

CHAPTER 6

METHODOLOGY FOR ESTABLISHING PHYSIOLOGICAL SUPPORT FOR HUMAN-ANIMAL INTERACTION THEORIES

6.1 Introduction

In order to support human-animal interaction theories, it was decided to study indicators which provide more information than those parameters used to determine benefits for humans with regard to cardiovascular diseases, stress and anxiety. Skin conductance tests, triglyceride and cholesterol plasma levels were thus not considered for measurement. Blood pressure was included in the test for two reasons. The one is that nowhere in the literature reference is made to blood pressure being measured in both species during the same positive interaction. A decrease in blood pressure was recorded in animals⁸⁹ and in humans,⁷¹ but not in a particular situation where the blood pressure of both species was taken during the same positive interaction. The second reason is that changes in blood pressure could be a measurable sign that other chemical-physiological changes could have taken place. This experiment will thus focus on neurochemicals or their metabolites which could play a role during positive human-animal interaction. Such measurements have as yet not been recorded on an interspecies basis.

Establishing physiological parameters as indicators of positive human-animal interaction does not exclude the circular argument completely. As indicated by Fisher¹⁵⁸ earlier, these chemicals do not cause the positive relationship, but are merely responsible for the feeling experienced during successful interaction. It will, however, provide parameters which could be measured, verifying their role objectively and repeatedly to indicate a biological feedback system.

6.2 Hypothesis

The measurement of plasma levels of specific neurochemicals and hormones will indicate physiological responses associated with positive human-dog interaction.

6.3 Benefits arising from the experiment

6.3.1 Positive results can support the interaction theories of human-animal interaction and thus link interaction theories of human-human and animal-animal interaction.

6.3.2 Such a theoretical basis can provide a rationale for animal-facilitated psychotherapy.

6.3.3 The parameters may serve as indicators for the successful application of animal-facilitated psychotherapy in psychiatry.

6.4 Materials and method

The type of research, the subjects and materials, the experimental design and procedures are as follows:

6.4.1 Type of research

Research with the objective to explain physiological influences and their effects on human-animal interaction is known as explanatory research. The main preconditions for such research are control over independent variables and the random assignment of subjects.¹⁷⁵

This study could also be described as basic research because the main purpose is to advance knowledge in disciplines¹⁷⁵ (physiology, psychiatry).

6.4.2 Pilot study

A pilot study was done to determine whether a decrease in blood

pressure could be used as an indication of neurochemical changes which are associated with positive human-dog interaction. Six people with six dogs, unfamiliar to them, were used in the same way as described under the experimental procedures (6.4.6), but in this case only PEA was determined as an example of interaction biochemical changes.

6.4.3 Sample selection

The samples consisted of the following human and dog subjects:

6.4.3.1 Human subjects

To recruit human subjects, an open invitation was sent via e-mail and by notices, stating that people were needed on a voluntarily basis to participate in the trial. The criteria for inclusion in the trial were:

- people should be healthy and not using prescription drugs;
- people should be over 18 years of age (adults);
- both sexes should be represented in the group;
- dog owners should "love" their dogs. Love was not defined, but it was left to the owners to decide what they thought their love for their dog meant. Ownership should have lasted for longer than one year;
- other people should be willing to interact with friendly dogs which had not been known to them before. These subjects should either have had a dog before or have a dog at home; and
- it should be practically possible for the people to attend the sessions.

6.4.3.2 Dog subjects and control group

For the animal subjects, dogs were chosen because dogs are, according to archaeological findings, the prototype of positive human-animal interaction.⁶⁷ Dogs are not only the first recorded companion animals, but are still the most popular and widespread pets among most communities globally.⁶³ Measurement of physiological changes in the dog as model for this investigation is thus appropriate and it also provides a practical model for collecting the necessary blood samples. Furthermore, the neurochemicals under investigation are present in both species.

The criteria for inclusion of dog subjects were:

- dogs should be healthy and not using prescription drugs;
- dogs should be over two years of age (adults);
- both sexes should be represented in the group;
- dogs should weigh > 15kg to draw blood easily and repeatedly from the vena cephalica (ie for the size and integrity of the veins);
- dogs with a known aggressive temperament were excluded; and
- bitches should not be in oestrus.

Dogs for the control group (non-owners) were from the Companion Animal Unit, Department of Veterinary Ethology, Faculty of Veterinary Science, University of Pretoria.

6.4.4 Experimental design

Three different controls were used in this experiment.

6.4.4.1 Experimental and control group

The experimental group consists of dog lovers interacting with their own dogs and the control group consists of dog lovers interacting with unfamiliar dogs.

6.4.4.2 Pre- and post-test control test (counter-balancing design) with different groups

A basic experimental design (pre- and post-test control group) was used because of the biological variables which may affect the interaction.¹⁷⁵ This meant that the effect of the interaction of every subject, human and dog, were measured against the subject's own baseline values (Fig 6.1):

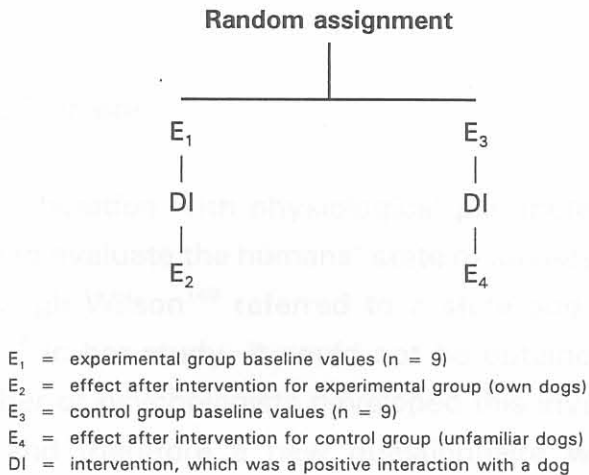


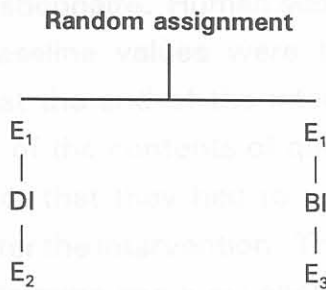
Fig 6.1: Basic pre- and post-test design with different groups

The experimental group had a long-term relationship with their own dogs and the control group had no relationship with the dogs used.

6.4.4.3 Pre- and post-test with different interventions

As a group, all human subjects were also exposed to different types of intervention also in a counter-balancing design.¹⁷⁵

Human subjects were exposed to positive human-dog interaction and the same subjects to quiet book-reading interaction (Fig 6.2):



- E₁ = same group (all humans) baseline values (n = 18)
- E₂ = effect after human-dog interaction
- E₃ = effect after human-book-reading interaction
- DI = intervention, human-dog interaction
- BI = intervention, book-reading

Fig 6.2: Pre-test and post-test with different interventions

6.4.4.4 Questionnaire

In combination with physiological parameters, a checklist was used to evaluate the humans' state of anxiety as another control. Although Wilson¹⁴⁹ referred to a state and trait anxiety inventory¹⁷⁶ in her study, it could not be obtained. It seems as if a number of psychologists developed this inventory for their own use and therefore a new questionnaire was compiled. The objectives of the questionnaire were to establish the subjects' feelings with regard to their:

- personal life (current and future life, health, finances)
- relatives or close friends (current and future relationships)
- work situation and country (or not at home)
- global affairs (eg global warming, ozone layer, pollution).

The questionnaire was pretested among three non-participants to ensure that the questions were clearly understood. A five-point scale was used to indicate the level of anxiety related to the issues in the questionnaire. Human subjects completed questionnaires before baseline values were taken for the human-dog interaction and at the end of the interaction. Subjects had no prior knowledge of the contents of questionnaires and were not aware of the fact that they had to complete a second, similar questionnaire after the intervention. The purpose of the questionnaire was to determine some psychological moods which could be linked to the physiology as well as to determine whether the interaction with the dog had any effect on such a mood on the very short term. Results of the questionnaire could also serve as a control with regard to the general state of anxiety of the group and their mean arterial blood pressure.

6.4.5 Experimental model

The experimental and control groups were randomly assigned to testing by drawing numbers from a hat. People from the experimental group and those from the control group were tested alternately. All the human subjects, in the same order (as far as practically possible) as during the dog interaction, were tested one week later for book-reading interaction and those first tested for book-reading, were tested in the same order for dog interaction (Table 6.1).

Table 6.1: Roster for experimental design

Specimen collection	Date 1	Date 2	Date 3	Date 4
A decrease in blood pressure was taken as indication to collect intravenous blood	4 dog owners 4 persons with other dogs	First group of 8 persons reading a book	Second group of 10 persons reading a book	5 persons with other dogs 5 dog owners

Experimental and control groups were tested over successive periods (Date 1 and 2 and Date 3 and 4) purely because of

logistical considerations. The week between the two types of intervention (dog or book) was also based on practical reasons, namely to ensure that the veins used for blood collection recovered well enough for further blood collection.

6.4.6 Experimental procedures

Hourly appointments were made with the participants. On arrival, participants were taken to a room, which was empty apart from two tables for the blood pressure apparatus and collection material, two chairs, one for a veterinary physiologist who took the blood pressure measurements and collected blood samples from the dogs, and one for the medical nurse, who collected blood samples from the humans. The curtains were drawn, but natural light filtered through to such an extent that the neon lights in the room could be switched off. The subjects sat on the floor for the sake of proper interaction with the dogs. Bedding was provided to make the area of contact comfortable. The first questionnaire was completed and placed in an envelope. Participants had about 10 minutes to adapt to the new environment before the baseline values were recorded.

The random assignment of participating subjects was done before commencement of the procedures. To minimise variables which could be present on a specific day, alternation of experimental and control group subjects was determined by the random assignment. Baseline values were taken as a covariate to ensure that individual differences in the dependent variables were considered when the effect of the interaction was evaluated.

The intervention with the dogs consisted of social gestures only, and included talking softly to the dog, stroking, low-key playing and scratching the body and ears of the dog. The person's attention was completely focused on the dog. The other intervention was to read a book quietly. The genre of the books was evaluated by a specialist in the field of language (qualified on doctorate level), as being the same. Three books in English and

three in Afrikaans, were available to choose from and the topic was "neutral" animal stories. Book-reading as a control was chosen because it had been used as a control for human-pet interaction before.¹⁴⁹

The procedures used for the collection of data were as follows:

- completion of first questionnaire and marking of the envelopes and allowing 10 minutes for adaptation;
- determining baseline blood pressure values by using an automatic Dinamap TP Blood Pressure Unit (Critikon Vital Signs Monitor 1846SX). Five readings were used to establish the baseline and the apparatus was applied to the non-active arm of the human and the base of the dog's tail;^{177,178,179,180}
- collection of blood samples, shortly after baseline values for blood pressure had been determined, into two 5ml heparised vacu-tubes which were treated with the following enzyme inhibitors: aprotonin (Sigma, USA) and soya bean tripsin inhibitor (Sigma, USA). All samples were properly labelled after collection;
- stable (five) blood pressure changes, i.e. a drop of at least 5-10%, were taken as an indication whether to collect further blood samples during the interventions. Time allowed for interventions was a maximum of 30 minutes;
- completion of second questionnaire and marking of the envelopes;
- blood samples were centrifuged at 4°C immediately after collection, transported to the Biochemistry Laboratory of the Department of Biological Sciences, Technikon Pretoria, where they were analysed in a polychrome diode array detector, high-performance liquid chromatography (HPLC) system (Varian 9065, SMM Johannesburg).¹⁸¹

The following measurements were taken:

- the mean score for the experimental and control groups' (n = 18) questionnaires before and after the interaction with dogs;
- the mean arterial blood pressure of all subjects (n = 36) before (baseline) and after the two interventions (effect);
- the analysis of plasma levels of beta-phenylethylamine metabolite, norepinephrine, dopamine, endorphins, oxytocin, prolactin and cortisol^{182,183,184,185,186,187,188,189,190,191,192,193,194} of all subjects (n = 36) before (baseline) and after the two interventions (effect).



Plate 6.1: A posed example of the experimental conditions

6.4.7 **Observation**

The total programme was organised, managed and observed by the researcher. Co-workers were selected on the basis of their expertise, namely a veterinary physiologist, a medical nurse, a biochemist and a chemist who determined the plasma levels of the neurochemicals. The questionnaire was compiled by the researcher and approved by a psychologist.

The researcher was not present during the actual interaction, because he was familiar with some of the people. The physiologist and nurse were the only co-workers present in the room and were unknown to all the subjects. Influences from persons knowing each other were thus excluded, and all subjects experienced the same test environment.

6.4.8 **Variables**

The variables of the study are defined as follows:

6.4.8.1 **Independent variables**

Independent variables consist of a limited positive interaction period, i.e. until the blood pressure drops sufficiently (maximum 30 minutes), during positive dog interaction or reading a book. The types of intervention were thus the same for all subjects, i.e. the human and dog interaction and humans reading books.

6.4.8.2 **Dependent variables**

Dependent variables consisted of a feeling or mood of anxiety as reflected in a questionnaire completed before and after the intervention; the mean arterial blood pressure of human and dogs (where applicable) before and during the intervention; and plasma levels of neurochemicals, as described above, in humans and dogs (where applicable) before and during the intervention.

6.5 Validity and reliability of the study

The validity of an experiment is the extent to which one measures what is supposed to be measured and reliability indicates the repeatability of results.¹⁷⁵

6.5.1 Validity

The internal validity of the experiment was kept as high as possible by controlling the following variables:

- representative subjects were randomly assigned to participate in the experiment to avoid ascertainment bias and satisfy probability requirements for appropriate statistical analysis;
- baseline values were used to control individual differences;
- the hypothesis was based on a *a priori* specification;
- subjects in the experimental and control groups were tested alternately;
- subjects were tested in two groups of which one first interacted with dogs and the other first read a book;
- the same room with the same environment was used for all interventions;
- the recording of blood pressure and collection of blood were done by the same people;
- the same staff analysed the chemicals in the same laboratory and the same apparatus, including the blood pressure apparatus, was used consistently;
- baseline values were taken on the same day as the intervention;

- the blood pressure apparatus was always applied on the non-active (non-stroking) arm and to the base of the dogs' tails;
- noise was limited outside the room on the days the experiment was executed;
- because the experiment was completed in three weeks, the weather conditions were fairly constant throughout the whole period (summer, $\pm 28^{\circ}\text{C}$);
- experiments on different days were all executed between 07:30 and 12:30;
- both people and dogs were adults, healthy and did not take prescription drugs. This definition helps to ensure consistency in physiological changes within normal ranges;
- during the pilot study it was found that in the dogs, it would have been more disturbing to insert a canula and withdraw blood with a syringe, than to use vacu-tubes. A needle puncture through the skin has a minimal disturbing effect when done by an efficient, professional person;
- although a choice of three books was offered, they had the same stimulation value from a literary point of view;
- three controls were used: baseline values versus effects, dog owners versus unfamiliar dogs, dogs versus books. A feeling of anxiety was compared to the mean arterial blood pressure;
- the subjects could not affect the plasma levels of the neurochemicals measured. Although the humans had some idea of what the experiment was about, it would have been impossible to manipulate the parameters (ie a "blind effect"); and
- the researcher was not involved during the interaction, collection of blood samples, the analysis of the neurochemicals

or the statistical analysis thereof. No bias towards, or manipulation of the results from the researcher was thus possible.

Despite all the measures taken to ensure high internal validity, two points should be considered: the experiment was executed in an artificial environment (the experimental room) with strange people (physiologist and nurse) taking measurements and collecting blood from the subject. Secondly, neurochemicals can be very sensitive to environmental changes over a very short period. What people (and to a lesser extent the dogs) experienced shortly (a few hours) before the test, as well as the experimental environment and experience, could affect the plasma levels of those chemicals. Because the experiment includes two biological entities (human and dog) interacting, it will never be possible to completely exclude the experimental environment and experience, as well as other unrelated emotional experiences.

6.5.2 Reliability

The reliability of the experiments were based on the following aspects:

- repeatability of results was aimed at using the largest sample practically possible, at least large enough for statistical analysis. Eighteen human subjects and 18 dog subjects were used and both sexes were represented in both species. The ages of the people and dogs varied from young adult to senior adult. Although emotion, blood pressure and neurochemicals can vary in people and dogs, the fact that all the subjects were healthy adults not using any medication, experience the same in a controlled environment, could limit unacceptable variation in order to make results reliable for similar groups in the population. The sample size could thus be accepted as adequate;

- dependent and independent variables were properly identified;
- the experiment and experimental procedures were described in detail; and
- statistical procedures were specified.

Obviously the reliability of this experiment will only be known after the results are replicated in similar studies by other researchers.

6.6 Ethics

The research protocol was approved by the Ethics Committee of the Faculty of Veterinary Science, University of Pretoria. The only invasive procedure was the collection of venous blood and this was done by a medical nurse and a veterinarian. Animals which people were interacting with, did so only in a positive manner under the supervision of a veterinarian. A medical doctor was available during every session of the experiment. The dogs were all well-tempered, whether they were own or unfamiliar dogs.

6.7 Statistics

The following measurements were statistically analysed;

- the scores of the questionnaires of humans before versus after interaction with a dog;
- the effect on individuals of the experimental and control groups before versus after the intervention for mean arterial blood pressure and the neurochemicals;
- the effect on all humans before and after the intervention versus the effect on dogs before and after the intervention for mean arterial blood pressure and the neurochemicals. This was

done because of it being an interaction study, evaluating the physiology of both species during the same contact;

- the effect of interaction with dogs versus book-reading on humans.

In order to test the effect of the interaction on the anxiety scores, neurotransmitters or hormone levels, the baseline values (i.e. before interaction measurements) are compared to the corresponding values after interaction. The before and after interaction measurements of a subject (human or dog) form a pair and are analyzed accordingly. A distribution-free statistical test, namely the signed rank test¹⁹⁴ was used. This test is also known as the Wilcoxon test for symmetry.¹⁹⁵ A distribution-free test is used when little to no information is available on the probability distribution of the population of measurements from which a sample is drawn. Fewer assumptions regarding statistical properties are needed than is usually the case with parametric tests, for instance, the well-known t-test.

The null hypothesis that the median difference between the measurements before and after interaction is zero, is tested against the alternative that the median difference is not zero at the 5% level of significance. In a study of this nature, where coherent information is not available, the alternative hypothesis is two-sided. The null hypothesis will be rejected when there is either a significant increase or decrease in the median biochemical levels after interaction. If the null hypothesis is rejected, it can be concluded that there is a statistically significant difference between the before and after treatment values, without specification of the direction of the difference. The direction of the difference can be observed in the data and suggests a tendency.

Note that the median difference is used. The median (50th percentile) is the central value of a data set. Half of the observations in a data set are smaller than the median. When the distribution of the data is symmetric, the median is equal to the mean. If outliers are present in the data, the median is a better

estimate of the central value of a data set, especially in the case of a small sample.

The tables in Chapter 7 (7.4 - 7.35) will give the following information:

- means before and after interaction.¹⁹⁶ Arithmetic mean of measurements before as well as after interaction. These values, together with the minimum, maximum and median are presented in the matching bar graphs, and are used to indicate the range and distribution of the measurements;
- standard deviations before and after interaction.¹⁹⁶ The standard deviation is an indication of the variability of the measurements before and after interaction;
- median before and after interaction.¹⁹⁶ As in the case of the means and standard deviations, the medians of the before and after interaction measurements is reported respectively; and
- p-value, the exceedance probability of the signed rank test.¹⁹⁷ Note that the p-value is not derived from the median values before and after interaction. These values were given to describe the observations before and after interaction took place.

The experimental (own dogs or owner) and control groups were also compared to test whether the familiarity level had any effect on the change in biochemical levels after interaction. In this case the two groups compared are considered to be independent. The Wilcoxon rank sum test¹⁹⁵ was used to test the null hypothesis that the median change in biochemical levels after interaction is the same in the experimental and control group versus the alternative hypothesis that there is a significant difference between the two groups' median change. A 5% level of significance was used.

The following chapter will present and discuss the results.