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Title:
The Effect of Real and Sham Acupuncture on Thermal Sensation and Thermal Pain Thresholds

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Running head
Acupuncture and Thermal Thresholds
Abstract

Objective: To compare the effect of real and sham acupuncture and a control intervention on thermal sensation and thermal pain thresholds.

Design: Single blind, randomized controlled, repeated measures, trial.

Setting: Laboratory.

Participants: Eighteen acupuncture naïve, healthy individuals; with no history of upper limb pathology or acupuncture contraindications were recruited and completed the study. Subjects were randomly assigned (blind card allocation) to one of the six possible orders of application of the interventions.

Interventions: 25 minutes each of control, real and sham acupuncture.

Outcome Measure: Thermal sensation and thermal pain thresholds measured with a Thermal Sensory Analyser before and after each intervention.

Results: Increases in cold and hot pain and cold sensation thresholds occurred with real acupuncture. The level of increase was not significantly different from the changes occurring with sham acupuncture and control.

Conclusion: While a trend of decrease in sensitivity to thermal pain and thermal sensation was observed with real acupuncture this was not significantly different from the changes with control or sham interventions. Therefore no support was provided for analgesic or placebo effects of acupuncture. The trend seen combined with the relatively low power of the inferential tests applied, however, does suggest further research is merited.

Key Words: Acupuncture, Placebo Effect, Sensory (Thermal) Thresholds
Acupuncture’s popularity in the West has increased in the last three decades with a wide variety of practitioners, including physiotherapists, nurses and general practitioners, now using the technique.\textsuperscript{1,2} The parallel growth in public perception of the limitations of orthodox medicine and concerns over side effects, has led to a substantial expansion of interest in and use of alternative and complementary medicines.\textsuperscript{3,4,5} In the West, acupuncture is most commonly used for the treatment of pain and musculoskeletal disorders.\textsuperscript{2} There is a call for acupuncture to be more widely available within mainstream clinical practice, however, for acupuncture to be accepted, a demonstrable evidence base is required.\textsuperscript{2}

The efficacy of acupuncture can be investigated from the basis of two contradictory theoretical models; Traditional Chinese Medicine or the Western scientific model.\textsuperscript{6} In Traditional Chinese Medicine vital energy (qi) is believed to flow through the body in specific channels. Illness arises where this flow is blocked and acupuncture needles inserted in certain points are believed to restore this flow and balance the system.\textsuperscript{3} Traditional Chinese Medicine also embraces a holistic view of a person’s health where all aspects of an individual’s life and how they interact with their surroundings are taken into account throughout treatment.\textsuperscript{3,7} Advances in neurophysiology, pharmacology and the Gate Theory of Pain, however, provide a possible alternative, more scientific, model for acupuncture where the possible analgesic action can be explained through neurophysiological processes attributable to both real and placebo effects of acupuncture.\textsuperscript{6,8,9}

Investigation of both possible real and placebo effects can be based upon the knowledge that pain is transmitted by thin myelinated, A delta and unmyelinated C nerve fibres to the dorsal horn in the spinal cord; nociceptive information is then conveyed, via the midbrain and thalamus, to the cerebral cortex where it is perceived.\textsuperscript{10} Acupuncture is hypothesised to work
at both segmental and non-segmental levels. The postulated segmental effects arise at the spinal cord level; acupuncture is believed to stimulate the A beta nerve fibres which then in turn block the transmission of pain to higher centres, ‘The Gate Theory of Pain’. Non-segmental effects, in general, occur at supraspinal levels. Acupuncture is believed to stimulate A delta and C fibres in addition to A beta and these activate three centres: the spinal cord, the midbrain (periaqueductal gray and nucleus magnum raphe) and the hypothalamic pituitary complex. These centres release various endogenous pain modulators (e.g. Beta-endorphins) and there are corresponding receptors distributed throughout the body. Additionally, the stimulation of supraspinal structures brings about diffuse noxious inhibitory control, whereby a noxious stimulant (acupuncture) in one area of the body can reduce the perceived intensity of pain produced by a noxious stimulant (tissue damage) in another area of the body.

It is also accepted that pain is a subjective, complex, multi dimensional event and involves not just a sensory discriminative component but also affective motivational and cognitive evaluative factors and can be influenced by a placebo effect. Various explanatory mechanisms have been proposed: psychological mechanisms may include both expectancy and motivational processes, which can lead an individual to gain relief from pain even if no ‘real’ treatment is applied. Psychological mechanisms can also mediate a physiological effect resulting in the release of some of the endogenous opioids that were referred to earlier.

The measurement of pain is therefore as complex as the phenomenon itself. One increasingly popular method is Quantitative Sensory Testing which is a psychophysical test permitting the quantitative assessment of temperature sensation and pain perception. It is known that the
same nerve fibre types convey both thermal and pain derived signals to the central nervous system.\textsuperscript{17,18} Measuring the sensitivity of thermal sensation and thermal pain thresholds, with Quantitative Sensory Testing, therefore indicates the perceptions arising from the activity of the common pathway and so can give insight into both the effectiveness and (possible) mode of action of any pain treatment.\textsuperscript{17,18} Quantitative Sensory Testing, using the method of limits algorithm, is an accepted method with which to measure thermal sensation and thermal pain thresholds.\textsuperscript{18,19} The existing literature on the effectiveness of acupuncture on both thermal sensation and thermal pain thresholds is conflicting.\textsuperscript{20,21,22,23} These studies cannot be considered a definitive body of work, however, due to both a paucity of studies in this area and the differing and sometimes limited methodologies employed. A common vulnerability arises from the difficulty in providing a credible placebo for acupuncture and various options have been applied: acupuncture at non-acupuncture points and superficial needling.\textsuperscript{22,23} Unfortunately with both techniques an analgesic effect could still occur via the diffuse noxious inhibitory control mechanism.\textsuperscript{24} Ashton et al\textsuperscript{25} applied a different placebo and compared subjects who received acupuncture with those given dissolved lactose (under the impression that it was aspirin). While this is an imaginative solution a placebo drug treatment is not a credible placebo for needleling. The recent introduction of a novel placebo needle – the Park Sham Device\textsuperscript{27} – may prove to be a major development in acupuncture research as individuals can be conditioned to expect pain relief with the application of needles.\textsuperscript{26,27} The device has been tested in one published study on acupuncture naïve individuals where it was found to be indistinguishable from a real needle and was applied as the placebo in the present study.\textsuperscript{28} The device gives the visual illusion that the needle penetrates the skin, while in reality no penetration occurs, as the shaft of the sham needle telescopes into the handle when downward pressure is applied (see Figure 1).
The aim of the present study was to address the lack of robust research of the analgesic and placebo effects of acupuncture through investigation of the effect of real acupuncture, sham acupuncture and a control intervention on thermal sensation and thermal pain thresholds. Cold sensation, warm sensation, cold pain and hot pain thresholds, measured by Quantitative Sensory Testing, before and after these interventions were employed as the outcome measures. The subjects’ experience of sensation with the acupuncture interventions and their identification of the types of acupuncture were also explored to aid evaluation of the effectiveness of the sham needles.
METHODS

Design

A single blind randomized controlled repeated measures (within subject) trial. The
independent variable was acupuncture (real and sham). The dependent variables were the
thermal sensation and thermal pain thresholds.

Subjects

A convenience sample of 18 healthy volunteers (12 females, 6 males: mean age 29.1 years,
standard deviation 9.24) was recruited from the student body of Queen Margaret University
College, by response to a general notice and word of mouth. All 18 subjects completed the
study. Approval was obtained from Queen Margaret University College Ethics Committee.
Exclusion criteria were: any upper limb pathology in the last five years, previous acupuncture
experience or acupuncture contraindications (metal allergy, fear of needles, skin sensation
problems, pregnancy, bleeding disorders, epilepsy, skin disorders, taking medication and
taken alcohol in previous 24 hours).29 Inclusion criteria: healthy, acupuncture naïve, no
cognitive, mental or communication impairment preventing informed consent.

Materials and apparatus

The Park Sham Device was employed to deliver both the real and sham acupuncture.27 A
thermal sensory analyser delivered the thermal stimuli and recorded the subjects’ response.
The stimuli were delivered via a thermode attached by a Velcro strap to the subject's thenar
eminence; the forearm was pronated - to allow access to the acupuncture points with the
subjects in a relaxed position. Using the method of limits algorithm, the stimuli were
presented with increasing intensity and the subjects were asked to indicate when they first
perceived the onset of a specific sensation.30 Subjects responded to the stimuli by pressing
the response button and the computer recorded the temperature; table one presents the test parameters of the thermal sensory analyser.

Insert Table 1 about here

**Procedure**

Two pilot studies were performed. Firstly, the researcher went through the testing procedure twice, so as to be familiar with the protocol. Secondly, the intra-rater reliability of assessment of the thresholds was measured. Five subjects were tested on three separate occasions (no more than one week apart). The results of the pilot work showed the reliability of the assessments were ‘substantial’ for the thermal sensations (cold, r=.68, warm, r=.77) and ‘almost perfect’ for the pain thresholds (cold, r=.98, hot, r=.91). On the basis of these findings it is reasonable to hypothesize that any changes observed in the thresholds, in the main study protocol, could be attributed to the intervention.

All subjects gave informed written consent. With three interventions, there are six possible orders in which they can be delivered. Subjects were randomly assigned, by blind card allocation, to receive one of those orders. For each intervention the subject lay supine on a plinth, with their right arm exposed from the elbow downwards. Their elbow was flexed to 90 degrees and supported on a pillow. They could not see the computer screen and as a consequence, the stimuli delivered and their previous responses. The thermode was attached to their right hand and they held the response button in their left hand. They were given a practice cycle of the four stimuli at the start of each testing session. Standardized instructions were given for each sensation.
Cold Sensation

‘Press the button as soon as you feel a change in resting temperature’.

Warm Sensation

‘Press the button as soon as you feel a change in resting temperature’.

Cold Pain

‘Let the stimulus go past the first sensation of cold, until it starts to become uncomfortable, press the button as soon as it becomes painful’.

Hot Pain

Let the stimulus go past the first sensation of warmth, until it starts to become uncomfortable, press the button as soon as it becomes painful’.

After the practice cycle, for all the interventions, the two acupuncture sites were cleaned with an alcohol wipe. Figure 2 shows the acupuncture points used, Triple Energizer [TE 5] and Large Intestine [LI 11].

Insert Figure 2 about here

In all interventions thermal sensation and thermal pain thresholds were recorded prior to intervention. In the sham acupuncture intervention two Park Sham Devices were used at the acupuncture points TE5 and LI11 for 25 minutes. In the real acupuncture intervention the same procedure was followed as for sham except that real acupuncture needles were used in the Park Sham Device carrier. In the control intervention the subjects lay for 25 minutes with no input or stimulus given. In all interventions thermal sensation and thermal pain thresholds were measured again immediately after the intervention period. Each subject experienced all three interventions within a three-week period, receiving one per week, always on the same
day and at the same time. After both sham and real acupuncture interventions the subjects were asked a standardised question, ‘Did you feel any sensations in your arm during the acupuncture’, with Yes/No response options and were given a list of possible sensations.32 After the third intervention they were asked to identify the different types of acupuncture. Figure 3 presents a CONSORT flow diagram illustrating the route taken by subjects entering the study.

**Data Analysis**

The thermal sensory software automatically calculated a mean figure (°C) for the 3 measurements of each thermal threshold over each experimental cycle. All values were expressed as change from the adaptation temperature of 32 °C. The two cold based absolute thresholds were calculated as: 32 – recorded value, and the two warm/hot as: recorded value – 32. These ‘absolute’ values of the threshold were employed to calculate change in threshold following control, sham or real acupuncture as threshold post intervention minus baseline. Thus, for all thresholds, positive values for change always represented an increase in the threshold and hence a decrease in sensitivity and negative values always represented a decrease in threshold and hence an increase in sensitivity. Differences in the baseline values, observed prior to each intervention, were investigated with a separate repeated measures ANOVA for each thermal threshold; for all thresholds there were no statistically significant differences between baselines. Differences in the changes in threshold that occurred (from pre to post intervention) were therefore investigated with a separate repeated measures ANOVA for each threshold. Normality of distribution was tested with the Shapiro-Wilk test; for thresholds where distribution was not within acceptable limits of normality (p<0.05) the
non-parametric equivalent - Friedman test - was employed. The statistical power of the inferential tests applied, as calculated by the SPSS (v11.0) software employed, is also reported. A Sign Test was employed to test frequency of correctness of identification of type of acupuncture.
RESULTS

Mean thermal sensation and thermal pain thresholds are presented in table 2.

Insert Table 2 about here

There were no statistically significant differences (p>0.05) between the baseline values of any of the four thermal thresholds recorded prior to application of each of the three interventions. Inferential comparisons were therefore made of the levels of change calculated as °C. The levels of change in thermal sensation and thermal pain thresholds are presented in table 3 as the mean of the changes calculated in °C.

Insert Table 3 about here

With the exception of warm sensation all thresholds increased with the application of real acupuncture representing a decrease in sensitivity. For cold sensation threshold, increases occurred under both control (0.08 °C) and real acupuncture (0.12 °C) and a decrease under sham (-0.21 °C); suggesting that real acupuncture increased threshold, to some degree, relative to sham, but only negligibly relative to control. In cold pain threshold an increase also occurred with real acupuncture (0.93 °C). This contrasted with a smaller increase of 0.18 °C with sham and a decrease with control (-0.43 °C); suggesting that real acupuncture increased threshold relative to both control and sham. For hot pain threshold an increase of 0.85 °C occurred with real acupuncture while under sham a -0.88 °C decrease occurred with no change under control. Again suggesting real acupuncture increased threshold relative to both control and sham. While this reveals an interesting trend to decreased sensitivity, in three of the four thresholds, with real acupuncture it must be noted that the levels of change observed were
small relative to the underlying variance in the data sets. The results of the inferential tests are presented in table 4.

Table 4 illustrates that none of the differences between the levels of change in threshold observed, following each of the three interventions, were statistically significant. It is, however, worthy of note that in all thresholds the Statistical Power of the tests was low. Indeed in all cases Power was well below the widely accepted optimum level of 0.80.33

The results must be considered in the context that 12 of the 18 subjects answered correctly when asked which type of acupuncture they had received. It is worthy of note, however, that these may well have been correct guesses rather than identifications as this is not a statistically significant departure (p=0.238) from the distribution expected had only chance lay behind the decision. Four subjects did not report experiencing any sensations with the real acupuncture while 8 experienced sensations with sham – interestingly 2 subjects therefore experienced a sensation with a type of acupuncture that they called sham – this further supports the argument that subjects may indeed have been guessing.


**DISCUSSION**

The present results show no significant differences in the level of change in thermal sensation and thermal pain thresholds occurring with the three interventions applied: real and sham acupuncture and control. Hence two inferences are made: firstly, real acupuncture did not have any significantly greater analgesic effect (as represented by change in thermal sensation and thermal pain thresholds) in comparison to sham and control. Secondly, as there was no significant differences between sham and control, there was no evidence of a placebo effect with acupuncture.

Meaningful comparison of the present results with those of previous published acupuncture research is problematic. Only Lundeberg et al\(^ {31} \) employed computerised quantitative sensory testing and in accord with the present results reported no significant differences between real and placebo acupuncture – placebo being defined as superficial needling. Man & Baragar\(^ {22} \) also found no analgesic or placebo effect of acupuncture on thermal sensations; unfortunately how thermal sensation was measured was not reported in that work and placebo was defined as acupuncture on non-acupuncture points. A similar placebo model was employed by Berlin et al\(^ {21} \) who, in contrast, found a significant difference between real and placebo acupuncture, in that real delayed onset of a pain terminating response to a heat source. In an extremely small and select sample (n=4, ‘scientists’), Day et al\(^ {20} \) reported no significant acupuncture effect on their subjects ability to discern between different intensities of heat from a calibrated heat dolorimeter. The present results counter those of Ashton et al\(^ {25} \) who reported a significant difference between acupuncture and a placebo drug treatment in terms of pain tolerance using the Cold Pressor Test and they concluded that acupuncture increased cold pain, ‘threshold’.
In the present study the low power of the inferential tests applied is worthy of note. The power of all the tests lay below 0.343, which is well below the minimum 0.80 recommended. It is therefore possible that a Type II error may have occurred and the trend seen here may reach significance if repeated in a future study conducted with a larger sample. It is equally possible, however, that the low power arose from a small effect size. While it is true that a trend to reduction of sensitivity to both thermal sensation and thermal pain thresholds with real acupuncture was observed, this trend was small relative to the underlying variance of the data sets. Consequently it could be argued that an analgesic effect of real acupuncture cannot be definitively ruled out on the basis of these results and further study is recommended.

12 of the 18 subjects correctly identified the type of acupuncture they were receiving; as this is not a significant (p=.238) departure from the distribution expected with random choice it appears these may in fact have been correct guesses rather than genuine identifications. As this decision was made after the subjects had experienced all three interventions there may also have been a question of recall accuracy. This interpretation is further supported by the fact that 8 subjects experienced a sensation with a form of acupuncture they later reported to be sham; it is therefore recommended that future research fully explore this issue. It is true that the production of valid placebo acupuncture is notoriously difficult and this finding may suggest that the Park Sham device is not a foolproof solution to this problem that plagues all acupuncture research. The efficacy of the Park Sham device is based on only one previous study that included hospital patients who had suffered ‘strokes’. Any resulting neurological deficits in these subjects may well have included sensory changes that may in turn have affected the results reported. Further work is therefore required to fully establish claims that the Park Sham device is a credible acupuncture placebo.
It is also important to note that four of the present subjects did not experience any sensation with the real acupuncture, which some may argue indicated that the acupuncture had not been applied correctly. The question of the necessity of subjects experiencing a sensation (commonly termed, ‘di-qí’; a numbness or toothache type ache/pain at the point) for acupuncture to be effective is debated among both clinical acupuncturists and acupuncture researchers and the question is by no means settled. Nonetheless, for interest, all inferential analysis was repeated on only the 14 subjects who perceived a sensation with real acupuncture and all tests remained non-significant. This suggests that experience of sensation was not an influencing factor on the present results.

The external validity of the present study may have been limited by the mode of delivery of the stimuli, via the thermode and its site of application: thenar eminence. The method of limits algorithm also has limitations, as verbal instructions must always be considered to be open to some degree of subjective interpretation. As pain has many components a wider range of outcome measures than were applied here may also be required to fully evaluate therapies aimed at pain modulation. This view is supported by the Sensory Decision Theory, which acknowledges that pain has a psychological component and takes into account measurement of both sensory and attitudinal components. Another methodological issue with the present study may be that the operator collecting the outcome measures was not blinded to the type of acupuncture delivered. Unfortunately due to financial constraints this model was not possible in the present study and it is recommended that future research employ two operators: one to deliver the intervention and one to collect outcomes.
CONCLUSIONS

No statistically significant differences were found between real and sham acupuncture and a control intervention on thermal sensation and thermal pain thresholds in this cohort. Consequently no support was provided for the analgesic and placebo effects of acupuncture. A trend to a reduction in sensitivity to all thresholds was observed with real acupuncture but the changes were small relative to the underlying variance. The low power of the inferential tests applied could have arisen from both a type II error or a small effect size and further study is recommended. Pain is a multi dimensional experience involving different processes: in future studies of the efficacy of acupuncture it may be appropriate to not only assess thermal sensation and thermal pain thresholds but also the wider spectrum of pain perception and experience, evaluation of coping strategies, attitude and functional abilities.

Acknowledgements

Andrew Grainger B.Sc. for technical support and Judith Lane M.Sc. M.C.S.P. for statistical advice.
References


Suppliers

a. TSA 2001® Medoc Advanced Medical Systems Ltd. 45 Ha’oren St. PO Box 423, Ramat Yishai, 30095 Israel
**Fig 1. Park Sham Device with sham and real needle**

1. Needle hand  
2. Guide Tube  
3. Guide O-Ring  
4. Park tube  
5. Flange  
6. Double sided tape  
7. Skin  
8. Dermis  
9. Muscle  
10. Dull tip of sham needle  
11. Sharp tip of real needle
Fig. 2 Location of acupuncture points
Fig. 3 CONSORT flow diagram illustrating the route taken by subject’s entering the study
**Table 1: Test parameters of the thermal sensory analyser**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order of sensation presentation</td>
<td>CS, WS, CP, HP*</td>
</tr>
<tr>
<td>Adaptation temperature</td>
<td>32°C</td>
</tr>
<tr>
<td>Rate of returning temperature</td>
<td>10°C/sec</td>
</tr>
<tr>
<td>Temperature range</td>
<td>0-50°C</td>
</tr>
<tr>
<td>Number of stimuli for each sensation</td>
<td>3</td>
</tr>
<tr>
<td>Rate of temperature change</td>
<td>0.5°C/sec</td>
</tr>
<tr>
<td>Interval between stimuli</td>
<td>4-12 seconds for CS and WS</td>
</tr>
<tr>
<td></td>
<td>4-6 seconds for CP and HP</td>
</tr>
</tbody>
</table>

* CS = Cold Sensation, WS = Warm Sensation, CP = Cold Pain, HP = Hot Pain
Table 2: Mean thermal sensation and thermal pain thresholds (°C) before (Base) and after (Post) control, sham and real acupuncture interventions, mean ± 1 SD

<table>
<thead>
<tr>
<th>TT</th>
<th>Control</th>
<th>Sham</th>
<th>Real</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base</td>
<td>Post</td>
<td>Base</td>
</tr>
<tr>
<td>CS</td>
<td>1.53±0.57</td>
<td>1.61±0.72</td>
<td>1.81±0.78</td>
</tr>
<tr>
<td>WS</td>
<td>2.29±1.75</td>
<td>1.63±0.98</td>
<td>2.27±1.94</td>
</tr>
<tr>
<td>CP</td>
<td>10.79±4.49</td>
<td>10.36±4.24</td>
<td>10.70±4.19</td>
</tr>
<tr>
<td>HP</td>
<td>10.19±3.54</td>
<td>10.19±3.44</td>
<td>10.59±3.16</td>
</tr>
</tbody>
</table>

TT = Thermal Threshold, CS = Cold sensation, WS = Warm sensation, CP = Cold Pain
HP = Hot Pain
Table 3: Change in thermal sensation and thermal pain thresholds, following control, sham or real acupuncture (post – baseline); mean of the changes observed in °C (± 1 SD)

<table>
<thead>
<tr>
<th>Thermal Threshold</th>
<th>Control</th>
<th>Sham</th>
<th>Real</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold Sensation</td>
<td>0.08±0.42</td>
<td>-0.21±0.64</td>
<td>0.12±0.69</td>
</tr>
<tr>
<td>Warm Sensation</td>
<td>-0.66±1.45</td>
<td>-0.72±1.35</td>
<td>-0.21±0.99</td>
</tr>
<tr>
<td>Cold Pain</td>
<td>-0.43±2.11</td>
<td>0.18±2.12</td>
<td>0.93±4.13</td>
</tr>
<tr>
<td>Hot Pain</td>
<td>0.00±0.79</td>
<td>-0.88±3.57</td>
<td>0.85±1.83</td>
</tr>
</tbody>
</table>
Table 4: Results of inferential tests of change in thermal sensation and thermal pain thresholds (°C), following control, sham or real Acupuncture interventions (post – baseline)

<table>
<thead>
<tr>
<th>Thermal Threshold</th>
<th>Norm</th>
<th>F</th>
<th>Chi²</th>
<th>d.f</th>
<th>p</th>
<th>Sig</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold Sensation</td>
<td>Yes</td>
<td>1.971</td>
<td>1.482</td>
<td>0.168</td>
<td>No</td>
<td>0.322</td>
<td></td>
</tr>
<tr>
<td>Warm Sensation</td>
<td>No</td>
<td>3.875</td>
<td>2</td>
<td>0.144</td>
<td>No</td>
<td>0.237</td>
<td></td>
</tr>
<tr>
<td>Cold Pain</td>
<td>Yes</td>
<td>1.146</td>
<td>2</td>
<td>0.330</td>
<td>No</td>
<td>0.235</td>
<td></td>
</tr>
<tr>
<td>Hot Pain</td>
<td>No</td>
<td>3.086</td>
<td>2</td>
<td>0.214</td>
<td>No</td>
<td>0.343</td>
<td></td>
</tr>
</tbody>
</table>

Norm = Data normally distributed and parametric test performed

F = Repeated measures ANOVA F value

Chi² = Friedman’s Chi² value

d.f = Degrees of freedom

Sig = Statistically significant at p<0.05

Power = Power for Repeated Measures ANOVA calculated on SPSS