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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.

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, 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 et al., 1971; Kraemer, 1997).

More recent attachment research has extended the attachment framework to the dog human-caregiver relationship. Topal et al., 1998 were the first to adapt the Ainsworth's Strange Situation Test (SST; Ainsworth and 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. Topal 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 described in terms of attachment between the dog

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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 (Topal 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" (, 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 et al., 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 Topál 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 et al., 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 and 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 and 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 and 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-h care from a human caregiver.

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

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+	35	47
Devra[1]	F	1	NA	NA	47
Gordon	M	1	21	35	47
Tilly	F	1	22	35	47

[1] Devra was unable to be tested at 3 and 5 weeks due to illness.
+ Last two episodes were excluded due to experimenter error.

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 Goodmann (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 h a day with the pups for the first 1.5–2 months of life, at which point the caregivers were present for approximately 16 h a day. Caregivers were also responsible for bottle-feeding the pups every 4–6 h 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 weeks 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^2) to which the pups had never previously been exposed. In each testing room, two 2 m-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

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