UNIVERSITY OF VETERINARY MEDICINE VIENNA

INTERDISCIPLINARY MASTER'S THESIS

Behavioral and Epigenetic Variations in Differentially Socialized Dogs
and
A Relational Approach to Aggression in Pet Dogs

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Declaration of Authorship

I, Katherine M Grillaert BS, declare that this thesis titled, “Behavioral and Epigenetic Variations in Differentially Socialized Dogs” *and* “A Relational Approach to Aggression in Pet Dogs” and the work presented in it are my own. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University.
- Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated.
- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
- I have acknowledged all main sources of help.
- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Signed:

Date: 27-Aug-2021

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In remembrance of Oliver, my shepherd during this season.

About this Thesis

This thesis is an interdisciplinary work in two parts.

Part One: The majority part, "*Behavioral and Epigenetic Variations in Differentially Socialized Dogs*", is in the Comparative Cognition Unit, supervised by Giulia Cimarelli PhD, Priv.-Doz Zsofia Virányi PhD, and Assoz.-Prof. Priv.-Doz. Friederike Range PhD.

Part Two: The minority part, "*A Relational Approach to Aggression in Pet Dogs*", is in the Ethics and Human-Animal Studies Unit, supervised by Univ.-Prof. Herwig Grimm.

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UNIVERSITY OF VETERINARY MEDICINE VIENNA

Abstract

Comparative Cognition Unit

Messerli Research Institute

Master of Science

Behavioral and Epigenetic Variations in Differentially Socialized Dogs

by Katherine M GRILLAERT BS

For a social species such as the dog, social relationships are important mediators of behavior. Close relationships influence exploratory and attachment behaviors and stress reactivity, likely through epigenetic methylation of associated genes. The body’s level of oxytocin, a hormone that plays a role in human-dog relationships, is mediated in part by the expression of the oxytocin receptor (OXTR) in the brain, and the gene for this receptor is variably methylated in dogs. Although previous studies have examined maternal caregiving effects within a single species, the human-dog relationship has been likened to that of a mother-child relationship, with the pet dog receiving caregiving behaviors. In this study, we are interested in possible inter-species effects of caregiving environments on canine behavior and epigenetic methylation of OXTR. We tested two groups of dogs, one group raised in conspecific packs and the other raised in pet homes, in a Strange Situation Test to identify attachment, and an open field test to test possible stress-buffering capabilities of attachment. Pet dogs were tested alone and with their owner, and pack dogs were tested alone, with their trainer, and with a canine partner from their pack. We also collected buccal cell samples to evaluate the methylation levels of OXTR. We hypothesized that pet dogs would show more attachment behaviors toward their owners than pack dogs would show toward their trainers, and that the pet dogs would behave more boldly in an exploration task in their owner’s presence than pack dogs accompanied by their trainer. We also predicted that pack dogs would behave the same whether they were tested with a canine or a human partner. Finally, we expected that pack dogs would have higher levels of OXTR methylation than pet dogs. We found that when their owner was present, pet dogs sniffed and explored more than pack dogs did with their trainer, and that after a stressful separation they returned to baseline levels of proximity to their owner, whereas pack dogs spent more time next to the trainer. We did not find behavioral differences between pack dogs tested with a canine partner and human partner. Pack dogs had higher levels of OXTR methylation than pet dogs, suggesting that the social experiences of pet dogs led to lowered methylation levels. The results of this study present preliminary evidence that the relationship between an extensively socialized pet dog and its owner yields stress-buffering effects, mediated by epigenetic methylation of OXTR, beyond that which can be provided by a close conspecific partner.

VETERINÄRMEDIZINISCHE UNIVERSITÄT WIEN

Abstrakt

Vergleichende Kognitionsforschung

Messerli Forschungsinstitut

Master of Science

Verhaltens- und epigenetische Variationen bei differentiell sozialisierten Hunden

von Katherine M Grillaert BS

Bei einer sozialen Spezies wie dem Haushund spielen soziale Beziehungen eine wichtige Rolle als Vermittler von Verhalten. Enge Beziehungen beeinflussen das Erkundungs- und Bindungsverhalten sowie die Stressreaktivität, wahrscheinlich hervorgerufen durch die epigenetische Methylierung der entsprechenden Gene. Der Spiegel an körpereigenem Oxytocin, einem Hormon, das in der Mensch-Hund-Beziehung eine große Rolle spielt, wird zum Teil durch die Expression des Oxytocinrezeptors (OXTR) im Gehirn beeinflusst und das spezielle Gen für diesen Rezeptor wird bei Hunden unterschiedlich methyliert. Obwohl frühere Studien die Auswirkungen mütterlicher Fürsorge nur innerhalb einer einzigen Spezies, dem Menschen, untersucht haben, wurde die Mensch-Hund-Beziehung mit der Mutter-Kind-Beziehung verglichen, wobei dem Hund als Haustier fürsorgliches Verhalten gegenüber gezeigt wurde. In der vorliegenden Studie interessierten uns die möglichen artübergreifenden Auswirkungen des Betreuungsumfelds auf das Hundeverhalten und die epigenetische Methylierung des OXTR. Wir haben zwei Hundegruppen getestet: eine Gruppe von Hunden, die in Artgenossen-Rudeln aufgewachsen sind und eine zweite Hundegruppe, die bei Hundehaltern aufgewachsen sind. Alle Hunde wurden in einem sog. Strange Situation Test getestet, um die Bindung zu bestimmen und zusätzlich noch in einem Außengehege-Test untersucht, um mögliche Stresspufferfähigkeiten durch die vorhandene Bindung zu untersuchen. Dabei wurden die Haushunde alleine und mit ihrem Halter getestet und die Rudelhunde wurden alleine, mit ihrem Trainer und mit einem anderen Hund aus ihrem Rudel zusammen getestet. Zudem wurden den Hunden Wangenzellproben entnommen, um den Methylierungsgrad des OXTR zu bestimmen. Unsere Hypothese war, dass die Haushunde ihren Haltern gegenüber mehr Bindungsverhalten zeigen würden als die Rudelhunde gegenüber ihren Trainern und dass sich die Haushunde bei einer Erkundungsaufgabe (Exploration) in Gegenwart ihres Halters furchtloser verhalten würden als die Rudelhunde in Begleitung ihres Trainers. Wir nahmen an, dass sich die Rudelhunde gleich verhalten würden, unabhängig davon, ob sie mit einem hündischen oder einem menschlichen Partner getestet werden würden. Zudem erwarteten wir, dass Rudelhunde höhere OXTR-Methylierungswerte aufweisen würden als Haushunde. Wir fanden heraus, dass die Haushunde in Anwesenheit ihres Halters mehr schnüffelten und die Umgebung erkundeten, verglichen zu den Rudelhunden mit ihrem Trainer anwesend und dass die Rudelhunde nach einer stressigen Trennung mehr Zeit in der Nähe des Trainers verbrachten als die Haushunde nahe zu ihrem Halter. Wir fanden keine Verhaltensunterschiede zwischen den Rudelhunden, die mit einem hündischen Partner und einem menschlichen Partner getestet wurden. Die Rudelhunde wiesen höhere OXTR-Methylierungswerte auf als die Haushunde, was darauf hindeuten könnte, dass die sozialen Erfahrungen von Haushunden zu niedrigeren Methylierungswerten führten. Die Ergebnisse dieser Studie sind ein vorläufiger Beweis dafür, dass die Beziehung zwischen einem umfangreich sozialisierten Haushund und seinem Halter durch die epigenetische Methylierung des OXTR eine stressabpuffernde Wirkung hat, die über die Wirkung eines engen Artgenossen hinausgeht.

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List of Abbreviations

ENOT	**E**xploration and **N**ovel **O**bject **T**ests
OT	**O**xy**t**ocin
OXTR	**O**xytocin **R**eceptor
SRT	**S**eparation and **R**eunion **T**est
SST	**S**trange **S**ituation **T**est

1. Introduction

1.1 Social Environment and Social Partners of Domestic Dogs

Dogs are social generalists, exhibiting a unique ability to thrive in a multitude of social environments, and forming relationships with both humans and conspecifics (Udell & Brubaker, 2016). Within these relationships, the dog's affiliative behaviors toward their partner, and the stress-buffering benefits they can receive, have been suggested to be similar regardless of the species of the partners (Cimarelli, Marshall-Pescini, Range, & Virányi, 2019). When challenged with a behavioral test (exploration of an unfamiliar field, followed by separation from and reunion with a partner, and lastly a novel object test), the dogs' stress, at a group level, was buffered to a similar extent by the presence of their human and dog partners. Importantly, however, the same study has also shown that this stress mediating effect of social partners greatly varies across relationships, and the authors have argued that the quality of the bond contributes to the functionality of the relationship (Cimarelli et al., 2019). Even if this study found no large differences between the stress buffering effects of intra- and interspecific relationships, another study did suggest a group-level difference in relationships of dog-dog and dog-human dyads: it has found that while most dog-human dyads met the criteria to be considered an infant-caregiver attachment bond, including stress and exploration behaviors, most dog-dog dyads did not (Sipple, Thielke, Smith, Vitale, & Udell, 2021). While the dog-dog dyads did exhibit other behaviors indicative of affiliation, the results of this study suggest that there may be qualitative differences between interspecific and intraspecific bonds (Sipple et al., 2021), with only human-dog bonds, at least in adult dogs, uniquely able to fulfill the criteria of an attachment bond, but see (Mariti, Carlone, Sighieri, Campera, & Gazzano, 2018).

Attachment theory was first proposed by psychologist John Bowlby (Bowlby, 1969) to describe the bond between infants and caregivers. This bond promotes a balance of protection and security of the caretaker with learning about the environment through exploration and the development of independence (Bowlby, 1973; Udell & Brubaker, 2016). There are four critical components of a secure attachment that can be observed in the infant: 1) proximity maintenance to the caregiver, 2) separation distress when the caregiver leaves, and the ability of the caregiver to function as a 3) "secure base" from which the infant can explore a new environment with confidence and as a 4) "safe haven" that provides security when the infant is stressed (Cassidy & Shaver, 1999). The Strange Situation Test (SST) was designed to elicit attachment-related behaviors under situations of high stress and low stress such that an overall pattern of attachment behavior can be deduced. The test begins with the child playing with the caregiver in the testing room. Over several three-minute phases, called episodes, both the caregiver and stranger enter and exit the room at prescribed times. An attached child will explore the environment and play while the caregiver is present, and be distressed by the caregiver's exit. The child will also greet the caregiver upon return, accept comfort from the caregiver, and will then resume play and exploration (Ainsworth & Wittig, 1969). Although the child may be comforted in part by the stranger, the child shows preferential behavior toward the caregiver. Several studies have found that children's attachment is associated with cortisol reactivity during the ASST, (Hertsgaard, Gunnar, Erickson, & Nachmias, 1995; Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996; Gunnar, Brodersen, Nachmias, Buss, & Rigatuso, 1996), suggesting that attachment is a mediating factor in the physiological response to stressful events.

Those who observed that dogs and their owners seemed to have a child-parent attachment relationship found confirmation in various behavioral tests that have since demonstrated that dogs show all behaviors necessary and sufficient to identify an infant-caregiver attachment bond with humans. They seek proximity to their caregiver rather than a stranger when stressed (Schöberl et al., 2012; Fallani, Previde, & Valsecchi, 2006; Topál, Miklósi, Csányi, & Dóka, 1998; Gácsi, Topál, Miklósi, Dóka, & Csányi, 2001), and they show distress when separated (Topál et al., 1998; Parthasarathy & Crowell-Davis, 2006; Prato-Previde, Custance, Spiezio, & Sabatini, 2003). Dogs explore a new environment more when in the presence of their owner (Horn, Huber, & Range, 2013), play more with a stranger when the owner is present rather than absent (Prato-Previde et al., 2003), and stress is reduced when in the presence of the owner (Gácsi, Maros, Sernkvist, Faragó, & Miklósi, 2013; Schöberl et al., 2012; Gácsi, Maros, Sernkvist, & Miklósi, 2009). Furthermore, when in the presence of the owner, dogs with stronger attachment had reduced cortisol reactivity (Schöberl et al., 2016).

Ever since dog-human attachment was described, it has been suggested to have evolved due to the unique domestication history of dogs, even though few studies have investigated if attachment behaviors developed during domestication in an anthropogenic environment, or if they were present in canine ancestors. When four-month-old hand-raised wolf puppies, identically hand-raised dog puppies, and pet puppies were tested with an SST paradigm, only the wolf puppies failed to show a preference for their handler over a stranger, falling short of the criteria for an attachment relationship with their hand-raiser (Topál et al., 2005). Other results, however, indicate that this lack of differentiation might have been due to the high interest of wolf pups in strangers (Ujfalussy, Kurys, Kubinyi, Gácsi, & Virányi, 2017), and that a preference for the hand-raiser may indeed be apparent in at least young wolves (Hall, Lord, Arnold, Wynne, & Udell, 2015; Wheat, Larsson, & Temrin, 2020). Even if dog-wolf comparisons have found somewhat contradictory results, most seem to agree that attachment to the human caregiver persists longer into adulthood in dogs than in human-raised wolves (Hall et al., 2015), but see (Lenkei, Újváry, Bakos, & Faragó, 2020).

When compared to wolves, dog puppies have a longer period of early development during which they are sensitive to social and environmental factors (Udell, Dorey, & Wynne, 2010). This may be advantageous for a species that inhabits a wide range of social environments, providing the attachment system with a greater window to receive input from the environment (Udell & Brubaker, 2016; Solomon, Beetz, Schöberl, Gee, & Kotrschal, 2019). While the Topal and colleagues' (Topál et al., 2005) puppies were tested after their first early socialization period (approximately 3-14 weeks of age (Freedman, King, & Elliot, 1961; J. Scott & Fuller, 1965), juvenile socialization continues for the first year of life (Battaglia, 2009; Serpell, Duffy, & Jagoe, 2016), which may provide the dog puppies with an advantage for forming attachments to human caretakers.

It is currently unknown, however, whether dogs have evolved a novel capacity of attachment due to their exposure to human care, as suggested by (Topál et al., 2005), or instead extended their intraspecific relationships (e.g., their bond to their mother or other fellow dogs) to humans. Few studies have investigated whether and to what extent dogs' relationships to other dogs can be characterized as attachment. For example, resembling a secure base effect, dogs spent more time investigating a novel object when tested with another dog versus alone (Moretti, Hentrup, Kotrschal, & Range, 2015). Furthermore, a comparative study of attachment behaviors between dogs separated from a cohabitant dog and separated from their owner demonstrated that dogs showed separation distress in both situations (Mariti et al., 2018). In this case, the higher level of stress shown by dogs separated from the cohabitant dog was concluded to represent a summation of the stresses from being separated both from the other dog and, necessarily, from the owner (Mariti et al., 2018). At the very least, dogs' attachment to humans seems to equal (Mariti et al., 2018) or even exceed ((Sipple et al., 2021); dog-human dyads have attachment bonds while dog-dog dyads do not) bonds to other dogs living in the same household. Such closer attachment to a human caregiver than to a cohabitant dog can easily be justified by the higher dependence of dogs on their human caregiver, and, therefore, possibly more comparable to dog pups' relationship to their mothers than to packmates.

Even fewer comparisons have been made between dogs' relationships to their owners and their biological mothers as compared to other conspecific companions. Nevertheless, one study demonstrated that puppies used social referencing when faced with a novel stimulus in the presence of a human showing positive or neutral emotion, a dog mother, and an unfamiliar dog partners, but showed behavioral regulation most often in the presence of the dog mother and the human showing positive emotion (Fugazza, Moesta, Pogány, & Miklósi, 2018). Further, a study of dogs tested in an SST with either their mother or unrelated older female dog found that while their behavior was overall highly similar, the dogs tested with an unrelated female showed a preference for the human stranger in the test, but those tested with the mother showed no preference, suggesting that dogs had a stronger bond with their mother than with an unrelated but close female conspecific (Mariti et al., 2017). While it seems likely that maternal interactions with puppies differ from that of packmates, especially as puppies age (Frederickson, 1952; Elliot & Scott, 1961; Ross, Scott, Cherner, & Denenberg, 1960; Pettijohn, Wong, Ebert, & Scott, 1977), it is an interesting finding here that puppies found the presence of some humans to be equally as influential as that of their mother. This might be because humans show extensive caring behavior toward puppies and dogs that even resembles that of their behavior toward human infants, including the use of "motherese" when speaking to adult dogs (Prato-Previde, Fallani, & Valsecchi, 2006;

Cimarelli, 2017; Herwijnen, Borg, Naguib, & Beerda, 2018). Combined with humans' obvious competence in an anthropogenic environment, this suggests that human caregiving toward dogs may even surpass that of mother dog caregiving toward her adult offspring, perhaps contributing to the basis of the strong attachment between humans and dogs that persists from puppyhood through adulthood. If so, it is an exciting question what kind of behavioral changes, especially stress-coping and learning about the environment, a close human bond may add to the natural behavioral repertoire of dogs that have only intraspecific relationships, and what kind of biological changes mediate these behavioral effects.

Investigating this question requires behavioral comparisons of dogs with close relationships to humans versus with conspecifics. As an example, in a previous study by Topal and colleagues (Topál et al., 2005), researchers raised one group of puppies with their dog mother and socialized them with humans, whereas the other group was separated from their mother and had a very close human hand-raiser who spent 24 hours per day with their assigned puppy and participated in extensive socialization. Although many of their behaviors with their human caregiver in an attachment test were highly similar, there were some behavioral differences between hand-raised and pet puppies indicative of activation of the attachment system. Hand-raised puppies with extensive human socialization discriminated more between the owner and a stranger: they played more with the owner, followed the owner leaving the pen, stood by the door more when separated from the owner, and showed more greeting behavior upon reunion than the pet puppies (Topál et al., 2005). Also, hand-raised puppies spent more time displaying passive behaviors and spent more time close to the door when their owners were separated (Topál et al., 2005), perhaps indicative of greater distress upon separation.

In the current study, we compared two groups of related dogs (two litters that shared a father) that had early and primary socialization with their biological mothers and human trainers under predictable and calm conditions. At the age of eight weeks, the litters were divided. Six puppies were adopted into traditional pet homes where they had a close human caregiver, and eight puppies remained in the dog pack with their mother and had regular interactions with their trainers. Our primary goal was to compare the dogs' relationships to their human partners between the two groups as well as the functions of these relationships in terms of effects of social support on stress, neophobia, and exploratory activity in an exploration and novel object test. In the pack group, by testing these dogs with both their trainer and a close canine group member, we also investigated how the functions of the interspecific relationships compared to the intraspecific relationships.

Given their extensive socialization with humans, we expected that the trainers can provide the pack dogs with as much support as their conspecific pack members. Importantly, however, we did expect differences between the two groups regarding their attachment to their human caregiver as well as the resulting stress-buffering effect of these relationships , demonstrating that living close to a human caregiver in a human home facilitates the development of a relationship that may benefit dogs' coping with stressful situations. In addition, to further investigate the biological effects of these different environments and a potential mechanism that may link living in a human caregiver's home to better stress coping, we investigated how the methylation level of the dogs' oxytocin-receptor gene (OXTR) developed in both groups pre- and post-adoption (up to 2.5 years of age).

1.2 Oxytocin Mediates Social Relationships and Stress-Coping

Oxytocin (OT) is a well-known neuropeptide and hormone that mediates many physiological processes and behavior in mammals (Herbeck, Gulevich, Shepeleva, & Grinevich, 2017; Jurek & Neumann, 2018). It plays a role in stress regulation, maternal behavior, pair bonding, social cognition, sociability, and attachment (Donaldson & Young, 2008; Insel, 2010; Thielke, Rosenlicht, Saturn, & Udell, 2017). It is produced by the hypothalamus and secreted into the brain and bloodstream by the posterior pituitary gland (Jurek & Neumann, 2018) in response to social situations (Rault, Munkhof, & Buisman-Pijlman, 2017; Uvnäs-Moberg, 1997) as well as both social and nonsocial stressors (Nishioka, Anselmo-Franci, Li, Callahan, & Morris, 1998; Olff et al., 2013). Endogenous oxytocin can be measured in brain tissue, plasma, cerebrospinal fluid (CSF), saliva, urine, and milk, and several studies have shown correlations between central and peripheral oxytocin levels and behavior. For example, the concentration of oxytocin in cerebrospinal fluid has been correlated with social behavior in rats and rhesus monkeys (Haller et al., 1996; Winslow, Noble, Lyons, Sterk, & Insel, 2003). In human infants, CSF OT concentration was associated with social-seeking behaviors at 0, 3, and 6 months of age (Clark et al., 2013), and in children CSF and plasma OT was correlated and associated with levels of anxiety (Carson et al., 2015). In parents, plasma and salivary OT concentrations were positively associated with parental engagement with their children (Feldman, Gordon, & Zagoory-Sharon, 2011; Gordon, Zagoory-Sharon, Leckman, & Feldman, 2010). Furthermore, parental salivary OT levels predicted salivary OT levels of their children (Weisman, Zagoory-Sharon, & Feldman, 2014; Feldman et al., 2011). A similar pattern of correlated oxytocin levels between social partners emerges in canine-human dyads as well. Affiliative interactions between dogs and humans result in an increase in blood plasma and salivary oxytocin in dogs (MacLean et al., 2017; Rehn, Handlin, Uvnäs-moberg, & Keeling, 2014) as well as in the urine of owners (Nagasawa, Kikusui, Onaka, & Ohta, 2009; Nagasawa et al., 2015), though see a cautionary review by Powell and colleagues (Powell et al., 2019).

Exogenous oxytocin, administered intranasally, intravenously, or injected into the brain, has been shown to affect behaviors modulated by stress, such as exploratory and fear behaviors. When compared to controls, rats administered oxytocin spent more time exploring a new environment, and showed fewer neophobic behaviors in response to a non-social auditory stressor (Windle, Judah, & Forsling, 1997). They also spent more time exploring an open arm of a maze test, suggesting decreased anxiety (Raadhika & Malathi, 2016). Socially induced stress is also mediated by oxytocin. For example, oxytocin administration in humans reduced negative self-appraisals and negative affect following a social challenge (Alvares, Chen, Balleine, Hickie, & Guastella, 2012; Kubzansky, Mendes, Appleton, Block, & Adler, 2012).

Oxytocin is also a critical neurotransmitter in attachment and bonding. Monogamous prairie voles, for example, recovered from a stressful event better when in the presence of their male partner, but this effect was prevented by the administration of an oxytocin receptor antagonist (Smith & Wang, 2014). Wild chimpanzees experience an increase in urinary oxytocin after a grooming bout, but this rise is mediated by the quality of the affiliation with their partner (Crockford et al., 2013). Dogs given intranasal OT showed higher affiliative behavior toward both other dogs and humans than those sprayed with a placebo (Romero, Konno, & Hasegawa, 2013), although in another study, intranasal OT did not increase owner-directed proximity and contact seeking behavior in an SST test, as had been expected (Thielke et al., 2017). It has been hypothesized that oxytocin mediates social behavior by reducing reactivity to stress (Buttner, 2016), which is emphasized again when considering that an important function of an attachment bond is to facilitate exploration of the environment.

These findings suggest that oxytocin may play a role in regulating stress responses and mediating the stress-buffering effect of a social partner, albeit sensitive to factors such as context and quality of the bond. An emerging pattern of differential behavioral responses to exogenous oxytocin may be due in part to the contribution of the oxytocin receptor gene (*OXTR*), which we will discuss next.

1.3 Regulation of the Oxytocin Receptor Gene

The oxytocin receptor is widespread throughout the brain (Jurek & Neumann, 2018), and plays a role in social attachment and coping with stress. In humans, polymorphisms and regulation of *OXTR* are associated with different socio-behavioral phenotypes (Kumsta, Hummel, Chen, & Heinrichs, 2013), increased maternal behavior (Bakermans-Kranenburg & IJzendoorn, 2008; Mileva-Seitz et al., 2013), and communication between partners (Schneiderman, Kanat-Maymon, Ebstein, & Feldman, 2014). Polymorphisms in *OXTR* are associated with dogs' proximity seeking toward people and their behavior toward strangers (Kis et al., 2014), as well as differential greeting behavior in two common dog breeds, Border Collies and German Shepherds (Kis et al., 2014; Kubinyi et al., 2017). Additionally, three single nucleotide polymorphisms (SNPs) of *OXTR* were found to have significant effects on dogs' behaviors in three components of attachment (Acceptance, Anxiety, and Attachment, as determined by factor analysis by Topal and colleagues (Topál et al., 1998) when challenged with the SST (Kovács, Virányi, Kis, Turcsán, & Hudecz, 2018). In addition to genetic variation in the oxytocin receptor gene itself, epigenetic gene methylation also mediates social and stress-related behaviors (Kumsta et al., 2013).

Epigenetic methylation is a regulatory mechanism that is sensitive to environmental factors, including the social environment and caregiving. It affects gene expression through a process that attaches a methyl group to the cytosine base, without altering the underlying DNA sequence. This cytosine is usually immediately followed by a guanine base (CpG site), and so-called CpG islands are particularly CpG rich areas often located near gene promoters (Saxonov, Berg, & Brutlag, 2006). High levels of methylation have been associated with little to no gene expression (Kusui et al., 2001), likely accomplished by blocking the assembly of transcription factors (Suzuki & Bird, 2008). Methylation patterns are often stable through cell division and inherited in offspring. In a recent study of seven genes that affect behavioral traits, including *OXTR*, researchers found shifts in methylation patterns that can be used to differentiate wolves and some dog breeds (Banlaki, Cimarelli, Viranyi, & Kubinyi, 2017). However, some genes can also be flexibly demethylated and methylated throughout an organism's lifetime, and methylation patterns can be influenced by environmental factors, including diet, environmental toxins, and the receipt of caregiving behavior (Zhang & Meaney, 2010; Dominguez-Salas, Cox, Prentice, Hennig, & Moore, 2012). A longitudinal study of callous-unemotional (CU) traits in children suggested two distinct pathways for developing CU based on *OXTR* methylation patterns over time, one centered on heritable methylation patterns and the other influenced by the social environment (C. A. M. Cecil et al., 2014). Finally, there is also evidence for short-term, dynamic changes of *OXTR* methylation in response to acute social stress. Human participants in a test of mental math and public speaking showed an increase in *OXTR* methylation in blood plasma 1 minute following the conclusion of the test, and then dropped below baseline when sampled 90 minutes later (Unternaehrer et al., 2012).

Taken together, this evidence suggests that epigenetic methylation of *OXTR* is affected by the social environment and influences the production of attachment and stress-related behaviors. Rats and adult men and women who received low levels of maternal care during their childhood were likely to have high levels of *OXTR* methylation (Beery, McEwen, MacIsaac, Francis, & Kobor, 2016; Unternaehrer et al., 2015), as well as experience stress and mental health difficulties later in life (humans) and produce higher levels of corticosteroids in reaction to a stressor (rats). In humans, lower levels of epigenetic methylation have been associated with increased emotional regulation in negative social situations (Puglia, Lillard, Morris, & Connelly, 2015), and better social functioning (N. Ebner et al., 2015). The first study of epigenetic methylation in pet dogs found that the behavioral response of the dog to the approach of a threatening stranger was associated with differential methylation of certain regions of the *OXTR* promoter (Cimarelli, 2017), further supporting the role of *OXTR* in differential stress-related behaviors. This study, however, failed to find a link between *OXTR* methylation and owner interaction styles. Additional positive results might have been found if it had sought for differences in the quality of dogs' relationship to their owners and such associations with *OXTR* methylation.

The role of oxytocin may change throughout critical periods of mammal development, perhaps mirroring incrementally greater exposure to the environment and maturation of social relationships. Indeed, researchers have identified patterns of transient *OXTR* expression in infant, pubertal, and adult humans (Grinevich, Desarménien, Chini, Tauber, & Muscatelli, 2015; Tribollet, Charpak, Schmidt, Dubois-Dauphin, & Dreifuss, 1989; Vaidyanathan & Hammock, 2017). While such patterns differ by species, they have also been observed in voles, rats, and mice (Hammock, 2015), although this has not been studied in dogs. One mechanism that may explain these observations is developmentally sensitive epigenetic methylation as a regulator of *OXTR* expression (Zhang & Meaney, 2010), granting the organism greater flexibility in responding to a changing environment and social relationships.

Domestic dogs have diverse and flexible social abilities, allowing them to form close social bonds both with conspecifics and humans. As discussed at the beginning of this introduction, dog-human bonds can even be considered attachment bonds, analogous to relationships between human mothers and infants. Caregiving in humans influences the infants' attachment and affects their later behavior as adults. Furthermore, caregiving in several other species, including humans, not only affects attachment, but along with other social and environmental factors, can affect the oxytocin system through epigenetic methylation. Methylation of *OXTR*, in turn, is associated with attachment bonds and decreased reactivity to stressful situations.

This thesis will take a step toward understanding such possible connections between human caregiving, attachment, stress coping, and *OXTR* methylation in dogs. Oxytocin receptor methylation is responsive to the social environment, and lower levels of *OXTR* methylation are associated with more attentive caregiving. Therefore, we predict decreased methylation levels of the *OXTR* promoter region in dogs taken care of by an individual owner in pet homes versus those living in the conspecific pack and engaging in frequent but more limited interactions with a human trainer.

1.4 Study Design and Predictions

This study followed 2 groups of genetically related dogs (N=14) that were born and raised by both their dog mother and human-raiser at the Wolf Science Center (WSC), Gamepark Ernstbrunn until 8 weeks of age. At that time, six dogs were adopted into pet homes, and eight dogs remained in the wildlife park in small captive packs. The Pet puppies lived in the owner's apartments, spent several hours a day together with them, and engaged in various activities together. The Pack puppies lived in large enclosures in groups of 2 to 7 dogs, where humans rarely intervened with their interactions. However, they had several weekly interactions with their trainers of about 10 to 90 minutes, such as obedience training, walks in the park, short interactions with unfamiliar visitors, and cognitive training and testing as part of the research at the WSC. To explore if these two socialization environments are associated with variations in methylation of the oxytocin receptor and differential attachment-related behaviors, we collected buccal samples from all animals pre- and post-adoption and at an adult age and tested the animals in two behavioral tests, an exploration and novel object test and an attachment test, between November 2016 and March 2017. Within the pack dogs, we compared how a preferred pack mate and a human trainer of the dogs facilitated exploration of a novel area and a novel object, as compared to facing these challenges alone. Across the pet and pack groups, we compared the animals' attachment to their human caregiver (owner in pet dogs and trainer in pack dogs). This included behavioral comparisons as well as stress reactivity, as we also collected salivary samples for cortisol analysis before and after the attachment test. Additionally, we measured the rise of *OXTR* methylation from before to after the attachment test, as *OXTR* methylation has been shown to change in response to social stress in humans (Unternaehrer et al., 2012).

Regarding the long-term changes of *OXTR* methylation, we expected both groups of puppies to have the same percentage of *OXTR* methylation before adoption, but after adoption, given individual human care and protection in a human home, that pet dogs would have lower methylation than pack dogs.

For the same reason, we also predicted that pet dogs will have a greater number of behaviors associated with attachment when tested with their human caregiver, as compared to pack dogs. Indicators of attachment include exploration in a new environment (moving around, sniffing, leaving the proximity of the human) in presence of the caregiver but distress when separated from her (standing at the door, vocalizing, stress signals), and greeting behavior upon reunion (proximity to and contact with the human). Following the attachment test, we expected that pet dogs would have lower cortisol reactivity than pack dogs, and that pack dogs would have a larger dynamic increase in *OXTR* methylation.

With respect to exploring a novel area and scary object, we expected both groups to spend more time active and sniffing their environment, more time approaching, sniffing, or manipulating the novel object, and fewer behavioral signs of stress (licking, yawning, shaking, paw raise) when tested with a partner versus alone. In pack dogs that were tested with two different partners, we expected no differences when accompanied by a human versus a canine packmate. However, when tested with the human caregiver, we did expect that the dogs in the pet group will show increased activity, increased sniffing, more time spent investigating the novel object, and fewer behavioral signs of stress than pack dogs, due to the stress-buffering effects of their strong attachment to their owner.

2. Methods

2.1 Ethics Statement

The study procedures were approved by the University of Veterinary Medicine Vienna Ethical Committee (Ref: 19/04/97/2014). All dog owners voluntarily participated in this study and gave their consent for behavioral testing and collection and analysis of buccal and salivary samples. As no invasive procedures as defined by Austria (Tierversuchsgesetz 2012 – TVG 2012) were performed for this study, no additional ethical permissions were necessary.

2.2 Subjects

Two litters of mixed breed puppies were born two weeks apart (March 21, 2014, and April 2, 2014) at the Wolf Science Center (WSC) in Ernstbrunn, Austria. Three females and four males were born in each litter, totaling 14 puppies (Table 2.1). The puppies were reared by the WSC puppy raising staff from the age of 10 days old. They were bottle-fed and spent their daytime hours with their litter and several hand-raisers. In the evenings, the puppies were returned to their mothers. At the age of 8 weeks old, 6 puppies (3 males, 3 females; 3 puppies from each litter) were adopted into pet homes. The remaining 8 puppies were integrated into captive dog packs at the Wolf Science Center. The animals in the captive packs live outside with access to shelters and an indoor warming house, and receive structured, daily positive training sessions with staff trainers.

TABLE 2.1: **Subjects**

Dog	Litter	Group
Banzai (m)	Layla	Pack
Enzi (m)	Layla	Pack
Gombo (m)	Nia	Pack
Hiari (m)	Nia	Pack
Imara (f)	Nia	Pack
Panya (f)	Layla	Pack
Pepeo (m)	Layla	Pack
Sahibu (f)	Nia	Pack
Cheza (m)	Layla	Pet
Jitu (m)	Nia	Pet
Kiatu (f)	Layla	Pet
Kisa (f)	Nia	Pet
Tuukka (f)	Nia	Pet
Zazu (m)	Layla	Pet

2.3 Buccal Cell Samples and *OXTR* Methylation Analysis

Samples were collected biweekly for the first four months of life, and monthly thereafter until the age of 1 year. At approximately age 2.5 years old, samples were again collected for 7-9 consecutive weeks. The mean number of samples per animal was 15.3, +/- 2.2. Additionally, buccal cell samples were taken immediately before the Strange Situation Test, and one hour thereafter, to detect possible short-term dynamic changes in epigenetic methylation.

Buccal epithelial cell samples were taken from each dog by inserting a cotton swab into the mouth and swiping the sides of the cheek several times. Samples were then frozen at -20°C until analysis. As 6 dogs were living in family homes, the owners collected the samples and froze them at -20°C, before transferring them to the laboratory for further storage. Samples were analyzed by partners in the Molecular Biology and Pathobiochemistry laboratory (Department of Medical Chemistry at Semmelweis University, Budapest, Hungary). The buccal DNA samples were extracted, bisulfite converted, amplified, and finally sequenced according to methods described in Cimarelli et al, 2017 (Cimarelli, 2017).

Four specific *OXTR* CpG islands were chosen for methylation analysis based on a previous study in canids which found these sites to be both highly variable (>10%) in methylation levels between individuals, and associated with social behavior (Cimarelli, 2017).

2.4 *OXTR* Methylation Data Analysis

Methylation analysis was reported as percentage methylation in triplicate for each sample. The standard deviation for each triplicate was calculated, and where it was over 4, the triplicate average was manually resolved by removing the outlying sample. In some cases, it was not possible to choose the outlying sample, or removal still resulted in a standard deviation greater than 4. These samples were removed from the analysis (see Table 2.2). The average methylation percentage for each sample was then used in the analyses.

Due to missing data in some weeks, the data were further prepared by separating the observations into three periods: pre-adoption period (4-8 weeks of age), post-adoption (9 weeks – 1 year of age), and adult (2.5-3 years of age). Samples were selected for each time period if there were at least 3 samples from Pack dogs and 3 samples from Pet dogs within the same week of sampling. These three periods were then used in statistical analyses.

TABLE 2.2: **Buccal Cell *OXTR* Methylation Samples at Four Loci**

	-1383	**-1371**	**-727**	**-751**
Buccal Samples	238	238	238	238
Samples Missing After Sequencing	8	10	21	25
Methylation Samples	230	228	217	213
Samples Outlier Removed to Resolve SD>4	41	21	100	71
Samples Removed SD>4	17	6	46	28
Total Samples After Selection for Analysis	103	78	60	66

2.5 Strange Situation Test

The SST was administered in an empty room (3.6m x 3.6m ft) within walking distance from the Wolf Science Center grounds, between November and January of 2017, when the dogs were approximately 2.5 years old.

Pack dogs were collected at their home enclosure, where salivary samples were immediately taken. They were then walked to the testing site, where an assistant took the buccal cell samples and explained the experimental procedure to the trainer. The trainer then entered the testing room, and the testing procedure began.

Pet dogs were walked from the parking lot to the testing site, where an assistant immediately took salivary and buccal cell samples and explained the experimental procedure to the owner. The owner then entered the testing room, and the testing procedure began.

The complete testing procedure of the SST consists of 7 phases, as outlined in Table 2.3. The tests were recorded by two video cameras, one main wide-angle camera was used in most of the analyses, and a second video camera was positioned as a backup and to capture any blind spots in the room. A third camera was used to monitor the room in real-time and was primarily used to assess the dog during separation so that the experiment could be halted in the case of undue stress. (See Figure 2.1.)

After the SST, another salivary sample and buccal cell sample were taken from all dogs. Pack dogs were walked back to their enclosure, where a third salivary sample was taken. Pet dogs were walked around the testing ground, and a third salivary sample was taken approximately 10 minutes after the conclusion of the SST.

The SST videos were assessed using a scheme developed by Kovács et al. to score subjects in three dimensions: Attachment, Stress, and Stranger (See Table 2.4). In this assessment, subjects could earn points in each dimension, based on whether they did or did not display a certain behavior, and in some cases, to what degree they displayed the behavior. For example, a dog that stood by the door none of the time earned 0 pts, versus some of the time (1 pt), or all the time (2 pts).

The Baseline, Separation, and Reunion phases were used for a second analysis, called here the Separation and Reunion Test (SRT). This analysis is based on findings from Thielke et al. (Thielke et al., 2017), which found these phases to be predictive of a dog's attachment security in the Strange Situation Test. The Separation and Reunion Test was coded from videos using Solomon Coder. The coding ethogram can be found in Table 2.5.

TABLE 2.3: **Strange Situation Test Procedure**

Phase	Length	Description
1. Baseline*	3 min	Owner and dog enter room, owner removes leash. Owner fills out questionnaire while sitting in Chair 1 for two minutes. Dog is free to explore. Owner get ups from the chair, picks up blocks from Table 1 and moves them one at a time to Table 2. When finished, Owner sits back in the chair and works on the questionnaire.
2. Owner, Stranger, and Dog	3 min	Stranger enters the room and sits down in Chair 2; does not interact with dog for 1 minute. Then the stranger attempts to play with the dog for 1 minute. The owner gets up and leaves the room, and the stranger continues attempts to play with the dog for 1 additional minute.
3. Stranger and Dog	3 min	Stranger fills out questionnaire while sitting in chair, and does not interact with the dog. After two minutes, the Stranger gets up and picks up block from Table 1 and moves them one at a time to Table 2. At the end of 3 minutes, the stranger leaves the room.
4. Dog Alone*	3 min	The dog is alone in the room for this phase. This phase would be ended if the dog showed excessive distress.
5. Owner and Dog	3 min	The owner enters the room, pausing for the first 5 seconds at the door while ignoring the dog. The owner then greets the dog for 5 seconds, and then sits down in Chair 1 and works on a questionnaire, ignoring the dog for the remainder of the phase. The owner then leaves the room.
6. Dog Alone	3 min	The dog is left alone in the room for 3 minutes. This phase would be ended if the dog showed excessive distress.
7. Stranger and Dog*	3 min	The stranger enters the room, pausing for the first 5 seconds at the door while ignoring the dog. The stranger then greets the dog for 5 seconds, and then sits down in Chair 2 and works on a questionnaire, ignoring the dog for the remainder of the phase.

The owner and stranger ignored the dog except where indicated otherwise. Phases marked with an asterisk (*) were used for the Separation and Reunion test analyses.

TABLE 2.4: **Strange Situation Test Scoring (Kovacs Rubric)**

	Behavior	Score
Attachment		
Owner present	Dog is close to O (closest body part is within 1m) - when it does not explore or play	1
	It does not stand at the door	1
	During the cube-carrying it watches or follows O	1
	When O first leaves it follows O to door	1
	When O leaves the second time it follows O to door	1
	When O enters it approaches at once and wags tail	1
Owner absent	Plays with S (at least 2s)	1
	Vocalizes	1
	Stand by or orients at door (sometimes - 1, almost all the time - 2)	2
	When S enters dog tries to sneak out the door instead of greeting	1
	Dog is mostly at the chair of O if it is not at the door	1
Anxiety		
Owner present	Stands at door (a little - 1, almost all the time - 2)	2
	Does not explore or play (only if it is obvious that dog would be interested)	1
	Dog (hides) under/behind O's chair	1
	As soon as O stands up dog approaches door	1
	Watches/approaches door while O is carrying cubes	1
	Vocalizes (if not asks for the ball)	1
Owner absent	Contact seeking with O before the separation	1
	At 1st separation dog vocalizes or runs around up and down or scratches door	1
	At 2nd separation dog vocalizes or runs around up and down or scratches door	1
	Follows S to the door when she leaves	1
	Plays or lie down comfortably (head down)	1
Acceptance		
Owner present	Approaches S when she first enters (at once, within reaching distance)	1
	Gets in physical contact and wags when the S first enters	1
Any time	Takes toy to S (not during play)	1
	Seeks physical contact (jumps on, snuggles up to, nudges) during the episodes	1
	Avoids S during play (stands off, avoids her touch)	1
Owner absent	Gets in physical contact and wags when the S enters second time	1
	During the cube carrying part dog mostly watches (1) and also follows (2) S	2
	Plays with her also during separation (a little - 1, a lot - 2)	2
	Dog is close (within 1m) to S during separation (a little - 1, almost all the time - 2)	2

Strange Situation Test scoring, reprinted from Kovács et al, 2018. S=Stranger, O=Owner.

TABLE 2.5: **SRT Ethogram**

Behavior	Description
Exploration	Dog is walking, trotting, or sniffing
Pace	Dog is moving in a stereotypical way, either back and forth in a line or in a loop
Passive	Dog is still in a lying, sitting, or standing posture
Play with Toy[1]	Dog plays on own with toy
Play with Owner[1]	Dog tries to engage owner in play with or without toy
Stand Door	Dog stands at door during Separation Phase
Look at Door	Dog looks at the door during Separation Phase
Vocalize	Bark, whine, growl
Lip Lick[2]	Dog extends tongue out of mouth and licks lips or nose
Yawn[2]	Dog opens mouth wide and then closes it
Contact	Dog is in physical contact with owner
Shake	Dog shook body as though wet.
Arousal[3]	Dog shows a displacement behavior such as mounting.
Proximity	Dog within 1 body length of owner

[1] Play with Owner and Play with Toy were combined post-hoc into one variable, but ultimately not evaluated as they occurred too few times (< 10 times total across all phases). [2] Lip Lick and Yawn were combined post-hoc into one variable. [3] Arousal was not evaluated as it occurred too few times (< 10 times total across all phases).

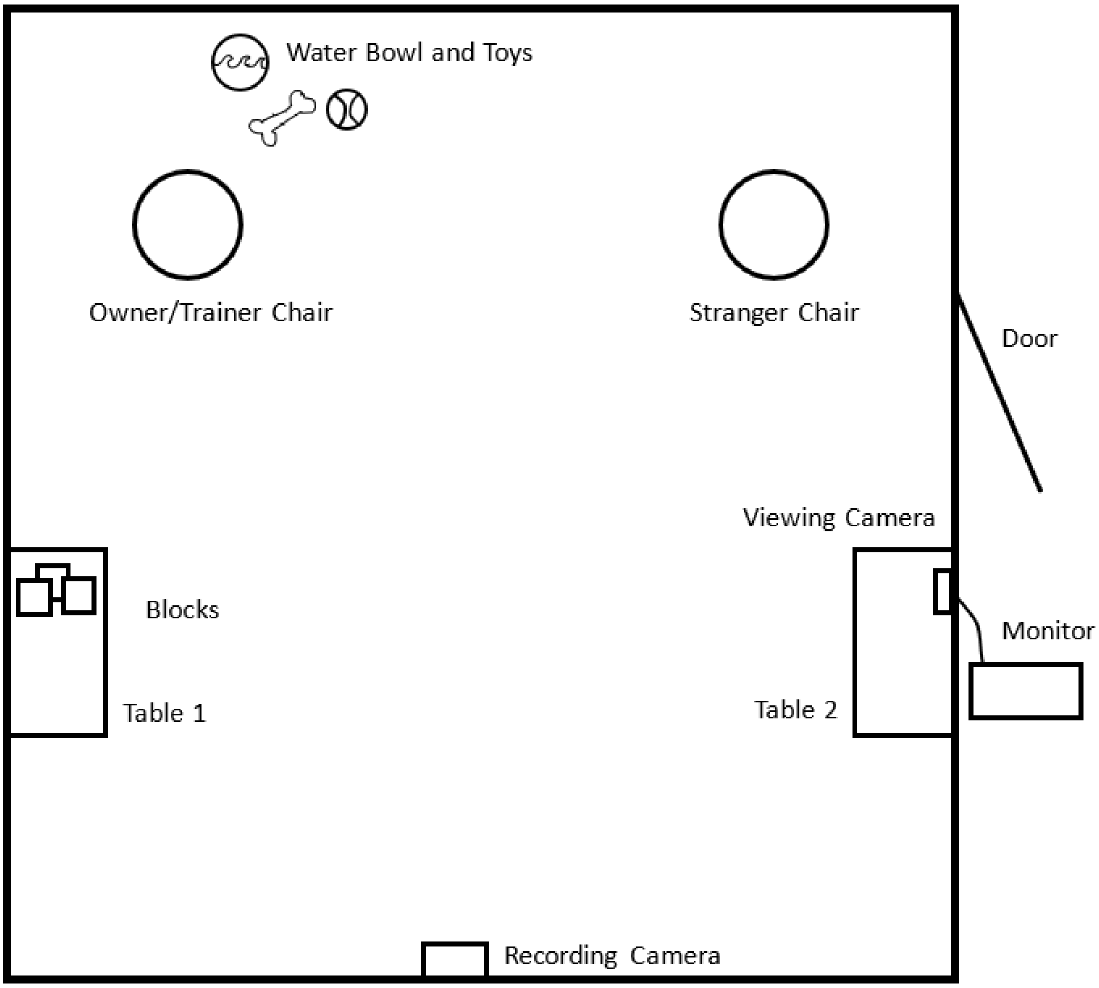

FIGURE 2.1: **Schematic of Strange Situation Test Room.**

2.6 Salivary Cortisol Analysis

Salivary samples for cortisol analysis were collected during the Strange Situation Test to verify a physiological stress response to the test conditions. Salivary samples were collected using Salivette® absorbent columns and tubes. In order to stimulate salivation, dogs were allowed to sniff, but not consume, food treats (Ligout et al., 2010). The absorbent cotton swab was placed in the dog's mouth for approximately ten seconds, and then frozen at -20°C before transport to the laboratory for analysis. Salivary cortisol analysis was performed by the student and partners in the Unit of Physiology, Pathophysiology and Experimental Endocrinology (Department of Biomedical Sciences, University of Veterinary Medicine Vienna, Austria) with a highly sensitive cortisol EIA kit (0.2 pg/well) (Palme, 1997). Samples were thawed on ice and centrifuged at room temperature at 3000 g for 15 minutes. Samples contaminated with blood or food were not analyzed. As many samples were of low salivary volume, they were diluted with buffer in a ratio of 1:10 in order to achieve 10 µL of saliva per well, resulting in a maximum sensitivity of 0.2 ng/mL. Again due to low salivary sample volume, only one well was analyzed per sample, rather than in duplicate. See Table 2.6 for sample information.

Samples were collected at three time points per subject:

1. within 3 minutes of leashing the dog to walk to the test site (Pack dogs) or within 3 minutes of arriving at the test site (Pet dogs), as previous studies have shown that salivary cortisol can rise due to handling within four minutes (Kobelt, Hemsworth, Barnett, & Butler, 2003)
2. immediately after the conclusion of the Strange Situation Test
3. approximately 25 minutes after the conclusion of the Strange Situation Test, as previous studies have shown that salivary cortisol should decrease within this time range (Beerda, Schilder, Janssen, & Mol, 1996)

TABLE 2.6: **Salivary Samples for Cortisol Metabolite Analysis**

Sample	No. Subjects	Notes
1	13	8 Pack / 5 pet
2	13	8 Pack / 5 pet
3	6	2 Pack / 4 pet; not all animals could be sampled
Total	32	Of 36 samples total, 4 had too low salivary volume for analysis

2.7 Exploration and Novel Object Test

We conducted a set of two open field tests, the first allowing free exploration of the enclosure, and then immediately following was the second test, which introduced a novel object. Each set of tests was conducted in three social conditions: alone, with a human, and with a close canine pack member.

The open field tests were conducted in outdoor enclosures at the Wolf Science Center in Ernstbrunn, Austria, from November through February 2016-2017, when the animals were approximately 2.5 years old. One subject completed the tests in March, due to owner illness. The enclosures measured approximately 2000 square meters. Five enclosures in total were used so that each test condition was performed in an enclosure unfamiliar to each subject. (See Figure 2.2.) Subjects participated in multiple conditions, and a different novel object was used for each condition (see Figure 2.3.). The enclosures and the novel objects were pseudo-counter-balanced across the two groups (see Table 2.7). Subjects participated in one test per day.

TABLE 2.7: **ENOT: Condition and Novel Object Order**

Dog	**Conditions**			**Novel Objects**		
	Test 1	*Test 2*	*Test 3*	*Test 1*	*Test 2*	*Test 3*
Banzai	Human	Canine	Alone	Circus Tent	Green Box	Colorful Baskets
Enzi	Canine	Alone	Human	Frog Toy	Circus Tent	Green Box
Pepeo	Alone	Human	Canine	Colorful Baskets	Circus Tent	Duck Toy
Panya	Alone	Human	Canine	Green Box	Duck Toy	Colorful Baskets
Hiari	Canine	Alone	Human	Duck Toy	Circus Tent	Green Box
Imara	Human	Canine	Alone	Green Box	Colorful Baskets	Circus Tent
Sahibu	Human	Alone	Canine	Circus Tent	Green Box	Frog Toy
Gombo	Canine	Human	Alone	Duck Toy	Colorful Baskets	Circus Tent
Jitu	Human	Alone		Green Box	Circus Tent	
Kisa	Alone	Human		Frog Toy	Colorful Baskets	
Cheza	Human	Alone		Circus Tent	Green Box	
Tuuka	Alone	Human		Colorful Baskets	Duck Toy	
Zazou	Human	Alone		Duck Toy	Green Box	
Kiatu	Alone	Human		Colorful Baskets	Frog Toy	

Each test consisted of two 3-minute phases: exploration of the enclosure, and exposure to a novel object. After the first phase, the dog was called back to a holding area so that he or she could not see the experimenter place the novel object in the enclosure before the second phase.

Pack dogs participated in three conditions of this 2-phase test. In one condition, the dog participated in the test while alone (“alone condition”). In the other two conditions, the dog participated with a social partner present in the enclosure. In the “human condition”, the dog was paired with a trainer with whom the dog shared a close relationship, as reported by the training staff. The human was instructed to casually walk around the enclosure in the same manner during each phase, and to acknowledge the dog only briefly should the dog solicit attention. In the “canine condition”, the dog was paired with an older dog from the pack with whom the subject dog shared a close relationship, as reported by the training staff. Table 2.7 shows each subject and their order of condition and novel objects.

The tests were video recorded by the experimenter and an assistant. Behavioral elements were then coded by the experimenter with the free software program Solomon Coder (Version beta 17.03.22, Andras Peter 2006-2017). We coded behavioral elements related to exploration of the environment, interaction with a novel object, interactions with the canine or human partner, and signs of stress. A list of the variables coded can be found in Table 2.8.

TABLE 2.8: **Exploration and Novel Object Tests Ethogram**

Behavior	**Description**
Active	Dog is walking, trotting, running
Sniffing	Dog is sniffing the ground or other environmental feature (not including novel object)
Proximity	Within two body lengths of partner
Contact	Dog makes physical contact with partner
Manipulate Object[1]	Manipulating object (contact with, mouthing, peeing, etc)
Sniffing Object[1]	Sniffing the novel object (within one body length)
Out of Sight	Dog is out of sight
Approach	Dog first sees object and approaches to within one body length
Stress Signals	Lip lick, paw raise, looking away from stimulus, yawn, sniff ground suddenly, shake-off, self-grooming/scratching
Gaze Partner[2]	Individual looks in the direction of the partner
Gaze Object	Individual looks in the direction of the object
Vocalization	Bark, whine, growl
Caution/Fear-Related Behavior	Tail lowered, head lowered with weight shifted backward, rounded back with hindquarters tucked, jumping away, risk assessment
Door Jump	Dog jumps on or scratches door
Proximity to Door	Dog within 2 body lengths of enclosure door

[1] These variables were combined for analysis due to few occurrences.

[2] This variable was not analyzed as it was too difficult to identify on video.

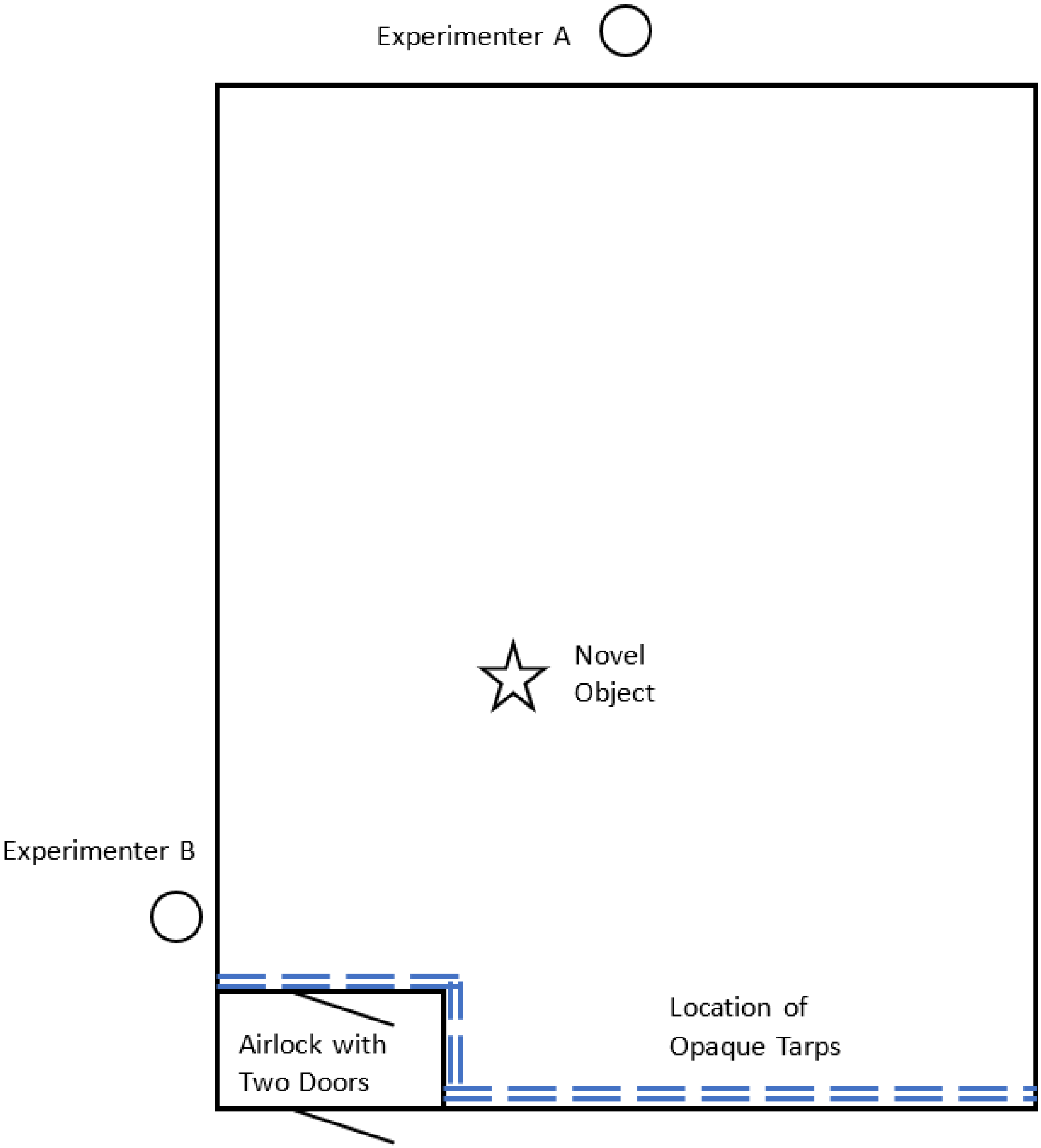

FIGURE 2.2: **Schematic of Exploration and Novel Object Test Field.**

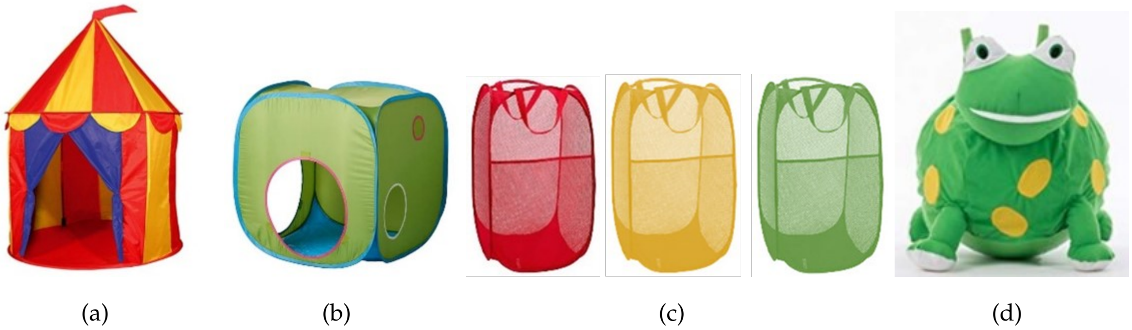

FIGURE 2.3: **Photographs of ENOT Objects.** (a) Circus Tent. (b) Green Box. (c) Colored Baskets. (d) Frog Toy; Duck Toy was the same general shape and size and was bright yellow. No animal was tested with both the Duck and the Frog toys.

(a) (b) (c)

FIGURE 2.4: **Photographs of SST and ENOT Testing Methods.** (a) Banzai with the stranger in the SST. (b) Panya exploring with a pack mate during the Exploration Test. (c) Sahibu investigates the novel object.

2.8 Statistical Analysis

All statistical tests were conducted using R version 3.1.1 (R Development Core Team). Plots of linear model residuals against fitted values were reviewed for homoscedasticity. Normality was assessed by review of the model residuals' q-q plots, and the data were transformed to achieve homoscedasticity and normality of the residuals when needed.

2.8.1 Longitudinal *OXTR* Methylation

Subject age was grouped into three ranges, Pre-adoption (4 weeks – 8 weeks old), Post-Adoption (9 weeks – 55 weeks old), and Adult (139 – 152 weeks). To investigate the level of methylation of the *OXTR* promoter regions in the Pet and Pack dogs across their life, we constructed Linear Mixed-Effects Models with Maximum Likelihood [R package lme4 (Bates et al., 2014), function lmer], with the methylation level as the dependent variable, litter as a fixed effect, group and age range at the time of sample as interaction terms, and subject as a random intercept.

We then constructed post-hoc models to investigate the interaction between group and age range. To investigate group differences within each specific age ranges we constructed models with the methylation level as the dependent variable, litter and group fixed effects, and subject as a random intercept, for each age range. We also constructed post-hoc models to investigate the change in methylation level over the entire time range within each group. The models were constructed with the methylation level as the dependent variable, litter and age at the time of sample as fixed effects, and subject as a random intercept.

2.8.2 Strange Situation Test

Kovacs Scoring

We fit Generalized Linear Mixed-Effects Models using a Poisson distribution [R package lme4 (Bates et al., 2014), function glmer]. Litter and Group were fixed effects, and Subject a random intercept.

Separation and Reunion Test

A second analysis was used to evaluate behavioral variables during three of seven phases from the Strange Situation Test: Baseline, Separation, and Reunion (Separation and Reunion Test). We fit a Linear Mixed-Effects Models with Maximum Likelihood [R package lme4 (Bates et al., 2014), function lmer] for the dependent variables Exploration, Passive, Contact, and Proximity, with Litter as a fixed effect, Group and Phase as an interaction term, and Subject as a random intercept. A centralization term was also created for Phase and fit as a random effect. Two versions of this model were compared with each other, one which allowed the random effects to be random slopes and random intercepts, and one which restricted the random effects to random intercepts only. We used the log-likelihood function logLik (R package MASS) to select the model with the largest score. This model was then compared with the null model using the anova function (R package car). If the model had more explanatory power than the null model, then it was further reduced by stepwise removing interaction terms with p-values > 0.05. Fixed effects remained in the final model, even if they were not statistically significant. Post-hoc linear models to investigate interactions were constructed with Litter as a fixed effect [built-in R function lm], and by separating the data sets by Phase or by Group, accordingly.

We fit Linear Models [built-in R function lm] for Stand at Door and Look at Door, which occurred only in the separation phase, with Litter and Group as the fixed effects. We fit Generalized Linear Mixed-Effects Models using a binomial distribution [R package lme4 (Bates et al., 2014), function glmer] for response variables Shake and Lip Lick/Yawn, with Litter as a fixed effect, Group and Phase as an interaction term, and subject as a random effect. The variables Play and Arousal had too few occurrences to model. We fit Generalized Linear Mixed-Effects Models using a Poisson distribution [R package lme4 (Bates et al., 2014), function glmer] for response variable Vocalization, with Litter as a fixed effect, Group and Phase as an interaction term, and subject as a random effect.

Salivary Cortisol Analysis

We conducted a two-way ANOVA for unbalanced designs [R package car (Fox et al., 2020), function Anova] to analyze the change in cortisol metabolites before and after the Strange Situation Test. For post-hoc analysis, we used Tukey's HSD to examine differences in group means before and after the SST [built-in R function TukeyHSD].

The dependent variable was the cortisol metabolite measurement, and the predictive variables were the group and the order of the sample (before or after the SST). This test was conducted with the outlier removed.

Dynamic *OXTR* Methylation After Social Stress

We fit a Linear Mixed-Effects Models with Maximum Likelihood [R package lme4 (Bates et al., 2014), function lmer] to analyze the change in methylation of the *OXTR* promoter regions in dogs before and after the Strange Situation Test. The dependent variable was the methylation level, and the predictive variable was the order of the sample (before or after the SST), with the subject as a random intercept.

We ran two models. The first time the model was fit with all animals that had a valid methylation sample taken immediately before and immediately after the SST. For the second model, we used only animals that also had a rise in salivary cortisol metabolites, as a control that the test induced a physiological stress response. A cortisol metabolite rise was defined as greater than 0.2 ng/mL, the sensitivity of our assay.

2.8.3 Exploration and Novel Object Test

We investigated whether behavioral variables associated with exploration and neophobia were affected by Group (i.e. Pet or Pack) and by the social partner Condition (i.e. tested Alone, with Human Partner, with Canine partner) by fitting linear models to the data. We constructed these models in two sets. The first set of comparisons (Human Partners) was between the dog tested alone and tested with a human partner (owner for the Pet dogs, and trainer for the Pack dogs). The second comparison (Pack) was within the Pack dogs, comparing their behavior when tested alone, with their trainer, and with their packmate. As Pet dogs did not have close, regular canine friends, we did not test Pet dogs with a canine social partner. Normality of model residuals' q-q plots was assessed for each response variable, and data transformed where appropriate (see Appendix).

We used Litter as a fixed effect, Group, Test (Exploration and Novel Object), and Condition (Alone, Canine Partner, Human Partner) as a three-way interaction term, and the Subject as a random effect. Centralization terms were created for Test and Condition, and included as random effects. Two versions of this model were compared with each other, one which allowed the random effects to be random slopes and random intercepts, and one which restricted the random effects to random intercepts only. We used the log-likelihood function logLik (R package MASS) to select the model with the largest score. This model was then compared with the null model using the anova function (R package car). If the model had more explanatory power than the null model, then it was further reduced by stepwise removing interaction terms with p-values > 0.05. If the 3-way interaction was not significant, we included Condition*Group and Group*Test but not the interaction term Condition*Test, as it was not relevant to the research question. Fixed effects remained in the final model, even if they were not statistically significant. For response variables "Activity", "Proximity to partner", "Proximity to door", "Sniffing", "Approaching the novel object", "Gaze at the novel object", and "Sniffing and manipulating the novel object", we fit Linear Mixed-Effects Models with Maximum Likelihood [R package lme4 (Bates et al., 2014), function lmer].

For the response variables "Caution and Fear" and "Stress" we fit Generalized Linear Mixed-Effects Models using a binomial distribution [R package lme4 (Bates et al., 2014), function glmer].

Four response variables were not modeled. There were not enough instances of "Contact" and "Vocalization" to construct a model. The residuals on the q-q plot for "Door Jump" could not be transformed. Finally, “Gaze partner” was not modeled as it was determined during the coding process that it was not possible to accurately discern the behavior on the videos.

Post-hoc models were constructed to further investigate significant interactions that emerged from the main models (three-way interaction between Group, Condition, and Test; two-way interactions between Group and Condition or between Group and Test), using Litter as a fixed effect [built-in R function lm], and by separating the data sets by Test or by Group, accordingly.

3. Results

3.1 *OXTR* Methyation

We found statistically significant interactions between the age of the dog and the group for all four loci (LMER, $p < 0.05$).

Post-hoc analyses were used to investigate interactions between group and age. During the pre-adoption time period there was no difference between Pet and Pack dogs in methylation percentage of OXTR, except at locus -751 where Pet dogs had a higher percentage of methylation (LMER, estimate (95% CL) = 7.32 (0.99, 13.64), t = 2.27, p = 0.03). During the post-adoption time period, Pet dogs had a lower percentage of OXTR methylation at loci -1383 (LMER, estimate (95% CL) = -2.65 (-4.85, -0.46), t = -2.37, p = 0.02) and -727 (LMER, estimate (95% CL) = -14.09 (-24.27, -3.90), t = -2.71, p = 0.01), and there were no differences between groups at loci -1371 and -751. When tested at 2.5 years of age, Pet dogs had lower percentage of OXTR methylation at locus -1371 (LMER, estimate (95% CL) = -1.59 (-3.03, -0.15), t = 2.16, p = 0.04), and there was no difference between groups at the other three loci.

We also investigated the longitudinal change in OXTR methylation level from puppyhood through 2.5 years of age within each group. There was no significant effect of time for Pack dogs except at locus -751, where Pack dogs had significantly higher methylation at 2.5 years than at preadoption (LMER, estimate (95% CL) = 5.77 (1.43, 10.10), t = 2.61, p = 0.01).

There was a significant effect of time in three loci for Pet dogs, with Pet dogs having lower methylation post-adoption versus pre-adoption (LMER locus -1383, estimate (95% CL) = -4.44 (-7.46, -1.43), t = -2.89, p = 0.004), (LMER locus -727, estimate (95% CL) = -21.09 (-32.67, -9.50), t = -3.57, p = 0.0004), (LMER locus -751, estimate (95% CL) = -10.34 (-18.67, -2.01), t = -2.43, p = 0.02), and lower levels at two years versus pre-adoption (LMER locus -1371, estimate (95% CL) = -2.61 (-3.84, -1.38), t = -4.16, p = 0.0001), (LMER locus -727, estimate (95% CL) = -17.60 (-30.60, -4.59), t = -2.65, p = 0.01). In Pet dogs, there was also a significant difference between age post-adoption and two years, with post-adoption having higher methylation levels (LMER locus -1371, estimate (95% CL) = 1.46 (0.19, 2.73), t = 2.26, p = 0.03).

TABLE 3.9: **Descriptive Statistics of *OXTR* Methylation Percentage at Four Loci**

	-1383	**-1371**	**-727**	**-751**
Min	80.0	76.0	11.3	5.5
Max	98.3	97.5	89.0	52.5
Median	87.0	85.0	41.0	28.3
Mean	87.2	85.2	40.1	28.9
SD sample	3.0	2.6	13.3	7.1

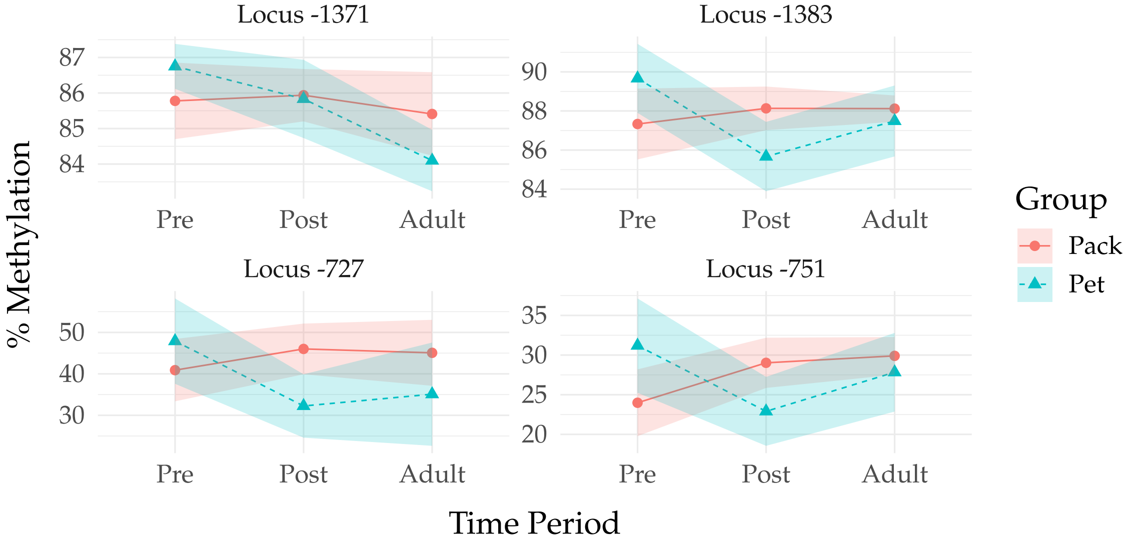

FIGURE 3.5: **Longitudinal *OXTR* Methylation**
Percentage of *OXTR* methylation at four loci for pre-adoption, post-adoption, and adult time periods.

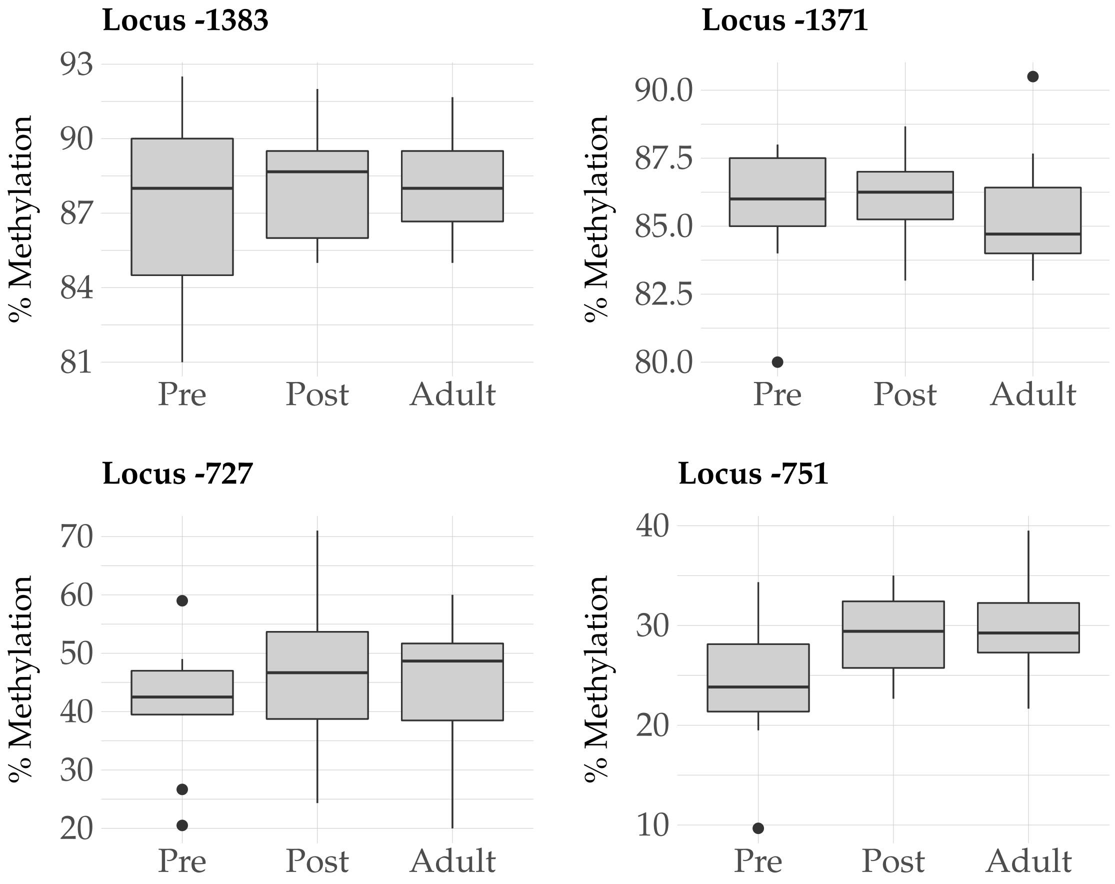

FIGURE 3.6: **Pack Dog Longitudinal *OXTR* Methylatio**n
Percentage of *OXTR* methylation at four loci for pre-adoption, post-adoption, and adult time periods in Pack Dogs.

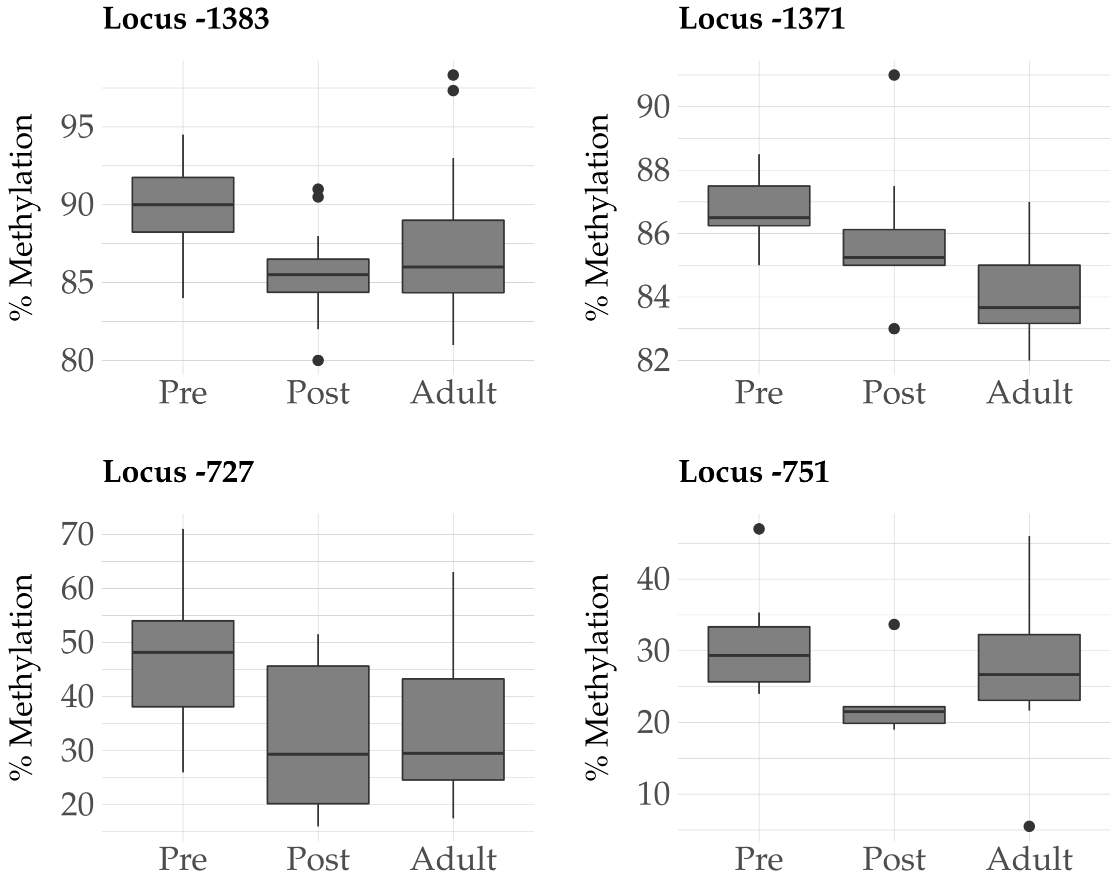

FIGURE 3.7: **Pet Dog Longitudinal *OXTR* Methylation**
Percentage of *OXTR* methylation at four loci for pre-adoption, post-adoption, and adult time periods in Pet Dogs.

3.2 Strange Situation Test

3.2.1 Kovacs Scoring

The generalized linear mixed-effects model was not statistically significantly different from the null model for all domains (Anxiety, Attachment, and Acceptance).

3.2.2 Separation and Reunion Test (SRT)

Below, I report those results with a significance of $p < 0.05$. Please see the Appendix for full results.

Explore

We found that all dogs explored more in the Baseline phase than the Separation phase (LMER, estimate (95% CL) = -6.02 (-8.04, -3.99), $p < 0.001$) and the Reunion phase (LMER, estimate (95% CL) = -5.68 (-7.63 -3.74), $p < 0.001$), with no significant differences found between Separation and Reunion phases.

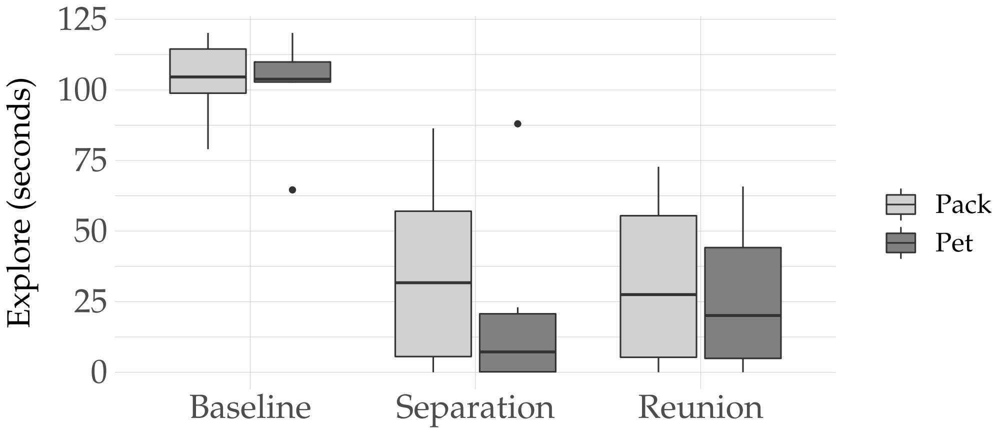

FIGURE 3.8: **Explore**
Time spent exploring during the Separation and Reunion Test.

Contact

All dogs had more contact with the human in the Reunion phase versus the Baseline phase (LMER, estimate (95% CL) = 14.01 (9.16, 18.87), p < 0.001).

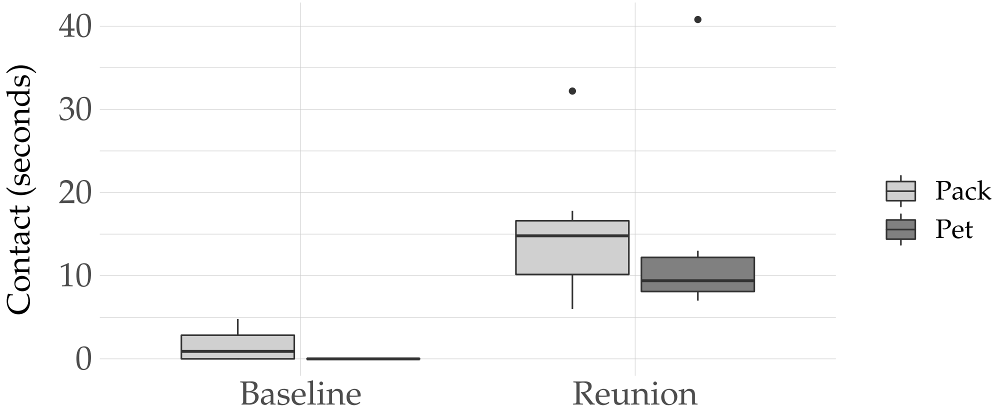

FIGURE 3.9: **Contact**
Time spent in contact with the human during the Separation and Reunion Test.

Passive

Dogs spent the most amount of time passive in the Separation phase when compared to the Baseline phase (LMER, estimate (95% CL = 79.9 (60.37, 99.43), p < 0.001) as well as more time passive in the Reunion phase compared to the Baseline phase (LMER, estimate (95% CL) = 57.31 (37.78, 76.85), p < 0.001). The difference between the Reunion and Separation phases is also statistically significant, with dogs spending less time passive in the Reunion phase (LMER, estimate (95% CL) = -22.59 (-42.12, -3.05), p < 0.05.)

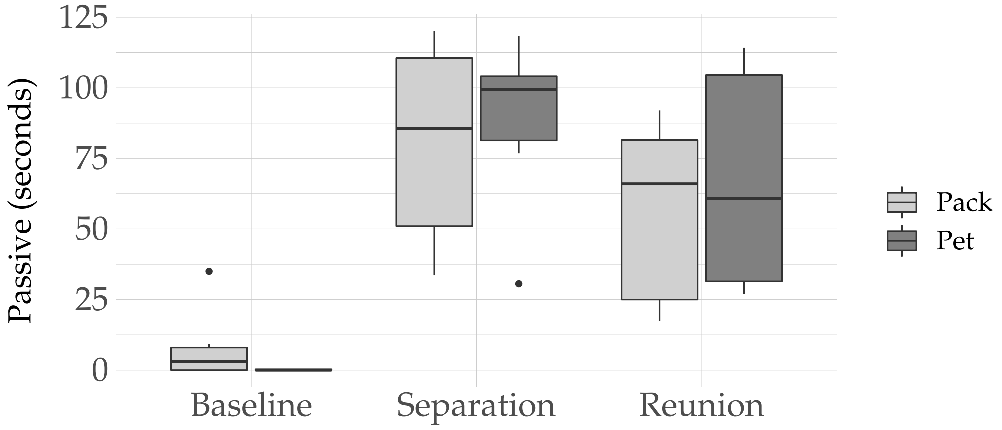

FIGURE 3.10: **Passive**
Time spent passive during the Separation and Reunion Test.

Proximity to Partner

There was a significant interaction between Group and Phase with respect to proximity to the owner/trainer (LMER, estimate (95% CL) = -27.93 (-54.21, -1.64), t = -2.08, p < 0.05) that we investigated with post-hoc models. We found that among Pack dogs only, subjects spent more time in proximity to the owner/trainer in the reunion phase (LMER, estimate (95% CL) = 32.12 (10.60, 53.66), p < 0.05), while there was no such change in the Pet dogs.

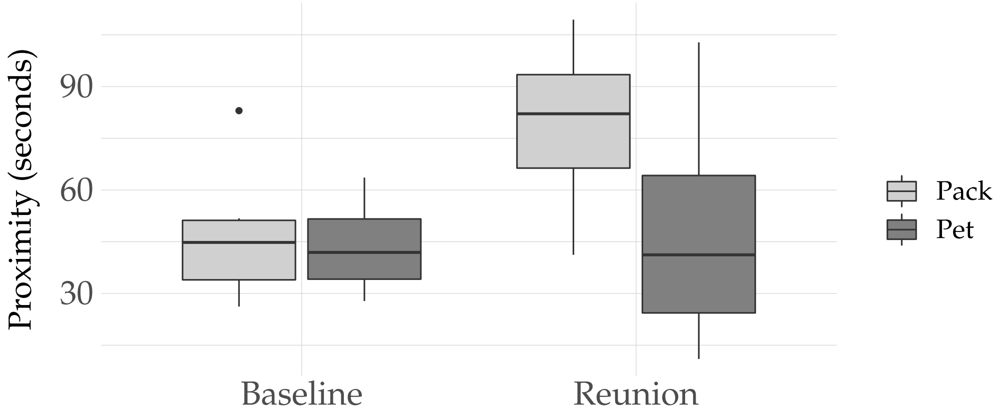

FIGURE 3.11: **Proximity to Partner**
Time spent in proximity to the owner/trainer during the Separation and Reunion Test.

Stand at Door

Pet dogs spent more time standing in front of the door in the Separation phase than did Pack dogs (LMER, estimate (95% CL) = 40.95 (6.00, 75.90), p < 0.05).

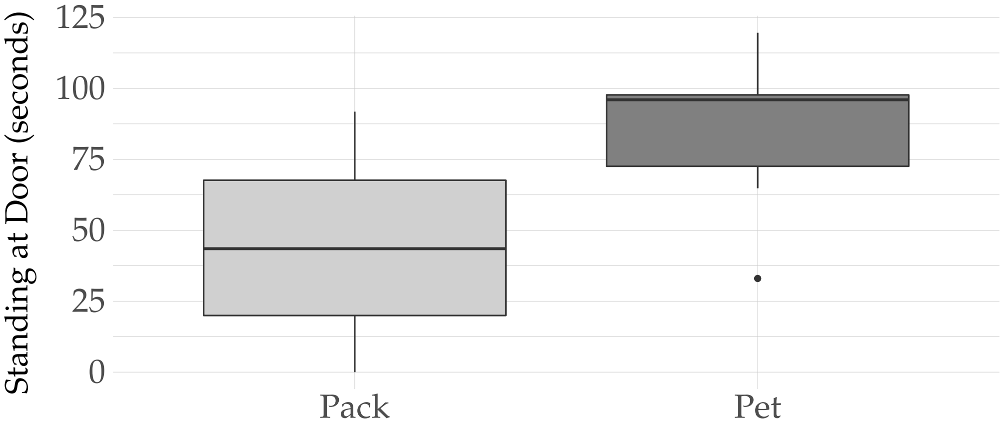

FIGURE 3.12: **Stand at Door**
Time spent standing at the door during the Separation phase of the Separation and Reunion Test.

Vocalization

There was a significant interaction of Group and Phase for the variable vocalization (LMER, $\chi^2 = 11.399 df = 2, p < 0.01$).

Pet dogs vocalized more than Pack dogs during the Separation phase (LMER, estimate (95% CL) = 16.67 (4.77, 28.56), p < 0.05).

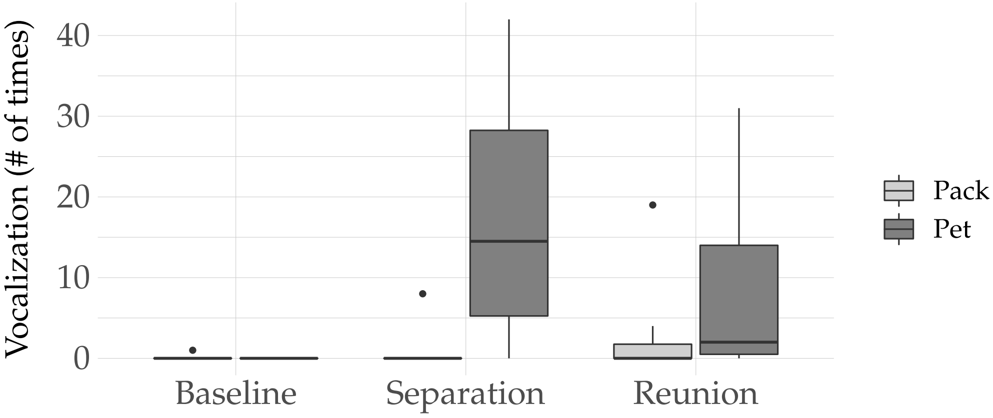

FIGURE 3.13: **Vocalization**
Number of vocalizations during the Separation and Reunion Test.

Pace

All dogs spent more time Pacing in the Reunion phase than in the Baseline phase (LMER, estimate (95% CL) = 24.90 (18.76, 31.04), $p < 0.001$). They also paced more in the Reunion phase than the Separation phase (LMER, estimate (95% CL) = 22.69(16.54, 28.82), $p < 0.001$). There was no statistically significant difference between the Baseline and Separation phases.

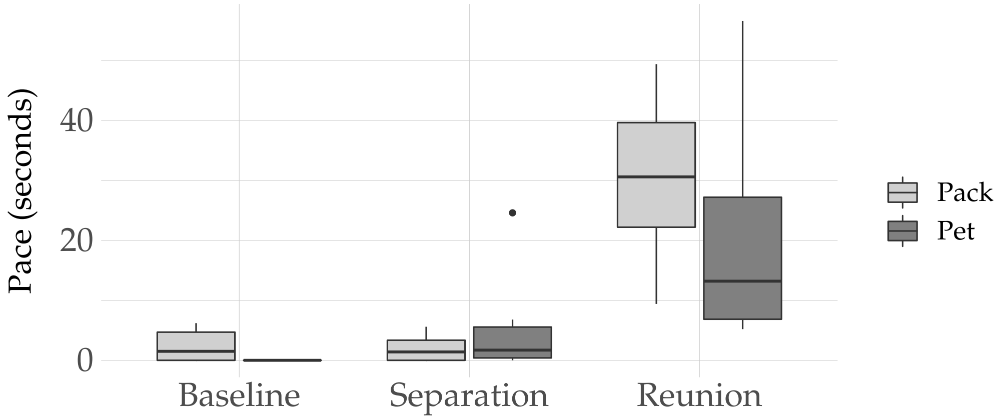

FIGURE 3.14: **Pace**
Time spent pacing during the Separation and Reunion Test.

Non-significant Variables

There were no statistically significant differences for the variables Looking at the Door, Shake, and Yawn/Lip Lick. Arousal and Play were not modeled due to too few occurrences. Please see the Appendix for full results.

See 3.10 for a summary of all significant results from the Separation and Reunion Test.

TABLE 3.10: **Separation and Reunion Test Summary**

Response Variable	
Explore	Baseline > Separation & Reunion
Contact	Reunion > Baseline
Pace	Reunion > Separation & Baseline
Passive	Separation > Reunion > Baseline
Proximity	Group:Phase
	Reunion > Baseline (within Pack dogs only)
Stand at Door	Pet > Pack
Vocalization	Group:Phase Interaction
	Pet > Pack in Separation phase

3.2.3 Cortisol Results

In total, 36 salivary samples were taken, 4 of which had too low of salivary volume and could not be extracted. There were only 6 animals for whom three consecutive salivary samples were available, due to experimenter error in the procedure, so these data were not analyzed. We analyzed cortisol metabolite data for 12 subjects for whom salivary samples were taken before the SST, and immediately after the SST. These results are in 3.11.

TABLE 3.11: **Individual Concentrations of Salivary Cortisol Metabolites (ng/mL)**

Subject	Before SST	After SST	Delta	Group
Banzai	0.2	0.9	0.7	Pack
Enzi	0.2	4.01	3.81	Pack
Gombo	0.2	0.78	0.58	Pack
Hiari	2.1	3.08	0.98	Pack
Imara	0.68	4.28	3.6	Pack
Pepeo	0.2	0.23	0.03	Pack
Sahibu	1.49	2.7	1.21	Pack
Panya*	6.37	0.2	-6.17	Pack
Jitu	0.2	0.2	0	Pet
Kiatu	0.2	0.2	0	Pet
Kisa	0.2	2.18	1.98	Pet
Zazu	0.2	0.2	0	Pet

*Our salivary cortisol metabolite assay is sensitive to 0.2 ng/mL.
Therefore, any concentration between 0.0 and 0.2 may be read as 0.2 ng/mL.

**Outlier

TABLE 3.12: **Mean Concentrations of Salivary Cortisol Metabolites Before and After SST**

Mean Cortisol Metabolites ng/ml	Pack	n	Pet	n	All	n
Before SST (mean ± SD)	0.72 ± 0.77	7	0.2 ± 0	4	0.53 ± 0.65	11
After SST (mean ± SD)	2.28 ± 1.64	7	0.66 ± 0.99	4	1.71 ± 1.60	11
Before/After Delta	1.56 ± 1.51	7	0.46 ± 0.99	4	1.17 ± 1.40	11
30 Min After	1.84 ± 1.95	2	0.45 ± 0.6	4	0.76 ± 0.15	6

**Values given with outlier (Panya) removed.

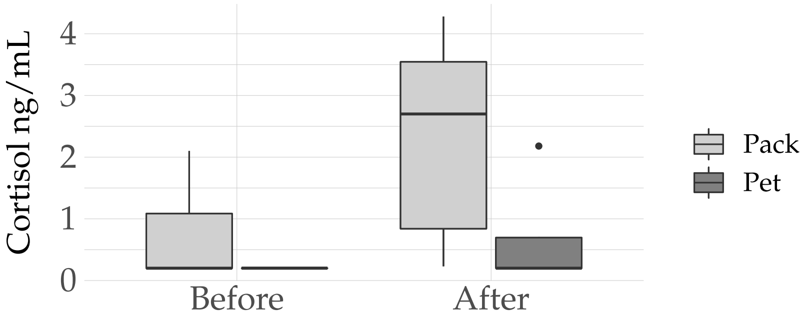

FIGURE 3.15: **Concentration of Salivary Cortisol Metabolites Before and After SST**
Cortisol metabolites for pack and pet animals taken before and after the SST. Outlier (Panya) removed.

We found that dogs had a significant difference in cortisol metabolite measurements before and after the SST (ANOVA (F(1, 11) = 5.95, p = 0.024), as well as between groups (ANOVA (F(1, 11) = 4.47, p = 0.048), with Pack dogs having a larger increase in salivary cortisol metabolite concentration after the SST than Pet dogs. There was not a significant interaction between group and sample order. A post-hoc Tukey's HSD showed no significant differences between Groups in the Before SST and After SST samples.

3.3 Dynamic *OXTR* Methylation in Response to a Social Stress Test

There was no statistically significant change in methylation of OXTR at any of the four loci when we fit our model with all paired samples.

When we categorized animals into two groups, one that showed a clear rise in cortisol metabolites and one that did not, we found a statistically significant interaction between sample order and cortisol category (LMER, estimate (95% CL) = -4.00 (-5.77, -2.25), t = -4.46, p = 8.32 e-6) at locus -1371, with the cortisol metabolite rise group having significantly higher methylation percentages after the test (paired t test, estimate (95% CL) = 2.34 (1.31, 3.36), t(4) = 6.35, p = 0.003), and no significant methylation change for the group without a cortisol metabolite rise.

We did not find any changes in methylation of OXTR at the other three loci.

For the -1371 model, we began with paired methylation samples for 9 dogs. Of these, 5 had a rise in cortisol metabolites following the SST, 2 did not, and 2 dogs did not have analyzable salivary samples. The average rise for the former group (n=5, all Pack dogs) was 36%, with a range of 0.7-3.81 ng/mL.

See 3.13 for a summary of all significant *OXTR* methylation results.

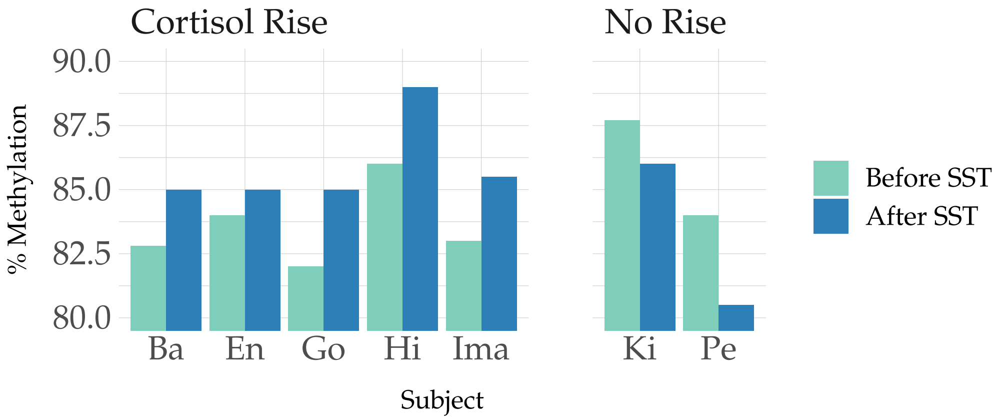

FIGURE 3.16: **Dynamic Methylation of OXTR Locus -1371**
Methylation levels of dogs that showed a rise in cortisol metabolites after the SST (left) and those that did not (right).

TABLE 3.13: ***OXTR* Methylation Summary**

	Interactions	Group (Post-hoc)	Age (Pet, Post-hoc)	Age (Pack, Post-hoc)	SST Test Order
-1383	Age:Group	Pet < Pack (Post-Adoption)	Pre-adoption > Post-adoption	ns	ns
-1371	Age:Group	Pet < Pack (Two Years)	Pre-adoption > Two Years Post-adoption > Two Years	ns	After > Before
-727	Age:Group	Pet < Pack (Post-Adoption)	Pre-adoption > Post-adoption Pre-adoption > Two Years	ns	ns
-751	Age:Group	Pet > Pack (Pre-Adoption)	Pre-adoption > Post-adoption	Two Years > Pre-adoption	ns

3.4 Exploration and Novel Object Tests

Below, I report those results with a significance of $p < 0.05$. Please see the Appendix for full results.

Active

There was a statistically significant interaction between Test and Group in the Human Partners comparison (LMER, estimate (95% CL) = -1.03 (-2.02, -0.04), t = -2.05, $p < 0.05$), with both groups being more active during the Novel Object test than during the Exploration test (LM Pet Dogs, estimate (95% CL) = 1.62 (0.52, 2.71), t = 2.89, $p < 0.01$), (LM Pack Dogs, estimate (95% CL) = 13.33 (1.51, 25.15), t = 2.21, $p < 0.05$). While this effect was stronger in Pack dogs than in Pet dogs, it was not significantly different.

The model for the Pack comparison was not significantly different from the null model.

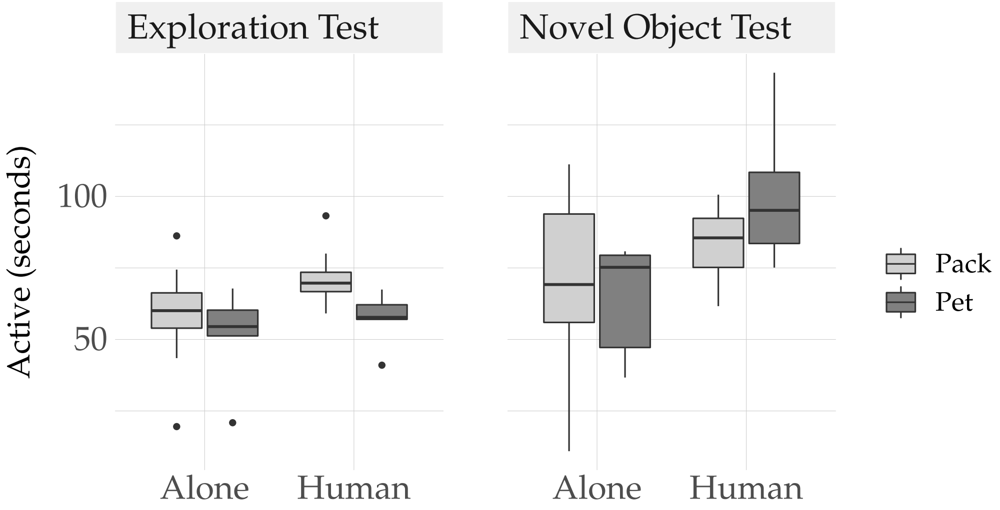

FIGURE 3.17: **Active**
Time spent active during the Exploration and Novel Object Tests.

Sniffing

In the Human Partners comparison, there were significant two-way interactions between Group and Condition (LMER, estimate (95% CL) = -1.61 (-3.12, -0.11), t = -2.10, p < 0.05). Post-hoc analysis showed that Pet dogs sniffed more when tested with a partner than when tested alone (LM Pet, estimate (95% CL) = 1.80 (0.67, 2.94), t = 3.12, p < 0.01), whereas there was no difference in Pack dogs tested alone, with a trainer, or with a canine partner. Pet dogs also sniffed more with their owners than Pack dogs did with their trainers (LM Group, estimate (95% CL) = 2.14 (1.07, 3.22), t = 3.91, p < 0.001), and Pet dogs sniffed more than Pack dogs in the Exploration Test (LM Group, estimate (95% CL) = 0.99 (0.18-1.80), t = 2.39, p < 0.015).

The Pack comparison model was not significantly different from the null.

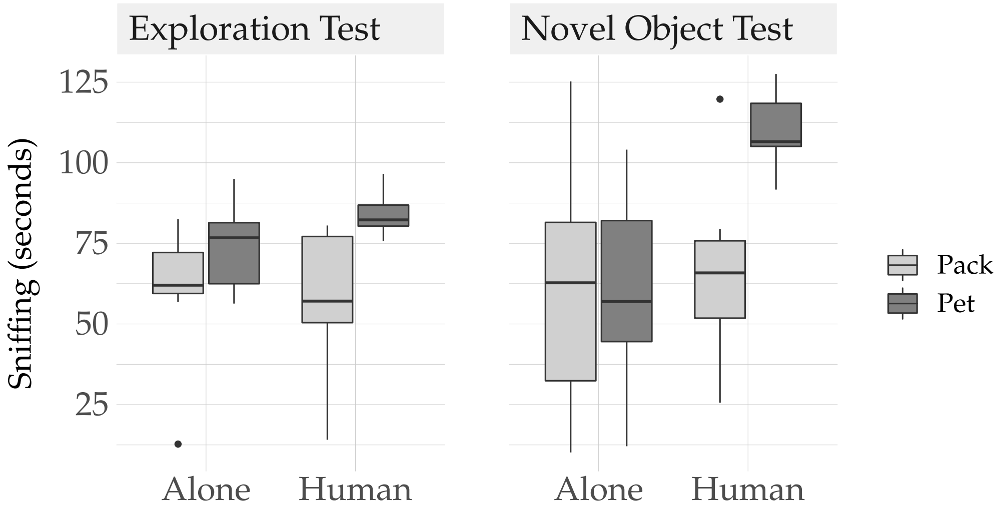

FIGURE 3.18: **Sniffing**
Time spent sniffing during the Exploration and Novel Object Tests.

Proximity to Door

In the Human Partners comparison, there was a significant interaction between Condition and Group (LMER, estimate (95% CL) = 1.00 (0.23, 1.78), t = 2.55, $p < 0.05$). Post-hoc analyses were not significant. Post-hoc analysis showed that in the Alone condition, Pack dogs spent less time near the door than did Pet dogs (LM Group, estimate (95% CL) = -1.22 (-2.03, -0.41), t = -2.96, $p < 0.01$). Within Pet dogs, they spent less time near the door when they were with their owner, than alone (LM, estimate (95% CL) = -1.44 (-2.15, -0.74), t = -4.00, $p < 0.001$).

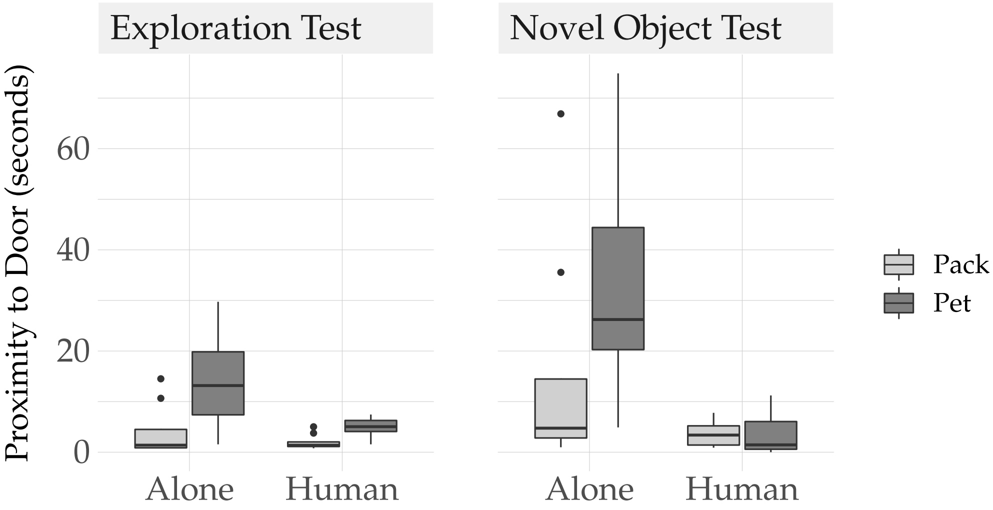

FIGURE 3.19: **Proximity to Door**
Time spent in proximity to the door during the Exploration and Novel Object Tests.

Stress Signals

All dogs showed fewer stress signals when they were tested a human partner rather than alone (GLMER, estimate (95% CL) = -3.06 (-5.70, -0.41), t = -2.26, $p < 0.05$). Pack dogs showed more signs of stress when with a canine partner than when tested alone (GLMER, estimate (95% CL) = -5.6 (-10.35, -0.85), t = -2.31, $p < 0.05$). In post-hoc analysis, Pack dogs showed fewer signs of stress than Pet dogs in the Exploration phase (GLM, estimate (95% CL) = -2.00 (-3.60, -0.40), t = -2.45, $p < 0.05$).

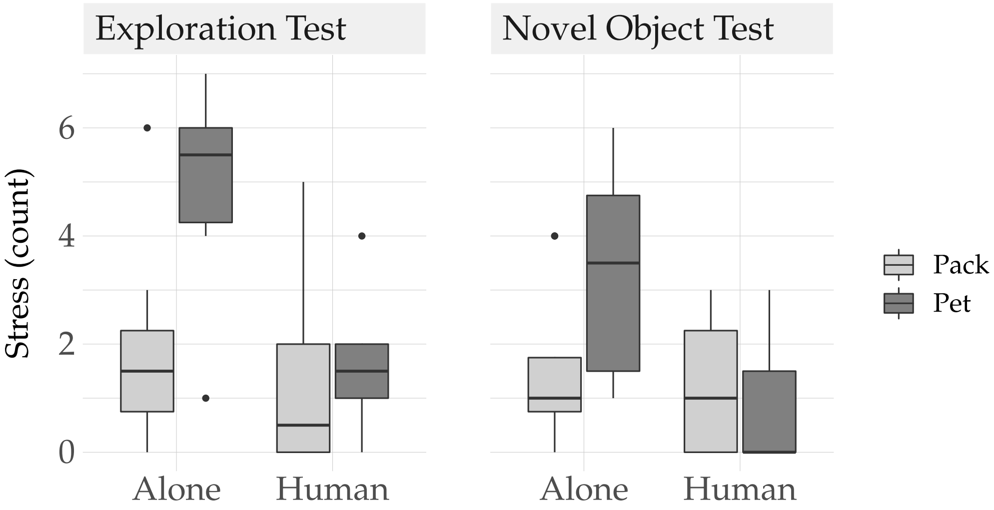

FIGURE 3.20: **Stress Signals**
Number of stress signals shown during the Exploration and Novel Object Tests.

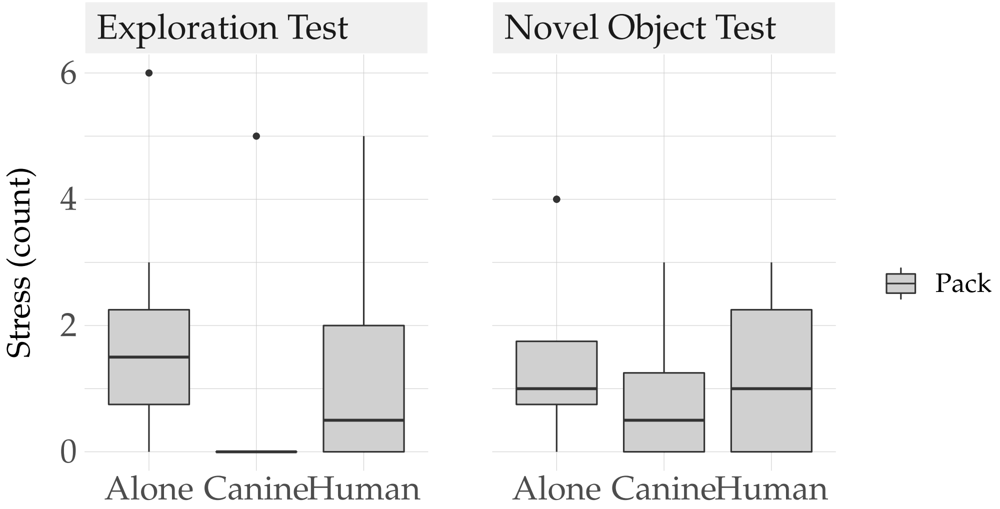

FIGURE 3.21: **Stress Signals in Pack Dogs**
Time spent active during the Exploration and Novel Object Tests in the pack dogs.

Non-significant Variables

No comparison models were statistically significantly different than the null model for the response variables Sniff and Manipulate Object, Caution and Fear, Gaze at Novel Object, Approach Novel Object, and Proximity to Partner.

See 3.14 for a summary of all significant results from the Exploration and Novel Object Tests.

TABLE 3.14: **Exploration and Novel Object Tests Summary**

Response Variable	**Human Partners** (Conditions: Partner, Alone)	**Pack Only** (Conditions: Human Partner, Canine Partner, Alone)
Active	Group:Test Interaction*	model ns
Proximity to Door	Group:Condition Interaction Pet > Pack (alone condition)	model ns
Sniffing	Group:Condition Interaction Condition:Test Interaction Pet > Pack (partner condition) Pet > Pack (Exploration phase) Owner > Alone (Pet dogs only)	model ns
Stress	Alone > Human Partner Pet > Pack (Exploration phase)	Alone > Canine Partner

*Post-hoc analyses were non-significant. †Variables not shown as model was not significantly different from the null model: Caution and Fear, Approach Object, Gaze Object, Sniff/Manipulate Object, and Proximity to Partner.

4. Discussion

This study explores the epigenetic *OXTR* methylation and the behavior of two groups of genetically related but differentially socialized dogs. In several species, the caregiving and socialization environment has been linked to *OXTR* methylation later in life; this is the first study to examine methylation levels longitudinally and dynamically in dogs. Additionally, we investigated several traits possibly associated with differential caregiving and *OXTR* methylation, including dimensions of attachment and stress-coping behaviors. We predicted that dogs living in pet homes would have lower methylation levels than dogs living in packs with more limited contact to humans, and that Pet dogs would show behavior associated with a strong attachment to a human partner, increased exploration and sniffing of a new environment, and decreased neophobia of the novel object.

In our longitudinal *OXTR* methylation analysis, we measured methylation of four loci at three separate time periods. We found evidence to support our hypothesis that differential socialization of Pet dogs is associated with lower *OXTR* methylation than in Pack dogs. Decreased methylation levels were seen during the Post-Adoption and Adult time periods only in Pet dogs. We found that post-adoption, Pet dogs had lower *OXTR* methylation than pet dogs at two loci (-1383 and -727). While at age two years these differences had dissipated, Pet dogs had lower methylation than Pack dogs at a third locus (-1371) at age two years. A previous study by Cimarelli et al (Cimarelli, 2017) found behavioral responses to the approach of a threatening stranger differentially associated with three of the four *OXTR* loci analyzed in this study (-1383 appeasement and passive behavior, -751 hiding behind the owner, and -727 reaction at the end of the test when the stranger is closest), indicating that these loci may have unique contributions to the behavioral phenotype.

While we observed, unexpectedly, that Pet dogs had higher *OXTR* methylation at one locus (-751), this was only at the pre-adoption time point, and the difference did not persist post-adoption and at age two years. It is unclear why Pet dogs may have had higher methylation levels than Pack dogs during puppyhood, as dogs in both groups were sharing the same environment at that time. Nevertheless, the disappearance of this difference post-adoption still supports our general prediction. Although at this locus there was no statistically significant difference between Pet and Pack dogs' methylation after adoption, the disappearance of the pre-adoption difference, due to Pet dogs' methylation levels lowering, indicates an additional effect of the socialization environment on decreased *OXTR* methylation. With only a couple exceptions, we found that the methylation of the Pack dogs remained stable throughout their lives, whereas the Pet dogs' methylation decreased during certain periods after adoption. This suggests that the social and environmental input that the Pet dogs received was instrumental in shaping their epigenetic profile.

Methylation of different *OXTR* loci may be independent and differentially sensitive to the environment, allowing the animal to have a greater range of behavioral flexibility at critical time periods. The periods that we tested are associated with sensitive periods of development in domestic dog puppies, during which time there may be differential demands placed on the developing social system. The first period is the initial socialization period of puppies, which occurs around 3-6 weeks of age (Socialization Period 3-10 weeks, (J. P. Scott & Marston, 1950)), and during which all subjects experienced the same environment. The Post-Adoption range includes two fear periods (one ranging around 10-16 weeks (Developmental Period, (J. P. Scott & Marston, 1950)), and the other around 20 weeks) and sexual maturity* (occurring between 6 and 12 months, (J. P. Scott & Marston, 1950)), both of which have a great effect on social responses. Finally, the Two Years age range coincides with social maturity*, which again influences social behavior. (*While sexual and social maturity depend on size and breed, these estimates are accurate for our mixed-breed, medium/large size subjects.) In humans, for example, it has been found that epigenetic methylation of one *OXTR* locus of interest varies longitudinally in infants, while in mothers the percentage of methylation remains relatively stable (Krol, Moulder, Lillard, Grossmann, & Connelly, 2019), differences in infant methylation were effected by maternal caregiving (Krol, Puglia, Morris, Connelly, & Grossmann, 2019) and were related to the processing of social information (Krol, Puglia, et al., 2019). Another study found that self-reports of attachment were correlated with the level of *OXTR* methylation, but was mediated by age (young adults vs older adults), indicating a developmental aspect related to social maturity in humans (N. C. Ebner et al., 2019). Future studies might investigate the potential sensitivity of *OXTR* methylation in canines during sensitive periods of development in finer detail.

In addition to longitudinal analysis, we assessed if dogs would demonstrate a dynamic *OXTR* methylation response to a socially stressful test. We did not observe any group differences in methylation at any of the four loci when we assessed all animals. However, a critical component of the test is that the individual should experience social stress. The reference studies conducted in humans found that the Trier Social Stress Test induced a cortisol response measured immediately after the test (10 minutes of anticipating the test and 10 minutes of performing the test) was 42-125% greater than baseline (taken at -10 min) cortisol levels (Liu et al., 2017). Therefore, we categorized our subjects into two groups, one group that demonstrated a clear rise in cortisol (n=5, all Pack dogs), and one group that did not (n=2, one Pet dog and one Pack dog) in response to our 21-minute Strange Situation Test. Subsequently, we found that one *OXTR* locus increased in methylation percentage for dogs that had a rise in cortisol, but did not increase for the other dogs. This dynamic methylation was found in the locus (-1371) for which Pack dogs had greater methylation levels at the Adult period. As our dynamic test was also administered during this Adult period, it provides additional evidence that this particular locus may be differentially sensitive during adulthood. Alternatively, it may be a locus that is sensitive to psychosocial stress rather than environmental experience, as has been suggested regarding a particular *OXTR* sequence in humans that responds differentially to a Trier Social Stress Test but not low maternal care (Unternaehrer et al., 2012, 2015).

We also administered behavioral tests related to attachment and coping with stress. Although we found no differences between Pack and Pet animals when scoring the animals in three behavioral dimensions (Anxiety, Attachment, and Acceptance) based on their behavior in the Strange Situation Test, we did find several behavioral differences between the groups of dogs when using a more sensitive coding analysis. Some are likely due to non-social environmental differences such as experience in the Wildlife Park where we tested the animals, and experience with doors in pet homes, while others seem to support our hypothesis that Pet and Pack dogs had behavioral differences indicative of the quality and functions of their social attachments.

Attachment security would mediate a stress response during Separation, and we would expect that Pack dogs would show more stress during Separation because they are rarely left alone without human or canine company, whereas Pet dogs are likely left alone for several hours when their owners leave the home for work. However, we saw few behavioral differences indicative of stress. We did find that Pet dogs spent significantly more time than Pack dogs near the door during the Separation phase, and Pet dogs vocalized more than Pack dogs. This could be explained by the experience that Pet dogs have with their caretaker response's to barking and whining, whereas Pack dogs are systematically trained not to vocalize (e.g. ignored when barking, given treats when silent), as well as general experience waiting at doors.

A hallmark of attachment is that following a separation the subject seeks a balance of contact with the returning attachment figure and a return to exploration of the environment (Udell & Brubaker, 2016). While we found no differences between groups in the amount of activity, exploration, or contact seeking, we found that Pack dogs spent more time in Proximity to the human partner than did Pet dogs during the Reunion phase of the Separation and Reunion Test. Care must be used when interpreting proximity, as it can be an indicator of both secure and insecure attachment, depending upon its qualitative presentation. Exaggerated proximity seeking may indicate a less secure attachment between dogs and their owners (Wanser & Udell, 2019; Thielke & Udell, 2019), although in this study the observed increase in proximity may be qualitatively different from "exaggerated proximity-seeking". Given that Pet and Pack dogs spent the same amount of time in Proximity to their human partner in the Baseline phase, it might be that the secure base of the Pet dogs facilitated their return to baseline levels of Proximity upon reunion , whereas the Pack dogs' stress led to increased proximity seeking. Although we considered that Pack dogs might have stayed near their partner due to disinterest in the environment, the two groups of dogs did not differ in their play, activity, exploration, or stress signals, so the evidence that Pack dogs were more quickly bored than Pet dogs is less compelling.

Like the current behavioral results, an earlier study by Topal and colleagues (Topál et al., 2005) had also found that pet and hand-reared puppies had remarkably similar behavior in an SST administered at 4 months of age. Our findings here may corroborate that even given an additional year of differential socialization, individual differences have a greater effect on behavioral phenotype than that of these particular socialization environments. Perhaps greater differences might be found, for example, if we investigated more extreme differences in socialization, such as those experienced by laboratory beagles or puppy mill dogs. Finally, it is possible that the SST is relatively insensitive at detecting the degree of attachment; for example, a study by Gacsi and colleagues found that 3 sessions of contact between a shelter dog and a new person were enough to induce behavior indicative of an attachment bond when challenged with the SST (Gácsi et al., 2001).

However, we did find that Pet dogs had a smaller rise in cortisol metabolite in response to the SST compared to Pack dogs. This may indicate that the close relationship of pet dogs to their owners helped the pet dogs cope with the mild stress of the SST. A previous study by Schöberl et al had also found that cortisol reactivity was associated with attachment style, with dogs with a strong attachment having lower cortisol reactivity as measured as the difference between cortisol metabolite concentration immediately before and immediately after administration of the SST (Schöberl et al., 2016). Alternatively, a couple of other factors might have contributed to higher cortisol in Pack dogs. Pack dogs display great eagerness when working with their trainers, as their interactions are more limited, and this eustress could contribute to a rise in cortisol. They may have also experienced stress from being separated from their packmates, however, this would have been reflected in the "Before SST" samples, as the walk to arrive at the testing location takes at least 15 minutes.

The behavioral and hormonal evidence of attachment that we found in our earlier tests was further confirmed in the Exploration and Novel Object tests. We found that Pet dogs Sniffed more with their human partner than did Pack dogs when tested with their trainer, which may be evidence that a strong attachment to their owner facilitated sniffing in the Pet dogs. Pet dogs also sniffed more when tested with a partner rather than alone. Conversely, Pack dogs' Sniffing was the same, whether tested alone, with a human partner, or with a canine partner. As sniffing can be a sign of exploration (Topál et al., 1998; Prato-Previde et al., 2003; R. Palmer & Custance, 2008; Valsecchi, Previde, Accorsi, & Fallani, 2010), we have evidence of an attachment effect of the human partner for Pet dogs, which was not seen in the Pack dogs.

We saw an effect of test for the variable Sniffing. We found that Pet dogs tested with a human sniffed more than Pack dogs in the Exploration phase, again evidence of a secure base. Interestingly, Pet dogs sniffed the most when tested with a human partner in the Novel Object phase. This is surprising in that both the expectation and general trend was that the animals would sniff less in the Novel Object phase, as this was their second round in the testing enclosure, reducing novelty. We did not find any Group differences with respect to approaching, sniffing, manipulating, or gazing at the novel object. However, the presence of the object could have signaled to the dogs that they should re-investigate the environment, even if they did not investigate the novel object itself. The confidence to undertake re-investigation by Pet dogs may have been facilitated by their post-adoption caregiving experience or relationship with their human partner. Our findings regarding sniffing were not congruent with those of activity, even though it was meant to be another variable measuring exploration. We observed no difference in activity when we compared the Human Partner tests with the Alone condition. Instead, Pet dogs were more active than Pack dogs independent of being alone or with their owner.

Although it has previously been demonstrated that a conspecific enhanced the approach to and exploration of a novel object (Moretti et al., 2015), we did not replicate this effect with a human partner (note: the study by Moretti et al did not investigate exploration or movement unrelated to the novel object, so we cannot make a direct comparison). However, this result is possibly influenced by the fact that in our study, the human walked in the testing field in the same way regardless of whether there was an object or not, and did not show interest in the object. For example, dogs are likely to look to their owners when determining if they should approach a novel object or not (Merola, Prato-Previde, & Marshall-Pescini, 2012) and they use owners' movement toward or away from an unfamiliar person to provide referencing information (Duranton, Bedossa, & Gaunet, 2016).

In the Exploration Phase, Pet dogs showed a greater number of stress signals than did Pack dogs, though possibly because Pack dogs had more experience with exploring the grounds of the Wolf Science Center in general (which may explain their generally lower activity, as well). As predicted, in our Human Partner comparison, we found both dogs in both groups had reduced stress signals when they were tested with a Human Partner, versus when each dog was tested Alone. Looking only within Pack dogs, they showed more stress signals when tested Alone than with a Canine Partner, but there was no significant difference between being the Alone and Human Partner conditions. Previous work by Cimarelli and colleagues (Cimarelli et al., 2019) found that the species of a partner (canine or human) was not as influential on stress reactivity as is the quality of the relationship. Our evidence here suggests that the Pack dogs' extensive socialization with their packmates provides for increased stress-buffering than their relationships with their trainers.

Although Pet dogs showed more stress signals than Pack dogs, it is unlikely that this supports a causal link between greater fear or stress and the increased amount of time they spent active and sniffing when compared to Pack dogs. A previous study found that general fearfulness in dogs was not correlated with olfactory exploration, and furthermore, that individual variation was significantly responsible for determining whether a dog increased or decreased activity in response to fear (Goddard & Beilharz, 1984). Pet dogs may feel freer to display, or even be rewarded for displaying, stress in the presence of their owner. Thus, it is more likely that despite their increased stress, the attachment between Pet dogs and their owners facilitated the dogs' exploration of the environment.

We found some differences between Pet and Pack dogs that are likely due to their different adult experiences, though not necessarily related to their attachment. In the Exploration and Novel Object test, we tested if Pack and Pet dogs would show behavioral differences when tested alone and when tested with a social partner. We did not find any differences between groups when dogs were tested Alone, with one exception. Pet dogs spent more time in proximity to the enclosure door when tested alone, possibly because of their previous experience with waiting at doors. Pet dogs have many daily opportunities to wait for their owners behind doors, and are often left alone behind doors for extended periods of time when their owner leaves the home. The Pack dogs are rarely left alone, and when they are returned to their enclosures by the trainers, their canine Packmates are available company. However, Topal and colleagues (Topál et al., 2005) found that hand-raised puppies spent more time near the door than did pet puppies, which was possibly indicative of greater attachment in the extensively socialized hand-raised puppies. Presumably, as they were all raised in homes, both groups of puppies had equal experience with doors.

Overall, we did not find any differences in behavior for Pack dogs tested with canine and human partners, suggesting that their socialization with trainers was sufficient to provide as much social support and stress-buffering capability as their preferred pack member. However, we found evidence that the attachment between the Pet dog and owner outperformed the attachment of the Pack dog to the trainer. We observed that Pet dogs might have had more stress than the Pack dogs in the testing environment, likely because Pack dogs live in the park and have had the opportunity to pass the testing enclosures during walks. However, Pet dogs showed more exploratory behavior, indicating that their relationship with their owner effectively buffered their greater stress and thus facilitated sniffing. Ultimately, it seems likely that the extensive time that Pet dogs spend in their human household translates to a stronger attachment, leading to increased support to explore their environment.

The findings here that 1) the relationship with owners conferred better stress-buffering function than that with trainers, but trainers were equally as effective as packmates, and 2) pet dogs had a lower methylation rate than pack dogs, indirectly suggests that humans may be able to provide dogs with care and social support, superseding the functionality of a relationship with a preferred conspecific partner. The extensive socialization provided by pet dog owners may have capitalized on the pre-existing potential of the pet dogs' attachment system, resulting in a more robust stress-buffering response. Further, better controlled, studies need to investigate this hypothesis, as in our study the differences between the human-dog relationships in the two groups were confounded with other factors in the dogs' physical and social environments. For instance, while the pack dogs lived outdoors in a wildlife park and had a very stable social environment, consisting of the same canine and human individuals over months and even years, pet dogs lived mainly indoors in urban environments and had a more fluid social environment, as they encountered unfamiliar people and dogs throughout their regular activities in public or hosting visitors in their home.

There are a few points to consider when interpreting the results of this study. By necessity, we were compelled to use noninvasive salivary samples to collect *OXTR*, and methylation levels can vary depending on the type of sample (salivary, plasma, various types of tissue). Previous studies of *OXTR* methylation and behavior have found significant sexual dimorphism (in humans (Gouin et al., 2017; Rubin et al., 2016), in dogs (Cimarelli, 2017)). The potential effects of this dimorphic phenotype could effectively reduce our sample size by 50%, so we may not have been able to detect sex-related effects. Additionally, we have seen that changes in methylation level vary with age, and across locus, and with the type of test (this study and Cimarelli (Cimarelli, 2017)). In addition to considering these factors, this study may have benefited from a sharper focus on individual aspects of social behavior, rather than the more broad and non-social behaviors of exploration and investigation. This observation is supported by previous studies which have found that early experiences with caregiving behavior and early childhood environment are associated with social functioning, including attachment, social anxiety, and anger (Gouin et al., 2017; Murgatroyd et al., 2009; Zhang, Labonté, Wen, Turecki, & Meaney, 2013; Champagne et al., 2006; Zhang & Meaney, 2010; Roth, Lubin, Funk, & Sweatt, 2009; Weaver et al., 2004; McGowan & Szyf, 2010); however, see a cautionary review by Cecil (C. A. Cecil, Zhang, & Nolte, 2020)). Finally, these same studies compared control or healthy individuals with those who had suffered severe isolation, neglect, and trauma. While our pet and pack dogs experienced very different social environments, to our knowledge there was never any form of abuse, neglect, or trauma associated with the treatment of these animals. Additionally, the differences that we found in behavior were relatively small (e.g. the Kovacs scoring method (Kovács et al., 2018) did not yield group differences, but the more sensitive video coding did). Populations with more divergent early experiences may yield results with greater effects.

5. Conclusion

In this study, we examined variations in behavior and epigenetic methylation of the oxytocin receptor in two groups of dogs with different socialization environments. These dogs were genetically related and differentially socialized after adoption at the age of 8 weeks old.

We found differential methylation between the two groups, with Pet dogs having lower levels of *OXTR* methylation at three different loci and at three different times post-adoption, which may have been mediated by their extensive socialization with their owners. The methylation level of Pack dogs did not change over time, perhaps due to their predictable social environment. Pet dogs, on the other hand, had lower levels of methylation after adoption, and these levels were variable over time, which may have been facilitated by adaptation to a more complex social environment.

We found evidence of attachment-mediated stress-buffering in Pet dogs, who sniffed and explored more in an exploration task with their owner present, when compared to dogs raised in conspecific packs and tested with their trainers and with their packmates. We also found that after a stressful separation from their owner, Pet dogs' proximity preference was comparable to the baseline measurement, whereas Pack dogs increased their proximity preference, spending more time near their trainer rather than returning to Baseline levels. These behaviors, together with lower cortisol reactivity in Pet dogs in response to the Strange Situation Test, may suggest that Pet dogs are more likely to experience a stronger attachment to their owner than Pack dogs to their trainer.

Our results suggest that differential socialization of dog puppies may affect epigenetic methylation of the oxytocin receptor, which in turn may mediate attachment behaviors and can buffer stress in challenging situations similar to those investigated in this study. Future studies need to investigate whether, by extensive socialization with a primary caregiver or by providing experience with a wide variety of social experiences, human care can better prepare dogs for coping with certain challenges, possibly going beyond the stress-buffering potentials of conspecific relationships.

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Appendix A

Appendix

A.1 Data Transformations

A.1.1 Separation and Reunion Test

TABLE A.1: **Data Transformations**

Separation and Reunion Test	Data Transformations
Exploration	Square Root
Pace	None
Passive	None
Play	None
Stand Door	None
Look at Door	Square Root
Vocalize	Square Root; post-hoc models by Phase None; post-hoc Pack and pet models Log1p
Yawn and Lick	Log1p
Contact	None
Shake	None
Proximity	None

A.1.2 Exploration and Novel Object Tests

TABLE A.2: **Data Transformations**

Exploration & Novel Object Tests	Data Transformations
Active	Human Partners comparisons: Square root Pack comparison: Log Post-hoc Pack only: None
Sniffing	Square Root
Proximity	Log1p
Sniff and Manipulate Object	Human Partners comparison: None Pack comparisons: Log1p
Approach Novel Object	Log1p
Stress Signals	None
Gaze Object	Log1p
Cautious and Fear-Related Behavior	None
Proximity to Door	Log1p

A.2 Model Comparisons to Null

A.2.1 Longitudinal *OXTR* Methylation

TABLE A.3: Model Comparisons, *OXTR* Methylation

	Df	Chisq	Chi Df	Pr(>Chisq)
null model	3			
-1383	9	12.99	6	0.0433*
null model	3			
-1371	9	18.96	6	0.0042**
null model	3			
-727	9	21.49	6	0.0015**
null model	3			
-751	9	14.24	6	0.0271*

A.2.2 Dynamic *OXTR* Methylation

TABLE A.4: Model Comparisons, Dynamic *OXTR* Methylation

	Df	Chisq	Chi Df	Pr(>Chisq)
null model	3			
-1383	6	7.63	3	0.054
null model	3			
-1371	6	16.20	3	0.001**
null model	3			
-727	6	4.17	3	0.244
null model	3			
-751	5	1.08	2	0.583

A.2.3 Strange Situation Test

TABLE A.5: Model Comparisons, Strange Situation Test

	Df	Chisq	Chi Df	Pr(>Chisq)
null model	2			
Anxiety	4	2.90	2	0.2344
null model	2			
Acceptance	4	3.00	2	0.22
null model	2			
Attachment	4	0.01	2	0.99

A.2.4 Separation and Reunion Test

TABLE A.6: Model Comparisons, Separation & Reunion Test

	Df	Chisq	Chi Df	Pr(>Chisq)
null model	3			
Contact	7	22.62	4	0.0002***
null model	5			
Explore	11	27.45	6	0.0001***
null model	5			
Pace	11	46.32	6	0.0000***
null model	5			
Passive	11	29.57	6	0.0000***
null model	3			
Proximity to Partner	7	13.20	4	0.0103*
null model	4			
Shake	10	13.97	6	0.0300*
null model	5			
Vocalization	11	16.92	6	0.0096**
null model	4			
Yawn and Lip Lick_ns	10	15.39	6	0.0174*

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
null model	13	0.76				
Look at Door	12	0.66	1	0.10	1.84	0.1998
null model	13	17910.72				
Stand at Door	12	12161.34	1	5749.38	5.67	0.0346*

A.2.5 Exploration and Novel Object Tests

TABLE A.7: Model Comparisons, Exploration & Novel Object Tests

	Df	Chisq	Chi Df	Pr(>Chisq)
null model	8			
Active (Human Partner)	16	22.82	8	0.0036**

TABLE A.7: Model Comparisons, Exploration & Novel Object Tests

	Df	Chisq	Chi Df	Pr(>Chisq)
null model	8			
Active (Pack Only)	14	6.72	6	0.3477
null model	3			
Approach (Human Partner)	7	1.37	4	0.8501
null model	3			
Approach (Pack Only)	6	4.33	3	0.2279
null model	7			
Caution & Fear (Human Partners)	15	8.19	8	0.4147
null model	7			
Caution & Fear (Pack Only)	13	15.87	6	0.0145*
null model	3			
Gaze at Novel Object (Human Partner)	7	0.47	4	0.9765
null model	3			
Gaze at Novel Object (Pack Only)	6	1.52	3	0.6766
null model	8			
Proximity to Door (Human Partner)	13	19.76	5	0.0014**
null model	8			
Proximity to Door (Pack Only)	12	9.61	4	0.0476*
null model	3			
Proximity to Partner (Human Partner)	7	8.06	4	0.0894
null model	5			
Proximity to Partner (Pack Only)	9	4.97	4	0.2907

TABLE A.7: Model Comparisons, Exploration & Novel Object Tests

	Df	Chisq	Chi Df	Pr(>Chisq)
null model	8			
Sniffing (Human Partner)	16	25.02	8	0.0015**
null model	8			
Sniffing (Pack Only)	14	3.67	6	0.7214
null model	3			
Sniff & Manipulate Object (Human Partner)	7	4.74	4	0.3146
null model	3			
Sniff & Manipulate Object (Pack Only)	6	4.29	3	0.2318
null model	6			
Stress (Human Partner)	7	10.30	1	0.0013**
null model	6			
Stress (Pack Only)	7	0.00	1	1.0000

A.3 Linear Model Results

A.3.1 Longitudinal *OXTR* Methylation

TABLE A.8: Linear Model Longitudinal *OXTR* Methylation

	-1383	-1371	-727	-751
Litter	0.86	0.89	11.02	3.46
	(-0.38,2.10)	(0.08,1.69)	(3.66,18.38)	(-0.74,7.66)
	t = 1.37	t = 2.16	t = 2.94	t = 1.61
	p = 0.18	$p = 0.04^{*}$	$p = 0.004^{**}$	p = 0.11
Age (Pre-Adopt)	-0.57	0.03	-0.73	-4.61
	(-2.81,1.67)	(-1.19,1.25)	(-11.08,9.62)	(-10.73,1.52)
	t = -0.50	t = 0.05	t = -0.14	t = -1.47
	p = 0.62	p = 0.96	p = 0.89	p = 0.15
Age (Two Years)	0.24	-0.16	3.34	1.13
	(-1.75,2.23)	(-1.48,1.17)	(-7.01,13.69)	(-4.24,6.49)
	t = 0.23	t = -0.23	t = 0.63	t = 0.41
	p = 0.82	p = 0.82	p = 0.53	p = 0.69
Group (Pet)	-2.48	-0.10	-14.10	-6.12
	(-4.84,-0.13)	(-1.39,1.18)	(-23.84,-4.36)	(-13.51,1.27)
	t = -2.07	t = -0.16	t = -2.84	t = -1.62
	$p = 0.04^{*}$	p = 0.88	$p = 0.005^{**}$	p = 0.11
Age:Group	$\chi^2 = 8.16$	$\chi^2 = 6.14$	$\chi^2 = 8.84$	$\chi^2 = 10.34$
	df = 2	df = 2	df = 2	df = 2
	$p = 0.02^{*}$	$p < 0.05^{*}$	$p = 0.01^{*}$	$p = 0.006^{**}$
Constant	87.50	85.27	37.96	27.29
	(85.69,89.31)	(84.24,86.31)	(29.72,46.21)	(22.02,32.57)
	t = 94.56	t = 161.73	t = 9.03	t = 10.14
	$p < 0.001^{***}$	$p < 0.001^{***}$	$p < 0.001^{***}$	$p < 0.001^{***}$
Observations	103	78	60	66

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.9: Linear Model *OXTR* Methylation of Locus -1383, Post-hoc Time

	-1383 Post-hoc Models		
	(Pre-Adoption)	(Post-Adoption)	(Two Years)
Litter	1.06	1.37	0.54
	(-1.51,3.64)	(-0.99,3.74)	(-1.26,2.35)
	t = 0.81	t = 1.14	t = 0.59
	p = 0.42	p = 0.26	p = 0.56
Group (Pet)	2.30	-2.65	-0.66
	(-0.29,4.89)	(-4.85,-0.46)	(-2.47,1.15)
	t = 1.74	t = -2.37	t = -0.72
	$p = 0.09^{+}$	$p = 0.02^{*}$	p = 0.48
Constant	86.84	87.21	87.88
	(84.73,88.94)	(85.02,89.40)	(86.43,89.33)
	t = 80.96	t = 78.15	t = 118.45
	$p < 0.001^{***}$	$p < 0.001^{***}$	$p < 0.001^{***}$
Observations	27	27	49

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.10: Linear Mode *OXTR* Methylation of Locus -1371, Post-hoc Time

	-1371 Post-hoc Models		
	(Pre-Adoption)	(Post-Adoption)	(Two Years)
Litter	0.19	0.71	2.05
	(-1.16,1.54)	(-0.75,2.18)	(0.60,3.50)
	t = 0.28	t = 0.96	t = 2.77
	p = 0.79	p = 0.34	p = 0.01**
Group (Pet)	0.98	-0.10	-1.59
	(-0.38,2.34)	(-1.38,1.17)	(-3.03,-0.15)
	t = 1.41	t = -0.16	t = -2.16
	p = 0.16	p = 0.88	p = 0.04*
Constant	85.68	85.40	84.77
	(84.52,86.83)	(84.02,86.78)	(83.65,85.89)
	t = 145.05	t = 121.36	t = 148.41
	p < 0.001***	p < 0.001***	p < 0.001***
Observations	27	28	23

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.11: Linear Model *OXTR* Methylation of Locus -727, Post-hoc Time

	-727 Post-hoc Models		
	(Pre-Adoption)	(Post-Adoption)	(Two Years)
Litter	17.54	5.61	11.67
	(7.54,27.55)	(-5.59,16.82)	(-2.88,26.22)
	t = 3.44	t = 0.98	t = 1.57
	p = 0.001***	p = 0.33	p = 0.12
Group (Pet)	6.28	-14.09	-14.30
	(-3.30,15.85)	(-24.27,-3.90)	(-28.97,0.37)
	t = 1.28	t = -2.71	t = -1.91
	p = 0.20	p = 0.01**	p = 0.06^{+}
Constant	35.04	41.97	41.21
	(27.68,42.40)	(31.54,52.39)	(30.92,51.50)
	t = 9.33	t = 7.89	t = 7.85
	p < 0.001***	p < 0.001***	p < 0.001***
Observations	17	27	16

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.12: Linear Model *OXTR* Methylation of Locus -751, Post-hoc Time

	-751 Post-hoc Models		
	(Pre-Adoption)	(Post-Adoption)	(Two Years)
Litter	7.56	-0.65	2.96
	(0.72,14.39)	(-6.05,4.74)	(-3.73,9.64)
	t = 2.17	t = -0.24	t = 0.87
	p = 0.04*	p = 0.82	p = 0.39
Group (Pet)	7.32	-6.12	-1.40
	(0.99,13.64)	(-11.57,-0.66)	(-8.18,5.38)
	t = 2.27	t = -2.20	t = -0.40
	p = 0.03*	p = 0.06^{+}	p = 0.69
Constant	21.72	29.35	28.63
	(17.17,26.26)	(24.87,33.82)	(23.27,33.98)
	t = 9.36	t = 12.85	t = 10.47
	p < 0.001***	p < 0.0001***	p < 0.001***
Observations	17	14	35

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.13: Linear Model *OXTR* Methylation of Locus -1383, Post-hoc Group

	-1383 Post-hoc Models	
	(Pet Only)	(Pack Only)
Age (Post-Adopt)	-4.44	0.77
	(-7.46,-1.43)	(-1.06,2.59)
	t = -2.89	t = 0.82
	p = 0.004**	p = 0.41
Age (Two Years)	-2.18	0.79
	(-4.79,0.43)	(-0.79,2.37)
	t = -1.64	t = 0.98
	p = 0.11	p = 0.33
Litter	1.78	0.13
	(-0.44,3.99)	(-1.21,1.47)
	t = 1.57	t = 0.18
	p = 0.12	p = 0.86
Age (Two Years to Post-Adopt)	-2.27	-0.02
	(-4.93,0.40)	(-1.65,1.60)
	t = -1.67	t = -0.03
	p = 0.10^{+}	p = 0.98
Constant	88.78	87.27
	(86.41,91.15)	(85.87,88.68)
	t = 73.36	t = 121.34
	p < 0.001***	p < 0.001***
Observations	46	57

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
***p<0.001, **p<0.01, *p<0.05, +p<0.1

TABLE A.14: Linear Model *OXTR* Methylation of Locus -1371, Post-hoc Group

	-1371 Post-hoc Models	
	(Pet Only)	(Pack Only)
Age (Post-Adopt)	-1.15	-0.03
	(-2.38,0.08)	(-1.34,1.28)
	t = -1.83	t = -0.05
	p = 0.07^{+}	p = 0.97
Age (Two Years)	-2.61	-0.20
	(-3.84,-1.38)	(-1.61,1.21)
	t = -4.16	t = -0.28
	p = 0.0001^{***}	p = 0.78
Litter	0.92	0.85
	(-0.12,1.96)	(-0.49,2.20)
	t = 1.73	t = 1.24
	p = 0.09^{+}	p = 0.22
Age (Two Years to Post-Adopt)	1.46	0.17
	(0.19,2.73)	(-1.28,1.63)
	t = 2.26	t = 0.23
	p = 0.03^{*}	p = 0.82
Constant	86.29	85.13
	(85.29,87.29)	(83.92,86.33)
	t = 169.50	t = 138.82
	p < 0.001^{***}	p < 0.001^{***}
Observations	35	43

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
***p<0.001, **p<0.01, *p<0.05, +p<0.1

TABLE A.15: Linear Model *OXTR* Methylation of Locus -727, Post-hoc Group

	-727 Post-hoc Models	
	(Pet Only)	(Pack Only)
Age (Post-Adopt)	-21.09	1.53
	(-32.67,-9.50)	(-8.44,11.50)
	t = -3.57	t = 0.30
	p = 0.0004***	p = 0.77
Age (Two Years)	-17.60	4.19
	(-30.60,-4.59)	(-6.30,14.67)
	t = -2.65	t = 0.78
	p = 0.01**	p = 0.44
Litter	13.79	8.99
	(-0.75,28.33)	(0.54,17.44)
	t = 1.86	t = 2.09
	p = 0.07^{+}	p = 0.04*
Age (Two Years to Post-Adopt)	-3.49	-2.66
	(-14.94,7.97)	(-12.63,7.31)
	t = -0.60	t = -0.52
	p = 0.56	p = 0.61
Constant	42.99	37.89
	(31.13,54.86)	(29.96,45.82)
	t = 7.10	t = 9.36
	p < 0.001***	p < 0.001***
Observations	27	33

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.16: Linear Model *OXTR* Methylation of Locus -751, Post-hoc Group

	-751 Post-hoc Models	
	(Pet Only)	(Pack Only)
Age (Post-Adopt)	-10.34	4.74
	(-18.67,-2.01)	(-0.60,10.07)
	t = -2.43	t = 1.74
	p = 0.02*	p = 0.09^{+}
Age (Two Years)	-5.39	5.77
	(-12.44,1.67)	(1.43,10.10)
	t = -1.50	t = 2.61
	p = 0.14	p = 0.01**
Litter	6.24	1.51
	(-2.45,14.93)	(-2.23,5.25)
	t = 1.41	t = 0.79
	p = 0.16	p = 0.43
Age (Two Years to Post-Adopt)	-4.95	-1.03
	(-12.17,2.26)	(-5.71,3.65)
	t = -1.35	t = -0.43
	p = 0.18	p = 0.67
Constant	30.12	23.53
	(22.69,37.55)	(19.83,27.23)
	t = 7.94	t = 12.47
	p < 0.001***	p < 0.001***
Observations	28	38

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

A.3.2 Dynamic *OXTR* Methylation

TABLE A.17: Linear Model Dynamic *OXTR* Methylation of -1371

	Dynamic OXTR Methylation of -1371
Order (Before SST)	1.73
	(0.12,3.33)
	t = 2.11
	p = 0.03*
Cortisol Group (Rise)	−1.47
	(-3.64,0.71)
	t = -1.32
	p = 0.19
Order (Before SST):Cortisol Group (Rise)	−4.01
	(-5.77,-2.25)
	t = -4.46
	p = 8.32e-06***
Constant	86.40
	(83.82,88.98)
	t = 65.69
	p = 2.2e-16***
Observations	14

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

A.3.3 Separation and Reunion Test

TABLE A.18: Linear Model Explore (SRT)

	Explore
Litter	0.665
	(-0.916,2.246)
	t = 0.825
	p = 0.410
Group (Pet)	-0.862
	(-2.460,0.735)
	t = -1.058
	p = 0.290
Phase (Reunion)	-5.684
	(-7.634,-3.735)
	t = -5.715
	p < 0.001***
Phase (Separation)	-6.016
	(-8.043,-3.989)
	t = -5.818
	p < 0.001***
Phase (Separation to Reunion)	0.332
	(-1.618,2.281)
	t = 0.333
	p = 0.739
Group:Phase	$\chi^2 = 0.598$
	df = 2
	p = 0.742
Group:Phase (Pet:Reunion)	-0.491
	(-4.409,3.426)
	t = -0.246
	p = 0.806
Group:Phase (Pet:Separation)	-1.551
	(-5.577,2.475)
	t = -0.755
	p = 0.451
Constant	10.168
	(8.438,11.897)
	t = 11.521
	p < 0.001***
Observations	42

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.19: Linear Model Contact (SRT)

	Contact
Litter	3.986
	(-0.874,8.845)
	t = 1.607
	p = 0.108
Group (Pet)	-1.067
	(-5.977,3.843)
	t = -0.426
	p = 0.671
Phase (Reunion)	14.014
	(9.155,18.874)
	t = 5.652
	p < 0.00001***
Group:Phase	$\chi^2 = 0.037$
	df = 2
	p =0.847
Group:Phase (Pet:Reunion)	0.967
	(-8.847,10.780)
	t = 0.193
	p = 0.847
Constant	-0.650
	(-5.355,4.055)
	t = -0.271
	p = 0.787
Observations	28

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.20: Linear Model Look at Door (SRT)

	Look at Door
Litter	-0.202 (-0.429,0.025) t = -1.743 p = 0.110
Group (Pet)	-0.172 (-0.401,0.058) t = -1.468 p = 0.171
Constant	0.347 (0.159,0.536) t = 3.615 p = 0.005**
Observations	14

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.21: Linear Model Pace (SRT)

	Pace
Litter	-5.355
	(-11.956,1.246)
	t = -1.590
	p = 0.112
Group (Pet)	-3.343
	(-10.013,3.326)
	t = -0.983
	p = 0.326
Phase (Reunion)	24.900
	(18.757,31.043)
	t = 7.944
	$p < 0.001^{***}$
Phase (Separation)	2.214
	(-4.127,8.555)
	t = 0.684
	p = 0.494
Group:Phase	$\chi^2 = 5.361$
	df = 2
	$p = 0.069^{+}$
Group:Phase (Pet:Reunion)	-7.175
	(-18.599,4.249)
	t = -1.231
	p = 0.219
Group:Phase (Pet:Separation)	6.275
	(-5.590,18.140)
	t = 1.037
	p = 0.300
Phase (Separation to Reunion)	22.686
	(16.542,28.829)
	t = 7.238
	$p < 0.001^{***}$
Constant	5.482
	(-0.794,11.758)
	t = 1.712
	$p = 0.087^{+}$
Observations	42

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.22: Linear Model Pace, Post-hoc Group (SRT)

	Pace (cont.)	
	Post-hoc Models	
	(Pack)	(Pet)
Litter	-4.967	-6.578
	(-10.945,1.012)	(-18.319,5.163)
	t = -1.628	t = -1.098
	p = 0.120	p = 0.291
Phase (Reunion)	27.975	20.800
	(20.653,35.297)	(6.420,35.180)
	t = 7.489	t = 2.835
	$p < 0.00001^{***}$	$p = 0.014^{*}$
Phase (Separation)	-0.475	5.800
	(-7.797,6.847)	(-8.580,20.180)
	t = -0.127	t = 0.791
	p = 0.901	p = 0.443
Constant	4.883	3.289
	(-1.095,10.862)	(-8.452,15.030)
	t = 1.601	t = 0.549
	p = 0.126	p = 0.592
Observations	24	18

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.23: Linear Model Pace, Post-hoc Phase (SRT)

	Pace			
	Main Model	*Post-hoc Models*		
		(Baseline)	(Separation)	(Reunion)
Litter	-5.355	-0.114	-3.057	-13.800
	(-11.956,1.246)	(-2.393,2.164)	(-9.795,3.680)	(-29.637,2.037)
	t = -1.590	t = -0.098	t = -0.889	t = -1.708
	p = 0.112	p = 0.924	p = 0.393	p = 0.116
Group (Pet)	-3.343	-2.400	3.875	-9.575
	(-10.013,3.326)	(-4.702,-0.098)	(-2.932,10.682)	(-25.576,6.426)
	t = -0.983	t = -2.043	t = 1.116	t = -1.173
	p = 0.326	p = 0.066^{+}	p = 0.289	p = 0.266
Phase (Reunion)	24.900			
	(18.757,31.043)			
	t = 7.944			
	p < 0.001***			
Phase (Separation)	2.214			
	(-4.127,8.555)			
	t = 0.684			
	p = 0.494			
Group:Phase	$\chi^2 = 5.361$			
	df = 2			
	p = 0.069^{+}			
Group:Phase (Pet:Reunion)	-7.175			
	(-18.599,4.249)			
	t = -1.231			
	p = 0.219			
Group:Phase (Pet:Separation)	6.275			
	(-5.590,18.140)			
	t = 1.037			
	p = 0.300			
Phase (Separation to Reunion)	22.686			
	(16.542,28.829)			
	t = 7.238			
	p < 0.001***			
Constant	5.482	2.457	3.454	37.275
	(-0.794,11.758)	(0.568,4.346)	(-2.133,9.040)	(24.144,50.406)
	t = 1.712	t = 2.549	t = 1.212	t = 5.564
	p = 0.087^{+}	p = 0.028*	p = 0.252	p = 0.0002***
Observations	42	14	14	14

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.24: Linear Model Passive (SRT)

	Passive
Litter	-3.752
	(-19.699,12.194)
	t = -0.461
	p = 0.645
Group (Pet)	3.361
	(-12.751,19.473)
	t = 0.409
	p = 0.683
Phase (Reunion)	57.314
	(37.784,76.845)
	t = 5.752
	p < 0.001***
Phase (Separation)	79.900
	(60.370,99.430)
	t = 8.018
	p < 0.001***
Group:Phase	$\chi^2 = 0.848$
	df = 2
	p = 0.655
Group:Phase (Pet:Reunion)	17.183
	(-21.890,56.257)
	t = 0.862
	p = 0.389
Group:Phase (Pet:Separation)	14.175
	(-24.898,53.248)
	t = 0.711
	p = 0.478
Phase (Separation to Reunion)	-22.586
	(-42.116,-3.055)
	t = -2.267
	p = 0.024*
Constant	4.621
	(-12.756,21.999)
	t = 0.521
	p = 0.603
Observations	42

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.25: Linear Model Play (SRT)

	Play
Litter	-0.288
	(-1.785,1.209)
	t = -0.377
	p = 0.707
Phase (Reunion)	0.288
	(-1.209,1.785)
	t = 0.377
	p = 0.707
Group (Pet)	2.079
	(-0.038,4.196)
	t = 1.925
	p = 0.055^{+}
Phase:Group (Reunion:Pet)	20.825
	(-46,092.080,46,133.730)
	t = 0.001
	p = 1.000
Constant	-2.793
	(-5.026,-0.561)
	t = -2.452
	p = 0.015*
Observations	28

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.26: Linear Model Play, Post-hoc Phase (SRT)

	Play (cont.)			
	Post-hoc Models			
	(Baseline)	(Reunion)	(Pet)	(Pack)
Litter	-0.143	0.000	-0.000	-0.083
	(-0.605,0.319)	(-0.365,0.365)	(-0.403,0.403)	(-0.247,0.080)
	t = -0.606	t = 0.000	t = -0.000	t = -1.000
	p = 0.557	p = 1.000	p = 1.000	p = 0.330
Phase (Reunion)			0.333	-0.125
			(-0.161,0.827)	(-0.325,0.075)
			t = 1.323	t = -1.225
			p = 0.208	p = 0.235
Group (Pet)	0.208	0.667		
	(-0.258,0.675)	(0.298,1.035)		
	t = 0.875	t = 3.546		
	p = 0.401	p = 0.005**		
Phase (Separation)			-0.333	-0.125
			(-0.827,0.161)	(-0.325,0.075)
			t = -1.323	t = -1.225
			p = 0.208	p = 0.235
Constant	0.196	-0.000	0.333	0.167
	(-0.186,0.579)	(-0.302,0.302)	(-0.070,0.737)	(0.003,0.330)
	t = 1.006	t = -0.000	t = 1.620	t = 2.000
	p = 0.337	p = 1.000	p = 0.128	p = 0.060+
Observations	14	14	18	24

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.27: Linear Model Shake (SRT)

	Shake
Litter	1.208
	(-0.972,3.388)
	t = 1.086
	p = 0.278
Phase (Reunion)	1.395
	(-0.415,3.205)
	t = 1.511
	p = 0.131
Phase (Separation)	-19.804
	(-17,775.220,17,735.610)
	t = -0.002
	p = 0.999
Phase (Separation to Reunion)	20.892
	(-5,655.815,5,697.599)
	t = 0.007
	p = 0.995
Group (Pet)	-0.762
	(-2.969,1.446)
	t = -0.676
	p = 0.499
Group:Phase	$\chi^2 = 1.716$
	df = 2
	p = 0.424
Phase:Group (Reunion:Pet)	-2.591
	(-6.468,1.286)
	t = -1.310
	p = 0.191
Phase:Group (Separation:Pet)	-0.483
	(-21,653.220,21,652.250)
	t = -0.00004
	p = 1.000
Constant	-1.659
	(-3.805,0.488)
	t = -1.515
	p = 0.130
Observations	42

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.28: Linear Model Proximity (SRT)

	Proximity				
	Main Model	*Post-hoc Models*			
		(Baseline)	(Reunion)	(Pack)	(Pet)
Litter	9.100	7.286	10.914	-2.075	24.000
	(-8.682,26.882)	(-9.541,24.113)	(-19.909,41.738)	(-23.605,19.455)	(-2.519,50.519)
	t = 1.003	t = 0.849	t = 0.694	t = -0.189	t = 1.774
	p = 0.316	p = 0.415	p = 0.503	p = 0.854	p = 0.110
Group (Pet)	-2.575	-2.575	-30.500		
	(-24.835,19.685)	(-19.576,14.426)	(-61.643,0.643)		
	t = -0.227	t = -0.297	t = -1.919		
	p = 0.821	p = 0.773	p = 0.082^{+}		
Phase (Reunion)	32.125			32.125	4.200
	(14.918,49.332)			(10.595,53.655)	(-22.319,30.719)
	t = 3.659			t = 2.925	t = 0.310
	p = 0.0003***			p = 0.012*	p = 0.764
Group:Phase (Pet:Reunion)	-27.925				
	(-54.209,-1.641)				
	t = -2.082				
	p = 0.038*				
Constant	41.625	42.532	72.843	47.213	31.600
	(24.554,58.696)	(28.580,56.484)	(47.285,98.400)	(28.567,65.858)	(8.634,54.566)
	t = 4.779	t = 5.975	t = 5.586	t = 4.963	t = 2.697
	p = 0.00001***	p = 0.0001***	p = 0.0002***	p = 0.0003***	p = 0.025*
Observations	42	14	14	16	12

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value. *** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.29: Linear Model Stand at Door (SRT)

	Stand at Door
Litter	-6.971
	(-41.561,27.619)
	t = -0.395
	p = 0.701
Group (Pet)	40.950
	(6.002,75.898)
	t = 2.297
	p = 0.043*
Constant	47.136
	(18.455,75.816)
	t = 3.221
	p = 0.009**
Observations	14

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.30: Linear Model Vocalization (SRT)

	Vocalization			
	Main Model	*Post-hoc Models*		
		(Baseline)	(Separation)	(Reunion)
Litter	-0.163	0.143	-3.714	-3.857
	(-1.072,0.746)	(-0.140,0.426)	(-15.487,8.058)	(-14.232,6.518)
	t = -0.351	t = 0.989	t = -0.618	t = -0.729
	p = 0.726	p = 0.345	p = 0.549	p = 0.482
Group (Pet)	-0.125	-0.125	16.667	5.833
	(-1.264,1.014)	(-0.411,0.161)	(4.772,28.561)	(-4.649,16.316)
	t = -0.215	t = -0.856	t = 2.746	t = 1.091
	p = 0.830	p = 0.411	p = 0.020*	p = 0.299
Phase (Reunion)	0.795			
	(-0.307,1.897)			
	t = 1.414			
	p = 0.158			
Phase (Separation)	0.229			
	(-1.058,1.515)			
	t = 0.348			
	p = 0.728			
Group:Phase	$\chi^2 = 11.399$			
	df = 2			
	p = 0.003**			
Phase (Separation to Reunion)	0.566			
	(-0.535,1.668)			
	t = 1.008			
	p = 0.314			
Group:Phase (Pet:Reunion)	1.312			
	(-0.371,2.994)			
	t = 1.528			
	p = 0.127			
Group:Phase (Pet:Separation)	3.345			
	(1.380,5.309)			
	t = 3.336			
	p = 0.001***			
Constant	0.206	0.054	2.857	4.929
	(-0.667,1.080)	(-0.181,0.288)	(-6.904,12.619)	(-3.674,13.531)
	t = 0.463	t = 0.447	t = 0.574	t = 1.123
	p = 0.644	p = 0.664	p = 0.578	p = 0.286
Observations	42	14	14	14

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.31: Linear Model Vocalization, Post-hoc Group (SRT)

	Vocalization (cont.)	
	Post-hoc Models	
	(Pet)	(Pack)
Litter	-1.085	0.567
	(-2.051,-0.119)	(-0.033,1.167)
	t = -2.201	t = 1.851
	p = 0.046*	p = 0.079^{+}
Phase (Reunion)	1.435	0.576
	(0.251,2.618)	(-0.159,1.311)
	t = 2.377	t = 1.535
	p = 0.033*	p = 0.141
Phase (Separation)	2.352	0.188
	(1.169,3.535)	(-0.547,0.923)
	t = 3.896	t = 0.501
	p = 0.002**	p = 0.622
Constant	0.542	-0.197
	(-0.424,1.508)	(-0.797,0.403)
	t = 1.100	t = -0.643
	p = 0.290	p = 0.528
Observations	18	24

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.32: Linear Model Yawn and Lick (SRT)

	Yawn and Lick
Litter	0.457
	(-2.364,3.278)
	t = 0.318
	p = 0.751
Phase (Reunion)	3.099
	(-0.694,6.892)
	t = 1.601
	p = 0.110
Phase (Separation)	-0.931
	(-7.218,5.356)
	t = -0.290
	p = 0.772
Phase (Separation to Reunion)	4.029
	(-0.626,8.685)
	t = 1.696
	p = 0.090^{+}
Group (Pet)	-1.673
	(-4.558,1.213)
	t = -1.136
	p = 0.256
Group:Phase	$\chi^2 = 0.00$
	df = 2
	p = 1
Phase:Group (Reunion:Pet)	18.337
	(-1,558.616,1,595.290)
	t = 0.023
	p = 0.982
Phase:Group (Separation:Pet)	0.757
	(-2,320.454,2,321.967)
	t = 0.001
	p = 1.000
Constant	-2.185
	(-6.414,2.043)
	t = -1.013
	p = 0.312
Observations	42

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

A.3.4 Exploration and Novel Object Tests

TABLE A.33: Linear Model Active, Human Partners (ENOT)

	Active
	(Human Partners)
Litter	-0.12
	(-0.71,0.47)
	t = -0.40
	p = 0.69
Condition (Owner)	1.09
	(0.24,1.95)
	t = 2.50
	p = 0.02*
Test (Novel Object)	1.63
	(0.88,2.38)
	t = 4.25
	p = 0.0001***
Group (Pack)	0.60
	(-0.15,1.34)
	t = 1.57
	p = 0.12
Condition:Test (Owner:Novel Object)	0.69
	(-0.25,1.63)
	t = 1.43
	p = 0.16
Condition:Group (Owner:Pack)	-0.26
	(-1.99,1.46)
	t = -0.30
	p = 0.77
Test:Group (Novel Object:Pack)	-1.03
	(-2.02,-0.04)
	t = -2.05
	p < 0.05*
ConditionOwner:TestNO:GroupWSC	-1.53
	(-3.34,0.28)
	t = -1.66
	p = 0.10^{+}
Constant	6.87
	(5.97,7.77)
	t = 15.02
	p < 0.001***
Observations	56

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.34: Linear Model Active, Pack Only (ENOT)

	Active (Pack Only)
Litter	0.04 (-0.14,0.22) t = 0.41 p = 0.68
Condition (Canine)	0.15 (-0.17,0.48) t = 0.93 p = 0.36
Condition (Owner)	0.28 (-0.17,0.73) t = 1.22 p = 0.23
Condition (Canine to OWner)	0.13 (-0.20,0.45) t = 0.76 p = 0.45
Test (Novel Object)	0.09 (-0.18,0.37) t = 0.66 p = 0.51
Condition:Test	$\chi^2 = 0.23$ df = 2 p = 0.89
Condition:Test (Canine:Novel Object)	0.09 (-0.29,0.47) t = 0.48 p = 0.64
Condition:Test (Owner:Novel Object)	0.05 (-0.33,0.43) t = 0.24 p = 0.81
Constant	3.97 (3.64,4.30) t = 23.72 p < 0.001***
Observations	48

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.35: Linear Model Active, Post-hoc, Condition (ENOT)

	Active Post-hoc Models	
	(Human Partners Condition)	(Alone Condition)
Litter	-0.41	0.97
	(-1.27,0.45)	(-0.25,2.19)
	t = -0.94	t = 1.56
	p = 0.36	p = 0.14
Group (Pack)	0.03	0.29
	(-0.84,0.89)	(-0.95,1.52)
	t = 0.06	t = 0.46
	p = 0.96	p = 0.66
Constant	8.96	7.02
	(8.17,9.74)	(5.91,8.13)
	t = 22.35	t = 12.35
	$p < 0.001^{***}$	$p < 0.001^{***}$
Observations	28	28

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.36: Linear Model Active, Post-hoc, Group & Test (ENOT)

	Active Post-hoc Models			
	(Pet)	(Pack)	(Exploration Test)	(Novel Object Test)
Litter	-0.42	9.93	0.91	0.42
	(-1.51,0.68)	(-1.89,21.75)	(-0.27,2.08)	(-0.85,1.70)
	t = -0.75	t = 1.65	t = 1.51	t = 0.65
	p = 0.47	p = 0.11	p = 0.15	p = 0.53
Test (Novel Object)	1.62	13.33		
	(0.52,2.71)	(1.51,25.15)		
	t = 2.89	t = 2.21		
	p < 0.01**	p = 0.04*		
Group (Pack)			-0.09	-0.35
			(-1.28,1.09)	(-1.63,0.94)
			t = -0.15	t = -0.53
			p = 0.88	p = 0.61
Constant	7.53	59.75	7.94	8.73
	(6.58,8.48)	(49.51,69.99)	(6.87,9.01)	(7.56,9.89)
	t = 15.56	t = 11.44	t = 14.50	t = 14.69
	p < 0.001***	p < 0.001***	p < 0.001***	p < 0.001***
Observations	24	48	28	28

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.37: Linear Model Approach Novel Object, Human Partners (ENOT)

	Approach Novel Object	
	(Human Partners)	(Pack Only)
Litter	-0.002	0.39
	(-0.50,0.50)	(-0.06,0.85)
	t = -0.01	t = 1.69
	p = 1.00	p = 0.10^{+}
Condition (Canine)		0.39
		(-0.17,0.95)
		t = 1.36
		p = 0.18
Condition (Owner)	-0.27	0.14
	(-1.03,0.49)	(-0.42,0.70)
	t = -0.69	t = 0.48
	p = 0.50	p = 0.64
Group (Pack)	-0.43	
	(-1.14,0.29)	
	t = -1.17	
	p = 0.25	
Condition:Group (Owner:Pack)	0.40	
	(-0.60,1.41)	
	t = 0.79	
	p = 0.44	
Constant	1.26	0.63
	(0.66,1.85)	(0.17,1.09)
	t = 4.16	t = 2.71
	p = 0.0001***	p = 0.01**
Observations	28	24

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
* **p<0.001, **p<0.01, *p<0.05, +p<0.1

TABLE A.38: Linear Model Caution and Fear, Human Partners (ENOT)

	Fear (Human Partners)
Litter	0.55
	(-0.89,2.00)
	t = 0.75
	p = 0.46
Condition (Owner)	-0.47
	(-1.75,0.81)
	t = -0.72
	p = 0.48
Test (Novel Object)	0.77
	(-0.82,2.36)
	t = 0.95
	p = 0.35
Group (Pack)	0.75
	(-0.97,2.46)
	t = 0.85
	p = 0.40
Condition:Test (Owner:Novel Object)	-0.05
	(-4.06,3.95)
	t = -0.03
	p = 0.98
Condition:Group (Owner:Pack)	-1.05
	(-5.33,3.23)
	t = -0.48
	p = 0.64
Test:Group (Novel Object:Pack)	1.54
	(-2.11,5.20)
	t = 0.83
	p = 0.41
Condition:Test:Group (Owner:Novel Object:Pack)	2.64
	(-3.16,8.44)
	t = 0.89
	p = 0.38
Constant	-1.55
	(-3.83,0.74)
	t = -1.33
	p = 0.19
Observations	56

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.39: Linear Model Caution and Fear, Pack Only (ENOT)

	Fear (Pack Only)
Litter	-0.13
	(-3.72,3.46)
	t = -0.07
	p = 0.95
Condition (Canine)	-2.80
	(-5.94,0.33)
	t = -1.75
	p = 0.08^{+}
Condition (Owner)	-0.37
	(-2.71,1.97)
	t = -0.31
	p = 0.76
Test (Novel Object)	2.75
	(-0.19,5.68)
	t = 1.84
	p = 0.07^{+}
Condition:Test	$\chi^2 = 2.87$
	df = 2
	p = 0.24
Condition:Test (Canine:Novel Object)	21.12
	(-19,300.48,19,342.72)
	t = 0.002
	p = 1.00
Condition:Test (Owner:Novel Object)	4.75
	(-0.75,10.25)
	t = 1.69
	p = 0.10^{+}
Constant	-1.46
	(-4.63,1.72)
	t = -0.90
	p = 0.37
Observations	48

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.40: Linear Model Gaze at Novel Object, Human Partners (ENOT)

	Gaze at Novel Object	
	(Human Partners)	(Pack Only)
Litter	0.03	0.39
	(-0.61,0.67)	(-0.32,1.10)
	t = 0.09	t = 1.07
	p = 0.93	p = 0.29
Condition (Canine)		0.25
		(-0.62,1.12)
		t = 0.56
		p = 0.58
Condition (Owner)	-0.20	-0.002
	(-1.18,0.78)	(-0.87,0.87)
	t = -0.39	t = -0.004
	p = 0.70	p = 1.00
Group (Pack)	0.09	
	(-0.83,1.00)	
	t = 0.18	
	p = 0.86	
Condition:Group (Owner:Pack)	0.20	
	(-1.10,1.49)	
	t = 0.30	
	p = 0.77	
Constant	1.15	1.06
	(0.39,1.92)	(0.35,1.77)
	t = 2.97	t = 2.93
	p = 0.004**	p = 0.004**
Observations	28	24

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
* **p<0.001, **p<0.01, *p<0.05, +p<0.1

TABLE A.41: Linear Model Manipulate and Sniff Object, Human Partners (ENOT)

	Manipulate and Sniff Object	
	(Human Partners)	(Pack Only)
Litter	0.61	0.21
	(-1.78,3.00)	(-0.41,0.83)
	t = 0.50	t = 0.67
	p = 0.62	p = 0.51
Condition (Canine)		-0.39
		(-1.14,0.37)
		t = -1.00
		p = 0.32
Condition (Owner)	1.45	-0.79
	(-2.20,5.09)	(-1.55,-0.04)
	t = 0.78	t = -2.06
	p = 0.44	p = 0.04*
Group (Pack)	2.74	
	(-0.67,6.15)	
	t = 1.58	
	p = 0.12	
Condition:Group (Owner:Pack)	-4.76	
	(-9.58,0.07)	
	t = -1.93	
	p = 0.06^{+}	
Constant	1.44	1.12
	(-1.41,4.28)	(0.50,1.74)
	t = 0.99	t = 3.56
	p = 0.33	p = 0.0004***
Observations	28	24

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
* **p<0.001, **p<0.01, *p<0.05, +p<0.1

TABLE A.42: Linear Model Proximity to Door, Human Partners (ENOT)

	Prox. to Door (Human Partners)
Litter	-0.22 (-0.67,0.23) t = -0.97 p = 0.34
Test (Novel Object)	0.81 (0.30,1.31) t = 3.13 p = 0.002**
Condition (Owner)	-1.05 (-1.70,-0.40) t = -3.16 p = 0.002**
Group (Pack)	-1.36 (-2.15,-0.57) t = -3.38 p = 0.001***
Test:Condition (Novel Object:Owner)	-0.82 (-1.41,-0.24) t = -2.78 p = 0.01**
Group:Condition (Pack:Owner)	1.00 (0.23,1.78) t = 2.55 p = 0.02*
Test:Group (Novel Object:Pack)	0.48 (-0.31,1.28) t = 1.20 p = 0.24
Test:Condition:Group (Novel Object:Owner:Pack)	1.02 (-0.09,2.13) t = 1.80 p = 0.08+
Constant	2.65 (1.99,3.31) t = 7.87 p < 0.001***
Observations	56

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.43: Linear Model Proximity to Door, Pack Only (ENOT)

	Proximity to Door (Pack Only)
Litter	-0.53 (-0.98,-0.07) t = -2.28 p = 0.03*
Condition (Canine)	-0.38 (-0.90,0.15) t = -1.41 p = 0.16
Condition (Owner)	-0.44 (-1.07,0.19) t = -1.36 p = 0.18
Condition (Canine to Owner)	-0.06 (-0.58,0.46) t = -0.24 p = 0.82
Test (Novel Object)	0.72 (0.20,1.25) t = 2.71 p = 0.01**
Condition:Test	$\chi^2 = 1.4237$ df = 2 p = 0.49
Condition:Test (Canine:Novel Object)	0.17 (-0.77,1.11) t = 0.36 p = 0.73
Condition:Test (Owner:Novel Object)	-0.39 (-1.33,0.55) t = -0.81 p = 0.42
Constant	1.54 (1.01,2.08) t = 5.62 p < 0.0001***
Observations	48

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.45: Linear Model Proximity to Door, Post-hoc (ENOT)

	Proximity to Door Post-hoc Models			
	(Human Partners Condition)	(Alone Condition)	(Pet)	(Pack)
Litter	-0.15	0.01	0.30	-0.49
	(-0.65,0.34)	(-0.79,0.81)	(-0.41,1.01)	(-1.01,0.03)
	t = -0.60	t = 0.02	t = 0.84	t = -1.84
	p = 0.56	p = 0.99	p = 0.42	p = 0.08^{+}
Group (Pack)	-0.22	-1.22		
	(-0.72,0.28)	(-2.03,-0.41)		
	t = -0.85	t = -2.96		
	p = 0.41	p < 0.01**		
Condition (Canine)				-0.38
				(-1.02,0.26)
				t = -1.15
				p = 0.26
Condition (Owner)			-1.44	-0.44
			(-2.15,-0.74)	(-1.08,0.20)
			t = -4.00	t = -1.34
			p < 0.001***	p = 0.19
Constant	1.49	2.86	2.71	1.89
	(1.04,1.95)	(2.13,3.59)	(2.10,3.32)	(1.36,2.41)
	t = 6.47	t = 7.68	t = 8.68	t = 7.07
	p < 0.0001***	p < 0.0001***	p < 0.0001***	p < 0.001***
Observations	28	28	24	48

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.44: Linear Model Sniffing, Human Partners (ENOT)

	Sniffing (Human Partners)
Litter	0.28 (-0.70,1.26) t = 0.56 p = 0.58
Condition (Owner)	1.05 (-0.26,2.35) t = 1.57 p = 0.12
Test (Novel Object)	-0.58 (-1.39,0.24) t = -1.38 p = 0.17
Group (Pack)	-0.55 (-2.03,0.94) t = -0.72 p = 0.48
Condition:Test (Owner:Novel Object)	1.44 (0.43,2.45) t = 2.79 $p < 0.01^{**}$
Condition:Group (Owner:Pack)	-1.61 (-3.12,-0.11) t = -2.10 $p = 0.04^{*}$
Test:Group (Novel Object:Pack)	0.11 (-1.19,1.41) t = 0.16 p = 0.88
Condition:Test:Group (Owner:Novel Object:Pack)	-1.75 (-3.69,0.19) t = -1.77 $p = 0.08^{+}$
Constant	8.21 (6.94,9.47) t = 12.72 $p < 0.001^{***}$
Observations	56

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.46: Linear Model Sniffing, Pack Only (ENOT)

	Sniffing (Pack Only)
Litter	0.02 (-1.26,1.30) t = 0.03 p = 0.98
Condition (Canine)	0.52 (-0.78,1.81) t = 0.78 p = 0.44
Condition (Owner)	-0.22 (-1.71,1.26) t = -0.29 p = 0.77
Condition (Canine to Owner)	-0.74 (-2.03,0.55) t = -1.12 p = 0.27
Test (Novel Object)	-0.15 (-1.44,1.13) t = -0.24 p = 0.82
Condition:Test	$\chi^2 = 0.62$ df = 2 p = 0.75
Condition:Test (Canine:Novel Object)	0.39 (-1.34,2.12) t = 0.44 p = 0.67
Condition:Test (Owner:Novel Object)	0.69 (-1.04,2.42) t = 0.78 p = 0.44
Constant	7.62 (6.28,8.96) t = 11.16 $p < 0.001^{***}$
Observations	48

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.48: Linear Model Sniffing, Post-hoc, Condition Test (ENOT)

	Sniffing Post-hoc Models			
	(Alone Condition)	(Human Condition)	(Exploration Test)	(Novel Object Test)
Litter	1.33	0.08	0.54	1.19
	(-0.09,2.75)	(-0.98,1.15)	(-0.26,1.34)	(-0.41,2.78)
	t = 1.84	t = 0.15	t = 1.32	t = 1.46
	p = 0.08^{+}	p = 0.89	p = 0.20	p = 0.16
Group (Pack)	-0.46	-2.14	-0.99	-1.03
	(-1.89,0.97)	(-3.22,-1.07)	(-1.80,-0.18)	(-2.65,0.58)
	t = -0.63	t = -3.91	t = -2.39	t = -1.25
	p = 0.54	p < 0.001***	p = 0.03*	p = 0.23
Constant	7.35	9.78	8.60	8.36
	(6.05,8.64)	(8.80,10.75)	(7.87,9.34)	(6.90,9.82)
	t = 11.13	t = 19.72	t = 23.02	t = 11.24
	p < 0.001***	p < 0.001***	p < 0.001***	p < 0.001***
Observations	28	28	28	28

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.49: Linear Model Sniffing, Post-hoc, Group (ENOT)

	Sniffing Post-hoc Models			
	(Pet)	(Pack)	(Pet)	(Pack)
Litter	0.50	0.66	0.50	0.66
	(-0.64,1.63)	(-0.31,1.63)	(-0.87,1.87)	(-0.31,1.63)
	t = 0.86	t = 1.33	t = 0.71	t = 1.33
	p = 0.40	p = 0.19	p = 0.49	p = 0.20
Condition (Canine)		0.71		
		(-0.48,1.90)		
		t = 1.17		
		p = 0.25		
Condition (Owner)	1.80	0.12		
	(0.67,2.94)	(-1.07,1.31)		
	t = 3.12	t = 0.20		
	p < 0.01**	p = 0.85		
Test (Novel Object)			0.08	0.20
			(-1.29,1.45)	(-0.77,1.18)
			t = 0.12	t = 0.41
			p = 0.91	p = 0.69
Constant	7.76	7.22	8.62	7.40
	(6.78,8.75)	(6.25,8.19)	(7.44,9.81)	(6.55,8.24)
	t = 15.49	t = 14.61	t = 14.23	t = 17.20
	p < 0.001***	p < 0.001***	p < 0.001***	p < 0.001***
Observations	24	48	24	48

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.47: Linear Model Stress, Human Partners (ENOT)

	Stress (Human Partners)
Litter	-0.16
	(-3.55,3.24)
	t = -0.09
	p = 0.93
Group (Pack)	-1.20
	(-4.68,2.27)
	t = -0.68
	p = 0.50
Test (Novel Object)	-0.81
	(-2.65,1.03)
	t = -0.87
	p = 0.39
Condition (Owner)	-3.06
	(-5.70,-0.41)
	t = -2.26
	p = 0.03*
Group:Test (Pack:Novel Object)	4.37
	(-0.88,9.63)
	t = 1.63
	p = 0.11
Group:Condition (Pack:Owner)	25.77
	(-962.44,1,013.98)
	t = 0.05
	p = 0.96
Test:Condition (Novel Object:Owner)	-1.00
	(-5.66,3.66)
	t = -0.42
	p = 0.68
Group:Test:Condition (Pack:Novel Object:Owner)	8.27
	(-1,127.10,1,143.64)
	t = 0.01
	p = 0.99
Constant	4.65
	(0.21,9.10)
	t = 2.05
	p = 0.05*
Observations	56

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.50: Linear Model Stress, Pack Only (ENOT)

	Stress (Pack Only)
Litter	-1.41
	(-7.97,5.14)
	t = -0.42
	p = 0.68
Condition (Canine)	-5.60
	(-10.35,-0.85)
	t = -2.31
	p = 0.03*
Condition (Owner to Canine)	2.78
	(-0.09,5.65)
	t = 1.90
	p = 0.06^{+}
Test (Novel Object)	2.26
	(-0.37,4.89)
	t = 1.68
	p = 0.10^{+}
Condition:Test	$\chi^2 = 1.83$
	df = 2
	p = 0.40
Condition:Test (Canine:Novel Object)	6.95
	(-3.16,17.05)
	t = 1.35
	p = 0.18
Condition:Test (Owner:Novel Object)	1.66
	(-4.51,7.82)
	t = 0.53
	p = 0.60
Condition (Owner)	-2.82
	(-6.46,0.82)
	t = -1.52
	p = 0.13
Constant	2.64
	(-2.72,7.99)
	t = 0.97
	p = 0.34
Observations	48

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.51: Linear Model Stress, Post-hoc, Group & Test (ENOT)

	Stress Post-hoc Models (cont.)			
	(Pet)	(Pack)	(Exploration)	(Novel Object)
Litter	0.08	-0.08	-0.64	0.29
	(-0.25,0.41)	(-0.37,0.20)	(-2.23,0.94)	(-1.01,1.58)
	t = 0.50	t = -0.57	t = -0.80	t = 0.43
	p = 0.63	p = 0.58	p = 0.44	p = 0.67
Test (Novel Object)	-0.25	0.17		
	(-0.58,0.08)	(-0.12,0.45)		
	t = -1.49	t = 1.14		
	p = 0.16	p = 0.26		
Group (Pack)			-2.00	-0.90
			(-3.60,-0.40)	(-2.21,0.42)
			t = -2.45	t = -1.34
			p = 0.03*	p = 0.20
Constant	0.88	0.50	3.57	1.94
	(0.59,1.16)	(0.25,0.75)	(2.13,5.02)	(0.76,3.13)
	t = 6.03	t = 3.96	t = 4.84	t = 3.21
	p < 0.0001***	p = 0.0003***	p = 0.0001***	p = 0.004**
Observations	24	48	28	28

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.53: Linear Model Stress, Post-hoc, Condition (ENOT)

	Stress Post-hoc Models	
	(Human Partners Condition)	(Alone Condition)
Litter	0.00	-0.00
	(-0.39,0.39)	(-0.26,0.26)
	t = 0.00	t = -0.00
	p = 1.00	p = 1.00
Group (Pack)	-0.02	-0.25
	(-0.41,0.37)	(-0.51,0.01)
	t = -0.10	t = -1.89
	p = 0.92	$p = 0.08^{+}$
Constant	0.58	1.00
	(0.23,0.94)	(0.77,1.23)
	t = 3.23	t = 8.37
	$p = 0.004^{**}$	$p < 0.001^{***}$
Observations	28	28

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.52: Linear Model Proximity to Partner, Human Partners (ENOT)

	Prox. to Partner (Human Partners)
Litter	-0.39
	(-1.16,0.39)
	t = -0.98
	p = 0.33
Test (Novel Object)	0.04
	(-0.73,0.82)
	t = 0.11
	p = 0.92
Group (Pack)	0.27
	(-0.51,1.05)
	t = 0.67
	p = 0.51
Test:Group (Novel Object:Pack)	0.12
	(-1.44,1.69)
	t = 0.15
	p = 0.88
Constant	1.44
	(0.63,2.24)
	t = 3.49
	p = 0.0005***
Observations	56

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.54: Linear Model Proximity to Partner, Pack Only (ENOT)

	Prox. to Partner (Pack Only)
Litter	-0.16
	(-0.54,0.21)
	t = -0.85
	p = 0.40
Condition (Owner)	-0.50
	(-0.95,-0.05)
	t = -2.16
	p = 0.04*
Condition (Canine to Owner)	-0.50
	(-0.95,-0.05)
	t = -2.16
	p = 0.04*
Test (Novel Object)	-0.22
	(-0.68,0.24)
	t = -0.93
	p = 0.36
Condition:Test (Owner:Novel Object)	0.41
	(-0.23,1.05)
	t = 1.26
	p = 0.21
Condition:Test (Canine to Owner:Novel Object)	0.41
	(-0.23,1.05)
	t = 1.26
	p = 0.21
Constant	3.55
	(3.17,3.93)
	t = 18.51
	p < 0.001***
Observations	32

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

TABLE A.56: Linear Model Proximity to Partner, Post-hoc, Condition & Test (ENOT)

	Prox. to Partner Post-hoc Models		
	(Human Partners Condition)	(Exploration)	(Novel Object)
Litter	-0.70	-0.53	-0.24
	(-1.21,-0.18)	(-1.64,0.57)	(-1.45,0.97)
	t = -2.63	t = -0.95	t = -0.39
	$p = 0.02^{*}$	p = 0.36	p = 0.70
Group (Pack)	0.63	0.21	0.33
	(0.11,1.16)	(-0.91,1.33)	(-0.89,1.55)
	t = 2.37	t = 0.36	t = 0.53
	$p = 0.03^{*}$	p = 0.72	p = 0.61
Constant	2.78	1.54	1.37
	(2.31,3.25)	(0.53,2.55)	(0.27,2.48)
	t = 11.54	t = 3.00	t = 2.44
	$p < 0.001^{***}$	$p = 0.01^{**}$	$p = 0.03^{*}$
Observations	28	28	28

Note: Reported values: estimate, 95% confidence limits, test statistic, p-value.
*** p<0.001, ** p<0.01, * p<0.05, + p<0.1

A.4 ANOVA and Paired T-Test Results

A.4.1 ANOVA Test of Salivary Cortisol Samples

TABLE A.55: ANOVA (Type III) Test of Salivary Cortisol Samples Taken Before and After SST

	Sum Squares	Df	F value	Pr(>F)
(Intercept)	7.2047	1	5.6738	0.028*
Group	5.6778	1	4.4714	0.048*
Sample Order	7.5524	1	5.9476	0.025*
Residuals	24.1265	19		

A.4.2 Tukey's HSD Test of Salivary Cortisol Samples

TABLE A.57: Tukey's HSD Test of Salivary Cortisol Samples Taken Before and After SST

Pet-Pack	Difference	Lower Bound	Upper Bound	P adjusted
Before SST	-0.52	-1.42	0.37	0.22
After SST	-1.59	-3.65	0.47	0.12

A.4.3 Paired T-Tests of Dynamic Methylation Samples

TABLE A.58: Paired T-Test of Dynamic *OXTR* Methylation Samples Taken Before and After SST

Group	Mean of Differences (95% CL)	t	Df	p value
Cortisol Rise	2.34 (1.32,3.36)	6.35	4	0.003**
No Cortisol Rise	-2.6 (-14.04,8.84)	-2.89	1	0.21

UNIVERSITY OF VETERINARY MEDICINE VIENNA

INTERDISCIPLINARY MASTER'S THESIS

A Relational Approach to Aggression in Pet Dogs

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UNIVERSITY OF VETERINARY MEDICINE VIENNA

Abstract

Ethics and Human-Animal Studies Unit

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Master of Science

A Relational Approach to Aggression in Pet Dogs

by Katherine M Grillaert BS

Clare Palmer's relational theory of animal ethics identifies duties that humans owe to animals based on morally relevant interactions and interferences in animal lives. Importantly, this approach may differentiate between scenarios that capacities-based approaches find indistinguishable, highlighting the moral importance of context. The complexity of the human-dog relationship illustrates the utility of such an approach for providing moral guidance regarding the treatment of dogs. In this paper, I will examine three paradigm cases of human-directed aggression in the domestic dog through Palmer's relational lens. These cases emphasize the effects of causal interactions, but also yield some surprising results that highlight dogs' vulnerability due to domestication. I conclude with an application of Palmer's proposal to account for domestication and comment on the successes and difficulties of applying her approach to my paradigm cases of aggressive pet dogs.

VETERINÄRMEDIZINISCHE UNIVERSITÄT WIEN

Abstrakt

Ethik der Mensch-Tier-Beziehung

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Ein relationaler Ansatz zur Aggression bei Haushunden

von Katherine M Grillaert BS

Die relationale Theorie der Tierethik Clare Palmer identifiziert Pflichten, die der Mensch dem Tier schuldet, basierend auf moralisch relevanten Interaktionen und Eingriffen in das Leben der Tiere. Wichtig ist, dass dieser Ansatz zwischen Szenarien differenzieren kann, die von kapazitätsbasierten Ansätzen als ununterscheidbar finden, was die moralische Bedeutung des Kontextes hervorhebt. Die Komplexität der Mensch-Hund-Beziehung veranschaulicht die Nützlichkeit eines solchen Ansatzes für die Bereitstellung moralischer Richtlinien für die Behandlung von Hunden. In diesem Beitrag werde ich drei paradigmatische Fälle von durch den Menschen ausgelöster Aggression beim Haushund durch Palmers relationale Linse untersuchen. Diese Fälle betonen die Auswirkungen kausaler Interaktionen, liefern aber auch einige überraschende Ergebnisse, die die Verwundbarkeit von Hunden aufgrund der Domestikation hervorheben. Ich schließe mit einer Anwendung von Palmers Vorschlag zur Erklärung der Domestizierung und kommentiere die Erfolge und Schwierigkeiten bei der Anwendung ihres Ansatzes auf meine paradigmatischen Fälle von aggressiven Haushunden.

List of Abbreviations

HAB	**H**uman-**A**nimal **B**ond
HAI	**H**uman-**A**nimal **I**nteractions
HAR	**H**uman-**A**nimal **R**elationship
HDR	**H**uman-**D**og **R**elationship

1. Introduction

1.1 A Relational Approach May Clarify Our Duties Toward Aggressive Dogs

The relational approach toward animal ethics developed by Clare Palmer (C. Palmer, 2010) exposes moral duties that arise from morally relevant interactions between humans and non-human animals. This approach may be especially applicable to the relationally rich situations that we encounter between humans and domestic dogs. Furthermore, it may be able to shed ethical light on situations where the relationship has suffered due to aggressive behavior exhibited by the dog. An exploration of the multi-faceted relations between humans and domestic dogs can be aided by the human-dog relationship (HDR), a conceptualization of the factors that both arise from and affect the HDR itself through a feedback loop. Aggressive behavior in dogs is a noteworthy phenomenon, as it can have significant effects on the HDR, and the HDR can also significantly affect the incidence and character of aggression. Furthermore, human-directed aggression in dogs can result in extreme and controversial consequences for the dog, including aversive training methods, loss of the home, and behavioral euthanasia. These outcomes are less socially acceptable, or indeed even unacceptable, in a behaviorally "normal" dog (for example, euthanasia of a physically healthy animal has the negative moniker "convenience euthanasia"), yet are much more prevalent and even accepted in the case of aggressive dogs.

A capacities-based moral approach would likely find that the moral elements of a given conflict between humans and dogs are identical whether a dog is aggressive or non-aggressive – after all, the morally relevant capacities do not change.[1]

However, given the complex HDR between humans and dogs, a relational approach may help us discover moral duties that are not generated by capacities-based approaches. Therefore, for my research question, I will apply Clare Palmer's theory to three theoretical paradigm cases of aggressive dogs to determine if the theory can discriminate between scenarios based on relational criteria. As a result of this process, I will ask if Palmer's theory informs us of duties that may be present when a dog is aggressive, and how this might reflect and inform our societal treatment of aggressive dogs.

First, I will examine what it means for a dog to be aggressive in the context of the human-dog relationship and human society. Next, I will provide a conceptualization of the human-dog relationship and center it within Clare Palmer's relational approach as a way to theorize our duties toward domestic dogs beyond capacities-based approaches. I then discuss three

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[1] This is not true, of course, if we consider "aggression" to be a capacity. I will briefly consider this scenario later in my paper.

paradigm cases of the human-dog relationship which have the potential to expose relational moral duties, and which may assist in our understanding of what we owe to dogs with human-directed aggression.

1.2 The Human-Dog Relationship

The evolutionary history of humans and dogs and their mutual relationship is appreciably interwoven, although it is difficult to construct a singular theory of canine domestication. Anthropological, archeological, and phylogenetic evidence support at least five non-exclusive theories. The truth is probably a combination of more than one of these propositions (Miklosi, 2007) and might be simplistically conceptualized as occurring in two phases: the divergence of the dog from a wild ancestor, and the development of the indigenous dog into recognizable and functionally distinct dog breeds (Zhang, Khederzadeh, & Li, 2020). This first phase may have occurred when scavenging canines found it advantageous to follow human activities (Koler-Matznick, 2002), or conversely that such human activities produced byproducts that conferred fitness to canines that began to scavenge (Coppinger & Coppinger, 2001). After establishing contact, elements of co-evolution may have taken place, as dogs and humans began to rely on each other for functionally different skills (Paxton, 2000). Then, humans may have selected specific individuals from the local population, over generations transforming tolerance into affiliation (Lorenz, 2002; Clutton-Brock, 1984; Paxton, 2000), and finally differentiating into breeds that could guard, herd, hunt, or pull sleds (Morey & Aaris-Sørensen, 2002; Guagnin, Perri, & Petraglia, 2018). This process, or parts of this process, likely occurred at several points in the historical timeline, and in several different locations across the globe (Miklosi, 2007).

When we arrive in modern times, we find that the domestic dog's natural environment is largely shared with humans to some degree (Miklosi, 2007), and they have developed remarkable inter-specific communication abilities, even performing better than chimpanzees (Topál et al., 2009; Elgier, Jakovcevic, Mustaca, & Bentosela, 2012). Dogs are sensitive to the visual awareness and attentional focus of humans (Pongrácz, Miklósi, & Csányi, 2001; Fukuzawa, Mills, & Cooper, 2005; Virányi, Topál, Gácsi, Miklósi, & Csányi, 2004), and follow human pointing gestures (Miklósi & Soproni, 2006), and gaze (Soproni, Miklósi, Topál, & Csányi, 2001). They respond to human emotional signals (Gácsi et al., 2013), employ referential emotion communication (Merola, Prato-Previde, Lazzaroni, & Marshall-Pescini, 2014), and there is evidence that they can imitate humans (Fugazza & Miklósi, 2014). Taken together, dogs' inter-species communication skills are compelling evidence of their inherent orientation toward relationships with humans.

For many dogs, this enhanced communication, along with increased human-directed sociability, has delivered them to the warm hearth of the human home, complete with family standing (Bonas, McNicholas, & Collis, 2000; Fifield, 2000). Indeed, the United States alone is home to 89 million owned dogs (Bedford, n.d.-a), with nearly half of all US households owning at least one dog (Bedford, n.d.-b).

Dog-assisted therapy, dog-assisted interventions, dog ownership, and even social interactions with someone else's dog, can confer physical and psychological benefits to humans. Studies have found a reduction in perception of minor health problems such as cough and headache (Serpell, 1991), improved cardiovascular health (Wilson, 1987; Vormbrock & Grossberg, 1988; Grossberg, Alf Jr, & Vormbrock, 1988), reduction in loneliness (Powell et al., 2019; Banks & Banks, 2005; Bernstein, Friedmann, & Malaspina, 2000), and reduced stress (Siegel, 1990; Fiocco & Hunse, 2017).[2] Some of the stress-reduction effects may be reciprocal, as well. Affiliative social contact with humans can reduce dogs' heart rates (McGreevy, Righetti, & Thomson, 2005) as well as cortisol levels (Tuber, Hennessy, Sanders, & Miller, 1996; Shiverdecker, Schiml, & Hennessy, 2013; Horváth, Dóka, & Miklósi, 2008), a hormonal indicator of stress.

Several studies have investigated the role of oxytocin, a neurohormone that mediates social behavior in mammals, including dogs (Kubinyi et al., 2017; Buttner, 2016; Jensen et al., 2016; Thielke et al., 2017). Administration of endogenous oxytocin caused dogs to orient, approach, and shower higher affiliation with their owners (Romero et al., 2013), and improves dogs' responses to human social cues of pointing and gaze (Oliva, Rault, Appleton, & Lill, 2015). There is also a positive oxytocin feedback loop between both partners in a dyad. When dogs and their owners are engaged in an affiliative encounter and gazing, plasma urinary oxytocin levels of both rise (Handlin, Nilsson, Ejdebäck, Hydbring-Sandberg, & Uvnäs-Moberg, 2012; Nagasawa et al., 2009,2015; Rehn et al., 2014; MacLean et al., 2017; Beetz, Uvnäs-Moberg, Julius, & Kotrschal, 2012; Miller et al., 2009), indicating that it may play a role in maintaining a close social bond between the pair.

Research in the field of human-animal interactions (HAI) broadly examines many types of interactions between humans and non-human animals. Within this field, we find the study of a subset of interactions termed the human-animal relationship (HAR). The HAR is the set of interactions between two individuals who are familiar with one another (Hinde, 1976; Hosey & Melfi, 2014). This definition has been expanded to allow for an individual to be substituted by a group, such that a human can have a relationship with a group of livestock (Waiblinger et al., 2006; Hemsworth & Coleman, 1998), where it might be difficult to have individual relationships with each animal, yet there is still a high level of familiarity. The HAR can take on a positive or negative valence depending upon the quality of the shared interactions (Hosey & Melfi, 2014). When additional conditions are met, a special case of the HAR is the human-animal bond (HAB), and this term is used most often in companion animal and laboratory animal literature, and less often when describing HAI of wild, zoo, and agricultural animals (Hosey & Melfi, 2014). The American Veterinary Medical Association defines the HAB as a *"mutually beneficial and dynamic relationship... influenced by behaviors that are essential to the health and well-being of both"* (Association, n.d.). Russow (Russow, 2002) proposed three criteria to distinguish a HAB from other types of HAR: 1) the relationship should be between a human and an individual animal, 2) the relationship persists over time and is reciprocal, and 3) it tends to increase the well-being of both members

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[2] Please see a review by Barker (Barker & Wolen, 2008) regarding further benefits, plus some cautionary notes.

of the relationship. It is clear from these definitions that most humans and pet dogs have an HAR (or, specifically, a human-dog relationship, HDR), and some may meet the criteria of an HAB.

We find further evidence for the HAB between people and dogs when we examine attachment research, but first I want to make a cautionary statement. The terminology that we use to describe the type of relationship between people and animals can influence our assessment of the motivations behind their behaviors and their performance in the partnership (Rault, Waiblinger, Boivin, & Hemsworth, 2020), as well as color our ethical attitudes (Anthony, 2003). It can also imply or exaggerate a positive valence, even in the absence of evidence. For this reason, I favor using the more neutral term HAR (or HDR, in the specific case of humans and dogs), placing the burden of proof on the human to demonstrate positive quality and improved well-being for each dyad in question, and within any given context. Nevertheless, there is substantial evidence for the ability of humans and dogs to form social bonds, and the quality of such a relationship affects their welfare and performance (Lefebvre, Diederich, Delcourt, & Giffroy, 2007).

One type of social bonding is attachment, which has been defined as a close and persistent relationship (Cohen, 1974), interdependency which is revealed through behavioral preferences (Wickler, 1976), and a behavioral system based on proximity seeking and proximity maintenance (Bowlby, 1969; Ainsworth & Wittig, 1969). Mary Ainsworth and John Bowlby proposed an attachment theory that described the relationship between infants and their caregivers (Bowlby, 1969; Ainsworth & Wittig, 1969). Observations that dogs seek support from their owners in a way similar to children with parents led to the adaptation of the attachment experiments performed with toddlers to instead be used with canine subjects (Topál et al., 1998). Several studies examining this relationship have found evidence that dog-human dyads can fulfill the four conditions characteristic of an attachment as defined by Ainsworth: the presence of the attachment figure reduces the dog's distress in a stressful situation (the safe-haven effect) (Gácsi et al., 2013), dogs explore more when in the presence of their attachment figure (the secure-base effect) (Horn et al., 2013; Mariti et al., 2012; R. Palmer & Custance, 2008), and therefore the dog aims to both maintain proximity to the attachment figure (Schöberl et al., 2012) and shows separation-distress-like behaviors when separated (Topál et al., 1998). Dogs' performances on social and cognitive tests are also be improved by the presence of the attachment figure to whom they are securely attached (Horn et al., 2013; Thielke & Udell, 2019).

Applied research has recognized that quantitative and qualitative evaluation of the human-dog relationship and attachment can be used to improve well-being of both partners (Wilson & Netting, 2012) and to identify at-risk relationships (Payne, Bennett, & McGreevy, 2015). The welfare of military working dogs was found to be positively correlated with the quality of the dog-handler relationship (Lefebvre et al., 2007). Owner's attitudes toward their dog are correlated with the dog's cortisol levels (Schöberl et al., 2012), and with the owner's perception of the dog's pain levels (Ellingsen, Zanella, Bjerkås, & Indrebø, 2010). Owners' expectations also contribute to the relationship. Overestimation of, and misunderstanding of, dogs' cognitive abilities (Horowitz, 2014; Howell, Toukhsati, Conduit, & Bennett, 2013), can lead to dissatisfaction within the relationship (Payne et al., 2015). The quality of the attachment can lead to negative behavioral expectations (Zilcha-Mano, Mikulincer, & Shaver, 2011), which may also bias the perception of the relationship.

Personality research has also begun to identify correlations between human personality, dog performance, dog cortisol, and owners' social attractiveness to their dogs (Schöberl et al., 2012; Kotrschal, Schöberl, Bauer, Thibeaut, & Wedl, 2009; Kis et al., 2012). However, high performance and dyad sociability are not always correlated, so more research is needed to understand how these factors affect the human-animal bond and dog welfare (Payne et al., 2015). Nevertheless, higher satisfaction in the human-dog relationship is correlated with owner personality traits of openness and agreeableness (Cavanaugh, Leonard, & Scammon, 2008), and when activities associated with openness and extraversion were enjoyed by both members of the dyad (Curb, Abramson, Grice, & Kennison, 2013). Humans also demonstrate several aspects of attachment to their dogs, including proximity maintenance (Prato-Previde et al., 2006; Kurdek, 2008), distress when separated (Planchon & Templer, 1996; Gerwolls & Labott, 1994; Osofsky, Osofsky, Kronenberg, Brennan, & Hansel, 2009), the ability of the dog to function as a secure base (Kurdek, 2008), and to a lesser extent as a safe haven (Kurdek, 2008).

While several tools measure aspects of the human-dog relationship, there is no single standard, comprehensive tool (Payne et al., 2015). Any such instrument should be used with caution and carefully selected to answer a specific question. It is important to note that 1) owner attachment to the dog and 2) dog attachment to the owner should be assessed separately, as they are not always congruent (Payne et al., 2015). It is likely that to thoroughly understand the human-dog relationship, we will need to include measurements of human behavior and attachment, dog behavior and attachment, human attitudes and perceptions, and dyadic factors such as cooperation and performance (Payne et al., 2015). As well-being is a critical aspect, resource-based and management-based measurements should also be assessed. While such measurements are used in welfare assessments for shelter dogs and working dogs (Arena, Berteselli, et al., 2019; Rooney, Gaines, & Hiby, 2009; Barnard et al., 2016) and livestock (Blokhuis, Miele, Veissier, & Jones, 2013), it is not often explicitly referenced in the human-dog bond literature. However, provision of resources, medical care, exercise opportunities, social opportunities, and enrichment are all critical components of well-being for both people and dogs. Although resource and management-based measurements are indirect welfare indicators, indicative of risk rather than diagnostic, they are still an important complement to animal-based (and human-based) measures of welfare. As we will see in the coming sections, the welfare damage caused by canine behavioral problems can be amplified by how such problems also negatively affect the animal's access to resources, and the animal's management restrictions. (Likewise, the human can experience decreased welfare due to how they must manage and provide for a dog with a behavioral problem, but that is beyond the scope of this paper.)

1.3 Disruption of the Dyad

Unfortunately, the human-dog relationship is not invulnerable to disruption. In the United States alone, approximately 3.3 million dogs enter the shelter system each year, 640,000 (20%) of which are euthanized (ASPCA, n.d.). Aggression towards people is one of the most common complaints (Tamimi, Malmasi, Talebi, Tamimi, & Amini, 2013; Beaver, 1994; Fatjo, Amat, Mariotti, Torre, & Manteca, 2007; Guy et al., 2001), and a significant risk factor for surrender to a shelter or rescue (Lambert, Coe, Niel, Dewey, & Sargeant, 2015; Siracusa, Provoost, & Reisner, 2017; Diesel, Brodbelt, & Pfeiffer, 2010) and for euthanasia (Hemy, Rand, Morton, & Paterson, 2017). Human-directed aggression is cited as the primary cause for surrender for 10% of all dogs relinquished to metropolitan shelters in the US (Salman et al., 1998), a figure similar to the 10-15% reported for dogs surrendered to Italian shelters (Mondelli et al., 2004; Arena, Berteselli, et al., 2019), and 6-10% reported in the UK (Diesel et al., 2010). Prevalence of human-directed aggression in shelter populations reflects both stray and surrendered dogs, and ranges from 5-25% percent (Arena, Berteselli, et al., 2019; Gates, Zito, Thomas, & Dale, 2018; Lord, Reider, Herron, & Graszak, 2008; Mornement, Coleman, Toukhsati, & Bennett, 2015; Wells & Hepper, 2000), the variation likely due to methods of evaluation and local factors.

Placement in a new home does not always end in success. Human-directed aggression can prevent the development of a bond between a shelter dog and a potential adopter (Jagoe & Serpell, 1996). Even after adoption, behaviorists warn that placement in a new home is not a panacea for behavior problems. If the new environment and contexts are not sufficiently different from those that historically triggered the problem behavior, the dog will likely continue to display aggressive behaviors (Overall et al., 1997). Indeed, Stephen and Ledger (Stephen & Ledger, 2007) found that adopted dogs that showed aggression toward unfamiliar people and veterinarians in their old home were likely to show this behavior in their new home as well, putting the dog's status at risk again. A follow-up survey of 67 shelter dogs found that after 13 months, 40% showed aggressive behaviors, despite passing a temperament test (Christensen, Scarlett, Campagna, & Houpt, 2007). A UK study found that dogs that displayed human-directed aggression were 11 times more likely to have a failed adoption than those without behavioral problems (Diesel, Pfeiffer, & Brodbelt, 2008). Of Australian shelter dogs that showed human-directed aggression within four weeks after adoption, 30% were returned to the shelter (Wells & Hepper, 2000).

Even with behavior modification and pharmacological interventions, human-directed aggression is not a problem that is ever considered "cured", it must be managed for life (Overall et al., 1997). If a rehabilitation program and lifelong management are not financially or practically feasible in a private home, dog owners may look to sanctuaries to provide a safe environment. The problem of limited enrolment aside, housing in a sanctuary is not guaranteed to deliver an acceptable quality of life, and such an option must be evaluated on both a species-specific scale and on an individual basis (Wells & Hepper, 2000; Timmins et al., 2007; Dalla Villa et al., 2013; Hiby, Rooney, & Bradshaw, 2006; Titulaer, Blackwell, Mendl, & Casey,

2013) There is little data on sanctuaries per se, but studies of long-term housing of sheltered dogs indicate that these environments can expose dogs to many stressors (Taylor, Mills, et al., 2007). Welfare consequences of these stressors can be measured using quantifiable physiological measurements, such as body condition and cortisol levels (Polgár, Blackwell, & Rooney, 2019; Sandri, Colussi, Perrotta, & Stefanon, 2015; Willen, Mutwill, MacDonald, Schiml, & Hennessy, 2017), as well as using qualitative behavioral assessments (Polgár et al., 2019; Arena, Wemelsfelder, Messori, Ferri, & Barnard, 2019). The longer that a dog spends in the shelter environment, the greater the risk for the development of mental or behavioral problems, or the intensifying of existing problems (Newbury et al., 2010; Wells & Hepper, 2000; Raudies, Waiblinger, & Arhant, 2021). Considering that some of the stressful stimuli of the shelter environment are correlated with a higher probability of aggression toward humans (Arena, Berteselli, et al., 2019), we can see the development of a destructive feedback loop. Not only does this make it more difficult for the dog to be placed in a home, but is itself one indicator of poor welfare for that individual animal (Arena, Berteselli, et al., 2019).

Finally, dogs with human-directed aggression are not only displaced from their homes but in jeopardy of euthanasia (Fatjo et al., 2007; Galac & Knol, 1997; Reisner, Erb, & Houpt, 1994). A study of Australian shelters reported that 11% of dogs were returned to the shelter for euthanasia due to human-directed aggression, with an additional 9% having bitten someone (Marston, Bennett, & Coleman, 2004). Human-directed aggression is also a significant contributor to owners' requests for euthanasia at a veterinary behavior clinic (Siracusa et al., 2017).

1.4 Defining Human-Directed Aggression in Pet Dogs

Practically defined, aggression occurs "any time an animal growls, snarls, snaps, or bites" (Hammerle et al., 2015). A working group of veterinary behaviorists and academically trained behaviorists further clarified aggression to be "*(1) actions that harm or intend to harm the other participant, or (2) threats and harmful actions that primarily serve to increase distance between themselves and the other participant*" (Jacobs, Coe, Widowski, Pearl, & Niel, 2018). This definition provides dog owners, veterinarians, and behaviorists with a plain-language, working definition to share when discussing an aggressive dog. Yet there is hardly a professional consensus (Jacobs et al., 2018; Bamberger & Houpt, 2006; Dewsbury, 1978; Galef Jr, 1976; Johnson et al., 1972), and one author writes that "*The solution to the problem of 'aggression' is simply to treat the word as a convenient, loosely defined aid to communication... recognizing that we cannot provide an adequate definition and that we are probably lumping together a number of diverse phenomena*"(Dewsbury, 1978). Nevertheless, it is necessary to start somewhere.

The lens through which we study aggression leads to different methods of categorization, all of which may have utility in specific contexts, and all of which are subject to revision (Frank & Dehasse, 2003; Miklosi, 2007). Ethological classification focuses on functions of behavior, such that it allows us to consider the organization of behavioral patterns and the pressures on sets of genes and to consider the proximate and distal mechanisms of evolution (Miklosi, 2007). Despite these guide rails, ethological classification can still be undertaken based on different features, and the approaches may be incongruent (Frank & Dehasse, 2003). Some classification schemes may be based on contextual features such as competition, territory, offspring defense, and so on, or based on underlying emotions or physical states such as fear-based or pain-based aggression (Frank & Dehasse, 2003; Camps, Amat, Mariotti, Le Brech, & Manteca, 2012). Aggression has also been categorized whether it serves to be distance-increasing or distance-decreasing between signaler and receiver, or by its morphology as a threat, inhibited attack, or attack (Feddersen-Petersen, 1991a). Statistical approaches have also been employed. One cluster analysis of owner questionnaires completed at a veterinary clinic found that aggression classifications collapsed into three functional categories, territorial aggression, hierarchical aggression, and resource-based aggression (Bamberger & Houpt, 2006). A factor analysis of nearly 2000 owner questionnaires yielded 11 factors related to aggression, which were later validated with a population of dogs seeking treatment at a behavior clinic (Hsu & Serpell, 2003).

Clinical animal behavior defines 13 categories (maternal, pain, fear, play, territorial, inter-dog, protective, re-directed, food-related, possessive, predatory, impulse-control, and idiopathic aggression based on differential diagnosis (Overall et al., 1997). This approach emphasizes the underlying mechanism, such that practitioners can develop and improve targeted behavioral interventions (Overall et al., 1997). However, Overall laments that veterinary behavioral medicine has been unwilling to develop a consensus terminology (Overall, 2005; Overall & Burghardt, 2006), which has resulted in the widespread classification of aggression based on the victim (Overall et al., 1997), such as we see with the terms human-directed and dog-directed aggression. Indeed, the term human-directed aggression does not indicate a function nor mechanism of the aggression, and for that reason, it is alone not useful in a clinical setting. However, continued use of the terms human-directed and dog-directed aggression, *at least in part*, by behavior practitioners, shelters, veterinarians, and researchers, indicates there is likely some value in differentiating aggression based on the victim, perhaps in that it quickly identifies the possible human and public health risks. I argue that this is further a useful construct when we consider that any category of aggression, when directed specifically at humans, likely damages the human-animal relationship more severely than aggression directed at another target. By identifying human-directed aggression, we identify human-dog dyads that are at greater risk for compounding disturbance.[3]

Despite the challenges of defining it, aggression is a normal part of the behavioral repertoire of social animals, including dogs (Miklosi, 2007). Additionally, aggression need not result in harm, either - “honesty” in signaling and ritualized aggression helps dogs avoid costly contests (Miklosi, 2007). While domestication has most likely selected dogs that have reduced aggression toward human partners (Miklosi, 2007), it may also have increased the probability of aggression by altering the signaling system (Miklosi, 2007) or changing the thresholds for

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[3] There is some evidence to support that a few subtypes of human-directed aggression are associated with lower risks and less damage to the HDR. However, as the general model of interaction between the HDR and aggression would be the same regardless of the underlying mechanism, I am not sure it would be helpful to separate the categories at this level, at least for this paper.

reactivity (Vas, Topál, Gácsi, Miklósi, & Csányi, 2005).[4] Dogs approached by a threatening stranger were classified as responsive or non-responsive, which was highly correlated with breed (Vas et al., 2005). Two theories of sociality are relevant here: one proposed by Fox (Fox, 1970) that the greater the sociality of the species, the more behavioral signals they will use. The second theory, proposed by Goodwin (Goodwin, Bradshaw, & Wickens, 1997), is that the greater the similarity in morphology that a dog breed shows to the wolf, the greater their signaling capability will be. This seems to be supported by a comparative study of poodle and wolf puppies that found the poodle puppies had a higher number of aggressive interactions than the wolf puppies (Feddersen-Petersen, 1991b). Another study of several dog breeds found differences in the number of aggressive signals that each breed demonstrated. These observed differences are likely due to a combination of morphological differences (i.e. ability of upright ears to signal better than floppy ears) as well as genetic mechanisms controlling the behavioral pattern. Finally, I would be remiss here if I did not at least mention that for the dog's signaling system to be effective, the receiver needs to both observe and understand the variety of signals, and most researchers and practitioners advocate for dog owner education regarding body language (Mariti et al., 2012; Wan, Bolger, & Champagne, 2012; Kerswell, Bennett, Butler, & Hemsworth, 2013).

When does "normal" aggression become a threat to the human-dog relationship? This is not a simple question, and there is no single answer. The first threshold to be met is if the behavior is considered a problem for the dog's owner.[5] Once a dog owner identifies a complaint, practitioners consider several factors when assessing the risk, which in turn influence the management decisions.

There are several risk factors to consider when assessing the severity of aggression, which fall into three general categories: companion-related factors (including the size of the animal), location and severity of the bite, and controllability of triggers (Overall et al., 1997). We also consider the home environment: who is the caretaker, and with whom the dog lives. As we assess and communicate the risk within the context of the HAR, it becomes more clear when "normal" aggression is indeed a threat to the human-dog relationship for the particular dyad.

1.5 Moral Considerability of Domestic Dogs

The keeping, rehoming, and euthanasia of aggressive dogs are controversial practices, and often provoke tense debates among dog owners, trainers, behaviorists, veterinary staff, shelter staff, and the public at large. The moral characteristics can be complicated: anyone who is in the contact zone of the dog may have some morally relevant responsibility for the dog's situation, as well as some degree of moral interest in the outcome. In practice, pragmatic

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[4] It is also likely that we have increased aggression in some lines where we have selected dogs for guarding and protection purposes (Miklosi, 2007).

[5] Note though that even if the owner does not consider it a problem, if the behavior is a problem for family members or the community, the owner will eventually be forced to consider the behavior a problem. Thus, in all but rare circumstances, the human-dog dyad must also be considered in societal context.

factors are considered when deliberating a course of action, including finances to support environmental and behavior modifications, technical ability to undertake such environmental and behavior modification, and inherent safety risks, among others. While the companion dog industry is refining practical applications and best practices for working with aggressive dogs, it is critical to consider the philosophical positions that underpin these decisions and actions. By now, it should be clear that there may be a great deal of particularism associated with the identification and treatment of aggressive dogs. However, if there are some universalizable tenets, we have an obligation to precipitate them using appropriate philosophical reasoning. If we endeavor to act ethically when working with a domestic dog with human-directed aggression, we need to understand our moral obligations toward domestic dogs, including the special case, or cases, of dogs demonstrating human-directed aggression.

1.5.1 Capacity Based Accounts of Moral Status for Dogs

Clare Palmer's central claim is that her relational approach to animal ethics is better equipped to deal with relevant contextual details that are necessary to work out our duties toward animals, than major accounts of animal morality that are largely based on moral capacities (C. Palmer, 2010, p. 25). Palmer sets the stage for understanding the strengths of her approach by providing an overview of three major capacities-based approaches of considering the moral status of animals, and noting what she observes as significant weaknesses. These approaches ascribe moral status based on the animal's ownership of certain relevant capacities, either through individual biology or species status. These capacities might be as simple as awareness of the environment, or as complex as friendship. Not all capacities are morally relevant or morally equal, and different approaches sometimes argue for the inclusion of different capacities.

Interest-based accounts assert that certain capacities, such as sentience, are morally significant. Peter Singer's utilitarianism is perhaps the most well-known, wherein competing interests can be summed and weighed to determine the action that results in the most good being realized. To have an interest is to have a relevant *capacity*; such a capacity allows one to have some sort of experience. Singer argues that the ability to suffer is the basis of having any interest at all (Singer, 2011, p. 55). Therefore, when considering the maximization of good, one must consider the interests of any being that can suffer, which would of course include our domestic dog. We see this approach underpinning many of our decisions involving dogs. For example, on an organizational level, a dog shelter might admit only those animals that are healthy or with easily curable ailments, and reject dogs that have diseases that are costly, difficult to cure, or terminal. In this way, they use their budget to maximize the good in terms of the number of healthy years accrued by their charges. One of the biggest problems that Singer's utilitarianism faces, in Palmer's opinion, is its inability to account for backward-looking duties: those that might require human assistance or reparations to an animal because of the human's past actions (C. Palmer, 2010, p. 31).

In response to the utilitarian movement, Tom Regan developed his moral framework for animal rights based on the concept of *subject-of-a-life*, whereby one is morally significant because of an inalienable, inherent worth (Regan, 2017). A subject-of-a-life is never a means to an end, but itself must be treated as ends-unto-themselves. Regan's argument moves beyond Singer's consequentialist theory. He posits that is not acceptable to consider the interests of one who can suffer yet still move to infringe upon those interests in order to maximize good. Rather, the subject-of-a-life simply cannot be sacrificed. This rights-based approach is rooted in deontology, whereby the action we take is not morally good or bad because of its consequences, but because it was good or bad in and of itself. Here, we see that our domestic dog has the capacities to be a subject-of-a-life: it has interests, preferences, emotions, and a sense that their life is going well or poorly for them. Therefore, we must not take any action that harms the dog. We see this approach reflected in the "no-kill" policy of some shelters and sanctuaries, which will accept any dog and has a policy firmly against euthanasia, upholding the dog's right-to-life. Regan's approach might be better suited than Singer's to deal with backward-looking duties (he makes a case for compensatory justice, p 99), Palmer points out that a significant weakness of his approach lies in the flow of moral duties from negative rights, to the exclusion of positive rights (C. Palmer, 2010, p. 35). While this avoids the problem of being overly demanding (for example, to require the rescue of every wild animal in peril), the result, in Palmer's opinion, is an under-commitment to moral duties. Regan himself hints at this when he discusses the potential of *acquired duties* toward humans and domestic animals (C. Palmer, 2010, p. 38), but does not flesh out the idea much further. In contrast, Palmer's relational theory uses contextual details to discuss potential acquired duties.

A third major movement is the capabilities approach, which values *flourishing* – species-specific (and even individual) means of fulfilling what it means to be a certain animal. These means are conceptualized as what an animal is meant or made to do, fulfillment of the telos or inherent purpose of the animal. This approach was adapted for animals by philosopher Martha Nussbaum (Nussbaum, 2011), who argued that in our moral concerns of animals, we must consider that they are harmed when prevented from fulfilling their capabilities. In this sense, capabilities are founded in capacities, and while not all capacities are valuable, those that assist in living a good life have inherent value. We see the capabilities approach when, for example, a dog shelter places term limits on how long they will keep a dog isolated in a kennel, as this practice harms his flourishing. While Nussbaum struggles with reconciling ideas of the sovereignty of species as a capability, she ultimately concedes that humans often have positive duties to provide aid to animals (C. Palmer, 2010, p. 42-43), which sets her approach apart from those of both Regan and Singer.

These three examples of major approaches to animal ethics share an important commonality: that there are relevant features inherent to the animal itself from which moral consideration is recognized. These features do not change from day to day, or situation to situation, as they are part of the very fabric of the animal's being. If we apply any one of these approaches to a consideration of the moral status of the dog, we will find that our moral duty is the same regardless of, for example, any interactions between the dog and a human. Although this may be somewhat of an overgeneralization, as *some* contextual detail is always present in any approach to moral theory, Palmer's relational approach emphasizes not simply the context of a given situation, but the *generalizable* moral features of the relations between animals and humans, and the duties that these features may confer (C. Palmer, 2010, p. 50). Therefore, individuals of the same species who find themselves in situations that are morally contextually dissimilar from other individuals of the same species may be treated differently.

1.5.2 A Relational Approach Toward Moral Duties

Clare Palmer argues that while a capacities-based approach provides an excellent foundation for moral status and resultant obligations, it incompletely captures our duties toward animals (C. Palmer, 2010, p. 44). Therefore, while Palmer's relational approach to animal ethics does not stand alone, it is an approach that layers on top of another philosophical foundation. (There may be varying degrees of compatibility between one's foundational approach and Palmer's relational approach. She does not necessarily promote any single foundation, but does note that it may work the best when layered with a rights view (C. Palmer, 2010, p. 55).) Palmer argues that we must consider what she calls "relational features of our contacts with animals."(C. Palmer, 2010, p. 44). A relational approach requires us to extend moral duties to animals to whom we have a morally significant relation or contact. These duties often necessitate the provision of aid or reparations.

Palmer's is not the first relational approach to ethics. For example, three major arguments for moral status have been made based on contractual relations (e.g. Budiansky (Budiansky, 1992) and Callicott (Callicott, 1988)), causal relations (e.g. Slote (Slote, 2007) and Rolston (Rolston, 2004)), and affective relations (e.g. Donovan and Adams (Donovan & Adams, 1996) and Callicott (Callicott, 1988)), from which moral duties would then flow (C. Palmer, 2010, p. 51). However, Palmer constructs a relational approach not as a foundation for determining moral status as some of these approaches do, but as an extension of capacities-based theories, whereby we may discover *additional* moral duties due to relations. Furthermore, she aims to avoid particularism, and is concerned with discovering which details are sufficient to confer universalizability (C. Palmer, 2010, p. 50).

While Palmer finds some existing relational approaches to be interesting and even useful, she endeavors to solve some of their drawbacks by developing her own approach. An affective relational approach draws upon our emotional and sentimental attachments to animals to substantiate moral duties. As Palmer notes, although emotions may play a role in ethical interactions with animals, this may not always provide appropriate guidance, as humans can purposefully or even unintentionally distance themselves from their attachments, such as is common with laboratory animals and confined animal feeding operations (C. Palmer, 2010, p. 52). Our intuition reflects this: if a child grows emotionally distant from their pet dog as they grow into their teenage years, we likely still believe that the duties the child owes the dog have not changed. Some affective approaches, such as ethics of care and affective communitarianism, recognize that humans and some animals share communities and have entangled lives. Furthermore, especially in the case of domestic animals, they are part of a relationship with a power imbalance (C. Palmer, 2010, p. 53). While these approaches usually use the concepts of these communities and relationships as sources of bonds and attachments from which moral duties flow, Palmer instead points to the *causal* rather than *affective* elements of these relationships and the concept of dependence as the sources of moral duties (C. Palmer, 2010, p. 63).

Palmer defines causal relations as those relations in which a human has at least some responsibility for the situation the animal finds itself in. Palmer is influenced by some approaches to animal ethics that recognize this as well. Regan, for example, proposes that we apply the concept of compensatory justice, reparations owed to humans based on past harms, to the situations of at least some animals, although he did not thoroughly develop this line of thinking (C. Palmer, 2010, p. 99). Holmes Rolston II had an even broader proposal when recognized that humans are causally responsible for the situations of and even the existence of domestic animals, and therefore we may have responsibilities toward domestic animals that we do not have toward wild animals (C. Palmer, 2010, p. 56). Contractualists have taken this even further by defining a "contract" between humans and domestic animals (C. Palmer, 2010, p. 57). There are significant problems with a contractual approach to animal ethics, as it is difficult to argue that animals can consent to a contract, to what they would consent if they could, and if it is valid to consent to a contract from which one possibly cannot exit (C. Palmer, 2010, p. 61). Furthermore, a peculiar situation arises in the sense that these domesticated animals would not even exist without such a contract to begin with, so it does not make sense to ask, even theoretically, if they would have been better off without a contract, as there is no other way for them to be (C. Palmer, 2010, p. 61). While Palmer does not rely on a contractual approach, her approach does likewise recognize that the roles that humans played in causing the actual creation of domestic animals are likely to be morally relevant (C. Palmer, 2010, p. 62). The existence of dependent and vulnerable animals is one special case of causal relations, and Palmer considers causal relations to be most likely to generate strong moral duties (C. Palmer, 2010, p. 62). She defines her use of the term relation as "*having an effect, potentially having an effect, or having had an effect on another, or the existence of an interaction*" (C. Palmer, 2010, p. 48). Those relations that are between morally considerable beings have the potential to be relations of ethical interest (C. Palmer, 2010, p. 49). This phenomenon is the "something else [that] is morally important along with animals' *capacities*."(C. Palmer, 2010, p. 49)

2. Discussion

2.1 Three Paradigm Cases of Aggression within the Human-Dog Relationship

I have discussed that our duties to dogs flow through capacities-based approaches, and if we are going to assume Palmer's perspective, then also our duties flow through relational contexts. Any time that a human intersects with a dog, there is the potential to generate relational duties. For example, if one hits a dog with his or her car, one is responsible for the dog's suffering, and therefore the duty to provide medical aid is generated. Likewise, if one chooses to move with their noise-sensitive dog from a quiet country home to a loud city home, then one is responsible for the dog's suffering due to the environment, and the duty is generated to provide behavior modification to reduce noise-related anxiety or environmental modification to reduce noise pollution.

The human-dog relationship (HDR) between dogs and humans is of particular relational interest: as briefly outlined above, we have co-evolved over millennia; we share homes, work, and play; we share communication systems; we can form rich and deep social bonds; we can affect each other's health and wellness. Given the influence of the HDR on the dog's welfare and well-being, and the impact of aggression on the HDR, it seems as though this conceptualization has the potential to yield morally relevant relations, and may clarify our duties when aggression disrupts the HDR (see 2.2.1). Of interesting note, this conceptualization seems not only to identify negative duties to not cause an animal to be harmed or suffer, but perhaps also generates positive duties (to be a social companion). Therefore, it comes to mind that we might also ask if the relational approach gives guidance on whether it may be considered ethical to *withhold* positive duties.

I am interested in exploring if Palmer's approach can justify the differential treatment of aggressive and non-aggressive pet dogs.[6] The consequences for dogs with aggressive behavior problems are often different than those dogs without such a behavior problem, including prolonged isolation, physical training methods, severely reduced welfare, and even death by euthanasia. This illustrates a paradigm in which two animals with seemingly similar capacities are treated very differently, and so drastically such that it seems as though the interests of aggressive dogs are at times completely overridden and all positive duties withheld. A capacities-based approach seems unable to examine how this might come to be, although in practice these outcomes are often even considered to be "ethical" by those involved. However, Palmer's relational approach might shed light on meaningful moral distinctions. In the next section, I will use three paradigm cases to explore this question.

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[6] For ease of reading, I will use the phrase "aggressive dog". In practice, it is more helpful to remember that aggression is a behavior shown by animals, and not a personality type or state.

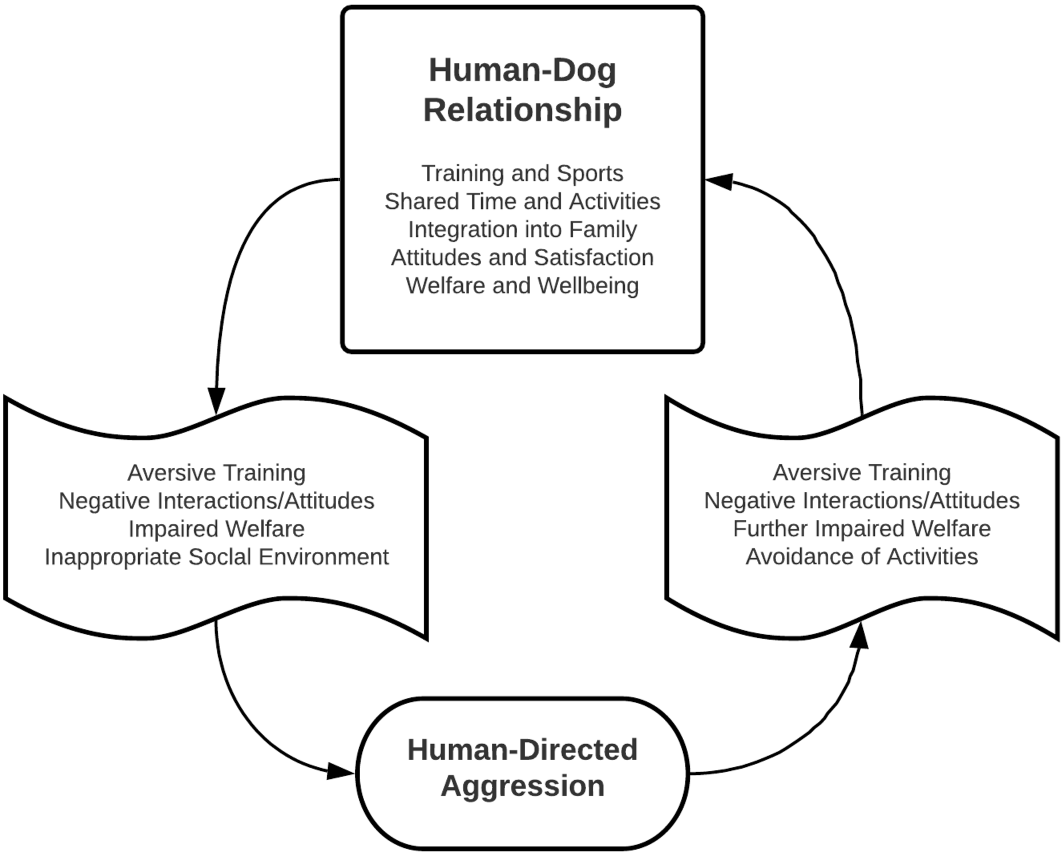

FIGURE 2.2.1: **Conceptualization of Aggression and the Human-Dog Relationship.** Good welfare, positive interactions, and companionship are important factors for both the human and the dog. Aggression can result from inappropriate or impaired execution of welfare and interactions, for example. Aggression can also trigger or exacerbate negative interactions and poor environmental conditions. *Note that this is a simple illustration and not comprehensive, and does not account for many other factors contributing to canine aggression including pain and medical issues.*

My paradigm cases explore three areas specifically related to aggression and where humans have direct contact with and interfere with their pet dog. Each factor (training, ownership, and human-dog bond) is prominent in the HDR conceptualization, and at least on the surface appears to have causal relevancy. For each factor, I will compare two theoretical scenarios involving an aggressive dog who has bitten a human, identical in most aspects except for one characteristic of the factor of interest.[7] I am interested in what we might find to be a morally justifiable ("allowable") consequence chosen for the dog by the owner. Therefore, I will pose the question: "Is the owner of the dog allowed to 1) abandon or refuse to repair the human-dog relationship, or does the owner 2) have the moral duty to provide behavioral modification?" The assumptions are thus: abandoning the relationship by rehoming the dog

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[7] It is of special note that I am setting aside the very real-life complications that occur when human welfare is also at stake, and these examples represent a narrow view of what might be morally acceptable but absolutely do not prescribe what actions should be undertaken in a given real-life situation.

is harmful (although in some situations, rehoming is arguably less harmful), refusing to repair the relationship is harmful, and that behavior modification is not harmful (although in some applications, it could arguably be harmful).

Although I will allude to an aggressive incident (dog bite), I am limiting my discussion to what the dog is owed, regardless of what the person may be owed or to what they may have a right. For example, while I think it is possible that a dog would be owed behavior modification and simultaneously a person owed an existence without being physically or emotionally threatened by the dog, I am only considering here what the dog is owed by the person. I will not try to reconcile all the human-dog conflicts and pragmatic considerations that must be taken into account in a real-life scenario. Instead, I intend to provide a starting point from which to consider the morality of certain consequences that the dog might face.

Finally, although aggressive dogs often face euthanasia, there are significant complexities with defending euthanasia that preclude it from this manuscript. If one finds that euthanasia is in some cases defendable, then my arguments below can be reconsidered with the option of euthanasia in mind. If I find that it is not acceptable to abandon or refuse to repair the human-dog relationship, then euthanasia would likewise also be ruled out. (Footnote: These cases are not presented from an omniscient viewpoint. The purpose of the exercise is not to compare the cases to discover a shared underlying factor (i.e. find the "true cause"), but rather to allow us to compare how we might think through real-life moral duties of two highly comparable cases that differ in one critical aspect.)

2.1.1 Case 1: Dog Training

Most dog training aims to condition a dog to respond reliably to cues given by the human, triggering behavior that the human considers desirable. Basic obedience and "manners" are considered essential foundations for any puppy or newly adopted dog, especially given the closely shared environment between humans and dogs. While the end goal may be to improve the human-dog relationship, the training method itself – the means to the end – is a key factor. Aversive training methods, such as pinch collars, electronic collars, and physically holding the dog down are more likely to result in aggressive behavior from the dog than non-aversive (appetitive) methods (see a review from Vieira de Castro and colleagues (Castro et al., 2020)). It is worth noting here that aggressive behavior is itself a contra indicator for using aversive training methods (Haug, 2008), rather than a sign that a more forceful or intense response should be employed. In addition to poor welfare during the application of such a training method (Ziv, 2017; Castro et al., 2020), there is the potential for lasting damage to the human-animal bond due to increased or intensified human-directed aggressive behavior (Herron, Shofer, & Reisner, 2009).

Training is indisputably an interference imposed on the dog by the human, and it is reasonable to infer that there are moral aspects to this type of relation. The training method is levied upon the dog, who has no control over the issue, by a moral agent. The method has the potential to harm the animal physically, emotionally, or psychologically. Even after the training is completed, the effects of the training method can continue to shape elements of the human-dog relationship, upon which the dog relies for basic needs such as shelter, food, water, and social interaction, as we have seen in the introduction of this paper. Here, I consider a paradigm case involving the training of a dog that later becomes aggressive.

> **Scenario A:**
> Anna is a new dog owner and understands that it is important to train her dog so that it can behave politely in public. Anna uses aversive training techniques in her obedience program – she uses a prong collar and applies collar corrections to punish unwanted behavior. Over time, her dog becomes aggressive and bites her.
>
> **Scenario B:**
> Anna is a new dog owner and understands that it is important to train her dog so that it can behave politely in public. Anna uses appetitive training techniques in her obedience program – she uses treats and toys to reward her dog for desired behavior. Over time, her dog becomes aggressive and bites her.
>
> *Is Anna allowed to 1) abandon or refuse to repair the human-dog relationship, or does she 2) have the moral duty to provide behavioral modification?*

In both situations, Anna interferes with the dog by applying a training method. In Scenario A, she applies an aversive method, which we understand is more likely to result in aggressive behavior than a non-aversive method. Anna is causally responsible for the situation of the dog, which resulted in episodic harm (decreased welfare during the training session). Furthermore, it could likely result in enduring harm, as the aggressive behavior may continue to negatively affect several facets of the HDR, including lowered owner satisfaction, less time spent together, fewer shared activities, and the continuation or escalation of aversive training methods.[8] Thus, she owes reparations (behavior modification) to the dog.

In Scenario B, Anna applied an appetitive training method, which is less likely to result in aggressive behavior. Therefore, although her training method in Scenario B is *an* interference, it is not likely to be *the* interference that caused the aggression. Thus, it may be morally justifiable to abandon or refuse to repair the relationship in Scenario B.

In reality, it might be that someone proceeds with an aversive training method after measuring it against the consequences that a dog faces if the behavioral problem is not solved. There is a myriad of moral components to such an equation, which I do not have space for in this essay. However, the relevant component for our purposes is that *even if* the aversive training is so justified, the relational duties generated from such an intervention would remain the same as those generated from an intervention that is “unjustified”, as Palmer’s relational approach focuses on the interaction itself, not the justification thereof.

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[8] One might be compelled to argue that we do not know for certain that Anna’s training method caused the aggression, and perhaps the dog was genetically predisposed toward aggression. As I was responsible for constructing this theoretical scenario, I will divulge that it is indeed the training method, not genetics, that led to the dog developing aggression. However, this objection speaks to the fact that in the real world it can be difficult to tease these elements apart so cleanly.

2.1.2 Case 2: Dog Adoption

Within the relationship between humans and dogs, each is expected to bring certain benefits (e.g. food, water, shelter, from the human; protection or other work and companionship from the dog). The human's moral duties include the provision of basic needs such that the dog enjoys good welfare and flourishes. A relational ethic posits that the interference of engaging in ownership with a dog constitutes a morally relevant interference. In this paradigm case, I explore a case of an aggressive dog that is adopted.

> **Scenario A:**
> Bo recently adopted a dog from the shelter. Bo is a new dog owner and was careful to request a dog that does not have aggression problems. However, after adoption, it became apparent that the dog was aggressive, and it bit Bo.
>
> **Scenario B:**
> Bo recently adopted a dog from the shelter. Bo is a new dog owner but agreed to take on a dog that has aggression problems. However, management quickly failed, and the dog bit Bo.
>
> *Is Bo allowed to 1) abandon or refuse to repair the human-dog relationship, or does Bo 2) have the moral duty to provide behavioral modification?*

In both Scenarios, Bo interfered by imposing ownership upon the dog. In Scenario A, Bo attempted to secure ownership of a dog without aggression problems, as Bo did not feel prepared to care for an aggressive dog. The dog that Bo adopted, however, was aggressive, which was not caused by Bo in any way: Bo did not commit any action that caused the dog to be aggressive. Therefore, in Scenario A, Bo is allowed to abandon, or refuse to repair, the human-animal relationship with this dog.

Similarly, in Scenario B, Bo did not commit any action that caused the dog to be aggressive. Therefore, again, we find that Bo is allowed to abandon, or refuse to repair, the human-animal relationship with this dog. This seems counterintuitive, as we might expect that Bo would have more duties in Scenario B than in Scenario A. Although Bo did not directly cause the aggression, what if we consider the sort of contract that Bo established when Bo agreed to adopt an aggressive dog, causing the dog to enter a human-animal relationship with Bo? Although the idea of contracts between animals and humans is deeply flawed, might we still find that the human promisor, as a moral agent, can be held morally responsible? While Palmer does not discuss contracts at length, she does highlight a contractual flaw on the part of the animal that could also be applied to the human side of the contract: the rational being entering into the contract must understand the terms of the contract (C. Palmer, 2010, p. 59). Although humans are considered rational beings whereas dogs are not, here we can emphasize the second element: "what does it mean to *understand* a contract?"

I argue that it would be difficult to prove that the average pet owner has enough knowledge to be considered able to consent to the terms of such a contract. Earlier in the paper, I have discussed that aggression is sometimes difficult to define, and that its perceived severity can be relative to contextual details. The average person, even one who has experience with dogs, does not seem particularly equipped to manage or train aggressive dogs. One study of dog owners, veterinarians, trainers, and non-owners found that people were remarkably poor at describing and interpreting dog behavior, and that performance was even worse when the observers tried to classify aggression (Tami & Gallagher, 2009). Another study found that parents were frequently unaware of factors that could contribute to a dog-child bite (Reisner & Shofer, 2008). A study of ten years of behavioral records from the Animal Behavior Clinic at Cornell University found that 60.6% of cases were diagnosed with human-directed aggression, with numbers trending upward over time (Bamberger & Houpt, 2006). Although there may be several reasons for this trend, such as a greater number of aggressive dogs in the population, or less tolerance of aggression by families, it nonetheless suggests that human-directed aggression is an issue that many dog owners cannot resolve without professional help. Furthermore, a study of pet owners who relinquished animals found significant knowledge deficits regarding basic pet behavior, and the authors wrote that this might lead to "unrealistic expectations and inappropriate actions by owners in an attempt to solve a problematic behavior" (New Jr et al., 2000). Taken together, we might conclude that the hypothetical "average" dog owner is not qualified to consent to the adoption of an aggressive dog. Instead, to consent to such a contract, one might need to have such substantial experience and education that they are no longer considered "average".

2.1.3 Case 3: Human-Dog Bonding

In the introduction to this paper, I have discussed the impressive abilities of dogs to communicate and form relationships with humans, even such that the human-dog relationship shares many features with the parent-child relationship. I have also presented a conceptualization of the human-dog relationship, illustrating the interconnectedness of human attitudes, personality, and behaviors and dog behaviors and temperament. The simple act of feeding and exercising one's dog contributes to a conditioned emotional response which can have feedback on the types and qualities of interactions that will be had in the future. Therefore, in this final paradigm case, I explore a scenario with an adopted dog that has a positive or a neutral human-dog relationship.

> **Scenario A:**
> Ray adopted a dog from a friend. He made sure to feed and exercise his dog as the veterinarian recommended. He also made a substantial effort to bond with the dog by spending time with and playing games together. The dog became aggressive and bit Ray.
>
> **Scenario B:**
> Ray adopted a dog from a friend. He made sure to feed and exercise his dog as the veterinarian recommended. He did not try to bond with the dog, did not spend extra time with the dog, and did not play games with the dog. The dog became aggressive and bit Ray.

> *Is Ray allowed to 1) abandon or refuse to repair the human-dog relationship, or does Ray 2) have the moral duty to provide behavioral modification?*

In Scenario A, Ray invested time and effort into developing a positive relationship with his dog. Despite this, the dog still became aggressive. While Ray did "interfere" with the dog, it was not a cause of the aggressive behavior, and Ray may choose to abandon or refuse to repair the relationship.

In Scenario B, there was no intervention. While Ray took excellent physical care of his dog, he chose not to invest time and effort into building a relationship. It would be difficult to argue that Ray has a relational duty to provide reparations flowing from a *non-intervention*. Therefore, we conclude that he may choose to abandon or refuse to repair the relationship.

This is a surprising result, as it seems that Ray from Scenario B was a "worse" dog owner than Ray from Scenario A – shouldn't Ray from Scenario B owe something more? But although we may see the second Ray as a "worse" dog owner, a moral value stemming from this judgement must be reasoned, not assumed.

My previous paradigm cases posed questions regarding what we ought to do in a scenario where we have infringed upon negative rights: the right to not be harmed. The dog had a right not to be harmed by harsh training, and the dog had a right not to be harmed by abduction into an unsuitable contract. In this third case, is it possible that we are instead questioning what we ought to do in a scenario where we have infringed upon the *positive* rights of the dog to be provided something necessary for its welfare?

This objection posits that the human-dog relationship generates a positive moral duty for the human to be a social companion to the dog, and to develop a positive human-dog bond. By not fulfilling this positive duty, Ray has harmed the dog. This seems to be an argument that is well-supported by our understanding of the duties generated by the human-dog relationship. However, in order to hold Ray accountable for the aggressive behavior of his dog, we must clearly find a link between the quality of the attachment and the aggressive behavior.

Emergent human-dog bond research suggests that bond quality, human personality, and human attachment might influence dog behavior, and predict whether the relationship will be successful or unsuccessful. Pet owners who relinquish their pets to shelters score lower on scales of attachment than those who do not relinquish their animals (Kwan & Bain, 2013). Owner attachment style can be associated with some dog behavioral problems (Konok et al., 2015), and can influence the dog's behavioral strategy in a challenging situation (Rehn, Beetz, & Keeling, 2017). Dogs showing different types of human-directed aggression were more likely to have owners with anxious and avoidant attachment styles when compared to dogs without aggression issues (Gobbo & Zupan, 2020). This research begins to suggest that several intrinsic factors of the owner may affect dog welfare. However, it remains difficult to ascertain a direct, causal link between Ray's lack of a bond with his dog and the dog's aggression. This missing or neutral bond is not a specific attachment style. And even if

Ray's reduced attachment predicts relinquishment, we have yet to place aggression in that equation.

A stronger argument for Ray's duties might be this: Ray did not invest much time into his relationship with his dog, and has decided to relinquish the dog because he does not feel emotionally close to the dog. In this case, we have substantially more evidence that sharing experiences such as training and activities increases owner satisfaction with dog ownership (Dwyer, Bennett, & Coleman, 2006; Payne et al., 2015; Herwijnen et al., 2018). In such a scenario, it seems that Ray would have a duty to repair his relationship with his dog.

It seems I have gotten a little closer to understanding our duties with respect to bonding with a dog, but this case has unearthed an even larger question: is the human-dog bond a capacities-based duty stemming from positive rights to have a social partner, or is it a relational duty stemming from the types of interactions that we have with dogs? I will now consider this in the discussion.

2.2 Discussion of Paradigm Cases

I have worked through three paradigm cases to demonstrate how Clare Palmer's relational theory might yield differential moral duties when considering the treatment of aggressive dogs. According to Palmer's theory, there is a suite of moral duties toward animals that cannot be weakened, as they are supported by a capacities-based foundation. However, additional relational duties toward animals may be generated by morally relevant interactions between humans and animals. We have seen in our Dog Training Case that harmful interactions require aid or reparations, and the Dog Adoption Case emphasized the need to explore critically what it means for an interaction or intervention to be causal. The Human-Dog Bonding Case illustrated that failure to deliver positive duties may also require aid or reparations to the deprived dog, but leaves us with some confusion whether this is a relational duty or a capacities-based duty. Finally, in both the Dog Adoption Case and the Human-Dog Bonding Case, we found some surprising results that were against our intuition, and perhaps even left us with the unsettled feeling that while perhaps the dog owner does not owe the dog something, the dog still *seems to be owed*. Somehow, the dog is living within the confines of the human-dog relationship yet does not seem to be supported such that it can achieve good, lasting welfare. These last two points are perhaps both related to the problem of domestication and moral responsibility.

The human-dog relationship goes beyond simply charting a web of interactions between the two – it highlights the responsibilities that humans have toward dogs because of domestication. Dogs are born into an environment heavily shaped by humans (towns and cities), often under direct human control (born in captivity or "rescued" from the streets), and intervention by humans is continually required by humans in most cases (access to food and other resources). Because his constitution has been irreversibly altered by domestication, letting a dog live free from human intervention is a harm to him in the sense that his capacities for self-sufficiency have been diminished, and to do so could be considered failure to provide positive duties.[9] Yet, in the provision of these duties, there is potential to harm the dog, for example, through harsh training or unsuitable home selection. It is conceivably harmful simply for the dog to be embedded in an environment where his interests may conflict with those of humans, yet there is little legal recognition or societal infrastructure to support that his interests are both direct *and* equal. Palmer recognizes this plight concerning aggressive dogs when she writes regarding their "humane killing":

> "This kind of humane killing seems to be underpinned by a judgment about the importance of comparative welfares: the actual or potential harm to human welfare from the animal aggression is regarded as being of more significance than the life of the animal." (C. Palmer, 2006, p. 175)

Domestication seems to have created a debt to the dog, that is not only difficult to fulfill without avoiding any harm, but furthermore, the identification of the debtor is unclear. The dog's needs for a social companion remain whether, or not, it happens to have an owner that can successfully form a bond with that dog. If this need exists regardless of relevant social interaction, is this better seen as a capacities-based characteristic? This might be a controversial statement. Palmer writes *"…domesticated animals are frequently dependent on and vulnerable to humans… and dependence is rightly understood to be a relational quality, not a capacity."* (C. Palmer, 2010, p. 53). While dependence itself may be too broad to define, I would argue that domestication has created a unique scenario where it is impossible to tease apart some qualities that are both capacity-based and relational in nature.

Dogs have been selectively bred for various purposes, including herding, hunting, guarding, sport, and for unique or attractive physical looks. In modern times, dogs have also been purpose-bred for the pet home, which generally means a character that is calm, easy-to-train, enjoys the company of people, is friendly, and is not aggressive.

We know that aggression is partly heritable, as we observe differences in aggression between recognized dog breeds as well as differences within the same breed bred for different types of work, such as field dogs versus ring dogs (Duffy, Hsu, & Serpell, 2008). Stranger-directed aggression in the dog has been found to have an among-breed heritability of between 0.68 (MacLean, Snyder-Mackler, VonHoldt, & Serpell, 2019).[10] The heritability of aggression has also been investigated in different breeds. For example, the heritability of "dominant-aggressive" cocker spaniels was found to be h^2= 0.20 for the sire and h^2= 0.46 for the dam (Pérez-Guisado, Lopez-Rodríguez, & Muñoz-Serrano, 2006), and in Golden Retrievers human-directed aggression has a heritability of h^2= 0.77 (Liinamo et al., 2007).[11] We can

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[9] Not all dogs have diminished capacities to survive on their own, of course, and many of the world's dogs survive and even thrive as feral or free-ranging animals, but this is not the population I am discussing in this paper.

[10] A heritability close to 1 means that a trait is highly heritable. A heritability close to 0 means that variation in a trait is due to the environment, rather than genetics. A heritability of 0.5 means that 50% of the genetic variation of a trait is due to genetics, and 50% due to environmental influence.

[11] The classification of "dominance-aggression" is based on a 5-part test designed by W.E. Campbell (Campbell, W.E., 1972. A behavior test for puppies selection. Mod. Vet. Pract. 12, 29–33). For more information about interpreting this test, see the original paper and also an evaluation by Pérez-Guisado and colleagues (Joaquín Pérez-Guisado, Andrés Muñoz-Serrano, and Rocío López-Rodríguez, 2018. Evaluation of the Campbell test and

see that a dog breeding program has the unique position of affecting the aggression of a dog by changing its *underlying capacity* for aggression. This makes for an interesting situation where we see a close intermingling of capacities and relational aspects. Dog breeding affects the quality of relationship that a dog might have with people, by changing the capacity (to some degree) for having this relation. Additionally, the act of breeding a dog is a relational interference, even though the dog technically does not yet exist.

We would find a similar situation given the measurement of friendliness, which can be as difficult to define as is aggression (Jones & Gosling, 2005), though is decidedly not simply the inverse or absence of aggression. In one study of canine personality, a panel of researchers agreed on seven temperament scales, which placed Friendliness/Sociability and Aggressiveness on separate spectrums (Jones & Gosling, 2005), and a further study found separate heritability scores for "sharpness" (aggression) and "affability" (friendliness) (Van Der Waaij, Wilsson, & Strandberg, 2008). Both friendliness and aggression are likely controlled by the expression of a variety of genes (Zapata, Serpell, & Alvarez, 2016), including those for oxytocin and the oxytocin receptor (M. Persson, Roth, Johnsson, Wright, & Jensen, 2015; M. E. Persson, Trottier, Bélteky, Roth, & Jensen, 2017), some of which may be breed-specific (M. E. Persson et al., 2017), and phenotypically mediated by other traits such as reactivity and fearfulness(Flint, Coe, Serpell, Pearl, & Niel, 2017; Kaneko, Arata, Takeuchi, & Mori, 2013). Like aggression, there is a myriad of opportunities for the *capacity* for friendliness to affect the human-dog relationship, and the *expression* of this capacity is a relational quality.

While this merits further discussion, it may not be necessary to resolve the question of capacities or relational qualities in order to move on in our discussion. Palmer recognizes that humans created this dependence and that a *"special relationship of this kind bring[s] ethical obligations."*(C. Palmer, 2006, p. 180), and she proposes a relational theory to explain who owns the moral obligations to animals.

Individuals, as we have seen, and as is generally common knowledge, have a first-level responsibility toward dependent animals. Palmer also recognizes a broader, second level of responsibility that she considers to be a social obligation toward dependent animals, that humans as a species have acquired through group membership. A weak argument of this nature posits that as a member of the human species, we have benefited in some way from the domestic dog, even if we have chosen not to have a dog. For example, we might wear wool from a sheep that was guarded by a dog or use medication that was tested on a beagle. However, this argument suffers in that it is difficult to ascribe duties stemming from a special relationship when one has not voluntarily entered such a relationship (C. Palmer, 2010, p. 95). A stronger argument recognizes a moral responsibility that, although weaker than direct ownership, flows from causal relations: the volunteerism of participating in pet ownership. That is, by participating in pet ownership, one joins a large group of humans that are generally responsible for the plight of domestic dogs.

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the influence of age, sex, breed, and coat color on puppy behavioral responses. Canadian Journal of Veterinary Research, 72(3), 269-277.)

I will consider a final scenario with this in mind. In Scenario A, Winnie adopts a dog from the shelter. She is generally opposed to pet ownership but realizes that this particular dog is vulnerable and dependent. The dog is aggressive and bites her. In Scenario B, Winnie adopts a dog from the shelter. She is in favor of dog ownership as a whole. The dog bites Winnie.

In neither scenario does Winnie have any particular interaction that causes the dog to show aggressive behavior. However, in Scenario B, Winnie is a member of a group that perpetuates the culture that is responsible for the plight of domestic dogs today and in the future. Due to this weak form of causal responsibility, Winnie is morally obligated to repair the relationship. In Scenario A, Winnie is making a deliberate effort to remove herself from the group responsible for the plight of domestic dogs. While one may argue if this is *actually* possible while owning a dog, at face value, it appears she is less causally responsible.[12] We might conclude that in this scenario, Winnie may abandon or refuse to repair the relationship.

This result is indeed interesting because it calls into question the conclusions of our previous scenarios. It now seems that there is at least a weak imperative for all people in cases 1-3 to attempt to repair their relationship with their dog, as they have a part in perpetuating the culture that makes domestication of dogs a problem for *this* dog.

Finally, I want to close with a cautionary note. Our knowledge about both canine aggression and the intricacies of and dependencies within the HDR will continue to grow. Where will we draw the line regarding what is an *ethical* causal factor, and how strong of a duty such a factor generates? Will Ray owe his dog reparations because he did not engage in a relationship-building canine hobby? If we find compelling scientific evidence that human personality plays a role in canine temperament, might we require personality coaching before allowing dog ownership? It is not too difficult to imagine a future where a relational approach, unchecked and unmodified, might become overly demanding of those who choose to engage in the culture of pet ownership. Furthermore, where would we draw the line trying to mitigate potentially negative factors? Would we restrict dog ownership to people who have the “correct” environment and housing, the “correct” financial situation, the “correct” hobbies, the “correct” education, or even the “correct” personal character? This seems to lead to a new set of questions where the interests and rights of humans may come into conflict with those of dogs.[13]

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[12] For example, if her dog is well-behaved and has a positive relationship with her, would it encourage more people to own a dog?

[13] Not entirely new questions, as shelters and rescue groups have long debated what appropriate restrictions on adopters are: Do they have a fenced yard? Can they afford a steep adoption fee? Should there be required training classes? These debates often bring into conflict claims of what is in the best interest of the dogs with claims of socioeconomic discrimination and biased frames of reference.

3. Conclusion

In this paper, I have explored three paradigm cases regarding how we ought to treat dogs that have shown aggressive behavior. In practice, it can often be difficult to ascertain the exact cause of aggression, not least of all as there can be more than one relevant factor, and factors may impact each other. This presents a significant challenge for a causal theory, which Palmer's relational theory relies heavily upon. However, Palmer does manage to address this by providing a causal explanation for domestication and the perpetuation of domestication, which holds some people responsible for the plight of the dog, namely those who choose to participate in the culture of pet ownership. Palmer's theory differentiates between duties based on relational aspects, and generalizable of some moral elements to situations with similar relevant features (choice of training method, interaction style, choice of becoming a dog owner) can be provide helpful future moral guidance.

Palmer's theory also allows us to tease apart different strengths of obligations based on our interactions with dogs. This perhaps reflects current societal views fairly well. The existence of animal shelters for dogs and cats (but not wild animals) themselves seem to indicate that there is a collective responsibility for domestic pets (C. Palmer, 2006, p. 180) and there is a broadly accepted viewpoint that pet owners should commit to training and caring for their animal. But there is also a rather accepted view that people do not owe dogs an unlimited number of resources, whether they be financial, temporal, or risk of bodily harm. Palmer's relational theory allows for some amount of flexibility given the relations in context, which is critical for guiding any serious real-life application of a moral theory. While Palmer's theory finds strength in its malleability to encompass a variety of real-life contextual details, in the future we might find that its lack of restrictions fails to give enough guidance when faced with growing scientific knowledge of causal factors related to aggression.

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