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Heart rate variability and saliva cortisol assessment in shelter dog: Human-animal interaction effects

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A B S T R A C T

The aim of this study is to investigate the effects of a human interaction program on shelter dogs and to determine the effect on canine heart rate variability, behaviour, and salivary cortisol levels. Twenty dogs were behaviourally (temperament tests) and clinically (full car-diologic examination) pre-tested and then matched in two homogenous groups. Ten dogs (group A) were submitted to a human interaction program and compared to a control group (group B). The study included four experimental sessions (T0, T1, T2 and T3). At T0 salivary cortisol samples (basal cortisol) were collected from all dogs. After 1 week, all dogs were submitted to the following procedures: electrocardiogram holter monitor unit application and recordings, saliva cortisol sample collection before behavioural test (pre-test cortisol), behavioural test application, and saliva cortisol sample collection after behavioural test (post-test cortisol). The above-mentioned experimental session was repeated every 4 weeks from the beginning of the study (T1, T2 and T3). All dogs were videotaped during all behavioural evaluations. Significant differences ($P<0.05$) between groups A and B were determined for heart rate variability (HRV) frequency domain (5min analysis): low frequency/high frequency ratio (LF/HF) at T1; LF/HF and standard deviation of time duration between two consecutive R waves (RR interval) of the electrocardiogram (SD RR) at T2; very low frequency (VLF) at T3. Overall behavioural test holter recordings showed statistical differences ($P<0.05$): LF/HF at T1 and T2, total number of interpolated

beats and total number of used beats at T3. Behavioural data showed significant improvements in sociability/diffidence and temperament in group A for some tests ($P<0.05$). A significant decrease ($P<0.05$) in salivary cortisol levels between T1 vs T2 and between T1 vs T3 has been reported. HRV and behavioural data reported significant correlations in some tests, as well as cortisol levels and behavioural data ($P<0.05$). These data suggest that human interaction supplement sessions have a positive effect upon the behaviour and they could affect the physiological indicators of animal welfare.

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1. Introduction

According to current Italian law (Legge 281/1991), stray dogs directly given to the rescue associations cannot be euthanized, but they are required to be kept in adequate facilities until they are adopted or until they die. Upon introduction into shelter housing, dogs experience a severe form of social isolation due to novel surroundings (Hennessy et al., 1997). Social isolation or restriction is regarded as a major stressor for a social species such as the dog (Hubrecht and Turner, 1998). With time, reduced human-animal and fellow dog interaction may lead to the disruption of physiological and behavioural homeostasis. Concern for the welfare of dogs housed in rescue shelters is increasing, promoting the need for optimized management of sheltered dogs that may be achieved by improving their housing conditions (Mertens and Unshelm, 1996), including social contact (Wells and Hepper, 2000; Hennessy et al., 2002; Wells, 2004; Coppola et al., 2006; Valsecchi et al., 2007; Normando et al., 2009).

Behavioural test, cortisol release and sympathetic nervous system activation can be used as ethological, physiological and hormonal indicators to assess and monitor animal welfare.

Heart rate variability (HRV) is a result of the relative balances of autonomic input to the sinoatrial node. An assessment of HRV can provide a non-invasive measure of cardiac sympathetic and vagal nervous system function (Akselrod, 1995). Spectral analysis of HRV frequency content can provide quantitative information on the relative contribution of the vagal and sympathetic nervous systems on the heart rate (HR) (Task force, 1996). High frequency component (HF; 0.15-0.5 Hz) of the HRV has been attributed to the vagal nerve. Low frequency component (LF; <0.15Hz) is thought to reflect both sympathetic and vagal influences. The spectrum is the area under the curve between the two frequency limits. In dogs at rest, the vagal effects on the heart predominate over sympathetic effects (Little et al., 1999). Canine HF is thus mainly a result of the influence of the vagal nerve on the sinoatrial node.

Previously, HRV has been applied in research to investigate modifications in sympathovagal balance related to pathological conditions (Calvert and Jacobs, 2000; Pomfrett et al., 2004), stress (Korte et al., 1999; Geverink et al., 2002; Mohr et al., 2002; Kuwahara et al., 2004; Rietmann et al., 2004), behavioural dysfunction (Bachmann et al., 2003), management practices (Langbein et al., 2004; Hagen et al., 2005), training regimes (Kuwahara et al., 1999; Physick-Sheard et al., 2000), temperament and emotional states (Visser et al., 2002; Désiré et al., 2004). HRV is a particularly good indicator for the non-invasive assessment of autonomic nervous system activity in response to psychophysiological stress (Tiller et al., 1996). Decreased HRV has often been reported during physically or emotionally stressful events (Sgoifo et al., 1999, 2001). High vagal tone has been linked to efficient autonomic regulatory activity which allows an organism to enhance its sensitivity and response to physiological and environmental challenges (Porges et al., 1996;

Friedman and Thayer, 1998). Positive emotions may significantly augment the HF component of a power spectrum (McCraty et al., 1995), whereas the opposite occurs with negative emotions. Therefore, cardiac vagal tone may also be an indicator of positive emotional states and recently patterns of HRV reduction have been studied in farm animals in reaction to stressors, behavioural disorders and in the context of cognitive appraisal (Bachmann et al., 2003; Désiré et al., 2004; Kuwahara et al., 2004).

Change in circulating cortisol concentration is considered a major indicator of altered physiological states that strongly correlates with stress. Salivary cortisol has been shown (1) to be a useful non-invasive measure of acute and chronic stress in dogs (Beerda et al., 1998, 1999); (2) well correlated with plasma concentrations in several species including humans (Kirschbaum and Hellhammer, 1989) and dogs (Vincent and Michell, 1992; Beerda et al., 1996); (3) to be a better measure of adrenal cortical function in term of physiological indicator of stress than plasma cortisol (Cook et al., 1996; Groschl et al., 2000) because it is a direct reflection of the biologically active portion of the total cortisol level (Cook et al., 1997). Salivary cortisol collection does not cause additional stress to the animal and these sample results may be more representative of the underlying welfare status of the dog. Even handling effect can be avoided if saliva samples are taken within 4min (Kobelt et al., 2003).

The aim of the present study was to investigate the effects of a human contact session implemented on long-term shelter dogs in order to verify its effect on canine heart rate variability, behaviour, and salivary cortisol levels.

2. Materials and methods

2.1. Ethical note

All the procedures and sample collections were conducted according to ethical guidelines down by the University of Torino as well as Torino Public Veterinary Service and Torino Humane Society (86/609/EEC).

2.2. Animals

A sample of long-term sheltered dogs was recruited at the Municipal Dog Shelter of the City of Turin, Italy, where the study took place from October 2005 through February 2006. At first the dogs had to fulfill the following criteria: aged between 18 months and 6 years, resident at the shelter for at least 7 months and not more than 3 years, no evidence of clinical disease for at least 1 year and not on any therapeutic protocol for at least 6 months. All data were obtained from medical records including a standard complete physical examination performed by the shelter veterinarian. On the basis of observation of dogs' behaviour by shelter staff and a certified

ECVBM-CA examiner (DVM, M.C.O.), dogs exhibiting aggression or obvious timidity were not entered into the study. Subjects were selected from among all those dogs that did not appear likely to bite (i.e., we did not choose dogs that barked aggressively at the front of the cage) because of the safety of the operators. We rarely chose dogs that either retreated to the back of the cage or trembled at our approach because these behaviours could be related to the manifestation of the symptoms associated with phobias and anxiety disorders, usually accompanied by an increase of sympathetic activity that could confound the HRV analysis. The clear majority of the dogs in the two rooms from which subjects were selected were highly socialized toward humans as evidenced by such behaviours as tail-wagging and licking. In all, the sample appeared to be quite representative of the population of dogs that might be considered for adoption at many public shelters.

Estimates of age were aided by inspection of dentition (presence of absence of puppy teeth, spacing and wear of teeth). Both sexually intact and gonadectomised males and females (non-lactating) were included. Consequently, the sample approximated the population of dogs commonly available at the shelter.

Then a pool of 42 potential dogs were submitted to cardiovascular screening (DVM, MsC Cardiology examiner; P.S.), consisting in electrocardiogram (ECG), blood pressure measurement, echocardiogram (Echo). A 5-min, 12 standard leads ECG (Esaote Archimed 4210, Firenze, Italy) was performed on unsedated animals, in right lateral recumbency (Kraus et al., 2002). Doppler method was used to measure blood pressure (Vettex Duo, Huntleigh Technology, Cardiff, United Kingdom). The Echo (Caris, 2.5-3.5 and 7.5-10 convex probes, Esaote Byomedica, Firenze, Italy) was performed according to the American Society of Echocardiography guidelines (Sahn et al., 1978; O'Rurke et al., 1984). Exclusion criteria were the presence of cardiovascular disease, arrhythmias, marked sinus arrhythmia, systemic hypertension or history of medical illness.

Electrocardiogram variables included: P wave duration (the first wave of the ECG corresponding to atrial depolarization), PR wave interval (the period of time from the onset of the P wave to the QRS complex; e.g. the time between atrial and ventricular depolarization), QRS complex duration (the QRS complex is the deflection in the tracing of the ECG, comprising the Q R, and S waves that represent the depolarization of the ventricles), QT wave interval (the time for both ventricular depolarization and repolarization to occur).

Echocardiogram was performed to identify dogs without congenital or acquired heart diseases which could lead to an activation of compensatory mechanisms, such as a catecholaminergic response, that could affect the sympato-vagal balance. Variables included: ejection fraction, shortening fraction, left atrium/aortic root ratio, end diastolic left ventricular diameter, end systolic left ventricular diameter, early diastolic filling wave (E wave) deceleration time, early/late diastolic filling of transmitral flow ratio

and E-point septal separation (E-point: maximum opening of the mitral valve leaflets during rapid ventricular filling in early diastole; E-point septal separation: the distance between E-point and the interventricular septum).

The final group of 20 dogs of various and mixed breeds underwent to the preliminary temperament test (Van der Borg et al., 1991-modified-) performed by a certified ECVBM-CA examiner (M.C.O.). All dogs were videotaped (Canon digital camera mv550i, Canon, Italy) during the entire test. According to scores and gender, two groups of dogs of similar temperament were determined. The dogs were randomly assigned to the study group (group A) or to the control group (group B). The two groups contained equal numbers of dogs (10 dogs each), sex (group A= six neutered female, four male; group B = six neutered female, four male, including one castrated). Salivary cortisol samples (basal cortisol) have been collected from all the dogs 1 week after the cardiovascular screening. Just before starting the experimental sessions, one dog (neutered female) was removed from the control group because she was adopted. All dogs included into the two groups were only handled by two shelter operators (E.P.; A.D.) for the duration of the study. These operators cared for the dogs providing them food and bringing the dogs to the exercise area, except for interaction. The behavioural intervention applied to the study group dogs was performed by the same handler (DVM, MsC Behaviour, L.O.) for the duration of the study. Throughout the study the normal routine of the dogs was respected and neither the composition of the couples nor their location within the kennels was in any way modified. All individuals involved with the study, except for L.O., and shelter staff working with the dogs was blinded as to the identity of the dogs included into the two groups.

2.3. Housing

At the Municipal Dog Shelter run by the City of Turin, Italy, dogs were housed in pairs. The shelter covers an area of 23,625 m^2 and is composed of units dedicated to the housing of cats and dogs, lecture halls, a clinic and offices. A field, measuring a further 10,000 m^2 and divided into seven fenced areas, is used as an exercise area for the dogs. Overall there are eight kennels dedicated to the housing of the dogs: six 'winter' and two 'summer'.

Each of the 'winter' kennels, contains 14 units, that occupy a total space of 320 m^2 divided into internal and external parts, of which 111 are internal. Each unit is made up of an inner area of roughly 2 m x 2 m and an external run of approximately 2 m x 7 m, partially covered by a roof; these areas are connected by a passage fitted with a sliding door measuring 55 cm x 70 cm to allow dog passage. The inner area contains dog beds, food and water and is maintained at a temperature between 12 °C and 14 °C, thus ensuring the animal's comfort even during the coldest periods. A central corridor in the unit allows the caregivers to distribute meals, and observe the dogs.

Twice daily the dogs are exercised in the open area where they are allowed to run without a leash.

Normally these exercise sessions occur between 8:00 and 17:00 h and last for 20min. The units are cleaned daily between 8:00 and 9:00 h when the dogs are outside. Feeding is provided daily between noon and 13:00h; it is composed of 80% dried food and 20% canned food for all dogs except those restricted to a specific diet. Fresh water was available ad libitum. In the 'winter' kennels dogs are confined to the heated indoor portion of their kennel overnight (17:00 to 8:00 h) then allowed free access to both areas for the remainder of the day.

2.4. Experimental design

The study included four experimental sessions (T0, T1, T2 and T3). At T0 salivary cortisol samples (basal cortisol) were collected from all dogs. After 1 week, all dogs were submitted to the following procedures: holter monitor unit application and recordings, saliva cortisol sample collection before behavioural test (pre-test cortisol), behavioural test application and saliva cortisol sample collection after behavioural test (post-test cortisol). The above-mentioned experimental session was repeated every 4 weeks from the beginning of the study (T1, T2 and T3). All dogs were randomly behaviourally tested (Lucidi et al., 2005) and the investigators/handlers were blinded to grouping.

Once the T1 session was complete, the control group (group B) continued to follow the standard management, while the study group (group A) was submitted to a behavioural intervention consisting in 20min sessions of supplemental human interaction and positive reinforcement based educational skill training occurring 3 days each week for a period 8 weeks.

2.5. Holter application and recordings

Electrocardiogram holter monitor recording equipment included a digital recording unit (Aria® Digital Holter Recorder, Del Mar Reynolds Medical, Hertford, United Kingdom) and four electrodes for three ECG channels (sampling rate: 128 samples/s). Prior to electrode application the skin surface was thoroughly cleansed with ether. In an effort to avoid detachment and artefact development the electrodes were not applied until the skin surface was completely dry. Both sides of the chest were clipped caudal to the front leg, allowing the placement of two electrodes for each emithorax. The electrodes wires were attached and the recording unit was kept in place by adhesive bandaging material (Tensoplast®, BNS Medical, Willerby, United Kingdom) and adhesive wrap (3 MT^{M} Vetrap^{T}M, 3M, Italy). One hour holter monitoring was recorded for all the dogs at each experimental session. All the dogs were well acclimatized to the recording device before the onset of behavioural data collection for a period of 20min at each experimental session. The time of the day was standardized for all the dogs. An event diary was recorded: beginning, behaviour tests and end times were noted.

2.6. Salivary cortisol collection and analysis

All the saliva samples (Kobelt et al., 2003-modified-) were collected at the same hour for each dog along the experimental sessions. Basal cortisol saliva samples were collected without any disturbance applied to the dogs. Pre-test cortisol samples were collected within 4-5 min of the onset of disturbance (e.g. holter application); post-test cortisol samples were collected immediately after the behavioural test was performed, but prior to the removal of the holter device. Saliva samples were collected using a cotton roll with no citric acid addition (Salivette, Sarst-edt, Verona, Italy) since concentrated acids can interfere with immunoassay (Kirschbaum and Hellhammer, 1994). To increase salivary flow the dogs were allowed to smell approximately one-half of a hot dog sliced into small pieces. The swab was gently put under the dog's tongue and in the cheek pouches for 60 s. Then the cotton roll was placed back in the labeled salivette tube, placed on ice and brought to the laboratory within 2 h from the collection. Samples were stored at -20 °C until time of assay. Saliva cortisol levels were measured by RIA analysis (Cortisol Ria Test, Pantec, Torino).

2.7. Behavioural tests and recording

Each dog was videotaped remotely (Canon digital camera mv550i, Canon, Italy) for the whole duration of the preliminary temperament test (Van der Borg et al., 1991-modified-) as well as during all the behavioural tests at the different times of the experimental sessions. The temperament test procedures included a set of 18 tests that were developed to test four problem-related characteristics: (I) aggression, (2) fear, (3) obedience and (4) separation anxiety. In addition a category of problem-related "miscellaneous behaviours" (e.g. pulling on leash, jumping up at or mounting people, chasing joggers) in shelter dogs was included in the assessment. The procedures in each test were standardized as far as possible and each dog was exposed to the same sequence of tests. On average it took 30 min to test each dog. The test procedure was carried out by three people: one female investigator that was the handler (ECVBM-CA, M.C.O.), one male (A.B.) and one female
(M.M.) investigator that assisted with the set-ups. Briefly, the test procedures consisted in the following tests. Outdoor. "Friendly approach by an unfamiliar person": the handler approached the dog from the front in its kennel, without staring at the dog and after 10 s called its name in a friendly tone, "Put on leash": the handler entered the kennel and put the leash on the dog, "Walk on leash": 5 min outdoor, "Basic commands": sit, down, come, follow, "Play with handler": i.e. ball, "Take away play object", "Tug of war", "Unfamiliar jogger": an unfamiliar jogger would run about 1 m from the dog
"Sound of a car horn". Indoor (small room, referred as a living room inside the shelter facilities, far from the visiting room and the public waiting room). (10) "Sound of the door-bell" (two different opular types), (II) "Exploration of the room(1 min)": the handler would hold the dog on the leash, "Left alone in the room (1 min)", "Threatening by person": the front of the dog was approached by a stranger, who stared at the animal for 10 s, then taking one step forwards in the dog's direction; the person neither

moved nor looked at the dog's direction for 10 s. Since dogs may be different in their reaction to man and woman (Lore and Eisenberg, 1986), this procedure was repeated with a woman (M.M.) and a man (A.B.) as stimulus, "Approach by doll (height: 120cm)": the dog was faced with a life-sized dressed doll on wheels, representing a child of 5-6 years old; at first the dog was free to interact with the doll, then the handler

Table 1

Preliminary temperament test evaluation (Van der Borg et al., 1991-modified-). The numbers in parentheses correspond to the number of the test used in the test procedure.

Temperament component	Test reference	Score
Aggressiveness toward people	Take away play object (6) Unfamiliar jogger (8) Threatening by person (13) Take away food-bowl while eating (15) Approach by doll (14)	0 positive ↓ 3 negative
Aggressiveness between dogs	Interaction with unfamiliar dog (18)	0 positive ↓ 3 negative
Hesitant toward stimuli	Sound of a car horn (9) Sound of the door-bell (10) Left alone in the room (12) Putting up of umbrella (16) Walk on leash in front of the cattery and confrontation with cats (17)	0 positive ↓ 3 negative
Attraction toward people	Friendly approach by unfamiliar person (1) Put on leash (2)	3 positive ↓ 0 negative
Exploration	Walk on leash (3) Exploration of the room: the handler holds dogs to the leash (11)	3 positive ↓ 0 negative
Training	Basic commands (4) Play with the handler (5) Tug of war (7)	3 positive ↓ 0 negative
Food motivation	Overall evaluation	3 positive ↓ 0 negative
Dominance	Overall evaluation	0 positive ↓ 3 negative

approached the doll, talked to it and made physical contact with it. "Take away food-bowl while eating", "Putting up of umbrella". From indoor to outdoor. "Walk on leash in front of the cattery and confrontation with cats in the kennels (2 min)", "Interaction with unfamiliar dog (same size and sex)".

Behavioural test procedures performed at T1, T2 and T3 consisted of the following tests.

(1) The handler approached the front of the dog in its kennel, without staring at the dog for 10 s,
(2) The friendly handler approached the dogs for 10 s,
(3) The handler entered the kennel, interacted with the dog and put the leash on the dog (30 s),
(4) The handler held the dog to leash and walks outside the kennel without any other stimulus to reach the exercise area (1 min),
(5) Basic commands in the exercise area with rewards (1 min),
(6) Coming back to the kennel and meeting an unfamiliar person (1 min),
(7) The dog entered the kennel; the handler interacted with the dog and put off the leash (30 s),
(8) The handler left the kennel and stared at the dog from distant (30 s).

In between each test a 2.5 min pause was performed, during which the handler neither moved nor stared at the dog to prevent any kind of interaction (she turned her shoulder frontally to the dog) and to better detach each test stimulation.

Both the preliminary temperament test data (Van der Borg et al., 1991-modified-) and the behavioural test data (Lucidi et al., 2005) at T1, T2 and T3 were analyzed and scored (Tables 1 and 2).

2.8. Human interaction program

The behavioural intervention consisted of human interaction and positive reinforcement based educational skill training that occurred 3 days each week for a period 8 weeks. Human interaction program with the dogs took place in their kennel and outside in a fenced enclosure. Briefly, the contact session included playing with the dog outside, walking on leash, grooming activities, playing inside with toys, obedience commands; treat rewards, and verbal and tactile contact. Each session lasted an average of 25 min (30-20 min) and was conducted by the same female human handler. The sessions varied in length because of individual animal variation in receptiveness, fear, socialization and

playfulness. The female handler for the program of human interaction (DVM, MsC Behaviour, L.O.) was trained prior to participating in the study by a certified behaviourist (ECVBM-CA, M.C.O.). This training involved all aspects of the handler's interactions with the dogs, including the

Table 2

Behavioural test used at the different experimental sessions T1, T2 and T3. Components, variables, and scores are reported. Reference test refers to each of the behavioural test procedures.

Reference test	Component	Variable	Behavioural description	Score
1	Initiative	The handler approach the dog's environment	The dog does not go close to the handler	0
			The dog jumps onto the fence repeatedly or stands on its hind legs against the fence for a long time	1
			The dog jumps onto the fence once or approaches the fence hesitantly	2
			The dog goes near the fence without jumping	3
2	Sociability/diffidence	The handler approaches the dog	The dog runs away	0
			The dog does not go near the handler or he approaches the handler then go back	1
			The dog rushes near the handler	2
			The dog approaches the handler	3
			The dog approaches the handler, wags its tail and licks the handler's hands	4
3	Sociability/diffidence	The handler goes into the kennel	The dog runs away	0
			The dog does not go near the handler	1
			The dog rushes near the handler or stands on its hind legs	2
			The dog approaches the handler	3
			The dog approaches the handler, wags its tail and licks the handler's hands	4
4	(a) Initiative	The handler holds the dog to leash and walks outside the kennel	The dog walks out ahead the handler	0
			The dogs walks out together with the handler	1
			The dog walks out after the handler	2
	(b) Walking on a leash	The handler walks with the dog on a leash	The dog does not walk	0
			The dog insistently draws on the leash	1
			The dog draws sometimes	2
			The dog walks without drawing	3
	(c) Elimination	Number of eliminations/marking behaviour	None	0
			1–3	1
			More than 3	2
	(d) Aggressiveness	Attitude toward other dogs when the dogs walks in front of their kennels	The dog rages against with anger	0
			Indifferent or afraid	1
			Friendly	2
5	(a) Temperament	The handler approaches the dog in a friendly way and touches it all over	The dog runs away or becomes restless or jumps on the handler	0
			The dog let the handler to pet him but he is hesitant or not interested	1
			The dog approaches the handler, licks the handler's hands and wags its tail	2
	(b) Basic commands	Comprehension of the command and ability to quickly execute the command given by the handler	The dog does not sit down	0
			The dog sits down with problems	1
			The dog sits down easily	2
6	(a) Sociability/diffidence toward unfamiliar male person	Unfamiliar male person friendly approaches the dog held by the handler	The dog runs away or is afraid	0
			The dog is indifferent	1
			The dog looks for the handler or is hesitant toward the unfamiliar male	2
			The dog approaches the unfamiliar male	3
			The dog is very friendly, licks the male's hands and wags its tail	4
	(b) Sociability/diffidence toward unfamiliar female person	Unfamiliar female person friendly approaches the dog held by the handler	The dog runs away or is afraid	0

Table 2 (*Continued*).

Reference test	Component	Variable	Behavioural description	Score
			The dog is indifferent	1
			The dog looks for the handler or is hesitant toward the unfamiliar female	2
			The dog approaches the unfamiliar female	3
			The dog is very friendly, licks the female's hands and wags its tail	4
7	Sociability/diffidence	The handler interacts with the dog before going out the kennel	The dog runs away	0
			The dog first goes away then approaches the handler	1
			The dog rushes toward the handler or jumps on the handler	2
			The dog stands	3
			The dog wags its tail, licks the handler's hands or sits down quietly	4
8	Initiative	The handler leaves the kennel and stares at the dog from distant	The dog goes to the inner part of the kennel and stays there	0
			The dog exhibits stereotypic behaviour (spinning or tail chasing, intense barking)	1
			The dog jumps onto the fence repeatedly or stands on its hind legs against the fence for a long time	2
			The dog goes near the fence without jumping and it stands watching or walks quietly then it goes into the kennel	3

manner in which the dogs were stroked and massaged, the handler's tone of voice in interacting with the dogs, the handler's positioning and posture in the kennel, and the manner in which commands were given and reinforcements were offered. To further ensure conformity during the study, the handler followed a systematic, detailed protocol for the program of human interaction.

2.9. Holter analysis and heart rate variability calculation

Electrocardiogram holter data were analyzed with a digital software system (CardioNavigator Plus, Impresario, Del Mar Reynolds, Hertford, United Kingdom). All the segments between two consecutive R waves (RR interval, ms) were automatically scanned and afterwards manually reviewed and edited by an experienced operator (DVM, MsC Cardiology; P.S.). All abnormal QRS complexes (ectopic beats and other arrhythmic events) and artifacts were excluded. According to the literature (Task force, 1996), the following time domain variables were calculated: the Standard Deviation of the Normal to Normal Intervals (SDNN, ms; NN intervals can also be referred as RR intervals); the Root Mean Square of the Standard Deviation (RMSSD, ms); the Standard Deviation of the Successive Differences (SDSD, ms); the Heart Rate Variability Index (HRV-I, ms); the Triangular Interpolation of the NN interval histogram (TINN, ms); the Mean RR interval (Mean RR, ms); the Mean Heart Rate (Mean HR, ms); the percentage

of the total number of all RR intervals of pairs of adjacent RR intervals differing more than 50 ms over the entire recording (Pnn50); the Minimum Interval Heart Rate Variability (Min interv HRV, ms); the Maximum Interval Heart Rate Variability (Max Interv HRV, ms); the Standard Deviation of NN Index (SDNN-I, ms); the Standard Deviation of the Average NN expressed in beats per minute (SDANN-bpm); the Standard Deviation of the Average NN expressed in ms (SDANN-ms).

The time domain analysis was performed on the whole holter recording session (mean duration = 60 min) and for the whole behavioural test procedures (mean duration =30 min).

The frequency domain analysis was done on the RR interval using a Fast Fourier Transform (FFT; sampling rate: 1024 s), applying a Hanning window, on the whole behavioural test session at the different experimental times. In addition, four replications of 300-s epochs were selected from the preliminary 24 min of the behavioural test session order to analyze 5 min of holter recording separately at the different experimental times. In detail, in the first 5 min the handler approached the dog from the front of its kennel, without staring at the dog for 10s then the friendly handler approached the dog for 10 s. In the second 5 min the handler entered into the kennel, interacted with the dog and put the leash on the dog (30 s); then the handler held the dog on a leash and walked outside the kennel without any other stimulus to reach the exercise area (1 min). In the third 5 min basic commands with rewards took place in the exercise area (1 min). In the fourth 5 min the dog came back from the exercise area to the kennel and met an unfamiliar person (1 min) then the dog entered the kennel; there the handler interacted with the dog and put off the leash (30 s). Spectral analysis of the whole behavioural test session holter recording was performed using an autoregressive method (Burg algorithm) with Cubic Spline interpolation, as well.

The following frequency domain variables were calculated: High Frequency HRV (HF, ms^2); Low Frequency

Table 3

Age, weight, blood pressure, electrocardiogram and echocardiography data of group A and group B dogs when they were enrolled in the study. These data refer to the cardiovascular screening the dogs underwent before receiving any behavioural intervention.

Variable	Group A (mean ± SEM)	Group B (mean ± SEM)
Age (year)	3.40 ± 0.37[a]	3.56 ± 0.34[a]
Weight (kg)	25.05 ± 1.51[a]	24.78 ± 1.64[a]
Pressure (mmHg)	167.50 ± 8.95	180.00 ± 8.12
P wave duration (ms)	45.60 ± 1.43	41.78 ± 0.70
PR wave interval (ms)	107.10 ± 4.11	106.11 ± 3.08
QRS complex duration (ms)	54.40 ± 2.08	52.89 ± 2.14
QT wave interval (ms)	184.10 ± 5.93	183.89 ± 5.27
Ejection fraction (%)	60.10 ± 4.64	62.44 ± 3.86
Shortening fraction (%)	32.50 ± 3.40	34.11 ± 2.69
Left atrium/aortic root ratio	1.27 ± 0.05	1.33 ± 0.09
End diastolic left ventricular diameter (cm)	3.87 ± 0.12	4.17 ± 0.10
End systolic left ventricular diameter (cm)	2.61 ± 0.17	2.75 ± 0.17
E-point septal separation (cm)	0.49 ± 0.04	0.44 ± 0.04
E wave deceleration time (ms)	110.20 ± 6.77	120.33 ± 12.67
Early/late diastolic filling of transmitral flow ratio	1.41 ± 0.10	1.26 ± 0.08

[a] Median values; P wave: the first wave of the electrocardiogram corresponding to atrial depolarization; PR wave interval: time between atrial and ventricular depolarization; QRS complex: the deflections in the electrocardiogram, comprising the Q, R, and S waves, that represent the depolarization of the ventricles; QT wave interval: time corresponding to ventricular repolarization; E-point (maximum opening of the mitral valve leaflets during rapid ventricular filling in early diastole) septal separation: the distance between the E point and the interventricular septum; E wave: early diastolic filling wave.

HRV (LF ms^2); Very Low Frequency HRV (VLF, ms^2); High frequency to Low frequency ratio (LF/HF); Maximum Frequency RR intervals (F Max RR, ms); Minimal Frequency RR intervals (F Min RR, ms); mean duration of RR intervals (Mean RR, ms); Standard Deviation RR intervals (SD RR, ms); Total Number of Interpolated Beats (N° INT BEAT); Total Number of Used Beats (N° TOT BEAT).

According to literature (Calvert and Wall, 2001), the variables measured in the frequency domain followed these specific ranges: VLF 0.0033-0.04 Hz; LF 0.04-0.15 Hz; HF 0.15-0.4 Hz.

2.1 *0. Statistical analysis*

Descriptive statistical analysis was used for age, weight, blood pressure, EGC, Echo data, holter, and cortisol data.

Shapiro-Wilk technique for normality was employed to check the Gaussian distribution of behavioural, holter and cortisol data.

Based on test responses (Van der Borg et al., 1991 -modified-) and on dogs' behavioural recorded observations, which included eight temperament components, the dogs were evaluated and scored during the preliminary temperament test.

Behavioural data at T1, T2 and T3 were analyzed (Lucidi et al., 2005). For each behavioural test procedure components, variables, behavioural descriptions and score were recorded for each dog at each time point. Score differences for each dogs included into the two groups throughout the experimental sessions have been used for statistical analyses. Behavioural data collected at T1, T2 and T3 have been statistically processed using Fisher's exact test and Bonfer-roni's correction.

On holter and cortisol data one-way ANOVA in a repeated measure and Kruskal-Wallis one-way analysis of variance by ranks were applied for each group throughout the experimental sessions. Unpaired Student's t-test and Wilcoxon rank sum tests have been used as post hoc tests and to compare data between the two groups at each experimental session, as well. In detail, one-way ANOVA for repeated measures and Kruskal-Wallis one-way analysis of variance were performed separately on each group (groups A and B) through the all study sessions (T1, T2 and T3) for each frequency and time domain variables of the HRV. Each variable of the HRV was compared at each session time (T1, T2 and T3) between each group (groups A and B) using unpaired t-test and Wilcoxon test.

The statistical analysis of the holter (FFT method and autoregressive method), the behavioural and cortisol data was completed by using the Kendall tau rank correlation coefficient.

All the analyses have been performed using the software R v. 2.3.0. All data were expressed as mean ± SEM, and the probability value was set at $P< 0.05$.

3. Results

3.1. Behavioural test

Behavioural data showed significant improvements in sociability/diffidence component in group A for test 2 (P=0.0031; Odds ratio = 39; i.c. = 1.720-884.310), test 6b (P=0.001; Odds ratio = 72; i.c. = 3.839-1350.500), test 7 (P=0.0031; Odds ratio = 39; i.c. = 1.720-884.310). Test 5a has been statistically improved in group A (P= 0.00185; Odds Ratio = 14; i.c. = 1.540-127.290).

3.2. Cardiovascular screening

Age, weight, blood pressure, ECG and Echo data of the two groups are reported in Table 3.

Twenty-two subjects were excluded from the original sample of 42 because of the presence of marked sinus arrhythmia (11 dogs), chronic mitral valve disease (seven dogs), first degree atrio-ventricular block (one dog), ventricular premature complexes (one dog), occult dilated cardiomyopathy (one dog) and systemic hypertension (one dog). One dog was excluded because of the adoption at the beginning of the study.

Table 4

Five minutes heart rate variability (HRV) variables in frequency domain analysis at the different experimental sessions T1, T2 and T3.

HRV variable	Timing	Group	T1 Mean ± SEM	T2 Mean ± SEM	T3 Mean ± SEM
HF (ms^2)	First 5 min	A	7880.06 ± 1998.43	7673.25 ± 1509.13	5686.38 ± 1854.13
		B	5290.11 ± 1093.53	5921.17 ± 2274.80	6337.00 ± 1949.67
	Second 5 min	A	3992.95 ± 900.21	7088.70 ± 2940.03	4805.39 ± 1383.98
		B	1723.39 ± 303.31	2574.92 ± 681.29	5003.97 ± 2189.51
	Third 5 min	A	2915.84 ± 717.77	1745.88 ± 299.79	2748.07 ± 771.70
		B	2029.98 ± 470.03	1975.58 ± 281.78	1597.90 ± 280.91
	Fourth 5 min	A	4048.87 ± 954.49	2574.19 ± 501.56	2425.93 ± 654.09
		B	1901.97 ± 425.84	1972.15 ± 339.07	1759.52 ± 342.36
LF (ms^2)	First 5 min	A	2425.49 ± 439.43	2761.83 ± 533.29	2166.06 ± 496.29
		B	2951.21 ± 551.80	3322.99 ± 955.56	3280.49 ± 822.47
	Second 5 min	A	1814.82 ± 302.57	2381.73 ± 785.53	2153.47 ± 532.83
		B	1671.93 ± 366.97	1845.38 ± 343.54	2394.31 ± 900.20
	Third 5 min	A	1972.06 ± 393.10	1243.26 ± 107.78	1862.62 ± 433.50
		B	1770.30 ± 388.73	1854.63 ± 310.42	1737.66 ± 230.25
	Fourth 5 min	A	1959.04 ± 340.97	1430.19 ± 291.32	1364.23 ± 288.31
		B	1907.74 ± 429.42	1759.19 ± 265.70	1680.12 ± 195.68
VLF (ms^2)	First 5 min	A	1671.64 ± 325.65	1604.64 ± 203.34	1838.57 ± 471.64
		B	1685.01 ± 302.57	1446.15 ± 451.76	1414.31 ± 314.13
	Second 5 min	A	1068.24 ± 341.91	1928.04 ± 581.27	1089.13 ± 187.09
		B	1414.29 ± 285.37	955.26 ± 214.25	1032.66 ± 352.84
	Third 5 min	A	594.17 ± 111.35	938.41 ± 202.58	565.15 ± 166.18[a]
		B	937.12 ± 266.66	1082.50 ± 277.50	1008.36 ± 150.64[b]
	Fourth 5 min	A	802.05 ± 135.08	464.38 ± 70.52	646.12 ± 174.33
		B	1022.57 ± 312.56	847.27 ± 249.44	851.84 ± 132.48
LF/HF	First 5 min	A	0.41 ± 0.08	0.44 ± 0.08	0.60 ± 0.13
		B	0.70 ± 0.16	0.95 ± 0.38	0.94 ± 0.26
	Second 5 min	A	0.68 ± 0.14	0.44 ± 0.10[a]	0.58 ± 0.11
		B	1.31 ± 0.38	1.19 ± 0.29[b]	0.74 ± 0.14
	Third 5 min	A	0.92 ± 0.15	0.87 ± 0.12	0.99 ± 0.19
		B	0.94 ± 0.14	0.98 ± 0.13	1.26 ± 0.20
	Fourth 5 min	A	0.68 ± 0.12[a]	0.70 ± 0.14	0.94 ± 0.21
		B	1.05 ± 0.13[b]	1.07 ± 0.16	1.09 ± 0.15
SDRR (ms)	First 5 min	A	146.30 ± 12.86	155.65 ± 9.86	132.66 ± 15.91
		B	144.63 ± 17.11	143.91 ± 17.91	144.53 ± 21.56
	Second 5 min	A	135.64 ± 13.94	146.38 ± 19.29	126.93 ± 15.56
		B	110.23 ± 6.13	120.17 ± 10.49	125.89 ± 18.10
	Third 5 min	A	110.25 ± 8.47	86.23 ± 4.20[a]	97.67 ± 9.77
		B	103.46 ± 9.43	108.92 ± 6.49[b]	102.80 ± 7.41
	Fourth 5 min	A	129.34 ± 14.32	115.35 ± 8.98	108.88 ± 9.54
		B	121.62 ± 9.07	122.20 ± 9.92	114.44 ± 6.60

[a,b]Different letter for a same variable indicates a significant difference between the two groups (unpaired Student's *t*-test and Wilcoxon; $P < 0.05$). HF: high frequency; LH: low frequency; VLF: very low frequency; LF/HF: low frequency/high frequency ratio; SDRR: standard deviation of time duration between two consecutive R waves (RR interval) of the electrocardiogram.

All physiological parameters recorded during the experimental sessions remained within normal ranges (Fox et al., 1999).

3.3. Holter analysis

One-way ANOVA for repeated measures and Kruskal-Wallis test showed no significant variations in time and frequency domain variables in groups A and B.

Unpaired t-test and Wilcoxon test reported statistical differences between group A and group B in frequency domain variables (Table 4).

In details, 5 min analysis showed the following statistical variations: (a) LF/HF at the fourth 5 min at T1 (P=0.0435); (b) LF/HF at the second 5 min (P = 0.0279) and SD RR at the third 5 min at T2 (P= 0.0109); (c) VLF at the third 5 min at T3 (P= 0.022). On the whole behavioural test session holter recording statistical differences (autoregressive method—Table 5) have been reported for the following frequency domain variables: LF/HF at T1 (P= 0.0172) and T2 (P = 0.0041). Total number of Interpolated Beats (P= 0.001) and Total Number of Used Beats (P= 0.0101) reported statistical differences in overall holter analysis at T3 (Table 5).

Heart Rate Variability (FFT method) and behavioural data derived by test responses reported significant positive correlation (Tau = 0.45; P= 0.02) atT2 test 5b and significant negative correlation (Tau = -0.37; P= 0.04) at T3 test 3.

3.4. Cortisol

Salivary cortisol was measured for all samples that could be obtained in an adequate quantity. Therefore, all the dogs had saliva samples collected at T0, T1 and at T2 pre-test

Table 5

Heart rate variability (HRV) variables recorded during the whole behavioural test session (autoregressive method—HF, LF and LF/HF) and during the whole holter recording session (N. INT. BEAT; N. TOT. BEAT) at the different experimental sessions T1, T2 and T3.

HRV variable	Group	T1 Mean ± SEM	T2 Mean ± SEM	T3 Mean ± SEM
HF (ms^2)	A	5497.21 ± 1102.98	5326.67 ± 1209.88	4225.84 ± 981.69
	B	3067.87 ± 4295.29	3537.59 ± 781.91	3745.76 ± 926.86
LF (ms^2)	A	2374.57 ± 381.01	2091.66 ± 347.19	2431.47 ± 422.72
	B	2383.01 ± 315.11	2629.74 ± 414.88	2623.28 ± 427.42
LF/HF	A	0.58 ± 0.12[a]	0.49 ± 0.09[a]	0.78 ± 0.19
	B	0.83 ± 0.09[b]	0.86 ± 0.11[b]	0.89 ± 0.13
N. INT BEAT	A	8317.40 ± 561.89	8288.50 ± 385.59	8889.10 ± 441.93[a]
	B	8011.67 ± 539.37	8078.11 ± 339.85	7376.89 ± 319.60[b]
N. TOT BEAT	A	7874.50 ± 557.87	7768.90 ± 364.43	8354.00 ± 279.20[a]
	B	7673.44 ± 569.07	7616.78 ± 378.15	6988.33 ± 338.90[b]

[a,b] Different letter for a same variable indicates a significant difference between the two groups (unpaired Student's *t*-test and Wilcoxon; $P<0.05$). HF: high frequency; LH: low frequency; LF/HF: low frequency/high frequency ratio; N. INT. BEAT: total number of interpolated beats; N. TOT. BEAT: total number of used beats.

condition. Seventeen dogs had T2 post-test condition saliva sample, 14 dogs had T3 pre-test condition sample and 12 dogs had T3 post-test saliva sample.

All results of cortisol measurements of pre- and post-test conditions in the two groups at the different experimental sessions are summarized in Table 6.

No significant variations have been reported in basal cortisol levels between group A (mean = 2.72 nmol/L± 0.21) and group B (mean = 2.34nmol/L±0.19), as well as in pre- and post-test cortisol levels at the different experimental sessions. One-way ANOVA for repeated measures and Kruskal-Wallis test showed no significant variations in group B, whereas significant differences were reported in group A cortisol pre-test levels (P = 0.009164). In details, Wilcoxon test showed a significant decrease in salivary cortisol levels between T1 vs T2 (P= 0.003598) and between T1 vsT3 (P = 0.001162).

Cortisol post-test levels and Heart Rate Variability (FFT method) data reported no significant correlations in both groups.

Cortisol post-test levels and behavioural data derived by test responses reported significant correlations in both groups. In details, group A showed a positive correlation (Tau = 0.73; P=0.03) at T3 in test 3. Group B showed significant negative correlations at T2 in test 1 (Tau=-0.73; P=0.03), test 4c (Tau=-0.71; P=0.049) and test 5b (Tau = -0.83; P= 0.016).

Table 6

Cortisol levels at basal condition (T0), pre-test and, post-test conditions of the two groups at the different experimental sessions T1, T2 and T3.

Cortisol (nmol/L)	Group A	Group B
T0 basal	2.72 ± 0.21	2.34 ± 0.19
T1 pre-test	6.09 ± 0.46[a,b]	4.93 ± 0.94
Post-test	3.30 ± 0.31	3.13 ± 0.37
T2 pre-test	3.21 ± 0.62	3.11 ± 1.12
Post-test	1.76 ± 0.22	1.79 ± 0.28
T3 pre-test	2.38 ± 0.40	2.65 ± 0.77
Post-test	2.43 ± 0.47	1.26 ± 0.08

[a] Statistically different from T2.
[b] Statistically different from T3.

4. Discussion

HRV measured under rest conditions in dogs has been studied for the diagnosis and prognosis of heart diseases (Minors and O'Grady, 1997; Calvert and Wall, 2001), but less is known about the relationship between HRV and behaviour. Both sympathetic arousal and vagal tone alterations resulting from confrontational environmental challenges can influence cardiovascular function and subsequent HR. In farm animals and horses cardiac changes are used as marker of psycho-physiological stress (von Borell et al., 2007), mental stress (Rietmann et al., 2004) and temperament traits (Visser et al., 2002), assuming that increased level of stress and emotionality are reflected by an increased HR. The primary goal of the present study was to verify whether the autonomic responses to behavioural test procedures could be evaluated by short-term measures of HR. The behavioural test procedures applied at the different times elicited HRV measurable changes in responses to different tests. However, changes in posture (Maros et al., 2008) and locomotion (Vincent and Michell, 1996; Palestrini et al., 2005) can affect HR. An enhanced cardiac activity was reported during periods of increased locomotion, while no significant variations in HRV were observed. During attentive/orienting state in dogs a higher SDNNs were reported (Maros et al., 2008). Petting the dog can produce both deceleration and acceleration in canine HR; its effectiveness can only be assessed if a parallel decrease in HR could also be measured (Kostarczyk and Fonberg, 1982). Petting can have variable effects on dogs; the outcome of this activity is related to the relationship between the dog and the petter and the context of the activity. Increased vagal activity has occurred following massage therapy (Diego et al., 2005). The concept of the "vagal brake" (e.g. decreasing vagal activity) was introduced as a potential mechanism underlying the individual differences in vagal reactivity and in response to social/attention tasks (Porges et al., 1996). According to this concept, higher vagal tone and lower reactivity vagal tone appear to predict optimal development in human infants. Conversely, vagal break theory suggesting that low vagal activity is optimal during attention tasks seems inconsistent with high baseline vagal activity being optimal for cognitive development in infants/humans. This polyvagal model could be combined with motivational theory, which contrasts activation with inhibition and explains wide individual differences (Beauchaine, 2001). Vagal activity interacts with stress reactivity system including the hypothalamic-pituitary-adrenal axis and the immune system (Porges, 2001). Correlations between increased cortisol and decreased vagal activity were reported (Cacioppo et al., 1995; Gunnar etal., 1995).

In the current study, investigation focused on shelter dog receiving human interaction supplement. The aim of this study was to use indirect measures of autonomic tone and determine the role it plays with regards to "psycho-physiological" reactivity in dogs. Analysis of 5 min segments in frequency domain showed significant differences for some variables at T1, T2 and T3. At T1 in the fourth 5 min (coming back from the exercise area to the kennel, meet an unfamiliar person and interaction

with the dog and put off the leash) LF/HF ratio in group A was decreased compared to group B.This trend (i.e. a decreased LF/HF ratio in group A compared to group B) was observed throughout the experimental sessions. Group A showed higher HF values compared to group B in the experimental sessions (Table 4). The same results and trends were observed for the spectral analysis of the whole behavioural tests session holter recording (autoregressive method—Table 5). LF/HF ratio in group A was significantly decreased compared to group B at T1 and T2 (Table 5). Since T1 corresponds to the first session of the behavioural test without any human interaction program supplement, this trend (i.e. group A higher vagal tone) could represent an internal variability of the sample. The LF/HF ratio reduction observed in group A (autoregressive method) at T2 could be related to the human interaction program effect.

Within each sample in the fourth 5 min, group A showed a decrease in HF and LF from T1 to T3; group B showed a decrease in LF, but HF increased between T1 and T2, and then decreased.

At T2 in the second 5 min (the handler enters into the kennel, interacts with the dog, puts the leash on the dog and walks outside the kennel without any other stimulus to reach the exercise area) LF/HF ratio is statistically different between the two groups, being decreased in group A and showing this trend throughout the experimental sessions. This trend could be related to the increase in HF and LF between T1 and T2 and to the decrease in HF and LF between T2 and T3 as reported for group A. Group B showed an increase in HF and LF throughout the experimental sessions. The handler interaction with the dog and the petting behaviour could be related to the higher vagal tone in group A, which was already shown in the human interaction supplement program. Additionally, various psychological stimuli can affect HF and LF differently.

At T3 the N° INT BEAT and the N° TOT BEAT in group A was significantly increased compared to group B in the whole holter analysis. These results could be related to dogs with higher vagal tone displayed less signs of anxiety and fear during the behavioural test procedure, resulting in a good quality (e.g. fewer artifacts) of the holter recording.

Behavioural data showed improvements in sociability/diffidence and temperament in group A (test 2, 6b, 7, 5a). These results could support that some environmental changes, such as an increase of human interaction and educational skills, have a positive effect upon the behaviour and the welfare of sheltered dogs, resulting in a more appropriate behaviour of the dogs to be adopted. No improvement has been noted for test 6a (sociability/diffidence toward unfamiliar male person). This fact could be related to the operator/handler female gender to which the dogs were exposed throughout the human interaction program. LF/HF ratio (FFT method) and behavioural data showed correlations at T2 test 5b (basic commands) and atT3 test 3 (sociability/diffidence). LF/HF ratio showed a positive correlation with the comprehension of the command and the ability to quickly execute the command given by the handler at T2, whereas at T3 LF/HF ratio showed an inverse relationship with the event in which the handler went into the kennel.

Some authors (Boxall et al., 2004) described two socialization programmes, which were designed to compare the HR and behaviour of dogs which had received different degrees of socialization, habituation and training. The above-mentioned study showed that there was little difference in HR between the groups, but that there were significant differences in the scores for key behaviours. Thus, it would seem that canine behaviour does not reflect cardiac response in a consistent way, assuming HRV as a good indicator for the assessment of autonomic nervous system activity in response to psycho-physiological stress.

According to literature (Jones et al., 1990), a large degree of cortisol individual variation was found. This could justify no statistical differences between the two groups and no correlations with LF/HF ratio, although saliva cortisol levels taken during the experimental sessions showed a reduction from T1 to T3 within each group. When the dogs were undisturbed in their kennels and no restrain or holter application were performed, basal cortisol samples were collected. They were similar to those at T3. Cortisol changes may be non-specific, occurring in response to various challenges such as activity and poor emotional states (Selye, 1950; Rushen and de Passille, 1992) particularly within different housing conditions (Ladewig, 1987). This fact could reflect variations in genetics which lead to a diversity in dog responses to many stimuli, including stressors.

The dogs of both groups were regularly exposed to the operator who collected the samples, so the novelty of the sampling procedure was less effective in activating stress response. This can be interpreted as a reduction in perceived stimulus severity or habituation to the stimulus and the animal deemed "less stressed" and its welfare "better". This reduction may be due to the intrinsic control mechanisms designed to prevent prolonged increases in corticosteroid concentrations. The stress signal at higher brain levels may still be present and the animal may still be experiencing the stimulus as aversive (Smith and Dobson, 2002). However, contact with humans can moderate or prevent both hypothalamic-pituitary-adrenal axis activation and autonomic response to acute stressors (Hennessy

et al., 1998). Cortisol placebo responses were reported in humans (Johansen et al., 2003; Colloca and Benedetti, 2005). The placebo effect seems to be a phenomenon that can be learned either consciously or unconsciously. In the first case, an increased expectation is likely to occur after repeated associations of contextual cues with the outcome (Rescorla, 1988). In the second case, a mechanism of Pavlovian conditioning is likely to be involved, in which contextual cues and outcomes are unconsciously associated because of their contiguity. Pavlovian conditioning is important in the placebo responses of animals (Herrnstein, 1962; Siegel, 2002), in which the conscious processes are supposed to be absent or at least much less important.

5. Conclusion

Results from the current study can suggest that some environmental changes, such as an increase of human-animal interaction and educational skills, have a positive effect upon the behaviour of sheltered dogs. Besides, these data can suggest that human interaction supplement sessions could affect some physiological indicators of animal welfare.

In spite of the obvious homogeneity of the dogs studied, HRV measures showed very little correlation with behavioural data. On the contrary, cortisol post-test levels showed better correlation with behavioural data in both groups. In group A an opposite correlation at T3 in test 3 has been reported between cortisol data and HRV data when related to behavioural results.

Therefore, further studies need to be performed to determine more precise information on the effects of human-dog interaction on the autonomic nervous system assessment and on the ethological and hormonal markers in long-term sheltered dogs.

Disclosure statement

All the authors disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within 3 years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

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