



Does the attachment system towards owners change in aged dogs?



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HIGHLIGHTS

- Aged and adult dogs showed clear-cut patterns of attachment during the Strange Situation Test.
- A significant effect of age group and test condition on behaviour was detected.
- Behavioural differences between adult and aged dogs were seen in base and separation conditions.
- Strange Situation Test induced an increased salivary cortisol response in aged dogs.
- Later in life, dogs cope less efficiently with emotional social distress.

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ABSTRACT

Changes during senescence can significantly affect both the emotional and relational needs of old individuals and the characteristics of the attachment system. In order to determine whether the emotional response of dogs is affected by old age, we compared the behavioural parameters of adult (AD <7 years of age, $n = 25$) and aged (AG ≥ 7 years of age, $n = 25$) dogs in a distressing situation, which gives rise to attachment behaviour patterns (Strange Situation Test, SST). The physiological response of dogs was assessed by measurement of salivary cortisol variations in samples collected both at the dogs' homes and at the study location, before and after the SST. Both groups of dogs expressed clear-cut patterns of attachment to their owners. During the initial part of the procedure, AG dogs sought more physical contact, but behaved more passively and showed less interest in an unknown person during separation from their owners. Compared with AD dogs, AG ones showed a significant increase in salivary cortisol concentrations after the SST. The combination of physiological and behavioural data of the present study supports the hypothesis that, later in life, dogs cope less efficiently with emotional distress caused by mild social challenge.

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1. Introduction

Emotional bonds are a fundamental aspect of the relationship between humans and companion animals, and very many studies have described the role of pet dogs in the emotional life of their caregivers [1–5]. Recently, scientific interest has been directed to the emotional bond of dogs with their owners [6,7].

The first experimental study in this sense described the dog–owner relationship as an attachment bond, resembling that between human infants and their mothers [7]. Attachment defines an enduring relationship with a particular other, which becomes apparent through distinctive behaviour, expressed under stress situations [8]. Activation of this behaviour-controlling system results in one individual seeking and maintaining proximity to the object of attachment, displaying confident

behaviour in the presence of the attachment figure ('secure base' effect) and showing distress upon involuntary separation.

To verify whether the construct of attachment applied to the dog–owner relationship, Topál et al. [7] adopted a modified version of the Strange Situation Test (SST), originally designed to elicit attachment responses in human infants in conditions of distress [9]. The same methodological approach was later employed in numerous studies concerning dogs' attachment to humans [6,10–18]. Although several studies provided valuable information on the role of attachment bonds in puppies [12,17], young adult [10,11,18] and adult dogs [6,7,13,14], no attention has been given to the characteristics of attachment bonds in dogs' later life.

Physiological changes during senescence can significantly affect the emotional and relational needs of old individuals. This aspect has been widely studied in humans, in whom the quality of attachment bonds has been associated with increased well-being [19,20] and lower levels of depression in old people [21]. The value of emotional bonds may be even higher when senescence is associated with disease [22]. For instance, cognitive impairment in early-stage dementia can result in weakened feelings of security and more frequent activation of attachment

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behaviour [23] and, in advanced stages of dementia, the ability to self-initiate attachment behaviour may be reduced [24]. Like humans, older dogs may also experience a decline in functions such as attention, learning, memory, perception and awareness [25–29]. More broadly, physiological changes in ageing may lead to sensory decline and increased physical and psychological frailty, resulting in behavioural changes and, in turn, making older pets more sensitive to changes and less able to cope with stress, including social-related stress [30–32].

In addition to a psychological perspective, understanding of attachment requires study of the physiological processes associated with social experience [33–36]. In this respect, the hypothalamic–pituitary–adrenal axis (HPA) is extremely sensitive to emotional experience [37] and emotions may mediate the intensity of the HPA axis in response to stressful and challenging situations [38,39]. In dogs, an increased level of salivary cortisol (a hormone released as a consequence of HPA system activation during stress) is a documented indicator of acute stress [40,41], if collection and assay procedures are carried out properly [42,43]. Salivary cortisol has been proved to be sensitive to dogs' interactions with humans [44–47] and, being non-invasive, it is increasingly used to assess the well-being of dogs [48–51]. Although measurement of salivary cortisol has been used to examine physiological processes triggered during SST in humans [52,53], this approach has never been applied to dogs.

The aim of this study was to assess whether old age in dogs gives rise to different physiological responses to SST distress and/or changes in the expression of attachment behaviour.

2. Materials and methods

2.1. Subjects

A sample of 50 dog–owner dyads was involved in this study. Participants were recruited by word of mouth and advertisements among the clients of local veterinary clinics and among the students and the employees of the University of Padova. The owners were unaware of the specific purpose of the study.

Recruitment ended when two groups of dogs had been composed according to age: adult dogs (AD: under 7 years of age, mean \pm SEM = 4.4 \pm 0.4 years, n = 25) and aged dogs (AG: 7 years or older, mean \pm SEM = 9.6 \pm 0.4 years, n = 25). The AD group was composed of 13 male and 12 female dogs, and the AG group contained 9 male and 16 female dogs. There were 9 mongrels and 16 purebred dogs in the AD group and 11 mongrels and 14 purebred dogs in the AG group. Breeds included German Shepherd (n = 8), Labrador Retriever (n = 8), Cocker Spaniel (n = 3), English Setter (n = 3), Maremmano-Abruzzese (n = 2), Maltese (n = 1), Border Collie (n = 1), Alaskan Malamute (n = 1), Australian Shepherd (n = 1), West Highland White Terrier (n = 1) and Vizsla (n = 1). Irrespective of breed, AD and AG dogs were equally distributed across classes of height at the withers (height at withers \leq 45 cm: n = 12, height at withers $>$ 45 cm: n = 13 in each age group).

In order to exclude overt medical conditions which might affect their behaviour during the test, all dogs underwent a standard physical examination and behavioural assessment, including history and direct observation. In addition, to ensure the formation of a stable social relationship within dyads, only dogs older than 18 months which had been living with their present owners for at least 6 months were enrolled. A minority of the dogs had been adopted as adults (AD = 4 and AG = 8) and all of them had only one previous owner.

Information was obtained about aspects of the dog's lifestyle, with particular regard to dog–human interactions, which were considered as relevant for the behaviour expressed during the SST. Selected aspects included: frequency of the dogs' encounters with non-family individuals; number of people in the household; hours spent by dogs with their owners during the day (from owner's waking up until bedtime); daytime hours spent in shared activities (walking/running or playing

together, petting, other owner activities during which the dogs follow and stay with their owners); and individual activities (activities not shared by the dyad, even though they were possible). Within shared activities, detailed data about hours spent playing were collected, since this behaviour was stimulated during the test. Lastly, to assess the level of owners' attachment to their dogs, the Lexington Attachment to Pets Scale (LAPS) [54] was adopted.

2.2. Strange Situation Test: setting and procedure

To elicit attachment responses, we used a modified version of the Strange Situation Test [7]. The test was conducted in a 5 \times 5 m unfurnished room, which only contained two chairs and some pet toys (a rope, a tennis ball, and a ball of the 'Kong' type). Throughout the study, the same experimenter, a 30-year-old woman, took part in the test as the unknown person (stranger). The test procedure consisted of a sequence of 2-min episodes, in which the dog could be in the experimental room with the owner, with the stranger, with both of them, or alone.

Owners and dogs were left in the experiment room for 30 s and the experimental episodes were then enacted, as follows:

- Episode 1: The owner sits on the chair and does not participate. The owner stimulates play after 90 s.
- Episode 2: The stranger enters the room and, after 30 s, initiates a conversation with the owner. One minute after entering the room, the stranger stimulates play. At the end of the episode, the owner leaves the room.
- Episode 3: The stranger continues to stimulate play, or petting, if the dog is not willing to play. Play is stopped after 1 min, but petting is allowed, if initiated by the dog.
- Episode 4: The owner enters and the stranger leaves as unobtrusively as possible. The owner greets the dog according to usual habits and is then free to comply with the dog's wishes throughout the episode. At the end of the episode, the owner tells the dog to "stay" and leaves.
- Episode 5: The dog is left alone in the room for the entire episode.
- Episode 6: The stranger enters, sits and waits for the end of greetings before being allowed to interact with the dog.
- Episode 7: The owner calls the dog before opening the door and enters, while the stranger leaves as unobtrusively as possible. The owner greets the dog as usual and is then free to comply with the dog's wishes throughout the episode.

All tests were live-monitored and video-recorded for later analysis by closed-circuit television (Panasonic WV-GP250, Panasonic, Osaka).

2.3. Collection of saliva and blood samples

Each dog underwent four saliva and one blood sampling. All samples were taken in the morning between 9:30 and 12:30 am. Two saliva samples were collected 30 min apart, 5 min before the beginning and 10 min after the end of the SST. A few days after the test, two further saliva samples were taken, 30 min apart, at the dogs' homes, to determine baseline concentrations of salivary cortisol and the effect of the sampling procedure. The last saliva sample at home was followed by a single blood collection in an EDTA tube (Venoject Terumo, Leuven, Belgium).

Whole unstimulated saliva samples were collected by the Salivette® system (Sarstedt, Nümbrecht, Germany) by rolling the swab round the dog's mouth for about 2 min. Owners were asked to prevent their dogs from eating or drinking for at least 1 h before sampling.

The swabs were refrigerated immediately after collection and transported in ice to the laboratory within 4 h. Samples were centrifuged at 2700 \times g for 15 min, to give clear saliva and plasma, which were then transferred to polythene tubes and frozen at -20 °C until analysis.

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