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Dogs' endocrine and behavioural responses at reunion are affected by how the human initiates contact

Therese Rehn [a,*], Linda Handlin [b], Kerstin Uvnäs-Moberg [a,b], Linda J. Keeling [a]

[a] Swedish University of Agricultural Sciences, Department of Animal Environment and Health, P.O. Box 7068, SE-750 07 Uppsala, Sweden
[b] University of Skövde, Systems Biology Research Centre, SE-54128 Skövde, Sweden

HIGHLIGHTS

- Reappearance of a familiar person increases dogs' oxytocin levels.
- Reappearance of a familiar person evokes dogs' approach behaviours.
- Physical and verbal contact promotes sustained increased oxytocin levels in dogs.
- Dogs' contact-seeking behaviour is determined by the person's behaviour.
- Dogs' cortisol levels decrease following reunion involving physical and verbal contact.

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ABSTRACT

For dogs, humans are likely to be the most important feature in their environment influencing their welfare. To investigate a commonly occurring human–dog interaction, behavioural and endocrine responses of 12 female beagle dogs were measured before, upon and after the return of a familiar person. Each dog was left by the person in a test arena to which it had been habituated prior to the experiment. Three different treatments were applied when the person returned and each dog experienced all these in a balanced design; the familiar person entered the test arena and 1) initiated physical and verbal contact in a calm and friendly way (PV), 2) there was verbal contact only (V) or, as a 'control', 3) the person ignored the dog (C). Interaction continued for 4 min during which the person behaved in a standardized way according to the treatment. Blood samples were collected to investigate oxytocin and cortisol levels. Upon return, oxytocin increased initially, probably because of the dog seeing the person entering the room and walking towards the area where the dog was housed. In treatment PV, where physical contact was applied, elevated levels of oxytocin were observed even after the interaction had ended. Cortisol levels showed a decreasing curve throughout the test, however this decrease was most pronounced in treatment PV, possibly as a consequence of the oxytocin release. Also, dogs in this treatment initiated more physical contact with the familiar person and expressed more lip licking upon reunion. The initial responses to reunion in treatment V were tail wagging and vocalisations. When dogs were ignored upon reunion in treatment C, they could have redirected their approach-behaviour towards an assistant (who was always situated in the room). To conclude, the type of interaction evidently affected the endocrine and behavioural responses of dogs in different ways. The mere return of the familiar person had a positive effect on oxytocin levels and induced contact-seeking behaviour, whereas physical contact was necessary in order to induce a sustained increase in oxytocin levels and to decrease cortisol levels in the period following reunion.

1. Introduction

Humans play a vital role for the welfare of companion animals. Although most studies using behavioural and physiological measures have investigated the effects of stressful situations, a calm and positive interaction, such as the dog being stroked by a human, has been shown to decrease cortisol levels [1] and blood pressure [2], while oxytocin is increased [2,3]. Nevertheless, compared to the number of studies on physiological responses, behavioural responses of dogs to positive human interactions are less investigated, as are the physiological responses to interactions initiated by humans that *do not* include petting or stroking the dog. To address this imbalance, and at the same time investigate one of the most common forms of positive human–dog interaction, we chose to examine the behavioural and endocrine responses of dogs being reunited with a familiar person. In this study, 'reunion' refers to the whole sequence of events, i.e.

* Corresponding author. Tel.: +46 18672108; fax: +46 18673588.
E-mail address: Therese.Rehn@slu.se (T. Rehn).

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from the visual reappearance of the person following a period of separation, to the interaction itself, which we varied to include or not the person stroking the dog.

Reunion between familiar primates is generally regarded as being a positive event [4]. Affiliative behaviour towards each other increases after separation [5,6] so reducing tension which probably results in reinforcement of the relationship [4]. Behaviour in dogs reunited with a human, however, is not well described in the scientific literature. Those descriptions of greeting behaviour that are available include parameters such as approach latency and proximity to owner at reunion (e.g. [7–9]), as well as tail wagging, lip licking and body shaking [10,11]. Although even here there is some evidence that contact-seeking and affiliative behaviour increase after longer periods of separation [11].

There have been studies of the physiological and endocrine responses in dogs during human interaction, such as studies on oxytocin and cortisol. Oxytocin is produced in the supraoptic nucleus and paraventricular nucleus (PVN) of the hypothalamus [12]. Oxytocin exerts hormonal actions in the circulation and effects in the brain after being released from oxytocinergic pathways emanating from the PVN. These project to several important regulatory sites in the brain. For example, oxytocin can be released into the amygdala, which is associated with increased approach behaviours and stimulation of social interactive behaviours so promoting attachment between individuals [13–16]. Oxytocin is often released in parallel into the brain and the circulation and therefore circulating oxytocin levels can be used as a proxy for centrally induced effects [14,17]. Oxytocin also decreases cortisol levels and blood pressure by its effects in the hypothalamus, the anterior pituitary and the brain stem region [18–22]. Oxytocin has been shown to play an important role in human–human interaction. Therefore it is interesting to study the role of oxytocin for behavioural and stress regulation also in human–animal interactions.

Owners interact in different ways with their dogs and even if interspecies interaction types are described [23–25], little is known about the actual effects of interactions initiated by the human. To better understand the behavioural and endocrine effects of how people interact with their dogs, we controlled the type of interaction initiated by a familiar human following their return after a period of separation. The person initiated physical and verbal contact in a calm and friendly way (treatment PV), there was verbal contact only (treatment V) or the person ignored the dog (treatment C). The PV treatment was selected because it is probably the most natural way of interacting upon reunion. Treatment V was perhaps slightly less familiar to the dogs, but gave the opportunity to investigate the dogs' behavioural and endocrine responses to the return of a known person *without* having any physical contact, something that has not been investigated previously. Treatment C was used as a control treatment in order to investigate the dogs' responses to the mere presence of the returning human. We chose to use both behavioural and endocrine measures as this has the advantage of allowing the investigation of the immediate behavioural responses and the longer lasting effects of an endocrine response, as well as possible associations between endocrine release and behavioural patterns.

2. Materials and methods

2.1. Subjects and settings

This study included twelve female beagle dogs at the age of 20 ± 0.2 months (mean ± SE), housed at the Swedish University of Agricultural Sciences. They were fed twice daily (at 07:30 and 16:00) and housed in large outdoor enclosures (approx. 145 m^2) between 08:00 and 15:30 every day. Indoors they were kept in groups of three individuals in an area of 24.3 m^2. They were taken for walks regularly by their caretaker. These dogs were kept especially for behavioural studies of positive emotional states and positive human–animal interaction experiments and they provided a standardized group of subjects for this investigation. Two women, very familiar with the dogs, participated in the study, each being responsible for 6 dogs. The familiar persons had known the dogs for approximately 1.5 years during which they had interacted with the dogs regularly in a positive and friendly way. Two female veterinary assistants were responsible for the blood sampling (see procedure below). They had first met the dogs a few weeks before the start of the experiment and, with the exception of training for blood sampling, they did not initiate interactions with the dogs. All dogs participated with the same familiar person and the same assistant in all treatments.

The test arena was 3.5 m^2 and included two chairs (one allocated to the familiar person and the other one for the assistant), blankets, water and a wooden toy. The arena consisted of three solid walls and a transparent plexi-glass wall including the entrance door, facing a 1.4 m wide and 9 m long room with a solid entrance door at the opposite end.

All tests were continuously video-recorded using two video cameras (Sony HDR-SR10E, Vivotek network camera, PT3124) placed opposite each other in order to cover the entire test arena.

2.2. Experimental setup

2.2.1. Preparations

One hour before the test started, dogs were shaved on their front leg where a local anaesthetic cream with prilocaine and lidocaine (EMLA®, AstraZeneca) was applied. The cream stayed on for 45 min, followed by insertion of an intravenous catheter into the cephalic vein. Catheter insertion was performed by experienced veterinary assistants. The dogs wore cones in order to prevent them from interfering with the catheter.

All dogs were habituated to the test arena before the experiment started and they were accustomed to wearing the cones. They had experienced the application of a catheter at least two times before the actual experiment. They were trained for the blood sampling procedure before the experiment in order to collect the blood as smoothly as possible and with minimal physical restraint. They were also used to the veterinary assistant sitting on a chair in the test room reading a book and ignoring the dog.

2.2.2. The test

The test lasted for 2 h during which time seven blood samples (BS1–7) were collected (Fig. 1). The test was divided in 5 different phases, beginning with a basal phase and separation from a familiar person. These were then followed by an appetitive state (familiar person returning and approaching the dog), a consummatory phase (the person entered the test arena where the dog was located and interacted with it according to treatment) and a post-consummatory phase (the period following reunion, which we call the relaxation phase).

2.2.2.1. Basal phase (35 min). Approximately 15 min after the application of the catheter, dogs were led through the experimental room by the familiar person and the veterinary assistant and they entered the test arena (time 0, start of the experiment). During this phase, the familiar person did not pay any attention to the dog, but sat on the floor reading a book. Two blood samples (BS-1 and BS-2) were collected at the end of the basal phase, at 30 and 35 min, respectively. During blood sampling, the familiar person gently held the dog in a stationary position while the veterinary assistant collected the blood (approximately 2 ml/sample).

2.2.2.2. Separation phase (25 min). Immediately after collecting the second blood sample, the familiar person left the room and walked out of sight. The dog was left in the room together with the assistant who returned to her chair and ignored the dog.

2.2.2.3. Approach phase (15 s). The familiar person entered the experimental room through the solid entrance door and approached the test arena where the dog was kept. Since the wall facing towards the experimental room was made out of plexi-glass, the dog could see the familiar person approaching.