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**Assessment of attachment behaviour to human caregivers in wolf pups (*Canis lupus lupus*).**

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**Abstract**

Previous research suggested that 16-week old dog pups, but not wolf pups, show attachment behaviour to a human caregiver. Attachment to a caregiver in dog pups has been demonstrated by differential responding to a caregiver compared to a stranger in the Ainsworth Strange Situation Test. We show here that 3-7 week old wolf pups also show attachment-like behaviour to a human caregiver as measured by preferential proximity seeking, preferential contact, and preferential greeting to a human caregiver over a human stranger in a modified and counterbalanced version of the Ainsworth Strange Situation Test. In addition, our results show that preferential responding to a caregiver over a stranger is only apparent following brief isolation. In initial episodes, wolf pups show no differentiation between the caregiver and the stranger; however, following a 2-min separation, the pups show proximity seeking, more contact, and more greeting to the caregiver than the stranger. These results suggest intensive human socialization of a wolf can lead to attachment-like responding to a human caregiver during the first two months of a wolf pup's life.

**Keywords:** Attachment, wolves, *Canis lupus*, Strange Situation Test, domestication

## 1. Introduction

Attachment behaviour refers to any “affectional tie” that one individual, be it human or non-human animal, displays towards another specific individual (Ainsworth and Bell, 1970). According to Ainsworth and Bell (1970, p. 50) “The behavioural hallmark of attachment is seeking to gain and to maintain a certain degree of proximity to the object of attachment, which ranges from close physical contact under some circumstances to inter-action or communication across some distance under other circumstances.” To help explain the origins and function of attachment behaviour, Bowlby and Ainsworth formulated a framework for attachment that posited the attachment to a caregiver is critical for the survival of infants of many species since caregiver proximity can function as protection against predators (Bowlby, 1958; Bowlby, 1982; for a review see Bretherton, 1992 or Kraemer, 1997). This perspective incorporated the findings from the primate literature that highlighted the importance of mother care for the healthy development of rhesus monkeys and the readiness with which infant monkeys will form attachments even to inanimate mother surrogates (Harlow, 1971; Kraemer, 1997).

More recent attachment research has extended the attachment framework to the dog human-caregiver relationship. Topál et al. (1998) were the first to adapt the Ainsworth’s Strange Situation Test (SST; Ainsworth & Bell, 1970) to assess whether adult pet dogs show attachment to their human owners. In the SST, the subject is brought into a novel room. Then, in a series of brief episodes, the presence of the

caregiver and a stranger is systematically manipulated. A brief isolation episode also occurs approximately halfway through the test, which typically leads to mild distress. Observers then score the subject's response to the presence and absence of the stranger and caregiver to assess attachment-related behaviours towards the human caregiver. Topál et al. (1998) recorded the amount of physical contact between the dog and owner and dog and stranger in addition to how often the dog engaged in play, exploration, passive behaviour, or waiting at the door in the owner's or stranger's absence. They found that the dog-owner relationship could be appropriately described in terms of attachment between the dog and owner, as some dogs showed the secure-base effect in which exploration increased in the presence of the owner compared to the stranger. In addition, dogs were shown to span a variety of attachment styles along the secure-insecure dimension, which is similar to human child attachment classifications (Topál et al., 1998).

Topál et al. (2005) explored the possible effects of domestication on dogs' formation of attachment to human caregivers by comparing the attachment behaviour of 16-week old hand-reared wolves, hand-reared dogs, and conventionally reared dogs (i.e. mother nursed in human homes) during an SST. Dogs that were raised in human homes (conventionally reared or hand-reared) showed greater responding to a human caregiver than a stranger, whereas hand-reared wolf pups showed equal responding to the caregiver and stranger. The authors of this study suggested that, through domestication, dogs might have evolved "a capacity for attachment to humans that is functionally analogous to that

present in human infants” (Topál et al., 2005, pp. 1373), whereas wolf pups did not appear to form this same attachment to their human caregiver.

However, recent research has brought to light the importance of socialization procedures, and experimental methodology in behavioural comparisons between dogs and wolves. For example, adult wolves, once thought to be incapable of following human’s points, are now known to be as responsive to human gestures and attentional state as pet dogs given equivalent rearing and testing conditions (Gacsi et al., 2009; Udell et al., 2008). Thus the hypothesis that dogs display a unique attachment mechanism to form attachments to humans, distinct from that displayed by other mammals (e.g. Cairns, 1966; Harlow, 1971; Kraemer, 1997) warrants further investigation.

Human infants start to use their mother as a secure base when exploring the environment at the age of eight months; however, from the second year on, their attachment behaviour becomes more flexible and they will be less dependent on the presence of their mother when interacting with others (Bowlby, 1969). Perhaps wolves may be more likely to show a caregiver preference in a novel situation at a younger age than the 16 weeks tested by Topal et al (2005). It’s unclear whether a wolf’s attachment to a human changes with age, but if wolves do form attachments to a human caregiver, it may be most apparent at a younger age when the wolves may require the presence of a caregiver to be comfortable and explore a novel situation. Thus attachment in wolves may be most apparent when wolves are first

starting to emerge from the den around three weeks of age (Packard, Mech, & Ream, 1992).

In addition, it is also important to note that at the time of testing, the wolves tested by Topál et al. (2005) were no longer living with their human caretaker, but had been relocated to a private wolf farm between 2-4 months of age (see Virányi et al., 2008). As a result, at the time of testing, interactions with their caretaker had been reduced to half a day twice per week (Virányi et al., 2008). Reduced levels of caretaker-wolf contact may have altered the attachment relationship during this period, which may have contributed to the study's findings (Udell & Wynne, 2010).

The aim of the present study was to investigate whether human-raised wolf pups, still experiencing around the clock interactions with their primary caregiver, would show an attachment response to that caregiver on the SST. Recent research with dogs in the SST has introduced a counterbalanced version of the SST controlling for the order in which the owner and stranger entered and exited the room (episode order; Palmer & Custance, 2008). While Palmer and Custance (2008) confirmed that adult dogs show attachment behaviours towards their owners, it was also found that episode order could significantly influence a dog's response towards their owner. Rehn et al. (2013) further investigated order effects within the SST in dogs by implementing a control condition in which two equally unfamiliar individuals entered and exited the room as they would in the normal SST. Here, the only difference between the two individuals was the order in which they entered and exited the room. Rehn et al. found that dogs displayed attachment-like

behaviour to one of the unfamiliar people simply as a function of the order in which the unfamiliar persons entered and exited. However, exploration was more susceptible to this order effect than proximity-seeking behaviours such as initiating contact.

In the present study, we therefore use a counterbalanced version of the SST to test 10 human-reared wolf pups' attachment-like behaviour to a human caregiver. Pups were tested three times, once each at 3, 5 and 7 weeks of age, throughout which time the pups were receiving near 24-hour care from a human caregiver.

## **2. Methods**

### *2.1 Subjects*

Ten wolf pups (*Canis lupus*) from two litters (one litter of four and one litter of six) participated in the present experiments. They were removed from the den when they were approximately 10 days of age and hand-reared according to the procedures outlined in Klinghammer and Goodman (1987) by two human-caregivers at Wolf Park in Battle Ground, IN (see Table 1 for subject information). The hand-rearing procedure involved the presence of a human caregiver in an indoor room for 24 hours a day with the pups for the first 1.5- 2 months of life, at which point the caregivers were present for approximately 16 hours a day. Caregivers were also responsible for bottle-feeding the pups every 4-6 hours until



the pups were able to eat solid foods. Testing procedures were approved by the University of Florida Institutional Animal Care and Use Committee.

## *2.2 General procedure*

Wolf pups were given a modified version of the Ainsworth Strange Situation Test (detailed below) during their 3rd, 5th and 7th week of life (see Table 1 for exact ages). At each age, a novel testing room and a novel stranger were used. The caregiver remained the same across ages.

In total, nine subjects were tested during week 3, nine during week 5, and ten during week 7. One subject was ill during week 3 and 5 and was only tested at 7 weeks of age. One additional subject's last two episodes from week 3 were excluded due to an experimenter error in which the episode order was inverted for the last two sessions.

Each novel testing room was an indoor space (approximately 18 m<sup>2</sup>) to which the pups had never previously been exposed. In each testing room, two 2m-diameter non-overlapping circles were marked on the floor with tape. The marked circles were used to code proximity to the caregiver or stranger by having the stranger and caregiver sit in the centre of each circle. Approximately six toys were distributed between the two circles. Toys were not included for the testing at 5 and 7 weeks of age for litter two due to experimenter error.

### *2.3 Modified Strange Situation Test*

The pup was brought to a novel room where the presence and absence of a caregiver and stranger were manipulated over six episodes each lasting 2 minutes. Each time the stranger and/or caregiver entered the room; they opened and closed the door, slowly walked to the centre of one of the circles and sat down on the floor. The circle the caregiver or stranger sat in was determined randomly prior to the start of the test. During the episode, the pups were free to move about the room without restriction. If the pup approached the caregiver or stranger in the circle and initiated contact, the caregiver or stranger would pet the pup. If the pup initiated play by bringing a toy to either the caregiver or stranger, the caregiver or stranger could engage in play. The stranger and caregiver, however, were instructed not to move outside their circle during an episode. To exit at the end of an episode, the caregiver or stranger stood up, turned to the door, and slowly walked towards it. Upon reaching the door, the caregiver or stranger said "goodbye" and exited.

To control for potential order effects, two counterbalanced sequences of the entering and exiting of the stranger and caregiver were utilized. Table 2 outlines these two episode orders and indicates whether the stranger alone, caregiver alone, stranger and caregiver, or neither was in the room with the pup. For Episode Order 1, the caregiver sat alone in the room with the pup for the first episode. After 2 mins, a stranger entered the room and sat in the adjacent circle to the caregiver for the second episode. Next, the caregiver left the room, leaving the stranger and pup alone in the room for Episode 3. For Episode 4, the strange left the room, leaving the pup

alone. Episode 5 the stranger returned to the room with the pup. In the final episode, the caregiver entered so the caregiver and stranger were present with the pup. Episode Order 2 followed a similar pattern except that it counterbalanced Episode Order 1 (see table 2). The episode order assigned for each pup was pseudo-randomly determined so that at each age, half of the pups were tested with each order. In addition, the order each pup was tested with was changed across the three testing weeks so that each pup was tested once with one episode order, and twice with the other episode order. In both orders, pups were exposed to their caregiver, a stranger and both the caregiver and stranger together prior to an episode of isolation, and then reintroduced to the caregiver and stranger separately after the brief isolation episode.

#### *2.4 Behaviour coding*

During each episode, the pups' behaviour was recorded on video for subsequent analysis. The behaviours scored, the behavioural definition and observer agreement scores are listed in Table 3. Briefly, during each episode we scored the amount of time the pup spent in proximity (within the 2m circle) and within physical contact of the caregiver and stranger. These two behaviours were not mutually exclusive: a pup could be in contact while also in proximity. Both proximity and contact behaviours were scored to assess the approach and investigative behaviour (proximity) as well as close contact seeking (contact). Both are important, as pups could prefer to approach and investigate one individual (high proximity), but prefer not to be touched (low contact). This would indicate a fearful

curiosity, whereas a high proximity high contact would be more indicative of comfort seeking. We also scored whether the pups greeted and followed the caregiver and stranger when entering or exiting the room, with a zero indicating no greeting or following, a one indicating a calm greeting or following, and a two indicating an excited greeting or follow (see Table 3). Scored episode times were approximately 2 min; however, due to minor variations in time taken for the human to enter, sit down or exit across episodes, all behaviours are reported as a proportion of the episode time, except for greeting and following which were rated categorically. A second observer scored 37% of the videos. Percent agreement was calculated for the continuous behaviours and Cohen's Kappa was calculated for the categorical scale by comparing the two raters' scores on an episode-by-episode basis. For the continuous behaviours, an agreement was defined as both observers scoring within two seconds (or 5% of the scored time) of each other. Any larger discrepancy was scored as a percent agreement by dividing the smaller scored time by the larger.

### *2.5 Statistical analyses*

Data were analysed using the statistical package R (R Core Team, 2012) and plotted with the R package *ggplot2* (Wickham, 2009). Linear mixed effects models using the *lme4* package (Bates, Maechler, Bolker & Walker, 2013) were used to assess the effects of the presence and absence of the caregiver and stranger on the subjects' behaviour. P-values for ANOVA tests and *t*-tests were generated from the *LmerTest* package (Kuznetsova, Brockhoff & Christensen, 2013) using a

Satterthwaite approximation for the degrees of freedom. Each model included random intercepts for the subject and litter variables. The subject term was nested in the litter term.

### 3. Results

Linear mixed effects models were run for each scored behaviour to investigate the effect of the episode and condition on that behaviour. To test whether pups engaged in differential levels of a scored behaviour towards the caregiver compared to the stranger, linear models included a dummy coded variable to indicate whether the behaviour occurred towards/ in the presence of the caregiver or the stranger.

#### *3.1 Proximity seeking to the caregiver and stranger*

Figure 1 shows the overall patterns of proximity to the caregiver and stranger across episodes separated by age and episode order, and an overall averaged summary across weeks. Overall, similar patterns of responding were observed at each age of testing (3, 5, 7 weeks); however, differences appeared in proximity to the stranger and caretaker across the episodes.

*3.1.1. Episodes 2 & 6:* During Episode 2 and Episode 6, both the caregiver and stranger were present in the room. A linear mixed effect model was used to test whether pups' proximity to a person was predicted by the familiarity of that person (caregiver vs. stranger), the pups' age (3, 5 or 7 weeks), the episode order (Order 1 or Order 2), a 2-way interaction between the episode (2 vs. 6) and the person

(caregiver vs. stranger), and a 2-way interaction between the episode and age. There was no interaction between age and episode ( $F_{(1,98.95)} = 0.05$ ,  $p = 0.82$ ), but there was a significant interaction between the episode and proximity to the caregiver and the stranger ( $F_{(1,98.95)} = 12.60$ ,  $p < 0.001$ ), indicating that preference for the caregiver and stranger changed from Episode 2 to Episode 6.

We therefore explored the pups' preference between the caregiver and stranger during Episode 2 and Episode 6. For Episode 2, there was no indication that pups had different preferences for approaching the caregiver over the stranger depending on the episode order (Person by episode order Interaction:  $F_{(1, 41.22)} = 0.11$ ,  $p = 0.74$ ). There was also no effect of age ( $F_{(1, 44.02)} = 0.40$ ,  $p = 0.53$ ), episode order ( $F_{(1, 47.75)} = 0.70$ ,  $p = 0.41$ ), or difference between the caregiver and stranger ( $F_{(1, 41.22)} = 2.61$ ,  $p = 0.11$ ). This contrasts the findings in Episode 6, which showed a significant interaction between the episode order and the person the pup approached ( $F_{(1, 46.98)} = 7.77$ ,  $p < 0.01$ ), indicating that pups' preference for the caregiver depended on the episode order. When looking at each episode order separately, there was a significant preference for proximity to the caregiver over the stranger ( $F_{(1, 24.99)} = 16.73$ ,  $p < 0.0001$ ) in Episode Order 1. For Episode Order 2 where the caregiver was already present in Episode 5, there was no significant difference in time spent with the caregiver and stranger in Episode 6 ( $F_{(1, 20.99)} = 0.05$ ,  $p = 0.82$ ). In both episode orders, there was again, no effect of age (Order 1:  $F_{(1, 24.99)} = 0.05$ ,  $p = 0.83$ ; Order 2:  $F_{(1, 20.99)} = 0.60$ ,  $p = 0.45$ ).

3.1.2. Episodes 1, 3 & 5: Given that there was no indication of an age effect across Episodes 2 and 6, the data were averaged across age to provide a complete within-subject data set. There was a significant effect of episode ( $F_{(2, 50.98)} = 8.00, p < 0.001$ ) showing that pups spent more time in proximity to both the caregiver and stranger in Episode 5 than Episode 1 ( $t_{50.98} = 3.99, p < 0.001$ ). When proximity was averaged across Episodes 1, 3 and 5, pups overall spent more time in proximity to the stranger ( $t_{50.99} = 2.07, p = 0.043$ ). When considering Episode 5 alone (the first reunion following isolation), however, there was no difference in proximity between the stranger and caregiver ( $t_{6.83} = 0.989, p = 0.36$ ).

### 3.2 *Contact between the caregiver and stranger*

Figure 2 shows the proportion of each episode the pup made physical contact (i.e. petting) with the caregiver and stranger across episodes for both episode orders across all three weeks of testing. The overall patterns are similar to proximity seeking, with contact changing as a function of the episode. Again, there appears to be little effect of testing across ages.

3.2.1 Episodes 2 & 6: Overall, contact seeking showed an identical pattern to proximity seeking. During Episode 2, pups showed no preference between the caregiver and stranger ( $F_{(1,48.98)} = 3.07, p = 0.09$ ). During Episode 6, however, preference between the caregiver and stranger depended on the episode order (Episode by person interaction:  $F_{(1,46.98)} = 6.93, p = 0.01$ ). Pups in episode Order 1 significantly preferred the caregiver ( $F_{(1,24.98)} = 20.79, p < 0.001$ ), whereas there was no difference in time with the caregiver and stranger for Order 2 when pups were

already re-united with the caregiver in Episode 5 ( $F_{(1,21)} = 0.05$ ,  $p = 0.83$ ). Similar to proximity, there was no effect of age ( $F_{(1, 98.95)} = 0.11$ ,  $p = 0.74$ ), or interaction between age and episode ( $F_{(1, 98.95)} = 0.05$ ,  $p = 0.82$ ).

3.2.2. Episodes 1, 3 & 5: Pups overall showed the same pattern of contact with the stranger and caregiver as they did for proximity. Pups overall showed significantly more contact in Episode 5 than Episode 1 ( $t_{50.97} = 4.44$ ,  $p < 0.0001$ ) and more contact with the stranger when averaged across episodes 1, 3, and 5 than with the caregiver ( $t_{50.98} = 2.05$ ,  $p = 0.046$ ). When looking at Episode 5 alone (the first reunion following isolation), however, there was no significant difference in time spent in contact with the caregiver or stranger ( $t_{8.90} = 0.30$ ,  $p = 0.77$ ).

### 3.3. *Greeting and following the caregiver and stranger*

Greetings were scored when the caregiver or stranger entered the room, which occurred during Episodes 2, 5 and 6. Following was scored when the caregiver or stranger exited a room, which occurred during Episodes 3 and 4. A mean greeting and following score was computed for each pup across all ages. Figure 3 shows the mean score for following and greeting both the stranger and caregiver. Overall, pups were not more likely to follow the caregiver over the stranger ( $F_{(1, 9.00)} = 1.18$ ,  $p = 0.31$ ). The pups, however, did show more excited greetings to the caregiver than they did to the stranger ( $F_{(1, 9.00)} = 6.40$ ,  $p = 0.03$ ) upon their return.



#### 4. Discussion

Overall, the pups showed differential responding to the caregiver when compared to the stranger. Pups were more likely to greet the caregiver with whines and ears back upon reunion than they did the stranger. In addition, pups showed an effect of reunion in Episode 6, seeking greater proximity and physical contact with the caregiver than the stranger. However, this effect was only evident when pups were tested with episode Order 1. This is likely due to the fact that the caregiver was already present during the post-isolation Episode 5 in Order 2. This provided time for the pups to engage in reunion behaviour during Episode 5, followed by increased exploration of other environmental features, including the unfamiliar human, in Episode 6. In fact, this is exactly what is predicted when a secure attachment is present, known as the secure base effect (Ainsworth & Bell, 1970). However in Order 1, Episode 6 was the first reunion with the caregiver, which led to a highly significant bias for the caregiver- an outcome also predicted in previous attachment literature (Ainsworth & Bell, 1970).

We did not observe many differences in preference between the caregiver and the stranger prior to isolation (Episode 4). Instead, pups were indifferent in Episode 2 when given a choice between these two people, and in fact showed a slight stranger preference for contact across Episodes 1, 3 and 5. However, after a brief 2-minute isolation phase, the pups showed a strong caregiver preference in Episode 6 if the caregiver returned, but the pups showed no preference for the stranger if the stranger returned in Episode 6. Interestingly, however, we did not

observe any differences in proximity and contact between the stranger and caregiver during Episode 5, the episode immediately after isolation. One possibility is that because this comparison was across episode orders (Order 1 vs. Order 2) and averaged across ages allowing order effects or minor age effects to potentially mask an effect. Another explanation is that isolation in Episode 4 was sufficiently stressful that it activated general proximity seeking to any available person, even if the only available person was not a caretaker. Importantly, however, if the caregiver was present in Episode 5, the pups showed indifference between the stranger and caregiver in Episode 6. If the stranger was present in Episode 5, the pups showed a dramatic caregiver preference in Episode 6 indicating that the effect of the presence of the caregiver or stranger in Episode 5 was not equivalent. Only if the caregiver was present in Episode 5, did the pups show the same pattern that was shown before isolation in Episode 2. This suggests that despite the pups showing similar time in contact and proximity with the caregiver and stranger in Episode 5, only contact and proximity with the caregiver in Episode 5 functioned to return the pup to baseline preferences- an indicator of a secure attachment/secure base.

The fact that caregiver preferences only occurred in Episode 6 exemplifies Ainsworth and Bell's (1970) hypothesis that attachment behaviour is heightened in situations perceived as threatening, which in the present case, was isolation. One difference; however, in our findings from those observed with human infants and dogs is that separation from the attachment figure alone was not sufficient to activate attachment behaviours (e.g. Ainsworth & Bell, 1970, Palmer & Custance, 2005; Topál et al., 1998; Topál et al., 2005). In the present study, complete isolation

(Episode 4) was required to activate attachment behaviours. However, this could have been a by-product of the rearing and socialization practices employed with the pups used in this study. The pups were regularly introduced to novel humans and environments (Klinghammer & Goodman, 1987), making it unsurprising that being in a room with a novel human was not, in itself, a strange or stressful situation.

Thus, this study provides evidence that the behaviour demonstrated by hand-raised wolf pups towards humans can be categorized as attachment (Ainsworth and Bell, 1970) in some cases, given early socialization to humans (Klinghammer and Goodman, 1987), with continued contact with the caregiver through the time of testing, and the implementation of methodological controls for known order effects (Palmer & Custance, 2008; Rehn et al., 2013). This of course does not mean that all wolf pups will necessarily show strong attachment behaviour towards humans (e.g., Topal et al., 2005), as early rearing history and differences in caretaker behaviour are known to influence both the initial formation of the attachment bond and the attachment style that develops between an individual and their caretaker (Ainsworth and Bell, 1970). The fact that the wolves tested by Topal et al. (2005) were older, less human dependent, and no longer living with their human caretaker at the time of testing could have altered their attachment behaviour.

At present, however, we cannot determine which if any of these factors contributed to the differences between the present study and Topal et al (2005). Age may be a significant factor (16 weeks vs. 3,5 & 7 weeks), however, differences in

the length of time spent with the pups on a daily basis, time spent overall during the subject's lifetime, or other unknown rearing differences could have contributed to the differences between the two studies. It is unknown what effect age may have on attachment to humans. In our limited age range of testing we saw no effect, however, we may have observed an effect had testing been carried out until 16 weeks of age. Future studies are necessary to determine the typical developmental stages of wolf attachment to humans and the rearing factors that may influence it. Early socialization and life experiences may likely influence attachment test performance for canids. In fact, many feral dogs actively avoid human contact in the absence of early socialization (Ortolani, Vernooij & Coppinger, 2009). Future studies on the development of attachment bonds in canids may carefully detail the ontogeny of attachment formation and the conditions that lead to its development and maintenance in later life allowing for further comparisons between wolves and dogs.

Overall, the results show that wolf pups will form attachments to their human caregivers. This is an important finding, as it suggests that capacity to form attachments to humans is not itself a product of domestication. This suggests that young non-domesticated canids can form attachments to humans. Our study also suggests that the conditions under which attachment behaviour is displayed, for example, that isolation is required to elicit attachment like responding, may differ between wolves and what is seen with dogs. Another area for future investigation is looking into the maintenance of attachment into adulthood. Although it's unclear whether the differences in results from Topal et al. (2005) and the present one is

due to the age of the subjects at testing, it's possible that domestication influenced how attachments are maintained throughout development and into adulthood. When tested, most adult dogs typically show attachments to their owners; it's not clear whether this would be the case for wolves or even all breeds of dogs. Thus, our results highlight that the ability to form attachments to humans likely proceeded domestication, but domestication may have changed the ease at which these attachments could be formed, the conditions under which they are shown, and how they are maintained as adults.

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Subject	Sex	Litter	Age in days (Week 3)	Age in days (Week 5)	Age in days (Week 7)
Kanti	M	2	23	37	50
Bicho	M	2	23	36	50
Mowgli	M	2	25	35	53
Pigeon	F	2	24	37	51
Bigboy	M	2	25	36	51
Fiona	F	2	24	35	50
Dharma	F	1	23 <sup>+</sup>	35	47
Devra <sup>1</sup>	F	1	NA	NA	47
Gordon	M	1	21	35	47
Tilly	F	1	22	35	47

Table 1. Subject information. Table gives sex and exact age at each testing week.

<sup>1</sup>Devra was unable to be tested at 3 and 5 weeks due to illness. <sup>+</sup>Last two episodes were excluded due to experimenter error.

Order	Episode 1	Episode 2	Episode 3	Episode 4	Episode 5	Episode 6
1	Caregiver	Caregiver + Stranger	Stranger	Isolation	Stranger Returns	Stranger + Caregiver
2	Stranger	Stranger + Caregiver	Caregiver	Isolation	Caregiver Returns	Caregiver+ Stranger

**Table 2.** Outline of the two episode orders. Each cell displays whether the caregiver, the stranger or both were present in the testing room with the pup.

Behaviour	Definition	Per cent Agreement	Cohen's Kappa
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### Behaviours During an Episode

Proximity seeking caregiver	Proportion of the episode in which the pup had at least 2 paws within the 2 m circle the caregiver/stranger was sitting in.	95.7%	
Proximity seeking stranger			

Contact caregiver	Proportion of the episode in which the pup engaged in physical contact with the caregiver/stranger (not mutually exclusive with proximity)	93.4%	
Contact stranger			

### Behaviour Between Episodes

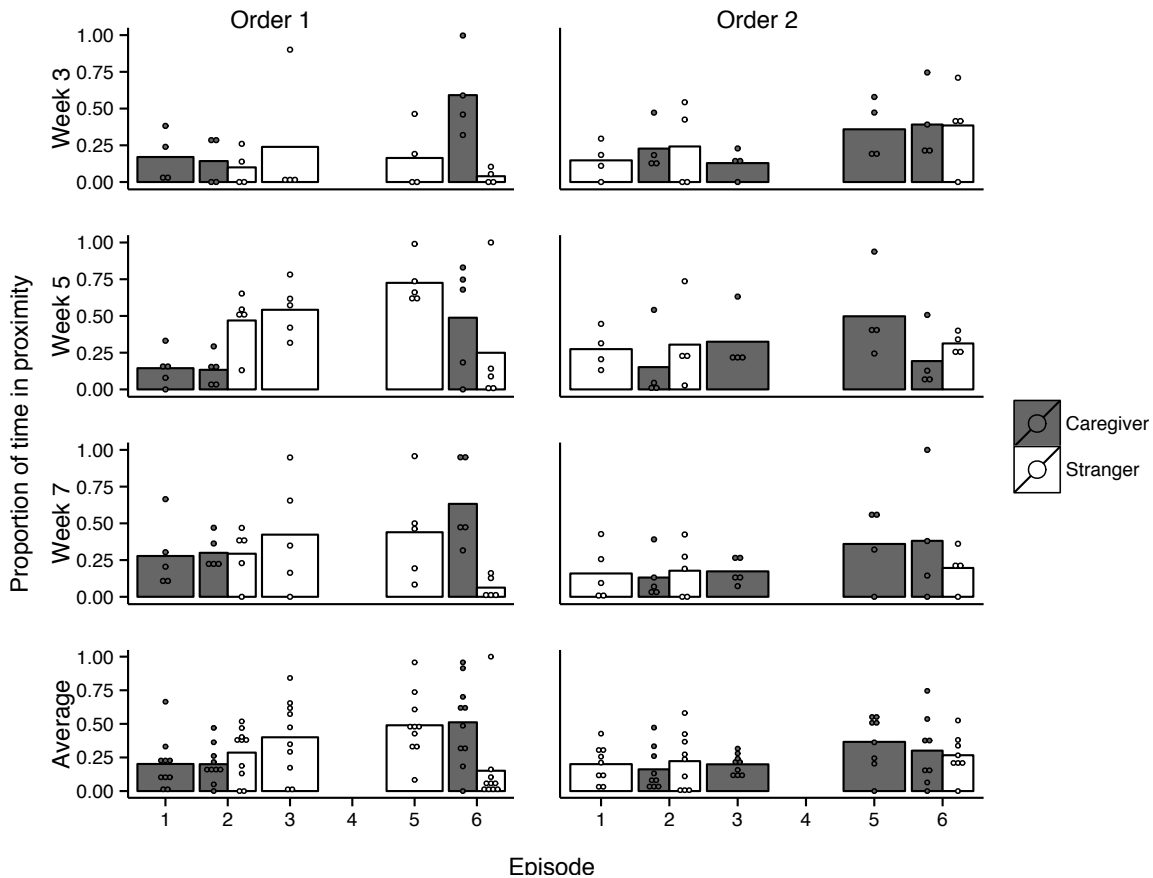
Greeting Caregiver	A score from 0-2 on the type of greeting that occurred within 15 s of the caregiver/stranger entering the room and sitting:		
Greeting Stranger	<p><b>0:</b> "No greeting- did not approach"</p> <p><b>1:</b> "Calm Greeting-approached but did not display ears back or whining"</p> <p><b>2:</b> "Excited greeting- approached with ears back and whining"</p>		0.71

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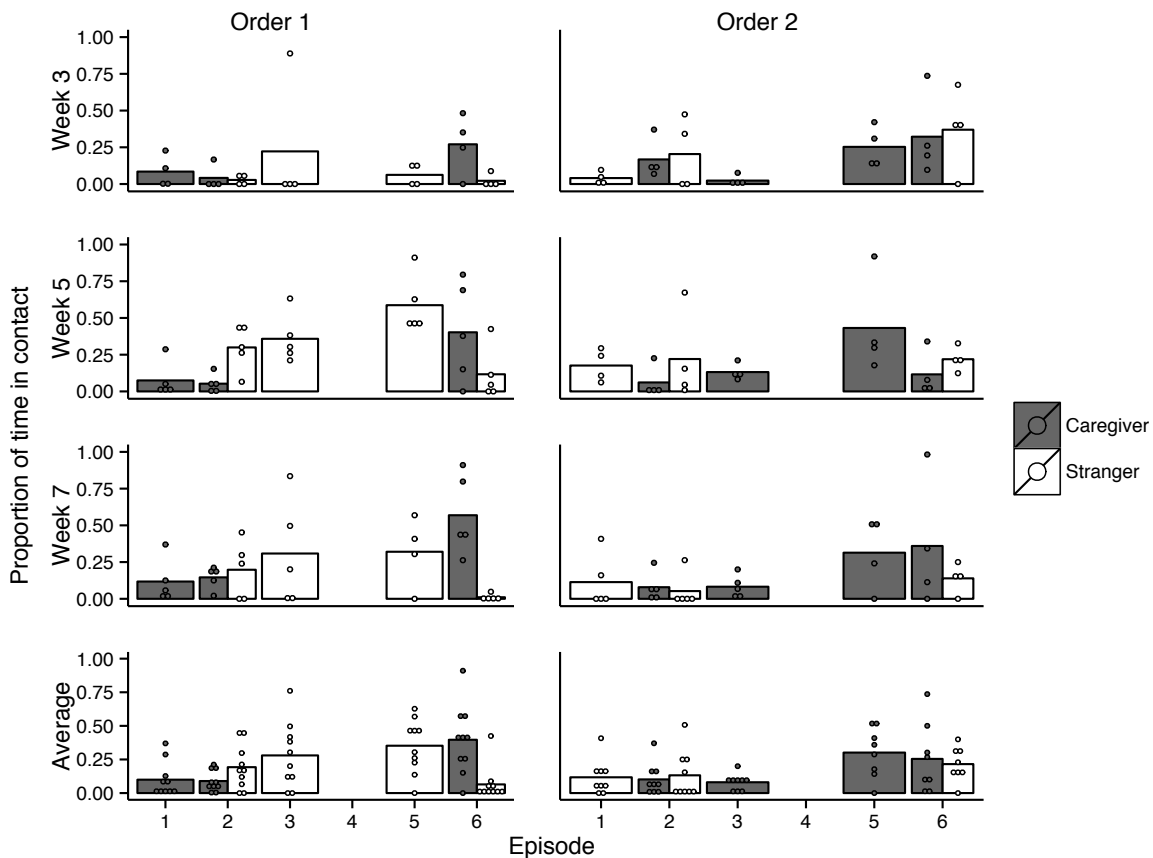
Following Caregiver	A score from 0-2 on the type of following that occurred within 15 s of the caregiver/stranger exiting the room.	0.63
	<b>0:</b> “No following”- did not approach leaving person or door	
	<b>1:</b> “Calm follow”-followed leaving person but did not try to follow through door, jump on door or	
Following Stranger	whine	
	<b>2:</b> “Excited follow”- followed person and tried to exit through door, jump at door or whined	

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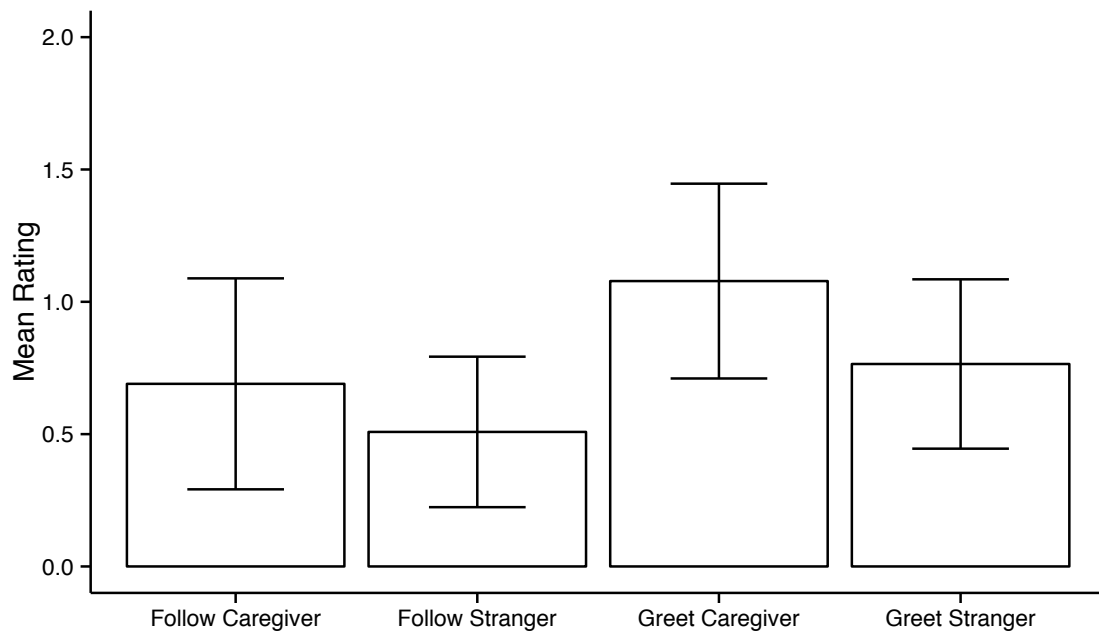
**Table 3.** Behavioural coding: definitions for each behaviour. For continuous variables, per cent agreement is reported from 36% of the videos double coded (10 of 28 videos). For categorical variables, Cohen’s Kappa is reported for the 36% of double coded videos.



**Figure 1.** Proportion of time in each episode spent in proximity to the caregiver and stranger in the Ainsworth SST procedure. The left column represents subjects experiencing Episode Order 1, the right column subjects experiencing episode order 2. Bars indicate the mean and dots show each data point. Each row shows the results for a different age and the final row shows the results averaged across all ages. Episodes 2 & 6 indicate proximity to both the caregiver and stranger as both were in the room (2 bars). Episodes 1, 3 and 5 indicate proximity to only the caregiver or stranger, as only one was present and the person present depended on the episode order. Episode 4 is blank, as neither the caregiver nor stranger was present (i.e. isolation). The Average row indicates each pups proportion in proximity averaged for the episode order in which they received twice.



**Figure 2.** Proportion of episode time spent in contact with the caregiver and stranger in the Ainsworth SST procedure. The left column represents subjects experiencing Episode Order 1, the right column subjects experiencing Episode Order 2. Bars indicate the mean and dots indicate each data point. Each row shows the results for a different age and the final row shows the results averaged across all ages. Episodes 2 & 6 indicate contact with both the caregiver and stranger as both were in the room (2 bars). Episodes 1, 3 and 5 indicate proximity to only the caregiver or stranger, as only one was present and the person present depended on the episode order. Episode 4 is blank, as neither the caregiver nor stranger was present (i.e. isolation). The Average row indicates each pups proportion in proximity averaged for the episode order in which they received twice.



**Figure 3.** Mean rating for following the caregiver and stranger as well as greeting the caregiver and stranger. See Table 3 for the scoring system. Bars show the mean score and error bars show the 95% confidence interval.