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Application of neurophysiological methodology in acupuncture research

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Abstract

Title: Application of neurophysiological methodology in acupuncture research.

Background: Increased popularity and acceptance of acupuncture also increases demand for scientific evidence of its effectiveness. In order to produce such evidence acupuncture research borrows methods from other scientific disciplines. Thus it is essential to validate the usefulness of such methods when they are employed specifically in physiological research of the acupuncture phenomenon. Confirmation of physiological changes due to acupuncture stimulation could increase engagement in acupuncture research and make direct comparisons with biomedical research possible.

Objectives: The main objective was to evaluate the usefulness of non-invasive neurophysiological methods in detecting physiological changes in response to manual acupuncture. It was not the purpose of this study to find a proof that acupuncture works or how it works, but to confirm that acupuncture can be successfully researched using objective, non-invasive neurophysiological methods.

Method: A rigorously designed, randomised controlled trial (RCT) was used to evaluate the effectiveness of some of the neurophysiological research methods. Three-point criteria was set up. Firstly, to investigate if manual stimulation of the acupuncture points can induce physiological changes in healthy subjects that are strong enough to be detected by non-invasive testing methods. Secondly, to investigate if physiological changes induced by acupuncture stimulation form a pattern, which is unique for every individual acupuncture point being tested. And thirdly, to investigate if physiological changes induced by acupuncture are more pronounced in subjects initially showing stronger signs of subjective / objective stress. Overall, sixty healthy female subjects were recruited for the study, and one hundred and twenty experimental sessions were completed in order to collect the data. Objective measurements by means of multichannel computerised recording were used to capture concurrently occurring physiological events.

Results: Acupuncture treatment used in group one (T1) using acupuncture point LU7 and in group three (T3) using acupuncture points LU7 and KD6 promoted deeper relaxation and light sleep in subjects showing higher levels of anxiety. In contrast, subjects in group two (T2) where only acupuncture point KD6 was stimulated, did not relax or easily fall into light sleep during the 40 minutes post-treatment. Usually Theta and Alpha "relaxation" waves were changed. Of the two treatment protocols T1 and T3, the T1 showed the strongest differences. This can be interpreted as indicating that acupuncture point LU7 promotes relaxation and light sleep. Stimulation of acupoint KD6 counteracted the relaxation effect promoted by acupoint LU7. Data from the experiments clearly showed that stimulation of two acupuncture points LU7 and KD6 did not influence heart beat in a strong way. The treatment effect was only detected in group T2 (acupoint KD6) where an increase in the sympathetic regulation may indicate that KD6 has a balancing and energising effect on heart rhythm. The respiration rate and respiration amplitude seems to be unchanged by acupuncture stimulation. There were no statistically significant differences in body temperature before and after the experiment. Systolic and diastolic blood pressure remained very stable and was not influenced by acupuncture stimulation of the two acupoints LU7 and KD6. Analysis of the electrodermal activity (EDA) data confirmed that changes in skin potential are very sensitive and specific to needle insertion and needle removal, but not specific to the site of the insertion. Physiological habituation seems to play significant role in diminishing the EDA response to manipulation of the acupuncture needle.

Conclusion: Insertion of a single needle invokes physiological changes that can be detected by neurophysiological methods. Certain acupoint-specific patterns of change can be seen in multichannel recordings. Results suggest that neurophysiological methods are appropriate for, and can be adopted in acupuncture studies. Furthermore, recommendations regarding research designs were formulated. They include a strong need for a separate control group in acupuncture studies and highlight the importance of continuous monitoring of multiple physiological parameters during experiments of that type. New methods of data analysis based on neural networks gave very promising results that may rival traditional statistical linear models. Overall the outcome of this project may serve as a call for standardisation of neurophysiological research protocols in acupuncture laboratories.

Chapter 1. Introduction

Acupuncture is a treatment modality that is now well accepted in Western society. It has its origin in the oriental systems of medicine, primarily in Traditional Chinese Medicine (TCM). The exact point which marks the beginning of acupuncture is unknown but various historical artifacts and early historical writings suggest a history of at least 3000 to 5000 years (Unschuld, 1990; Chen, 1997). Some authors even suggest that the Chinese borrowed the theories of acupuncture from yet another even more ancient civilization which preceded them (Wolfson, 2003).

One of the prominent Western writers on traditional Chinese medicine, Dr Kaptchuk, states that Chinese medicine views health and disease quite differently from Western medicine (Kaptchuk, 1983). For example, illness is viewed in TCM as disharmony of the whole organism and the purpose of any treatment intervention is to restore this harmony. Acupuncture practitioners developed over the centuries, not only unique therapeutic techniques to maintain this harmony, but they also refined the theoretical framework underpinning their work, including functions of acupuncture points and the theory of meridians.

The practice of acupuncture in Western countries took many turns in the last thirty years and its acceptance and popularity is growing. This acceptance by the general public is, however, not necessarily supported by the existing biomedical model of medicine. There is an increasingly strong call for the scientific evidence of acupuncture effectiveness and an explanation of acupuncture mechanisms.

1.1 The context of the investigation

In order for the practice of acupuncture to prosper in Western society in a similar fashion to medical practice, some fundamental conditions need to be met. One such condition is, of course, the provision of an effective and safe treatment for known diseases, in particular those health problems for which biomedical medicine has no remedy available. The second condition is the acknowledgment of the demand for evidence based medicine (EBM) in all areas of health service delivery: in the hospitals, in general practice (GP), and in complementary and alternative medicine (CAM). Patients are now well informed about available choices and are able to consider various options before committing to any one treatment. Evidence is becoming a key factor in the acceptability of old and new treatments. Practitioners of acupuncture and researchers need to develop more collaboration and they need to implement randomized controlled trials (RCT) as a routine way to measure the effect of acupuncture interventions. Finally, new models for acupuncture research need to be developed; models that are better suited to the theoretical and practical framework of acupuncture practice.

The research project presented in this thesis aims at adding a better understanding of the role which acupuncture research should play in the practice of acupuncture, and in particular, the application of non-invasive neurophysiological methodologies.

1.1.1 The use and acceptance of acupuncture as a treatment modality

Acupuncture is considered a relatively safe treatment modality. There are very few reported incidences of serious side effects (Ernst, 2003). At the time when many biomedical and pharmacological treatments carry the danger of moderate-to-severe side effects, acupuncture enjoys a status of high safety. Patients in Australia use acupuncture routinely for such complaints as back and neck pain, headaches and menstrual disorders. They also try acupuncture for various others disease conditions for which biomedical medicine has little to offer.

Acupuncture is used both as a complementary and as an alternative “medicine”. In the first instance acupuncture may help to reduce the side effects of pharmacological treatment in cancer management (Johnstone, et al. 2002). In the second instance, it may become a true alternative to surgical or pharmacological interventions as is the case in musculoskeletal problems and in the control of arthritic pain (Soeken, 2004). Despite the fact that the 90 % of the costs of CAM consultations in UK were purchased privately, according to the survey conducted in 1998, researchers concluded that CAM therapies made a “measurable contribution” with an estimated 22 million visits to practitioners of six major CAM modalities (Thomas, et al. 2001). Recent studies in Australia revealed that demographics, patients' attitudes and the postmodern values about health are correlated with the use of CAM modalities. Active involvement in the healing process and patient's belief about natural remedies should not be ignored in the evaluation of CAM usage (O'Callaghan and Jordan, 2003).

Popularity of acupuncture and other CAM therapies is consistently reported in many countries. Approximately 30% of patients with allergies and asthma in Europe reported the use of complementary and alternative medicine. Only a few modalities account for the majority of use, acupuncture being one of the primary choices (Schafer, 2004). A survey conducted in Italy in 1999-2000 confirmed wide spread use of CAM therapies. Almost 9 million Italians (15.6%) used at least one CAM therapy in 1999, the most popular being homoeopathy (8.2%), manual treatments (7.0%), herbal medicine (4.8%) and acupuncture (2.9%) (Menniti-Ippolito, et al. 2002). The cost effectiveness of acupuncture in the treatment of chronic headaches was evaluated positively in a study conducted in the UK. A one year follow up revealed that acupuncture improved health related quality of life at a small additional cost (Wonderling, et al. 2004).

The integration of acupuncture with biomedical medicine is also finding its way into hospitals. For example, out of 13 hospitals affiliated with the prestigious Harvard Medical School, eight had acupuncture available in the ambulatory clinics (Highfield, et al. 2003). One Norwegian study found big discrepancies, however, in attitudes towards CAM therapies between oncology professionals. Only 4% of physicians as compared with 33% of nurses and 32% of radiographers described their reaction to CAM as positive (Risberg, et al. 2004).

A review of the literature shows that the overall acceptance of CAM therapies, including acupuncture, is increasing and that CAM is gaining popularity among primary health care providers. A study conducted in United Kingdom revealed that there is “considerable interest” among professionals in either using CAM modalities or referring patients to CAM therapists (van Haselen, et al. 2004). Interestingly, the main reason for medical doctors making referrals to therapists were direct patient request (68%) , failure of conventional treatment (58%), and existing evidence for CAM effectiveness (36%).

Also, more than half the health care professionals in that survey considered participating in studies investigating CAM (56%), with the greatest preference being given to research in acupuncture (41%), rather than homoeopathy (30%) or massage (24%).

1.1.2 Misunderstanding between scientific researchers and TCM practitioners

Clinical practice guidelines are becoming an accepted method of quality assurance in medical practice. General practitioners use them every day in order to ensure that their patients receive the best possible diagnosis and treatment. Without practice guidelines, the practice of medicine could slide into dangerous individualism, unethical experimentation, the use of unproven methods, or it may even decay into the routine prescription of drugs long abandoned by modern medical practitioners.

The role of clinical practice guidelines in complementary and alternative medicine was examined in 1995 by a panel of experts established by the National Institute of Health in the Unites States . The panel concluded that "CAM practices currently are unsuitable for the development of evidence-based practice guidelines, in part because of the lack of relevant outcome data from well-designed clinical trials" (NIH, 1997, page 154). The Cochran Library contains only a handful of systematic reviews of CAM therapies. In 2003 it included 20 reviews of herbal medicines, 7 of acupuncture, 3 of homoeopathy, 2 of manual therapies and 2 of other modalities (Ernst, 2003).

Such a situation, if continued may well jeopardize the growing acceptance of acupuncture and other CAM therapies. A call for more research is only partially answered by researches and practitioners. There is limited research being conducted, but the methodological limitations and poor quality of RCTs in CAM make much of this effort invalid (Veal, 2004). In fact even case study reports, which are of much less value than RCTs, are difficult to find in the published literature. They may be useful in describing events such as rare and serious conditions, adverse effects or the effectiveness of certain styles of practice, but practitioners generally do not publish the results of their work. Some of the acknowledged reasons are lack of time, working in isolation and not having enough experience in writing case study reports (White, 2004b).

Much of the research in acupuncture seems to be conducted by professional scientists rather than clinical practitioners. Typically the 'proof of effectiveness', or lack of it, is presented to an audience of other researchers rather than acupuncture practitioners, and in the form of articles in scientific journals rather than as practice guidelines. At the end of the day it is clinically relevant data that counts in improving the clinical practice of acupuncture. A survey conducted among medical acupuncturists revealed that clinical details of the research were the most important focal points in the evaluation of published papers (Claraco, et al. 2003).

Far too many published papers on acupuncture consist of polemics and affirmations regarding the position of acupuncture in the modern health care system. A search of Medline reveals more than four thousands papers mentioning acupuncture, yet only a few hundred of them are randomized controlled trials. A paradoxical situation has therefore emerged where researchers and practitioners are not communicating well. What practitioners want is practice guidelines that are based on evidence, the evidence that the researchers seem unable to supply. Guidelines ideally should be in a form of high quality RCT from which clinical practice can be synthesized. The general impression remains the same; that there are very few acupuncture practitioners who have motivation, sufficient understanding of research methodologies, and the necessary know-how to conduct acupuncture research; and there are certainly few researchers who are acupuncture practitioners, or who have an interest in developing long-term acupuncture studies.

1.1.3 The need for experimental and clinical research in acupuncture

Clinical research in acupuncture already exists and the results of new studies are published every year. Reviews of these studies and meta analysis of the results indicate however, that there are very deep systemic problems. Reviewers agree that “the methodological rigor of acupuncture clinical trials has generally been poor and that higher quality clinical trials are necessary” (Birch, et al. 2004, page 468). Reported effectiveness of acupuncture varies enormously depending on the type and quality of the study and disease conditions.

A review of asthma studies published in 1998 revealed that there is insufficient support for the inclusion of acupuncture in the management of this disease (Davis, et al. 1998). A further study published four years later only confirmed this conclusion (Martin, et al. 2002), and yet another meta-analysis published the same year extended this conclusion to major CAM modalities (Steurer-Stey, et al. 2002).

How can we then say that acupuncture is a valid treatment option for patients suffering, for example, from asthma? What clinical value is in such statements as "LU7 *Lieque*, stimulates descending and dispersing of Lung *Qi*, circulates the *Wei Qi* and releases the exterior; opens the *Ren Mai* with KD6 Z*haohai*" (MacPherson, 1997)? Possibly thousands of acupuncture practitioners read such statements and apply them in everyday practice believing in their effectiveness. But where is the evidence that acupoints LU7 and KD6 actually do what is assigned to them in acupuncture manuals?

The need for quality research in acupuncture is obvious and much depends on the willingness of researchers to reconsider their current approach. Even hundreds of new RCTs will not produce clinical guidelines if RCTs are of poor quality. And no number of speculative discourses on the possible origins of acupuncture will replace well designed physiological/neurophysiological investigations into biological and/or energetic mechanisms of acupuncture.

1.2 The rationale for the project

How can one doctoral research project change the way the research in the field of acupuncture is conducted. It is certainly a challenging question but in order to claim any degree of rationality in any scientific project this question needs to be addressed. Research presented in this thesis can be characterized as a pragmatic study into the physiological effects of stimulation of selected acupuncture points. It used non-invasive neurophysiological methods of monitoring changes in the physiology of healthy volunteers and employed a randomized and controlled experimental design to gather data. In its experimental component the project aimed to provide methodologically coherent findings and in its analytical component it aimed to demonstrate how different aspects of the research paradigm can affect research outcomes in acupuncture studies.

1.2.1 The use of acupuncture stimulation to induce physiological changes

Needle stimulation is an essential component of acupuncture treatment. A call for evaluation of various treatment techniques in acupuncture is essentially a call for the validation of acupuncture as a therapeutic intervention (Cummings, 2001). Traditional or classical acupuncture uses metal needles which are manually inserted into the skin and underlying tissues. The manipulation of the needle can take many forms such as turning, thrusting, pushing and pulling. The needle is then left inserted in the patient's body and in principle it works therapeutically by changing the flow of *Qi* in the acupuncture channels. Stimulation is sometimes modified by the application of moxibustion (heating the needle by burning dried artemisia herb). In contrast to more traditional acupuncture, the electroacupuncture technique applies active electrical current to acupuncture points. This electrical current stimulates surrounding tissues and nerve endings. Thus the recent trend in acupuncture research of replacing manual stimulation with electroacupuncture can raise many concerns as to the mechanisms of such stimulation. So called laser acupuncture, which in a strict traditional sense is not acupuncture at all, should be considered a different technique to manual acupuncture and should be used in acupuncture studies with caution.

In order to map the relationship between acupuncture stimulation and changes in the physiology of healthy subjects, the treatment intervention should be simple and the therapeutic outcomes clearly defined. In this doctoral research project, treatment intervention was defined as manual stimulation of the acupuncture needle inserted into the acupuncture point. The expected outcomes were defined as changes in the physiology of the major functional systems of the human body; cardio-pulmonary system (CPS), central nervous system (CNS) and autonomic nervous system (ANS).

The benefits of such an approach can easily be foreseen. Results of studies based on the measurement of known physiological functions are easily interpreted in biomedical terms. They can also be interpreted in terms of the 'physiology of *Qi*' and associated with known facts extracted from the clinical practice of acupuncture. For example, most TCM clinicians will know that the area on the skin surrounding the acupuncture needle frequently becomes red when the needle is stimulated. What does it mean? Is the redness restricted only to the surface of the skin or is it due to the increase of blood flow in the entire muscle underneath the skin? The blood flow in the skin and muscles can be objectively measured and compared with the baseline values. In one recently published study, researchers discovered that deep muscle stimulation with an acupuncture needle produced an increase in blood flow in both the skin and the muscles when compared with baseline measurements (Sandberg, et al. 2004).

Unfortunately there are very few scientific papers published in the English language that use objective physiological measurements in human TCM studies. The vast majority of research papers describe physiological changes in animals under invasive surgical procedures. One such study for example describes changes in the production of free radicals in brain tissues and improved learning in the water labyrinth as a result of acupuncture and medication treatment. Subjects of the study were rats suffering from experimentally induced dementia. One treatment group received electroacupuncture and another group nimodipine medication (Wang, et al. 2004). Even if that study was 'methodologically sound' the results are still difficult to interpret. In contrast, another study conducted on 63 human dementia patients using a battery of physiological and functional measurements concluded that "acupuncture treatment was superior in immediate therapeutic effect on senile vascular dementia to drug treatment". Despite the fact that there were imperfections in the methodology of this study the results can at least be considered as a good pilot for future randomised controlled trials.

1.2.2 The application of neurophysiological methodology in acupuncture research

A careful review of the acupuncture literature confirms that acupuncture has not yet developed its own unique research paradigm. Theoretically, any investigative method could be used in acupuncture studies as long as it delivers objective measurements of physiological and psychological conditions or evaluates treatment outcomes such as quality of life or reduction of pain. Potential evaluation methods vary enormously from biochemical analysis of blood samples to functional magnetic resonance imaging (fMRI). Not all methods however are suitable for acupuncture research and there are important reasons for this. Firstly, acupuncture treatment is based on stimulation and movement of *Qi*, and the 'integrity' of *Qi* is essential in fostering the body's response to acupuncture stimulation. Any additional disturbance of *Qi*, either through illness or experimental procedure should be taken into consideration. Secondly, a conscious human subject may feel stress and discomfort from the experimental procedure itself, such as having blood samples taken, application of cold water stressors or strong forces applied to pressure points. Mind and emotions also have their own influence on physiology and are regularily reported in psychophysiological literature (Pankseep, 2005; Davidson, 2003; Gordon, 2001).

In animal studies the application of acupuncture together with invasive surgical procedures have been used frequently (Klide, 1992). However, concern for the wealthfare of the animal should be sufficient to reject animal research models in acupuncture. An in-depth analysis of published RCTs (Backer, et al. 2004; Boutouyrie, et al. 2001; Martin, et al. 2002; Xue, et al. 2002; Meng, et al. 2003) indicate that in order to improve the quality of acupuncture research in physiological studies on human subjects the methodology should have the following important properties:

1. Sensitivity to detect small changes in the functioning of major organs and physiological systems. Acupuncture is a gentle treatment intervention and it is likely that the physiological changes, if they occur at all, would be of small magnitude with a tendency to accumulate during repeated acupuncture treatments over time.
2. Continuous monitoring of the physiological parameters for a duration equivalent to, or exceding, an acupuncture treatment session in a clinical setting, which is about 30-60 minutes. Ability to maintain this continuity of monitoring over 24 hours or even several days will improve assessment.
3. Simultaneous recording of physiological parameters from multiple physiological systems with the ability to analyse data for correlations and interdependencies between physiological events.
4. Non-invasivness as an essential property for ethical as well as for safety reasons, leaving insertion of the acupuncture needle as the only invasive procedure.

5. Straightforward interpretation of the results and compatibility with methods of biomedical sciences, both in physiological interpretations and statistical analysis.
6. Standardized research protocols and ease of replicating experiments by researchers in other centers.

Good examples of sucessful adaptation of neurophysiological methodologies can be found in recent studies. For example, electroencephalography (EEG) that measures electrical activity of the brain was used to evaluate sedative effects of acupuncture stimulation of the *yintang* acupoint (Litscher, 2004). In another study EEG and evoked potential (EP) methods were used to compare the effects of acupuncture and *Qi-Gong* (Xu, et al. 1998). Patients with Parkinson's disease were evaluated by a method of auditory evoked potential (Wang, et al. 2002). The electrocardiogram (ECG) together with heart rate variability (HRV) analysis was used to determine the balance between sympathetic and parasympathetic regulation. Researchers concluded that stimulation of S*ishencong* acupoints (four points around GV20 acupoint) “enhanced cardiac vagal and suppressed sympathetic activities in humans” which could contribute to the reduction of physiological symptoms of stress (Wang, et al. 2002).

On the cautionary note, the application of neurophysiological research methods does not imply that the therapeutic effects of acupuncture are based on neurophysiological mechanisms. These are two different concepts and the distinction should be made clear to avoid confusion presented in some reviews (Shen, 2001).

1.2.3 The search for the optimal research model in acupuncture studies

The link between research questions and the research model that is chosen to answer these questions is of primary importance. Different questions may require the use of different research models, and this is rather independent from the actual choice of measurement methods. For example we may be interested in detecting differences between the pre-treatment baseline and post-treatment results in a group of subjects with known disease conditions. On another occasion we may be more interested in monitoring health improvements over a longer period of time, lets say every 3 months for the duration of 12 months. In each case the design of the study will be different despite the fact that the same health outcomes or physiological changes are being measured.

The review of the acupuncture research literature indicates that there is widespread confusion as to how different research models may be applied in acupuncture. Issues of control versus treatment groups, issues of randomization, issues of active treatment, placebo and sham acupuncture, or issues of repeated measurements, sample sizes and power of experiments are not addressed appropriately in the majority of acupuncture studies. For example, the review of nonoperative treatments for patellofemoral pain syndrome which also included acupuncture treatment, concluded that “most RCTs reviewed contained qualitative flaws that bring the validity of the results into question, thus diminishing the ability to generalize the results to clinical practice” (Bizzini, et al. 2003). In another review, the quality of research papers reporting non pharmacological treatment for osteoarthritis were compared with papers reporting pharmacological treatments. Overall assessment quality was lower for non-pharmacological papers mainly due to the absence of placebo groups and not 'blinding' patients and care providers participating in the studies (Boutron, et al. 2003). Numerous examples of poor experimental designs can be given such as small group size in the study of migraine (Backer, et al. 2004) or questionable logic in the study of the effects of acupuncture in exercise stress (Li, et al. 2004). There is some optimism, however, as more and more attention is given to these issues in the last few years (Kaptchuk, 2001).

The optimal research model can be defined as a model that complies with known canons of scientific research and provides the shortest and most logical pathway to answer research questions. More details regarding research models and methodologies used in the research presented in this thesis are outlined in Chapter 3 Methodology (general). All essential aspects of the research model are presented including randomization, experimental variables, control and treatment groups, and three different stages of statistical analysis. Additionally there are also separate sections on methodology in Chapters 4 to 7 but they are more concerned with measurement techniques rather than with general experimental design.

1.3 The experimental aims

The overall purpose of the research project presented in this thesis was to investigate the possibility of applying neurophysiological methodologies to acupuncture research. The doctoral thesis presents experimental laboratory based research which closely resembled the setting of an acupuncture clinic. Such an approach could improve the understanding of research methodologies and could also clarify theoretical and practical issues in acupuncture research.

1.3.1 Aim 1: To investigate degree and direction of physiological changes

Aim 1 involved the investigation of the manual stimulation of specific acupuncture points to determine whether physiological changes occur in healthy subjects, and to investigate whether the degree and the direction of physiological change can be precisely monitored by known neurophysiological methods and explained by biomedical theories.

Implications: When physiological changes are detected, the next natural question to ask is why such changes occur. If the findings are such that they cannot be fully explained by known sympathetic/parasympathetic nervous system regulation in the case of autonomic functions, or by arousal/inhibition principles in the case of CNS functions, then new theoretical propositions may need to be developed. It can be postulated that there are other, not yet known, regulatory mechanisms which may be responsible for the regulation of physiological processes in response to acupuncture stimulation. Traditional acupuncture theories regarding interactions between various organs and systems of the body (*Zang-Fu*, Five Elements, Triple Energizer) may be suggested as an alternative explanation of the physiological changes due to acupuncture.

1.3.2 Aim 2: To investigate if physiological changes form a pattern

Aim 2 involved the investigation of physiological changes induced by acupuncture stimulation to determine whether patterns of change are associated with individual acupuncture points being tested. Knowing that all body systems are interconnected, it is reasonable to expect that physiological changes induced by different acupuncture points may form unique clusters. These clusters of physiological parameters may significantly differ between each other and be dependent on the type of acupuncture points being used as well as the stimulation techniques used.

Implications: The differences between patterns of physiological change may indicate the differences in the dynamic action of the acupuncture points. Simultaneously induced changes in more than one physiological system may suggest that acupuncture stimulation can influence higher and more complex regulatory mechanisms (eg. neuronal or hormonal). If this is confirmed then all major acupuncture points could be 'tested' using a similar research model, and only then can the overall analysis of physiological properties of the acupuncture points reveal possible similarities with traditional classifications of acupuncture points as outlined in manuals of Chinese medicine.

1.3.3 Aim 3: To investigate if physiological changes are stress related

Aim 3 involved the investigation of physiological changes induced by acupuncture treatment to determine whether changes are more pronounced in subjects showing stronger signs of stress.

Implications: If the physiological effects of acupuncture stimulation are small in healthy subjects as compared to stressed subjects than it can be assumed that acupuncture works mainly through mechanisms of regulation and re-balancing rather than through mechanisms of direct control of specific physiological systems. Determining the relationship between the level of stress and the magnitude of physiological changes due to acupuncture stimulation may have a major implication on clinical practice. It would confirm the correctness of assumptions made by acupuncture practitioners that stressed patients need to be treated differently to patients with primarily physical symptoms. Emotional stress could than be viewed as a contributing factor in disease manifestation.

1.4 The research questions

Data collected in experiments may have multiple dimensions and may be influenced by many factors. It may form a complex matrix of related variables, particularly in the experiments using multichannel recording. The analysis of findings may only be possible if it is 'question or hypothesis driven'. The more precise the research question, the easier it is to produce a selective and precise answer.

In the project presented in this doctoral thesis research questions were grouped into four major categories, all related to the way in which biomedical sciences define the human body and its response to the environment. The cardio-pulmonary system represents two major organs, heart and lungs which are fundamental to life itself. The central nervous system produces consciousness, regulates sleep and alertness, and has higher coordinating functions. The autonomic nervous system, with its sympathetic and parasympathetic branches, coordinates all vegetative functions of the body. Finally there are psychological and life style factors that influence the three previously mentioned systems through life style choices and environmental stressors.

The physiological role of each system is discussed briefly together with expected changes in recorded measurements. These expected changes are defined as functional changes such as heart rate, respiration rate or changes in electrical activity of the brain. Some physiological changes are slower to manifest, such as an increase or decrease of white blood cells, so immune function was not measured in this study.

1.4.1 Cardio-pulmonary system and its response to acupuncture

The heart and the lungs have a primary role in human physiology. Health status depends very much on the coordinated functioning of these two major organs. There is an overwhelming amount of research on the physiology of the cardio-pulmonary system; however selection of research which is relevant to acupuncture studies is not easy (Middlekauff, 2004). On the one hand acupuncture research itself does not have a good track record in creating quality RCTs. Only a few single studies are published on heart rate and heat rate variability, and on lung capacity in papers on asthma. On the other hand there are numerous studies in cardiology which describe the results of various interventions and their affect on microbiology and physiology of the cardio-pulmonary system.

The rhythmic activity of the heart and lungs seems to be relevant to acupuncture practice. Heart rate and heart rhythm are of primary clinical importance. The diagnostic procedure of pulse 'taking' forms an essential component of Traditional Chinese Medical practice. The respiration rate is also very important, in particular the ratio between heart beats and respiration. TCM interprets such results and explains the interdependencies using traditional theories. It can also be interpreted in terms of biomedical knowledge.

Recording heart rate and respiration rate is relatively easy in healthy subjects. There are also well developed computational techniques which allow processing of these two important physiological signals. Calculation of heart rate (HR), heart rate variability (HRV), respiration rate (RESP), and the ratio between heart beats and single respiration (HR/RESP) have become standard measures of cardio-pulmonary evaluation (Sandercock, et al. 2005; Kleiger, et al. 2005). Variability of heart rate and respiration rate is largely dependent on regulation from the autonomic nervous system (ANS) however they have their own intrinsic rhythm influenced by blood pressure, exercise, emotions and genetic makeup. Additionally, respiration can be influenced by conscious breath control, and can show periods of no-activity called apnea.

It is unlikely that there would be immediate morphological changes in response to acupuncture stimulation simply because it takes time to develop such changes. Thus measuring parameters of the blood, hormone levels or mucosa of the lungs seems to be rather counterproductive. It is reasonable to expect, however, that the regulatory mechanisms of heart rate and respiration rate can be influenced by acupuncture stimulation. They are sensitive to levels of exertion (Perini and Veicsteinas, 2003). They change in response to pain and to negative and positive emotional states. Most importantly they also change according to the effects of different disease conditions (Milliani and Montano, 2002). It is expected that HR, HRV, RESP will change in response to acupuncture stimulation and that this response will be different for different acupuncture points. It is also expected that by monitoring the differences and similarities between baselines in control and treatment groups, that conclusions can be reached regarding possible placebo effects.

1.4.2 Central nervous system and its response to acupuncture

The concept of the central nervous system is not well developed in traditional Chinese medicine. The brain is considered as 'bone marrow' and the consciousnesses (Spirit) resides in the Heart. Only relatively recent clinical applications of acupuncture has placed emphasis on the primary role of the CNS. Many researchers go even so far as to attribute the therapeutic effects of acupuncture to the regulation of the CNS, abandoning the concept of *Qi* altogether.

Biomedical sciences view the CNS as a coordinating 'computer' for the whole body. Numerous centers in the brain and spinal cord are responsible for neuronal and hormonal regulation. The brain is the place where human consciousness is maintained. Regulation of sleep and awakening, attention and memory, perception and cognitive functions are all attributed to the CNS.

There are literally thousands of research papers where the central nervous system has been investigated in humans and in animals. The anatomical structures are examined using histological and X-ray imaging techniques. Neurological and psychological functions are investigated with neuropsychological tests, behavioral experiments and, more recently, with functional magnetic resonance imaging. However, acupuncture research is again very limited as far as investigations of the CNS are concerned. Anatomical and neurophysiological experiments are performed on animals using anesthesia and invasive surgical procedures, with little generalities to human studies. Studies performed on human subjects are so limited that it is hard to even find a single example of a well designed RCT. Electroencephalography (EEG) is the most frequently used method, sometimes supported by evoked potentials technique or measurement of nerve conduction.

The commonly used technique of EEG is suitable for the study of acupuncture. Electrical signals from the electrodes placed on the surface of the head can be amplified and processed. The signals are split into component frequencies called 'brain waves'. Numerous studies reveal the existence of links between brain waves and physiological and psychological states. Over the last 40 years knowledge has been accumulated from EEG studies bringing more understanding into the role of the CNS in physiology and health (Darvas, et al. 2004).

It is expected that brain waves of healthy subjects will change in response to acupuncture stimulation. At present there is insufficient reliable evidence from past acupuncture research to speculate about the possible direction of such changes, but sensitivity of the CNS to external stimulation gives hope that such changes can occur. It is essential to analyse associations between brain waves and different stages of acupuncture treatment such as baseline preparation, active stimulation of the acupuncture needle and the post-treatment phase. Conclusions about the effects of acupuncture on levels of alertness, stress and relaxation will be drawn from the study.

1.4.3 Autonomic nervous systems and its response to acupuncture

Self regulation and maintenance of homeostasis is an essential property of every living organism. The more complex the organism, the more complex and specialised the form of homeostatic control. In many animals, and in humans, that regulation reached the point where specialised nerve centers are entirely responsible for that regulation. Body temperature, skin temperature, blood pressure and heart rate are all at least partially controlled by the autonomic nervous system. ANS works in the background and does not require intervention of the conscious mind (Marieb, 1998).

There are two main divisions within the autonomic nervous system. The sympathetic division is responsible for the 'fight or flight' responses and the parasympathetic division plays a role in relaxation, regeneration and rest. Balance between these two branches indicates that the body is able to sustain health and can also quickly and efficiently respond to the challenges of environmental stressors (Marieb, 1998).

Monitoring the status of the autonomic nervous system is relatively simple and can be performed using non-invasive methods. Not all body systems respond equally strongly to environmental change. Multichannel recording allows monitoring of changes in the sympathetic and parasympathetic divisions of the ANS as they influence major body systems. That influence can be observed, for example, as a variability in heart rate, as changes in skin temperature, vasodilation of capillaries in peripheral blood circulation, and arterial blood pressure or electrodermal activity (EDA).

It is expected that the ANS will respond to acupuncture stimulation. Possibly not all body organs will respond equally strongly and possibly not all at the same time. Body temperature and blood pressure will most likely remain unchanged but electrodermal activity (skin potential) may respond to the insertion of the acupuncture needle. The sensitivity of the various methods is yet to be verified and it is hoped that this randomised controlled study will contribute valuable information to our understanding of the influence of acupuncture on the ANS.

1.4.4 Psychological and lifestyle factors affecting acupuncture outcomes

Psychological factors such as anxiety and depression can affect the physiology of the body. Short and long term effects are reported frequently in psychophysiological and psychosomatic research (Tennant and McLean, 2001). It is not clear how anxiety can influence the way in which the human body responds to acupuncture. Subjective feeling of stress, restlessness or lack of sleep may also play a role in the response to acupuncture. The review of current acupuncture research unfortunately provides no answers. Despite the fact that many patients seek acupuncture treatment for health conditions which can be clearly defined as psychosomatic in origin, no acupuncture research exists in this area (Biondi and Portuesi, 1994).

It is relatively easy to measure the anxiety and stress level by using validated self evaluation questionnaires (Spielberger 1983; Craig, et al. 1996). By asking questions about the frequency and intensity of stressful events and the nature of thoughts and feelings it is possible to assess the baseline psychological status of the person. Increased anxiety and stress levels are well correlated with numerous physiological conditions. There is strong experimental evidence linking anxiety and stress with heart disease, duodenal ulcers, migraine headaches and psychosomatic illness (Muller, et al. 2005).

It is expected that subjects characterised by more pronounced anxiety levels will also show a stronger response to acupuncture stimulation. The direction of the physiological changes is hard to predict as there is almost no acupuncture research in this area. The primary assumption would be that subjects who are very relaxed will somehow increase in sympathetic tonus, and subjects already alert or restless will counterbalance this towards more relaxed “parasympathetic” status.

1.5 The noninvasive measurement of physiological changes

The fundamental differences between the oriental and the western approach to health and healing may be attributed to the ways in which medical practitioners acquire their knowledge about disease conditions. For centuries western medical practitioners dissected the bodies and investigated the ever smaller building blocks of life: first organs and tissues, than cells and now DNA and individual molecules. In contrast, oriental medicine emphasized observing external signs and symptoms, never interfering by using surgical procedures on internal organs or investigating diseases on the cellular level. Oriental practitioners were able to observe the mysterious *Qi* at work in every healthy and unhealthy person. They evaluated the effectiveness of acupuncture or herbal treatment by observing the external symptoms. There was never the need to study the diseased organs or to see red blood cells in order to diagnose and to treat a sick person (Kaptchuk 1983) .

Western biomedical sciences, by rejecting the notion of life force, energy or *Qi*, concluded that all causes of disease must have a material origin and that only material, tangible signs and symptoms are of any importance. With the advance of science and technology this never ending quest for more detailed analysis is now reaching the point where scientists can see more and more 'trees”, and at the same time they are losing sight of the forest. Introduction of the notion of a biological basis of acupuncture is a clear example of science concentrating only on the material evidence (Ma, 2004).

1.5.1 Biological basis of acupuncture (NIH approved CAM projects)

The National Institute of Health (NIH) in the United States has been a long time advocate of research in complementary and alternative medicine. With over 30 clinical trials listed on the website of the National Center for Complementary and Alternative Medicine (NCCAM), the increasing trend toward acupuncture research is clear. The majority of these trials however have a dual goal. They attempt to confirm the usefulness of acupuncture in the management of known disease conditions and at the same time, they try to increase understanding of acupuncture mechanisms. From the detailed analysis of these trials it is obvious that researchers are focusing on biological mechanisms only. In fact the whole research into mechanisms seems to have a very well defined but hidden agenda; that is, to establish acupuncture as a legitimate medical treatment if two conditions are met. One, acupuncture is able to improve patients conditions, and two, acupuncture works through biological mechanisms such as endorphins, the nervous system, or by means of hormonal regulation.

It would be pointless to argue about mechanisms of acupuncture, biological or *Qi*-based, if this treatment modality is not really effective in disease management. So the primary goal of acupuncture research remains unchanged for both western and oriental researchers. At this stage it can only be postulated that much more effort will be needed in the future to discover *'how it works'*, possibly long after the question of *'does it work'* has been positively answered. After all one century of biomedical research was not enough to answer the questions about how aspirin or codeine works (Tarnawski and Caves, 2004; Chung and Chang, 2002).

1.5.2 Role of the nervous system in creating therapeutic effects of acupuncture

Many researchers who are not familiar with the fundamental notions of acupuncture are comfortable with attributing acupuncture mechanisms to biological causes. Such an approach certainly removes the dichotomy between oriental and western approach. The advancement in neuroscience and popularity of neurophysiological methods make the choice very obvious – it is the nervous system that mediates and/or creates the acupuncture effect. The NIH states that “It is proposed that acupuncture produces its effects through regulating the nervous system, thus aiding the activity of pain-killing biochemicals such as endorphins and immune system cells at specific sites in the body” (NIH, 2005). And despite the fact that the vast majority of acupuncturists practice traditional acupuncture, there is a strong tendency between researchers to dispose of the old beliefs. In one of the reviews on traditional versus evidence-based acupuncture, the author states that “healing is not achieved by manipulating *Qi* but rather by neuro-electric stimulation for the gene expression of neuropeptides” (Ulett, et al. 1998a). Is there evidence for such a strong statement? Certainly not but it sounds very “scientific”. Research supports the view that the nervous system is involved in acupuncture effects. Pain relief induced by acupuncture is attributed to causes such as axon reflexes, propriospinal inhibition, supraspinal mechanisms as well as cortical, psychological and “placebo” mechanisms (Carlsson, 2002). Involvement of endorphins in pain relief was also confirmed in electroacupuncture research (Han, 2004; Ulett, et al. 1998b). Brain structures such as hypothalamus (Hsieh, et al. 2001), cerebellum and dorsomedial nucleus of thalamus (Yoo, et al. 2004) and somatosensory area of the cerebral cortex (Zhang, et al. 2003) all show increased activity during acupuncture stimulation. Overall, the role of the nervous system is so important to the understanding of acupuncture that it will be discussed in more detail. For now it is essential to acknowledge that the nervous system must be part of the investigation into the effects of acupuncture.

1.6 Contribution of this study to acupuncture research

However small is the contribution of the PhD project to the overall pool of scientific knowledge, one thing remains certain – it must contribute something which can be clearly defined and easily categorised. This doctoral research project contributes in three areas: in implementing neurophysiological methodology into the field of acupuncture research, in testing new hypotheses that are relevant to clinical practice, and in evaluating research models best suited to acupuncture.

The future of evidence based acupuncture practice depends on acupuncture research, but the future of acupuncture research depends on the choices made now regarding methods, models, and approaches relevant to the phenomenon of acupuncture. All branches of medical sciences such as neuroanatomy, histology, pharmacology and many others have built their strength by optimising research methods to their own unique requirements and by learning to formulate precise research hypotheses. In comparison acupuncture research in still in its infancy.

1.6.1 Implementing new methodology in acupuncture research

The research project presented in this thesis has the characteristics of exploratory research. It aims at investigating physiological changes due to acupuncture stimulation in healthy human subjects. New methodology has been developed and tested specifically for this acupuncture study. The methodology has a few unique features which makes it different to any other acupuncture research. Firstly, it employs multichannel recording of many vital physiological parameters such as heart and respiration rate and brain waves, commonly used in psychophysiological research (Wientjes, 1992). This approach allows evaluation of the functions of three major physiological systems at the same time. Secondly, it uses new software that allows for full automation of the experiments. As a result, the researcher can concentrate on the acupuncture treatment protocol rather than on operating measuring equipment, making the experiment 'feel' more like a treatment session in an acupuncture clinic. Thirdly, it utilizes on-line processing of physiological data and automatic reformatting of the results into spreadsheets compatible with statistical software. This allows for a significant increase in the number of statistical comparisons and depth of analysis traditionally limited by the time spent on retyping or cutting and pasting numerical values into statistical software.

The potential importance of such an approach became obvious when the 120 experimental sessions required for the completion of the project were completed within a period of just 6 months. Initial time of around 2 years spent on the development of the computer software could pay off in just 2-3 research projects of similar size to this PhD project. Additionally this new methodology reduces the number of errors, biases, and frustrations normally associated with manual pre-processing of experimental results.

1.6.2 Testing new hypotheses

The hypotheses tested in this research project are closely linked to clinical practice. The choice of two acupuncture points LU7 and KD6 tested in the experiment is based on the traditional acupuncture theories of Eight Extra Meridians (Larre and Rochat, 1997). Clinical literature postulates that this therapeutic pair has synergistic effects (Ross, 2002). For that reason physiological changes in major functional systems of the body are being treated as clusters which could be unique for each acupuncture point or combination of points. Magnitude and the direction of changes is assumed to be always within the normal physiological range. Three research hypotheses formulated in this project reflect the gradual advancement in questioning the recorded phenomenon: 1) do changes occur in response to manual acupuncture? if yes then 2) what is the magnitude and direction of these changes? and 3) do these changes form any physiological patterns specific to each of the acupuncture points being tested? Hypotheses formulated in such a way contrast with hypotheses carelessly formulated in many acupuncture studies. Research questions such as “Is acupuncture good for asthma?” cannot be treated as research hypotheses. They are too broad, they lack precision, and they cannot lead to clinically valid conclusions.

The advantage of having well formulated hypotheses is obvious when the results of the research project are being used to design the follow-up study. Unnecessary repetitions are easily avoided and new experiments are built upon knowledge gained from previous studies. The sense of continuity can be fostered and new 'fields of study' can emerge such as research into clinical outcomes, treatment techniques or mechanisms of acupuncture.

1.6.3 Evaluating research models

Traditional acupuncture can best be classified as the practical application of energy medicine with its unique set of theoretical principles and treatment techniques. By ignoring the notion of *Qi* and by disregarding the concept of acupuncture channels, researchers can only pretend that they conduct studies in acupuncture. In this research project such an approach is entirely rejected. All fundamental concepts underpinning acupuncture practice should not be ignored or misrepresented, rather they should be accepted 'a priori' and then tested in a scientific way. Research models currently used in acupuncture studies are more suitable for the pharmacological studies, where 'active' is compared with 'non-active' treatment. In order to improve the situation a new 'differential approach' has been proposed: instead of comparing the action of acupuncture points with placebo or sham points, points are compared with each other. In simple terms apples are compared with apples, not with oranges. It means that physiological changes due to acupuncture point stimulation are measured not in absolute units but in relation to each other. After all, that is what acupuncture is all about, detecting subtle differences between the actions of single acupuncture points and then choosing the most effective point combinations for specific disease conditions.

Data collected in this research project is analysed using three different research statistical techniques. Firstly, a factorial analysis was used to discover if there were any physiological changes with significant magnitude. Secondly, data from each treatment group was compared one by one against the control group. In the third step the control group was removed from the analysis altogether and only differences between each of the treatment groups were analysed ('differential approach'). The neural network analysis complements that analysis and serves as an example of the non-linear approach to detecting patterns in physiological changes.

1.7 Scope of the project

The PhD project is by design limited both in its scope and time frame. It serves as a proof that the candidate for the doctoral degree is proficient in the cannons of scientific investigations. Sometimes special time considerations may apply to projects that span two or more scientific disciplines. The project presented in this thesis can be classified as multidisciplinary. The modern neurophysiological methodologies were blended with traditional Chinese medicine. The candidate's degree in clinical psychology provided a knowledge of these research methodologies, while a degree in acupuncture helped to formulate the research hypotheses, and programming skills helped in the creation of the computer software that makes this experimental research possible.

1.7.1 Theoretical issues and limitations

Setting limits over what can be done and what should be done in this project was not easy. Research questions were chosen very selectively and were focused only on physiological changes in healthy human subjects. Naturally many more questions can be put forward particularly if another arm in the RCT was to be established, allowing patients with specific disease conditions to enter. This of course would double the size of the project and render it impossible to achieve in the alloted time.

Manual acupuncture of only two points was used in this study. It could be argued that many more acupuncture points 'deserve' to be tested and that they may have been even more important in regulating *Qi* in the human body. Introduction of each new acupuncture point, however, increased time and effort by an additional 25%. It also would make the analysis more complex and that may not be a desired outcome at such an early stage in acupuncture research.

1.7.2 Practical and clinical implications

The time frame of this project was limited to a 10 semesters part-time study. Four out of ten semesters were used to design, develop and test computer software which was used to automate the experiments. The size of the control and the experimental group was limited to 30 subjects in each of four groups. The calculated sample size based on heart rate variability data needed to provide 80% statistical power indicated a minimum of 27 subjects in each group. That did not leave any time to explore individual differences between subjects. Practical limitations of time and available funding, as well as the focus on evaluation of methodologies rather than clinical outcomes, limited the study to 120 experimental sessions (4 groups times 30 subjects).

It was important to design the acupuncture laboratory in such way that it resembled as closely as possible the setting of a typical acupuncture clinic. It was also considered essential to limit the intrusiveness of the research protocol on feelings and comfort of the subjects enrolled in the experiments. This was achieved by manufacturing custom-built sensors to continuously monitor respiration activity and by conducting experiments in a quite and air conditioned room.

1.8 Structure of the thesis

This thesis consists of eight chapters. The introduction to the field of study and background information are presented in the first two chapters. The methodology section is distributed between a few chapters. The main section describing methodologies and the research models is presented in Chapter 3, and the remaining information on methodologies relevant to individual physiological systems is included in sub-sections of relevant chapters. The analysis of results is presented in four chapters covering the cardio-pulmonary system in Chapter 4, the central nervous system in Chapter 5, and the autonomic nervous system in Chapter 6. The new analytical method of neural networks is presented in Chapter 7. The discussion of the research findings and conclusions are found in Chapter 8.

1.8.1 Formal structure

The structure and the outline of the thesis complies with recommendations of the Faculty of Science, University of Technology. The main table of content is placed at the beginning of the thesis, followed by eight chapters, references and appendices.

1.8.2 Narrative vs scientific style

The scientific style of writing is maintained through all chapters of this thesis. On a few occasions, however, a more narrative style is adopted in order to provide better understanding of the research dilemmas. Traditional Chinese medicine contains a wealth of scientific information and much of it is presented in narrative form. Case studies based on real life examples have been long recognised as an excellent tool in education. It is obvious that not every detail of the research methodology can be fully described without turning the thesis into a technical manual, and not every change in the research results is worth mentioning. Thus by using numerous examples and by referencing other sources for detailed information, an attempt was made to bring balance to the writing style in this thesis.

Chapter 2. Background of the study

The field of studies is defined here as a scientific investigation into the phenomenon of acupuncture. It specifically targets the possibility of using well established scientific methods in acupuncture research. It concentrates only on acupuncture research on human subjects and only on the application of non-invasive measurement methods such as those used in neurophysiology.

A review of the literature revealed that there are at least three types of publications associated with acupuncture research, that is research into clinical outcomes (Is acupuncture good for disease X, Y, Z ?), research into acupuncture mechanisms (How does it work?) and research about utilisation of acupuncture as complementary and/or alternative medicine. The focus of this project is primarily on mechanisms, both energetic and physiological.

The present study concentrates on methodology and research models as these seem to be essential components of any scientific discipline. The body of literature related to acupuncture and TCM is examined and presented here. The purpose of this background review is to establish links between the current status of acupuncture practice, developments in acupuncture research and the requirements of evidence based medicine.

2.1 Constant changes in medical methods

The old and new methods and approaches seems to be mixed together in almost all specialties of medicine. In the past two decades the acceptance of complementary and alternative medicine by the general public added to the mix of therapeutic methods used in medicine. It is safe to conclude that there will always be a mixture of medical methods, old and new, proved and unproved. Science should learn to deal with that mix more creatively.

2.1.1 Evaluation of current methods (Evidence Based Medicine)

A new trend in medicine, called evidence based medicine, has its origin in clinical practice and in medical research. As more and more research is available in the form of objective RCTs it is becoming obvious that some medical methods are therapeutically more effective than others; some are more cost effective than others, and some are just accepted without any solid scientific proof. After the initial hesitations in the early 1990's EBM now has its strong promoters. Clinical guidelines are published regularly and EBM is becoming a standard benchmark in some medical specialties such as cardiology.

It could be expected that complementary and alternative medicine would adopt similar standards to those of biomedical medicine, but it appears not to be that simple. First of all the objectivity of the systematic reviews is questioned (Linde and Willich, 2003). Different inclusion criteria for RCTs, different methods for data extraction, and the number of included studies are the primary factors. These factors and other tasks in the review processes can have a major impact on the outcomes of systematic reviews.

The clinical recommendation regarding usefulness of acupuncture are also questioned. Despite the fact that the National Institutes of Health in US Acupuncture Consensus Development Panel published its conclusions supporting the use of acupuncture in 14 medical conditions there is little data to support such conclusions (Mayer, 2000). The road towards EBM in TCM may be very long indeed mainly due to the lack of well designed RCTs. Without experimental evidence the principles of EBM cannot be used. Acupuncture is an empirical discipline on the long journey towards evidence-based medicine (Xiao, et.al. 2001)

2.1.2 Re-discovering the pre-scientific past

The current 'age of reason' deals sometimes very harshly with the pre-scientific past. China almost lost its traditional medicine during the so called 'cultural revolution' (Unschuld, 1990). In Europe and United States the naturopathic methods were almost entirely replaced by pharmacological medicine in the mid 50's. And it is hard to believe that homeopathic medicine was once the most popular system taught in American medical schools at the end of 19^{th} century (Jonas, et al 2003).

So what brings people back to the principles of healthy living, what forces them to 'open' old medicinal cabinets with herbal remedies and what makes them think that the body is more than just amalgamation of biological cells? It is probably the same 'age of reason', which recognises the successes and failures of modern medical methods. If alternative medicine does help with labor pain without the need to resort to medication, why not to use it to help women during the delivery (Smith, et. al. 2003). If acupuncture (Sok, et. al. 2003) and acupoint massage (Tsay, et. al. 2003) help to relieve insomnia why not use them as a complementary treatment to reduce sleeping pill medication.

Study of the history of the Chinese medicine and historical records of achievements of individual physicians clearly indicates that TCM is a very potent therapeutic system of medicine (Wai, 2004). It is also relatively safe. Acupuncture in particular has an excellent safety record. New and improved safety protocols in clinical practice are proposed to reduce further the risk of adverse effects (White, 2004a). The proof of effectiveness through randomised controlled trials is of course needed but there is already some work done in this area and knowledge is accumulating on how to design phase I, II and III acupuncture clinical trials (Berman, 2001)

In 1988, the US Congress recommended the establishment of the National Center for Complementary and Alternative Medicine. Initially NCCAM supported research based on clinical trials but in the last few years it also supports basic and pre-clinical research. For example, a wide range of therapeutic modalities has been recommended for the review of their effectiveness in cardiovascular diseases (Wong and Nahin, 2003). With the baby boomer in the western population now starting to retire, interest in CAM is on an increase (Williamson, et al. 2003). The older population may still rely heavily on pharmacological medicine but the high costs of such treatments has forced health authorities and policy makers to look for alternatives. Even the Food and Drug Administration (FDA) in United States is getting involved by establishing a working group within its own ranks to guide the policies on using botanicals as drugs (Eskinazi and Hoffman, 1998). The pre-scientific methods are numerous and their practical usefulness was evaluated over many centuries. It does not mean however that they are all good or that they are all safe. Their clinical effectiveness and safety need to be evaluated in the same fashion as it is required in biomedical medicine.

2.1.3 Complementary, alternative and integrative medicine

Currently there are robust discussions about the most effective models of service delivery in medicine. These discussions are divided into at least three major groups. One is concerned only with the biomedical model, that is pharmacological and surgical. It is dominated by medical practitioners, pharmacists and by nurses, and backed-up by the pharmaceutical industry. It is present in hospitals, emergency departments and in general practice. The second group represents the complementary and alternative medicine practitioners, comprising herbalists, acupuncturists and naturopaths. It represents non-pharmacological methods of treatment and ranges from dietary systems to the well established systems such as traditional Chinese medicine. This group's biggest professional concern is about being recognised and registered by state health authorities. There is also a third group which is emerging from within the medical fraternity and strongly advocating the integrative model of medicine. These are registered medical practitioners who see themselves as holistic practitioners of modern medicine, able to integrate into one medical system all the methods that help and benefit the patient.

The integration of medical methods may encounter many potential barriers (Barrett, 2003). It may be equally opposed by the majority of medical practitioners and some groups of CAM practitioners. The only group which is certain to welcome the integration and is certain to benefit from it are patients. The evidence-based and multidisciplinary practice together with a patient-oriented approach will most certainly improve the current system of health care delivery which suffers from fragmentation and over specialisation. The first attempts to integrate complementary and alternative medicine into conventional medical education have already been made (Haramati and Lumpkin, 2001). Some hospitals are already opening their first outpatient clinics and medical centers where integrated medicine is freely practiced (Oberski, 2003). Some physicians are already trying to integrate alternative methods into the hospital setting using well designed randomised controlled trials as a proof of effectiveness. For example a study conducted on 210 healthy pregnant women supports the view that acupuncture can be beneficial in the reduction of labor pain. It may reduce the need for the use of epidural analgesia which carries serious risks of side effects in some women (Skilnand, et al. 2002). In another study it was revealed that most surgical patients use some form of CAM treatment and that they are willing to accept CAM as part of their management program (Wang, et al. 2003).

Acupuncture in particular has very promising prospects of being integrated into the holistic practice of medicine. For example, the results of RCTs support the view that acupuncture is very useful in the treatment of lower back pain in elderly patients when combined with standard pharmacological treatment (Meng and Wang, 2003) or combined with exercise in younger patients (Yeung, 2003). Post-stroke treatment and rehabilitation is another area where acupuncture shows promising results. One study, in which single-photon emission computed tomography (SPECT) was used, revealed that acupuncture can activate some parts of the brain (Lee, et al. 2003), and another study confirmed anti-spastic effects (Moon, et al. 2003). The preventative role of acupuncture is also very important. A study conducted on 179 patients demonstrated that acupuncture could be useful in preventing migraine attacks in 35 % of patients, the same level of effectiveness as in the group receiving sumatriptan medication and twice as effective as the placebo injection (Melchart, et al. 2003).

Integrating acupuncture with pharmacological management of chronic obstructive asthma was investigated on 41 patients by researchers from Taiwan. The clinically significant improvement in the quality of life was reported with recommendation for the supplementary role of acupuncture in the treatment of asthma (Maa, et. al. 2003). The recommendation for the combined use of medication with acupuncture also came from the researchers investigating nausea during chemotherapy treatment of rheumatic diseases. Both the severity and the frequency of vomiting were reduced as compared with pharmacological (ondansetron) therapy (Josefson and Kreuter, 2003). All such results simply indicate a very strong potential for acupuncture to become a treatment method which can perfectly blend into hospital wards and outpatient departments.

2.2 What constitutes the proof of effectiveness

Evidence based medicine demands a proof of effectiveness, but what is it? Current bias towards the biological bases of acupuncture has placed pressure on acupuncture and traditional Chinese medicine to comply with the same standards as biomedical medicine. The question regarding the 'base' upon which the evidence of acupuncture effectiveness is 'based' is not easy to answer (Hammerschlag, 2003). It is further complicated by the lack of good quality RCTs and the tendency not to publish trials with null results (Ezzo, 2003). Despite the fact that the National Institute of Health (US) Consensus Conference in 1998 confirmed the usefulness of acupuncture in the treatment of many disease conditions, the conclusions in the published report did not go beyond recommendations of "usefulness", "complementary", "anecdotal evidence", "promising" or "acceptable alternative" (NIH, 1998). Almost six years later the situation is very much the same: what is available in the published literature as evidence is frequently rejected in systematic reviews and what is needed as credible evidence is frequently missing. Further, the results of acupuncture research are spread over many journals and many databases (Pilkington and Richardson, 2004) and systematic reviews in acupuncture are very limited in numbers. There are, however, at least some constructive suggestions coming from the users of the Cochran libraries, combined with the calls for action (Manheimer and Berman, 2003). Without action supporting better research outcomes the lack of evidence of effectiveness will continue to hinder acupuncture practice. For example one of the most frequently treated conditions in acupuncture practice, musculoskeletal tension and pain, still awaits 'evidence' of effectiveness to emerge (Hanada, 2003). In the meantime patient expectations are focused on symptom relief, improved quality of life and most importantly on the wider availability of such therapies as acupuncture, osteopathy and homoeopathy (Richardson, 2004). Proof of effectiveness strategies will now be discussed.

2.2.1 Seeing is believing

It is certainly easier to believe in the therapeutic effects of acupuncture if the results are immediate and visible with the naked eye. The breech presentation of the fetus during pregnancy is certainly a call for concern as it justifies elective Cesarean section.

Traditional Chinese medicine recommends stimulation of the acupuncture point BL67 for such a condition. In two separate studies researchers confirmed the clinical importance of this recommendation. In a study involving 226 Italian women the proportion of recommendations for Cesarean section dropped from 53% in the control group to 36% in the group receiving acupuncture treatment (Neri, et. al. 2004). In another study involving 67 pregnant women, the correction of fetal breech occurred in 76% of cases when acupuncture was used, as compared to 45% of cases with spontaneous conversion (Habek, et. al. 2003).

Another visible proof of acupuncture effectiveness may be in a form of self reported symptoms. In a study of seasonal allergic rhinitis, 85% of patients receiving acupuncture treatment and herbs reported improvements as compared to 40% in the control group (Brinkhouse, et. al. 2004). Yet another visual way may be by using complex computerized system such as positron emission tomography (PET). Stimulation of the acupuncture point ST36 (Zusanli) increased glycometabolism in the vegetative nerve centers of the brain of six healthy volunteers (Yin, et. al. 2003). The metabolically active areas of the brain were visible on the computer screen, and despite the fact that a very complex computer system was used to make the detection of these active areas possible, the link between acupuncture treatment and physiological effects was able to be illustrated.

After all, seeing is believing and thus the simplest way to convince others that *Qi* exists is either to show that *Qi* is a visible substance or to show the effects of *Qi* in action. The first one may be impossible at present, but the second option should be possible in a laboratory or clinical setting in a controlled trial.

2.2.2 Physical changes

Detection of physical and observable changes can become an instrument to detect therapeutic effects of acupuncture treatment. It may even shed some light on the mechanisms involved in creating such effects. For example the biomechanical response of human tissues to acupuncture stimulation was measured by means of computer-controlled apparatus. The well known phenomenon of 'grasping the needle' was confirmed by measuring the pullout force necessary to withdraw the needle (Langevin, et. al. 2001).

The visual evaluation of motor function can be useful in measuring improvements in physical performance or in rehabilitation. In a study of 33 children with cerebral palsy, researchers confirmed statistically significant improvements in gross motor functions after a short course of tongue acupuncture (Sun et. al. 2004). Cerebral palsy is certainly an incurable congenital disease but any therapy which reduces spasticity and improves motor functions is of benefit to the patients and their carers. A similar situation exists in patients with cerebrovascular dementia. The condition is incurable but therapeutic interventions that increase the cerebral blood flow help patients to continue with their independent performance of daily tasks. One case study reported improvements in the clinical status and cognitive function in 77 year old woman after just four acupuncture sessions (Schwarz, et. al. 2004).

Unfortunately sometimes researchers are simply trying to find visible proof where proof may not exist. In one such study a number of physiological methods were used in order to detect physiological changes in the gastrointestinal system. The electrogastrography (EGG) was used, together with measurements of pancreatic polypeptides and gastrin levels. Electroacupuncture and atropine were applied as interventions to show that therapeutic stimulation of the acupuncture point ST36 cannot counteract effects of atropine (Chang, et. al. 2002). A very similar study was published by the same team of researchers one year later, this time it was focused more on physiological mechanisms of action of the acupuncture point ST36. Once again it is difficult to see what kind of therapeutic effects are being examined in this study and what types of hypotheses are being tested (Chou, et. al. 2003). This is a rather clear example of research driven by the requirements of the pharmacological model where the action of a known agent such as atropine is counteracted by acupuncture stimulation. As a result neither acupuncture mechanisms are explained nor is the clinical usefulness of acupuncture confirmed.

2.2.3 Physiological changes

The locations and energetic characteristics of acupuncture points are well documented in the literature. They form the basis from which students learn how to practice acupuncture and how to design therapeutic interventions (Rogers and Rogers, 1990). The relationships between acupuncture stimulation and changes in the flow of *Qi* are also described in various manuals and handbooks of traditional Chinese medicine (Deadman and Al-Khafaji, 1998). However the relationship with the physiology of the body as understood in modern biomedical sciences is established only at the level of external signs and symptoms. Various attempts to 'discover' how acupuncture works lead to numerous physiological experiments. The vast majority of these experiments are conducted on animals using invasive surgical techniques (Wu, et al. 2004). Published results are fragmented, inconclusive and frequently based on very questionable logic. For example the effect of electroacupuncture on duodenal motility was examined using anesthetised and artificially ventilated rats. This is equivalent to the human patient during full anesthesia, on a life support system and undergoing open abdominal surgery. Researchers however concluded that "the inhibitory response of duodenal motility elicited by EAS (electroacupuncture) to the abdominal area is a spinal reflex response involving splanchnic inhibitory efferent nerves" (Noguchi, et al. 2003). Another problem with physiological studies on animals is presented in experiments where the disease condition all artificially induced. For example, in a study on ulcerative colitis experimental rats were forced to have this condition through the application of 'immunological methods and local stimulation' (Wu, et al. 2004). The use of acupuncture and other methods to treat such artificially induced conditions raises questions about the validity of such research models and most importantly about the generalisability of the findings.

In human studies there is very little attention given to physiological research simply because the majority of RCT studies concentrate on clinical outcomes rather than acupuncture mechanisms. For example, in one study the specific analgesic effect of acupuncture was examined using neurophysiological technique called somatosensory evoked potentials. When sixteen healthy volunteers were anesthetised, a painful electric stimulus was applied to their forefinger. Electroacupuncture was able to significantly reduce the amplitude of the evoked potentials suggesting that electroacupuncture may have a moderate analgesic effect. (Meissner, et al. 2004). The immune-modulatory effect of electroacupuncture via the autonomic nervous system was also suggested in another study from Japan. The results were rather inconclusive but indicated that acupuncture may normalise the pattern of leukocytes (Mori, et. al. 2002).

One of the most fundamental problems with the generalisation of the results in physiological experiments relate to the ways in which acupuncture is defined. The titles and abstracts of the published papers frequently consolidate all acupuncture points simply as “acupuncture”. Stimulation of one acupuncture point constitutes acupuncture treatment, as do two or more points. It is as if all medications were reduced to “pharmacological treatment' without giving due attentions to the pharmaco-dynamics of individual drugs. Such generalizations are very common even if unintentional, as in the study that compared the effects of different forms of acupuncture such as traditional Chinese, ear acupuncture and Korean hand acupuncture, on cerebral blood flow (Litscher, 2002). Another study, this time showing no therapeutic effects of 'traditional Chinese acupuncture' on asthma, made two incorrect generalizations. First in relation to 'acupuncture' when only a few points were actively used, and second in relation to 'asthma' when in fact only the 'total airway resistance' of the lungs was measured (Malmstrom, et. al. 2002). It is obvious that there are many indicators and outcome measures that help to evaluate the status of asthma, and airway resistance is just one of many (Blaiss, 2005).

2.2.4 Outcome measures

Acupuncture and other modalities of CAM are frequently used by patients with chronic illness. Such patients, apart from the primary disease, have additional symptoms for which they are being treated. The outcomes of such interrelated treatments are not easy to define and are not easy to measure. Outcomes can be both qualitative and quantitative and relate to improvement over time or improvement due to specific therapeutic intervention or both (Peterson and Britten, 2003). The increased demand for acupuncture and homeopathy by chronically ill patients was reported in one German study where prospective documentation of 5000 acupuncture and 900 homeopathy patients was examined (Guthlin, at. al. 2004). The results showed evidence of a subjective benefit for patients from acupuncture and homeopathy. This, according to the researchers, may explain the increase in demand for alternative medicine, particularly when patients are dissatisfied with conventional biomedical treatment.

From the patient perspective it is not unreasonable that importance is focused on Quality of Life, defined in many different ways such as reduction of pain, improved mobility or preservation of physical independence. As long as this quality of life is maintained and supported, patients will continue to use complementary and alternative medicine. Such is the case with the use of acupuncture in the treatment of post-chemotherapy fatigue (Vickers, et al. 2004a), reduction of osteoarthritic pain (Stener-Victorin, et. al. 2004) or management of migraine headaches using acupuncture treatment (Vickers, et al. 2004b; Vickers, et al. 2004c). The discussion about outcome measures should also include the definition of the 'minimum clinically important differences' which are essential in the evaluation of clinical trials. Not every statistically significant difference between outcomes is of clinical importance to the patient and the healing process. This fact should be taken into account when clinical decisions are made based on the results of clinical trials (Strand and Kelman, 2004).

The development of practical data collection systems that allow the evaluation of outcomes of clinical practice has already been undertaken. For example, outcome measures such as pain reduction and physical and mental health were evaluated in the CAM clinic in Connecticut (US). The system proved to be successful in evaluating positive outcomes in patients treated with acupuncture, chiropractic or naturopathy (Secor, et. al. 2004). In another study 201 cases with a soft tissue shoulder disorder were retrospectively re-examined using outcome measures such as levels of pain, mobility and disability. In 60% of patients, symptoms entirely disappeared, 33% reported remarkable improvement and 6% simple improvement. Only one patient (0.5%) reported no improvement. Researchers concluded that such outcome measures are indicative of acupuncture and moxibustion usefulness in diminishing symptoms, shortening duration of discomfort, and improving functional ability of the shoulder (Guerra, et. al. 2003). In a similar way, application of outcome measures was reviewed in dentistry with such proposed categories as control of anxiety, control of increased saliva production or control of gagging reflex just to name a few (Vachiramon, et al. 2004).

2.3 “Cause and effect” in acupuncture

Researchers believe that what is the cause and what is the effect in the observable phenomenon will determine the direction of his or her thinking (Arocha, et al. 2005). The fundamental principles of biomedical science are firmly based in inductive/deductive thinking. In that respect the oriental way of thinking and the oriental way of medical reasoning contrasts to western 'Aristotelean' methods. These fundamental contradictions are at the core of the disputes between western and oriental practitioners (MacPherson, 2001)

The biomedical researcher will certainly accept the following logic: event A is causing event B, event B is causing event C, event C is contributing to the occurrences of event D, thus event A contributes to event D. All events must be known and no 'breaks' longer than one element in the chain of events can be accepted. It is clearly a mechanistic approach. Following such logic all that the American Cancer Society can say about breast cancer is: “We do not yet know exactly what causes breast cancer, but we do know that certain risk factors are linked to the disease” (ACS, 2005).

In oriental thinking such logic is also fully accepted but there is also plenty of room for other options. The internal factors causing breast cancer in women are described in the following way: “women rely on the *Chong* and *Ren* vessels as the Root, which depends on the Essence-*Qi* of the Kidneys. Deficiency of Vital *Qi* (*Zheng Qi*), internal damage due to seven emotions, obstruction of Spleen *Qi*, and Deficiency of the Liver and Kidneys will disturb *Qi* and Blood and cause disharmony of the *Chong* and *Ren* vessels. When the functions of the *Zang-Fu* organs are debilitated and the immune functions weakened, *Qi* and Blood will stagnate, and Phlegm will congeal. Pathogenic Toxins will eventually accumulate in the network vessels of the breast, resulting in cancer“ (Peiwen, 2003 p.436).

Two different ways of reasoning with two different chains of 'causes and effect' finally leading to two very different approaches to treatment. Biomedical medicine will try to remove the cancerous cells and prevent them from spreading, Chinese medicine will try to revitalize the essential body organs, remove stagnations and invigorate blood, leaving the rest of the healing effort to the intrinsic wisdom of the body itself. The strength of clinical findings and the high frequency of remarkable therapeutic results produced by traditional Chinese medicine forces advocates of integrative medicine to get seriously involved in the translation process. Of course not in the linguistic translations of medical text only, but in translation and explanation of diseases using both western biomedical and oriental *Qi*-energetic interpretations.

However, the theories about purely biological causes of disease are now being challenged from many directions. Epidemiologists and social scientists suggest environmental and social causes, psychologists and mental health workers emphasize emotional and stress factors. Recent advancements and discoveries in human genomics add yet another dimension of inheritance and genetic predisposition. Complementary and alternative medicine adds its own theoretical and practical framework. Chinese medicine, in comparison with western biomedical medicine, stands very strongly on its own, being almost too big and too self sufficient for the integration to occur. For that reason integration of TCM with biomedical medicine may never happen by way of one system absorbing the other. It may as well lead to a new approach to health and healing in the West, something similar to what now exists already in mainland China, 'one country, two systems', medical systems that is. As Asia is rapidly adopting discoveries in modern medicine and biomedical sciences, it also gives away its own medical secrets in return (Lu, et al. 2004). The process of exchange is bilateral with great benefits to both sides.

2.3.1 Oriental approach: *Qi* and healing, meridians and *Zang-Fu* physiology

The term 'energy medicine' is used to describe treatment modalities which rely on the concepts of energy in explanation of their therapeutic effects (Cassidy, 2004). In Chinese medicine not only a basic concept of *Qi* is used but it is expanded into an elaborate system similar to western physiology. It is postulated that there are many types and grades of *Qi* in the body and that transformation of one form of *Qi* into another is governed by strict rules. The interaction between the *Qi* of the body and the *Qi* of the environment is also described in the form of canons or principles. Unfortunately many contemporary practitioners of acupuncture reject such rules and theories of *Qi* as archaic and search for the biomedicaly explainable processes which may account for the therapeutic effects (Kaptchuk, 2002). The exclusion of *Qi* from acupuncture research seems almost inevitable because research is going in that direction (Ernst, 2006). There is more and more evidence supporting the biomedical model. The cause and effect in acupuncture is simply described as 'acupuncture stimulation invoking physiological change'. The need for the medium through which these effects are created is no longer needed because it has been decided *a priori* that it is the nervous system which does the work. Such views are supported by research in which methods such as fMRI are used where acupuncture point stimulation is shown to activate specific parts of the brain (Li, et al. 2003a; Li, et al. 2003b).

The theoretical framework of traditional acupuncture is firmly based on the physiology of *Qi* and the concept of channels. *Zang* ('solid') and *Fu* ('hollow') organs of the body; that is Lungs, Kidneys, Liver, Spleen, Heart in the first group and Large Intestine, Bladder, Gall Bladder and Stomach in the second group. These organs constitute the basic physiological system where *Qi* is produced and transformed. The network of 14 major channels and countless numbers of collaterals distribute this *Qi* to every part of the body including skin, muscles, tissues and *Zang-Fu* organs (Maciocia, 1989). The relationship between the physical body and the *Qi* is one of interdependency. The physical body is built of *Qi,* a solid form of *Qi*. The more fluid form of *Qi,* together with the blood, interpenetrates every organ and tissue of the body and invigorate them with vitality. The concept of vitality is crucial to Chinese medicine (Kaptchuk, 1983). The level and the state of vitality can be diagnosed and then treated, if necessary, by acupuncture, moxibustion and herbal remedies.

Despite the fact that acupuncture has already been used with success by medical doctors in general practice (Joseph, 2002) and in gynecology (Nestler and Dovey, 2001) there is still strong debate about its legitimacy (Ernst, 2006). The crucial point in this dispute should be focused around *Qi* and its role as a causative factor in treatment. Without *Qi* and channels there is nothing else in the framework of traditional acupuncture that can account for its therapeutic effects. A call for the scientific basis of acupuncture is essentially a call for a material and biological basis (Cao, 2002).

2.3.2 Western medical approach: sensory stimulation, nervous system and endorphins

The role of the central and autonomic nervous systems in mediating the therapeutic effects of acupuncture has been emphasized by many researchers. For example it was confirmed that certain brain centers such as the Anterior Cingulus, the left Superior Frontal Gyrus, and the right Medial and Inferior Frontal Gyri are being activated during acupuncture stimulation and may be responsible for the analgesic effects (Biella, et. al. 2001). Positive differences were also noticed between stimulation of real and sham acupuncture points. In a study on 15 human volunteers "an increase in activation in both secondary somatosensory cortical areas, frontal areas, the right side of the thalamus and the left side of the cerebellum" was observed when acupuncture points LR3 and GB40 were stimulated. No such effects were detected when a sham point was stimulated (Fang, et. al. 2004). Such experimental findings strongly support the view that the CNS is involved in acupuncture efficacy. It does not however necessarily imply that the CNS is a source of therapeutic effects. In fact the CNS may have a less direct role such as being a mediator of neuronal impulses transmitted through the thalamus in the brain or, as postulated by some researchers, through the limbic system (Esch, et al. 2004).

In recent years a concept of endorphins has been rather popular as an explanation of therapeutic effects of acupuncture (Ku and Chang, 2001). The mechanisms of analgesia in particular are consistent with the way in which endorphins influence the body and perception of pain. Other proposed mechanisms through which acupuncture may work have been proposed, such as the interaction between neuropeptides and cytokines (Bonta, 2002). The response of the immune system to acupuncture has also been postulated as the pathway through which acupuncture may exert its therapeutic effects. Research in this area is however very inconclusive, mostly due to methodological faults, such as small group sizes as is the case in the study on polymorphonuclear leukocytes (Karst et. al. 2002). New theories of acupuncture channels were also proposed by biomedical sciences, based on the propagation of nerve impulses along major peripheral nerves and the spinal cord. In recent years, however, new explanations were advanced including the possible role of perivascular space (PVS) in creating the phenomenon of acupuncture meridians (Ma, et al. 2003)

Despite a growing interest in TCM very few clinical facts and theoretical considerations are actually passed to the practitioners of biomedical medicine such as medical doctors and nurses (Davidson, et. al. 2003). Thus it is easy for medical practitioners to criticize traditional techniques and approaches in acupuncture if they never practice them, if they have never been treated by them, and without having a fundamental knowledge about acupuncture principles as viewed in Traditional Chinese Medicine.

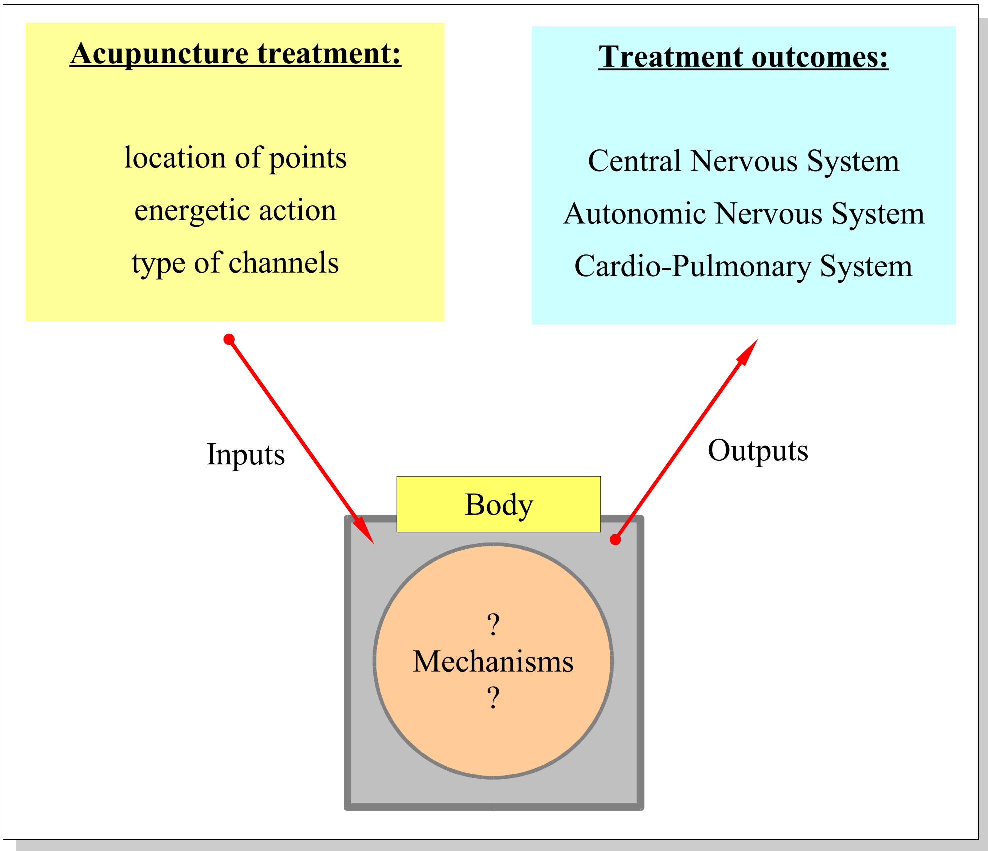
A lack of agreement between researchers regarding the nature of the acupuncture mechanism and a lack of credible research make a fertile ground for speculation. Basically any medical hypothesis can be proposed as an explanation of mechanisms of acupuncture because there are so many therapeutic claims made by traditional acupuncture (Ernst, 2006). According to acupuncture handbooks an insertion and manipulation of the acupuncture needle may lead to such therapeutic effects as reduction of pain and reduction of inflammation, improved digestion and increased immunity. It is obvious that to offer a mechanism for so many physiological effects researchers will need to go beyond the boundaries of just one physiological system (Maciocia, 1989).

For example, it is true that endorphins and the central nervous system may be credited with the analgesic effects of acupuncture, but certainly not credited to the immune response. In response to such assumptions it may be argued that the CNS can have an influence on the immune system as well (Biber, et al. 2002). Such thinking however will lead to the confirmation of the accepted medical law, that every system in directly or indirectly connected and influenced by any other system. And this is the very conclusion that Traditional Chinese Medicine has postulated for a very long time: however the medium through which all systems are connected is called *Qi*.

2.3.3 True skeptic's approach: black box with inputs and outputs.

The true skeptic of biomedical science and medicine does not care about the speculations regarding possible mechanism until he or she is truly convinced that there is a link worth explaining. The effects of a given phenomenon such as acupuncture should be objectively measured first, and repeated in controlled conditions. In other words, it is preferable that the link between what is believed to be the cause or trigger on one hand, and the effect or outcome on the other hand be established first before any questions being asked regarding the mechanisms.

Figure 1. Possibility of using 'black box' approach to acupuncture research.



The history of science tells us that all major discoveries in physics regarding electricity and magnetisms are based on such an approach (Daintith, 2005). Observation of the flow of electrical current in the electrical coil every time the magnet was moved in and out of that coil lead to the conclusion that there must be a link between the properties of the magnet and the properties of the electrical coil. This link or mechanism was initially called “induction” and served as an explanation of the “electro-magnetic” phenomenon (Daintith, 2005). A similar approach can be adopted in acupuncture studies. In the above example, manipulation of the acupuncture needle is represented by the movement of the magnet, and the body is represented by the sensitive electrical coil. Thus by examining the nature of the stimulation (inputs) and the nature of induced effects (outputs), physicists were able to discover new properties of electro-magnetic materials and formulate new laws which describe with mathematical precision relationships between magnetic and electrical fields. A similar approach in acupuncture research may lead to the acceptance of *Qi* as the 'energy' medium through which acupuncture needles induce changes in the physiology of the body without the need to specify the exact role of the central nervous system or other biological factors such as endorphins.

2.4 Theoretical propositions

What can be done in acupuncture research in order to retain its original notion of *Qi* and the concept of acupuncture channels? What can be done in order to prove specificity of individual acupuncture points as described in manuals of traditional acupuncture? What can be done in order to explain the mechanisms through which manipulation of the acupuncture needle induces healing processes in the physical body? These and many other questions still remain without answers.

The usefulness of acupuncture in the treatment of disease conditions and the cost -effectiveness of acupuncture interventions and the health politics behind the acceptance of acupuncture, have a decisive influence on the choices of research topics and research methodologies. Without asking detailed questions however, and without persistent effort in finding the answers, the true nature of acupuncture phenomena may never be revealed in scientific investigations. It may be postulated that acupuncture researchers should perhaps stop being too shy in formulating their research hypothesis. Acupuncture, after all, is a truly independent system of oriental medicine with a long history of clinical practice and many documented successes. Asking questions about *Qi* may be as valid as asking questions about the role of endorphins and the central nervous system in creating the phenomenon of acupuncture.

The following section of 'theoretical propositions” aims at addressing a few issues essential to acupuncture research. This may serve as a reminder of what are the main features of traditional acupuncture and what the relationship between clinical practice and research could be in conditions where no limits or methodological restrictions are present.

2.4.1 The nature of acupuncture treatment

After reviewing many published papers in Chapters 1 and 2, a conclusion could be reached that a number of important limitations are present in acupuncture research. Research methodologies are not well adapted to the acupuncture paradigm; controlled and randomised studies are frequently conducted on groups too small to secure sufficient statistical power and statistical analysis is often inappropriately applied. There are however other even larger limitations. First, the research into mechanisms of acupuncture is driven more by curiosity than by well defined research hypotheses and second, research hypotheses are frequently not supported by the theoretical framework of traditional Chinese acupuncture.

The focal characteristics of traditional acupuncture can be summarised in a few important points which have strong implications for acupuncture research. These are:

1. Acupuncture treatment consists of inserting metal needle into the skin and underlying tissues. Puncturing the skin and reconnecting 'inner' with the 'outer' may be the allegorical description of this essential procedure of acupuncture.

2. An acupuncture point is the point on the surface of the body where access to the energy channels is possible and where manipulation of the needle gives an effective way to influence the flow of *Qi* in these channels (meridians).

3. Channels are the invisible to the naked eye pathways through which *Qi* circulates in the body. There are 14 major channels and a fixed number of acupuncture points on each channel.

4. Each acupuncture point has a different influence on the energy flow in the channel. Individual acupuncture points have their own specific energetic characteristics which

differentiate them from other points. The differences can be relatively big or relatively subtle, depending on the type of acupuncture point, the channels to which they belong, and the type of technique used in needle manipulation.

5. Several acupuncture points can be combined in one treatment session in order to increase their therapeutic effectiveness. This is achieved by mixing the balancing, reinforcing and reducing actions of each point in the prescribed combination of points, to best address the imbalance of *Qi* in the patient's body.

This re-statement of the main principles of acupuncture could serve as a reminder of what is and what is not included in traditional acupuncture. It is hoped that this list of possible checkpoints will help to maintain clarity in the design and evaluation of acupuncture research. The research described in this thesis did not use electro or laser acupuncture techniques, nor did it use sham acupuncture and sham acupuncture points.

2.4.2 Determinants of the physiological state of the person

Biomedical sciences seek to discover ever more details about structures and functions of the human body. Current discoveries are now focused at the genetic and molecular level. In contrast, oriental medicine has never been interested in small details, rather it focused on the relationships between different organs of the body and the relationship between the body itself and the external environment.

In order to remain within the framework of oriental medicine, researchers need to resist the temptation of going into great details in their investigations. The reason for this is simple. The theoretical framework of acupuncture does not contain such concepts as biological cells, neurotransmitters, antibodies or free radicals. Its 'modus operandi' is concerned with the production, transformation and transportation of *Qi* and Blood. It is the “organ” and “channel” level of abstraction. Such an approach allows for simple, coherent and deterministic evaluation of the physiological status of the patient under medical care. Involvement in any more details will cloud the picture of signs and symptoms, will introduce doubt into the practitioner's mind and, in consequence, could lead to an incorrect diagnosis and incorrect treatment.

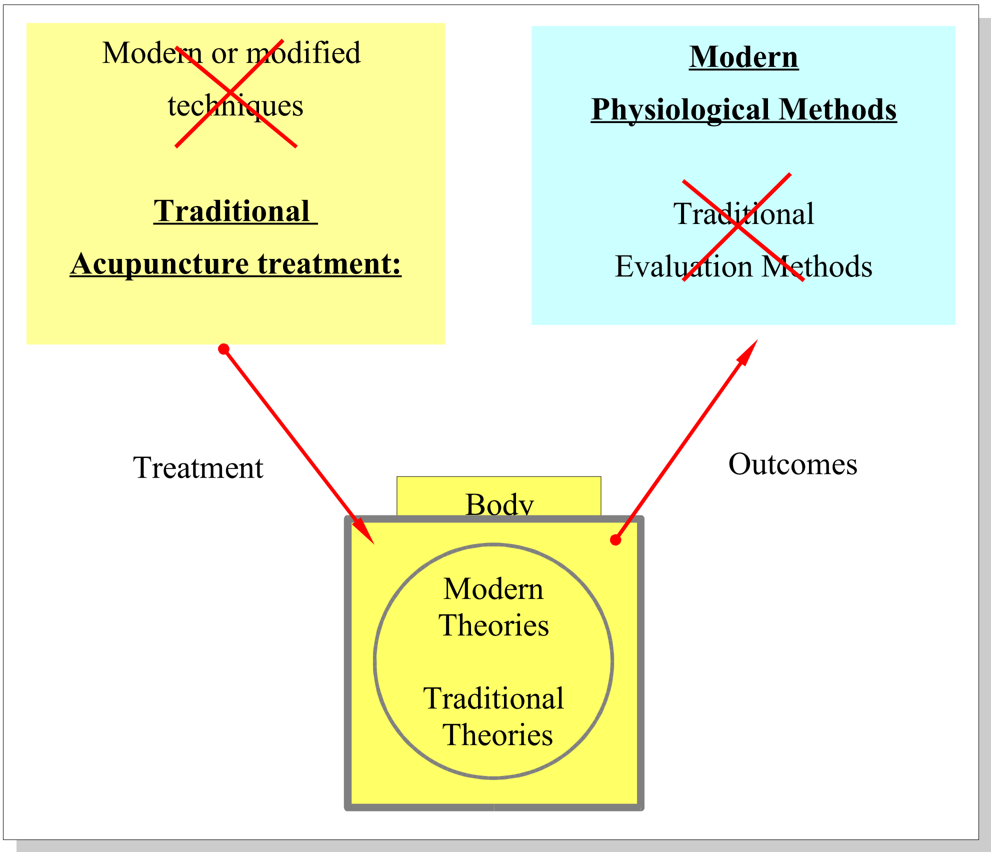
Practitioners of Traditional Chinese Medicine use four diagnostic techniques to evaluate the physiological and *Qi* status of the person, that is inspection, listening, smelling, inquiry and palpation (Deng, 1999, p.1-164). The well known procedure of pulse diagnosis is almost always included as it is an essential component in diagnosis and in the evaluation of treatment outcomes. All that the individual practitioner can say at the end of such an evaluation is that the *Qi* of the body, the *Qi* of the individual organs and the *Qi* in the channels are either healthy and balanced, or unhealthy and unbalanced. The practitioner can also specify the strength and the direction of each imbalance.

When a 'traditional' clinical approach is used in acupuncture research then all evaluations and measurements are based on the subjective assessments made by the individual practitioner. And this is exactly the major point of criticism coming from biomedical sciences – subjectivity of measurements. The first attempts to formalize this evaluation processes have already been made by creating computer software that guides the practitioner in the evaluation of energetic imbalances and suggests options in the treatment protocol (Radu, et. al. 2001). However the measurements are still subjective even if the process of collection is formalised and guided.

Evidence based medicine is interested only in physiological changes that are measured in an objective and scientific way. Then why measure anything else in the research setting. The solution to the dilemma of acupuncture research may be very simple indeed. If only one element is replaced in the chain of: "traditional treatment techniques" => "traditional theories" => "traditional evaluation methods" then the model may still be valid and retain its traditional meaning. In particular, if the traditional evaluation methods are replaced by modern biomedical methods this model may become a fertile ground for the validation of traditional acupuncture and at the same time produce results that are fully accepted by evidence based medicine.

Figure 2 illustrates that it is quite conceivable to adopt this clinical model into acupuncture research without abandoning traditional treatment techniques and traditional theories, on condition that the evaluation of treatment outcomes and the physiological status of the person can be performed using modern scientific methods. In the research presented in this thesis such an approach has been used and various neurophysiological measurements were performed in order to determine the physiological status of the individual.

Figure 2. Application of modern evaluation methods in acupuncture.



2.4.3 Scientific implications of finding physiological changes due to acupuncture

The demand for evidence that acupuncture can induce physiological changes in the human body is already partially fulfilled. There are many studies which conclude that such changes do occur (Josefson and Kreuter, 2003; Maa, et al. 2003; Melchart, et al. 2003). These studies however do not supply the evidence that can be easily turned into clinical practice guidelines. The situation in acupuncture research is similar to the situation in Chinese herbal medicine 30-40 years ago where the only methods used to evaluate the effectiveness of herbal remedies were subjective methods, based on the evaluation of symptoms and the status of *Qi*. As soon as the biomedical sciences focused on individual herbs and their pharmacological properties, that approach was changed dramatically. First of all the chemical analysis of herbs was used to distinguish active and non-active biological agents in herbal remedies (Chen and Chen, 2001). Secondly, the effect of these active components on the human physiology was evaluated using various objective methods such as physiological measurements of heart, lungs and brain, blood analysis and histological findings. At present most of the pharmacopoeias of Chinese herbs contain both traditional *Qi* based characteristics of each herb as well as pharmacological and biochemical data (Chen and Chen, 2001). There are few problems with this approach as both sets of data are very useful in clinical practice and in research.

Figure 3. Dual characteristics of acupuncture points similar to herbs.

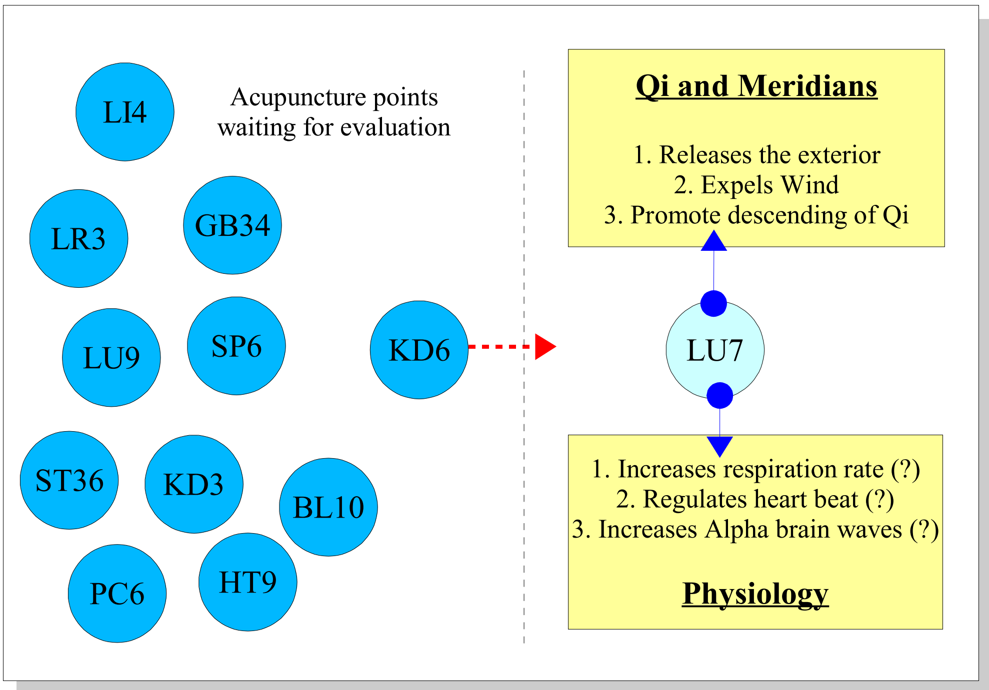


Figure 3 illustrates that a similar situation could be established in the field of acupuncture. Each individual acupuncture point may be evaluated in the laboratory for its ability to influence or regulate the physiology of various body systems. Each point will thus be characterised by its ability to regulate heart rhythm, respiration rate, brain waves, homeostatic temperature regulation, or morphology of the blood. At the same time the traditional *Qi*-based and meridian-based characteristics will be listed side-by-side with the physiological ones.

The major challenge addressed by the research presented in this thesis is in breaking the stereotypes surrounding the phenomenon of acupuncture. Traditional acupuncture theories state that acupuncture influences the flow and transformation of *Qi*. That *Qi* however also influences the physiology of the body and thus influences the balance between health and illness. With the use of appropriate research methods all acupuncture points could be assessed in regards to their ability to make such physiological changes. The task of evaluating over 360 acupuncture points may be very laborious indeed but not necessarily difficult if the right physiological measurement methods are being used. Thus finding appropriate research methodologies and measurement methods is becoming a task of primary importance.

Once the data regarding the physiological effects of acupuncture points starts to accumulate, the gap between physiological findings and clinical findings may well be bridged. Better understanding of the "physiology" of individual acupuncture points may help to formulate better point combinations for use in clinical practice, the same way that modern pharmacological knowledge about individual herbs made the use of herbal prescriptions safer and more effective.

The scope of the research project presented in this thesis only allows for testing two acupuncture points, used individually and also as a pair. Most important however is the evaluation of the research methodologies and calibration of measurement techniques. The success of this project will be judged by two outcomes. One, the ability to demonstrate that chosen neurophysiological methodologies are both stable and sensitive enough to detect physiological changes in healthy human subjects, and two, the ability to induce physiological changes by manipulation of a single acupuncture needle.

Chapter 3. Methodology (general)

Methodology used in the experimental research constitutes the essential base upon which the whole experimental design rests. It is driven by the need to find the evidence which supports or rejects the research hypothesis. It is restricted by the available material resources and know-how. So far acupuncture research has not established its own methodological framework despite the fact that much discussion has taken place in the recent years (Frank, 2001). A vastly different theoretical and philosophical framework of Traditional Chinese Medicine (TCM), as compared with biomedical sciences, makes the situation even more difficult.

Various attempts to demonstrate the effectiveness of acupuncture and other modalities of traditional Chinese medicine can be categorized into three distinctive groups. One group is the research into clinical outcomes related to specific disease conditions. In this type of research, methodology is the same as methodology used in similar trials with pharmacological treatment, such as medication versus acupuncture in the treatment of headaches (Allais and De Lorenzo, 2002). Studies in which two or more different treatment protocols are compared, by definition use the same or very similar experimental designs. The second group is the "case study" approach, representative of, and certainly frequently used in Traditional Chinese Medicine. Single patient history or small groups of patients is included in the evaluation of effectiveness. Naturally, the results are difficult or impossible to generalize, and control of extraneous variables is not maintained. Single-case studies are most strongly criticized by biomedical researchers as they represent a perfect conglomerate of all the possible errors that a scientific researcher can make. The third group is mostly concerned with the biological basis of individual therapies such as acupuncture, spinal manipulation or herbal treatment. Researchers concentrate on detecting changes in the physiology of individual organs or the body as a whole. Both animal and human studies are included in this group. Physiological (Xing, et. al. 2004), neurophysiological (Yun, et. al. 2002) and biochemical (Karst, et. al. 2003) methodologies are widely used. The results of such studies are easily accepted by the scientific community however the validity of such methodologies in TCM research has not yet been confirmed.

The research methods that are used in the third group of traditional Chinese medicine studies are most frequently borrowed from other well established scientific disciplines. In particular, neurophysiology and neuropsychology offer a comprehensive range of research tools that allow noninvasive investigations on human subjects. The research project described in this thesis is representative of the third group and is focused on the physiological changes in the healthy human subject due to acupuncture point stimulation. The basic requirement of the modern biomedical sciences is to conduct research in such a way that the results can be generalised to broader populations and that the experiments can be repeated by other groups of researchers. In the last few years the methodological issues are at least being addressed in the discussions about best research models in acupuncture (Molsberger, et. al. 2004). Much effort has been made by the universities and teaching colleges of Traditional Chinese Medicine to educate undergraduate student about the basic principles of research methodology. Two aspects of research methodology are of primary importance here, that is the randomisation of subjects and the control of experimental variables.

3.1 Foundations of physiological measurements

The complexity and interdependency of the major physiological systems in a human subject becomes obvious when the decision needs to be made regarding specific physiological measurements to be used in the studies. Only a limited number of measurements can be made at any one time, so it is essential that what is measured during the experimental session has physiological meaning and is an important indicator of the health status of the subjects.

A biomedical approach to acupuncture assumes that the nervous system is responsible for the therapeutic effect of acupuncture. This may or may not be true, but it is certainly true that the nervous system responds to a vast amount of internal and external stimuli. Both the central and autonomic branches of the nervous system are involved in regulation of the physiology of the major body organs. Standard neurophysiological recording methods are used to evaluate the objective indicators of physiological changes.

3.1.1 Multi channel recording

Application of multichannel recording techniques provides accurate observation of concurrent changes in human physiology. Each physiological system has its own self regulatory properties and intrinsic rhythm, but at the same time is dependent on all other systems. Heart for example regulates it own rhythm in a very automated way, but in addition also has influence from both sympathetic and parasympathetic branches of the autonomic nervous system.

In order to capture all physiological events in a given experimental setting various technological solutions are employed. In the past recording was done using polygraphs and magnetic tape recorders. Currently modern computerised systems were used in this doctoral study to record, process and analyse the physiological data.

The study that is described in this thesis also required the development of new data acquisition and data analysis software, together with two computers and an array of biomedical amplifiers and sensors. The successful completion of the project was very much dependent on the design and implementation of 'software based' solutions. Six software applications had to be developed by the candidate in order to record and analyze electroencephalography (EEG), electrocardiography (ECG), electrooculography (EOG), electrodermal activity (EDA), and respiration data. All on-line results were simultaneously displayed on eight monitors to allow the researcher not only to evaluate the progress of the experimental session, but also to view the physiological parameters in the unprocessed raw form. The timing of experimental events such as insertion or manipulation of the needle was fully automated by the software, leaving the experimenter free to concentrate on the acupuncture procedure.

The arrangement of the equipment in the acupuncture laboratory and the use of screening curtains was designed to promote in the subjects a feeling of participation in the scientific experiment. When the recording session commenced the movable curtains were used to separate the treatment couch from the computer equipment giving subjects more privacy and thus recreating the conditions of a clinical practice.

Figure 4 shows a photograph of the monitors that were used to display physiological data from on-line analysis.

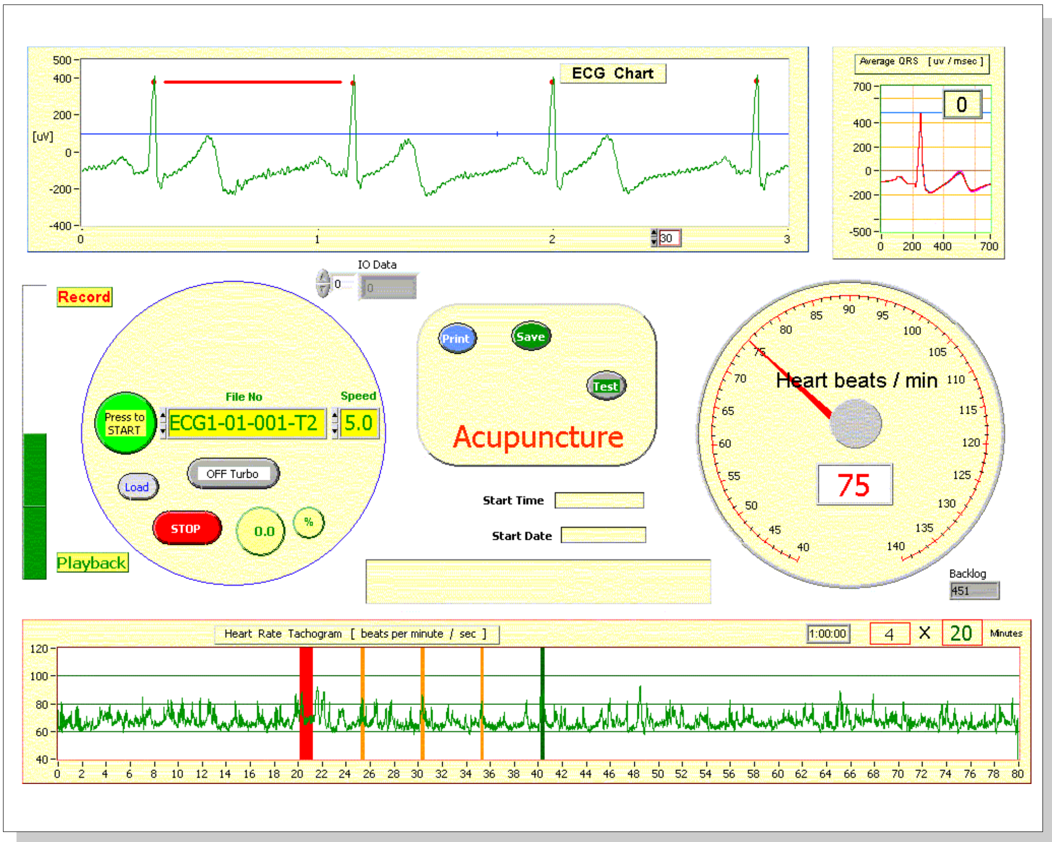
Figure 4. Photograph presenting simultaneous recording of multiple physiological parameters

The software was designed in such way that the experimenter could print the results in a form of graphs and tables immediately after the experiment ended. It was also possible to 'play' back a previously recorded file and stop the analysis at any given time. The researcher could review the data by simply choosing the file name from the list and pressing the Play button. Examples of printouts are included in Appendix B. Copies of the original raw data files were transferred to the removable hard disk drive and to the CD-R disks for a safe storage.

3.1.2 Cardio-pulmonary system

Three computer programs have been created specifically for recording heart and lung physiology, that is: *ECG Recorder* (see Figure 5), *HRV Analyser* and *RESP Analyser.* This software allowed for automated recording of the rhythmic activity of heart and lungs. The results of the analysis were displayed on the computer screens, printed on paper and also converted into spreadsheets in a format suitable for statistical analysis.

Figure 5. Screenshot of the ECG Recorder software created for the recording and analysis of cardio-pulmonary activity.

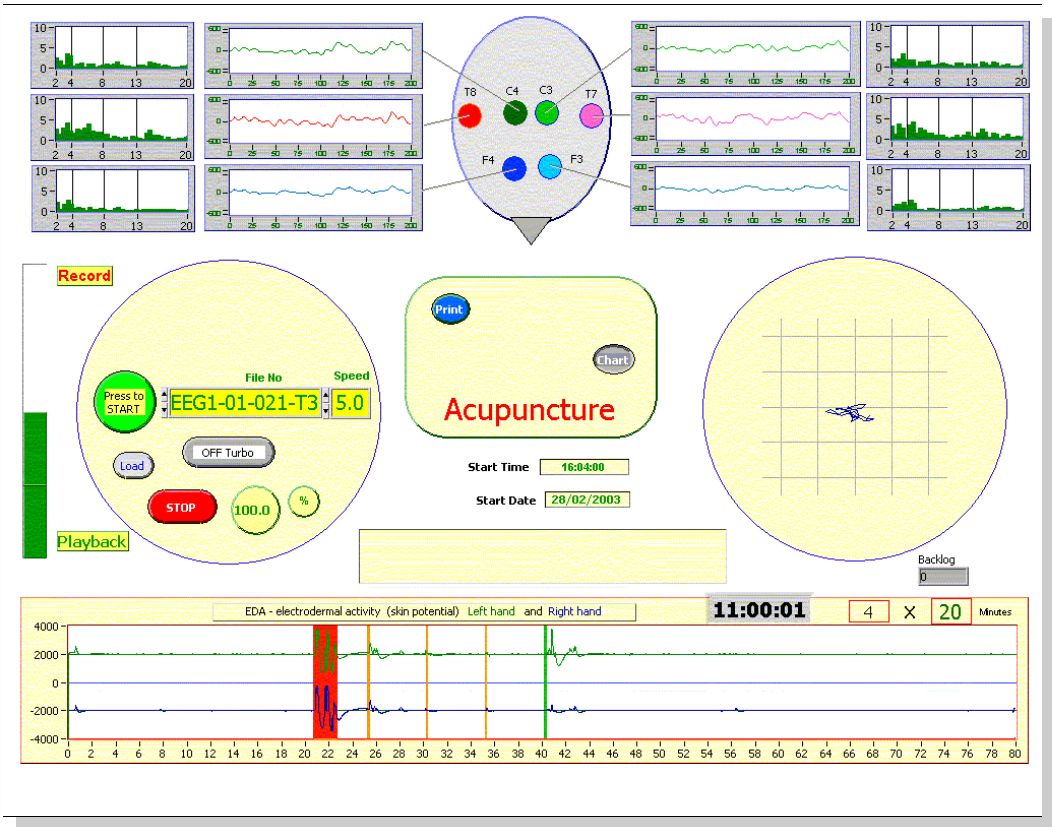


Some aspects of the data analysis for respiration were performed off line due to the fact that shallow breathing left some artifacts in the recorded signals. These artifacts were infrequent in the majority of sessions but some subjects displayed prolonged apnea or short periods of shallow and irregular breathing. Thus visual examination of respiratory signals was needed in order to determine true respiratory movements of the chest and abdomen. Details of this methodology are presented in Chapter 4.

3.1.3 Central Nervous System

Two computer programs were created for the purpose of recording and analysing brain waves, that is *EEG Recorder* (see Figure 6) and *EEG Analyzer*. Both programs combined together allowed for automated continuous evaluation of brain electrical activity during the 80 minute experimental session. Each EEG signal was processed using digital filtering techniques, routinely used in neurophysiological research to

Figure 3. Screenshot of the EEG Recorder software used in experiments to record brain wave activity.



distinguish four major frequency components, that is Delta, Theta, Alpha and Beta waves. The frequency spectrum of electrical signals from each of the electrodes was displayed on the computer screen. The results of the experiments were also printed on paper as well as converted into spreadsheets ready for statistical analysis. Eye movements were recorded by an electrooculogram They were also used to evaluate the generalised state of restlessness and/or sleep in subjects during the experimental session. Details of the EEG methodology used in this study are presented in Chapter 5.

3.1.4 Autonomic Nervous System

The evaluation of functions of the autonomic nervous system was based on direct physiological recordings such as electrodermal potential and on data analysis of other physiological parameters such as heart rate variability. The influence of the ANS on various organs and physiological systems were seen by observing changes in physiological parameters. The more parameters being recorded simultaneously, the more accurate were the assumptions regarding the influence of ANS.

In the study presented in this thesis four types of ANS influences were simultaneously analysed:

- heart rate variability and the balance between sympathetic and parasympathetic innervation described in Chapter 4 on cardio-pulmonary system.
- electro dermal activity (EDA) in the form of skin potential being indicative of the autonomic tone
- body temperature being indicative of homeostatic regulation and stress
- peripheral blood pressure (BP) being indicative of cardiovascular tension

A balance between sympathetic and parasympathetic branches of the autonomic nervous system is of crucial importance in physiological studies. Many disease conditions are reflected in a lack of such balance. Thus simultaneous recording and analysis of physiological events that are influenced by the ANS gave the opportunity to address questions regarding the mediating role of that system in the phenomenon of acupuncture. Details of methodologies are described in Chapter 6.

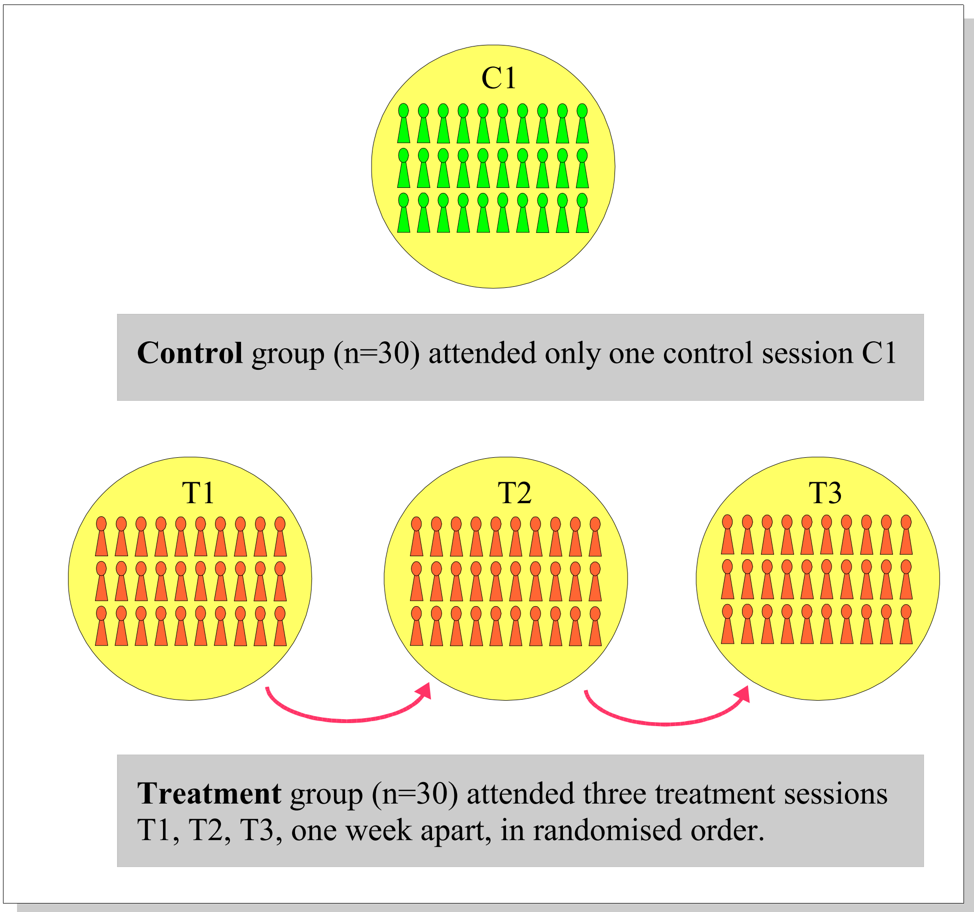
3.2 Definition of research model

The research model employed in this project can be described as an RCT model with repeated measurements. Each experimental session took 2.5-3.0 hours. Separate control and treatment groups were recruited from the general public. Long term follow up of the acupuncture treatment was not used and no post-trial data collection was attempted.

3.2.1 Control vs. Treatment groups

Two separate groups of subjects were recruited for the study. The control group consisted of 30 female subjects who attended the experimental session only once. The treatment group consisted of 30 female subjects who were asked to attend the experimental sessions three times, one week apart, at the same time of day as illustrated in Figure 7. The allocation of the sequence of treatment sessions T1, T2 and T3 was

Figure 7. Allocation of subjects to either control or treatment group

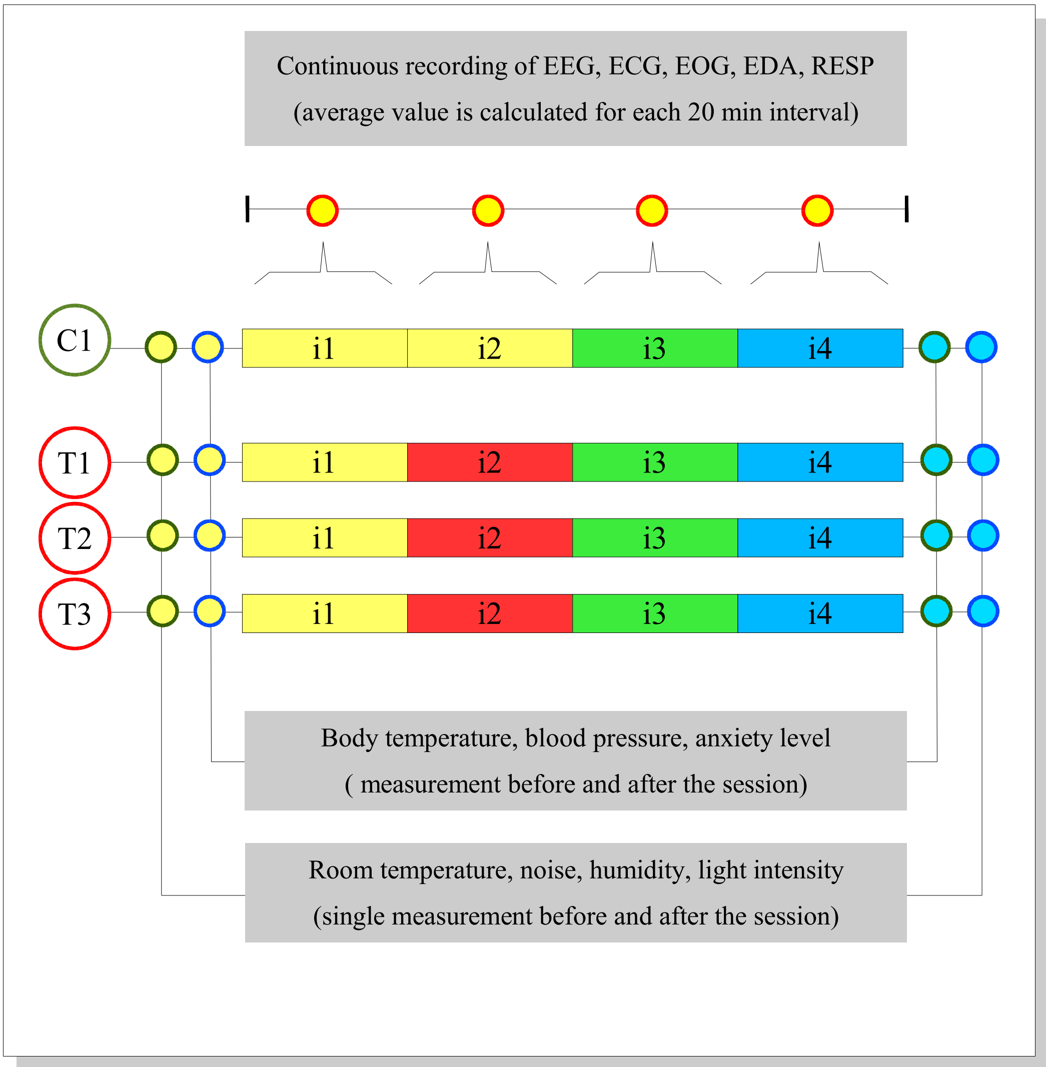


randomised. The same experimental protocol was followed for each group. Subjects were aware of the group allocation. Subjects in the treatment group received acupuncture treatment during three sessions. The LU7 acupuncture point was used in session T1, the KD6 acupuncture point was used in session T2, and both points combined together in session T3. The control group did not receive sham acupuncture but was exposed to the same amount of practitioner-subject interaction.

3.2.2 Repeated measurements

The research model that was adopted in the design of the experiments in this research project utilised two levels of repeated measurements. First, each experimental session was divided into four intervals of 20 minute duration. Despite the fact that there was a continuous recording during the whole 80 minutes, each 20 minute interval was treated

Figure 8. Division of control C1 and treatment sessions T1-T2-T3 into four equal 20 minutes intervals (i1, i2, i3, i4)



as a separate measurement ('within" group repeated measurements). Secondly, subjects in the treatment group attended three sessions T1, T2 and T3. The measurements taken in each session were considered as repeated measurements 'between' groups.

Figure 8 shows the repeated measurements model. The average values for the main experimental variables were calculated to represent individual 20 minute intervals. For the variables that were measured only twice, that is before and after the experimental session, only two average values were calculated. The environmental variables such as room temperature, noise, humidity and light intensity were also estimated at two points, before and immediately after the session, and were considered as repeated measurements in statistical analysis.

3.3 Experimental variables

Four groups of variables were used in the analysis, each representing a different set of influences present during the experimental sessions:

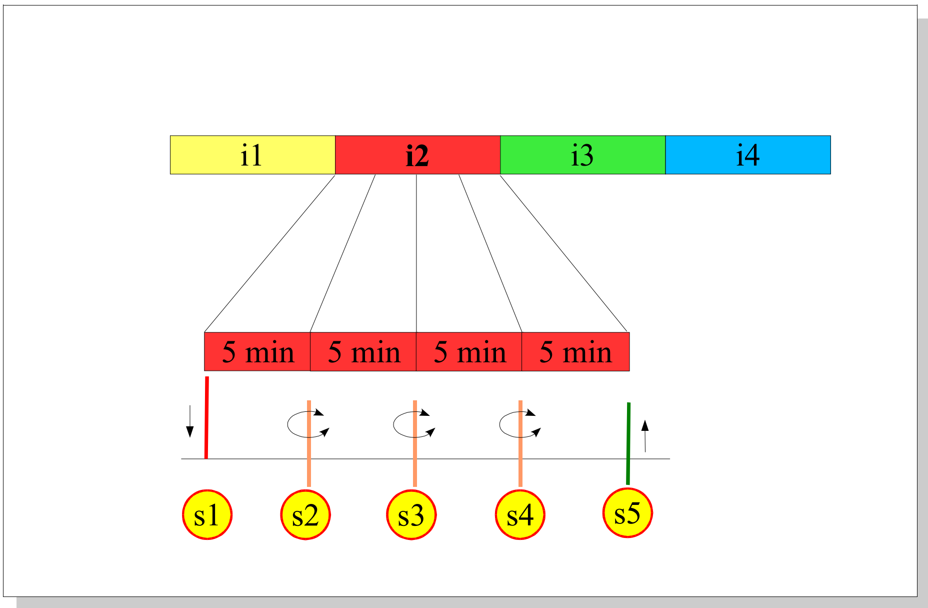
- independent variables
- dependent variables
- factorial variables
- environmental variables

Each group of variables has certain common characteristics that make such grouping possible. Clear definition and examples for each group are presented below.

3.3.1 Independent variables

The independent variables are the only variables that can be considered as being fully controlled variables. In the experiments described in this thesis the acupuncture treatment which was performed during the second 20 minute interval is considered an independent variable. However the time when the needle was inserted or manipulated should also be considered as an independent variable. It was used in the analysis of the electrodermal activity, but not in the analysis of other dependent variables. Figure 9 illustrates how interval 'i2' is marked with five separate events such as the insertion, manipulation and withdrawal of the acupuncture needles. The data that were collected from the 5 minute intervals that followed each of the events were used as measurements supposedly to be triggered by that event. Statistical analysis was used to either confirm or reject such assumptions.

Figure 9. Sub-division of interval i2 into five independent variables (insertion, manipulation x 3, and removal of the needle)



3.3.2 Dependent variables

A range of dependent variables was used to evaluate the physiological status of the experimental subjects. They are grouped in logical clusters that represent the functioning of the major body systems. The cardio-pulmonary system was evaluated by such variables as heart rate, respiration rate, heart rate variability and ratio between heart rate and respiration. The central nervous system was evaluated by measuring the magnitude of electrical brain waves and autonomic nervous system by such variables as skin electrical potential, peripheral blood pressure and body temperature.

The psychological variables, such as anxiety level were also recorded before and immediately after the experimental sessions. They served as indicators of the emotional status of the subjects and also as a factor in the MANOVA analysis of the other dependent variables. Table 1 presents names, acronyms and the descriptions of major dependent variables.

Table 1. Division of dependent variables into four major groups.

Group	Measure	Acronim	Description
Cardio-pulmonary system	ECG + RESP	HR	Heart rate
		HRV-Symp	Heart rate variability: ”sympathetic”
		HRV-Para	Heart rate variability: “parasymp”
		HRV-Ratio	Ratio: sympathetic / parasympathetic
		HR/RESP	Ratio: heart beats / one respiration
		RESP	Respiration rate
Central nervous system	EEG	D	Brain wave 2.0- 3.5 Hz Delta
		T	Brain wave 3.5- 7.5 Hz Theta
		A	Brain wave 7.5-13.0 Hz Alpha
		B	Brain wave 13.0-30.0 Hz Beta
		A1	Brain wave 7.5-10.0 Hz Alpha1
		A2	Brain wave 10.0-13.0 Hz Alpha2
		B1	Brain wave 13.0-18.0 Hz Beta1
		B2	Brain wave 18.0-30.0 Hz Beta2
		“8”	Brain wave 7.0- 9.0 Hz “8”
		DF	Brain wave 0.5- 3.5 Hz Delta (full)
	EOG	EOG(H) Max	Eye movement horizontal maximum
		EOG(H) Ave	Eye movement horizontal average
		EOG(V) Max	Eye movement vertical maximum
		EOG(V) Ave	Eye movement vertical average
Autonomic nervous system	EDA	EDA(L) Max	Skin potential left hand maximum
		EDA(L) Ave	Skin potential left hand average
		EDA(R) Max	Skin potential right hand maximum
		EDA(R) Ave	Skin potential right hand average
	TEMP	TEMP	Body temperature (ear)
	BP	BP	Peripheral blood pressure (left arm)
Psycho-logical factors	Self Assess. Scale	Anx Gen	Trait anxiety level (time of enrolment)
		Anx Pre	State anxiety level (before session)
		Anx Post	State anxiety level (after session)

3.3.3 Factorial variables

The main factorial variables used in the study are group / session / interval variables. The 'group' factor allows the division of experimental results into separate groups, that is either control group (C) or the treatment group (T1/T2/T3). The 'treatment' factor divides the results from the treatment sessions into three subgroups according to the type of treatment received, that is LU7 acupuncture point (T1), KD6 acupuncture point (T2) or both points combined together (T3). The 'interval' factor splits the results from the 80 minute sessions into four intervals; that is 0-20 minute (i1), 20-40 minute (i2), 40-60 minute (i3) and 60-80 minute (i4).

Psychological and the life style variables are also being used as factors in the analysis of the experimental results. State and trait anxiety levels were used to distinguish between subjects who exhibited relatively lower or relatively higher levels of pre- and post experimental anxiety. Other factors such as familiarity with acupuncture were also used in the analysis. There were also factors derived from other dependent variables such as heart rate and respiration rate. For example in one factorial analysis all subjects were categorized according to heart rate level at baseline, in order to see if that can be correlated with outcomes of the experiments.

3.3.4 Environmental variables

There were four environmental variables monitored during the experimental session. These were room temperature, humidity, noise level and light level. All could be used as factors in the analysis as well as dependent variables in the evaluation of stability of the laboratory environment in which the experiments were conducted. The need to monitor the environment of the research laboratory comes from the acknowledgment that noise level may affect the subject's arousal, relaxation or sleep. It may also trigger a sudden startled response. Humidity and room temperature may produce discomfort and lower the ability to retain a quiet position during the experimental session. The light level may induce either relaxation or, if excessive, may keep the subjects artificially alert.

3.4 Components of statistical analysis

The raw data collected during the experimental sessions were processed and then analysed using statistical tools. The type of analysis that was performed and type of comparisons being made depended entirely on the research questions and hypotheses. This study was designed in such a way that it could provide choices in analysis of the experimental data and demonstrate the effects of such choices on research outcomes.

One of the common mistakes that is made in acupuncture studies is associated with logical reasoning about cause and effect, or more specifically, about the relationship between dependent and independent variables. Firstly, the overall results from the treatment group can be compared with the results from the control group assuming that 'active' is compared with 'non-active'. Secondly, two or more different treatment protocols can be compared, one by one, against the control group. Thirdly, different treatment protocols can be compared between each other, without reference to the control group. More comparisons are possible if multiple measurements are made in each group at different points in time, such as before and after the treatment, or at regular intervals during the experimental session.

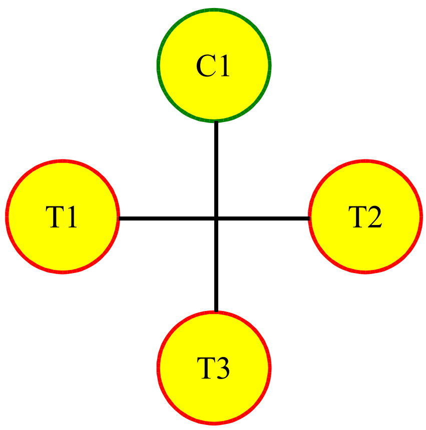
It is obvious that individual research questions will require a clearly defined logical plan about how to arrive at an answer. Unfortunately this is a source of many research errors in acupuncture studies. For example a comparison between results from inappropriately matched measurements may lead to incorrect findings, and the conclusions based on such findings may also be incorrect.

Very few physiological measurements are considered important if based on their absolute values. Individual differences between human subjects as well as the influence of other physiological conditions makes many physiological measurements 'relative' and influenced by other dependent variables. In the study presented in this thesis all major measurements were repeated four times during the experiential sessions (i1 – i4) as well as repeated four times for different experimental groups (C1, T1, T2, T3). The major types of possible comparisons between these measurements, and the expected outcomes, are presented in Figures 10, 11, 12.

3.4.1 Stage 1 - Initial analysis (Factorial ANOVA)

The main difference between experimental groups is based here on the presence or absence of therapeutic intervention, in this case acupuncture stimulation. The control group represents the neutral or baseline results, while the treatment groups represent the active interventions. A different treatment intervention was used three times in this study, each time with a different acupuncture point combination. Results from each treatment group were divided into four 20 minute intervals, thus care must be take in using the comparisons only between the results that come from the same measurement intervals, for example [C1(i1) and T1(i1) and T2(i1) and T3(i1)], or [C1(i2) and T1(i2) and T2(i2) and T3(i2)] and so on.

Figure 10. Comparisons between different groups in Stage 1 of data analysis



Stage 1 analysis

1. Groups treated independently
2. No repeated measurement between groups acknowledged
3. No repeated measurements within groups acknowledged
4. Factorial ANOVA test
5. T-test between any two groups
6. Detecting only major differences between groups

Expected outcomes: ability to detect large differences between measurements that are indicative of larger physiological changes. All measurements in corresponding intervals i1-i4 and groups (sessions) C1, T1, T2, T3 are assumed to be independent of each other, which in real life this is not the case. Stage 1 presents a good overview of the data that were collected in the experiment. It can also be used to calculate the effect sizes and the required sample sizes at the given level of the power of experiment.

The arrangement of variables in the spreadsheet is presented in Table 2. The subject's identification (ID) is listed in column 1, and factorial variables 'group' and 'interval' are listed in columns 2 and 3. Dependent variables are listed in columns with names that refer to their physiological meaning. For example, column 4 contains a numerical value that represents the magnitude of the brain wave called Delta (D), recorded from electrode C4. A correct placement of variables is essential for statistical analysis.

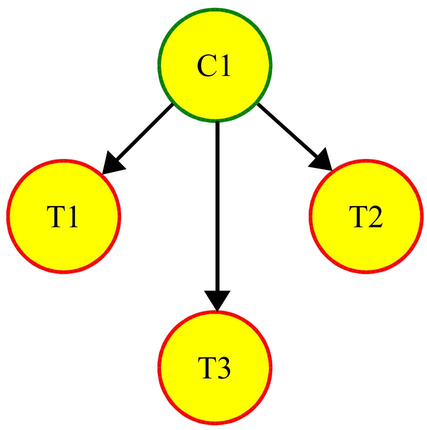
Table 2. The arrangement of variables in the data spreadsheet for the Stage 1 analysis (example of EEG data)

	Factors		Electrode C4											
ID	Group	Interval	D	T	A	B	A1	A2	B1	B2	“8”	DF	D	T
1		1												
1	T1	2												
1		3												
1		4												
1		1												
1	T2	2												
1		3												
1		4												
1		1												
1	T3	2												
1		3												
1		4												
.	.													
.	.													
.	.													
.	.													
31		1												
31		2												
31		3												
31		4												
.		.												
.	C1	.												
.		.												
.		.												
60		1												
60		2												
60		3												
60		4												

3.4.2. Stage 2 - Repeated Measures (one level) MANOVA

The control group represents the normal or baseline results, and four intervals within that group represent the natural change in the recorded physiological measurements over time. Different treatment interventions are represented by group T1, T2, and T3. The analysis is focused on control vs. treatment. Analysis is done independently for each pair C1-T1 or C1-T2 or C1-T3. Care must be taken in using the comparisons only between the results that come from the same measurement intervals, for example [C1(i1) and T1(i1)] or [C1(i2) and T1(i2)] or [C1(i3) and T1(i3)]. Statistical test MANOVA with one-way repeated measures and with post-hoc analysis was used in order to detect differences between the control and each treatment group.

Figure 11. Comparisons between different groups in Stage 2 of data analysis



Stage 2 analysis

1. Groups treated as independent
2. No repeated measurements between groups
3. Repeated measurements within groups, at time intervals i1-i4
4. MANOVA test with one level repeated measures
5. Detecting differences between control vs. treatment groups

Expected outcomes: ability to detect small differences between measurements that are indicative of small physiological changes. Measurements in corresponding intervals i1-i4 are treated as being acquired from the same subject, which is the case. The comparison [C1(i1) and T1(i1)] will show how similar are the measurements in the first 20 minutes before acupuncture treatment is applied, and comparison [C1(i4) and T1(i4)] will show the differences at the end of the session.

The arrangement of variables in the spreadsheet for Stage 2 analysis is presented in Table 3. The measurements in each set of the four columns correspond to intervals i1-i4. For example columns 3-4-5-6 contain numerical values that represents the magnitude of brain wave called Delta (D), recorded from electrode C4, and acquired in consecutive time intervals i1-i4. The correct placement of variables is essential for the statistical analysis when one set of 4 columns represents one set of repeated measurements.

Table 3. The arrangement of variables in the data spreadsheet for the Stage 2 analysis (example of EEG data)

	Factors	Electrode C4													
		Delta				Theta				Alpha				Beta	
ID	Group	i1	i2	i3	i4	i1	i2	i3	i4	i1	i2	i3	i4	i1	i2
1	T1														
2	T1														
3	T1														
.	.														
.	.														
.	.														
28	T1														
30	T1														
31	C1														
32	C1														
33	C1														
.	.														
.	.														
59	C1														
60	C1														

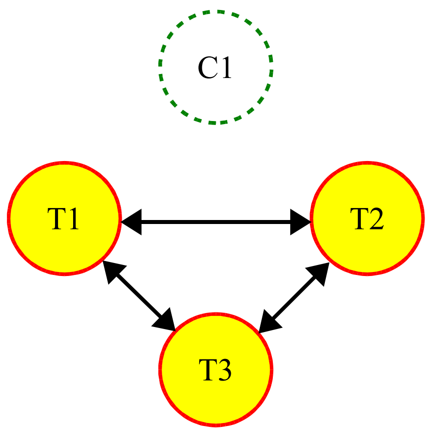
Repeated measurements

D	T	A	B	A1	A2	B1	B2	8	DF		D	T	A	B	A1	A2	B1	B2	8	DF
Electrode C4											Electrode T8									
---------------------------->>>																				

3.4.3 Stage 3 - Repeated Measures (two level) MANOVA

The comparisons that are being made only between treatment groups with an exclusion of the results from the control group help to isolate 'pure' treatment effects. Because the conditions in all treatment groups are identical, the possible placebo effect, if present, must also be identical. Thus all detected differences between treatment groups could be attributed primarily to the influence of the acupuncture. Care must be take in using the comparisons only between the results that come from the same measurement intervals, for example [T1(i1) and T2(i1) and T3(i1)] or [T1(i2) and T2(i2) and T3(i2)] and so on. Statistical test MANOVA with two-way repeated measures and with post-hoc analysis was used in order to detect differences between each treatment.

Figure 12. Comparisons between different groups in Stage 3 of data analysis



Stage 3 analysis

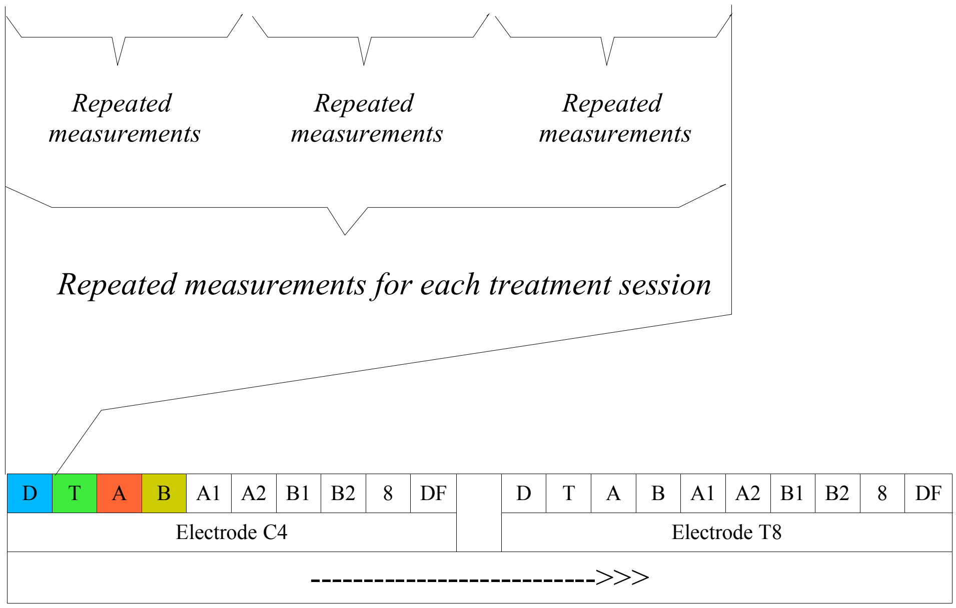
1. Groups treated as dependent (same subjects).
2. Repeated measurements between groups T1,T2,T3 and within groups, at intervals i1-i4
3. MANOVA test with two levels repeated measures
4. Detecting differences between effects of different treatments

Expected outcomes: ability to detect small differences between measurements in treatment groups that are indicative of 'pure' acupuncture effects. Such results could be easily compared with data from similar experiments where any number of other acupuncture points are being tested for their physiological action. The departure from the control vs treatment model will allow for a greater freedom in formulating research hypotheses once the doubt regarding the placebo effect is removed.

The arrangement of variables in the spreadsheet for Stage 3 analysis is presented in Table 4. The measurements in each set of the four columns correspond to intervals i1-i4. One set of four columns represents measurements for one treatment. Overall, the set of twelve measurements is repeated for each of the dependent variables. The correct placement of variables is essential for the statistical analysis when one set of 12 columns represents two levels of repeated measurements (3 treatments X 4 intervals)

Table 4. The arrangement of variables in the data spreadsheet for the Stage 3 analysis (example of EEG data)

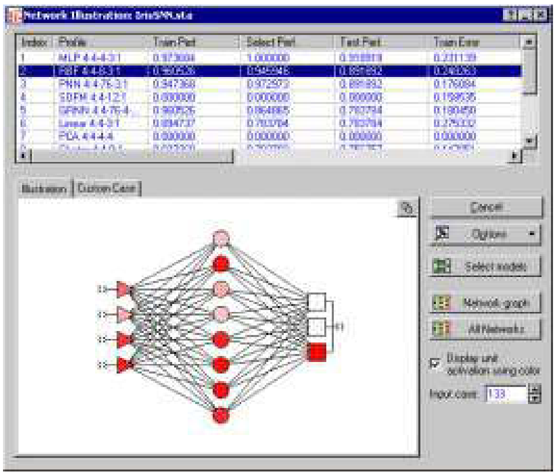
	Electrode C4															
	Delta												Theta			
	T1				T2				T3							
ID	i1	i2	i3	i4	i1	i2	i3	i4	i1	i2	i3	i4	i1	i2	i3	i4
1																
2																
3																
.																
.																
.																
28																
29																
30																



3.4.4 Neural Networks nonlinear analysis

Research in artificial intelligence (AI) in the 1960s-1980s introduced a new concept of systems that are fault tolerant and have a capacity to learn (Widrow and Lehr, 1990). The concept of neural networks came from mimicking the biological neural systems. A neuron is a single biological unit of the brain. It fires only when the total strength of the received signals exceeds a certain level. In similar fashion, the logical neuron of the neural networks fires only when the threshold of the sum of received signals is exceeded. It was proved possible to construct simple models of neural networks that can be trained to respond to certain patterns of input data. Figure 13 shows interconnections between logical neurons and their ability to converge the results into one output.

Figure 13. Neural networks mimicking the architecture of the brain



Training the Neural Network (NN) to respond to certain patterns of input data is made by 'exposing' the network to the subset of data from the real experiments. Training allows it to establish the threshold levels for all input neurons. When the network is exposed to different types of new data, the logical neurons will 'fire' when the sum total of the new inputs exceeds previously learned levels. Despite the fact that AI concepts are already used in the processing of the biomedical data, the interpretation of the results is still subject of debate. Differences between statistical (general linear model) and non-linear methods of data analysis will be further discussed in Chapter 7.

3.5 Experiments

There have been a number of processes involved in the implementation of the experiments described in this thesis. They included recruitment of the subjects, obtaining consent and Ethics Committee clearance, conducting experimental sessions and analysis of the results. In order to avoid confusion regarding the way the experiments were conducted, definitions of the essential aspects of the research design are presented below.

3.5.1 Terminology and format

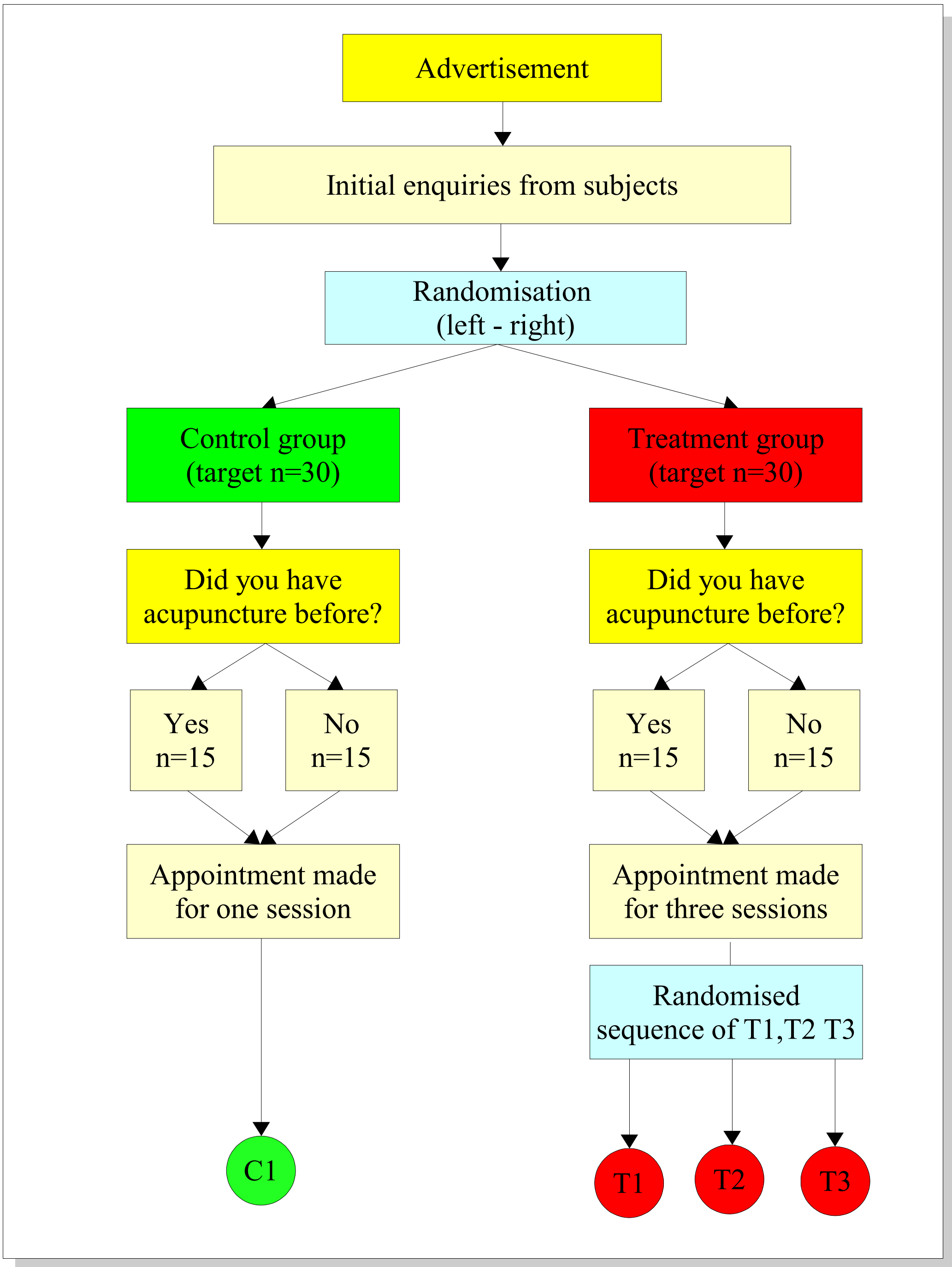
Experimental sessions lasted for about 2.5 hours and included preparatory work with electrodes and biomechanical sensors, completing questionnaires, participating in the recording session and finally removal of the electrodes and completing one more questionnaire. The recording session of 80 minute duration was started when all preparatory work was completed. Recording physiological parameters was divided into four 20 minute intervals. The baseline recording within each session consisted of the first 20 minutes of the recording session (interval i1). This was followed by 20 minutes of acupuncture stimulation (interval i2). Intervals i3 (40-60 minutes) and i4 (60-80 minutes) were considered as the post treatment interval. Subjects in the control group consisted of 30 female volunteers who attended one experimental session and did not receive any acupuncture treatment. Subjects in the treatment group consisted of 30 female volunteers who attended three experiential sessions, one week apart, and received a different acupuncture treatment at each session.

The multichannel recording of physiological parameters was conducted using a computerised system that consisted of electronic amplifiers, data acquisition devices with analog-to-digital converters, computer hardware and software. Custom designed software allowed for full automation of the laboratory setting and simultaneous recording of many physiological parameters. The role of the researcher was concentrated on the acupuncture treatment and the welfare of the volunteers participating in the experiments. A set of electronic switches was provided to allow precise marking of the real-time events during the experimental session such as insertion of the needles.

3.5.2 Recruitment process

The subjects for the study were recruited from students and staff of the University of Technology, Sydney. Overall 63 subjects were enrolled in the study, however data from three of them was excluded due to the presence of ectopic beats in the ECG. Figure 14 illustrates the steps taken in the recruitment process and two stage randomisation.

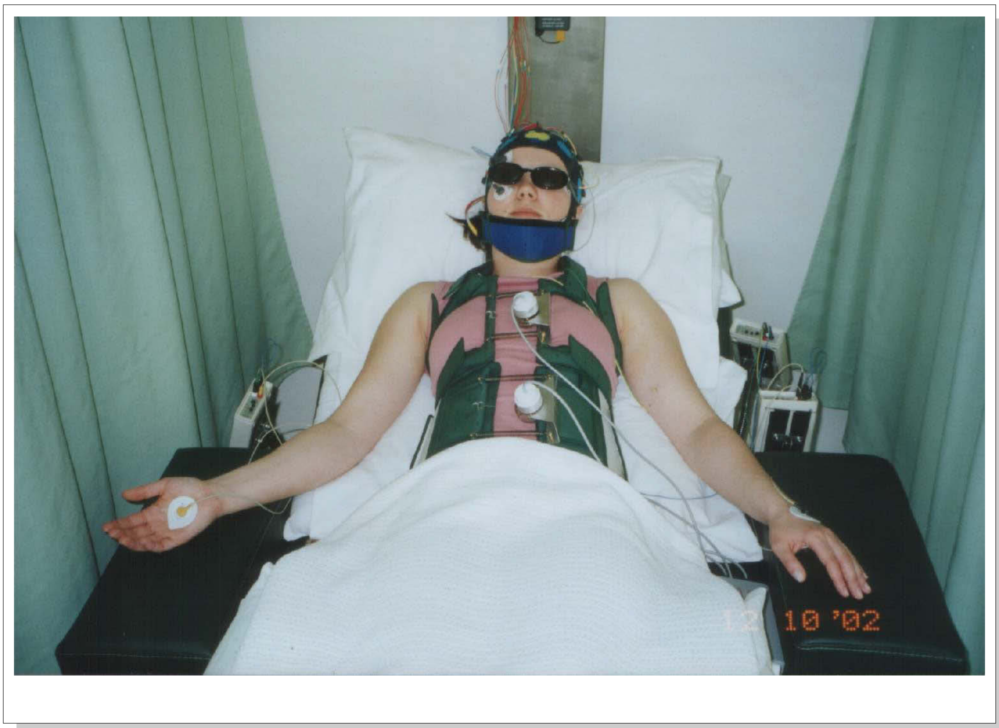
Figure 14. Diagram representing steps in the recruitment process.



3.5.3 Data collection procedure

During the 80 minute recording session subjects were positioned on the couch. Pillows and blanket were used to secure a comfortable condition for each of the participants. The EEG cap with electrodes was placed on the head, EOG electrodes on the face and ECG electrodes on the chest of the subject. The electrodes for EDA (skin potential) were attached to the dorsal side and the palm of each hand. Additionally the vest with mechanical sensors around chest and abdomen were used to monitor the respiratory movements. All electrodes and sensors were connected to the electronic amplifiers. Figure 15 shows a photograph of the participant with all electrodes and sensors attached before the recording session started.

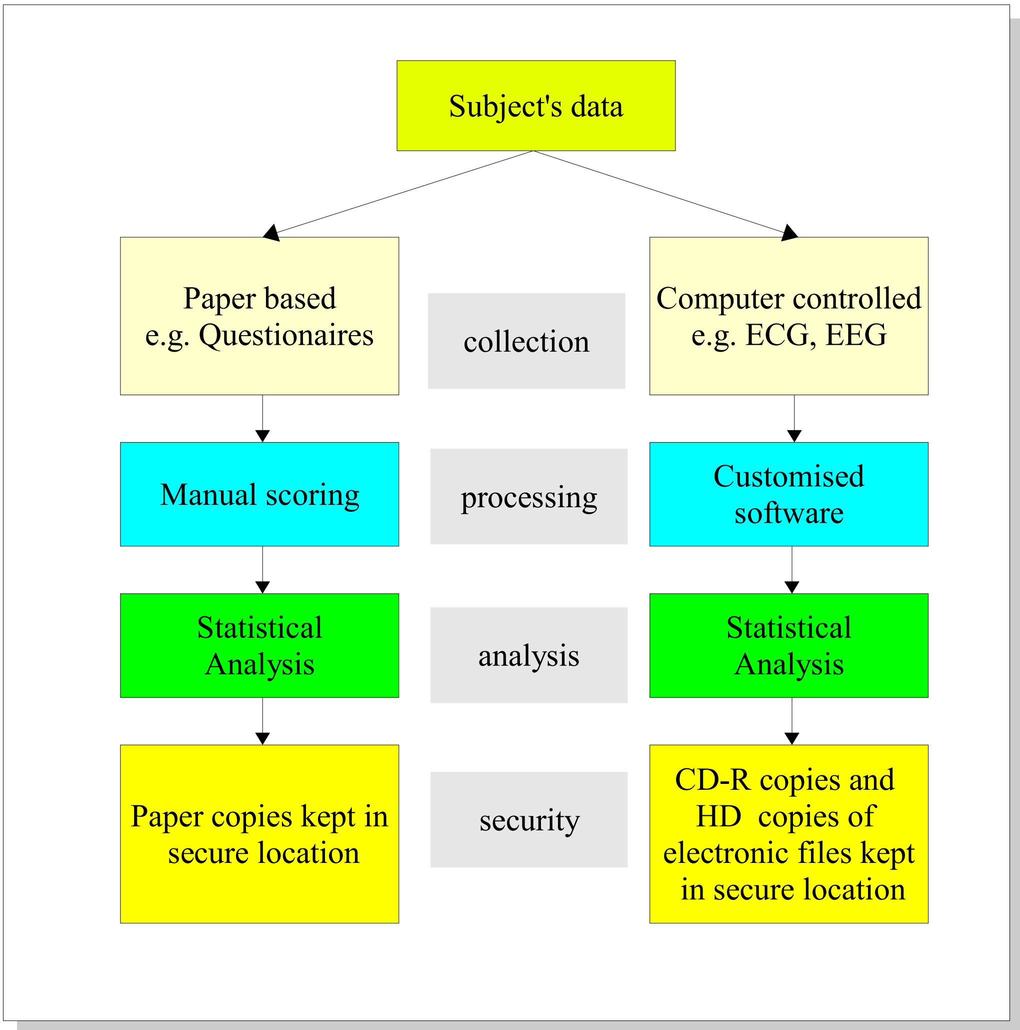
Figure 15. Volunteer with electrodes and sensors ready for the recording



The blood pressure and body temperature were recorded just before and immediately after the recording session. The data about the environment of the lab was also collected. Room temperature, relative humidity of the air, light level and noise level were recorded before and after the recording session.

The essential steps of the data collection and analysis are represented in Figure 16. The distinction between paper-based and computer-based procedures illustrates how different are the processes and tools involved in each activity.

Figure 16. Diagram representing steps in data collection, processing, analysis and maintaining data security



3.5.4 Instruments

The spectrum of measurements conducted during the experiments consisted of paper-based questionnaires, electronic amplifiers and computer software. The software played the key role in this experimental setting. Software was designed for both data acquisition as well as for the automation of the experiments. The physical arrangement of the acupuncture laboratory is presented in Figure 17. Within the reach of the experimenter's hands were both electronic and software based instruments. The computer monitors on the right side displayed physiological signals and the results of data analysis. All electronic instruments were placed on the left side in close proximity to the couch on which subjects were positioned. A movable curtain was used to visually separate the subject from the environment of computers and monitors.

Figure 17. Photograph of the instruments and monitors used in the acupuncture laboratory during recording sessions.

During the first few minutes, just before the beginning of the recording session, the researcher checked the placement of the electrodes and the contact of these electrodes with the subject's skin. Once the equipment and electrodes were checked, the recording sessions was started by simply clicking a 'start' button on the computer screen. Timing of the experiment was programmed into the software. The insertion and manipulation of the needles was confirmed by the researcher by means of pressing the switch placed on the right side of the curtain. Table 5 lists all the tools used in the experiments.

Table 5. Instruments used for the measurement and analysis of experimental variables.

Paper based instruments			
	Measurement	Name	Supplier
1	Anxiety	"STAI Form Y-1" scale	Charles D. Spielberger
2	Anxiety	"STAI Form Y-2" scale	Charles D. Spielberger
3	Lifestyle	"Lifestyle Appraisal Questionnaire"	Prof. Ashley Craig
Electronic instruments			
	Measurement	Name	Supplier
4	Body temperature	Infrared termometer "ThermoScan 6012"	Braun
5	Blood pressure	Digital blood presure monitor "Omron M4"	Omron
6	ECG	High sensitivity BioAmplifier "BIO-2"	Contact Precision Instruments U.K.
7	EEG / EOG	High sensitivity BioAmplifier "EEG-8"	
8	EDA	High sensitivity BioAmplifier "BIO-2 "	
9	Respiration	Respiration sensors and harness	Mechanical Workshop Faculty of Science UTS
10	Environmental	Universal meter (noise, temperature, humidity and light)	
Computer software			
	Measurement	Name	Supplier
11	HR / HRV	*ECG Recorder*	Software was developed by the author of the thesis using LabView developmental platform (Apr 2000 – Oct 2002)
12	EEG / EOG	*EEG Recorder*	
13	HRV	*HRV Analyser*	
14	EEG	*EEG Analyser*	
15	EDA	*EDA Analyser*	
16	Respiration	*RESP Analyser*	
17	MANOVA Analysis	*STATISTICA* – General Linear Model	StatSoft, US
18	Neural Networks analysis	*STATISTICA*– Neural Networks	

3.6 Subject characteristics

All subjects enrolled in the study described in this thesis were given written information about the nature of the experiments and the risks involved in participation (Appendix A-3). It was explained to them that the purpose of the study was advancement in understanding of the physiological effects of acupuncture. Informed consent was obtained from all participants (Appendix A-1), accompanied by an agreement that they could withdraw from the study at any time without giving any reasons. Approval of University's Ethics Committee was obtained before the commencement of the recruitment process (Appendix A-4). Because individual differences between human subjects may contribute to the variability of the research results, all participants in this study were asked to fill in questionnaires regarding age, medical conditions, smoking, drinking, diet and exercise.

3.6.1 Sample size

Sixty healthy female volunteers were recruited to the study, in two groups, each containing 30 subjects. The calculated sample size (Welkowitz, et al. 1982) from a previous pilot study for HRV was n=27 in order to achieve a power of experiment of at least 0.7. Current practice in acupuncture research where reported group sizes in many RCT are just 10-20 subjects and power estimates are not reported. The important issues of sample sizes and power in acupuncture experiments will be further discussed in Chapter 8.

3.6.2 Representativeness of the sample

Subjects recruited into this study were randomly allocated to either the control or treatment group. All subjects were is good health, without any known heart conditions. Only females were invited to participate. This on one hand may reduce the generalisability of the findings, but it also limited the influence of individual differences. The fact that a pair of acupuncture points that was used in the treatment was recommended in the traditional literature as more appropriate for women rather than men, had a contributing influence on limiting subjects to one gender.

The experimental sessions were conducted at a fixed time during the day. Sessions started every 3 hours, beginning at 9 am, then 12pm, 3pm and 6pm. Allocation to the session was dictated by the subject's available time, but in the case of the treatment group all three sessions were scheduled for each subject in the same time slot, in order to reduce the influence of day / night rhythm.

3.7 Strengths and limitations of the research methodology

Much work had been done during the planning and implementation phase of this project in order to conduct this research in a scientific way using modern methodologies. Some major weaknesses of the acupuncture research were addressed. Also an effort was made to simplify the model of research design almost to the level of a 'teaching' laboratory. This was done in hope that the results of this projects may contribute to a better understanding of acupuncture research and to breaking the deadlock in inefficient and misguided physiological studies in acupuncture.

3.7.1 Life studies

Ability to conduct a life study on human subjects adds to the value of this project. The fact that emotions, stress and environmental factors may interfere with the outcomes makes the study even stronger because it imitates the conditions of real clinical practice. It also has the risks that variability of these data could be too big to detect small changes when groups sizes are limited. The psychological factors associated with apprehension or fear of acupuncture needles were considered. It has been acknowledged that there can only be one single session where the subject is truly 'naive' to acupuncture procedure. However each subject in the study has been asked about previous experience.

3.7.2 Healthy subjects

Serious adverse effects of acupuncture are certainly reduced in healthy subjects, however the distribution of *Qi* is not as strongly disturbed as is in the case in the sick person. It can be assumed that in healthy subjects needle stimulation can only have a limited influence on the flow of *Qi* and thus on the physiology of the body, however this promotes an opportunity to observe how undisturbed *Qi* can respond to the needling phenomenon – a factor almost entirely ignored in acupuncture research. An understanding of the status of health and disease can only come from comparative studies, therefore conducting research on generally healthy subjects is of crucial importance to acupuncture studies. Similar projects should be repeated on subjects with known disease conditions.

3.7.3 Multi parameter recording

By monitoring multiple physiological events simultaneously the researcher can draw conclusions not only about changes in one physiological system, but also about possible correlations between many systems. Such data are of extreme value to the understanding of the mechanisms of acupuncture. No single organ works in isolation. According to TCM reasoning, all organs and body systems are interconnected and analysis of a single processe in isolation would have no clinical sense.

3.7.4 Internal baseline

Each subject in the treatment group was exposed to the same level of patient-practitioner interaction and the same environmental conditions. Additionally the first 20 minutes of each recording session in both groups was considered as an internal baseline. Participants were encouraged to relax and to 'settle' in their new role as subjects in the acupuncture experiment. All active interventions were synchronized. The direct comparisons were possible between consecutive intervals within the session and the same intervals between groups.

3.7.5 Control group

It is obvious that many physiological parameters are subject to rhythmic fluctuations, even in such a short period of time as 80 minutes. The control group is in a sense the baseline for each of the treatment sessions. Having a separate control group of subjects helped to remove doubts about true progression of the physiological changes during the 80 minutes of recording session.

3.7.6 Randomisation

Two levels of randomisation were used in the study, that is randomisation of control or treatment group, and randomisation of the sequence of three treatment sessions. RCTs are generally considered to provide stronger evidence of outcomes than single case studies or studies that are not randomised (Greenhouse and Meyer, 1991)

3.7.7 Psychological and lifestyle factors

The design of this study took into account some major psychological factors that could occur in the experimental setting where human subjects are present. Stress and anxiety are the most common factors but other lifestyle factors have also been considered, such as previous use of acupuncture. Of course there are many potential influences but the main purpose was to examine at least a few of them and thus further validate the research model.

Chapter 4. Cardio-pulmonary system

The heart and the lungs are essential organs in human physiology (Marieb, 1998). This view is shared equally by both western and oriental medicine. Biomedical science considers these two organs as separate functional units, but also groups them together as belonging to the cardio-pulmonary system, entirely enclosed and protected by the chest cavity. Its function is to supply oxygen, remove carbon dioxide and distribute blood to all organs and tissues of the body (Marieb, 1998).

In Traditional Chinese Medicine the lungs are considered to be the organ from which the Qi of the body starts to circulate through meridians. The physiology of Qi and Blood involves all major organs, each contributing to the process of transformation and movement. The heart has a more complex function than lungs because it not only governs the blood but is also strongly associated with Spirit, the oriental equivalent for Souls. Together they participate in the continuous movement of Qi and Blood, externally manifested as rhythmic inhalation-exhalation movement and heart beat.

4.1 Heart rate (HR) and heart rate variability (HRV)

The definition of the heart rate is rather simple - it is the rate at which the cardiac muscle contracts in a given period of time. It is expressed as the number of heart beats per minute. In clinical practice it is referred to as pulse. Heart rate variability is defined as the degree of change from the baseline of the heart rate (Kleiger, et al. 2005). Various data processing techniques and statistical methods are used to evaluate this degree of change and to make it a standardised measure of variability. Heart rate can change very rapidly due to an increase in physical activity, strong emotions and in response to certain drugs and substances. It can also have lower or higher than normal baseline values over a longer period of time. In healthy individuals at rest the heart rate is in the range of 72-80 bpm (females) and 64-72 bpm (males) . When it is persistently elevated above 100 bpm it is called tachycardia and when it is below 60 bpm it is called bradycardia (Marieb, 1998)

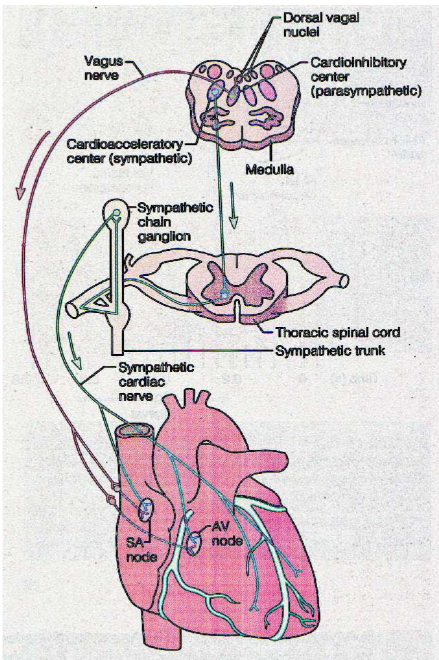
4.1.1 Introduction

An electrocardiogram constitutes the basic form of assessment of heart function. It contains rich information about electrical activity of the heart and it is closely related to the heart's mechanical actions. In a healthy person one contraction of the cardiac muscle is accompanied by one P-QRS-T complex of electro-cardio-graphic signal (ECG). In the studies of human health the measurement of heart rate and heart rate variability are the most frequently used parameters describing heart functions.

4.1.1.1 Physiological importance of heart rate

The cardiac conduction system is rather complex and consists of the sinoatrial node (SA), atrioventricular node (AV), Purkinje fibres, and inervation from sympathetic and parasympathetic branches of the autonomic nervous system. Heart rate can be easily changed by a combination of influences: sympathetic nerves make the heart beat faster, while parasympathetic nerves relax the heart and slow it down (Marieb, 1998).

Figure 18. Inervation of heart from autonomic sympathetic and parasympathetic nervous system(adapted from Marieb (1998)



4.1.1.2 New methods in evaluation of heart rate variability

One of the challenges in the field of ECG measurement was to develop methods which will quantify the influence of the autonomic nervous system on heart rate and heart rate variability. New techniques are available that objectively evaluate changes in the heart rhythm. The most important method currently used in neurophysiological and psycho-physiological research is called power spectrum analysis of heart rate variability. It allows extraction from the tachogram of heart beat changes of two spectral components: a low frequency component (LF=0.05-0.15 Hz) that is strongly associated with heart rate changes due to the influence of the sympathetic branch of the autonomic nervous system, and the high frequency component (HF=0.15-0.45 Hz) that is associated with the parasympathetic influence (Kleiger, et al. 2005).

4.1.1.3 Correlations between HRV and disease states

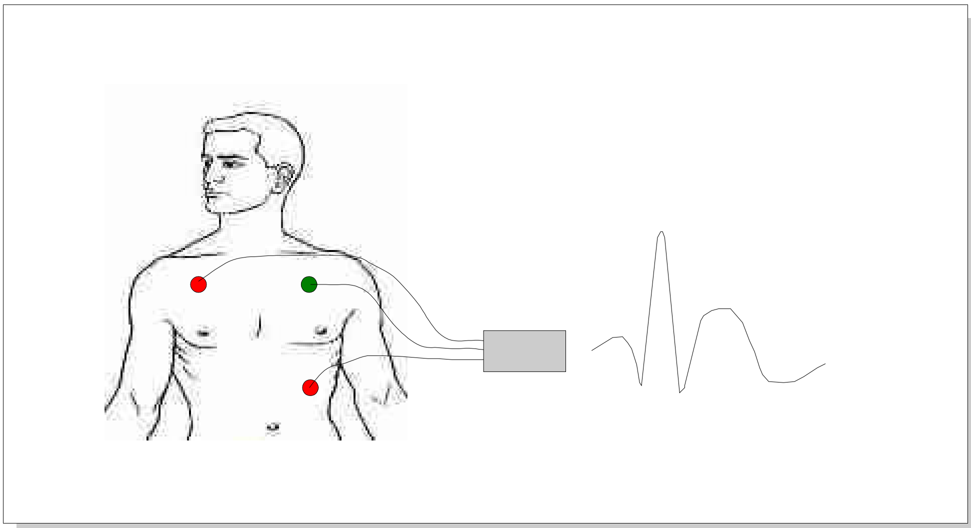
The correlation between changes in HRV and heart disease are well established. The American College of Cardiology (ACC) as far back as 1993 stated that "heart rate variability may offer information about sympathetic and parasympathetic autonomic function and could serve as a measure of risk stratification for serious cardiac arrhythmias and possible sudden cardiac death" (ACC, 1993 p.1). Correlations with other disease conditions were investigated in numerous studies and links were found with hyperglycemia (Schachinger, et al. 2004), depression (Carney, et al. 2000), obstructive sleep apnea (Gula, et al. 2003), and various aspects of psychological stress (Mazzacappa, et al. 2001).

Normal physiological states are also linked to changes in HRV, but exposure to various stressors seems to be very strong. This comes as no surprise because the heart is, after all, the center of human emotions. The meditative techniques (Peng, et al. 2004), prayer (Bernardi, et al. 2001) and even reflexology (Zhen, et al. 2004) are all linked to the changes in sympathetic and parasympathetic regulation of heart beat.

4.1.2 Methodology

A standard ECG recording procedure was adopted for all experimental and control sessions. Subjects were placed on the treatment table in the supine position. The Lead II ECG electrode positioning system was used with Ag/Ag-Cl electrodes placed directly on the skin. Skin was prepared by cleaning with alcohol swabs and without using any abrasive procedures. Figure 19 illustrates the essential aspects of the ECG recording.

Figure 19. ECG recording procedure with electrodes placed according to the Lead II



4.1.2.1 Acquisition of ECG

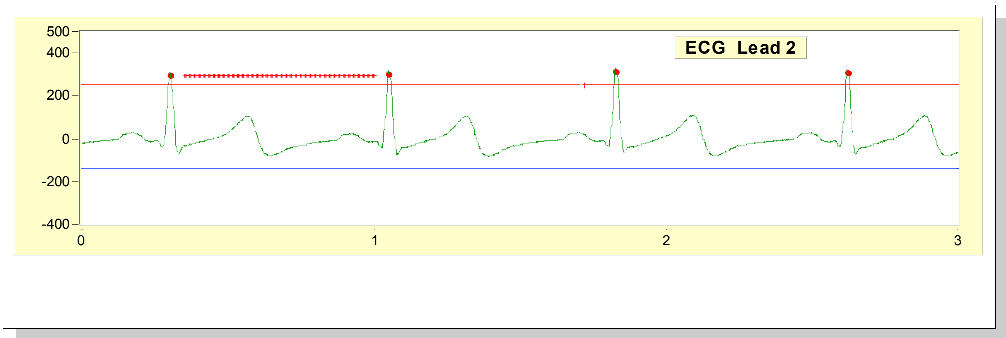
The electrical ECG signal was amplified and filtered using Bio2 amplifier (Precision Instruments, UK). Gain was set to x5000 and analog filters to low-pass=100Hz and high-pass=1Hz. The ECG signal was then digitized using 12-bit A/D converter (PCI-6028E, National Instruments, USA). Sampling rate for the digital converter was setup to 1000 samples per second which is the recommended rate for high resolution ECG recording.

The on-line recording and analysis of ECG signals was performed using custom built software. This software was created by the author of this research project. The LabView G programming language (LabView v.6.1, National Instruments, USA) was used to develop the *Virtual ECG Recorder* software which emulates on the computer screen all functions of the electronic ECG equipment.

The *Virtual ECG Recorder* allowed simultaneous recording and analysis of the ECG signal. It performed a series of general operations to control the experimental session in accordance with the requirements of the protocol described in Chapter 2. It also performed specific functions required for the analysis of heart rate (HR) and heart rate variability (HRV). All the essential steps of this analysis are described below.

The raw ECG signal, free of artifacts and noise, was first processed using the peak detector algorithm as presented in Figure 20. All "R" peaks in QRS complexes were marked and distances between consecutive peaks automatically calculated for all heart beats within a 20 minute period. The resolution in the measurement of R-R intervals was 1 msec.

Figure 20. The R-R interval between two consecutive QRS complexes were measured continuously.

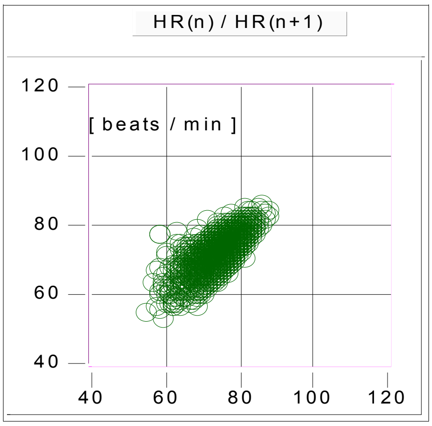


Each R-R interval represented the delay between two consecutive heart beats. For example intervals of 1 second duration would represent a heart rate of precisely 60 beats per minute. Shorter intervals represented heart rate higher than 60/min and longer intervals represented heart rate lower than 60/min.

4.1.2.2 Tachogram of Heart Rate and Heart Rate Variability

Naturally the healthy heart never beats with constantly the same rhythm. Heart beat is influenced by many physiological and psychological factors. This variability can be easily observed on the scatter plot when the value for one beat is plotted against value of the heart beat which follows as presented on Figure 21. The area covered by the multiple dots represents the majority of the heart beats occurring during specified time (e.g. 20 minutes). Single dots outside this area mark heart beats which are of different intervals: either a “short” beat followed by a “long” beat, or vice verse. Such visual examination quickly reveals that a small, tide area represents small variations from the baseline heart beat. The bigger area represents more “scattered” heart beats thus bigger variability.

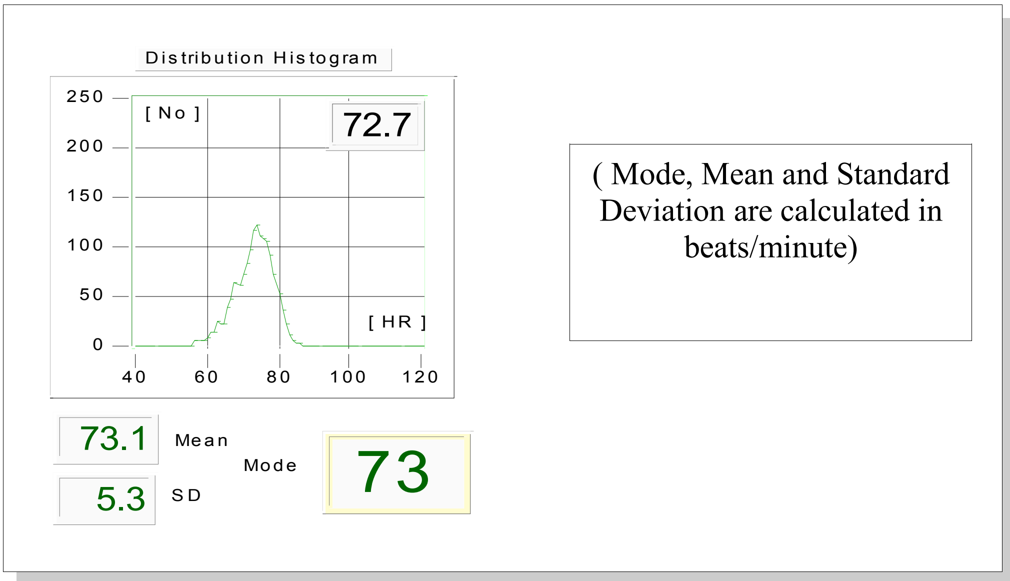
Figure 21. The scatter plot representing variability of heart beat



(each dot represents heart beat within 20 minutes of recording (Subject 021-T3)

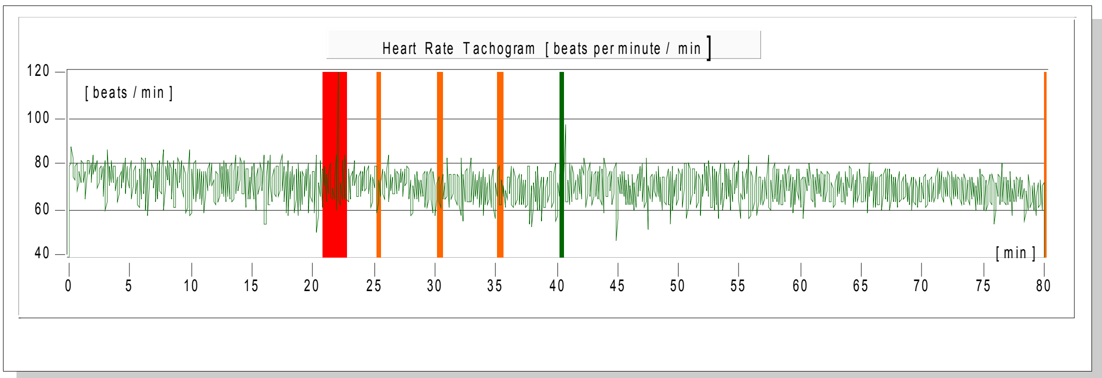
The distribution of individual heart beats was also evaluated by building the histogram as represented on Figure 22. Each R-R interval between two consecutive heart beats was converted to beats per minute value and then placed in the corresponding “bin” of 0.1 beats/min resolution. The vertical axis of the histogram represents the number of heart beats with corresponding beats/minute value. The statistical analysis of the distribution was performed on-line and values for Mode, Mean and Standard Deviation displayed on the screen and transferred into the data spreadsheet.

Figure 22. Histogram representing distribution of all heart beats within 20 minutes period



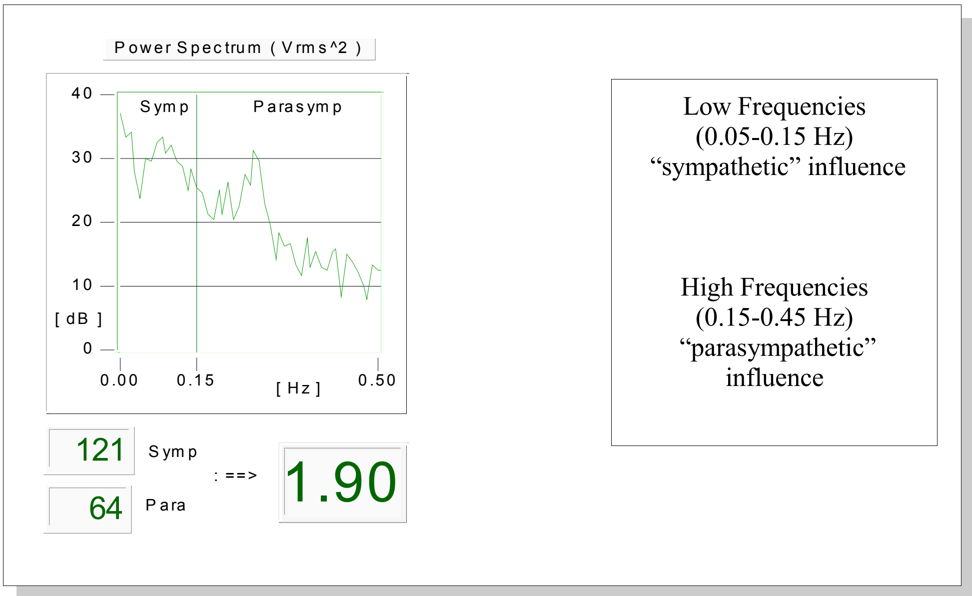
The tachogram is a graph representing changes in heart rate over a period of time. An interval between two consecutive heart beats was measured in msec and then converted into equivalent measure of beats per minute. Every time the new heart beat appeared, a new data point was added to the graph. Figure 23 shows tachogram constructed from all heart beats collected over the period of 80 minutes. The vertical lines mark the time when acupuncture needles were used. Color red indicates insertion of the needle(s), orange indicates time of stimulation and green marks the removal of the needles.

Figure 23. Tachogram representing changes in heart beat over a period of 80 minutes



One way of describing the power spectrum (PS) analysis as applied to heart rate variability is to compare the heart rate tachogram with a voice recording. If the waveform in the previous Figure 23 was representative of an acoustic signal, it could be filtered into component frequencies. In the case of the human voice these component frequencies will be spread in the range between 100 Hz to 6000 Hz. If similar filtering is applied to the signals generated by the brain, the range of the component frequencies will be say between 2 Hz to 30 Hz. Heart rate variability is very slow compared to acoustic signals or even brain signals. Its component frequencies spread over the range of 0.05 Hz to 0.45 Hz. Digital filtering techniques were used in on-line analysis of the heart rate variability. Well known algorithms of Fast Fourier Transforms (FFT) were used to calculate power spectrum of component frequencies. Figure 24 shows the spectrogram of heart rate variability in a narrow range of frequencies. The magnitude of low band and high band was calculated (values 121 and 64) together with ratio between these two measurements (value 1.90 in the example below)

Figure 24. Frequency spectrum representing heart rate variability over the period of 20 minutes



The ratio between the Low and High part of the spectrum was used to represent the relative balance between sympathetic and parasympathetic components in heart rate variability. This ratio is independent of the baseline levels of LF and HF components of frequency spectrum.

4.1.3 Results

Table 6 presents the dependent variables used in the analysis of heart function. All major parameters of heart rate and heart rate variability were analysed during the experimental sessions. The 25%-75% range and standard deviations are included in the table. The ratio between heart rate and respiration rate was calculated for each 20 minute interval after the experiments were completed.

Table 6 . Dependant variables used in the analysis of the heart function

Measurement	Description	25% - 75%	SD	Unit
HR (Mean)	Mean value of Heart Rate for each 20 minutes interval	54 - 68	9.1	BPM
HR (Mode)	Mode value of Heart Rate for each 20 minutes interval	52 - 67	9.6	BPM
HR (SD)	Standard Deviation of heart rate histogram of 20 min.	3.8 - 6.2	1.8	BPM
HRV (LF)	Low Frequency component of the HRV power spectrum	21 - 74	56	V/Hz2
HRV (HF)	High Frequency component of the HRV power spectrum	30 - 103	63	V/Hz2
HRV (Ratio)	Ratio between LF and HF of HRV power spectrum	0.46 - 1.17	0.7	n
HR/RES (Ratio)	Number of heart beats per one respiration	3.4 - 4.9	1.1	n

The analysis of the results was performed in three stages in accordance with the general methodology outlined earlier in Chapter 3. Each stage had a different hypothesis to resolve and a different capacity to answer the research questions. Table 7 highlights the main features of each stage.

Table 7. Three stages of data analysis for HR and HRV

Data analysis	Groups	Repeated measures	
Stage 1	T1-T2-T3 C1	none	30 + 30 + 30 30
Stage 2	C1 - T1	Intervals	30 + 30
	C1 - T2		30 + 30
	C1 - T3		30 + 30
Stage 3	T1-T2-T3	Intervals & Groups	30 + 30 + 30

Stage 1 of HR and HRV analysis was focused on factorial ANOVA with main factors being "group" and "interval" within the experimental sessions. All measurements were treated as independent measurements to reveal the main effect sizes for each variable.

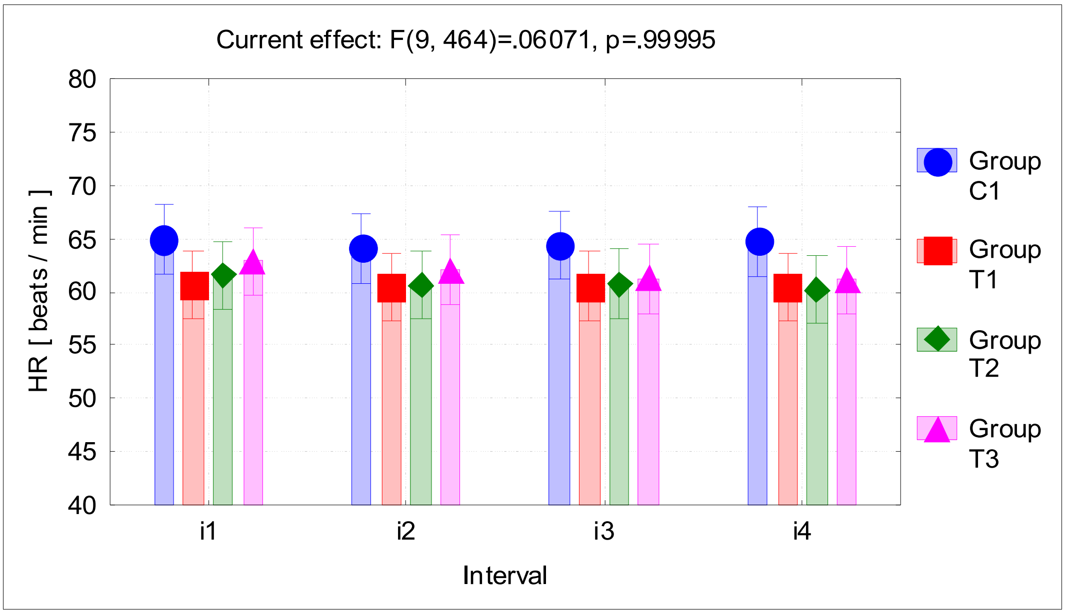
Stage 2 concentrated on individual comparisons between groups, that is each of the experimental groups T1 or T2 or T3 against the control group C1. The main purpose was to compare the "active" with non-active" influences on experimental subjects. It was expected that the control group would reveal how healthy subjects perform during 80 minute experimental sessions and how significant are the naturally occurring trends.

Stage 3 analysis focused only on the "active" groups that is T1 and T2 and T3, leaving the control group aside. It was assumed that placebo effects, if they exist in the study, would be significantly nullified. Only the differences between the influences of treatment protocols were taken into account. It was expected that stage 3 analysis would be the strongest in detecting significant differences in the action of individual acupuncture points.

4.1.3.1 Stage 1 Analysis: **Heart Rate**

The heart rate results for all groups (C1-T1-T2-T3) and all intervals (i1-i2-i3-i4) are shown in Figure 25. Each bar represents mean values for heart rate in 20 minute intervals. The range of values is typical for healthy subjects at rest. The heart rate appears to be stable in all groups during the 80 minute experimental session.

Figure 25. Heart Rate analysis using factorial ANOVA test.

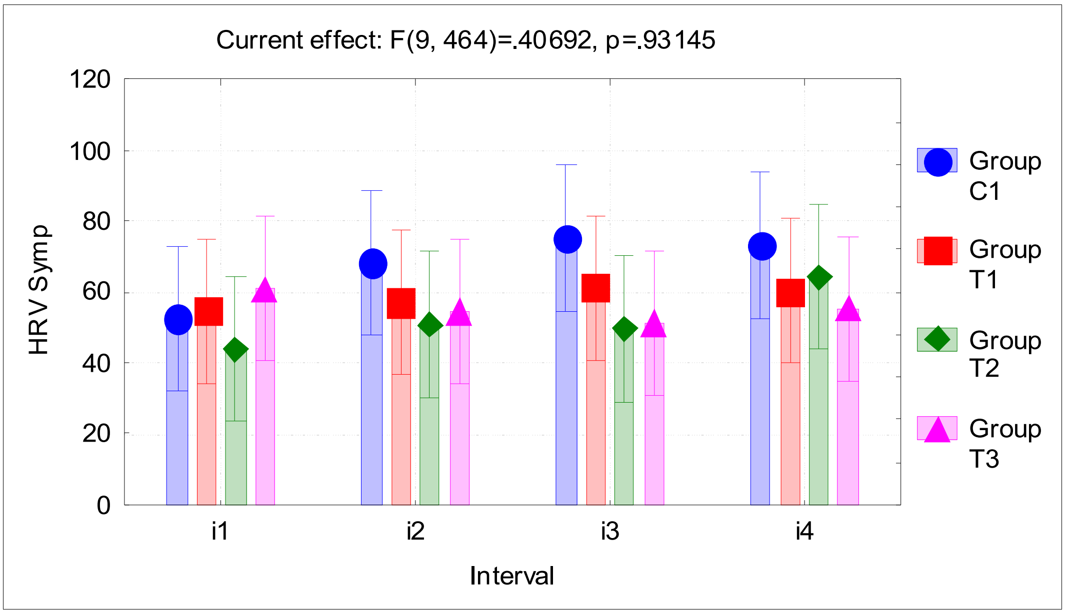


There are no statistically significant differences between heart rate in intervals and groups. There is however a consistent bias between the control group and all three treatment groups where the mean value for heart rate is lowered by 4-5 beats per minute, and remains lower throughout the course of the experimental session. That difference is not statistically significant but it points to the possibility of having some sort of psychological influence, possibly based on the subject's expectations. It is unlikely however that this bias is due to the anxiety response as this will tend to increase rather than decrease heart rate. Overall, no major changes in heart rate have been detected due to the acupuncture stimulation that was used in sessions T1, T2 and T3. The results confirm however that heart rate is a very stable physiological parameter not easily influenced by environmental and internal factors.

4.1.3.2 Stage 1 Analysis: **Heart Rate Variability (Sympathetic)**

The Heart Rate Variability (Sympathetic) results for all groups (C1-T1-T2-T3) and all intervals (i1-i2-i3-i4) are shown in Figure 26. Each bar represents the mean value for HRV in 20 minute intervals. The range of values is typical for a healthy subject at rest. The HRV sympathetic component shows fluctuations during the 80 minute session.

Figure 26. HRV (Sympathetic) analysis using factorial ANOVA test

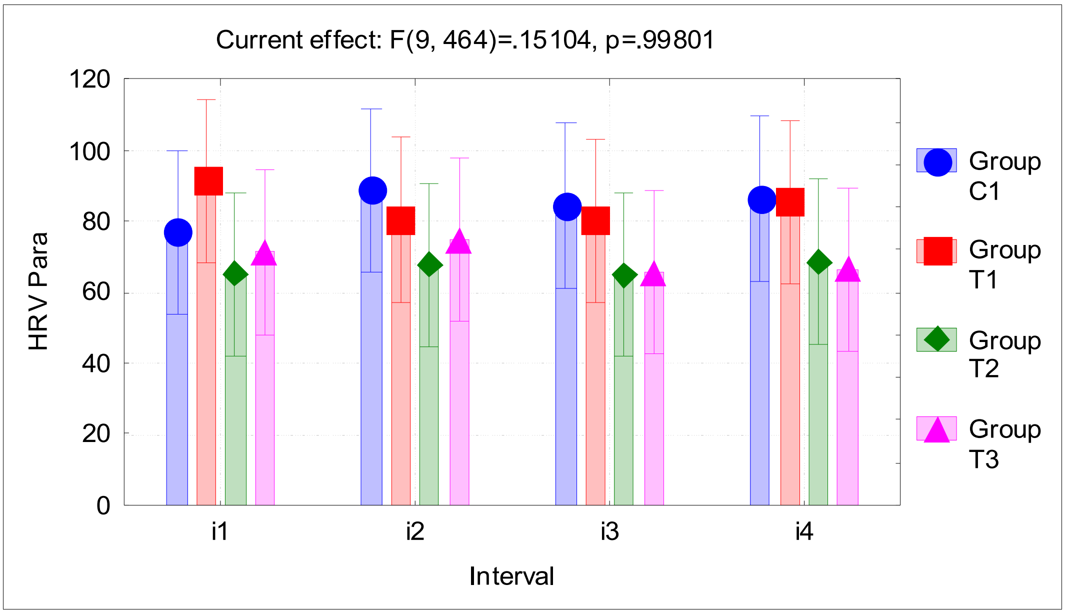


There are no statistically significant differences between the sympathetic component of HRV in intervals and groups. The mean values for the individual intervals show clear differences in trends between the control group and treatment groups. The control group remains somehow more 'sympathetic' which can be interpreted as being more alert or perhaps less relaxed during the experimental session. The differences in means for interval i1 in groups T1-T2-T3 (same subjects) may indicate that there are some other factors such as expectation anxiety that may influence heart rate variability other than individual differences. Overall, no major changes in the sympathetic component of heart rate variability have been detected due to the acupuncture stimulation that was used during interval i2 in sessions T1, T2 and T3. Heart rate variability seems to be a much less stable physiological parameter than heart rate alone.

4.1.3.3 Stage 1 Analysis: **Heart Rate Variability (Para-sympathetic)**

The Heart Rate Variability (Para-Sympathetic) results for all groups (C1-T1-T2-T3) and all intervals (i1-i2-i3-i4) are shown in Figure 27. Each bar represents the mean value for the HRV Para in 20 minute interval. The HRV para-sympathetic component shows strong fluctuations during the 80 minute experimental session.

Figure 27. HRV (Para-Sympathetic) analysis using factorial ANOVA



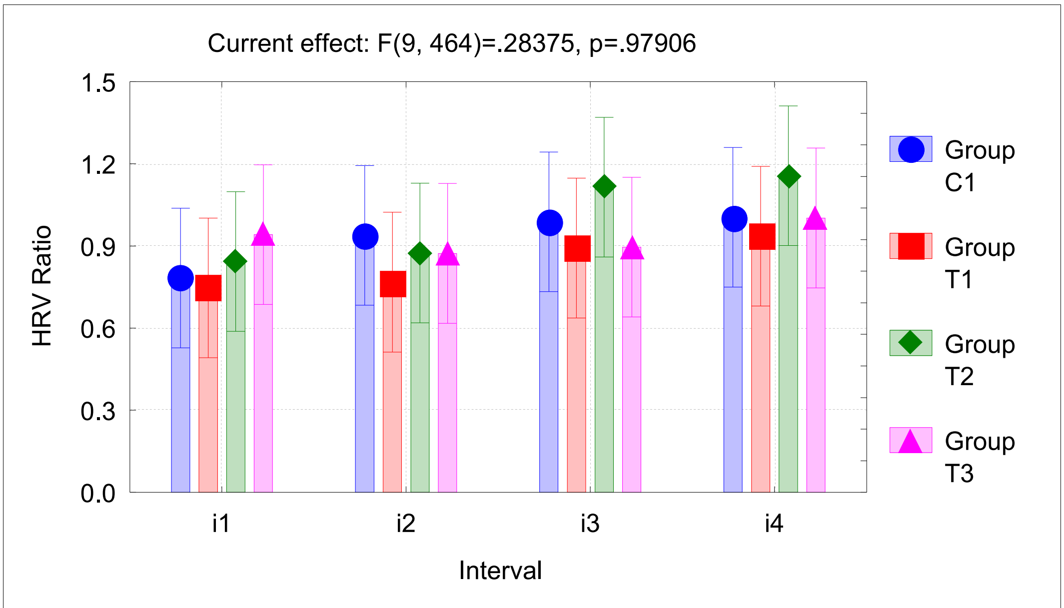
There are no statistically significant differences between the para-sympathetic component of HRV in intervals and groups. The mean values for the interval i1 (internal baseline) are between 65.2 to 91.4, with standard deviation between 53.4 to 74.0, which indicates that there are strong internal factors that influence the HRV para-sympathetic component.

Overall, no major changes in the para-sympathetic component of heart rate variability have been detected due to the acupuncture stimulation that was used during interval i2 in sessions T1, T2 and T3. Heart rate variability seems to be a much less stable physiological parameter than heart rate alone, and in comparison the para-sympathetic component is less stable than the sympathetic component.

4.1.3.4 Stage 1 Analysis: **Heart Rate Variability (Ratio)**

The Heart Rate Variability (Ratio) results for all groups (C1-T1-T2-T3) and all intervals (i1-i2-i3-i4) are shown in Figure 28. Each bar represents the mean value for the HRV Ratio in 20 minute intervals. The HRV Ratio shows fluctuations during the 80 minute experimental session.

Figure 28. HRV (Ratio) analysis using factorial ANOVA



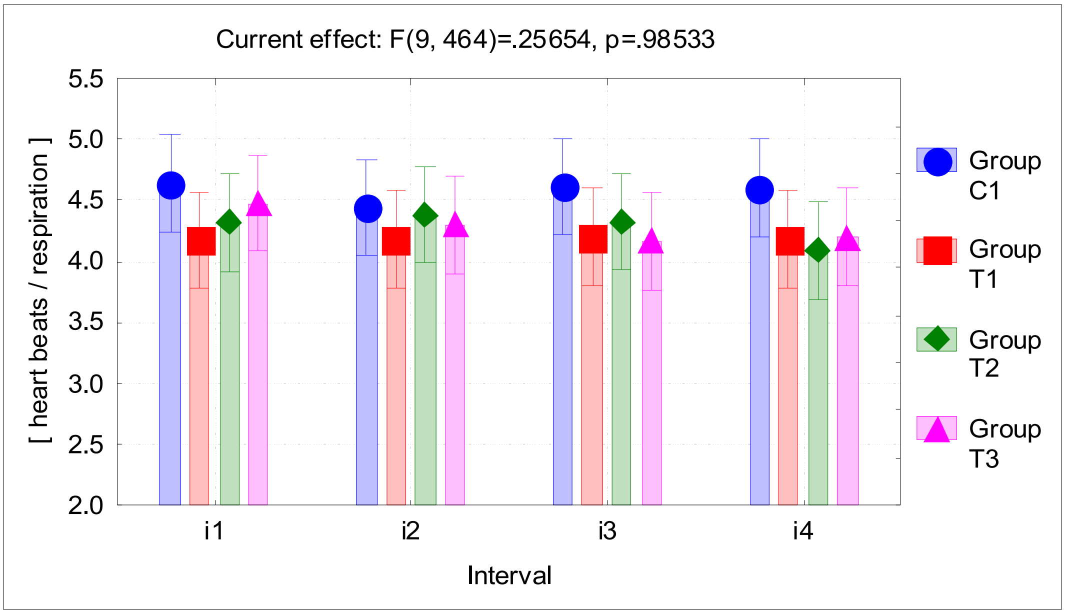
There are no statistically significant differences between HRV Ratio in intervals and groups. HRV Ratio represents the balance between sympathetic and para-sympathetic components of HRV. Results indicate that there is a strong trend in the T2 group for the HRV Ratio to increase after the acupuncture treatment in intervals i3 (40-60 min) and i4 (60-80 min).

Overall, no major changes in the HRV Ratio have been detected due to the acupuncture stimulation that was used in sessions T1, T2 and T3. The results confirm however that the HRV Ratio is a stable physiological parameter, much more stable than the sympathetic and parasympathetic components alone.

4.1.3.5 Stage 1 Analysis: **Heart Beats per one Respiration**

The Heart Beats per respiration results for all groups (C1-T1-T2-T3) and all intervals (i1-i2-i3-i4) are shown in Figure 29. Each bar represents the mean value for the average number of heart beats in each respiratory cycle. This ratio between HR and Respiration rate shows minor fluctuations during the 80 minute experimental session.

Figure 29. Heart Beats per Respiration analysis using factorial ANOVA



There are no statistically significant differences between the number of heart beats per one respiration in intervals and groups. There is however a consistent bias between the control group and all three treatment groups where the control mean value is higher by about 5-10 %. That bias is not statistically significant but it highlights the possibility of some psychological influence, possibly based on the subject's expectations regarding what is going to happen during the experimental session, e.g. acupuncture vs. no-acupuncture. Overall, no major changes in the number of heart beats per one respiration have been detected due to the acupuncture stimulation that was used in sessions T1, T2 and T3. The results confirm however that this ratio between heart and respiration rate is a reasonably stable physiological parameter not easily influenced by environmental and internal factors.

The overall results for Stage 1 factorial analysis is presented in Table 8. Both the mean values and standard deviations are presented for each variable according to group and interval allocation. These values will be used later on in the discussion regarding required samples sizes and calculations of the power of experiment in acupuncture research.

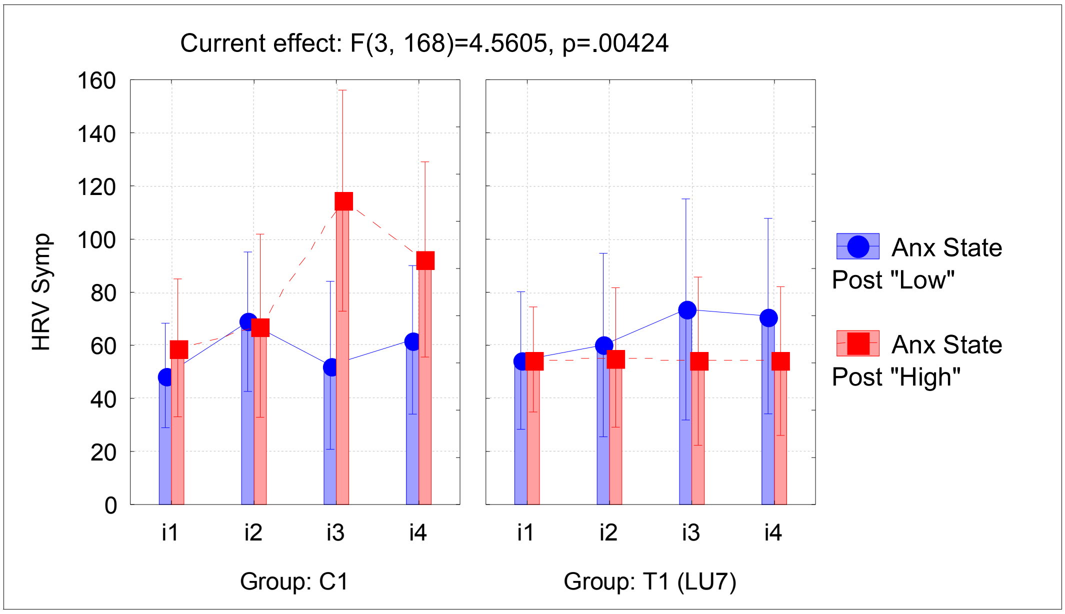
Table 8. Mean values and SD for all groups and all intervals of variables used in evaluation of the heart activity.

	Interval i1		Interval i2		Interval i3		Interval i4		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
C1	65.0	10.3	64.1	10.3	64.4	9.9	64.8	9.9	Heart Rate
T1	60.7	6.7	60.5	7.5	60.6	6.8	60.4	7.1	
T2	61.6	9.5	60.6	8.6	60.8	8.7	60.2	8.8	
T3	62.9	10.1	62.0	9.8	61.3	9.4	61.1	9.8	
C1	52.5	38.4	68.3	56.6	75.2	77.0	73.2	50.9	HRV Symp
T1	54.5	45.9	57.1	55.7	61.1	65.3	60.3	69.7	
T2	43.8	49.4	50.8	52.6	49.6	46.8	64.2	65.1	
T3	60.8	63.3	54.4	59.0	51.2	54.0	55.3	50.7	
C1	77.0	53.4	88.8	81.0	84.4	72.4	86.3	71.5	HRV Para
T1	91.4	74.0	80.5	60.7	80.3	62.7	85.4	80.3	
T2	65.2	53.4	67.8	60.7	64.9	61.4	68.6	53.3	
T3	71.3	50.7	74.6	63.8	65.6	59.1	66.4	60.2	
C1	0.78	0.46	0.94	0.62	0.99	0.69	1.00	0.58	Ratio
T1	0.75	0.44	0.77	0.42	0.89	0.86	0.94	0.66	
T2	0.84	1.01	0.87	0.61	1.11	1.10	1.16	1.06	
T3	0.94	0.73	0.87	0.57	0.90	0.44	1.00	0.60	
C1	4.64	1.21	4.44	0.88	4.61	1.12	4.60	0.98	HR / RESP
T1	4.18	0.87	4.18	0.85	4.20	0.89	4.18	0.92	
T2	4.32	1.18	4.38	1.52	4.32	1.18	4.09	0.82	
T3	4.48	1.53	4.30	1.34	4.17	1.09	4.20	1.06	

4.1.3.6 Stage 2 analysis: **Heart Rate Variability (sympathetic)**

Figure 30 presents the results of a comparative analysis between the control group C1 and individual treatment groups. The measurements for intervals i1, i2, i3, i4 are treated as repeated measures ("within" group factor). Each bar represents the HRV Sympathetic component for two sub-groups of subjects, those showing lower (blue) and higher (red) levels of state anxiety at the end of the experiment.

Figure 30. HRV Symp in C1-T1 comparison at Anx State Post 0/1 levels (statistically significant differences at p=0.004)

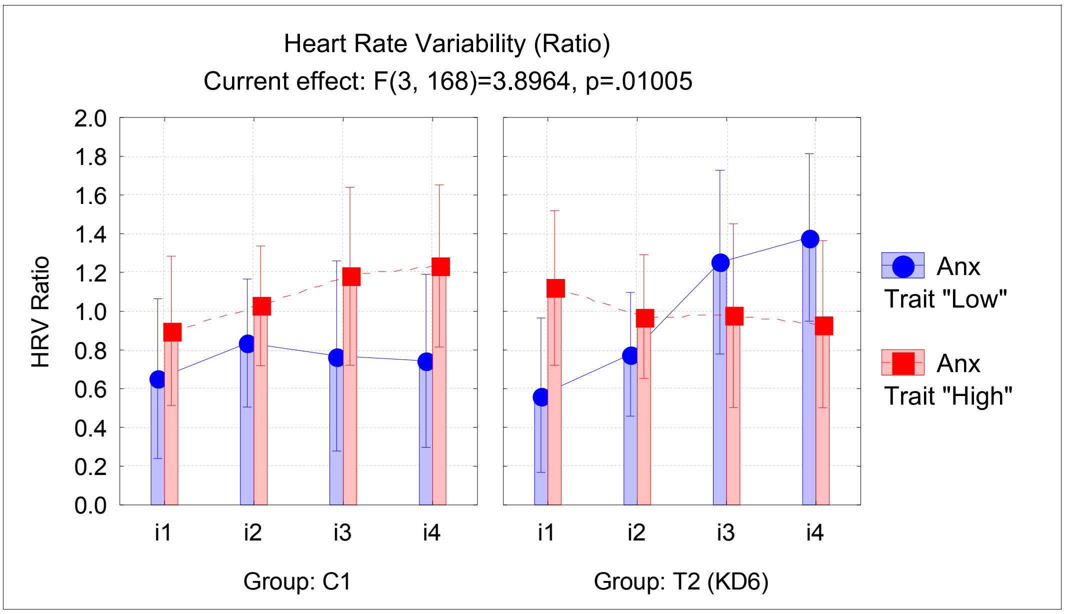


There is a statistically significant difference between the control group C1 and treatment group T1 (LU7). In the control group the higher anxiety level is associated with a significant increase in the HRV Sympathetic component. In the treatment group however, despite reporting higher levels of state anxiety subjects do not show increased HRV in the sympathetic component. Such a finding can be interpreted as a presence of cognitive anxiety without accompanying physiological response from the sympathetic branch of the autonomic nervous system. Similar results were obtained when data for group T2 and T3 was analysed, suggesting that acupuncture may have an important harmonizing influence on heart activity.

4.1.3.7 Stage 2 analysis: **Heart Rate Variability (Ratio)**

An example of the harmonizing effect of treatment received by group T2 is presented in Figure 31 in the form of a comparative analysis between the control group C1 and treatment group T2. The measurements for intervals i1, i2, i3, i4 are treated as repeated measures ("within" group factor). Each bar represents the HRV Ratio for two sub-groups of subjects, those showing lower (blue) and higher (red) levels of trait anxiety.

Figure 31. HRV Ratio C1-T2 comparison at Anx Trait 0/1 levels (statistically significant differences at p=0.01)



There are statistically significant differences between control C1 and the treatment group T2. Those subjects in the treatment group that reported lower levels of trait anxiety at the beginning of the experiment show a very strong increase in the HRV Ratio. Subjects in the control group do not show similar changes.

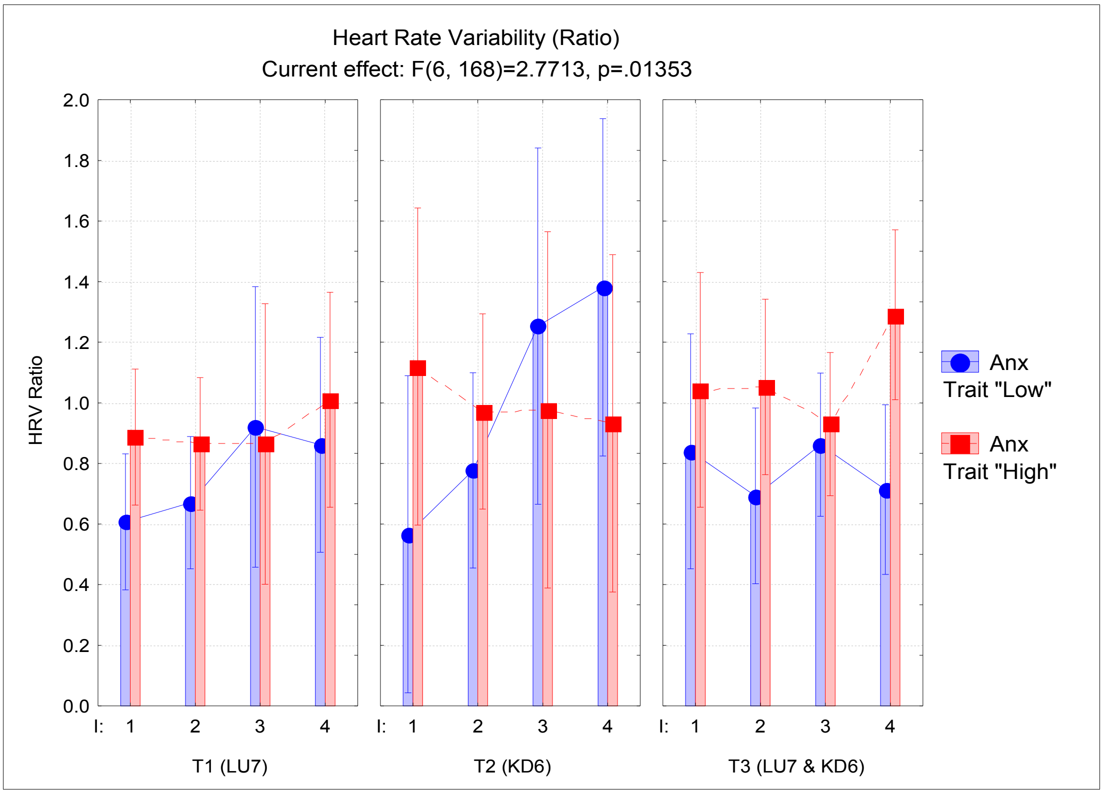
The results could be interpreted as a regulatory and balancing effect of KD6 acupuncture point. By increasing the sympathetic influence upon the heart, the KD6 acupoint may have an energizing or invigorating effect on the heart rhythm. Interestingly, subjects in group T1 (LU7 acupoint) and T3 (LU7 and KD6) did not show similar changes.

4.1.3.8 Stage 3 analysis: **Heart Rate Variability (Ratio)**

In order to capture smaller variations in dependent variables, data in Stage 3 was analysed using two level repeated measures MANOVA. The variability of the control group was thus removed from the analysis but variability between the treatment sessions was fully taken into account. Also additional factors were used such as Trait Anxiety.

Figure 32 shows measurements for intervals i1, i2, i3, i4 that are treated as repeated measures ("within" group factor). Each bar represents HRV Ratio for two sub-groups of subjects, those showing lower (blue) and higher (red) levels of trait anxiety.

Figure 32. HRV Ratio T1-T2-T3 comparison at Anx Trait 0/1 levels (statistically significant differences at p=0.01)



These findings suggest that only very relaxed subjects (low anxiety) are influenced by stimulation of KD6 acupoint. Stimulation of acupuncture point LU7 or KD6 combined with LU7 do not show such effects. The statistically significant change in the HRV

Ratio can be interpreted as an increase in the influence from the sympathetic branch of the autonomic nervous system. In terms of TCM it can be considered as a stimulating effect and confirms the general observation that subjects became less restless when the single KD6 acupoint alone is stimulated. The stimulating effect is delayed by about 20 minutes and does not manifest during the time when the needle is inserted and manually manipulated, but rather after the needle is removed.

4.1.4 **Conclusions.**

As expected, there were no statistically significant differences in variables related to heart rhythm in Stage 1 analysis. When variables for each interval are treated independently and the number of subjects in the groups are small, only very large changes could be detected as significant. Data from the experiments clearly show that stimulation of two acupuncture points LU7 and KD6 do not influence heart beat in such a strong way.

However, in Stage 2 analysis when intervals are treated as repeated measures, that is belonging to the same subject, and when additional factorial variables are introduced, methodology is sensitive enough to detect statistically significant differences. The level of anxiety seems to have the most significance here. Subjects reporting an increased state of anxiety after the experiments showed no increased sympathetic physiological response from the heart despite such a response in the control group.

In Stage 3 analysis the external influencing factors are reduced to a minimum and instead of comparing the treatment group with the control, comparisons are made only between treatments. Results show that the clear treatment effect exists only in group T2 (acupoint KD6). The increase in the sympathetic influence in subjects with reported low trait anxiety is very interesting, and may indicate that KD6 acupuncture point has a balancing effect on heart rhythm. When the HRV Ratio is low the acupuncture stimulation of KD6 seems to increase it, but only when the anxiety is low.

4.2 Lung respiratory rate and amplitude

The respiration rate of a healthy human ranges from 8-20 per minute. It can change very rapidly due to an increase demand for oxygen, but can also change in response to emotions such as fear or excitement. Respiration is also under voluntary control and can be changed at will. This mixture of automatic and voluntary control of respiration creates certain obstacles in the measurement process as it is difficult to distinguish these two components. The mechanical movement of the chest is augmented in respiration by movement of diaphragm and abdominal muscles. Dynamic changes between chest respiration and abdominal movement is often determined by other factors such as physical positioning of the body, relaxation versus physical effort or the general state of health.

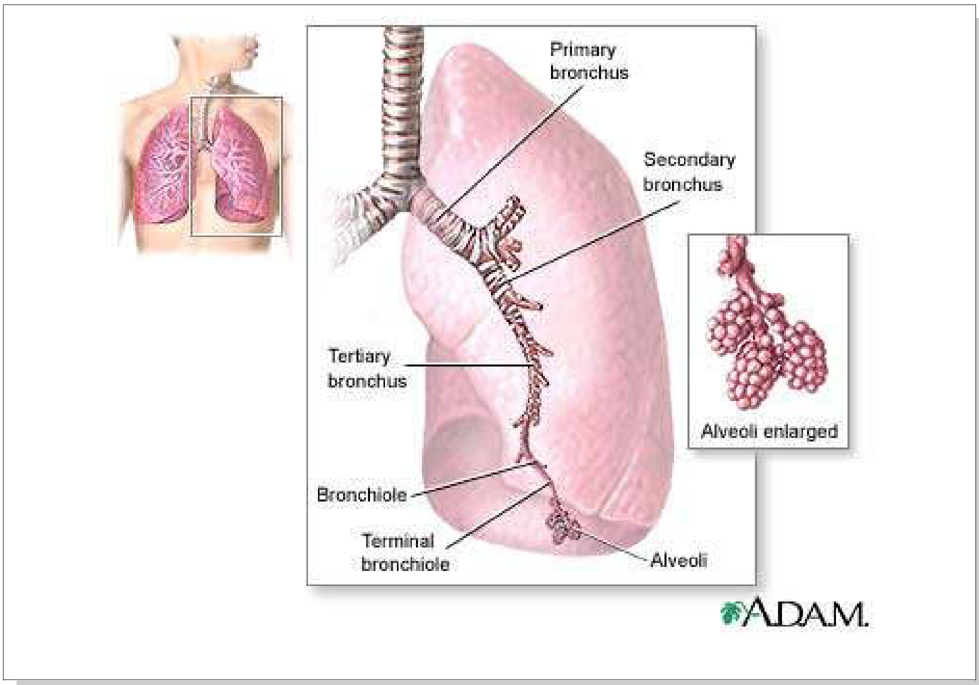
4.2.1 Introduction

Respiratory movements can be measured using a variety of techniques. The most accurate are methods that employ sensors monitoring changes in circumsphere of the chest or abdomen. Changes in physical dimensions of the chest closely correspond to inhalation and exhalation of air. By examining the recorded waveforms it is possible to evaluate the frequency and the amplitude of the respiratory effort.

4.2.1.1 Physiological importance of the respiratory rate

Regular inhalation and exhalation from the lungs plays a critical role in maintaining not only good health but also securing life itself. Oxygen is the most important gas because it keeps the body alive. An average person can not survive without oxygen for more than 2-3 minutes. The exhalation of carbon dioxide, a by-product of cell respiration also has essential importance in health physiology (Marieb, 1998). An accumulation of carbon dioxide leads to respiratory acidosis which is a disturbance of the acid-base balance. In consequence the body fluids become excessively acidic. Figure 33 illustrates the main anatomical structures of the lungs.

Figure 33 . Structure of the lungs (adopted from MedlinePlus)



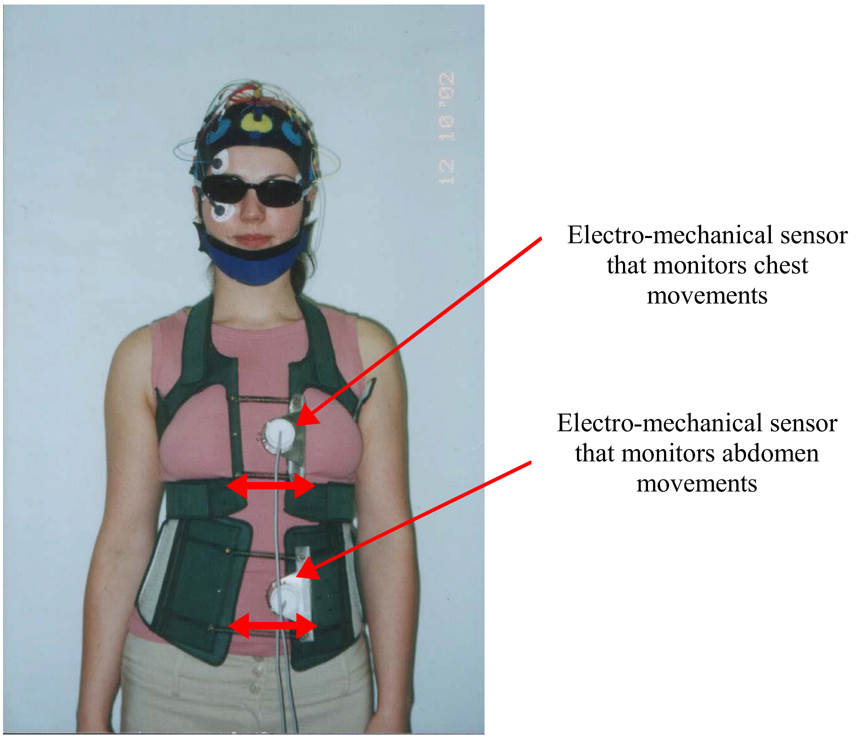
4.2.1.2 Correlations between respiratory physiology and disease states

Almost all physiological processes depend in some way on proper oxygenation of blood and removal of waste products. In this respect the lungs can be classified as one of the most important organs of the body (Marieb, 1998). Respiratory acidosis and respiratory alkalosis are the conditions emerging from the disturbance of the respiration pattern. Hyperventilation, that is fast and deep breathing, is usually associated with fear and panic attacks, but rapid and shallow breathing should also be considered as a medical emergency. Respiratory stress can be caused by a variety of conditions such as asthma, chronic obstructive pulmonary disease, pneumonia or pulmonary embolism. Traditional Chinese medicine regards the lungs as the primary organ where *Qi* starts its journey through the body. Disturbances of Lung *Qi* is recognized as either the cause or contributing factor in a much broader range of diseases than in western medicine. Symptoms such as excessive sweating, sinusitis, or blood stasis are all related to the physiology of the lungs.

4.2.2 Methodology

Custom made sensors were used in the measurement of respiratory movements for this study. Three different size harnesses were designed and manufactured so that subjects could wear a harness of appropriate size for their body frame. Tension of the springs was carefully adjusted to secure maximum comfort during the experiment. Figure 34 illustrates how the harness was placed and adjusted for each subject. The procedure allowed for complete freedom of movement and no restrictions were placed on how the subject should breath during the experiments. The ability of the sensors to measure the direct current allowed monitoring of even prolonged periods of apnea, which is normally not possible with popular piezoelectric sensors.

Figure 34. Respiration recording procedure with sensors being placed around chest and abdomen.



4.2.2.1 Monitoring respiratory movements in healthy subjects

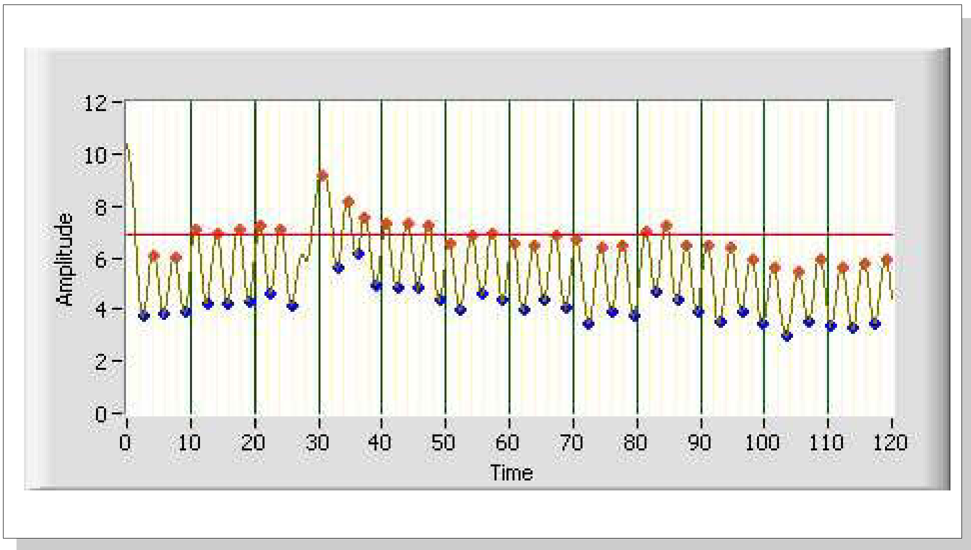
During rest the respiration can slow down significantly (van den Aardweg and Karemaker, 1991). The regularity and amplitude of inhalation and exhalation is normally reduced when compared to the standing position or active exercise. Changes in the circumference of the chest or abdomen can be as small as 1-3 mm when sensors are placed on the top of the clothing. In the experiment described in this thesis the custom made electromechanical sensors were designed by the author and manufactured in the Faculty of Science mechanical workshop. The linear movements of the plastic string were transferred into rotational movement of the electronic sensor. Electrical signals were than amplified and filtered before entering the analog-to-digital converters. Signals representing respiration movements were then digitized using a 12-bit A/D converter (PCI-6028E, National Instruments, USA). Sampling rate for the digital converter was set to 200 samples per second and digital filtering was used to remove noise associated with electromagnetic interference.

4.2.2.2 Peak-to peak and peak-to-valley analysis of respiratory signals

The on-line recording and analysis of respiration movement was performed using custom built software. This software was created by the author of this research project. The LabView G programming language (LabView v.6.1, National Instruments, USA) was used to develop the *Virtual RESP Recorder* and *RESP Analyser* software which emulate on the computer screen all functions of the electronic equipment.

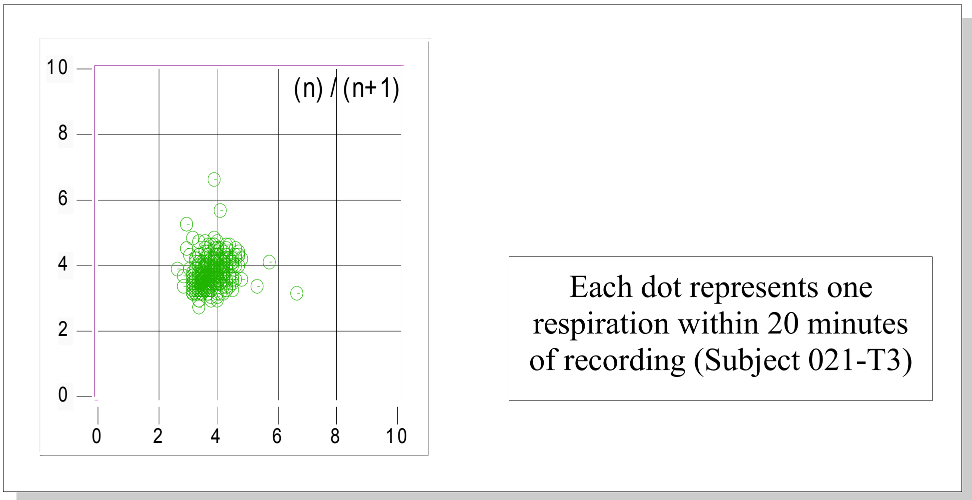
The *Virtual RESP Recorder* allowed for recording the electronic signals generated by electromechanical sensors. The timing of this software was linked with the *Virtual ECG Recorder* that performed a series of general operations to control the experimental session in accordance with the requirements of the protocol described in Chapter 2. The analysis of the respiration rate and respiration amplitudes was based on the measurement of peaks and valleys in the respiration signal. Figure 35 illustrates how the maximum and minimum amplitudes were automatically marked by the software. However some points had unresolved ambiguities that had to be reviewed manually as shown by the signal at around 28 seconds on Figure 35.

Figure 35. The peak-to peak intervals between two consecutive repiration movements were measured continuously



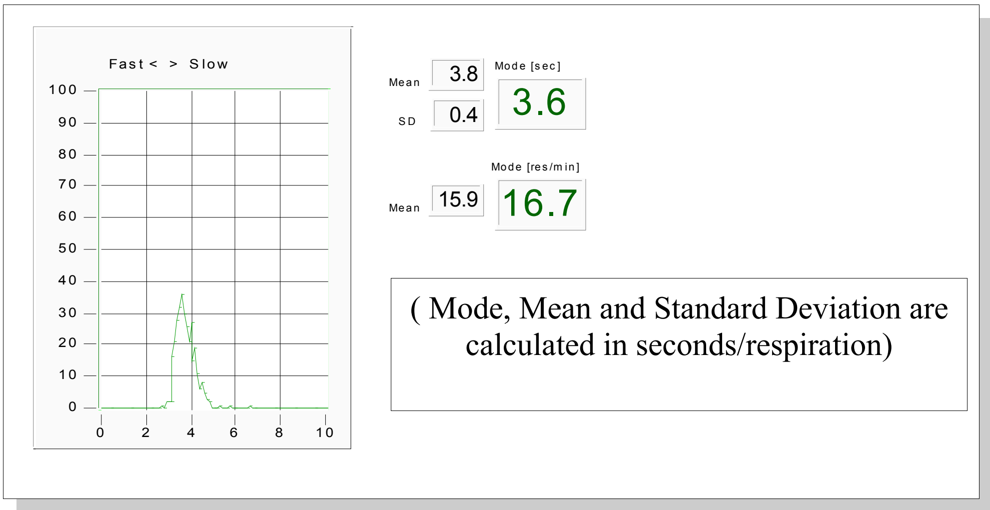
The irregularity of the respiration rhythm can best be viewed on the scatter plot where each dot represents one full respiratory cycle, as illustrated by Figure 36. A short respiration time is sometimes followed by a long respiration time or vice versa, but on average the time of one cycle is about 4 seconds. That corresponds to around 15 respirations per minute.

Figure 36. The scatter plot representing variability of time required for one respiration



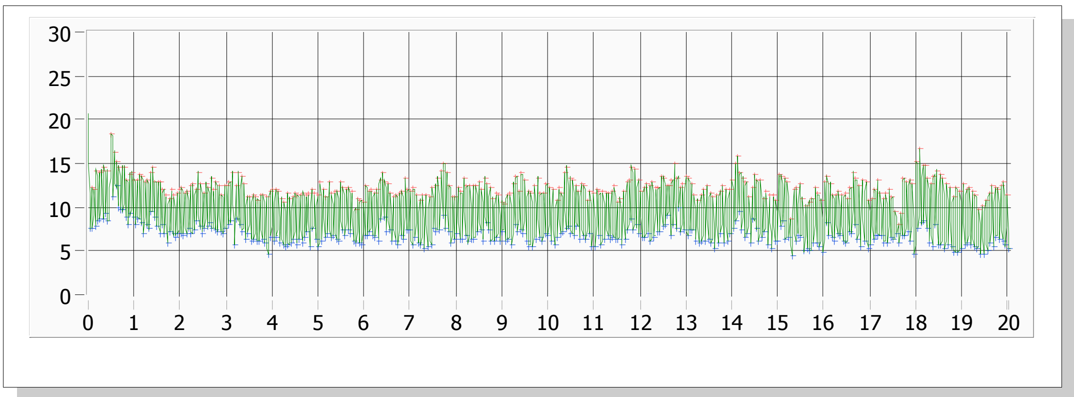
The distribution of respiratory cycles can also be viewed in the histogram. The exact values for mean, mode and standard deviation were calculated for each of the 20 minute intervals as presented on Figure 37.

Figure 37. Histogram representing distribution of respiration movements within 20 minutes period



The maximum and minimum amplitudes for chest respiratory movements are presented in Figure 38. The mean value and histograms for differences between peaks and valleys of corresponding respiratory cycles were calculated for each 20 minute interval. The analysis of amplitudes was similar to the analysis of time per each cycle.

Figure 38. Tachogram representing changes in amplitude of chest respiration



4.2.3 Results

The dependent variables used in the analysis of frequency and amplitudes of respiration are presented in Table 9. All major parameters of respiration rate and respiratory movement were computed after the experimental sessions ended. The 25%-75% range and standard deviations are included in the table.

Table 9 . Dependant variables used in the analysis of the respiration

Measurement	Description	25 – 75 %	SD	Unit
Resp dT (Mean)	Mean value of respiration cycle for each 20 min interval	3.6 – 4.5	0.94	second
Resp dT (Mode)	Mode value of respiration cycle for each 20 min interval	3.4 – 4.2	0.81	second
Resp dT (SD)	Standard deviation of respiration cycle histogram	0.5 – 1.3	0.87	second
Resp dA (Mean)	Mean value of respiration amplitude for 20 min interval	1.7 – 5.0	2.37	mm
Resp dA (Mode)	Mode value of respiration amplitude for 20 min interval	1.2 – 4.4	2.24	mm
Resp dA (SD)	Standard deviation of amplitude histogram	0.7 – 2.8	1.64	mm
Resp Freq (Mean)	Mean value of respiration frequency for 20 min interval	13.2 – 16.5	2.71	Rep / min

The analysis of the results was performed in three stages in accordance with the general methodology outlined earlier in Chapter 3. Each stage had a different hypothesis to resolve and a different capacity to answer the research questions. Table 10 highlights the main features of each stage.

Table 10. Three stages of data analysis for the frequency and amplitude of respiratory movements

Data analysis	Groups	Repeated measures	
Stage 1	T1-T2-T3 C1	none	30 + 30 + 30 30
Stage 2	C1 - T1	Intervals	30 + 30
	C1 - T2		30 + 30
	C1 - T3		30 + 30
Stage 3	T1-T2-T3	Intervals & Groups	30 + 30 + 30

Stage 1 of respiration analysis was focused on factorial ANOVA with main factors being “group” and “interval” within the experimental sessions. All measurements were treated as an independent measurement to reveal the main effect sizes for each variable.

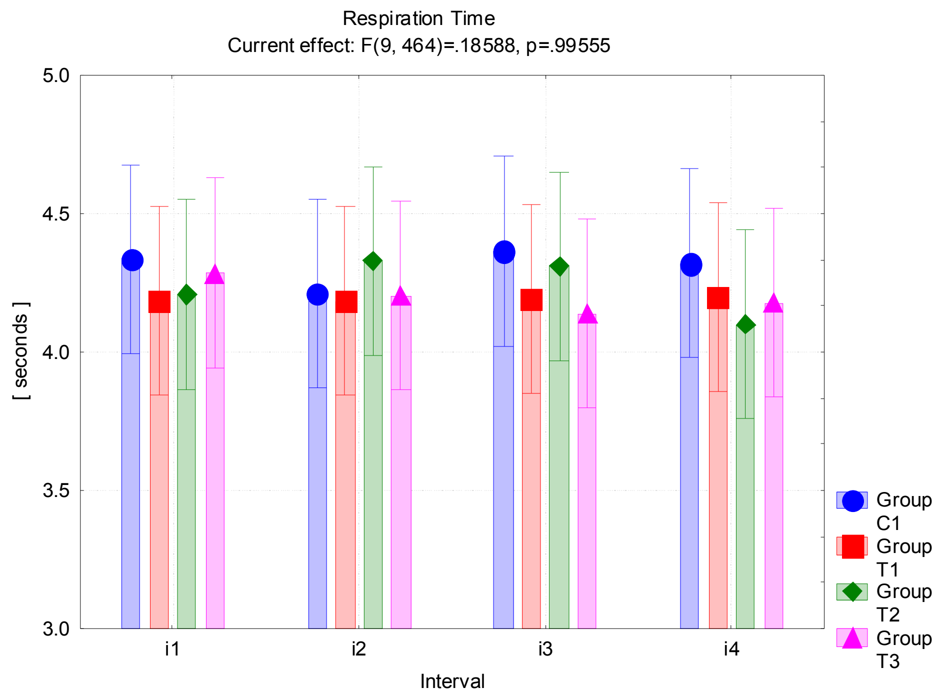
Stage 2 concentrated on individual comparisons between groups, that is each of the experimental groups T1 or T2 or T3 against the control group C1. The main purpose was to compare the “active” with 'non-active” influences on experimental subjects. It was expected that the control group may reveal how healthy subjects respond to the environment and procedures of the experimental laboratory during 80 minute experimental sessions and how significant are the naturally occurring trends.

Stage 3 analysis focused only on the “active” groups, that is T1 and T2 and T3, leaving the control group aside. It was assumed that placebo effects, if they exist in the study, would be significantly nullified. Only the differences between the influences of treatment protocols were taken into account. It was expected that stage 3 analysis would be the strongest in detecting significant differences in the action of individual acupuncture points.

4.2.3.1 Stage 1 Analysis: **Respiration time**

The results representing the respiration time for all groups (C1-T1-T2-T3) and all intervals (i1-i2-i3-i4) are shown in Figure 39. Each bar represents the mean value of the time required for one respiration cycle in a 20 minute interval. The range of values is typical for healthy subjects at rest. The time required for one respiration is stable in all groups during the 80 minute experimental session.

Figure 39. Respiration time dt in all groups and all intervals shows no statistically significant differences

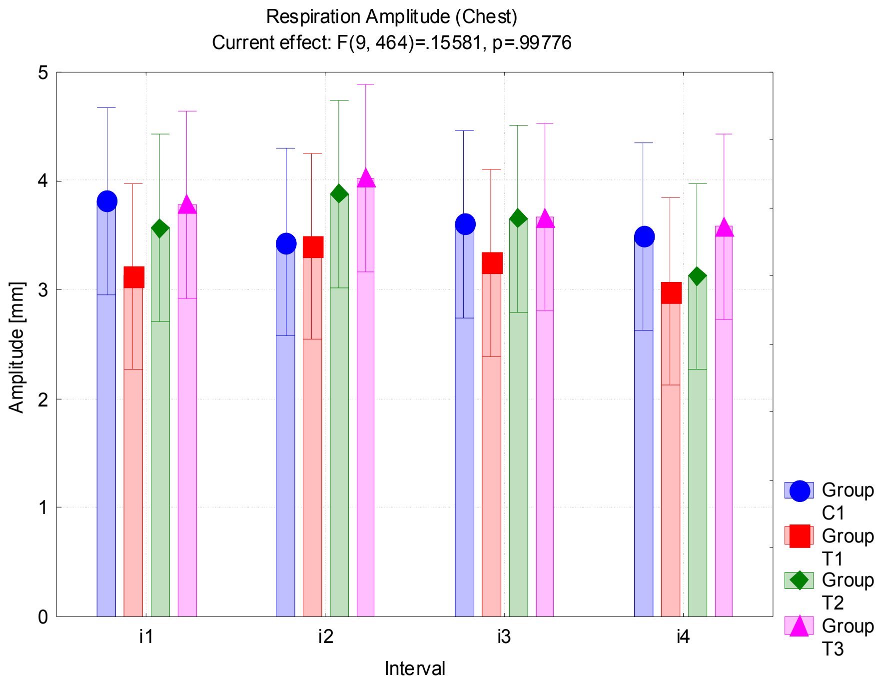


The analysis of the data revealed that there are no statistically significant differences between intervals and groups in the time required for one respiration cycle. There is a certain bias in group C1 intervals i1, i3 and i4 indicating that the respiration was slower than in the treatment groups, however differences were not statistically significant. The respiration time appears to be a stable physiological parameter not easily influenced by internal or environmental factors. Overall no major changes were detected in respiration time due to acupuncture stimulation in sessions T1, T2 and T3.

4.2.3.2 Stage 1 Analysis: **Respiration Amplitude**

The amplitude of the chest respiration movement for all groups (C1-T1-T2-T3) and all intervals (i1-i2-i3-i4) are shown in Figure 40. Each bar represents the mean value of displacement between maximum and minimum expansion of the chest for one respiration cycle in a 20 minute interval. The range of values is typical for healthy subjects at rest. The amplitudes show substantial variability in all groups during the 80 minute experimental session indicating the naturally occurring phenomenon of both shallow and deep breathing patterns.

Figure 40. Respiration dAmplitude in all groups and all intervals show no statistically significant differences

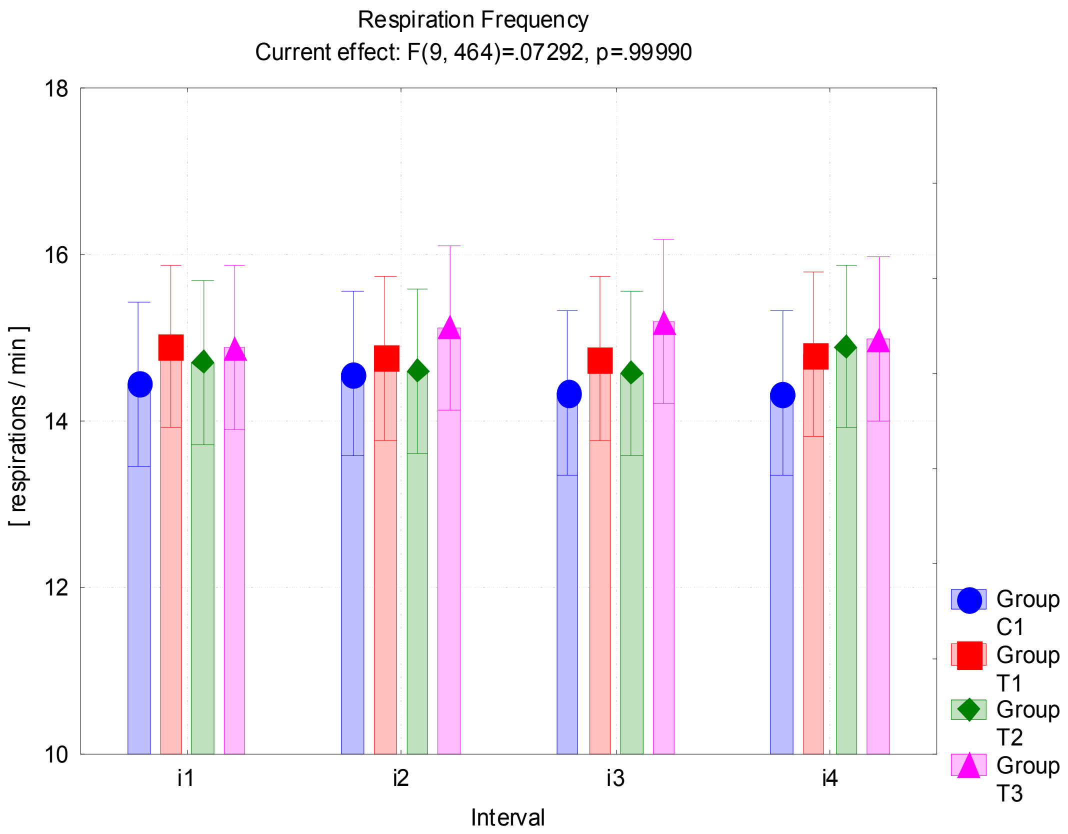


There were no statistically significant differences between amplitudes of respiration in different groups and intervals. The lower values for the group T1 in interval i1 may indicate some sort of bias due to psychological factors. Overall, no major changes have been detected due to the acupuncture stimulation that was used during interval i2 in sessions T1, T2 and T3. Amplitude of respiration seems to be a much less stable physiological parameter than respiration time.

4.2.3.3 Stage 1 Analysis: **Respiration Frequency**

The results representing the respiration frequency for all groups (C1-T1-T2-T3) and all intervals (i1-i2-i3-i4) are shown in Figure 41. Each bar represents a mean value of respiration frequency calculated by adding all respiration movements occurring during the interval and then dividing it by 20 minutes. The range of values is typical for healthy subjects at rest.

Figure 41. Respiration Frequency in all groups and all intervals shows no statistically significant differences



The analysis of the data revealed that there were no statistically significant differences between intervals and groups. The respiration frequency appears to be a stable physiological parameter not easily influenced by internal or environmental factors. Overall no major changes were detected in respiration frequency due to the acupuncture stimulation in sessions T1, T2 and T3.

The overall results for Stage 1 factorial analysis are presented in Table 11. Both the mean values and standard deviations are presented for each variable according to group and interval allocation. These values will be used later in a discussion regarding required samples sizes and calculations of the power of experiments in acupuncture research.

Table 11. Mean values and SD for all groups and all intervals of variables used in evaluation of the respiratory activity.

	Interval i1		Interval i1		Interval i1		Interval i1		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
C1	4.34	1.05	4.21	0.66	4.36	1.01	4.32	0.85	RESP dt
T1	4.19	0.95	4.18	0.76	4.19	0.78	4.20	0.90	
T2	4.21	0.83	4.33	1.17	4.31	1.08	4.10	0.60	
T3	4.28	1.22	4.20	1.12	4.14	1.00	4.18	0.98	
									RESP dA
C1	3.82	2.81	3.44	2.16	3.61	2.47	3.50	2.17	
T1	3.13	2.05	3.40	2.22	3.25	2.21	2.98	1.92	
T2	3.57	2.25	3.88	2.82	3.65	2.55	3.13	1.58	
T3	3.78	2.78	4.03	2.72	3.67	2.57	3.58	2.65	
C1	14.4	2.9	14.6	2.2	14.3	2.7	14.3	2.4	RESP Freq
T1	14.9	2.8	14.8	2.5	14.7	2.5	14.8	2.6	
T2	14.7	2.6	14.6	3.0	14.6	2.8	14.9	2.0	
T3	14.9	3.5	15.1	3.4	15.2	3.1	15.0	2.8	

4.2.3.4 Stage 2 analysis: **Respiration frequency and amplitude**

The analysis in Stage 2 did not detect differences in respiration frequencies between control group C1 and any of the treatment group T1, T2, or T3. There were also no differences in respiration amplitudes. Factors such as state and trait anxiety or Life Appraisal Questionaire (LAQ) did not affect the outcomes. The results could be interpreted as a lack of acupuncture influence on respiratory rhythm, when acupuncture points LU7 and KD6 are being used. The control group showed similar trends as the treatment groups in all 20 minute intervals.

4.2.3.5 Stage 3 analysis: **Respiration frequency and amplitude**

The Stage 3 analysis did not reveal any differences in respiration frequencies between any of the treatment group T1, T2, or T3. There were also no differences in respiration amplitudes. Factors such as trait anxiety or LAQ did not affect the outcomes. The results could be interpreted as a lack of acupuncture influence on respiratory rhythm, when acupuncture points LU7 and KD6 are being used. This comes as a surprise because one of the acupuncture points used in the study was traditionally associated with lung functions. Results however indicate that respiration was not affected during the experimental session and appeared to be a very stable physiological parameter.

4.2.4 **Conclusions.**

Data from the experiments clearly show for the subjects assessed in this doctoral study that stimulation of two acupuncture points LU7 and KD6 do not influence respiratory system in a decisive or strong way. As expected, there were no statistically significant differences in variables related to respiration in Stage 1 analysis. That means that when the variables for each interval are treated independently and the number of subjects in the groups are small, only very large changes could possibly be detected.

In Stage 2 analysis where each treatment group was compared individually with the control group, and where variables from consecutive intervals were treated as repeated measures, that is belonging to the same subject, methodology failed to detect changes. This must raise a question regarding the sensitivity of the measurement methods on one hand , and on the other hand, a question regarding the magnitude of the physiological changes in the respiratory system due to acupuncture stimulation in healthy subjects. When additional factorial variables were introduced, such as level of trait anxiety, subjects did not show significant changes in respiration frequency nor amplitude.

In Stage 3 analysis the comparisons were made only between treatments groups, leaving the control group aside. Results show that there were no clear treatment effects in groups T1, T2 and T3. The respiration frequency and amplitude seems to be unchanged by acupuncture stimulation. Future research may show if other acupuncture points have stronger regulatory influence on the lungs, or give different results in diseased subjects.

Chapter 5. Brain and somatic system

The central nervous system in humans and in animals consists of two major components, that is spinal cord and brain. Receiving sensory information and transfer of the motor information towards muscles and glands is performed by the peripheral nervous system (PNS). The brain receives sensory information from the spinal cord and cranial nerves, and in response initiates and coordinates motor outputs. Much of the brain function is however, devoted to the processing of information (Marieb, 1998). The cerebral cortex is the outer layer of the brain and is involved in many essential processes such as perception, memory, thinking, speech and consciousness. Different regions of the cortex are responsible for processing sensory information, for processing information coming from other areas of the brain, and finally for integrating information in so called “association” areas. All these complex functions are performed in a form of parallel processing with simultaneous involvement of hundreds of thousands of neurons (Marieb, 1998)

5.1 Brain electrical activity and EEG

Nerve conduction is associated with changes in electrical potentials along the axon of the neuronal cell. When many neuronal cells in the cortex discharge electricity in close proximity to each other, change in the local electrical field can be detected. Hans Berger is widely credited for the discovery of electroencephalography which dates back to 1929. Since then advances in electronic instrumentation and research in neurology and psychology have increased our understanding of the relationship between electrical activity of the brain and various physiological functions. With recent developments in computational methods, the precise analysis of the electrical field of the brain is now possible (Thakor and Tong, 2004). Historically, there are only four major brain waves recognised in the EEG recordings, that is: Delta, Theta, Alpha and Beta. Each wave is associated with different physiological and psychological states. Currently a large pool of scientific literature exists that deals with the correlation of various brain waves and psycho-physiology of both humans and animals. The EEG methods are being used to diagnose pathologies of the nervous system such as epilepsy, Alzheimer's and Parkinson's disease (Smith, 2005; Jeong, 2004; Bodis-Wollner, 2003).

5.1.1 Introduction

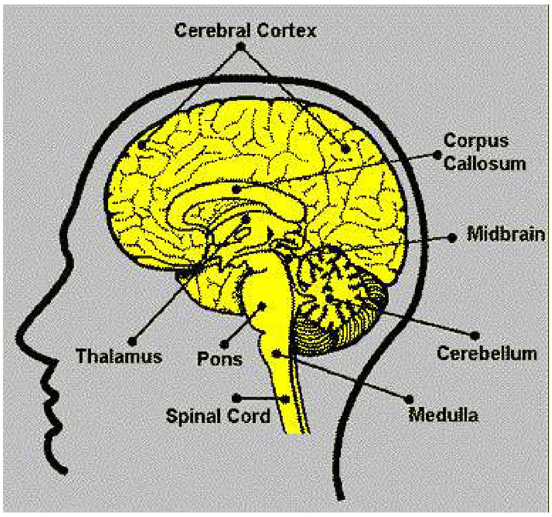
The association between electrical activity of the brain and different physiological states give grounds for the use of the EEG in diagnostics and in research. The recording techniques are now sufficiently simplified and available in many laboratories. EEG is a non-invasive method that is well suited for human studies. At present computerised equipment is used to amplify the weak electrical signals from the surface electrodes and to separate different component frequencies into separate “bands” or brain waves. Complex quantitative studies using EEG techniques that make comparisons between physiological and psychological findings possible, are frequently published in the scientific literature (Bullock, 2002). The use of EEG methods in complementary medicine studies is still in its early stages and certainly is not as popular as in the fields of neurology, psychiatry and cognitive sciences.

5.1.1.1 Physiological importance of brain waves

The four major brain waves each has its own physiological and psychological correlates (Fisch, 1999). Much research effort has been spent on defining the demarcation lines between these associations in order to make the EEG useful as a diagnostic tool in clinical practice (Fisch, 1999). Delta waves are the slowest with a frequency range below 3Hz. This dominant rhythm is normal when present in infants up to one year of age. In adults delta waves appear only in stages 3 and 4 of sleep. Theta waves with a frequency 3.5 to 7.5 Hz are indicative of deep relaxation or when consciousness slips toward drowsiness. They are related to the level of arousal. Their presence is normal during sleep and it is believed that they are associated with deep structures of the brain such as the limbic system and hippocampal regions. Healing and the integration of mind/body is also associated with an increased presence of theta waves (Xu, 1994).

Alpha waves are those with frequency between 8.0 to 13.0 Hz. They are brought about by a state of general relaxation and with closing the eyes. Some researchers claim that alpha is characteristic of the “empty mind” rather than a relaxed mind. Alpha is an indicator of alertness, but not of actively processing complex information. The fastest are the Beta waves with a frequency above 14 Hz; that are present when a person is alert, anxious and has their eyes open (Schurmann and Basar, 2001). They are also present at a time of active thinking, solving analytical problems and focusing on external stimulus. Figure 42 shows the regions of the human brain and spinal cord responsible for the transmission, processing of information, and generation of the brain waves.

Figure 42 . Central nervous system (adapted from Medline Plus)



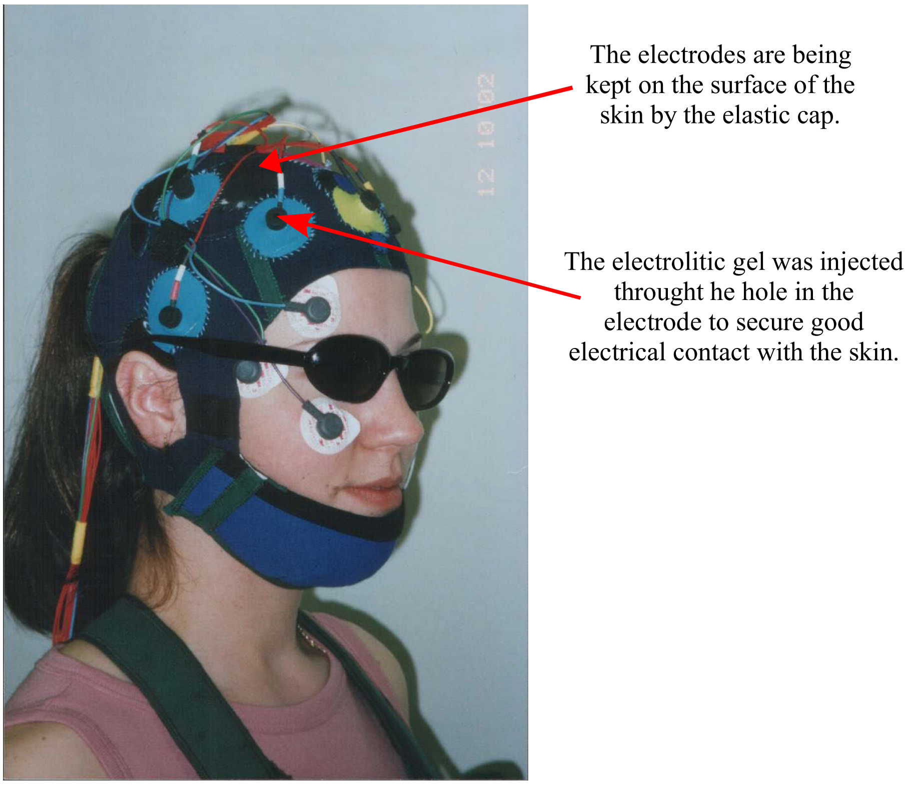
5.1.1.2 New methods in the evaluation of brain waves

At present, the digital filtering techniques that are used to process EEG signals are based on Fast Fourier Transforms (FFT) – the mathematical algorithm that allows the decomposition of waveforms into their component frequencies (Cerna and Harvey, 2000). The average values for the frequencies within the spectrum of Delta, Theta, Alpha and Beta brain waves are calculated either on-line or as a part of post experimental analysis. In more complex experiments, maps of electrical activities of the brain are correlated with findings from functional MRI. In the experiments presented in this thesis custom built software was used to automate the recording of EEG and to automate the analysis of the brain waves. Also, it is important to mention that the conditions under which the EEG recording was made were free from interference such as traffic noise that could cause artifacts in the recorded signal or introduce cognitive bias.

5.1.2 Methodology

The EEG cap was used to hold the electrodes in position close to the surface of the skin. It has been specifically designed for this PhD project to allow maximum comfort to the subject. Caps were made in three different sizes to accommodate for individual differences. Figure 43 illustrates how the cap was placed on the head and how its position was secured with additional chin support.

Figure 43. EEG recording procedure with electrodes being placed around the head.



The back of the cap had an opening through which the hair could be moved, thus improving the comfort of subjects during the 80 minute long experimental session. All cables were tied together and attached to the cap at the vertex to allow unrestricted sideway movement of the head without triggering artifacts in the recorded EEG.

The Ag/AgCl electrodes that were used in this research were of the 'sintered' type (Neuroscan, US) that guarantees low noise and small offset potentials. They come with a rubber-based housing. This 10 mm diameter housing allowed for the the electrolytic gel to be kept firmly between electrode and the surface of the skin, and prevented the electrodes from move. As a result, the EEG signals recorded during the experimental sessions were relatively artifact free. A visual examination of the recorded signals was performed routinely during and after the experimental session.

5.1.2.1 Acquisition of EEG signals

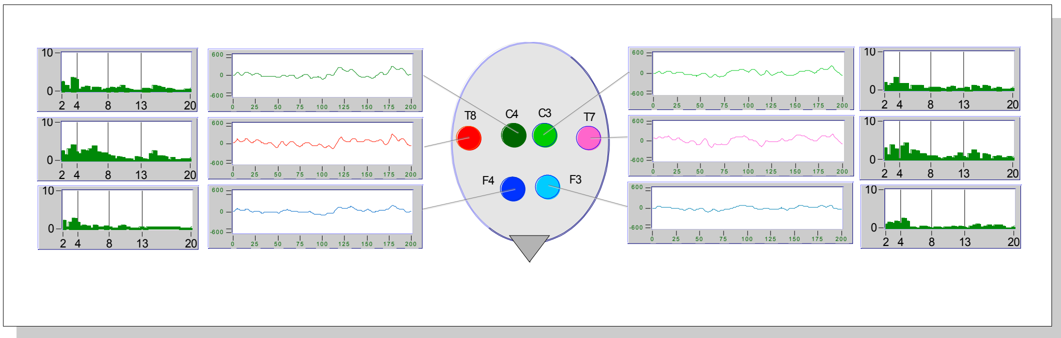
The EEG was recorded from six electrodes that were positioned according to the 10-20 system (Fisch, 1999). Measurement from locations C4, T8, F4 on the right side and C3, T7, F3 on the left side were made with reference to the Fz electrode placed in the mid-line. The weak EEG signals were first amplified and filtered using BioAmplifier EEG-8 (Precision Instruments, UK). Gain was set to x100 000 and analog filters to low-pass=30Hz and high-pass=1Hz. The EEG signal was then digitized using a 12-bit A/D converter (PCI-6028E, National Instruments, USA). Sampling rate for the digital converter was set to 1000 samples per second and then was reduced by averaging techniques to 200 samples per second.

The on-line recording and analysis of the EEG signals was performed using custom built software. This software was created by the author of this research project. The LabView G programming language (LabView v.6.1, National Instruments, USA) was used to develop the *Virtual EEG Recorder* software which emulates on the computer screen all functions of the electronic EEG paper recorder (see App.B). This software allowed for simultaneous recording and analysis of the EEG signal. It also allowed automatic control of the tasks required in the experimental session in accordance with the requirements of the protocol described in Chapter 2.

The raw EEG signal, relatively free from artifacts and noise, was first processed using digital filtering techniques. Each of the six EEG signals was subjected to the FFT analysis that allowed for splitting the signal into the component frequencies. On-line calculations were made every second, using segments of 3 second of EEG signals.

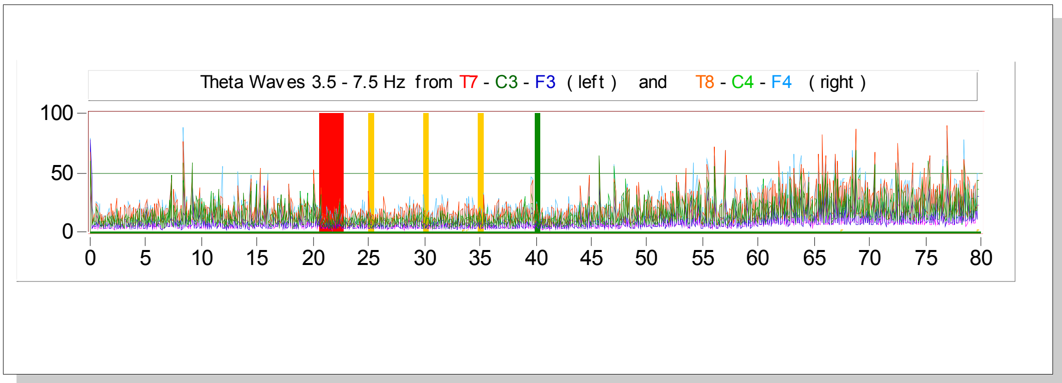
Figure 44 illustrates this process and shows the position of the electrodes on the head.

Figure 44. Digital filtering of EEG signals into brain waves.



The on-line processing of the EEG signals was continuous during the 80 minute sessions. A continuous plot of the magnitudes of four major brains waves was stored on the hard disk drive of the computer and printed as a paper record. Figure 45 presents such plot for the Theta waves as they appeared in the EEG of all six electrodes.

Figure 45. Brain waves were measured continuously during 80 minute experimental sessions.



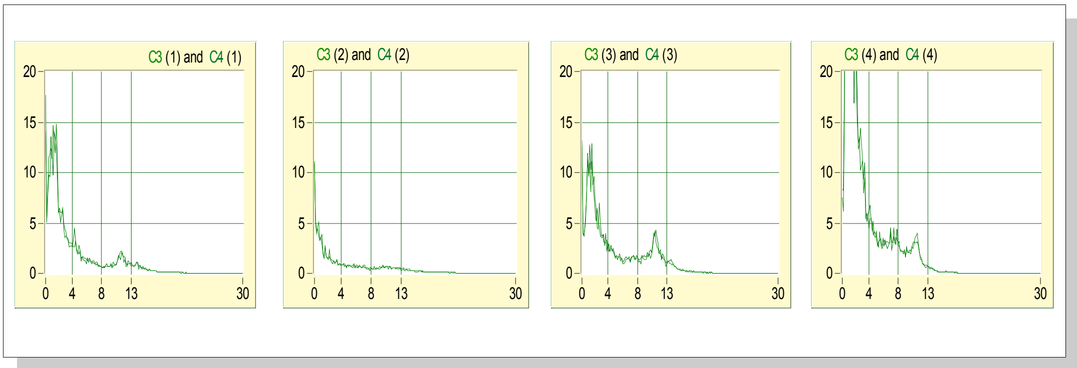
The overlapping traces on the graph indicate general trends in the magnitude of the brain wave. In the example above it is easy to see that theta waves were significantly lower during interval i2 (20-40 min) as compared to interval i4 (60-80 min). The vertical lines on the graph mark the time when acupuncture needles were used. The color red indicates insertion of the needle(s), orange indicates needle stimulation and green marks the removal of the needles.

5.1.2.2 The dominant brain waves in experimental intervals

The EEG signals were processed once again at the end of the experiments. This time the FFT analysis was applied to each segment of 20 minute EEG recording. This allowed for the accurate detection of the overall pattern of dominant frequencies that characterized particular experimental intervals. Values for each interval were considered as repeated measurements in statistical analysis.

The combined spectrum for each interval was then divided into separate "bins" that included four major brain waves Delta, Theta, Alpha and Beta. Spectrum from the left and right hemispheres were simultaneously presented on the same graphs in order to detect possible asymmetry. Figure 46 presents an example of eight spectrograms, two for each graph (left and right hemisphere) and four for intervals i1 to i4.

Figure 46. Frequency spectrum of brain activity for electrodes C3 and C4.



The vertical lines that divide the graph into sections at 4, 8 and 13 Hz mark the boundaries for Delta, Theta, Alpha and Beta waves. The overlap of two curves on each graph indicates that there were only very small differences in the EEG spectrum between symmetrically positioned electrodes for left and right hemispheres. Similar graphs have been plotted for each of the four remaining EEG electrodes.

5.1.3 Results

Table 12 presents the dependent variables used in the analysis of brain wave activity . All major parameters of the EEG were calculated by the *EEG Analyser* software at the end of the experimental sessions. The 25%-75% range and standard deviations are included in the table. All values describing the power of the brain waves are reported as per 0.5 Hz bins. In order to calculate the magnitude for the whole spectrum the results should be multiplied by 3 bins for Delta, 9 bins for Theta, 11 bins for Alpha and 33 bins for Beta.

Table 12 . Dependant variables used in the analysis of brain waves

Measurement	***Description***	***25 % - 75 %***	***SD***	***Unit***
Delta	EEG spectrum 2.0- 3.5 Hz	1.24 – 5.61	5.95	$uV^2/0.5Hz$
Theta	EEG spectrum 3.5- 7.5 Hz	0.69 – 1.92	1.33	$uV^2/0.5Hz$
Alpha	EEG spectrum 7.5-13.0 Hz	0.42 – 1.24	0.62	$uV^2/0.5Hz$
Beta	EEG spectrum 13.0-30.0 Hz	0.06 – 0.13	0.06	$uV^2/0.5Hz$
Alpha 1	EEG spectrum 7.5-10.0 Hz	0.41 – 1.4	0.93	$uV^2/0.5Hz$
Alpha 2	EEG spectrum 10.0-13.0 Hz	0.32 – 0.96	0.55	$uV^2/0.5Hz$
Beta 1	EEG spectrum 13.0-18.0 Hz	0.11 – 0.31	0.16	$uV^2/0.5Hz$
Beta 2	EEG spectrum 18.0-30.0 Hz	0.02 – 0.05	0.03	$uV^2/0.5Hz$
“8”	EEG spectrum 7.0- 9.0 Hz	0.45 – 1.39	1.05	$uV^2/0.5Hz$
Full Delta	EEG spectrum 0.5- 3.5 Hz	3.04 – 11.45	13.9	$uV^2/0.5Hz$

The analysis of the results was performed in three stages in accordance with the general methodology outlined earlier in Chapter 3. Each stage had a different hypothesis to resolve and a different capacity to answer the research questions. Table 13 highlights the main features of each stage.

Table 13. Three stages of data analysis for EEG

Data analysis	Groups	Repeated measures	
Stage 1	T1-T2-T3 C1	none	30 + 30 + 30 30
Stage 2	C1 - T1	Intervals	30 + 30
	C1 - T2		30 + 30
	C1 - T3		30 + 30
Stage 3	T1-T2-T3	Intervals & Groups	30 + 30 + 30

Stage 1 of the EEG analysis was focused on factorial ANOVA with the main factors being “group” and “interval” within the experimental sessions. All measurements were treated as independent measurements to reveal the main effect sizes for each variable.

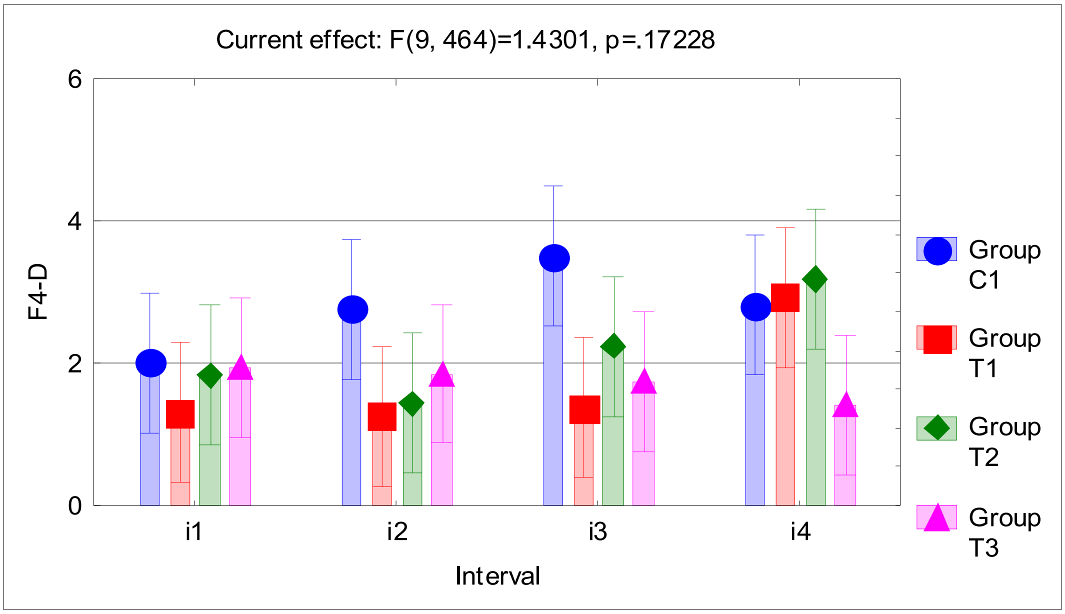
Stage 2 concentrated on individual comparisons between groups, that is each of the experimental groups T1 or T2 or T3 against the control group C1. The main purpose was to compare the “active” with non-active” influences on experimental subjects. It was expected that the control group may reveal how healthy subjects perform during 80 minute experimental sessions and how significant are the naturally occurring trends.

Stage 3 analysis focused only on the “active” groups that is T1 and T2 and T3, leaving the control group C1 aside. Only the differences between the influences of treatment protocols were taken into account. It was expected that stage 3 analysis would be the strongest in detecting significant differences in the action of individual acupuncture points.

5.1.3.1 Stage 1 Analysis: Delta waves

The results representing Delta waves for all groups (C1-T1-T2-T3) and all intervals (i1-i2-i3-i4) are shown in Figure 47. The EEG was obtained from electrode F4 placed in the frontal area. Each bar represents the value for Delta waves recorded during each 20 minute interval. The magnitude of Delta waves from electrode F3 and F4 were very similar therefore only F4 is illustrated on the graph.

Figure 47. Magnitude of the Delta waves from electrode F4.

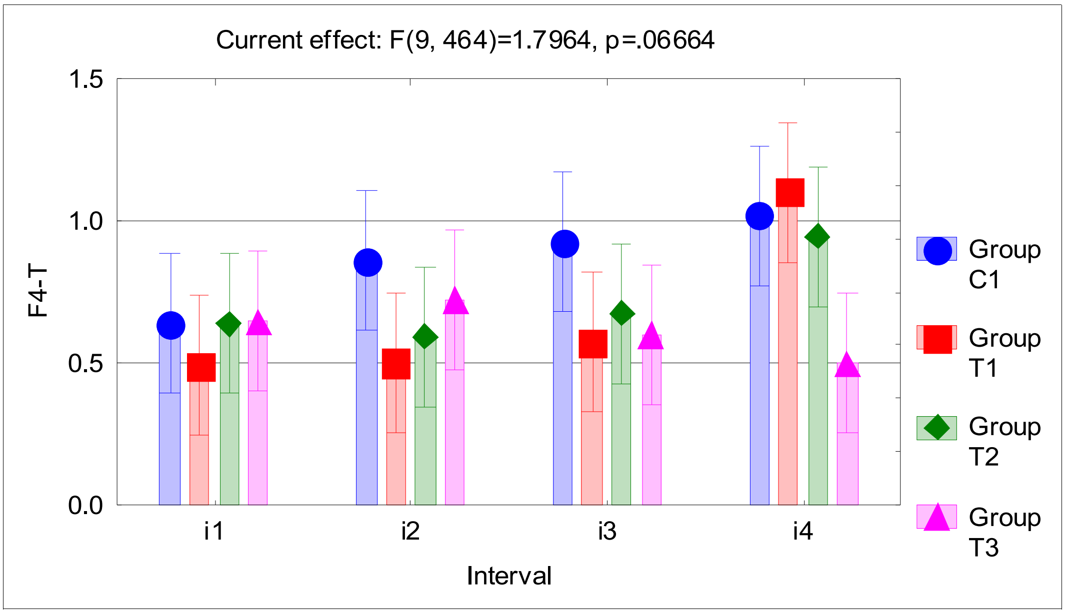


There are no statistically significant differences between the magnitude of Delta waves in intervals and groups. There is however a consistent bias between the control group and all three treatment groups during intervals i2 and i3. That difference is not statistically significant but it suggests that there is a possible psychological or environmental influence. It could be based on subject expectations. The strong presence of Delta waves in the control group may indicate much stronger "drifting" of the subjects towards drowsiness and sleep. Overall, no major changes in Delta have been detected due to the acupuncture stimulation that was used in sessions T1, T2 and T3. The results confirm that Delta waves are a very dynamic physiological parameter quite easily influenced by environmental and internal factors.

5.1.3.2 Stage 1 Analysis: Theta waves

The results for EEG Theta waves obtained from electrode F4 that was placed in the frontal area, for all groups (C1-T1-T2-T3) and all intervals (i1-i2-i3-i4) are shown in Figure 48. Each bar represents the mean value for Theta waves during each 20 minute interval. The magnitude of Theta waves from all other electrodes was very similar.

Figure 48. Magnitude of the Theta waves from electrode F4

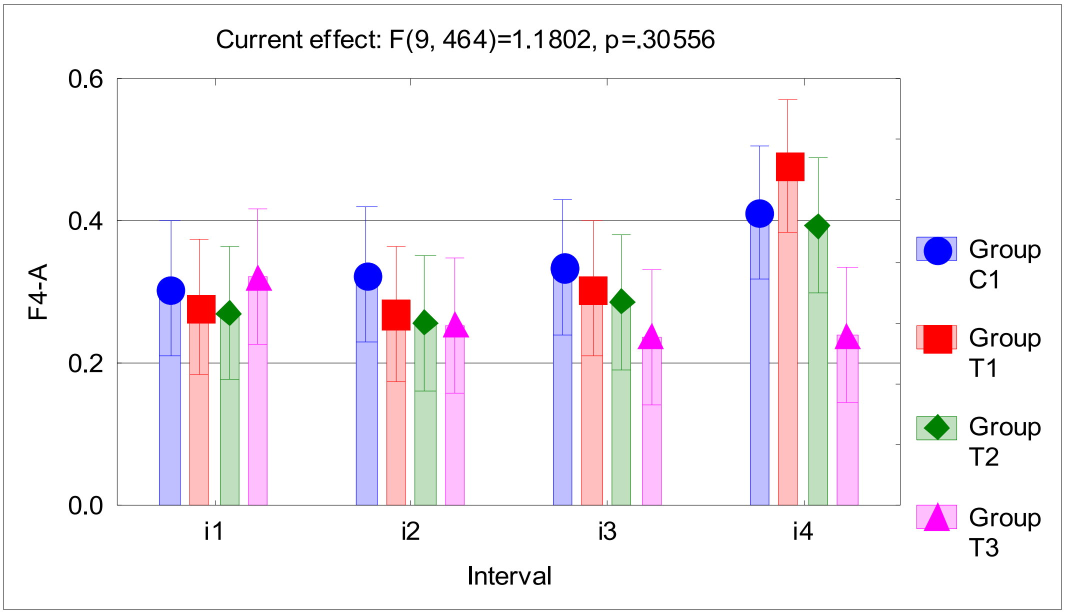


There were no statistically significant differences between the magnitude of Theta waves in intervals and groups. The bias between the control group and all three treatment groups is clearly visible during intervals i2 and i3, and is similar to the Delta results. It may indicate that the subjects in the control group were less alert and possibly experiencing light sleep. Only during the last 20 minutes of the experiment Theta waves matched in their amplitude the level that is present in the control group, and only in groups T1 and T2. Overall however, no statistically significant changes in Theta waves were detected due to the acupuncture stimulation that was used in sessions T1, T2 and T3. Analysis of EEG from other electrodes revealed similar values and trends. The results confirm that Theta waves are a sensitive physiological indicator and that they can be quite potentially influenced by environmental and internal factors.

5.1.3.3 Stage 1 Analysis: Alpha waves

The results for EEG Alpha waves obtained from electrode F4 that was placed in the frontal area, for all groups (C1-T1-T2-T3) and all intervals (i1-i2-i3-i4) are shown in Figure 49 Each bar represents the mean value for Alpha waves during each 20 minute interval. The magnitude of Alpha waves from all other electrodes was very similar.

Figure 49. Magnitude of the Alpha waves from electrode F4

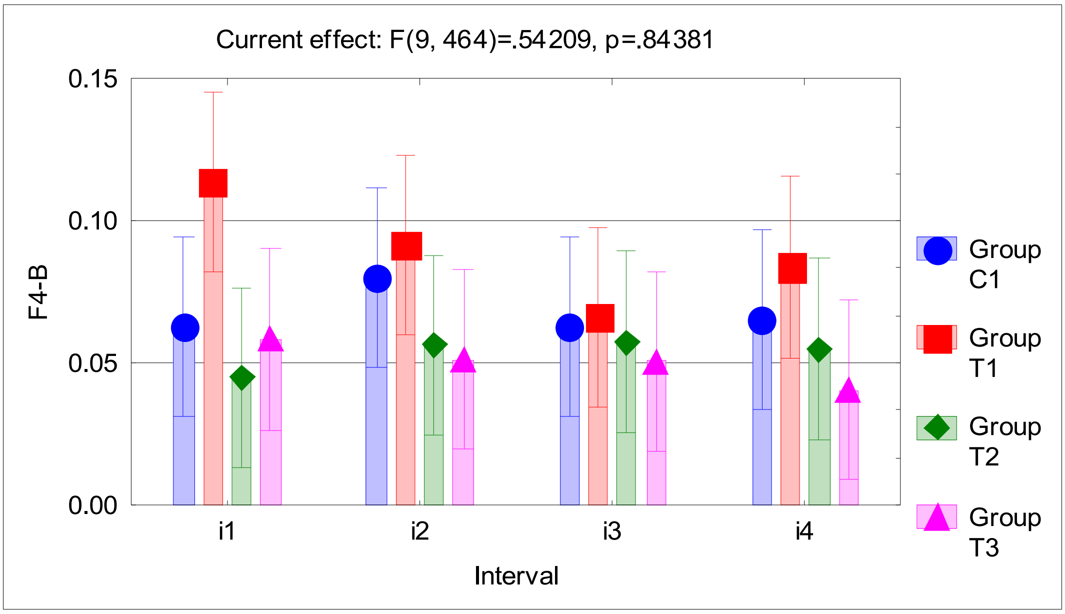


There were no statistically significant differences between the magnitude of Alpha waves in intervals and groups. Only in interval i4 the amplitude of Alpha waves in groups T1 and T2 matches or exceeds values in the control group C1. This trend may indicate that subjects in group T1 and T2 were as relaxed as those in the control group. When treatment T3 was used, subjects did not show such increased level of Alpha waves. Overall however, no statistically significant changes in Alpha waves have been detected due to the acupuncture stimulation that was used in sessions T1, T2 and T3. Analysis of the EEG from other electrodes revealed similar values and trends for Alpha waves. The results suggest that Alpha waves are not immediately influenced by acupuncture stimulation. A trend towards increased magnitude in interval i4 may indicate the presence of a delayed response, possibly due to the relaxation effect on the central nervous system.

5.1.3.4 Stage 1 Analysis: Beta waves

The results representing Beta waves for all groups (C1-T1-T2-T3) and all intervals (i1-i2-i3-i4) are shown in Figure 50. EEG was obtained from the electrode F4 placed in the frontal area. Each bar represents the value for Beta waves recorded during 20 minute interval. The magnitude of Beta waves from electrode F3 and F4 were very similar thus only F4 is illustrated in the graph.

Figure 50. Magnitude of the Beta waves from electrode F4



There were no statistically significant differences between the magnitude of Beta waves in intervals and groups. There is however a consistent bias between one of the treatment groups (T1) and the other two treatment groups during interval i1. That difference was not statistically significant but it indicates that there is a possible psychological or procedural influence. It could be based on subject expectations and perception of safety. Subject were fully informed about location of the acupuncture points, e.g. around wrist area and around the ankle. The strong presence of Beta waves in that group may indicate greater alertness of the subjects to the environmental events. Overall, no major changes in Beta waves were detected due to the acupuncture stimulation that was used in sessions T1, T2 and T3.

The calculated effect sizes for all EEG variables are presented in Table 14. Despite the fact that some of the effects are quite strong (>0.5), the overall factorial MANOVA analysis confirmed only statistically significant differences in Delta and Theta waves as described in Stage 2 and 3 of the analysis.

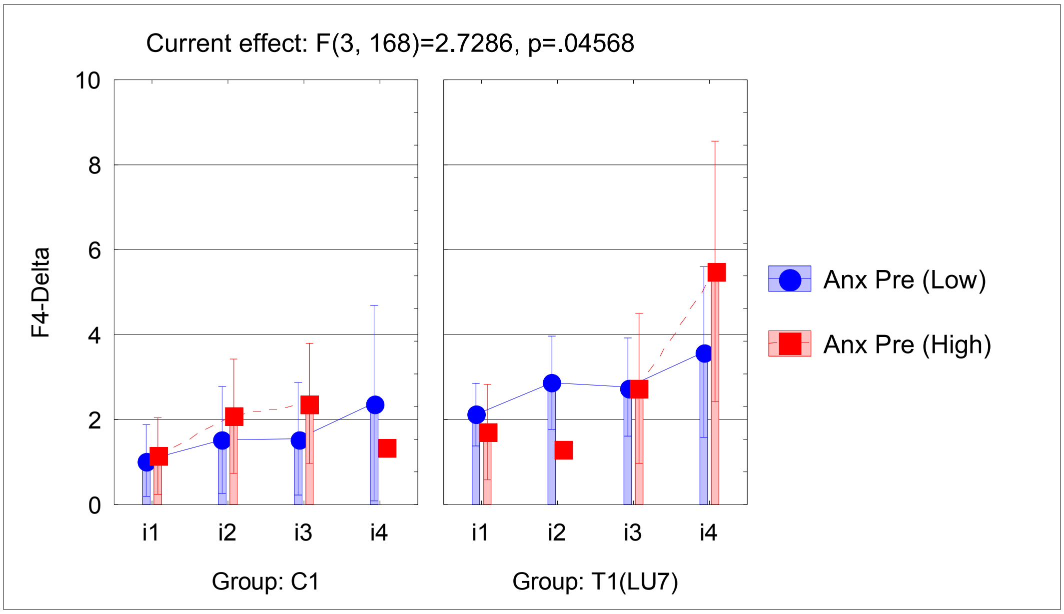
Table 14. Calculated effect sizes for EEG variables in all groups and intervals

	Group	C1(n=30) to T1(n=30)				C1(n=30) to T2(n=30)				C1(n=30) to T3(n=30)			
	Interval	i1	i2	i3	i4	i1	i2	i3	i4	i1	i2	i3	i4
	C4-D	0.48	0.11	0.24	0.37	0.38	-0.05	0.15	0.27	0.49	-0.27	0.02	0.25
	C4-T	0.31	0.07	0.19	0.34	0.28	-0.08	0.15	0.23	0.14	-0.52	-0.02	0.25
	C4-A	-0.42	-0.39	-0.32	-0.25	-0.21	-0.39	-0.16	-0.22	-0.33	-0.26	-0.24	-0.06
	C4-B	0.15	-0.29	-0.12	-0.02	0.16	-0.30	-0.09	-0.08	0.03	-0.35	-0.16	
	C4-A1	-0.56	-0.57	-0.25	-0.26	-0.31	-0.41	-0.04	-0.20	-0.44	-0.30	-0.30	-0.17
	C4-A2	-0.14	-0.11	-0.32	-0.19	-0.01	-0.20	-0.32	-0.15	-0.13	-0.14	-0.14	0.07
F4	C4-B1	0.24	-0.22	0.00	0.11	0.21	-0.28	-0.02	-0.05	0.11	-0.45	-0.10	0.12
	C4-B2	-0.25	-0.43	-0.55	-0.35	-0.11	-0.20	-0.19	-0.10	-0.13	-0.08	-0.27	0.01
	C4-8	-0.35	-0.40	-0.02	-0.12	-0.16	-0.27	0.10	-0.17	-0.32	-0.28	-0.22	-0.09
	C4-FD	-0.26	-0.10	0.12	0.30	-0.32	-0.28	0.12	0.19	-0.33	-0.79	-0.18	0.21
	T8-D	0.46	0.05	0.16	0.35	0.35	-0.09	0.13	0.26	0.51	-0.41	0.01	0.26
	T8-T	0.21	0.02	0.18	0.29	0.24	-0.14	0.11	0.20	0.09	-0.63	-0.07	0.26
	T8-A	-0.40	-0.21	-0.36	0.04	-0.31	-0.28	-0.11	-0.06	-0.19	-0.12	-0.17	0.19
	T8-B	0.07	-0.39	-0.23	-0.17	0.19	-0.29	-0.10	-0.14	0.09	-0.53	-0.15	-0.04
	T8-A1	-0.72	-0.34	-0.42	-0.05	-0.71	-0.35	-0.15	-0.22	-0.45	-0.12	-0.26	-0.01
C4	T8-A2	0.15	0.00	-0.14	0.26	0.25	-0.11	-0.01	0.19	0.27	-0.12	-0.02	0.42
	T8-B1	0.17	-0.36	-0.07	0.00	0.20	-0.33	-0.15	-0.13	0.16	-0.69	-0.25	0.07
	T8-B2	-0.26	-0.25	-0.73	-0.55	0.09	-0.10	-0.02	-0.08	-0.10	-0.21	-0.02	-0.24
	T8-8	-0.61	-0.39	-0.12	-0.03	-0.55	-0.50	0.02	-0.22	-0.51	-0.34	-0.25	-0.03
	T8-FD	0.05	-0.12	0.07	0.26	0.01	-0.38	0.09	0.15	0.16	-1.02	-0.30	0.09
	F4-D	0.41	0.18	0.25	0.37	0.31	-0.01	0.18	0.24	0.31	-0.71	-0.07	0.18
	F4-T	0.26	0.18	0.31	0.39	0.27	0.01	0.18	0.27	0.10	-0.31	-0.03	0.26
	F4-A	0.22	0.03	0.06	0.24	0.18	-0.23	0.04	0.18	0.16	-0.56	-0.12	0.29
	F4-B	0.13	-0.21	-0.19	-0.02	-0.12	-0.04	-0.20	0.09	-0.17	-0.25	-0.94	-0.07
	F4-A1	-0.01	-0.04	0.07	0.12	-0.11	-0.22	0.09	-0.00	-0.12	-0.29	-0.17	0.13
T8	F4-A2	-0.01	-0.04	0.07	0.12	-0.11	-0.22	0.09	-0.00	-0.12	-0.29	-0.17	0.13
	F4-B1	0.22	-0.14	-0.05	0.05	0.09	-0.08	-0.14	0.06	-0.05	-0.47	-0.57	-0.01
	F4-B2	0.03	-0.30	-0.39	-0.09	-0.34	-0.00	-0.23	0.10	-0.24	-0.08	-0.94	-0.10
	F4-8	0.03	-0.02	0.17	0.16	-0.07	-0.22	0.17	0.02	-0.11	-0.27	-0.11	0.13
	F4-FD	-0.26	-0.04	-0.13	0.25	-0.54	-0.52	0.03	0.14	-0.49	-2.45	-0.76	0.10

5.1.3.5 Stage 2 Analysis: Delta waves (state anxiety subgroups)

The results of comparative analysis between group C1 and individual treatment groups are presented in Figure 51. The measurements for intervals i1, i2, i3, i4 are treated as repeated measures ("within" group factor). Each bar represents the magnitude of Delta waves from electrode F4 in two sub-groups of subjects - those showing lower (blue) and higher (red) levels of anxiety at the beginning of the experiment.

Figure 51. Magnitude of Delta waves in sub-groups with lower (blue) and higher (red) state anxiety levels

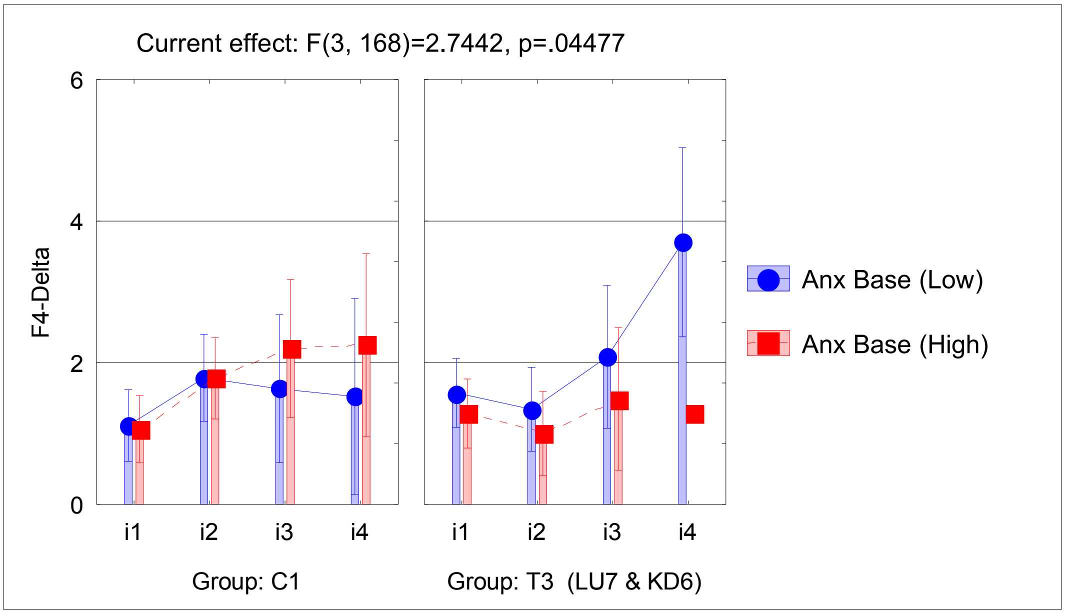


The statistically significant differences in the level of Delta waves is visible during interval i4, but only for a sub-group of subjects with higher anxiety levels. Delta waves are believed to be typically associated with drowsiness and sleep (Lal and Craig, 2002). The results support the hypothesis that stimulation of the acupuncture point LU7 (group T1) can induce a relaxation. The question why it applies only to more anxious subjects may possibly be explained by assuming that these subjects need more relaxation in order to counteract the anxiety. These data also indicate that a separate control is essential in order to make an evaluation of naturally occurring trends in recorded variables. Treatment in groups T2 and T3 did not have a similar affect on subjects reporting a higher state of anxiety before the experimental session.

5.1.3.6. Stage 2 Analysis: Delta waves (trait anxiety subgroups)

The differences in results between the control group C1 and treatment group T3 are presented in Figure 52. The measurements for intervals i1, i2, i3, i4 are treated as repeated measures ("within" group factor). Each bar represents the magnitude of Delta waves from electrode F4 in two sub-groups of subjects - those reporting lower (blue) and higher (red) levels of anxiety.

Figure 52. Magnitude of Delta waves in sub-groups with lower (blue) and higher (red) anxiety levels

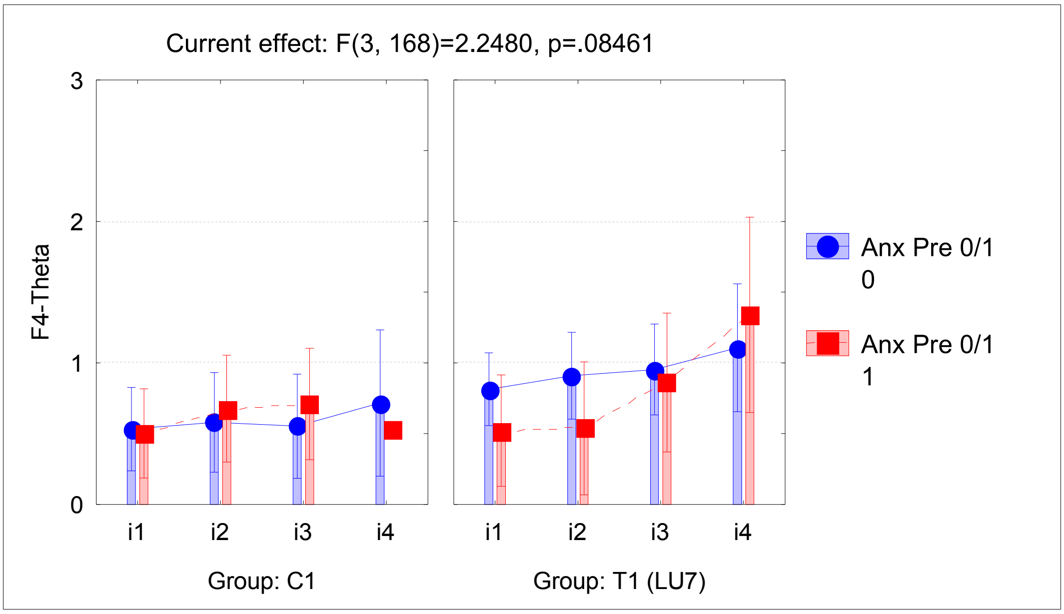


There were statistically significant differences between the magnitude of Delta waves during interval i4 in the sub-group of subjects that reported lower levels of trait anxiety. The results were opposite to changes in Delta waves in group T1 presented in Figure 51. Delta waves are believed to be present in adults only during light sleep and relaxation (Lal and Craig, 2002). The stimulation of the acupuncture points LU7 and KD6 (group T3) can help to promote stronger relaxation in subjects with lower anxiety. Results presented in Figure 51 and 52 may suggest that the "state" (current) and "trait" (baseline) anxiety are two very different psychological conditions, influencing the physiology of the healthy subject in a different way. Treatment in groups T1 and T2 did not have a similar affect on subjects reporting lower anxiety before the experimental session.

5.1.3.7 Stage 2 Analysis: Theta waves ("state" anxiety subgroups)

The results of comparative analysis between the control group C1 and individual treatment groups are presented in Figure 53. The measurements for intervals i1, i2, i3, i4 are treated as repeated measures ("within" group factor). Each bar represents the magnitude of Theta waves from electrode F4 in two sub-groups of subjects - those showing lower (blue) and higher (red) levels of 'state" (current) anxiety at the beginning of the experiment.

Figure 53. Magnitude of Theta waves in sub-groups with lower (blue) and higher (red) state anxiety levels

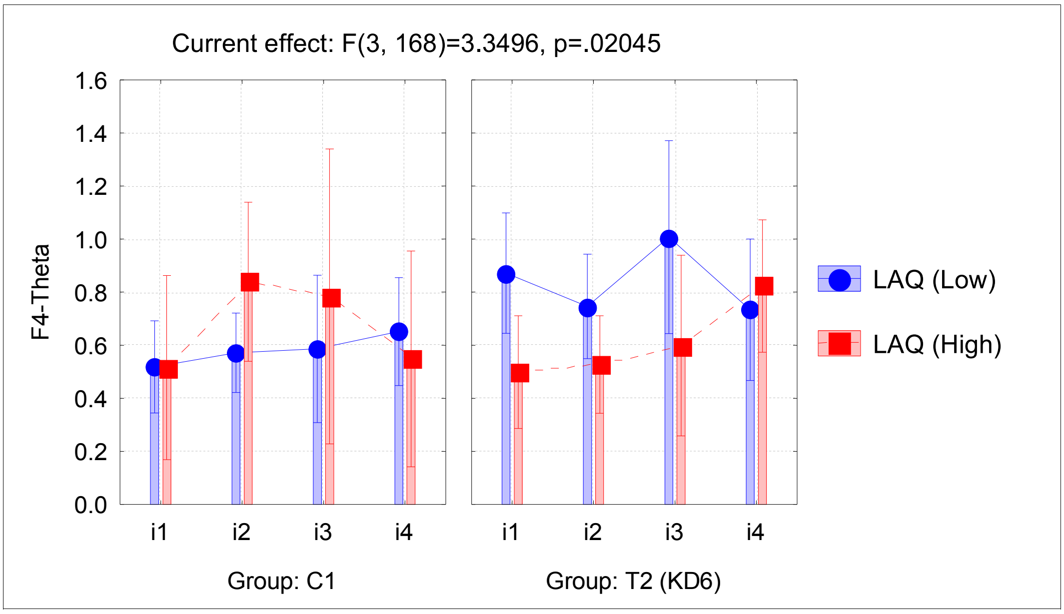


The strong trend towards an increased level of Theta waves is clearly visible during interval i4, but only for the sub-group of subjects with higher state anxiety levels. Theta and Delta waves are typically associated with drowsiness and sleep (Lal and Craig, 2002). In a similar way to the results presented in Figure 51, the increase in Theta waves supports the hypothesis that stimulation of the acupuncture point LU7 (group T1) can induce relaxation. Treatment in groups T2 and T3 did not have a similar affect on subjects reporting higher state anxiety.

5.1.3.8 Stage 2 Analysis: Theta waves (LAQ subgroups)

The differences in results between the control group C1 and treatment group T2 are presented in Figure 54. The measurements for intervals i1, i2, i3, i4 are treated as repeated measures ("within" group factor). Each bar represents the magnitude of Theta waves from electrode F4 in two sub-groups of subjects - those reporting lower (blue) and higher (red) levels of Life Appraisal Questionnaire scores.

Figure 54. Magnitude of Theta waves in sub-groups with lower (blue) and higher (red) LAQ scores

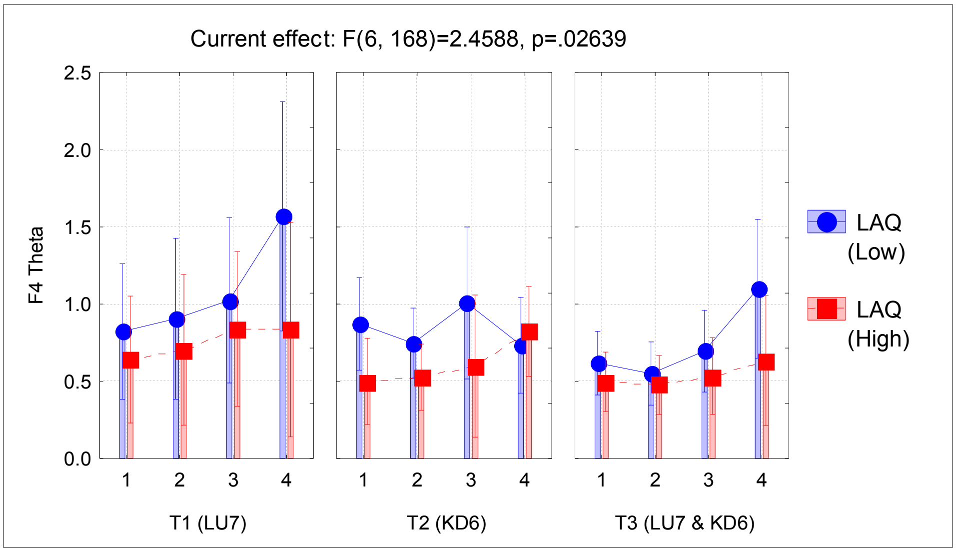


The statistically significant differences in the level of Theta waves is clearly visible during intervals i2 and i3, but only for a sub-group of subjects with higher LAQ scores ("stressed"). In the control group C1 levels of Theta waves are very different depending on the LAQ score. However this tendency to increased Theta in intervals i2 and i3 is not present when subjects received acupuncture treatment at acupoint KD6. Treatment in groups T1 and T3 did not have a similar effect. These results may indicate that higher lifestyle stress scores have different modifying effect on physiology to that of an anxiety levels. Strong bias in interval i1 in group T2 also points to possible environmental influence.

5.1.3.9 Stage 3 Analysis: Theta waves (LAQ subgroups)

Data in Stage 3 was analysed using two level repeated measures ANOVA. The control group was thus removed from the analysis and the differences were measured only between treatment groups T1, T2 and T3. Thus the variability between the treatment sessions was fully taken into account. Figure 55 shows measurements for intervals i1, i2, i3, i4 that are treated as repeated measures ("within" group factor) and three treatment groups that were also considered as repeated measures ("between" group factors). Each bar represents Theta waves for two sub-groups of subjects, those showing lower (blue) and higher (red) scores in the Life Appraisal Questionnaire.

Figure 55. Increased level of Theta waves in subject with lower LAQ scores

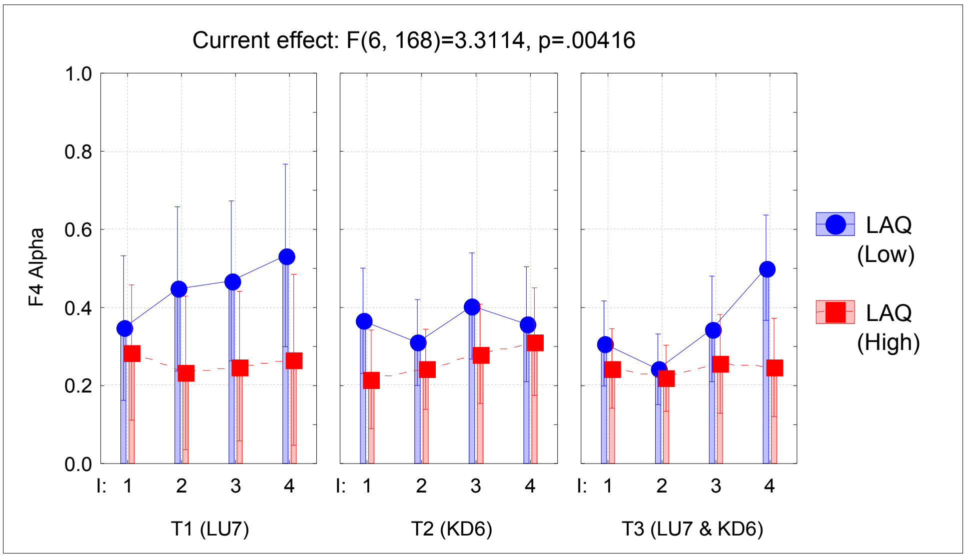


There are statistically significant differences in the levels of Theta waves between subjects that reported lower LAQ scores (less stressed subjects). The difference is most pronounced in interval i4 and in group T1 and T3 where both groups received acupuncture at LU7 point (group T3 also received treatment at KD6 acupuncture point). In group T2, where only the KD6 point was stimulated, the level of Theta waves is unchanged for both subgroups. The general perception that subjects in group T1 and T3 were much more ready to relax and fall into light sleep seems to be confirmed by this data. The absence of such effects in group T2 may indicate that subjects are either less relaxed or more alert.

5.1.3.10 Stage 3 Analysis: Alpha waves

Data in Stage 3 was analysed using two level repeated measures ANOVA. The control group was thus removed from the analysis and the differences were measured only between treatment groups T1, T2 and T3. Thus the variability between the treatment sessions was fully taken into account. Figure 56 shows measurements for intervals i1, i2, i3, i4 that are treated as repeated measures ("within" group factor) and three treatment groups that are also considered as repeated measures ("between" group factors). Each bar represents Alpha waves for two sub-groups of subjects, those showing lower (blue) and higher (red) levels of Life Appraisal Questionnaire scores.

Figure 56. Increased level of Alpha waves in subject with lower LAQ scores



There are statistically significant differences in levels of Alpha waves between subjects reporting lower LAQ scores, that is the less stressed subjects. The difference is most pronounced in group T1 that received acupuncture at LU7 point, but also significant in group T3 where LU7 and KD6 acupuncture points were used together. In group T2 where only the KD6 point was stimulated, the level of Alpha waves is unchanged. These increases are similar to increases in Theta waves presented previously in Figure 55. The increase in Alpha waves may be associated with deeper relaxation. But the question, why the subjects with lower LAQ scores relax more easily when treated, remains unanswered.

5.1.4 Conclusions

The overall results from the EEG analysis indicate that there are statistically significant differences in levels of brain wave activity in subjects treated with different acupuncture points. The EEG method seems to be sensitive enough to detect changes attributed to the stimulation of a single acupuncture point.

Stage 1 analysis did not result in any statistically significant results, indicating that without use of repeated measures there is insufficient resolution in the methodology. The analysis in Stage 2 however, revealed that Delta and Theta waves were influenced by acupuncture stimulation. That influence is highly modified by the internal condition of the subject such as anxiety level and stress levels associated with lifestyle. Significant effects are present only in certain treatment groups. This raises the possibility of undertaking comparative studies between different acupuncture points.

Acupuncture treatment used in group T1 (LU7) and in group T3 (LU7 and KD3) seems to promote relaxation and light sleep in subjects showing higher levels of anxiety. In contrast, subjects in group T2 (KD6) did not relax or fall into a light sleep easily. This finding was confirmed in Stage 3 analysis which revealed that it is mainly Theta and Alpha "relaxation" waves that change. Of the two treatment protocols T1 and T3, T1 shows the strongest differences. It can be interpreted as an indicator of LU7 acupuncture point promoting relaxation and light sleep.

It is important to notice that the modulatory effect of LU7 is not instantaneous. The physiological effects induced in the central nervous system gradually accumulate over the period of 40 minutes after the withdrawal of the acupuncture needles. Such results are in full agreement with observations in the clinical setting where patients often show a delayed response to acupuncture treatment. The implication of such findings for acupuncture research methodology is very clear – enough time should be given for the therapeutic effect to develop. It is quite possible that the major physiological effects take hours to develop, and that the statistically significant changes found in this study form only the beginning of the accumulative effect. Further implications will be discussed in Chapter 8.

5.2 Eye movement and electrooculogram

Eye movement is the expression of both voluntary and involuntary activity of the nervous system through motor division of the peripheral nervous system. In order to compensate for large head movements during normal visual perception, the eyes must also move to allow for clear vision. There are four extra-ocular muscles attached to each eyeball (Marieb, 1998). Their coordinated effort make smooth rotation of the eyes possible without conscious involvement of the person. The coordinated voluntary eye movement in a conscious person allows for precise targeting of the objects within the field of vision. The involuntary eye movements are also possible during sleep. They are noticeable during dreams, when eyes seems to follow imaginary object (Marieb, 1998).

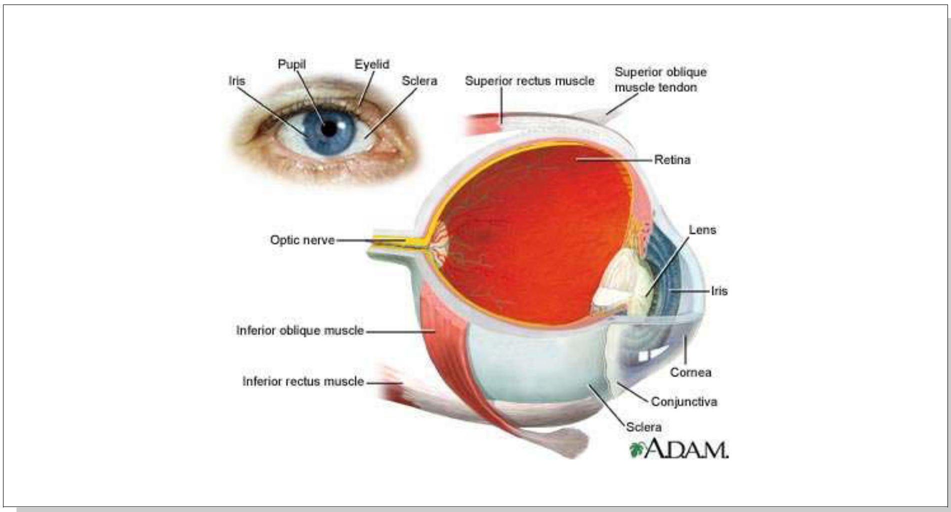
5.2.1 Introduction

The physiological limitation in slow processing of the electrical signals generated by the retina requires compensatory eye movement in order to trace moving objects. The electrical phenomenon associated with the retina can be measured and used in opthalmologic diagnosis (Marmor and Zrenner, 1993). The resting potential of the retina is used to evaluate the so called Adren ratio, that is the ratio between electrical potential in light and in dark conditions. The electrical potential of the retina is also reflected when measuring electrodes are placed on the surface of the skin, on each side of the eye. When the eyes move towards one electrode and away from the other electrode, the differential potential between electrodes is proportional to the angle of rotation. Thus with two sets of electrodes both vertical and horizontal movements can be recorded quite accurately.

The electrooculogram (EOG) can be used in two different settings. In an opthalmology clinic, EOG is used as a measure of the light response of the retinal pigment epithelium (Marmor and Zrenner, 1993). In psycho-physiology the EOG is used as a measure of horizontal and vertical movements of the eyes. At rest, even with the eyes being closed, small eye movements can be observed (Porte, 2004). Detection of eye movement can supply valuable information about the state of the central nervous system, in particular it can be used to detect rapid eye movements during sleep and dreaming (Marieb, 1998).

Figure 57 illustrates some essential anatomical structures involved in eye movement.

Figure 57. The anatomy of the eye and the extra-occular muscles (adapted from MedlinePlus)



5.2.2 Methodology

The noninvasive measurement of eye movement was performed by using the electrophysiological method. Two pairs of Ag/AgCl surface electrodes were placed on the face, one pair for vertical and one pair for horizontal movements, as illustrated in Figure 58. The electrical cables from the electrodes were attached to the EEG cap and were included in the bundle of cables connected to the amplifier. The EOG signals were first amplified and filtered using BioAmplifier EEG8 (Precision Instruments, UK). Gain was set to x10000 and the analog filters to low-pass=30 Hz and high-pass=0.01Hz. The EOG signal was digitized using 12-bit A/D converter (PCI-6028E, National Instruments, US). Sampling rate for the digital converter was setup to 1000 samples per second and then was reduced by averaging techniques to 200 samples per second.

The on-line recording and analysis of the EOG signals was performed using custom built software. This software was created by the author of this research project. The LabView G programming language (LabView v.6.1, National Instruments, USA) was used to develop the *Virtual EOG Recorder* software which emulates on the computer screen all functions of the electronic EOG paper recorder.

5.2.2.1 Monitoring eye movements in healthy subjects.

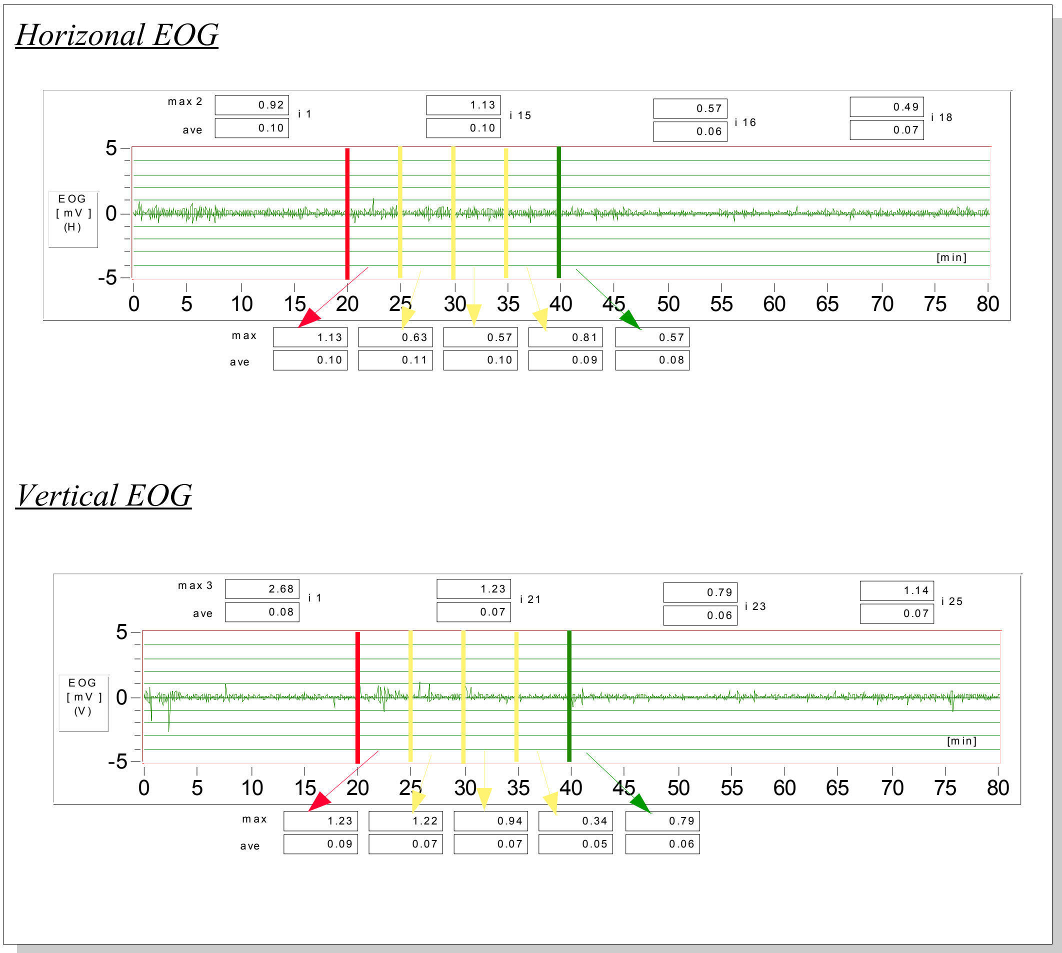
It was essential to maintain maximum comfort for the subjects during the duration of the physiological experiments. It is possible that placement of electrodes on the face may produce adverse emotional reactions from the subject. Small neonatal ECG electrodes were therefore used to record EOG because they had non-irritating adhesives. Out of the 120 experimental sessions none of the subjects complained of discomfort or skin irritation on the face. Figure 58 shows the subject with both EEG and EOG electrodes in place. Before the experimental session began subject were asked to keep their eyes closed and not to pay too much attention to the surroundings. Curtains were drawn around the treatment table where the subject was resting in order to reduce any unnecessary visual stimulation.

Figure 58. Positioning of two pairs of electrodes for EOG recording

5.2.2.2 Horizontal and vertical components of EOG

There are two distinctive components of the EOG recording, horizontal and vertical. They represent the X and Y axis of the eye movement. In fact, the X-Y graph was plotted continuously during the experiment session allowing the experimenter to "trace" the eye movements of the subject without looking at the person. For the purpose of the qualitative analysis however, horizontal and vertical components of the EOG were treated separately as two independent signals. Figure 59 shows two recorded signals from EOG surface electrodes. Numerical values above the graph represent maximum peak amplitude and average (RMS) voltage for each of the four 20 minutes intervals. Numerical values below the graph also represent maximum and average values of the EOG signal for consecutive 5 minutes intervals marked by insertion of the needle(s) (red), manual stimulation of the needle (yellow) and removal of the needle(s) (green).

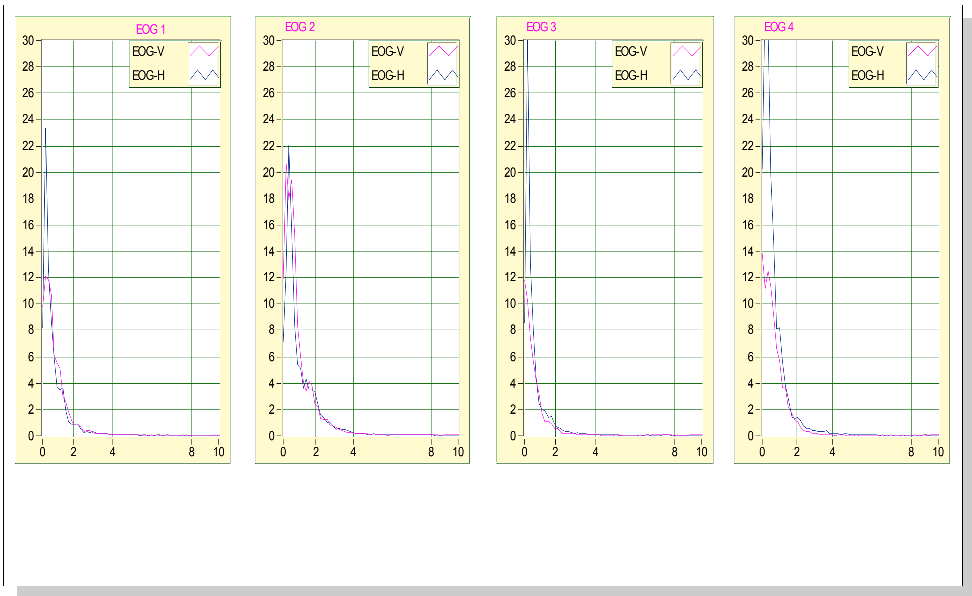
Figure 59. Horizontal and vertical components of EOG



5.2.2.3 Spectral analysis of EOG signals

In order to estimate further possible interference of the EOG signals on the EEG recordings, the spectral analysis of the EOG signal was performed in a similar fashion to that of the EEG signals. It was expected that low frequency components in the EOG signals would be identical in all four intervals and that they would not correlate with changes in the frequency spectrum of the EEG signals. Figure 60 shows an example of results for both the vertical and horizontal EOG within the spectrum of 0.05 to 10.0 Hz.

Figure 60 . An example of frequency spectrum for EOG signals



Data obtained for each of the 20 minute experimental intervals was analysed using a three stage process described in previous sections on the EEG. This somewhat cautious approach and extra steps in the EOG analysis were considered necessary in order to eliminate potential mistakes in the interpretation of results from brain wave analysis. Transferance of artifacts from the EOG to the EEG is generally considered as constant in any given experimental setting, thus estimation of variability of the EOG signals could be beneficial in the estimation of the quality of the EEG recordings.

5.2.3 Results

Data presented in Table 15 describes dependent variables used in the analysis of electrooculograms. All major parameters of EOG activity were calculated by the *EEG Analyser* and *EOG Analyser* software at the end of the experimental sessions. The 25%-75% range and standard deviations are included in the table. All values describing peak and average amplitudes of horizontal and vertical EOG are presented in millivolts. All values describing the magnitude of the frequency spectrum are reported as per 0.5 Hz bins. In order to calculate the magnitude for the whole spectrum the results should be multiplied by 3 bins for Delta, 9 bins for Theta, 11 bins for Alpha and 33 bins for Beta.

Table 15 . Dependant variables used in the analysis of electrooculograms

Measurement	*Description*	*25 % - 75 %*	*SD*	*Unit*
EOG-H Max	Peak value for horizontal movement	0.59 – 1.63	1.08	mV
EOG-H Ave	Average value for horizontal movement	0.06 – 0,10	0.03	mV
EOG-V Max	Peak value for vertical movement	0.94 – 2.63	1.32	mV
EOG-V Ave	Average value for vertical movement	0.06 – 0.09	0.04	mV
Delta	EOG-V spectrum 2.0- 3.5 Hz EOG-H spectrum 2.0- 3.5 Hz	0.15 – 0.78 0.12 – 0.40	3.30 0.66	uV^2/0.5Hz
Theta	EOG-V spectrum 3.5- 7.5 Hz EOG-H spectrum 3.5- 7.5 Hz	0.03 – 0.11 0.04 – 0.10	0.70 0.11	uV^2/0.5Hz
Alpha	EOG-V spectrum 7.5-13.0 Hz EOG-H spectrum 7.5-13.0 Hz	0.01 – 0.03 0.02 – 0.03	0.16 0.02	uV^2/0.5Hz
Beta	EOG-V spectrum 13.0-30.0 Hz EOG-H spectrum 13.0-30.0 Hz	0.002-0.004 0.003-0.004	0.029 0.001	uV^2/0.5Hz

The analysis of the results was performed in three stages in accordance with the general methodology outlined earlier in Chapter 3. Each stage had a different hypothesis to resolve and a different capacity to answer the research questions. Table 16 highlights the main features of each stage.

Table 16. Three stages of data analysis for EOG

Data analysis	Groups	Repeated measures	
Stage 1	T1-T2-T3 C1	none	30 + 30 + 30 30
Stage 2	C1 - T1	Intervals	30 + 30
	C1 - T2		30 + 30
	C1 - T3		30 + 30
Stage 3	T1-T2-T3	Intervals & Groups	30 + 30 + 30

Stage 1 of EOG analysis was focused on factorial ANOVA with the main factors being "group" and "interval" within the experimental sessions. All measurements were treated as independent measurements to reveal the main effect sizes for each variable.

Stage 2 concentrated on individual comparisons between groups, that is each of the experimental groups T1 or T2 or T3 against the control group C1. The main purpose was to compare the "active" with non-active" influences on experimental subjects. It was expected that the control group might reveal how healthy subjects perform during the 80 minute experimental sessions and how significant are naturally occurring trends.

Stage 3 analysis focused only on the "active" groups, that is T1 and T2 and T3, leaving the control group C1 aside. It was assumed that a placebo effect, if it existed in the study, would be significantly nullified. Only the differences between the influences of the treatment protocols were taken into account. It was expected that stage 3 analysis would be the strongest in detecting significant differences in the action of individual acupuncture points.

5.2.3.1 Stage 1 Analysis: Peak and average amplitudes of EOG

Calculated mean values and standard deviations for EOG amplitudes are presented in Table 17. The factorial Anova analysis revealed that there were no statistically significant differences between EOG amplitudes in various groups and intervals. As expected, the vertical eye movements elicited much higher peak amplitudes than horizontal movements, however the average (RMS) values were very similar.

Table 17. Peak and average amplitudes for horizontal and vetical EOG

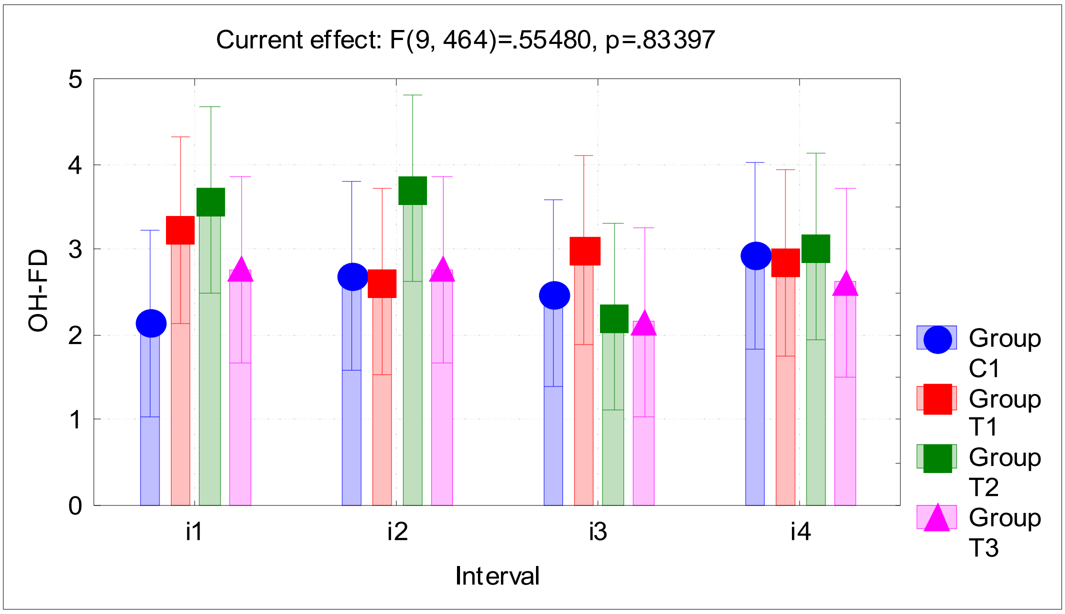
	Interval i1		Interval i2		Interval i3		Interval i4		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
C1	1.18	0.94	1.37	1.07	1.22	1.08	1.23	1.21	EOG-H Max
T1	1.14	0.90	1.39	1.00	1.09	0.73	1.63	1.53	
T2	1.08	0.87	1.22	0.88	1.37	1.20	1.47	1.21	
T3	1.09	0.95	1.70	1.30	1.34	1.04	1.40	1.21	
C1	0.09	0.03	0.08	0.03	0.07	0.03	0.07	0.04	EOG-H Ave
T1	0.08	0.03	0.09	0.02	0.08	0.02	0.07	0.02	
T2	0.10	0.02	0.10	0.03	0.09	0.03	0.08	0.04	
T3	0.09	0.02	0.09	0.03	0.08	0.03	0.07	0.02	
C1	1.82	1.33	2.22	1.57	2.09	1.56	2.01	1.70	EOG-V Max
T1	1.82	1.18	1.71	1.11	1.53	1.08	2.09	1.38	
T2	1.66	1.01	2.13	1.31	1.80	1.30	2.32	1.60	
T3	1.75	1.10	2.21	1.17	1.81	1.16	1.91	1.32	
C1	0.07	0.02	0.07	0.02	0.07	0.03	0.08	0.06	EOG-V Ave
T1	0.07	0.02	0.07	0.02	0.07	0.02	0.08	0.05	
T2	0.08	0.02	0.08	0.03	0.08	0.05	0.08	0.02	
T3	0.09	0.05	0.08	0.04	0.08	0.05	0.08	0.04	

These results are also consistent with general observations coming from the graphical evaluation of the recorded EOG and EEG signals during 80 minutes of experimental sessions. Peak amplitudes generated by eye movements were uniformly spread over time and were very similar in all experimental groups.

5.2.3.2 Stage 1 Analysis: Frequency spectrum of EOG signals

The results representing spectral analysis of the horizontal EOG for all groups (C1-T1-T2-T3) and all intervals (i1-i2-i3-i4) are shown in Figure 61. Each bar represents the value for Delta frequencies recorded during each 20 minute interval. The magnitude of Delta frequencies obtained from the vertical EOG was very similar thus only one set of results is presented on the graph.

Figure 61. Magnitude of frequencies 0.5-3.5 Hz in horizontal EOG

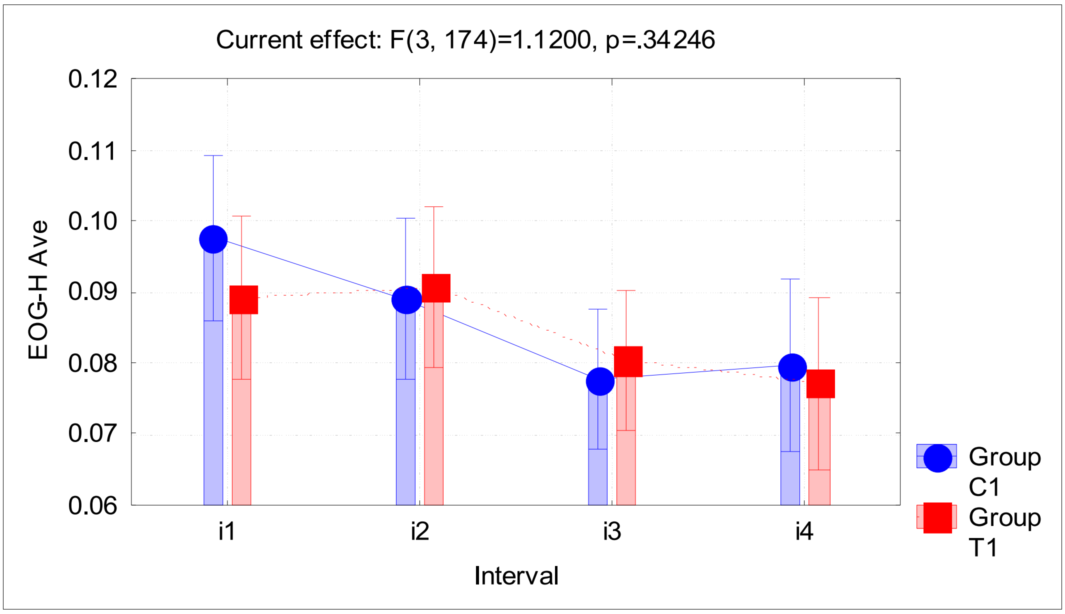


Analysis revealed that there were no statistically significant differences between the magnitude of delta frequencies in EOG for all intervals and groups. There was however a consistent bias between the results in treatment group T2 and control group C1 during the first two intervals. This bias is not statistically significant but it may suggest that there is possible psychological or procedural influence. It could be based on subject expectations and perception of safety. A stronger presence of Delta in EOG signals can be interpreted as increased eye movement, possibly due to nervousness or curiosity. Overall, no major changes in Delta have been detected due to the acupuncture treatment received during the experimental sessions. The EOG can be assumed to be identical in all groups and during all intervals.

5.2.3.3 Stage 2 Analysis: Peak and average amplitudes of EOG

The results of a comparative analysis between the control group C1 and individual treatment groups is presented in Figure 62. The measurements of average (RMS) amplitudes in horizontal EOG for intervals i1, i2, i3, i4 were treated as repeated measures ("within" group factor). The results of analysis for comparisons C1-T2 and C1-T3 were very similar and thus not included on the graph.

Figure 62. Average amplitudes in horizontal EOG for group C1-T1

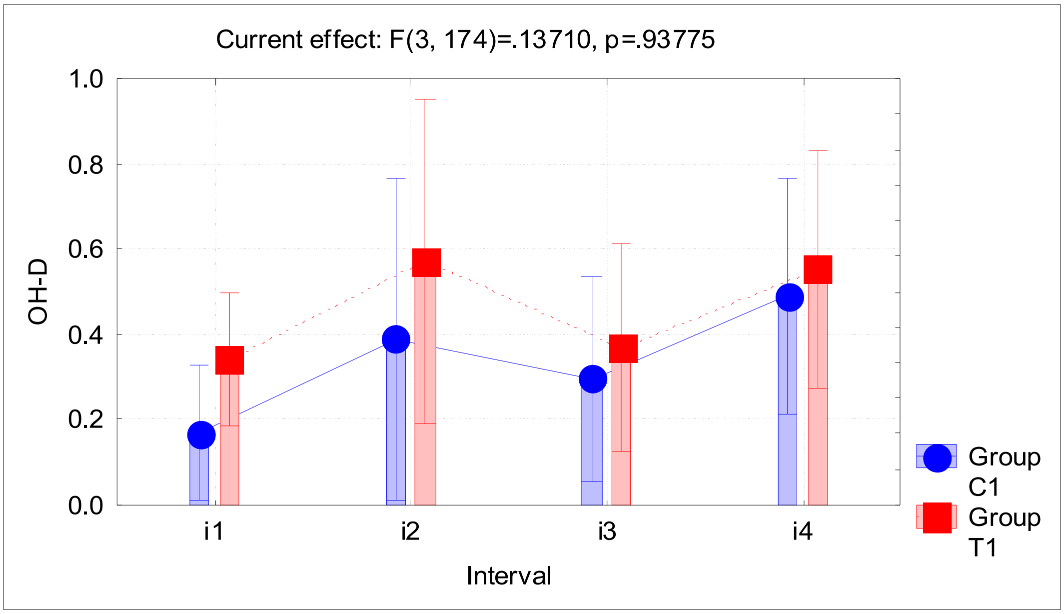


There were no statistically significant difference between average EOG amplitudes in the control group C1 and any of the treatment groups T1, T2 or T3. There was a tendency towards decreased average amplitudes during intervals i3 (40-60 minute) and i4 (60-80 minute). This may indicate the general relaxation that naturally occurred in the control group. It is important to notice that there was no significant increase in amplitudes that would be consistent with rapid eye movement (REM). This significant finding confirms that subjects were relaxed and possibly feeling drowsy, but not falling into sleep with REM episodes. These findings were unchanged even when factorial analysis was performed with factors such as anxiety or stress level. This further strengthens the assumption regarding the absence of sleep.

5.2.3.4 Stage 2 Analysis: Frequency spectrum of EOG signal

The differences in magnitude of Delta frequencies in the EOG spectrum between the control group C1 and treatment group T2 are presented in Figure 63. The measurements for intervals i1, i2, i3, i4 are treated as repeated measures ("within" group factor). The magnitude of Delta frequencies for comparisons C1-T2 and C1-T3 were very similar, therefore not included in the graph.

Figure 63. Magnitude of Delta frequencies in horizontal EOG (group C1-T1)

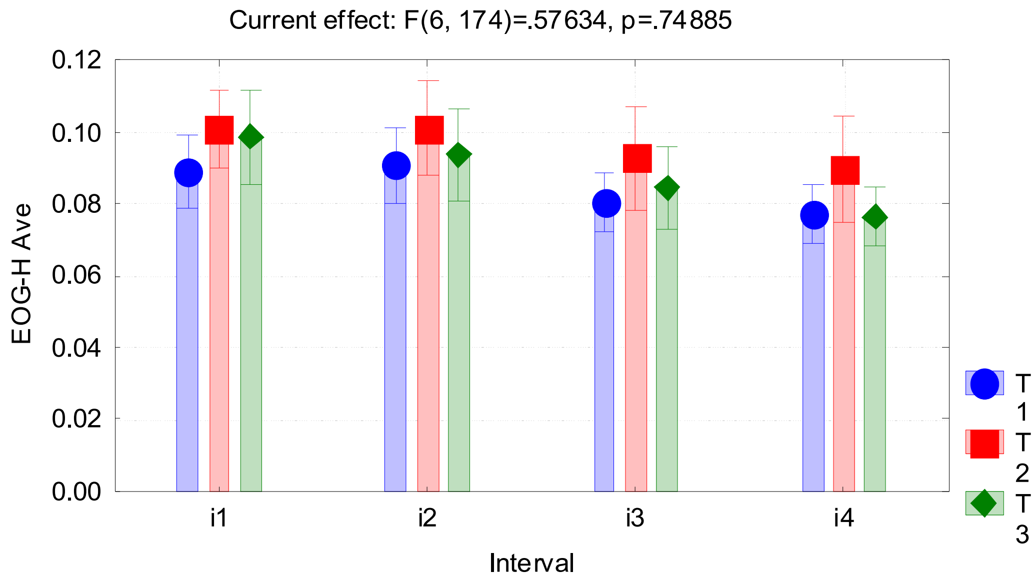


The naturally occurring trends in the control group were closely followed by similar changes in the treatment group. Analysis revealed that there was no statistically significant difference between the control group and each of the treatment groups. The differences between intervals were also not statistically significant. This finding suggests that the level of EOG activity was similar in all groups and consistent with naturally occurring trends. This gives an additional certainty that subjects were not falling into deep sleep during intervals i3 and i4. It also strengthens the assumption that the subjects were not overly stimulated by cognitive activities that required visual focusing and attention.

5.1.3.5 Stage 3 Analysis: Peak and average amplitudes of EOG

Data in Stage 3 was analysed using two level repeated measures MANOVA. The control group was thus removed from the analysis and the differences were measured only between treatment groups T1, T2 and T3. Figure 64 shows measurements for intervals i1, i2, i3, i4 that were treated as repeated measures ("within" group factor) and three treatment groups that were also considered as repeated measures ("between" group factors). Each bar represents the average amplitude of EOG during 20 minute intervals. Results for vertical EOG had a similar profile and therefore they are not shown on the graph.

Figure 64. Average amplitudes in horizontal EOG for groups T1-T2-T3

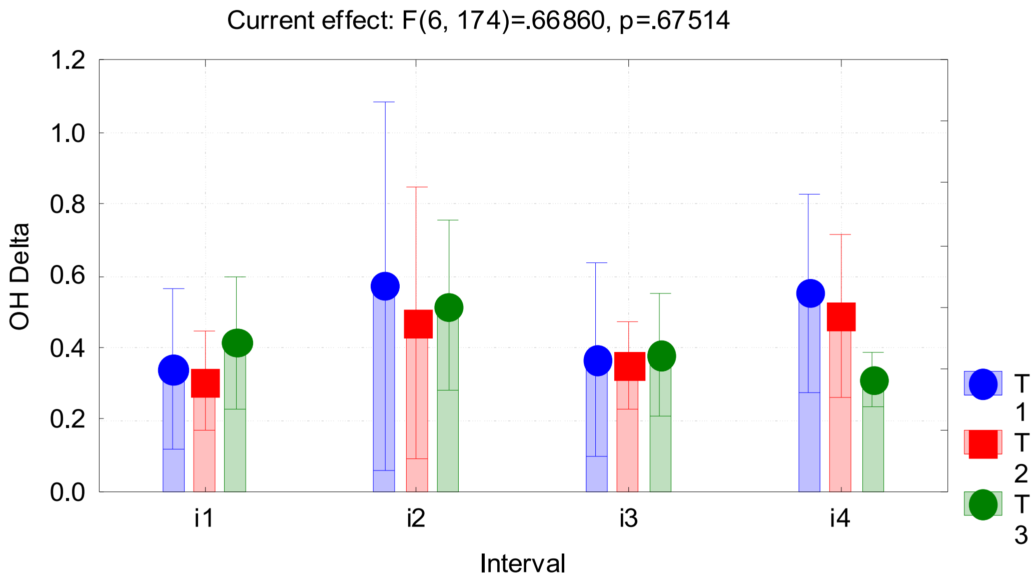


Analysis revealed that there were no statistically significant differences between groups and intervals. Average amplitudes for EOG remained the same despite changes in other physiological systems. These data support the view that subjects were not falling asleep during the experiments. There were also no indicators that the type of acupuncture treatment made any difference in recorded EOG. This suggests that subjects were, on one hand not overloaded with visual stimulation during different stages of the experiment, and on the other hand, that they were comfortable with their eyes closed.

5.2.3.6 Stage 3 Analysis: Frequency spectrum of EOG signals

Results of spectral analysis of the horizontal EOG are presented in Figure 65 below. Two level repeated measures MANOVA were performed on the set of data representing groups T1, T2 and T3, leaving out control group C1. Measurements for intervals i1, i2, i3, i4 were treated as repeated measures ("within" group factor) and three treatment groups were also considered as repeated measurements ("between" group factors). Delta frequencies were by far the most prevalent in the spectrum as they represented eye movement within normal range.

Figure 65. Magnitude of Delta frequencies in horizontal EOG (T1-T2-T3)



There were no statistically significant differences between Delta frequencies in all groups and intervals. The mean values are very similar in each of the experimental intervals. There is an increased variability of measurements in interval i2, possibly due to the fact that some of the subjects could not resist watching the acupuncture needling procedure that was performed within that interval. As expected, there was no significant increase of Delta frequencies in interval i4. This indicates that any potential artifact due to the transference of EOG signals into EEG signals remains constant for all experimental groups.

5.2.4 Conclusions

The overall results of EOG analysis suggests that there were no statistically significant differences in levels of eye movements in subjects treated with stimulation of different acupuncture points. The analysis further confirmed that the potential transference of EOG induced artifacts into EEG signals would be similar in all experimental groups.

The question about possible interference between EOG and EEG recording is always present in physiological research. Thus extra effort has been directed into frequency spectrum analysis of EOG signals, in particular very low frequency band Delta. Data analysis in all three stages confirmed that there were no significant differences between groups and individual intervals within groups. The implication of such a finding is important for the interpretation of the EEG results, in particular attributing changes in magnitude of Theta and Alpha brain waves to acupuncture stimulation. Such changes in brain waves could be induced by acupuncture treatment, by excessive artifacts associated with EOG interference, and by full sleep. The frequency and amplitude analysis of EOG eliminates these last two possible causes.

Peak amplitudes of EOG signals remained unchanged during all experimental sessions. The average amplitudes (RMS) of both horizontal and vertical EOG also remained unchanged. An important implication of this finding is in confirming that subjects were not experiencing stages of deep sleep with rapid eye movements. They reported states of relaxation, “floating”, drowsiness and even light sleep, but no state of deep sleep.

In should be further emphasised that data used in the analysis represents naturally occurring physiological events and subject behavior. At no point during the experiment were subjects prevented from falling asleep or from opening their eyes. They behaved the same way as patients in the acupuncture clinic, with the same amount of restrictions (or freedoms). Also, the recording time for all physiological parameters, including EOG, was extended to 4x20 minutes. This eliminated errors associated with mistaking transient physiological events for continuous events. This is a particularly important issue in the analysis of EOG and EEG data which is, by its nature, very dynamic.

Chapter 6. Autonomic nervous system

Control of the functions of the major body organs such as heart, lungs, intestines or liver is complex and involves many regulatory centers. Hormonal control through the endocrine system is generally slow in action. Much faster regulation comes from the nervous system, in particular from the autonomic nervous system, which forms part of the peripheral nervous system. It consists of two major components, that is the sympathetic and parasympathetic branches, which influence all vital processes in the body including respiration, heart beat, blood pressure, sweating and body temperature.

The decision to separate the description of the results from the cardio-pulmonary system in a separate section (Chapter 4) rather than including it in this chapter on ANS regulation was dictated by the following. First, both heart and lung have their rhythmic activity strongly determined by specialised regulatory centers. Secondly, sympathetic and parasympathetic regulation plays only a small part in modulation of their activity. Therefore this present chapter will describe other physiological parameters that are regulated by the ANS and cannot be assigned to any other physiological system. The electrodermal activity, body temperature (TEMP) and peripheral blood pressure (BP) were chosen as the major measurements influenced by the autonomic nervous system.

6.1 Skin potential as expression of electro-dermal activity

The term 'electrodermal activity' (EDA) is used in published literature (Critchley, 2002) to describe a number of physiological measurements such as galvanic skin response (GSR), skin conductance (SC) or skin potential (SP). They all share similar characteristics such as having tonic and phasic components. The tonic component is the baseline level of skin conductance (or skin potential), and phasic component indicates changes when certain events take place. Spontaneous changes are also possible and typically their frequency is between one to three per minute. There is general consensus that EDA is associated with changes in the activity of sweat glands of the skin (Critchley, 2002). This explanation was first proposed by Edelberg (1972) and is still used, despite facts that contradict such a theory.

6.1.1 Introduction

It is possible to utilize the EDA as a measure of psycho-physiological processes in social and physiological experiments. Such states as attention, vigilance, emotional expressions or pain, all have a strong impact on either tonic or phasic components of EDA. Their impact cannot be consciously controlled by the individual. Electrodermal activity is generally considered as being a sensitive indicator of the "importance" of specific events, either external or internal. Its professional use is probably best known as the so called "lie detector".

Application of the EDA in acupuncture research is certainly very new and little is yet known about its physiological correlates (Lewith, 2003). It is expected that the experiments conducted in this PhD project will help to validate the EDA method as an important indicator of the functional status of the autonomic nervous system during acupuncture treatment.

6.1.1.1 Physiological importance of EDA

The use of electrodermal activity in response to stimuli, as a measure of sympathetic reactivity, is frequently reported in psychophysiology (Guinjoan et.al. 1995). EDA phasic response is non-specific, which means that a similar response can be induced by a vastly different stimulus, either external or internal. What is important, however, is its sensitivity, far beyond the conscious level of human perception. A person may not perceive an external stimulus but the EDA has already registered a change in the autonomic nervous system. The EDA is used intensively in various psycho-social and physiological experiments as well as in biofeedback training. The literature suggests that there are three possible neurological pathways involved in the mechanisms of EDA which are beyond the currently understood regulation from the autonomic nervous system (Sequeira, et al. 1995). They may include cortical as well as limbic structures, hypothalamus, amygdala, hippocampus, basal ganglia and the reticular system. All these structures are part of the "old" brain, which humans share with their early ancestors (Marieb, 1998). This may indicate the primary importance of responses elicited by these structures and by the autonomic nervous system, and captured in a form of EDA.

6.1.1.2 Skin Potentials and GSR

The distinction between two forms of EDA, that is skin potential and skin conductance, is poorly acknowledged in the literature (Edelberg, 1983). On many occasions both terms are used as equivalent when in fact they are not. In the case of skin conductance it is postulated that changes in sweat glands modulate the skin's electrical resistance, and that changes can be measured with electronic devices. Skin also displays another phenomenon however, that is electrical polarization. The difference in electrical potentials can be recorded when electrodes are placed on the finger, or on opposite surfaces of the hand. There is no theory that explains how this skin potential is generated by sweat glands, or in fact if sweat glands are involved at all. Recent studies confirmed that spontaneous bursts of positive skin potential can be observed in healthy subjects during sleep. When electrodermal activity was recorded using skin conductance method on the same subjects, no such bursts were observed (Shiihara, et al. 2000). Such facts may lead to better explanations of EDA mechanisms and involvement of the so called human energy field, widely postulated in traditional Chinese medicine as *Qi*.

6.1.2 Methodology

The EDA recorded in this PhD project was based on skin potential, concentrating on the electrical polarization phenomenon, rather that skin conductance. Subjects undergoing preparation for the experimental session were fitted with the EEG cap, respiration sensors and EOG electrodes. After being positioned on the treatment table in a supine position, ECG and EDA electrodes were attached to the skin. The surface of the skin was cleaned with alcohol swabs, without using any abrasive procedures. Two pairs of Ag/AgCl electrodes were used, one pair for each hand. The “positive” electrode was placed in the center of the palm, and the “negative/reference” electrode was placed on the dorsal surface of the hand. The continuous recording of EDA was done simultaneously for both left and right hand, as it was essential to determine if the EDA has a symmetrical appearance on both sides of the body. It was expected that changes in skin potential would be similar on both sides, despite the fact that acupuncture needling was performed only on the left side. Figure 66 shows how the electrodes were placed on the subject's hand.

Figure 66. Placement of the EDA electrodes on the palm of the hand

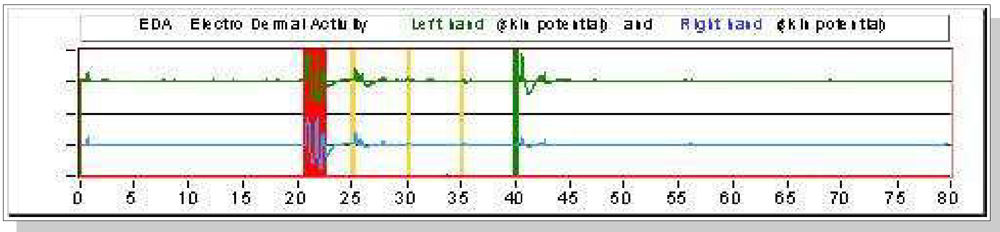
6.1.2.1 Acquisition of skin potentials

The skin potentials captured by surface electrodes were amplified and filtered using Bio2 amplifiers (Precision Instruments, UK). Gain was set to x1000 and analog filters to low-pass=30Hz and high-pass=0.01Hz. The EDA signals from the left and right hands were simultaneously digitized using 12-bit A/D converter (PCI-6028E, National Instruments, USA). Sampling rate for the digital converter was set to 1000 samples per second and then reduced to 200 samples per second by averaging. It is essential to stress that the Bio2 amplifiers were not designed to measure true DC (direct current) potentials. A 100-second time-constant (equivalent of 0.01 Hz) however was sufficient for the precise recording of very slow phasic changes in the EDA.

The on-line recording and analysis of the EDA (skin potentials) was performed using custom built software. This software was created by the author of this research project. The LabView G programming language (LabView v.6.1, National Instruments, USA) was used to develop the *Virtual EDA Recorder* software which emulates on the computer screen functions of the electronic polygraph.

The example of recorded EDA from the left and right hand is shown in Figure 67. The first 20 minutes constitute the baseline recording followed by acupuncture treatment. The vertical red line marks the time during which acupuncture needles were inserted. The yellow lines mark needle manipulation, and green line indicates removal of the needle(s) at the end of the second interval (i2=20-40 minute).

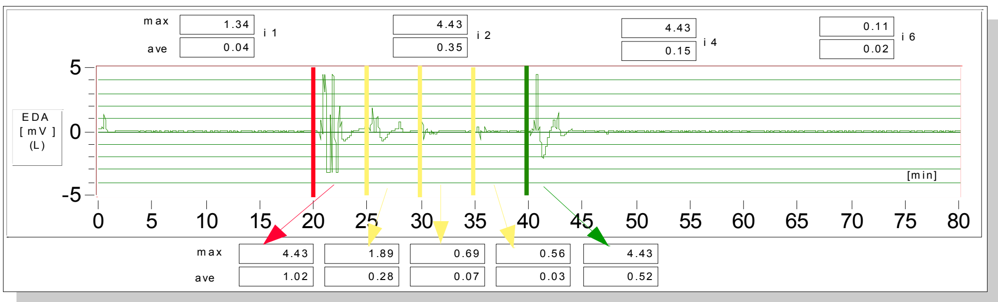
Figure 67. Simultaneous recording of EDA from both hands



6.1.2.2 Peak and average values of skin potential

The quantitative analysis of the EDA was based on two parameters, that is, peak values and average (RMS) values of skin potentials for each experimental interval. Figure 68 shows an example of such analysis. The numerical values placed above the graph represent peak (MAX) and average (AVE) values for each of the 20 minute intervals Additional analysis was also performed for each of the 5 minute intervals that occurred only during acupuncture treatment, and numerical values were placed at the bottom of the graph. At the end of the experiment all numerical values were automatically transferred to the spreadsheet ready for statistical analysis.

Figure 68. Numerical analysis of EDA (peak and average values)



6.1.3 Results

The analysis of the results was performed in three stages in accordance with the general methodology outlined in Chapter 3. Each stage had a different hypothesis to resolve and a different capacity to answer the research questions. Table 18 highlights the main features of each stage.

Table 18. Three stages of data analysis for EDA

Data analysis	Groups	Repeated measures	
Stage 1	T1-T2-T3 C1	none	30 + 30 + 30 30
Stage 2	C1 - T1	Intervals	30 + 30
	C1 - T2		30 + 30
	C1 - T3		30 + 30
Stage 3	T1-T2-T3	Intervals & Groups	30 + 30 + 30

Stage 1 of the EDA analysis was focused on factorial ANOVA with the main factors being "group" and "interval" within the experimental sessions. All measurements were treated as independent measurement to reveal the main effect sizes for each variable.

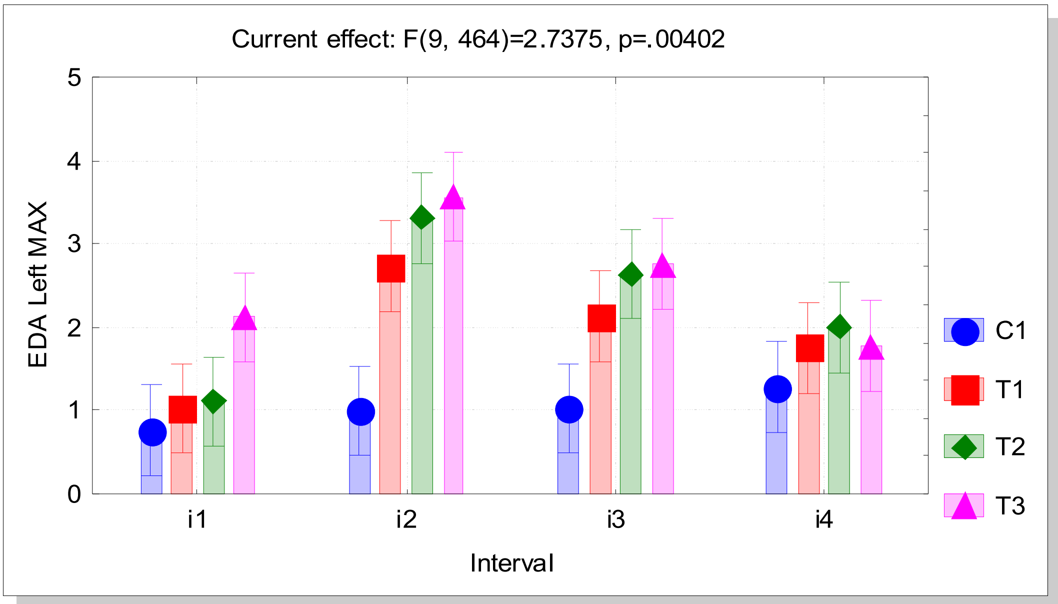
Stage 2 concentrated on individual comparisons between groups, that is each of the experimental groups T1 or T2 or T3 against the control group C1. The purpose was to compare the "active" with the "non-active" influences on experimental subjects. It was expected that the control group might reveal how subjects perform during the 80 minute experimental sessions and how significant are naturally occurring trends.

Stage 3 analysis focused only on the "active" groups, that is T1 and T2 and T3, leaving the control group C1 aside. It was assumed that a placebo effect, if it existed in the study, would be nullified. Only the differences between the influences of treatment protocols were taken into account. It was expected that stage 3 analysis would be the strongest in detecting significant differences in the action of individual acupuncture points.

6.1.3.1 Stage 1 Analysis: Peak and average amplitudes of EDA

The results representing peak amplitudes of skin potential for all groups (C1-T1-T2-T3) and all intervals (i1-i2-i3-i4) are shown in Figure 69. Each bar represents the maximum value in mV recorded during each 20 minute interval. Skin potentials on both hands were similar and they followed the same pattern of changes, therefore only results for one side are presented on the graph.

Figure 69. Peak amplitudes of skin potential recorded from the left hand

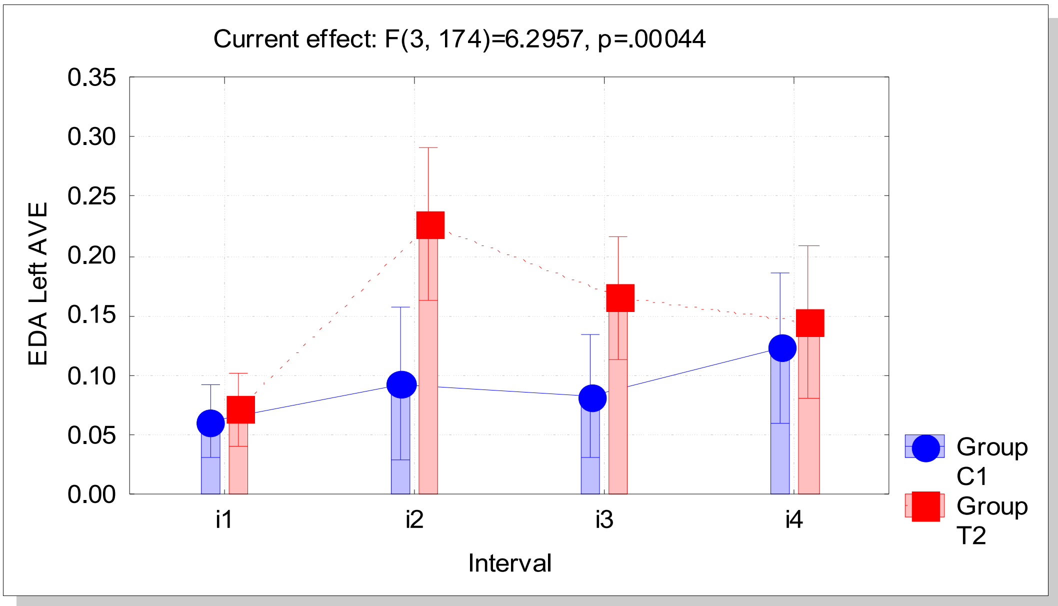


There were statistically significant differences between the peak values of skin potentials. The control group continued with unchanged values throughout the experiment, while the treatment groups showed a three-fold increase during interval i2 (20-40 min) and i3 (40-60 min). An increase in skin potential is an equivalent of decrease in skin resistance. These significant differences suggest that phasic components of the EDA are strongly influenced by acupuncture treatment. Corresponding values for both left and right hand were similar, not only in peak values but also in the shape of the waveforms which closely resembled each other. Such findings have important implications for the study. They confirm that EDA is not just a local somatic response to needling, but that it is a complex and delayed response transferred to the opposite side of the body. Further analysis using factorial variables such as anxiety or stress levels did not reveal any further significant differences.

6.1.3.2 Stage 2 Analysis: Peak and average amplitudes of EDA

The results of comparative analysis between the control group C1 and the individual treatment group T2 are presented in Figure 70. The measurements of average amplitudes (RMS) of skin potentials for intervals i1, i2, i3, i4 were treated as repeated measures ("within" group factor). The results of analysis for comparisons C1-T1 and C1-T3 were very similar and therefore are not included on the graph.

Figure 70. Differences in skin potentials between groups C1-T2

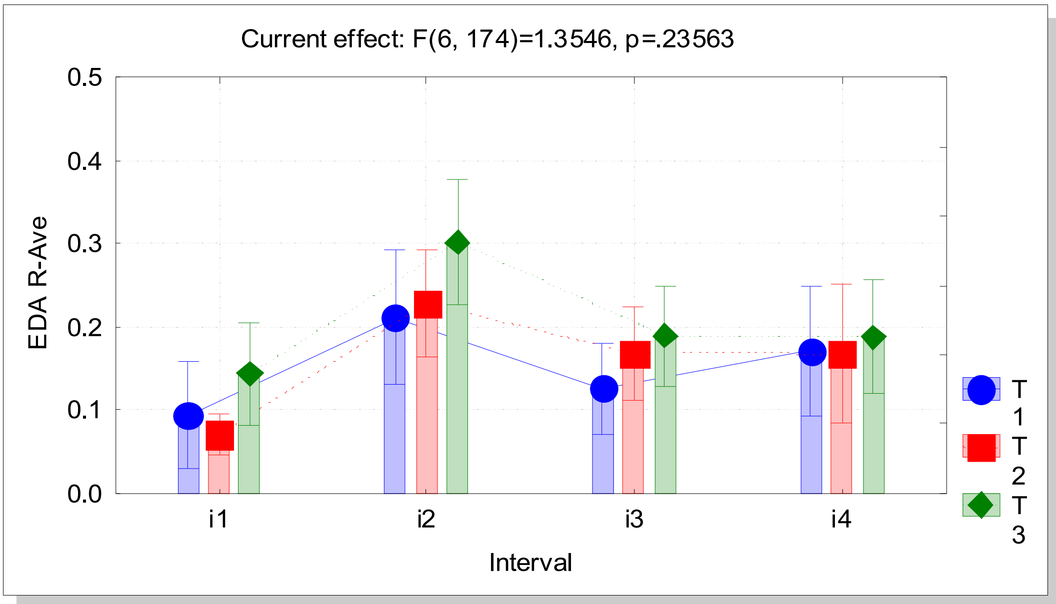


Data analysis revealed that there were statistically significant differences between average (RMS) values of skin potentials between the control C1 and the treatment group T2 (and T1 and T3). During interval i2 (20-40 minutes) and interval i3 (40-60 minutes) the increase of the EDA was very pronounced. The results for the left and right hand were similar despite the fact that needle insertion and manipulation was performed only on the left side of the body. Further analysis using factorial variables such as anxiety and LAQ scores did not reveal any further significant differences. Overall, the results confirm the importance of EDA as a measure of the responsiveness of the autonomic nervous system to acupuncture procedures. At this stage it is difficult to speculate about the nature of the triggering events, which may be related to needle insertion, somatic stimulation, expectation anxiety or some other factors.

6.1.3.3 Stage 3 Analysis: Peak and average amplitudes of EDA

Data in Stage 3 was analysed using two level repeated measures MANOVA. The control group was thus removed from the analysis and the differences were measured only between treatment groups T1, T2 and T3. Figure 71 shows measurements for intervals i1, i2, i3, i4 that were treated as repeated measures ("within" group factor) and three treatments groups that were considered as repeated measures ("between" group factors). Each bar represents the average amplitude of EDA during each 20 minute interval. Results for the left hand had a similar profile and thus they are not shown on the graph.

Figure 71. Average skin potentials (right hand) for groups T1-T2-T3

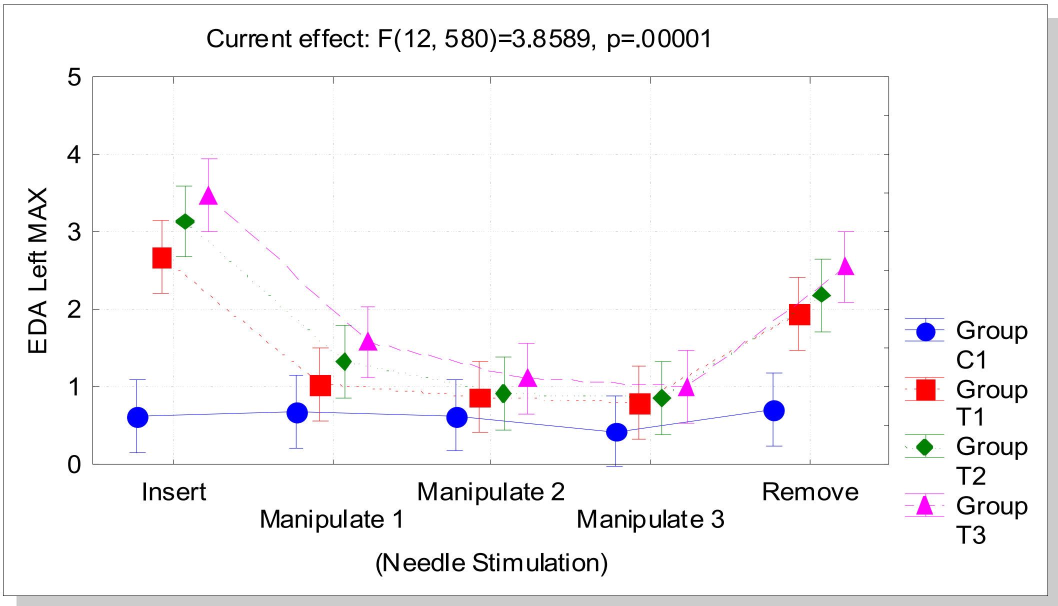


Analysis of the data revealed that there were no statistically significant differences between treatment groups. Average values for skin potentials in interval i2 (20-40 minutes) were higher than in other intervals, but very similar for each treatment group. This finding may indicate that EDA is nonspecific to the type of therapeutic action of acupuncture point or combination of points, and that it is specific to skin penetration. Needling of two points that occurred during treatment in group T3 (LU7 and KD6) did not show a significant increase in average EDA amplitudes as compared with needling of a single point. This observation is certainly worth further analysis and possible experimentation with a number of different acupuncture points.

6.1.3.4 EDA events related to needle manipulation

Detailed analyses of peak values of skin potential are presented in Figure 72. Each point on the graph represents the maximum value of EDA in mV that occurred during consecutive intervals of 5 minutes, and marked as “Insert” (20-25 min), “Manipulate 1” (25-30 min), “Manipulate 2” (30-35 min), “Manipulate 3” (35-40 min), “Remove” (40-45 min). Corresponding values for the control group C1 are marked with blue dots. Values for the right hand were very similar therefore only one set of data is presented.

Figure 72. Peak amplitudes of EDA during acupuncture treatment



There were statistically significant differences between EDA responses to acupuncture procedures. First of all, as expected the control group did not show any significant response. In contrast, all three treatment groups showed a very strong response to the insertion of the acupuncture needle(s). The manual manipulation of the needle(s) correlates with only limited EDA response, which seems to decrease with each consecutive manipulation. This finding may be consistent with well known physiological processes of habituation to external or internal stimulus (Broman and Hetta, 1994). The removal of the needle triggered changes in the skin potential, but was less marked than needle insertion. Insertion and manipulation of two needles, that occurred in group T3, did not show a significant increase in peak amplitudes of EDA when compared with single needle.

6.1.4 Conclusions

Analysis of various parameters of electrodermal activity in healthy subjects indicate that acupuncture can trigger changes in skin potential. These changes are statistically significant and can be attributed to insertion, manipulation and removal of a single acupuncture needle. Changes in the EDA are almost identical on the left and right side despite the fact that the acupuncture procedure was always performed on the left side of the body. This finding confirms that the EDA recorded in these experiments is not just a local somatic response to acupuncture, but that it is a much more complex response, possibly involving the central nervous system. Additionally, symmetrical response on both sides of the body further indicates that recorded EDA is a true physiological phenomenon rather than an experimental artifact.

The peak amplitudes of the phasic component of skin potential can reach 5 mV. There is a noticeable delay of 5-10 seconds from the beginning of the acupuncture procedure to the first increase in skin potential. Naturally occurring spontaneous changes in skin potentials can also be visible in recorded EDA, but they have much smaller peak amplitudes only reaching up to 1 mV. They were more frequent and were not found to be asociated with acupuncture procedures. A small number of subjects (around 20 %) did not show a strong EDA response. The recorded data however, does not indicate the reason for such a diminished response. One possible explanation could be a lack of precision in needle insertion into the area that is considered to be a true "acupuncture point". Such an explanation would be very favorable for acupuncture (Lund and Lundeberg, 2006). Another reason could be based on individual differences between subjects, in a similar way as distinguishing between type A and type B subjects in psychosomatic medical research (Fusilier and Manning, 2005)

Analysis of the EDA data confirms that the EDA is strongly associated with needle insertion and needle removal, but not specific to the site of the insertion. Physiological habituation seems to play significant role in diminishing the EDA response to manipulation of the acupuncture needle. Other factors such as anxiety or stress levels do not influence the outcome. Overall, these finding indicate that EDA may become a very important parameter in future acupuncture studies, as it is influenced by acupuncture .

6.2 Body temperature (TEMP)

Homeostatic regulation of the human body assures that the temperature of blood and internal organs remains constant. Small variations in body temperature are possible but only within certain narrow limits. A physiological tolerance to lower body temperatures is well developed in humans, however tolerance to increased temperatures is very limited. An internal temperature of 44 degree Celsius is very dangerous to life itself. A normal range of temperatures inside the body (core temperature) oscillates around 37.0 deg C, and may vary as much as +/- 1 deg C. The surface temperature of the skin depends largely on environmental conditions and coverage of the skin with clothing. Additionally, the temperature of fingers and toes that are distant from the torso are dependent on vascular dilation. The direct measurements of body temperature, both core and peripheral, can be utilised in physiological and psychophysiological experiments, as they provide vital indicators of the body status (Marieb, 1998)

6.2.1 Introduction

Body temperature can be measured in a non-invasive way using simple electronic devices. It is a popular measurement in any clinical setting and medical practice. It helps in the detection of fevers, hyper- and hypothermias and diseases with impaired thermo-regulation. Evaluation of body temperature is also performed in TCM practice, however without the use of measurement devices. The practitioner may ask questions regarding perception of heat or coldness, tolerance to changes in surrounding temperatures or type of stimulus that helps to relieve pain. The practitioner may also record various body signs that correlate with the temperature of the body, such as skin and lip color, pulse rate, sweating, and intensity of respiratory effort. In acupuncture research preference is given to objective methods of temperature measurements (Kuo, et al. 2004)

6.2.1.1 Physiological importance of body temperature

The stable core body temperature is an indicator of the body's ability to constantly generate heat that warms the body, and at the same time an ability to dissipate excess heat that may be damaging to the internal organs and tissues (Marieb, 1998). An ability to maintain such balance is important to health and survival. Heat generation is based on burning sugars during cell metabolism. Dissipation of heat is based on mechanisms of sweating, dilation of peripheral capillaries and increased respiration. When limits of normal temperatures are not maintained, the body may go into shock, causing many essential processes to shut down.

6.2.1.2 Correlations between body temperature and physiology

There are a number of known factors that influence core body temperature. For example day-night rhythms and seasonal changes may influence temperatures, not only by variations of external temperatures but also by changes in physical activity and diet. Generally, body temperature is lower during sleep and when physical activity is reduced. Changes in hormone levels may also modulate body temperature such as during ovulation in women. Certain metabolic disorders and dysfunctions of the thyroid gland may cause variations in body temperature (Marieb, 1998).

Traditional Chinese medicine recognises a number of disharmonies in the function of internal organs that may lead to changes in body temperature. An explanation of the mechanisms is very different to biomedical sciences, but strong emphasis is placed on the recognition that all factors that may change temperature balance. It remains an open question however, whether changes in core temperature due to acupuncture treatment are possible.

6.2.2 Methodology

Two distinctive methods are generally used in physiological studies to measure body temperature. One method concentrates on measuring peripheral temperature in uncovered parts of the body such as hands and feet. Another method concentrates on measuring only core body temperature such as inside the mouth or inner ear. One true drawback in measuring peripheral temperatures is lack of clarity in interpretation of the results. Unless changes in blood flow in the peripheral capillaries are measured at the same time, it is difficult to interpret temperature changes (Kuo, et al. 2004). Core temperature is much more stable and less dependent on peripheral circulation. The method chosen in this PhD project is based on temperature measurement in the ear. It was expected that the length of the experiment (80 minute) was sufficient to allow for slow changes in temperature to manifest if there was a physiological reason for the change.

6.2.2.1 Normal range of body temperature

It was expected that all subjects participating in the study would have body temperature in the normal range when the experiment started. Two subjects however had to cancel their sessions because they felt feverish and expected the onset of flu. They were not excluded from the study but their sessions were re-booked for the following week.

6.2.2.2 Measurement of body temperature using an infrared thermometer

The digital thermometer used in the study was the “infrared” type (Thermo-Scan 6012, Braun). It measured heat from the eardrum and surrounding tissues. The temperature probe consists of a small cone-shaped plastic cover that is inserted into the ear canal. Three measurements were made one-by-one without removing the probe from the ear. The average value was calculated to provide the temperature before the experiment. At the end of the session the three measurements were repeated, and their average value provided body temperature after the experiment (Chapter 3, General Methodology). All measurements were then manually entered into the spreadsheet in a format ready for statistical analysis.

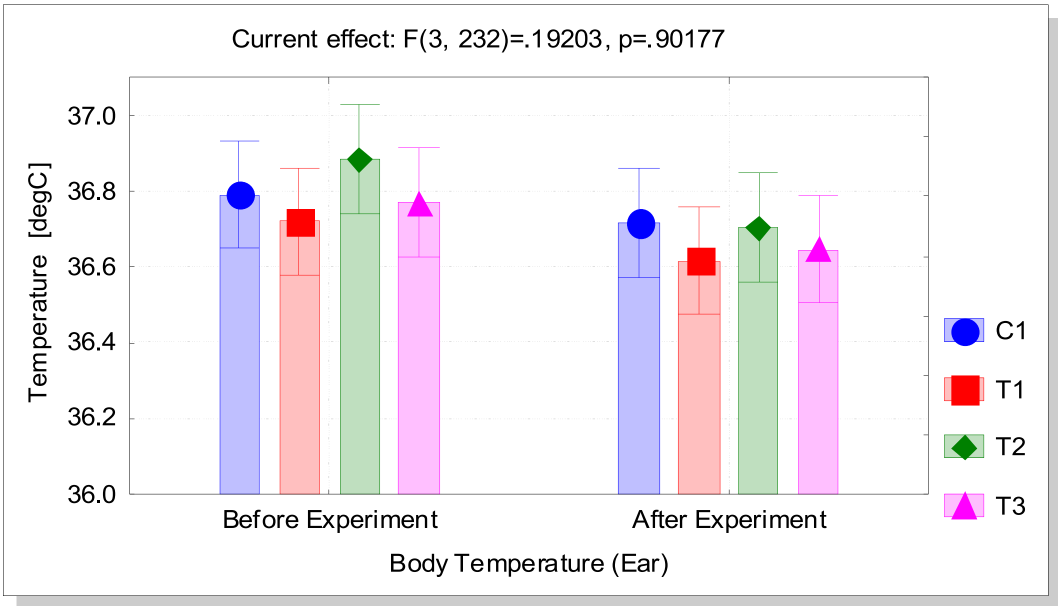
6.2.3 Results

The analysis of the results was performed in three stages in accordance with the general methodology outlined in Chapter 3. Each stage had a different hypothesis to resolve and a different capacity to answer the research questions. Measurements before and after the experiment were treated as repeated measures (“within” group). Accordingly, measurement done in different sessions were considered as repeated measurements (“between” group).

6.2.3.1 Stage 1 Analysis: Body temperature (ear)

The results representing body temperature for all groups (C1-T1-T2-T3) at two points during the session, that is before and after the experiment, are shown in Figure 73. Each bar represents the average value for three consecutive temperature measurements. Temperature in calibrated in degrees Celsius.

Figure 73. Body temperature before and after the acupuncture experiment

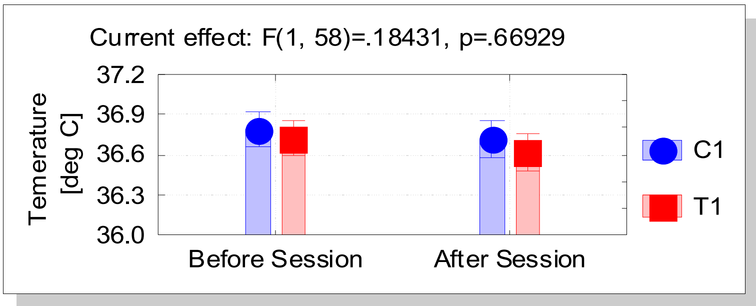


The analysis of the results reveal that there were no statistically significant differences in body temperature between groups and between "before-after" measurements. Body temperature remained extremely stable during the experimental session, and was not influenced by acupuncture treatment. Small variations between groups and a trend towards lower temperature after the experimental session were not statistically significant. The implications of such findings are twofold. A stable body temperature indicates efficient thermal regulation in the experimental subjects. Lack of a significant drop in body temperature indicates that subjects remain comfortable as far as temperature is concerned, despite being motionless for 1.5 hour. This is an expected result which confirms the necessity of measuring body temperature in the control group during the experiment where subjects movement and water intake is restricted.

6.2.3.2 Stage 2 Analysis: Body temperature (ear)

As illustrated in Figure 74, no statistically significant changes were detected in comparisons between control group C1 and treatment groups T1, using one-level repeated measures ANOVA. Core body temperature remains stable and is not changed by acupuncture treatment. Results for comparisons C1-T2 and C1-T3 were very similar and do not show statistical significance. Additional analysis revealed that factors such as anxiety or stress levels did not influence outcomes. Individual differences played an minor role in temperature measurement in healthy subjects.

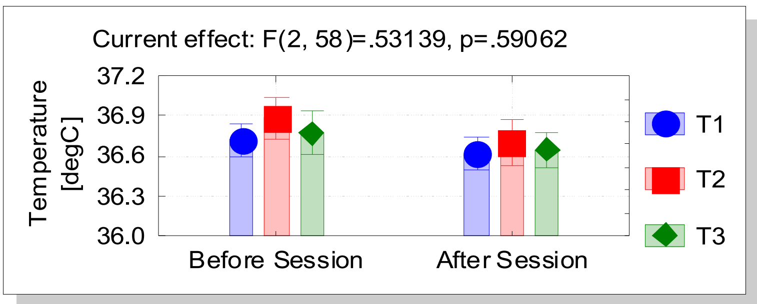
Figure 74. Comparison of body temperature between groups C1-T1



6.2.3.3 Stage 3 Analysis: Body temperature (ear)

Results of two-level repeated measures ANOVA for body temperature are presented in Figure 75. There were no statistical differences between treatment groups and before-after measurements. Such findings indicate that body temperature was not influenced by specific acupuncture treatment and remains stable during the length of the experiment.

Figure 75. Comparison of body temperature between groups T1-T2-T3



6.2.4 Conclusions

All three stages of statistical analysis indicate that there were no statistically significant differences in body temperature between the control group C1 and treatment groups T1, T2 or T3. The core body temperature remained stable and was not influenced by acupuncture stimulation of two acupoints LU7 and KD6. Such results were expected as they indicate that core temperature is regulated by a very efficient homeostatic system.

It is important to mention that all experiments were carried out in an air conditioned laboratory. The variations in room temperature did not exceed +/- 2 deg C. Subjects were covered with a light blanket to keep them warm and comfortable. It would be ideal to correlate ear temperature measurements with temperature at the periphery, for example on the palms of the hands. However, as mentioned previously, such measurements will also require the measurement of blood flow in the capillaries in order to distinguish cooling/warming affects of vascular dilution from changes in the core temperature. Unfortunately the appropriate sensors to allow such parallel measurements were not fully completed at the time when this PhD project was started.

The duration of 80 minutes for the experimental session is probably sufficient to capture temperature changes even at the core level, as it usually takes about 20-30 minute for the body to either cool down or warm up when faced with strong environmental temperature changes. However, experimentation on healthy subjects may be an insufficient model to discover the influence of acupuncture treatments on either the generation of heat or heat dissipation.

6.3 Blood pressure (BP)

Blood pressure, in a similar way to body temperature, heart rate, and respiration rate, is a fundamental physiological parameter that characterizes health and disease. It is defined as pressure that is exerted on the internal surface of the blood vessels. Rhythmic contractions of the heart muscle force the blood to circulate through a network of arteries, capillaries and veins. The peak pressure that occurs during contraction of the heart is called systolic pressure, and pressure during the resting phase of the heart is called diastolic (Marieb, 1998). Typical arterial blood pressure in healthy adults is around 120 mmHg systolic and around 80 mmHg for diastolic. Blood pressure is largely regulated by the autonomic nervous system, and does not remain fixed at one level. It may vary substantially depending on physical activity, stress levels, hormonal and nutritional factors, as well as pharmacological and narcotic substances taken by the person. Overall agreement exists that the measurement of systolic and diastolic blood pressure has primary importance in the diagnostic process and in the evaluation of physiological performance of the cardio-vascular system.

6.3.1 Introduction

Measurement of blood pressure can be performed using invasive and non-invasive methods. Invasive methods are most accurate but they involve placing a cannula into the artery. Of course in human studies only non-invasive procedures are used. They are less accurate however, and based on the application of an inflatable cuff around the arm. The cuff is filled with air until the arteries are completely occluded. When the air is slowly released, it is possible to detect Korotkoff sounds that indicate the return of the flow of blood in the artery. At that precise moment pressure of the air in the cuff is equal to the pressure of the blood in the artery (Marieb, 1998).

For long term measurements of blood pressure the oscillometric method is frequently used in which the cuff is inflated automatically. Detection of the return blood flow is achieved by an electronic device. Usually, systolic and diastolic blood pressure are displayed in digital form, together with frequency of the heart beat. Clinical guidelines specify in detail how blood pressure should be measured in order to avoid inconsistent or erroneous reading (Myers, 2004). They include recommendations for allowing the person to rest for at least 5 minutes before measurements are taken, repeating the measurement three times and using an average value. Smoking, eating and medication should also be taken into account when BP measurements are made (Appel, et al. 2006).

6.3.1.1 Physiological importance of blood pressure

There must be sufficient blood pressure in the vessels in order for circulation to occur against total peripheral resistance. Such factors as cardiac output and elasticity of the arteries influence blood flow. Physiological mechanisms behind blood pressure regulation are very complex and not completely understood by science (Marieb, 1998). So far only three such mechanisms have been well documented, that is baroreceptor reflex, rennin-angiotensin system and aldosterone release (steroid hormone). Through baroreceptor reflex the autonomic nervous system exerts its regulatory influence. Baroreceptors can be found in many organs of the body as well as in major blood vessels, and their function is to detect local changes in blood pressure. When required, the ANS may respond to such changes by modifying frequency and force of heart contraction. Total peripheral resistance which consists of resistance of many thousands of small arterioles in the body, can also be altered by the autonomic nervous system (Marieb, 1998).

6.3.1.2 Correlations between blood pressure and physiology

Persistent high blood pressure called hypertension can become a serious medical problem. It puts the arterial walls under increased mechanical stress leading to unhealthy tissue growth (atheroma). Continuous overload of the heart muscle may progress its thickening and enlargement. It may also lead to stroke and result in disability due to damage to the brain (Marieb, 1998). Lower than normal blood pressure, called hypotension, may also be a sign of disease. If the blood supply to the brain is insufficient it may cause dizziness and fainting. Other symptoms associated with low blood pressure are chest pain, shortness of breath, irregular heart beat and headaches. In all cases they require medical attention (Munver and Volfson, 2006).

Direct measurement of blood pressure is not used in acupuncture or traditional Chinese medicine. Practitioners may however evaluate the strength and other qualities of the pulse during patient examination. Both acupuncture and herbal medicine offer effective prescriptions for patients suffering from high blood pressure. Some of the acupuncture points are credited with regulatory action on blood vessels, cardiac output and heart rhythm. Thus it is reasonable to expect that stimulation of acupuncture point(s) may influence systolic and diastolic blood pressure even in healthy subjects (Backer, et al. 2003).

6.3.2 Methodology

Accurate measurement of peripheral blood pressure requires not only use of an accurate electronic device but also adherence to the clinical guidelines on how blood pressure should be measured. Correct positioning of the cuff on the subject's arm is very important as well as the resting position of the body. The Royal College of General Practitioners in Australia recommends taking three BP measurements and averaging the reading. Patients should be seated or resting in a supine position. As blood pressure could be easily elevated by stress, it is essential that BP measurements are made when the patient's emotions are calm. Stimulants such as coffee or alcohol should be avoided, as they may increase both systolic and diastolic BP. All the measurements of blood pressure collected in this project were acquired according to these recommendations.

6.3.2.1 Normal range of blood pressure

Only subjects with normal blood pressure were accepted into the study. Information regarding basic health requirements for participation were clearly defined in the advertising material. Before the experiment started all subjects enrolled in the study were asked to fill in a questionnaire regarding their health status and known medical conditions. This process was designed to recruit only healthy subject. The “before experiment” blood pressure measurement was performed when subjects were already fully prepared for the experiment and resting on the treatment table. In all cases no abnormal blood pressure was detected either at the beginning or at the end of experiment, placing all subjects into the “normal” range (RACGP, 2006).

6.3.2.2 Measurement of blood pressure

The measurement of systolic and diastolic blood pressure was performed using non-invasive oscillometric methods (Digital Blood Pressure Meter - Omron M4). Three measurements were made one-by-one without removing the cuff, but allowing about one minute between measurements for the arm to rest. The average value was calculated to provide blood pressure before the experiment. At the end of the session the three measurements were repeated, and their average value provided blood pressure after the experiment (Chapter 3, General Methodology). All measurements were then manually entered into the spreadsheet in a format ready for statistical analysis.

6.3.3 Results

The analysis of the results was performed in three stages in accordance with the general methodology outlined in Chapter 3. Each stage had a different hypothesis to resolve and a different capacity to answer the research questions. Measurement before and after the experiment were treated as repeated measures ("within" group). Accordingly, measurements done in different sessions were considered as repeated measurements ("between" group). Table 19 highlights the main features of each stage.

Table 19. Three stages of data analysis for blood pressure

Data analysis	Groups	Repeated measures	
Stage 1	T1-T2-T3 C1	none	30 + 30 + 30 30
Stage 2	C1 - T1	Intervals	30 + 30
	C1 - T2		30 + 30
	C1 - T3		30 + 30
Stage 3	T1-T2-T3	Intervals & Groups	30 + 30 + 30

6.3.3.1 Stage 1 Analysis: Systolic and diastolic blood pressure

The results representing peripheral arterial blood pressure for all groups (C1-T1-T2-T3) at two points during the session, that is before and after the experiment, are shown in Table 20. Average values from three consecutive measurement were used in calculations. Mean values and standard deviations for systolic and diastolic blood pressure are included in the table.

Table 20. Systolic and diastolic BP "before" and "after" experiment

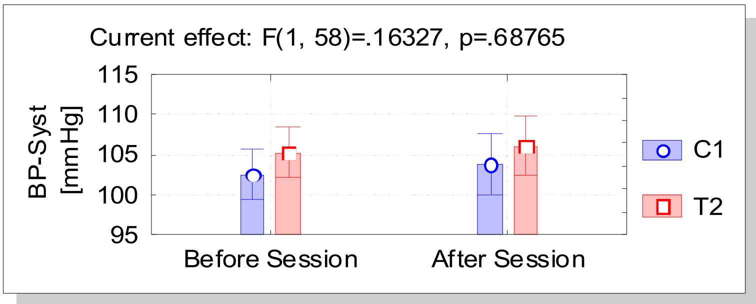
	Before Session				***After Session***			
	Systolic BP		Diastolic BP		Systolic BP		Diastolic BP	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C1	**102.5**	10.6	**66.1**	6.2	**103.8**	12.3	**65.6**	7.8
T1	**105.3**	7.1	**67.1**	5.9	**107.0**	7.5	**68.9**	5.9
T2	**105.3**	6.8	**67.8**	6.6	**106.1**	7.8	**69.1**	7.1
T3	**104.7**	7.0	**67.8**	6.6	**105.5**	7.3	**68.8**	6.5

Analysis of the results revealed that there were no statistically significant differences in systolic blood pressure between groups, and "before-after" measurements. Similarly, there were no significant differences in diastolic blood pressure. Blood pressure remained extremely stable during the experimental session and was not changed by acupuncture treatment. The control group showed consistently lower readings than the treatment groups, but that trend was not statistically significant. Standard deviations were small indicating small individual differences between subjects. Lack of significant change in blood pressure before and after the experiment indicates that BP is a stable physiological parameter in healthy subjects, not easily influenced by acupuncture treatment at points LU7 and KD6, or by environmental factors. Results from control group C1 show that blood pressure was not changed due to physical inactivity and rest of 1.5 hour duration. This outcome was expected and confirms the importance of having reference measurements from a control group in the initial stage of data analysis.

6.3.3.2 Stage 2 Analysis: Systolic and diastolic blood pressure

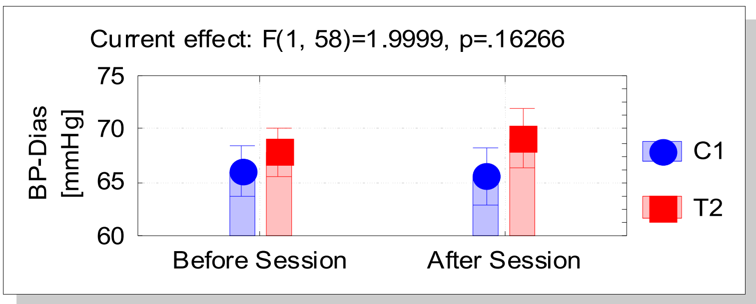
There were no statistically significant differences detected between control group C1 and any of the treatment groups T1 or T2 or T3. Figure 76 presents results from one-level repeated measures ANOVA for comparison C1-T2 in systolic blood pressure recorded before and after the acupuncture experiment. Outcomes for comparisons C1-T1 and C1-T3 were very similar and also do not show statistical significance. The individual differences were very small which was expected in healthy subjects.

Figure 76. Comparison of systolic BP between groups C1-T2



Analysis of the diastolic blood pressure revealed that there were no statistically significant differences between the control group C1 and any of the treatment groups T1 or T2 or T3. Figure 77 shows results from one-level repeated measures ANOVA for comparison C1-T2 in diastolic blood pressure recorded before and after the acupuncture experiment. Outcomes for comparisons C1-T1 and C1-T3 were very similar and also do not show statistical significance.

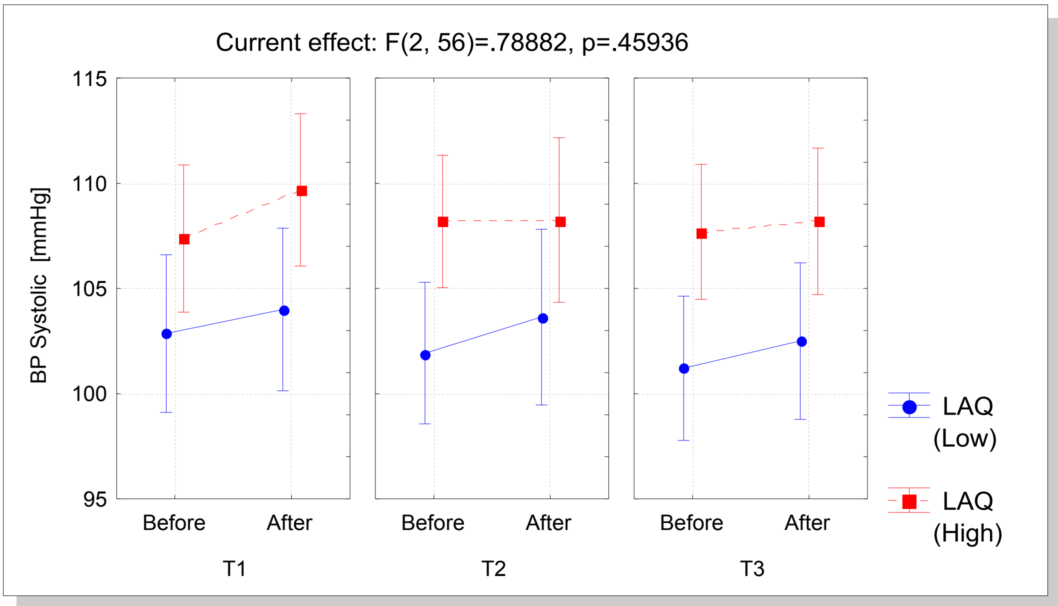
Figure 77. Comparison of diastolic BP between groups C1-T2



6.3.3.3 Stage 3 Analysis: Systolic and diastolic blood pressure

Analysis using two-level repeated measures ANOVA confirmed that there were no statistically significant differences between treatment groups T1 and T2 and T3. Both systolic and diastolic blood pressure remain the same before and after the acupuncture experiment. Such findings indicate that blood pressure is a very stable physiological parameter, and that it is not easily influenced by acupuncture stimulation of acupoints LU7 and/or KD6, but despite the fact that there were only small variations due to individual differences, introduction of additional factors into the analysis revealed an interesting occurrence as illustrated in Figure 78.

Figure 78. Systolic BP in subjects wih high (red) and low (blue) stress level



There was a consistent non-significant elevation of systolic blood pressure in subjects with higher stress level (defined by higher scores on LAQ test). The difference between means is around 5%. It is important to emphasize that a group size of n=30 was too small to provide sufficient power to detect differences at such a small level as 5%, thus statistical analysis did not confirm that such differences were significant. Nevertheless, this finding further raises the question about the importance of internal factors such as stress and anxiety, and their potential ability to modulate physiological outcomes in healthy subjects.

6.3.4 Conclusions

All three stages of statistical analysis indicate that there were no statistically significant differences in peripheral blood pressure between the control group C1 and treatment groups T1, T2 or T3. The systolic and diastolic blood pressure remained very stable and was not influenced by acupuncture stimulation of two acupoints LU7 and KD6. There was also no specific difference between any of the three treatment interventions T1 or T2 or T3 in terms of blood pressure variation.

The BP measurement methods adopted in this PhD project are similar to methods used in ambulatory and hospital settings, thus results could be used for direct comparisons. All subjects recruited for the study displayed good general health and their BP was in the normal range. It should be emphasized that BP was measured only at two points in time, that is, at the beginning and at the end of the experiment. Any potential changes in BP during the 80 minute session were not subjects of this investigation. In fact, continuous measurement of BP is possible but it requires frequent inflation of the measurement cuff placed around the arm or the wrist. Such a procedure may cause medium intensity discomfort to the person and prevent that person from relaxing or falling into light sleep. This of course could significantly change experimental results and was avoided.

The 80 minute duration of the experiment may not be sufficient to allow for long-term effects of acupuncture treatment to occur. It is essential to remember that only the baroreflex mechanism of BP regulation is able to respond quickly to external or internal stimulus. The remaining mechanisms are slow in action and their involvement cannot be evaluated in a relatively short period of time (Marieb, 1998).

Overall, measurement of systolic and diastolic blood pressure provides significant information about the physiological status of a person. For a group of healthy subjects it confirms that they are in good cardiovascular health. For hypertensive subjects it would be an indicator of the severity of blood pressure imbalance. Future research with hypertensive patients and the use of more specific acupuncture point combinations, may help to investigate further possible regulatory effects of acupuncture interventions.

Chapter 7. Neural Network analysis

Traditional statistical tools are widely employed to model and analyse data that represent problems that are linear or approximately linear. If the problem has linear characteristics then it would be counterproductive to use much more complex non-linear methods such as neural networks to analyse them. Neural networks analysis will not perform any better in solving linear problems and are prone to the errors of over fitting the data. In situations when non-linearity of recorded phenomenon is obvious however, such as in physiological models, non-linear methods may provide exceptional benefits (Higuchi, et al. 2006).

7.1 Introduction

There is a growing popularity in using neural networks in many areas of science and research. Wherever the linear statistical tools fail to solve complex problems, neural networks seem to step in with ease and offer solutions, in particular when issues of classification, control and prediction are of major importance. In the last decade neural networks were successfully employed in engineering, finance, geology and medicine.

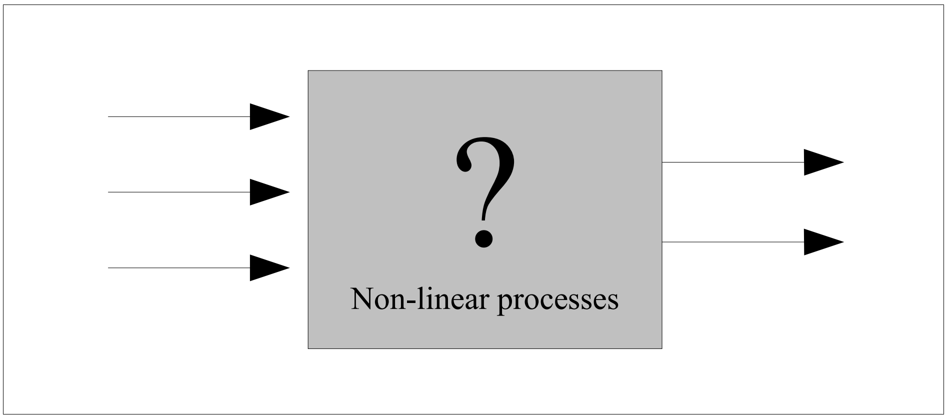
7.1.1 Complexity of the data with multiple dependent variables

Data collected in physiological experiment can be quite complex. Typically, not only multiple variables are recorded simultaneously, but they are recorded from different physiological systems. This complexity represents an enormous challenge for statistical tools such as ANOVA or MANOVA which require that linear approximations are valid. For example the dynamic changes of the heart rhythm in response to therapeutic intervention may not have the same pattern as changes in respiration. The brain waves may represent complex patterns of activity but only in certain parts of the brain and only under certain experimental conditions. Physiological experiments may produce very complex data sets that contain hundreds or even thousands of variables. Neural network modelling techniques provide solutions when modelling very complex functions is required, and when a large number of variables is provided (Malmagren, 2000).

7.1.2 Black Box approach in physiological research

It is frequently difficult to provide an accurate hypothesis in regards to the nature of physiological processes. It is much easier to describe and control experimental variables than to define accurately processes involved and to describe the outcomes. The so called "black box" approach has been successfully used in engineering and medicine to model the unknown phenomenon. The black box has inputs and outputs, and unspecified "processes" that link inputs and outputs as represented in Figure 79.

Figure 79. Black box approach to modeling physiological processes.

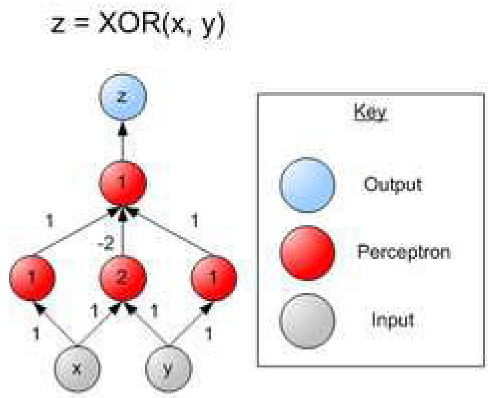


In order to utilize neural network techniques, the experimenter must know, or at least suspect, that there is a causal relationship between changes in inputs, that are known, and changes in outputs that are unknown. This is normally not an issue in physiological experiments when correlations between variables can be assumed. The flexibility of not having to specify the exact nature of the relationship between different variables gives the researcher a chance to include in the analysis a wide range of variables. When an advanced analysis is required, some of the variables can be continuous and some can be categorical, mixed together in the same research model (Papik, et al. 1997).

7.1.3 Neural Network way of processing data

Neural networks learn from examples that are given to them. At first a set of representative data is given to the neural network. By invoking training algorithms the networks automatically learn the structure of the input data. It is assumed that the learning process is similar to processes used in cognitive systems and that they mimic neurological functions of the central nervous system. Thus neural networks acquire the ability to predict the nature of new observations from the observations done during the training process (Malmagren, 2000). The most common approach is supervised training, where the training data set contains both input and output data. Training data may consist of previous records, or it may be acquired in the pilot studies or baseline measurements. The neural network learns to discover the relationship between variables by adjusting the weights and thresholds of its own "neuronal" components, in order to minimize the error in its predictions of outcomes. After a number of repetitions during the training phase, the neural network acquires an ability to classify new and previously unknown events. Figure 80 shows a simple model of the neuron that has the ability to adjust sensitivity of its own inputs.

Figure 80. Model of a simple neuron when used in Neural Networks



Adapted from http://en.wikipedia.org/wiki/Artificial_neural_network

The biological inspiration for neuronal networks architecture is more symbolic than practical. Real networks of biological neurons are immensely more complex and still subject to speculation rather than scientific fact (Malmagren, 2000). Nevertheless, simple neuronal models are quite adequate to mimic the behaviors of systems that can regulate their own response in accordance to previous experience.

7.2 Methodology

The scope of this PhD project only allowed for limited involvement in non-linear analysis. The major component of the data analysis so far is based on traditional ANOVA / MANOVA statistical tools as described in Chapters 4-5-6. Thus, in order to illustrate the possibilities of neural network analysis in acupuncture research, only well known and accepted algorithms and approaches have been used. Current knowledge regarding applications of neural network is so extensive that software development companies such as StatSoft usually supply templates, as part of the package, for the less advanced users (Statistica Neural Networks, StatSoft, US).

7.2.1 Intelligent Problem Solver

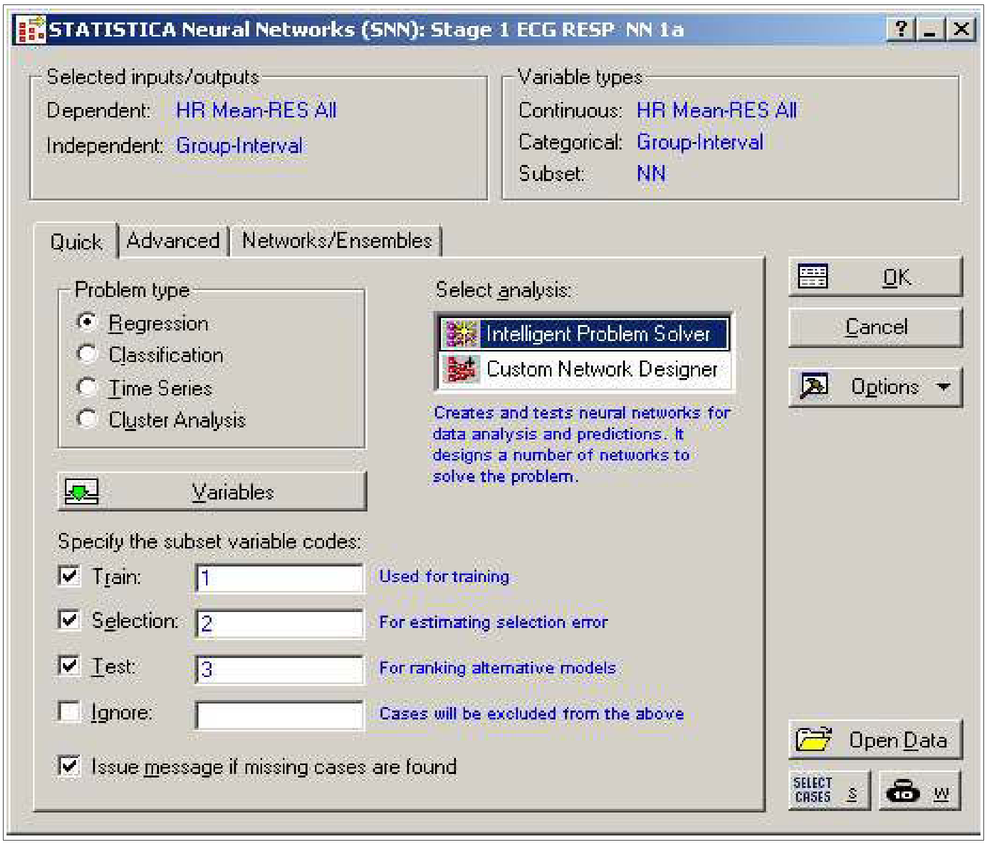
The steps in preparing data for neural network analysis is similar to steps in statistical analysis. One clear difference is when creating data tables. All variables should be marked with the selection codes so they can be grouped into training and testing sets accordingly. Values from the training set will be used to train the network, which is done primarily by making required estimates for the network's weights and other parameters. The testing set of data is used for an independent check of the final network performance. Sets must be kept separate in order to avoid over fitting network performance into specific data sets (Statsoft, 2006)

The Intelligent Problem Solver is a "specialized tool to analyze the data and generate neural networks ... requiring minimal interventions .... and conducting all necessary phases of the analysis" (StatSoft, 2006). Without this tool the majority of researchers will not be able to benefit from neural network analysis because it would be too complex and too time consuming to experiment with various options available.

All aspects of analysis that are specified by the Intelligent Problem Solver are based on the extensive experience of neural network designers, and encapsulated in the ready to use algorithms. The more advanced users have a choice of building new individual networks to fit exact specifications. Such advanced work however is exceeding the scope of this research project.

Figure 81 shows the panel of the user interface that allows the selection of all essential parameters for neural networks such as categorical and output variables, type of the problem to be solved and the coding for the subsets used in training and testing. The Intelligent Problem Solver trains simultaneously several networks with different architectures, and then computes predictions from each of them.

Figure 81. User interface of the Intelligent Problem Solver in Statistica NN.



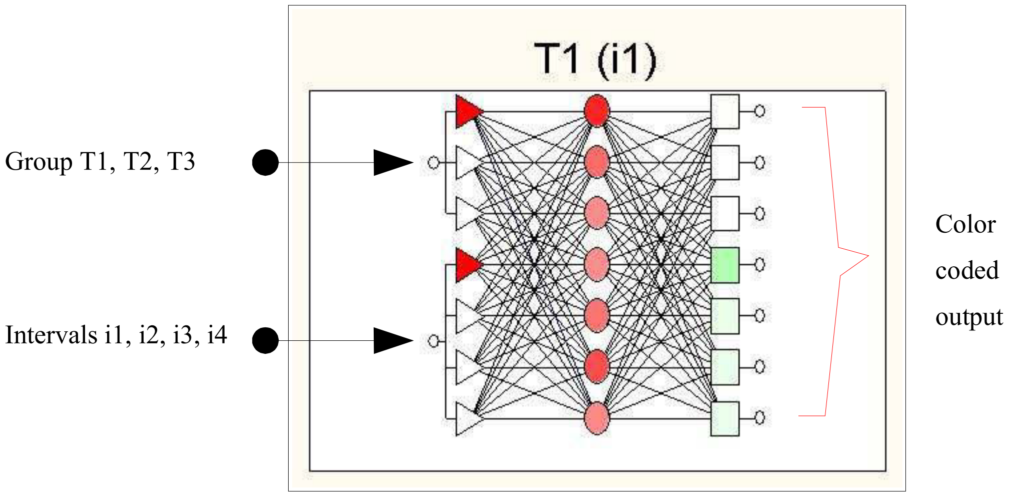
7.2.2 Setting parameters for Neural Networks analysis

Two data sets have been prepared for neural network analysis. The first set contained seven continuous dependent variables and two independent categorical variables representing the cardio-pulmonary system. The second set contained sixty continue dependent variables representing EEG and two independent categorical variables. In each analysis the training subset included data from control group C1. Data from treatment groups T1-T2-T3 were equally divided into selection subset and testing subset. The reminder of the required parameters were set up automatically by the Intelligent Problem Solver and were not altered. The ten best neural networks with the smallest errors were displayed automatically as results of the analysis.

7.2.3 Graphical representation of the results

The way in which neural networks deal with the data is of course different to traditional statistical methods. Because of this, the way in which performance of neural networks is illustrated also differs significantly. Neural networks do not display means, standard deviations or p-levels. Instead, they display results as color coded outputs. These outputs represent differences between what the network has learned to "see" as normal during the training process, and what are the actual outputs from new data sets. Figure 82 illustrates an example of NN analysis from the cardio-pulmonary data. The dependent variables are not visible on the graph, but categorical variables such as group and interval are represented as inputs on the left side. The color coded output can be considered as a simplified chart. By comparing outputs from the same network, but under different input conditions, the researcher is able to see the overall differences.

Figure 82. Example of graphical representation of NN analysis



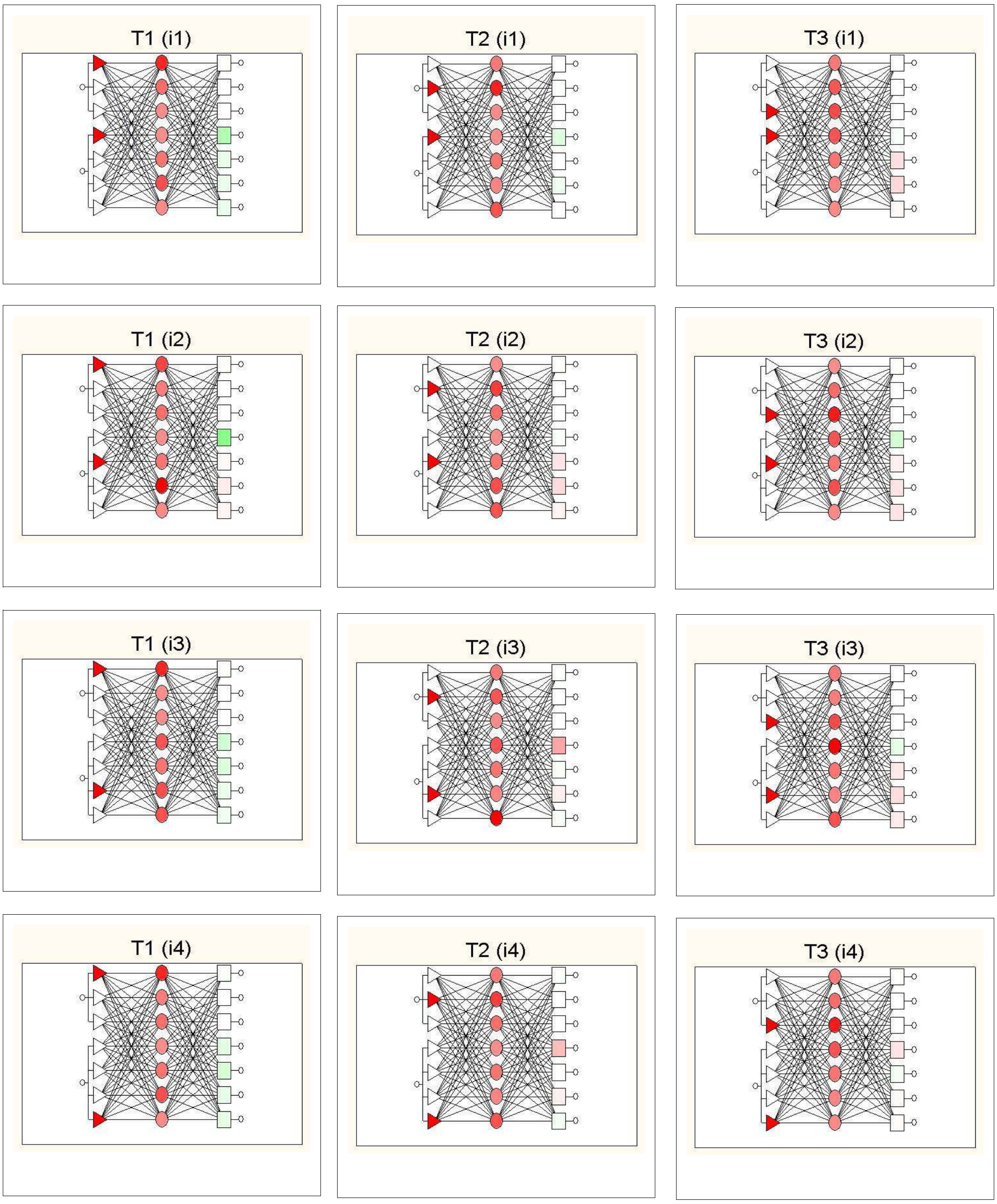
The color coded outputs can not be directly linked to individual variables, but instead they represent the network's 'convergent' response to complex events described by dependent and categorical variables. It is the responsibility of researcher to associate meaning with individual patterns, which in physiological research is an advantage.

7.3 Results

7.3.1 Case 1 - Neural networks analysis for cardio-pulmonary data set

An example of Neural Networks analysis of cardio-pulmonary data is presented in Figure 83. Separate graphs are shown for each pair of categorical variables, that is “group” and “interval” in accordance with the design outlined in general methodology.

Figure 83 Results from Neural Networks analysis of cardio-pulmonary data.



7.3.2 Case 2 - Neural networks analysis for brain waves data set

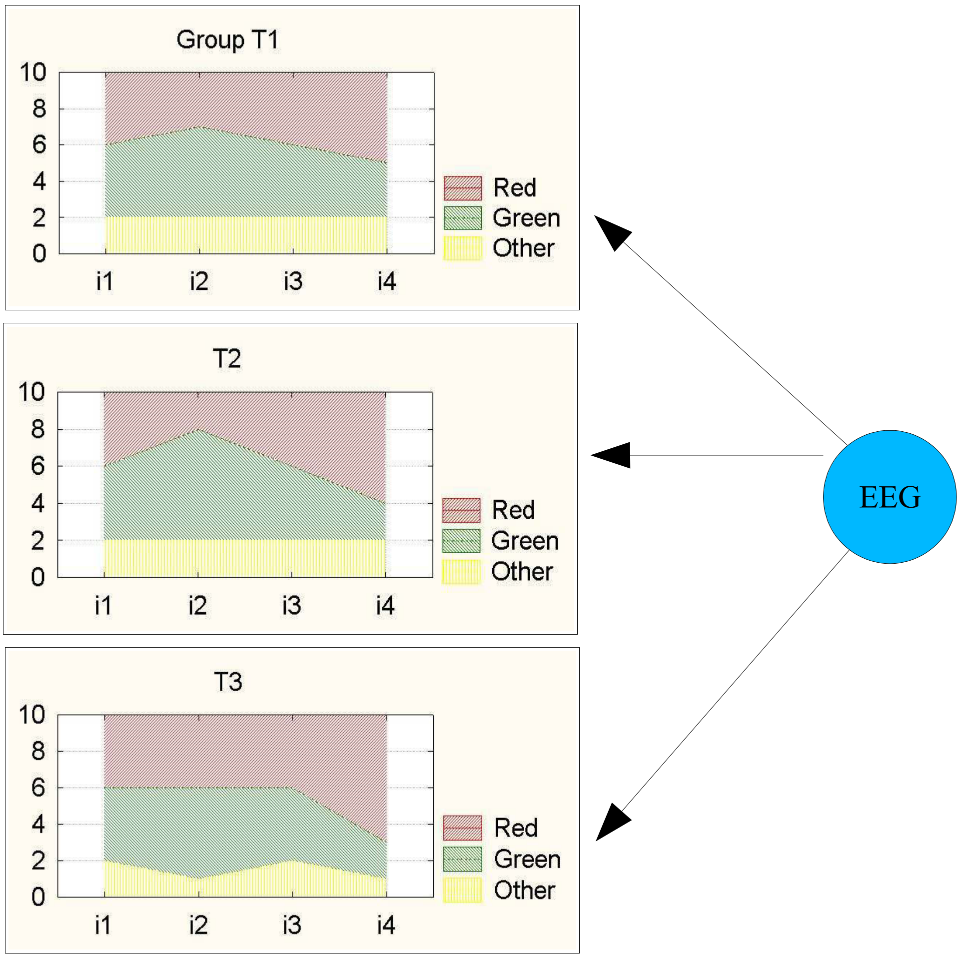
An example of Neural Networks analysis of brain waves is presented in Figure 84. Separate graphs are shown for each pair of categorical variables, that is “group” and “interval” in accordance with the design outlined in general methodology.

Figure 84 Results from Neural Networks analysis of brain waves.

7.3.3 Cumulative results from Neural Networks analysis

The results of Neural Networks analysis from Figures 83 and 84 may be considered as "accumulative" results and thus represented on sequential/stacked 2D plot. Because the network's output is in this case represented by 10 color squares, it is possible to plot the ratios between red / green / other colors.. For example Figure 84 showed that NN results for group T1/i1 were 4/10 red, 4/10 green and 2/10 others. It is assumed that such ratios could represent the magnitude of the brain's electrical activity during the experimental sessions. Figure 85 shows three stacked plots for each treatment group. In order to appreciate this summary of results for the EEG signal, it is necessary to remember that it is a summary of 60 dependent variables. This is the "overall" view of the EEG activity during the 80 minute experiment, as seen by trained Neural Networks.

Figure 85 EEG results after Neural Networks analysis



7.4 Conclusions

Application of Neural Networks analysis in acupuncture studies is certainly very novel. It required additional preparation of raw data and use of specialized software to conduct the analysis. Legitimate questions can be asked here. Why do we need Neural Networks analysis at all? The answer is very simple – because traditional linear statistical tools do not give sufficient answers. For example when dealing with single dependent variables in ANOVA analysis it is easy to distinguish between significant and non-significant differences. However when there are ten variables and each of them follows different trends over time, it is not easy at all to combine such results. Such a dilemma is certainly encountered in EEG sleep studies when complex patterns of brain activity must be detected first in order to divide sleep into meaningful physiological states such as rapid eye movement (REM) sleep.

The main question that the acupuncture researcher is interested in may be formulated as follows: Is there a difference in physiological activity of the organ or body system in response to acupuncture treatment? Or more specifically, is overall activity of the heart (brain, lungs, etc.) any different after acupuncture intervention. Traditional linear models do not easily provide such answers. They can show that, for example, sympathetic nervous system influence heart rate variability and at the same time respiration remains unchanged. However when 10 or 20 variables simultaneously form a pattern, it is very difficult to decide if such a physiological pattern is any different to a pattern from another treatment group or the control group.

Results presented in Figure 83 for cardio-pulmonary analysis indicate that the pattern of physiological changes in group T2/i4 is different to the rest of the recorded intervals. This comes as a welcome surprise, because a similar conclusion has been reached after complex and laborious ANOVA / MANOVA analysis. Results presented in Figure 84 and 85 are even more interesting because they represent activity of 60 variables from EEG recordings. Initial patterns of EEG activity during interval i1 seems to be similar in all three treatment groups. However in the last interval of the experiment i4 (60-80 minute) differences between groups are clearly visible. Further implications of such findings will be discussed in Chapter 8.

Chapter 8. Discussion

The results of the study that were presented in previous chapters will now be evaluated from the perspective of the aims and research hypotheses that were used to guide this project. Overall, 60 healthy subjects were recruited for the study, and 120 experimental sessions were completed in order to collect these data. It took nearly six months of intensive laboratory work to complete all experimental sessions. This effort was preceded by almost two years of software programming in order to build an automated neurophysiological laboratory that was able to collect and analyse such a large amount of data. No major methodological difficulties were encountered during this period and the experiments for this PhD project were completed according to schedule.

There are a number of practical and theoretical issues that will be discussed in this chapter, ranging from evaluation of research aims to recommendations regarding optimal research protocols for the acupuncture laboratory. It is hoped that such a discussion will not only highlight the main features of this current study but also improve future research projects in acupuncture.

8.1 Aims of this study

The main objective for this PhD project was to examine possible applications and evaluate the usefulness of non-invasive neurophysiological methods in detecting physiological changes in response to manual acupuncture. It is essential to state that the purpose of this study was not focused on finding proof that acupuncture works in a clinical setting or how it works. The aim was to confirm that acupuncture can be successfully researched using objective neurophysiological methods.

A rigorously designed RCT was used to evaluate the effectiveness of selected non-invasive neurophysiological methods. Three-point criteria was set up. Firstly, to investigate if manual stimulation of the acupuncture points can induce physiological changes in healthy human subjects, and to investigate if the degree and the direction of physiological changes, if they occur, can be consistently monitored by neurophysiological methods. Secondly, to investigate if physiological changes induced by acupuncture stimulation form a pattern, which is unique to each individual acupuncture point being tested. Thirdly, to investigate if physiological changes induced by acupuncture treatment are more pronounced in subjects initially showing stronger signs of subjective / objective stress.

8.1.1 Why do we need physiological research in acupuncture

Review of the acupuncture research conducted at the beginning of this doctoral project revealed that only a small fraction of the published research uses physiological measurements as indicators of objective outcomes. In contrast, there are many examples of biomedical research using objective physiological measurements, either at organ, tissue or cellular level. This makes comparisons of effectiveness of acupuncture with other treatment modalities very difficult. It is true that acupuncture research of clinical outcomes concentrates on the subjective wellbeing of the patients, on improved functioning of the major body organs and on decreasing pain and discomfort. Such measures are also used in biomedical research. However, acupuncture research does not often ask questions about physiological changes that occur simultaneously with subjectively reported 'wellbeing".

When critics say that acupuncture has no scientific basis, they do not say that the acupuncture phenomenon does not exist. All they are saying is that there is no evidence, within the framework of objective science, to either accept or deny its existence. That is why we need physiological reseach in acupuncture. We need to confirm in objective ways the existence of changes that can be independently recorded by unbiased observers. This goal has been achieved in this research project by showing that physiological changes in major physiological systems accompany the acupuncture phenomenon.

8.1.2 Can acupuncture research rely on neurophysiological methodologies?

The question regarding sensitivity of the measurement methods is essential in any objective study. It is clear that the method must be able to not only detect changes but to do it with the required resolution and within the range defined in the study. When subjects in the study are healthy individuals as in this research project, and when they remain at rest during the experimental session, the naturally occurring changes in physiological parameters are much more subtle. This, on one hand is very helpful, because it reduces variability. On the other hand it leaves some unanswered questions about the readiness of the body to respond to subtle acupuncture procedures. The results of this study confirmed that the methods employed to perform physiological measurements were sensitive enough to monitor changes in heart rhythm, respiration rate, brain waves, eye movements and in electrodermal activity. The resolution was sufficient for the creation of smooth distribution histograms. One exception was the measurement of core body temperature which remained very stable and almost unchanged in all experimental subjects.

The neurophysiological methods have many advantages when applied in acupuncture research. They offer compatibility and cross referencing with biomedical studies, they are objective, and they produce data that can be easily processed by computer systems. Also, an automation of the laboratories that use such methods is now common practice. The results of the study conducted in this PhD project clearly show that such methods provide outcomes that can be utilized is randomised trials of acupuncture.

8.2 Principal findings

Three major physiological systems have been evaluated in this study, that is the cardio-pulmonary system, the brain and somatic system, and the autonomic nervous system. The physiological response to acupuncture was clearly detected and the results of analysis confirmed that stimulation of a single acupuncture point can invoke physiological changes. Anxiety and stress levels influenced the outcomes in a significant way.

8.2.1 Single acupuncture needle makes the difference

One of the most important findings in this research were changes in physiological measurements in response to stimulation of a single acupuncture point. These changes were statistically significant and are present in all three physiological systems. For example, acupuncture treatment used in group T1 (LU7) and in group T3 (LU7 and KD3 together) promotes deeper relaxation and light sleep in subjects showing higher levels of anxiety. In contrast, subjects in group T2 (KD6) do not relax or fall into light sleep that easily. This finding was confirmed in Stage 3 analysis which reveled that it is mainly Theta and Alpha “relaxation” waves that change predominantly. Of the two treatment protocols T1 and T3, the T1 shows the strongest differences. It can be interpreted as an indicator of a leading role of LU7 acupuncture point in promoting relaxation and light sleep. The physiological effects induced in the central nervous system gradually accumulate over the period of 40 minutes after the withdrawal of acupuncture needles. Such results are in agreement with observations in clinical setting.

The EEG method seems to be sensitive enough to detect changes attributed to the stimulation of a single acupuncture point. Stage 1 analysis did not reveal statistically significant results, indicating that without the use of repeated measures there is insufficient resolution in the methodology. However analysis in Stage 2 confirmed that Delta and Theta waves were influenced by acupuncture stimulation. That influence is highly modified by internal conditions of the subject such as anxiety level and stress levels associated with lifestyle. In other words, acupuncture tends to normalize the subject's condition.

8.2.2 Stress and anxiety as modulators of acupuncture effect

The role of emotions has been always acknowledged in traditional Chinese medicine. The results from this study confirm that the emotional state of the subject plays important role in modulating acupuncture effects. For example, data from the experiments clearly showed that stimulation of two acupuncture points LU7 and KD6 in subjects reporting an increased state of anxiety after the experiments, does not increase sympathetic physiological response from the heart, despite such a response in the control group. A differential analysis in stage 3 indicated that statistically significant treatment effects existed only for the acupoint KD6. The increase in the sympathetic regulation in subjects with reported low trait anxiety is very interesting, and may indicate that KD6 has a balancing effect on heart rhythm. Also, when the heart rate variability ratio between sympathetic and para-sympathetic is low, the acupuncture stimulation of KD6 seems to increase it, but only when the anxiety is low. This once again indicates a normalization effect of acupuncture.

8.2.3 Synergy between two acupuncture points has not been confirmed

The traditional functions associated with the acupuncture points LU7 and KD6 are not easily translated into biomedical terms. They describe energetic properties rather than external physiological effects. It has been expected however that at least the LU7 point will have a noticeable effect on respiration, possibly increased by synergistic influence when combined with KD6 in one treatment. This expectation however has not been confirmed by objective neurophysiological measurements in this study. The frequency and amplitude of respiratory movements remanded the same over an 80 minute experimental session. It is very possible, however, that the expected changes were manifested with a considerable delay of some few hours, thus escaping detection during the experiment. It is also possible that the expected outcome would occur with the addition of the acupoint LI4 – the traditional combination of acupoints to increase lung functions.

It is essential to mention that the acupuncture points LU7 and KD6 used in this study belong to the group of points associated with eight extra meridians. Their synergistic energetic action is mentioned in many clinical texts (Ross, 1995). Results of this study however do not confirm the existence of physiological synergism within three major physiological systems. On the contrary, stimulation of the acupuncture point KD6 appears to have nullified effects on relaxation induced by the acupoint LU7. Long term study would need to be conducted in order to further clarify such results. It should be noted however that the traditional role of these two points, used in combination, is mainly to regulate hormonal cycles in women, in treatment of lung and kidney problems, and in problems of emotional balance such as panic attacks, phobias and depressions (Ross, 1995).

8.3 Research model

Acupuncture is considered a safe and effective form of complementary and alternative medicine. However the evidence regarding its effectiveness and mechanisms is very limited. Available research is of poor quality with contributing factors being a lack of reliable methodologies and incompatibility of research models with theoretical framework of acupuncture. Acupuncture research is still in its early stages despite the growing number of publications.

The neurophysiological methods that are used in acupuncture research have been borrowed from other scientific disciplines, such as physiology (ECG), neurology (EEG) and psychophysiology (EDA). Through the efforts of many researchers in the past, they were tested and evaluated for implementation into specific fields of study such as cognitive psychology, psychiatry or sport medicine. Despite frequent attempts to use these methods in acupuncture research, little has been done in order to validate these methods on human subjects. The study described in this project is one of the first of its kind, concentrating primarily on validation of the research models in acupuncture.

8.3.1 Need for objective measurements and RCT model

The methodological requirements of this research project where closely aligned with requirements for randomised controlled trials in biomedical research. A separate control group of subjects was recruited for the study in order to observe naturally occurring changes in physiological parameters over time. Subjects in the treatment group were asked to attend three separate sessions, one for each different type of acupuncture treatment. The sequence of these sessions was randomly allocated. Only female subjects participated in the experiments in order to reduce gender-based variability of physiological data. Psychological and life style factors such as stress and anxiety levels were evaluated through self reporting questionnaires.

8.3.2 Choice of acupuncture points

The review of previously published acupuncture research revealed that few acupuncture points have been tested in physiological experiments. The same points are used in different studies, preference being given to PC6, ST36, LI4 and LR3. Point combinations have not been tested at all, except when combined in the treatment of specific disease conditions. It has been acknowledged in this research project that any acupuncture point can be tested, but the choice was governed by two principles. One, that the points may have possible synergistic / antagonistic actions, and second, that they are routinely used in clinical practice. The limitation of time and resources has forced this project to be limited to testing just one pair of acupuncture points. Future research may extend these physiological investigations to other points.

8.3.3 Evaluation of stress and anxiety levels

Biomedical research recognises stress and anxiety as factors in the creation of a physiological imbalance. An adaptive response to stress may have both beneficial and detrimental effects on health. A number of illnesses are correlated with psycho-somatic conditions and good evidence exists supporting such associations. In traditional Chinese medicine emotions were always recognised as one of the primary factors in the creation of disease (Ross, 1995; Kaptchuk, 1983).

In order to evaluate the impact of stress and anxiety on outcomes in acupuncture treatment, all subjects in this research project were asked to complete psychological tests. Such initial assessment of lifestyle and anxiety allowed analysis of the physiological data with additional factorial variables. For example, results indicated that stimulation of acupoint KD6 increases the sympathetic affect on the heart, but only in subjects with an initial low anxiety level as measured before the experiment. In similar ways, subjects with a lower score on LAQ test (less stressed) showed a statistically significant increase of Theta brainwaves in response to stimulation of LU7 acupuncture point.

The size of this study was not sufficient to precisely define such associations, but the findings should have a positive impact on acupuncture research methodology. In simple terms it means that it is incorrect to conduct acupuncture experiments without obtaining at least basic data about the psychological state of subjects involved in the experiments.

8.4 Data collection process

One of the major shortcomings of acupuncture research in general is its inability to deal with patterns or clusters of physiological changes. Experimental research frequently reports only individual physiological characteristics in isolation from other physiological variables. Comparisons are frequently made between active and non-active 'sham' acupoints rather than between active points only. Such limited models of research are prone to errors of assigning naturally occurring changes as active properties of acupuncture points.

8.4.1 Simultaneous recording of multiple physiological variables

One of the aims of this project was to monitor simultaneously multiple independent and dependent variables, and then discover if specific patterns of change can be assigned to individual acupuncture points. It has been postulated in the chapter on general methodology that neurophysiological methods can only be classified as useful if they can make the distinction between patterns of physiological changes in response to the stimulation of different acupuncture points. An introduction of objective measurements and multichannel recording into acupuncture research allowed the capture of many concurrently occurring physiological events. There are only few other published studies to date which address acupuncture methodology with such detail and precision. This is certainly an achievement of this thesis.

8.4.2 Non-invasiveness of measurement methods

Discomfort or pain associated with experimental settings was reduced to a minimum. It has been acknowledged however that the experiment required a certain degree of patience and acceptance of experimental protocol from the subjects. The preparation for the experiment and experimental session itself was certainly a novel experience to all participants, however none of the subjects complained of difficulties during the experiment.

The nature of the acupuncture phenomenon is strongly linked with the notion of Qi and meridians through which Qi circulates in the body. Insertion of the needles into acupuncture points triggers only a small sensory effect, and the process of needle manipulation can hardly be noticed by most patients. It is obvious that in order to research such subtle phenomenon, measurement methods cannot be either painful or traumatic to the subject. Therefore the choice of non-invasive measurement methods is important. Electronic instruments allows monitoring simultaneous activity in many physiological systems. The acupuncture research laboratory that was established as part of this PhD projects now allows measurement of physiological activity of the heart, lungs, brain and sensory nerves, autonomic nervous system and eye movements.

The non-invasiveness of the methods used in this acupuncture research has one more advantage. Such methods can be used not only in research laboratories but also in acupuncture clinics. The amount of simultaneous measurements will be reduced for practical reasons, but the objective measurement of even one physiological variable such as ECG or pulse could provide important information about the patient's health. It can be proposed that the new concept of acupuncture practice will emerge in future, where the practitioner-researcher occupies a central role in creating evidence that supports clinical practice. Such a model has long been established in general medical practice, where doctors participate in trials and supply data for clinical research.

8.5 Data analysis

The analysis of data has been performed in three stages, each representing a different approach to data comparisons, such as treatment vs. control, or treatment vs. treatment. Analysis in Stage 1 used no repeated measures, but in Stage 2 and Stage 3 one level repeated measures and two-levels repeated measures were used respectively. The benefits of such approach can be clearly seem when for example analysis in Stage 1 failed to detect any changes in brain waves in response to acupuncture treatment, but analysis in Stage 3 did distinguish between actions of two different acupuncture points. In Stage 1, when only factorial analysis was performed, methodology was not sensitive enough for acupuncture research, and the study groups were two small to secure sufficient values for the 'power of experiment'. In Stage 2, when data from control group was compared against data from each of three treatment sessions, the methodology was sensitive to detect changes. Concurrent changes over time in the control group indicated that only a 'differential' approach such as in Stage 3 can have any real value in acupuncture research. In Stage 3, treatment groups were compared with each other. Methodology proved to be sensitive enough to detect changes between different treatments protocols, despite naturally occurring baseline variations.

8.5.1 Towards "differential" model of data analysis

It may seem as if substantial effort has been spent in order to illustrate differences between Stage 1, 2 and 3 analysis. These differences however are very important because they highlight the fact that measurements of absolute values are probably of little importance. In clinical practice only knowledge about similarities and differences between actions of acupuncture points most likely have any value. The review of previously published acupuncture research shows that vast majority of research is still trapped in Stage 1or Stage 2 model of analysis, and that they are looking at control vs. treatment or sham vs. treatment comparisons.

The "differential" approach has been implemented in this project. The analysis in Stage 3 revealed that the detection of the physiological patterns is not only possible but provides interesting correlations between activity of the major physiological systems of the body. It confirms that the physiological effects of stimulation of different acupuncture points can be distinguished and that a sham acupuncture group is not necessary in order to detect the effects of acupuncture stimulation.

8.5.2 Neural networks analysis as an alternative to MANOVA

A Neural Networks analysis has been introduced in this study. Neural Networks have the ability to detect complex physiological patterns. This method of analysis requires complex preparation of data sets and the use of specialised software tools. Once the initial preparations and training of the NN is done, analysis is extremely simple. The significant differences between physiological patterns are displayed in a form of color coded charts rather than means, standard deviations and p-values. For the acupuncture researcher this could be a very valuable model of analysis.

Non-linear analysis of the experimental data by using Neural Networks gave very interesting results. It has to be emphasised that it is a novel method even in well established biomedical laboratories. It is obvious that the most important question in the mind of the acupuncture researcher is first and foremost the question regarding overall change. The initial investment in developing NN analysis could pay off very quickly in laboratories where many acupuncture points are going to be tested under the same experimental conditions.

8.5.3 Assessment of overall physiological status is possible

It is certainly an achievement of this research project to successfully introduce a set of consistent physiological measurements into acupuncture research. Traditional pulse diagnosis and subjective evaluation of the health status of the subjects were deliberately omitted from the experimental protocol. The rationale for such an approach was not based on disrespect for traditional diagnostic methods, but rather on the need for clarity and objectivity in the evaluation of experimental outcomes. Some of the major criticisms of acupuncture research in the past have been focused on inappropriate use of research models, overuse of subjective evaluation methods and too strong a reliance on case studies. The results of this study confirmed that the physiological status of the healthy subject can be monitored, and comparisons could be made between pre and post treatment physiological conditions.

8.6 Future research

The main aims of this project have been achieved. The overall evaluation of the findings confirmed that neurophysiological measurements are appropriate for the acupuncture laboratory. The outcomes of the study showed that stimulation of a single acupuncture point can trigger physiological changes. These changes can be consistently recorded and used to distinguish between the actions of different acupuncture points.

Such objective physiological research can be extended into three important areas. First, research into mechanisms that are involved in acupuncture phenomenon. This could support teaching and advance basic knowledge. Second, research into the physiological effects of acupuncture could promote a better understanding of the action of individual acupuncture points. It may also help to verify the traditional understanding of acupuncture. Third, research into the clinical effectiveness of acupuncture in disease states. Normally this is the most difficult to conduct and most expensive to run. It requires collaborative agreement between practitioners and patient groups.

8.6.1 Possibility of testing all major acupuncture points

Insertion and manipulation of a single acupuncture needle can invoke physiological changes. If the insertion and manipulation of the needle constitutes an acupuncture treatment then the conclusion from this study is straightforward – acupuncture treatment can induce physiological changes. However the results of the study suggest that the combined effect of two or more points may not be immediate. It took about 20-40 minutes from the time of needle insertion for physiological effects to manifest in the central nervous system.

There are over one hundred frequently used acupuncture points. Each of these points could be tested in an acupuncture research laboratory for its physiological effects. It is possible to imagine that once the knowledge about different points starts to accumulate, new classifications could be created, based on physiological characteristics rather than on energetic characteristics. Such a possibility of testing numerous acupuncture points could have a very important and positive influence on acupuncture.

8.6.2 Creation of physiological markers for acupuncture research

One of the most important findings of this research project are changes in electrodermal activity recorded from the palms of both hands. Results of the analysis clearly confirmed that acupuncture triggers changes in skin potential. Changes in EDA are almost identical on the left and right side despite the fact that the acupuncture procedure was always performed only on the left side of the body. EDA seems to be a good indicator of some sort of subtle perception and alertness. This is an interesting finding which was not expected before the start of the experiments. It would certainly be interesting to see if similar responses could be associated with laser acupuncture.

Skin potential measurements are very sensitive but not specific to the location of the acupuncture point. Results are however limited to only two acupuncture points and reveal once again limitations of studies with a small scope of research. In order to be certain about generalisability of EDA findings, possibly another 20-30 acupuncture points should be tested using measurement of skin potentials.

8.6.3 Towards the evidence-based acupuncture practice

The theory and practice of traditional acupuncture survived for many centuries, but now it is challenged by new ways of thinking. Will acupuncture theory and practice survive the scrutiny of modern scientific evaluation and successfully enter the “age of reason”? To answer this question we need to ask another question: what kind of research will be used in order to accept or reject acupuncture as a treatment modality?

The theory and the practice can be considered as two essential dimensions along which acupuncture has been developed over a long period of time. In modern times there is a constant need for the evaluation of health outcomes, for reasons that are not always medical, but also ethical, sociological and economical. Current trends towards evidence based medicine add one more essential dimension to acupuncture, that is, acupuncture research. The interdependency between theory, practice and research will determine the future of acupuncture and its acceptance in health care.

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Appendix A-1

Consent Form for participation in the study

Consent Form - Student research

I ………………………………………………….. agree to participate in the research project

"An investigation of neuropsychological profiles of acupuncture points"

The effect of stimulation of LU7 and KD6 acupuncture points on Heart Rate Variability, Respiratory Rate, Electroencephalograph and Electro Dermal Activity in healthy human subjects"

Being conducted by:	Mr. Victor Vickland, PhD student	(ph.9514-7861)
	Adjunct Professor Carole Rogers	(ph.9514-7853)
	Professor Ashley Craig	(ph.9514-1358)

At the location: College of Traditional Chinese Medicine, University of Technology Level 4 / 645 Harris Street, Ultimo (Sydney), NSW 2500.

I understand that the purpose of this study is to evaluate the effect of acupuncture on heart rate, respiratory rate, brain wave and skin electrical potential.

I have read the subject/participant information sheet and understand that there may be some minor risks associated with the acupuncture procedure such as a bruise or slight discomfort/pain. I understand that this research will involve the applicatcording electrodes to my torso and head and there may be some slight discomfort in preparing the skin for ation of retachment of the electrodes. I also understand a vest for monitoring respiratory rate will be fastened around my chest. If at any time during the procedure I wish to discontinue the procedure the process will be stopped immediately and all acupuncture needles withdrawn.

I understand I will be debriefed at the completion of the study and that I have the opportunity to receive three free acupuncture treatments from the Acupuncture Clinic UTS at anytime in the next twelve months.

I am aware that I can contact Mr. Victor Vickland if I have any concerns about the research. I also understand that I am free to withdraw my participation from this research project at any time I wish and without giving a reason.

I agree that Mr Victor Vickland and/or Adjunct Professor Carole Rogers have answered all my questions fully and clearly.

I agree that the research data gathered from this project may be published in a form that does not identify me in any way.

I state that I do not suffer from any known cardiac (heart) conditions.

________________________ ___/____/____ _______________________ ___/___/___

Signed by Witnessed by

This study has been approved by the University of Technology, Sydney Human Research Ethics Committee. If you have any complaints or reservations about any aspect of your participation in this research which you cannot resolve with the researcher, you may contact the Ethics Committee through the Research Ethics Officer, Ms Susanna Davis (ph: 02 - 9514 1279, Susanna.Davis@uts.edu.au). Any complaint you make will be treated in confidence and investigated fully and you will be informed of the outcome.

Appendix A-2
Demografic Information

University of Technology, Sydney
Demographic Information

Project: An investigation of neurophysiological profiles of acupuncture points.
The effect of stimulation of LU7 and KD6 acupuncture points on Heart Rate Variability, Respiratory Rate, Electroencephalograph and Electro Dermal Activity in healthy human subjects.

Please provide following information:

Name:____

Age:____

Weigh:____

Height:____

Contact Number (home):____________________________________

Contact Number (work):____________________________________

Do you suffer from heart disease / dysfunction or high blood pressure:___________

____

Do you take any medication:____

____

The information contained above will be kept secure and confidential. No identifying data will be published or released. The information will be available only to researchers directly associated with this project.

Office use only **Date:** ___________

Assigned code number:_____________________

Appendix A-3
Information for the subjects about the study

UNIVERSITY OF TECHNOLOGY, SYDNEY
SUBJECT/PARTICIPANT INFORMATION SHEET

An investigation of neurophysiological profiles of acupuncture points.

The effect of stimulation of LU7 and KD6 acupuncture points on Heart Rate Variability, Respiratory Rate, Electroencephalograph and Electro Dermal Activity in healthy human subjects.

The aim of this study is to evaluate the effect of acupuncture on heart rate, respiratory rate, brain waves and skin electrical activity. You will be asked to attend either one session (control group) or three sessions (treatment group) depending on random allocation at the beginning of the study. Each recording session will take approximately one hour and thirty minutes

Recording Your Heart Rate

The recording of your heart rate is attained through a process called electrocardiography (ECG). This requires the application of three electrodes to your chest. We will be recording your heart rate for 80 minutes during which you will be lying on a couch. Your skin will be prepared for electrodes using an alcohol swab. Following this procedure some gel is applied and the electrodes attached. This method is non-intrusive, that is, the skin is not punctured.

Recording of Your Respiratory Pattern

To record your respiratory pattern you will be required to wear a vest over the top of your clothing. The vest has a sensor attached over the chest and abdomen to measure the movement of your chest and abdomen during breathing. This method is non-intrusive, that is, the skin is not punctured.

Recording of Your State of Alertness

To measure your state of alertness an electroencephalograph (EEG) “brain waves” and electrooculograph (EOG) “eyes movement” will be recorded using the electrodes positioned on your scalp and your face. Your will be asked to wear an EEG cup with electrodes attached to it. The researches will also attach four electrodes around your eyes. Your skin will be prepared for the electrodes using an alcohol swab. Following this procedure some gel is applied and the electrodes attached. This method is non-intrusive, that is, the skin is not punctured.

Recording of Your Electro Dermal Activity

To measure how emotions and other factors are affecting the electrical potential of the skin four electrodes will be attached to your hands. Your skin will be prepared for the electrodes using an alcohol swab. Following this procedure some gel is applied and the electrodes attached. This method is non-intrusive, that is, the skin is not punctured.

Acupuncture Procedure

If you are allocated to the treatment group you will receive acupuncture treatment during each of the three sessions. The acupuncture point LU7 will be used in Session A, the acupuncture point KD6 will be used in Session B, and both points together will be used in Session C. The sessions will be one week apart and the sequence of the session will be randomly allocated. The needle will puncture the skin in an area on your wrist (LU7) or ankle (KD6), or both, and will remain in place for approximately 20 minutes.

There may be some minor risks associated with acupuncture, for example a bruise, however all effort will be taken to minimise such risks. The acupuncture needles are sterilized and disposable for single use only. During acupuncture the needle will be stimulated every five minutes by rotating the needle for a 10 second period. A visual analogue scale (questionnaire) will used to assess the degree of stimulation felt by the subject. The acupuncturist is an experienced and qualified practitioner.

Preparation for the Sessions

In order to attain accurate and valid recording of your heart rate we ask that you refrain from eating, smoking or drinking alcohol for two hours prior to commencing the session. We also ask you to refrain from receiving acupuncture one week prior to the day of the session. If you are unable to meet these criteria please notify the researcher and we will reschedule your session for another day.

Qualified practitioner will provide the acupuncture. If at any stage you feel discomfort or pain and wish to withdraw from the study, please inform the researchers immediately and the procedure will be terminated. You will be asked whether you want to continue the experiment or withdraw completely from the project. You are not required to give a reason for withdrawal.

You will be asked to fill out two questionnaires at the beginning and one at the end of the study to evaluate the level of stress in your life. Also you will be asked to complete one short questionnaire at the beginning of each session. Your identity will remain confidential.

At the completion of the project you will be informed as to the outcome of the study. To thank you for your participation in the study you are offered three free acupuncture treatments at the UTS acupuncture clinic.

Contact Details

Victor Vickland (PhD student) victor.vickland@uts.edu.au

(w) 9514-7861 (m) [redacted]

Appendix A-4

UTS Ethics Committee approval

16 July 2002

Adjunct Professor Carole Rogers
College of Traditional Chinese Medicine
Faculty of Science
4/645 Harris Street
Ultimo

Dear Carole

UTS HREC 02/31 - ROGERS, Adjunct Professor Carole, CRAIG, Professor Ashley, (for VICKLAND, Mr Victor - PhD student) – "An investigation of neurophysiological profiles of acupuncture points"

Thank-you for your response to my letter of 17 April 2002. I have no hesitation in approving your application subject to the consent form being printed on the formal UTS letterhead, as found on this letter.

Your approval number is HREC 02/31A.

The *National Statement on Ethical Conduct in Research Involving Humans* requires us to obtain a report about the progress of the research, and in particular about any changes to the research which may have ethical implications. The attached report form must be completed at least annually, and at the end of the project (if it takes more than a year), or in the event of any changes to the research as referred to above, in which case the Research Ethics Officer should be contacted beforehand.

I also refer you to the AVCC guidelines relating to the storage of data. The University requires that, wherever possible, original research data be stored in the academic unit in which they were generated. Should you submit any manuscript for publication, you will need to complete the attached *Statement of Authorship, Location of Data, Conflict of Interest* form, which should be retained in the School, Faculty or Centre, in a place determined by the Dean or Director.

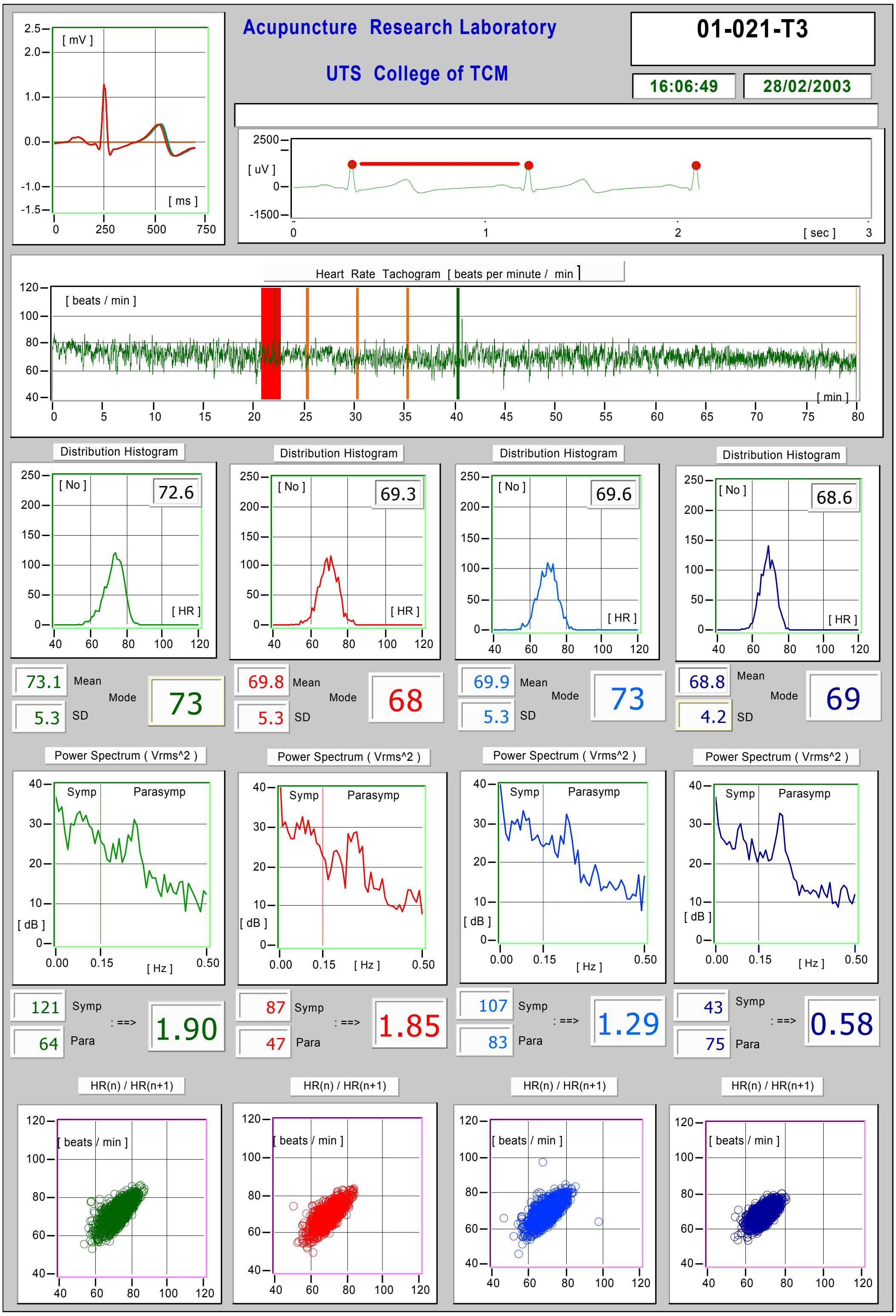
Please complete the attached (green) report form at the appropriate time and return to Susanna Davis, Research Ethics Officer, Research Office, Broadway. In the meantime, if you have any queries please do not hesitate to contact either Susanna or myself.

Yours sincerely,

Associate Professor Jane Stein-Parbury
Chair
UTS Human Research Ethics Committee

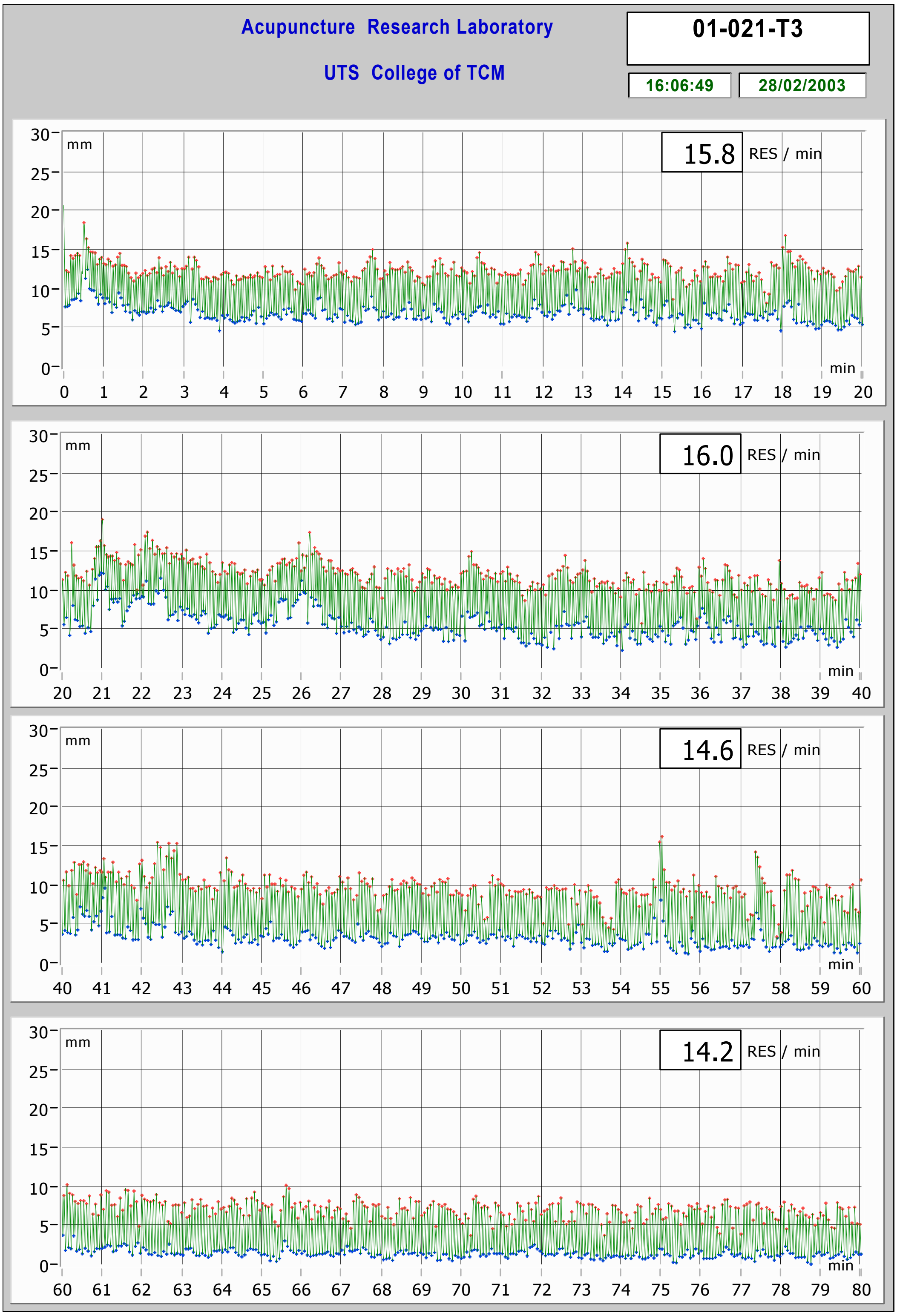
Appendix B-1

Printout of results from ECG analysis



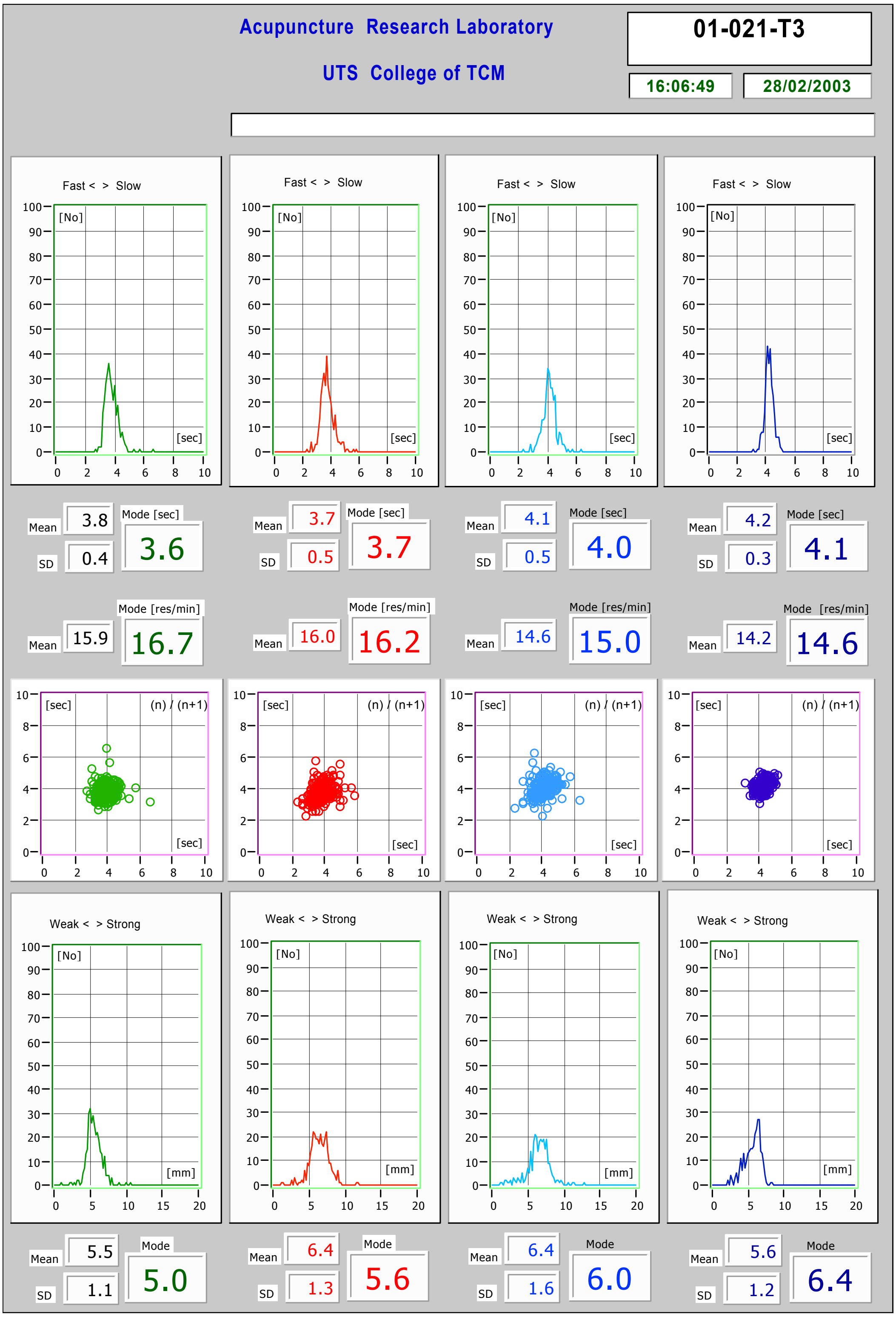
Appendix B-2

Printout showing respiratory activity



Appendix B-3

Printout of the results from respiratory analysis



Appendix B-4

Printout of the results from EEG and EDA analysis

Appendix B-5

Printout of the results from EEG analysis

Acupuncture Research Laboratory

UTS College of TCM

01-021-T3

16:04:00 | **28/02/2003**

C3 - C4

	i1 - i2 [%]		i1 - i3 [%]		i1 - i4 [%]	
D	-75.9	-76.63	-5.24	-10.95	168.52	156.31
T	-59.77	-63.80	-3.46	-3.98	98.66	101.86
A	-43.97	-45.09	81.82	110.17	140.67	135.75
B	-34.05	-42.01	-3.04	-2.77	-52.59	-51.87
A1	-31.65	-30.61	91.20	119.89	265.61	266.23
A2	-50.55	-52.38	76.81	105.27	74.03	70.03
B1	-42.49	-51.68	3.10	3.45	-50.76	-49.55
B2	0.27	-1.89	-27.98	-28.56	-60.05	-61.51
8	-35.56	-40.93	69.01	70.07	274.66	243.53
S	-68.89	-66.96	-22.54	-15.36	215.99	250.94

	C3	C4	i1 -----> i2		i2 -----> i3		i3 -----> i4	
D	5.79	5.57	1.40	1.30	5.49	4.96	15.54	14.29
T	1.96	1.98	0.79	0.72	1.89	1.90	3.90	4.00
A	0.98	0.98	0.55	0.54	1.79	2.06	2.37	2.31
B	0.17	0.17	0.11	0.10	0.16	0.16	0.08	0.08
A1	0.75	0.72	0.51	0.50	1.44	1.59	2.75	2.64
A2	1.18	1.19	0.58	0.57	2.08	2.45	2.05	2.03
B1	0.45	0.46	0.26	0.22	0.47	0.48	0.22	0.23
B2	0.05	0.05	0.05	0.05	0.03	0.03	0.02	0.02
8	0.87	0.88	0.56	0.52	1.47	1.49	3.27	3.02
S	9.11	8.38	2.84	2.77	7.06	7.09	28.80	29.41

F3 - F4

	i1 - i2 [%]		i1 - i3 [%]		i1 - i4 [%]	
D	-71.28	-71.99	2.89	-6.21	232.18	218.47
T	-44.40	-44.89	3.49	9.15	176.67	218.92
A	-54.23	-58.60	85.60	130.09	112.61	122.28
B	-36.55	-35.31	-1.56	4.60	-54.73	-54.66
A1	-28.21	-21.08	63.85	95.63	246.66	290.12
A2	-66.40	-73.97	95.78	144.19	49.86	53.55
B1	-46.14	-47.12	7.24	13.18	-52.86	-51.88
B2	-10.19	0.64	-25.79	-21.51	-59.88	-63.12
8	-31.49	-30.89	39.97	55.37	252.91	276.63
S	-49.81	-43.17	-11.77	-3.50	190.59	202.00

	F3	F4	i1 -----> i2		i2 -----> i3		i3 -----> i4	
D	1.92	1.82	0.55	0.51	1.98	1.70	6.38	5.79
T	0.55	0.52	0.30	0.29	0.56	0.57	1.51	1.66
A	0.36	0.37	0.17	0.15	0.67	0.85	0.77	0.82
B	0.05	0.05	0.03	0.03	0.05	0.06	0.02	0.02
A1	0.25	0.24	0.18	0.19	0.42	0.46	0.88	0.92
A2	0.45	0.48	0.15	0.12	0.89	1.17	0.68	0.74
B1	0.13	0.14	0.07	0.07	0.14	0.16	0.06	0.07
B2	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01
8	0.31	0.30	0.21	0.21	0.43	0.47	1.09	1.13
S	3.58	3.49	1.80	1.98	3.16	3.37	10.42	10.55

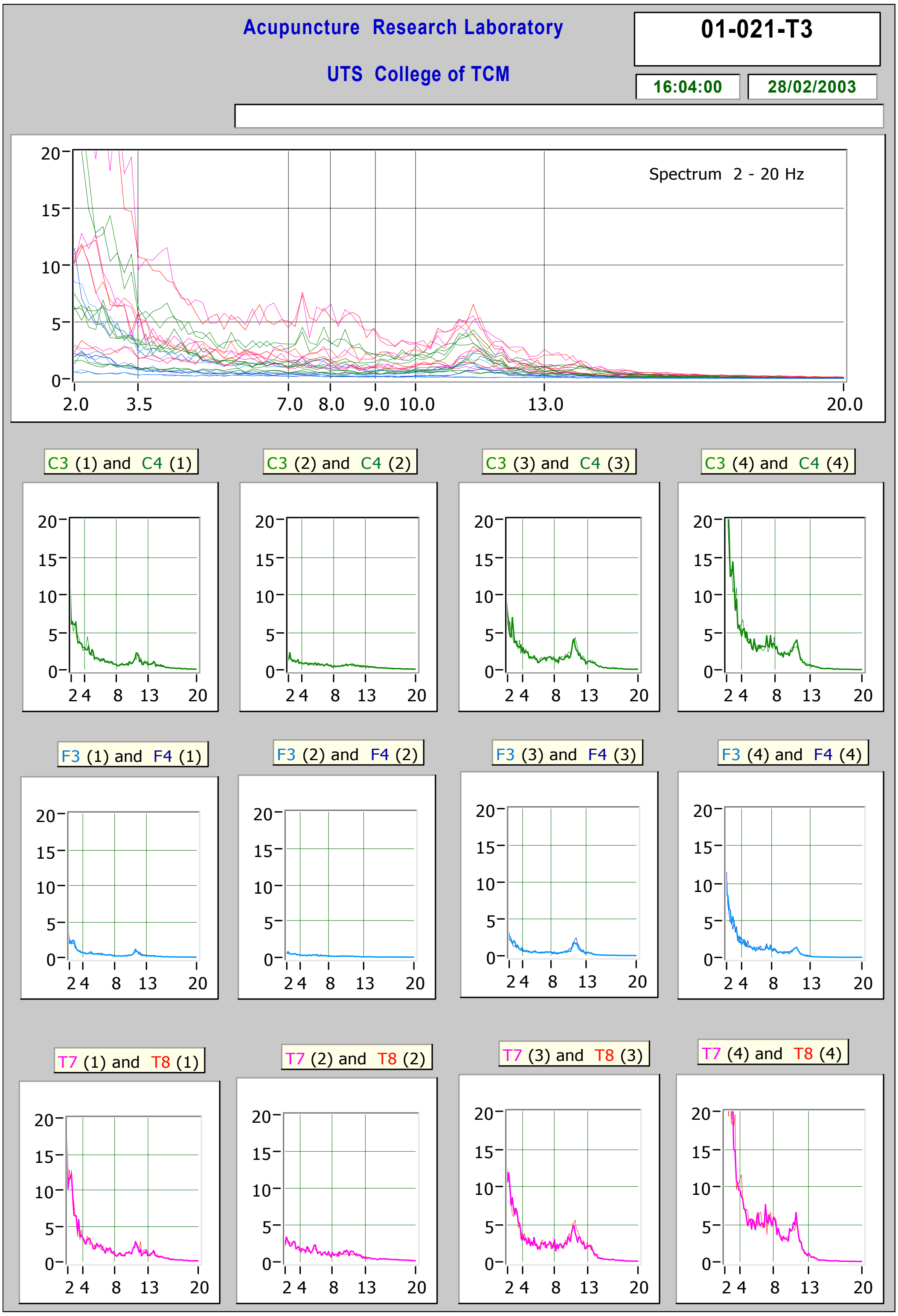
T7 - T8

	i1 - i2 [%]		i1 - i3 [%]		i1 - i4 [%]	
D	-72.38	-73.27	-7.43	-12.96	175.36	152.90
T	-39.11	-42.96	6.01	4.56	148.69	163.25
A	-21.68	-35.20	91.71	91.98	176.11	156.23
B	-16.49	-30.83	4.70	1.82	-55.60	-60.04
A1	-12.07	-14.53	83.86	82.26	277.93	292.45
A2	-27.93	-46.51	96.83	97.31	109.82	81.66
B1	-37.38	-41.68	12.62	8.51	-53.86	-59.51
B2	65.92	13.71	-26.53	-25.64	-62.44	-62.24
8	-24.54	-25.74	58.82	56.02	274.00	291.33
S	-67.31	-66.84	-27.01	-22.04	177.85	203.12

	T7	T8	i1 -----> i2		i2 -----> i3		i3 -----> i4	
D	9.56	9.87	2.64	2.64	8.85	8.59	26.32	24.96
T	2.62	2.63	1.60	1.50	2.78	2.75	6.53	6.92
A	1.38	1.48	1.08	0.96	2.65	2.84	3.82	3.79
B	0.23	0.25	0.19	0.17	0.24	0.25	0.10	0.10
A1	1.20	1.15	1.06	0.98	2.21	2.10	4.54	4.51
A2	1.54	1.75	1.11	0.94	3.03	3.45	3.23	3.18
B1	0.61	0.67	0.38	0.39	0.69	0.73	0.28	0.27
B2	0.06	0.07	0.11	0.08	0.05	0.05	0.02	0.03
8	1.44	1.38	1.08	1.02	2.28	2.15	5.37	5.40
S	17.76	16.06	5.81	5.33	12.96	12.52	49.35	48.68

Appendix B-6

Printout of the results for EEG spectrum analysis



Appendix B-7

Printout of the results for EOG and EDA analysis