The effects of a single session of chiropractic treatment on pain processing

Aalborg Universitetshospital

Study synopsis

Protocol no.: SPINAL 26-2-2015

Expected start of the study: 01/03/2015
Expected end of the study: 01/06/2016

Objectives

To investigate brain alterations after a single session of chiropractic treatment in the subclinical pain population.

Protocol signature page

Investigator's statement: I have read and understand the foregoing protocol entitled "**The effects of a single session of chiropractic treatment on pain processing**", protocol no.: **SPINAL-26-2-2015** and agree to conduct the study in compliance with Good Clinical Practice (CPMP/ICH/135/95), designated Standard Operating Procedures, the Danish Health and Medicines Authority, the Research Etchics Committee in Denmark, and within the principles of the Declaration of Helsinki (amended by the 52nd General Assembly, Edinburgh, Scotland, October 2000, clarified by the General Assembly in Washington 2002, Tokyo 2004, Seoul 2008, and Fortaleza 2013 as outlined herein).

Asbjørn Mohr Drewes	Professor, Dr. Med., Ph.D
Principal investigator (Asbjørn M. Drewes)	Principal investigator's title
23 March 2015	
Date	Principal investigator's signature

Table of Contents

1. Glossary

CRF	Case Report Form
EEG	Electroencephalography
EMG	Electromyography
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference of Harmonization
IFCN	International Federation of Clinical Neurophysiologists
MMP	Multichannel Matching Pursuit
PSIS	Posterior Superior Iliac Spine
SCP	Sub-clinical Pain
SEP	Somatosensory Evoked Potential

2. Participants and Study Centre

Sponsor, clinically responsible investigator:
Asbjørn Mohr Drewes, Professor, Dr.Med.
Mech-Sense
Dept. Gastroenterology & Hepatology
Aalborg Hospital
9000 Aalborg
Denmark
Telephone: +45 99 32 62 28
E-mail: amd@mech-sense.com

Sub investigator:
Dina Lelic, MSc. (Biomedical Engineering), PhD
Mech-Sense
Dept. Gastroenterology & Hepatology
Aalborg Hospital
9000 Aalborg
Denmark
Telephone: +45 97 66 35 20 / +45 97 66 35 24
E-mail: dl@mech-sense.com

Collaborators:
Imran Khan Niazi, MSc., PhD
New Zealand College of Chiropractic
Auckland 1060
New Zealand
Telephone: +64 9 526 2105
E-mail: imran.niazi@nzchiro.co.nz

Heidi Haavik, Dr., PhD
New Zealand College of Chiropractic
Auckland 1060
New Zealand
Telephone: +64 9 526 2105
E-mail: Heidi.Haavik@nzchiro.co.nz

Kelly Holt, Dr., PhD
New Zealand College of Chiropractic
Auckland 1060
New Zealand
Telephone: +64 9 526 2105
E-mail: Kelly.Holt@nzchiro.co.nz

Study initiation:

This study was initiated by Dr. Asbjorn Mohr Drewes and Dina Lelic (PhD) from Mech-Sense in Aalborg University Hospital.

Study centre:

Dept. Gastroenterology & Hepatalogy
Aalborg Hospital
9000 Aalborg
Denmark
Telephone: +45 99 32 62 44

Laboratory

Physical examinations, spinal manipulations, and experimental testing will be conducted in the Mech-Sense research laboratory at Aalborg University Hospital.

3. Conduct of study

The present clinical study will be conducted in compliance with this protocol, the guidelines of the World Medical Association Declaration of Helsinki in its revised edition (Fortaleza, Brazil, 2013), the guidelines of International Conference on Harmonization (ICH) GCP (CPMP/ICH/135/95), and designated Standard Operating Procedures.

4. Time Schedule

The study is expected to be initiated in **March 2015** and be concluded by **June 2016**. A detailed overview is given in the diagram below:

	Spring 2015	Summer 2015	Fall 2015	Winter 2015	Spring 2016
Submitting protocol					
Recruiting subjects					
Conducting study					
Data analysis					
Writing and submitting articles					

5. Background Information

Over the past decade there has been a growing body of evidence to suggest that neural plastic changes occur following chiropractic adjustments (Haavik and Murphy, 2012b). Investigators utilizing techniques such as transcranial magnetic stimulation and somatosensory evoked electroencephalographic potentials (EEG) have suggested that neuroplastic brain changes occur in structures such as the primary sensory cortex, primary motor cortex, basal ganglia and cerebellum (Daligadu et al., 2013;Haavik-Taylor and Murphy, 2007b;Taylor and Murphy, 2008;Taylor and Murphy, 2010a). However, the evidence for the involvement of these brain structures is indirect. Although EEG measures neuronal activity directly (with high millisecond time resolution), it has poor spatial resolution, making it difficult to know exactly where in the brain the changes are occurring. Studies with only a few recording EEG electrodes (Haavik-Taylor and Murphy, 2007a;Taylor and Murphy, 2010b) allow investigation of evoked potential amplitudes and latencies, but do not allow identification of the brain generators underlying the evoked signals.

In recent decades efforts have been made to improve the spatial resolution of EEG. Recently, we showed that a multichannel matching pursuit (MMP) technique, which decomposes the instantaneous data into its contributing time–frequency components, works with high accuracy even under conditions of increasing noise level and increasing number of sources(Lelic et al., 2011b). The investigation of brain generators of these evoked potentials has shown that at least 60 recording electrodes are required for a reliable result (Michel et al., 2004). We set out to utilise this new technique (Lelic et al., 2012b) to explore the active brain sources and the cross-communication that occurs prior to, and after, spinal adjustments. We hypothesize that MMP, in combination with inverse modelling of evoked brain potentials, could be used to study cerebral pain processing and connectivity in a subluxated group that fall into the category of subclinical pain (n=25) following a single session of chiropractic treatment and that the treatment will change the cross-communication/source organisation within the involved brain networks.

We will also look at how people that fall in the category of subclinical pain process nociceptive reflex electromyography (EMG) information, as this is a spinal phenomenon. By better understanding how people who fall into the category of subclinical pain process nociceptive information, our group hopes to identify objective brain markers present in this population as compared to a healthy control group. By testing these brain and muscle markers pre/post a non-invasive treatment options such as spinal manipulation we hope to identify markers that can change with improved function.

6. Potential Risks and Benefits

6.1 Risks Related to Chiropractic Treatment

Chiropractic treatment may involve a variety of manual therapy procedures including manipulation or mobilisation which have a small risk of causing physical harm (Gouveia et al., 2009a). Adverse events associated with chiropractic treatment are generally transient and involve mild musculoskeletal soreness(Gouveia et al., 2009b;Hurwitz et al., 2004b;Thiel et al., 2007c). The risk of serious adverse events due to chiropractic treatment is low or very low (Gouveia et al., 2009c;Thiel et al., 2007b). A recent prospective survey that followed almost 29,000 chiropractic treatment consultations reported no serious adverse events (Hurwitz et al., 2004a;Thiel et al., 2007a). Serious adverse events that have been linked to spinal manipulation include conditions such as cerebrovascular injury, vertebral disc extrusion and fractures.

The serious adverse events most commonly linked to spinal manipulation are cerebrovascular complications, in particular vertebrobasilar artery dissection following cervical manipulation. The largest study to investigate this link reported that in patients under the age of 45 who had suffered from a vertebrobasilar artery stroke there was a positive association with chiropractic visits before the stroke occurred(Cassidy et al., 2009). The investigators also found the same association existed with primary contact physician visits prior to the stroke. They concluded that the increased risk of vertebrobasilar artery stroke associated with chiropractic and primary contact physician visits is likely due to patients with headache and neck pain from vertebrobasilar artery dissection seeking care before the stroke occurred. Our proposed study will not include anyone in any form of current pain (i.e. see definition of subclinical pain population), thus we will be excluding any participant that would potentially fall into the possibly vertebrobasilar artery dissection category seeking relief of symptoms.

6.2 Benefits

As this study will be carried out in a subclinical pain population, and the data compared to a healthy control group, this study will potentially advance our basic science knowledge about how the brains of people who are potentially developing more chronic pain syndromes process somatosensory information compared with the healthy control group. This may help us identify objective neurophysiological brain measures that can be used as markers to help determine the benefits of different interventions in clinical populations in future studies.

This study will also potentially advance our understanding about how spinal manipulation improves function. This study will add to a growing body of research that has demonstrated that chiropractic adjustments alter brain function, by being able to document with much greater clarity exactly what structures within the brain cross-communicate before and after a single chiropractic adjustment session. These findings can have far reaching effects on scope of practice, funding for, and access to chiropractic treatment.

7. Trial Population

Twenty-five sub-clinical spinal pain patients will be included; An estimate of n = 25 is based on an estimated difference of 20 and 25% in the EMG power parameters (standard deviation between subjects) and with a type I and type II errors of 5 and 20% and a coefficient of variation of 25% (Farina and Merletti, 2000). This requires approx. 20-25 people when using paired comparisons (eg. ANOVA) and to ensure that the models are robust. In addition, we expect some drop off, as some people might find intramuscular electrodes uncomfortable and want to stop in the middle of the trial. For these reasons, we have listed 25 people as the ideal number. However, if we have enough volunteers who have completed the trial for statistical significance, we will stop before including 25 patients.

8. Trial Objectives and Purpose

The overall objectives of this study are to:

1) Reveal the brain areas involved in processing nociceptive information in subclinical pain volunteers and how these brain areas are modified following chiropractic treatment.

2) Study what changes in the EMG signals recorded at tibialis anterior occur in subclinical pain volunteers following a single session of chiropractic treatment.

The overall purpose of this study is to:

To gain a much better understanding about how spinal dysfunctions and adjustments affect nervous system function.

9. Trial Design

9.1 Study Design

To address our hypotheses we will use a quasi-experimental study design. The subclinical pain group will attend two sessions and act as their own controls in a cross-over experimental design. The two different groups' data will be compared separately to look for brain cross-talk differences and EMG patterns between the two groups. The subclinical pain group will receive either a control intervention or a chiropractic adjustment intervention on the two sessions they attend. The order of which intervention they receive will be randomized. The interventions have been described below in detail.

9.2 Full Spine Chiropractic Adjustment Assessment and Intervention for the Sub-clinical Pain (SCP) Group

The subclinical pain group will attend two intervention sessions in random order, an experimental session where they will receive chiropractic treatment, and a control session where they will receive no adjustments but will be moved around as if the chiropractor was going to adjust the spine (i.e. cervical, thoracic and lumbar setups) to act as a physiological control for time, as well as the vestibular, cutaneous and muscular afferent discharge changes involved in moving and touching the subject when preparing for the adjustments. Care will be taken to ensure no forces are applied to individual segments during the control intervention.

Full spine adjustments will be carried out during the experimental session. The entire spine and sacroiliac joints will be assessed for spinal dysfunctions, and adjusted where deemed necessary by a registered chiropractor with at least ten years of experience. The spinal dysfunction indicators that will be used prior to and after each spinal adjustment intervention include assessing for tenderness to palpation of the relevant joints, manually palpating for restricted intersegmental range of motion, assessing for palpable asymmetric intervertebral muscle tension, and any abnormal or blocked joint play and end-feel of the joints. All of these biomechanical characteristics are known clinical indicators of spinal dysfunction (Fryer et al., 2004). These findings will be documented prior to and after each spinal adjustment intervention. The improvements in segmental function following spinal adjustments will also be recorded for each subject.

9.3 Spinal Dysfunction Indicators

The most reliable cervical spine dysfunction-indicator is tenderness with palpation of the dysfunctional joint(Hubka and Phelan, 1994b;Jull et al., 1988a). Cervical joint restriction has also been shown to have good interexaminer reliability(Hubka and Phelan, 1994a;Jull et al., 1988b). Therefore, for the purpose of this study spinal dysfunctions will be defined as the presence of both restricted intersegmental range of motion and tenderness to palpation of the joint in at least one cervical spine segment. For the thoracic spine good interexaminer reliability has also been shown for motion palpation(Cooperstein et al., 2010). For the lumbar spine, intersegmental range of motion has also been shown to have acceptable reliability, particularly for the lower lumbar segments(Strender et al., 1997). Although it is recognized that clinical

tests of sacroiliac joint function have questionable reliability(Herzog et al., 1989;Potter and Rothstein, 1985), these tests are still widely used clinically, and Flynn et al(Flynn et al., 2002) have adopted them as one of the criteria for a clinical prediction rule of whether a patient is likely to benefit from sacroiliac manipulation. For the purpose of this study lumbopelvic dysfunction has been defined as the presence of both restricted intersegmental range of motion and tenderness to palpation of at least one lumbopelvic spinal joint segment.

9.4 Spinal Assessment

For the cervical spine functional assessment the chiropractor will gently move the subjects head passively from the neutral position to the maximal range of lateral flexion in the coronal plane, while palpating over each segment and applying gentle pressure to both the left and the right sides. If this movement appears restricted, the examiner will apply additional gentle pressure to the joint, while watching for signs of discomfort from the subject. The examiner will also ask the subject if the pressure to the joint elicited discomfort or pain.

To assess the function of the lumbar segments, the examining chiropractor will palpate the movement of individual lumbar segments while the participant's spine is laterally flexed to the right and left. Where the movement feels restricted, the examiner will apply gentle pressure to the joint and surrounding soft tissues, while watching for signs of discomfort from the subject. The examiner will also ask the subject if the pressure to the joint elicited pain and/or tenderness.

To assess the function of the sacroiliac joints, subjects will be asked to walk up and down on the spot with their knees flexed to assess the movement of each ilium relative to the sacrum while the assessor holds their thumbs on the inferior margin of either the right or the left posterior iliac spines and the adjacent aspect of the sacrum. When the posterior superior iliac spine (PSIS) and the sacrum moves together, the joint will be considered to be restricted in this plane. Subjects will also be asked to bend sideways while the examiner's thumbs contact the right and left PSIS's. Subjects where the sacrum does not shift toward the contralateral side will be considered to be restricted in the lateral flexion plane. When apparent movement dysfunction is identified on one side, the clinician will then palpate over the sacroiliac joints and ask the participant if the palpation elicited tenderness over the joint. The clinician will also palpate the musculature adjacent to the sacroiliac joint on each side, assessing for palpable differences in muscle tension.

9.5 Spinal Adjustments

All of the spinal adjustments to be carried out in this study will be high-velocity, low-amplitude thrusts to the spine. This is a standard adjustment technique used by manipulative physicians, physiotherapists, and chiropractors. The mechanical properties of this type of central nervous system perturbation have been investigated; and although the actual force applied to the subject's spine depends on the therapist, the patient, and the spinal location of the adjustment, the general shape of the force-time history of spinal adjustments is very consistent(Hessell et al., 1990) and the duration of the thrust is always less than 200 milliseconds. The high-velocity type of adjustment was chosen specifically because previous

research(Herzog et al., 1995) has shown that reflex EMG activation observed after adjustments only occurred after high-velocity, low-amplitude adjustments (as compared with lower-velocity mobilizations). This adjustment technique has also been previously used in studies that have investigated neurophysiological effects of spinal adjustments (Haavik and Murphy, 2012a).

After each individual segmental adjustment the spine will be re-checked in order to determine if the subsequent levels identified as subluxated still require an adjustment.

For cervical segments the thrust will be applied to the spine held in lateral flexion, with slight rotation and slight extension.

Thoracic spine adjustments will be carried out either in the supine or prone position. For supine thoracic adjustments the flexed hand of the chiropractor will contact the relevant thoracic segments over the spinous processes, so that the spinous processes lie in the groove between the chiropractors flexed fingers and the thenar area of their thumb. The subjects arms will be flexed and folded over their chest. The thrust will be applied over the subjects arms which will be positioned over the chiropractors contact hand held under the subject (see Figure 1 below for this setup). For prone thoracic segment adjustments the chiropractor will contact either side of the subjects spinous process with both thenar areas of their thumbs. The thrust will be applied in a posterior to anterior and inferior to superior direction.

Lumbar or sacroiliac joint adjustments will be carried out with the subject positioned in the lateral decubitus position (see Figure 2 below). The free superior leg will be flexed at the knee and the pelvis so as to flex the lumbar spine. The pisiform bone of the clinician's inferior (in relation to the subjects head) hand will contact the relevant lumbar spinal segment over the spinous process, or the PSIS of the sacroiliac joint and an adjustive thrust will be applied in a posterior to anterior, and lateral to medial direction for the lumbar spine, or along the plane of the ilium with an inferior and lateral line of drive for the sacroiliac joint.

Figure 1. supine thoracic adjustment setup.

Figure 2. Lumbopelvic adjustment setup.

9.6 Control Intervention

The control group will not receive the actual spinal adjustments. The control intervention will consist of passive and active movements of the subject's head, spine and body that will be carried out by the same chiropractor who pre-checks the subjects for spinal dysfunctions and who performs the adjustments in the experimental intervention session. This control intervention will involve the subjects being moved into the adjustment setup positions where the chiropractor would normally apply a thrust to the spine to achieve the adjustments. However, the experimenter will be particularly careful not to put pressure on any individual spinal segments. Loading a joint, as is done prior to spinal adjustments has been shown to alter paraspinal proprioceptive firing in anesthetised cats(Pickar and Wheeler, 2001), and will therefore be carefully avoided by ending the movement prior to end-range-of-motion when passively moving the subjects. No spinal adjustments will be performed during any control intervention. This control intervention is not intended to act as a sham adjustment but to act as a physiological control for possible changes occurring due to the cutaneous, muscular or vestibular input that will occur with the type of passive and active movements involved in preparing a subject/patient for an adjustment. It also acts as a control for the effects of the stimulation necessary to collect the dependent measures of the study, and acts as a control for the time required to carry out the adjustment intervention.

9.7 Neurophysiological Assessment of Spinal Manipulation

The EEG will be recorded from 62-scalp electrodes using the extended 10-20 system montage (Quick-Cap International). The subject will be seated comfortably in a supine position with eyes open throughout the entire recording. The neurophysiological assessment will be done in three different ways: 1. Spontaneous resting EEG, 2. EPs following nociceptive withdrawal reflex stimulation on the right foot, and 3. Tonic pain /

conditioning pain modulation, while the subject's left hand is in cold water for two minutes. These different stimulation paradigms are explained next.

9.7.1 Resting spontaneous EEG

Spontaneous EEG will be recorded for two minutes while the volunteer lays in a supine relaxed position with eyes open.

9.7.2 Nociceptive Withdrawal Reflex

The withdrawal reflex is induced by electrical stimulation on the foot, reflecting activity at the spinal cord mainly. First the nociceptive withdrawal reflex threshold will be found and then 18 stimulations will be given in order to elicit clear Eps. The 18 stimulations will be at three different intensities given at random: the nociceptive withdrawal reflex threshold, 1.3 * the threshold, and 1.6 * the threshold. During these 18 stimulations, both EEG and EMG will be recorded. The EMG will be recorded at tibialis anterior.

9.7.2 Conditioning Pain Modulation

Conditioning pain modulation reflecting the downstream pain inhibition will be measured using the cold pressor test. First the heat pain tolerance threshold will be found and the pain rating of five seconds of heat at this threshold will be given. Then the volunteer will be asked to keep their hand in cold water (2 degrees) for two minutes. After the hand has been in the cold water for one minute, the pain score of the cold water will be taken. After 90 seconds, 5 seconds of heat at pain tolerance threshold will be given again and the pain score will be taken. After two minutes, the pain score of the cold water will be taken and the volunteer will be asked to take the hand out of the cold water. Thirty seconds following this, final five seconds of the heat stimulus at the pain detection threshold will be given and the subject will be asked to rate the pain on scale from 0-10.

During this process, the EEG will be recorded. One minute before the subjects puts the hand in the cold water, the EEG recording will be started to have a baseline measure. After one minute of EEG recording, the subject will be asked to put the hand in the cold water and once the hand is out of the water, the EEG recording will be stopped.

9.8 Source data

Source documents (including all demographic information), Case report Form (CRF), and a copy of the signed informed consent form (ICF) indicating the protocol number and title) for each volunteer in the study will be maintained by the investigator. Subject identification list is also considered as a source document. A source data list will be created and stored in the trial master file. Source documents must be

available to document the existence of the subject and substantiate the integrity of study data collected. The source data are available in case of an audit/inspection.

9.9 Recording of Data

All data will be entered directly into the CRF or saved electronically. All electronically saved data will also be stored on an external hard-disk as back-up (copy from the computers used for the data collection to the external hard-disk) and in the CRF a mark will be made for all electronically saved data. All data from the CRF will be entered electronically into datasheets at the study site by trained study personnel. The investigator is required to ensure the continued storage of the documents, even if the investigator, for example, leaves the clinic/practice or retires before the end of required storage period. Sponsor undertakes to store originally completed CRFs and separate copies of the above documents for the same period, except for source documents pertaining to the individual investigational site, which are kept by the investigator only.

10. Study Group

10.1 Subject Recruitment

We have a long list of people who have participated in previous experiments that want to participate again. Several of them have friends and acquaintances that also want to participate, thereby extending the list all the time. If more subjects are needed, they will be recruited through "http://www.forsoegsperson.dk/" and through Aalborg University volunteer recruitment facebook group called "volunteers". The following is the text that will be used to recruit the volunteers on the facebook group and http://www.forsoegsperson.dk/ :

We are looking for participants for experiments in our lab at Aalborg University Hospital. In this experiment we will be studying the effect of chiropractic on experimental pain. The experiment consists of two visits of 1-2 hours each. You will receive 150 kr per hour for your participation. This amount is taxable. If you are interested, please contact me at dile@rn.dk or 97663523 for further information.

And in Danish:

Vi søger raske deltagere til forsøg i vores laboratorium på Aalborg Universitetshospital. I forsøget vil vi undersøge effekten af kiropraktik på eksperimentelle smerter. Forsøget består af to besøg af 1-2 timers varighed. Du modtager 150 kr. i timen som ulempegodtgørelse. Beløbet er skattepligtigt. Hvis du er interesseret i at deltage kan du kontakte Dina Lelic på mail dile@rn.dk eller telefon 97663523 for nærmere information.

10.2 Subject Inclusion and Exclusion Criteria

Subjects for the subclinical pain group will be eligible for inclusion if they are aged 18-50, and have some history of recurring spinal dysfunction such as mild pain, ache, and/or stiffness with or without a history of known trauma. Subjects for this group will be ineligible to participate if they exhibit no evidence of spinal dysfunctions, have absolute contraindications to spinal adjustment, have experienced previous significant adverse reactions to chiropractic treatment, or if they have sought treatment for the subclinical pain symptoms. The inclusion criteria will also include fluent understanding of both written/spoken English and written/spoken Danish.

10.3 Withdrawal Criteria

A healthy volunteer should be withdrawn from trial, if at any time:

- It is the wish of the healthy volunteer (or their legally acceptable representative) for any reason
- The investigator judges it necessary due to medical reasons
- Severe non-compliance to protocol as judged by the investigator

If a healthy volunteer does not turn up for a scheduled visit, every effort should be made to contact the healthy volunteer.

In any circumstance, every effort should be made to document healthy volunteer status.

10.4 Information about the Study

The subjects will be informed orally and in writing. Before the information interview the written information is sent to the subjects ("Deltagerinformation"). Investigator will give the information interview according to the North Denmark Region Committee on Health Research Ethics guidelines. The interview will take place in our laboratory at Aalborg Hospital without disturbances. During the interview, the subject will be further informed about the project and any questions from the subject will be answered. The subject has the right to consider if he is willing to participate in the trial after the information interview, and they are allowed to have an assessor with them to the interview. The participant is informed that 48 hours for reflection are allowed prior to possible acceptance of participation. Before the trial is started the subjects give their informed consent and proxy statement in writing. In the informed consent, the subject can choose not to get any essential information about his/her state of health during the trial. The information will be given in either Danish or English (as the subject chooses) and the experiment will be carried out in English.

10.5 Honorarium of subjects

The subjects in this trial are honored with 150 DKK for each hour they attend during the experiment. Costs for transportation to and from Aalborg University Hospital will be held in accordance with government tariffs. Current rates can be found on www.statenstakster.dk.

11. Data Analysis

11.1 EP Analysis

The analysis of EPs from each session will be carried out offline. Latencies and amplitudes of the main SEP peaks will be analyzed.

11.2 Brain Source Identification

Brain sources of EPs recorded on the scalp reflect the upstream activation of brain activity in different centres. Brain sources are identified by inverse modelling of the evoked brain activity(Lelic et al., 2011a).

11.3 Brain Networks

The network between brain sources can be assessed using decomposition of the evoked brain potentials(Lelic et al., 2012a). By applying algorithms developed by our research group it is possible to detect brain activity on a millisecond scale. Furthermore, the waveform of the neural signals can be determined using multi-channel matching pursuit (MMP) (Lelic et al., 2009). Hence, information about the brainstem and limbic system mainly involved in pain processing and descending inhibition can be revealed in real-time. As the methods provide qualitative information (waveform and frequency) regarding the involved brain centres with a sufficient temporal resolution, connectivity analysis can determine the communication between them – the so-called “brain web”

11.4 Spontaneous EEG and Tonic Pain EEG Analysis

Spontaneous EEG and tonic pain EEG will be analysed offline. The data will be analysed in frequency domain as well as its underlying brain sources.

11.5 EMG Analysis

EMG will be analysed offline. EMG signals will be assessed in terms of area under curve, peak-to-peak amplitude, and root-mean-square.

11.5 Statistical Analysis

Descriptive statistics will be reported as mean ± SD. To assess the effect of chiropractic adjustments on the dependent variables a multifactorial repeated measures ANOVA will be used for each of the dependent measures (EMG area under the curve, peak-to-peak amplitude, root-mean-square, EEG frequencies and brain sources, EP peak amplitudes, latencies and MMP components), with ‘TIME’ (pre and post intervention measures) and ‘INTERVETION’ (Chiro vs control) as factors. A priori pairwise comparisons of the pre and post intervention data will be carried out when an interactive effect is significant. All statistical analysis will

be carried out using IBM SPSS Statistics Version 21.0.0.0 software (IBM Corp, Armonk, NY). Significance will be set at $P \leq 0.05$.

12. Quality Control and Quality Assurance

An independent audit at the study site may take place at any time during or after the study. The study will be monitored by an independent company/person not otherwise involved in the project. Furthermore, monitoring visits to the trial site will be made periodically during the trial to ensure that all aspects of the protocol are followed. Source documents will be reviewed for verification of agreement with data on CRFs. The Investigator/institution guarantees direct access to source documents to appropriate regulatory agencies. The trial site may also be audited (quality assurance) or inspected by appropriate regulatory agencies. It is important that the Investigator and their relevant personnel are available during the monitoring visits and possible audits and that sufficient time are devoted to the process.

12.1 Quality Control

Quality Control is defined as the operational techniques and activities undertaken within the quality assurance system to verify that the requirements for quality of the study related activities have been fulfilled. Quality Control should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

12.2 Quality Assurance

Quality Assurance is defined as the planned and systematic actions that are established to ensure that the study is performed and the data are generated, documented (recorded) and reported in compliance with GCP and the applicable regulatory requirements.

13. Ethics

This clinical study will be conducted in compliance to this protocol, and in accordance with the provisions of the guidelines of the World Medical Association Declaration of Helsinki in its revised edition (Fortaleza, Brazil, 2013), the guidelines of International Conference on Harmonisation (ICH) GCP (CPMP/ICH/135/95), designated Standard Operating Procedures, and is submitted to the North Denmark Region Committee on Health Research Ethics. In addition, this study will be undertaken in accordance with the Protocol and Good Clinical Practice on the conducting and monitoring of clinical studies. The Independent Ethics Committee/Institutional Review Board (IEC/IRB) must be constituted according to the local laws/guidelines. The trial will only be initiated once the approval from the North Denmark Region Committee on Health Research Ethics. Information about the volunteers is protected by the "Lov om behandling af personoplysninger og Sundhedsloven". All volunteers will be informed verbally and in writing before they decide whether or not they will take part in the study. Furthermore, they will be informed that they are allowed to withdraw from the study at any given time without giving any reason. The healthy volunteer can contact Dina Lelic (+45 97 66 35 20) if he/she wants to know the results or any other information about the project.

There are no immediate benefits for the subject. However, it can be uncomfortable for the subjects to undergo the electrical stimulation of the median nerve or the spinal manipulation.

There are used safe electrical, heat, and cold stimuli. The group has extensive experience in working with experimental pain stimuli, and has never observed serious complications as a result of these stimuli.

The EMG methods used are all thoroughly tested and has over the last 10 years been used at the Center for Sensory-Motor Interaction at Aalborg University and over the last 5 years at Mech-Sense, Aalborg University Hospital. During this period there have been no reported side effects or risks of the methods used. The procedures have been used previously, and the North Denmark Region Committee on Health Research Ethics has previously approved similar experimental studies for recording EMG (eg. VN 200 845).

14. Data Handling and Record Keeping

The study will be submitted to the Danish Data Protection Agency through the umbrella application of the North Denmark Region ("Region Nordjyllands Paraplyanmeldelse ved Datatilsynet – Sundhedsvidenskabelig forskning i Region Nordjylland (2008-58-0028)"). The study will comply with Danish Act on Processing of Personal Data and Danish Health Legislation.

For each subject a CRF is kept in which data for the subject is entered.

All data will be anonymous and confidential. Data will be stored at Aalborg Hospital, Department of Medical Gastroenterology, for 5 years under the responsibility of the investigator, Asbjørn Mohr Drewes. All forms are filled out during (or immediately after) the assessment of a subject and must be legible. Errors are crossed out, corrections are added and next to the changes date and initials are applied.

Patient Identification list containing patient number, full name, social security number, study medication and treatment codes for all persons included in the study will be created. The list is populated and updated by a project nurse or other competent person and stored at Aalborg Hospital, Department of Medical Gastroenterology, under the responsibility of the investigator, Asbjørn Mohr Drewes.

Principal investigator must maintain complete and accurate records to ensure that the execution of the study is fully documented and the study data can be subsequently verified. These documents should be classified in 2 separate categories: (1) researcher Trial Master File and (2) study/subject's clinical source documents (CRF). The trial master file must contain the protocol/amendments, correspondence with the North Denmark Region Committee on Health Research Ethics, informed consent, staff curriculum vitae, forms and other appropriate documents/correspondence etc. Investigator, Asbjørn Mohr Drewes, allows direct access to all source data and documents at monitoring, auditing and inspection from the North Denmark Region Committee on Health Research Ethics or from other countries' health authorities.

15. Finance and Insurance

This study is economically supported by a local grant at Aalborg University Hospital comprising kr 15.000, the New Zealand College of Chiropractic (NZCC) comprising kr 75,000 and the NZCC Research Supporters Programme comprising kr 45,000.

All subjects are covered by the hospital's patient insurance for the experiments conducted at Aalborg University Hospital.

16. Publication

Results, positive as well as negative and inconclusive, will be published in scientific journals. Results may also be used in submission to regulatory authorities. The first author will be appointed according to the Vancouver system.

The investigator will inform the North Denmark Region Committee on Health Research Ethics after the termination of the trial. Published articles are sent to the North Denmark Region Committee on Health Research Ethics.

17. Initiation

Before initiating a study, the Investigator should have written and dated approval from the North Denmark Region Committee on Health Research Ethics for the study protocol (and any amendments), written informed consent form, consent form updates, and any other written information to be provided to healthy volunteers. Approval will be indicated in writing with reference to the final protocol number and date. During the study the investigator should provide all documents that are subject to review.

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