CHAPTER 7

RESULTS OF ESTABLISHING PHYSIOLOGICAL PARAMETERS DURING HUMAN-ANIMAL INTERACTION

7.1 Biographical details of subjects

An analysis of first the human subjects and then the dog subjects will be given.

7.1.1 Humans

Humans (n = 18) were of European origin and the number of 18 was the total number of people who indicated that they are willing to participate in the experiment. All participants completed the experiment as arranged.

Half of the human subjects had their own dogs and nine interacted with unfamiliar dogs. Eight of the humans were male and 10 were females, of which four males and five females had their own dogs while four males and five females had dogs unfamiliar to them.

The ages of the subject ranged from 19 to 55. The average age for males (n = 8) was 32,4 and for females (n = 10) 28,1 years, while the average for all humans was 30 years.

7.1.2 Dogs

All the control (unfamiliar) dogs (n = 9) were Beagles. The experimental dogs (n = 9) were two Labradors, Labrador-cross, Border Collie, Border Collie-cross, Cocker Spaniel, Dachshund (standard, smooth hair), Staffordshire Bull Terrier and a Bull Dog-cross. All dogs completed the experiment as arranged.

Seven of the dogs were males of which three were castrated, and 11 were females of which five were spayed.

The ages of the dogs ranged from two years to 12 years. The average age for the males (n = 7) was 5,4 and for females (n = 11) 7,0 years, while the average age for dog subjects was 6,4 years.

The following table summarizes the characteristics of the subjects (Table 7.1):

Table 7.1: Characteristics of human and dog subjects participating in human-dog interaction (n = 36)

Humans	Gender	Age in years	Ethnic orientation
(n = 18)	8 males 10 females	19-55 30 average	European
Dogs	**Gender**	**Age in years**	**Breeds**
(n = 18)	7 males 11 females	2-12 6,4 average	9 Beagles (control) 3 Labradors 2 Border Collies 1 Cocker Spaniel 1 Dachshund 1 Staffordshire Bull Terrier 1 Bull Dog

7.2 Questionnaire to determine the state of anxiety of human subject

The average for all participants before and after interaction (n = 18 + 18 = 36) was 2,72 on a scale where the median is 3,00. The range of scores were 1,0 to 3,8. The results are reflected in Table 7.2.

Table 7.2: State of anxiety scores on a 5-point scale questionnaire before and after positive dog interaction

Groups	Average before dog interaction	Standard deviation	Average after dog interaction	Standard deviation
Whole group (n = 18)	2,72	0,81	2,68	0,77
Experimental group (n = 9)	2,58	1,00	2,64	0,88
Control group (n = 9)	2,87	0,60	2,72	0,70

Differences in the scores before and after dog interaction as well as differences (after - before) of the control and experimental groups were compared. The differences were not significant on a 95% confidence level ($p > 0,05$).

7.3 Interpretation of the results

It is not expected that the state of anxiety will change dramatically in less than an hour, except in cases where a pertinent threat is experienced during such a period. This was obviously not the case during the experiment. A slight decrease in the state of anxiety after intervention, however, could be an indication of emotional feelings being affected even after a very brief positive interaction with a dog. It may have also contributed to the decrease in blood pressure experienced by the subjects.

A mean score of 2,72 for the whole group is slightly lower than the median of 3,00 of the scale. None of the participants showed an unacceptable high score (i.e. >4) on the anxiety scale and this could be an indication that the participants who were healthy, adult, dog-loving persons, had no unacceptable levels of anxiety during the experiment. In other words, there were indications that from an emotional point of view, the participants complied with the definition for the human subjects. The absence of extreme anxiety on the day of the experiment can also contribute to the external validity of the results if one would consider to generalize the findings to the rest of balanced, healthy, dog-loving adults of the human population.

7.4 Pilot study

A pilot study was conducted in order to establish the feasibility of the experiment.

7.4.1 Aims and method

The aims of the pilot study were as follows:

- to establish whether a decrease in blood pressure could be taken as an indicator for neurochemical changes associated with positive human-dog interaction. Based on the pioneering work of Liebowitz[154] on positive human-human interaction, it

was decided to use plasma PEA[182,200,201,202,203,204,205] as an example of the neurochemicals to be investigated during positive human-animal interaction; and

- to establish whether an experiment of such a nature would have been logistically feasible.

The same method as for the main experiment was used, except that the blood pressures of the dogs were not recorded. Samples from the dogs were taken at the same time as those from the humans, that is after the humans' blood pressure has decreased.

All participants used their own dogs with which they had a positive relationship for more than a year. Only one of the participants in the pilot study, was used again for the main experiment. A different laboratory, namely from the Dept of Pharmacology, Faculty of Medicine, Medical University of Southern Africa, than the one used for the experiment, was used as well as a different analysis.[204] Plasma concentrations of ß-phenylethylamine was detected directly on a high performance liquid chromatography system (Microcep, Waters, USA).

7.4.2 Subjects of pilot study

Six adult humans (22-46 years) participated on a voluntary basis. Three were males and three females. The dog subjects were also adults (2-8 years), three females and three males. One of the males was castrated and 2 females were spayed. The breeds or cross breeds were 3 Labradors, 1 Bull Terrier, 1 German Shepherd Dog and 1 Collie.

7.4.3 Results of pilot study

Results indicated that statistically significant increases of PEA were recorded in the human subjects ($p < 0,04$) according to the t-test[197] as well as in the dog subjects ($p < 0,01$) (Table 7.3).

Table 7.3: Phenylethylamine (PEA) values in human and dog subjects before and after positive interaction (n = 6)

Subject	PEA(pg/100mℓ) before interaction	Standard deviation	PEA (pg/100mℓ) after interaction	Standard deviation
Humans	540		966	
	0		2780	
	0		2890	
	370		857	
	430		381	
	0		1930	
Average	223,3	250,6	1364,0	129,9
Dogs	0		6160	
	0		2540	
	780		3750	
	510		1904	
	1350		2850	
	390		2700	
Average	505,0	512,4	3317,3	174,5

7.4.4 Conclusion of pilot study

The pilot study indicated that decreased blood pressure could serve as an indicator to collect blood plasma for neurochemical analyses during positive human-dog interaction at least for the human subjects. It also proved that an experiment of this nature is possible from a practical point of view.

7.5 The main experiment

The independent variable is the positive interaction between healthy, dog-loving, adult humans and healthy, well-tempered, adult dogs. Controls are before versus after measurements (counter-balancing) of own dogs (experimental group) versus unfamiliar dogs (control group), dog interaction versus quiet book reading, followed by neurochemical and hormonal changes.

7.5.1 Results

The results of the following eight parameters as dependent variables are reported in histograms, which include the range, mean and median of blood pressure, phenylacetic acid (metabolite

of ß-phenylethylamine), dopamine, beta-endorphins, norepinephrine, oxytocin, prolactin and cortisol. The tables show the mean, standard deviation and median and the statistical significance of the values ($p < 0,05$). The block on the right hand side of every figure is explained as follows:

- □ Mean = Topline of the histograms
- \- Max = Maximum value
- \- Min = Minimum value
- ■ Median = Median of values.

7.6 Changes in MAP and selected biochemicals in humans interacting positively with dogs

MAP which was used as indicator for biochemical changes will be reported first, followed by plasma levels of the neuropeptides and hormones.

7.6.1 Changes in MAP

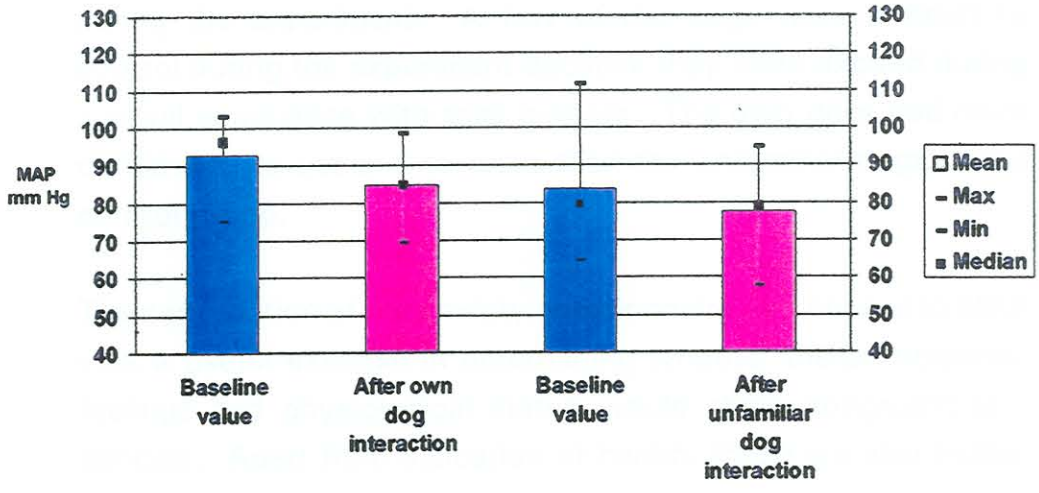

Figure 7.1: Mean arterial blood pressure (MAP) of humans before and after interacting positively with own (n = 9) and unfamiliar dogs (n = 9)

Table 7.4: Mean arterial blood pressure of humans (mmHg) before and after interacting positively with own dogs (n = 9) and unfamiliar dogs (n = 9)

MAP (own dogs)	Before	After	p-value	MAP (unfamiliar dogs)	Before	After	p-value
Mean	92,5	84,5	-	Mean	83,4	77,3	-
Standard deviation	9,0	8,2	-	Standard deviation	14,7	14,2	-
Median	96,2	84,5	-	Median	79,6	78,6	-
Significance	-	-	0,01	Significance	-	-	0,06

The time taken for MAP to decrease by 5-10% for blood collection, was six to 18 minutes for the owners and five to 24 minutes for human subjects with unfamiliar dogs.

From the results of the anxiety state questionnaire it was assumed that all the participants were healthy, non-anxious adults. The results of the blood pressure measurements supported this assumption, because the MAP of both groups fell within normal range. A higher baseline MAP for people in the experimental group could be attributed to their dogs' behaviour during the experiment. A few of the dogs were difficult to control during the experiment because they were excited during a novel experience with their owners. The own dogs had more confidence to act and to react than the unfamiliar dogs in the control group.

The contribution of the anxiety questionnaire as a control to MAP was a useful exercise in determining whether the participants' feelings and physiological status would show congruent tendencies. Apart from indication of health, these are also indications of the body-mind unity, and this in turn supports the theory that one physiological change can be used to measure other biochemical changes.

7.6.2 Changes in plasma neuro transmitters

7.6.2.1 Phenylacetic acid as metabolite of phenylethylamine

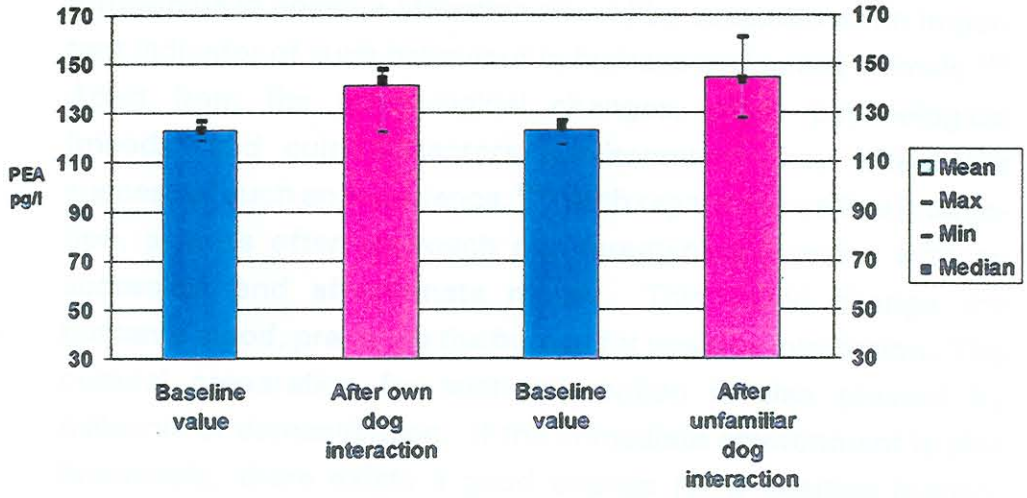

Figure 7.2: Plasma phenylacetic acid (PEA) of humans before and after interacting positively with own (n = 9) and unfamiliar dogs (n = 9)

Table 7.5: Concentrations of phenylacetic acid (pg/ℓ) in the plasma of humans before and after interacting positively with own dogs (n = 9) and unfamiliar dogs (n = 9)

PAA (own dogs)	Before	After	p-value	PAA (unfamiliar dogs)	Before	After	p-value
Mean	122,8	140,7	-	Mean	123,0	144,1	-
Standard deviation	2,4	7,7	-	Standard deviation	3,6	11,7	-
Median	123,0	143,0	-	Median	124,0	143,0	-
Significance	-	-	0,00	Significance	-	-	0,00

The results indicate that ß-phenylethylamine showed a significant increase ($p < 0,05$) after interaction.

Phenylethylamine is responsible for a feeling of elevated mood and decreased fatigue, because it is an amphetamine-like chemical. The feeling tends to happen instantaneously and voluntarily and it is of short duration, often as a first positive

reaction.[153] It is present in the limbic system where it acts as an emotion-regulator.[152,156]

Although other neurotransmitters such as dopamine and serotonin received more attention in recent literature, the role of phenylethylamine in positive affiliation should be accepted as an important indicator of such behaviour in humans and social animals.[158] Apart from the physiological changes, other psychological (moods) and cultural factors (environment) may affect the success of such an experience.[153] With regard to positive interaction, animals often approach non-threatening humans with an accessible and affectionate mood. This could change the human's mood, preparing the human for positive interaction. The cultural preparation for such interaction is also present by millennia of domestication. If the immediate environment is also favourable, there exists a good change for a positive human-animal interaction to succeed and to produce phenylethylamine effects.

7.6.2.2 Dopamine

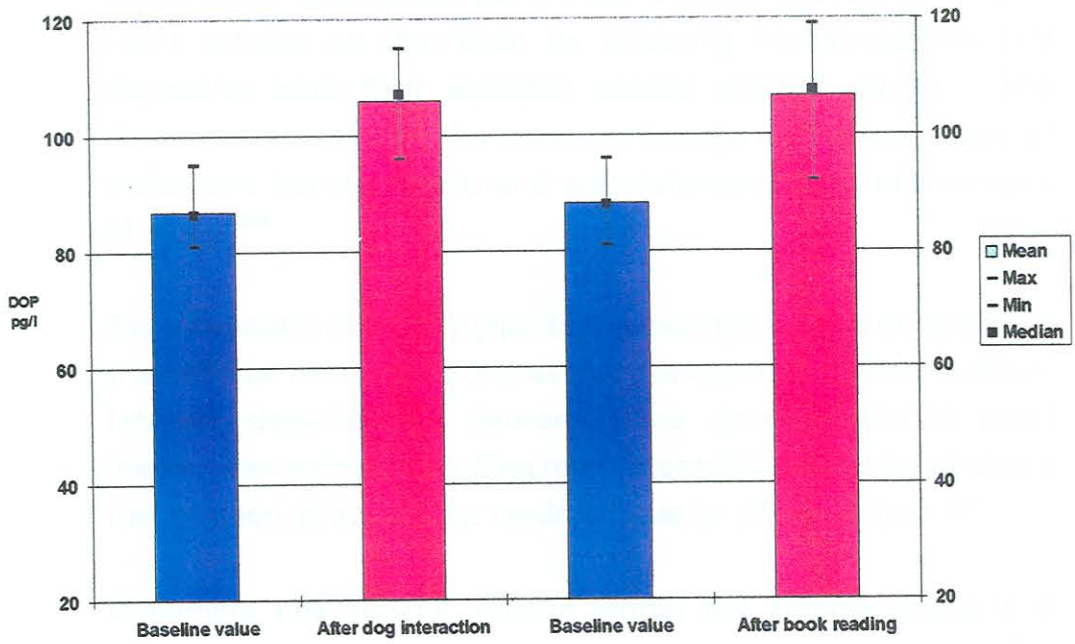

Figure 7.3: Plasma dopamine (DOP) of humans before and after interacting positively with own (n=9) and unfamiliar dogs (n=9)

Table 7.6: Concentrations of dopamine (pg/ℓ) in the plasma of humans before and after interacting positively with own dogs (n = 9) and unfamiliar dogs (n = 9)

DOP (own dogs)	Before	After	p-value	DOP (unfamiliar dogs)	Before	After	p-value
Mean	87,8	106,3	-	Mean	85,6	105,0	-
Standard deviation	4,8	5,2	-	Standard deviation	4,3	5,9	-
Median	87,0	108,0	-	Median	86,0	104,0	-
Significance	-	-	0,00	Significance	-	-	0,00

Positive human-dog interaction showed a significant increase ($p < 0,05$).

Dopamine is contained in relatively few neurons. These neurons are grouped together in restricted regions of the brain, mainly the raphe nuclei, the locus coeruleus and the substantia nigra respectively. These neurons project to virtually all regions of the central nervous system. Dopamine is synthesized from the amino acid tyrosine and it plays a role in regulating emotional responses. It also plays a crucial role in the control of complex movements. Dopamine appears to facilitate pleasurable sensations and is known to mediate the exhilaration that people seek in taking cocaine and amphetamines. These drugs produce their excitement actions on the brain by releasing norepinephrine and dopamine from their synaptic vesicle storage points. The dopamine neurons of the pons participate in the regulation of transitions between sleep and wakefulness and between stages of sleep.[205]

Experimental evidence supports the hypothesis that dopamine is a functional neuromodulator at many levels of the visual system. Intrinsic dopaminergic neurons were characterized in most mammalian retinas, including man. Contrast sensitivity of vision has to been shown to be modified due to this affection.[206]

Dopamine has several actions within the kidney where it is produced in the proximal tubule and dopaminergic neurons in the kidneys. It acts as a vasodilator, increases the renal blood flow, inhibiting renin secretion and inhibits NaCl and water reabsorption in the proximal tubule.[207]

Mesencephalic dopamine-containing neurons that innervate limbic regions, notable nucleus accumbens, are thought to be involved in the control of a variety of species-typical behaviours such as male copulatory behaviour, feeding and drinking. Their precise function remains, however, a source of conjecture.[208]

7.6.2.3 ß-endorphin

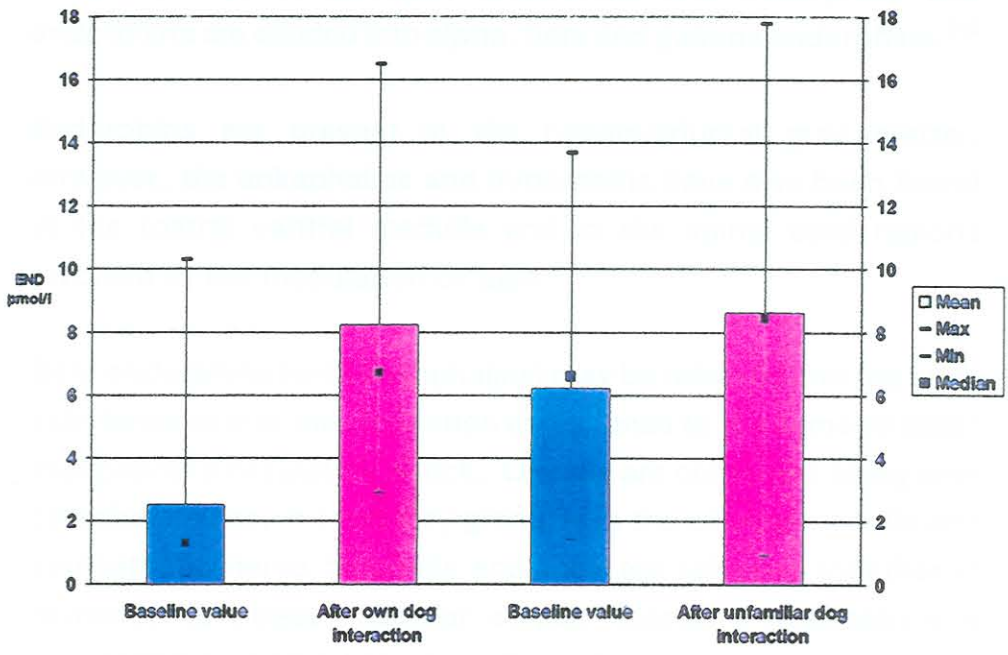

Figure 7.4: Plasma beta-endorphin (END) of humans before and after interacting positively with own (n=9) and unfamiliar dogs (n=9)

Table 7.7: Concentrations of ß-endorphin (pmol/ℓ) in the plasma of humans before and after interacting positively with own dogs (n = 9) and unfamiliar dogs (n = 9)

END (own dogs)	Before	After	p-value	END (unfamiliar dogs)	Before	After	p-value
Mean	2,5	8,2	-	Mean	6,2	8,5	-
Standard deviation	3,1	4,8	-	Standard deviation	3,9	5,3	-
Median	1,3	6,7	-	Median	6,6	8,4	-
Significance	-	-	0,00	Significance	-	-	0,00

The significant increase ($p < 0,05$) in endorphin concentrations in plasma, could indicate that it plays a role in stress-relief reported during human-animal interaction.[71]

The endorphins are endogenous opioids and are derived from the precursor protein ß-lipotropin. They have been found to elicit similar biological reactions as morphine, namely, analgesia, euphoria, respiratory depression and to slow the release of transmitters that activate contraction of intestinal muscles. There are three major classes of endogenous opioid peptides in mammals, namely, enkephalins, endorphins and dynorphins and endorphins are divided into alpha, beta and gamma endorphins.[209]

Endorphins are present in the periaquaductal gray matter, however, the enkephalins and dynorphins have also been found in the rostral ventral medulla and in the spinal cord regions involved in the modulation of pain.[209]

Beta-endorphins (with enkephalins) may be released into the brain substance or into the circulation in response to the same stresses that provoke circulatory shock. Opioids are contained along with catecholamines, in secretory granules in the adrenal medulla and sympathetic nerve terminals and they are released together in response to stress. Similar stimuli release ß-endorphin and adrenocortico-tropic hormones from the rostral pituitary gland. Opioids depress the brain stem centres that mediate some of the compensatory anatomic adaptations to blood loss, endotoxemia and other shock-provoking stresses. Hypothalamo-hypophyseal tracts can integrate multiple simultaneous pituitary responses with each other and regulate pituitary function in accordance with change in temperature, energy needs, or fluid balance. ß-endorphin acts as neurotransmitter for efferent impulses to the median eminence. These impulses regulate the discharge of releasing hormones and inhibiting hormones into the adjacent capillaries. Dopamine and ß-endorphin also modulate efferent hypothalamic outflow by transmitting signals between areas of the hypothalamus.[209]

The release of ß-endorphin from the anterior pituitary gland is stimulated by serotonin and norepinephrine.[210]

Behavioural interaction in monkey social groups can be divided into four categories, namely, investigation, aggression, affiliation and sexual interaction. Among these, grooming is an important affiliative behaviour and as sexual behaviour also has a substantial affiliative grooming component, it is likely that such activities may also be accompanied by an increase of ß-endorphin. Nalaxone (morphin antagonist) will decrease affiliative as well as sexual activities. This is not to imply that the endorphins are the "affectional" peptides, because they have been shown to influence the release of other peptide transmitters in the limbic system (oxytocin, vasopressin) as well as the classical neurotransmitters (noradrenalin, acetylcholine). Oxytocin and vasopressin are closely linked to pair-bonding in other mammals.[211]

7.6.2.4 Norepinephrine

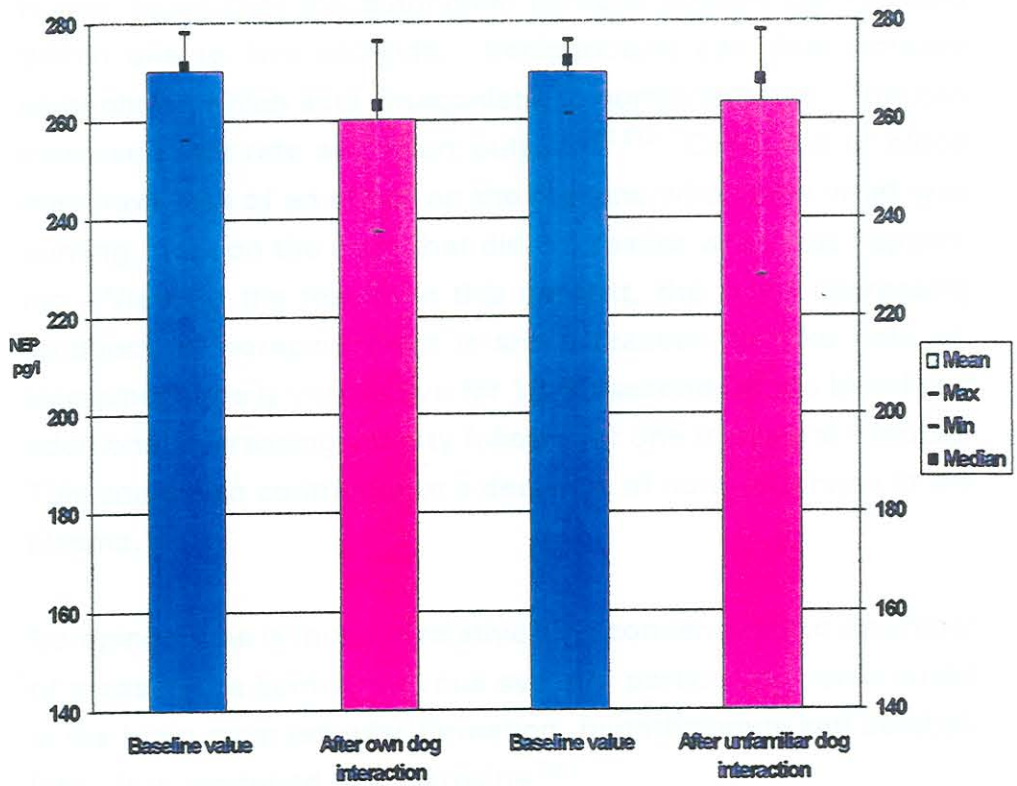

Figure 7.5: Plasma norepinephrine (NEP) of humans before and after interacting positively with own (n=9) and unfamiliar dogs (n=9)

Table 7.8: Concentrations of norepinephrine (pg/ℓ) in the plasma of humans before and after interacting positively with own dogs (n = 9) and unfamiliar dogs (n = 9)

NEP (own dogs)	Before	After	p-value	NEP (unfamiliar dogs)	Before	After	p-value
Mean	270,0	259,8	-	Mean	269,5	263,2	-
Standard deviation	6,6	14,0	-	Standard deviation	5,3	15,4	-
Median	271,0	263,0	-	Median	272,0	268,0	-
Significance	-	-	0,066	Significance	-	-	0,67

Norepinephrine did not change significantly with human-dog interaction, but a tendency to decrease was found and more so in humans with their own dogs. Norepinephrine may be more sensitive than the other neurochemicals to the process of blood collection.

It was found that the autonomic nervous system can respond within one to two seconds. Venipuncture can thus increase epinephrine which acts antagonistic to norepinephrine. This can increase heart rate and heart output.[209,212] Collection of blood may have less of an effect on the humans who knew what was coming, than on the dogs that did not realize what was happening. Viewing the results in this context, the slight decreasing tendency of norepinephrine is still a reaction to take note of. Norepinephrine is very active for 10-30 seconds in the blood and additional decreasing activity follows for one to several minutes. This could also contribute to a decrease of norepinephrine in the plasma.[157]

Norepinephrine is found in relative high concentrates in a number of areas in the central nervous system, particularly some nuclei in the brain stem reticular formation, hypothalamus and cerebellum. It is produced from tyrosine.[213]

Norepinephrine acting as a neurotransmitter in the brain appears to play a complex role in initiating and stopping ingestion

to play a complex role in initiating and stopping ingestion behaviour and the regulation of body temperature. The locus coeruleus in the pons is a concentrated collection of cell bodies of neurons that secrete the neurotransmitter norepinephrine which triggers emotional arousal. Excessive norepinephrine in the brain over a too long period of time is implicated in severe stress reactions, while too little results in depression. Norepinephrine may also play a part in producing feelings that an organism experiences as pleasurable.[209]

It is presumed that the norepinephrine system especially, and perhaps the serotonin system as well, normally function to provide motor drive to the limbic system to increase a sense of well-being, happiness, contentment, appetite, appropriate sex drive, psychomotor balance, although too much can cause mania. In support of this concept is the fact that the pleasure and reward centres of the hypothalamus and surrounding areas receive large numbers of nerve endings from noradrenergic neurons.[157]

Despite extensive research, the biochemical abnormalities underlying the predisposition to and the pathogenesis of affective disorders remain to be clearly established. Efforts to study norepinephrine output and function point to a dysregulation of the noradrenergic neurons. Depressed patients excrete relatively more norepinephrine in urine and its major extraneural metabolite, normetanephrine, than control subjects.[214]

7.6.2.5 Oxytocin

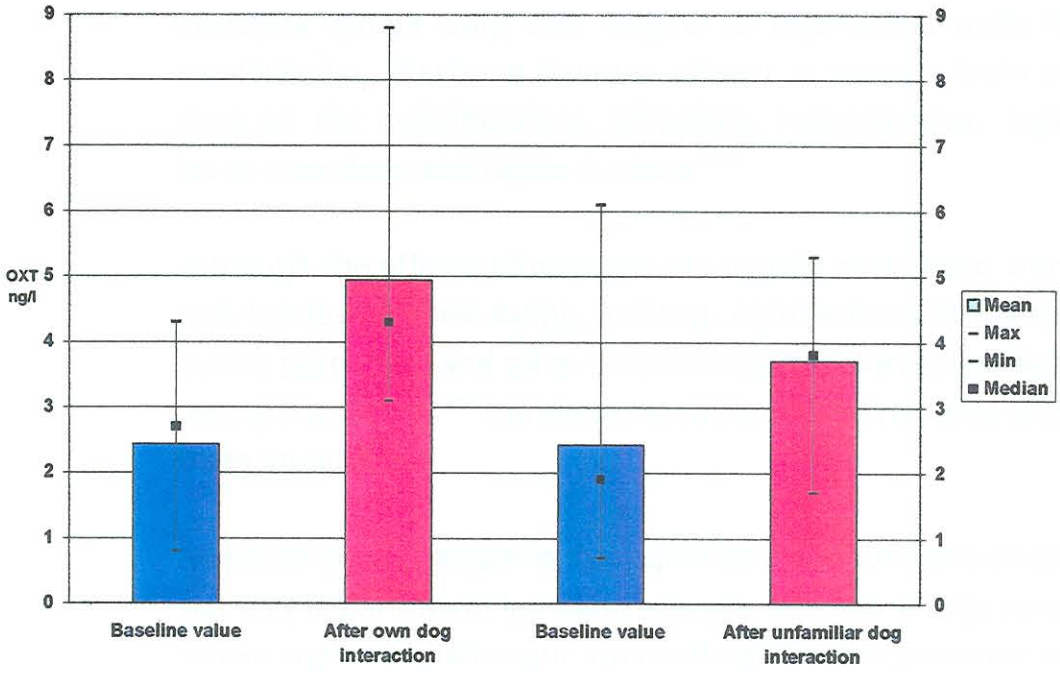

Figure: 7.6: Plasma oxytocin (OXT) of humans before and after interacting positively with own (n=9) and unfamiliar dogs (n=9)

Table 7.9: Concentrations of oxytocin (mg/ℓ) in the plasma of humans before and after interacting positively with own dogs (n = 9) and unfamiliar dogs (n = 9)

OXT (own dogs)	Before	After	p-value	OXT (unfamiliar dogs)	Before	After	p-value
Mean	2,4	4,9	-	Mean	2,4	3,7	-
Standard deviation	1,3	1,9	-	Standard deviation	1,7	1,1	-
Median	2,7	4,3	-	Median	1,9	3,8	-
Significance	-	-	0,00	Significance	-	-	0,01

Only during the past decade has oxytocin, acting as a neurotransmitter, been implicated in positive intraspecies interaction.[164,166,167,168] It has been called the "happiness" hormone. The tendency for oxytocin to increase in both human groups with dog interaction was highly significant ($p < 0,05$).

Oxytocin is a polypeptide (nonapeptide) hormone which is synthesized in the paraventricular and supraoptic nuclei of the hypothalamus and is secreted by the neurohypophysis. The structure differs only with regard to two amino acids from vasopressin. Oxytocin neurons project to various brain areas such as the hypothalamus, amygdala, hippocampus, septum, locus coeruleus and raphe nucleus.[213]

Although the effects of oxytocin are usually associated with the milk-let-down reflex during suckling, contractions of the uterus during parturition and other smooth muscle contractions such as during copulation,[157] its role as neurotransmitter has only recently been studied.

Morphological changes in the supraoptic nucleus were observed not only in parturient, lactating animals, but also in virgin animals where maternal behaviour was induced by the presence of rat pups. The supraoptic nuclei of lactating and maternally behaving virgin animals had a higher incidence of dendritic bundling relative to non-maternal virgin animals.[215]

Oxytocin is not confined to the hypothalamo-neurohypophysial system but present elsewhere in the body, however, the significance of the peptide in such peripheral sites is unclear. Oxytocin was found to be a male gonadal hormone and is regulated by factors which alter gonadal function.[216]

Two types of oxytocin binding sites have been detected. One, widely distributed throughout the central nervous system, is comparable to the uterine type receptor. A second, sexually dimorphic slightly different type, is found in the ventromedial nucleus of the limbic system. This is the case for the influence of vasopressin on social communication, temperature regulation, epilepsy, and barrel rotation which may be an animal model of febrile convulsions, and some aspects of the central regulation of the cardiovascular system and for oxytocin on sexual behaviour,

social communication, and grooming. Nonendocrine C-terminal conversion products seem to exert their effects exclusively on other parts of the brain. These neuropeptides modulate learning and memory processes, social recognition, and rewarded behaviour. The neuroendocrine and neuropeptide effect of vasopressin, oxytocin and related neuropeptides often exert their central nervous system effects in an opposite way. Neurochemical and electrophysiological studies suggest that norepinephrine, dopamine, serotonin, and glutamate are the neurotransmitters involved in the function. It appears that adequate amounts of vasopressin and oxytocin to induce these effects are released at the appropriate sites of action. It is postulated that the mix of neuropeptides released in the brain in response to environmental changes qualifies the behavioural, neuroendocrine, and immune response and the response of the autonomic nervous and vegetative systems of the organism.[217]

Oxytocins' visceral functions include the regulation of the cardiovascular and gastro-intestinal systems. It facilitates maternal, grooming and feeding behaviour and is reported to both stimulate and inhibit sexual behaviour under different circumstances. Oxytocin appears to have an amnesic effect, inhibiting memory processes and also regulates water excretion.[210]

A combination of behavioural, neuroanatomical and pharmacological evidence underscores a critical and interactive role for oxytocin and gonadal steroids in mammalian sociosexual behaviour. Underlying mechanisms of oxytocin action includes the activation of neurotransmitter networks that affect oxytocin gene expression and secretion, or homologous modulation of oxytocin neurosecretion.[219]

7.6.2.6 Prolactin

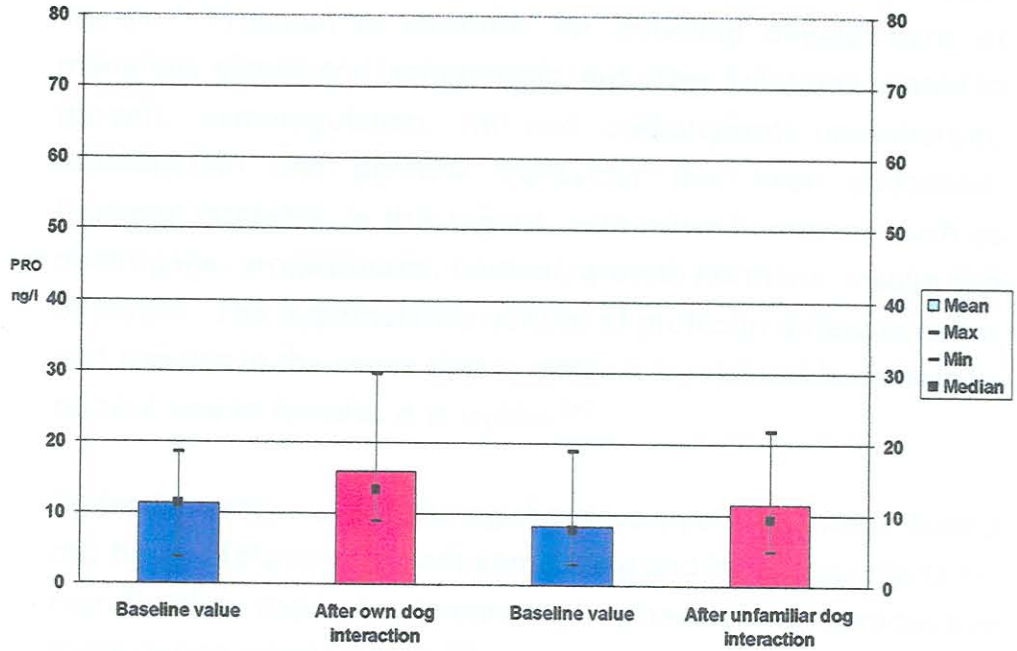

Figure 7.7: Plasma prolactin (PRO) of humans before and after interacting positively with own (n = 9) and unfamiliar dogs (n = 9)

Table 7.10: Concentrations of prolactin (ng/ℓ) in the plasma of humans before and after interacting positively with own dogs (n = 9) and unfamiliar dogs (n = 9)

PRO (own dogs)	Before	After	p-value	PRO (unfamiliar dogs)	Before	After	p-value
Mean	11,1	15,8	-	Mean	8,2	11,3	-
Standard deviation	4,4	6,5	-	Standard deviation	5,5	5,9	-
Median	11,3	13,3	-	Median	7,8	9,4	-
Significance	-	-	0,00	Significance	-	-	0,01

Prolactin increased significantly after interaction. On an intra-species basis it was indicated that this neurochemical could play a role in social bonding.[163]

Dopamine acts as a prolactinstatin while serotonin, endorphins and thyroliberin stimulate secretion of prolactin.[213] Prolactin is produced in lactotroph (mammotroph) cells in the adenohypophysis. Prolactin is essential for initiating development of mammary glands and lactogenesis, but other functions related to growth, osmoregulation, fat and carbohydrate metabolism, reproduction and parental behaviour has been described. Prolactin interacts, in this regard, with other hormones such as oestrogens, progesterone, cortisol, growth hormone, insulin and oxytocin. The hypothalamic control of prolactin differs in males and females in the sense that in males it is released in an acyclic pattern and in females it is cyclic.[210]

Prolactin reaches peaks during the sleep cycle and drops during day time. Prolactin has both stimulating and inhibiting effects on reproduction, depending in part on the phase of the reproductive cycle during which it acts.[209]

Prolactin, a trophic hormone, is also required for normal development and growth of the prostate as well as other tissues (e.g. haemopoietic tissues). In males, oxytocin regulates prostate citrate production.[220]

At physiological levels prolactin is also trophic for lymphocytes. Either too much, or too little prolactin may be immunosuppressive. Lymphocytes produce a prolactin-like substance and prolactin may play a role in a number of auto-immune processes.[221,222]

In mammals, prolactin is associated with learning, reduction of body temperature and increased corticosterone secretion. Secretion is strongly stimulated in the female rat on exposure to pups.[223]

7.6.2.7 Cortisol

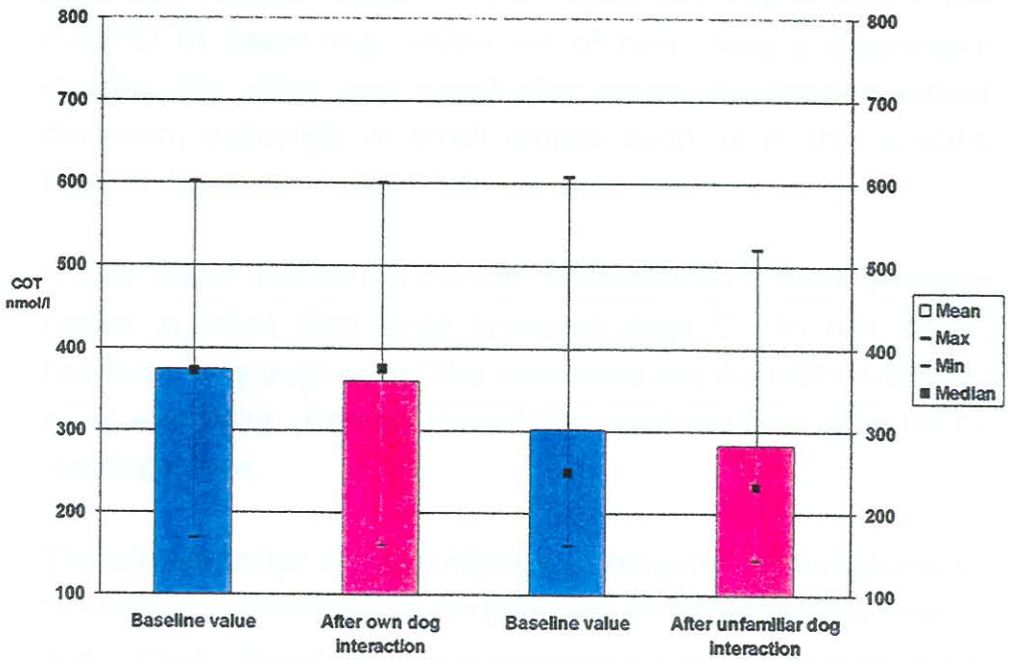

Figure 7.8: Plasma cortisol (COT) of humans before and after interacting positively with own (n = 9) and unfamiliar dogs (n = 8)

Table 7.11: Concentrations of cortisol (nmol/ℓ) in the plasma of humans before and after interacting positively with own dogs (n = 9) and unfamiliar dogs (n = 9)

COR (own dogs)	Before	After	p-value	COR (unfamiliar dogs)	Before	After	p-value
Mean	373,1	359,2	-	Mean	301,6	281,1	-
Standard deviation	155,7	154,9	-	Standard deviation	146,1	128,0	-
Median	372,0	374,0	-	Median	248,0	232,0	-
Significance	-	-	0,01	Significance	-	-	0,01

Cortisol is a well-known parameter in stress measurement. The significant decrease in cortisol values ($p < 0,05$) in both groups is indicative that this hormone has an effect during positive interaction with dogs. Companion animal ownership is often associated with better general health, because it may serve as a stress reliever.[6,82,128] These results support this idea on a physiological level, other than a decrease in blood pressure.

Despite the fact that the standard deviation is large in both groups, the significance could still be meaningful according to the statistical method used.[195] The reason for this is that if the majority of cases, e.g. seven out of nine, have a meaningful change, the other two cases may cause the large standard deviation, especially in small groups such as in this specific experiment.

It was found that serum cortisol concentrations were generally higher in small than large breed of dogs.[224] In this study, however, only dogs over 15kg were used and the maximum body mass was 28kg. Cortisol should thus not have been affected by the dogs' size.

The adrenal cortex secretes mineralocorticosteroids and glucocorticosteroids. Examples of the latter are corticosterone, cortisone and cortisol. Stress leads to an increase in corticoliberin secretion from the hypothalamus and corticotropin secretion from the adenohypophysis. This in turn stimulates secretion of glucocorticosteroids. They affect many bodily functions, namely through their role in carbohydrate, protein and fat metabolism. Cortisol increases blood glucose concentrations by stimulating glyconeogenesis and by inhibiting the effect of insulin. Catabolism of proteins, especially in bone, skin and muscle, is stimulated by cortisol. Glucocorticoids are also active in lipolysis of fat, together with other hormones such as thyroid hormone, growth hormone and the catecholamines which stimulate hormone sensitive lipase. In some regions in the body, glucocorticoids may have an opposite effect, namely lipogenesis and fat accumulation.[213]

The secretion of cortisol by the zona fasciculata is controlled by corticotropin (ACTH), the secretion of which in turn is controlled by corticoliberin. All the factors that influence ACTH secretion will affect cortisol secretion. Plasma levels of the latter follow the former by 15-30 minutes. Thus cortisol like ACTH exhibits distinct diurnal variation with a peak just before the subject awakens in the morning and the lowest levels just after the

subject falls into slow-wave sleep. Cortisol increases the rate of glomerular filtration by decreasing preglomerular resistance and increasing glomerular plasma flow. The hormone is also essential for rapid excretion of a water load through its inhibitory effect on vasopressin (ADH).[209]

Cortisol also facilitates maturation of the foetus, decreases connective tissue, inhibits inflammatory and immune responses and maintains cardiac output, increases arterial tone and decreases endothelial permeability. Cortisol plasma levels are higher during pregnancy and it plays a role in parturition with other hormones. There is an intimate anatomic and physiological relationship between the adrenal medulla and cortex.[207]

Adrenal glands are complexed multifunctional endocrine organs that are essential for life. The cortex (80-90% of the gland) is derived from mesodermal tissue and is the source of corticoid hormones. The medulla is derived from neuroectodermal cells of the sympathetic ganglia and it is the source of the catecholamine hormones. There are three cortical zones which differ histologically. The outermost zona glomerulosa is thin and consists of small cells, the middle zona fasciculata is the widest and consists of columnar cells and the innermost zona reticularis consists of a network of interconnecting cells. Glucocorticoids are largely synthesised in the zona fascilulata with some contribution from the zona reticularis. Cortisol is the dominant glucocorticoid and it is synthesised from cholesterol. The medulla is the source of the circulating hormone epinephrine and small amounts of norepinephrine, nominally a neurotransmitter, which in select circumstances may also function as a hormone. The adrenal medulla essentially represents an enlarged and specialised sympathetic ganglion, but the neural cell bodies of the medulla do not have axons and they discharge their catecholamine hormones directly into the bloodstream. Its function is thus rather endocrine than nerve cells. Apart from the close anatomical relationship of the cortex and medulla, physiological they both play a role in stress reactions. In acute stress, the medulla via the peripheral sympathetic system produces in the "fight or flight" reaction,

while in continuous stress the cortex, via glucocorticoids produces a complexed metabolic reaction to adapt or survive.[209]

Cortisol modulates excitability, behaviour and mood of individuals by influencing the electric activity of neurons. Glucocorticoid receptors of both type I and II are present in various areas of the brian, particularly in the limbic system and hippocampus.[207]

7.7 Statistical difference between experimental and control human groups

The only statistical difference was oxytocin ($p < 0,05$), which was higher in the own dog group. The other parameters between the groups did not differ significantly.

7.8 Changes in MAP and selected biochemicals in dogs

7.8.1 Changes in MAP

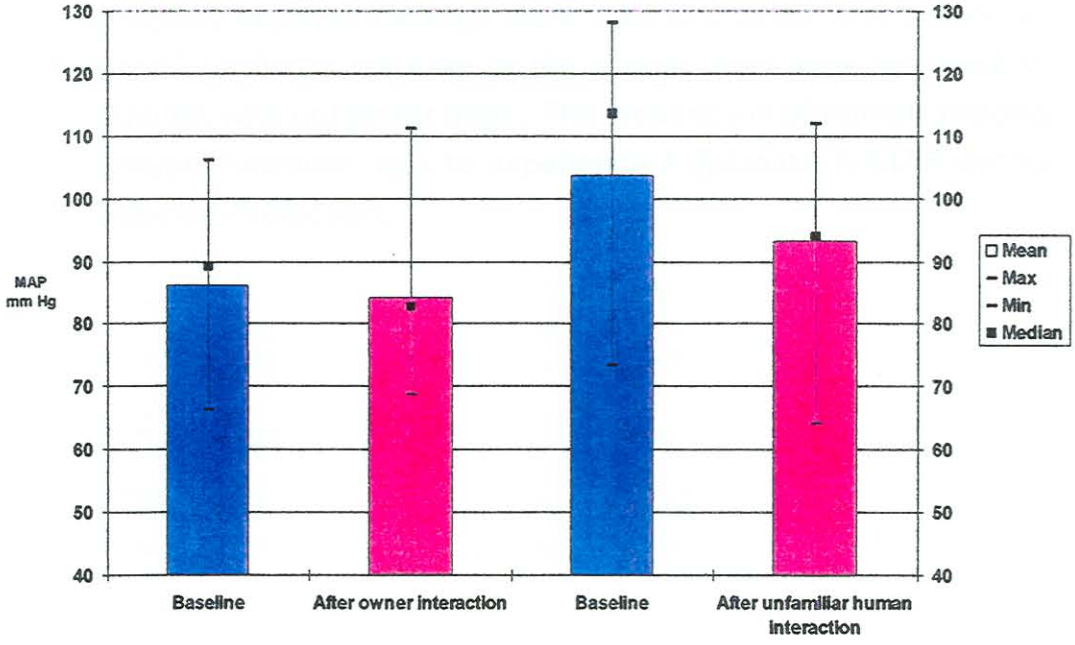

Figure 7.9: Mean arterial blood pressure (MAP) of dogs before and after interacting positively with owners (n=9) and unfamiliar dog lovers (n=9)

Table 7.12: Mean arterial blood pressure (mmHg) of dogs before and after interacting positively with owners (n = 9) and unfamiliar dog lovers (n = 9)

MAP (owners)	Before	After	p-value	MAP (unfamiliar people)	Before	After	p-value
Mean	86,1	84,0	-	Mean	103,5	93,1	-
Standard deviation	12,9	12,8	-	Standard deviation	19,5	16,7	-
Median	89,2	82,6	-	Median	113,6	94,0	-
Significance	-	-	0,17	Significance	-	-	0,01

The time taken for MAP to decrease sufficiently for blood collection, was five to 19 minutes for own dogs and five to 23 minutes for unfamiliar dogs.

There was a significant decrease in the MAP of those dogs interacting with unfamiliar people, but not in those interacting with their owners. This phenomenon could be explained in terms of the dogs' behaviour as described below Table 7.4. The placid dogs of the control group did better than the dogs with owners, probably because the dogs were used to interact with unfamiliar people whilst in the case of the people, they were not used to interact with unfamiliar dogs. The tendency in all human and dog groups, however, was to experience a decrease in MAP during positive interaction.

7.8.2 Changes in plasma neurotransmitters

7.8.1 Phenylacetic acid as metabolite of phenylethylamine

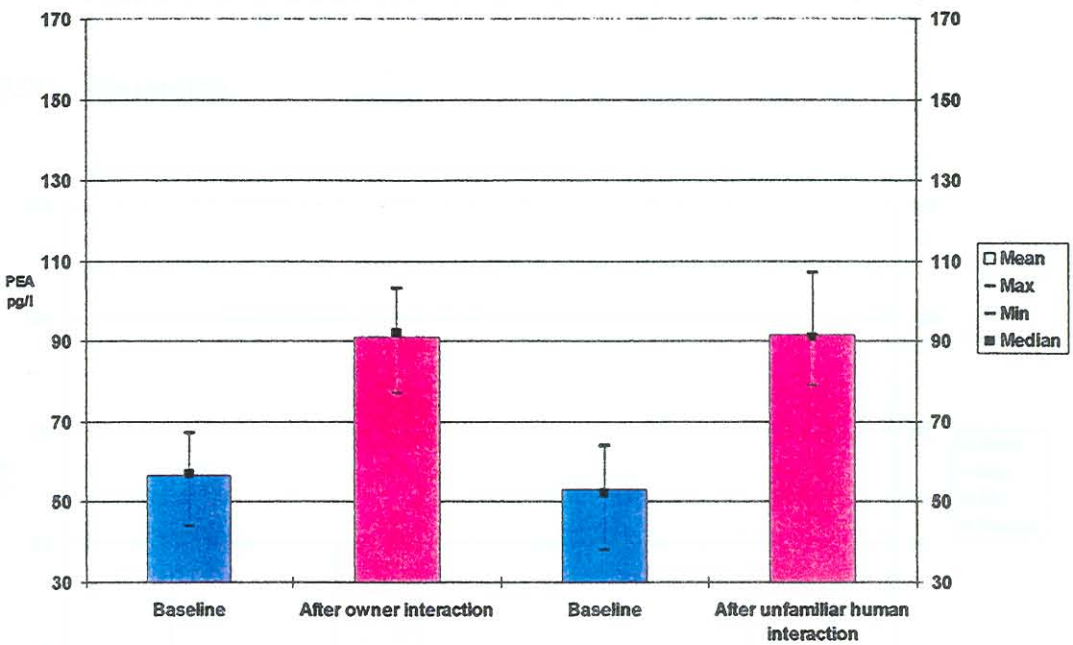

Figure 7.10: Plasma phenylacetic acid (PEA) of dogs before and after interacting positively with owners (n=9) and unfamiliar dog lovers (n=9)

Table 7.13: Concentrations of phenylacetic acid (pg/ℓ) in the plasma of dogs before and after interacting positively with owners (n = 9) and unfamiliar dog lovers (n = 9)

PAA (owners)	Before	After	p-value	PAA (unfamiliar people)	Before	After	p-value
Mean	56,3	91,0	-	Mean	52,8	91,5	-
Standard deviation	7,4	7,5	-	Standard deviation	8,5	8,0	-
Median	57,0	92,0	-	Median	52,0	91,0	-
Significance	-	-	0,00	Significance	-	-	0,00

This highly significant result correlates that found in humans. The observation is as important as the similar observation in humans. The fact that ß-phenylethylamine increased in the same fashion indicates that human-dog interaction is beneficial to both species. It also supports the theory that the dog, as highly social animal, not only can act as a substitute for human positive interaction, but that the effect is reciprocal. This in turn could

explain the human-dog interaction over the centuries in so many different peoples. As the main indicator of positive interaction on a physiological level, this finding can also be seen as the main support for the *attentionis egens* theory on an interspecies level.

7.8.2.2 Dopamine

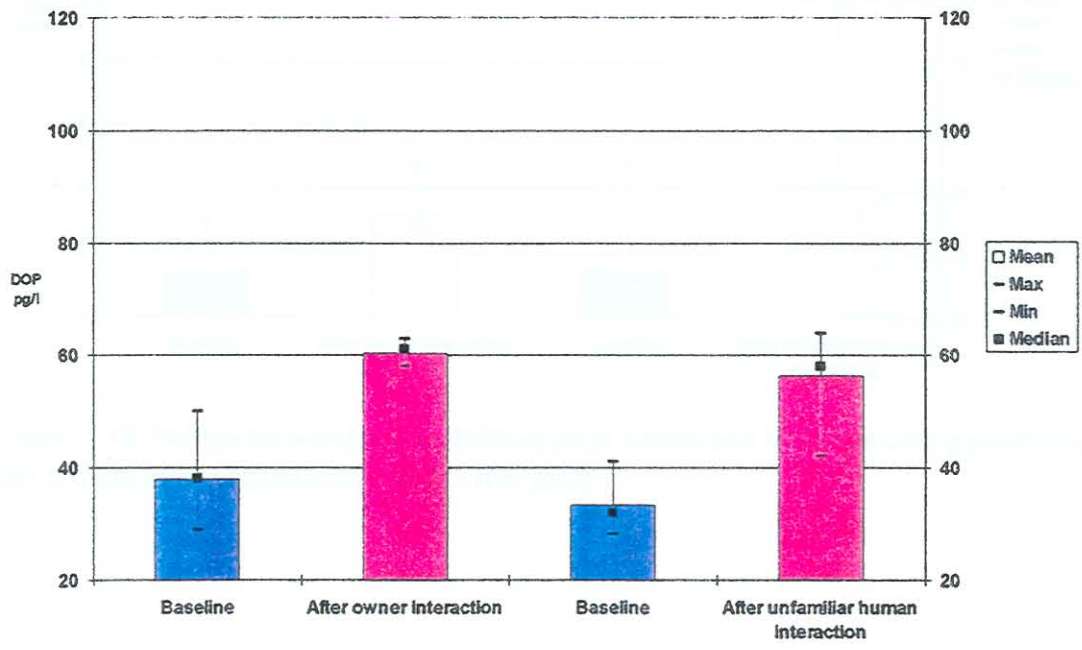

Figure 7.11: Plasma dopamine (DOP) of dogs before and after interacting positively with owners (n = 9) and unfamiliar dog lovers (n = 9)

Table 7.14: Concentrations of dopamine (pg/ℓ) in the plasma of dogs before and after interacting positively with owners (n = 9) and unfamiliar dog lovers (n = 9)

DOP (owners)	Before	After	p-value	DOP (unfamiliar people)	Before	After	p-value
Mean	37,8	60,3	-	Mean	33,2	56,2	-
Standard deviation	6,5	1,9	-	Standard deviation	4,5	7,2	-
Median	38,0	61,0	-	Median	32,0	58,0	-
Significance	-	-	0,00	Significance	-	-	0,00

As in the case of humans, the dopamine also increased significantly and once more it is an indication of similar physiological effects during positive interaction.

7.8.2.3 ß-endorphin

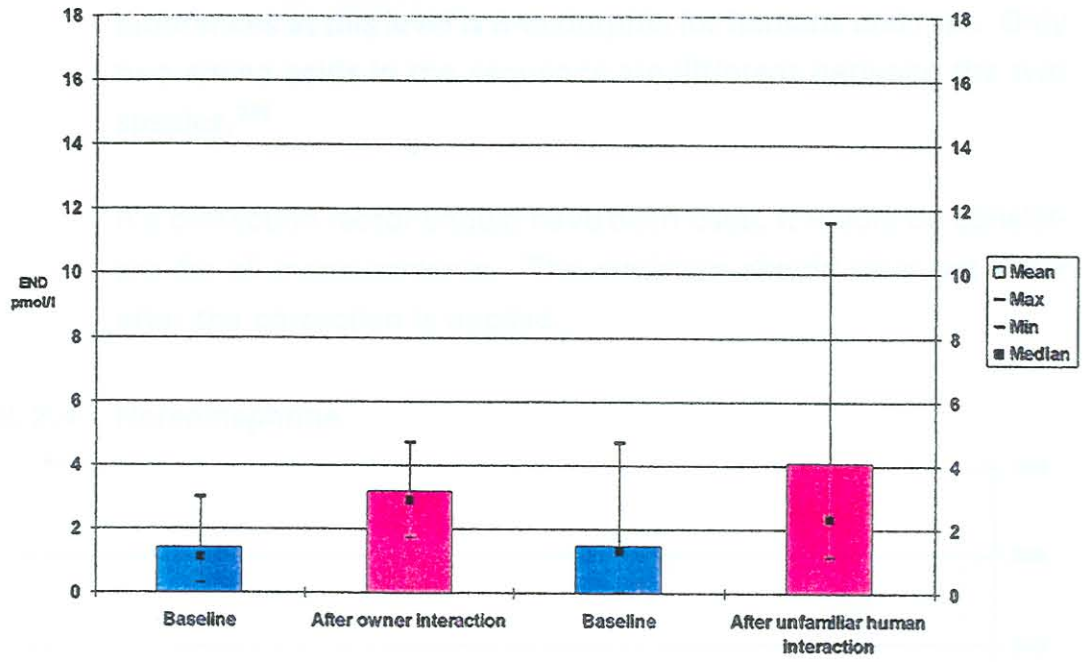

Figure 7.12: Plasma beta-endorphin (END) of dogs before and after interacting positively with owners (n=9) and unfamiliar dog lovers (n=9)

Table 7.15: Concentrations of ß-endorphin (pmol/ℓ) in the plasma of dogs before and after interacting positively with owners (n = 9) and unfamiliar dog lovers (n = 9)

END (owners)	Before	After	p-value	END (unfamiliar people)	Before	After	p-value
Mean	1,4	3,1	-	Mean	1,4	4,0	-
Standard deviation	0,9	1,0	-	Standard deviation	1,3	3,8	-
Median	1,1	2,9	-	Median	1,3	2,3	-
Significance	-	-	0,00	Significance	-	-	0,00

The observations followed the same tendency as in humans. However, the Laboratory indicated that another neuropeptide may have interfered with the reading obtained. The retention time for humans was 12,78 minutes and for dogs 11,05 minutes. Retention time refers to the time peaks develop on the HPLC apparatus. For quantification of canine ß-endorphin, human standards were used. The peak closest to the human peak was

thus taken as dog ß-endorphin, however, there is a slight chance that it could be another peptide. An example of known species differences at this level is ß-endorphin for humans and rats. Only two amino acids in the sequence are different between the two species.[225]

If a correction factor should have been used, it would be consistent for all measurements. The statistics should thus not differ after the correction is applied.

7.8.2.4 Norepinephrine

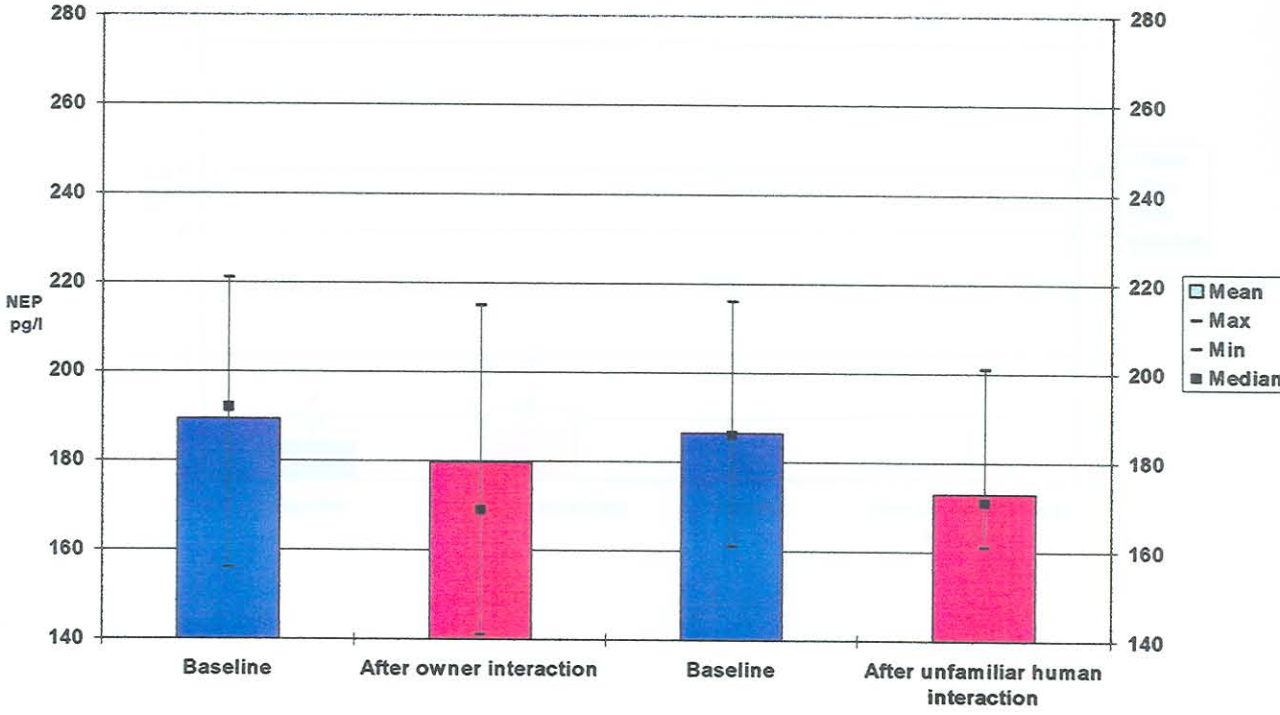

Figure 7.13: Plasma norepinephrine (NEP) of dogs before and after interacting positively with owners (n = 9) and unfamiliar dog lovers (n = 9)

Table 7.16: Concentrations of norepinephrine (pg/ℓ) in the plasma of dogs before and after interacting positively with owners (n = 9) and unfamiliar dog lovers (n = 9)

NEP (owners)	Before	After	p-value	NEP (unfamiliar people)	Before	After	p-value
Mean	189,3	179,5	-	Mean	186,4	172,8	-
Standard deviation	22,7	22,5	-	Standard deviation	19,2	12,3	-
Median	192,0	169,0	-	Median	186,0	171,0	-
Significance	-	-	0,08	Significance	-	-	0,06

These results are not statistically significant and it could be ascribed to factors such as venipuncture. In humans the same procedure had a lesser effect because, unlike dogs, they knew what to expect.

7.8.2.5 Oxytocin

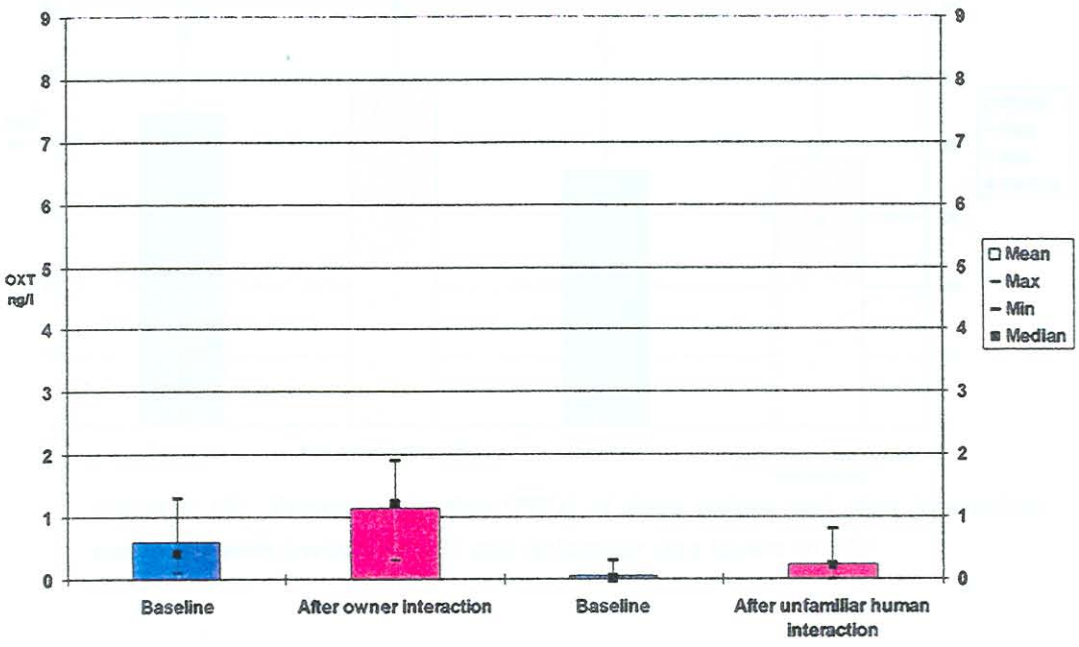

Figure 7.:14: Plasma oxytocin (OXT) of dogs before and after interacting positively with owners (n = 9) and unfamiliar dog lovers (n = 9)

Table 7.17: Concentrations of oxytocin (ng/ℓ) in the plasma of dogs before and after interacting positively with owners (n = 9) and unfamiliar dog lovers (n = 9)

OXT (owners)	Before	After	p-value	OXT (unfamiliar people)	Before	After	p-value
Mean	0,5	1,1	-	Mean	0,04	0,2	-
Standard deviation	0,4	0,5	-	Standard deviation	0,10	0,2	-
Median	0,4	1,2	-	Median	0	0,2	-
Significance	-	-	0,00	Significance	-	-	0,09

In the group where a long relationship was established between dog and owner, the oxytocin increased in a highly significant manner. Although still significant, the difference was less marked in the contact between people with unfamiliar dogs.

7.8.2.7 Prolactin

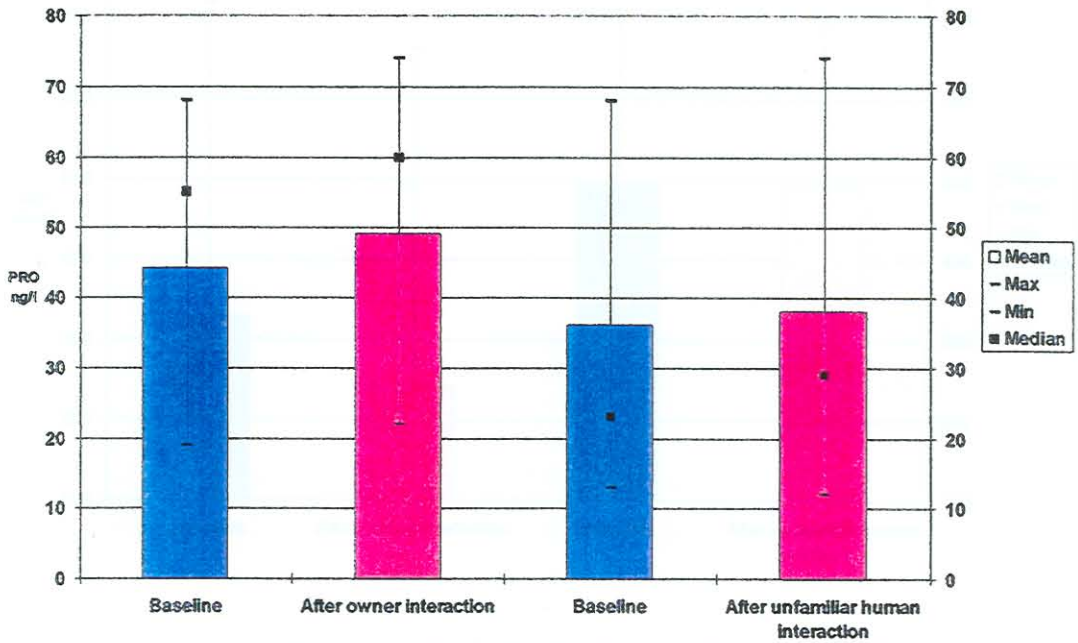

Figure 7.15: Plasma prolacting (PRO) of dogs before and after interacting positively with owners (n = 9) and unfamiliar dog lovers (n = 9)

Table 7.18: Concentrations of prolactin (ng/ℓ) in the plasma of dogs before and after interacting positively with owners (n = 9) and unfamiliar dog lovers (n = 9)

PRO (owners)	Before	After	p-value	PRO (unfamiliar people)	Before	After	p-value
Mean	44,2	49,1	-	Mean	36,1	38,1	-
Standard deviation	19,9	21,7	-	Standard deviation	21,6	23,5	-
Median	55,0	60,0	-	Median	23,0	29,0	-
Significance	-	-	0,03	Significance	-	-	0,20

As in humans, a significant increase in value was obtained following interaction between owners and their dogs, but not with the unfamiliar people group. Other substances which have the same retention time as prolactin, may be present as the peak area for dogs was larger than that for human standards. This finding could be normal for the dog, but on the other hand,

another peptide with a similar retention time may be involved.

7.8.2.8 Cortisol

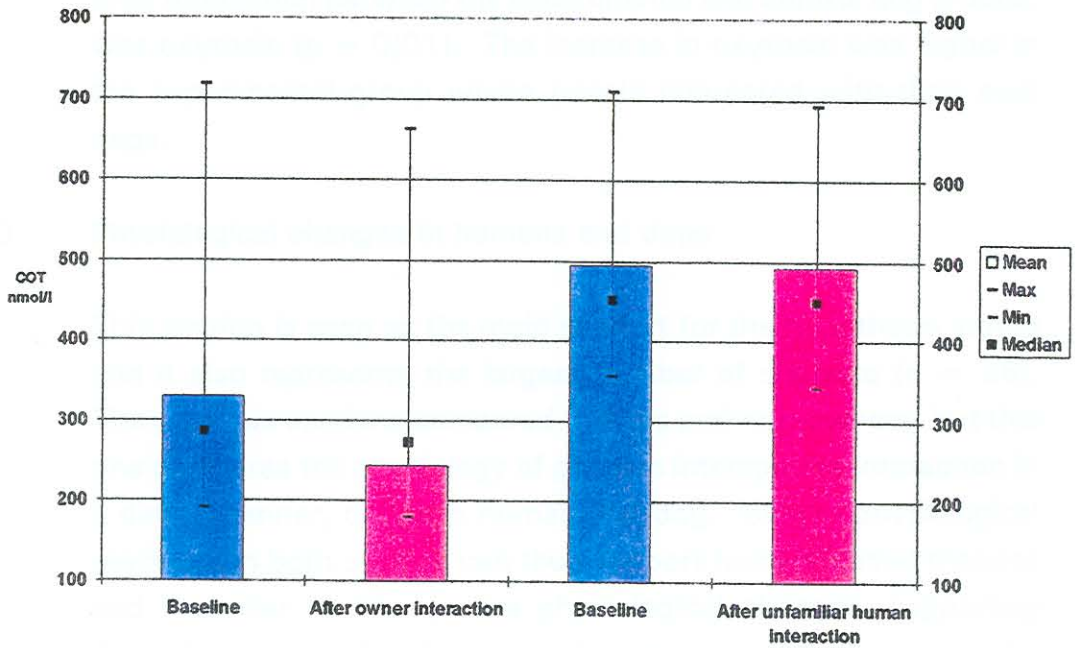

Figure 7.16: Plasma cortisol (COT) of dogs before and after intracting positively with owners (n = 9) and unfamiliar dog lovers (n = 9)

Table 7.19: Concentrations of cortisol (nmol/ℓ) in the plasma of dogs before and after interacting positively with owners (n = 9) and unfamiliar dog lovers (n = 9)

COR (owners)	Before	After	p-value	COR (unfamiliar people)	Before	After	p-value
Mean	330,0	243,0	-	Mean	493,5	490,7	-
Standard deviation	156,0	160,9	-	Standard deviation	125,0	122,5	-
Median	286,0	273,0	-	Median	452,0	449,0	-
Significance	-	-	0,44	Significance	-	-	0,44

Cortisol levels did not decrease significantly in dogs. Factors such as the circadian cycle and diet of the dogs could have affected the results as well as the unfamiliar experimental environment. The tendency, however, was for cortisol levels to decrease.