Social support in wolves (*Canis lupus*) and dogs (*Canis familiaris*). The stress-ameliorating effects of human and conspecific partners in hand-raised and mother-raised pups.

verfasst von
Katrin Hann

angestrebter akademischer Grad
Magistra der Naturwissenschaften (Mag.rer.nat.)

Wien, 2013
In loving memory to my grandparents
Liselotte Kampleitner and Friedrich Hann.
Table of Contents

LIST OF TABLES ................................................................................................................. 3
LIST OF FIGURES .................................................................................................................. 3
ZUSAMMENFASSUNG (GERMAN SUMMARY) ................................................................. 4
ABSTRACT ............................................................................................................................. 5

1 INTRODUCTION ............................................................................................................... 6
   1.1 SOCIAL SUPPORT ......................................................................................................... 6
       1.1.1 Definition and characteristics of social support ......................................................... 6
       1.1.2 Interspecific social support and its relatedness to social bonding ......................... 8
       1.1.3 Human social bonding in wolves and dogs ................................................................. 10
   1.2 HYPOTHESES AND RESEARCH QUESTIONS .................................................................. 12

2 MATERIAL AND METHODS ............................................................................................ 14
   2.1 THE STUDY GROUPS .................................................................................................... 14
       2.1.1 Subjects .................................................................................................................... 14
       2.1.2 Socialisation procedure ........................................................................................... 15
           2.1.2.1 Hand-raising ........................................................................................................ 15
           2.1.2.2 Pet dogs ............................................................................................................... 16
   2.2 EXPERIMENTAL PROCEDURE ...................................................................................... 19
       2.2.1 General experimental arrangement ........................................................................ 19

3 TASK 1: NEOPHOBIA ...................................................................................................... 20
   3.1 METHODS ..................................................................................................................... 20
       3.1.1 Experimental arrangement ....................................................................................... 20
           3.1.1.1 Alone condition .................................................................................................. 22
           3.1.1.2 Partner condition ............................................................................................... 22
       3.1.2 Behavior categories ................................................................................................. 23
       3.1.3 Data analysis ........................................................................................................... 26
   3.2 RESULTS ....................................................................................................................... 28
       3.2.1 The effect of human presence (raiser or owner). A comparison of the test conditions “alone” and “with partner”. ................................................................. 28
           3.2.1.1 Exploration of the object ...................................................................................... 28
           3.2.1.2 Escape behavior ................................................................................................ 30
           3.2.1.3 Stress-related behavior ...................................................................................... 31
           3.2.1.4 Searching for human proximity (cameraperson) ................................................. 33
           3.2.1.5 Standing by the door .......................................................................................... 33
           3.2.1.6 Activity ............................................................................................................... 33
           3.2.1.7 Passive behavior (freezing) ............................................................................... 34
           3.2.1.8 Object-directed play ........................................................................................ 34

Social Support in Wolves and Dogs
3.2.2 The effect of a human partner in regard of her proximity towards the object (phase 1 versus 2 and 3) and communicative interaction with the subject (phase 3) .......................................................... 34
3.2.2.1 Exploration of the object ........................................................................................................ 34
3.2.2.2 Searching for proximity to the human partner ...................................................................... 36
3.2.2.3 Approaching the object out of human proximity .................................................................. 38
3.2.2.4 Escape behavior .................................................................................................................... 39
3.2.2.5 Stress-related behavior ......................................................................................................... 41
3.2.2.6 Activity .................................................................................................................................. 41
3.2.2.7 Passive Behavior .................................................................................................................. 42
3.2.2.8 Object-directed play ............................................................................................................. 42

3.3 DISCUSSION .................................................................................................................................. 43

4 TASK 2: SOCIAL SEPARATION ........................................................................................................ 49
4.1 METHODS .................................................................................................................................. 49
4.1.1 Experimental arrangement ...................................................................................................... 49
4.1.1.1 Phase 2, Condition raiser: Raiser as partner ........................................................................ 51
4.1.1.2 Phase 2, Condition peer: Peer as partner ............................................................................. 51
4.1.2 Behavior categories .................................................................................................................. 52
4.1.3 Data analysis ............................................................................................................................. 53

4.2 RESULTS .................................................................................................................................... 54
4.2.1 Comparison of stress ameliorating effects with (phase 2) and without human and conspecific social support (phase 1 and 3) ................................................................. 54
4.2.1.1 Escape behavior .................................................................................................................. 54
4.2.1.2 Stress-related behavior ....................................................................................................... 55
4.2.1.2.1 Vocalization .................................................................................................................... 56
4.2.1.3 Searching for human proximity (cameraperson) ................................................................. 56
4.2.1.4 Standing by the door .......................................................................................................... 57
4.2.1.5 Activity ................................................................................................................................ 57
4.2.1.6 Passive Behavior ............................................................................................................... 57
4.2.1.7 Playing .................................................................................................................................. 58

4.2.2 Comparing the effectiveness of a raiser (human or mother-dog, respectively) with a peer .... 58
4.2.2.1 Searching for proximity towards the partner ....................................................................... 58

4.2.3 The influence of a peer as a function of its behavior on the stress behavior of the subjects .... 59

4.3 DISCUSSION ................................................................................................................................. 60

5 GENERAL DISCUSSION ............................................................................................................... 63
5.1 LIMITATIONS AND POTENTIAL SUPPLEMENTS OF THE STUDY DESIGN ......................... 66

6 REFERENCES .................................................................................................................................. 68

ACKNOWLEDGEMENTS .................................................................................................................. 81

CURRICULUM VITAE ....................................................................................................................... 83
List of Tables

**TABLE 1.** The hand-raised wolves of the WSC (N = 12), their sex (M = male, F = female), birth date, birthplace and their relatives in the pack. ..................................................................................................................................... 15

**TABLE 2.** The dogs of the WSC (N = 14), their sex (M = male, F = female), birth date, birthplace and their relatives in the pack. ............................................................................................................................................................. 15

**TABLE 3.** The mother-reared dogs (N = 6), their sex (M = male, F = female), breed, birth date, birthplace, their peers and the litter size during testing. .......................................................................................................................................................... 17

**TABLE 4.** Comparison of environmental and social features that hand-raised and mother-raised pups were confronted with at the age of 3 to 8 weeks. ......................................................................................................................................................... 17

**TABLE 5.** The sequence of testing for the Neophobia Task within the three study groups. ..................................................................................................................................................... 23

**TABLE 6.** Coding Table for the Neophobia Test and the Social Separation Test. ................................................................................................................................................................................................. 24

**TABLE 7.** Relative duration of total time the subject spent in close proximity to (< 50 cm) coded locations of the testing room in the Neophobia Task and the Social Separation Task. ........................................................................................................................................................................................................ 25

**TABLE 8.** The number of tested individuals included in the final analyzes of the Social Isolation Test. ..................................................................................................................................................................................................................... 52

**TABLE 9.** Behavioral score of the peer in the partner condition (phase 2) of the Social Separation Task. ........................................................................................................................................................................................................ 53

List of Figures

**FIGURE 1.** Testing conditions of the Neophobia Task at the age of week 5 and 7. ................................................................................................................................................................................................. 21

**FIGURE 2.** Schematic illustration of the test room in the Neophobia Task. I ..................................................................................................................................................................................................................... 22

**FIGURE 3.** Relative time the subjects spent with exploring the object in the Neophobia Task when tested alone and in the presence of a human partner. ........................................................................................................................................................................................................ 29

**FIGURE 4.** Relative time the subjects spent with escaping behavior when tested alone or in the presence of a human partner in the Neophobia Task. ........................................................................................................................................................................................................ 31

**FIGURE 5.** Frequency of stress-related behavior in the Neophobia Task when tested alone or in the presence of a human partner. )........................................................................................................................................................................................................ 32

**FIGURE 6.** Relative time of exploration in the Neophobia task when a human partner was present. ........................................................................................................................................................................................................ 36

**FIGURE 7.** Relative time the subjects spent in close proximity to the human partner in the Neophobia Task. ................................................................................................................................................................................................. 38

**FIGURE 8.** Relative time the animals spent with escaping in the presence of a human partner in the Neophobia Task. ........................................................................................................................................................................................................ 40

**FIGURE 9.** Frequency of stress-related behavior in the presence of a human partner in the Neophobia Task. ........................................................................................................................................................................................................ 41

**FIGURE 10.** Testing conditions of the Social Isolation Task at the age of 5 weeks. ........................................................................................................................................................................................................ 50

**FIGURE 11.** Schematic illustration of the test room in the Isolation Task at the age of 5 weeks. ........................................................................................................................................................................................................ 50

**FIGURE 12.** Relative time spent with escape behavior when tested alone and with a partner in the Separation Task. ........................................................................................................................................................................................................ 54

**FIGURE 13.** Frequency of stress-related behavior when tested alone and with a partner in the Separation Task. ........................................................................................................................................................................................................ 55

**FIGURE 14.** Frequency of vocalization when tested alone and with a partner in the Separation Task. ........................................................................................................................................................................................................ 56

**FIGURE 15.** Relative time spent close to the partner in phase 2 of the Social Separation Task. ........................................................................................................................................................................................................ 59

**FIGURE 16.** The influence of a peer’s behavior on stress behavior of the subject in phase 2 of the Separation Task. ........................................................................................................................................................................................................ 59
Zusammenfassung

Abstract

Empirical evidence shows that the relationship between dogs and their owners may resemble a strong affectional bond, and that domestic dogs react to separation from their owner similarly to human children being separated from their mother. In contrast, Topál et al. (2005) failed to find similar attachment of 4 months old wolf pups to their hand-raisers at the age of 4 months. However, it is known that the mere presence of a social ally – and even more so the attachment figure - can ameliorate stressful events. In order to investigate the effectiveness of a human partner we compared human social support to that of a conspecific. We furthermore tried to reveal the impact of different socialisation and therefore, compared social support by a human hand-raiser to that of the mother-dog. Therefore, dog puppies (N=14) and wolf pups (N=12) were reared under same conditions and their behavior was compared to pet dogs puppies fostered by their own mothers (N=6). The social support effect of their raisers or owners, respectively, was compared in two test situations. At the age of 5 and 7 weeks pups were confronted with novel objects (yellow sponge ball and ringing alarm clock) either alone, or in the presence of their human raiser or owner, respectively. Furthermore, a separation task was conducted where after an isolation period of 5 minutes, the pup was accompanied by a partner before being separated again. Hand-raised wolves and dogs were tested in the presence of their human raiser whereas pet dog pups were accompanied by their mothers. In a second task all tested subjects were tested with a peer. Behavioral stress reaction was videotaped for coding behavior. Our results show that independently from the rearing-history all subjects showed less behavioral stress when accompanied by a human or conspecific partner. However, human social support was more effective than either the mother or a peer. We found greater behavioral stress-responses in the hand-reared animals compared to pet dog pups and wolves generally showed more stress-related behavior, increasing with age, in the relatively stressful clock task. Thus, our findings provide evidence for a synergistic-hypothesis (Gácsi et al. 2009) suggesting that affiliative bonding in dogs to humans developed due to evolutionary and ontogenetic processes. Although wolves may become independent faster during development our data clearly shows that also hand-raised wolf pups accept their human hand-raisers as haven of safety, profiting from social support provided by a familiar human partner in stressful situations.

Keywords: social support, hand-raising, dog, wolf, human partner
1 Introduction

1.1 Social Support

1.1.1 Definition and characteristics of social support

A beneficial aspect of living in socially bonded groups is that group members may support each other in stressful environmental events (Kikusui et al. 2006). Thus, the effects of social bonding may positively affect life history traits such as reproductive success (e.g. successfully raising of offspring) and enhanced survival (Cooke et al. 1995). According to von Holst (1998) stressors are defined as „all social and non-social stimuli that are challenging or threatening to the survival, health, and reproductive success of animals and that are therefore, an essential part of natural selection“. Thus, social support is defined as a stress-ameliorating process provided by the presence of a familiar social partner” (van Holst 1998). While ‘active’ social support intends active interactions in agonistic encounters by social allies (e.g. Horrocks & Hunte, 1983, Pereira 1992), ‘passive’ support describes the emotional stress-buffering effects. Due to the presence of a social ally during a stressful event, endocrine switches may affect motivational effects (Lamprecht 1986a) that modulate individual stress management (Scheiber et al. 2005). In humans, perceived support is suggested to be strongly influenced by the recipients’ judgments on the supporter’s effectiveness in modulating stress (Lakey et al. 1996). Hence, supportive potential itself may emerge from a unique supporter-recipient dyadic relationship (Lakey et al. 1999). The prime model for such emotional social support is the attachment of human babies to their primary caregivers (summarized particularly for human-animal in Julius et al. 2012).
So far, the phenomenon of emotional social support has been investigated in primates (e.g. Horrocks & Hunte 1983; Bernstein & Erhardt 1985, 1986; Pereira 1992), non-human primates (e.g. Coe et al. 1987), non-primate mammals (rats: Davitz & Mason 1955, tree-shrew: von Holst 1986, guinea pigs: Hennessy & Ritchey 1987, alpine marmots: Arnold & Dittami 1997, geese: e.g. Scheiber et al 2009) and humans (e.g. Thorsteinsson et al. 1978).

Social isolation is known to evoke moderate to considerable stress on endocrine and behavioral levels (e.g. rats: Hatch et al. 1965, mice: Valzelli 1973, human children: Clancy & McBride 1975, monkeys: Noble et al. 1976) and novelty (e.g. cats: Maesserman 1943, goats: Lidell 1950, rats: Davitz & Mason 1955, monkeys: Noble et al. 1976).

Since companionship may provide protection from environmental threats, social isolation can be a striking stressor to social living animals when confronted with unfamiliar stimuli. For example, goat kids showed higher tolerance towards a novel environment when accompanied by its mother than when being alone (Lidell 1950). In humans, sub-optimal attachment and social isolation are known co-variates in physiological and psychological pathogenesis (Rabkin & Struenig 1976, West et al. 1986), whereas bonding partners will generally support salutogenesis (Cobb 1976, Ell 1996).

Crucial factors for the evolution of stress buffering systems in social living animals are (1) complex organization, (2) long-term relationships and (3) matrilines (von Holst 1998). Besides mammals like primates or humans these criteria by von Holst also apply to social birds such as Greylag goose, Anser anser, which form flocks and show complex relationships (Lorenz 1979; Rutschke 1982; Schneider & Lamprecht 1990; Fox et al. 1995, Kotrschal et al. 2005, 2010). Engaging in long term pair and family bonds, juvenile geese remain with their parents until the next breeding season (Lorenz et al. 1978, Lamprecht 1987, Hemetsberger 2001). Offspring benefits from parental alertness and assistance in encounters with other flock members (Black & Owen 1986, 1989; Kotrschal et al. 1993, 2010). Furthermore, even if not actively interfering, juveniles and females won more agonistic interactions if the gander was nearby and social support is the prime mechanism explaining long-term monogamy and family cohesion in geese (Weiss et al. 2004, Scheiber et al. 2005, Scheiber et al. 2009, Scheiber et al. 2009 in press, Wascher et al. 2011, Kotrschal et al. 2010).

Individuals in wolf packs, Canis lupus, also engage in complex social relationships and potentially, bonding (Fox 1980), similar to human society, regarded as an extended family that mainly consists of a breeding pair and their offspring (Mech 1999, Gadbois 2002, Packard 2003). Wolves move in exclusive home ranges with territory cores defended against
strangers from neighboring packs (Mech & Boitani 2003). The usually monogamous breeding pair gives birth to 4-6 pups once a year. Offspring either disperse at 10-36 months or remain with their parents until social environment commits breeding at average 3 years of age (Mech & Boitani 2003).

Starting at the age of 3 weeks (20 – 24 days) pups socialize with pack members (dogs: Scott & Fuller 1965). Then, motor skills allow to explore the surrounding of the den (Mech 1970, Ryon 1977, Ballard et al. 1987, Fuller 1989a) and to get into contact with the other pack members (Murie 1944, Ryon 1977, Fentress & Ryon 1982). After weaning at around 8-10 weeks (Mech 1970, Packard et al. 1992) pups become increasingly independent and are regularly left alone at so called rendezvous sites when the pack is hunting (Mech 1970), but are only fully independent when they would be able to hunt successfully even on their own.

However, besides the mating pair also male and female yearlings (Packard et al. 1992, Mech 1995d) deliver food and care for the offspring (Packard et al. 1992, Mech et al 1999). Permanent interaction including regular touching and resting in body contact may provide comfort and strengthen affiliative bonding. (Klinghammer & Goodmann 1987). Pups are moved between home sites and protected against environmental threats (Mech et al. 1999), which in turn, may increase their environmental awareness. Thus, the unique social environment of a wolf group buffers the pups against physical (e.g. weather or landscape) and social (e.g. hostile wolves, prey species or animals that threaten wolves) dangers of the surrounding in a variety of ways (Packard 2003), making wolves the top-predators wherever they may occur.

In contrast to the wolves, in feral dogs group structure varies strongly (Miklósi 2011). Even when in West Indian feral dogs, females and males were observed to feed and protect their pups (Pal et al. 1998, 2003, 2004), it has often been described, that females give birth alone on the periphery of the territory (Kleiman & Malcolm 1981, Daniels & Bekoff 1989, Boitani et al. 1995) and neither the father nor other familiar pack members contribute to the raising of the young (Miklósi 2007).

1.1.2 Interspecific social support and its relatedness to social bonding

A number of mainly primate studies explained emotional social support on the basis of close kin (e.g. Kawai 1958, Berman 1980, Horrocks & Hunte 1983, Bernstein & Erhardt 1985, 1986, Netto & van Hoof 1986). However, considering that social support not only evolved in the context of group living but more basically and probably, in connection with
parental care (Eibl-Eibesfeldt 2004), this suggests that the underlying mechanisms should also affect interspecific relationships. Frigerio et al. (2003) showed that hand-raised graylag geese hormonally and behaviorally responded to the presence of a familiar human in social interactions with conspecifics. The mere presence of a human increased the access to feeding resources as well as the chance of winning agonistic interactions and modulated energy management in terms of individual glucocorticoid levels. Thus, a parent-mimicking social ally seems to provide similar benefits to the offspring as goose parents (e.g. Weiß & Kotrschal 2004, Scheiber et al. 2005, 2009). Therefore, a human raiser is suggested to be a valid (Frigerio et al. 2003) and functional (Hemetsberger et al. 2010) model for conspecific parenthood. In line with these findings, Van Ijzendoorn et al. (2008) investigated that orphaned chimpanzee infants, *Pan troglodytes*, receiving intensive human care according to their typical socio-emotional and communicative development (Van Lawick-Goodall, 1968) gain more advantage of a human raiser in the Strange Situation Test (Ainsworth 1969) compared to peers receiving standard care. Considering that this test originally was developed to investigate human mother-infant-bonding, this implicates that sensitive care-giving promotes strong social bonding towards the partner not only in human children but also in infant chimpanzees (Van Ijzendoorn et al. 2008). Although hand-raising may constrain learning (Hinde & Stevenson-Hinde 1973) it is proposed not to change behavior in a fundamental way (Lorenz 1988). That human foster parents are similarly (and at times, even better) suited than conspecific parents is less a miracle as it may seem; it is now known that social birds and mammals share the essential parts of the social brain and other physiological mechanisms (reviewed in Julius et al 2012, chapter 2) and also, the mechanisms of bonding and care-giving, and of similar social needs during early development. If hand raising is done correctly, i.e. in a socially empathic way, considering the species-specific needs, this results in socially competent, impulse-controlled and confident adults, which are mainly different from conspecific parent-raised control animals by their much more robust stress management (i.e. being less excitable in all kind of stressful situations; Hemetsberger et al. 2010). According to Mech (1970) the general development in wolf pups is similar to dogs (Scott & Fuller 1965) of dogs. Thus, socially involved hand-rearing of wolves and dogs during the “critical period” of socialisation (Freedman et al. 1961), as provided to the animals at the Wolf Science Center Ernstbrunn, that is in the Lorenzian tradition, results in cooperative and calm animals for experiments, but is also a model situation to look into the question of whether human raisers could be emotional social supporters to dog and wolf pups in a stressful situation.
1.1.3 Human social bonding in wolves and dogs

Domestication is generally viewed as an evolutionary process that developed under human controlled influence (Price 1984). About 17,000 years of genetic selection for mainly tameness shifted allele frequencies away from their wild ancestors and created cooperative (Hare & Tomasello 2005. Miklósi et al. 1998) and enculturable animals within the human society (Herre & Röhrs 1973, Miklósi 2007). Based on genetic evidence it has been concluded that the wolf is the ancestor of the dog (Gray et al. 2010, Larson et al. 2012), originating from south central China, around 16,000 years ago (Pang et al. 2009). Kotrschal (2012) suggests that commonalities in ecology (i.e. similar prey and habitat) and complex social orientation (i.e. cooperative hunting and raising offspring) may have caused a mutual approach of humans and wolves in a gradual manner. Thus, the domestication of the dog reflects socio-economic changes of human society that finally sophisticated wolves’ cooperative skills towards humans. Although several studies underline the dog’s unique competence concerning communication with humans (Miklósi et al. 1998, 2000; McKinley & Sambock 2000, Agnetta et al. 2000, Hare et al. 2002, Soproni et al. 2001, 2002), the adaptability of dogs towards human surrounding may be rather explained due to the common mammalian “social toolbox” rather than a specific change of human-directed social skills in dogs (Hare & Tomasello 2005). However, in order to figure out to which extent genetic predispositions are responsible (i.e. to what dogs are now different from wolves), it is necessary to compare the behavior of dogs to wolves (Miklósi 2004). In general, it is assumed that learning on the social environment takes place within the first weeks of life, resulting in increased fear responses towards novel (social) stimuli after the social reference system has fully established (Miklósi 2011). Several studies showed that the sensible period in wolves is shorter than in dogs (Fentress 1967, Frank & Frank 1982 a, Zimen 1987). Klinghammer & Godman (1987) proposed that wolves can engage in human bonding only when socialisation starts before eye-opening (11.-12.day of life). In dogs, however, even when socialisation was delayed up to 8-14 weeks, puppies easily engaged in human bonding after initial avoidance behavior decreased (Scott & Fuller 1965). These findings are in line with those of Belyaev et al. (1985). They investigated that foxes, Vulpes fulvus, not selected for affiliative interactions with humans showed increased anxious behavior at the age of 5 weeks when confronted with an unfamiliar environment. In contrast, selectively bred foxes showed fear responses at the age of 10 weeks. Forty years of selection seemed to extend this phase, similar to that of the suggested socialisation period in domesticated dogs with 8-12 (-14) weeks (Freedman et al.
11 and Scott & Fuller 1965). In a social preference test intensively human-socialized wolves (starting at the age of 4-6 days of life) did not show preference for a human when a dog was present (Gácsi et al. 2005). In contrast, similar raised dog puppies clearly preferred a human over a conspecific individual. Currently, the social bonds between dogs are unclear (Rajecki et al. 1978). In 2-month old dog puppies the mother only played a minor role in reducing stress signal when pups were socially isolated (Frederickson 1952, Ross et al. 1960, Elliott & Scott 1961). Furthermore, in a choice task, pups did not prefer their mother over an unfamiliar female dog (Pettijohn et al. 1977, Hepper 1994). When confronted with a novel environment, the stress-level of pet dogs was only reduced in the presence of a familiar human but not in the presence of a familiar dog (Pettijohn et al. 1977). However, Topál et al. (2005) tested 4-months old hand-raised wolves and dogs in the attachment-paradigm of Ainsworth (1969) and compared their attachment behavior to that of mother-reared dog pups of the same age. According to their results, dogs showed attachment behavior towards their human bonding partner whereas wolves did not. In general, attachment is considered be a behavioral control system that evokes care-giving in the attachment figure (Bowlby 1969). Although originally investigated in the human mother-infant bonding (Bowlby 1969, Ainsworth 1969), the social brain hypothesis (Byrne & Whiten, 1988; Dunbar 1998, 2007) suggests that the biological aspects of attachment and care-giving systems may act similar within various vertebrates, particularly mammals (summarized especially for human-animal in Julius et al. 2012). Thus, the main features of attachment (provision of security and stress reduction) may also act on interspecific relationships. In adult dogs, patterns of attachment to humans were already investigated (Topál et al. 1998, Prato-Previde et al. 2003, Gácsi et al. 2001, 2003; Marston et al. 2005a, Fallani et al. 2006). However, Prato-Previde (2003) argued that due to the test arrangement the results of Topál et al. (2005) do not show real attachment behavior in dog pups. Hence, also the results on wolf behavior have to be interpreted carefully. In adult dogs, however, behavioral stress responses towards novel objects were ameliorated when accompanied with an emotionally calm familiar person (Merola et al. 2012). Consistent with the findings of Gácsi et al. (2009) that showed similar ability of using human communicative cues in hand-reared dogs and wolves at the age of 8 weeks, Range & Virányi (2011) concluded that human-oriented communication skills in intensively socialized wolf pups as old as 14 weeks indicate a predisposition for increased attention and cooperation with humans. Thus, also wolf pups may possess similar mechanisms to benefit from a familiar human partner in an emotionally stressful situation.
1.2 Hypotheses and research questions

In continuation of the latter studies, I aimed to investigate experimentally mechanisms and functions of human-related social alliances in dogs and wolves. To my knowledge, present study is the first experimental contribution that investigates human-dog bonding on the basis of human and conspecific social support considering socialisation processes at very early age. Using the same experimental method, the aim was to examine whether environmental effects (rearing history) and/or inheritance (genetic background) are more important. Therefore, the behavior of hand-raised wolf and dog pups was compared to mother-reared pet dog puppies of the same age. All groups were confronted with a moderate stressful neophobia task (5 and 7 weeks of life) and a social separation task (5 weeks of life). We investigated whether or not subjects show attachment behavior and whether stress responses are ameliorated in the presence of human and conspecific partners.

According to the synergistic hypothesis (Gácsi et al. 2009) we suggest that both genetic background and rearing conditions are acting on behavioral phenotypes. In general, the domestication hypothesis claims that independently of the socialisation procedure (hand-raising or mother-raising), dogs differ in their behavior compared to wolves as a result of specific genetic changes selected for during domestication. The socialisation hypothesis, however, suggests that if the same environmental conditions (hand-raising) are given, no behavioral differences related to human-bonding should occur within hand-raised wolves and dogs. In contrary, environmental differences as we find in hand- and mother-reared pups should lead to distinct bonding behavior.

Within these hypotheses I tried to figure out following research questions:

(1) Can a human raiser provide similar social support to wolves as to dogs?

Latest research suggests that differences in early bonding towards humans in wolves and dogs may be a result of domestication that caused disintegration of trait linkage in dogs, suggesting that different traits develop at different pace in wolves and dogs (see Belyaev et al. 1985). For example, wolves are able to follow the pointing clies of their hand-raisers only from 9 months of age, whereas similar raised dogs do already from 4 months of age (Virányi & Range, in press). Thus, for the present study it is expected that different traits develop at
different pace in dogs and wolves. Based on the findings of Belyaev et al. (1985) in foxes, Vulpes fulvus, that the window of learning about social partners closes at the age of 5 weeks, this entails greater fear responses in wolves at the age of 7 weeks. Gácsi et al. (2005) found no maturation effect of behavior when comparing motor abilities in 3, 4 and 5 week old hand-raised wolf and dog pups, however they argue that wolf pups may develop faster in other respects. However, although Topál et al. (2005) failed to show attachment behavior in wolves, based on the findings of Hemetsberger et al. (2010) that hand-raising creates cooperative partners, thus, also wolves should engage in social bonding and profit from human support. Overall, I hypothesize that all three tested groups (hand-raised wolves and dogs and mother-reared dogs) will benefit from both, active and emotional social support of a human ally even at this early age. Thus, I assume less stress-related behaviors and expect more attachment behaviors when a human partner is present.

(2) **Does the presence of a human and conspecific partner modulate stress-related behavior and attachment behavior?**

As dogs showed attachment behavior to humans independently from their raising conditions (Topál et al. 1998, 2005; Prato-Previde 2003) they may similarly react to human social support. In regard of conspecific social support, a social stress test conducted by Elliot & Scott (1961) showed that the female had no effect on reducing stress behavior in mother-reared dog puppies. Furthermore, in a preference study, May et al. (2009) found, that 8 week old dog pups show no preference for their mother over a sibling. Thus, I suggest that social support of a human ally will modulate stress behavior more effectively in dog puppies than that of the mother-dog or a peer. Referring to the listed literature und conclusions in research question (1) I suggest that also in 5 week old hand-raised wolves, a human social ally will be more effective in modulating stress behavior than a peer. However, under natural conditions, wolf pups are regularly left alone when the pack is hunting (Mech 1970). Thus, pups spend most on their time with their littermates which could involve a strong bonding towards their peers, since group formation effectively protects against potential environmental threats (Kikusui et al. 2006). Thus, peers could be more effective in ameliorating stress in hand-raised wolves, than in hand-raised and mother-raised dogs.

However, in general I assume that both, human and conspecific alliances are able to modulate stress behavior in wolves and dogs.
2 Material and methods

2.1 The study groups

2.1.1 Subjects

Twelve hand-raised wolf pups, 14 hand-raised dog puppies and 6 mother-raised dog pups at their age of 5 to 7 weeks participated in the study. The wolves were timber wolves (*Canis lupus occidentalis*) from 7 unrelated litters born in different captive facilities in Europe and the USA (Table 1). The hand-raised dogs were picked up from Hungarian shelters and were all mongrels from 7 litters (Table 2). The mother-raised dogs belong to 3 litters of different breeds (Table 3). The sex, birth date and relatedness of the animals are described in Tables 1 to 3.

The wolves were raised in 3 groups, in summer 2008 in the Cumberland Game Park, Grünau, Oberösterreich whereas in summer 2009 and 2010 in Wildpark Ernstbrunn, Niederösterreich because the Wolf Science Center moved in April 2009. The hand-raised dog puppies (*Canis familiaris*) were reared in 2 groups in winter 2010 and autumn 2010 respectively. Because of their originating from shelters, before we moved them to the Wolf Science Center at their age of 6 to 8 weeks, they were all kept in a private flat in Hungary. Since most of them were in a bad condition when getting them from the shelters, they received special medical treatment until they were in good health.

The pet dog puppies were born in 2010 or 2011 and lived in 3 different households (Table 3).
Table 1. The hand-raised wolves of the WSC (N = 12), their sex (m = male, f = female), birth date, birthplace and their relatives in the pack.

<table>
<thead>
<tr>
<th>Subject (Abbreviation)</th>
<th>Sex</th>
<th>Birth date</th>
<th>Birthplace</th>
<th>Relatives</th>
<th>Pack size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apache (p)</td>
<td>m</td>
<td>19.05.2009</td>
<td>Zoo Basel, Swiss</td>
<td>Brother of c</td>
<td>6</td>
</tr>
<tr>
<td>Aragorn (a)</td>
<td>m</td>
<td>04.05.2008</td>
<td>Game park Herberstein, Austria</td>
<td>Brother of s</td>
<td>4</td>
</tr>
<tr>
<td>Cherokee (c)</td>
<td>m</td>
<td>19.05.2009</td>
<td>Zoo Basel, Swiss</td>
<td>Brother of p</td>
<td>6</td>
</tr>
<tr>
<td>Geronimo (g)</td>
<td>m</td>
<td>02.05.2009</td>
<td>Tripple D Farm, Montana, USA</td>
<td>Brother of y</td>
<td>6</td>
</tr>
<tr>
<td>Kaspar (k)</td>
<td>m</td>
<td>04.05.2008</td>
<td>Game park, Herberstein, Austria</td>
<td>Brother of i</td>
<td>4</td>
</tr>
<tr>
<td>Kenai (e)</td>
<td>m</td>
<td>01.04.2010</td>
<td>Parc Safari, Hemmingford, Canada</td>
<td>Brother of w</td>
<td>2</td>
</tr>
<tr>
<td>Nanuk (n)</td>
<td>m</td>
<td>28.04.2009</td>
<td>Tripple D Farm, Montana, USA</td>
<td>No Sibling</td>
<td>6</td>
</tr>
<tr>
<td>Shima (s)</td>
<td>f</td>
<td>04.05.2008</td>
<td>Game park Herberstein, Austria</td>
<td>Sister of a</td>
<td>4</td>
</tr>
<tr>
<td>Tatonga (t)</td>
<td>f</td>
<td>21.04.2009</td>
<td>Tripple D Farm, Montana, USA</td>
<td>No Sibling</td>
<td>6</td>
</tr>
<tr>
<td>Tayanita (i)</td>
<td>f</td>
<td>02.05.2008</td>
<td>Game park Herberstein, Austria</td>
<td>Sister of k</td>
<td>4</td>
</tr>
<tr>
<td>Wapi (w)</td>
<td>m</td>
<td>01.04.2010</td>
<td>Parc Safari, Hemmingford, Canada</td>
<td>Brother of e</td>
<td>2</td>
</tr>
<tr>
<td>Yukon (y)</td>
<td>f</td>
<td>02.05.2009</td>
<td>Tripple D Farm, Montana, USA</td>
<td>Brother of c</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2. The dogs of the WSC (N = 14), their sex (m = male, f = female), birth date, birthplace and their relatives in the pack.

<table>
<thead>
<tr>
<th>Subject (Abbreviation)</th>
<th>Sex</th>
<th>Breed</th>
<th>Birth date</th>
<th>Birthplace</th>
<th>Relatives</th>
<th>Pack size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alika (a)</td>
<td>f</td>
<td>Mongrels</td>
<td>30.11.2009</td>
<td>Shelter, Hungary</td>
<td>Sister of r</td>
<td>8</td>
</tr>
<tr>
<td>Asali (s)</td>
<td>m</td>
<td>Mongrels</td>
<td>13.09.2010</td>
<td>Shelter, Hungary</td>
<td>Brother of n</td>
<td>6</td>
</tr>
<tr>
<td>Bashira (b)</td>
<td>f</td>
<td>Mongrels</td>
<td>13.09.2010</td>
<td>Shelter, Hungary</td>
<td>Sister of h</td>
<td>6</td>
</tr>
<tr>
<td>Binti (n)</td>
<td>f</td>
<td>Mongrels</td>
<td>13.09.2010</td>
<td>Shelter, Hungary</td>
<td>Sister of s</td>
<td>6</td>
</tr>
<tr>
<td>Doa (d)</td>
<td>f</td>
<td>Mongrels</td>
<td>30.11.2009</td>
<td>Shelter, Hungary</td>
<td>Sister of j</td>
<td>8</td>
</tr>
<tr>
<td>Hakima (h)</td>
<td>m</td>
<td>Mongrels</td>
<td>13.09.2010</td>
<td>Shelter, Hungary</td>
<td>Brother of b</td>
<td>6</td>
</tr>
<tr>
<td>Imani (i)</td>
<td>m</td>
<td>Mongrels</td>
<td>08.12.2009</td>
<td>Shelter, Hungary</td>
<td>Brother of u</td>
<td>8</td>
</tr>
<tr>
<td>Jini (j)</td>
<td>f</td>
<td>Mongrels</td>
<td>30.11.2009</td>
<td>Shelter, Hungary</td>
<td>Sister of d</td>
<td>8</td>
</tr>
<tr>
<td>Kilio (k)</td>
<td>m</td>
<td>Mongrels</td>
<td>18.12.2009</td>
<td>Shelter, Hungary</td>
<td>Brother of m</td>
<td>8</td>
</tr>
<tr>
<td>Maisha (m)</td>
<td>m</td>
<td>Mongrels</td>
<td>18.12.2009</td>
<td>Shelter, Hungary</td>
<td>Brother of k</td>
<td>8</td>
</tr>
<tr>
<td>Meru (e)</td>
<td>m</td>
<td>Mongrels</td>
<td>01.10.2010</td>
<td>Shelter, Hungary</td>
<td>Brother of t</td>
<td>6</td>
</tr>
<tr>
<td>Rafiki (r)</td>
<td>m</td>
<td>Mongrels</td>
<td>30.11.2009</td>
<td>Shelter, Hungary</td>
<td>Brother of a</td>
<td>8</td>
</tr>
<tr>
<td>Tana (t)</td>
<td>m</td>
<td>Mongrels</td>
<td>01.10.2010</td>
<td>Shelter, Hungary</td>
<td>Brother of e</td>
<td>6</td>
</tr>
<tr>
<td>Uzima (u)</td>
<td>f</td>
<td>Mongrels</td>
<td>08.12.2009</td>
<td>Shelter, Hungary</td>
<td>Sister of i</td>
<td>8</td>
</tr>
</tbody>
</table>

2.1.2 Socialisation procedure

2.1.2.1 Hand-raising

The hand-raised wolves and dogs were socialized in the same way. Both groups were hand-reared by a group of humans after being separated from their mothers 3-12 days after birth (before eye-opening). A team of 8 to 10 experienced people (including students, PhD students, trainers and scientists) fostered the pups which were kept in peer groups (Table 2
and Table 3). The first 4 months of life the animals spent in close human contact 24 hours a day. A raiser slept together with the group of pups during nights. The pups were bottle-fed (type of milk powder). From the age of 4-5 weeks they were additionally hand-fed. Meat and moist food for dogs were gradually introduced into their diet. During the first weeks of raising, the pups were kept in a 20 m² room where they spent most of the time. The room was equipped with toys and clothes of various materials which were regularly exchanged and altered to the playing properties of the growing pups. As old as 4 weeks they had controlled access to a garden (~20 m²) three times a week. At the age of 5 weeks they had daily free access to the yard. Three to four times a week the pups were exposed to unfamiliar people (Table 4). Since they participated in cognitive and behavioral experiments, they were confronted with novel objects and situations (e.g. experiencing unfamiliar rooms and noises, being alone, …) about every other day. Furthermore they participated in training with positive reinforcement at least once a day. At the age of 6 weeks they were taken out for a walk on leash 2 to 3 times a week. They weekly experienced social contact to 3-4 familiar adult dogs of various breeds to which they developed close relationships. As a result of this socialisation regimen the wolves showed no sign of wariness or avoidance of humans but were keen to interact with unfamiliar persons. The basic principle of socialisation was adjusted to the need of the wolves, that is its aim was to build up exclusively positive associations with humans. Accordingly, we avoided competitive situations and conflicts with the animal, and did not use inhibition during their raising and training.

2.1.2.2 Pet dogs

The pet dog puppies were reared by their mothers until 8 - 9 weeks of age. At the time of the tests they lived in human households with close contact towards the owner, the mother-dog and their peers (Table 4). All pups had regular contact throughout the day to familiar and unfamiliar persons and lived together with 2 to 5 familiar dogs. Furthermore they had irregular contact to unfamiliar dogs as well as cats and horses (2 to 3 times a week). As old as 4 weeks the pups were able to move around without constrictions. That is, they were confronted with novel environments, situations and objects daily.
Table 3. **The mother-reared dogs** (N = 6), their sex (m = male, f = female), breed, birth date, birthplace, their peers and the litter size during testing.

<table>
<thead>
<tr>
<th>Subject (Abbreviation)</th>
<th>Sex</th>
<th>Breed</th>
<th>Birth Date</th>
<th>Birthplace</th>
<th>Relatives</th>
<th>Litter Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheeky (c) f</td>
<td>f</td>
<td>Pinscher-Terrier-Mix</td>
<td>12.06.2011</td>
<td>Sollenau, Austria</td>
<td>Sister of g</td>
<td>7</td>
</tr>
<tr>
<td>Gigolo (g) m</td>
<td>m</td>
<td>Pinscher-Terrier-Mix</td>
<td>12.06.2011</td>
<td>Sollenau, Austria</td>
<td>Brother of c</td>
<td>7</td>
</tr>
<tr>
<td>Joey (j) m</td>
<td>m</td>
<td>Malteser</td>
<td>23.04.2010</td>
<td>Ernstbrunn, Austria</td>
<td>Brother of k</td>
<td>3</td>
</tr>
<tr>
<td>Kloey (k) f</td>
<td>f</td>
<td>Malteser</td>
<td>23.04.2010</td>
<td>Ernstbrunn, Austria</td>
<td>Sister of j</td>
<td>3</td>
</tr>
<tr>
<td>no name (a) f</td>
<td>f</td>
<td>Collie</td>
<td>10.06.2011</td>
<td>Schachau, Austria</td>
<td>Sister of b</td>
<td>6</td>
</tr>
<tr>
<td>no name (b) m</td>
<td>m</td>
<td>Collie</td>
<td>10.06.2011</td>
<td>Schachau, Austria</td>
<td>Brother of a</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 4 shows differences and similarities of the rearing conditions.

**Table 4. Comparison of environmental and social features** that hand-raised and mother-raised pups were confronted with at the age of 3 to 8 weeks.

**Comparison of the raising conditions**

<table>
<thead>
<tr>
<th>Contact to human raiser / mother-dog</th>
<th>Estimated frequency of social and environmental encounters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hand-raising</td>
</tr>
<tr>
<td></td>
<td>Almost permanent throughout the day, intensive care-giving started already within the first days after birth</td>
</tr>
<tr>
<td></td>
<td>Weaning decreasing over time, removed by hand-feeding meat; physical contact slightly decreasing during development</td>
</tr>
<tr>
<td></td>
<td>Positive only (no aggressive interaction)</td>
</tr>
<tr>
<td>Human Contact</td>
<td></td>
</tr>
<tr>
<td>Familiar people</td>
<td>Frequently throughout the day</td>
</tr>
<tr>
<td></td>
<td>Soft handling (no rough play, no intensive petting)</td>
</tr>
<tr>
<td>Unfamiliar people</td>
<td>Infrequently about every day or every second day (e.g. vet, visitors, new)</td>
</tr>
<tr>
<td>Canid contact</td>
<td>Familiar dogs/wolves</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Students, test situations</strong></td>
<td>Soft handling only (e.g. no rough play, intense petting)</td>
</tr>
<tr>
<td><strong>Mainly contact if the pups initiate approach (except in test situations)</strong></td>
<td>Mainly contact if the pups initiate approach (except in test situations)</td>
</tr>
<tr>
<td><strong>Option to withdraw in stressful situations (except in test situations)</strong></td>
<td>Option to withdraw in stressful situations (except in test situations)</td>
</tr>
<tr>
<td><strong>Canid contact</strong></td>
<td>2 to 3 times a week to various dogs (wolf pups also had contact to adult wolves about every second day)</td>
</tr>
<tr>
<td><strong>Environmental features</strong></td>
<td>Human controlled but rarely intervened (e.g. to interrupt too aggressive behavior towards the pups)</td>
</tr>
<tr>
<td><strong>Environmental features</strong></td>
<td>Partially option to withdraw in stressful situations (except in test situations)</td>
</tr>
<tr>
<td><strong>Environmental features</strong></td>
<td>2 to 3 times a week several scientific tests per day (5 to 6 days a week) including intensive handling</td>
</tr>
<tr>
<td><strong>Environmental features</strong></td>
<td>Novel environments 2 to 3 times a week (mainly in test situations)</td>
</tr>
<tr>
<td><strong>Environmental features</strong></td>
<td>Novel objects every second day</td>
</tr>
<tr>
<td><strong>Environmental features</strong></td>
<td>Frequently confronted with loud noises throughout the day</td>
</tr>
<tr>
<td><strong>Environmental features</strong></td>
<td>No comparable handling except usual vet treatment</td>
</tr>
<tr>
<td><strong>Environmental features</strong></td>
<td>No transport from birth-place until the age of 8 weeks</td>
</tr>
</tbody>
</table>
2.2 Experimental procedure

In order to reveal the effectiveness of human and conspecific social support, each study group participated in two different tasks - a neophobia test (Figure 1) and a social isolation test (Figure 10).

2.2.1 General experimental arrangement

The tests were performed between 8 am and 8 pm, and the raisers fed the pups with milk at least 2 hours earlier. Each pup was tested in the Neophobia Task and in the Social Isolation Task in 2 different rooms. The rooms were empty and about 3 x 3 m of size. The two tasks were never conducted on the same day. The pups behavior was continuously recorded throughout the tests with a video camera (Handycam DCR SR35 Sony) held by an experimenter who was positioned in a corner of the room, out of reach of the subjects.
3 Task 1: Neophobia

3.1 Methods

3.1.1 Experimental arrangement

Two neophobia tasks were conducted at each of the ages of 5 and 7 weeks. In the 2 conditions of each task the pups were confronted with one of 2 novel objects either alone (Figure 1; Picture A) or in presence of a human partner (Figure 1, Pictures B-D). In case of the hand-reared wolves and dogs the social partner was their hand-raiser, whereas the mother-raised dogs were tested with their owner. The objects were an alarm clock (Figure 1; Picture A) and a yellow sponge ball (Figure 1; Pictures B-D). Each pup was first tested in the ball and then in the clock test. In each test, each subject was tested both in the ”alone” and in the ”with human partner” condition, in a counterbalanced order. Both tests were conducted in the same test room and each individual was tested in all 4 conditions (ball; clock; alone; with partner) on the same day. The tasks were conducted one after another with elapsing a few minutes between tests and conditions. Since saliva samples were taken the conduction of the last clock condition was delayed for 40 minutes in week 5.

At the age of 7 weeks all four conditions were repeated in the same room. The order of tests remained the same, whereas the order of conditions within tests was changed for each subject.
Task 1: Neophobia  

Methods

Neophobia Task (5 and 7 Weeks)

<table>
<thead>
<tr>
<th>Condition 1</th>
<th>Condition 2</th>
</tr>
</thead>
</table>
| **Ball/Clock**  
  **Alone**  
  **(1 min)** | **Ball /Clock with Human**  
  **(2,30 min)** |
| **Phase 1** | **Phase 2** | **Phase 3** |
| Human distant  
  passive  
  **(1 min)** | Human close  
  passive  
  **(30 sec)** | Human close  
  active  
  **(30 sec)** |

| Figure 1. Testing conditions of the Neophobia Task at the age of week 5 and 7. Wolves and dogs were confronted with a ball and a clock either alone (Condition Alone; Picture A), and in the presence of a human partner (Condition Partner; Pictures B-D). * Hand-reared dogs and wolves were tested with their raiser, mother-reared dogs were tested in the presence of the breeder or owner, respectively. |

The test room was always empty. Only the cameraperson (C) remained outside the testing area while recording the behavior of the subject (Figure 2). She was totally separated from the animal either by sitting high in at least 1 m distance or by hiding behind a visual barrier. Experimenter one (E1) was located on the opposite side of (C), either outside the room (e.g. by using the entrance) or at least outside the testing area. While confronting the subjects with the objects (O) during testing, she hid behind a 1 m visual barrier. Both stimuli were adhered to a string. The sponge ball was slowly moved like a pendulum at eye level of the animal, the alarm clock was put immobile on the ground, while ringing loudly for 10 seconds after appearance. Before the tests started, a familiar experimenter (E2) carried the subject into the test room.
### Task 1: Neophobia Methods

#### Neophobia Task (5 and 7 Weeks)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Alone</th>
<th>Partner</th>
</tr>
</thead>
<tbody>
<tr>
<td>i)</td>
<td><img src="image1.png" alt="Figure 1, Picture A" /></td>
<td><img src="image2.png" alt="Figure 1, Picture B" /></td>
</tr>
<tr>
<td></td>
<td><img src="image3.png" alt="Figure 1; Pictures C and D" /></td>
<td><img src="image4.png" alt="Figure 1; Pictures C and D" /></td>
</tr>
</tbody>
</table>

(see Figure 1, Picture A) (see Figure 1, Picture B) (see Figure 1; Pictures C and D)

**Figure 2.** Schematic illustration of the test room in the Neophobia Task, in the alone condition (i), and the partner condition (ii and iii). (O) indicates the position of the objects (ball or clock) which the experimenter 1 (E1) exposed to the subject. During the test, the cameraperson (C) stood on the opposite side of room, outside the testing area. In the alone condition, which lasted 1 minute, (E2) placed the subject (S) 1.5 m distant in front of (O). In phase 1 (ii) of the partner condition (E2) put the pup into the humans (H) lap who was passively sitting 1.5 m distant to (O) for 1 minute. In phase 2 and 3 (iii); (H) was sitting 0.3 m close to (O), remaining passive in phase 2 and trying to attract the subject to (O) in phase 3, with each phase lasting 30 seconds.

#### 3.1.1.1 Alone condition

In the alone condition (E2) placed the subject 1.5 m distant to the object (Figure 2; i), held it for a second making it orient toward the object, and then let it go. Then she left the test room. After 1 minute of observing the subject’s behavior, the (E2) entered the testing area and tried to attract the pup’s attention by gently trampling with her feet. If the pup approached her, she picked the pup up without talking to it. If the pup did not react to the entering E2 within 10 seconds, she slowly approached the subject, stroked the animal and lifted it up. Video recording was stopped after filming the subject’s reaction to the handling.

#### 3.1.1.2 Partner condition

The partner condition consisted of three different phases (Figure 2). In phase 1 the human partner (H) was sitting in a 90 ° position (with its back towards the wall) in 1.5 m distance to the object (Figure 2; ii). The humans’ position (right or left side from the object) was counterbalanced for each subject across tests. (E2) put the subject into the human’s lap and left the testing area. When the object appeared the human partner released the pup and
remained motionless, quietly facing the subject. After 1 minute (H) moved 0.3 m close to the object, again sitting calmly on the floor in phase 2 (Figure 2; iii). In phase 3, (H) tried to attract the subject to the object while gently talking to the subject and touching the object. After further 30 seconds the test was finished.

Due to health problems of the animals and losing some video files the actual number of individuals varied in the tests, so in each case we give the exact number of the animals whose data were analysed (Table 5).

### Table 5. The sequence of testing for the Neophobia Task within the three study groups. The age of testing, the conditions (Condition 1: whether tested alone or with a partner present; Condition 2: whether tested with a ball or a clock), and the number of tested individuals are listed.

<table>
<thead>
<tr>
<th>Neophobia Test</th>
<th>Hand-raised wolves</th>
<th>Hand-raised dogs</th>
<th>Mother-raised dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Weeks</td>
<td>Ball alone: N = 11</td>
<td>Ball alone: N = 14</td>
<td>Ball alone: N = 6</td>
</tr>
<tr>
<td></td>
<td>Ball with partner: N = 10</td>
<td>Ball with partner: N = 14</td>
<td>Ball with partner: N = 6</td>
</tr>
<tr>
<td></td>
<td>Clock alone: N = 10</td>
<td>Clock alone: N = 14</td>
<td>Clock alone: N = 6</td>
</tr>
<tr>
<td></td>
<td>Clock with partner: N = 11</td>
<td>Clock with partner: N = 14</td>
<td>Clock with partner: N = 6</td>
</tr>
<tr>
<td>7 Weeks</td>
<td>Ball alone: N = 11</td>
<td>Ball alone: N = 13</td>
<td>Ball alone: N = 6</td>
</tr>
<tr>
<td></td>
<td>Ball with partner: N = 12</td>
<td>Ball with partner: N = 13</td>
<td>Ball with partner: N = 6</td>
</tr>
<tr>
<td></td>
<td>Clock alone: N = 12</td>
<td>Clock alone: N = 11</td>
<td>Clock alone: N = 6</td>
</tr>
<tr>
<td></td>
<td>Clock with partner: N = 12</td>
<td>Clock with partner: N = 13</td>
<td>Clock with partner: N = 6</td>
</tr>
</tbody>
</table>

### 3.1.2 Behavior categories

All videos were coded by using the Solomon Coder (Version Solomon beta 100926, copyright Andras Peter) in accordance with the below ethogram (Table 6). Mostly the same behaviors were recorded in the Neophobia and Social Separation Tasks, with exception of 2 behaviors that were coded only in the Neophobia Task.
Table 6. Coding Table for the Neophobia Test and the Social Separation Test. Following behaviors were defined to analyze the subject’s behavior in both conducted task. The abbreviations NT (Neophobia Task) and SST (Social Separation Task) indicate in which test the behavior was coded, D (Duration) and F (Frequency) refer to the coded entity of each behavior.

<table>
<thead>
<tr>
<th>Coding Table</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
</tr>
<tr>
<td><strong>General activity</strong></td>
</tr>
<tr>
<td>Active</td>
</tr>
<tr>
<td>Escape</td>
</tr>
<tr>
<td>Passive</td>
</tr>
<tr>
<td><strong>Stress-related behavior</strong></td>
</tr>
<tr>
<td>Defecation</td>
</tr>
<tr>
<td>Freezing</td>
</tr>
<tr>
<td>Licking</td>
</tr>
<tr>
<td>Panting</td>
</tr>
<tr>
<td>Peeing</td>
</tr>
<tr>
<td>Scratching</td>
</tr>
<tr>
<td>Shaking</td>
</tr>
<tr>
<td>Vocalizing</td>
</tr>
<tr>
<td>Yawning</td>
</tr>
</tbody>
</table>
Neophobia Methods

Playing with object
To run around the object, snapping, jumping, pawing or barking at it (play bow), accompanied with erected ears and often tail wagging.

Playing with others
To run around in the testing area, including self-play, play bow and often tail wagging.

Playing with partner
To run around the partner, jump on it maybe snap or bite without pressure to cause injury at it, further perform an invited chase accompanied with play bow and tail wagging.

In both tests we furthermore measured the relative duration of the total time the subject spent in close proximity (< 30 cm with the entire body) to its human or conspecific partner and the cameraperson (Table 7). Additionally, in the Separation Test we further recorded proximity to the exit of the test room. In the Neophobia Task we additionally calculated the time spent close to the object. The category “Object/Partner” indicates that the subject is between the human partner and the object (Neophobia Task, phase 2 and 3) and thus, closer than 30 cm to both the partner and the object.

Table 7. Relative duration of total time the subject spent in close proximity (< 50 cm) coded locations of the testing room in the Neophobia Task and the Social Separation Task.

<table>
<thead>
<tr>
<th>Location</th>
<th>Description</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Door</td>
<td>The time spent in close proximity to the door.</td>
<td>SST</td>
</tr>
<tr>
<td>Cameraperson</td>
<td>The time spent in close proximity to the cameraperson. The subject is oriented towards the human, does climbing movements in front of her or directs any other behavior towards her.</td>
<td>NT,SST</td>
</tr>
<tr>
<td>Object</td>
<td>The time spent in close proximity to the object.</td>
<td>NT</td>
</tr>
<tr>
<td>Object / Partner</td>
<td>The time spent between the human partner and the object.</td>
<td>NT</td>
</tr>
<tr>
<td>Partner</td>
<td>The time spent in close proximity to the human partner.</td>
<td>NT,SST</td>
</tr>
</tbody>
</table>

Since recording always focused on the subject it was not possible to keep the partner constantly within the camera perspective. Thus, gaze alternating between subject and partner was excluded from coding. Without having both simultaneously in view, adequate gaze coding of the subject cannot be warranted.
3.1.3 Data analysis

For both tests we analysed the data by using the program R 2.15.2 (R Core Team 2012). Graphs were created with IBM SPSS Statistics 18. Inc.

Comparison of alone and partner condition:

We investigated whether “object-directed exploration behavior”, “escaping”, “stress-related behavior”, “searching for human proximity (cameraperson)”, “standing by the door”, “activity”, “passive behavior”, and “object-directed play behavior” were influenced by the presence or absence of a human partner, by the animals age (5 or 7 week), the group (mother, and hand-raised dogs, and wolves), or the test (ball, clock). Analysing the exploration behavior we calculated first a generalized linear mixed model (GLMM) using the binomial distribution to investigate the influence of the factors mentioned above on the likelihood of its occurrence. In a second analysis we investigated whether the same factors were influencing the duration of an animal’s exploration behavior if it occurred by calculating a linear mixed effect model (lme). Since the residuals were not normal distributed we used a square root transformation. Furthermore, analysing the duration of escaping behavior, searching for human proximity, and the duration of activity we calculated a lme, the residuals were not normal distributed, therefore, we used a square root transformation in case of the escaping behavior and in case of searching for human proximity (cameraperson) and we squared the activity. The frequency of emitting stress-related behavior we analysed by calculating a glmm using a poisson distribution and to analyze standing by the door, passive behavior, and the occurrence of play behavior calculating another glmm using a binomial distribution.

Comparison of the three phases within the partner condition:

We investigated whether “object-directed exploration behavior”, “proximity towards the partner”, “escaping behavior”, “approaching the object out of human proximity”, “stress-related behavior”, “activity”, “passive behavior” and “play behavior” were influenced by the animals age (5 or 7 week), the group (mother, and hand-raised dogs, and wolves), or the test (ball, clock). Analysing the exploration behavior we calculated first a generalized linear mixed model (glmm) using the binomial distribution to investigate the influence of the factors mentioned above on the likelihood of its occurrence. In a second analysis we investigated
whether the same factors were influencing the duration of an animal’s exploration behavior if it occurred by calculating a linear mixed effect model (lme). Since the residuals were not normal distributed we used a square root transformation. Furthermore, analysing the duration of being close to the human partner, the escaping behavior, and the activity we calculated linear mixed effect models. In case of escaping and activity the residuals were not normal distributed and we used a log transformation (escaping), and we squared the activity. The frequency of emitting stress-related behavior we analysed by calculating a glmm using a poisson distribution and to analyze the occurrence of approaching the object out of human proximity, passive behavior, and play behavior we used a binomial distribution.
3.2 Results

3.2.1 The effect of human presence (raiser or owner). A comparison of the test conditions “alone” and “with partner”.

3.2.1.1 Exploration of the object

The likelihood to explore the object did not differ between 5 and 7 weeks of age (nlme: $F_{1,235}=1.02$, $p=0.31$). However, the animals were more likely to explore the ball than the clock (nlme: $z=3.517$, $p<0.001$). Furthermore, we found an interaction between test group and condition (nlme: $F_{1,235}=4.500$, $p=0.010$). When the animals were tested alone hand-raised dogs were less likely to explore the object than mother-raised dogs (nlme: $z=2.530$, $p=0.011$), and there was a tendency that hand-raised dogs were less likely to explore the object than wolves (nlme: $z=1.942$, $p=0.052$). However, when the animals were tested in the human presence we found no difference between hand-raised and mother-raised dogs (nlme: $z=-0.31$, $p=0.76$), but wolves were more likely to explore the object than hand-raised dogs (nlme: $z=3.002$, $p=0.003$).

The animals that explored the object were included into the analysis of the relative duration in exploring the object (Figure 3). We found a difference between the two tests (lme: $F_{1,135}=20.489$, $p<0.001$). The animals explored the ball longer than the clock. Additionally, we found an interaction between test group and age (lme: $F_{1,135}=5.045$, $p=0.008$). If the animals explored the object, at 5 weeks of age hand-raised dogs spent more time exploring than wolves did (lme: $t=-2.010$, $p=0.048$). However, there was no difference between hand-raised and mother-raised dogs (lme: $t=0.002$, $p>0.99$). In contrast, at the age of 7 weeks the hand-raised dogs spent less time in exploring the object than wolves (lme: $t=2.113$, $p=0.0452$) and we found a tendency that hand-reared dogs even explored shorter than the mother-raised dogs (lme: $t=1.841$, $p=0.077$). Furthermore, we found no influence of the test condition on the animals duration exploring the object (lme: $F_{1,134}=0.55$, $p=0.46$).
Figure 3. Relative time the subjects spent with exploring the object in the Neophobia Task when tested alone and in the presence of a human partner. Pups were tested at the age of 5 and 7 weeks (hr = hand-raised, mr = mother-raised).
3.2.1.2 Escape behavior

Over all the animals showed more escaping behavior in the age of 7 weeks than 5 weeks (lme: F_{1,89}=6.73, p=0.011; Figure 4).

Analysing the ball condition, we found an interaction between group and condition (lme: F_{2,85}=7.92, p<0.001). When tested alone there was no difference between wolves and hand-raised dogs (lme: t=-1.09, p=0.28), but hand-raised dogs showed more escaping behavior than mother-raised dogs (lme: t=-2.660, p=0.013). However, when tested with a human partner, we found that wolves showed more escaping behavior than hand-raised dogs (lme: t=2.530, p=0.017), but there was no difference between hand-raised and mother-raised dogs (lme: t=-1.50, p=0.14).

In the clock condition, the animals showed more escaping behavior when tested alone than with a partner (lme: F_{1,79}=29.280, p<0.001). Furthermore, we found an interaction between group and age (lme: F_{2,80}=4.880, p=0.010). Wolves showed more escaping behavior at the age of 7 weeks than at the age of 5 weeks (lme: F_{1,30}=5.700, p=0.020), however hand-raised and mother-raised dogs showed no difference between the two ages (lme: hr dogs: F_{1,36}=1.07, p=0.30; mr dogs: F_{1,22}=0.05, p=0.80).
3.2.1.3 Stress-related behavior

Wolves showed significantly more stress-related behavior than hand-raised dogs did, there was no difference between mother- and hand-raised dogs (wolves / hand-raised dogs: nlme: $z=2.116, p=0.034$; mother-raised dogs / hand-raised dogs: nlme: $z=-0.60, p=0.55$; Figure 5). Independent from their age and the object presented all three groups showed less stress behavior when a human partner was present (nlme: $F_{1,212}=8.930, p=0.003$). We found no influence of age (nlme: $F_{1,217}=2.15, p=0.14$) and whether tested with the clock or ball (nlme: $F_{1,210}=0.03, p=0.86$).
Figure 5. Frequency of stress-related behavior in the Neophobia Task when tested alone or in the presence of a human partner. Pups were tested at the age of 5 and 7 weeks (hr = hand-raised, mr = mother-raised).
3.2.1.4 Searching for human proximity (cameraperson)

When the animals were tested alone they spent more time next to the cameraperson in the clock than in the ball condition (lme: F1,87=4.554, p=0.036) and more time with 7 than with 5 week of age (lme: F1,88=9.472, p=0.002). Furthermore, the group the animals belonged to differed (lme: F2,29=3.728, p=0.036). Hand-raised dogs spent more time seeking for proximity towards the cameraperson than mother-raised dogs did (lme: t=-2.612, p=0.014) but we found no difference between wolves and hand-raised dogs (lme: t=-0.402, p=0.691).

Even in the presence of a partner, subjects searched for proximity to the cameraperson. When the animals were tested with a human partner, they spent more time with the cameraperson at the age of 7 than 5 weeks (lme: F1,118=19.329, p<0.001), but there was no difference between the two tests (lme: F1,118=1.38, p=0.24). Furthermore, the three test groups differed (lme: F2,117=19.329, p<0.001). Mother and hand-raised dogs did not differ (lme: t=-0.47, p=0.64), but wolves spent more time with the cameraperson than hand-raised dogs did (lme: t=6.807, p<0.001).

3.2.1.5 Standing by the door

Wolves were more likely to be in close proximity to the door (where humans go in and out) than hand-raised dogs (nlme: z=-4.166, p<0.001) but we found no difference between hand and mother-raised dogs (nlme: z=-1.42, p=0.16). Furthermore, the animals were more likely to stay at the door when tested alone compared to when a human partner was present (nlme: F1,211=9.800, p=0.002). However, we found no influence of age, and test (nlme: age: F1,214=2.610, p=0.11; test: F1,209=2.40, p=0.12).

3.2.1.6 Activity

We found that the animals were more active in the ball than in the clock test (nlme: F1,211=47.221, p<0.001). However, we found no influence of age, test condition, and group (nlme: age: F1,214=2.74, p=0.10; condition: F1,209=0.65, p=0.42; group: F2,29=1.69, p=0.20).
3.2.1.7 Passive behavior

Passive behavior rarely occurred. However, we found no influence of week (nlme: $F_{1,214}=1.44$, $p=0.23$), but we found that the animals were more likely to be passive when they were tested alone than with a human partner (nlme: $F_{1,209}=4.400$, $p=0.037$). However, we found an interaction between group and test (nlme: $F_{2,208}=4.010$, $p=0.019$). When tested with the ball, we found no influence of the test group on the likelihood to be passive (nlme: $F_{2,29}=0.29$, $p=0.70$). However, when tested with the clock, mother-raised dogs were more likely to be passive than hand-raised dogs (nlme: $z=3.272$, $p=0.001$), but we found no difference between hand-raised dogs and wolves (nlme: $z=-1.14$, $p=0.25$).

3.2.1.8 Object-directed play

The test animals were more likely to play in the ball than in the clock test (nlme: $F_{1,194}=12.210$, $p<0.001$). However, there was no difference whether they were tested alone or with a human partner (nlme: $F_{1,194}=0.81$, $p=0.37$). Furthermore, we found an interaction between age and test group (nlme: $F_{2,199}=4.430$, $p=0.010$). When tested with 5 weeks of age we found no difference between the test groups (nlme: $F_{2,20}=1.80$, $p=0.20$). Hence, at the age of 7 weeks mother-raised dogs were more likely to play with the object than hand-raised dogs (nlme: $z=2.156$, $p=0.031$), but we found no difference between hand-raised dogs and wolves (nlme: $z=1.46$, $p=0.14$).

3.2.2 The effect of a human partner in regard of her proximity towards the object and communicative interaction with the subject

3.2.2.1 Exploration of the object

In the presence of a human partner, no difference in the likelihood to explore the object within the three test groups was found between the age of 5 and 7 weeks (nlme: $F_{1,342}=0.73$, $p=0.39$);
However, we found a difference between the three groups when exploring the object (nlme: $F_{2,28}=3.440, p=0.050$). Wolves were more likely to explore the object than hand-raised dogs (nlme: $z=2.409, p=0.016$) but no difference between hand-raised and mother-raised dogs was found (nlme: $z=-0.28, p=0.78$). Furthermore, in the ball test they were more likely to explore the object than in the clock test (nlme: $F_{1,333}=15.340, p<0.001$). In addition, we found an influence of the phase on the individual’s likelihood to explore the object (nlme: $F_{2,333}=3.110, p=0.050$). In phase 1 and 2 we did not find a difference (nlme: $z=1.41, p=0.46$) however, in phase 3, when the human partner is encouraging, the animals were more likely to explore the object than in phase 2 (nlme: $z=2.518, p=0.012$).

Analysing the relative duration of animals that explored the object, we found a difference between the two tests (lme: $F_{1,157}=17.571, p<0.001$). The animals explored more in the ball than clock condition. However, we found no influence of age, phase, and group (lme: age: $F_{1,161}=0.53, p=0.47$; phase: $F_{2,153}=1.58, p=0.21$; group: $F_{2,31}=0.58, p=0.56$).
3.2.2.2 Searching for proximity to the human partner

Independent from the test group the animals spent more time close to the partner at an age of 5 weeks than 7 weeks (lme: $F_{1,335}=7.177, p=0.008$; Figure 7). Furthermore, we found an interaction between test group and test phase (lme: $F_{4,327}=2.446, p=0.046$) and between test group and test (lme: $F_{2,327}=3.165, p=0.043$).

Hand-raised dogs spent more time close to the human partner during the clock test than during the ball test (lme: $F_{1,145}=18.230, p<0.001$). Furthermore, they spent more time close to the human partner in phase 1 than 2 (lme: $t=-2.633, p=0.009$) and longer in phase 3 than 1(lme: $t=-3.114, p=0.002$).

Mother-raised dogs showed no difference between the two tests in time spending close to the human partner (lme: $F_{1,68}=0.12, p=0.73$). We found no difference between phase 1 and 2 in time the mother-raised dogs spent close to the human partner (lme: $t=-0.20, p=0.84$).
However, they spent less time with the human partner in phase 3 than in phase 1 (lme: \( t=3.478, p=0.001 \)).

We found a tendency that wolves spent more time close to the human partner in the clock than in the ball condition (lme: \( F_{1,120}=3.089, p=0.081 \)). We found no difference between phase 1 and 2 in time the wolves spent in close contact with the human partner (lme: \( t=-0.98, p=0.33 \)). However, the wolves spent less time close to the human partner in phase 3 than in phase 1 (lme: \( t=-2.638, p=0.009 \)).
3.2.2.3 Approaching the object out of human proximity

We found no influence of group on the likelihood to approach the object over the human partner (nlme: F_{2,28}=0.19, p=0.82). However, the animals were more likely to use human background for approaching the object at the age of 5 than 7 week (nlme: F_{1,28}=4.320, p=0.040). Furthermore, they were more likely to approach the ball than the clock out of human proximity (nlme: F_{1,333}=3.83, p=0.050), and more likely in phase 3 than in phase 2, where the human partner is similar close to the object but remaining silent (nlme: Phase 2 vs. 3: z=-6.023, p<0.001).

Looking only at phase 3, where the human partner tries to attract the animals towards the object, we found that the animals were longer between object and partner when tested at the age of 5 than at 7 weeks of age (lme: F_{1,93}=7.843, p=0.006). However, we found no influence of test group and test (nlme: group: F_{2,29}=0.48, p=0.62; condition: F_{1,90}=2.02, p=0.16).
3.2.2.4 Escape behavior

In the overall analysis, the escaping behavior did not differ whether the animals were tested in the age of 5 or 7 weeks (lme: $F_{1,339}=0.31$, $p=0.58$; Figure 8). The animals showed more escaping behavior in the clock than in the ball experiment (lme: $F_{1,334}=18.749$, $p<0.001$). Furthermore, the animals showed more escaping behavior in phase 1 than 3 but there was no difference between phase 2 and 3 (lme: phase 1 vs. 3: $t=6.259$, $p<0.001$; phase 2 vs. 3: $t=0.95$, $p=0.34$). In addition, hand-raised dogs showed more escaping than mother-raised dogs and wolves showed more escaping than hand-raised dogs (lme: mr dogs vs. hd dogs: $t=-2.384$, $p=0.024$; hd dogs vs. wolves: $t=2.34$, $p=0.026$).

Analysing the ball condition, we found no influence of age on the escaping behavior (lme: $F_{1,152}=7.92$, $p=0.11$). The animals showed more escaping behavior in Phase 1 than 3 (lme: $t=-4.241$, $p<0.001$), but there was no difference between phase 2 and 3 (lme: $t=-1.06$, $p=0.29$). Furthermore, we found a tendency that hand-raised dogs showed more escaping behavior than mother-raised dogs (lme: $t=2.040$, $p=0.051$), but we found no difference between hand-raised dogs and wolves (lme: $t=-1.58$, $p=0.12$).

When tested in the clock condition, we found more escaping behavior in phase 1 than 3 but no difference between phase 2 and 3 (lme: phase 1 vs. 3: $t=5.125$, $p<0.001$; phase 2 vs. 3: $t=0.20$, $p=0.84$). Furthermore, we found a tendency for an interaction between age and test group (lme: $F_{2,67}=2.427$, $p=0.092$). At the age of 5 weeks, hand-raised dogs showed more escaping behavior than mother-raised dogs but we found no difference between hand-raised dogs and wolves (lme: hr vs mr dogs: $t=-2.649$, $p=0.013$; hr dogs vs. wolves: $t=1.47$, $p=0.15$). At the age of 7 weeks, however, we found no difference between hand-raised and mother-raised dogs but wolves showed more escaping behavior than hand-raised dogs (lme: hr vs. mr dogs: $t=-1.10$, $p=0.28$; hr dogs vs. wolves: $t=3.282$, $p=0.003$).
Figure 8. Relative time the animals spent with escaping in the presence of a human partner in the Neophobia Task. Pups were tested over the three phases at the age of 5 and 7 weeks (hr = hand-raised, mr = mother-raised).
3.2.2.5 Stress-related behavior

Wolves emitted more stress-related behavior than hand-raised dogs did (nlme: $z=2.612$, $p=0.009$) and hand-raised dogs more than mother-raised dogs (nlme: $z=-2.794$, $p=0.005$; Figure 9). Furthermore, the animals showed more stress during phase 1 than 3 (nlme: $z=2.764$, $p=0.006$) and more during phase 2 than 3 (nlme: $z=3.387$, $p=0.001$). However, we found no influence of age and test (nlme: condition: $F_{1,336}=2.18$, $p=0.14$; age: $F_{1,339}=1.81$, $p=0.18$).

![Figure 9. Frequency of stress-related behavior in the presence of a human partner in the Neophobia Task. Pups were tested over the three phases in the ball and clock test at the age of 5 and 7 weeks (hr = hand-raised, mr = mother-raised).](image)

3.2.2.6 Activity

We found no influence of the test group on the duration of being active (lme: $F_{2,29}=0.40$, $p=0.67$). However, at the age of 5 weeks the animals were active for longer than at the age of 7 weeks (lme: $F_{1,338}=6.230$, $p=0.013$). Furthermore, the animals were more active in the ball
than in the clock test ($F_{1,333}=38.721$, $p<0.001$) and more active in phase 3 than 2 and 1
($F_{1,333}=38.721$, $p<0.001$). 

3.2.2.7 Passive Behavior

We found a tendency that the animals were more likely to behave passively at the age of 7
weeks than at 5 weeks of age ($F_{1,340}=2.930$, $p=0.088$), and they were more likely to
behave passive in the clock than in the ball condition ($F_{1,336}=8.010$, $p=0.005$). Furthermore, mother- raised dogs were more likely to behave passively than hand-raised dogs
($z=2.718$, $p=0.007$), but there was no difference between hand-raised dogs and wolves
($z=-0.57$, $p=0.57$). There was no difference between the 3 phases ($F_{2,333}=1.28$, $p=0.28$)

3.2.2.8 Object-directed play

We found an interaction between test phase and test ($F_{2,330}=4.940$, $p=0.008$). We
found an influence of phase in the ball but not in the clock test ($F_{2,148}=8.950$, $p<0.001$; clock: $F_{2,151}=1.05$, $p=0.40$). In the ball test the animals were more likely to play with
the object in phase 3 than 2 ($z=3.095$, $p=0.002$) but we found no difference between
phase 1 and 2 ($z=-0.58$, $p=0.56$). Furthermore, we found no influence of test group and
age on the likelihood that the animals were playing with the object ($F_{2,27}=0.21$, $p=0.81$; age: $F_{1,331}=0.08$, $p=0.78$).
3.3 Discussion

Social bonding to a familiar partner can decrease stress responses (e.g. von Holst 1998) in dependence of stressor severity and the relationship between the bonding partners. Our data clearly shows that a human partner can be a strong social ally that successfully ameliorates stressful events in both, wolves and dogs. I regard of attachment it may also increase exploration behavior (children: Bowlby 1969, dogs: Topál et al. 2005, chimps: Van Ijzendoorn 2008). My study reveals that all tested groups do use a human partner as a safe haven (Ainsworth 1969). Similar to the findings of Topál et al. (2005) we found that dogs, independently from their rearing history, similarly profit from human social support in a stressful situation when confronted with a novel object. However, against the findings of Topál et al. that 4-months old wolf pups do not show attachment towards their human raiser, our data clearly shows that hand-raised wolves as old as 5 and 7 weeks, similarly gain profit of a human partner as mother-raised and hand-raised dogs do. In contrary, they even Independently from rearing-history, all test groups showed less stress-related behavior and escaping behavior in the presence of a human partner compared to when tested alone. These findings are in line with the assumption that a familiar social ally ameliorates stress (van Holst 1998). However, do address whether or not stress was really ameliorated or whether just stress behavior decreased our Cortisol data will provide further insight.

In general, regardless whether the human partner was present or not, the ball test evoked less stress than the clock test. All subjects were more likely to explore it, play with it, explored it longer than the clock and were more active in terms of neutral environmental exploration. In contrast, the clock evoked more stress and escaping behavior. Overall, escaping behavior seemed to be a more informative indicator for stress than stress-related behavior (e.g. yawning, licking, vocalizing, etc.). Independently of age and test situation all groups emitted more stress-related behavior when tested alone. Interestingly, overall subjects showed more stress at the age of 7 weeks than 5 weeks, though habituation would be expected. In contrast, whereas exploration behavior decreased with age in dogs, wolves showed more exploration behavior at the age of 7 weeks. It seems that exploration behavior is differently linked with fear in dogs and wolves. Whereas dogs decrease their exploration behavior with increasing fear because they probably rely more on the human person, wolves might become more independent leading to inspection of the environment whether a human partner is present or not. Under natural conditions, wolves at the age of 5 weeks are merely close to just starting to explore the environment (Mech 1970). However, at the age of 7 weeks
they are already regularly left alone when the pack is hunting. Thus, it might be useful to increase exploration behavior with age in order to cautiously inspect potential threats. In contrast, dogs, which are mainly growing up under safe environmental conditions in a human controlled environment. Regarding human support, also the cameraperson seemed to provide advantages to some extent. Although she was standing outside the testing area, she was in close proximity to the subjects. Thus, with increasing fear over age, all subjects spent more time next to the cameraperson at the age of 7 weeks and more when tested alone than in the presence of a human partner. This indicates that besides dog, also wolves are seeking for human contact in order to ameliorate a stressful event. In line with this, all groups were more likely to wait for help at the door where humans were walking in and out, when tested alone. These findings are in contrast to those of Topál at al. (2005) who found that wolves are less likely to wait at the door when tested in the isolation part of the attachment test. In further contrast to the study of Topál et al. (2005), in our study all test groups were more likely to be passive when tested alone regardless of age. As suggested in geese (Frigerio et al. 2003) this indicates that the presence of a human ally motivates environmental interaction. In line with the findings of Gácsi et al. (2005) we found no significant difference in the general activity level between groups and age. Thus, it can be excluded, that behavioral differences are related to motor abilities.

However, in regarding evolutionary background as well as socialisation we found some differences between groups.

**Wolves** showed more stress-related behavior than dogs did. In the alone condition, wolves explored less likely than pet dog puppies but still more than hand-raised dogs. In the presence of a human partner wolves were more likely to explore compared to dogs. In contrast to dogs, they explored longer in week 7 where all subjects showed more stress behavior. Compared to dogs, wolves escaped more in the presence of a human partner and their escaping behavior increased in the scarier clock test with age. Furthermore, they even searched more for proximity to the cameraperson than dogs did, when accompanied by their raiser. In accordance wolves were more likely to wait at the door than dogs independently whether a human is present or not.

Compared to hand-reared animals, when tested alone, mother-reared dogs were more likely to explore, they escaped less long and spent less time next to the cameraperson and they were more likely to behave passive even in the more stressful clock test. Independently
whether alone or not, they were more likely to play with the ball in week 7 although all test groups showed more stress compared to week 5.

Regardless of rearing history dogs explore more likely when alone (pet dogs puppies more than hand-raised puppies) and in contrast to wolves exploration behavior towards the object decreases with age. They escape less in the presence of a human partner and show less stress-related behavior in general. In the presence of a partner, dogs spend less time next to the cameraperson and are less likely to wait at the door, independently whether a human is present or not.

**In sum** this indicates that due to different socialisation mother-reared dogs seem to be less stressed – as judged by their behavior - compared to hand-reared animals. Greater escape responses in hand-raised animals when tested alone either suggests that mother-reared dogs are less threatened because of different socialisation or they engage in a less intensive bonding towards their owner compared to hand-raised individuals and their raiser (Frigerio et al. 2003, Hemetsberger et al. 2010). Thus the lack of the human partner may evoke more stress in hand-raised animals.

In terms of evolutionary differences wolves emitted more stress behavior and these responses even increased during development. That they explored more likely in human presence indicates that they use the save haven function of the human raiser (Ainsworth 1969). However, they seemed to behave more independently in terms of human affiliative behavior than dogs did. That they were more likely to wait at the door or spent more time next to the cameraperson in the presence of the human might be explained best that if there is coming no solution from the social partner, they search for alternatives on their own. Thus, faster growing in combination with more independent behavior could further explain why they escaped more in the clock test at the age of 7 weeks compared to dogs, although a human partner was present.

In general, **independently of the phases**, all groups spent more time in close proximity to the humans at the age of 5 weeks. This indicates that human proximity seems to be negatively correlated with fear at least to some extent, since overall stress responses increased over time whereas human proximity decreased with age. However in wolves, decreasing proximity was correlated with increasing exploration behavior at the age of 7 weeks, whereas in dogs exploration decreased over time. Anyhow, there was no influence of the humans behavior on the time exploring the object or the likelihood to explore it. Overall, wolves spent less time in
proximity to the human partner than dogs did but there was a tendency that they were more seeking for contact in the scarier clock test. This indicates also wolves do use human proximity for ameliorating a stressful event. However, regardless whether less scary (ball) or more scary (clock) pet dog puppies did not differ in the length being close to the owner. In contrast, similar to wolves, hand-raised dogs searched more for human proximity in the scarier clock test. Anyhow, wolves as dogs were more likely to approach the object out of human proximity at the age of 5 weeks and in the less scary ball test, which may result from the fact that ball was generally more explored by all subjects although even with a human partner, it evoked more escaping behavior in hand-raised wolves and dogs. As already mentioned above, also in the presence of a human raiser, wolves showed more escaping behavior at the age of 7 weeks compared to dogs. In accordance with these findings, also in the presence of a human partner wolves emitted more stress-related behavior than hand-raised dogs. In line with the socialisation theory mother-raised dogs evoked less stress-related behavior and wolves as hand-reared pups escaped more in the less scary ball condition. However, neither stress-related behavior nor passive behavior was influenced by the humans’ behavior.

In regard of **phase 1 and 2** where the partner is passive and either distant (phase 1) or close (phase 2) to the object we found that wolves and hand-raised dogs spent more time in close proximity to the human partner in phase 1 where the test started with the puppies sitting in the humans lap than in phase 2. However, mother-reared dogs did not discriminate between the 2 phases which might indicate that they probably left the humans lap faster. Wolves were more likely to explore the object than dogs. However, there was no difference whether the human partner was sitting close or distant for none of the groups. All subjects showed more escaping behavior when the human partner was distant (phase 1), but they did not differ whether the human was close to the object remaining passively (phase 2) or actively encouraging (phase 3). This may either indicate that subjects were more escaping due to the fact that they were confronted with the scary objects in phase 1 the first time, or this may indicate that they relaxed more due to the fact, that the reliable ally approached the object. This further suggests, as already found in dogs (Vás et al. 2005, Merola et al. 2012) also wolves engage in social referencing towards humans. However, there was no difference in the likelihood to explore the object, to play with it and there was no difference in the general activity in none of the test groups.
Compared to the passive behavior in phase 1 and 2, the emphasis of friendly encouraging subjects to approach the scary object in phase 3 had a positive effect on all test groups. In dogs it has been shown that they are positively influenced by a human demonstrator talking in both, a social learning task (Pongrácz et al. 2004) and in a classic two-object pointing task (2012), and that they are sensitive to the tone of voice (gentle vs. harsh) used by a human in an obedience task (Fukuzawa et al. 2003) and pointing task (Scheider et al. 2011). In line with the findings in human children, that approach “noisy/movable scary toys” more when the mother delivered a positive message (Mumme et al. 1966, Walden & Ogan 1988, Kim et al. 2010) present study reveals that besides dogs also wolves experience the test objects as less intimidating when the raiser is friendly encouraging. Besides the ball, all subjects were more likely to approach the even scarier clock. Interestingly, wolves and mother-raised dogs spent less time in close proximity compared to hand-raised dogs in this phase. However, compared to the passive human behavior in phase 2, when the partner is active all subjects reached closer to the object out of human proximity in phase 3. That is, besides dogs also wolves used the secure effect of human proximity to interact with a scary object. Between-group comparison showed no difference in the time exploring the object. Although the escaping behavior of all groups decreased when the human reached closer to the object, friendly human interaction in phase 3 had only minor effect in lowering stress behavior. However, although escape behavior increased over age still used human vicinity when more scared at the age of 7 weeks. In line with all these findings, we furthermore found, that “active” human social support increased general activity in terms of neutral general exploration. Finally, human encouraging enhanced play in all test groups even in the scarier clock test.

Generally, decreasing distance (phase 1 vs. phase 2) of the human partner towards the object did not influence the subjects behavior in terms of approaching the object or decreasing stress-related behavior, although escaping behavior decreased in phase 2 and 3. However, this indicates, that when the human partner remained passive this might have delivered unclear message about the value of the object. In phase 3 however, when the human partner was encouraging, wolves and dogs regulated their behavior in accordance to the expressed emotion of the human. In sum, these results are also in line with the findings of Pettijohn et al. (1977) indicating more activity and play when a human was interacting with 4-8 week old dog puppies. Also hand-raised chimpanzees investigated a novel object more when the raiser delivered a positive message about it (Russell at al. 1997). Although we suggested a less intensive bonding towards the owner in pet dog puppies, besides hand-raised animals also
mother-reared subjects reacted towards the friendly behavior of their human caretaker. Our data further suggests, that hand-raised dogs seem to engage in the most intensive human bonding compared to hand-raised wolves and pet dog puppies as they show intermediate behavior. Similar to wolves and pet dogs they were more likely to explore in phase 3, but in contrast to the other two test groups they reacted with remaining in close proximity towards the human when she was encouraging, and when tested alone they were least likely to explore.

However, we provide evidence, that besides dogs also wolves interact with a scary object when a human partner delivers positive message on it through friendly facial and vocal means. They not only adapt their behavior to the friendly encouraging of their human raiser, they also use the security of human vicinity to cope with the stressful situation. Although wolves may differ from dogs in their way of expressing human social bonding with humans, similar to dogs they guide their behavior according to the emotional expression of their human ally towards a novel object (Merola et al 2012). In addition they seemed to express greater environmental orientation compared to dogs. Thus, in contrast to the widely spread assumption that dogs evolved due to direct selection for human social environment (e.g. Coppinger & Coppinger 2002) we suggest that the ability of social interaction with humans may be a wolf predisposition that decreased in the course of domestication due to disintegration of trait linkage (Belyaev 1979) in dogs. For example, less environmental orientation enabled dogs to become better suited to human social surroundings compared to their ancestors.
4 Task 2: Social separation

4.1 Methods

4.1.1 Experimental arrangement

We conducted a social separation task at the age of 5 weeks. The test consisted of three phases: after an isolation period of 5 minutes (Figure 10; Phase 1), the pup was accompanied by a social partner for 5 minutes (Figure 10; Phase 2), before being separated for another 5 minutes (Figure 10; Phase 3). Each subject was tested in 2 conditions in a counterbalanced order: with their raiser (for hand-reared dogs and wolves with their human raiser (Figure 10; Picture B), whereas for mother-reared dogs their mother (Figure 10; Picture C) and with a peer as a partner (Condition 2; Figure 10; Picture D). Each individual was tested in both tasks on the same day, and in the same room with 30 minute time between tests.
Figure 10. **Testing conditions of the Social Isolation Task at the age of 5 weeks.** Wolves and dogs were isolated for 5 minutes in phase 1 (Picture A). In phase 2 a human or conspecific partner was present for 5 minutes. Hand-reared dogs and wolves were accompanied by their raiser (Picture B), mother-raised dogs were accompanied by their mum (Picture C). In a second condition all three study groups were tested in the presence of a peer (Picture D). Pups were isolated for another 5 minutes in phase 3 (Picture E).

The test room was always empty except the cameraperson (C) who recorded the behavior of the subject outside the testing area (Figure 11). She was completely separated from the subject either by a visual barrier or by sitting high in at least 1 m distance. A familiar experimenter (E) carried the subject into the test room. Video recording started after she set the pup down in the middle of the room (Figure 10; A). (E) then left the room. After 5 minutes of isolation (Phase 1) the pups were accompanied by a partner for another 5 minutes (Figure 11; Phase 2).

---

**Social Separation (5 weeks)**

| Phase 2, Condition 1 |  
|----------------------|---|
| i) Partner: Hand Raiser | ii) Partner: Mother-dog |
| ![Partner: Hand Raiser](Figure 10, Picture B) | ![Partner: Mother-dog](Figure 10, Picture C) |

**Figure 11. Schematic illustration of the test room in the Isolation Task at the age of 5 weeks.** When hand-reared dogs and wolves were attended with their hand raiser (H) in phase 2 (i), she was sitting in the middle of the room, friendly interacting with the subject whenever it approached her. In case of the mother-reared dogs, the mother-dog (M) was leashed either alongside the wall or in the middle of the test room (ii), depending on the structure of the room. The cameraperson (C) was partially covered standing outside the testing area.
4.1.1.1 Phase 2, Condition raiser: Raiser as partner

After the subject spent 5 minutes alone in the room, its raiser entered. The hand-raised dogs and wolves were attended by their human raiser (H); (Figure 10; Picture B), who sat down in the middle of the room (Figure 11; i). Whenever the pup was approaching her, she was allowed to talk to the subject and pet it without restricting the pup in its movements. After 5 minutes she silently left the room.

The mother-reared dogs were tested with their mum (Figure 10, Picture B) who was led into the test room by the owner. In order to create a comparable testing situation to the human raiser, the owner fixed the mother-dog on a short leash. The length of the leash restricted the dogs’ mobility but not its possibility to interact with the pup. Depending on the structure of the test room, the mother-doges were leashed alongside the wall or in the middle of the room (Figure 11; ii) where a good camera perspective was provided. Before leaving the testing room the owner instructed the mother-dog to lie down. When the mother-dog got up during testing, the cameraperson silently tried to make her lie down again by giving a visual commando (hand gesture). After 5 minutes the owner silently entered the room, and lead the mother-dog out without interacting with the subject.

4.1.1.2 Phase 2, Condition peer: Peer as partner

The peer which was least active at the time of the test was chosen as partner. Since all of the hand-raised pups were kept together continuously, relatedness was not taken into account when selecting a partner.

After the subject spent 5 minutes alone in the test room, (E) silently entered carrying the peer partner in her hand and placed it in the middle of the test room. Afterwards she silently left the test room. The peer was not restrained during the test. When 5 minutes were over (E) silently entered, quickly picked the peer up and left without interacting with the subject.

After a further isolation period of 5 minutes in Phase 3 (Figure 10; Picture D), (E) entered the testing area and tried to make the pup approach her as described in 3.1.1.1 Alone condition (Neophobia Task).
The number of animals whose data were analysed is given in Table 7. Due to illness one of the wolf pups missed both conditions (human partner and peer) of the Social Separation Task.

Table 8. The number of tested individuals included in the final analyzes of the Social Isolation Test.

<table>
<thead>
<tr>
<th>Social Separation Test</th>
<th>Hand-raised Wolves</th>
<th>Hand-raised Dogs</th>
<th>Mother-raised Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 12</td>
<td>N = 14</td>
<td>N = 6</td>
</tr>
<tr>
<td>5weeks</td>
<td>Raiser as partner: N = 11</td>
<td>Raiser as partner: N = 14</td>
<td>Raiser as partner: N = 6</td>
</tr>
<tr>
<td></td>
<td>Peer as partner: N = 11</td>
<td>Peer as partner: N = 14</td>
<td>Peer as partner: N = 6</td>
</tr>
</tbody>
</table>

4.1.2 Behavior categories

As described in the Neophobia Task (3.1.2 Behavior categories) also the videos of the Social Separation Task were encoded by using the Solomon Coder (Version Solomon beta 100926, copyright Andras Peter) in accordance with the ethogram (Table 6). Except the category “Playing with Object” the same behavioral elements were recorded.

Again the relative duration of the total time the subject spent closer than 30 cm with the entire body to its partner (peer and raiser), the exit and the cameraperson was calculated (Table 7).

In accordance to the Neophobia Task, gaze altering between the subject and its partner was excluded from coding.

In order to investigate whether and to what extent the behavior of the peer was influencing the behavior of the subject during the Social Separation Test (phase 2), also the behavior of the peer was scored (score 0-5; see Table 9). Since the mother-dogs were fixed within the test-room and did not show relaxed behavior, their behavior was comparable to that of the human partner. Thus, no behavioral index was scored for the mother-doges.
Table 9. Behavioral score of the peer in the partner condition (phase 2) of the Social Separation Task.

<table>
<thead>
<tr>
<th>Score</th>
<th>Behavior of the Peer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>To sleep or rest. The pup doesn’t move a lot and stays most of time on the same place.</td>
</tr>
<tr>
<td>2</td>
<td>To be “neutral”, that is the animal sits, walks or rests but does not explore the environment very intensively. It neither shows signs of stress nor play.</td>
</tr>
<tr>
<td>3</td>
<td>To be very active. The peer walks, runs around and intensively explores the environment. This includes play (with partner and / or self-play) but no signals of stress.</td>
</tr>
<tr>
<td>4</td>
<td>To walk around, infrequently showing slight signs of stress (defecating, licking, panting, peeing, scratching, shaking, vocalizing, yawning).</td>
</tr>
<tr>
<td>5</td>
<td>To run around nervously while permanently showing intensive signs (defecating, licking, panting, peeing, scratching, shaking, vocalizing, yawning) of stress as well as escape behavior.</td>
</tr>
</tbody>
</table>

4.1.3 Data analysis

In accordance to the Neophobia Task also for the Social Separation Task we analysed the data by using the program R 2.15.2 (R Core Team 2012). Graphs were created with IBM SPSS Statistics 18 .Inc

Comparison between the phases:

We investigated whether “escaping”, “activity”, “passive behavior”, “stress-related behavior”, “vocalization”, “searching for human proximity (cameraperson)”, “standing by the door”, and “play” were influenced by the presence or absence of a human or conspecific partner, the group (mother- and hand-raised dogs, and wolves), or the test phases. Analysing the duration of escaping behavior, activity, and passive behavior we calculated a lme, the residuals were only normal distributed for activity for the other two variables there were not normal distributed, therefore, we used a square root transformation for escaping, and passive behavior. The frequency of emitting stress-related behavior and vocalization we analysed by calculating a glmm using a poisson distribution and searching for human proximity, standing by the door and play we used a binomial distribution.

Influence of social support in Phase 2:

We investigated whether “searching for proximity towards the partner” was influenced by the presence or absence of a human partner respectively of a peer, or the group (mother, and hand-raised dogs, and wolves). We calculated a lme, the residuals were not normal distributed, therefore, we squared in case of the time spent with the partner. In addition, we analysed the influence of the peer’s behavior on the frequency of stress-related behavior in the focal animal with a glmm using a poisson distribution.
4.2 Results

4.2.1 Comparison of stress ameliorating effects with (phase 2) and without human and conspecific social support (phase 1 and 3)

4.2.1.1 Escape behavior

When comparing the escape behavior over the three phases there was no difference found between wolves and hand-reared dogs (lme: $t=1.49$, $p=0.15$; Figure 12). Mother-raised dogs showed more escaping behavior than hand-raised dogs (lme: $t=2.522$, $p = 0.018$). Furthermore, we found longer escape behavior when tested with a peer compared to when tested with a raiser (lme: $F_{1,151}=4.556$, $p=0.034$). Independent of who was present we found less escape behavior in phase 2 than 1 (lme: $t=7.132$, $p<0.001$) and less in phase 2 than 3 (lme: $t=5.848$, $p<0.001$).
4.2.1.2 Stress-related behavior

No differences of stress-related behavior was found between the test groups (wolves / hand-raised dogs: nlme: $t_{28}=1.14$, $p=0.16$; wolves / mother-raised dogs: nlme: $t_{28}=-1.11$, $p=0.27$; Figure 12). Animals showed less stress when accompanied by a partner in phase 2 (phase 1 / phase 3: nlme: $t_{152}=-1.41$, $p=0.16$, phase 1 / phase 2: nlme: $t_{152}=-3.951$, $p<0.001$). There was no difference found whether test groups were tested in the presence of a raiser or a peer (nlme: $t_{151}=-0.90$, $p=0.37$).

Figure 13. Frequency of stress-related behavior (mean value) when tested alone (phase 1 and 3) and with a partner (phase 2) in the Social Separation Task. In the raiser condition wolves and hand-raised dogs (hr dogs) were accompanied by their human raiser while mother-reared pups (mr-dogs) were tested in the presence of the mother-dog. In a second condition all subjects were tested in the presence of a peer (phase 2).
4.2.1.2.1 Vocalization

When analysing vocalization separately from the rest of the stress-related behavior, no difference was found between test groups (wolves / hand-raised dogs: nlme: t\textsubscript{28}=1.47, p=0.15; wolves / mother-raised dogs: nlme: t\textsubscript{28}=-1.50, p=0.15; Figure 14). All animals were vocalizing less in phase 2 (phase 1 / phase 3: nlme: t\textsubscript{152}=-1.41, p=0.16, phase 1 / phase 2: nlme: t\textsubscript{152}=-2.440, p<0.016). Furthermore, no difference was found whether a raiser or a peer was present (nlme: t\textsubscript{151}=-0.66, p=0.51).

Figure 14. Frequency of vocalization (mean value) when tested alone (phase 1 and 3) and with a partner (phase 2) in the Social Separation Task. In the raiser condition wolves and hand-raised dogs (hr dogs) were accompanied by their human raiser while mother-reared pups (mr-dogs) were tested in the presence of the mother-dog. In a second condition all subjects were tested in the presence of a peer (phase 2).

4.2.1.3 Searching for human proximity (cameraperson)

We found an influence of the phase on the likelihood to be close to the cameraperson. The animals were more likely to be close to the cameraperson when tested alone in phase 1 than 3 and more likely in phase 3 than 2 where a partner is present (nlme: phase 1 vs. 3: z=2.358,
p=0.018; phase 2 vs. 3: z=-4.981, p<0.001). However, we found no influence of group and condition (nlme: Group: F_{2,28}=0.83, p=0.40; Condition: F_{1,151}=0.02, p=0.90)

### 4.2.1.4 Standing by the door

We found no influence of test group on the likelihood to be next to the door (lme: F_{2,28}=0.30, p=0.74). The animals were more likely to be at the door in phase 1 than 3 where they are alone and more likely in phase 3 than 2 when accompanied by a partner (lme: phase 1 vs 3: z=2.812, p=0.005; phase 2 vs 3: z=-2.349, p=0.019). Furthermore, animals tested with a peer were more likely to be at the door than when tested with a human raiser or the mother (lme: F_{1,152}=5.320, p=0.010).

### 4.2.1.5 Activity

We found no influence of test group and condition (lme: group: F_{2,28}=0.27, p=0.77; condition: F_{1,151}=0.005, p=0.94). However, we found an influence of phase on the individuals activity (lme: F_{2,152}=51.992, p<0.001). When a partner is present in phase 2, the animals were more active than in phase 1 and they were more active in phase 1 than in phase 3 (lme: phase 1 vs 2: t=2.890, p=0.004; phase 1 vs 3: t=-7.044, p<0.001).

### 4.2.1.6 Passive Behavior

We found an interaction between group and phase as well as between group and condition (lme: group vs. phase: F_{4,145}=3.393, p=0.011; group vs. condition: F_{2,145}=5.345, p=0.006). Therefore, we analysed the three groups separately.

Wolves showed no difference between the two conditions (lme: F_{1,61}=0.08, p=0.25). However, we found more passive behavior in phase 2 than 3 but we found no difference between phase 3 and 1 (lme: phase 1 vs. 3: t=0.37, p=0.72; phase 2 vs. 3: t=2.826, p=0.006).

Hand-raised dogs showed more passive behavior in phase 3 than in phase 1 and there was a tendency for more passive behavior in phase 3 than phase 2 (lme: phase 1 vs. 3: t=-3.618, p=0.001; phase 2 vs. 3: t=-1.803, p=0.076). Furthermore, they showed more passive behavior in the raiser condition than in the condition with a sibling partner (lme: F_{1,67}=8.494, p=0.005).
In the mother-raised dogs, we found no influence of condition on the duration of passive behavior (lme: $F_{1,27}=0.07$, $p=0.79$). However, the three phases did differ (lme: $F_{2,28}=5.807$, $p=0.007$). The mother-raised dogs showed more passive behavior in phase 3 than the other phases (lme: phase 1 vs. 3: $t=-3.377$, $p=0.002$; phase 2 vs. 3: $t=-2.086$, $p=0.046$).

4.2.1.7 Playing

We found no influence of the group the animals belonged to ($F_{2,28}=0.49$, $p=0.62$). However, we found an interaction between phase and condition ($F_{2,149}=7.23$, $p=0.001$). The animals were more likely to play in phase 1 than 3 and there was a tendency that they were more likely to play in phase 2 where a raiser (human or mother-dog, respectively) was present than in phase 3 (nlme: phase 1 vs. 3: $z=2.186$, $p=0.029$; phase 2 vs. 3: $z=1.926$, $p=0.054$). When tested with a peer we found no difference between phase 1 and 3 in the likelihood to play but they were more likely to play in phase 2 than 3 (nlme: phase 1 vs. 3: $z=0.36$, $p=0.72$; phase 2 vs. 3: $z=4.798$, $p<0.001$).

4.2.2 Comparing the effectiveness of a raiser (human or mother-dog, respectively) with a peer (phase 2)

4.2.2.1 Searching for proximity towards the partner

Hand-reared wolves and dogs spent more time with the raiser than mother-reared dogs (mother-raised dogs / hand-raised dogs: nlme: $t_{2,28}=-2.249$, $p=0.032$; wolves / hand-raised dogs: lme: $t_{2,28}=-0.05$, $p=0.96$; Figure 15). Furthermore, we found a tendency that wolves spent more time with the peer than hand-raised dogs did (lme: $t_{2,28}=1.962$, $p=0.060$), but we found no difference between dogs (lme: $t_{2,28}=-0.06$, $p=0.96$). Hence, hand-raised dogs spent more time with the raiser than with the peer (nlme: $F_{1,13}=23.024$, $p<0.001$). However, we found no difference in being close to a human partner or peer in wolves and mother-reared dogs (nlme: mother-raised dogs: $F_{1,10}=0.11$, $p=0.75$; wolves: $F_{1,20}=2.82$, $p=0.11$).
4.2.3 The influence of a peer as a function of its behavior (stressed or relaxed) on the stress-related behavior of the subjects

There was no difference within the three study groups found (mother-raised dogs / hand-raised dogs: nlme $t_{27}=-0.14$, $p=0.89$; wolves / hand-raised dogs: nlme $t_{27}=1.49$, $p=0.15$; Figure 16). Subjects neither showed any difference in their behavior (nlme: $t=-0.29$, $p=0.77$) nor in their escaping behavior (lm: $F1=0.33$, $p=0.57$) when attended by a stressed or relaxed peer.
4.3 Discussion

In line with prior studies that mainly investigated yelping (Causey 1956, Ross et al. 1959, Pettijohn et al. 1977) and stressed “activity” including biting, licking and clawing (Ross et al. 1959) as a potential indicators for emotional distress also present study underlines that social isolation in dog and wolf pups evokes stress responses. When investigating stress related behaviors like escaping and general stress-related behavior including vocalization, wolf and dog pups show strong stress responses when socially isolated. However, independently from their rearing history, all animals showed less stress behavior when a social ally was present in phase 2 compared to the two isolation periods (phase 1 and phase3). Similar to the findings of Pettijohn et al. (1977) none of the social allies had persistent effect that resulted in a reduction of stress behavior in phase 3. Thus, a potential habituation effect of the pups to the test situation can be excluded and more importantly, this suggests that our results reflect effective methods of ameliorating separation distress in the pups.

Anyhow, when comparing the effectiveness of human and conspecific allies in ameliorating stress we found some differences between the test groups. In general, pet dog puppies showed more escaping behavior than hand-raised dogs and wolves did when tested with a partner. However, all groups escaped more in the presence of a peer than a raiser (human or mother-dog, respectively). These results indicate the within the raiser condition, the human was more effective than the mother-dog. Pettijohn et al. (1977) also showed that the presence of a human partner was more effective in alleviating stress responses in 4 to 8 week old pups than the own mother-dog was. Furthermore, these findings are in line with the suggestions of May et al. (2008) that 8 week old dog puppies do not prefer their mother over a sibling in a 2-choice task. Although a peer ameliorated escaping behavior less effective than a raiser (human or mother-dog, respectively) our results are in alignment with the findings of Fredericson (1952) that still, the presence of a peer can lower the level of escaping behavior.

Regarding stress-related behavior (including vocalization) all test groups showed the same frequency of stress. There was no difference found whether a raiser (human or mother-dog, respectively) or a peer was present. Even when analysing vocalization separately we could not find the effect suggested by Pettijohn et al. (1977) that a human is more effective in reducing yelping than the mother. Furthermore, our results are in contrast to Elliott & Scott (1961) that showed higher effect of a sibling over the mother-dog when reducing distress vocalization.
In alignment with the results above that a human raiser is more effective in buffering stress, wolves and hand-raised dogs spent more time in close proximity towards their raiser than pet dog spent towards the mother-dog in phase 2. Since the mother-dogs were physically restricted to investigate the room on their own, the same amount of interaction with the puppies was provided as in the human-raiser condition. Human and conspecific raiser either passively accepted the pups’ advances or actively interacted with them (human: petting and friendly talking; mother-dog: licking, nursing and vocal calls). Comparing the effectiveness of a peer, wolves spent more time with a peer than dog pups. This could result from the fact that under natural living conditions, 5 week old wolf pups mainly spent time with littermates (Mech 1970). High attentiveness towards conspecifics may be crucial for survival in threatening situations. In contrast, dogs that adapted to the safe conditions of human society during domestication (e.g. Coppinger & Coppinger 2002) may not rely on conspecifics to that extent anymore even at very young age. These suggestions could be underlined by our results that wolf and pet dog pups do not distinguish between a peer and the human or conspecific raiser when searching for proximity, respectively. In contrast, hand-reared dogs clearly spent more time next to the human raiser than to a peer.

In regard of our general findings, that all test groups gain profit from social support this is underlined by the fact that the subjects showed more active behavior in form of exploring the environment when accompanied by a human and conspecific partner, respectively than alone.

Although the test situation evoked rarely any play, interestingly, all test groups show more play behavior in the presence of a peer than in the presence of a raiser. This could indicate that pups discriminate differently between a raiser and a peer. In our tests, pet dog puppies were often nursed by the mother-dog, indicating that similar to a conspecific raiser also a human raiser may have different functions (e.g. providing food) compared to a peer.

Finally, regarding human social support in a more far way, all test groups were searching for proximity to the cameraperson who was standing outside the test area when tested alone in phase 1 and 3 which disappeared in phase 2. In line with these findings, all test groups waited next to the door (where the humans walk in and out) more frequently when they were alone. There was no difference whether a human partner or the mother was present in phase 2, however, the subjects were more frequently waiting at the door when a peer was present. This could further indicate, that subjects search for human support since a peer might be less effective in ameliorating a stressful situation.
In contrast to the findings of Winslow et al. (2003) our results show little evidence that hand-raising may reduce the possibility to gain advantage of conspecific social support due to the lack of social interaction. For hand-reared wolves and dogs as well as pet dog puppies a peer was less effective although wolves spent more time next to the peer than hand- and mother-reared dog pups did. Furthermore all groups played more in the presence of a peer than in the presence of a human or conspecific raiser, respectively.

In conclusion, regarding the stress ameliorating effectiveness of human and conspecific social support we find a similar ranking order as Pettijohn et al. (1977) for all test groups independently from their raising condition: human raiser > mother-dog > peer.
5 General discussion

According to the synergistic-hypothesis (Gácsi et al. 2009) present study reveals that behavioral differences in wolves and dogs arise from a combination of rearing-history and genetic divergences. In regard of socialisation hand-raised dogs and wolves showed more behavioral stress than mother-reared dogs did. According to the postnatal-handling-theory, originally investigated in rats, it is supposed that environmental stimuli during early stages of life can alter behavioral and endocrinological stress responses (Meaney et al. 1991, Anisman et al. 1998). In line with this assumption, Hemetsberger et al. (2010) showed that hand-raised geese emitted less stress responses compared to their conspecific reared. Thus, one may assume that hand-raising also decreases emotional reactivity in wolves and dogs. Our data show, that in various cases hand-raising similarly influences the behavior in wolves and dogs. However, although hand-raised individuals are confronted with a series of testing from very early age on, from personal experience I dare to say that the tested pet dog puppies were raised under more “rough” environmental conditions. That is, they experienced less soft handling (e.g. rough handling by children) and were daily confronted with different stimuli (e.g. loud farming machines, trampling horses, etc.). However, an additional explanation might be that in pet dogs these stimuli gradually appear in the course of every-life and more importantly, in most of the cases they can decide whether to approach or withdraw. In contrast, hand-raised wolves and dogs are frequently confronted with stressful situations, without the possibility to withdraw. Thus, different rearing-history and the way of experiencing stimuli may generally increase emotional reactivity.

Independently from rearing-history both pet dog puppies, and hand-raised dogs and wolves showed less stress behavior when accompanied by a human partner than when tested alone. According to the concept of a “social toolbox” (Kotrschal et al. 2009) the ability to socialize across-species may arise from a ancient convergent vertebrate brain structures and functions (i.e. Goodson 2005, Panksepp 2005). When comparing human social support to conspecific support, humans seemed to be even more effective than the mother-dog or a peer resulting in less emotional stress behavior in hand-raised wolves and dogs and more proximity seeking towards a human partner compared to a conspecific. Thus, similar to hand-raised geese (Frigerio et al. 2003), also in wolves and dogs, a human ally seems to be a valid parent-mimicking model. Various studies demonstrated that being stroked by the owner, increased oxytocin and decreased cortisol levels in the dog (Odendaal 200, Handlin 2010, Handlin et al.
As some of our data seem to indicate, a human raiser may be even a “hyperoptimal” parent compared to the mother-dog. As mammals, pups enjoy physical contact (e.g. being touched and stroked) which aligns with the typically primate-like sticky attitude. Thus, being touched by the hands of a human may be better in satisfying the social templates in the brain of the pups compared to canine mothers with their more distant attitude and tongues.

As suggested by Gácsi et al. (2009) also our data suggest that despite similar amount of early socialisation the role of humans as social partners seems to be different for wolves and dogs. Similar to pet dogs, hand-raised dogs are less stressed in the presence of their human partner, they spent more time in close proximity to the human and are less likely to search for alternative help when stressed. In contrast, wolves seem to behave more independent and are more likely to search for alternative solutions even if a human partner is present. Wolves clearly do gain profit from human social support, however, this advantage is not necessarily connected to human vicinity compared to dogs. Ontogenetic differences in terms of a greater general stress level that even increased during development, suggests faster growing in wolves (Fentress 1967, Frank & Frank 1982a, Zimen 1987). In turn, this implicates decreased emotional stress reactivity in dogs through indirect selection during domestication. In combination with increasing exploration behavior when getting older, it seems unlikely that wolves as old as 4 months are able to react similar to the attachment paradigm as dogs do (Topál et al. 2005). However, although current research still argues whether real attachment behavior is restricted to human bonding only (e.g. romantic relationships, parent-child), Fraley and Shaver (2000) argued that the utility of attachment theory is not restricted to human bonding only. They further stated that relationships which do not meet all four of the attachment criteria (secure base, haven of safety, maintenance of proximity and negative emotions when separated) as defined by Ainsworth (1991) can nevertheless fulfill attachment-related functions. Since security and stress reduction are among the main features of attachment (for a review see Julius et al. 2012) our results could also be interpreted in terms of attachment behavior. Thus, our study provides evidence for the very first time, that besides dogs (Topál et al. 2005, Prato-Previde et al. 2003, Gácsi et al. 2001) also wolves showed attachment behavior to a human partner. They even used the human partner to a greater extent as safe haven (i.e. increased exploration) than dogs did. In contrast, dogs rather decreased exploration behavior but searched more for human proximity (secure base). These findings are in line with current leash walking experiments (Hampl Karina, Master-thesis in progression) suggesting that bonding dispositions to humans are even less expressed in dogs compared to wolves. In line with present study, her data further shows decreased...
environmental orientation in dogs. In contrast to the assumption that the human-dog-bond evolved due to direct selection for social skills towards humans (Hare & Tomasello 2005) our findings provide evidence that domestication lead to a disintegration of traits (Belyaev 1979) in dogs. For example, wolves were able to follow the pointing clies of their hand-raisers only from 9 months of age, whereas similar raised dogs do already from earlier age on (Virányi & Range, in press).

However, whether or not present data resemble attachment-like relationships in wolves and dogs may remain uncertain. Anyhow, we provide evidence that wolves similarly engaged in human bonding as found in chimps (Van Ijzendoorn 2008) and dogs (Topál et al 1998, 2005, Gácsi 2001), and were even more supported by the presence of a human ally compared to dogs.
5.1 Limitations and potential supplements of the study design

Indisputably, the present study underlines the significance of social support in modulating stressful events by a social ally and points out basic mechanisms of this phenomenon. However, environmental and genetic origin of social buffering cannot clearly be distinguished according to the present results. Thus, parts of the study design might be improved or extended, respectively, in order to even increase its scientific output.

First, to get more insight in how far environmental features are influencing social behavior, in the course of this study the daily routine of hand-raised and mother-raised subjects from the age of 4 to 8 weeks was recorded. For example, whereas pet dog puppies seemed to experience all kinds of social and non-social stimuli (e.g. noisy animals, loud machines, etc.) hand-raised pups passed through a series of behavioral and cognitive tests from very early age on (4 weeks). Due to the big amount of videos these data were not analysed yet.

Second, the establishment of an ethogram that mainly focuses on the interactions between subjects and their social environment can help examining the kind of affiliative bonding that may distinctly develop towards the raiser, the mother-dog, the owner and the peers. In turn this might help interpreting the results concerning social support of different partners.

Third, to get more insight to which extent two closely related species as the dog and the wolf differ in their perception of human and conspecific social support controlling for endocrine mechanisms in terms of glucocorticoids might provide further insight. Thus, in the course of this study saliva samples were selected on two different purposes. To evaluate the general stress level of the test groups, saliva was taken from all subjects in arrangement with the video recording schedule of everyday life. Furthermore, in order to investigate stress levels during testing, salivary samples were taken according to the “Trier Social Stress Test” conducted by Kirschbaum et al. (1993) during the Neophobia and Social Separation Task. In the first case, samples were not analysed yet. Based on already conducted pre-analysations wolf and dog saliva cortisol seem to differ in their basic cort-values, which makes an adequate comparison problematic. Verifying these findings would underline the hypothesis of Richter et al. (1959) that selective processes during domestication (e.g. reduced fearfulness towards humans) also reduced the function of the adrenal glands and thus, the excretions of glucocorticoids. In the second case, samples were analysed but were not interpreted yet due to various analysation problems. For example, taking saliva samples of puppies entails the
difficulty to collect enough saliva for running the immune-enzyme-assay. Thus, due to the lack of the appropriate amount of saliva, most samples could not be analysed.

Finally, the sample size of tested individuals should be increased in order to exclude individual features from the results. In the present study 12 hand-reared wolf, 14 hand-reared dog and 6 mother-raised dog pups were tested. Although the results may provide insight in basic outcomes of stress behavior derived from different social background, clearer interpretations may be provided by repeating the tests with additional puppies. Furthermore, it has to be kept in mind that some of the hand-reared dog pups were in bad physical conditions when adopted from the shelters. Since they were only a few days old it should be taken into account that illness at this early age may have had effects on their behavior later on. Although unlikely, it should be considered, that medical treatment may have influenced their behavior to some extent. The transport of hand-raised dog pups from Hungary to Austria at the age of 6 – 7 weeks may have resulted in a generally increased stress level. Changing environmental conditions might be a conspicuous determinant in effecting the pups’ stress management behavior.

In conclusion, the study design entails great potential in investigating social support in affiliative (interspecific) partners and thus, is worth proceeding.

“Each solved problem immediately gives birth to its offspring.”
Prof. Dr. Konrad Lorenz
6 References


Hanson, H.C. 1953. Inter-family dominance in Canada geese. Auk 70: 11-16.


Social Support in Wolves and Dogs


Acknowledgements

Thank you to …

… my parents Fritz and Veronika Hann for always supporting my dreams and goals! You are the most important people in my life, I love you! Special hugs to my mum who even helped me collecting data for my thesis! Thank you to my whole family for being interested in my work.

… to Dr. Friederike Range, Dr. Zsófia Virányi and Ao. Univ.-Prof. Mag. Dr. Kurt Kotrschal for offering me the possibility to write my thesis at the Wolf Science Center, Ernstbrunn and teaching me scientific skills. The unique contact to your dogs and wolves fundamentally influenced my personality as well as my professional development in a positive way!

… to Ao. Univ.-Prof. Dr. med. vet. Erich Möstl and colleagues from the Department of Biochemistry of the Veterinary University of Vienna for giving advises in analysing cortisol from saliva samples (which are unfortunately not part of the thesis). Further thanks to Ao. Univ.-Prof. Dr. Eva Millesi and staff. Enzyme immune assays were conducted in the laboratories of the Department of Ethology, University of Vienna, by Anna Schöbitz.

… the animal trainers Bea Belényi and Rita Takács for considering my observations, helping me with the tests and giving me insight into animal training.

… to M.Sc. Marianne Heberlein for her everlasting social support, for patiently answering all my questions, for helping me with the statistical analysis, for being a role model in so many different ways and most importantly, for being an unique friend.

… to M.Sc. Marion Heszle for being “Se Cracker”. Friendship!

… to Erik Kersting for enabling me to absolve his apprenticeship on dog training. The basic knowledge that I gained on dog and wolf behavior helped me arranging my thesis to some extent. Furthermore thank you to M.Sc. Marleen Hentrup for initiating the collaboration and providing her car “Hugo” for the common trips to Germany.

… to the dog owners Jutta Waxenegger (Schachau 6, A-3281 Oberndorf an der Melk, http://sonnenschein.heimat.eu), Martina Scheifinger (Reitstall „Spirit-Horse“, Wr. Neustädterstr. 16, 2601 Sollenau, Austria; http://www.spirit-horse.at) and Dr. Mathilde Buchmayer (Dipl. Veterinary, A-2115 Steinbach, Steinbach 10, http://www.tierpraxis-buchmayer.at) who and participated at my thesis. Special thanks to the whole family Buchmayer for kind of integrating me as a part of their family during the testing period, to Carina Buchmayer for helping me taking data at M Scheifingers place, and again to Dr. Mathilde Buchmayer for vetting the ill WSC wolf and dog pups.


… to my colleague and friend Mag. Ülker Utku for her belief in me and her tireless positive words, as well as her husband Erkan Utku for pimping up my technical equipment and providing a second life to my computers!

… to my employer Jens König and my colleagues Mag. Ülker Utku and Mag. Venetia Mexi to organize our job in a way that simultaneously enabled me to work at my thesis.

Finally, special acknowledgements to all participating wolves and dogs.
Curriculum Vitae

Education

1988 – 1992  Primary School (Otto-Glöckel Volksschule), St. Pölten, Austria
1992 – 1996  Private Secondary School (Institut der Englischen Fräulein), St. Pölten, Austria
2001 – 2010  Diploma’s Study of Biology/Zoology at the University of Vienna
Focus: Ethology and Human-Animal-Relationships
2004 - today  Employee of Generali Group, Vienna
2010 – 2013  Apprenticeship as Consultant for Dog Behavior and Education, at the dog training center “Canis –Familiaris”, Germany
2012 -2014  Vocational Preparation Course for „Animal Assisted Therapy and Animal Assisted Affirmative Action” of the Association “Tiere als Therapie”, University of Veterinary Medicine Vienna
2013  Qualification for Animal Training of the Association “Tiere als Therapie”, University of Veterinary Medicine Vienna

Practical courses and work experience

2001  1 month internship at the Kindergarten of the Clinical Center St. Pölten, Austria
2002 - 2006  3 years Private Educational Aid for Primary and Secondary School Children with physically and psychologically special needs, St. Pölten, Austria
2008  2 months animal care attendant internship at the Zoological Garden „Schönbrunn“, Vienna, Austria
2006 - 2008  several months of scientific internship at the Zoological Garden „Schönbrunn“, Vienna, Austria
Preparing the speech “Human-Elephant-Conflicts” for Dr. Harald Schwammer, Acting Director Zoo Schönbrunn, Vienna

Personal data

Katrin Hann

Qualifications

Knowledge of business management
International language courses in England, France and Italy
Curriculum Vitae

2008 – 2010  2 years internship at the Veterinary Specialist for Small Animals, Mag. Karl Zuser, St. Pölten, Austria

2009 - 2012  3 years internship at the Wolf Science Center, Ernstbrunn, Austria
Hand-raising of several generations of dog and wolf pups
Data collection for diploma thesis
Organisation und implementation of the guided tours
Assistance at scientific investigations

2010  3 weeks internship at Parc Safari, Quebec, Canada
Hand-raising and transfer of 2 wolf pups to the Wolf Science Center, Ernstbrunn, Austria

2010  Canine Science Forum, Vienna, Austria
Poster-teaser „Human Social Support in hand-reared wolf “Canis lupus lycaon” und dog pups “Canis familiaris”.
Furthermore, assistance in the administration of the CSF

2010  5. International Symposium of the Association “Tiere als Therapie”, University of Veterinary Medicine, Vienna, Austria

2012  6. International Symposium of the Association “Tiere als Therapie”, University of Veterinary Medicine, Vienna, Austria

2011 - 2014  Center for International Languages, University of Vienna, Austria
Acquisition of the Austrian Sign Language

Language kills

German: first language
English: advanced skills in reading, writing and speaking
Italian: basic knowledge
French: basic knowledge
Austrian sign language: basic knowledge

IT skills

Very good command of Microsoft OfficeTM tools
(WordTM, ExcelTM and Power Power PointTM)
Basic knowledge of graphic design applications
(PhotoshopTM, Adobe IllustratorTM, SPSS)

Membership

Viennese Society for Animal Protection,
Foundation “Hunde-helfen-leben”, Germany

Interests

Literature
Nature
Music
Drawing
Photography
Playing Guitar