Hand-reared wolves show similar, or stronger, attachment toward human caregivers compared to hand-reared dogs

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Abstract

Domesticated animals are generally assumed to display increased sociability towards humans compared to their wild ancestors. Dogs (*Canis familiaris*) have the ability to form lasting attachment, a social bond based on emotional dependency, with humans and it has specifically been suggested that this ability evolved post-domestication in dogs. Subsequently, it is expected that dogs but not wolves (*Canis lupus*), can develop attachment bonds to humans. However, while it has been shown that 16-weeks-old wolves do not discriminate in their expression of attachment behaviour toward a human caregiver and a stranger when compared to similar aged dogs, wolves at the age of eight weeks do. This highlights the potential for wolves to form attachment to humans, but simultaneously raises the question if this attachment weakens over time in wolves compared to dogs. Here we used the Strange Situation Test (SST) to investigate attachment behaviour expressed in hand-reared wolves and dogs toward a human caregiver at the age of 23 weeks. Both wolves and dogs expressed attachment toward a human caregiver. Surprisingly, wolves, but not dogs, discriminated between the caregiver and a stranger by exploring the room more in the presence of the caregiver compared to the stranger and greeting the caregiver more than the stranger. Our

results thereby suggest that wolves can show attachment toward humans comparable to that of dogs at later developmental stages. Importantly, our results indicate that the ability to form attachment with humans did not occur post-domestication of dogs.

Introduction

Animals have been a prominent part of human society ever since the dog (*Canis familiaris*) was domesticated as the first species at least 15,000 years ago (Driscoll et al., 2009). Domestication, the evolutionary process in which species are selected to live in human-controlled environments (Price, 2002), has significantly impacted behavioural expression in animals (Belyaev et al., 1985; Künzl & Sachser, 1999; Trut, 1999; Himmler et al., 2013). For instance, domesticated animals are generally assumed to display increased sociability towards humans compared to their wild ancestors (Belyaev, 1979; Bentosela et al., 2016) and it has specifically been suggested that domestication affects interspecific attachment in human-animal relationships (Topál et al., 2005).

Attachment is a social bond based on emotional dependency, which is formed between two individuals and endures over time (Ainsworth & Bell, 1970). Originally described as the bond between a human infant and its mother, attachment behaviour constitute any type of behaviour performed by the emotionally dependent individual to promote proximity or contact to the individual of attachment (Bowlby, 1958; Ainsworth & Bell, 1970). Ainsworth's Strange Situation Procedure (SSP) is a highly influential method developed to empirically investigate the attachment bond between human infants and their primary caretaker (Ainsworth & Bell, 1970). Based on the assumption that the attachment system is only activated in challenging situations (Rehn et al., 2013; Prato-Previde & Valsecchi, 2014), the

SSP examines an infant's attachment behaviour toward its primary caregiver under standardized conditions of low and high emotional stress, which includes separation, reunion and the presence of a stranger (Ainsworth & Bell, 1970). Attachment is quantified as the behavioural discrimination between a primary caretaker and a stranger (Ainsworth, 1989; Topál et al., 1998; Rehn et al., 2013). Attachment behaviours animated by the SSP include increased exploration and play when the attachment figure is present (i.e. secure base effect) and proximity maintenance and contact seeking behaviour, in which the infant actively seek physical contact with the attachment figure (Ainsworth & Bell, 1970). In 1998, Topál et al. adapted the SSP to dogs (i.e. the Strange Situation Test (SST)), and demonstrated that the human-dog bond is comparable to that of a parent-child attachment bond. Since then multiple studies have used the SST to demonstrate the presence of attachment behaviour toward humans across different groups of dogs, including guide dogs (Valsecchi et al., 2010), shelter dogs (Gácsi et al., 2001) and pet and working dogs (Mariti et al., 2013).

While research on human-dog attachment is abundant, less than a handful of studies (Gácsi et al., 2005; Topál et al., 2005; Hall et al., 2015; Ujfalussy et al., 2017) have included attachment between humans and wolves (*Canis lupus*), the closest extant relative of dogs. Of these, two have used the SST to quantify attachment (Topál et al., 2005; Hall et al., 2015;). In 2005, Topál et al. demonstrated that hand-raised dogs, but not wolves, discriminate between a familiar person and a stranger in the SST at 16 weeks of age, and concluded that dogs have evolved a unique ability to show attachment toward humans. Yet, another study using the SST showed that hand-raised wolf puppies up to the age of eight weeks express attachment, in the form of proximity and contact seeking behaviour, toward human caregivers, thereby demonstrating the potential for attachment between humans and wolves (Hall et al., 2015). While a possible explanation for these contrasting results could be that age and increased

independence during ontogeny generally affect the attachment toward caregivers (Mongillo et al., 2013; Hall et al., 2015), it has been suggested that the attachment bond in guide dogs is only fully formed once the dogs reach maturity (Valsecchi et al., 2010) and that adult dogs tested in the SST show attachment to their owners (Palmer & Custance, 2008; Prato-Previde et al., 2003). Thus, the affectional bond between a wolf and a human might not endure over time. However, the 16-weeks-old wolves and dogs in the study from 2005 (Topál et al., 2005) were hand-raised individually by different caregivers, and the wolves had been relocated to an animal park up to two months before the test was conducted (Virányi et al., 2008). Therefore, at the time of testing, dogs were still living with their caregivers, but wolves were not. This unequal socialization of wolves and dogs could bias the results of this study. Therefore, additional studies on equally socialized wolves and dogs are needed to address the domestication effects on attachment behaviour, and if this behaviour changes with age in wolves.

Here we investigate attachment toward a human-caregiver in wolves and dogs at a later ontogenetic stage than previously tested. Dogs and wolves were reared under identical conditions, using standardized methods for both hand-raising and socialization (Klinghammer & Goodman, 1987; Udell & Wynne, 2008; Range & Virányi, 2011) as well as testing, using the SST adapted to canids (Topál et al., 1998; Topál et al., 2005). The enormous effort necessary to hand-raise, socialize and test wolves and dogs combined with limited animal availability, highlights a fundamental challenge in domestication research on canids, namely inherently small sample sizes rarely exceeding N = 11 for wolves and N = 13 for dogs (Miklósi et al., 2003; Topál et al., 2005; Gácsi et al., 2005; Udell & Wynne, 2008; Moretti et al., 2015; Range et al., 2015; Marshall-Pescini et al., 2017). However, contemporary studies, with small sample sizes, are essential in furthering our understanding of the behavioural

consequences of domestication, and therefore standardizing and replicating behavioural studies on wolves and dogs becomes critical. This study is a replication of the study by Topál et al. (2005), using identical testing protocols and analyses. However, to adequately control for environmental effects, the animals in our study were raised within litters under identical conditions and equally socialized prior to testing. Furthermore, to gain insight into the presence of attachment behaviour at a later ontogenetic stage, our animals were tested at 23 weeks of age. While, young wolf puppies have been shown to form attachment with human caregivers (Hall et al., 2015), we expected this attachment to weaken with age in wolves, and thus predicted that dogs, but not wolves, would show attachment behaviour towards their human caregiver when tested in the SST. Specifically, we predicted that wolves would not discriminate between their caregiver and a stranger, by expressing similar levels of attachment behaviours regardless of which person was present.

Materials and Methods

Ethical statement

Daily care and all experiments were performed in accordance with guidelines and regulations under national Swedish Law. The experimental protocols in this study were approved by the Ethical Committee in Uppsala, Sweden (approval number: C72/14). Facilities and daily care routines were approved by the Swedish National Board of Agriculture (approval number: 5.2.18-12309/13). As required by national law in Sweden, all hand-raisers working with the puppies were ethically certified and trained to handle animals.

Study animals

Twelve Alaskan huskies and 10 European grey wolves where included in this study. The dogs, four females and eight males, came from two different litters. The wolves, five females and five males, came from three different litters. To minimize environmental bias, including maternal effects, which is well-documented to affect the development of behavioural patterns (Clark & Galef, 1982; Wilsson & Sundgren, 1998; Bray et al., 2017), puppies were hand-raised and extensively socialized under standardized conditions from the age of 10 days. Dogs and wolves were raised with their littermates and socialized with 24-hour presence of human caregivers for the first two months. Caregiver presence was decreased with a few hours a day from the puppies were two months, and this decrease was gradually increased so that they at four months of age would spend every other night without a caregiver present. All wolf and dog litters were reared under standardized conditions. Puppies were reared in identical indoor rooms until they were five weeks old and here after given access to smaller roofed outdoor enclosures. At six weeks of age, after a week of habituation to the roofed outdoor enclosure, puppies were given access to a larger fenced grass enclosure. From the age of six weeks the puppies had free access to all three enclosures during the day and access to the indoor room and the roofed enclosure during the night. At three months of age puppies were moved to large outdoor enclosures (2,000 square meters), in which they remained for the rest of the study period. Dogs and wolves were kept separate throughout the entire period. Behavioural observations were initiated immediately at 10 days of age and behavioural testing was initiated at six weeks of age. Caregiving, socialization procedures, enrichment, testing procedures and exposure to the new environments were standardized over all three years. Puppies were not disciplined or trained. From the age of eight weeks puppies were gradually introduced to strangers through the fence, always with the support of one or more caregivers.

Experimental design

All wolves and dogs were tested at the age of 23 weeks (dogs: 23 weeks ± 0; wolves: 23 weeks ± 0.3). The experimental design was identical to that of Topál et al. (2005). Briefly, the Strange Situation Test adapted to dogs consists of seven experimental episodes, each lasting two minutes, in which the presence and absence of a familiar person and a stranger in a test room with the focal animal is alternated (Table 1).

Table 1. Strange Situation Test procedure. In the seven episodes in the Strange Situation Test a familiar person (F) and/or a stranger (S) is present in the test room with the focal animal (expect for episode 5 where the animal is alone). Each episode is structured differently. The procedure is identical to the study of Topál et al. (2005).

Episode	Present	Minutes	Structure of episode
1	F	0-2	F leads the animal into the test room, closes the door and sits down in one of two chairs and reads from a paper in silence. After 1 min F initiates play with the animal. F stops playing after 2 min as S enters the room.
2	F+S	2-4	S enters the room and stops for up to 5 s, allowing the animal to greet, and then sits down in the vacant chair. After 30s S initiates a friendly chat with F. After 30s S stops chatting with F, stands up and initiates play with the animal. F then leaves as quite as possible.
3	S	4-6	S continues to play/initiates play with the animal. After 1 min S stops playing and returns to the chair. If the animal initiates contact, S is allowed to reciprocate physical contact by petting it.
4	F	6-8	F calls the animal from outside the room. After entering the room, F stops for up to 5s to allow the animal to greet and then goes to the chair and sits down. S leaves. F initiates play with the animal for 1 min and then returns to the chair. If the animal initiates contact, F is allowed to reciprocate physical contact by petting it. At the end of the episode F says 'I must go, stay here' and leaves the room.
5	-	8-10	The animal is alone in the room.
6	S	10-12	S enters the room and stops for up to 5s to allow the animal to greet, and then initiates play with the animal. After 1 min S sits down in the chair. If the animal initiates contact, S is allowed to reciprocate physical contact by petting it.
7	F	12-14	F calls the animal from outside the room. After entering the room, F stops for up to 5s to allow the animal to greet. S leaves the room while F stimulates the animal to play for 1 min and then sits down in the chair. If the animal initiates contact, F is allowed to reciprocate physical contact by petting it.

The familiar person was a primary caregiver, who had raised all the litters from 10 days of age and was the hand-raiser who had spent most time with the animals. The stranger had never met the dogs or the wolves prior to the experiment. The same familiar person and stranger were used in all tests. In the 6x6 meter test room, two chairs were placed in the middle of the room, 1.5 m from each other and facing in the same direction. Seven toys from the animal's home enclosure, such as balls, rope and rubber toys, were distributed across the floor in the test room. Tests were filmed with two diagonally mounted GoPro cameras (model Hero, 3, 3+, 4, GoPro Inc.).

Behavioural scoring

Following the procedures in Topál et al. (2005) a total of seven behaviours expressing secure base effects, and proximity and contact seeking were scored using an ethogram (Table 2). These seven behaviours were divided into a) continuously measured behaviours, which included exploration, passive behaviour, physical contact, social play and standing by the door, and b) scored behaviours, which included following and greeting (Table 2, Table S1).

Behavioural scoring was carried out using the software program BORIS v. 2.97 (Friard & Gamba, 2016). Of the recorded tests, 25% were independently coded by both LL and CHW. Cronbach's Alpha was calculated and inter-observer reliability was high for all continuous behaviours (exploration: 0.986; passive behaviour: 0.978; social play: 0.985; stand by door: 0.989; physical contact: 0.987).

Table 2. Ethogram. Behavioural categories coded following Topál et al. (2005), including a) Continuously measured behaviours and b) Scored behaviours. All continuous behaviours were scored as both frequency and duration. The type of attachment behaviour is given for each behavioural category as well as the sampling method.

Behaviour	Definition	Type
a) Continuously measured behaviours		
Exploration	Activity directed towards non-movable aspects of the test room (not including toys), including sniffing, distal visual inspection (staring or scanning), close visual inspection or oral examination, while F and/or S are present and during episode 5 when the animal is alone.	Secure base
Passive behaviour	Sitting, standing or lying down without any orientation towards the environment (this includes grooming), while F and/or S are present, and during episode 5 when the animal is alone.	Secure base
Physical contact	Bodily contact initiated by F or S (e.g. petting, touching) or the animal.	Proximity/ contact
Social play	Motor activity performed when interacting with F or S; including running, jumping, active physical contact and chasing toys.	Secure base
Stand by the door	Standing within 1 meter of the door and facing towards the door.	Proximity/ contact
b) Scored behaviours		
Following	Conditional scoring between 0 and 3 of following F and S leaving the room while the other person stays behind. **Score 0:** no orientation towards the leaving person at all, or only for less than 1s. **Score 1:** orientation towards the leaving person for more than 1 s. **Score 2:** following the leaving person to the door. **Score 3:** trying to get through the door or standing by the door for more than 1s. The mean based on scores from episode 3, 4 and 7 is used as total score.	Proximity/ contact
Greeting	The behaviour of the animal towards the entering F or S, scored using one of five categories: **approach initiation (+1):** the animal moves towards the entering person; **full approach (+1):** the animal approaches the entering person until physical contact is made; **avoidance (−1):** avoidance behaviour towards the entering person, such as backing or getting out of the way of the entering person; **durable physical contact upon greeting (+1/2):** the animal spends more than 3s in bodily contact with the entering person; **delay of approach (−1/2):** when F or S enters, the animal hesitates to initiates any form of approach for more than 5s. Scores are summed up to a total score (maximum 5 since each person entered twice)	Proximity/ contact

F = Familiar person, S = Stranger.

Statistical analyses

Because our study was a replication of the study by Topál et al. (2005), we performed all our statistical analyses using similar methods. For all statistical analyses we used the software SPSS Statistics version 25. Graphs were made in GraphPad Prism version 7.04. To account for slight variations in durations of episodes across tests (because of the time it took for the test persons to enter and exit the room), we used the relative proportion of the time spent on each behaviour for every episode for all individuals. Upon testing for normality using a Shapiro-Wilk test (Razali & Wah, 2011), we found that the two variables passive behaviour and social play were not normally distributed. We therefore arcsine transformed these two variables prior to statistical testing (Table S1). Arcsine transformation is commonly used for proportional data (Cohen and Cohen 1983; McKillup, 2012). For one wolf the test was aborted prematurely and as a result episode 6 and 7 were excluded for this individual. Thus, sample sizes for episode 6 and 7 were $N_{dog} = 12$ and $N_{wolf} = 9$. We present the mean and SE for the untransformed data in our figures (McDonald, 2009, Table S2). All behaviours were divided into two main categories: 1) 'In the presence of the familiar person', which refers to those episodes in which the familiar person was present (1, 2, 4, 7), and 2) 'In the presence of the stranger', which refers to those episodes where the stranger was present (2, 3, 6). We used a General linear model (GLM) for repeated measurements where the proportions of the presence of the familiar person and the stranger were classified as the within-subject factor, and dogs and wolves as the between-subject factor (Table S3). In post-hoc comparisons, we used t-tests for between-groups comparisons and paired t-tests for within-group comparisons (Table S4).

Results

Activity

To evaluate whether a familiar person functioned as a secure base, we compared the activity level in wolves and dogs in the presence of the familiar person with the activity level in the presence of the stranger.

Exploration was significantly more common in the presence of the familiar person than in the presence of the stranger ($F_{1,20}$=7.968, P=0.011, Figure 1, Table S3). There was no species difference in the expression of explorative behaviour ($F_{1,20}$=1.928, P=0.180, Figure 1) and no interaction between species and test person ($F_{1,20}$=0.056 P=0.815). Upon testing whether wolves and dogs explored less in the presence of the stranger we found this to be the case in wolves (paired t-test: t_{10}=2.888, P=0.018, Figure 1, Table S4) but not in dogs (t_{12}=1.622, P=0.133).

The expression of passive behaviour was low in both wolves and dogs and not affected differently by the presence of the familiar person and the stranger in either species ($F_{1,20}$=0.053, P=0.820, Table S3). There was no interaction effect ($F_{1,20}$=0.112, P=0.741), but overall dogs were significantly more passive than wolves ($F_{1,20}$=14.393, P=0.001), both in the presence of the familiar person (t=2.862, P=0.014, Table S4) and in the presence of the stranger (t=3.151, P=0.008).

The expression of social play was not affected differently by the presence of the familiar person and the stranger ($F_{1,20}$=0.371, P=0.549, Table S3). There was no interaction effect ($F_{1,20}$=0.461, P=0.505), but overall dogs were significantly more playful than wolves ($F_{1,20}$=12.543, P=0.002), and played more than wolves with both the familiar person (t=4.650, P=0.001, Table S4) and the stranger (t=3.149, P=0.009).

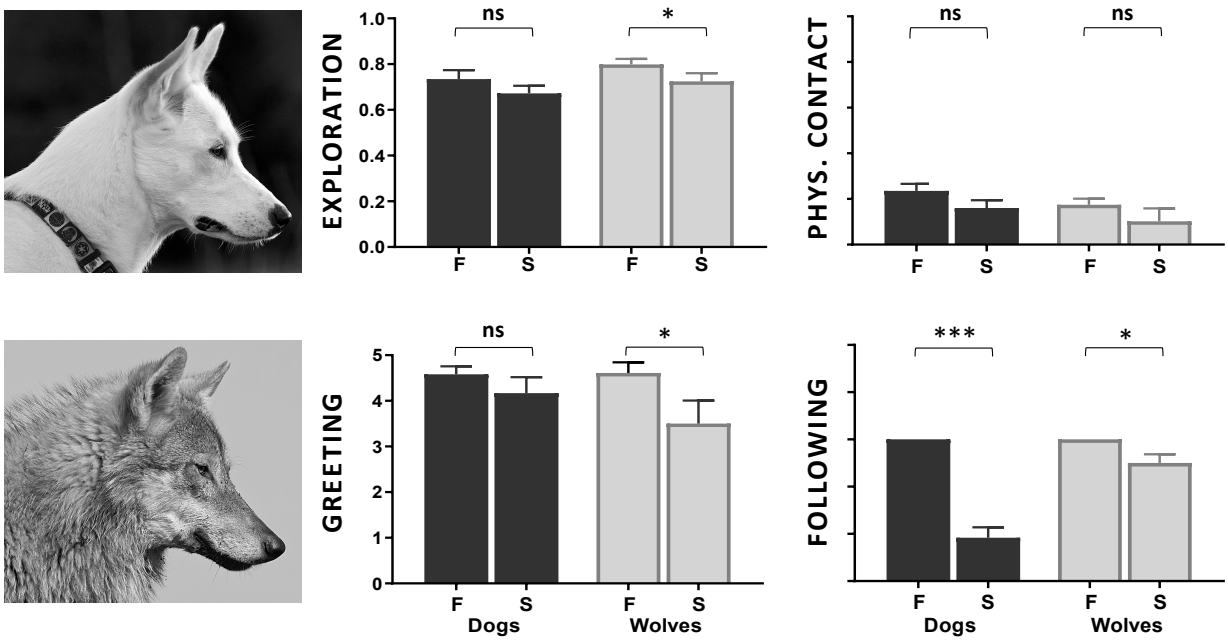

Figure 1. Attachment behaviours, discrimination between familiar person and stranger. Mean proportion (+SE) of time dogs and wolves spent on exploration and physical (phys.) contact in the presence of a familiar person (F) and a stranger (S), and mean score (+SE) for dogs and wolves for greeting and following a familiar person and a stranger. Significance levels are given for discrimination between familiar person and stranger in dogs and wolves (not significant = ns, <0.05 = *, <0.01 = ** and <0.001 = ***, Table S4). Note that species differences not relating to the ability to discriminate between familiar person and stranger are not indicated in this figure.

Proximity and contact seeking

To evaluate if dogs and wolves expressed more proximity and contact seeking with a familiar person, we investigated if the behaviours following, standing by the door, physical contact and greeting to a higher degree were expressed toward the familiar person compared to the stranger.

The occurrence of physical contact was not affected differently by the presence of the familiar person and the stranger ($F_{1,20}$=3.183, P=0.090, Figure 1, Table S3). There was no species difference in time spent in physical contact with the familiar person and the stranger ($F_{1,20}$=3.109, P=0.093) and no interaction effect ($F_{1,20}$=0.001, P=0.970).

Greeting behaviour was scored when the familiar person or the stranger entered the room, which occurred during episode 2, 4, 6 and 7. Greeting was higher for the familiar person than for the stranger ($F_{1,19}$=10.225, P=0.005, Table 3, Figure 1, Table S3). There was no difference between dogs and wolves in their expressions of greeting behaviour ($F_{1,19}$=0.637, P=0.435) and no interaction effect ($F_{1,19}$=2.113, P=0.162). Upon testing whether wolves and dogs greeted the stranger less than the familiar person we found this to be the case in wolves (paired t-test: t_{10}=2.403, P=0.043, Figure 1, Table S4) but not in dogs (t_{12}=1.820, P=0.096).

The familiar person was followed at a higher degree when leaving the room compared with when than the stranger left the room ($F_{1,19}$=73.134, P<0.001, Figure 1, Table S3). We found a species difference in the proportion of following ($F_{1,19}$=27.473, P<0.001), where wolves followed the stranger more than dogs (t_{19}= -5.241, P<0.001, Table S3). While we found a significant interaction between test person and species ($F_{1,19}$=27.473, P<0.001), there was no species difference in following the familiar person as all wolves and dogs followed the

familiar person when she left the room. We found a significant interaction between test person and species ($F_{1,19}$=27.473, P<0.001). Upon testing whether wolves and dogs followed the stranger less than the familiar person we found this to be the case in both wolves (paired t-test: t_{10}=2.683, P=0.028, Figure 1, Table S4) and dogs (t_{12}=9.449, P<0.001).

Overall, the animals stood less by the door when a familiar person was in the room compared to when a stranger was in the room ($F_{1,20}$=18.346, P<0.001, Table S3). Wolves stood more by the door compared to dogs ($F_{1,20}$=5.391, P=0.031). There was a significant difference between wolves and dogs in standing by the door when a familiar person was present with wolves standing more by the door (t=3.301, P=0.006, Table S4). However both species stood more by the door in the presence of the stranger (paired t-test: t_{10}=-2.626, P=0.028, Table S4) and dogs (t_{12}=-3.534, P=0.005) and there was no species difference in the presence of the stranger (t=1.201, P=0.244, Table S3) and no interaction effect ($F_{1,20}$=1.050, P=0.318).

Activity when alone

In episode 5 the animal was alone in the room. The behaviours expressed during this time were exploring the room and standing by the door. There was no significant difference between wolves and dogs in explorative behaviour (mean proportion of time exploring +SE: Dogs: 0.44 +0.06, N=12, wolves: 0.58 +0.06, N=10, t-test: t= -1.624, P=0.120, Table S2, S4) or in standing by the door (mean proportion of time standing by the door +SE: Dogs: 0.48 +0.07, wolves: 0.42 +0.06, t-test: t= 0.687 P=0.500, Table S2, S4) during episode 5.

Discussion

It has been suggested that the ability to show attachment behaviour toward a human caregiver evolved post-domestication in dogs (Topál et al., 2005). Subsequently, it is expected that in spite of intense socialization, dogs but not wolves, can develop attachment to humans. However, while it has been shown that 16-weeks-old wolves do not discriminate in their attachment behaviour toward a human caregiver and a stranger when compared to similar aged dogs (Topál et al., 2005), wolves aged eight weeks do (Hall et al., 2015). This highlights the potential for wolves to form attachment with humans, but simultaneously raises the question if the attachment in wolves weakens over time compared to dogs. Here we demonstrate that both wolves and dogs show attachment toward a human caregiver as late as 23 weeks of age when subjected to the SST. Surprisingly, we found that wolves, but not dogs, expressed secure base effects by exploring the room more in the presence of the caregiver, and proximity and contact seeking by greeting the caregiver more than the stranger. Our results provide the first evidence that wolves show attachment toward humans comparable, if not stronger, to that of dogs at later developmental stages and further suggest that the ability to form attachment to humans did not evolve post-domestication.

The attachment behaviour expressed in our wolves toward a familiar person are comparable to attachment behaviours reported in adult dogs (Topál et al., 1998; Gácsi et al., 2001; Prato-Previde et al., 2003), chimpanzees (van IJzendoorn et al., 2009) and human infants (Ainsworth & Bell, 1970) when subjected to the SST or SSP. Importantly, our results suggest that the attachment system towards the familiar person was activated in dogs, but more so in wolves, during the test. In contact seeking, reunion behaviours (e.g. greeting between attached individuals) are arguably the most relevant type of behaviour for quantifying attachment (Ainsworth & Bell, 1970; Rehn et al., 2013). Greeting of a familiar person after separation

reliably measures comfort seeking (Rehn et al., 2013) and while our wolves and dogs showed same levels of greeting intensity only wolves discriminated between the familiar person and the stranger, by greeting the the familiar person significantly more. Furthermore, similar to dogs expressing attachment behaviour towards a primary caregiver (Topál et al., 1998; Gácsi et al., 2001; Prato-Previde et al., 2003), both wolves and dogs followed the familiar person, but not the stranger, every time she left the room. Additionally, both wolves and dogs stood less by the door when a familiar person was in the room compared to when a stranger was in the room, thereby suggesting that both species attempted to maintain proximity when involuntarily separated from the familiar person (Ainsworth & Bell, 1970; Topál et al., 1998).

The discrimination between a familiar person and a stranger in wolves in our study suggest that wolves show attachment to their human caregiver. This contrasts with the results of Topál et al. (2005) in which dogs, but not wolves, were found to show attachment toward human caregivers at 16 weeks of age in the SST. Rather, our results are in agreement with Hall et al. (2015) who found that wolf puppies at the age of eight weeks express attachment toward human caregivers. While increased independence with age in wolves could explain the discrepancy in expressed attachment behaviour between younger and older wolves (Mongillo et al., 2013; Hall et al., 2015), our results directly contradict this explanation as we show that wolves as old as 23 weeks show attachment toward a human caregiver. Importantly, the wolves in the study by Topál et al. (2005) did not receive the same amount of socialization prior to the test as the dogs. The wolves were returned to a wolf park up to two months prior to the test and only received visits from their human caretakers once or twice a week, whereas the dogs remained with their caretakers until testing (Kubinyi et al., 2007; Virányi et al., 2008). Because environmental factors significantly affect behavioural development (Zimen, 1987) and experimental outcomes in studies comparing wolves and dogs (Hare, 2002; Udell

& Wynne, 2008), this environmental bias is substantial. The lack of intense contact with an attachment figure for a prolonged period of time, together with the drastic change of environment, could therefore be a likely explanation for the lack of attachment toward a human caregiver in the 16-weeks-old wolves compared to dogs. Indeed, our results indicate that continuous socialization can elicit attachment behaviour in wolves and thereby suggest that the capacity to form attachment with humans is not exclusive to dogs.

The expression of passive behaviour and social play was limited in both wolves and dogs. Though dogs expressed significantly higher degrees of both behaviours compared to wolves, the behavioural expression was not dependent on the presence of the familiar person or the stranger in either species. The limited occurrence of passive behaviour and social play during the test might have impaired our ability to adequately detect a difference in these behavioural expressions in the presence of a familiar person compared to a stranger (Rehn et al., 2013). Furthermore, wolves engaging in play with a human are likely rare (Hansen Wheat & Temrin, 2020) and wolf hybrids show significantly decreased expression of human-directed play behaviour when compared with dogs (Hansen Wheat et al., 2018). It is therefore likely that the wolves' limited engagement in social play during the SST is based in a general lack of interest in human-directed play, and does not necessarily represent an expression of low attachment (i.e. secure base effect) to the familiar person. On the contrary, the significant increase in exploratory behaviour in wolves, but not dogs, in the presence of the familiar person compared to the stranger confirm that the wolves indeed used the familiar person as a secure base, and thus an attachment figure (Ainsworth, 1989), during the test.

While wolves did follow the stranger when she left the room to a larger degree than dogs, we do not find it likely that this behavioural difference is founded in a weaker attachment to the

familiar person in wolves compared to dogs. Unlike dogs, wolves do not seem to generalize familiarity with human hand-raisers to strangers (Zimen, 1987; Lord, 2013) and even though both wolves and dogs had met strangers before (though not the person acting as the stranger in the test), this was limited to greeting through the fence. Thus, the situation created during the SST was unusual for both wolves and dogs. We therefore have no reason to believe that the wolves viewed the stranger as a potential attachment figure and further note that like dogs, wolves did follow the familiar person significantly more than the stranger. This suggests that wolves discriminated between the two persons in the room during the test. Additionally, wolves stood more by the door than dogs in the presence of either test person. However, wolves were visibly more motivated to exit the room compared to dogs during tests, and we therefore find it plausible that standing by the door from which they entered the room at the beginning of the test, as well as following the leaving stranger, was based in a motivation to exit the room. Therefore, as in the case with following behaviour, we find that standing by the door while the familiar person was in the room is unlikely to reflect weak attachment to the familiar person. This is also supported by wolves, as well as dogs, stood more by the door after the familiar person had left the room and the animals were alone with the stranger.

In sum, wolves discriminated between a human caregiver and a stranger in in our study, which suggests attachment expressed toward a human caregiver. Thereby, our findings do not support the hypothesis that the ability to form attachment with humans was developed post-domestication in dogs (Topál et al., 2005). Instead, we show that continuously socialized wolves express attachment to human caregivers as late as 23 weeks of age, which indicates that attachment behaviour does not weaken with age in wolves.

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