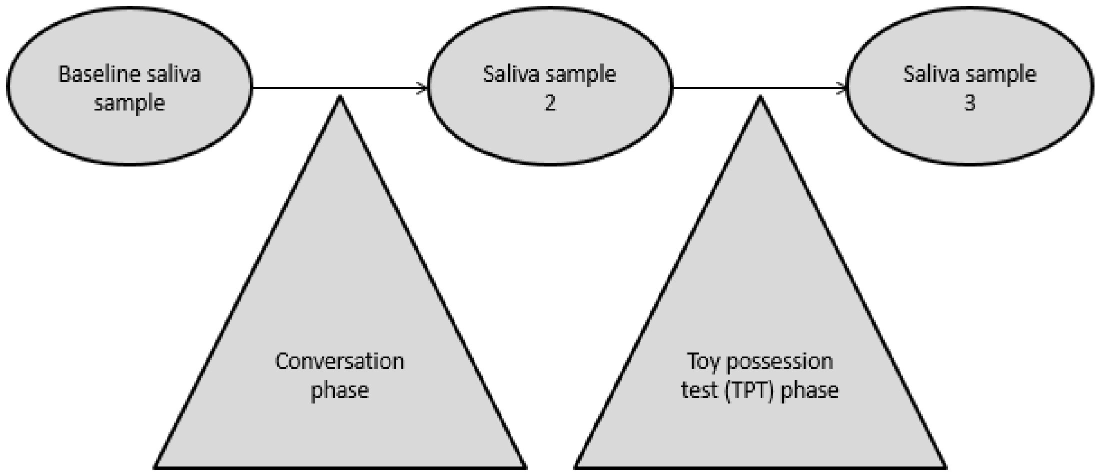

Fig. 2.1: Basic format of the visit for data collection in homes with two or three dogs.

The research assistant attempted to capture all animals on the video or, when that was not possible, focussed on the dog closest to the main researcher. If dogs left the area in which the main researcher and their owner were located, the research assistant remained within the owner's field of view, even if that prevented some or all dogs from being filmed.

Filming stopped while the owner collected the second saliva sample, which was also placed in the freezer. After the second saliva sample was taken, filming resumed and the toy possession test (TPT) was conducted. This test used two different rubber toys: one that squeaked and one filled with a treat. Each toy was used in three alternating trials (total of six trials); the first toy used was alternated between homes. For each trial, the owner was instructed to call the dogs, allow them to sniff the toy and throw it to a point equidistant from all animals. If some dogs went after the toy and some not, the research assistant was instructed to film the animals that sought the toy. The outcomes of each TPT trial were coded from the video.

Approximately 10 minutes after the TPT had finished, a third saliva sample was taken from each dog. At the end of the visit, owners were asked to comment on how their dogs organized themselves socially, which comprised the owner-reported dominance variable. Specifically, the questions were: “Do you think that your dogs organize themselves according to a hierarchy? Is one of them more dominant?”. Owners were not instructed on definitions of hierarchy or dominance, and may have had different interpretations for these terms based on popular use and their own experience. These questions were designed to solely assess owners’ impressions on the possible social organization of the dogs under their care. This topic was not discussed before the other data collection procedures so that owners would not focus on their own beliefs about their dogs’ social organization when answering the questionnaires and participating in the TPT.

Saliva samples were transported in a cooler containing ice packs to a freezer at Memorial University of Newfoundland and stored there at -20^{o}C.

Toys were washed with unscented anti-bacterial hand soap after each visit to prevent the transmission of diseases between dogs of different homes and to minimize the scent of other dogs on the toy.

2.2.2.4. Homes with four or more dogs

For homes with four or more dogs, the methodology was adapted to reduce the amount of work an owner would have as a study participant.

Owners were contacted via email or telephone and received a kit containing the necessary material for data collection. This kit included (1) a data collection instruction sheet for homes with four or more dogs (Appendix IX), (2) a consent form for homes with four or more dogs (Appendix X), (3) an individual questionnaire including general questions and the MCPQ-R (Appendix XI), (4) one set of behavioural questions (Appendix XII), (5) a social interaction questionnaire (Appendix XIII), (6) a sheet containing the standard operating procedures for saliva sampling (Appendix VII), (7) a saliva sampling record sheet for homes with four or more dogs (Appendix XIV), (8) one Salimetrics children's swab, and one Salimetrics swab storage tube for each dog.

The first change in the methodology was the separation of the behavioural questions from the individual questionnaire. The behavioural questions remained the same, but owners were asked to fill out only one set of these questions, instead of a separate set for each dog. The behavioural questions were customized for each home, such that for each question owners could select the animal(s) that had performed the behavior in question from the list of names of all dogs in the home presented as possible answers (see Appendices XII and XIII, colours are used instead of dog names).

The second change was the inclusion of the social interaction questionnaire, which was also customized for each home with the names of dogs as possible answers. This questionnaire was designed to substitute for the TPT because this test would be impractical to perform. This questionnaire asked owners to predict the results of an imaginary toy possession test using their previous knowledge of their dogs and how they interact.

The third change in the methodology was the reduction of the number of saliva samples per dog and the separation of sampling from the visit. In the data collection instructions, owners were asked to collect one saliva sample from each of their dogs, not sampling more than two animals at a time to avoid possible changes in hormone levels because of the sampling procedure itself. They were also asked to collect samples at approximately the same time of day for all dogs while staying within the 1 to 9 pm timeframe.

After owners had filled out the questionnaires and collected the samples, a visit was scheduled. During the visit, the main researcher discussed the questionnaires with the owner and confirmed that all questions had been clearly answered while the research assistant filmed the dogs. The research assistant was instructed to try to capture as many animals as possible in the video while focusing on the one(s) closest to the main researcher. At the end of the visit, owner impressions of which dog was more dominant within each home group were obtained for all homes. The home visits for households with four or more dogs lasted approximately 30 minutes.

2.2.3. Saliva sample analyses

Owners were asked to place baseline saliva samples in a freezer immediately after collection. These were collected during the visit and transported in a cooler with icepacks to a -20^{o}C freezer. All saliva samples were shipped immersed in dry ice to Salimetrics LLC (State College, Pennsylvania, USA) for single enzyme immunoassay analysis for cortisol (values expressed in µg/dL, sensitivity: <0.007 µg/dL) and testosterone (values expressed in pg/mL, sensitivity: 1 pg/mL). Priority was given to obtaining cortisol levels when the amount of saliva did not allow for both analyses.

2.2.4. Video

In homes with two or three dogs, video footage obtained during the TPT was used to evaluate: (1) dog participation in each trial (indicated by movement towards the toy after it was thrown by the owner) and (2) which dog got first possession of the toy in each trial.

2.2.5. Variables

Tables 2.1 to 2.6 show all the variables analyzed in this study with a brief description and the source of the data.

In addition to using absolute age and personality scores for analyses, it was considered relevant to examine a dog's relative age or personality scores in the context of its home group. For example, a 5-year-old dog might be the oldest in its home group, but another dog of the same age might be the youngest in its home group. To be able to evaluate the relationship between a dog's relative age or personality score within its home group and other variables, mean age and mean personality scores were calculated for each home. These values were subtracted from age and from each personality score for each dog in the household to obtain relative age, relative Extraversion, relative Motivation, relative Training Focus, relative Amicability and relative Neuroticism.

Table 2.1: List of TPT variables used in this study with description and source. Range shown in parentheses where appropriate.

Toy possession test variables		
Variable	**Description**	**Source**
TPT treat tot	Total points won in trials using toy with treat (0-3)	Video
TPT squeaky tot	Total points won in trials using squeaky toy (0-3)	
TPT participation total	Total trials in which dog moved towards the toy (0-6)	
TPT total	Total points won during whole toy possession test considering all trials (0-6)	
TPT atleastonedog prop	Proportion of available points won in trials in which at least one dog moved towards the toy	Calculated from other TPT variables
TPT win comp prop	Proportion of available points won in trials in which at least two dogs moved towards the toy	
TPT high score	Yes/no depending on whether dog got highest number of TPT points compared to other dogs in house	Obtained from a comparison between TPT scores of dogs in the same house

Table 2.2: List of variables related to owner-reported behaviours used in this study with description and source. Range shown in parentheses where appropriate.

Owner-reported behaviour variables		
Variable	**Description**	**Source**
Q01 – play with	Yes/no, asked whether dogs had done the behaviour with/to other dogs in the house in the previous 6 months. A 'yes' counts as a score of 1 for the totals below.	Questionnaire
Q02 – initiate play with		
Q03 – sleep with		
Q04 – fight with		
Q05 – initiate fight with		
Q06 – mark object / area		
Q07 –over-mark object / area		
Q08 – eat other dog's food		
Q09 – guard food		
Q10 – guard object		
Q11 – mount		
Q12 – get attention from people interacting with other dog		
Q13 – lick muzzle		
Q14 – bite muzzle		
Q15 – growl at		
Q16 – bare teeth at		
Q17 – lay paw over back or belly of other dog		
Bark first	Yes/no, when stranger comes to the door	
Bark longest	Yes/no, when stranger comes to the door	
Affiliation total	Affiliative behaviours total: Q1, Q3; (0-2)	Calculated from other behavioural question variables
Agonism total	Agonistic behaviours total: Q4, Q14-16; (0-4)	
Resource-guarding total	Resource-guarding behaviours: Q6-10, Q12; (0-6)	

Table 2.3: Owner-reported dominance variable used in this study with description and source.

Owner-reported dominance variable		
Variable	**Description**	**Source**
Dominant owner opinion	Yes/no, question asked orally at the end of visit	Video

Table 2.4: List of demographic variables used in this study with description and source.

Demographic variables		
Variable	**Description**	**Source**
Age	In months at the time of the visit	Questionnaire
Relative age	Mean age for all dogs in house subtracted from age of individual	Calculated from age
Weight	In kg	Questionnaire
Height	In cm	Questionnaire

Table 2.5: List of hormone variables used in this study with description and source.

Cortisol variables		
Variable	**Description**	**Source**
Cort1	Pre-visit cortisol level, baseline	Saliva sample
Cort2	Mid-visit cortisol level	
Cort3	Cortisol level after the TPT	
Logcort1, 2, 3	Log_{10} transformed cort 1, 2, 3	Calculated from other hormone variables
React cort 12	Cort 1 subtracted from cort 2, reactivity during the conversation phase	
React cort 23	Cort 2 subtracted from cort 3, reactivity during the TPT	
Mean cort	Average: cort 1, 2, 3	
Log mean cort	Log_{10} transformed mean cort	
React cort 12 increase	‘Yes’ when react cort 12 positive, ‘no’ when negative	Obtained from other hormone variables
React cort 23 increase	‘Yes’ when react cort 23 positive, ‘no’ when negative	
Testosterone variables		
Testost1	Pre-visit testosterone level, baseline	Saliva sample
Testost2	Mid-visit testosterone level	
Testost3	Testosterone level after the TPT	
React testost 12	Testost 1 subtracted from testost 2, reactivity during the conversation phase	Calculated from other hormone variables
React testost 23	Testost 2 subtracted from testost 3, reactivity during the TPT	
Mean testost	Average: testost 1, 2, 3	
React testost 12 increase	‘Yes’ when react testost 12 positive, ‘no’ when negative	Obtained from other hormone variables
React testost 23 increase	‘Yes’ when react testost 23 positive, ‘no’ when negative	
Z testost 1, 2, 3, z react testost 12, 23, and z mean testost	Z-scores calculated using sex and gonadectomy subgroup means (spayed females, intact females, neutered males, and intact males).	Calculated from other hormone variables using sex and gonadectomy variables to generate subgroups

Table 2.6: List of personality variables used in this study with description and source.

Personality variables		
Extraversion	Obtained using the results of the Monash Canine Personality Questionnaire - Revised	Questionnaire
Motivation		
Training Focus		
Amicability		
Neuroticism		
Relative Ext	Mean Extraversion for all dogs in house subtracted from individual Extraversion score	Calculated from personality variables
Relative Mot	Mean Motivation for all dogs in house subtracted from individual Motivation score	
Relative Trai	Mean Training Focus for all dogs in house subtracted from individual Training Focus score	
Relative Amic	Mean Amicability for all dogs in house subtracted from individual Amicability score	
Relative Neurot	Mean Neuroticism for all dogs in house subtracted from individual Neuroticism score	

Missing hormone data and outlier removal

Whenever possible, both cortisol and testosterone were analyzed for each saliva sample collected. Of 152 total saliva samples, 5% (N = 8) did not have enough saliva for either test (no hormonal results), 5% (N = 8) did not have enough saliva for both tests, so were only analysed for cortisol, and 2% (N = 3) showed no detectable testosterone. These cases are entered in the data sheet as missing data.

Hormone outliers (scores outside the range delimited by mean ± 3SD) were removed and entered as missing data. One cortisol baseline and one cortisol sample 2 value were removed as outliers. For testosterone measurements, mean and SD were calculated for each sex/gonadectomy group (spayed females, intact females, neutered males, and intact males), and the values obtained for each dog were compared to its own group. In the spayed female group, one testosterone baseline (3 % of baseline samples for that group) and one testosterone sample 2 value (5 % of samples 2 for that group) were removed as outliers.

Hormone output and reactivity variables

New variables representing hormone output and reactivity were calculated using cortisol and testosterone measurements obtained from the three sample points. Because this study depended on home visits and, consequently, owners’ schedules and home routines for saliva collection, time intervals between samples varied from one home to another. Therefore, area under the curve values (Khoury et al., 2015), which are the result of a multiplication of average hormone level for two samples and time between these samples, are not used here. However, Khoury et al. (2015) showed that area under the curve with respect to ground is highly correlated with mean hormone level. Hence, to represent total hormone output, mean cortisol and mean testosterone levels across samples were calculated. Khoury et al. (2015) also showed that reactivity scores (subtracting final from initial hormone measurements) are highly correlated with area under the curve values with respect to change. To represent hormone change, reactivity scores were calculated for the conversation phase and the TPT phase.

Log-transformed cortisol

All scale variables were tested for normality using the Kolmogorov-Smirnov test, and many were not normally distributed. Log_{10} transformation of absolute cortisol values and mean cortisol was performed (Khoury et al., 2015; Mehta & Josephs, 2010) to reduce positive skew and make data more suitable for parametric analyses. Cortisol reactivity in the conversation phase was not normally distributed, but log transformation did not help reduce the skew. Also, cortisol reactivity during the TPT was normally distributed, so none of the cortisol reactivity variables were log-transformed.

Testosterone groups and testosterone z-scores

Testosterone production takes place in large quantities in the testes of males, and to a lesser extent in the adrenal cortex of both sexes and ovaries of females (Costanzo, 2014; Smith et al., 2013). Because individuals of different sexes and gonadectomy statuses possess different testosterone producing organs, subjects were separated into the four different sex/gonadectomy groups previously described: spayed females, intact females, neutered males, and intact males. Testosterone variables were converted to z-scores per testosterone group distributions to analyze relationships between testosterone variables and cortisol, personality, and TPT variables including all possible subjects (Mehta & Josephs, 2010).

Toy possession test (TPT) variables

From video analysis, TPT participation in and results for each trial were recorded, and total points won in squeaky toy trials (0-3), treat-stuffed toy trials (0-3) and all trials (0-6) were tallied.

Total participation (TPT participation total, 0-6) in the toy possession test was calculated by adding up all trials in which each dog participated (moved towards the toy after it was thrown by the owner). Proportion of wins in trials in which at least one dog participated (TPT at least one dog prop, 0-1) and proportion of wins in trials in which at least two dogs participated (TPT win comp prop, 0-1) were also compiled. In the context of their own home groups, dogs were also classified as having achieved the highest score or not (TPT high score, categorical variable).

Owner-reported behaviour totals

Affirmative responses to some of the behavioural questions asked in the questionnaire were compiled into three different variables: affiliative behaviours (Q1, 3), agonistic behaviours (Q 4, 14, 15, 16), and resource-guarding behaviours (Q6 – 10, 12). Other questions (Q2, 5, 11, 13, 17, bark first and bark longest) were analyzed separately. All these questions referred to the interaction between cohabiting dogs, not between dogs and owners.

Data from 4-5 dog homes

Owners from homes in which there were more than 3 dogs did not collect saliva samples 2 and 3, and the TPT was not performed with these dog groups. Thus, analyses involving hormone levels from samples 2 and 3, and TPT results did not include data from homes with 4-5 dogs.

2.2.6. Data analyses

Statistical analyses were performed with IBM SPSS Statistics 23, except for Fisher's Exact probability tests for tables bigger than 2 x 2: these were performed with VassarStats (http://vassarstats.net). Scale variables were tested for normality using the Kolmogorov-Smirnov test, and many of them were not normally distributed. Log_{10} transformation was performed on absolute cortisol measurements and mean cortisol to reduce positive skew (Khoury et al., 2015; Mehta & Josephs, 2010) and make them more suitable to parametric analyses. Other non-normal variables were subjected to non-parametric analyses. Exploration of the relationship between demographic, hormone, personality, TPT, owner impression, and behavioural question variables was done with correlations, group comparison tests, and chi square tests for independence, as appropriate. Analyses were two-tailed, with a significance level (α) of 0.05. The Bonferroni method of correcting for multiple comparisons was not used since the objective of this study was to explore the relationships between characteristics, and the correction would be too restrictive (Bender & Lange, 2001; Ottenheimer Carrier et al., 2013). This means that significant findings should be interpreted cautiously, and used as a basis to generate further hypotheses for future research, due to the higher likelihood of Type II error.

General linear mixed models (GLMMs) with restricted maximum likelihood estimation were used to examine the relationship between each hormone (cortisol and testosterone) and sample type (baseline, sample 2, and sample 3), sex, gonadectomy status, and interaction between sex and gonadectomy status. Hormone level measurements were entered as the dependent variable, while sample type, sex, gonadectomy status and the interaction between sex and gonadectomy status were fixed factors. Cortisol levels were log-transformed to reduce positive skew. Random intercepts and slopes were added to the analysis, and goodness of fits for the different models were compared using the log likelihood ratio test (Barr, Levy, Scheepers, & Tily, 2013). If two models had statistically different fits, the one with best fit was used. If two models did not show statistically different fits, then the simplest model was selected.

To investigate whether testosterone levels relate to TPT results and how cortisol might be involved in this interaction, hierarchical multiple regressions were run using TPT results as dependent variables as per Mehta and Josephs (2010). In this analysis, different blocks of independent variables are evaluated, and an estimate of how much better a certain block predicts variation in the dependent variable compared to previous blocks is generated. Sex/gonadectomy group of each dog and age were used as predictors in the first block, log-transformed cortisol baseline and testosterone baseline converted to z-scores in the second block and log-transformed cortisol baseline multiplied by testosterone baseline z-score (cortisol and testosterone interaction term) in the third block.

Cortisol baseline was log-transformed to reduce positive skew, and testosterone baseline was converted to z-scores considering sex/gonadectomy groups, so that all four groups could be included in the same analysis.

Other models were run using the same explanatory variable structure, i.e., hierarchical multiple regressions with personality scores and behavioural question totals as dependent variables and a logistic regression with owner impression of dominance as the dependent variable.

2.3. Results

This section will present results for each of the different methods of characterizing a dog's relative social status within its home group (Method 1: TPT, Method 2: owner-reported behaviours, and Method 3: owner-reported dominance). Their relationships to dog demographics, hormones, personality, and to each other are reported. After this, other relationships of hormones and personality variables are also reported.

2.3.1. Method 1: Toy possession test (TPT)

Total points obtained in trials using the treat-stuffed toy and the squeaky toy were positively correlated ($r_s(44)=0.696$, $p<0.001$; Fig. 2.2). A Wilcoxon signed-ranks test showed there was no statistically significant difference between the scores for the two different toys. Thus, all further analyses use scores for the combined trial types.

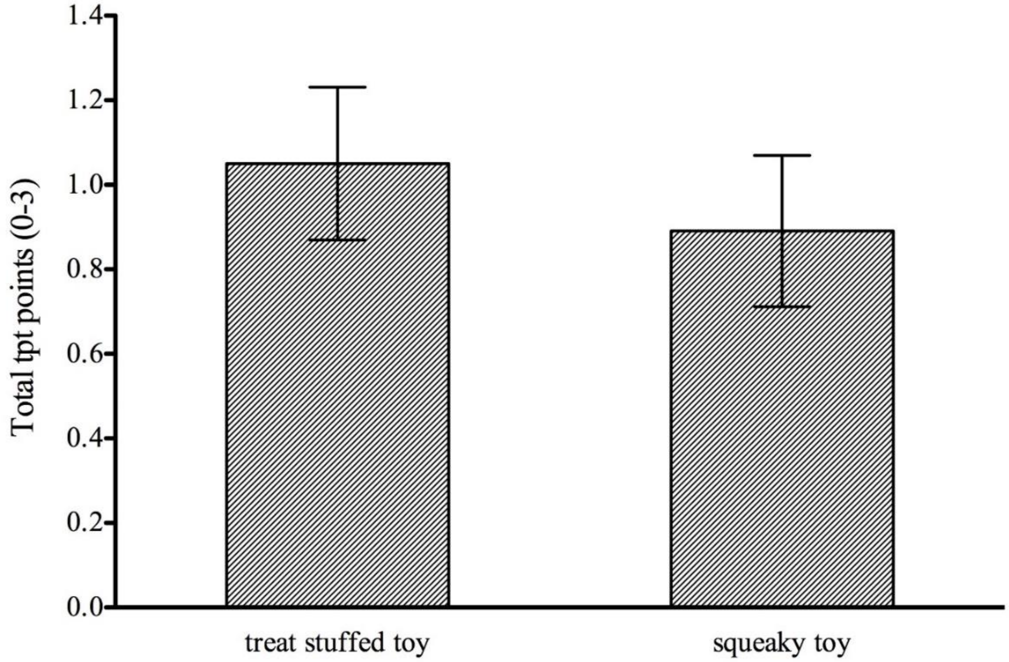

Fig. 2.2: Mean (± SE) points won in treat-stuffed toy and squeaky toy trials (N =44).

2.3.1.1. TPT and dog demographics

For each of the 18 two- or three-dog homes, there were six TPT trials, totalling 108 trials. There was no participation in 10 (9.3%) of these, and participation of at least one dog in 98 (90.7%) trials. Of the TPT trials with dog participation, at least two dogs competed for the toy in 64 (64/98 = 65.3%) trials.

TPT participation total per dog (4.06 ± 0.36, mean ± SE; N = 44), proportion of points won in trials in which at least one dog participated (TPT atleastonedog prop; 0.405 ± 0.065; N = 42), and proportion of points won in trials in which at least two dogs participated (TPT win comp prop; 0.34 ± 0.064; N = 44) were examined using bivariate Spearman correlations in relation to dog age, relative age, height, and weight. Relative age was significantly and negatively correlated with the proportion of points won in trials in which at least one dog participated ($r_s(42) = -0.338$, $p = 0.029$), such that relatively younger dogs won more points than relatively older dogs.

Mann-Whitney U tests to examine whether age, relative age, weight, and height differed by TPT high score showed that dogs receiving the TPT high score for their households were relatively younger compared to their housemates (Mann–Whitney U = 129, p = 0.021, $mean_{yes} = -9.17$, $mean_{no} = 9.16$). TPT high score did not differ by absolute dog age, weight, or height.

TPT variables did not differ significantly with sex/gonadectomy group. However, the proportion of TPT high scores differed marginally by sex with females getting high scores more often than males (Chi-square: χ^2 (1, n=42) =3.857, p=0.050) and did not differ by gonadectomy status (Chi-square) or sex/gonadectomy group (Freeman-Halton extension of Fisher's exact probability test).

2.3.1.2. TPT and hormones

In order to investigate whether TPT variables were correlated with hormone variables, Spearman correlations were carried out. Correlations between TPT and cortisol variables were examined for all cases, while correlations between TPT and testosterone variables were evaluated for each sex/gonadectomy group.

TPT participation total, i.e., number of trials in which a dog participated, was negatively correlated with both cortisol baseline levels ($r_s(41) = -0.374$, $p = 0.016$) and mean cortisol levels ($r_s(39) = -0.343$, $p = 0.032$). There were no other significant correlations between TPT variables and cortisol. For testosterone, baseline testosterone was significantly positively correlated with proportion of points won in trials in which at least one dog participated for intact females ($r_s(5) = 0.949$, $p = 0.014$). For neutered males, testosterone sample 2 (prior to TPT) was significantly negatively correlated with TPT participation total ($r_s(10) = -0.740$, $p = 0.014$), but not with points won. Spayed females showed no statistically significant correlations between TPT and testosterone variables. Intact males could not be examined because of low sample size.

Independent samples t-tests to examine cortisol and testosterone variables showed no statistically significant differences between the dog in the household that obtained a TPT high score and those that did not.

As described earlier, hierarchical multiple regressions were run using TPT variables as dependent variables. For the proportion of points won in trials in which at least one dog participated as the dependent variable, the inclusion of the cortisol and testosterone interaction term produced a statistically significant model ($F(5,31)=4.062$, $p=0.006$) with an adjusted R^2 of 29.8%. Inclusion of the cortisol X testosterone interaction generated a statistically significant improvement in model fit compared to the second block of predictors ($\Delta R^2=20.2\%$, $\Delta F(1,31)=10.34$, $p=0.003$). In this model, baseline testosterone z-scores ($p=0.008$), baseline cortisol X baseline testosterone interaction ($p=0.003$) and relative age ($p=0.018$) were significantly related to the proportion of points won in trials in which at least one dog participated, with younger dogs and dogs with higher levels of testosterone obtaining more TPT points. To better understand how the cortisol and testosterone interaction affected the dependent variable, a median split was used to separate dogs into high and low groups for cortisol and testosterone. Figure 2.3 shows that dogs with higher baseline testosterone levels and lower baseline cortisol levels won more points than any other combination of hormone levels.

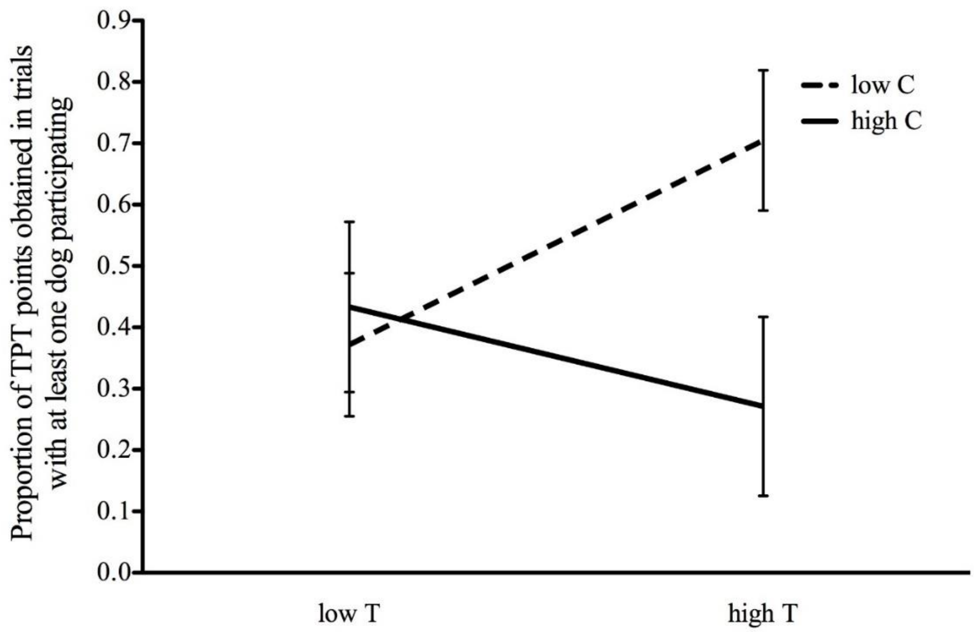

Fig. 2.3: Interaction effect between cortisol baseline (C) and testosterone baseline z-scores (T) on proportion of points won in trials in which at least one dog participated (Mean±SE). Low and high hormone groups were obtained by median split.

Interestingly, neither total TPT participation nor proportion of points won in trials in which at least two dogs participated were significantly affected by any of the variables used as predictors, and the different blocks of predictors did not show significantly different model fits.

2.3.1.3. TPT and MCPQ-R personality dimensions

To investigate whether TPT variables were correlated with personality and relative personality variables, Spearman correlations were carried out. The proportion of points won in trials in which at least one dog participated was significantly positively correlated with Extraversion ($r_s(42) = 0.399$, $p = 0.009$), relative Extraversion ($r_s(42) = 0.384$, $p = 0.012$) and relative Motivation ($r_s(42) = 0.386$, $p = 0.011$). The proportion of points won in trials in which at least two dogs participated was also significantly positively correlated with Extraversion ($r_s(42) = 0.446$, $p = 0.003$), relative Extraversion ($r_s(42) = 0.421$, $p=0.006$), Motivation ($r_s(42) = 0.373$, $p = 0.015$) and relative Motivation ($r_s(42) = 0.417$, $p = 0.006$). Total TPT participation was not significantly correlated with any of the personality variables.

Mann-Whitney U tests to compare personality and relative personality dimension scores by TPT high score ($n_{yes} = 20$, $n_{no} = 21$) showed that, compared to other dogs in their household, dogs that got the highest TPT score had higher scores for Extraversion (Mann–Whitney $U = 110$, $p=0.009$), relative Extraversion (Mann–Whitney $U = 111.5$, $p=0.010$), Motivation (Mann–Whitney $U = 114.5$, $p=0.013$), and relative Motivation (Mann–Whitney $U = 116$, $p=0.014$) (Figs. 2.4, 2.5).

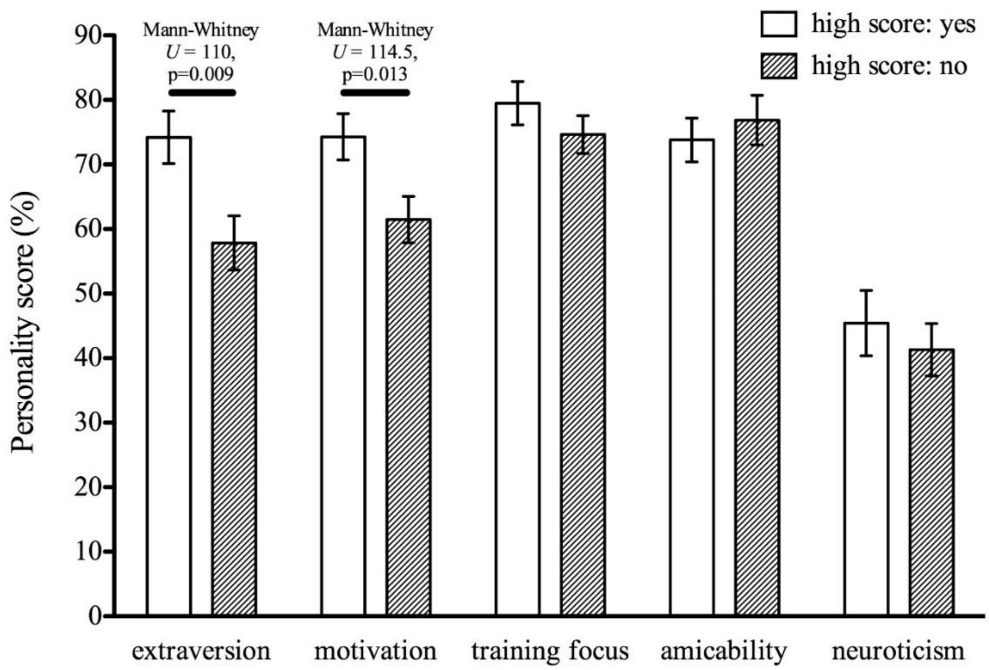

Fig. 2.4: MCPQ-R personality dimension scores (Mean ± SE) for dogs either obtaining or not obtaining the TPT high score.

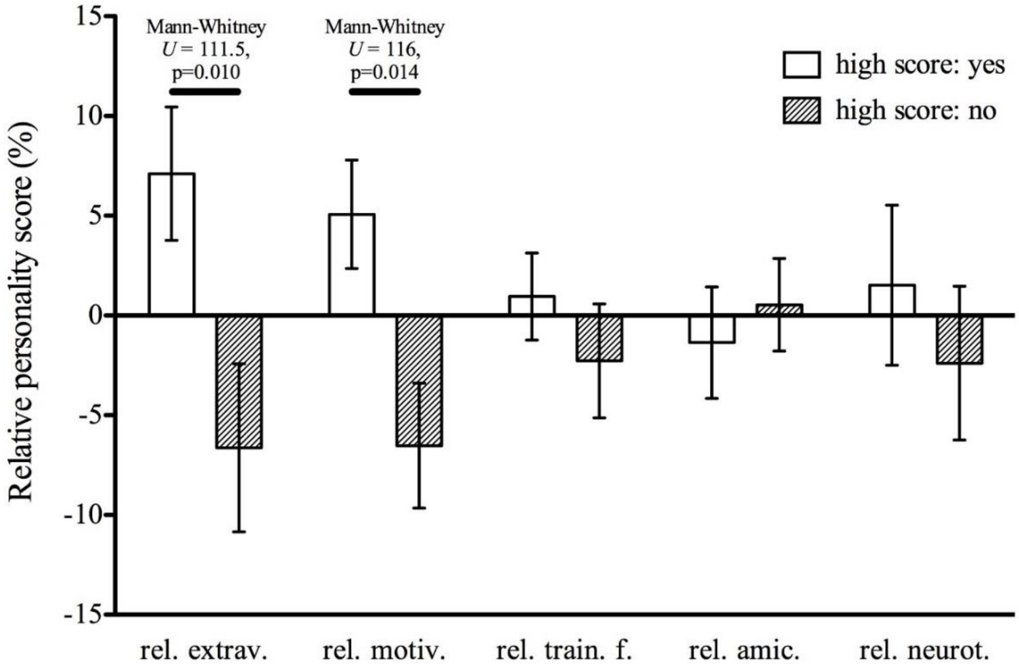

Fig. 2.5: Relative MCPQ-R personality dimension scores (Mean±SE) for dogs either obtaining or not obtaining the TPT high score.

2.3.1.4. TPT and owner-reported dominance

The examination of TPT variables by owner-reported dominance with Mann-Whitney U tests found no significant relationships.

2.3.2. Method 2: Owner-reported behaviours

Totals for affiliative, agonistic and resource-guarding behaviours were tallied from the questionnaires and examined for inter-correlations (Spearman's rho). Total agonistic and resource-guarding scores were positively correlated ($r_s(66) = 0.326$, $p = 0.008$).

2.3.2.1. Owner-reported behaviours and dog demographics

Spearman's rho correlations of affiliative, agonistic and resource-guarding behaviour totals with age, relative age, weight and height showed only a negative correlation between total agonistic behaviours and age ($r_s(66)=-0.288$, $p=0.019$). There were no differences for affiliative, agonistic and resource-guarding question totals as a function of sex or gonadectomy status (Mann-Whitney U tests).

Included in this study's behavioural questions were behaviours that have been previously used for the evaluation of dominance (e.g., Qs 4, 8, 11,13, 14, 17, bark first, bark longest; see Bauer & Smuts, 2007; Bonanni et al., 2010; Cafazzo et al., 2010; Pongrácz et al., 2008) and the initiation of play and fighting (Q2, & 5). Table 2.7 shows the relationships between owner responses to questions about these behaviours and age, relative age, height and weight. It also includes information on percentage of dogs that show the behaviour.

Table 2.7: Significant differences in age and relative age between dogs that perform and do not perform behaviours previously associated with social status, as reported by owners. Results of analyses not significantly related to any of the questions, such as weight and height, are not shown.

Question	**Owners answered YES to the question for dogs that were:**
Q04 – fight with (n_{yes} = 13, n_{no} =48, $n_{n/a}$=5, $\%_{yes}$=21.3)	no significant relationship with age, relative age, height or weight.
Q08 – eat other dog's food (n_{yes} = 28, n_{no} = 30, $n_{n/a}$=8, $\%_{yes}$=48.3)	no significant relationship with age, relative age, height or weight.
Q11 – mount (n_{yes} = 18, n_{no} =42, $n_{n/a}$=6, $\%_{yes}$=30)	no significant relationship with age, relative age, height or weight.
Q13 – lick muzzle (n_{yes} = 23, n_{no} = 43, $n_{n/a}$=0, $\%_{yes}$= 34.8)	younger (Mann–Whitney U = 133.5, p=0.000, $mean_{yes}$=40.74, $mean_{no}$=94.02)
	relatively younger (Mann–W U = 259, p=0.002, $mean_{yes}$=-18.9, $mean_{no}$=10.1)
Q14 – bite muzzle (n_{yes} = 17, n_{no} = 44, $n_{n/a}$=5, $\%_{yes}$=27.9)	younger (Mann–Whitney U = 166, p=0.001, $mean_{yes}$=48.53, $mean_{no}$=85.98)
	relatively younger (Mann–Whitney U = 238.5, p=0.029, $mean_{yes}$=-14.9, $mean_{no}$=5.8)
Q17 – lay paw over back or belly of other dog (n_{yes} = 22, n_{no} = 44, $n_{n/a}$=0, $\%_{yes}$=33.3)	younger (Mann–Whitney U = 273.5, p=0.004, $mean_{yes}$=50.6, $mean_{no}$=87.9)
	relatively younger (Mann–Whitney U = 228.5, p<0.001, $mean_{yes}$=-18.4, $mean_{no}$=9.2)
Bark first (n_{yes} = 20, n_{no} = 46, $\%_{yes}$=30.3)	no significant relationship with age, relative age, height or weight.
Bark longest (n_{yes} = 22, n_{no} = 44, $\%_{yes}$=33.3)	older (Mann–Whitney U = 317.5, p=0.024, $mean_{yes}$=92.2, $mean_{no}$=67.1)
	relatively older (Mann–Whitney U = 276.5, p=0.005, $mean_{yes}$=18.9, $mean_{no}$=-9.4)

Questions 2 (initiation of play) and 5 (initiation of fight) were not included in affiliative, agonistic, and resource-guarding totals, nor have they been used as an indicator of dominance. The responses to these questions were examined as above. There were no differences among these variables for the responses to Q5 (initiate fight: n_{yes} = 11, n_{no} =46, $n_{n/a}$=9). However, for Q2, dogs who initiated play (n_{yes} = 51, n_{no} = 15, $n_{n/a}$=0) were younger (age: Mann–Whitney U = 185, p=0.003, $mean_{yes}$=66.65, $mean_{no}$=105.4) and relatively younger (relative age: Mann–Whitney U = 216, p=0.011, $mean_{yes}$=-6.55, $mean_{no}$=22.27).

Chi square and Fisher's exact tests for independence were used to examine all questions above (Q 2, 4, 5, 8 11, 13, 14, 17, bark first, and bark longest) by sex, gonadectomy status, and sex/gonadectomy group. The only significant finding was that gonadectomised dogs were reported to display the muzzle licking behaviour (Q13) less than intact dogs (χ^2 (1, n=66) =6.242, p=0.012).

2.3.2.2. Owner-reported behaviours and hormones

Spearman's rho correlations of affiliative, agonistic and resource-guarding behaviour totals with hormone variables showed positive correlations between total affiliative behaviours and baseline cortisol (r_s(60)=0.344, p=0.007) and z-scores of baseline testosterone (r_s(55)=0.444, p=0.001). Total agonistic behaviour score was positively related to cortisol reactivity during the conversation phase (r_s(40)=0.340, p=0.0328), z-scores of testosterone sample 3 (r_s(38)=0.366, p=0.024) and z-scores of mean testosterone levels (r_s(31)=0.505, p=0.004).

Hierarchical multiple regressions were run using the owner-reported behaviour totals as dependent variables. Sex/gonadectomy subgroup and age were used as predictors in the first block, baseline cortisol and baseline testosterone converted to z-scores in the second block, and cortisol baseline multiplied by testosterone baseline z-score (cortisol X testosterone interaction term) in the third block.

For the affiliative behaviour total, both the second ($F(4,49)=5.082$, $p=0.002$; adjusted $R^2=23.6\%$) and third ($F(5,48)=4.983$, $p=0.001$; adjusted $R^2=27.3\%$) blocks of predictors resulted in statistically significant models, but only the change from first to second block of predictors was statistically significant ($\Delta R^2=24.6\%$, $\Delta F(2,49)=8.54$, $p=0.001$). In the second block, baseline cortisol ($p=0.014$) was significantly related to the affiliative behaviour total, with higher affiliative behaviour totals in dogs showing higher cortisol baseline. Agonistic behaviour totals and resource-guarding totals were not significantly predicted by the dependent variables examined.

The results for questions 2, 4, 5, 8, 11,13, 14, 17, bark first, and bark longest were examined for their relationship to hormone variables. Dogs who ate another dog's food had higher testosterone reactivity during the TPT (Mann–Whitney $U = 14$, $p=0.029$, $mean_{yes}=1.817$, $mean_{no}=-0.032$).

2.3.2.3. Owner-reported behaviours and personality

Spearman's rho correlations of affiliative, agonistic and resource-guarding behaviour totals with personality variables showed a positive correlation between affiliative total and Training Focus ($r_s(66)=0.251$, $p=0.042$). Agonistic total was positively related to relative Motivation ($r_s(66)=0.274$, $p=0.026$), and negatively correlated with both Amicability ($r_s(66)=-0.263$, $p=0.033$) and Neuroticism (($r_s(66)=-0.255$, $p=0.039$).

Table 2.8 shows the relationships of owner responses to questions 4, 8, 11,13, 14, 17, bark first, bark longest, with personality variables.

Table 2.8: Significant differences in personality variables between dogs that perform and do not perform behaviours previously associated with social status, as reported by owners.

<table>
<tr><th>Question</th><th>Owners answered YES to the question for dogs that had:</th></tr>
<tr><td>Q04 – fight with (n_{yes} = 13, n_{no} =48, $n_{n/a}$=5, $\%_{yes}$=21.3)</td><td>no significant relationship with any personality variables.</td></tr>
<tr><td>Q08 – eat other dog's food (n_{yes} = 28, n_{no} = 30, $n_{n/a}$=8, $\%_{yes}$=48.3)</td><td>no significant relationship with any personality variables.</td></tr>
<tr><td>Q11 – mount (n_{yes} = 18, n_{no} =42, $n_{n/a}$=6, $\%_{yes}$=30)</td><td>no significant relationship with any personality variables.</td></tr>
<tr><td rowspan="2">Q13 – lick muzzle (n_{yes} = 23, n_{no} = 43, $n_{n/a}$=0, $\%_{yes}$= 34.8)</td><td>more extraverted (Mann–Whitney U = 312.5, p=0.014; $mean_{yes}$=72.5, $mean_{no}$=59)</td></tr>
<tr><td>relatively more extraverted (Mann–Whitney U = 346.5, p=0.046, $mean_{yes}$=7.07, $mean_{no}$=-3.5)</td></tr>
<tr><td>Q14 – bite muzzle (n_{yes} = 17, n_{no} = 44, $n_{n/a}$=5, $\%_{yes}$=27.9)</td><td>more motivated (Mann–Whitney U = 245.5, p=0.038, $mean_{yes}$=76.9, $mean_{no}$=67.3)</td></tr>
<tr><td rowspan="2">Q17 – lay paw over back or belly of other dog (n_{yes} = 22, n_{no} = 44, $n_{n/a}$=0, $\%_{yes}$=33.3)</td><td>more extraverted (Mann–Whitney U = 298, p=0.011, $mean_{yes}$=73, $mean_{no}$=59)</td></tr>
<tr><td>relatively more extraverted (Mann–Whitney U = 293, p=0.009, $mean_{yes}$=8.8, $mean_{no}$=-4.2)</td></tr>
<tr><td>Bark first (n_{yes} = 20, n_{no} = 46, $\%_{yes}$=30.3)</td><td>relatively less amicable (Mann–Whitney U = 260.5, p=0.005, $mean_{yes}$=-7.9, $mean_{no}$=3.1)</td></tr>
<tr><td rowspan="2">Bark longest (n_{yes} = 22, n_{no} = 44, $\%_{yes}$=33.3)</td><td>less amicable (Mann–Whitney U = 318, p=0.024, $mean_{yes}$=66.1, $mean_{no}$=77.3)</td></tr>
<tr><td>relatively less amicable (Mann–Whitney U = 256.5, p=0.002, $mean_{yes}$=-9.4, $mean_{no}$=4.3)</td></tr>
</table>

There were no differences among personality variables for the responses to Q2 (initiate play) and Q5 (initiate fight).

2.3.2.4. Owner-reported behaviours and TPT

Spearman's rho correlations between TPT scale variables and affiliative, agonistic and resource-guarding totals showed no significant relationships. There were no differences for affiliative, agonistic and resource-guarding question totals as a function of TPT high score (Mann-Whitney U tests).

Mann-Whitney U tests of TPT scale variables by results of questions 2, 4, 5, 8, 11,13, 14, 17, bark first, and bark longest showed no significant differences.

Chi square and Fisher's exact tests for independence were used to examine all questions above (Q 2, 4, 5, 8 11, 13, 14, 17, bark first, and bark longest) by TPT high score. No significant relationships were found.

2.3.2.5. Owner-reported behaviours and owner-reported dominance

There were no differences for affiliative, agonistic and resource-guarding question totals as a function of owner impressions of dominance (Mann-Whitney U tests).

Chi square and Fisher's exact tests for independence were used to examine questions 2, 4, 5, 8 11, 13, 14, 17, bark first, and bark longest by owner-reported dominance and no significant relationships were found.

2.3.3. Method 3: Owner-reported dominance

Each dog in the 20 (of 23) households was categorized by their owner as being either the dominant member of the group or not (n_{dom} = 20, $n_{\text{not dom}}$ = 39). In the remaining three (13%) participating homes, owners did not believe that any of their dogs was more dominant than the others, so these homes were excluded from owner-reported dominance analyses.

2.3.3.1. Owner-reported dominance and demographics

Mann-Whitney U tests of age, relative age, height and weight by owner-reported dominance showed no significant results.

The relationship between owner-reported dominance and sex and gonadectomy status was investigated with Fisher's Exact tests and there were no statistically significant differences. Using the Freeman-Halton extension of the Fisher's Exact test, there were no differences in owner-reported dominance by sex and gonadectomy group.

2.3.3.2. Owner-reported dominance and hormones

Mann-Whitney U tests of hormone variables by owner-reported dominance showed no significant results.

A binary logistic regression with owner-reported dominance as the dependent variable was carried out. Sex/gonadectomy subgroup and age were used as predictors in the first block, baseline cortisol and baseline testosterone converted to z-scores in the second block, and baseline cortisol baseline multiplied by baseline testosterone z-score (cortisol X testosterone interaction term) in the third block. No significant relationships were found.

2.3.3.3. Owner-reported dominance and personality

Personality variables were examined by owner-reported dominance with Mann-Whitney U tests. Three relative personality variables were significantly different among dogs classified as dominant or not dominant by owners. Relative Motivation (Mann–Whitney $U = 252$, $p=0.027$) and relative Training Focus (Mann–Whitney $U = 249.5$, $p=0.024$) were higher for dogs that were viewed as more dominant by owners, while relative Amicability (Mann–Whitney $U = 224.5$, $p=0.008$) was lower (Fig. 2.6).

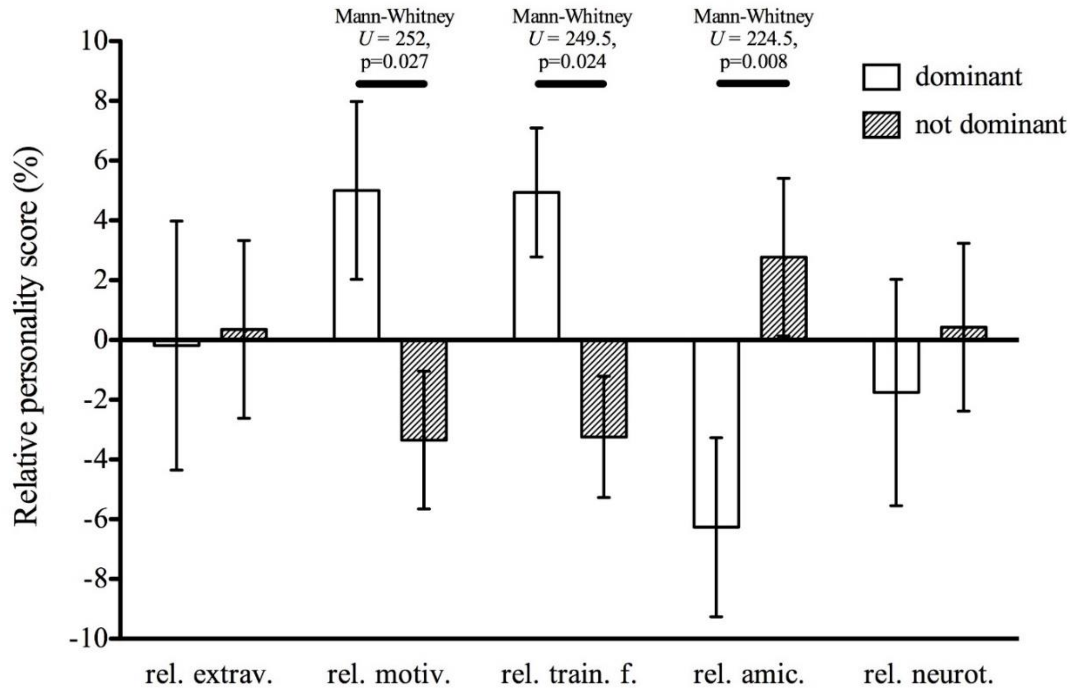

Fig. 2.6: Mean±SE relative MCPQ-R personality dimension scores according to owner-reported dominance.

2.3.4. Hormones

2.3.4.1. Cortisol

Appendix XV shows range and sample sizes for cortisol variables (µg/dL). Fig. 2.7 contains mean cortisol levels, back-transformed, by sample type and mean, including 95% confidence intervals. Fig. 2.8 presents mean and standard error for cortisol reactivity during the conversation and the TPT phases.

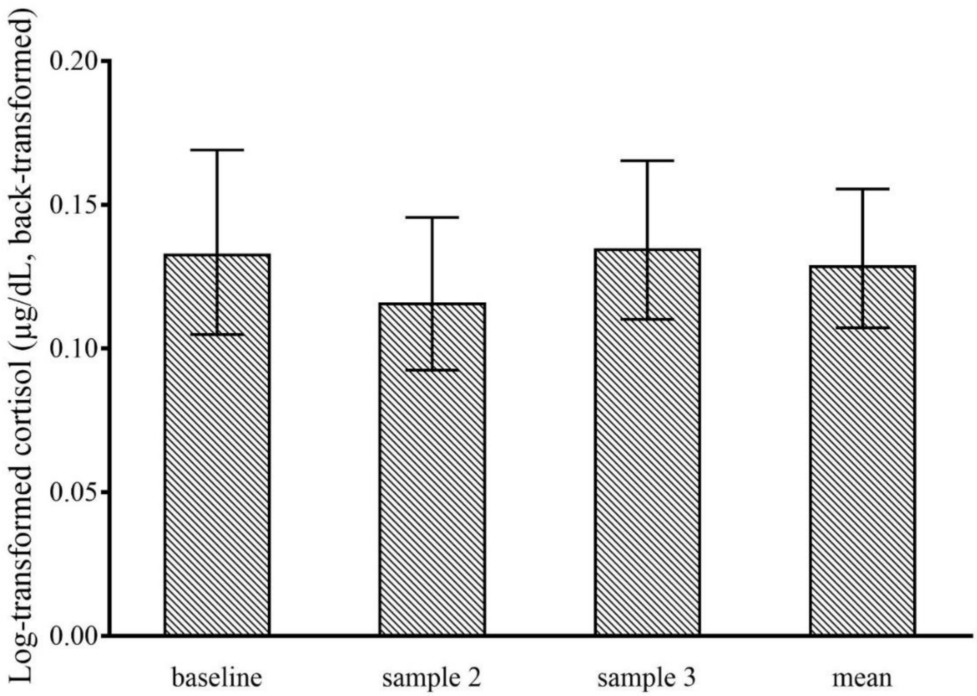

Fig. 2.7: Back-transformed mean cortisol values for each sample time. Error bars represent 95% CIs for the means. Mean cortisol did not differ significantly over samples.

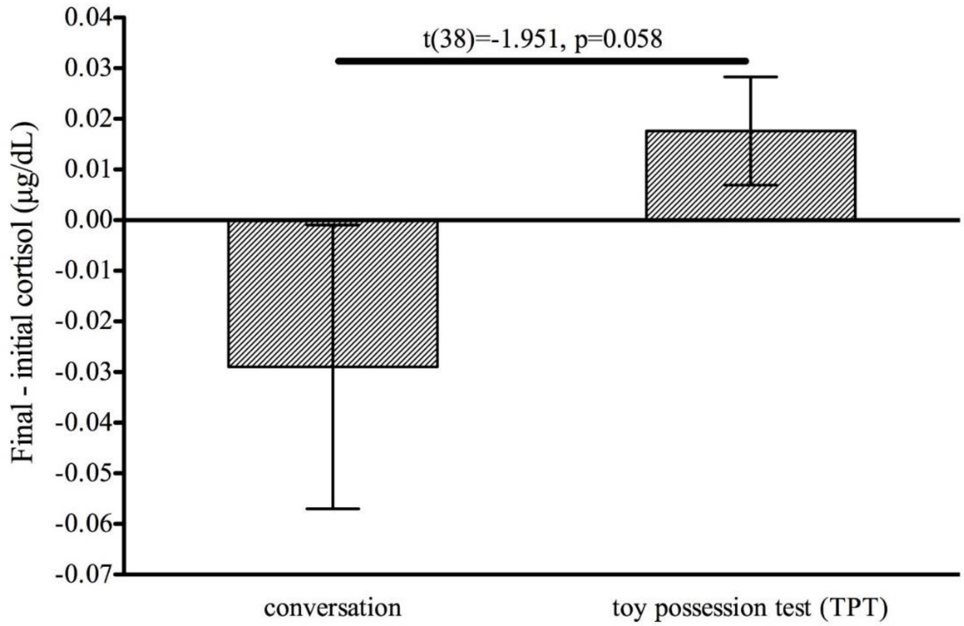

Fig 2.8: Mean ±SE cortisol reactivity for the conversation and the TPT phase. There was a marginally significant difference between the two measurements.

Pearson r correlations were calculated between log-transformed cortisol baseline, sample 2 and sample 3. There was a statistically significant positive correlation between log cortisol 2 and 3 ($r(40)=0.751$, $p<0.001$). Untransformed cortisol baseline, sample 2 and sample 3 correlations with each other were also examined with Spearman's rho correlations, and significant, positive monotonic relationships were found in all cases: baseline and sample 2 ($r_s(40)=0.525$, $p=0.001$), baseline and sample 3 ($r_s(40)=0.444$, $p=0.004$), sample 2 and sample 3 ($r_s(40)=0.751$, $p<0.001$).

GLMMs with restricted maximum likelihood estimation were carried out to examine the relationship between cortisol (the dependent variable), sample type (baseline, sample 2, and sample 3), sex, gonadectomy status, and interaction between sex and gonadectomy status. There were no significant effects.

Cortisol reactivity in the conversation phase was positively correlated with cortisol reactivity in the TPT ($r(39)=0.418$, $p=0.008$). A paired samples t-test showed a marginally significant difference between cortisol reactivity in the two different parts of the visit ($t(38)=-1.951$, $p=0.058$, Fig. 2.8). Specifically, the average change in cortisol was negative in the conversation phase and positive in the toy possession test, reflecting generally lower cortisol measurements at sample 2, followed by an overall increase at sample 3.

Between baseline and sample 2 (conversation phase), 18 dogs (45%) showed an increase in cortisol, while between samples 2 and 3 (TPT phase), 25 dogs (62.5%) showed an increase in cortisol. A Chi-square test examining frequencies of increase and decrease in cortisol in the conversation and the TPT phases showed no statistically significant difference. Of the 39 dogs that had cortisol reactivity measurements for both the conversation and the TPT phases, 4 (10%) dogs showed a decrease in both phases, 17 (44%) showed a decrease in the conversation phase and an increase in the TPT phase, 10 (26%) showed an increase in the conversation phase and a decrease in the TPT phase, and 8 (20%) showed an increase in both phases.

2.3.4.2. Cortisol and dog demographics

To investigate whether any of the cortisol variables used in this study were correlated with age, Pearson r correlations were calculated between age and log-transformed cortisol levels (Fig. 2.7) at baseline, sample 2, and sample 3, log-transformed mean cortisol, and cortisol reactivity (Fig. 2.8) during the conversation phase and the TPT phase. There were no statistically significant correlations.

To assess the possible effects of sex and gonadectomy status on cortisol variables, each of the following were compared between males and females and between gonadectomised and intact dogs using independent samples t-tests: log-transformed cortisol values, cortisol reactivity during the conversation phase and the TPT, and log-transformed mean cortisol. There were no statistically significant differences. One-way ANOVAs showed no significant differences for these cortisol variables across the four sex/gonadectomy subgroups (spayed females, intact females, neutered males, and intact males).

The same cortisol variables were examined with an independent samples t-test comparing the effect of number of dogs in the house (sample sizes: samples 1-3=41, reactivity variables=39, mean=40; two and three-dog homes only). There were no significant differences. Similarly, a one-way ANOVA including homes with two, three, four or five dogs showed no effect of number of dogs in the house on baseline cortisol.

Chi-square tests examining increase and decrease in cortisol reactivity in the conversation phase and the toy possession test by total number of dogs in the house, sex, gonadectomy status, and sex/gonadectomy subgroups did not show any statistically significant relationships.

2.3.4.3. Testosterone

Appendix XVI shows range and sample sizes for testosterone variables (pg/mL) by sex/gonadectomy groups. Fig. 2.9 contains mean testosterone levels by sample type and mean for each sex/gonadectomy group, including standard error. Fig. 2.10 presents mean and standard error for testosterone reactivity during the conversation and the TPT phases by sex/gonadectomy group.

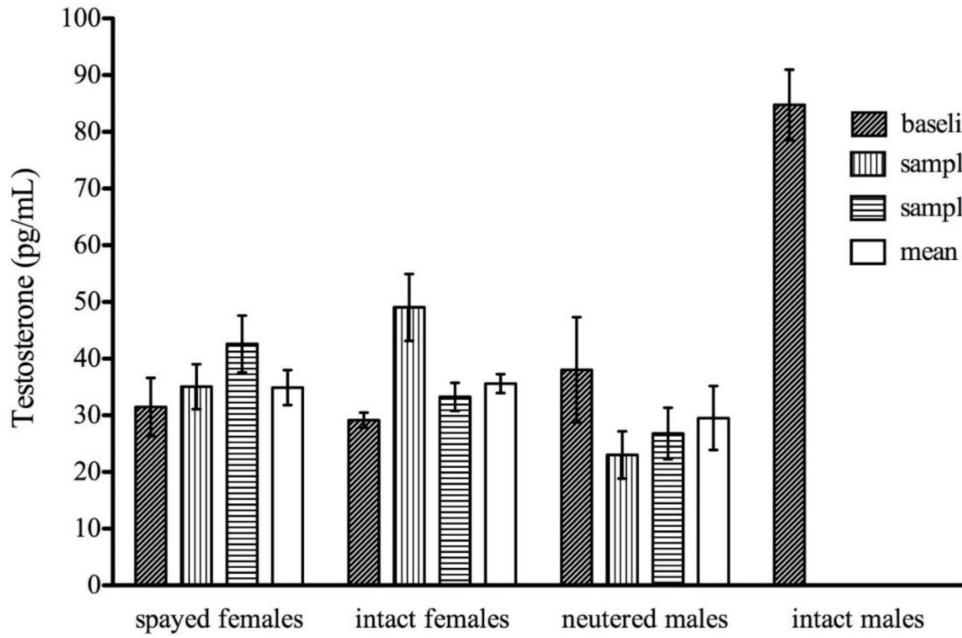

Fig 2.9: Mean ± SE testosterone level by sex/gonadectomy subgroup for each sample and mean. Only the baseline sample is shown for intact males (n=7), because the sample size for intact males for the other values is too low (n=2).

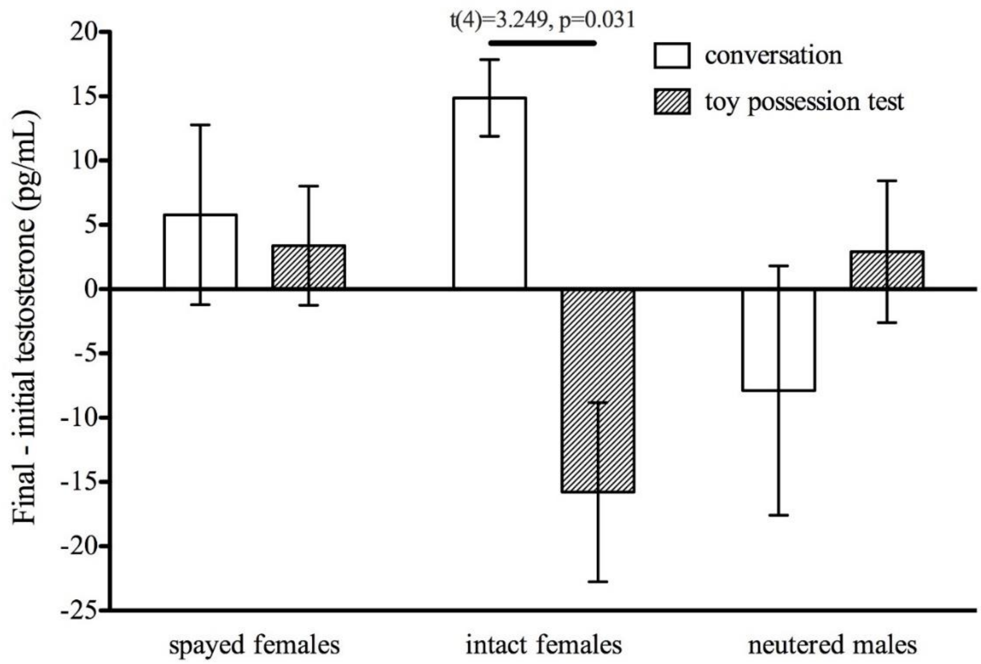

Fig. 2.10: Mean ± SE testosterone reactivity during the conversation phase and the toy possession test. Intact males are not included in this figure because of the low sample size for this group.

Since only the single baseline saliva sample was collected in homes with four or five dogs, the following Pearson r correlations were performed using only data obtained from homes with two or three dogs.

Pearson r correlations were carried out between testosterone measures for each sex/gonadectomy subgroup. Spayed females showed a statistically significant correlation between testosterone baseline and sample 3 ($r(19)=0.516$, $p=0.024$). There were no statistically significant correlations for intact females and neutered males, and correlations for intact males could not be performed because of the limited sample size ($n=2$).

Pearson r correlations were carried out between testosterone reactivity during the conversation phase and the TPT. Spayed females showed a statistically significant negative correlation between the two phases ($r(16)=-0.683$, $p=0.004$), intact females and neutered males showed no statistically significant correlations, and the correlation using data for intact males could not be performed because of the low sample size ($n=2$). Paired sample t-tests using the same variables for each sex/gonadectomy subgroup showed that testosterone reactivity for intact females in the conversation phase was more positive than the reactivity during the TPT ($t(4)=3.249$, $p=0.031$). There were no statistically significant differences between the two variables for spayed females and neutered males. Intact males were not examined because of low sample size.

During the conversation phase, 64% of dogs ($n=21$) showed an increase in testosterone levels, while during the TPT phase, 35% ($n=15$) showed an increase. Fisher's Exact tests to examine whether there were differences in the frequency of increase and decrease in testosterone by conversation or TPT phase showed that more intact females showed an increase in testosterone during the conversation phase than during the TPT ($p=0.002$). The other groups (spayed females, neutered males and intact males) did not show any differences. Of the 31 dogs that had testosterone reactivity measurements for both the conversation and the TPT phases, 2 (6%) dogs showed a decrease in both phases, 8 (26%) showed a decrease in the conversation phase and an increase in the TPT phase, 17 (55%) showed an increase in the conversation phase and a decrease in the TPT phase, and 4 (13%) showed an increase in both phases.

2.3.4.4. Testosterone and dog demographics

Pearson r correlations between testosterone variables and age were performed for each sex/gonadectomy subgroup (spayed females, intact females, neutered males, and intact males). For spayed females, intact females, and neutered males, Pearson r correlations between age and testosterone measurements, mean testosterone, and testosterone reactivity in the conversation phase and the TPT were not statistically significant. Testosterone baseline was not significantly correlated with age in intact males, but since there were only two intact males in homes with two or three dogs (the other five intact males were in homes with four or five dogs and did not provide samples 2 and 3), the correlation of the other testosterone variables with age could not be examined.

Independent samples t-tests of testosterone measures, testosterone reactivity in the conversation phase and the TPT, and mean testosterone did not show any significant differences by sex or gonadectomy status. A one-way ANOVA showed significant differences for testosterone baseline ($F(3,51)=10.936$, $p<0.001$) by sex/gonadectomy subgroup (spayed females, intact females, neutered males, and intact males), and post hoc comparisons using the Fisher LSD test showed that intact males have higher testosterone baseline than spayed females ($p<0.001$), intact females ($p<0.001$), and neutered males ($p<0.001$). ANOVAs comparing testosterone sample 2, sample 3, mean testosterone, and testosterone reactivity during the conversation and the TPT phases by sex/gonadectomy subgroups did not include intact males because of the limited sample size for this group ($n=2$) for these variables. Testosterone 2 showed a statistically significant difference

($F(2,32)=5.170$, $p=0.011$), being higher in intact females than in neutered males ($p=0.003$).

GLMMs with restricted maximum likelihood estimation were carried out to examine the relationship between testosterone measurements, the dependent variable, and fixed factors: sample type (baseline, sample 2, and sample 3), sex, gonadectomy status, and interaction between sex and gonadectomy status. Random intercept and random slopes for sample type, sex, and gonadectomy status were included in the model of best fit. There were statistically significant effects of sex ($p=0.011$), gonadectomy status ($p<0.001$), and the sex and gonadectomy status interaction ($p<0.001$), but not of sample type ($p=0.417$), on testosterone levels. Specifically, males had higher testosterone than females, and gonadectomy affected testosterone levels in differently in males and females: while intact males had higher testosterone than neutered males, intact females and spayed females did not show different testosterone levels (Fig. 2.11).

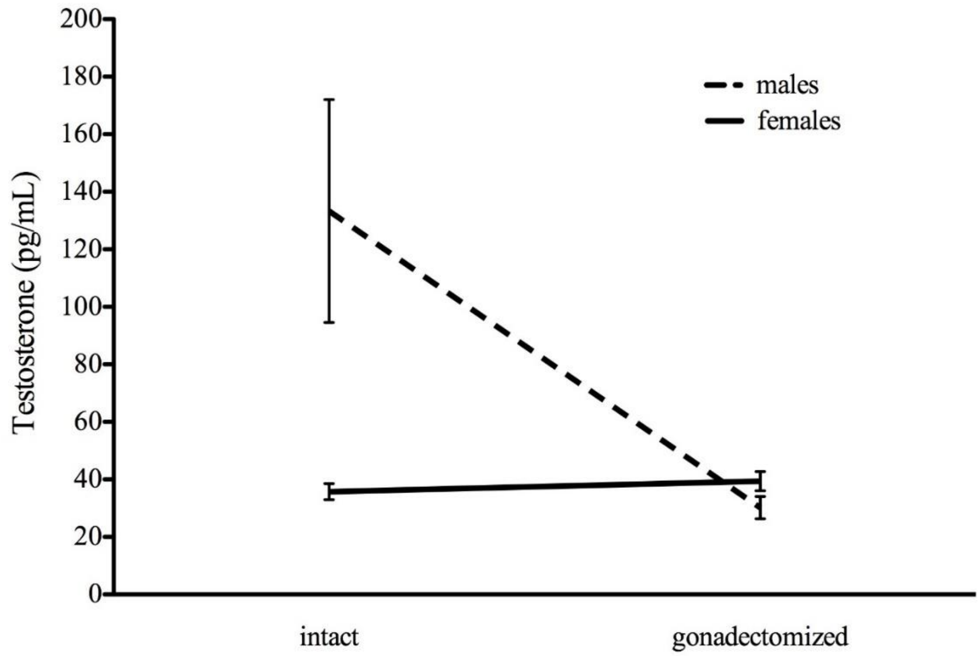

Fig. 2.11: Effect of gonadectomy status on testosterone (Mean ± SE) for males and females.

Fisher's Exact probability tests were carried out to examine the frequencies of testosterone increase and decrease during the conversation and the TPT phases. Testosterone increase and decrease by sex and gonadectomy group during the conversation phase was marginally different (p=0.051), and significantly different during the TPT (p=0.025). Follow up Fisher's Exact (2 x 2) probability tests comparing sex and gonadectomy groups showed that more intact females had an increase in testosterone levels during the conversation phase than neutered males (p=0.03), while more neutered males showed an increase in testosterone during the TPT phase compared to intact females (p=0.03).

2.3.4.5. Inter-relationships between cortisol and testosterone

Bivariate Pearson r correlations between cortisol variables and testosterone z-scores by sex/gonadectomy subgroups were performed. Z testosterone sample 2 was positively correlated with cortisol sample 2 ($r(36)=0.488$, $p=0.003$), cortisol sample 3 ($r(37)=0.500$, $p=0.002$), and mean cortisol ($r(36)=0.329$, $p=0.050$). Z testosterone sample 3 was positively correlated with cortisol sample 2 ($r(37)=0.332$, $p=0.044$) and cortisol sample 3 ($r(38)=0.373$, $p=0.021$). Other analyses resulted in correlations that were not statistically significant.

2.3.5. Personality

Means (± standard error) for Extraversion (range=19.44-100%), Motivation (range=36.67-100%), Training Focus (range=33.33-100%), Amicability (range=23.33-100%), and Neuroticism (range=16.67-95.83%) are presented in Fig. 2.12.

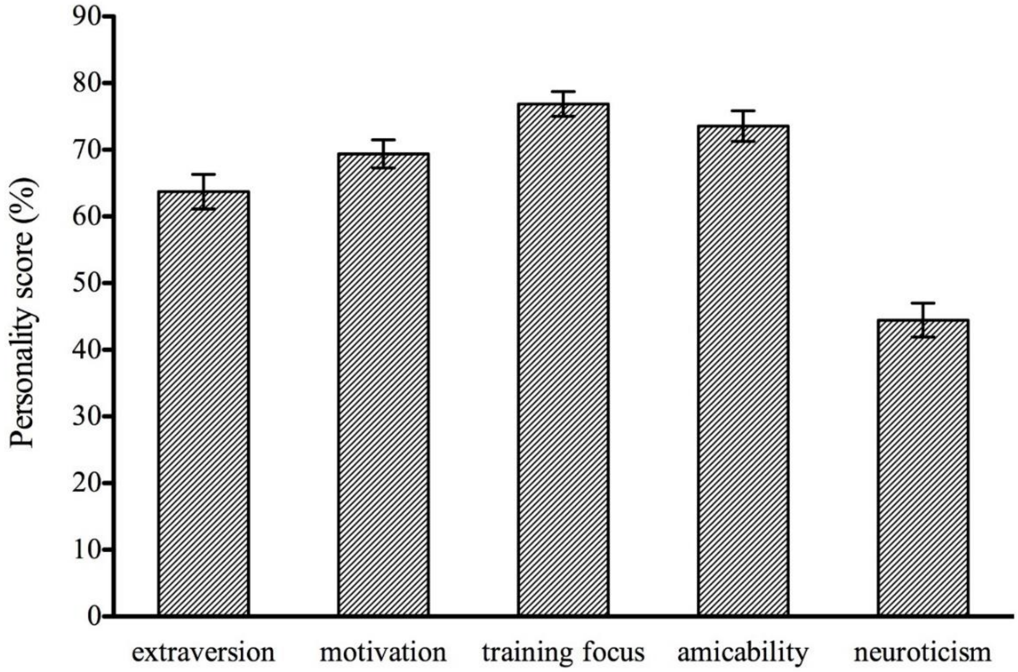

Fig. 2.12: Mean ± SE personality scores. Includes all subjects (n=66).

2.3.5.1. Personality and dog demographics

Extraversion, Motivation, Training Focus, Amicability, and Neuroticism were examined for their relationship to age. There were statistically significant negative correlations between age and both Extraversion ($r_s(66)=-0.565$, $p<0.001$) and Motivation ($r_s(66)=-0.276$, $p=0.025$). There were no statistically significant differences in each of the personality variables by sex, or by gonadectomy status (independent t-tests). One-way ANOVAs also showed no statistically significant differences in personality variables by sex/gonadectomy subgroups (spayed females, intact females, neutered males, and intact males) or by number of dogs in the house.

2.3.5.2. Personality and hormones

Hierarchical multiple regressions were run using personality dimension scores as dependent variables. Sex and gonadectomy subgroup and age were used as predictors in the first block, cortisol baseline and testosterone baseline converted to z-scores in the second block, and cortisol baseline multiplied by testosterone baseline z-score (cortisol and testosterone interaction term) in the third block. For Extraversion, all three blocks resulted in statistically significant models but adding variables in blocks 2 and 3 did not significantly increase model fit (first block: $F(2,51)=11.742$, $p<0.001$). Age was the only variable with a significant effect on Extraversion ($p<0.001$), i.e., older dogs were less extraverted. None of the other personality variables (Motivation, Training Focus, Amicability, and Neuroticism) produced significant models.

Bivariate Pearson r correlations between personality variables (Extraversion, Motivation, Training Focus, Amicability, and Neuroticism) and hormone variables including cortisol variables and testosterone z-scores were performed. Dogs that scored higher in Neuroticism showed increased cortisol reactivity during the TPT ($r_s(40)=0.328$, $p=0.039$). Testosterone reactivity z-score during the TPT was more positive in dogs that scored higher in Extraversion ($r_s(35)=0.466$, $p=0.005$) and Motivation ($r_s(35)=0.355$, $p=0.037$).

2.4 Discussion

The three methods for investigating the social status of cohabiting pet domestic dogs used in this study were a toy possession test, whether each dog performed behaviours reported in the literature to indicate dominance, and owner-reported dominance. Even though these methods purportedly measure the same characteristic, i.e., dominance in dogs, no significant relationships were found between TPT variables, owner-reported behaviours and owner-reported dominance. This failure of convergence indicates that the three methods may be measuring different sets of characteristics and are not necessarily either reliable or valid for determining social status differences among dogs. There were, however, some similarities across the significant relationships found between age and personality variables that were significantly related to the three different methods of evaluating social status in dogs, although the nature of these relationships is somewhat different for each, as discussed below.

2.4.1 Toy Possession Test

A competitive behavioural task, i.e., the TPT, was used by Lisberg and Snowdon (2009) to assess the social status of cohabiting dogs; dogs that won more trials were characterized as having a higher status. In the present study, dogs that had a higher proportion of points won in trials in which at least one dog participated were relatively younger than their housemates, had higher baseline testosterone levels, and were described by their owners as relatively more extraverted and motivated than their housemates. In addition, the interaction between cortisol and testosterone significantly predicted the proportion of toy possessions that dogs obtained in the TPT for trials in which at least one dog sought possession of the toy, i.e., dogs with low baseline cortisol and high baseline testosterone scored proportionately more points than dogs with all other cortisol and testosterone level combinations.

The lack of a similar relationship between proportion of points won in trials in which at least two dogs participated and the cortisol X testosterone interaction could be a consequence of fewer trials with the participation of two or more dogs leading to a loss of statistical power. Total participation in the TPT also does not show a relationship to testosterone or age, but low cortisol is associated with increased participation. So, participation in a (potential) competition and winning when there is more than one competitor seem to be affected by different characteristics. However, both total participation and TPT points won were negatively correlated with baseline cortisol levels. Thus, individual dogs with high baseline cortisol appear both less likely to participate in a competitive task and less likely to win if they do so.

To date, the dual-hormone hypothesis has not been examined in animals other than humans. Some studies have found evidence for an interaction of cortisol and testosterone on status-relevant behaviours in humans (Mehta & Prasad, 2015). For example, Mehta and Josephs (2010) found the same pattern of social status related to high testosterone levels when cortisol levels were low in a study investigating the effects of these hormones on observers' impressions of how dominantly people behaved in a leadership task. The dominance measurement was obtained from observers who rated videotaped interactions of study participants on a Likert scale for adjectives related to dominance.

A dog's MCPQ-R personality dimensions Extraversion and Motivation, relative to these scores for other housemates, were positively correlated with the proportion of TPT wins (i.e., successful toy possession) for trials in which at least one dog participated, as well as trials in which at least two dogs participated. Curiously, the list of adjectives used by observers in the Mehta and Josephs (2010) study to characterize human subjects' dominance included items which represented ideas very similar to those found in the MCPQ-R: "engaged", "bored", and "energetic" were similar to the adjectives in the Extraversion dimension of the MCPQ-R, while "leader-like", "confident", "assertive", and "directive" matched adjectives for the Motivation dimension. Adjectives such as "shy/timid", "anxious" and "hesitant" were similar to the adjectives in the MCPQ-R Neuroticism dimension. This overlap suggests that the dominance measurement used by Mehta and Josephs (2010) is closely related to the MCPQ-R assessment of Extraversion, Motivation and Neuroticism personality dimensions in dogs.

Results of this study revealed a relationship between these personality dimensions, hormones and toy possession test scores. Testosterone reactivity during the TPT was higher in more extraverted and more motivated dogs. Additionally, Neuroticism, although unrelated to TPT scores, was higher in dogs that responded to the TPT with increased HPA axis activation (cortisol reactivity). It is possible that cortisol and testosterone have joint effects on the appearance of a certain behavioural profile characterized by higher Extraversion, higher Motivation and lower Neuroticism, and that this behavioural profile is commonly associated with increased social or dominance status in a group of conspecifics.

Mehta and Josephs (2010) also found that observers gave human males higher dominance scores than females, but the relationship between sex and TPT high score in this study showed that female dogs got a high score more often than males. There could be a few explanations for this. First, there are likely species differences between humans and dogs. Second, TPT variables were obtained directly from results of interactions, while Mehta and Josephs (2010) used ratings by observers watching interactions. It is possible that human observers are biased towards assigning increased dominance to human males (one of the adjectives indicating higher dominance in the study by Mehta & Josephs, 2010 was “masculine”), and coding toy possession test results is less prone to this type of bias. Third, the sample of dogs in this study was composed of a high proportion of females and few intact males, and this skew could influence the relationship between TPT variables and sex/gonadectomy status, i.e., if more intact males had been included, the overall outcome related to sex might be different.

As described earlier, dogs with higher baseline cortisol and higher overall average cortisol values participated in fewer competitive interactions with household conspecifics (i.e., had lower total TPT participation scores). Unlike the outcomes of TPT trials described above, total TPT participation was not predicted by age, testosterone, the interaction of cortisol and testosterone, or any MCPQ-R personality dimension. This indicates that increased stress or arousal can make dogs less willing to engage in a competitive task, and is consistent with the dual hormone hypothesis: if increased cortisol blocks or inverts the influence of testosterone levels on social status, engaging in competition with high cortisol levels might decrease chances of success, and individuals might be less prone to engage in competition in this situation as a result.

The TPT was not related to owner-reported behaviours that have been used to assess dominance in dogs in previous studies, with one exception: dogs whose owners reported them to most often eat another dog’s food had higher testosterone reactivity during the TPT. TPT outcomes were also not related to the owner’s perception of which dog in their household was dominant.

Therefore, the outcome of the competitive task seems to reflect a dog’s age, the MCPQ-R personality dimensions Extraversion and Motivation, baseline testosterone levels and the interaction between baseline cortisol and testosterone. The question remains as to whether it is possible to generalize successful TPT outcomes as a characteristic of more dominant individuals. The TPT examines results of competitions leading to preferential access to toys only. To more fairly characterize an individual as more dominant in the household, the relationship between the interaction of cortisol and testosterone levels, age, sex, personality and preferential access to other resources such as food, space and attention from owner should be examined.

2.4.2 Owner-reported behaviours

Dogs’ behavioural characteristics have been related to social status (as described earlier, e.g., Bauer & Smuts, 2007; Bonanni et al., 2010; Cafazzo et al., 2010; Pongrácz et al., 2008). In this study, a behavioural questionnaire was created to examine behaviours potentially associated with dominance relationships in domestic dogs.

The total number of different affiliative behaviours that the owner reported the dog to engage in was positively related to baseline cortisol and baseline testosterone, as well as the personality dimension Training Focus. The fact that dogs exhibiting more affiliative behaviours scored higher on the MCPQ-R dimension Training Focus may suggest that owners, who probably encourage affiliative behaviours between their dogs, are more successful in reinforcing those behaviours in dogs that are more susceptible to training.

The relationship between cortisol and affiliative behaviours has been examined in studies about the ‘tend-and-befriend’ response to stress, in which increased stress (i.e., higher cortisol levels) leads to increased affiliation with one’s social group (Taylor et al. 2000). The tend-and-befriend response was originally found in women (Taylor et al. 2000), but Berger, Heinrichs, von Dawans, Way, and Chen (2016) have since found that cortisol reactivity during a stressful situation increased affiliation in men, as well. Furthermore, Steinbeis, Engert, Linz, and Singer (2015) related higher baseline cortisol in men with increased trust.

In agreement with the tend-and-befriend response, dogs with higher cortisol levels didn’t tend to engage in the competition for the toy. Increased affiliation with one’s social group in stressful situations could be an adaptive response to increase group cohesion and maximize survival in social species like humans (Berger et al., 2016; Geary & Flinn, 2002; Steinbeis et al., 2015; Taylor et al., 2000) and, as these data suggest, domestic dogs.

Owner-reported agonistic behaviours were positively related to mean testosterone and cortisol reactivity during the conversation phase of the home visit, and were negatively related to dog age. Dogs who mounted a bigger stress response to the part of the visit in which strangers came into their home appear to be more likely to generally display agonistic behaviours towards cohabiting dogs than those with a smaller stress response. A relationship between cortisol and the development of agonistic behaviour has been shown by Wommack and Delville (2007) in golden hamsters; hamsters receiving exogenous cortisol during early puberty transition from play fighting to adult aggression earlier during puberty. While maturation of agonistic behaviours is not the focus of this study, their results support the idea that there is a relationship between cortisol levels and agonism. The relationship found in this study indicates that dogs that have a higher stress response to strangers coming into their homes exhibit more agonistic behaviours towards their cohabiting dogs.

Additionally, older dogs had lower total agonistic behaviour scores, indicating that aggressive behaviours decline with age. This decrease might be a consequence of decreased motivation for agonistic interactions as a dog ages, cumulative effects over time of owners discouraging aggressive behaviours, or a combination of both.

A higher agonistic behaviour total also co-occurred with more resource-guarding behaviours, indicating that the tendency to monopolize resources and aggression are linked. It is possible that agonistic behaviours are a means for maintaining priority in access to resources. Additionally, dogs that showed more resource-guarding behaviours were rated as less amicable, less neurotic, and more motivated on the MCPQ-R.

Resource-guarding behaviours and other behaviours reported by owners that have been presumed to indicate dominance were mostly related to one or more of the personality dimensions of Extraversion, Motivation, Amicability, and Neuroticism, and/or age. Since a dog's Amicability score relates to how a dog is perceived to tolerate other individuals, it is not surprising to find that dogs that guard their resources from others are perceived as less amicable. The Neuroticism dimension relates to how cautiously a dog is perceived to behave, so it would be expected that more cautious dogs show less resource-guarding behaviours, avoiding confrontations with their cohabiting partners. A higher score in the Motivation dimension, which reflects a dog's persistence in the face of distractions, is also understandably related to resource-guarding behaviours, since more motivated individuals will probably be more willing to guard resources even if other dogs or humans resist their attempts to do so.

Age and personality were often related to individual behaviours used to assess social status, and are probably underlying factors affecting the social organization of cohabiting pet domestic dogs. However, the direction of observed relationships is not consistent. Some analyses showed a direct relationship between age and increased social status (muzzle licking and barking longest), while other analyses (muzzle biting and paw laying, as well as TPT scores) showed an inverse relationship between age an social status. The same tendency can be found in the relationships of behaviours with Extraversion: there was an inverse relationship between Extraversion and presence of muzzle licking, but a positive relationship with paw laying.

Additionally, owner reports that one dog eats a cohabiting dog’s food were related to higher testosterone reactivity during the social challenge of the toy possession test. Both the TPT and eating another dog’s food can be considered competitive tasks, so it is not surprising that testosterone may be related to both.

With the exception of this relationship between eating another dog’s food, testosterone and the TPT, owner-reported behaviours did not show any significant relationships with TPT variables or owner-reported dominance.

2.4.3 Owner-reported dominance

Multi-dog owners obviously have their own beliefs regarding social status relationships that exist between their cohabiting dogs. Not all owners believed that their dogs might have different social statuses. In 13% of the participating homes, owners did not report that any of their dogs was more dominant than the others. In contrast, higher status dogs, as reported by the other 87% of owners, were rated as more motivated and more focused on training, as well as less amicable. This result suggests that owners who believe that there is a dominance relationship between their dogs may assign a different social status to each of them based on these three personality characteristics of Motivation, Training Focus and Amicability.

2.4.4 Age relationships

Many studies relate increased age to increased social status in domestic dogs (Bauer & Smuts, 2007; Cafazzo et al., 2010; Trisko & Smuts, 2015). Van der Borg et al. (2015), however, found no relationship between age and social status, but their sample included a very low proportion of adult dogs. In this study, higher TPT scores tended to be obtained by younger dogs, while relationships between age and owner-reported behaviours varied in direction. The relationships between muzzle licking, barking longest and age support the idea that increased age leads to higher social status, while the relationships of age with muzzle biting and paw laying indicate the opposite. Lower age might lead to increased competitive ability and the appearance of some of the behaviours commonly associated with dominance relationships, but possibly not to increased social status.

2.4.5 Other personality relationships

The personality dimensions Extraversion and Motivation were correlated with age in this study sample, with younger dogs being assessed as more extraverted and more motivated. However, in a hierarchical regression adding sex, gonadectomy and hormone information, only the relationship between Extraversion and age was supported beyond the influence of the other factors. It is not surprising to find that Extraversion and Motivation, being related to age, also commonly appear as related to other variables related to age, such as TPT scores and specific owner-reported behaviours.

2.4.6 Other hormone relationships

Cortisol

Neither cortisol measurements nor cortisol reactivity in this study were related to age, sex, gonadectomy status, or number of cohabiting dogs in a household. Cortisol values did not differ consistently across the conversation phase or the TPT. Nonetheless, cortisol sample 2 and cortisol sample 3 were strongly positively correlated, which indicates that cortisol measurements minutes after the arrival of researchers and following the competitive toy test change similarly in different subjects.

The lack of a linear relationship between baseline cortisol samples and the other two cortisol samples, along with the monotonic relationship found between all cortisol samples, may be due to the high variance in baseline measurements compared to the other two. That is, temporal and contextual differences may explain the increased variability in baseline cortisol measurements, because the amount of time and the events taking place during this time immediately before this sample was taken were not specified by the visit protocol. So, dogs might have been engaged in different activities and have been in different emotional and motivational states as the baseline sample was taken. The other two samples were taken after the visit had started and under more controlled conditions, so it would be expected that they would be less variable.

Cortisol reactivity during the conversation phase and the TPT phase was positively correlated, which supports the idea that cortisol changes in similar ways in different situations, even when situations vary in the type of challenge they present to a dog. Recall that the researchers were strangers introduced to the dogs' environment during both conversation and TPT phases, and never interacted with the dogs directly during either phase, only with owners. During the TPT, however, dogs were additionally presented with a challenge, or novel situation. So, it is possible that most dogs perceived the conversation phase as not only a less threatening context, but also as a familiar context in which their owners interact with non-threatening strangers coming into their home.

Cortisol levels during the conversation phase decreased for over half (55%) of dogs, indicating a possible decrease in arousal and stress levels during that part of the visit. Yong and Ruffman (2014) found that dogs exposed to human baby babbling did not show a statistically significant change in cortisol levels before and after the intervention. It is possible that human conversation, regardless of whether it is babbling or conversation among adult humans, is a comfortable context for most dogs that leads to no change in or decreasing stress and arousal and, consequently, stable or decreasing cortisol levels.

The TPT, however, was likely a social challenge for the cohabiting dogs that could be perceived by dogs as relatively more stressful or arousing than the conversation phase. Indeed, cortisol increased for 62.5% of dogs following this phase, consistent with the evidence that unfamiliar situations can be related to increasing cortisol in dogs (Ottenheimer Carrier et al., 2013). Hence the marginally significant, lower cortisol reactivity in the conversation phase as compared to the TPT could reflect the different challenges these two parts of the visit posed to dogs. But since there was no statistically significant difference in the frequencies of increase and decrease in cortisol levels between the two parts of the visit, differential effects of conversation phase and TPT on cortisol reactivity are not fully supported.

Testosterone

Analyses involving the relationships of testosterone variables to each other, to demographic information, and to toy possession test results were performed by sex and gonadectomy subgroup. Not surprisingly, most of the significant relationships were found in the spayed females group because this was the group with highest number of participants. The low sample sizes of intact female, neutered male, and intact male groups made it more difficult to detect effects due to low power. Because of that, relationships between testosterone and other types of variables (cortisol, personality, owner impressions and behavioural questions) were examined using z-scores, so all cases could be grouped for analyses.

Neither testosterone levels nor reactivity were related to age or sample type. Sex, gonadectomy status and their interaction influenced testosterone levels in that intact males had higher testosterone than neutered males and both intact and spayed females. This result can be easily explained, since intact males are the only group with testes that produce large quantities of the hormone.

In spayed females, testosterone baseline and the third sample levels, taken minutes after the end of the toy possession test, were positively correlated, indicating that testosterone levels at both points changed similarly among subjects in this group. In the same group, testosterone reactivity during the conversation phase and the TPT were negatively correlated, with a more positive reactivity during the conversation phase than during the TPT. This trend was not observed in any of the other groups, possibly because the lower sample size led to reduced power for these analyses. Nonetheless, testosterone measures and reactivity profiles behaved differently for each sex and gonadectomy subgroup, suggesting that sex and gonadectomy status might affect the function of the HPG axis, and possibly also its interaction with the HPA axis.

More extraverted and more motivated dogs had higher testosterone reactivity during the competitive toy possession test. These results, along with the relationships found between the same personality variables and testosterone on toy possession test results, indicate that there might be a relationship between testosterone levels and testosterone reactivity, higher toy possession test score, Extraversion and Motivation in domestic dogs.

Cortisol and testosterone relationships

In intact females, testosterone increased significantly more during the conversation phase than during the TPT. Since the HPA and HPG axes of the endocrine system interact with each other in that increased HPA activity has a negative feedback onto the secretions of sex steroids (Gardner & Shoback, 2011), more negative testosterone reactivity in intact females during the TPT could be a consequence of increased HPA activity in the same phase, as demonstrated by the marginally higher cortisol reactivity during that phase. Thus, the new social opportunities created by the arrival of non-threatening strangers could lead to an increase in testosterone secretions during the conversation phase, while the stress and arousal associated with the competition and novelty of the TPT could lead to a decrease in the same hormone.

It is interesting to note that while the interaction between HPA and HPG axes can explain the pattern of testosterone reactivity in intact females, the same pattern does not seem to apply to the other sex and gonadectomy groups. This could be a result of the low sample size in these groups leading to low power of analyses. But although neither testosterone reactivity variables differed by sex and gonadectomy group, the distribution of testosterone increase and decrease by sex and gonadectomy groups was marginally different during the conversation phase and significantly different in the TPT. More intact females had a testosterone increase from baseline during the conversation phase than neutered males, while more neutered males showed an increase in testosterone from baseline during the TPT than females. It is possible that sex differences in the HPA and HPG axes interactions (Goel et al., 2014; Juster et al., 2016) and the disturbance of the normal pattern of interaction for each sex caused by removal of the gonads are related to this difference in testosterone reactivity patterns of different sex and gonadectomy groups.

Although dogs of different sexes and gonadectomy statuses seem to vary in their pattern of interaction between HPA and HPG axes, analyses of cortisol and testosterone relationships performed by grouping all subjects and using testosterone z-score showed support for a positive correlation between cortisol and testosterone levels obtained after the arrival of researchers and after the competitive toy possession test.

In conclusion, while it is probable that general trends in the pattern of interaction between HPA and HPG axes in domestic dogs exist, further examination of the effects of sex, gonadectomy status, and individual differences on this interaction is necessary before its influence on behavioural characteristics, personality and social status can be fully explained.

2.4.7 Study limitations

Recruitment and participants

Participation in this study required not only a considerable time commitment from owners, but also that researchers be allowed into their homes for a filmed visit. Despite this intrusiveness, which made recruitment difficult, 23 multi-dog home-owners completed data collection. Most homes had two cohabiting dogs, with number of households decreasing as the number of dogs in the household increased. There was a large proportion of female primary caregivers, female dogs, and gonadectomised dogs. Intact males represented a small proportion of the sample. Most dogs had been acquired from breeders, followed by rescue organizations and other homes. It is not possible to evaluate whether sampling for this study was skewed because information about the composition of the population of multi-dog owners and their dogs is not available. A larger, more balanced sample including similar numbers of male, female, gonadectomized, and intact dogs would help confirm the relationships found in this study, and clarify the interaction between HPA and HPG axes in these different groups.

Behavioural questions and owner impressions

In this study, measurements related to some behavioural characteristics of the dogs and owner-reported dominance were obtained from owners. These owner reports on behaviour and social organization might be subject to distortions caused by owners' previous experiences, opinions, and expectations. Many of these questions had been used in prior studies, but the reliability and validity of these questions had not been previously assessed and were taken at face value for this study. It is crucial for future work to more carefully determine the validity of any of these behaviours for predicting dominance in domestic dog relationships (e.g., through factor analysis of behavioural questions).

Owner-reported dominance information was obtained following the toy possession test, and, therefore, could have been influenced by TPT outcomes. However, because owner-reported dominance and TPT results were not related, knowledge of the TPT outcomes probably did not play a significant role in the results.

3. General conclusions

Domestic dogs are social animals that share recent common ancestors with grey wolves. In contrast to wolves, dogs live in close association with humans, often in human-directed environments, and have evolved under different constraints for many thousands of years. Thus, any assumption that the two species should exhibit the same pattern of social organization is likely incorrect. In fact, the different contexts in which domestic dogs are found (e.g., human households, dog day-care environments, shelters, research facilities and feral groups) all vary in terms of degree of human involvement, temporal patterns of conspecific association, and availability of resources. These different contexts undoubtedly influence the social behaviours expressed by dogs, the meaning and consequences of those behaviours, and the social structure, if any, which emerges among dog groups. Indeed, wolves have been shown to organize themselves in different social structures depending on their living contexts, and the same is likely true for domestic dogs.

Given that the question of whether a "top dog" exists in domestic dog groups is one of the more controversial topics in recent discourse among animal behaviour researchers (e.g., Bradshaw et al., 2009, Schilder, et al., 2014; see also Bekoff, 2016), surprisingly few empirical studies have been carried out to date. In fact, the current study is the first to examine whether dominance in dogs living in permanent social groups in multi-dog households exists. If dominance is detectable among dogs, it can be argued that it should be the most detectable within permanent groups (vs. temporary social groups, such as those found in dog daycares or dog parks). As well, in Westernized countries, most dogs live as companion animals in human-directed households. While studies on the social structure of feral dogs and group-housed dogs living in kennel or shelter environments are critical for both theoretical and welfare reasons, the context of the current study is arguably more relevant for many dogs. Thus, this study evaluated dominance in cohabiting pet domestic dogs using three different methods: first, the toy possession test, a competitive behavioural task in which dogs that win more trials have been characterized as more dominant; second, an owner-completed behavioural questionnaire comprised of behaviours commonly associated with dominance in domestic dogs in prior studies; third, owner-reported dominance. The relationships among the outcomes of each method for evaluating dominance, and their additional relationships with demographic factors (e.g., age, sex), personality dimensions, and hormones were examined to elucidate the underpinning and applicability of the concept of dominance to cohabiting dogs, and raise further questions for future research that, once addressed, might clarify how to more effectively assess social behaviours and organization in domestic dogs.

3.1. Does the TPT measure social dominance in dogs?

A dog's competitive performance on the TPT was related to higher testosterone levels, the interaction between testosterone and cortisol (low cortisol and high testosterone positively impacted TPT score), relative age of the dog, and the personality dimensions of Extraversion and Motivation. Importantly, the low cortisol/high testosterone interaction pattern related to TPT performance is similar to the relationship found with observer-rated dominance in humans (Mehta & Josephs, 2010). In most species, the outcomes of competitive interactions (e.g., wins) can be used by individuals to increase or to maintain high social status (e.g., Cafazzo et al., 2010). This is the first study to report a relationship between a cortisol X testosterone interaction and competitive task outcomes in dogs. A major difference between the TPT and the dominance measurement used by Mehta and Josephs (2010) is that TPT results, having been obtained from competition scores, are quantitative measures less prone to observer bias. However, whether a high TPT score is indicative of higher social status in dogs is still currently unknown.

Investigation of the associations between hormone levels and interactions, competitiveness and personality provides important information about the physiology underlying social status behaviours. With this information, breeders may be able to select dogs with specific hormone profiles that lead to desirable behavioural characteristics for reproduction. Furthermore, comparing the differences between these associations in dogs and wolves can provide clues about how dogs and wolves evolved into separate groups from a common ancestor.

Toy possession test results appear to relate to how active and motivated a dog is, characteristics that are inversely related to age in the current study population. Younger dogs obtained more TPT points and were both more extraverted and motivated than older dogs. However, these characteristics are not necessarily related to dominance per se. While an individual's resource holding potential, i.e., its physical ability to win a dispute (see Parker, 1974, for definition and Bradshaw, 2009, for a discussion on application to domestic dog social structure) might be higher compared to its housemates, different levels of motivation towards the resource being disputed will influence competition results. So, it is possible that competition results would be different if other resources, towards which dogs have increased motivation, were being disputed.

Although adult dogs play, increased motivation for play of all types, including object/toy play, is typical of younger dogs (Bradshaw, Pullen, & Rooney, 2015). However, the same might not be true for motivation for winning disputes involving other resources such as food, water, attention, or shelter. Whether a dog competes for a certain resource may depend on factors unrelated to any dominance relationship among dogs including: 1) how valuable that particular resource is to the dog, which will likely vary between individuals, 2) the associatively-learned history of interactions between dogs during competitions and competitive outcomes for a similar resource, and 3) possible differential reinforcement of the dogs by the owner for exhibiting competitive behaviours (discussed in Bradshaw et al., 2016; Bradshaw et al., 2009).

Thus, in the current study, both age and a hormone profile characterized by low cortisol and high testosterone appear to be related to competitive ability in domestic dogs. However, this competitive ability might not translate directly into increased dominance in the "winner". Acquiring and maintaining preferential access to resources in a group of cohabiting pet domestic dogs, if possible, might be better achieved through other strategies, such as keeping a close relationship with the owner, and responding appropriately to his or her cues. Specifically in the context of cohabiting pet domestic dogs, Training Focus might play an important role in the establishment of status relationships, as owners reported dogs that have higher Training Focus scores than other dogs in the household as being more dominant. However, what owners mean by “more dominant” is not entirely clear; certainly, as discussed below, it appears that those owners who believe there is a social structure among their dogs may be basing their assessments on relative personality differences among their dogs.

While a higher TPT score was associated with younger dogs in this study, other work on domestic dogs examining the relationship between age and social status as measured by formal dominance and submission displays have found that age is positively correlated with social status (Bauer & Smuts, 2007; Cafazzo et al., 2010; Trisko & Smuts, 2015). Not surprisingly, many of the behaviours investigated through the behavioural questionnaire were also related to age, but the direction of the relationship varied across behaviours (see below). Younger dogs may have increased competitive ability and higher Extraversion scores, leading to higher TPT scores and the appearance of some of the behaviours that have been commonly associated with dominance. Another possible explanation for the relationship between age and TPT scores in this study involves the older dogs “permitting” younger housemates access to the toy during the TPT tests. Indeed, it is known that older group members in many species allow juveniles to have feeding priority, and this resource access is unrelated to social rank (discussed in Cafazzo, et al., 2010). Whether TPT scores and owner-reported behaviours are valid indicators of differences in social status, and/or measure other characteristics, such as inherent age, behavioural and physiological differences, remains an open question.

3.2. Dominance-associated behaviours in dogs

Agonistic behaviours along with formal dominance and submission displays have been used to identify social hierarchies in free ranging domestic dogs (Cafazzo et al., 2010) and in pet domestic dogs interacting at a dog day care facility (Trisko & Smuts, 2015). At the day care facility, such behaviours indicated a one-way relationship in less than one-third of dyads, with submissive displays being the most consistent (Trisko & Smuts, 2015). In contrast, more than 70% of the dyads among the feral dogs studied by Cafazzo et al. (2010) showed unidirectional submission behaviours. Such data suggest that among the pet dogs, behaviours associated with social status may not be widely used, raising the question as to how important dominance is among pet dogs, particularly for those not part of a permanent social group and with few resources to contest. While it is possible that some dominance-associated behaviours might be related to increased social status in feral dogs, and that some behaviours exhibited within dyads reveal unequal social status among some pet dogs, such behaviours appear to not be used widely by all pet dogs (both in the current study and in Trisko & Smuts, 2015), raising the question as to why this is the case.

One reason that social behaviours might be poor indicators of status in cohabiting pet dog groups involves owner behaviour. As noted above, it is likely that particular behaviours are actively discouraged by owners in pet dogs, and, thus, do not appear reliably in their behavioural repertoires. This does not mean, however, that behaviour cannot be used at all to predict social status differences among pet dogs. It is still possible that status relationships between cohabiting pet domestic dogs are related to behaviours that are less conspicuous to owners, such as changes in body posture, ear and tail position (discussed by Schilder et al., 2014; van der Borg et al., 2015). Being more subtle these behaviours might be less likely to be observed and/or interfered with by the owner. Thus, based on the current work, it is still not clear that any dominance-associated behaviours measured are indeed related to social status differences amongst dogs that live together in a human household. In fact, any assumption that such behaviours function to maintain dominance in dogs is premature, since such behaviours could be related to other factors not measured in the Cafazzo et al. (2010) and Trisko & Smuts (2015) studies, such as personality and hormonal differences.

3.3. The role of canine personality in dog-dog relationships

The MCPQ-R personality dimensions Extraversion and Motivation, which were higher in younger dogs, were also related to some of the behaviours commonly associated with social status. Dogs that performed muzzle licking and laying paw were more extraverted, and dogs that were reported to bite muzzle were more motivated. Additionally, lower Amicability was a characteristic of dogs that barked first and longest.

Owner-reported dominance was also related to dog personality characteristics. Relatively more motivated dogs were perceived by owners as having a higher status than other cohabiting dogs. Motivation might be an important trait in determining whether dogs form dominance relationships with other dogs, and Motivation was closely related to Extraversion and age in this study sample. Other personality dimensions were also related to owner-reported social status: dogs relatively higher in Training Focus and relatively lower in Amicability were also perceived as having increased social status. It is important to note, however, that 13% of the owners believed that none of their dogs had a higher social status than the others.

Personality dimensions were also related to the different methods for assessing social status of cohabiting pet domestic dogs, but not consistently. Bradshaw et al. (2016) suggest that asymmetries in personality characteristics can lead to the formation of dominance relationships in domestic dogs. It is possible that individual personality characteristics of dogs will contribute to which specific behaviours are exhibited in the group, and that these may be related to social status. Individuals' personalities have been shown to influence the pattern of social organization in another species: in a group of wild baboons in which more aggressive males were killed by a tuberculosis outbreak, less aggressive males became predominant in the group and a more affiliative culture was passed on from generation to generation (Sapolsky & Share, 2004).

3.4. Summary and future directions

The current study indicates that there is little agreement among results of toy possession tests, owner-reported dog behavioural characteristics, and owner-reported dominance in cohabiting pet dogs. These different methods that have previously been used to assess a dog's social status appear to measure different behavioural and/or physiological profiles, and it is not clear which one, if any, accurately assesses dominance relationships in this context, should there be actual dominance relationships formed among household dogs. While using behavioural displays can lead to the detection of dominance relationships among the majority of dyads in feral domestic dogs, the same does not seem to apply to cohabiting pet domestic dogs.

The human influence in multi-dog households might be important in decreasing the likelihood of dominance relationships being established, since access to resources is likely mainly controlled by the owner, and resources are unlikely to be scarce. Additionally, dominance relationships between cohabiting pet domestic dogs might be better detected by other behaviours not assessed in this study, such as body posture, ear and tail position.

This study shows that assessing social status in cohabiting pet domestic dogs is not an easy task, and that some methods that have been previously used for this purpose likely do not assess the same behavioural characteristics. In recent years, ideas about wolf social organization had to be revisited once it was recognized that wolves in different contexts organize themselves differently. The same might have to be done for our understanding of the social organization of domestic dogs. Cohabiting pet domestic dog social organization is probably different from the organization found in other types of dog groups, and assumptions derived from wolf and feral dog studies must be evaluated carefully before being accepted as valid for groups of domestic dogs in different contexts.

Dominance relationships might not be the rule for cohabiting domestic dogs since they do not have many opportunities to contest resources as a consequence of increased human involvement. Cohabiting domestic dogs might vary in their motivation towards different types of resources and their behaviours associated with social status might be more subtle than the same behaviours in feral dogs due to the increased human involvement. Owners possibly discourage behaviours perceived as unfair or aggressive, thus affecting the social status behaviours of dogs under their care. Cortisol and testosterone levels, personality characteristics and age seem to be related to cohabiting domestic dog social organization, but further work is necessary to confirm the relationships found in this study.

Future work investigating dominance relationships and social status in cohabiting pet domestic dogs should aim to: 1) perform a factor analysis of owner-reported behaviours to examine which of them may be more predictive or reliable in determining social status, leading to the creation of a more accurate questionnaire of owner-reported behaviours, 2) test specific hypotheses about the relationships between social behaviours, hormonal, demographic, and personality traits, in order to reduce the likelihood of Type II error from which these more exploratory analyses might suffer; 3) investigate whether current results concerning the influence of a cortisol X testosterone interaction on competitive outcomes among social groups of cohabiting pet dogs can be reproduced, specifically, whether the interaction between cortisol and testosterone levels predicts specific behaviours (especially other competitive task outcomes, agonistic and resource guarding behaviours) in a larger sample with more power; and 4) examine how subtleties of domestic dog communication and other behavioural indicators, such as tail base position, ear and body posture, are related to the characteristics examined in the present work through coding of postural changes during dog interactions.

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APPENDICES

Appendix I: Canine Research Unit recruitment poster for this project.

Appendix II: Canine Research Unit ad on a free classified advertisement website

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Over 5,193,607 Free Local Classifieds

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Browse Categories | Search | in | All Ads | Go | My Loc

Kijiji Newfoundland > St. John's > pets > other > Ad ID 364817543 | Back to

Wanted: Volunteers to participate in dog research

Watch | Share |

Date Listed	11-Sep-12
Price	**Free**
Address	St John's, NL, Canada View map

Canine Research Unit (CRU)

As animal behaviour and cognitive researchers in the Department of Psychology at Memorial University, we believe that there is much to learn about dogs since until fairly recently, they were rarely the subjects of scientific studies.

We are especially interested in the behaviour and cognition of all types of dogs (domestic dogs and wild canids). Our main work is focused on dog social behaviour, specifically how do dogs communicate and interact with each other, and the factors that affect these social interactions.

We are also interested in how factors like a dog's temperament, or personality, influence social behaviours, and whether measuring stress-related hormones, such as cortisol, can give us insight into how these factors interact with each other.While some of our research involves testing dogs in their homes and yards, much of what we do happens in our local dog parks.

If you would like to volunteer your time and participate in our research please contact us.

Also, feel free to check out our website, and to get in touch with us at any time if you have any questions or comments!

http://dogsbody.psych.mun.ca/cru/Canine_Behaviour_Lab/CRU_Home.html

Appendix III: Breed and height of participating dogs

Breed	**Height at withers (cm; mean±SD for n>1)**	**Sample size**
Miniature Dachshund	21.9±1.8	3
Chihuahua	22.9±3.6	4
Scottish Terrier	25.4±0.0	3
Papillon	25.4	1
West Highland White Terrier	26.2±1.4	3
Miniature Schnauzer	27.9±2.6	3
Cocker Spaniel	30.5	1
Standard Wire-haired Dachshund	31.5±1.4	5
Shetland Sheepdog	37.2±3.9	3
Beagle	40.7±3.6	2
Boston Terrier	41.9±1.8	2
Welsh Springer Spaniel	44.5±1.8	2
Dalmatian	48.3±4.6	4
Australian Shepherd	48.9±1.7	3
Mixed-breed	49.8±11.6	15
Eurasier	55.9	1
Labrador Retriever	57.6±1.4	3
Portuguese Water Dog	61	1
Bernese Mountain Dog	63.5	1
Doberman Pinscher	65.7±1.6	4
Leonberger	73.7	1
Great Dane	83.8	1

Appendix IV: Instruction sheet for homes with 2-3 dogs

Dear Participant,

Thank you for your interest in participating in the study "*Do cortisone and testosterone levels covary with social role in domestic dogs?*". This document contains the instructions you need to follow in order for the data collection to happen as smoothly as possible. Please follow the steps as outlined below and feel free to contact me *(*contact information removed*)* if you have any questions during the process.

Cheers,

Mariana Kroll

DATA COLLECTION INSTRUCTIONS

Content of the collection kit:

This data collection kit contains:

This data collection instruction sheet;

1 consent form;

1 questionnaire for each of your dogs;

1 standard operating procedure (SOP) sheet for saliva collection;

1 saliva sampling record sheet for each of your dogs;

5 (4+1 extra) saliva collection swabs for each of your dogs;

3 saliva collection vials for each of your dogs;

Data collection steps

Please follow these steps in the order in which they appear in these instructions.

1. **Consent form**
   Please read the consent form and sign it if you agree to participate in the study. I will sign it in the beginning of the visit.
2. **Dog information, behaviour and personality questions**
   You will need: 1 questionnaire for each of your dogs.
   Preparation: none
   Procedure: Fill out one questionnaire for each of your dogs. The completed questionnaires will be collected with the saliva samples after the visit is over.
3. **Practice saliva collection**

You will need: SOP sheet, 1 saliva sampling record sheet for each of your dogs, 1 saliva collection swab for each of your dogs, a pen or pencil, 1 treat for each of your dogs (optional).
Preparation: read the saliva collection instructions on the SOP sheet.
Procedure: Perform steps 1 through 4 of the SOP sheet on each of your dogs. Complete the necessary fields on the saliva sampling record sheet for each of your dogs.

4. **Day and time of the visit**

   Please send me an email *(*contact information removed*)* or call me *(*contact information removed*)* to arrange the visit. It needs to take place between 1 and 9 pm, preferably on a weekday when the members of the household usually follow a routine. Write down the arranged day and time below.

   Day: **Time:**

5. **Preparation for the visit**

   Please do not exercise or feed your dog for 3 hours prior to the visit.

   Choose an area of the house (backyard, living-room, kitchen, etc…) where the toy-possession test can take place. The whole visit will take place in that location. It is important to avoid distractions and interruptions during the visit as much as possible so that dogs' hormone levels are not affected by events other than the data collection procedure.

6. **Pre-visit saliva sample**

   You will need: SOP sheet, 1 saliva sampling record sheet for each of your dogs, 1 saliva collection swab for each of your dogs, 1 collection vial for each of your dogs, a pen or pencil, 1 treat for each of your dogs (optional).
   Preparation: read the saliva collection instructions on the SOP sheet.
   Procedure: Up to 30 minutes before the arranged visit time, follow steps 1 through 5 of the SOP sheet on each of your dogs and fill in the necessary fields on each of your dog's saliva sampling record sheet. Place the samples in a refrigerated area.

7. **Visit**

   The visit will consist of the steps outlined below. The researchers will let you know when to perform each of them:
   a. Arrival
   b. Conversation about consent form, questionnaire, saliva sampling and environment
   c. Second saliva sample

d. Toy-possession test

e. Third saliva sample

f. Interview on opinions about dog behavior

All parts of the visit will be filmed and it will take approximately 60 – 90 minutes. Saliva samples and questionnaires will be collected at the end.

Appendix V: Consent form for homes with 2-3 dogs

Consent Form

Project Title: Do cortisol and testosterone levels covary with social role in domestic dogs?

Researchers:

Mariana Castro, Master of Science Candidate, Cognitive and Behavioural Ecology Programme, Canine Research Unit, Department of Psychology*(*contact information removed*)*

Carolyn Walsh, PhD, Assistant Professor, Canine Research Unit, Department of Psychology; *(*contact information removed*)*

You are invited to take part in a research project entitled ***"Do cortisol and testosterone levels covary with social role in domestic dogs?"***.

This form is part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included here, you should feel free to ask. Please take the time to read this carefully and to understand any other information given to you by the researcher.

It is entirely up to you to decide whether to take part in this research. If you choose not to take part in the research, or if you decide to withdraw from the research once it has started, there will not be any negative consequences for you, now or in the future. If you decide to withdraw from the study after some data have already been collected, these data may still be used for the study at the researchers' discretion.

Introduction:

We are interested in investigating whether pet dogs living in small permanent groups form consistent social roles and what factors influence this process. The hormones cortisol and testosterone have been shown to be related to an individual's social status, and can be easily measured via saliva sampling. With your help, we intend to investigate the relationship between 1) videotaped and owner- reported dog behaviors, 2) dog personalities, as reported by owners, and 3) hormone levels of testosterone and cortisol. We expect our study to inform the debate about the social structure of dog groups and add to the growing body of scientific knowledge about dog behaviour.

Purpose of study:

To examine the relationship between cortisol and testosterone levels, social role, behaviours exhibited during social interactions and personality traits in pet dogs living in multi-dog households. Additionally, we would like to evaluate whether owner reports on their dogs' behaviours are good predictors of any of the abovementioned factors.

What you will do in this study:

As the dog's owner, you will be asked to do four things: 1) complete a written questionnaire about your dogs, their behaviours and their personalities, 2) allow two researchers into your home for an (approximately) one-hour long visit during which we will stimulate dog-dog interaction using a novel toy and videotape your dogs, 3) answer questions about your views on domestic dog social structure, which will be recorded, and 4) take saliva samples from your dog according to the collection schedule given and with the kits that we provide. We will provide you with instructions on you how to take these saliva samples from your dog.

Four dog saliva samples will be required: 1) a practice sample taken a day or more before the arranged visit, 2) a sample taken up to 30 min before researchers' arrival on a visit day, 3) a sample taken approximately 30 minutes into the visit, and 4) a sample taken at the end of the visit.

All saliva samples will need to be taken in the afternoon/evening between the hours of 1-9 pm.

Length of Time:

Your time commitment will be the time it takes you to get each saliva sample (approximately 2-3 minutes for each of the four samples per dog), approximately 30 minutes to fill out the written questionnaire, and the 60 min visit to your home.

Possible Benefits:

Your participation in this study will help us better understand how hormonal levels (cortisol and testosterone), social behaviours and dog personality are related to a dog's social role within its permanent social group. This will lead to a clearer picture of the possible social structure of permanent pet dog groups. We expect to add to the growing body of scientific knowledge about domestic dog behavior and, by leading to a better understanding of dogs in general, increase their welfare. We also hope that the unique opportunity to participate in this study with your dog will benefit the relationship that you and your dog share.

Possible Risks:

Your main risk in this study would be having your dog physically struggle when you are taking the saliva sample from him/her. We will provide you with instructions (including an on-line video) and suggestions about how to take the sample with minimal "bother" to your dog. There is also the possibility that your dogs engage in physical struggle when interacting with the toy. However, dogs most frequently resolve conflicts without physical aggression. We ask that you only allow your dogs to participate in the toy-possession test if you believe they are unlikely to be physically aggressive towards each other.

Confidentiality:

Your identity will be kept strictly confidential, and your contact information and address will remain locked in a filing cabinet. We will ask you mainly information about your dogs. The only personal information that will be collected from owners will be their opinions about dog social behaviours recorded during the visit. Your dogs' filmed behaviours obtained in your home and your opinions about dog social interactions will be viewed by researchers at the Canine Research Unit at Memorial University, and if you consent to its use for the purpose of scientific conferences, etc., your identity will never be revealed. Electronic files will be stored on a password protected computer.

Anonymity:

Your name shall never be identified in any reports or publications arising from our research. As stated above, should we wish to use any videotaped footage of your dog for scientific or educational purposes, your identity will never be revealed.

Reporting of Results:

This data we collect will primarily be used for Mariana Castro's Master's thesis in the Cognitive and Behavioural Ecology Programme, Faculty of Science. All Master's theses are maintained in the Centre for Newfoundland Studies at the Queen Elizabeth II Library at Memorial University. The data will likely also be incorporated into a paper that will be submitted for publication to a scientific journal intended for animal behaviour researchers, veterinarians, and so forth. Our data will be published without identifying information on the owners or dogs, in aggregated form, and possibly in the form of case studies, where warranted. Occasionally, videotape footage may be used for the purposes of teaching, and/or scientific conferences. You are free to decline consent for the use of your dogs' video/images for such purposes, should you wish, by leaving the box below unchecked.

Storage of Data:

The data (questionnaires, videos, recorded answers and hormonal values) and participant information will be stored indefinitely at the Canine Research Unit in the Department of Psychology in a locked filing cabinet. Electronic files will be stored on a password protected computer. Dog owner names, addresses and contact information will be kept separately from the collected data.

Questions:

You are welcome to ask questions at any time during your participation in this research. If you would like more information about this study, please contact:

Mariana Castro, *(*contact information removed*)*

Dr. Carolyn Walsh, *(*contact information removed*)*

The proposal for this research has been reviewed by the Institutional Animal Care Committee and the Interdisciplinary Committee on Ethics in Human Research and found to be in compliance with Memorial University's ethics policy. If you have ethical concerns about this research (such as the way you have been treated or your rights as a participant), you may contact the Chairperson of the ICEHR at icehr@mun.ca or by telephone at 864-2861.

Consent:

Your signature on this form means that:

You have read the information about the research.

You have been able to ask questions about this study.

You are satisfied with the answers to all of your questions.

You understand what the study is about and what you will be doing.

You understand that you are free to withdraw from the study at any time, without having to give a reason, and that doing so will not affect you now or in the future.

If you sign this form, you do not give up your legal rights, and do not release the researchers from their professional responsibilities.

The researcher will give you a copy of this form for your records.

Your Signature:

"I have read and understood the description provided; I have had an opportunity to ask questions and my questions have been answered. I consent to participate with my dog in the research project, understanding that I may withdraw my consent at any time. A copy of this Consent Form has been given to me for my records if I have indicated a desire to have such."

"I agree to release and waive liability for all claims that I or others in my household have, or may in the future have, against Memorial University of Newfoundland, its agents, servants and employees or any person(s), entities or organization(s) associated in any way with this study, from any and all liability for any loss, damage, injury or expense that I or others in my household may suffer as a result of this study."

Specifically, I consent to:

☐ Allowing my dogs to be videotaped in my home

☐ Allowing my dogs to participate in the toy-possession test

☐ Taking saliva samples from my dogs for research purposes

☐ Allowing the videotape of my dogs to be used for research and educational purposes

______________________________ ____________________

Signature of Participant Date

Researcher's Signature:

"I have explained this study to the best of my ability. I invited questions and gave answers. I believe that the participant fully understands what is involved in being in this study with his/her dog, any potential risks of the study and that he or she has freely chosen to be in the study."

______________________________ ____________________

Signature of Investigator Date

Telephone #: *(*contact information removed*)*

E-mail address: *(*contact information removed*)*

Appendix VI: Questionnaire for homes with 2-3 dogs

Questionnaire

This questionnaire should be filled out by the primary caregiver of the dogs. We define the primary caregiver as the person who, most of the time, feeds the dogs. We would like to ask that questionnaires about dogs living in the same household be filled out by the same person to avoid interpersonal differences in interpretation. If you have any questions about the content of this questionnaire, please contact Mariana Castro *(*contact information removed*)*.

Identification

(This part will be filled in by the researcher at the time of the visit)

House number: Dog number:

Was this questionnaire filled out 1st, 2nd or 3rd? (Please circle appropriate number)

Dog information

1. Dog name:
2. Dog birth date (approximate if the exact date is not known):
3. Sex:
4. Breed:
5. Weight:
6. Height:
7. Neutered or spayed? YES or NO

   If yes, how old was he/she when the surgery was performed?
8. Where did he/she come from (kennel, shelter, rescued by an organization, from another house, etc...)
9. How old was he/she when you got him/her?
10. Were any of your other dogs (the ones participating in this study) already living in your home before the arrival of this one?
11. Does he/ she have any current health issues? If so, which one(s)?
12. Is he/she taking any medication? If so, please list medication names:

13. Has this dog undergone any training? If so, please give a short description.

14. Do you participate in any regular activities with this dog (e.g., walks, dog park visits, dog sports such as agility, showing)?

15. Other relevant comments (Any “dog-friends”? Does he or she interact with dogs from other houses regularly?):

Behavioural Questions

1. In the last six months, have you seen this dog exhibit any of the following behaviours? Please circle N/A if that situation is not possible due to, for example, size differences amongst your dogs in case of mounting, or the fact that they don’t eat in the same area in the case of eating another dog’s food.

BEHAVIOUR				For owners of 3+ dogs: IF YES, WHICH OTHER DOG(S)?
Playing with any of your other dogs	YES	NO	N/A	
Initiating play with any of your other dogs	YES	NO	N/A	
Sleeping with any of your other dogs (on the same surface, not more than 1 metre apart)	YES	NO	N/A	
Fighting with any of your other dogs	YES	NO	N/A	
Initiating fights with any of your other dogs	YES	NO	N/A	
Marking objects/areas with urine (including in the yard)	YES	NO	N/A	
Over-marking an object/area recently urinated on by any of your other dogs	YES	NO	N/A	
Eating any of your other dogs’ food	YES	NO	N/A	
Guarding his/her food from your other dogs	YES	NO	N/A	

Guarding an object (e.g., toy) or area (e.g, sleeping spot) from your other dogs	YES	NO	N/A	
Mounting any of your other dogs	YES	NO	N/A	
Attempting to get attention from people who are interacting with any of your other dogs	YES	NO	N/A	
Licking any of your other dogs' muzzles	YES	NO	N/A	
Biting any of your other dogs' muzzles	YES	NO	N/A	
Growling at any of your other dogs	YES	NO	N/A	
Baring teeth at any of your other dogs	YES	NO	N/A	
Laying his/her paw over any of your other dogs' backs or bellies	YES	NO	N/A	

2. Is there anything this dog does towards any of your other dogs that you don't like? If so, do you try to avoid it? If so, how?

3. If a stranger arrives at your door, is this dog the most likely to bark first, before your other dog(s)? Is this dog the most likely to bark the longest?

Monash Canine Personality Questionnaire – Revised

Please rate your dog's personality using the Monash Canine Personality Questionnaire.

Please rate how well each word describes your dog's personality by marking the appropriate box.

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

	Really does not describe my dog					Really describes my dog
friendly	1	2	3	4	5	6
persevering	1	2	3	4	5	6

nervous	1	2	3	4	5	6
energetic	1	2	3	4	5	6
attentive	1	2	3	4	5	6
easy going	1	2	3	4	5	6
independent	1	2	3	4	5	6
trainable	1	2	3	4	5	6
non-aggressive	1	2	3	4	5	6
hyperactive	1	2	3	4	5	6
submissive	1	2	3	4	5	6
determined	1	2	3	4	5	6
relaxed	1	2	3	4	5	6
tenacious	1	2	3	4	5	6
timid	1	2	3	4	5	6
biddable*	1	2	3	4	5	6
active	1	2	3	4	5	6
intelligent	1	2	3	4	5	6
sociable	1	2	3	4	5	6
restless	1	2	3	4	5	6
fearful	1	2	3	4	5	6
obedient	1	2	3	4	5	6
lively	1	2	3	4	5	6
reliable	1	2	3	4	5	6
assertive	1	2	3	4	5	6
excitable	1	2	3	4	5	6

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

Appendix VII: Standard operating procedure sheet for saliva sampling

Standard Operating Procedure for Saliva Sampling of Domestic Dogs

Procedure:

1) Refer to your "Saliva Sampling Record Sheet" provided in the sampling kit and look at the information you will need to write down for the sample you are taking (practice, pre-visit, second or third samples)

2) Call or approach your dog and show him/her a small "high value" treat (e.g. cheese, hot dog piece), but do not let him/her have it. You will then ask your dog to sit or lie down. Feel free to hold your dog's collar, but do not restrain your dog too vigorously. You should try to be as relaxed as possible while doing this. Sometimes, a second person can be helpful (as seen in the video here: http://dogsbody.psych.mun.ca/cru/Canine_Behaviour_Lab/Cortisol.html)

3) With one hand, insert the swab into the corner of the dog's mouth while making sure to hold the other end firmly. Keep the swab in the dog's mouth for approximately 120 seconds in total, using a timer or clock if necessary. This time should be sufficient to totally saturate the swab. If the top one-third of the swab does not seem to be really wet with saliva, insert it for a few seconds more (some dogs naturally have more saliva than others!). It is OK if your dog chews on the swab a bit, just be sure that he/she doesn't bite it off.

4) After removing the swab please give your dog the treat and lots of verbal praise. We want him/her to associate this sampling procedure with good things!

5) Place the wet end of the sponge into the swab storage tube and press the sponge into the tube until it is completely inside it. Close the tube cap. Place the tube in your freezer (preferably inside a freezer bag or baggie) until it can be delivered to the researcher on the visit day.

The total handling time should be between 3-4 minutes for each saliva sample that you take.

Should your dog show a lot of resistance to the saliva collection procedure, or if you feel your safety or the safety of your dog is being jeopardized, *__stop the procedure immediately__* and make a note on the record sheet.

These methods are based on Dreschel, N.A. & Granger, D.A. (2009). Methods of collection for salivary cortisol measurement in dogs. Hormones and Behavior, 55: 163-168.

Appendix VIII: Saliva sampling record sheet

Saliva Sampling Record Sheet

Dog name:

Practice saliva sample:

Did you have any difficulties following the procedure? If so, what happened?

Pre-visit saliva sample

Was this sample taken 1st, 2nd or 3rd? (Please circle appropriate number)

Vial #: Time of collection:

Is there visible blood contamination on the sample? YES or NO

Were there any problems during collection? If so, please describe:

Second saliva sample (during visit):

Was this sample taken 1st, 2nd or 3rd? (Please circle appropriate number)

Vial #: Time of collection:

Is there visible blood contamination on the sample? YES or NO

Were there any problems during collection? If so, please describe:

Third saliva sample (during visit):

Was this sample taken 1st, 2nd or 3rd? (Please circle appropriate number)

Vial #: Time of collection:

Is there visible blood contamination on the sample? YES or NO

Were there any problems during collection? If so, please describe:

Appendix IX: Data collection instructions for homes with 4 or more dogs

Dear Participant,

Thank you for your interest in participating in the study "*Do cortisone and testosterone levels covary with social role in domestic dogs?*". This document contains the instructions you need to follow in order for the data collection to happen as smoothly as possible. Please follow the steps as outlined below and feel free to contact me *(*contact information removed*)* if you have any questions during the process.

Cheers,

Mariana Castro

DATA COLLECTION INSTRUCTIONS

Content of the collection kit:

This data collection kit contains:

This data collection instruction sheet;

1 consent form;

1 individual questionnaire for each of your dogs;

1 set of behavioural questions;

1 social interaction questionnaire;

1 standard operating procedure (SOP) sheet for saliva collection;

1 saliva sampling record sheet;

1 saliva collection swabs for each of your dogs;

1 saliva collection vial for each of your dogs;

Data collection steps

Please follow these steps in the order in which they appear in these instructions.

1. **Consent form**

    Please read the consent form and sign it if you agree to participate in the study. I will sign it in the beginning of the visit.

2. **Dog information, behaviour and personality questions**

You will need: 1 individual questionnaire for each of your dogs, 1 set of behavioural questions and 1 social interaction questionnaire.

Preparation: none

Procedure: Fill out the questionnaires . The completed questionnaires will be collected with the saliva samples after the visit is over.

3. **Saliva collection**

You will need: SOP sheet, saliva sampling record sheet, 1 saliva collection swab and 1 vial for each of your dogs, a pen or pencil, 1 treat for each of your dogs (optional).

Preparation: read the saliva collection instructions on the SOP sheet.

Procedure: Please collect all samples between 1 and 9pm. Perform steps 1 through 5 of the SOP sheet on each of the dogs being sampled that day. Please do not sample more than 2 dogs per day. Complete the necessary fields on the saliva sampling record sheet. Place the samples in the freezer. They will be collected by the researcher on the day of the visit.

4. **Day and time of the visit**

Please send me an email *(*contact information removed*)* or call me *(*contact information removed*)* to arrange the visit.

Day: **Time:**

5. Preparation for the visit

Please do not exercise or feed your dog for 3 hours prior to the visit.

Choose an area of the house (backyard, living-room, kitchen, etc…) in which all dogs can be together. The whole visit will take place in that location. We will go over the filled-out questionnaires and more detailed information will be requested by the researcher where applicable.

Appendix X: Consent form for homes with 4 or more dogs

Consent Form

Project Title: Do cortisol and testosterone levels covary with social role in domestic dogs?

Researchers:

Mariana Castro, Master of Science Candidate, Cognitive and Behavioural Ecology Programme, Canine Research Unit, Department of Psychology; *(*contact information removed*)*

Carolyn Walsh, PhD, Assistant Professor, Canine Research Unit, Department of Psychology; *(*contact information removed*)*

You are invited to take part in a research project entitled ***"Do cortisol and testosterone levels covary with social role in domestic dogs?"***.

This form is part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included here, you should feel free to ask. Please take the time to read this carefully and to understand any other information given to you by the researcher.

It is entirely up to you to decide whether to take part in this research. If you choose not to take part in the research, or if you decide to withdraw from the research once it has started, there will not be any negative consequences for you, now or in the future. If you decide to withdraw from the study after some data have already been collected, these data may still be used for the study at the researchers' discretion.

Introduction:

We are interested in investigating whether pet dogs living in small permanent groups form consistent social roles and what factors influence this process. The hormones cortisol and testosterone have been shown to be related to an individual's social status, and can be easily measured via saliva sampling. With your help, we intend to investigate the relationship between 1) videotaped and owner- reported dog behaviors, 2) dog personalities, as reported by owners, and 3) hormone levels of testosterone and cortisol. We expect our study to inform the debate about the social structure of dog groups and add to the growing body of scientific knowledge about dog behaviour.

Purpose of study:

To examine the relationship between cortisol and testosterone levels, social role, behaviours exhibited during social interactions and personality traits in pet dogs living in multi-dog households. Additionally, we would like to evaluate whether owner reports on their dogs' behaviours are good predictors of any of the abovementioned factors.

What you will do in this study:

As the dog's owner, you will be asked to do four things: 1) complete written questionnaires about your dogs, their behaviours and their personalities, 2) allow two researchers into your home for an (approximately) 40 minute long visit during which we will videotape your dogs, 3) answer questions about your views on domestic dog social structure, which will be recorded, and 4) take saliva samples from your dogs according to the collection schedule given and with the kits that we provide. We will provide you with instructions on you how to take these saliva samples from your dog. One saliva sample per dog will be required. Samples are to be taken on the same time of day for all dogs, with a maximum of two dogs being sampled per day. The time of collection should be in the afternoon/evening between the hours of 1-9 pm and be kept constant for all sampling days.

Length of Time:

Your time commitment will be the time it takes you to get each saliva sample (approximately 2-3 minutes for each dog), approximately 45 minutes to fill out the written questionnaires, and the 40 min visit to your home.

Possible Benefits:

Your participation in this study will help us better understand how hormonal levels (cortisol and testosterone), social behaviours and dog personality are related to a dog's social role within its permanent social group. This will lead to a clearer picture of the possible social structure of permanent pet dog groups. We expect to add to the growing body of scientific knowledge about domestic dog behavior and, by leading to a better understanding of dogs in general, increase their welfare. We also hope that the unique opportunity to participate in this study with your dogs will benefit the relationship that you and your dogs share.

Possible Risks:

Your main risk in this study would be having your dog physically struggle when you are taking the saliva sample from him/her. We will provide you with instructions (including an on-line video) and suggestions about how to take the sample with minimal "bother" to your dog.

Confidentiality:

Your identity will be kept strictly confidential, and your contact information and address will remain locked in a filing cabinet. We will ask you mainly information about your dogs. The only personal information that will be collected from owners will be their opinions about dog social behaviours recorded during the visit. Your dogs' filmed behaviours obtained in your home and your opinions about dog social interactions will be viewed by researchers at the Canine Research Unit at Memorial University, and if you consent to its use for the purpose of scientific conferences, etc., your identity will never be revealed. Electronic files will be stored on a password protected computer.

Anonymity:

Your name shall never be identified in any reports or publications arising from our research. As stated above, should we wish to use any videotaped footage of your dog for scientific or educational purposes, your identity will never be revealed.

Reporting of Results:

This data we collect will primarily be used for Mariana Castro's Master's thesis in the Cognitive and Behavioural Ecology Programme, Faculty of Science. All Master's theses are maintained in the Centre for Newfoundland Studies at the Queen Elizabeth II

Library at Memorial University. The data will likely also be incorporated into a paper that will be submitted for publication to a scientific journal intended for animal behaviour researchers, veterinarians, and so forth. Our data will be published without identifying information on the owners or dogs, in aggregated form, and possibly in the form of case studies, where warranted. Occasionally, videotape footage may be used for the purposes of teaching, and/or scientific conferences. You are free to decline consent for the use of your dogs' video/images for such purposes, should you wish, by leaving the box below unchecked.

Storage of Data:

The data (questionnaires, videos, recorded answers and hormonal values) and participant information will be stored indefinitely at the Canine Research Unit in the Department of Psychology in a locked filing cabinet. Electronic files will be stored on a password protected computer. Dog owner names, addresses and contact information will be kept separately from the collected data.

Questions:

You are welcome to ask questions at any time during your participation in this research. If you would like more information about this study, please contact:

Mariana Castro, *(*contact information removed*)*

Dr. Carolyn Walsh, *(*contact information removed*)*

The proposal for this research has been reviewed by the Institutional Animal Care Committee and the Interdisciplinary Committee on Ethics in Human Research and found to be in compliance with Memorial University's ethics policy. If you have ethical concerns about this research (such as the way you have been treated or your rights as a participant), you may contact the Chairperson of the ICEHR at icehr@mun.ca or by telephone at 864-2861.

Consent:

Your signature on this form means that:

You have read the information about the research.

You have been able to ask questions about this study.

You are satisfied with the answers to all of your questions.

You understand what the study is about and what you will be doing.

You understand that you are free to withdraw from the study at any time, without having to give a reason, and that doing so will not affect you now or in the future.

If you sign this form, you do not give up your legal rights, and do not release the researchers from their professional responsibilities.

The researcher will give you a copy of this form for your records.

Your Signature:

"I have read and understood the description provided; I have had an opportunity to ask questions and my questions have been answered. I consent to participate with my dog in the research project, understanding that I may withdraw my consent at any time. A copy of this Consent Form has been given to me for my records if I have indicated a desire to have such."

"I agree to release and waive liability for all claims that I or others in my household have, or may in the future have, against Memorial University of Newfoundland, its agents, servants and employees or any person(s), entities or organization(s) associated in any way with this study, from any and all liability for any loss, damage, injury or expense that I or others in my household may suffer as a result of this study."

Specifically, I consent to:

☐ Allowing my dogs to be videotaped in my home

☐ Taking saliva samples from my dogs for research purposes

☐ Allowing the videotape of my dogs to be used for research and educational purposes

________________________________ ______________________

Signature of Participant Date

Researcher's Signature:

"I have explained this study to the best of my ability. I invited questions and gave answers. I believe that the participant fully understands what is involved in being in this study with his/her dog, any potential risks of the study and that he or she has freely chosen to be in the study."

________________________________ ______________________

Signature of Investigator Date

Telephone #: *(*contact information removed*)*

E-mail address: *(*contact information removed*)*

Appendix XI: Individual questionnaire for each dog of homes with 4 or more dogs

Individual Questionnaire

This questionnaire should be filled out by the primary caregiver of the dogs. We define the primary caregiver as the person who, most of the time, feeds the dogs. We would like to ask that questionnaires about dogs living in the same household be filled out by the same person to avoid interpersonal differences in interpretation. If you have any questions about the content of this questionnaire, please contact Mariana Castro *(*contact information removed*)*.

Identification

(This part will be filled in by the researcher at the time of the visit)

House number: Dog number:

Dog information

1. Dog name:
2. Dog birth date (approximate if the exact date is not known):
3. Sex:
4. Breed:
5. Weight:
6. Height:
7. Neutered or spayed? 8. If yes, how old was he/she when the surgery was performed?
9. Where did he/she come from (kennel, shelter, rescued by an organization, from another house, etc...)
10. How old was he/she when you got him/her?
11. Were any of your other dogs (the ones participating in this study) already living in your home before the arrival of this one?
12. Does he/ she have any current health issues? If so, which one(s)?
13. Is he/she taking any medication? If so, please list medication names:
14. Is there anything this dog does towards any of your other dogs that you don't like? If so, do you try to avoid it? If so, how?

Monash Canine Personality Questionnaire – Revised

Please rate your dog's personality using the Monash Canine Personality Questionnaire.

Please rate how well each word describes your dog's personality by marking the appropriate box.

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

	Really does not describe my dog				Really describes	my dog
friendly	1	2	3	4	5	6
persevering	1	2	3	4	5	6
nervous	1	2	3	4	5	6
energetic	1	2	3	4	5	6
attentive	1	2	3	4	5	6
easy going	1	2	3	4	5	6
independent	1	2	3	4	5	6
trainable	1	2	3	4	5	6
non-aggressive	1	2	3	4	5	6
hyperactive	1	2	3	4	5	6
submissive	1	2	3	4	5	6
determined	1	2	3	4	5	6
relaxed	1	2	3	4	5	6
tenacious	1	2	3	4	5	6
timid	1	2	3	4	5	6
biddable*	1	2	3	4	5	6
active	1	2	3	4	5	6
intelligent	1	2	3	4	5	6
sociable	1	2	3	4	5	6
restless	1	2	3	4	5	6
fearful	1	2	3	4	5	6
obedient	1	2	3	4	5	6
lively	1	2	3	4	5	6
reliable	1	2	3	4	5	6
assertive	1	2	3	4	5	6
excitable	1	2	3	4	5	6

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

Appendix XII: Behavioural questions for all dogs in homes with four or more dogs. Colours represent dog names. This questionnaire was customized with dog names for each home.

Behavioural Questions

House number: (This part will be filled in by the researcher at the time of the visit)

For part A, please check all appropriate names. Check N/A if that situation is not possible due to, for example, size differences amongst your dogs in case of mounting, or the fact that they don't eat in the same area in the case of eating another dog's food.

A. In the last six months, which of your dogs have you seen:

1. **Playing** with any of your other dogs?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

2. **Initiating play** with any of your other dogs?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

3. **Sleeping** with any of your other dogs (on the same surface, not more than 1 metre apart)?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

4. **Fighting** with any of your other dogs?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

5. **Initiating a fight** with any of your other dogs?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

6. **Urinating** on specific objects / areas (including in the yard)?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

7. **Urinating** on an object/area recently urinated on by any of your other dogs?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

8. **Eating** any of your other dogs' food?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

9. **Guarding** his/her **food** from your other dogs?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

10. **Guarding an object** (e.g., toy) or **area** (e.g, sleeping spot) from your other dogs?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

11. **Mounting** any of your other dogs?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

12. **Attempting to get attention** from people who are interacting with any of your other dogs?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

13**. Licking** any of your other dogs' **muzzles**?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

14. **Biting** any of your other dogs' **muzzles**?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

15. **Growling** at any of your other dogs?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

16. **Baring teeth** at any of your other dogs?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

17. **Laying** his/her **paw** over any of your other dogs' backs or bellies?

□ N/A

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

For part B, please check only one name.

B. When a stranger arrives at your door:

1. Which of your dogs **barks first**?

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

2. Which of your dogs **barks longest**?

□ Blue □ Red □ Green □ Yellow

□ White □ Black □ Pink □ Purple

Appendix XIII: Social interaction questionnaire for homes with four or more dogs. Colours represent dog names. This questionnaire was customized with dog names for each home.

Social Interaction Questionnaire

House number: (This part will be filled in by the researcher at the time of the visit)

For the following questions, imagine all your dogs are in the same area and you throw ONE toy for them to play with.

1. Stuffed Kong Scenario

1A. If you threw one **rubber toy stuffed with treats** (like a Kong toy):

Would this dog be interested in the toy?	How certain are you of this dog's interest in the rubber toy stuffed with treats? Please circle the number which best indicates your level of certainty of your answer.
Black □Yes □No	not at all certain uncertain certain totally certain 1-------------------2-------------------3-------------------4
Blue □Yes □No	not at all certain uncertain certain totally certain 1-------------------2-------------------3-------------------4
Green □Yes □No	not at all certain uncertain certain totally certain 1-------------------2-------------------3-------------------4
Red □Yes □No	not at all certain uncertain certain totally certain 1-------------------2-------------------3-------------------4
Yellow □Yes □No	not at all certain uncertain certain totally certain 1-------------------2-------------------3-------------------4

1B. Which of your dogs do you think would **get the rubber toy stuffed with treats** first (please check only one name)?

□ Black □ Blue □ Green

□ Red □ Yellow

How certain are you of this answer?

not at all certain uncertain certain totally certain

1-------------------2-------------------3-------------------4

1C. Would the dog you selected in the previous question be able to **keep the rubber toy stuffed with treats undisturbed** for 1 minute?

□Yes □No

How certain are you of this answer?

not at all certain uncertain certain totally certain

1--------------------2--------------------3--------------------4

If you have answered yes to this question, please go straight to question 2. If you have answered no, please answer questions 1D. and 1E. as well.

1D. Which of your dogs would **try to take the rubber toy stuffed with treats away from** the dog that got the toy first?

Would this dog try to take the toy away from the dog that got the toy first?	How certain are you of this dog's attempt to recover the toy from the dog that got the toy first? Please circle the number which best indicates your level of certainty of your answer.
Black □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Blue □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Green □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Red □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Yellow □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4

1E. Which of your dogs would **succeed in taking the rubber toy stuffed with treats away from** the dog that got it first?

Would this dog succeed in	How certain are you of this dog's success in recovering the toy from the dog that got the toy first? Please circle the number which best indicates your level of certainty of your answer.

taking the toy away?	
Black □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Blue □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Green □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Red □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Yellow □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4

2. Squeaky Toy Scenario

2A. If you threw one **squeaky rubber toy**:

Would this dog be interested in the toy?	How certain are you of this dog's interest in the **squeaky rubber toy**? Please circle the number which best indicates your level of certainty of your answer.
Black □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Blue □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Green □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Red □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Yellow □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4

2B. Which of your dogs do you think would **get the squeaky rubber toy** first (please check only one name)?

□ Black □ Blue □ Green

□ Red □ Yellow

How certain are you of this answer?

not at all certain uncertain certain totally certain

1--------------------2--------------------3--------------------4

2C. Woud the dog you selected in the previous question be able to **keep the squeaky rubber toy undisturbed** for 1 minute?

□Yes □No

How certain are you of this answer?

not at all certain uncertain certain totally certain

1--------------------2--------------------3--------------------4

If you have answered yes to this question, please go straight to question 3. If you have answered no, please answer questions 2D. and 2E. as well.

2D. Which of your dogs would **try to take the squeaky rubber toy away from** the dog that got it first?

Would this dog try to take the toy away from the dog that got the toy first?	How certain are you of this dog's attempt to recover the toy from the dog that got the toy first? Please circle the number which best indicates your level of certainty of your answer.
Black □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Blue □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Green □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Red □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Yellow □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4

2E. Which of your dogs would **succeed in taking the squeaky rubber toy away from** the dog that got it first?

Would this dog succeed in taking the toy away?	How certain are you of this dog's success in recovering the toy from the dog that got the toy first? Please circle the number which best indicates your level of <u>certainty</u> of your answer..
Black □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Blue □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Green □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Red □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Yellow □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4

3. Both Scenarios

3A. During any of the situations proposed above, would there be a fight?

□Yes □No

How certain are you of this answer?

not at all certain uncertain certain totally certain

1--------------------2--------------------3--------------------4

3B. If you have answered <u>yes</u> above, please indicate which of your dogs would be involved in the fight:

Would this dog be involved in the fight?	How certain are you that this dog would be involved in the fight? Please circle the number which best indicates your level of <u>certainty</u> of your answer.
Black □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Blue □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4

Green □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Red □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4
Yellow □Yes □No	not at all certain uncertain certain totally certain 1--------------------2--------------------3--------------------4

Appendix XIV: Saliva sampling record sheet for homes with 4 or more dogs.

Saliva Sampling Record Sheet

House number: (This part will be filled in by the researcher at the time of the visit)

Dog's name:

Was this the 1st or 2nd sample of the day? (Please circle appropriate number)

Vial #: Time of collection: Day of collection:

Is there visible blood contamination on the sample? YES or NO

Were there any problems during collection? If so, please describe:

Dog's name:

Was this the 1st or 2nd sample of the day? (Please circle appropriate number)

Vial #: Time of collection: Day of collection:

Is there visible blood contamination on the sample? YES or NO

Were there any problems during collection? If so, please describe:

Dog's name:

Was this the 1st or 2nd sample of the day? (Please circle appropriate number)

Vial #: Time of collection: Day of collection:

Is there visible blood contamination on the sample? YES or NO

Were there any problems during collection? If so, please describe:

Dog's name:

Was this the 1st or 2nd sample of the day? (Please circle appropriate number)

Vial #: Time of collection: Day of collection:

Is there visible blood contamination on the sample? YES or NO

Were there any problems during collection? If so, please describe:

Appendix XV: Range and sample sizes for cortisol variables (μg/dL).

Cortisol variable	**Minimum**	**Maximum**	**Sample Size (n)**
Log-transformed baseline	-2.52	0.06	60
Log-transformed sample 2	-2.15	-0.41	41
Log-transformed sample 3	-1.44	-0.32	41
Log-transformed mean	-1.44	-0.32	39
Reactivity - conversation	-0.9	0.3	40
Reactivity - TPT	-0.18	0.16	40

Appendix XVI: Range and sample sizes for testosterone variables (pg/mL) by sex and gonadectomy groups and z-scores.

Group or transformation	Testosterone variable	Minimum	Maximum	Sample Size (n)
Spayed females	Baseline	4.13	185.08	28
	Sample 2	2.05	59.2	19
	Sample 3	1.39	96.32	21
	Mean	20.18	67.29	16
	Reactivity – conversation	-74.29	53.29	18
	Reactivity – TPT	-22.19	45.74	16
Intact females	Baseline	13.57	42.66	9
	Sample 2	30.98	74.23	6
	Sample 3	27.02	41.88	6
	Mean	29.03	37.89	5
	Reactivity – conversation	5.14	23.07	5
	Reactivity – TPT	-43.14	-0.39	6
Neutered males	Baseline	11.02	103.36	12
	Sample 2	10.74	48.05	10
	Sample 3	11.88	50.38	9
	Mean	14.41	59.87	8
	Reactivity – conversation	-77.48	23.99	9
	Reactivity – TPT	-18.85	28.74	9
Intact males	Baseline	16.54	366.38	6
	Sample 2	76.43	93.29	2
	Sample 3	47.79	75.75	2
	Mean	67.58	86.67	2
	Reactivity – conversation	-2.09	2.3	2
	Reactivity – TPT	-28.64	-17.54	2
z-scores	Baseline	-1.9	4.08	55
	Sample 2	-1.91	1.9	37
	Sample 3	-1.79	2.34	38
	Mean	-1.78	2.61	31
	Reactivity – conversation	-2.78	1.65	33
	Reactivity – TPT	-1.6	2.15	35