

THE EFFECT OF ACUPUNCTURE ON ALPHA-MOTONEURON EXCITABILITY

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Abstract:

The effect of short duration and sustained manual acupuncture on α -motoneuron excitability was studied using the Soleus H-reflex. The acupuncture points GB-34 and SP-9 were needled and stimulated manually. Normalised H-reflex and M-wave recruitment curves were constructed and the ratio of the slope of the ascending part of the H-reflex recruitment curve (H_{slp}) to the slope of the ascending part of the M-wave recruitment curve (M_{slp}) was used for assessing changes in α -motoneuron excitability. Sustained manual acupuncture caused significant reduction of α -motoneuron excitability, while short duration manual acupuncture had no effect. The reduction of α -motoneuron excitability following sustained manual acupuncture occurred fifteen minutes after cessation of acupuncture and was still present at 30 minutes. The relationship between perceived intensity of acupuncture sensation (Deqi) and changes in α -motoneuron excitability was also studied, but no significant correlation was found. This study indicates that manual acupuncture would be useful in clinical conditions associated with increased α -motoneuron excitability and that when using acupuncture for these conditions, higher intensity of perceived acupuncture sensation (Deqi) may not result in greater degree of reduction in α -motoneuron excitability.

KEY WORDS: Manual acupuncture; H-reflex; Motoneuron excitability; Deqi; Recruitment curve; Acupuncture sensation

INTRODUCTION

Research into the scientific basis of acupuncture has been mainly focussed on analgesic effects and neurohumoral connections [1,2,3,4,5]. There has been little research into the effect of acupuncture on motor control. The final pathway of motor control is via nerve fibres which arise from large neurons, known as α -motoneurons, located in the anterior horns of the spinal cord. It is possible that some of the pain modulating pathways activated by acupuncture could also influence the excitability of these α -motoneurons. This hypothesis is supported by the discovery of multitudinous synaptic inputs to motoneurons from both spinal and supraspinal sources [6,7,8,9]. Stimulation of sensory afferents would be expected to influence α -motoneurons at the level of the spinal cord in which they enter and synapse. Acupuncture applied to effect a response at a particular spinal cord segment may be termed segmental stimulation.

Studies on reflex inhibition of spinal motoneurons during muscle fatigue have provided an important link between acupuncture and motor control [10,11]. The majority of sensory axons leaving the skeletal muscle are from free nerve endings within the muscle [12]. These fibres are small myelinated (Group III) or unmyelinated (Group IV) and have slower conduction velocities as opposed to the larger myelinated and higher conductive Group I axons from the muscle spindle primary endings and Golgi tendon organs and Group II axons from the spindle secondary endings. Group III and group IV free nerve endings have been shown to react vigorously to muscle contraction and to muscle metabolites that accumulate with fatigue [13,14]. The afferent limb of the fatigue associated inhibitory reflex is known to involve the group III and group IV muscle afferents [15]. Group III and group IV fibres are also implicated in the conduction of afferents input to the central nervous system during acupuncture [16,17]. Both acupuncture and motor control appear to involve similar structures within the central nervous system such as the cerebral cortices, limbic system, basal ganglia and brainstem structures and the spinal cord. It is, therefore, possible that acupuncture can have an effect on α -motoneuronal excitability through modulation of activity in these structures. Neurotransmitters such as serotonin, noradrenaline, GABA, cholecystokinin, acetylcholine and neuropeptides might also be involved in both acupuncture and motor control [5,9,18,19,20,21]. Thus, it appears that there may be a significant commonality between the neural structures involved in acupuncture analgesia and in motor control.

Studies investigating the physiological effects of acupuncture have utilised a variety of methods. These include the changes in EMG responses, and the alteration of muscle reflexes such as the stretch reflex, tonic vibration reflex and the Hoffmann-reflex [22,23,24,25,26,27,28,29]. The Hoffmann-reflex (H-reflex) is an artificially induced response evoked by stimulating a mixed peripheral nerve to a muscle [30] (Fig.1.1). The Ia afferent fibres in the nerve are stimulated preferentially at low intensities. These synapse essentially monosynaptically with homonymous α -motoneurons in the anterior horn of the spinal cord. The impulse generated is then transmitted in the motor axon to the recipient muscle which responds with a contraction. The electromyogram (EMG) of this muscle contraction elicited by the mixed peripheral nerve stimulation is called the H-reflex [30,31]. With increasing intensity of stimulation, more and more α -motoneurons are excited to firing threshold, a process known as recruitment. The greater the number of α -motoneurons recruited, the larger the amplitude of the H-reflex. At higher intensity of stimulation, the motor axons in the peripheral nerve are also excited and these evoke

direct stimulation of the muscle endplates, leading to the appearance of a second wave in the EMG. This is at a shorter latency than the H-reflex and is called the M-wave [30]. In simple terms, the M-wave involves stimulation of the motor axons, the neuromuscular junctions, and the muscle. It can be affected by the state of these anatomical areas and the stimulation site itself. The amplitude of the H-reflex, in addition, involves the afferent Ia fibres, their synapses with the α -motoneurons, and the excitability of the α -motoneurons themselves. The amplitude is also influenced by the state of these anatomical areas and the stimulation site. In the H-reflex methodology for assessing α -motoneuron excitability after an intervention, M-waves are recorded before and after the intervention to ensure peripheral stability, so that any changes in the H-reflex can be ascribed to changes in the central nervous system [32,33,34]

The purpose of the present study was to evaluate the effect of segmental, short and long duration manual acupuncture on α -motoneuron excitability using the H-reflex and to evaluate whether a correlation exists between the intensity of perceived acupuncture sensation (deqi) and the effect of acupuncture on motoneurons.

MATERIALS AND METHODS

Subjects

Twenty (20) subjects participated in the study. There were 8 males and 12 females with age between 24 to 56 years (mean = 36.7). Fourteen (14) participants had previous experience with acupuncture. Subjects were excluded from the study if they had a history of previous spinal surgery, systemic disease, neurological disease, or persistent pain in the lower back or lower limbs. Subjects were requested to abstain from taking aspirin, caffeine or alcohol [35] and vigorous exertion [36] on the day of the experiment. Ethical approval was obtained from the Auckland University of Technology Ethics Committee. Written and verbal explanations of experimental procedures were provided to subjects prior to testing. Each subject provided informed consent.

H-reflex and M-wave Recordings (Fig.1, 2a, 2b)

The experiments were carried out in a quiet environment within the Human Neurophysiology Laboratory of the Physical Rehabilitation Research Centre, Auckland University of Technology. During the experimental procedure, subjects were seated in a dental chair with head rest and adjustable back, leg and foot supports. The leg of the subject was strapped to the leg support to reduce the variation in serial recording of the H-reflex [37]. A foot rest maintained the foot at an angle of 80° to the leg. To avoid the influence of tonic neck reflexes, the subject was asked to keep the head in the midline during the experiment [38]. The tibial nerve was stimulated via two 9-mm Silver/Silver Chloride disc electrodes coated with Ten 20 Conductive EEG Paste (D.O. Weaver & Co., Aurora, USA). The cathode was positioned over the tibial nerve at the popliteal crease, secured with Fixomull® Stretch Tape (Beiersdorf AG, Hamburg, Germany) and further fixed with a piece of foam held by elastic bandage. The anode was positioned on the knee just lateral to the patella using Fixomull® Stretch Tape. The H-reflexes and M-waves were recorded with a disposable self-adhesive bipolar EMG bar electrode (Noro-trode™, Naromed Inc., Seattle, WA) placed in the midline of the Soleus muscle parallel to the muscle fibres, three quarters of the distance distal between the popliteal crease and calcaneum [39]. The electrode

was determined to be correctly placed when the H-reflex had a similar configuration to the M-wave [31,40].

LabVIEW computer software (National Instruments Inc., USA) drove a Digitimer DS7 constant current stimulator (Digitimer Ltd, Hertfordshire, UK) which delivered a 0.5 – 1.0 ms duration square wave pulse to the tibial nerve every 6 seconds [31] [41]. The intensity of stimulation was increased stepwise from below the threshold for eliciting an H-reflex (H_{th}) to that producing a maximum M-wave (M_{max}). Three stimuli were given at each intensity level to account for any variability in the H-reflex amplitude at rest [42]. Electromyographic signals generated in the Soleus muscle were sampled at a rate of 5 kHz, then amplified and filtered with a bandwidth of 1 Hz-5 kHz by a Grass P5 Series Amplifier. LabVIEW computer software was used to display digitised data on the computer screen for direct scrutiny. Recordings were stored and the peak-to-peak amplitudes of the H-reflexes and M-waves were later analysed.

Recruitment Curve Recording Protocol

Reflexes were recorded in response to a progressive increase in stimulus intensity over 14 steps from pre-threshold H-reflex to maximal M-wave in order to obtain a recruitment curve. A recruitment curve reflects the number of α -motoneurons firing at each stimulus intensity. The same range of stimulus intensities was used for determining the H-slope (H_{sp}) in each trial of the same experiment.

Experimental Design

The effects of two acupuncture interventions of different durations, 5 minutes and 20 minutes, were compared using a repeated-measures, crossover design. Each subject received both interventions. To minimise any carry-over effects, the interventions were delivered at least two weeks apart. To control for the learning effect, the order of short duration acupuncture and sustained acupuncture was randomised for each subject.

Two H-reflex and M-wave recruitment curves (Trials 1 and 2) were recorded 5 minutes apart before the acupuncture intervention and were used as controls. Further trials were then performed to assess the change in α -motoneuron excitability immediately after acupuncture (Trial 3) and 15 minutes after acupuncture (Trial 4). In a proportion of participants a further trial was taken 30 minutes after acupuncture (Trial 5) to assess the delayed effects of acupuncture.

Acupuncture interventions

Sterile, single use disposable acupuncture needles with a gauge of 20 x 40 and with guide-tubes (Suzhou Medical Instruments, Suzhou, China) were used. The acupuncture points GB-34 (Yanglingquan) and SP-9 (Yinlingquan) were selected for this study. It was expected that acupuncture at these two sites would cause afferent impulses to travel to the L4, L5, S1 and S2 segments of the spinal cord and then to higher centres within the nervous system.

The subject was asked to indicate when acupuncture sensation (deqi) occurred after needle insertion. "Deqi" is defined as "the arrival of qi and refers to soreness, numbness or a distending feeling around the point after the needle is inserted to a certain depth" [43]. At each acupuncture point, the needle was manipulated to maintain the acupuncture sensation for at least 1 minute. The needles were then left in place.

For the short duration acupuncture no further manipulation of the needles was undertaken and the needles were removed 5 minutes after the first arrival of acupuncture sensation (deqi). For the sustained acupuncture intervention, the needles were manipulated at 5 minutes intervals. On each occasion, acupuncture sensation was maintained for at least 1 minute. The needles were then removed 20 minutes after the first arrival of acupuncture sensation (deqi).

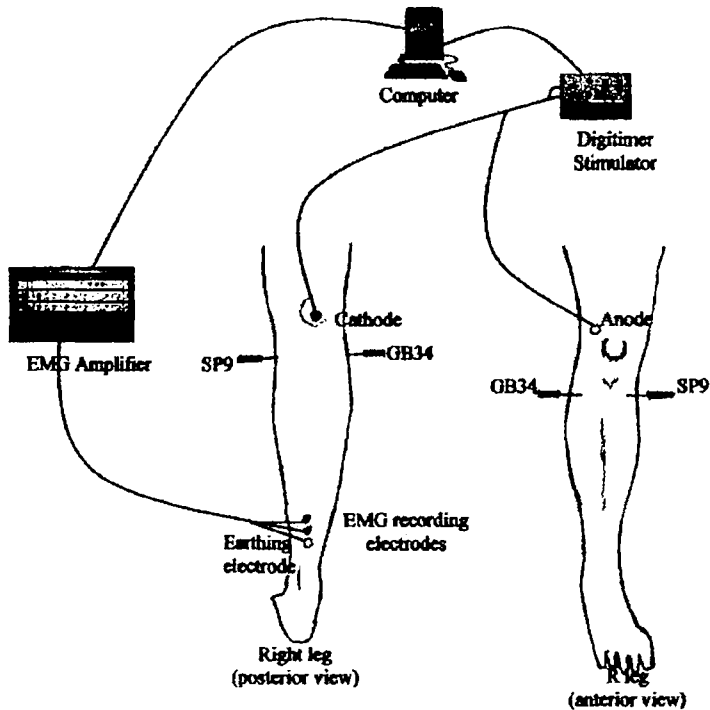


Fig.1 Experimental setup of the H-reflex stimulation and recording system. A computer programme triggers the Digitimer stimulator to cause an electrical stimulation of the Soleus Ia afferent fibres in the Popliteal fossa. These fibres synapse essentially monosynaptically with the homonymous α -motoneurons in the spinal cord, resulting in an impulse traveling down the motor nerve to the Soleus muscle which then contracts. The contraction of the Soleus muscle is recorded with EMG electrodes in the lower calf area and stored in the computer for further analysis.

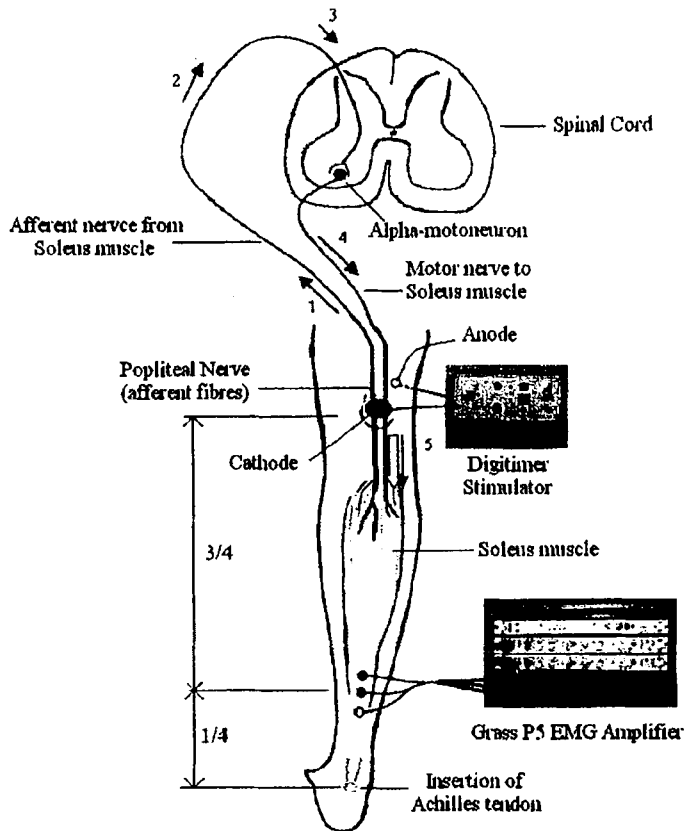


Fig. 2a Positioning of EMG recording electrodes and neural connections of H-reflex and M-wave. The EMG electrodes were positioned in the posterior aspect of the leg at the junction of the upper $\frac{3}{4}$ and lower $\frac{1}{4}$ of the distance between the popliteal crease and the Achilles tendon insertion on the calcaneum. The block arrow \Rightarrow indicates the flow of electrical impulses from the stimulating electrode (cathode) directly to the Soleus muscle via the motor nerve fibres. This causes a contraction which is labelled as the M-wave in the EMG. The black arrows (1-5) \rightarrow indicate the flow of electrical impulses in the muscle afferent nerves from the stimulating electrode (cathode) to the anterior horn of the spinal cord where they synapse with the α -motoneurons. The impulses then travel down the motor nerve fibres to the Soleus muscle causing a contraction which is known as the H-reflex in the EMG.

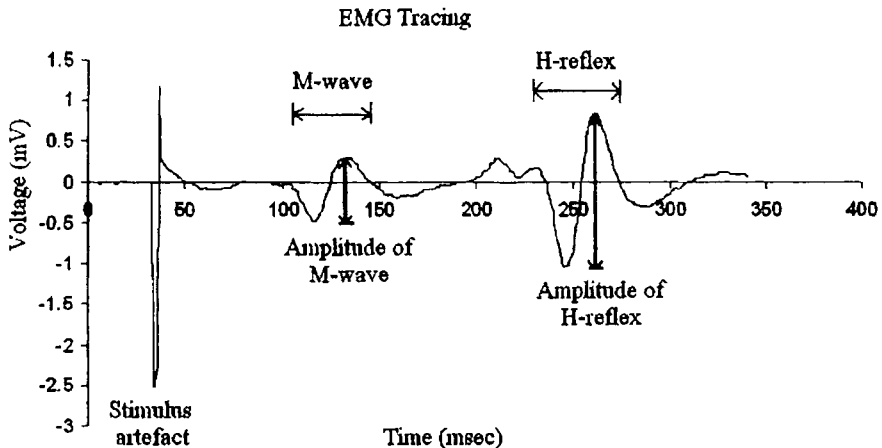


Fig. 2b Electromyographic tracing showing the stimulus artefact, followed by the M-wave and H-reflex. The M-wave has a shorter latency after the stimulus artefact because it is the result of direct stimulation of the muscle via the motor nerve fibres while the H-reflex has a longer latency because it is the result of a reflex stimulation via the afferent fibres which synapse with the spinal α -motoneurons. The peak-to-trough amplitude of M-wave reflects the number of motor fibres directly stimulated while that of the H-reflex reflects the number of spinal α -motoneurons reflexly excited during an experiment.

Data analysis

The peak-to-peak amplitudes of all H-reflexes and M-waves were measured by LabVIEW computer software. The amplitudes of H-reflexes and M-waves to three consecutive stimuli of the same intensity were averaged. To reduce inter-individual differences and variability in absolute H-reflex and M-wave sizes, and to allow data to be pooled, amplitudes of H-reflexes and M-waves and the corresponding stimulus intensities were normalised according to established protocol [32,44,45,46]. Recruitment curves were plotted with normalised averaged H-reflex or M-wave amplitudes on the y-axis and normalised stimulus intensities on the x-axis for all experiments.

Linear Regression Analysis of H-reflex & M-Wave Recruitment Curves (Fig.3)

Linear regression analysis was applied to the linear portion of the ascending part of the H-reflex recruitment curve using EXCEL (Microsoft ® Excel 2002 SP-1, Copyright © Microsoft Corporation, 1985-2001). All data measured on the rising part of the recruitment curve at stimulus intensities less than the threshold of the M-response (M_{th}) were included for this analysis. The slope of the line described the increase in H-reflex amplitude per unit increase in stimulus intensity and was called H-slope (H_{slp}). A change in H_{slp} will represent a change in the

excitability of the α -motoneuron pool [32,47]. Linear regression analysis was applied in the same manner to the ascending slope of the M-wave recruitment curve. The slope of the line described the increase in M-wave amplitude, and, indirectly, the number of muscle fibres recruited per unit increase in stimulus intensity. This was called M-slope (M_{slp}).

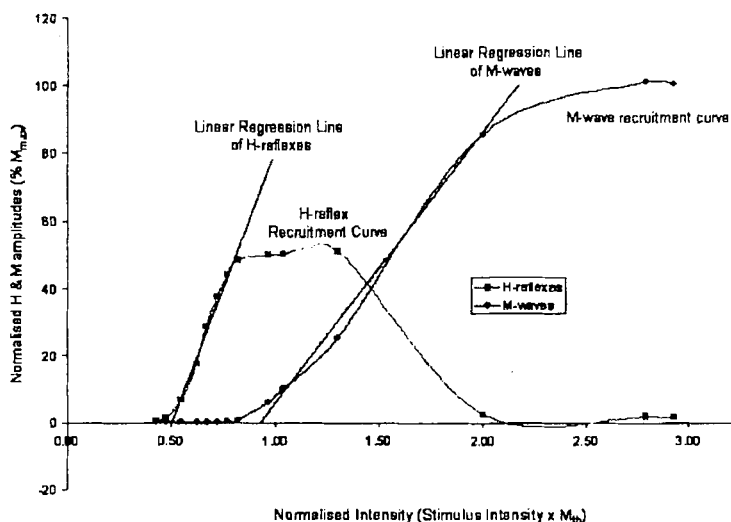


Fig. 3 Normalised H-Reflex and M-Wave Recruitment Curves. Each data point represents the average of 3 response amplitudes at the same stimulus intensity. Linear regression lines were fitted to data points on the linear ascending portion of the H-reflex and M-wave recruitment curves.

Analysis of Pre-intervention H-slopes and Overall M-slopes Stability

To screen and confirm the consistency of the pre-intervention control H-slopes and M-slopes across the trials, intraclass correlation coefficient (ICC) was calculated using SPSS (version 11.0.1, Copyright ©SPSS Inc. 1989-2001). Intraclass correlation is sensitive to any systematic change in the means of the scores [48] and thus can be used to give an overall index of repeatability and stability. An ICC of 0.95 or above was chosen as the criteria for acceptance. In addition, the M-slope intercept method was used to establish M-wave stability [34], with the exception that if the difference of the lower and upper bound 95% confidence intervals of a pair of non-overlapping y-intercepts was less than 1% of the mean of the two values, the set of data would still be accepted. When M-slope stability had been confirmed, changes in α -motoneuron excitability could then be ascribed to a change in central nervous system excitability.

Assessment of Motoneuron Excitability by H_{slp}/M_{slp}

The H-slope/M-slope ratio (H_{slp}/M_{slp}) has been used as a parameter for assessing changes in α -motoneuron excitability in previous studies [32,33], whereby the M_{slp} is used as a reference

property of motoneurons to evaluate the reflexive H_{slp} [44]. An increase in H_{slp}/M_{slp} indicates an increase in α -motoneuron pool excitability while a decrease in H_{slp}/M_{slp} indicates a reduction. H_{slp}/M_{slp} data for all participants were pooled according to trials. The means and standard deviations of H_{slp}/M_{slp} from each trial were calculated. The mean of H_{slp}/M_{slp} in Trials 1 and 2 was referred to as baseline or control trial mean. The difference between the H_{slp}/M_{slp} in the post-acupuncture trials (Trials 3, 4 or 5) and the control trial mean for each stimulus intensity was designated a change score and expressed as a percentage of the control trial mean by the following formula:

$$\text{Percentage Change Score} = \frac{\text{Trial Mean (3,4 or 5)} - \text{Control Trial Mean}}{\text{Control Trial Mean}} \times 100$$

The percentage change score allows assessment of percentage change from the control trial mean and comparison of the effect of time at different intervals after acupuncture in addition to the effect of different durations of acupuncture.

Assessment of Intensity of Perceived Acupuncture Sensation (deqi)

Intensity of acupuncture sensation (deqi) was assessed by using a 100 mm horizontal Visual Analogue Scale, divided into 10 equal sections. Zero (0) on the extreme left represented no acupuncture sensation, and 10 on the extreme right represented the strongest imaginable acupuncture sensation. The subjects were asked to average out and grade the intensity of the sensation afterward each episode of needle manipulation.

RESULTS

Effects of Short Duration Acupuncture

Data from 16 subjects with stable M-slopes across the trials (ICC = 0.9740) and stable pre-intervention H-slopes (ICC = 0.9784) were included for final analysis in the short duration acupuncture group. The percentage changes of H_{slp}/M_{slp} in Trials 3 ($p = 0.438$) and 4 ($p = 0.168$) were not significant when subjected to One-Sample T-Test analysis, indicating there was no significant change in α -motoneuron excitability up to 15 minutes after short duration acupuncture.

Effects of Short Duration Acupuncture (extended observation)

To determine the delayed or continual effects of short duration acupuncture on α -motoneuron excitability, 10 subjects receiving short duration acupuncture had H-reflexes monitored up to 30 minutes after acupuncture. Data from 8 subjects with stable M-slopes across the trials (ICC = 0.9717) and stable pre-intervention H-slopes (ICC = 0.9728) were included for final analysis in the short duration acupuncture, extended observation group. The percentage changes of H_{slp}/M_{slp} in Trial 3 ($p = 0.693$), Trial 4 ($p = 0.215$) or Trial 5 ($p = 0.233$) were not significant when subjected to One-Sample T-Test analysis, indicating there was no significant change in α -motoneuron excitability up to 30 minutes after short duration acupuncture.

Effects of Sustained Acupuncture

Data from 15 subjects with stable M-slopes across the trials (ICC = 0.9662) and stable pre-intervention H-slopes (ICC = 0.9662) were used for final analysis of the effects of sustained acupuncture on α -motoneuron excitability. There appeared to be a negative percentage change in H_{slp}/M_{slp} immediately after sustained acupuncture (-10.9%) followed by a further reduction 15 minutes later (-12.3%). The percentage changes of H_{slp}/M_{slp} in Trials 3 and 4 were subjected to One-Sample T-Test analysis. There was a significant change in percentage score in Trial 4 ($p = 0.004$), but not in Trial 3 ($p = 0.069$). This indicated that after sustained acupuncture there was a significant reduction in α -motoneuron excitability at 15 minutes after completion of acupuncture.

Effects of Sustained Acupuncture (extended observation)

To determine delayed and/or continual effects of sustained acupuncture on α -motoneuron excitability, 10 participants receiving sustained acupuncture had H-reflexes monitored up to 30 minutes after acupuncture. Data from 8 subjects with stable M-slopes across the trials (ICC = 0.9581) and stable pre-intervention H-slopes (ICC = 0.9855) were included for final analysis in the long duration acupuncture, extended observation group. There also appeared to be a negative percentage change in H_{slp}/M_{slp} immediately and 15 minutes after sustained acupuncture followed by a further reduction 30 minutes later. The percentage changes of H_{slp}/M_{slp} in Trials 3 to 5 were subjected to One-Sample T-Test analysis. The percentage change of H_{slp}/M_{slp} in Trial 4 ($p = 0.043$), and Trial 5 ($p = 0.010$) were significant but not in Trial 3 ($p = 0.053$). This indicated that, after sustained acupuncture, there was a significant reduction in α -motoneuron excitability from 15 minutes and the effects were still apparent 30 minutes following the intervention.

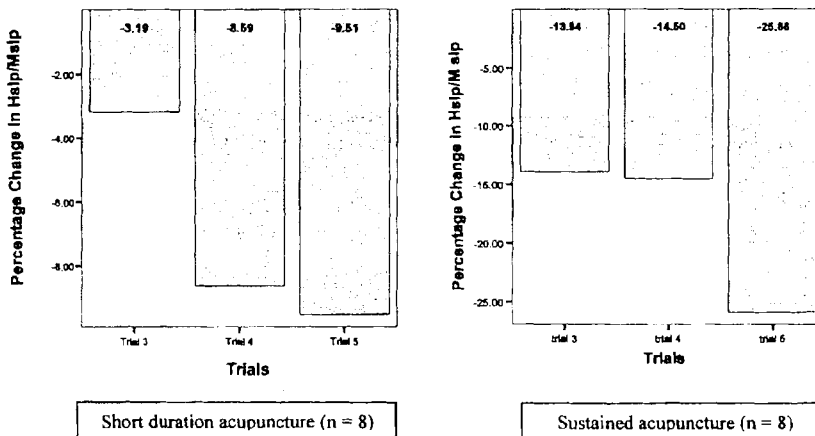


Fig. 4. Bar charts showing the different effects of short duration and sustained acupuncture on α -motoneuron excitability (represented by H_{slp}/M_{slp}) up to 30 minutes after withdrawal of the acupuncture needles immediately prior to Trial 3.

Relation of perceived intensity of acupuncture sensation to changes in α -motoneuron excitability

The relationship between the perceived intensity of acupuncture sensation (deqi) and changes in motoneuron excitability in this study was explored using Spearman's Rank Order Correlation. There was no significant correlation between the perceived intensity of acupuncture sensation (deqi) and percentage changes in H_{slp}/M_{slp} after short duration (in Trial 3, correlation coefficient = - 0.065, $p = 0.810$; in Trial 4, correlation coefficient = - 0.201, $p = 0.456$) or sustained acupuncture (in Trial 3, correlation coefficient = - 0.495, $p = 0.061$; in Trial 4, correlation coefficient = - 0.470, $p = 0.077$), indicating that the intensity of perceived acupuncture sensation (deqi) has no significant correlation to the changes in α -motoneuron excitability as a result of acupuncture.

DISCUSSION

The most significant findings from the present study were that sustained manual acupuncture caused a significant reduction in α -motoneuron excitability. The effect was statistically significant at 15 minutes and was maintained for at least up to 30 minutes after withdrawal of needles. On the other hand, short duration manual acupuncture had no effect on the excitability of the α -motoneuron pool when measured using the H-reflex. Further, the intensity of sensation produced by acupuncture (deqi) was not related to the inhibitory effect.

The findings of this study confirmed previous work whereby acupuncture was found to have no statistically significant effect on the excitability of the motoneuron pool, measured by stretch or H-reflexes immediately after the intervention [23,28,49]. While Milne et al. [23] and Dawson et al. [49] observed a transient inhibition during segmental electroacupuncture, Chang et al. (2001) found no change in the soleus H-reflex during and following extra-segmental manual acupuncture delivered to the contralateral hand for 15 minutes. In contrast this current study found segmental manual acupuncture delivered for 30 minutes had a significant inhibitory effect which persisted after removal of the needles. There are several reasons centered around study design that could explain the differences between this experiment and the work of Chang et al. These include the measurement of H-reflex in the same limb, the inclusion of an extended period of observation, sustained manual acupuncture stimulation, the use of a different parameter in the analysis and possibly the inclusion of acupoint GB-34 (Yanglingquan).

By applying the acupuncture intervention to the same leg from which H-reflex recordings were recorded, the measurements reflected the sum of effects of acupuncture on α -motoneuron excitability via mechanisms which could have taken place in the spinal cord, in addition to those elicited in the midbrain, pituitary-hypothalamus and cerebral cortex when acupuncture was given to the contralateral hand. It is possible that these effects are synergistic and thus greater than the supraspinal effects alone as in the case of acupuncture analgesia [5]. Timing of the post-intervention H-reflex recording could also be an important factor explaining the differences between this and previous work. Here, significant change was only identified at 35 minutes after insertion of needles in the sustained acupuncture experiments. This time span allowed for delayed humoral effects from acupuncture which may appear 30 minutes after the insertion of needles [21,50] and may explain the significant findings not observed in the previous work where stimulation times and post experimental recording times were shorter.

The difference in findings may also be due to the difference in intensity and mode of stimulation of the acupuncture needles. During sustained acupuncture in the present study, two acupuncture points were stimulated for 20 minutes. Chang et al (2001) stimulated a single acupuncture point for a shorter period of time. The greater amount of afferent input in this study might result in summation at the spinal cord and a greater response from α -motoneurons. Experimental evidence using electroacupuncture at the same acupuncture points in rats has shown an increase in analgesia occurring with increase in stimulation intensity at the same frequency [51,52]. Higher intensity of transcutaneous electrical stimulation of acupuncture points was also shown to result in a greater reduction in post-operative analgesia than low intensity stimulation [53].

The use of manual acupuncture in this study could also have influenced the results. Dawson et al. [49] found the inhibitory effect on the reflex response to muscle stretch with electroacupuncture began to fade during the intervention although it was still demonstrable up to 30 minutes post-intervention. Hsieh et al [29], however, did not find any effect of 2 Hz electroacupuncture at the acupuncture point Zusanli (ST-36) on H-reflex in healthy subjects. These results could be explained by different neural pathways and brain mechanisms involved in electroacupuncture and manual acupuncture. A recent functional magnetic resonance imaging (fMRI) study demonstrated that manual and electroacupuncture affected different parts of the brain [54]. Electroacupuncture mainly produced signal increases in precentral gyrus, postcentral gyrus/inferior parietal lobule, and putamen/insula; in contrast, manual needle manipulation produced prominent decreases of fMRI signals in posterior cingulate, superior temporal gyrus, and putamen/insula [54]. Electroacupuncture also increased the concentrations of calcitonin gene-related peptide (CGRP) in cerebrospinal fluid and the frontal cortex of the brain in rats while manual acupuncture had no effect [55]. Experiments performed in rats using electroacupuncture demonstrated increased levels of substance P (SP)-, neuropeptide Y (NPY)-, and neurokinin A (NKA)-like immunoreactivity (-LI) in the hippocampus, and NPY-LI in the occipital cortex, while manual acupuncture did not evoke change in any one of the neuropeptides tested [56]. The different physiological responses of the body to manual compared to electroacupuncture may, therefore, result in differential responses of α -motoneuron excitability after the respective intervention.

Different methods of analysis of the H-reflex could also contribute to different findings between authors using this methodology. In this study, a range of stimulation intensities was used to elicit the H-reflexes from which recruitment curves were constructed and the rate of change in the ascending part of the recruitment curve was measured. In previous studies [23,28,49], the change in magnitude of the reflex in response to a single electrical stimulation intensity to the peripheral nerve was used to assess α -motoneuron pool excitability. A single stimulation intensity would be expected to evoke responses from a smaller pool of α -motoneurons in the spinal cord than the methods used in this study. A range of stimulation intensities will reflect the recruitment of α -motoneurons from small to large according to the "size principle" [57], and would, therefore, be more sensitive to changes in spinal cord activity than experiments using a single stimulation intensity. In addition, different parameters for measuring change in motoneuron excitability could also account for the lack of significant effect revealed in other studies. For example, the use of the greatest H/M ratio parameter by Hsieh et al. [29] demonstrated no significant change 30 minutes after electroacupuncture in the ipsilateral or contralateral leg. It may be that the

H_{slp}/M_{slp} ratio is more sensitive in identifying changes in the H-reflex than the H_{max}/M_{max} parameters [33,44].

Different acupuncture points are purposely used in clinical situations to elicit different therapeutic effects [43,58]. It has been shown by fMRI that different areas of the brain are activated by stimulation of specific acupuncture points. For example, BL-67 (Zhiyin), BL-65 (Shugu), and BL-60 (Kunlun) activated the primary visual cortex, while the adjacent BL-66 (Zutonggu) along the same meridian did not [59]; and stimulation of ST-36 (Zusanli) activated the hippocampus while GB-34 (Yanglingquan) activated the hypothalamus, insula and the motor cortex [60]. LI-4 (Hoku) and LI-11 (Quchi) were the acupuncture points used in the other studies on healthy subjects [23,28,49], while SP-9 (Yinlingquan) and GB-34 (Yanglingquan) were used in this study. The different acupuncture points used might have resulted in different effects on the central nervous system. Further studies on the specificity of acupuncture points will be required to clarify the issue.

The results of the present study concurred with that of a study investigating changes in α -motoneuron excitability in stroke patients [61], in which significantly increased H-reflex recovery time was found after acupuncture indicating a decrease in motoneuron excitability. However, in the Yu et al study [61] the onset of the inhibitory effect occurred immediately after acupuncture rather than as a delayed response as found in this current work. While this could be related to the increased afferent input from use of five acupuncture points as opposed to two used in the present study, a higher baseline α -motoneuron excitability in stroke patients could also contribute to the difference in response. Acupuncture is generally identified as a form of regulatory therapy which encourages a change towards homeostasis [22,58,62]. Thus, in subjects who have near normal baseline levels, like the normal subjects used in this study, the changes after acupuncture may not be as marked as in subjects with pathological conditions.

The significant inhibition of α -motoneuron excitability in this study was not likely to be a result of the subjects lying inactive for 35 minutes. Previously, Dawson et al. [49] demonstrated no change in α -motoneuron excitability even 1 hour after commencement of the experiment. In this present study, no significant effect occurred in the 8 subjects who were assessed 35 minutes after short duration acupuncture. Hence the different effects must be attributed to the different duration of the acupuncture intervention rather than the period of inactivity. The significant inhibition shown after sustained acupuncture, but which was absent in short duration acupuncture, could be the result of greater sensory input from the longer period of needle stimulation eliciting greater response from the central nervous system.

Perceived Intensity of Acupuncture Sensation and α -Motoneuron Excitability

The presence of acupuncture sensation (deqi) is considered to be a pre-requisite for effective acupuncture by traditional acupuncturists [43,58]. However, difference in the subjective perception of needling sensation can occur among individuals and at different points on the same individual [63]. Despite this, Li et al [63] reported a close correlation of needling sensation with needling effect. They found strong needling sensation was associated with marked elevation of pain threshold and depression of somatosensory evoked potentials. The present study, however, showed no significant correlation between the intensity of subjective acupuncture sensation (deqi) and the changes in α -motoneuron excitability in short or sustained acupuncture. This

concur with the finding by Roth et al. [64] that deqi sensation did not correlate with the rise in serum cortisol level after manual acupuncture. Although moderate negative correlations were demonstrated with the percentage changes in H_{slp}/M_{slp} after sustained acupuncture, these were not significant. The results could not, therefore, support the long held belief that the efficacy of acupuncture is related to the subjective perception of a strong acupuncture sensation (deqi), at least in the response of α -motoneuron excitability. It is possible that the relationship between perceived intensity of acupuncture sensation (deqi) and body response, even if present, is not a strong one since slowly conducting muscle afferent units, which presumably are responsible for the transmission of acupuncture input, could be activated by mechanical stimuli at threshold intensity which was not perceived as painful by volunteers [65]. Further study will be needed for better definition of the correlation.

Possible Mechanisms of Acupuncture Effect on α -motoneuron Excitability

During fatigue from a sustained maximal voluntary contraction, mean motoneuron discharge rates declines [10]. The increased inhibition of homonymous or heteronymous motoneurons was ascribed to increased discharges of groups III (A δ) and IV (C) muscle afferents during muscle fatigue [11]. The action was presumably mediated by inhibitory interneurons in lamina VII of the spinal cord [66]. Further, both motor cortical and spinal motoneuron excitability were shown to be suppressed by tonic muscle pain [67], which was also thought to be mediated by activation of group III (A δ) and IV (C) muscle afferents. Since A δ and C muscle afferents transmit acupuncture stimuli to the central nervous system, it is likely that acupuncture will also lead to inhibition of the motoneurons through activation of these inhibitory afferents.

The time course of the changes in α -motoneuron excitability in this study showed a delayed and progressive inhibitory effect even after cessation of acupuncture. From this observation it is postulated that the modulating effect of acupuncture also involves a humoral mechanism such as the release of neuropeptides or endorphins in the spinal cord or higher centres in the brain stem and hypothalamus [68]. Although it has been shown that naloxone did not attenuate exercise-induced H-reflex suppression, which was hypothesized to involve endogenous opioid regulation [69], it could be that the dosage of naloxone used (10 mg) was too low. Sandrini et al [70] demonstrated a marked facilitation of the H-reflex amplitude in normal subjects, but not in paraplegics, by administration of a higher dose of naloxone (1.66 mg/Kg). This suggested the existence of tonic inhibitory modulation of the H-reflex in normal subjects. Acupuncture stimulation could have enhanced this tonic inhibition via release of endorphins from the hypothalamus and brainstem. Neurons from the brainstem project to and modulate excitability of the α -motoneurons in the spinal cord [8]. Activation of the brainstem motor inhibitory systems and inactivation of the brainstem motor facilitatory systems may underlie acupuncture-induced inhibition of α -motoneuron excitability [71,72]. Further study is required to assess the role which endorphins might play in the process. It is important to stress that the exact mechanism of acupuncture causing inhibition of α -motoneuron excitability is not known from this study.

Clinical Implications

This study only demonstrated the physiological effects of acupuncture on α -motoneuron excitability in normal subjects. It may be postulated that acupuncture alters α -motoneuron excitability which, in turn, may influence motor unit activation and hence muscle activity. One has to be cautious when applying the information obtained to clinical situations.

In this study, sustained acupuncture was shown to have an inhibitory effect on α -motoneuron excitability in normal subjects. Therefore, conditions where there is a pathological increase in α -motoneuron excitability may theoretically benefit from the administration of acupuncture. This is encountered in patients with spasticity related to strokes [73]. Since sustained acupuncture for 20 minutes is more effective than short acupuncture for 5 minutes, sustained acupuncture should be preferably applied. The patients should also be informed of the lapse of time between acupuncture and the onset of a significant effect, so that they can have the right expectation from the outset.

This study showed only a moderate but insignificant correlation between the perceived intensity of acupuncture sensation (deqi) and the inhibitory effect on α -motoneuron excitability. Therefore, when acupuncture is administered, it is not essential to attempt to obtain the strongest bearable acupuncture sensation (deqi) if a decrease in α -motoneuron excitability is the goal of the treatment.

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