CHAPTER 2
MATERIALS AND METHODS

CHAPTER CONTENTS:

1. Introduction

All the methods used in the study, physiological as well as psychological, will be dealt with in this chapter. Before the onset of the study it was necessary to test the sensitivity and reliability of heart rate recordings as well as the spectral analysis of heart rate variability. The technique evaluation for this study is presented in Chapter 3. The protocol was presented to the Faculty of Health Sciences Research Ethics Committee, University of Pretoria on 21/10/2003 and accepted (ethical clearance number: S234/2003).

In this study, the psychological profile of the patients was assessed in terms of the attachment style of the patient. To achieve this objective, the Experiences in Close Relationships-questionnaire (ECR-R) was used (1). The physiological health was evaluated by means of a Patient Health Questionnaire (PHQ), gathering information on the patient's past health problems, operations and accidents. The Review of Current Symptoms-questionnaire (RCS) evaluated the patient's present health complaints. The components of health status that are believed to be most affected by fibromyalgia, (e.g. pain, fatigue and depression) were evaluated by means of the Fibromyalgia Impact Questionnaire (FIQ) (2). Neurological parameters assessed as part of the physiological profile of the patients, were hemispheric dominance and autonomic nervous system function. The Herrmann Brain Dominance Instrument (HBDI) determined whether a person prefers to think with either his left or right hemisphere, or with his cerebral versus limbic brain structures (3). Autonomic nervous system function was assessed by spectral analysis of the patient's heart rate variability. ELISA provided a way of measuring cortisol levels in the saliva (4). This gave information on the HPA-axis function of the patient.

2. Summary of tests and techniques used

I. TECHNIQUE EVALUATION (see Chapter 3)

- Technique reproducibility
- Interpersonal variation
- Intrapersonal variation
- Sensitivity and response to stressors

II. PSYCHOLOGICAL PROFILE

Experiences is Close Relationships - Revised (ECR-R)

Fibromyalgia Impact Questionnaire - depression score

Fibromyalgia Impact Questionnaire - anxiety score

III. PHYSIOLOGICAL PROFILE

Heart rate variability (HF, LF, HF/LF etc.)

Salivary cortisol level

Patient Health Questionnaire (PHQ)

Review of Current Symptoms (RCS)

Fibromyalgia Impact Questionnaire (FIQ)

Preferred mode of thinking (HBDI)

3. Experimental subjects

The study group consisted of 31 subjects:

I. Patient group: Fibromyalgia patients presently being treated. Although this could be considered a confounding factor, the purpose of this study was not to investigate the origin of the disease but the status quo, in other words, to put together a profile for fibromyalgia patients irrespective of their therapies (n=16).

II. Control group: Sex- and age-matched healthy controls (n=15).

Patients were selected and clinically evaluated by a physician from the Department of Family Medicine (University of Pretoria) who runs a fibromyalgia clinic. Fibromyalgia patients were subsequently sub-diagnosed with chronic fatigue syndrome (CFS) if they fulfilled the Fukuda diagnostic criteria for chronic fatigue syndrome (see Table 3.2.). Potential control subjects were evaluated to ensure that they did not have fibromyalgia or chronic fatigue syndrome. All subjects gave written informed consent to the experimental procedure. The inclusion and exclusion criteria are set out in Table 3.1:

Table 3.1. *The inclusion and exclusion criteria for the two study groups*

Subject group	Inclusion	Exclusion
Patient group	▪ Patient must meet the 1990 American College of Rheumatology (ACR) classification criteria for FM (see Table 3.3.) ▪ FM must have been confirmed to be present for at least 3 months	▪ Patients with any current psychiatric illnesses diagnosed in addition to FM other than mood disorders of the depressive spectrum ▪ A FIQ score less than 35
Control group	▪ Healthy persons ▪ Body mass index close to that of the patient	▪ Persons suffering from any chronic disease ▪ Persons with current psychiatric illness ▪ A FIQ score larger than 30

Table 3.2. *Fukuda diagnostic criteria for CFS*

1. Unexplained, persistent, or relapsing fatigue lasting six or more consecutive months:
   - that is of new or definite onset
   - is not substantially relieved by rest
   - is not the result of ongoing exertion
   - results in substantial reduction in previous levels of occupational educational social personal activities

2. Four or more of the following symptoms occurring concurrently:
   - impairment of short term memory or concentration
   - sore throat
   - tender cervical or axillary lymph nodes
   - muscle pain, or multijoint pain
   - headaches
   - unrefreshing sleep
   - post exertional malaise (5).

Fukuda, K./ Annals in International Medicine 1994;121:953-959 (5).

Table 3.3. *American College of Rheumatology Criteria for Classification of FM (1990)*

1. History of widespread pain (i.e., presenting at all of the following sites):
   - Right and left sides of body (including shoulders and buttocks)
   - Above and below waist
   - In axial skeleton (i.e., cervical spine or anterior chest)

2. Pain on digital palpation (performed with about 4kg of force) in 11 or more of the following 18 tender points (bilateral points at each site):
   - Occiput: at suboccipital muscle insertion
   - Low cervical: at anterior aspects of intertransverse spaces at C5-C7
   - Trapezius: at midpoint of upper border
   - Supraspinatus: at origins, above scapula spine near medial border
   - Second rib: at second costochondral junctions, just lateral to junctions on upper surfaces
   - Lateral epicondyle: 2cm distal to epicondyles
   - Gluteal: in upper outer quadrants of buttocks in anterior fold of muscle
   - Greater tronchanter: posterior to trochanteric prominence
   - Knee: at medial fat pad proximal to joint line (6).

~In this definition, low back pain is considered segment pain.

~Patient must state the palpation is painful; tenderness is not considered pain.

Ang, D./ Comprehensive therapy 1999;25:221-227 (7).

4. Psychological assessments

4.1. Experiences in close relationships (ECR-R)

4.1.1. Development and validation of questionnaire

The Experiences in close relationships questionnaire consists of 36 items reviewing the individual's 'attachment style', classifying him/her into a secure or insecure attachment group (on a scale of continuity). The questionnaire was filled out while the subject were connected to the Polar heart rate monitor (after an initial baseline recording was completed). This way the questionnaire served as a psychological stressor on the autonomic nervous system.

In early attachment research, the association between individual differences in adult attachment and people's perceptions about their relationships, and their childhood memories

about their relationships with their parents, were studied. Hazan and Shaver (1987) were the first researchers to develop an uncomplicated questionnaire to measure these individual differences (8). The simple questionnaire (based on Ainsworth observations of the 'strange situation) involved three type-descriptions that subjects had to read and indicate which paragraph describes their behaviour in close relationships best:

I. "I am somewhat uncomfortable being close to others; I find it difficult to trust them completely or to allow myself to depend on them. I am nervous when anyone gets too close, and often, others want me to be more intimate than I feel comfortable being."

II. "I find it relatively easy to get close to others and am comfortable depending on them and having them depend on me. I do not worry about being abandoned or about someone getting too close to me."

III. "I find that others are reluctant to get as close as I would like. I often worry that my partner does not really love me or won't want to stay with me. I want to get very close to my partner, and this sometimes scares people away." (8)

The work of Hazan and Shaver (1987) was useful in the study of the association between attachment styles and relationship functioning, but their questionnaire classified subjects into three attachment-style prototypes or categories. These authors did not keep track with additional work done through discriminate analysis by Ainsworth, which stated that the infant attachment types identified in her 'strange situation' should be scored on a continuous rating scale (9). Soon researchers realized that the three major attachment types could be conceptualised as regions in a two-dimensional space, the two dimensions being avoidance and anxiety. The three type-descriptions were broken up into 'agree-disagree' items, which could be factor-analysed, and then presented on continuous scales (10).

Kim Bartholomew (1991) organised these two dimensions conceptually on a two-dimensional, four-category conceptual scheme of individual differences in adult attachment and labelled the two dimensions 'model of self' and 'model of others'. The 'model of self' relates to anxiety and the 'model of others' to avoidance (11). Figure 4.1. demonstrates Bartholomew's four-category scheme:

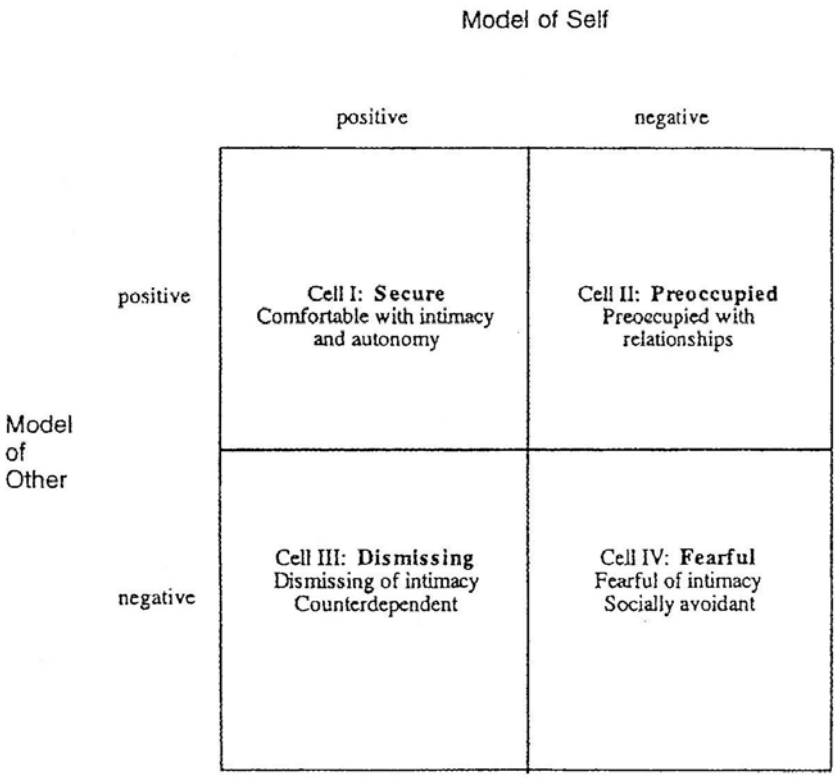

Figure 4.1.1. Bartholomew's (1990) four-category diagram. Model of self – individuals with a high score for this variable tend to be concerned about their partners' availability, attentiveness and responsiveness. A low score is associated with security in relationships. Model of other/partner – individuals on the high end of this dimension, prefer independence. Individuals on the low end tend to be more comfortable with intimacy. Figure taken from *Brennen, K.A./ Attachment theory and close relationships. New York: The Guilford Press; 1998. p. 46-76 (10).*

Brennan *et al.* conducted a large-sample study in an effort to incorporate the findings of various authors actively working on attachment into a comprehensive measuring tool. Out of a pool of 482 (extracted from attachment literature) they selected 323 items from which 60 subscales scores was computed. These subscales were factor-analysed to produce two essentially independent factors that corresponded to the 'anxiety' and 'avoidance' dimensions. After clustering subjects into four groups based on their anxiety and avoidance scores, the groups corresponded to Bartholomew's four types. These findings led to the development of a multi-item measure of adult romantic attachment called the 'Experiences in close relationships' questionnaire (10).

In this study the ECR-R was used to measure attachment as it provides continuous scores on the two dimensions, excluding true attachment typology, as there is no evidence for distinct attachment classes (1).

4.1.2. Contents of questionnaire

Table 4.1.2 includes the questions constituting the Experiences in close relationships-questionnaire (ECR-R). The fist 18 questions form the attachment-related anxiety subscale of the ECR-R (Table 4.1.2.a). Table 4.1.2.b. contains the questions forming the attachment-related avoidance subscale. During the evaluation of subjects, these two subscales are merged into a single questionnaire.

Table 4.1.2.a. *The attachment-related anxiety subscale of the ECR-R*

1. I am afraid that I will lose my partner's love.
2. I often worry that my partner will not want to stay with me.
3. I often worry that my partner doesn't really love me.
4. I worry that romantic partners won't care about me as much as I care about them.
5. I often wish that my partner's feelings for me were as strong as my feelings about them.
6. I worry a lot about my relationships
7. When my partner is out of sight, I worry that he/she might become interested in someone else.
8. When I show my feelings for romantic partners, I'm afraid they will not feel the same about me.
9. I rarely worry about my partner leaving me.
10. My romantic partner makes me doubt myself.
11. I do not often worry about being abandoned.
12. I find that my partner(s) don't want to get as close as I would like.
13. Sometimes romantic partners change their feelings about me for no apparent reason.
14. My desire to be very close sometimes scares people away.
15. I am afraid that once a romantic partner gets to know me, he/she won't like who I really am.
16. It makes me mad that I don't get the affection and support I need from my partner.
17. I worry that I won't measure up to other people.
18. My partner only seems to notice me when I am angry. (10)

Table 4.1.2.b. *The attachment-related avoidance subscale of the ECR-R*

1. I prefer not to show my partner how I feel deep down.
2. I feel comfortable sharing my private thoughts and feelings with my partner.
3. I find it difficult to allow myself to depend on romantic partners.
4. I prefer not to be too close to romantic partners.
5. I get uncomfortable when a romantic partner wants to be very close.
6. I find it relatively easy to get close to my partner.
7. It's not difficult for me to get close to my partner.
8. I usually discuss my problems and concerns with my partner.
9. It helps to turn to my romantic partner in times of need.
10. I tell my partner just about everything.
11. I talk things over with my partner.
12. I am nervous when partners get too close to me.
13. I feel comfortable depending on romantic partners.
14. I find it easy to depend on romantic partners.
15. It's easy for me to be affectionate with my partner.
16. My partner really understands me and my needs.
17. I don't feel comfortable opening up to romantic partners.
18. I am very comfortable being close to romantic partners. (10)

4.1.3. Scoring of questionnaire

The scoring criteria for the ECR-R are published in 'An item response theory analysis of self-report measures of adult attachment.' by Fraley, Waller, and Brennan (2000). The two subscales in table 4.1.a) and b) are answered on a 7-point scale where 1 = strongly agree and 7 = strongly disagree. Certain of these questions are stated in the negative, and need to be reversed before scoring. For the anxiety-related subscale the reversed questions are question 9 and 11. For the avoidance-related subscale, questions 2, 6-11, 13-16 and 18 need to be reversed. After these questions are reversed the scores (on scale ranging from 1 to 7) for each subscale are added together and divided by 18 (the number of questions in subscale). This way a mean anxiety and avoidance score is calculated for each subject. Because of the undersized study group in this study, the scores were not multiplied by the item parameter estimate as proposed when item response theory is applied in analysis (1).

5. Physiological assessments

5.1. Patient health questionnaire (PHQ)

5.1.1. Development of questionnaire

The questionnaire gathers information regarding the medical history of the patient and lists the medication presently utilised. The occurrence of a major traumatic incident, which could have been a possible trigger to the persisting symptoms, was also recorded. Various questionnaires (developed by physicians working with fibromyalgia or other diseases in the multiple subjective complaints spectrum) were combined in order to set up the PHQ. The purpose for the development and inclusion of this questionnaire in the study was to collect information regarding the demographic variables of patients.

5.1.2. Contents of questionnaire

Items on the questionnaire included the following:

- Personal information (age, weight, height, marital status, highest academic qualification, occupation)
- Current medical problems
- Past illnesses and medical problems
- Duration of fibromyalgia complaints
- Previous hospitalisations, surgeries, accidents, major psychological traumatic event with the year in which it occurred
- How fibromyalgia started. Here the patient could choose between the following responses: following an accident, operation or illness; after a time of over-exertion; gradually; without preceding provoking events; following a significant psychological stressor
- Changes in symptoms – whether it be better, more painful locations, higher pain intensity, unclear or no change at all
- Major complaint
- Description of pain
- Treatment. The patient indicated whether he/she make use of an exercise program, physiotherapy, medication, and/or non-allopathic treatment
- List of current medications
- Factors that influence symptoms. Possible factors were exercise, alcohol, stress, time of day, humidity, sleep, caffeine, season, heat, barometric pressure, certain

foods, salt, sunlight, cold. For each of these factors, the patient was expected to state whether it changes their symptoms for better or worse.

- The patient's drinking and smoking habits
- Fitness level
- Disability compensation (this item was included in the questionnaire to discern whether patients exaggerated in reporting symptoms to gain financially from disability compensation)

5.2. Review of current symptoms (RCS)

5.2.1. Development of questionnaire

The RCS-questionnaire verified which symptoms were present, as well as the extend to which patients experience these symptoms. Various internet websites were explored for clinics that treat fibromyalgia. Most of these clinics have a form available on their website that prospective patients need to complete before their treatment program begins. This way the physician can constitute a patient profile before the first appointment. All of these surveys were combined to set up a comprehensive questionnaire assessing all the possible symptoms the patients in this study could present with.

5.2.2. Contents of questionnaire

The total of 100 symptoms, commonly associated with fibromyalgia and chronic fatigue syndrome, were grouped together in categories. The 15 categories were:

- constitutional symptoms, e.g. fatigue
- skin, eyes, ears
- nose/throat
- mouth
- lymph nodes
- breasts
- respiratory symptoms
- gastrointestinal symptoms
- reproductive system function
- thyroid function and
- neuropsychiatric symptoms.

For each symptom the patient has, the patient was expected to state whether he/she experience the symptom as being mild, moderate or severe. Table 5.1 lists the 15 categories with the symptoms associated with that specific organ system:

Table 5.2.2. *The Review of current symptoms (RCS) questionnaire*

Constitutional:	**Breast:**	**Joints:**	**Thyroid:**
fatigue	lumps	ache/pain	mass or lump in neck
weight change	cystic breasts	stiff	cold or heat tolerance
fever/chills/sweats	discharge	swelling	history of x-ray to neck
appetite change	swollen		
abnormal thirst		**G.U. and Hormonal (Female):**	
difficulty sleeping	**Lungs:**		
light-headed	cough	severe menstrual cramps	**Neuropsychiatric:**
	wheezes	severe premenstrual cramps	headache (mild/ moderate)
Skin:	shortness of breath	menstrual irregularity	headache (severe)
itching	- at rest	herpes	depression/apathy
flushing	- on exertion	frequent vaginal discharge	anxiety/irritable
rashes	can't get full breath	yeast or candida infection	hyperactive
hives	hyperventilation	painful or difficult urination	learning disability
dry/rough skin	phlegm/mucus/ bronchitis	pressure/urgency/ itching	"brain fog"/difficulty concentrating
acne	chest pain on exertion	vaginal rash	mood swings
nail/hair problem	other chest pain or distress	sexual problem	suicidal
	palpitations/rapid, slow or irregular heart rate/rhythm		homicidal
Eyes:	ankle swelling	**G.U. (male):**	numbness, tingling
vision	calf pain on exercise	difficulty voiding	faints/blackouts
tearing	sore tender legs	prostate problem	seizures/convulsions
itching	high blood pressure	lump on testis	
feels heavy		sexual problem	**Gastrointestinal:**
allergic shiners	**Mouth:**	herpes	nausea
	sores/fissures		blenching, bloating, or passing gas
Ears:	herpes or frequent cold sores	**Muscles:**	heartburn or stomach pain
itching	gum/tooth problems	tight/stiff	diarrhea
hearing problem	tongue problem	ache-sore-pain	constipation
blocked ears		- neck	cramps or aches
ringing in ears	**Lymph nodes:**	- shoulder	rectal pain or itching
sensitive to sounds	swollen	- upper back	blood or black stools
dizziness/vertigo	sensitive	- low back	worms or parasites
		- extremities	
Nose/Throat:		weakness	
stuffed/runny nose			
postnasal drip			
sore throat			
tight/swollen throat			
hoarse voice			
trouble swallowing			

5.2.3. Scoring of questionnaire

For each subject, the average response to each symptom, average number of symptoms in an organ category, total number of symptoms, and the most severe symptoms, were calculated.

5.3. Fibromyalgia Impact Questionnaire (FIQ)

5.3.1. Development and validation of questionnaire

Burckhardt, Clark & Bennett (1991) developed the Fibromyalgia Impact Questionnaire (FIQ) to be utilized as an assessment and evaluation instrument, measuring fibromyalgia patient symptom status, progress and outcome (2). This brief, self-administered instrument has been designed to measure the components of health that are most affected by fibromyalgia. The FIQ is composed of 10 items, providing scores for physical impairment, well-being, work status, pain, fatigue, stiffness, sleep, anxiety and depression. The items for the questionnaire were derived from clinical interactions with patients, publications on the major characteristics of the syndrome and from existing rheumatology health status instruments like the Health Assessment Questionnaire (HAQ) and the Arthritis Impact Measurement Scales (AIMS) (12,13).

In 1991 Burckhardt *et al* published an article on the validation of the FIQ (2). The AIMS were chosen as the comparison instrument of the psychometric properties of the FIQ as it is a thorough instrument (both psychometrically and clinically) for measuring health status in rheumatic disease; and is more comprehensive than the HAQ. The objectives of the authors to determine the reliability, content validity and construct validity of the FIQ, were met in the following way:

- Reliability (which items of the AIMS yielded valuable information in patients with fibromyalgia)

  The percentage of patients signifying impairment in response to each of the physical function items in the AIMS, were calculated. A cut-off criterion of > 25% impairment responses were set to indicate a valid item.

- Content validity

  The percentage of missing data was calculated.

- Construct validity

  After evidence was gathered for the construct validity of the AIMS and FIQ respectively, correlations were done between the two instruments by associating measures of symptom severity and comparable scales. The authors also attempted to establish whether the 11 sub-items of item 1 would lead to one single factor.

The authors ascertained that the FIQ has test-retest reliability, that there are significant correlations between the items on the FIQ and the comparable scales of the AIMS (indicative of convergent construct validity), and that the content of the instrument is relevant to the syndrome (2).

5.3.2. Contents of the questionnaire

Each of the ten items has a maximum score of 10, with a higher score indicating a greater impact of the syndrome on the patient. The average fibromyalgia patient usually scores about 50, severely afflicted patients 70 plus (the maximum possible score is 100). The questions asked in the FIQ are listed in the following table:

Table 5.3.2. *The Fibromyalgia Impact Questionnaire (FIQ)*

1. Were you able to:
   - Do shopping?
   - Do laundry with a washer and dryer?
   - Prepare meals?
   - Wash dishes/cooking utensils by hand?
   - Vacuum a rug?
   - Make beds?
   - Walk several blocks?
   - Visit friends or relatives?
   - Do yard work?
   - Drive a car?
   - Climb stairs?

Patients were expected to answer these questions on a scale ranging from 0 (always) to 3 (never).

2. Of the 7 days of the week, how many days did you feel good?
3. How many days last week did you miss work, because of fibromyalgia?

For questions 2 and 3, patients had to encircle the number of days ranging from 0 – 7.

4. When you worked, how much did pain or other symptoms of your fibromyalgia interfere with your ability to do your work, including housework?
5. How bad has your pain been?
6. How tired have you been?
7. How have you felt when you get up in the morning?
8. How bad have your stiffness been?
9. How nervous or anxious have you felt?
10. How depressed or blue have you felt?

Questions 4 to 10 were answered by indicating the severity of the problem on a 100mm horizontal visual analog scale ranging from 0 to 10 (2).

5.3.3. Scoring criteria

Table 5.3.3. *The scoring criteria for the FIQ*

No.	Scale	Items	Recode	Score range	Normalization
1	Physical impairment	11	No	0 – 3	Raw score * 3.33
2	Feel Good	1	Yes	0 – 7	Raw score * 1.43
3	Work Missed	1	No	0 – 7	Raw score * 1.43
4	Do Job	1	No	0 – 10	None
5	Pain	1	No	0 – 10	None
6	Fatigue	1	No	0 – 10	None
7	Rested	1	No	0 – 10	None
8	Stiffness	1	No	0 – 10	None
9	Anxiety	1	No	0 – 10	None
10	Depression	1	No	0 – 10	None (2)

The questionnaire is scored in the following manner:

I. The physical functioning scale is made up by the first 11 questions, assessing the patient's ability to perform large muscle tasks. As mentioned above, each of the 11 questions is rated on a 4-point Likert type scale: 0 – always, 1 – most, 2 – occasionally or 3 – never. These scores were then summed. Since it is possible that the patient do not do a specific task at all (not because of impairment caused by fibromyalgia), the patients were given the option to delete the questions that is not applicable. The summed score was then divided by the number of questions answered. The highest possible score for the physical functioning scale is 33. The raw score was normalized (to count out of 10) by multiplying it by 3.33 (see Table 5.3.3.).

II. The score for item two needed to be reverse so that the higher number indicated impairment. The reversed score was then multiplied by 1.43 (see Table 5.3.3.).

III. This score was also normalized by multiplying it by 1.43 (see Table 5.3.3.).

IV. The items 4 – 10 are visual analogue scales marked in 10 increments on which the patients marked the severity of their pain, fatigue, stiffness, anxiety and depression. No normalization needed to be done for these items as the scale already ranges from 0 – 10 (2).

6. Neurological assessments

6.1. Herrmann Brain Dominance Instrument

6.1.1. Background on the assessment of hemispheric dominance

The scientific techniques occasionally used to assess hemispheric dominance include electro-encephalograph measures (EEG), tachistoscope measures, eye movements, dichotic listening and self-administered questionnaires (14). A short description of each technique and the principle it relies on, is presented in Table 6.1.1. Naturally the assumption can be made that physiologically based testing would be the most reliable in hemispheric dominance assessment, but this assumption is not necessarily correct. EEG recordings probably provide the most dependable measurement tool, but could not be used due to a lack of accessibility to the EEG apparatus and expertise to perform the recordings. The other measurement instruments presently being employed have their own limitations (see Table 6.1.1.a).

The practice of assessing an individual's tendency towards right- or left-brain laterisation is common in the corporate sector (in the process of personal selection and training). The validity of these techniques for the measurement of hemispheral laterisation is not well established, though. A couple of self-administered questionnaires have been developed that seems to perform just as well as physiological measures (Table 6.1.1.b). From a financial point of view, as well as availability of instrumentation, these questionnaires offer the most feasible option for the testing of hemispheric dominance. Reviewing the self-administered questionnaires available to assess hemispheric dominance, the Herrmann Brain Dominance Intrument (HBDI) was noticeable the best alternative for reasons that will become apparent in Section 6.1.2.

Table 6.1.1.a *Different techniques for the study of laterisation*

Dichotic listening	
Description	**Principle**
Using stereo-phonic earphones, different sounds (tunes or words) are sent to either the left or the right or both ears simultaneously. The respondent then needs to perform a certain task in response to the signal (14).	Information sent to the one ear will be processed with the opposite hemisphere. For instance: Tunes sent to the left ear seems to be recognized better than tunes sent to the right ear (15). Only a limited number of studies attempted to cross-validate this technique to other measures of hemispheral dominance (14).

Table 6.1.1.a *Different techniques for the study of laterisation – continued*

Electro-encephalographic measures (EEG)	
Description EEG recordings provide a method for the psycho-physiological measurement of the electrical activity of the brain. By placing electrodes on the unopened scull, it is possible to signify variations in brain potential. In studies assessing hemisphere laterisation, electrodes are placed on the left and right frontal region, as well as on the left and right rear side of the scull (14).	**Principle** During rest, the brain exhibits alpha waves from 7 – 12 Hz, where as cognitive activity generates beta waves from 12 – 24 Hz. In the experimental setup, the subject will be asked to perform a certain task, and if the individual is relying more on the on hemisphere than on the other, it will be evident in the electrical activity of that specific brain hemisphere/quadrant. Increased beta waves in this particular hemisphere will be indicative of the individual's preference towards a specific hemisphere (14,15,15).
Tachistoscope measures	
Description A respondent is expected to fix his attention on a particular point. Information is then brought into either the left or the right visual field. Afterwards the respondent is supposed to tell what he saw (what the test material was) (14). This technique was first used by Sperry (1973) in split-brain studies (16).	**Principle** The tachistoscope relies on the principle of human vision that when an object appears in the one visual field (whether is the left or the right), the information is initially transferred to the opposite hemisphere (15). This measure is not that reliable though, because normal individuals will probably transmit the information from the one hemisphere to the other shortly after the initial exposure to the visual field (14).
Eye movements	
Description Different types of questions are asked to the respondent, and his different lateral eye-movements are then observed.	**Principle** Kinsbourne (1972) recorded that with verbal type of questions, the respondents tend to move their eyes to the right, whilst other type of questions results in left lateral eye-movements (17). The validity of this method is questionable, though.

Table 6.1.1.b *Self-administered questionnaires*

Example of questionnaire	**Reference**
▪ Richardson's verbaliser-visualiser dimensions	Richardson, 1977
▪ The Hansen-Lundsgaard lateralisation index	Hansen & Lundsgaard, 1981
▪ The Donegan test	Donegan, 1979
▪ The Herrmann brain dominance instrument (HBDI)	Herrmann, 1979

6.1.2. Development and validation of the Hermann Brain Dominance Instrument

Ned Herrmann, the father of the Herrmann brain dominance instrument, spent 30 years in active research to develop the instrument. In his search for a way to measure brain dominance, he had two main objectives. He wanted to develop an instrument that would be able to provide a scale for measuring preference in mental functioning, similar to the model used to measure handedness. In other words, he wanted the instrument to measure and express laterisation on a continuum from left to right (18).

It is important to note that Ned Herrmann defined laterisation/ hemisphere dominance in terms of the individual's preferred thinking style or his 'preferred modes of knowing' like Herrmann called it. The specific thinking style used by an individual was determined by assessing the individual's tendency to use faculties characteristic of each hemisphere (i.e. analytic thinking for the left hemisphere or holistic thinking for the right). This way, Herrmann's second objective for the model was reached: the model had to relate measures of brain dominance to specific thinking and learning styles (3).

The first step in the development of the instrument was to find some kind of measuring device to supply the data for the individual preferences in thinking styles. Herrmann started the search by performing biofeedback experiments utilizing a bimodal EEG apparatus. In these experiments different tasks were performed to see which hemisphere was activated during those tasks (18).

The success with the initial biofeedback experiments led to comprehensive EEG research, referred to as the 'Berkeley brain tests' where a 'mind mirror', providing an analogue display of the frequency states in both hemispheres at once, were also used in conjunction with the digitised autogenic EEG apparatus (14,18). The initial results were confirmed but this method still did not offer an ideal way in which individuals could be tested (for practical and financial reasons).

This was the motivation to develop the instrument in the form of a questionnaire, the items of which were validated with EEG-measures and factor-analised to determine what factors explained the correlations among different items. The instrument was cross-validated with selected psychological tests (19). In this factor-analytical study, seven factors were extracted from the 18 variables (16 factors from the psychological tests and the left and the right score from the HBDI profile). The correlations between these 16 factors and the left and right score of the HBDI were all under 0.4 except for the 'sensing-intuition' and the judging-perceiving' score of the Myers-Briggs instrument (14,19).

6.1.3. Composition of instrument

The instrument is based upon a questionnaire in which subjects:

- indicated their preferred job activities out of 60 alternatives;
- selected eight self-descriptive items among 25 possibilities;
- reported on preferred hobbies from 23 alternatives;
- had to choose among 24 self-descriptive adjective pairs;
- had to rate 20 Likert-type self-descriptive items;
- indicated their own perception of their degree of introversion vs. extroversion
- reported handedness
- had to indicate whether they have tendencies towards motion sickness (3,14).

6.1.4. Scoring of the instrument

In this study, the scoring of the instrument involved that the subject's responses to the questions above were captured with software provided by Ned Herrmann International (Africa). The data were then sent to Ned Herrmann International USA to be scored in a standardized, rather complicated manner. Only the patients' responses were scored because of insufficient funds. The patients' brain profile scores was then compared to data obtained from over 500 000 scored HBDI surveys (published in 'The Creative Brain' by Ned Herrmann).

The HBDI determined the subjects' tendency towards right versus left hemisphere, and cerebral versus limbic brain structure thinking. An individual's thinking style were described in terms of a score for each one of the following quadrants: the so-called cerebral left, cerebral right, limbic left and limbic right (each one of these quadrants is referred to as quadrant A, quadrant D, quadrant B and quadrant C respectively). In addition to the

quadrant scores, percentages for the left and right hemisphere (mode) as well as the cerebral and limbic structures were calculated by adding the scores for quadrant A and B together for a 'left mode' value; quadrant C and D together for a 'right mode' value; quadrant A and D for a cerebral structure value; and B and C for a limbic structure value. The scores for each quadrant were drawn in a figure like the one shown in Figure 6.1.4. In the figure it is suggested that a total of 27 different individual types can be distinguished based on the scores for each quadrant/dimension (3). These types of profiles are referred to as 'generic codes' or 'profile codes'.

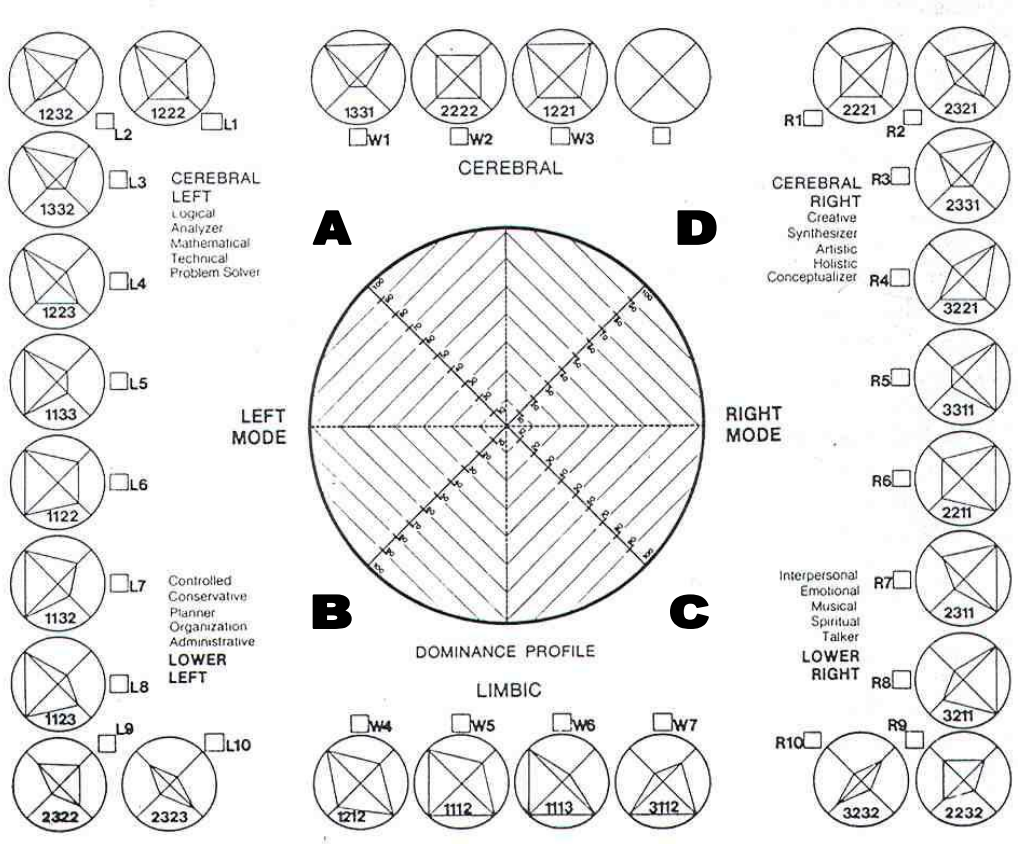

Figure 6.1.4. Scoring scheme for the Herrmann brain dominance instrument. Typical examples obtainable from the four HBDI quadrants are shown around the central scoring scheme. Abbreviations: **A**, quadrant A; **B**, quadrant B; **C**, quadrant C; **D**, quadrant D. Figure taken from *Hansen, F./ Journal of Economic Psychology 1984;5:49-70 (14).*

Generic codes are described by various combinations e.g. 2-1-3-1, 2-1-1-1 or 3-2-1-1 (as seen in Figure 6.1.4.). These combinations are representative of the four HBDI quadrants in the following arrangement: A-B-C-D ('A' referring to quadrant A, 'B' to quadrant B, 'C' to quadrant C and 'D' to quadrant D). In these combinations a '1' indicates a primary (very strong) preference, which means that the person obtained a score of 67 and higher for the specific quadrant. A '2' refers to a secondary preference (intermediate), with scores

between 34 and 66 for the particular quadrant. '3' is a tertiary (low) preference, indicative of scores less than 34. Thus, the generic code 3-2-1-1 actually means: quadrant A (low preference) – quadrant B (intermediate preference) – quadrant C (very strong preference) – quadrant D (very strong preference).

An interesting feature of the HBDI is the score calculated for what is referred to as 'adjective pairs'. This score is calculated from a range of responses on the questionnaire where the person is forced to choose between adjective pairs of self-descriptive words. In other words, the person must select the word (from the adjective pair) that he/she feels describe him/herself the best, even if the person feels that he/ she doesn't relate hundred percent to that word. Apparently this score, also expressed in terms of the four HBDI quadrants, is an indication of how a person will react or behave in stressful situations.

Before the onset of the study the MSc candidate underwent training in the administration of the HBDI as well as in the interpretation of the results obtained by the instrument.

6.2. Heart rate variability

6.2.1. Heart rate variability (HRV)

The technique evaluation for the recording and analysis of R-R intervals (heart rate variability) were completed before the onset of the fibromyalgia study. During the technique evaluation, technique reproducibility, interpersonal variation, intrapersonal variation and the technique's sensitivity in response to stressors were evaluated. The technique evaluation can be found in Chapter 3. The physiological basis as well as the mathematical analysis of heart rate variability is also discussed in that chapter.

6.2.2. The recording of R-R intervals

R-R intervals were recorded using the Polar S810 Heart Rate Monitor. The recording itself relies on a few simple steps:

- The transmitter is put around the subject's chest after a water-based gel had been applied to the electrodes.
- The wrist receiver is put around the subject's wrist.
- With the press of the OK button on the wrist receiver the subject's heart beat per minute are displayed on the screen.
- With a second press of the OK button, the stopwatch and exercise recording start.

During the first session, basal recordings were done, followed by a physical stressor (subject lied down, sat upright, and were then required to stand up). On the second study day, a basal recording was done followed by a psychological stressor (subject was required to fill out the attachment (ECR-R) questionnaire whilst connected to the monitor).

To terminate the recording, the stop button was pressed. The stopwatch and other calculations stopped. The heart rate measurement continued until the stop button is pressed a second time. The exercise data could then be downloaded to the computer by means of an interface using an infrared connection.

6.2.3. Analysis of data

The procedure followed in the analysis of the R-R interval data are set out in Chapter 3. Similar to the technique evaluation, data was analysed with advanced HRV Analysis Software 1.1, developed by The Biomedical Signal Analysis Group, University of Kuopio, Finland. Time- and frequency domain parameters were then calculated at five-minute intervals. Each 30-minute recording period were segmented into ten-minute segments, separating supine, sitting, standing and ECR-R recordings. In the frequency domain, only fast Fourier analysis was used to study the sympathetic-parasympathetic balance and the amount of variability in heart rate, since the technique evaluation proved it to be more reliable than autoregression transformation analysis.

The variables applicable to the assessment of autonomic balance and the amount of variability were:

- Time domain results:
  - mean heart rate (HR)
  - standard deviation of the mean heart rate (mean HR (STD)
- Frequency domain results:
  - low frequency (LF)
  - LF normalised units
  - high frequency (HF)
  - HF normalised units
  - LF/HF ratio
  - total power.

These parameters are described in detail in chapter 3.

7. Endocrinological assessment (salivary cortisol)

7.1. Enzyme-linked immunosorbent assay (ELISA)

7.1.1. Salivary cortisol

The cortisol level in saliva represents the concentration of biologically active free cortisol (4). As no venous puncture had to take place by drawing blood, the DRG Salivary Cortisol ELISA (purchached from AEC Amersham (PTY) LTD) provided a reliable method for the determination of free cortisol. This way the stress experienced by the subjects was minimised, and dependable values could be obtained for the level of cortisol.

7.1.2. Saliva collection

During the day, there is fluctuation in cortisol levels, with the highest level in the morning and the lowest level at night (20). For this reason, samples were taken at the same time of the day and the exact time the samples were taken was recorded to be able to take circadian rhythms into account. Each subject delivered ±10 ml of unstimulated saliva into a sterile centrifuge tube. The saliva was centrifuged at 3500 rpm for 10 min at 4 ˚C, the clear supernatant removed and stored at –70 °C until use.

7.1.3. The assay

7.1.3.1. Principle of the test

The solid phase enzyme immunoassay for cortisol is based on the competition and microplate separation principle. An unknown amount of cortisol present in the sample and a fixed amount of cortisol conjugated with horse-radish peroxidase (HRP-cortisol) compete for the bindings sites of a polyclonal cortisol-antiserum, coated onto the wells of the microstrips. An hour incubation time follows. Once the competitive immuno-reaction has occurred, the microtiterplate is washed to stop the competition reaction. After the substrate solution is added, the HRP-cortisol fraction bound to the antibody in the solid phase is converted to a blue compound. The cortisol is inversely proportional to the optical density of this compound measured at 450 nm (4).

7.1.3.2. Validity of method

AEC Amersham LTD evaluated their technique for determining salivary cortisol by means of ELISA by calculating the specificity, precision and accuracy of the test and finding a lower limit of detection (4).

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Specificity

The specificity of the DRG Cortisol kit was assessed according to Abraham's method. The specificity of the kit for corticosterone is 29.0%, 60.0% for prednisolone, and 100.0% for cortisol.

- Precision

  The inter assay variation coefficient for a sample size of 19 is 5.88% and 4.73% for n = 21.

  The intra assay variation coefficient for a sample size of 18 is 5.14% and 3.65% for sample size of 20.

- Accuracy

  The accuracy of the assay was evaluated by recovery and dilution tests. The recovery tests proved that the kit's percentage recovery (depending on the concentration cortisol) ranges from 98.6 to 107.7%. According to the dilution test the percentage recovery ranged from 91.2 to 107.8%.

- Lower limit of detection

  The lower limit of detection is defined as the cortisol concentration given by the mean absorbance of the zero calibrator minus two standard deviations. It has been found to be approximately 1.14 ng/ml (3.14 nmol/l) (4).

7.1.3.3. Assay procedure

Table 7.1.3.3. *The ELISA procedure*

1.	Bring all reagents to room temperature.
2.	Leave sufficient strips in the strip holder to enable the running of standards, controls and samples in duplicate, plus one well for chromogen blank. Place the remaining strips and the desiccant into the transparent plastic pouch and seal it properly.
3.	Pipette 50 µl of standards and samples into the appropriate wells of the strips.
4.	Add 250 µl of HRP-cortisol conjugate to each well in sequence.
5.	Incubate for 60 minutes at room temperature without covering the plate.
6.	Washing: discard the incubation solution, rinse the wells three times with the washing solution, and remove any residual
7.	Promptly pipette 100 µl of the chromogen/substrate mixture into the rinsed wells.
8.	Incubate for 15 minutes at room temperature.
9.	Stop the reaction by pipetting 100 µl of stop solution into the wells with the same sequence adopted to dispense the chromogen/substrate mixture.
10.	Shake the microplate gently, being careful not to let the content come out from the wells and read at 450 nm within 30 minutes from stopping.

7.1.3.4. Calculation of results

The cortisol level of each sample was then obtained as followed (4):

$$B/Bo * 100 \quad \frac{A - Ac}{Ao - Ac} \quad * 100$$

- The mean absorbance of the standards and the samples (A) were calculated.
- The absorbance of the chromogen blank (Ac) was subtracted from all the means.
- Then, the corrected mean absorbance obtained was divided by the corrected mean absorbance of the zero calibrator (Ao), and multiplied by 100.
- A standard curve was constructed by plotting the average absorbance of each reference standard against its corresponding concentration. The average absorbance of each serum sample was used to determine the cortisol concentration value by simple interpolation from the standard curve. The cortisol concentration was calculated in ng/ml, and this value was then compared to the normal controls as well as normative values for the time the samples were taken.

8. Statistical calculations

Statistical calculations (descriptive and inferential) for each of the evaluations mentioned above were done in collaboration with staticians from the Department of Statistics, University of Pretoria. Descriptive statistics involved the generation of contingency tables as well as the calculation of means and standard deviations for all the respective variables. Inferential statistical estimates provided p-values for the differences between the patients and controls. Finally, Pearson coefficients (correlations) and model R-square estimations (obtained through regression analysis) were calculated to aid in setting up a psychoneurological profile for the fibromyalgia patients.

9. Schematic representation of daily procedures

Diagnosis of patient (ACR classification 'tender point' assessment)

Evaluation of control (according to inclusion criteria)

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Study Day 1:

- Saliva sample taken (for patient and control group)
- Explanation of protocol and informed consent to subject
- Heart rate variability recording (HRV):

  Physical stressor:
  - 10 minutes supine
  - 10 minutes sitting
  - 10 minutes standing
- Explanation of questionnaires (HBDI, PHQ, RCS, FIQ) – filled out in own time

↓

Study day 2:

- Heart rate variability recording (HRV):

  Psychological stressor:
  - 10 minutes supine
  - 10 minutes sitting
  - 15 minutes filling out ECR questionnaire
  - 10 minutes supine
- Patient hand back completed questionnaires

Patients were visited at their homes to minimize stress and discomfort.

The questionnaires were completed in the patient's own time, and collected on the final study day.

All the evaluations were done during a 07:30 to 9:00 timeslot. The precise time each determination was done, were recorded on the following sheet:

PATIENT PROTOCOL

Patient no:…….
Date:…….

Session 1

				Time allocated	Time
1. Introduction				5 min	:
2. Saliva sample				10 min	:
3. Heart Rate Variability	Supine: (10:00)	BP	Pulse		:
	Sit: (10:00)				:
	Stand: (10:00)				:
4. Questionnaires – explain each scale	Complete in own time				

Session 2

Date:…….

				Time allocated	Time
1. Introduction				5 min	:
2. Heart Rate Variability	Supine: (10:00)	BP	Pulse		:
	Sit: (10:00)				:
	ECR: (15:00)				:
	Supine: (10:00)				:
3. Review questionnaires	In own time				

(The same protocol was followed for each of the controls)

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