Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Applied Animal Behaviour Science 94 (2005) 75–88
APPLIED ANIMAL BEHAVIOUR SCIENCE
www.elsevier.com/locate/applanim

Heart rate and behavioural responses of dogs in the Ainsworth's Strange Situation: A pilot study

Clara Palestrini [a,*], Emanuela Prato Previde [b], Caterina Spiezio [b,c], Marina Verga [a]

[a] *Istituto di Zootecnica, Facoltà di Medicina Veterinaria, Università degli Studi di Milano, Via Celoria 10, 20133 Milano, Italy*
[b] *Istituto di Psicologia, Facoltà di Medicina e Chirurgia, Via Pini 1, 20134 Milano Italy*
[c] *International School for Advanced Studies, Via Beirut 2-4, 34014 Trieste, Italy*

Accepted 17 February 2005
Available online 31 March 2005

Abstract

The possibility of linking physiology and observable behaviour is of great importance in gaining a better understanding of the dog's reactions to environmental changes and potential stressors. Many studies of human–dog interactions explored the issues concerning attachment of people to their pets, whereas only few studies investigated the nature of the dog–human relationship or the dog's level of attachment to its owner. The aim of this study was to investigate dog's reactions to different emotional situations integrating physiological (heart rate) and behavioural measures. Seventeen adult dogs were tested in a 'strange' environment using a modified version of Ainsworth's Strange Situation Test. The procedure consisted of an introductory episode followed by eight consecutive experimental episodes in which the dogs were placed in an unfamiliar environment, introduced to an adult stranger and subjected to separations from the owner. During each observational session the behaviour of each dog was videorecorded and heart rate was measured in order to allow a comparison between heart rate and behaviour. The level of activity of each dog in each experimental episode was assessed recording 12 different behavioural categories. The heart rate values during the first experimental episode were analysed to obtain a baseline for each subject and the dogs' heart rate across episodes was assessed and compared to the baseline values. Furthermore, the effect of specific events (stranger's entrance and owner's return) on dogs' heart rate was evaluated.

* Corresponding author. Tel.: +39 02 5031 8042; fax: +39 02 5031 8030.
E-mail address: clara.palestrini@unimi.it (C. Palestrini).

0168-1591/$ – see front matter
doi:10.1016/j.applanim.2005.02.005

The results of the present pilot study show that socioemotional conditions induce changes in both behaviour and heart rate in adult dogs and that these changes, especially those at the behavioural level, indicate emotional stress.

Keywords: Dogs; Behaviour; Heart-rate; Attachment; Stress

1. Introduction

In recent years most attention has been devoted to the human–dog relationship (Barba, 1995; Menache, 1998) due to the fact that dogs are more and more becoming a true part of the human pack (Voith, 1985). Dogs have been part of the human society for longer than any other domestic species (Clutton-Brock, 1999) and have been selected to form strong social bonds with humans (Serpell, 1995; Wilson and Turner, 1998). Recent empirical evidence show that the relationship between dogs and their owner is a strong affectional bond and that domestic dogs react to separations from their owner like human infants and infant chimpanzees (Topal et al., 1998; Prato Previde et al., 2003). In the dog's behavioural development the formation of affectional bonds is related to the so-called 'sensitive period for socialisation', lasting from the third to the twelfth week of age, during which the dog is able to generalise the socialisation to the human beings (Scott and Fuller, 1974; Scott, 1992).

The available literature clearly shows that modern pet owners are strongly bonded to their dogs and, quite often, tend to view and treat them as a child-like or child surrogates as their behaviour patterns, like those of children, seem especially designed to elicit care and affection (Voith, 1985; Askew, 1996; Overall, 1997). Thus, like all affectional bonds the dog–human relationship involves specific individuals and is emotionally significant.

Heart rate has a long history as a psychophysiological measure of animals' affective and cognitive responses and several studies have investigated heart rate responses of dogs *(Canis familiaris)* to different stimuli and environmental conditions (Murphee et al., 1967; Fox, 1978; Beerda et al., 1997). Heart rate represents an accessible, quantifiable, physiological measure underlying emotional responses in dogs and the possibility of linking physiology and observable behaviour is of great importance in gaining a better understanding of the dog's reactions to environmental changes (Stohr, 1988; Engeland et al., 1990; Kostarczyk, 1992; Mason and Mendl, 1993; Beerda et al., 1998; Palestrini et al., 2001; Casey, 2003).

Both behaviour and heart rate are considered useful indicators to evaluate stress reactions in dogs (Kostarczyk, 1992), due to the interaction among central nervous system and neuro-endocrine system (Henry and Ely, 1976; Beerda et al., 1998).

The aim of this study was to investigate dogs' reactions to different emotional situations integrating physiological and behavioural measures, and to assess to what extent dogs' heart rate could be treated as a correlate of behaviour. To reach this goal, we used a modified version of Ainsworth's Strange Situation Test (Prato Previde et al., 2003). This laboratory procedure was originally designed to investigate mother–infant attachment under conditions of low and high stress (Ainsworth and Bell, 1970; Ainsworth et al., 1978) but has also been used for studying affectional bonds in other species (Bard, 1991; Topal et al., 1998; Prato Previde et al., 2003).

The Strange Situation procedure involves conducting controlled observations of a subject's response to being placed in an unfamiliar room, introduced to an unfamiliar adult stranger and subjected to short episodes of separation from the attachment figure. Therefore it reproduces situations that dogs are likely to encounter in their everyday life, such as being in a new environment, meeting a stranger, and being separated from their owner for short periods.

2. Materials and methods

2.1. Subjects

The subjects were seventeen adult dogs (9 females and 8 males) whose ages ranged from 13 months to 9 years (mean = 4.64 years, STD = 1.29). Of the 17 recruited, 11 dogs were pure breeds and 6 mixed-breeds. All the dogs were kept exclusively for companionship and lived within the human household: they were accustomed to being taken out of doors with their owner and encountering human strangers.

2.2. Procedure

The dogs were tested in a standardised and controlled environment, as similar as possible to Ainsworth's Strange Situation Test (see Prato Previde et al., 2003). The strange environment was an unfamiliar and relatively bare room (3.00 m × 5.30 m) equipped with two chairs, one each for the 'stranger' and 'owner', a selection of dog toys, a water bowl and two video cameras (Sony Handycam Video HI 8, Fig. 1).

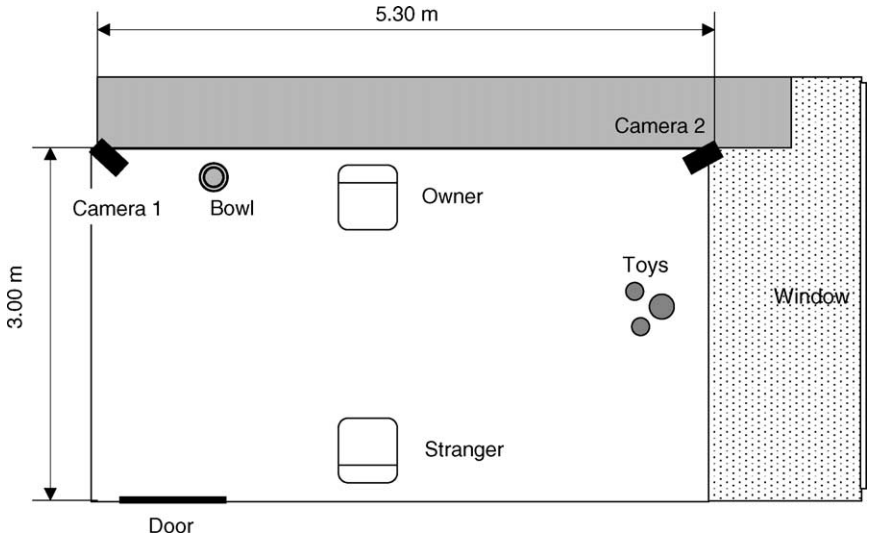

Fig. 1. Visual representation of the experimental room equipped with two chairs ('stranger' and 'owner'), dog toys, a water bowl and two video cameras.

The same woman always played the stranger who had never met the dog before.

The entire procedure comprised two consecutive phases: pre-experimental phase and experimental phase.

During the pre-experimental phase the owner and dog were escorted to a waiting room. The procedure was briefly described to the owner and after gaining his/her permission a lightweight heart monitor (Polar® Vantage NV) was strapped around the dog's chest. Then, the two video cameras in the experimental room were activated and both the dog and owner were led to the experimental room.

The entire protocol lasted approximately 27 min and consisted of an introductory episode followed by eight 3-min experimental episodes. During the introductory episode, owner and dog were escorted to the experimental room and the heart rate recorder was started in the 5 s interval mode (Vincent and Leahy, 1997) and synchronised with video recording. The owner was asked to sit on his/her chair, the experimenter took the dog's lead and walked out of the room leaving the owner with his/her dog. The eight experimental episodes are detailed in Table 1. After each session, the experimental room, water bowl and toys were washed using a non-toxic, weakly scented disinfectant.

Table 1
Description of the Strange Situation Procedure

Episode	Description
1: owner and dog	The owner sat quietly participating only if the dog sought attention; the dog was free to explore the room
2: owner, dog and stranger	The stranger entered the room, sat quietly for 1 min, conversed with the owner for the second minute, approached the dog and attempted to stimulate play during the last minute. At the end of this episode the owner left the room unobtrusively
3: stranger and dog (1st separation episode)	The stranger continued to play with the dog if it was willing; if it was inactive or distressed, the stranger attempted to distract it with play or by providing comfort
4: owner and dog (1st reunion episode)	The owner entered the room and greeted and/or comforted the dog as usual. The stranger quietly exited the room. The owner had been told that she/he could play with the dog throughout the episode. At the end of this episode the owner left the room
5: dog alone (2nd separation episode)	The dog was left alone for three minutes, but was constantly observed by the owner and researchers on the monitor in the adjacent room
6: stranger and dog	The stranger entered the room and followed the same protocol as in episode 3. Before exiting she left her shoes near her chair and an article of clothing on the chair
7: owner and dog (2nd reunion episode)	The owner entered the room and greeted and/or comforted the dog as usual. The stranger left the room unobtrusively. At the end of this episode the owner left his/her shoes near his/her chair and an article of clothing on the chair before exiting the room
8: dog and objects (3rd separation and reunion)	The dog remained alone with the objects and monitored as in episode 5. At the end of the episode the owner returned and the experimenter arrived and officially terminated the procedure

2.3. Data collection and analysis

The behaviour of each dog during the eight experimental episodes was videorecorded and heart rate was measured using a Polar® Vantage NV system (Vincent and Leahy, 1997) in order to allow a comparison between heart rate and behaviour. The heart rate device was activated at the start of the introductory episode and synchronised with video recording of behaviour in order to have a perfect match between behavioural and physiological data.

2.3.1. Behaviour analyses

The videotaped sessions were analysed by two trained observers, and 12 mutually exclusive categories of behaviour were recorded. Table 2 contains the list of the mutually exclusive categories and their definition. To compare behaviour with heart rate, which was recorded every 5 s, a 5 s 'instantaneous sampling' method was used a to describe the dog's

Table 2
Behavioural categories

	Behavioural category	Definition
Dynamic behaviours	Exploration	Activity directed toward physical aspects of the environment, including sniffing, close visual inspection, distal visual inspection and gentle oral examination such as licking
	Locomotion	Walking, pacing or running around without exploring the environment or playing
	Individual play	Any vigorous or galloping gaited behaviour directed toward a toy when clearly not interacting with the owner or stranger; including chewing, biting, shaking from side to side, scratching or batting with the paw, chasing rolling balls and tossing using the mouth
	Social play	Any vigorous or galloping gaited behaviour performed when interacting with the owner or stranger; including running, jumping, active physical contact and chasing toys
	Following	Following the person around the room or to the door
	Scratch the door	All active behaviours resulting in physical contact with the door, including scratching the door with the paws, jumping on the door, pulling on the door handle with the forelegs or mouth
	Drink	Drinking water from the bowl
	Other behaviours	Any activity not included in the behavioural catalogue, such as self-grooming, self-scratching
Static behaviours	Passive behaviour	Sitting, standing or lying down without any obvious orientation toward the physical or social environment
	Oriented to door	Sitting, standing or lying down staring fixedly at the door, either when close to it or from a distance
	Oriented to person	Sitting, standing or lying down staring fixedly at the owner or stranger, regardless of whether the behaviour was reciprocated
	Oriented to chair	Sitting, standing or lying down staring fixedly at the owner's or stranger's empty chair

activity. Furthermore, as vocal behaviour is reported to be an indicator of stress and anxiety (Fox, 1978; Overall et al., 1999; Appleby and Pluimakers, 2003) the number of vocalisation bouts during the test were recorded using a continuous recording method.

Interobserver reliability was assessed by means of independent parallel coding of a random sample of videotaped sessions (10%) using percentage agreement: percentage agreement was always more than 97%.

In order to have an overall view of the dogs' level of activity during the test the behaviour of each dog in each episode of the experimental phase was then divided into 'dynamic' and 'static' according to whether the subject was engaged in grossly observable physical activity or was sitting, standing or lying down and therefore movement was almost absent or very limited (see Table 2).

The degree of activity of each dog in each episode of the experimental procedure was evaluated by considering the proportion of sample points spent in dynamic versus static behaviours, and therefore the amount of time spent performing dynamic behaviours. Differences in dogs' overall degree of activity and in specific behaviours across episodes were evaluated using non-parametric and two-tailed statistical tests (Siegel and Castellan, 1992).

2.3.2. Heart rate analyses

The heart rate values during the first experimental episode (owner and dog) in which no social interactions occurred were analysed to obtain a baseline for each subject. Heart rate during the experimental procedure was analysed considering two measures: mean heart rate values in each episode and changes in mean heart rate expressed as percentages with respect to the baseline. The dogs' heart rate across episodes were analysed and the effect of specific events such as the reunions with the owner (episodes 4 and 7) and the entrances of the stranger (episodes 2 and 6) were assessed comparing the mean heart rate during the event occurrence with the mean heart rate of the previous episode. Differences in heart rate were analysed using parametric statistical tests.

3. Results

The results presented here reflect average tendencies rather than individual differences. There were some individual differences among the dogs, but a detailed exposition of these is not within the scope of the present paper.

3.1. Behaviour

The analysis of dogs' behaviour in successive episodes, carried out comparing the overall proportion of sample points spent performing dynamic behaviours in the different episodes, showed the existence of highly significant differences in overall activity levels (Friedman test: $X^2 = 40.442$, $p < 0.0001$). As Fig. 2 shows the overall amount of time spent by the subjects in dynamic behaviours was higher when the dogs were with their owner (episodes 1, 4 and 7) than with the stranger (episodes 3 and 6) or alone (episodes 5 and 8).

The comparison between the proportions of sample points spent in dynamic behaviours showed no significant differences in dogs' activity levels between episode 1 (owner and

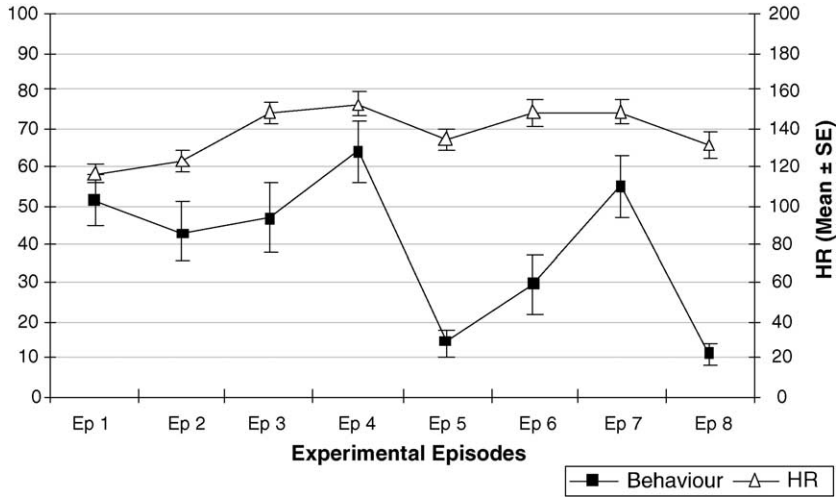

Fig. 2. Percentage of sample points spent in dynamic behaviours and mean heart rate of the subjects during the different episodes.

dog, baseline) and the first and second reunion with the owner (Student–Newman–Keul test -SNK- for multiple comparisons: episode 1 versus episodes 4 and 7, p = ns).

Similarly, no significant differences emerged between episode 1 (owner and dog) and episodes 2 (owner, dog and stranger) or 3 (stranger and dog).

However, as Fig. 2 clearly shows, activity levels decreased remarkably the second time the dogs remained alone with the stranger (SNK test for multiple comparisons: episode 1 versus episode 6, $p < 0.05$) and during the two isolation periods (SNK test for multiple comparisons: episode 1 versus episodes 5 and 8, $p < 0.05$). There were no differences in activity levels between the two isolation episodes (episodes 5 and 8): however, overall the dogs tended to spend more time near the owner's chair in episode 8, in the presence of his/her belongings (shoes and article of clothing).

Some dynamic behaviours occurred very rarely and some almost exclusively in certain episodes (i.e. scratching). Locomotion varied across episodes (Friedman test: $X^2 = 21.599$, $p = 0.003$) and was significantly higher in episodes 1, 5 and 8 than in all other episodes (SNK test for multiple comparisons: episodes 1, 5 and 8 versus all other episodes, $p < 0.05$). Exploration decreased significantly over time (Friedman test: $X^2 = 54.04$, $p < 0.0001$) and was high only in episodes 1 and 2 (SNK test for multiple comparisons: episodes 1 and 2 versus all other episodes, $p < 0.05$). Individual play was very rare and only two dogs engaged in this activity to some extent in episodes 1 and 2. Social play occurred in those episodes characterised by the presence of a social partner (2, 3, 4, 6 and 7) but varied significantly (Friedman test: $X^2 = 12.07$, $p = 0.017$); overall the dogs preferred to play with their owner rather than the stranger (episodes 3, 6 versus episodes 4,7, $p < 0.05$); there was no difference in play between the two reunion episodes with the owner, but the dogs played significantly less the second time they remained with the stranger (episode 3 versus episode 6, $p < 0.05$). Vocalisations were rare in the presence of

the owner, increased in the presence of the stranger and especially during isolation (Friedman test: $X^2 = 110.33$, $p < 0.0001$; episodes 5 and 8 versus episodes 1,2,4,7, $p < 0.05$). During the isolation episodes the dogs' overall activity was remarkably lower in comparison to episode 1 (baseline) in which no social interaction occurred but the owner was sitting in the experimental room (see Fig. 2). In fact, most dogs reacted to separation from their owner (i.e. to remaining alone in the experimental room) by remaining oriented to the door most of the time (Friedman test: $X^2 = 26.235$, $p < 0.0001$; episode 1 versus episodes 5 and 8, $p < 0.05$). During episodes 5 and 8 exploration was almost absent, locomotion was not significantly different from episode 1, and some dogs scratched the door. Many subjects vocalised at least to some extent (SNK, episode 1 versus episodes 5 and 8, $p < 0.05$).

3.2. Heart rate

The analysis of both mean heart rate values (Fig. 2) and changes in mean heart rate (HR) expressed as percentages with respect to the baseline showed that heart rate varied significantly across experimental episodes (ANOVA for repeated measures, mean HR: $F = 14.530$, $p < 0.0001$; HR changes: $F = 13.797$, $p < 0.0001$). Overall heart rate values varied in relation to overall activity level, being higher in those episodes characterised by a more intense physical activity and interaction with the owner and the stranger.

The comparison between episode 1 (baseline) and episode 2 showed that the presence of the stranger when the owner was in the room had no significant effect on heart rate (SNK test for multiple comparisons: episode 1 versus episode 2, p = ns). Heart rate increased significantly when the dogs remained alone with the stranger in episodes 3 and 6 (SNK test for multiple comparisons: episode 1 versus episodes 3 and 6, $p < 0.05$) and when they were with their owner in episodes 4 and 7 (SNK test for multiple comparisons: episode 1 versus episodes 4 and 7, $p < 0.05$).

During isolation episodes (episodes 5, 8) heart rate was significantly lower than when the dogs were with the stranger (episodes 3 and 6) or with their owner (episodes 4 and 7) and significantly higher than the baseline (SNK test for multiple comparisons: episode1 versus episode 5, $p < 0.05$; episode 1 versus episode 8, $p < 0.05$). No differences in heart rate were found between the two isolation episodes (episodes 5 and 8). As shown by Fig. 2 there were no significant differences in HR between episodes 3 and 4 and episodes 6 and 7 and between the first and second reunion with the owner and the first and second encounter with the stranger.

The effects of the entrance of the stranger at the beginning of episodes 2 and 6 and the return of the owner at the beginning of episodes 4 and 7 were assessed by comparing mean HR during these events with mean HR of the immediately previous episode.

The first time the stranger entered in the room (episode 2) mean heart rate increased but not significantly (paired t test: $t = 1.861$, $p = 0.081$). However, when the stranger entered the second time (episode 6) after the first isolation period heart rate increased significantly (paired t test: $t = 3.317$, $p = 0.004$, Fig. 3a).

An increase in heart rate was also observed in episode 4 (first reunion with the owner, paired t test: $t = 3.366$, $p = 0.004$) and to a lesser extent in episode 7 (second reunion with the owner, paired t test: $t = 2$, $p = 0.062$, Fig. 3b).

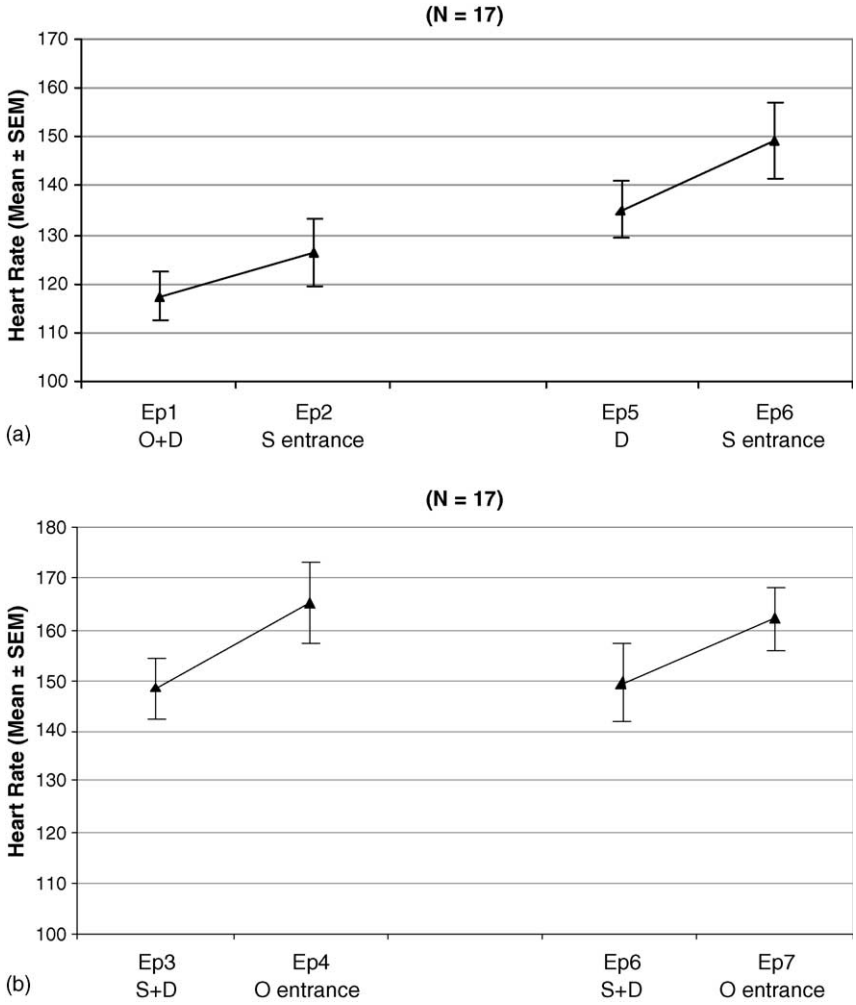

Fig. 3. (a) Stranger's entrance; (b) owner's return.

3.3. Heart rate and behaviour

As Fig. 2 shows heart rate was higher than the baseline in episodes characterised by a high level of physical activity (% of dynamic behaviour) and lower than the baseline in episodes characterised by a low level of activity on the part of the dogs.

However, during episodes 5 and 8 heart rate was significantly higher than during episode 1 (baseline), even though the dogs were overall clearly less active than during the baseline (see Fig. 2). In fact, during isolation episodes the dogs spent most of the time performing static behaviours (85.6 and 88.8% of the time, respectively) and engaged in static behaviours significantly more than during episode 1 (48.67% of static behaviours).

During all these episodes, characterised by the lack of social interactions with the owner or the stranger, the repertoire of possible dynamic behaviours was more limited and, thus, more easily comparable; an analysis of the dynamic behaviours showed that exploration was higher during episode 1 (baseline), locomotion and drink were comparable across these episodes whereas scratching the door occurred during isolation only. During the separation episodes the dogs vocalised more than during the baseline (Friedman Test: $X^2 = 13.897$, $p = 0.001$) and the dogs that vocalised more frequently when alone showed higher heart rate values (Spearman Rank Correlation: episode 5 $\rho = 0.711$, $p = 0.004$; episode 8 $\rho = 0.586$, $p = 0.02$).

4. Discussion and conclusions

The aim of this study was to investigate dogs' reactions to different emotional and potentially stressful situations recording both physiological and behavioural measures, and to assess to what extent dogs' heart rate could be treated as a correlate of behaviour. To do this, we used a standardised procedure, an adapted version of Ainsworth's Strange Situation test (Ainsworth and Bell, 1970), aimed at testing the reaction of free moving pet dogs to environmental situations that could be stressful to the animal, such as being in an unfamiliar environment, meeting an unfamiliar person and being separated from the attachment figure.

A number of studies show that in social animals the manipulation of the social environment, such as brief separations from the mother or from other social partners, induce behavioural and physiological signs of arousal that suggest psychological stress (Boissy and Le Neindre, 1997; Syme and Elphick, 1982; Mendoza and Mason, 1986; Mal et al., 1991; Wolfle, 2000). This has been reported for dogs as well and there is evidence that a number of environmental events, including interacting with unfamiliar persons and being separated from the owner, are likely to be accompanied by profound behavioural and physiological changes (Kostarczyk, 1992; Palestrini et al., 2001; Flanningan and Dodman, 2001). In particular, recent studies have shown that dogs are emotionally bonded to their owners and react to brief separations from him/her showing behaviours that suggest stress and anxiety and resemble those observed in human infants and chimpanzees (Topal et al., 1998; Prato Previde et al., 2003).

Under stress conditions an individual can react both by freezing and remaining inactive or by engaging in frantic activity; similarly, at the physiological level heart rate acceleration or deceleration (bradychardia) may be shown (Fox, 1978; Duncan and Filshie, 1979).

Overall our results suggest that emotional stress is accompanied by changes in behaviour and in heart rate.

Our data indicate that at the behavioural level the experience of being separated from the attachment figure and of remaining alone or with an unfamiliar person can be considered a source of distress for adult dogs. In our study many subjects played exclusively with their owner and refused to play with the stranger in the absence of the attachment figure (especially during the second encounter). Furthermore, when separated from the owner (episodes 3, 6, 5 and 8) the dogs showed both passive and active proximity seeking and

discomfort related behaviours, such as staring at the door, pacing around, or scratching the door, whining/barking; In particular, when alone the dogs spent most of their time performing static behaviours (in particular sitting, standing or lying down staring fixedly at the door) and vocalizing.

Although each of these behaviours does not necessarily imply distress, various studies suggest that their occurrence may indicate discomfort, distress and anxiety (McCrave, 1991; Lund and Jørgensen, 1999; Flanningan and Dodman, 2001; Horwitz, 2003).

Heart rate varied significantly across episodes, increasing in episodes characterised by a high level of physical activity (% of dynamic behaviour) and decreasing in episodes characterised by a lower activity on the part of the dogs. Therefore, it changed in relation to the overall amount of physical activity of the subjects. As in our study the dogs were moving freely and different behaviours occurred in different episodes, a comparison between heart rate and ongoing behaviour was not possible. However, episodes 1 (baseline), 5 and 8 (in which no social interaction occurred and the dogs' activity consisted of a limited number of dynamic behaviours) could be compared to some extent. During the two isolation episodes the dogs' were significantly less active than during the baseline: in fact, in these two episodes the dogs spent most of their time sitting, standing and lying down staring at the door, and the amount of time spent in dynamic behaviours was quite low. In particular, exploratory behaviour (which was very high in episode 1) was almost absent and locomotion did not increase significantly. However, during these episodes heart rate remained significantly higher than the baseline. This might suggest that a drastic reduction in motor activity might not be accompanied by a decrease in heart rate. Although there is evidence that cardiac responses depend on motor and respiratory activity (Coote, 1975; Obrist, 1976) it has also been reported that emotional states during stress can be accompanied by evident cardiovascular responses with minor motor activity (Galosy and Gabelein, 1977; Duncan and Filshie, 1979). This might explain why during isolation in a strange environment (a potentially stressful condition), despite a remarkable decrease in activity, our dogs showed a significantly higher value of heart rate; however, it is also possible that the observed differences in heart rate between the baseline and the isolation episodes were related to the presence of vocalisations or scratching. The lack of significant differences in behaviour and in heart rate between the two isolation episodes seems to suggest that in our sample of dogs the items left behind by the owner and the stranger had no particular effect in reducing discomfort. However, it is interesting to note that the dogs showed a tendency to spend more time next to the chair of their owner when his/her items were in the room.

Changes in heart rate during specific events, namely the entrance of the owner and the stranger cannot be easily interpreted. The appearance of the owner (attachment figure) and the stranger both resulted in an increase in heart rate and the cardiac response to the second stranger's entrance was similar to that observed towards the owner; therefore, it is possible that in both cases the increase in heart rate could be due to the excitement for the reunion with the attachment figure or with a friendly person. This would suggest that the distressing event for the dogs was the separation from the owner in an unfamiliar environment and that the stranger was not perceived as a stressful stimulus 'per se'.

However, there is evidence that emotional responses to potentially dangerous stimuli are often associated with cardiac acceleration (i.e. defence reactions, Spinks and Siddle, 1983;

Turpin, 1983, 1986); therefore, it cannot be completely ruled out that the entrance of the stranger might have represented a source of stress.

In summary, our findings show that brief separations from the owners and interactions with a stranger induce clear changes in both behaviour and heart rate in adult dogs and that these changes, especially those at the behavioural level, indicate emotional stress.

Acknowledgements

We are grateful to Prof. Marcello Cesa Bianchi and to Prof. Marco Poli for allowing us to carry out the work in the Psychology Institute of Università di Milano and to Prof. Corrado Carenzi for his useful suggestions. We thank Barbara Rotta for her invaluable help in data collection and scoring and Dr. Debora Groppetti for her help in heart rate analysis. Finally, a special thank to all the owners and dogs that participated as volunteers.

References

Ainsworth, M.D.S., Bell, S.M., 1970. Attachment, exploration, and separation: illustrated by the behaviour of one-year-olds in a strange situation. Child Dev. 41, 49–67.

Ainsworth, M.D.S., Blehar, M.C., Waters, E., Wall, S., 1978. Patterns of Attachment: a Psychological Study of the Strange Situation. Lawrence Erlbaum Associates, Hillsdale, NJ.

Appleby, D., Pluimakers, J., 2003. Separation anxiety in dogs: the function of homeostasis in its development and treatment. Vet. Clin. Small Anim. 33, 321–344.

Askew, H.R., 1996. Treatment of Behaviour Problems in Dogs and Cats. Blackwell Science, London.

Bard, K., 1991. Distribution of attachment classifications in nursery chimpanzees. Am. J. Primatol. 24, 88.

Barba, B.E., 1995. A critical review of research on the human-companion animal relationship: 1988 to 1993. Anthrozoos 8, 9–15.

Beerda, B., Schilder, M.B.H., van Hooff, J.A.R.A.M., de Vries, H.W., 1997. Manifestations of chronic and acute stress in dogs. Appl. Anim. Behav. Sci. 52, 307–319.

Beerda, B., Schilder, M.B.H., van Hooff, J.A., de Vries, H.W., Mol, J.A., 1998. Behavioural, saliva cortisol and heart rate responses to different types of stimuli in dogs. Appl. Anim. Behav. Sci. 58, 365–381.

Boissy, A., Le Neindre, P., 1997. Behavioral,cardiac and cortisol responses to brief peer separation and reunion in cattle. Physiol. Behav. 61 (5), 693–699.

Casey, R., 2003. Fear and stress. In: Horwitz, D., Mills, D., Heath, S. (Eds.), BSAVA Manual of Canine and Feline Behavioural Medicine. BSAVA, Gloucester, pp. 144–153.

Clutton-Brock, J., 1999. A Natural History of Domesticated Mammals. Cambridge University Press, Cambridge.

Coote, J.H., 1975. Physiological significance of somatic efferent pathways from skeletal muscle and joints with reflex effects on heart and circulation. Brain Res. 87, 139–144.

Duncan, I.J.H., Filshie, J.H., 1979. The use of radio telemetry devices to measure temperature and heart rate in domestic fowl. In: Amlaner, C.J., MacDonald, D.W. (Eds.), A Handbook of Biotelemetry and Radio Tracking. Pergamon, Oxford, pp. 575–585.

Engeland, W.C., Miller, P., Gann, D.S., 1990. Pituitary-adrenal adrenomedullary responses to noise in awake dogs. Am. J. Physiol. 258, R672–R677.

Flanningan, G., Dodman, N.H., 2001. Risk factors and behaviors associated with separation anxiety in dogs. J. Am. Vet. Med. Assoc. 219, 460–466.

Fox, M.W., 1978. The Dog: its Domestication and Behaviour. Garland STPM Press, New York and London.

Galosy, R.A., Gabelein, C.J., 1977. Cardiovascular adaptation to environmental stress: its role in the development of hypertension, responsible mechanism, and hypothesis. Biobehav. Rev. 1, 165–175.

Henry, J.P., Ely, D.L., 1976. Biologic correlates of psychosomatic illness. In: Grenell, R.G., Gabay, S. (Eds.), Biological Foundations of Psychiatry 2. Raven, NY.

Horwitz, D.F., 2003. Separation-related problems in dogs. In: Horwitz, D., Mills, D., Heath, S. (Eds.), BSAVA Manual of Canine and Feline Behavioural Medicine. BSAVA, Gloucester, pp. 154–163.

Kostarczyk, E., 1992. The use of dog–human interaction as reward in instrumental conditioning and is impact on dogs' cardiac regulation. In: Davis, H., Balford, D. (Eds.), The Inevitable Bond: Examining Scientist–Animal Interaction. Cambridge University Press, Cambridge.

Lund, J.D., Jørgensen, M.C., 1999. Behaviour patterns and time course of activity in dogs with separation problems. Appl. Anim. Behav. Sci. 63, 219–236.

Mal, M.E., Friend, T.H., Lay, D.C., Vogelsang, S.G., Ienkins, O.C., 1991. Behavioral responses of mares to short-term confinement and social isolation. Appl. Anim. Behav. Sci. 31, 13–24.

Mason, G., Mendl, M., 1993. Why there is no simple way of measuring animal welfare? Animal Welfare 2, 301–319.

McCrave, E.A., 1991. Diagnostic criteria for separation anxiety in the dog. Veterinary Clinics of North America. Small Animal Prac. 21, 247–255.

Menache, S., 1998. Dogs and Human Beings: A Story of Friendship. Soc. Anim. 6 (1), 67–86.

Mendoza, S.P., Mason, W.A., 1986. Contrasting responses to intruders and to involuntary separations by monogamous and polygamous new world monkeys. Physiol. Behav. 38 , pp. 795–801.

Murphee, O.D., Peters, J.E., Dykman, R.A., 1967. Effect of person on nervous, stable and cross bred pointer dogs. Cond. Reflex 2 (4), 273–276.

Obrist, P.A., 1976. The cardiovascular-behavioural interaction: as it appears today. Psychophysiology 13, 95–107.

Overall, K.L., 1997. Clinical Behavioural Medicine for Small Animals. Mosby, St. Louis.

Overall, K.L., Agulnick, L., Dunham, A.E., et al., 1999. Proceedings of the Second World Meeting on Ethology. Qualitative and quantitative differences in vocalizations by dogs affected with separation anxiety and unaffected dogs using sonografic analysis 108–113.

Palestrini, C., Prato Previde, E., Custance, D.M., Spiezio, C., Sabatini, F., Verga, M., 2001. Heart rate and behavioural responses of dogs (*Canis familiaris*) in the Ainsworth's Strange Situation Test: a pilot study. In: Overall, K.L., Mills, D.S., Heath, S.E., Horwitz, D. (Eds.), Proceedings of the 3rd International Congress on Veterinary Behavioural Medicine, Vancouver, Canada, pp. 89–91.

Prato Previde, E., Custance, D.M., Spiezio, C., Sabatini, F., 2003. Is the dog–human realationship an attachment bond? An observational study using Ainsworth's Strange Situation. Behaviour 140, 225–254.

Scott, J.P., 1992. The phenomenon of attachment in human–nonhuman relationships. In: Davis, H., Balfour, D. (Eds.), The Inevitable Bond: Examining Scientist–Animal Interaction. Cambridge University Press, Cambridge, pp. 72–91.

Scott, J.P., Fuller, J.L., 1974. Dog Behaviour: the Genetic Basis. University of Chicago Press, Chicago.

Serpell, J., 1995. The Domestic Dog: its Evolution, Behaviour and Interactions with People. Cambridge University Press, Cambridge.

Siegel, S., Castellan, N.J., 1992. Non parametric Statistics for the Behavioural Sciences, 2nd ed. McGraw-Hill, London, p. 399.

Spinks, J.A., Siddle, D., 1983. The functional significance of the orienting response. In: Siddle, D. (Ed.), Orienting and habituation: Perspectives in Human Research. Wiley, New York, pp. 237–313.

Stohr, W., 1988. Long term heart rate telemetry in small mammals. Comprehensive approach as a prerequisite for valid results. Physiol. Behav. 43, 567–576.

Syme, L.A., Elphick, G.R., 1982. Heart-rate and the behaviour of sheep in yards. Appl. Anim. Ethol. 9, 31–35.

Topal, J., Miklosi, A., Csanyi, V., Doka, A., 1998. Attachment behavior in dogs (*Canis familiaris*): a new application of Ainsworth's (1996) strange situation test. J. Comp. Psychol. 112 (3), 219–229.

Turpin, G., 1983. Unconditioned reflexes and the autonomic nervous system. In: Siddle, D. (Ed.), Orienting and habituation: Perspectives in Human Research. Wiley, New York, pp. 1–69.

Turpin, G., 1986. Effects of stimulus intensity on autonomic responding: the problem of differentiating orienting and defense reflexes. Psychophysiology 23, 1–14.

Vincent, I.C., Leahy, R.A., 1997. Real-time non-invasive measurement of heart rate in working dogs: a technique with potential applications in the objective assessment of welfare problems. Vet. J. 153, 179–184.

Voith, V.L., 1985. Attachment of people to companion animals. Vet. Clin. North Am.: Small Anim. Prac. 15 (2), 289–296.

Wolfle, T.L., 2000. Understanding the role of stress in animal welfare. In: Moberg, G.P., Mench, J.A. (Eds.), The Biology of Animal Stress: Basic Principles and Implication for Animal Welfare. CABI Publishing, pp. 335–368.

Wilson, C., Turner, D., 1998. Companion Animals in Human Health. Sage Publications.