THE EFFECT OF NECK MANIPULATION
ON EXCITABILITY OF
THE MOTOR CORTEX.

Thesis submission to Auckland University of Technology in partial

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the qualification of any other degree or diploma of a university or other institution of higher leaning, except where due acknowledgment is made in the acknowledgments.
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Abstract

Neck manipulation is commonly used in the management of some musculoskeletal disorders to reduce pain and improve movement. There is, however, little understanding about the underlying mechanism. Recent research has alluded to a neurophysiological mechanism mediated through supraspinal pathways in the central nervous system, that may alter motor activity. The purpose of this study was to determine the effect of neck manipulation on the excitability of cortical motoneurons by means of activating corticospinal pathways to the flexor carpi radialis (FCR) muscle in an active motor system using the transcranial magnetic stimulation (TMS) technique.

Motor evoked potentials (MEPs) were elicited by TMS and recorded in 20 normal subjects using established procedures. The peak-to-peak amplitude of MEPs were measured both before and after C6/7 manipulation and before and after neck positioning. Both interventions were applied to the normal subjects in random order on two different days. MEPs were recorded immediately after the intervention, then seven minutes and 14 minutes later to assess the time course of the effect. Five neck pain subjects participated in the manipulation experiments. The effects of manipulation, resulting in joint cavitation, were also explored. Two trials were undertaken before the intervention and these served as control measures. MEP data was represented in two ways. Firstly, 40 MEPs were averaged over 120 seconds; secondly, 15 MEPs were average over 60 seconds. A percentage change calculation was used to express the data relative to the baseline. Alterations in cortical excitability before and after manipulation were analysed by repeated measure analysis of variance (ANOVA) on the MEP data, and percentage change scores.
Cortical neurons projecting to FCR were significantly facilitated up to 60 seconds after the manipulation of the non-painful segment, relative to baseline values and the positioning control. A small but significant latent increase in excitability was also observed 15 minutes after manipulation. The response to manipulation of the painful cervical segment was significantly different from that of the non-painful segment. When manipulation of the painful segment did not result in joint cavitation, an inhibitory effect was observed. In contrast, however, cortical motoneurons were facilitated when joint cavitation was associated with manipulation.

In conclusion, motor activity is enhanced with neck manipulation when cortical motoneuron excitability is measured by TMS in human subjects performing a voluntary contraction. This may explain the clinically observed improvement in spinal motion and motor control with spinal manipulation. Further, joint cavitation may be important in signifying the success of the joint manipulation. The excitatory effect on cortical motoneurons is probably mediated through a transcortical pathway by means of the activation of muscle afferents with the manipulative thrust.

These findings assist in understanding the neurophysiological mechanism underlying the effect of spinal manipulation.
Chapter One  Introduction

1.1  Manual Therapy

1.1.1  Background

Manual therapy is a specialised field in physiotherapy where a variety of procedures are used to assess and treat musculoskeletal disorders. Manual therapy techniques used in the management of spinal pain may include joint mobilisation, traction and manipulation and are often termed spinal manual therapy (SMT).

Cervical spine pain is a common musculoskeletal complaint that is frequently treated with spinal manipulation (Bronfort, 1999; Di Fabio, 1999). Recent studies have shown cervical spine manipulation may reduce pain, increase range of motion, and hence improve motor control (Di Fabio, 1999; Nansel, Waldorf, & Cooperstein, 1993; Suter & McMorland, 2002; Whittingham & Nilsson, 2001). However, despite its wide usage, the mechanism by which spinal manipulation may produce clinical benefits has not been unequivocally demonstrated in the literature. Understanding the mechanism of the effect of manual therapy is vitally important because, without such an understanding, it is hard to state that its effect is any greater than the psychological interplay between therapist and patient.

There is evidence of the effectiveness of SMT in the treatment of acute lumbar spine pain (Shekelle, 1994). In New Zealand, the Government has endorsed the clinical use of manipulation in the management of acute low back pain (ACC Low Back Pain Guide, 1997). Investigations of the cervical spine have,
however, been less conclusive. One recent extensive study of acute neck pain found rapid improvements with SMT compared to exercise-based treatments or general medical management (Hoving et al., 2002). In contrast, (van Tulder, Koes, & Bouter, 1997) found a lack of clear evidence to support SMT in the treatment of chronic neck pain.

On the other hand, a recent meta-analysis has found a paucity of quality double-blinded randomised controlled trials, and this may limit the conclusions that can be drawn (Koes et al., 1991). There is some supportive evidence for the use of SMT in acute and chronic musculoskeletal neck pain (Di Fabio, 1999; Gross et al., 2002; Hurwitz, Aker, Adams, Meeker, & Shekelle, 1996; Koes, Assendelft, van der Heijden, & Bouter, 1996). For example, mobilisation and manipulation were found to be slightly better than control interventions (Di Fabio, 1999). Spinal manipulation may also be more effective than spinal mobilisation treatment (Hurwitz et al., 1996). From the recent literature it could be concluded that SMT may provide some short-term benefits in the treatment of musculoskeletal neck pain.

1.1.2 Manual Therapy Techniques

The manual therapy techniques of mobilisation and traction are distinguished from manipulation by the technique’s speed of application. Manipulation involves a high velocity but low amplitude thrust delivered at the end range of spinal joint motion, which the patient cannot prevent (Di Fabio, 1999). Mobilisation entails a repetitive low velocity (no thrust), graded, passive movement applied to the spinal joint, which can be prevented by the patient (Di Fabio, 1999; Hing, Reid, & Monaghan, 2003).
Forces during cervical spine manipulation have been estimated to vary between 40 and 120 Newtons applied over a 30 – 120 millisecond period (Kawchuk & Herzog, 1993). A short-lever thrust is applied by direct contact of the therapist’s hand with the spinal joint (Hing et al., 2003). Short-lever thrust techniques are recommended for neck manipulation, because the spine is positioned so that forces are localised to one joint without undue stress being placed on other adjacent joints.

1.1.2.1 Cavitation

Joint manipulation frequently generates a popping or cracking sound, which is commonly referred to as joint cavitation. Initial investigations into joint cavitation were largely based on manipulation experiments to metacarpophalangeal joints. Radiological studies indicated cavitation was linked with an increase in joint space, and an increase in gas within that space (Meal & Scott, 1986; Mierau, Cassidy, Bowen, Dupuis, & Noftall, 1988; Roston & Haines, 1947; Unsworth, Dowson, & Wright, 1971). A mechanism within the joint was thought to cause the sound, due a drop in internal joint pressure (Meal & Scott, 1986; Roston & Haines, 1947; Unsworth et al., 1971). The gas within the joint was found to be predominantly carbon dioxide, which is miscible in synovial fluid (Unsworth et al., 1971). Further joint cavitation was not possible within a refractory period of approximately 15 to 30 minutes, which was also consistent with the time frame for re-absorption of gas into synovial fluid (Meal & Scott, 1986; Mierau et al., 1988; Roston & Haines, 1947; Unsworth et al., 1971). From the above investigations it was concluded that the cavitation sound resulted from either the release of gas with the joint gapping on joint fluid or due to considerable stretch on the joint capsule and ligaments (Brodeur, 1995; Chen & Israelachvili, 1991).
In the lumbar spine Crammer et al. (2000), using the sensitive MRI scanning technique, demonstrated that rotational manipulation significantly gapped the lumbar zygapophyseal joint space. In contrast, using X-ray and computed tomography with cervical spine manipulation, there was no evidence of either an increase in cervical zygapophyseal joint space, or gas in the joint space (Cascioli, Corr, & Till, 2003).

Furthermore, the significance of the cavitation sound is still debated in the literature, with no firm supportive evidence of its cause or its effect. In a recent review of the literature Evans (2002) stated that, if cavitation was not important, then there would be ongoing benefits of repeated manipulation even after cavitation occurred, such as during the refractory period. This, however, has not been observed clinically (Evans, 2002).

1.1.3 Mechanisms Underlying the Effect of Manual Therapy

Early theories of the mechanism underlying the effect of SMT were based on clinical observations; joint hypomobility was the traditional clinical indicator. Scientific research initially focused on mechanical changes to the joint and surrounding tissue (Katavich, 1998). Many biomechanical theories have been proposed to explain the observed changes with manipulation. For example, the mechanical pressure generated by manipulation could in turn lead to changes in the local chemical environment, or stimulate healing of an injured joint (Evans 2000). The precise biomechanical mechanism, however, has yet to be elucidated.

Recent experimental research has indicated that the mechanism for the effect of manipulation may be mediated through the central nervous system (CNS)
(Sluka & Wright, 2001; Vicenzino, Collins, & Wright, 1996). Investigations in the rat demonstrated that hyperalgesia around the injured joint was reversed with mobilisation of a more proximal joint (Sluka & Wright, 2001). The authors suggested that this was caused by a centrally mediated mechanism, as no mechanical pressure was applied to the site injury to influence the local healing environment (Sluka & Wright, 2001).

The underlying rationale for a neurophysiological mechanism for the effects of manipulation is based on the CNS’s ability to modulate sensory afferent information. CNS input from afferents residing in paraspinal tissue, activated by the mechanical thrust of manipulation, may influence nociceptive transmission and may also exert an effect on motor function (Wright, 1995; Zusman, 1986).
1.2 Neurophysiological Mechanisms

Sensorimotor systems in the CNS are organised generally in a hierarchical and functional order, with each level having various operational conditions. The essentially hard-wired spinal cord deals with rapid responses to a wide range of sensory input, while the more adaptive supraspinal centres of the brain stem and cortex integrate sensory information to plan and to develop movement strategies (Loeb, Brown, & Cheng, 1999). Accordingly, sensory input at each level of the CNS is essential in the regulation of movement. This is also emphasised by the fact that different sensory environments can elicit different motor strategies (Loeb et al., 1999; Rudomin, 1999). It is likely that manipulation evokes and, therefore, may modulate neural activity at a spinal level, supraspinal level or both, to affect motor output.

1.2.1 Spinal Cord Modulation

Input from large-diameter afferents, such as those that SMT would activate, may modulate the excitability of spinal motoneurons through direct or indirect spinal pathways. The Ia-afferent pathway is the most direct pathway, with monosynaptic connection to the α-motoneuron (Brown & Fyffe, 1981). Most spinal circuits are polysynaptic with one or more interneurons in between the sensory terminal and the α-motoneuron. Interneurons are considered important sites of modification as they receive both descending information and segmental sensory feedback (Loeb et al., 1999). Spinal α-motoneurons also receive synaptic input from propriospinal neurons arising at other spinal segments (Lundberg, Malmgren, & Schomburg, 1977; Rekling, Funk, Bayliss, Dong, & Feldman, 2000; Riddell & Hadian, 2000).
Egger (1978) demonstrated that repetitive stimulation of large-diameter afferents led to initial motor excitation followed by a period of motor inhibition, a phenomenon termed habituation. This phenomenon is known to be intrinsic to the spinal cord (Dimitrijevic & Nathan, 1970, 1971). Habituation occurs with repetitive afferent stimulation; and it is therefore more likely to be associated with the mechanism underlying the oscillatory movement of joint mobilisation rather than manipulation.

Large-diameter afferents mediated by group I and II fibres are also thought to inhibit activity of small-diameter afferents, transmitted by group III and IV fibres, as hypothesised by the gate control theory of pain (Melzack & Wall, 1965). Further, Pickar and Wheeler (2001) demonstrated that spinal manipulation activated group I and II afferents in the cat. On this basis, the barrage of sensory input evoked with SMT may induce inhibitory effects at a spinal cord level and reflexively influence motor output.

The gate control theory, however, also proposed that modulation could occur through descending pathways arising from higher centres, affecting dorsal horn neurons and pathways projecting to spinal α-motoneurons (Mendell, 1966; Siddall & Cousins, 1997). It is not, therefore, clear whether spinal pathways or central pathways or both are responsible.

### 1.2.2 Supraspinal Modulation

#### 1.2.2.1 Cortical Pathways

At a cortical level, the strong link between sensory and motor processing is indicated by direct synaptic connections and close anatomical and functional organisation between motor and sensory areas (Svensson, Miles, McKay, &
Ridding, 2003; Terao et al., 1995). Staining studies in the cat demonstrate cortical motoneurons receive monosynaptic connections from the somatosensory cortex (Ghosh & Porter, 1988; Porter, Sakamoto, & Asanuma, 1990). In addition, the motor cortex also receives monosynaptic links from other sensory processing areas, such as the thalamus, that relays information from cutaneous, joint and muscle afferents (Ghosh & Porter, 1988; Guyton & Hall, 2000). There are also direct connections between the thalamus and the somatosensory cortex (Guyton & Hall, 2000). Human and animal studies have shown that passive joint movement activates the motor and somatosensory cortices as well as the supplementary motor cortex (Fetz, Finocchio, Baker, & Soso, 1980; Weiller et al., 1996). Afferent input by SMT stimulation could, therefore, be relayed through any number of these pathways.

Moreover, recent studies have demonstrated that various sensory stimulation can significantly influence central motoneuron excitability, with both excitatory and inhibitory effects observed on motoneurons (Baldissera & Leocani, 1995; Clouston, Kiers, Menkes, Sander, Chiappa & Cros, 1995; Ridding, McKay, Thompson, & Miles, 2001; Tokimura et al., 2000). The effect of afferent stimulation was demonstrated as being mediated through the motor cortex, owing to the fact that the somatosensory cortex, the brainstem and the peripheral nervous system remained unchanged (Kaelin-Lang, Luft, Sawaki, Burstein, Sohn, & Cohen, 2002).

1.2.2.2 The Corticospinal Tract

The motor cortex may significantly modulate spinal α-motoneurons via the corticospinal tract. Cortical motoneurons form direct monosynaptic connections with α-motoneurons as well as indirect synapses through related interneurons.
(Fetz, Cheney, & German, 1976; Fetz et al., 1980). The corticospinal tract is the largest and considered the most important descending motor pathway in humans (Rothwell, 1994). Further, corticospinal collaterals diverge extensively onto a variety of ventral horn neurons (Cheney & Fetz, 1985)

1.2.2.3 **Transcortical Long-Loop Reflex**

Spinal manipulation may influence cortical pathways through a long-loop transcortical pathway activated by muscle stretch. There has been some debate, however, whether Ia-afferents generate motor output changes through a polysynaptic spinal pathway, or through long-loop reflex pathways that traverse the motor cortex. Recent evidence from animal studies and neurophysiological experiments in humans overwhelmingly favoured a transcortical reflex mechanism (Christensen, Petersen, Andersen, Sinkjaer, & Nielsen, 2000; Marsden, Rothwell & Day, 1983). Further, corticospinal cells with monosynaptic projections to spinal α-motoneurons were activated by stretch, with a latency corresponding to a long-loop stretch muscle response (Cheney & Fetz, 1984; Day, Riescher, Struppler, Rothwell, & Marsden, 1991).

1.2.2.4 **Brainstem and Accessary Pathways**

Spinal manipulation may influence motor output through indirect brainstem pathways. Neurons originating from the dorsal and ventral periaqueductal grey (PAG) matter of the midbrain, project through brainstem nuclei onto spinal neurons (Fields & Basbaum, 1994; Reynolds, 1969). Serotonergic and adrenergic projections from the brainstem nuclei are known to enhance spinal motoneuron activity. They are the serotonergic raphe-spinal projection and the noradrenergic coeruleo-spinal projection (White, Fung, Jackson, & Imel, 1996).
Further, projections from the PAG matter have been linked to non-opioid nociceptive inhibition, sympathetic facilitation as well as α-motoneuron excitation (Bandler & Shipley, 1994; Cannon, Prieto, Lee, & Liebeskind, 1982; Lovick, 1991). Wright (1995) suggested that the mechanism for the effect of SMT may be mediated through the PAG area, as numerous studies have demonstrated that SMT also exerts a similar co-ordinated effect on nociceptive, autonomic and motor function (Sterling, Jull, & Wright, 2001; Terrett & Vernon, 1984; Vicenzino, Collins, & Wright 1996). In addition, pharmacological studies in animals have shown that joint manipulation can lead to a non-opioid form of analgesia, mediated through serotonergic and noradrenergic receptors in the spinal cord (Skyba, Radhakrishnan, Rohlwing, Wright, & Sluka, 2003; Zusman, 1989).
1.3 Manual Therapy Effects on the Motor System

1.3.1 Effect of Manipulation on Peripheral Afferents

Manipulation is likely to activate peripheral receptors and afferent pathways in a different manner than that of mobilisation and traction, due to the high-velocity delivery of the manipulative thrust.

Afferents that reside in paraspinal tissues, such as skin, muscle, tendons, zygapophyseal joint capsule, ligaments and discs, could be activated by spinal manipulation. In the cervical spine these structures are all extensively innervated with afferents that relay sensory information to the CNS (Bolton, 1998). Activation of all classes of sensory afferents (group I, II, III and IV fibres) from any one of these structures could theoretically occur as their receptors are likely to have mechanical thresholds below that of the applied load during spinal manipulation (Gillette, 1987).

Numerous animal studies have examined the discharge properties of afferents with receptive fields in paraspinal tissue, in response to mechanical stimuli (Bolton & Holland, 1998; Pickar & McLain, 1995; Pickar & Wheeler 2001). Multi-afferent firing, in response to spinal manipulative forces, has also been observed in humans during spinal surgery (Colloca, Keller, Gunzburg, Vandeputte, & Fuhr, 2000).

1.3.1.1 Joint Afferents (group II, III and IV)

Large diameter joint afferents are known to respond to passive joint motion at the end of joint range (Burke, Gandevia, & Macefield, 1988; Grigg & Greenspan, 1977). Further, in animals mechanical loading of zygapophyseal joints was also shown to increase activity in small diameter, group III and IV
joint afferents (Abrahams, Lynn, & Richmond, 1984; Cavanaugh, el-Bohy, Hardy, Getchell, Getchell, & King, 1989; Pickar & McLain, 1995). In the cervical spine the majority of group III afferents have high mechanical thresholds, thus may be more likely to be activated with manipulation (Abrahams et al., 1984). However, it remains to be seen whether the mechanical loads used in the animal experiments replicate forces required for spinal manipulation. At present, no studies have directly investigated the effects of spinal manipulation on small fibre afferents (Pickar, 2002).

1.3.1.2 Muscle Afferents (group I, II)

Pickar and Wheeler (2001) recorded in the cat discharge properties of single-unit activity from primary muscle afferents with receptive fields in paraspinal tissue. They also observed changes in group I and II afferents when a mechanical force replicating manipulation was applied to the spine. Golgi Tendon Organs (GTO) were activated during the thrust phase, whereas, muscle spindle firing increased just before the thrust.

1.3.1.3 Cutaneous Afferents

In humans, cutaneous afferents activated by spinal manipulation were not thought to contribute to the observed changes in central neural activity, as anaesthetised skin failed to affect outcomes (Murphy, Dawson, & Slack, 1995). This is in contrast to cutaneous afferents activated by cervical traction which were found to influence central neural excitability (Bradnam, Rochester, & Vujnovich, 2000).

1.3.1.4 Nociceptor Afferents

Manipulation type loads in the spine of the cat have been demonstrated to activate nociceptors. In contrast, slower mobilisation type loads did not trigger nociceptors (Pickar & Wheeler 2001).
In the pain state, processing of afferent input in the dorsal horn is known to change (Siddall & Cousins, 1997). Wide dynamic range neurons, which normally respond only to non-noxious mechanical stimuli, start to respond to innocuous mechanical stimuli arising from the injured area (Rang & Urban, 1995; Siddall & Cousins, 1997) and from the uninjured surrounding tissue (secondary hyperalgesia) (Blumberg & Janig, 1994; Hoheisel, Mense, Simons, & Yu, 1993). The changes that occur in the dorsal horn are known as central sensitisation (Siddall & Cousins, 1997). With increased nociceptive input into the dorsal horn, substance P is also known to spread, sensitising neurons of the segments above and below (Mense, Hoheisel, & Reinert, 1996).

In other words, sub-threshold mechanical stimuli may signal nociceptive input to the CNS because dorsal horn neurons have become sensitised. Because of sensitisation, manipulation of a pathological joint may stimulate normally quiescence afferents that lie in and around the paraspinal tissue (He, Proske, Schaible, & Schmidt, 1988; Schaible & Schmidt, 1988; Schaible, Schmidt, & Willis, 1987; Yamashita, Minaki, Oota, Yokogushi, & Ishii, 1993).

### 1.3.2 Assessing Motor Effects

There are many ways of scientifically examining the effects of spinal manipulation on the human motor system. Measuring the change in the frequency and rate of firing in single motor units using EMG indicates the effect of an intervention on individual motor units (De Luca, 1997). This method does not, however, provide evidence of how the population of motor units would respond, thus conclusions are limited to the level of the single motor unit only.
By way of comparison, the Hoffman or H-reflex technique can measure the relationship between sensory input and motor output in a population of motoneurons (Hugon, 1973). The H-reflex, therefore, provides a more useful indication of the effect of the intervention on the motor system. The H-reflex method involves electrical stimulation of a peripheral nerve, resulting in a nearly synchronised volley in la-afferents, which terminate monosynaptically on homonymous spinal motoneurons. The biphasic motor response evoked in the muscle supplied by those α-motoneurons is recorded using EMG. Changes in the amplitude of the EMG response can, therefore, provide an objective measure of changes in the excitability of α-motoneurons in response to an intervention (Hugon, 1973).

1.3.3 Effect of Manipulation on the Motor System

1.3.3.1 Pain-Spasm-Pain Cycle

Initial neurophysiological theories proposed that manipulation reduced motoneuron excitability, which was abnormally heightened due to pain, and thereby reduced muscle spasm (Zusman 1986). This was based on clinical and anecdotal observations that spontaneous motor unit firing was reduced with spinal manipulation in symptomatic subjects (Grice, 1974; Herzog, Scheele, & Conway, 1999; Thabe, 1986). This theory presumes that pain leads to an increase in motor activity, and spinal manipulation reduces motor activity.

In support of this theory, H-reflex studies have demonstrated sacroiliac, cervical and lumbar spine manipulation attenuated α-motoneuron excitability in normal subjects (Dishman & Bulbulian, 2000; Dishman & Burke, 2003; Murphy et al., 1995). Although, the type of SMT technique did not seem to matter, as both mobilisation and manipulation reduced α-motoneuron excitability (Bradnam et
Studies have looked at the response of motor activity to spinal manipulation, using EMG to record motor unit firing in symptomatic subjects. Lehman, Vernon and McGill (2001), investigating lower back pain subjects, found mechanical pressure applied to the painful zygapophyseal joint increased EMG responses. Whereas, there was no change in EMG responses when pressure was applied to the non-painful joint. Moreover, manipulation appeared to attenuate EMG responses to applied mechanical pressure (Lehman et al., 2001).

In direct contrast, EMG investigations in both symptomatic and asymptomatic subjects have found spinal manipulation increased motor activity (Colloca & Keller, 2001; Herzog et al., 1999). Manipulation consistently elicited a brief reflexive firing of motor units (Colloca & Keller, 2001; Herzog et al., 1999; Suter, Herzog, Conway, & Zhang, 1994). In addition, animal studies have also indicated that joint displacement produces an increase in motor unit firing (Wyke, 1979), with high loads considerably increasing the magnitude of the EMG motor unit response (Holm, Indahl, & Solomonow, 2002; Solomonow, Zhou, Harris, Lu, & Baratta, 1998).

### 1.3.3.2 Effect of Pain on Motor Output

The excitatory effect of nociceptive stimulation on the motor system has been well documented in animal studies. Noxious input from joints, cutaneous and, in particular, muscle afferents, has been demonstrated to increase motor unit
firing recorded in the flexor muscles (He et al., 1988; Woolf, 1984; Woolf & McMahon, 1985; Woolf & Wall, 1986).

On the other hand, there is also substantial evidence from numerous animal and human studies that nociceptive input may lead to depression of the motor system. An inhibitory effect has been demonstrated in animal studies at a spinal cord level (Holmqvist & Lundberg, 1961; Kniffki, Schomburg, & Steffens, 1981) and brainstem level (Westberg, Clavelou, Schwartz, & Lund, 1997). Furthermore, in humans suppression of cortical motor pathways has been demonstrated with acute experimental pain (Farina et al., 2001; Kofler et al., 1998; Le Pera et al., 2001; Valeriani et al., 1999). The mechanism for this suppression is thought to be mediated supraspinally through cortico-cortical circuits, and then subsequently through concurrent spinal cord mechanisms (Farina et al., 2001; Le Pera et al., 2001; Svensson et al., 2003).

1.3.3.3 Effect of Manipulation on Motor Output

Recent investigations have also indicated that spinal manipulation may actually facilitate or reduce inhibition of motor pathways in a depressed system. Motor depression observed in neck pain subjects was shown to be reversed with neck manipulation (Suter & McMorland, 2002). These investigators used the twitch interpolation technique to assess muscle activation. The authors found greater inhibition of the biceps muscle during elbow flexion in neck pain patients, compared with normal control subjects. Immediately after cervical spine manipulation, however, biceps muscle activation significantly increased (Suter & McMorland, 2002). The same group of investigators demonstrated similar results with axial-skeleton manipulation for peripheral joint disorders (Suter, McMorland, Herzog, & Bray, 1999, 2000).
In short, the neural mechanism underly ing motor dysfunction evoked by pain, has yet to be elucidated (for review see Graven-Nielsen, Svensson, & Arendt-Nielsen, 2000; Hodges, 2000). There is also no conclusive opinion on the effect of manipulation on motor output. The neurophysiological effect of manipulation maybe dependent on the state of the motor system at the time that the manipulation stimulus is applied.

On the other hand, investigations of the effects manipulation on the motor system have led to contradictory findings, therefore the effects may not have been fully identified. Excitatory effects on motor unit firing have been observed with spinal manipulation, whereas inhibitory effects on populations of α-motoneuron have been observed using H-reflex monitoring (Dishman & Bulbulian, 2000; Herzog et al., 1999; Murphy et al., 1995). Discrepancies in research findings may be accounted for by the use of different measurement techniques. Inherent difficulties have been reported with the use of EMG to measure motor unit firing, with many confounding intrinsic and extrinsic variables (De Luca, 1997). In contrast, the H-reflex has been considered to be more reliable as the technique can assess excitability of a population of α-motoneurons more accurately at a spinal cord level (Dishman & Bulbulian, 2000; Katavich, 1998).

1.3.3.4 Transcranial Magnetic Stimulation

A new technique has recently been used to examine the influence of sensory modulation on motor output at a supraspinal level. Transcranial Magnetic Stimulation (TMS) is a non-invasive and pain-free method of stimulating the motor cortex to assess cortical neuronal input onto the spinal α-motoneuron pool via the corticospinal tract.
At present, one published study has investigated cortical motoneuron excitability changes with manipulation. Dishman et al. (2002) used the TMS method to measure the excitability of cortical neurons projecting to lower limb muscles and found spinal manipulation significantly increased cortical excitability.

This is in frank contrast to the inhibitory effect of manipulation on spinal motoneuron pool excitability when measured using the H-reflex technique. The influence of afferent stimulation on higher centres can be more readily assessed using the TMS technique (Nielsen, Morita, Baumgarten, Petersen & Christensen, 1999). Thus, the TMS technique may be a superior method of assessing the effect of manipulation on motor output compared to the H-reflex.

To date, the effect of manipulation of the cervical spine has not been investigated using the TMS technique. In theory, neck manipulation should generate a greater neurophysiological response than lumbar spine manipulation as the upper limb has stronger corticospinal projections compared to that of the lower limb (Brouwer & Ashby, 1990). Furthermore, measuring cortical excitability using TMS, via the corticospinal pathway, is more straightforward in the upper limb as the site of stimulation on the motor cortex for the upper limb lies just beneath the scalp; whereas the lower limb site is deeper, requiring stronger magnetic stimulation (Allison, McCarthy, Luby, Puce, & Spencer, 1996). Thus a more accurate measure of neurophysiological modulation on motor output should be identified with neck manipulation.
The Dishman et al. (2002) study only explored excitability changes with manipulation in a motor system at rest. Sensory stimulation, however, appears to have less of an effect in an active motor system. Other TMS studies, which have evaluated motor cortical excitability changes with stimulation of sensory afferents, have demonstrated the observed changes seen at rest no longer apply when excitability changes are assessed during voluntary muscle contraction (Clouston et al., 1995; Ridding & Rothwell, 1997). For example, Clouston et al (1995) found the reduction in muscle responses evoked with cutaneous afferent stimulation, were no longer seen when the target muscle was contracted. Furthermore the motor cortex is concerned with movement and is more active just before and during muscle contraction (Chen & Hallett, 1999). Therefore, it would be of greater functional value to examine cortical motoneuron changes during motor activity.

In addition, no researchers have investigated the effect of manipulation on cortical excitability with TMS on symptomatic subjects, yet spinal manipulation is widely utilised in the treatment of musculoskeletal pain and/or stiffness (Di Fabio 1999).

Examining the supraspinal effects of cervical spine manipulation in a motor system during an activity in symptomatic and asymptomatic subjects using the TMS technique should, therefore, reveal more useful information regarding the neurophysiological mechanism underlying manipulation on the motor system as a whole.
1.4 Transcranial Magnetic Stimulation

TMS was introduced by Barker, Jalinous and Freeston (1985) and is regarded as a valuable research tool. It has been extensively used in neurophysiological investigations and to investigate many aspects of brain function (Hallett, 2002; Rothwell, 1998). Single pulse TMS has been established as a safe and sensitive method of assessing changes in cortical excitability (Wassermann, 1998).

TMS stimulation of the motor cortex by a single magnetic pulse evokes a reflex muscle response in the contralateral target muscle, which can be measured by EMG as motor evoked potentials (MEPs). Magnetic stimulation, at an intensity just above the discharge threshold for motoneurons, results in the activation of cortical motoneurons that project to the spinal motoneurons of the target muscle (Rothwell, 1997). The amplitude of the MEP response reflects both cortical and spinal motoneuron excitability (Edgley & Lemon, 1999). The TMS single pulse technique does not differentiate between cortical and spinal motoneuron changes. Other techniques are required to evaluate spinal cord effects, such as brainstem input by transmastoid stimulation technique.

1.4.1 Methodology

During TMS a large capacitor connected to a stimulation coil generates a magnetic field. The coil is placed on the scalp; a rapid alternating current passing through the insulated coil of wire generates a magnetic pulse. The magnetic pulse passes unimpeded through the scalp to induce an electrical current in the superficial cortex of the brain. The induced current flows in the horizontal plane to the stimulating coil (Terao & Ugawa, 2002). In other words, when the coil is placed at a tangent to the vertex of the scalp, the induced
current preferentially activates horizontally orientated neural elements (Branston & Tofts, 1990; Day et al., 1989). This results in excitatory or inhibitory post-synaptic potentials in these horizontal cortical neural elements, such as interneurons and cortico-cortical fibres from other cortical areas, but not the vertically orientated corticospinal cells themselves (Cracco & Cracco, 1999; Siebner & Rothwell, 2003; Terao & Ugawa, 2002). Other elements, such as axon collaterals of pyramidal tract neurons and afferent fibres from the motor thalamus, have been largely discounted (Patton & Amassian, 1954; Sakai, Ugawa, Terao, Hanajima, Furubayashi, & Kanazawa, 1997).

The exact location of cortical neurons activated using TMS remains unclear; it may also depend on other factors such as the individuals’ physiology (Christensen et al., 2000). It is, however, generally accepted that TMS activates the corticospinal fibre trans-synaptically (Amassian & Cracco, 1987; Cracco & Cracco, 1999; Day et al., 1989; Patton & Amassian, 1954). Consequently, due to this indirect activation of the corticospinal fibre, the TMS technique is able to evaluate changes in cortical excitability.

1.4.1.1 Cortical Excitability in an Active Motor System

An increase in cortical neuronal activity has been observed with voluntary muscle contraction using the TMS technique. Reduced latency of motor response and motor threshold (or firing threshold), as well as a significantly larger MEP amplitude, have been commonly observed (Fuhr, Agostino, & Hallett, 1991; Wu, Goto, Taniwake, Kinukawa, & Tobimatsu, 2002). The increase in excitability of cortical neurons projecting to the contracting muscle reflects activation of a greater number of descending corticospinal fibres (Mills & Kimiskidis, 1996). The change in excitability was thought to be as a result of an
alteration in the balance of excitatory and inhibitory pathways synapsing on
cortical neurons projecting to the contracting muscle (Reynolds & Ashby, 1999).

1.4.2 Reliability with TMS

The change in MEP amplitude following various experimental interventions has
been attributed to an alteration in cortical excitability due to the intervention
(Rossini et al., 1994). Excitability of cortical neurons, however, is known to
fluctuate spontaneously at rest and during motor activity (Burke, Hicks,
Furthermore, the reproducibility of any biological system will be limited by the
“interference” or “noise” introduced by the experimental measuring system
(Burke et al., 1995). In other words, variability in the MEP amplitude to a
constant TMS stimulus, may result from natural fluctuations in cortical neuronal
excitability, experimental error or other unknown factors.

1.4.2.1 Firing Thresholds

Variability is also a function of the proportion of neurons not consistently
recruited by TMS stimulation (Kiers et al., 1993). Firing threshold (or motor
threshold) is the minimum intensity of magnetic stimulation required to activate
a MEP response (Rossini et al., 1994). The amplitude of the MEP response
reflects the number of motoneurons raised above firing threshold by the
magnetic stimulus. This includes a proportion of motoneurons that are resting
below firing threshold, which will fire if a spontaneous change in excitability
coincides with the magnetic pulse (Rossini et al., 1994).

1.4.2.2 Within-Subject Variability

MEP amplitudes, elicited by a constant TMS stimulus, have been estimated to
vary by a factor of approximately 33% over time which, in part, is thought to be
due to spontaneous oscillations in both cortical motoneuron or spinal
motoneuron excitability (Burke et al., 1995; Ellaway, Davey, Maskill, Rawlinson, Lewis, & Anissimova, 1998). Further, a constant TMS stimulus is known to activate a variable combination of cortiospinal pathways (Burke et al., 1995; Di Lazzaro et al., 1998). The exact mechanism responsible for the variation of the MEP amplitude within individuals, however, is unknown (Kiers et al., 1993).

1.4.2.3 Variation between Individuals

Carroll, Riek, & Carson (2001) and Wassermann (2002) observed greater variability in the normal population between individuals than within individuals. Approximately 60% of variability between individuals in MEP amplitude was estimated to be due to unknown, but relatively stable, biological differences between people, such as physiological differences in neural properties and anatomy. Additional sources of variability, which are difficult to verify experimentally, include circadian variations in arousal, circulating hormone levels and other factors affecting neural function that could influence corticospinal excitability (Wasserman, 2002). Whereas, variability in MEP responses between individuals has been shown to be independent of age, sex, cardiac cycle, menstrual cycle, and left or right handedness (Ellaway et al., 1998).

Variation in amplitudes of MEPs could be related to experimental application (Wasserman, 2002). A main source of experimental error, when using the TMS technique, is the control of the three-dimensional placement of the stimulating coil in relation to the subject’s head (Wasserman, 2002). A change in the coil position is known to contribute to variability of MEPs amplitude, either by altering the population of cortical neurons recruited or the magnitude of induced current under the coil (Amassian, Cracco, & Maccabee, 1989; Brasil-Neto,

1.4.3 Methods Used to Reduce Variability

Rossini et al. (1994) and Kiers et al. (1993) demonstrated that variability in MEP amplitude, due to motoneuron fluctuation around the critical firing level, was reduced by both voluntary muscle contraction and raising the TMS intensity. If, however, the intensity of TMS is too high, experimentally-induced changes in neuronal excitability could be masked, due to the higher number of neurons already recruited by TMS (Lemon, Johansson & Westling, 1995). In the literature, using a TMS intensity of 20% above motor threshold has been commonly adopted (Rossini et al., 1994). Muscle contraction has the effect of raising the number of motoneurons to firing threshold and, consequently, fewer motoneurons are prone to spontaneous activation (Kiers et al., 1993). Muscle contraction as low as 5% of maximum voluntary contraction (MVC) has been shown to reduce variability (Kiers et al., 1993). Further, maximum amplitudes of MEPs can be reached with contraction levels ranging between 5-10% of MVC (Wu et al., 2002). For that reason, only a small contraction would be required for an optimal MEP muscle response (Wu et al., 2002).

In an active motor system, background EMG from the muscle contraction may confound identification of the MEP amplitude. When measuring MEP amplitude at motor onset, however, there is little time for movement preparation, thus background EMG can be kept to a minimum (Chen & Hallett, 1999). The MEP amplitude, as a consequence, can be clearly identified (Rossini et al., 1994).
1.4.3.1 Optimal Site

Each muscle is supplied by cortical neurons distributed throughout the motor cortex. However, there is an optimal site on the motor cortex that has a higher concentration of cortical neurons for a specific muscle. At this optimal site the lowest TMS stimulation intensity, and the shortest response latency, has been observed (Brasil-Neto et al., 1992; Fuhr et al., 1991). Furthermore, variability in the MEP amplitude increased the further away the stimulation magnetic pulse was from the optimum site (Brasil-Neto et al., 1992). The suggested reason for this effect was possibly due to the lower density of cortical neurons and excitatory fibres projecting to the target muscle (Fuhr et al., 1991). Marking the optimal site on a cap has been used to reduce error when positioning the coil (Rossini et al., 1994).

1.4.3.2 Averaging

To overcome the variability caused by natural fluctuations in cortical motoneuron excitability, and to obtain a true representation of motoneuron excitability, increasing the number of TMS stimulations, and averaging the number of MEPs amplitudes, has been recommended (Brasil-Neto et al., 1992; Kiers et al., 1993; Rossini et al., 1994). In the current literature, averaging has ranged anywhere from ten to forty MEP amplitudes (Brasil-Neto et al., 1992; Burke et al., 1995; Rossini et al., 1994).

1.4.3.3 Percentage Change Calculation

The wide variation between individuals’ MEP amplitudes makes comparison between groups more difficult (Wasserman, 2002). Some investigators have overcome this methodological problem by expressing the amplitudes of MEPs elicited after the experiment intervention, as a percentage of trials before the intervention (Le Pera et al., 2001; Farina et al., 2001).
1.4.3.4  

Coil Type

Kiers et al. (1993) found the MEP amplitude elicited by TMS stimulation with a hand-held circular coil to be less variable than other coils, such as the figure-8 coil. The circular coils are thought to stimulate a wider cortical area and therefore consistently stimulate a larger proportion of descending corticospinal pathways. This has the consequence of reducing variability of MEP amplitudes elicited in the target muscle (Kiers et al., 1993).
1.5 Objectives

The main objective of this study was to investigate the effect of neck manipulation on the excitability of cortical motoneurons in human subjects performing an active movement. The TMS technique was chosen as it was considered to be a superior method compared to the H-reflex for measuring changes in cortical and spinal motoneuron activity. An active motor system was chosen as it is considered more functionally relevant. The cervical spine was examined because motor cortex excitability changes with manipulation have not been investigated in the upper limb.

Another objective of the study was to consider the effect that joint cavitation has on cortical excitability. The consequences of whether spinal manipulation results in cavitation are currently unknown.

Lastly, the effect of manipulation was examined in a small group of symptomatic subjects. The objective was to explore trends of the effect of manipulating the painful joint on cortical motoneuron excitability; as well as the estimation of the number of neck pain subjects that would be required to determine a significant result for future research.

The knowledge gained from this study would enhance understanding of the underlying mechanism of the effect of manipulation. Accordingly, improved knowledge would lead to better clinical reasoning behind the application of spinal manipulation. In physiotherapy today, there is a demand for evidence-based practice to replace anecdotal and recipe-based treatment regimes. It is
of the utmost importance to prove scientifically that manipulation is more than the psychological interplay between therapist and patient.

1.5.1 Hypothesis

It was hypothesised that:

- Neck manipulation will facilitate excitability of the motor cortex in an active motor system.
- Neck positioning will not have an effect on the excitability of the motor cortex in an active motor system.
- Manipulation resulting in joint cavitation may have a greater effect on cortical excitability than a non-cavitating joint.
- Spinal manipulation in symptomatic subjects may have a different effect than in asymptomatic subjects.
Chapter Two  Methodology

2.1 Introduction

Cortical motoneuron excitability was assessed by TMS. MEPs were evoked by TMS, at onset of muscle contraction (10% of maximum force), and recorded from the right FCR muscle. The MEPs elicited in the FCR muscle were recorded electromyographically. The amplitude of the MEP was used to measure motor cortex excitability. This was quantified by measuring peak-to-peak electromyographic (EMG) response in the test muscle. Two interventions took place, manipulation and neck positioning. All subjects acted as their own control. The MEP amplitudes in FCR were recorded at: two-time intervals before the intervention (baseline), and at three time intervals after the intervention. Normal, healthy subjects participated in both interventions. For each subject, the experiments using either intervention were recorded at least two days apart. Five normal subjects also participated in experiments where no intervention was applied. In order to explore trends with neck pain patients, a small number of symptomatic subjects participated in the manipulation intervention only. MEP data was collected and stored for later computer analysis.

All experiments took place in the Human Neurophysiology Laboratory of the Physical Rehabilitation Research Centre, at Auckland University of Technology. Unnecessary sounds and movement were kept to a minimum.
2.2 **Selection of Subjects**

In accordance with requirements of the Auckland Ethics Committee, subjects were invited to participate on a voluntary basis. Written and verbal explanations of the experimental procedures were provided and written informed consent was obtained before testing (Appendices I & II). All subjects were interviewed and excluded if they had:

- A history of epilepsy.
- Electronic or metal implants.
- Exposure to medications that might affect cortical excitability (Rossini et al., 1994).
- A general medical history of diabetes, peripheral vascular disease, or neurological disease.
- Any sign or symptoms of vertebrobasilar insufficiency (VBI) as stated by the Australian Physiotherapy Association’s Clinical Guidelines for Pre-manipulative Procedures for the Cervical Spine (Magarey, Coughlan, & Ribbeck, 2000).
- Contraindications to manipulation (Maitland, 1986).

2.2.1 **Normal Subjects**

A group of 20 normal volunteer subjects were studied following recruitment by advertisement at the Auckland University of Technology (AUT). All subjects satisfied the inclusion criteria of:

- Absence of musculoskeletal pain in the neck within the last 6 months; and
- Absence of musculoskeletal pain in the right arm.
2.2.2 Symptomatic Subjects

Five volunteer subjects with right-sided neck pain were studied following recruitment by advertisement at local musculoskeletal physiotherapy clinics. Physiotherapists within the clinics identified subjects diagnosed with right-sided lower cervical spine somatic dysfunction; criteria for inclusion were:

- Intermittent pain stemming from the lower cervical spine.
- Loss of range of motion of the lower cervical spine.
- Pain that was not easily aggravated by moderate activity.
- In acute cases, no extensive muscle guarding.
- No history of surgery to the cervical spine.
- Normal deep tendon reflexes, muscle strength, and sensation in the right upper limb indicating integrity of all cervical nerve roots.
- Improving symptoms.
- Absence of upper cervical spine symptoms.

Subjects who met these criteria were invited to participate in the study. Subjective and objective examinations were conducted on each subject (Appendix III), to confirm the diagnosis of somatic dysfunction of the lower cervical spine, to exclude VBI pathology and other contraindications to manipulation (Magarey et al., 2000; Maitland, 1986). The guidelines described by Hing et al. (2003) were used in selecting the subjects suitable for manipulation. On the day of testing a qualified manipulative physiotherapist collected the clinic data.

The purpose of the subjective examination was to determine the history and characteristics of the symptoms the subject experienced. This was undertaken
according to the methodology described by Hing et al. (2003), and was used to establish a somatic dysfunction diagnosis of mechanical origin. The medical history and medications were recorded. Pain at rest was measured on a visual analogue scale (VAS) (1 – 10 mm) and recorded before and immediately after manipulation (trial 3).

The purpose of the objective examination was to determine the physical characteristic of each subject’s dysfunction. This was undertaken using the principals described by Hing et al. (2003). The objective examination was used to confirm the diagnosis of lower cervical spine dysfunction with pain on palpation of the C6/7 zygapophyseal joint. Pain provocation testing has been demonstrated to be a reliable way of identifying symptomatic zygapophyseal joints in the cervical spine (Hubka & Phelan, 1994; Jull, Bogduk, & Marsland, 1988). A neurological examination of deep tendon reflexes, muscle strength, and sensation in the right upper limb was undertaken to test the integrity of all cervical nerve roots.

Pain at the end of right side bend was measured on VAS (0 – 10 mm) and recorded before the manipulation experiments (trial 1) and again at the end of the manipulation experiments (trial 5). Data sheets for both normal and symptomatic subjects are contained in Appendix IV.
2.3 Equipment

2.3.1 Flexor Frame

A flexor frame (University of Auckland, Auckland, New Zealand), used in a previous study (Vujnovich, 1999) provided rigid fixation of the forearm and preferential resistance to the FCR muscle during each isometric contraction.

The base of the flexor frame was screwed to the metal support of the right armrest. The proximal end of the hand rest was connected to the forearm support by a multiaxial joint. On either side of the forearm rest, two lateral arms of a perspex scaffold were mounted at a 30° angle. A hollow perspex box was fixed to the superior aspect of the lateral arms of the scaffold (Figure 2.1).

A load cell (Tedea ™, Huntleigh, UK) with a maximum load capability of 5 kg was attached to the perspex box superiorly. An inextensible wire was attached at one end to the force transducer mechanism and, at the other end, screwed to the distal end of the hand rest. A D1119 force transducer (Gentech Industries, Hastings, New Zealand) was connected to the load cell. The force transducer was calibrated to produce an output of 1 V/0.5kg and the output balance without load was set to 0 Vdc. When the subject contracted the forearm flexors, there was no observable movement of the wrist and the contraction was isometric.

2.3.2 Oscilloscope

The flexor frame was constructed to provide specific resistance to wrist flexion. Lim and Yiannikas (1992) demonstrated how consistent muscle contraction reduced variability in muscle responses to TMS. Therefore special attention was paid to produce consistent isometric contractions of forearm flexor muscles. An
oscilloscope (20 MHz, PS 200 UL®, Dick Smith Electronics, Auckland, NZ.) was used to give the subjects visual feedback in order to gauge the force required to evoke a contraction at 10% of maximal force. The oscilloscope was positioned directly in front of the subject.

The torque output from the force transducer during a muscle contraction was projected as a beam onto the oscilloscope monitor. The force production from the best three maximal efforts was chosen and calibrated using labVIEW software (National Instruments Inc, Austin, Texas). The level of muscle contraction that produced 10% of the subject’s maximal force was calculated in labVIEW and projected as a stationary beam on the oscilloscope. A second beam, responsive to force produced during each muscle contraction, was also displayed on the oscilloscope. Subjects were instructed to look at the oscilloscope and perform a rapid wrist flexion movement, sufficient to align the two beams as precisely as possible. Subjects were able to practice this in preliminary trials.

During the experiments, the labVIEW computer program generated a tone at a randomised time (between 2 – 4 seconds), which signalled to the subject to make a muscle contraction. When 10% of the subject’s maximal force was generated by the contraction, the computer program was set to trigger the magnetic stimulus to evoke a MEP.
Figure 2.1  The flexor frame and force transducer. The photograph shows the lateral view of the flexor frame demonstrating the force transducer mounted to the superior aspect of the hollow perspex box. The inferior aspect of the perspex box is attached to the two lateral arms of scaffolding, which is fixed to the forearm support. A multiaxial joint connects the hand rest to the forearm support. An inextensible wire runs from the force transducer to the distal end of the handrest. The purpose of the force transducer mechanism was firstly to trigger the TMS magnetic pulse at 10% of maximal force of an isometric contraction, and secondly to provide the subjects’ feedback so that consistent isometric contractions could be produced.
2.4 Subject Position

For each experiment the subject was seated comfortably in a modified dental chair (Figure 2.2) with an adjustable back, and with legs supported. The body position, head position, arm position and hand position were standardised across all subjects and all experiments. The adjustable back of the chair was tilted to an angle of 120° and the subjects were instructed to sit as far back in the chair as possible. The left forearm was supported on the armrest of the chair. The right elbow was flexed to an angle of 140°. The forearm was supported in pronation on the forearm rest of the flexor frame, and the hand was positioned palm down on the hand rest. In addition, the right hand was aligned so that the trapezoid to hamate reference was aligned with the proximal edge of the perspex hand rest. The thumb and index finger tip positions were marked on the non-slip mat for easy realignment.

To minimise unwanted movement, the forearm and hand were restrained with elastic velcro straps, and the hand lay on a non-slip mat. For either the manipulation or the positioning intervention the subject was reclined to an angle of 160°, which was the maximum limit of the chair (Figure 2.3).

To maintain head position, subjects were instructed to keep their head in the midline. In order to align their head, a reference point was placed on the wall directly in front of their line of sight. Each subject’s head position was also visually checked so that the chin was in line with the sternal notch.
**Figure 2.2** Subject position with TMS of the motor cortex. The photograph shows the frontal view of the subject’s position and the experimental set-up. Subjects were seated comfortably in an adjustable dental chair, at an angle of 120°. The subject’s right arm was supported on the flexor frame and secured with velcro straps. The subject’s hand was placed on a non-slip mat on the hand rest. The magnetic stimulating coil was placed on the subject’s head; the TMS capacitor is seen on the subject’s left.
Figure 2.3 Reclined position for the interventions. The photograph of the lateral view of the adjustable chair shows the 160° limit.
2.5 TMS Technique

Single pulse TMS was delivered to the motor cortex to stimulate cortical neurons projecting to FCR motoneurons. The magnetic pulse was delivered by a 90 mm circular coil using a Magstim 200 Magnetic stimulator (Magstim Co, Whitland, Dyfed, UK.).

MEPs were evoked at the onset of the muscle contraction, as opposed to during a sustained contraction, as the former method was considered to limit the amount of EMG that could contaminate the MEP amplitude (Chen & Hallett, 1999).

2.5.1 MEP Measurement

EMG signal was used to record the MEPs elicited by TMS. Changes in cortical excitability, from the EMG signal, are commonly assessed in two ways. Firstly, the EMG signal is assessed by calculating the area of the MEP amplitude, by taking the integral of the rectified MEP wave (Carroll et al., 2001; Uozumi, Tsuji, & Murai, 1991; van der Kamp, Zwinderman, Ferrari, & van Dijk, 1996). Secondly, the EMG signal is assessed by measuring the peak-to-peak of the MEP amplitude. Previous investigators have demonstrated that the MEP area, and the MEP peak-to-peak amplitude are qualitatively similar and statistically equivalent (Latash, Danion, & Bonnard, 2003). It was, therefore, decided to use peak-to-peak amplitude measurements. This was measured in millivolts as the difference between lowest and highest value of the EMG signal.

2.5.2 Protocol for Establishing Motor Threshold

The threshold of cortical neurons at motor onset, was defined according to conventional criteria. The minimum intensity required to produce a MEP
amplitude of at least 50 μV on 5 out of 10 consecutive trials was taken as motor threshold. The stimulation intensity was adjusted to a level where MEPs were produced consistently and then lowered incrementally until the MEP frequency fell below 5 out of 10 (Rothwell et al., 1999; Wassermann, 1998).

2.5.3 Recording TMS responses

2.5.3.1 Skin Preparation

For optimal electrical conduction, the skin was prepared just before the electrodes were placed. Hair was shaved from the right medial aspect of the elbow and the antero-medial aspect of the forearm using a disposable razor. The skin was abraded with Green Prep (Mavidon ™, Lake Worth, USA). To remove any residual paste the area was cleaned with alcohol and then wiped dry with a paper towel. Surface electrodes were placed over the muscle belly of FCR and an earth electrode over the medial epicondyle of the elbow joint.

2.5.3.2 Recording Electrodes Placement

A disposable, surface bipolar self-adhesive EMG bar electrode (Noro-trode ™, Naromed Inc, Seattle, WA.) recorded the MEP responses. Each bar contained two 9 mm-diameter silver-silverchloride electrodes separated by 20 mm. The tendon of FCR was palpated at the point of insertion to the medial humeral epicondyle. The muscle belly of FCR was identified distal to the tendon by palpation during resisted wrist flexion. The recording electrode was placed over the FCR muscle belly, longitudinally on the upper third of the antero-medial aspect of the forearm, 10 mm distal to the crease of the elbow joint. The earth, a single, disposable, self-adhesive electrode (Red Dot™, 3M Healthcare, Canada) was placed over the bony point, the medial epicondyle of the elbow joint. The skin resistance was checked using an Ohm metre (Dick Smith Electronics, Auckland, NZ.) and kept below 5 kΩ (Rossini et al., 1994).
2.5.4 Protocol for Establishing Coil Placement

2.5.4.1 Head Set-up

Each subject wore a snug fitting plastic cap. To ensure no movement occurred during each intervention, the cap was secured to the head by a tie under the chin. A latex lattice grid of 5 mm$^2$ sections was attached to the cap with tape. The subject's head was measured to establish the central point of the head. The conventional central point, termed Cz, was calculated as being the vertex of the scalp between the nasion-inion line and the interaural line (Rossini et al., 1994).

2.5.4.2 Stimulating Coil Position

The coil was placed centrally over the vertex and tangential to the scalp with the centre of the coil approximately 40 mm anterior to Cz and 60 mm lateral. This position was preferential for evoking motor responses in the FCR muscle (Pascual-Leone, Cohen, Brasil-Neto, & Hallett, 1994). The coil was placed with the “A” side up, which induced an anticlockwise intracellular current to stimulate preferentially the left hemisphere (Rossini et al., 1994).

When the coil position was optimal for eliciting a MEP at onset of a FCR contraction, the position was marked on the scalp cap in four places. This accounted for the anterior-posterior and medial-lateral coordinates. Special attention was paid to controlling the superior-inferior angle of tilt of the stimulating coil. A clinical goniometer (MIE Medical Research Ltd, Leeds, UK) was mounted to the superior surface of the stimulating coil. This meant that the experimenter had visual feedback for repositioning the coil in the same position during subsequent trials (Figure 2.4).
Figure 2.4  TMS stimulating coil position and optimal site. The photograph shows the superior-posterior aspect of the stimulating coil placement on the subject’s head. The clinical goniometer was mounted to the superior surface of the stimulating coil to give the experimenter visual feedback of the coil position. The red dot marks the optimal site for stimulating the FCR muscle. Three other marks (not shown here) were also used for coil re-positioning in subsequent trials.
2.5.5 Data Acquisition System

The EMG signals, used to record the MEP responses, were sampled at 5 kHz, amplified and filtered to a bandwidth between 0.3 Hz - 3 kHz by a Grass P5 Series Amplifier (Grass Instrument Co., Rhode Island, USA). LabVIEW computer software converted the EMG recordings from analog to digital. The digitalised data was displayed on the monitor of a Cyclone Pentium 75MHz CPU computer (Southmark Computers, Auckland, NZ) for immediate scrutiny and stored on disc for later analysis.

2.5.6 Safety

Magnetic stimulation was applied according to established protocols (Rossini et al., 1994) and the manufacturers’ instructions. Single pulse TMS is a safe low-frequency technique that is capable of generating a pulse no more than once every few seconds (Wassermann, 1998). During the experiments a low intensity was used that ranged from 30% - 60% of stimulator output.
2.6 Experimental Design

A repeated measures, cross-over design was used to compare the effects of the manipulation and positioning interventions. Twenty normal subjects participated in these experiments. The normal subjects received both interventions, and the positioning intervention was used as the control. The repeat measures design was used as it was considered a robust method when using small subject numbers, and reduced the variation between subjects. To explore the effect of manipulation on the excitability of the motor cortex in neck pain subjects, a small number of symptomatic subjects participated in the manipulation intervention only.

The experimental design evaluated the influence of each intervention on cortical motoneuron excitability, in comparison to baseline controls. Additionally the design assessed the time course of the effect. To control any learning effects that may confound a repeated measures design, the order of the interventions was randomised for each subject. A counterbalance randomisation process was used, so that there were even numbers of subjects in each group. Half the subjects were randomly assigned to participate in the positioning protocol first and the other half participated in the manipulation protocol first. To minimise any carry-over effects, the interventions were delivered at least two days apart. To establish stability of cortical motoneuron excitability, at the onset of muscle activity and in the absence of an intervention, a further five experiments were undertaken on five normal subjects.
2.7 Experimental Protocol

During each experiment five trials were performed. For each trial 40 MEPs were elicited over 120 seconds. To establish the stability of excitability of the motor cortex over time, two separate trials were recorded (trials 1 and trial 2), before intervention, five minutes apart. These were denoted as baseline trials. To assess the effect of the intervention on cortical motor excitability, and to establish the time course of the effect, a further three trials were recorded. These were taken immediately after the intervention (trial 3), and then at five minutes intervals (trial 4 and trial 5) at seven and 14 minutes respectively. Each experiment took 27 minutes to complete. The experimental protocol is summarised in Figure 2.5. For the experiments without any intervention, trials 1-5 were undertaken with the subjects in the upright position using the same protocol.
Figure 2.5 Experimental protocol. The schematic representation above depicts the experimental protocol for all manipulation and positioning experiments. 40 MEPs were collected over 120 seconds for each trial and all trials were recorded five minutes apart. Trials 1 and 2 were deemed as baseline trials to compare post intervention trials. The bold line represents either the manipulation or positioning interventions. Trial 3 was recorded immediately following manipulation. Trials 4 and 5 were collected after the interventions to ascertain the time course of the effects of the interventions.
2.7.1 Experimental Intervention

Two experimental interventions were used: manipulation was applied to the lower cervical spine and the lower cervical spine was positioned. A third experimental procedure without any intervention was undertaken on five normal subjects.

2.7.1.1 Manipulation Intervention Procedure:

An upslope manipulation, a common technique used by manual therapists, was performed according to established procedures (Monaghan, 2001, p56). A qualified manipulative therapist delivered the short-lever, spinal manipulative thrust technique to the C₆/₇ cervical segment.

Prior to the experiment the C₇ spinous process was palpated and marked. The subject was reclined to an angle of 160° in the dental chair, the articular pillar of C₇ vertebra was palpated lateral to the spinous process, then the subject’s head was taken into right side-bend and left rotation to the C₆ level, then an upslope manipulation was performed (high-velocity low-amplitude).

2.7.1.2 Positioning Intervention Procedure:

The same procedure was used with the same amount of applied manual hand contact, except instead of a manipulative thrust, the right side-bend left rotation position was held for the same length of time as was used with the manipulation intervention.

Figure 2.5 demonstrates the position of the subject’s head for both the interventions. The head was placed back in the neutral position after the interventions.
2.7.1.3 Experiments without Intervention:

Five normal subjects participated in experiments without any intervention. Five trials were carried out with the subjects in the upright position and without movement of the head.
Figure 2.6 Position of the subject’s head for both interventions. The photograph shows a superior view of the subject’s head position. Cervical spine right side bend and left rotation was applied at the level of the C\textsubscript{6/7} zygapophyseal joint for both the positioning and manipulation interventions.
2.8 **Data Analysis**

The peak-peak MEP amplitude of each EMG signal was measured by mobile cursors. The data was represented in two ways. Firstly, an average was taken of the 40 amplitudes of MEPs recorded in the first 120 seconds of each trial. Secondly, an average was taken of the first 15 amplitudes of MEPs recorded in the first 60 seconds of each trial.

2.8.1 **Reliability Testing of Baseline Trials on Normal Subjects**

The first two trials of each experiment were used to establish that TMS recordings were consistent before any intervention was applied. To examine the degree of consistency or repeatability for all subjects across these baseline trials, the intra-class correlation coefficient (ICC, the two-way random effect model) was calculated, using SPSS for Windows software (version 11.0.1, Copyright ©SPSS Inc. 1989-2001). The ICC is also sensitive to systematic change in scores (Shrout & Fleiss, 1979) and gives an index of repeatability and stability of the TMS recording across the two baseline trials. To establish consistency across scores the ICC was set a 0.85 recommended by convention (Polit & Hungler, 1991; Shrout & Fleiss, 1979).

Consistency in the TMS recordings for the experiments on the five normal subjects without intervention was also established. This was assessed using repeated measures, analysis of variance on the averaged MEP data.

2.8.2 **Percentage Change Calculation**

A percent change calculation was used to help reduce variability between subjects. For each subject the mean of trial 1 and trial 2 was calculated and termed mean baseline. Then the trials recorded after the interventions (trials 3-
5) were expressed as a percentage of the mean baseline, for that subject, using the formula:

\[
\text{Percentage change score} = \frac{\text{Trial Mean (3,4 or 5)} - \text{Baseline Mean}}{\text{Baseline Mean}} \times 100
\]

The percentage change scores, therefore, expressed the degree of change, in mean amplitudes of MEPs, due to the intervention.

2.8.3 Examination of the Effect of the Interventions on Normal Subjects

The effects of the intervention were analysed in two ways. Firstly, to detect change from baseline measures, each intervention was analysed separately, using a repeated measures ANOVA and individual t-tests. Here, both the MEP data and percentage change data was analysed. Secondly, manipulation was compared to the positioning control using a repeated measures ANOVA on the percentage change data.

2.8.3.1 Cross-over Design

Learning effects or other confounding variables may alter the outcome in cross-over design rather than the intervention. To minimise any carry-over effects, the interventions were delivered at least two days apart (Rossini et al., 1994). The cross-over design was also examined by ANOVA analyses, comparing the experiments delivered first with the experiments delivered second, regardless of intervention. In this way, if there was a significant difference between experiments, it would be due to the order of delivery rather than the intervention.
2.8.4 Examination of the Effect of Manipulation on Symptomatic Subjects

Exploratory analyses were performed on the percentage change data taken from the five symptomatic subjects. Repeated measures ANOVA and t-tests were used to assess the effect of the manipulation intervention. Changes in both VAS resting pain levels and end of range pain level were also examined for trends.

A power analysis was performed to predict the number of symptomatic subjects that would be required to demonstrate a significant change in cortical motoneuron excitability greater than 20%. The power analysis explored the variability of the symptomatic subjects’ response to manipulation, using standard deviations of the percentage change MEP data.

The symptomatic subjects were also compared to the 10 normal subjects, who had the manipulation delivered as the first intervention. These normal subjects were used as they were unaffected by the cross-over design.

2.8.5 Statistical Analysis

Statistical analysis was performed using SPSS for Windows software program. Repeated measures ANOVA was used to compare trials and interventions using the univariated test, Huynh Feldt to determine statistical significance. The statistical level of significance was set at p< 0.05. Pairwise comparisons were performed with Bonferroni correction, where necessary.
Chapter Three  Results

3.1 Introduction

Twenty normal subjects and five symptomatic subjects participated in the study. The normal subjects received, in random order, a manipulation intervention and a positioning intervention. The interventions were separated by at least a two-day interval. The symptomatic subjects received only the manipulation intervention.

Changes in cortical motoneuron excitability were evaluated using the TMS technique. The amplitude of the motor evoked potentials elicited by TMS, provides an indication of cortical motoneuron excitability (Wassaman, 1998). MEPs were elicited and recorded using established criteria. Changes in MEP amplitude were assessed before and after each intervention. An increase in MEP amplitude indicated an increase in cortical motoneuron excitability, whereas a decrease in MEP amplitude would indicate a reduction in excitability (Barker, Olivier & Lemon, 1994; Rossini et al., 1994).

Five trials were undertaken in each experiment. During each trial 40 MEPs were elicited by TMS. The MEP responses were recorded in the right FCR muscle at the onset of muscle activity in forearm flexor muscles. The peak-to-peak amplitude of each MEP was measured. The data was represented in two ways. Firstly, an average was taken of the 40 amplitudes of MEPs recorded in the first 120 seconds of each trial. Secondly, an average was taken of the first 15 amplitudes of MEPs recorded in the first 60 seconds of each trial.
Two trials (trials 1 and trial 2) were recorded before each intervention. These were deemed baseline values. Three trials were then recorded after each intervention (trial 3, trial 4 and trial 5). The baseline served as a control value against which the subsequent trials 3, 4 and 5 were compared. Trial 3 was used to detect any immediate changes in cortical motoneuron excitability, following each intervention. Whereas, trials 4 and trial 5 established the time course of change, as a consequence of each intervention. Trial 4 was recorded seven minutes and trial 5 was recorded 14 minutes, after each intervention.

To ensure that cortical motoneuron excitability did not fluctuate at the onset of muscle activity, in the absence of an intervention, a further five experiments were undertaken on five normal subjects. These experiments were also used to establish both the reproducibility of the TMS and the experimental technique at the onset of muscle activity in forearm flexor muscles.
3.2 **Experiments without Intervention on Normal Subjects**

To ensure that cortical motoneuron excitability was stable over time, MEP amplitudes were recorded at the onset of muscle activity in forearm flexors in the absence of an intervention, in five normal subjects.

### 3.2.1 Cortical Motoneuron Excitability Stability (40 MEPs)

The mean peak-to-peak amplitudes of 40 MEPs collected in trials 1 – 5, were calculated for each subject, then pooled for all subjects (n = 5), according to trial and the group means calculated (Table 3.1). To determine any significant differences between the mean amplitudes of MEPs, a repeated measures analysis of variance (ANOVA) was performed on trials 1 – 5. No significant difference was observed between the mean amplitudes of MEPs collected in trials 1 – 5 (p = 0.54) (Figure 3.1).

### 3.2.2 Cortical Motoneuron Excitability Stability (15 MEPs)

The mean peak-to-peak amplitudes of 15 MEPs collected at trials 1 – 5 were calculated for each subject, then pooled for all subjects, according to trial and the group means calculated (Table 3.1). To determine any significant differences between the mean amplitudes of MEPs, a repeated measures analysis of variance (ANOVA) was performed on trials 1 – 5. No significant difference was seen between the mean amplitudes of MEPs collected in trials 1 – 5 (p = 0.88) (Figure 3.2).
Table 3.1 The tables contain the descriptive statistics for experiments without interventions on five normal subjects. The tables depict the MEP data represented in two ways; first, 40 MEPs were averaged over 120 seconds (top table). Second, the first 15 MEPs were averaged over 60 seconds (bottom table).

<table>
<thead>
<tr>
<th>40 MEPs (n = 5) (mV)</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
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<td>0.15</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
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<tr>
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<td>0.49</td>
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<tr>
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<td>0.08</td>
<td>0.12</td>
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<table>
<thead>
<tr>
<th>15 MEPs (n = 5) (mV)</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
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<td>0.20</td>
<td>0.21</td>
<td>0.21</td>
<td>0.20</td>
</tr>
<tr>
<td>Maximum</td>
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</tr>
<tr>
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<td>0.34</td>
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<td>0.37</td>
</tr>
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<td>+/- . SD</td>
<td>0.17</td>
<td>0.13</td>
<td>0.09</td>
<td>0.16</td>
<td>0.16</td>
</tr>
</tbody>
</table>
Figure 3.1: Pooled mean MEP amplitudes for experiments without intervention on five normal subjects. The line graph displays the 40 MEPs averaged over 120 seconds and pooled for all subjects according to trial (1 - 5). The error bars depict the standard deviations. No significant difference was found between trials (p = 0.54). The mean amplitudes of MEPs are clearly consistent between trials.
Figure 3.2 Pooled mean MEP amplitudes for experiments without intervention on five normal subjects. The line graph displays the first 15 MEPs averaged over 60 seconds and pooled for all subjects according to trial (1 - 5). The error bars depict the standard deviations. No significant difference was found between trials ($p = 0.88$). The mean amplitudes of MEPs are clearly consistent between trials.
3.2.3 Conclusion

These results indicated that the mean amplitudes of MEPs recorded at the onset of a repeatable contraction, in the absence of an intervention, were consistent in the first 120 seconds of recordings and in the first 60 seconds of recordings. This also provided evidence for the reproducibility of MEPs using the TMS technique to measure cortical motoneuron excitability, at the onset of muscle contraction of forearm flexor muscles.

The excitability of cortical neurons projecting to forearm flexor muscles, at the onset of a repeatable contraction was, therefore, considered stable over time. A significant change in the mean amplitude of MEPs with an intervention could, therefore, be considered to reflect the intervention’s effect on the activation of cortical neurons projecting to contracting muscles.
3.3  Interventions’ Effects on Normal Subjects (40 MEPs)

A change in the mean amplitude of MEPs was considered to reflect a change in excitability of cortical neurons projecting to the contracting muscles. Twenty normal subjects received, in random order, a manipulation intervention and a positioning intervention at least 2 days apart. The effect of each intervention on mean amplitudes of MEPs was evaluated. The interventions were assessed separately and then compared statistically.

In each experiment, five trials were undertaken and in each trial 40 MEP amplitudes were elicited by TMS over a 120 second period. The mean amplitudes of the 40 MEPs, collected in trials 1 – 5, were calculated for each subject.

3.3.1  Effect of Positioning

The mean amplitudes of 40 MEPs collected before and after positioning were examined.

3.3.1.1  Analysis of Stability of Trial 1 and Trial 2.

In order to determine the baseline excitability of cortical neurons projecting to forearm flexors at the onset of a repeatable contraction, MEP amplitudes recorded before the positioning intervention were compared. MEPs collected in trial 1 and trial 2 before positioning were deemed baseline values. Trial 3, trial 4 and trial 5, recorded after positioning were then compared to baseline. Establishing stability in the baseline values was considered important, so that the change in mean amplitude of MEP from baseline could then be attributed to a change in excitability of cortical neurons projecting to forearm flexors as a result of positioning.
The single intra-class correlation coefficient was used to examine the reliability of mean amplitudes of MEPs in baseline trials. This correlation coefficient indicated the extent to which the mean amplitudes of MEPs in trial 1 and trial 2 were repeatable in each subject, compared for all subjects and thus gave an overall index of repeatability. The single intra-class correlation coefficient of trial 1 and trial 2 was demonstrated to be 0.88, above the 0.85 recommended by convention. The mean amplitudes of MEPs collected prior to positioning were, therefore, considered to be repeatable and reliable.

Reliability of the baseline values was examined further using a paired-sample t-test. The t-test was used to determine whether there was a systematic increase or decrease in cortical excitability between trial 1 and trial 2. The mean amplitudes of MEPs collected at trials 1 and trial 2 were pooled for all subjects according to trial, and the group means of trial 1 and trial 2 were calculated. There was no significant difference between the mean amplitudes of MEPs collected at trial 1 and trial 2 when compared using the paired-sample t-test (\( p = 0.06 \)). Figure 3.3 illustrates the range and median of trials 1 and 2, where consistency in baseline trials was observed.

The baseline excitability of cortical neurons projecting to forearm flexor muscles, at the onset of a repeatable contraction was, therefore, considered stable before the intervention. On this basis, the mean amplitude of MEPs of trial 1 and trial 2 was calculated for each subject and referred to as the baseline for that subject. The baseline was used as a control value to which MEPs collected after positioning could be compared for that subject.
Figure 3.3 MEP data pooled for all normal subjects (n = 20) for baseline trials 1 and 2. The boxplots display the range and median of the trials before the positioning intervention, when 40 MEPs were averaged over 120 seconds. The intra-class coefficient was 0.88 and the difference in the means was non-significant (p = 0.06), demonstrating stability of the baseline trials.
3.3.1.2 Change in MEP Responses after Positioning

Figure 3.4 demonstrates the average trace recordings of the mean amplitude of MEPs in a typical subject collected before and after positioning. A small change in mean amplitude of MEPs was observed at trial 3. Trial 4 and trial 5 were relatively similar to baseline. Figure 3.5 shows the mean amplitude of MEPs in individual subjects before and after positioning, for all subjects, where individual variation is observed.

The mean baseline amplitudes of MEPs were pooled for all subjects and the group mean calculated. The mean amplitudes of MEPs, collected at trial 3, trial 4 and trial 5 were pooled for all subjects and the group means calculated (Table 3.2). To determine a significant difference in the mean amplitudes of MEPs before and after positioning, repeated measures ANOVA was used to compare the means of baseline, trial 3, trial 4 and trial 5. No significant difference was observed between the mean amplitudes of MEPs collected at baseline, trial 3, trial 4 or trial 5 (p = 0.07).

The results indicated that the mean amplitudes of MEPs, recorded at the onset of a repeatable contraction were, therefore, consistent before and after positioning. However, due to the marked variation in each individual’s response to positioning, small to moderate differences between the trials may not have been detected.
Figure 3.4 Raw traces of MEPs for a typical subject before and after the positioning intervention. Mean baseline, trials 3 trial 4 and trial 3 are shown. Each trace depicts the average of 40 MEPs (collected over 120 seconds). The mean baseline was calculated from trial 1 and trial 2. The interrupted lines indicate the difference in amplitude of the MEPs. The arrow indicates the positioning intervention. The MEP traces are relatively similar for all trials with a small reduction at trial 3. Minimal change in MEP amplitude is demonstrated with the positioning intervention.
**Figure 3.5** MEP recordings for individual subjects (n = 20), for the positioning intervention. The line graph depicts the individual responses before and after the positioning intervention, when 40 MEPs were averaged over 120 seconds. The mean baseline was calculated from trial 1 and trial 2. Trial 3, trial 4 and trial 5 were recorded after the positioning intervention. Variability of individuals’ responses is demonstrated.
Table 3.2: The tables contain descriptive statistics for the intervention experiments when 40 MEPs were averaged over 120 seconds. The tables depict the pooled MEP data for all normal subjects (n = 20). The top table shows the positioning intervention and the bottom table shows the manipulation intervention.

<table>
<thead>
<tr>
<th>40 MEPs Positioning (n = 20) (mV)</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Mean Trials 1 &amp; 2</th>
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<th>Trial 4</th>
<th>Trial 5</th>
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<td>Minimum</td>
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<td>+/- SD</td>
<td>0.20</td>
<td>0.16</td>
<td>0.18</td>
<td>0.14</td>
<td>0.17</td>
<td>0.15</td>
</tr>
</tbody>
</table>
3.3.1.3 Percentage Change in MEPs after Positioning

Recent studies have suggested that there is considerable variation between different subjects in their responses to TMS. This variation is thought to be largely due to physiological differences between individuals (Wasserman, 2002). Small to moderate changes in MEP responses to positioning may, therefore, not be detected due to the variable individual response.

To help reduce variability between subjects, a percentage change calculation was used to represent the change in MEP amplitude from baseline. The baseline, for each subject, served as a control against which the effects of the intervention could be compared. The baseline would, therefore, equal zero and the percentage change in MEP amplitude would consequently express the degree of change from the baseline.

The mean amplitudes of MEPs at trial 3, trial 4 and trial 5 were calculated, for each subject, as a percentage of that subject’s baseline. For each subject, therefore, the MEP responses, elicited after the intervention, were expressed as the individual’s own control value and as a consequence, comparison could be made more readily between subjects. The percentage change calculation is described in Methodology 2.9.2.

The main criterion for using the percentage change calculation is that the percentage change values are independent from baseline data. In other words, there should be no correlation between the percentage change values and the baseline. The relationship between the two variables was, then examined in all subjects using a scatter plot. A random distribution in the correlation between
the positioning percentage change values and the mean baseline in all subjects was demonstrated (Figure 3.6). Independence was confirmed and as a consequence, the MEP responses to positioning could be expressed as a percentage change from baseline. The percentage change from baseline for trial 3, trial 4 and trial 5, were then pooled for all subjects according to trial. The means and standard deviations were calculated for each pooled trial and shown in Table 3.3.

To determine a significant difference in the percentage change in mean amplitudes of MEPs after positioning, a repeated measures ANOVA was used to compare the means of trial 3, trial 4 and trial 5. A significant difference was demonstrated between the means at trial 3, trial 4 and trial 5 (p = 0.01). A pairwise comparison was performed in order to clarify where the significant difference had occurred. When multiple post hoc comparisons were performed, a more stringent criterion for significance was used. This avoided finding a significant difference when the significant difference may have only occurred due to chance (type I error). When the more rigorous Boniﬁerroni adjustment was performed a significant difference was found between trial 3 and trial 5 (p = 0.03).

The mean percentage change from baseline to trial 3, trial 4 and trial 5 was +9.5%, +3.8 % and -6.1% respectively. This indicated a decrease in cortical excitability over time that was significant between trial 3 and trial 5. Figure 3.7 illustrates the range and median of the percentage change from baseline after positioning where a significant reduction in MEP amplitude was observed between trial 3 and trial 5.
A one-sample t-test was used to determine whether there was a significant difference in the percentage change values for each trial before and after positioning. The baseline was equal to zero and, therefore, the one-sample t-test assessed whether the mean percentage change value at each trial was significantly different from zero. No significant difference was observed from zero to trial 3, or trial 4, or trial 5 ((p = 0.18), (p = 0.42) (p = 0.27) respectively).

In summary, no significant change in MEP amplitude was recorded in the first 120 seconds of each trial, at the onset of a repeatable contraction with the positioning intervention. However, when MEP responses to positioning were expressed as a percentage change from baseline, a significant reduction in MEP amplitude after positioning was demonstrated between trial 3 and trial 5. In contrast, this did not occur when trial 3, trial 4 and trial 5 were independently compared to the baseline.

The percentage change data was, therefore, considered to be a more sensitive method of detecting change in MEP response with the intervention.
Figure 3.6 Correlation between percentage change MEP data and mean baseline, for all normal subjects (n = 20). The scatterplot depicts the relationship between the percentage change MEP data and the mean baseline for the positioning intervention, when 40 MEPs were averaged over 120 seconds. The mean baseline is the average of trial 1 and trial 2. A random distribution is shown, confirming independence of percentage change scores from the mean baseline. The percentage change calculation, therefore, can be used to normalise the post positioning trials to the mean baseline.
Table 3.3  The tables contain descriptive statistics of percentage change MEP data for the intervention experiments. The tables depict the pooled percentage change MEP data for all normal subjects (n = 20), when 40 MEP amplitudes were averaged over 120 seconds. The top table shows positioning intervention and the bottom table shows manipulation intervention.

<table>
<thead>
<tr>
<th>40 MEPs Positioning (n = 20) (%)</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>- 27.2</td>
<td>- 31.5</td>
<td>- 36.4</td>
</tr>
<tr>
<td>Maximum</td>
<td>105.2</td>
<td>47.6</td>
<td>65.0</td>
</tr>
<tr>
<td>Mean</td>
<td>9.5</td>
<td>3.8</td>
<td>- 6.1</td>
</tr>
<tr>
<td>(SD +/−)</td>
<td>30.2</td>
<td>20.5</td>
<td>23.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>40 MEPs Manipulation (n = 20) (%)</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>- 45.4</td>
<td>- 32.4</td>
<td>- 39.0</td>
</tr>
<tr>
<td>Maximum</td>
<td>60.9</td>
<td>52.6</td>
<td>48.1</td>
</tr>
<tr>
<td>Mean</td>
<td>11.1</td>
<td>3.0</td>
<td>- 1.0</td>
</tr>
<tr>
<td>(SD +/−)</td>
<td>25.9</td>
<td>20.2</td>
<td>23.3</td>
</tr>
</tbody>
</table>
Figure 3.7 Percentage change MEP data for the positioning intervention for all normal subjects (n = 20). The boxplots show the range and median of 40 MEPs averaged over 120 seconds for the post intervention trials (3, 4 and 5). Zero on the y-axis represents the mean baseline. The boxplots represent the degree of change in MEP amplitude from the baseline due to the positioning intervention, expressed as a percentage. The circles are the outliers. A significant reduction in mean amplitude was found between trial 3 and trial 5 (p = 0.03) with Boniferroni adjustment. No significant difference was observed from zero to trial 3 (p = 0.18), or trial 4 (p = 0.42), or trial 5 (p = 0.27).
3.3.2 **Effect of Manipulation**

A change in mean amplitudes of MEPs before and after manipulation was examined in the same manner as the positioning experiments.

### 3.3.2.1 Analysis of Stability of Trial 1 and Trial 2.

In order to determine the baseline excitability of cortical neurons projecting to forearm flexors, at the onset of a repeatable contraction, MEP amplitudes recorded before the manipulation intervention were compared. The single intra-class correlation coefficient was used to examine the reliability or repeatability of the baseline trials in the absence of an intervention. The single intra-class correlation coefficient of trial 1 and trial 2 was demonstrated to be 0.92. The mean amplitudes of MEPs collected prior to manipulation were, therefore, considered to be repeatable and reliable.

Reliability of the baseline measures was examined further, using a paired-sample t-test. The mean amplitudes of MEPs, collected in trials 1 and trial 2 were pooled for all subjects, according to trial and the group means of trial 1 and trial 2 were calculated. No significant difference was observed between the mean amplitude of MEPs collected at trial 1 and trial 2 when compared using the paired-sample t-test ($p = 0.38$). Figure 3.8 illustrates the range and median of trials 1 and 2, where consistency in baseline trial was observed.

The baseline excitability of cortical neurons projecting to forearm flexor muscles at the onset of a repeatable contraction was, therefore, considered stable in the absence of an intervention. On this basis, the mean amplitudes of MEPs of trial 1 and trial 2 was calculated for each subject and referred to as the baseline for
that subject. The baseline was used as a control value to which MEPs, collected after manipulation, could be compared for that subject.
Figure 3.8 MEP data pooled for all normal subjects (n = 20) for baseline trials 1 and 2. The boxplots display the range and median of the trials before the manipulation intervention, when 40 MEPs were averaged over 120 seconds. The intra-class coefficient was 0.92 and the difference in the means was non-significant (p = 0.38), demonstrating stability of the baseline trials.
3.3.2.2 Change in MEPs after Manipulation

Figure 3.9 demonstrates the average trace recordings of the mean amplitudes of MEPs in a typical subject collected before and after manipulation. A small increase in mean amplitudes of MEPs from mean baseline was noted at trial 3 and trial 4. A reduction in mean amplitude from mean baseline was observed at trial 5. Figure 3.10 shows the mean amplitudes of MEPs collected in individual subjects before and after manipulation for all subjects. A difference in the individual’s response to manipulation was observed.

The baseline amplitudes of MEPs were then pooled for all subjects and the group mean calculated. The mean amplitudes of MEPs, collected at trial 3, trial 4 and trial 5, were then pooled for all subjects and the group means calculated (Table 3.2). To determine significant differences in the mean amplitudes of MEPs collected before and after manipulation, a repeated measures ANOVA was used to compare the means of baseline, trial 3, trial 4, and trial 5. No significant difference was observed between the mean amplitudes of MEPs collected at baseline, trial 3, trial 4, and trial 5 (p = 0.38).

The results indicated that mean amplitudes of MEPs recorded at the onset of a repeatable contraction did not significantly change after manipulation.
Figure 3.9 Raw traces of MEPs for a typical subject before and after the manipulation intervention. Mean baseline, trials 3, trial 4 and trial 5 are shown. Each trace depicts the average of 40 MEPs (collected over 120 seconds). The mean baseline was calculated from trial 1 and trial 2. The interrupted lines indicate the difference in amplitude of the MEPs. The arrow indicates the manipulation intervention. The MEP traces show an increase in amplitude at trial 3 and at trial 4 with a drop off at trial 5. A non-significant increase in MEP amplitude is demonstrated with the manipulation intervention.
Figure 3.10 MEP recordings for individual subjects (n = 20), for the manipulation intervention. The line graph depicts the individual responses before and after the manipulation intervention, when 40 MEPs were averaged over 120 seconds. The mean baseline was calculated from trial 1 and trial 2. Trial 3, trial 4 and trial 5 were recorded after the manipulation intervention. Variability of individuals’ responses is demonstrated.
3.3.2.3 Percentage change in MEPs after Manipulation

Due to the wide individual responses, small to moderate changes in the mean amplitudes of MEPs with manipulation, may not be detected. To help reduce variability between subjects, a percentage change calculation was used to represent the change in MEP amplitude from baseline. The mean amplitudes of MEPs, collected at trial 3, trial 4 and trial 5, were calculated for each subject, as a percentage of that subject’s baseline.

The relationship between the percentage change values and mean baseline MEP data was examined for independence in all subjects using a scatter plot. A random distribution in the correlation between the manipulation percentage change values and the baseline in all subjects was demonstrated (Figure 3.11). Independence was confirmed and as a consequence, the MEP responses to manipulation could be expressed as a percentage change from baseline. The percentage change from baseline at trial 3, trial 4 and trial 5, was then pooled for all subjects according to trial. The means and standard deviations were calculated for each pooled trial and are shown in Table 3.3.

To determine significant differences of the percentage change in mean amplitude of MEPs collected after manipulation, a repeated measures ANOVA was used to compare the means of trial 3, trial 4, and trial 5. No significant difference was observed between the means of trial 3, trial 4 or trial 5 (p= 0.06). The mean percentage change from baseline to trial 3, trial 4 and trial 5, was +11.1 %, +3.0 % and -1.0 % respectively. This indicated a decrease over time, which did not reach a significant level. Figure 3.12 illustrates the range and median of the percentage change values after manipulation. The median MEP
amplitude for each trial was observed to reduce over time. However the larger range at trial 5 may explain why statistical significance was not reached.

A one-sample t-test was used to determine whether there was a significant difference in the percentage change values for each trial before and after manipulation. The baseline was equal to zero and, therefore, one-sample t-test assessed whether the mean percentage change value in each trial was significantly different from zero. No significant difference was observed from zero to trial 3, or trial 4, or trial 5 ((p = 0.07), (p = 0.52), (p = 0.85) respectively).

In summary, these results indicated that there was no significant change in the mean amplitudes of MEPs recorded in the first 120 seconds of each trial at the onset of a repeatable contraction with manipulation. Further, no significant latent effect of manipulation was seen. In other words, manipulation had no effect on the excitability of cortical neurons projecting to contracting forearm flexor muscles. However, variation in each individual’s response to manipulation may have prevented a significant finding.
Figure 3.11 Correlation between percentage change MEP data and mean baseline, for all normal subjects (n = 20). The scatterplot depicts the relationship between the percentage change MEP data and the mean baseline for the manipulation intervention, when 40 MEPs were averaged over 120 seconds. The mean baseline is the average of trial 1 and trial 2. A random distribution is shown, confirming independence of percentage change scores from the mean baseline. The percentage change calculation, therefore, can be used to normalise the post manipulation trials to the mean baseline.
Figure 3:12 Percentage change MEP data for the manipulation intervention for all normal subjects (n = 20). The boxplots show the range and median of 40 MEPs averaged over 120 seconds for the post intervention trials (3, 4 and 5). Zero on the y-axis represents the mean baseline. The boxplots represent the degree of change in MEP amplitude from the baseline due to the positioning intervention, expressed as a percentage. The circles are the outliers. No significant difference was found between trials (p = 0.06). No statistical significance was found from zero to trial 3 (p = 0.07), or trial 4 (p = 0.52), or trial 5 (p = 0.85).
3.3.3 Comparison between Positioning and Manipulation

The positioning and manipulation interventions were compared using a repeated measures ANOVA on the percentage change data collected at trial 3, trial 4 and trial 5. The percentage change MEP data was used as it was considered to help reduce between subject variability in the MEP response to the interventions. Positioning served as a control to compare the effects of manipulation. The positioning and manipulation interventions were compared by two factors, and trials 3 – 5 were compared by three factors.

No significant difference in the mean percentage change values was observed between interventions at trial 3, trial 4 or trial 5 (p = 0.55). Figure 3.13 compares the effects of the interventions with the mean percentage change values and illustrates the similar effect of positioning and manipulation in each trial.

A significant difference between trial 3, trial 4 and trial 5, was observed regardless of the intervention (p = 0.01), indicating both manipulation and positioning influenced the outcome of post intervention trials. A pairwise comparison with Bonferroni adjustment demonstrated that the significant difference was seen between trial 3 and trial 5 (p = 0.01). This was examined further, and the interventions were considered separately. Manipulation demonstrated a significant difference between trial 3 and trial 5 (p = 0.05). Positioning also demonstrated a significant difference between trial 3 and trial 5 (p = 0.03). Hence, the effect of manipulation on percentage change values was not significantly different from the positioning control. Figure 3.13 also shows
the MEP amplitudes from the baseline had significantly reduced from trial 3 to trial 5 for both interventions.

In summary, these results indicated that the effect of manipulation, on the mean amplitudes of MEPs, recorded at the onset of a repeatable contraction, was not significantly different from positioning, in the first 120 seconds of each trial. Both interventions significantly influenced the post intervention mean amplitudes of MEPs between trial 3 and trial 5. Therefore, there appears to be a reduction in mean amplitudes of MEPs after both manipulation and positioning, at trial 5. This effect may have been caused by the influence of the vestibular system on cortical excitability or other confounding variables.
Figure 3.13  Comparison between manipulation and positioning of the percentage change MEP data for all normal subjects (n = 20). The bar graph depicts the mean change in MEP amplitude from baseline to the post intervention trials (3,4 and 5), when 40 MEPs were averaged over 120 seconds. Zero on the y-axis represents the mean baseline. The error bars represent the standard deviations. No statistical difference was found between the positioning and manipulation interventions (p = 0.55). A significant reduction in mean amplitude MEPs over time, from trial 3 to trial 5 (p = 0.01) was observed in both interventions.
3.3.4 Conclusion

There was no evidence that the excitability of cortical neurons projecting to forearm flexor muscles at the onset of a repeatable contraction, significantly changed after manipulation when compared to positioning, when MEP recordings were averaged over the first 120 seconds of each trial. There was, however, a latent effect in both interventions on the excitability of cortical motoneurons. There was a significant reduction in excitability of cortical motoneurons after either manipulation or positioning from at least 120 seconds to at least 16 minutes.
3.4 *Interventions’ Effects on Normal Subject (15 MEPs)*

The first 15 MEPs were averaged over the first 60 seconds of each trial. The effect of each intervention on mean amplitudes of MEPs was statistically evaluated in the same manner as when the first 40 MEPs were averaged.

3.4.1 *Effect of Positioning*

The mean amplitudes of the first 15 MEPs, collected in trials 1 – 5 were calculated for each subject. A change in mean amplitudes of MEPs before and after positioning was examined.

3.4.1.1 *Analysis of Stability of Trial 1 and Trial 2.*

In order to determine the baseline excitability of cortical neurons projecting to forearm flexors at the onset of a repeatable contraction, MEP amplitudes recorded before the positioning intervention were compared. The single intra-class correlation coefficient was used to examine the reliability or repeatability of the baseline trials in the absence of an intervention. The single intra-class correlation coefficient of trial 1 and trial 2 was demonstrated to be 0.88. The mean amplitudes of MEPs collected prior to positioning were, therefore, considered to be repeatable and reliable.

Reliability of the baseline measures was examined further using a paired-sample t-test. The mean amplitudes of MEPs collected at trials 1 and trial 2 were pooled for all subjects according to trial and the group means of trial 1 and trial 2 were calculated. No significant difference was observed between the mean amplitude of MEPs collected in trial 1 and trial 2 when compared, using the paired-sample t-test ($p = 0.23$). Figure 3.14 illustrates the range and median of trials 1 and 2, where consistency in baseline trial was observed.
The baseline excitability of cortical neurons projecting to forearm flexor muscles at the onset of a repeatable contraction was, therefore, considered stable in the absence of an intervention. On this basis, the mean amplitudes of MEPs of trial 1 and trial 2 was calculated for each subject and referred to as the baseline for that subject. The baseline was used as a control value to which MEPs, collected after manipulation, could be compared for that subject.
Figure 3.14 MEP data pooled for all normal subjects (n = 20) for baseline trials 1 and 2. The boxplots display the range and median in the trials before the positioning intervention, when the first 15 MEPs were averaged over 60 seconds. The intra-class coefficient was 0.88 and the difference in the means was non-significant (p = 0.32), demonstrating stability of the baseline trials.
3.4.1.2 Change in MEPs Responses after Positioning

Figure 3.15 demonstrates the average trace recordings of the mean amplitudes of MEPs in a typical subject collected before and after positioning. Trial 3 and trial 4 are relatively similar to baseline, demonstrating no observable change in mean amplitude of MEPs. At trial 5 there is a small, notable reduction in mean amplitude of MEPs compared to the baseline. Figure 3.16 shows each individual’s mean amplitude of MEPs collected before and after positioning. Once again, a difference in each of the individual’s response to positioning was observed.

The baseline amplitudes of MEPs were then pooled for all subjects and the group mean calculated. The mean amplitudes of MEPs collected at trial 3, trial 4 and trial 5 were then pooled for all subjects and the group means calculated (Table 3.4). To determine significant differences in the mean amplitudes of MEPs collected before and after positioning, a repeated measures ANOVA was used to compare the means of baseline, trial 3, trial 4, and trial 5. No significant difference was observed between the mean amplitudes of MEPs collected at baseline, trial 3, trial 4, and trial 5 (p = 0.47).

The results indicated that the mean amplitudes of MEPs recorded at the onset of a repeatable contraction were consistent before and after positioning.
Mean Baseline                Trial 3                        Trial 4                          Trial 5

1 mV

1 ms

1 mV

1 ms

Figure 3.15  Raw traces of MEPs for a typical subject before and after the positioning intervention. Baseline average, trials 3, trial 4 and trial 3 are shown. Each trace depicts the average of 15 MEPs (collected over 60 seconds). The mean baseline was calculated from trial 1 and trial 2. The interrupted lines indicate the difference in amplitude of the MEPs. The arrow indicates the positioning intervention. The MEP traces are relatively similar for all trials with a small reduction at trial 5. Minimal change in MEP amplitude is demonstrated with the positioning intervention.
Figure 3.16 MEP recordings for individual subjects (n = 20), for the positioning intervention. The line graph depicts the individual responses before and after the positioning intervention, when the first 15 MEPs were averaged over 60 seconds. The mean baseline was calculated from trial 1 and trial 2. Trial 3, trial 4 and trial 5 were recorded after the positioning intervention. Variability of individuals’ responses is demonstrated.
Table 3.4  The tables contain descriptive statistics for the intervention experiments when the first 15 MEPs were averaged over 60 seconds. The tables depict the pooled MEP data for normal subjects (n = 20). The top table shows positioning intervention and the bottom table shows manipulation intervention.

<table>
<thead>
<tr>
<th>15MEP Positioning (n = 20) (mV)</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Mean Trials 1 &amp; 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>0.10</td>
<td>0.11</td>
<td>0.11</td>
<td>0.09</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.62</td>
<td>0.50</td>
<td>0.56</td>
<td>0.75</td>
<td>0.49</td>
<td>0.72</td>
</tr>
<tr>
<td>Mean (SD +/-)</td>
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<td>0.26</td>
<td>0.29</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>0.11</td>
<td>0.12</td>
<td>0.18</td>
<td>0.13</td>
<td>0.17</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>15 MEPs Manipulation (n = 20) (mV)</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Mean Trials 1 &amp; 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>0.10</td>
<td>0.08</td>
<td>0.09</td>
<td>0.12</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.65</td>
<td>0.66</td>
<td>0.65</td>
<td>0.84</td>
<td>0.85</td>
<td>0.84</td>
</tr>
<tr>
<td>Mean (SD +/-)</td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
<td>0.35</td>
<td>0.3063</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>0.14</td>
<td>0.13</td>
<td>0.18</td>
<td>0.20</td>
<td>0.18</td>
</tr>
</tbody>
</table>
3.4.1.3 Percentage Change in MEPs after Positioning

Due to the wide individual responses, small to moderate changes in the mean amplitudes of MEPs with positioning, may not be detected. To help reduce variability between subjects, a percentage change calculation was used to represent the change in MEP amplitude from baseline. The mean amplitudes of MEPs, collected at trial 3, trial 4 and trial 5, were, therefore, calculated for each subject as a percentage of the individual’s baseline.

The relationship between the percentage change values and mean baseline MEP data was examined for independence in all subjects using a scatter plot. A random distribution in the correlation between the positioning percentage change values and the baseline in all subjects was demonstrated (Figure 3.17). Independence was, therefore, confirmed and consequently the MEP responses to positioning could be expressed as a percentage change from baseline. The percentage change from baseline to trial 3, trial 4 and trial 5 was then pooled for all subjects according to trial. The means and standard deviations were then calculated for each pooled trial and are shown in Table 3.5.

To determine significant differences in the percentage change in mean amplitudes of MEPs collected after positioning, a repeated measures ANOVA was used to compare the means of trial 3, trial 4, and trial 5. No significant difference was observed between the means of trial 3, trial 4 or trial 5 (p= 0.59). The mean percentage change from baseline in trial 3, trial 4 and trial 5, was +4.9%, +2.3% and -2.1% respectively, This indicated a small, but non-significant, decrease over time. Figure 3.18 illustrates the range and median of
the percentage change from baseline after positioning, where the medians were similar.

A one-sample t-test was used to determine whether there was a significant difference in the percentage change values for each trial before and after positioning. The baseline was equal to zero and, therefore, the one-sample t-test assessed whether the mean percentage change value at each trial was significantly different to zero. No significant difference was observed from zero to trial 3, or trial 4, or trial 5 ((p = 0.37), (p = 0.73), (p = 0.72) respectively).

In summary, these results indicated that there was no significant change in the mean amplitudes of MEPs recorded in the first 60 seconds of each trial, at the onset of a repeatable contraction with positioning. The positioning intervention could be used to compare the manipulation intervention.
Figure 3.17 Correlation between percentage change MEP data and mean baseline, for all normal subjects (n = 20). The scatterplot depicts the relationship between the percentage change MEP data and the mean baseline for the positioning intervention, when 15 MEPs were averaged over 60 seconds. The mean baseline is the average of trial 1 and trial 2. A random distribution is shown, confirming independence of percentage change scores from the mean baseline. The percentage change calculation, therefore, can be used to normalise the post positioning trials to the mean baseline.
Table 3.5 The tables contain descriptive statistics of percentage change MEP data for the intervention experiments. The tables depict the pooled percentage change MEP data for all normal subjects (n = 20), when the first 15 MEPs were averaged over 60 seconds. The top table shows positioning intervention and the bottom table shows manipulation MEP data.

<table>
<thead>
<tr>
<th>15 MEPs Positioning (n = 20) (%)</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>-36.7</td>
<td>-32.4</td>
<td>-45.0</td>
</tr>
<tr>
<td>Maximum</td>
<td>54.0</td>
<td>84.7</td>
<td>87.7</td>
</tr>
<tr>
<td>Mean (SD +/-)</td>
<td>4.9</td>
<td>2.3</td>
<td>-2.1</td>
</tr>
<tr>
<td></td>
<td>23.6</td>
<td>28.3</td>
<td>29.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>15 MEPs Manipulation (n = 20) (%)</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>-8.5</td>
<td>-24.4</td>
<td>-21.0</td>
</tr>
<tr>
<td>Maximum</td>
<td>73.4</td>
<td>66.0</td>
<td>81.8</td>
</tr>
<tr>
<td>Mean (SD +/-)</td>
<td>34.1</td>
<td>10.6</td>
<td>15.1</td>
</tr>
<tr>
<td></td>
<td>23.3</td>
<td>23.4</td>
<td>28.1</td>
</tr>
</tbody>
</table>
Figure 3:18 Percentage change MEP data for the positioning intervention of all normal subjects (n = 20). The boxplots show the range and median of 15 MEPs averaged over 60 seconds for the post intervention trials (3, 4 and 5). Zero on the y-axis represents the mean baseline. The boxplots represent the degree of change in MEP amplitude from the baseline due to the positioning intervention, expressed as a percentage. The circles are the outliers. No statistical significance was found between trials (p = 0.59). No statistical significance was found from zero to trial 3 (p = 0.37), or trial 4 (p = 0.73), or trial 5 (p = 0.72).
3.4.2 **Effect of Manipulation**

A change in mean amplitudes of MEPs before and after manipulation was examined in the same manner as the positioning experiments.

3.4.2.1 **Analysis of Stability of Trial 1 and Trial 2.**

In order to determine the baseline excitability of cortical neurons projecting to forearm flexors at the onset of a repeatable contraction, MEP amplitudes recorded before the manipulation intervention were compared. The single intra-class correlation coefficient was used to examine the reliability or repeatability of the baseline trials, in the absence of an intervention. The single intra-class correlation coefficient of trial 1 and trial 2 was demonstrated to be 0.90. The mean amplitudes of MEPs collected prior to manipulation were, therefore, considered to be repeatable and reliable.

Reliability in baseline measures was examined further using a paired-sample t-test. The mean amplitudes of MEPs collected at trials 1 and trial 2 were pooled for all subjects according to trial and the group means of trial 1 and trial 2 were calculated. No significant difference was observed between the mean amplitude of MEPs collected in trial 1 and trial 2 when compared using the paired-sample t-test (p = 0.81). Figure 3.19 illustrates the range and median of trials 1 and 2, where consistency in baseline trial was observed.

The baseline excitability of cortical neurons projecting to forearm flexor muscles at the onset of a repeatable contraction was therefore, considered stable in the absence of an intervention. On this basis, the mean amplitude of MEPs of trial 1 and trial 2 was calculated for each subject and referred to as the baseline for that subject.
Figure 3.19 MEP data pooled for all normal subjects (n = 20) for baseline trials 1 and 2. The boxplots display the range and median for the trials before the manipulation intervention, when the first 15 MEPs were averaged over 60 seconds. The intra-class coefficient was 0.90 and the difference in the means was non-significant (p = 0.81), demonstrating stability of the baseline trials.
3.4.2.2 Change in MEPs after Manipulation

Figure 3.20 demonstrates the average trace recordings of the mean amplitudes of MEPs in a typical subject collected before and after manipulation. An increase in mean amplitudes of MEPs from baseline was noted at trial 3 and trial 4. The mean amplitude at trial 5 was observed to be relatively similar to baseline. Figure 3.21 shows the mean amplitudes of MEPs collected in individual subjects before and after manipulation for all subjects.

The baseline amplitudes of MEPs were then pooled for all subjects and the group mean calculated. The mean amplitudes of MEPs collected at trial 3, trial 4 and trial 5, were then pooled for all subjects and the group means calculated (Table 3.4). To determine significant differences in the mean amplitudes of MEPs collected before and after manipulation, a repeated measures ANOVA was used to compare the means of baseline, trial 3, trial 4, and trial 5.

A significant difference was found between the mean amplitude of MEPs at baseline, trial 3, trial 4, and trial 5 ($p = 0.002$). A pairwise comparison with Bonferroni adjustment demonstrated that the difference was between baseline and trial 3 ($p = 0.05$). The mean amplitudes of MEPs from baseline to trial 3 were 0.2632 mV and 0.3510 mV respectively. The significant difference in mean amplitudes of MEPs was observed from baseline to trial 3.

These results demonstrated a significant increase in the mean amplitude of MEPs recorded at the onset of a repeatable contraction collected at trial 3.
Figure 3.20 Raw traces of MEPs for a typical subject before and after the manipulation intervention. Baseline average, trials 3, trial 4 and trial 5 are shown. Each trace depicts the average of 15 MEPs (collected over 60 seconds). The mean baseline was calculated from trial 1 and trial 2. The interrupted lines indicate the difference in amplitude of the MEPs. The arrow indicates the manipulation intervention. The MEP traces show an increase at trial 3 and at trial 4, with a drop off at trial 5. An increase in MEP amplitude is demonstrated with the manipulation intervention.
Figure 3.21 MEP recordings for individual subjects (n = 20), for the manipulation intervention. The line graph depicts the individual responses before and after the manipulation intervention, when the first 15 MEPs averaged over 60 seconds. The mean baseline was calculated from trial 1 and trial 2. Trial 3, trial 4 and trial 5 was recorded after the manipulation intervention.
3.4.2.3 Percentage Change in MEPs after Manipulation

To help reduce variability between subjects, a percentage change calculation was used to represent the change in MEP amplitude from baseline. The mean amplitudes of MEPs collected in trial 3, trial 4 and trial 5 were calculated for each subject as a percentage of the individual’s baseline.

The relationship between the percentage change values and mean baseline MEP data was examined for independence in all subjects using a scatter plot. A random distribution in the correlation between the manipulation percentage change values and the baseline in all subjects was demonstrated (Figure 3.22). Independence was, therefore, confirmed and consequently, the MEP responses to manipulation could be expressed as a percentage change from baseline. The percentage change from baseline to trial 3, trial 4 and trial 5 was then pooled for all subjects, according to trial. The means and standard deviations were calculated for each pooled trial and are shown in Table 3.5.

To determine significant differences in the percentage change in mean amplitudes of MEPs collected after manipulation, a repeated measures ANOVA was used to compare trial 3, trial 4, and trial 5. A significant difference was observed between the means of trial 3, trial 4 and trial 5 (p= 0.006). Pairwise comparisons with Bonferroni adjustment demonstrated the significant difference was between trial 3 and trial 4 (p = 0.01).

The mean percentage change from baseline in trial 3, trial 4 and trial 5 were +34.1%, +10.6% and +15.1% respectively. This indicated an increase immediately after manipulation that was significant. Figure 3.23 illustrates the range and median of the percentage change values after manipulation. There
was an observed increase in MEP amplitude at trial 3 and then a significant reduction in MEP amplitude by trial 4.

A one-sample t-test was used to determine whether there was a significant difference in the percentage change values in each trial, before and after manipulation. The baseline was equal to zero and, therefore, one-sample t-test assessed whether the mean percentage change value at each trial was significantly different from zero. A significant difference was observed from zero to trial 3 ($p = 0.000$). No significant difference was found from zero to trial 4 ($p = 0.06$). There was, however, a significant difference from zero to trial 5 ($p = 0.03$).

In summary, these results indicated that there was a significant change in mean amplitudes of MEPs at the onset of a repeatable contraction before and after manipulation up to 60 seconds after manipulation. There also appears to be a significant latent effect by 15 minutes, collected at trial 5. In other words, manipulation had an effect on the excitability of cortical neurons projecting to contracting forearm flexor muscles, immediately after manipulation and again at 15 minutes.
Figure 3.22  Correlation between percentage change MEP data and mean baseline, for all normal subjects (n = 20). The scatterplot depicts the relationship between the percentage change MEP data and the mean baseline for the manipulation intervention, when 15 MEPs were averaged over 60 seconds. The mean baseline is the average of trial 1 and trial 2. A random distribution is shown, confirming independence of percentage change scores from the mean baseline. The percentage change calculation, therefore, can be used to normalise the post manipulation trials to the mean baseline.
Figure 3.23 Percentage change MEP data for the manipulation intervention for all normal subjects (n = 20). The boxplots show the range and median of 15 MEPs averaged over 60 seconds for the post intervention trials (3,4 and 5). Zero on the y-axis represents the mean baseline. The boxplots represent the degree of change in MEP amplitude from the baseline due to the manipulation intervention, expressed as a percentage. The circles are the outliers. The asterisk indicates significant differences from the baseline. A significant difference was found between trial 3 and trial 4 (p = 0.01). A significance difference was also found from zero to trial 3 (p = 0.00), and zero to trial 5 (p = 0.03); whereas, no significant difference was found from zero to trial 4 (p = 0.06).
3.4.3 Effect of Cavitation

It has been suggested in the literature that cavitation is required as a measure of a successful manipulation (Brodeur, 1995). To establish if there was a change in MEP amplitudes dependent on whether the manipulation resulted in joint cavitation, the manipulation intervention for all normal subjects was examined. Fourteen manipulations resulted in joint cavitation and six manipulations did not cavitate.

The percentage change MEP data for the manipulation intervention was analysed using a repeated measures ANOVA. Trials 3 – 5 were assessed by three factors and joint cavitation was evaluated as a between subject factor. The means and standard deviations were calculated for the cavitators and non-cavitators (Table 3.6). No significant difference was observed between the means of the cavitators and non-cavitators (p = 0.21). Figure 3.24 compares the effects of cavitation on MEP amplitude. A trend was observed of greater mean amplitudes of MEPs for the manipulations that resulted in joint cavitation compared to the manipulations that did not cavitate. The means were not, however, significantly different.

A one-sample t-test was used to compare each trial, before and after manipulation. A difference in response between the cavitators and non-cavitators was found at trial 5. The cavitators were significantly different from zero (p = 0.04), whereas, the non-cavitators were not significantly different from zero (p = 0.46). However caution needs to be applied when interpreting the results due to low numbers of non-cavitators and comparisons may be undesirable.
Table 3.6  The table contains descriptive statistics of percentage change MEP data for manipulations that resulted in either cavitation or non-cavitation, for all normal subjects (n = 20). The table depicts the pooled percentage change MEP data, when the first 15 MEPs were averaged over 60 seconds.

<table>
<thead>
<tr>
<th>Manipulation (%) Mean (%/SD)</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cavitators (n = 14)</td>
<td>37.0 (26.1)</td>
<td>13.3 (27.0)</td>
<td>18.8 (30.7)</td>
</tr>
<tr>
<td>Non-Cavitators (n = 6)</td>
<td>27.2 (14.7)</td>
<td>4.3 (11.3)</td>
<td>6.8 (20.3)</td>
</tr>
</tbody>
</table>
Figure 3.24 Comparison between manipulations that resulted in either cavitation \((n = 14)\) or non-cavitation \((n = 6)\), of the percentage change MEP data, for all normal subjects \((n = 20)\). The line graph depicts the mean change in MEP amplitude for trials 3, 4, and 5 recorded after manipulation, when 15 MEPs were averaged over 60 seconds. Zero on the y-axis represents the mean baseline. No statistical difference was found between the manipulation that resulted in cavitation or non-cavitation \((p = 0.21)\). A significance difference was seen from zero to trial 5 for the joints that did cavitate \((p = 0.04)\). In contrast, no significant difference was observed from zero to trial 5 for the joints that did not cavitate \((p = 0.46)\).
3.4.4 *Comparison between Positioning and Manipulation (15 MEPs)*

The positioning and manipulation interventions were compared using repeated measures ANOVA on the percentage change data collected at trial 3, trial 4 and trial 5. Positioning served as a control to compare the effects of manipulation. The positioning and manipulation interventions were compared by two factors, and trials 3 – 5 were compared by three factors.

A significant difference was observed between interventions and trials in the percentage change in mean amplitudes of MEPs ($p = 0.05$). A pairwise comparison with Bonferroni adjustment demonstrated the significant difference between manipulation and positioning occurred at trial 3 ($p = 0.003$). No significant difference was found between manipulation and positioning at trial 4 ($p = 0.69$) or at trial 5 ($p = 0.06$). Figure 3.25 compares the effects of the interventions on the percentage change values from baseline. The significant difference between manipulation and positioning at trial 3 is illustrated, with an increase of +34.1% and +4.9% respectively. At trial 5 there was a small non-significant rise again in mean amplitude with manipulation.

The interventions were also analysed separately at trial 3, trial 4 and trial 5. There was a significant difference between trials 3 and 4 ($p= 0.012$) with manipulation. Whereas, no significant difference was seen between trial 3, trial 4 or trial 5 with positioning. Figure 3.25 also demonstrates the significant difference of the percentage change in mean amplitude of MEPs for manipulation between trial 3 and trial 4.
In summary, these results indicated that the effect of manipulation on the mean amplitudes of MEPs recorded at the onset of a repeatable contraction was significantly different from positioning at trial 3, within the first 60 seconds of recordings. Therefore, there appears to be an increase in mean amplitude of MEPs up to at least 60 seconds after manipulation.

The significant increase in the mean amplitude of MEPs observed after manipulation at trial 3 had significantly reduced by trial 4. This was seen in the significant difference between trials 3 and 4 in the manipulation experiments, but no significant difference was observed between manipulation and positioning at trial 4. There was a trend towards an increase in mean amplitude of MEPs at trial 5, however, this was not significantly different from the positioning control.
Figure 3.25 Comparison between manipulation and positioning of the percentage change MEP data for all normal subjects (n = 20). The bar graph depicts the mean change in MEP amplitude in the post intervention trials (3, 4 and 5), when 15 MEPs were averaged over 60 seconds. Zero on the y-axis represents the mean baseline. The error bars represent the standard deviations. A significant difference was found between the positioning and manipulation interventions at trial 3 (p = 0.003). A significant difference was also found between trial 3 and trial 4 for the manipulation intervention (p = 0.01).
3.4.5 Examination of the Cross-over Design

The use of a repeated measures cross-over design could lead to practice or learning effects, which in turn could influence the outcome. Therefore, the order in which the interventions were delivered was also examined. If there was a significant learning effect it would be expected that the experiments delivered first would influence the outcome of the experiments delivered second, regardless of intervention.

Means and standard deviations of the percentage change MEP data were calculated for each trial for the experiments delivered first and second, regardless of the intervention (Table 3.7). The learning effect was analysed by comparing the experiments delivered first, with the experiments delivered second, using a repeated measures ANOVA on the percentage change data. The experiments were compared by two factors and the trials by three factors.

A significant relationship was found between experiments ($p = 0.02$). This relationship indicated that the order in which the subjects had received the interventions, had a significant influence on MEP amplitude, regardless of whether the intervention was manipulation or positioning. Figure 3.26 compares the experiments that were delivered first with the experiments that was delivered second and demonstrates the significant learning effect. This learning effect is illustrated by the greater change in mean amplitudes of MEPs when the experiments were delivered a second time. It is notable that at trial 3 only a small increase in MEP amplitude was observed when the second experiments were delivered.
3.4.5.1 Further Exploration of Learning effect

The learning effect was examined further. All normal subjects were divided into two groups according to whether they had received the second experiment within three days (n = 10) or three days and over (n = 10). All group one subjects received the experiments two days apart. The majority of subjects in group two had received the second experiment within a week, however, three subjects received the experiments between one and six months apart. Group one and group two subjects were then analysed separately by a repeated measures ANOVA, in the same manner as before.

3.4.5.2 Group One Learning Effect

When the effect of receiving the experiments two days apart was analysed, a significant relationship was observed between the experiments (p = 0.01). This indicated that the order in which the subjects received the interventions had a significant influence on MEP amplitude. In other words, there was a significant learning effect if a wash-out period of two days was used between experiments (Figure 3.27, top graph).

3.4.5.3 Group Two Learning Effect

When the effect of receiving the second experiment three days or more after the first experiment was analysed, no significant relationship was observed between experiments (p = 0.79). This indicated, the order in which the subjects received the interventions, had no significant influence on MEP amplitude. In other words, there was no significant learning effect if a wash-out period of three days or more was used between experiments (Figure 3.27, bottom graph).

In summary the results indicated there was a learning effect with the use of the TMS technique in an active motor system. There was a significant carry over
effect from the first experiments to when the experiments were delivered a second time, regardless of whether manipulation or positioning was delivered. When a longer wash-out period was used between experiments the learning effect did not appear to be significant.
Table 3.7  The table contains descriptive statistics of experiments delivered first and second, regardless of intervention. The table depicts the percentage change MEP data of experiments that were delivered first and second, when the first 15 MEPs were averaged over 60 seconds, for all normal subjects (n = 20).

<table>
<thead>
<tr>
<th>15 MEPs Mean (+/-SD) (%)</th>
<th>1ˢᵗ Experiments</th>
<th>2ⁿᵈ Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>17.4 (30.0)</td>
<td>21.5 (25.4)</td>
</tr>
<tr>
<td>Trial 2</td>
<td>0.0 (22.9)</td>
<td>12.8 (27.9)</td>
</tr>
<tr>
<td>Trial 3</td>
<td>2.6 (15.0)</td>
<td>18.0 (32.2)</td>
</tr>
</tbody>
</table>
Figure 3.26 Percentage change MEP data demonstrating the learning effect for all normal subjects (n = 20). The line graph shows the learning effect, when 15 MEPs were averaged over 60 seconds. The lines depict the experiments that were delivered first and second, regardless of intervention. Zero on the y-axis represents the mean baseline. A significant increase in MEP amplitudes was demonstrated for the experiments that were delivered second (p = 0.02). Of note is that at trial 3 only a small increase in MEP amplitudes was observed.
Figure 3.27 Percentage change MEP data demonstrating the learning effect for group one (n = 10) and group two (n = 10) subjects. The line graphs depict the learning effect dependant upon the wash-out period, where 15 MEPs were averaged over 60 seconds. The lines depict the experiments that were delivered first and second, regardless of intervention. Zero on the y-axis represents the mean baseline. The top graph demonstrates a significant learning effect when the experiments were delivered two days apart (p = 0.01). The bottom graph shows a non-significant learning effect (p = 0.79) when the experiments were delivered three days or more apart.
3.4.6 Conclusion

These experiments indicate that the excitability of cortical motoneurons projecting to forearm flexor muscles, at the onset of a repeatable contraction, significantly increased 60 seconds after manipulation at trial 3, when compared to positioning. This increase in excitability had significantly decreased again by trial 4. By way of comparison positioning had no significant effect on the excitability of cortical neurons projecting to contracting forearm flexor muscles.

There was some evidence of an after effect, 15 minutes after manipulation. During trial 5, manipulation was significantly different from zero baseline, however, the after effect of manipulation on cortical excitability was not sufficiently significant to be statistically difference from positioning.

There was no significant difference between manipulations that resulted in joint cavitation compared to no joint cavitation. However, a trend of higher excitability was noted with the manipulation that did result in cavitation. Evidence of this was further observed at trial 5 when the cavitators were significantly different from zero baseline, whereas, this was not observed with the non-cavitators.

There was a significant carry-over effect due to the cross-over design, with a significant increase in MEP amplitude observed when the intervention was delivered the second time. However, this carry-over effect appeared to be non-significant when the wash-out period was three or more days.
3.5 Intervention Effects on Symptomatic Subjects (15 MEPs)

A small number of symptomatic subjects (n = 5) were examined to determine trends in the changes that occur in the excitability of cortical neurons projecting to forearm flexors at the onset of a repeatable contraction in response to manipulation. Symptomatic subjects received the manipulation intervention only.

During each trial 40 MEPs amplitudes were elicited by TMS. The effect of manipulation, on peak-to-peak amplitude of the first 15 MEPs averaged over the first 60 seconds of each trial, was statistically evaluated in the same manner as the manipulation experiments on normal subjects.

3.5.1 Subject Description

One male and four female symptomatic subjects volunteered to participate in the manipulation intervention experiments. All subjects were diagnosed with acute episode, right-sided, somatic dysfunction of the lower cervical spine, and with pain provocation on palpation of the C6/7 zygapophyseal joint. Of the five subjects, four subjects had a history of episodic neck pain. A summary of the subjective findings is displayed in Table 3.8.

3.5.2 Effect of Manipulation

Figure 3.28 shows the mean amplitudes of MEPs collected in individual subjects before and after manipulation for all subjects. A difference in the individual’s response to manipulation was observed. Mean amplitudes of MEPs for individual subjects were pooled for all subjects and the group mean calculated, according to trial (Table 3.9). Due to the small subject numbers stability of baseline trials was assumed.
The percentage change MEP data was used for statistical analysis as it was considered to reduce variability between subjects. The means and standard deviations on the percentage change data were calculated for each trial (Table 3.9). The effect of manipulation on the symptomatic subjects was evaluated using a repeated measures ANOVA on the percentage change MEP data collected at trial 3, trial 4, and trial 5. No significant difference was observed between the means of trial 3, trial 4 or trial 5 (p = 0.42). The mean percentage change in trial 3, trial 4 and trial 5 were -8.8%, -27.7% and -9.0% respectively. This indicated a non-significant decrease between trial 3 and trial 4, with an increase again at trial 5. Figure 3.29 illustrates the range and median of the percentage change values after manipulation.

A one-sample t-test was used to determine whether there was a significant difference in the percentage change data before and after manipulation. There was no significant difference from zero to trial 3, or trial 4, or trial 5 ((p = 0.53), (p = 0.08), (p = 0.29) respectively).

In summary, no significant difference in the excitability of cortical motoneurons projecting to contracting forearm flexor muscles was observed with manipulation on symptomatic subjects. However, variation of individual responses and the small subject numbers, were likely mask any significant findings. This is particularly evident by the results observed at trial 4. Even though a notable reduction in excitability had occurred (-27.7%), no significant difference from the baseline was found between trials.
Table 3.8 Symptomatic subjects’ history. The table contains a summary of the history of the complaint for each of the five symptomatic subjects. All subjects had the diagnosis of right-sided lower cervical somatic dysfunction. The subjective history includes: the duration of current symptoms, any past episodic symptoms and whether the subject had experienced manipulation of the neck within the last five years.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis Description</th>
<th>Episodic History</th>
<th>Manipulation History (&lt;5 yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>32</td>
<td>2 wks</td>
<td>2 yrs</td>
<td>&gt;5x</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>33</td>
<td>2 wks</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>29</td>
<td>2 wks</td>
<td>4 yrs</td>
<td>&gt;5x</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>29</td>
<td>1 wk</td>
<td>2 yrs</td>
<td>1x</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>47</td>
<td>1 wk</td>
<td>10 yrs</td>
<td>1x</td>
</tr>
</tbody>
</table>
Figure 3.28 MEP recordings for individual symptomatic subjects (n = 5), for the manipulation intervention. The line graph depicts the individual responses before and after the manipulation intervention, when the first 15 MEPs averaged over 60 seconds. The mean baseline was calculated from trial 1 and trial 2. Trial 3, trial 4 and trial 5 was recorded after the manipulation intervention. Variability of individuals’ responses is demonstrated.
Table 3.9 The tables contain descriptive statistics for the manipulation intervention on the five symptomatic subjects, when the first 15 MEPs were averaged over 60 seconds. The top table shows the pooled MEP data and the bottom table displays the pooled percentage change MEP data.

<table>
<thead>
<tr>
<th>15 MEP data Manipulation (n = 5) (mV)</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Mean Trials 1 &amp; 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>0.26</td>
<td>0.24</td>
<td>0.26</td>
<td>0.18</td>
<td>0.14</td>
<td>0.22</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.66</td>
<td>0.73</td>
<td>0.73</td>
<td>0.91</td>
<td>0.61</td>
<td>0.64</td>
</tr>
<tr>
<td>Mean</td>
<td>0.46</td>
<td>0.45</td>
<td>0.45</td>
<td>0.44</td>
<td>0.36</td>
<td>0.41</td>
</tr>
<tr>
<td>(SD +/-)</td>
<td>0.16</td>
<td>0.21</td>
<td>0.18</td>
<td>0.29</td>
<td>0.20</td>
<td>0.17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>15 MEP Manipulation (n = 5) (%)</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>- 38.3</td>
<td>- 51.3</td>
<td>- 25.5</td>
</tr>
<tr>
<td>Maximum</td>
<td>31.6</td>
<td>1.3</td>
<td>18.8</td>
</tr>
<tr>
<td>Mean</td>
<td>- 8.6</td>
<td>- 22.4</td>
<td>- 8.7</td>
</tr>
<tr>
<td>(SD +/-)</td>
<td>28.7</td>
<td>22.5</td>
<td>17.3</td>
</tr>
</tbody>
</table>
Figure 3.29 Percentage change MEP data for the manipulation intervention for all symptomatic subjects (n = 5). The boxplots show the range and median of 15 MEPs averaged over 60 seconds for the post intervention trials (3, 4 and 5). Zero on the y-axis represents the mean baseline. The boxplots represent the degree of change in MEP amplitude from the baseline due to the manipulation intervention, expressed as a percentage. The circle depicts the outliers. No statistical significance was found between trials (p = 0.42). No significant difference was seen from zero to trial 3 (p = 0.53), or trial 4 (p = 0.08) or trial 5 (p = 0.29).
3.5.3 Cavitation

Due to the small subject numbers no inferential statistics were performed to assess the cavitation phenomenon. Two manipulations, on the symptomatic subjects, resulted in cavitation compared with three manipulations that did not cavitate. An interesting observation occurred at trial 3, where the cavitators and non-cavitators demonstrated different trends. The change in mean amplitude of MEPs was +19.9% and -28.0%, respectively (Table 3.10). The trends over all the trials are shown in Figure 3.30. At trial 3, joint cavitation tended to increase MEP amplitude, whereas, a reduction in MEP amplitude was observed with the non-cavitators. No notable differences were observed at trials 4 and 5.

3.5.3.1 Effect of Joint Cavitation on Pain Measures

Subjects’ resting pain level was subjectively measured on a VAS scale and recorded immediately before and after manipulation. In two subjects, where stiffness was the main problem, no resting pain was reported. Cavitation did not appear to have an effect on resting pain levels as both an increase and a decrease was reported immediately after manipulation.

An end of range VAS score was also taken before trial 1 and after trial 5. Of the two subjects that cavitated, one subject’s end of range pain level did not change. While in the other subject, a reduction in end of range pain was observed, as well as the greatest increase in range of motion (Table 3.11).
Table 3.10 The table contain descriptive statistics of percentage change MEP data for manipulations that resulted in cavitation or non-cavitation for all symptomatic subjects (n = 5). The table depicts the pooled percentage change MEP data, when the first 15 MEPs were averaged over 60 seconds.

<table>
<thead>
<tr>
<th>Manipulation (%) Mean (%)</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cavitators (n = 14)</strong></td>
<td>19.9 (16.6)</td>
<td>-26.3 (21.0)</td>
<td>-14.2 (10.0)</td>
</tr>
<tr>
<td><strong>Non-Cavitators (n = 7)</strong></td>
<td>-28.0 (11.9)</td>
<td>-20.3 (27.2)</td>
<td>-5.5 (21.5)</td>
</tr>
</tbody>
</table>
Figure 3.30 Comparison between manipulations that resulted in cavitation (n = 2) or non-cavitation (n = 3) of the percentage change MEP data for all symptomatic subjects (n = 5). The bar graph depicts the mean change in MEP amplitude for trials (3,4 and 5) recorded after manipulation, when 15 MEPs were averaged over 60 seconds. Zero on the y-axis represents the mean baseline. The error bars represent the standard deviations. A trend is seen at trial 3 where joint cavitation results in an increase in MEP amplitude, whereas, the opposite effect is observed with the joints that did not cavitate.
Table 3.11 The table contains the change in the objective data before and after manipulation for the five symptomatic subjects. The visual analogue scale (VAS) was used for intensity of pain description. The change in VAS was recorded immediately before and after manipulation (trial 3). Changes in the cervical spine right side bend, range of motion (ROM) and the pain level at the end of this movement (VAS EOR) were recorded before trial 1 and after trial 5.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cavitation</th>
<th>Change in VAS</th>
<th>Change in VAS EOR</th>
<th>Change in ROM (r) SB (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>-</td>
<td>↓ -4</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>↑ +1</td>
<td>↓ -2</td>
<td>↑ +12°</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>↓ -2</td>
<td>-</td>
<td>↑ + 2°</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>↓ -4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>-</td>
<td>↑ +1</td>
<td>-</td>
</tr>
</tbody>
</table>
3.5.4 Power Analysis

A power analysis was performed to predict the number of symptomatic subjects required, for future research, to demonstrate a significant effect of neck manipulation on cortical excitability. The power analysis explored variability in the response of five symptomatic subjects immediately after manipulation (trial 3). The standard deviation of the symptomatic subjects' response at trial 3 was used to describe variability, and was approximately 30% (Table 3.9).

The null hypothesis was a predicted change in cortical excitability of 0% (mean 0). The alternative hypothesis was a predicted change of at least 20% in cortical excitability (mean 1). A two-sided one-sample t-test was used to assess the difference between the means of the null hypothesis and the alternative hypothesis.

To achieve 80% power, a sample size of 20 symptomatic subjects would be required to detect a difference in cortical motoneuron excitability of 20% (Table 3.12).
Table 3.12  The table contains the power analysis using the standard deviation of the response of symptomatic subjects immediately after manipulation (trial 3). The table depicts the power analysis for a predicted change of at least 20% in cortical excitability. This was termed the alternative hypothesis (mean 1). The null hypothesis was a predicted change in cortical excitability of 0% (mean 0). Power is definite as the probability of rejecting a false null hypothesis and should be close to one. Alpha is the probability of rejecting a true null hypothesis and should be small. Beta is the probability of accepting a false null hypothesis and should be small. N is the number of subjects predicted to demonstrate a significant change in cortical excitability of at least 20%.

<table>
<thead>
<tr>
<th>Power</th>
<th>SD</th>
<th>Mean 1</th>
<th>Mean 0</th>
<th>Alpha</th>
<th>Beta</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.81</td>
<td>30%</td>
<td>20%</td>
<td>0.0%</td>
<td>0.50</td>
<td>0.19</td>
<td>20</td>
</tr>
</tbody>
</table>
3.5.5 Conclusion

Analyses of the effect of manipulation on symptomatic subjects indicated that there was no significant change in the mean amplitudes of MEPs at the onset of a repeatable contraction after manipulation. In other words, spinal manipulation of the painful zygapophyseal joint had no effect on the excitability of cortical neurons projecting to contracting forearm flexor muscles. However, due to small subject numbers and variation of each individual’s response to manipulation, a significant finding may have been missed.

An interesting observation occurred when the cavitators and non-cavitators were considered separately. Immediately after manipulation, cortical motoneuron excitability for the cavitators notably increased (+20%), whereas, the non-cavitators substantially decreased (-28%). This indicated that cavitation may be important for increasing cortical excitability in symptomatic subjects. In contrast, the effects of the manipulation resulting in joint cavitation on pain measures were inconsistent; therefore, noteworthy trends cannot be seen.
3.6 Comparison between Symptomatic and Asymptomatic (15 MEPS)

3.6.1 Effect of Manipulation

To establish whether the normal subject group and the symptomatic subject group responded differently to neck manipulation a repeated measures ANOVA was performed.

The effect of manipulation on normal subjects and symptomatic subjects was compared using repeated measures ANOVA on the percentage change data collected in trial 3, trial 4 and trial 5. The percentage change MEP data was used as it was considered to reduce between subject variability in the MEP response to the interventions. The trials 3 – 5 were assessed by three factors and normal subjects and symptomatic subjects were analysed as the between subject factor. The symptomatic group (n = 5) had received only the manipulation intervention. Therefore, only the subjects that had received the manipulation intervention first were selected from the normal subject group for analysis (n = 10). Thus, there was no influence from the cross-over design.

There was a significant difference in the response to manipulation between groups (p = 0.006). Figure 3.31 compares the effects of manipulation on the percentage change values from baseline and illustrates the significant difference between the normal subject group and the symptomatic group at trial 3. An increase in percentage change in mean amplitude MEP was seen with manipulation in the normal subject group. Whereas, a reduction in percentage change in mean MEP amplitude was seen with manipulation on the symptomatic subject group. At trial 3, trial 4 and trial 5, the mean percentage
change for manipulation of normal subjects was +37.8%, +7.8% and +5.2% respectively. This is compared to -8.8%, -22% and -9.0% respectively, for symptomatic subjects.

These results indicated that the effect of manipulation, on the mean amplitudes of MEPs, recorded at the onset of a repeatable contraction, differed significantly between normal and symptomatic subjects, in the first 60 seconds of recordings. There is some evidence, therefore, that the effect of manipulation on cortical motoneuron excitability in symptomatic subjects is significantly different to that of normal subjects.

3.6.2 Conclusion

Manipulation of the painful zygapophyseal joint did not significantly change cortical excitability. Yet the response of symptomatic subjects to manipulation was significantly different to normal subjects. The effect of manipulating the painful zygapophyseal joint on cortical motoneuron excitability was likely to be missed due to small subject number and the variation of individual responses.
Figure 3.31 Comparison between normal subjects (n = 10) and symptomatic subjects (n = 5) for the percentage change MEP data. The bar graph depicts the mean change in MEP amplitude for trials (3, 4 and 5) recorded after manipulation, when 15 MEPs were averaged over 60 seconds. Zero on the y-axis represents the mean baseline. The error bars represent the standard deviations. A significant difference was found between normal and symptomatic subjects (p = 0.006). MEP amplitude increased with neck manipulation in normal subjects, whereas symptomatic subjects demonstrated a reduction in MEP amplitude with neck manipulation.
3.7 Summary of Main Findings

Table 3.13 show a summary of the main results on the percentage change data, when 40 MEPs were averaged over 120 second, and when 15 MEPs were averaged over 60 second. The percentage change data was found to be a more sensitive method of detecting change in MEP response with the interventions.

In normal subjects, there was no evidence that the excitability of cortical neurons projecting to the forearm flexor muscle, at the onset of a repeatable contraction, significantly changed after manipulation, when 40 MEPs were averaged over 120 seconds.

There was, however, a significant increase in the excitability of cortical neurons projecting to the contracting forearm flexor muscle, when 15 MEPs were averaged of 60 seconds. The significant increase in excitability was observed at trial 3, up to at least 60 seconds after manipulation. In comparison, no significant change in the excitability of cortical neurons projecting to the contracting forearm flexor muscle was observed with positioning and baseline measures.

No significant difference in the excitability of cortical neurons projecting to the contracting forearm flexor muscle was found between the manipulations that resulted in cavitation or non-cavitation.

In symptomatic subjects, manipulation of the painful joint had no significant effect on the excitability of cortical neurons projecting to the contracting forearm
flexor muscle, when 15 MEPs were averaged of 60 seconds. On the other hand, due to small subject numbers a significant finding may have been missed. Comparisons between normal subjects and symptomatic subjects, however, demonstrated a significantly different response to manipulation, where cortical excitability was observed to increase in normal subject and reduce in symptomatic subjects.
Table 3.13 The table contains a summary of the main results on the percentage change data, for normal subjects and symptomatic subjects. 40 MEPs were averaged over 120 second and 15 MEPs were averaged over 60 second. A significant result is demonstrated when $p \leq 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>Normal Subjects</th>
<th>Positioning vs Manipulation</th>
<th>$p = 0.55$</th>
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</tr>
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<tbody>
<tr>
<td>40 MEP (%)</td>
<td></td>
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<tr>
<td>15 MEP (%)</td>
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<tr>
<td>Symptomatic Subjects</td>
<td>Normal subjects vs Symptomatic</td>
<td></td>
<td>$p = 0.006$</td>
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Chapter Four  Discussion

4.1 Major Findings

The most significant finding in this study was that cervical spine manipulation led to a significant increase in motoneuron excitability in normal human subjects performing a voluntary contraction. This was demonstrated in two ways on the data when 15 MEPs were averaged over 60 seconds. Firstly, manipulation significantly increased MEP amplitude from baseline trials. Secondly, the effect of manipulation was significantly different from mere positioning alone. This suggests that manipulation evokes facilitation of either cortical motoneurons, or spinal motoneurons, or both.

The effect of manipulation was considered to be centrally mediated, as the change was measured in cortical neurons projecting to the muscles, whose sensory afferents projected to the manipulation site. This is the first time that a significant change in motoneuron excitability has been demonstrated with manipulation of the cervical spine. Additionally, this is the first time that the effect of manipulation has been evaluated in an active motor system.

The observed increase in central motor excitability is likely to be a consequence of the summation of inputs from a variety of sensory afferent receptors arising from spinal and paraspinal tissue, stimulated as a result of the high velocity manipulative thrust. Further, there was some evidence of a delayed effect of manipulation, with a significant increase in motoneuron excitability 15 minutes after manipulation.
Other major findings of the study were:

- The effect of manipulation on motoneuron excitability was not evident when 40 MEP amplitudes were averaged over the first 120 seconds.
- The response of symptomatic subjects to manipulation was significantly different to that of normal subjects.
- In normal subjects, there was no significant change in motoneuron excitability regardless of whether the manipulation resulted in cavitation or not. There was, however, a trend towards higher motoneuron excitability observed with the subjects that did cavitate.
- A significant learning effect was also revealed with the cross-over design, with some indication that the “wash-out period” should be greater than two days.

4.1.1 Consistency of Control Trials

In order to determine that the observed change in motoneuron excitability was due to the effect of manipulation, consideration of other factors that may contribute to alterations of the MEP amplitude elicited by TMS stimulation is required. For example, an alteration in MEP amplitude could be influenced by natural fluctuations in cortical motoneuron excitability, or peripheral changes such as movement of the recording electrodes or stimulation coil. To be confident that manipulation did in fact mediate the observed changes in MEP amplitude, procedures were put in place to limit possible peripheral changes and create consistent stimulation and recording environments throughout the experiments. The control procedures, such as the positioning intervention and baseline measures, were used to minimise the contribution of natural fluctuations in cortical excitability and other extraneous variables. In addition,
the statistical analyses used took into account confounding variables (for example, the repeated measures cross-over design).

4.1.1.1 Stimulating Coil

Movement of the hand-held coil from the optimum site for eliciting a muscle response in the FCR muscle could alter the population of cortical neurons being stimulated and hence potentially change the MEP amplitude. This could occur in two ways. Firstly, the experimenter could inadvertently alter the hand-held coil position in anterior-posterior, medial-lateral or superior-inferior tilt coordinates. Secondly, the subject’s head could move in relation to the coil.

In the current study, during each trial great care was taken to position the stimulating coil precisely. The cap was secured to the subject’s head so no movement could occur. The optimum site for eliciting a MEP response in the FCR muscle was clearly marked on the cap. The stimulating coil was centred over the marked optimum site and four marks were made on the cap, for coil realignment. This accounted for the anterior-posterior and medial-lateral coil orientations. Previous investigations found the superior-inferior angle of tilt to be the most variable coordinate (Dishman et al., 2002). Special attention was therefore paid to controlling the stimulating coil in this direction. A clinical goniometer was used to help replicate the angle of the superior-inferior tilt coordinate during each trial. The goniometer was fixed to the superior surface of the coil, to allow visible feedback for the experimenter to adjust and maintain the coil position over the optimal site.

Previous studies examining reproducibility have, however, demonstrated that 5 – 10 mm deviation in coil position from the optimum muscle stimulation site did
not result in any significant alteration in MEP amplitude (Brasil-Neto et al., 1992; Kasai, Kawai, Kawanishi, & Yahagi, 1997). Furthermore, these studies used a more focal figure-of-eight coil, which is considered to produce more variable MEP amplitudes than the circular coil (Kier et al., 1993). Dishman et al. (2002) used an optical 3-D tracking system to monitor the three-dimensional position of the stimulating coil in relation to the subject’s head. The authors found coil position to be repeatable over trials, even when the subject was repositioned after manipulation. Even the most variable co-ordinate (the superior-inferior tilt), which ranged between 0°-12° degrees of movement, was found to be insignificant (Dishman et al., 2002).

4.1.1.2 Subject Movement for the Intervention

A potential source of experimental error may also have arisen from the need to recline the subject during the intervention. Precautions to limit the amount of variability due to subject movement included the following:

The subjects were asked to align their line of vision with a fixed marker on the wall directly in front of them. Additionally, the experimenter visually checked that the subject's chin was aligned with their sternal notch.

The chair was blocked at the possible limit of the reclined angle.

Markers were used to realign the subject’s wrist and thumb, and then their forearm was secured with velcro straps.

The subject was instructed to sit upright, in the same position with their lumbar spine as far against the back of the chair as possible and legs supported.

Dishman et al. (2002) demonstrated that whole body positioning did not significantly change MEP amplitude. In that study subjects were required to roll from supine to side lying for the interventions, then back to supine (Dishman et
Furthermore, Carrol et al. (2001) observed only slightly less consistency in MEP amplitude across different sessions, where both recording electrodes and stimulation coil were repositioned. Thus, it is likely that the same or similar populations of corticospinal neurons and muscle fibre units are stimulated in the same individual in different experiments or in the same experiment where different positions are used (Carroll et al., 2001).

**4.1.1.3 Recording Electrodes**

Movement of the recording electrodes during the course of the experiment could alter the motor units measured. The securing of the recording electrodes was confirmed by the difficulty of removing the electrodes after the experiment. An alteration in MEP amplitude was, therefore, unlikely to be due to movement of the recording electrodes.

**4.1.1.4 Stability at Motor Onset**

Assessing stability of recording conditions before any interventions were applied was necessary to ensure reliability of the TMS technique in an active motor system. Stability of MEP amplitudes was examined in two ways. Firstly, the intra-class correlation coefficient (ICC) and the paired-sample t-test were used to inspect baseline trials for consistency before any intervention took place. The baseline trials before intervention were found to be consistent and comparable with baseline trials in the Dishman et al. (2002) study. Secondly, five subjects participated in separate experiments to establish stability in MEP amplitudes over five trials without an intervention. Stability of the MEP amplitudes across five trials was demonstrated by analysis of variance. These results allowed confidence in the interpretation that changes in MEP amplitudes were due to the intervention.
4.2 Effect of Manipulation on Normal Subjects

4.2.1 15 MEPs Averaged over the First 60 seconds

The main finding in this study was that cervical spine manipulation led to a significant increase in amplitude of MEPs elicited by TMS in the forearm flexor muscles. These results indicate that manipulation has a facilitatory effect on the central nervous system at either a cortical or spinal level. This conclusion concurs with the findings of Dishman et al. (2002) who found lumbar spine manipulation increased motor excitability of cortical neurons projecting to the lower limb muscles in human subjects at rest. In the studies described here, the significant increase in excitability was, however, transient and was only observed in the first 60 seconds after manipulation.

To date, no other investigations have examined changes in cortical motoneuron excitability with manipulation of the cervical spine. In theory, neck manipulation should generate a greater neurophysiological response than lumbar spine manipulation for two main reasons. Firstly, the upper limb has stronger corticospinal projection compared to that of the lower limb (Brouwer & Ashby 1990). Secondly, it has been reported that the cervical spine is more densely populated with proprioceptors and mechanical joint receptors than the lumbar spine (Amonoo-Kuofi, 1982; Bolton, 1998). Greater summation of afferent input would be expected, therefore, with cervical spine manipulation generating potentially greater motoneuron excitability than that following lumbar spine manipulation (Dishman et al., 2003).

However, lumbar spine manipulation appears to evoke greater excitation of cortical motoneurons projecting to gastrocnemius muscle than that evoked in
FCR muscle by cervical manipulation. Dishman et al. (2002) observed an approximate 44% increase in cortical motoneuron excitability within 60 seconds (as estimated from the graphically displayed data). This compares with the 34% increase in excitability seen in the study described here, within the same time frame.

The difference in the magnitude of change in motoneuron excitability with lumbar compared to cervical spine manipulation has also been observed in experiments using the H-reflex technique (Dishman & Burke 2003). Lumbar spine manipulation attenuated the H-reflex to a greater degree and for a longer period than neck manipulation (60 seconds and 20 seconds respectively). Dishman & Burke (2003) proposed that the processing of afferent input may be more refined in the cervical spinal cord compared to that of the lumbar spine. The cervical spine, therefore, would be less sensitive to sudden aberrant sensory stimulation, such that manipulation would produce (Dishman & Burke 2003).

Methodological differences between the use of TMS to evoke responses in the upper compared to the lower limb must also be considered. The difference may be due to the fact that the lower limb requires greater stimulation to evoke MEPs and therefore more motoneurons would be recruited with the higher stimulation intensity (Brouwer & Ashby 1990; Rossini et al., 1994). This was observed in the Dishman et al. (2002) study where stimulation intensities reached 100% of the maximal output of the TMS stimulator. In the study described here, stimulator output was always set 20% above motor threshold and ranged between 30 and 60% of maximal output. Subtle changes in the
modulation of motor cortex excitability have been demonstrated at lower TMS stimulus intensities, which were not observed at higher stimulus intensities (Lemon et al., 1995). Thus, more reliable information may be gained by assessing changes in cortical excitability with manipulation of the cervical spine, with the TMS technique.

For the first time, the findings described in the current study have demonstrated the effect of spinal manipulation on cortical motoneuron excitability in subjects during muscle contraction. This is significant as previous TMS studies have indicated that changes observed to afferent stimulation in a resting motor system may not be present in an active motor system (Clouston et al., 1995; Ridding et al., 2001). This may be because the motor cortex is less active with subjects at rest and, therefore, more likely to show changes in excitability in response to experimental or other inputs. Furthermore, changes in excitability in an active motor system are more applicable to human subjects performing normal movement.

In summary, the results in the current study suggest that there was a significant, albeit transient, neurophysiological change with neck manipulation. This is in agreement with the findings of one other study involving manipulation of the lumbar spine (Dishman et al., 2002).

4.2.2 40 MEPs Averaged over the First 120 seconds

In the study described here, when cortical excitability was examined by averaging MEP amplitudes over the first 120 seconds, the significant effect of manipulation was not apparent. After 120 seconds the effect of manipulation was not significantly different from baseline trials or the positioning control. This
is in direct contrast to the Dishman et al. (2002) study, where a significant increase in excitability from baseline was still observed in the first 120 seconds. The major difference between the Dishman and the current study was the number of MEP amplitudes averaged during the 120-second time period. In the current study, 40 MEPs were averaged over the 120-second period, whereas Dishman et al. (2002) averaged 10 MEPs over this period. In the current study, it was decided to average 40 MEPs amplitudes over 120 seconds, as this was considered to reduce the variability in MEPs collected in the same subject (Kier et al., 1993). However, the effect of manipulation was also lost when averaging over such a large number of MEPs, collected over an extended time period.

In the current study, a significant reduction in motoneuron excitability was observed 16 minutes after positioning. The manipulation intervention demonstrated the same trend, though was not statistically significant. Kiers et al. (1993) found that the amplitude of MEPs fluctuated when TMS was applied in subjects at rest. However, unlike the study described here, no systematic reduction in MEP amplitude was observed over time (Kiers et al., 1993). A plausible explanation for the observed reduction in excitability over time could be that the motor task of wrist flexion became learnt (Siebner & Rothwell 2003). Over time the excitability of the motor cortex may have accommodated. The repetitive motor task may, therefore, have been controlled by subcortical areas (Lemon et al., 1995).

Experiments in the present study performed on five normal subjects over five trials where no intervention was applied, did not support the supposition of a reduction in CNS excitability with learnt motor tasks. This is because no
significant change was observed in cortical excitability at the onset of a repeatable muscle contraction over the five trials (27 minutes). One possible explanation for this lack of change in the non-intervention group is that the statistical method of analysis may not have been rigorous enough to detect a significant change. This may have been because the variability between subjects was too high and the subject numbers were low. A more stringent statistical method, such as the intra-class correlation coefficient, however, could not be used with these experiments due to small subject numbers. Additional research on a larger number of subjects would be required to clarify this point.

The effect of the reclined position used on each subject also needs to be considered, due to the fact that both positioning and manipulation interventions reduced motoneuron excitability, whereas the non-intervention group remained stable. The reclining movement would have had an effect on the vestibular system. However, the vestibular system did not appear to exert any influence on motoneuron excitability when MEP amplitude was averaged over 60 seconds. As the positioning control intervention was not stable over the collection time period, there are too many confounding variables to draw any firm conclusions.

In summary, when assessing cortical motoneuron excitability in an active motor system, averaging 15 MEPs over 60 seconds for the FCR muscle is a superior method, compared to averaging 40 MEPs over 120 seconds.
4.3 Effect of Manipulation on Symptomatic Subjects

The response to manipulation was significantly different between symptomatic subjects and normal subjects, although, no significant change in motoneuron excitability was observed after manipulation of the painful spinal joint. The lack of significant change in excitability was most likely due to the small numbers of subjects examined. Nevertheless, a trend of reduced motoneuron excitability was observed. This finding concurs with previous investigations that have examined the effect of pain using TMS (Farina et al., 2001; Le Pera et al., 2001; Valeriani et al., 2001; Valeriani et al., 1999). In those studies, experimental pain had an inhibitory effect on cortical motoneuron excitability. In the study described here, the excitatory effect of manipulation observed in normal subjects was not observed with symptomatic subjects. These studies indicate that manipulation of the painful spinal joint may, in itself, produce motor depression, in a similar manner to nociceptive stimulation.

On the other hand, it could be argued that the inhibitory effect of manipulation on cortical motoneuron activity may be beneficial. Traditionally, pain was thought to alter sensory processing and induce excessive muscle activity (or muscle spasm). Abnormal sensory processing, by central neurons already sensitised by tissue injury, was thought to lead to changes in muscle recruitment patterns. The abnormal recruitment of motor units was also thought to drive the sensitisation process and to self-perpetuate the pain-muscle spasm-pain cycle. Accordingly, it was hypothesised that the inhibitory effect of manipulation on spinal α-motoneurons, reduced muscle spasm by altering the abnormal recruitment of motor unit and hence broke the pain-spasm-pain cycle (Dishman et al., 2000; Zusman 1986). On this basis, the inhibitory effect of
manipulation on motor output may indicate a reduction in muscle spasm. A reduction of motor unit firing with manipulation of the painful spinal joint has been observed in several studies (Herzog et al., 1999; Thabe 1986). However, these clinical trials need to be interpreted with caution, due to limited number of subjects and poor methodologies. Furthermore, the effect of pain on motor control is not fully understood, and pain does not necessarily result in an increase in muscle activity (Lund, Stohler, & Widmer, 1993).
4.4 Effect on the Vestibular System

It is possible that the vestibular system influenced the excitability of cortical motoneurons projecting to the FCR muscle. Vestibular reflexes originating from the inner ear operate to maintain the head vertical with respect to gravity; they are evoked by a change in head position regardless of neck motion (Goldberg & Hudspeth 2000). These include vestibulocollic and vestibulospinal reflexes, which influence muscle activity of the neck and limbs, respectively (Wilson, 1984).

In the current study, the vestibular system could have been activated in two ways. Firstly, in both the positioning and manipulation interventions, whole body tilt occurred when the subjects were reclined and returned to upright sitting. Vestibular activation with whole body tilt has been reported to have both excitatory and inhibitory effects on spinal α-motoneurons (Aiello, Rosati, Serra, Tugnoli, & Manca, 1983; Chan & Kearney, 1982, 1984). Secondly, the movement of the head with neck rotation and side bend to the right, in both interventions, would evoke receptors of the otolith organs and the vertical semicircular canals (Wilson & Schor, 1999). This would have an effect of stimulating both vestibulocollic and vestibulospinal reflexes. Short latency (disynaptic and trisynaptic) pathways have been demonstrated between semicircular canal/otolith receptors and spinal motoneurons (Wilson & Schor, 1999).

Excitability of the right FCR α-motoneurons has been observed to increase significantly with right-sided neck movement, as measure by the H-reflex (Sabbahi & Abdulwahab, 1999). Both neck and vestibular reflexes have been
shown to converge on vestibular nuclei and produce complex inhibition and excitation effects on extensively distributed motoneurons pools (Goldberg & Hudspeth 2000).

In the current study, vestibular afferent activation from body tilt did not exert any significant influence on motoneuron excitability. This can be assumed as the positioning control was demonstrated to be stable. It is not, however, possible to differentiate between the influences of vestibular or neck reflexes on motoneurons, as both reflexes are sensitive to velocity of movement (Wilson, 1984). Thus, both vestibular and neck reflexes could have been affected by the speed of application the manipulative thrust.
4.5 Cavitation

Often the success of a manipulation is judged by a cracking sound that is commonly accepted to be a consequence of joint cavitation (Brodeur, 1995). In the current study, when 15 MEP amplitudes were averaged over 60 seconds, there was a trend towards an increased motoneuron excitability in the group of 14 normal subjects in which neck manipulation resulted in cavitation. Yet, when compared with the group of six subjects that did not cavitate, no significant difference was found in motor excitability. There is, however, some evidence that joint cavitation may exert an effect on cortical excitability. Fifteen minutes after manipulation, motor excitability of the group that did cavitate was found to be significantly different from baseline trials, whereas no significant change was observed in the group that did not cavitate.

This finding is in contrast with previous neurophysiological investigations in normal subjects, as the cavitation phenomenon appeared to have no effect on reflex muscle responses to spinal manipulation (Dishman et al., 2000; Suter et al., 1994). For example, EMG measured muscle responses were found to be dependent on the speed of application of the manipulation, regardless of joint cavitation (Suter et al., 1994). Furthermore, in H-reflex studies both joint mobilisation and manipulation significantly influenced muscle responses (Bradnam et al., 2000; Dishman et al., 2002), therefore, it could be assumed that joint cavitation was not relevant.

4.5.1 Cavitation in Symptomatic Subjects

Of the five symptomatic subjects, two manipulations resulted in joint cavitation. The immediate response of the subjects who cavitated with manipulation was opposite to subjects who did not cavitate. The two subjects that cavitated
demonstrated an increase in cortical excitability, in the first 60 seconds after manipulation, to a similar extent as that observed in the normal subjects (20% and 34% respectively). Whereas, symptomatic subjects who did not cavitate with neck manipulation demonstrated a substantial reduction in cortical excitability (-28%). The reduction in cortical excitability when the painful cervical spine joint was manipulated, but did not cavitate, was similar to that observed in research where acute pain was induced experimentally (La Pera et al., 2001; Farina et al., 2001). The increase in cortical excitability in the two subjects that did cavitate, suggests cavitation may be a measure of the success of spinal manipulation.

4.5.1.1 The Analgesic Effect of Manipulation

An analgesic effect of manipulation with concurrent modulation of motor output has been observed with spinal manipulation in symptomatic subjects (Cassidy, Lopes, & Yong-Hing, 1992; Lehman et al., 2001; Vicenzino, Collins, Benson, & Wright, 1998). In the current study, however, the analgesic effect of manipulation cannot be determined using subjective pain measures as subject numbers were to small and their responses were to variable.

The results in the present study suggest that cavitation may be an indicator of the success of the manipulation, despite the lack of evidence of a concurrent reduction in pain. Further investigation into manipulation of the painful segment is warranted using a larger subject group.
4.6 Site

Most neurophysiological studies have investigated the segmental effects of SMT. Motor unit firing has been reported to increase in both segmental and non-segmentally related muscles with manipulation (Herzog et al., 1999; Suter et al., 1994). Manipulation, however, does not appear to have a global motor response. Two recent studies demonstrated that neck manipulation had no effect on lumbar spine H-reflex, as measured in the calf muscle (Dishman et al., 2002; Dishman & Burke, 2003).

In the current study, manipulation of the C6/7 zygapophyseal joint induced a segmentally mediated, distal muscle response. It cannot, however, be determined whether the response was purely segmental for three reasons. Firstly, no other motoneuron pool was examined external to the segmental supply. Secondly, it is likely that afferent input, evoked by manipulation, may not have been solely derived from the C6/7 spinal segmental nerve supply, and thirdly, it is also not possible to state with any accuracy that the C6/7 joint manipulated.

The current study is the first to investigate a centrally mediated mechanism for spinal manipulation in symptomatic subjects. The results suggest manipulation of the painful segment may inhibit motor activity, in a similar manner to nociceptive input into the CNS. In contrast, neck manipulation in normal subjects facilitates motor activity. On this basis, there is evidence to suggest that manipulation may be more therapeutically beneficial when directed at the non-painful zygapophyseal joint. Maitland (1986) has always recommended
that SMT should be performed on the joint away from the painful movement direction. However, further research is needed to clarify this proposition.
4.7 Duration of the Effect

In the current study, the significant changes in motoneuron excitability lasted up to at least 60 seconds after the manipulation, which concurred with the findings of Dishman et al. (2002). Transient neurophysiological changes have also been observed, with cervical and lumbar spine manipulation using the H-reflex method of measuring $\alpha$-motoneuron excitability, lasting between 20 and 60 seconds (Dishman & Bulbulian, 2001; Dishman & Burke, 2003). The implication of this transient change is not known, however, it seems noteworthy that cortical motoneuron excitability changes were demonstrated to be of longer duration that of the manipulation itself.

In the study described here, there was also some evidence for a delayed effect of manipulation. A significant increase in excitability was found 15 minutes after the manipulation, although the magnitude of the change was not significantly different from the positioning control. Murphy et al., (1995) also demonstrated a change in spinal motoneuron excitability 15 minutes after manipulation using the H-reflex technique.
4.8 Limitations

A significant learning or practice effect was found due to the cross-over design. The learning effect was significant for the intervention delivered second, regardless of whether it was manipulation or positioning, with an increase in cortical motoneuron excitability observed. These results indicate that the full effect of manipulation on excitability may not have been observed. It is noteworthy, however, that in trial 3 the learning effect was small in comparison to the other trials. Therefore, it could be said that the significant learning effect did not undermine the significant increase in cortical excitability observed immediately after manipulation. Accordingly, the time course of the effect of manipulation may not have fully demonstrated. Therefore, it cannot be determined accurately from this study whether or not neck manipulation had a latent effect on motoneuron excitability, greater than 60 seconds.

Further exploration of the cross-over design found there was no significant learning effect when the subjects received the interventions three or more days apart, which also concurs with McKay Ridding, Thompson, & Miles (2002). This indicates that a greater wash-out period may reduce the learning effect, on cortical motoneuron excitability, of the subjects participating in the TMS experiments more than once.

The lack of statistical results in the symptomatic subjects could be explained by the small number of subjects and the variation in individual responses to manipulation. On this basis, the finding that neck manipulation had no effect on excitability should be interpreted with caution. The power analysis indicates that it would be expected that 20 symptomatic subjects would be required to
reach a significant change in cortical excitability of approximately 20%. In other words, 20 symptomatic subjects would need to be examined to conclude that manipulation had any effect on motoneuron excitability.

Research of the cavitation phenomenon has been found to be particularly problematic, even when external sound and vibration measurement methodologies are used to detect joint cavitation (Herzog, Zhang & Conway, 1999). The symptomatic subjects selected for the present study demonstrated pain provocation on palpation of the C₆/₇ zygapophyseal joint. Intra-tester and inter-tester reliability in detecting the painful zygapophyseal joint has been demonstrated (Hubka & Phelan, 1994; Jull et al., 1988). It is not possible, however, to state with accuracy that the C₆/₇ zygapophyseal joint actually cavitated with the manipulated. Moreover, in the current study, because the number of symptomatic subjects and normal subjects in the non-cavitating group were small, caution needs to be applied when drawing conclusions.
4.9 Mechanism For the Effect of Manipulation

4.9.1 Peripheral Afferents Activated

It has been well documented that sensory afferent stimulation can modulate motor output (Kaelin-Lang et al., 2002; Ridding et al., 2001; Stefan, Kunesch, Benecke, Cohen, & Classen, 2002; Tokimura et al., 2000). In the current study, it was likely that the passive movement of manipulation modulated motoneuron activity by activating afferents that resided within spinal and paraspinal tissue. Furthermore, velocity sensitive afferents are most likely to contribute to the excitatory effect on motor output of manipulation. This can be assumed because the positioning intervention had no significant effect on motoneuron pathways. The excitatory effect of manipulation may, as a consequence, be associated with the manipulative thrust. To clarify this point, however, further experimentation would be required to demonstrate, for example, the effect of spinal joint mobilisation as opposed to positioning.

4.9.1.1 Joint Afferents

Manipulation was localised specifically to the lower cervical spine, with the combined rotation and side flexion movement. Therefore, sudden stretch, at end range, would have occurred on the C6/7 joint capsule and ligaments, as well as other surrounding tissues. Large diameter, group II joint afferents (mechanoceptors/A-beta fibres) are known to be activated with rapid end range stretch, as well as approximately one-third of small diameter afferents of group III (Burke et al., 1988; Clarke, 1975; Grigg & Greenspan, 1977; Schaible & Grubb, 1993). No studies at present have directly investigated the effects of spinal manipulation on joint afferents, primarily because they are difficult to identify (Pickar, 2002).
4.9.1.2 Muscle Afferents

As stretch of the musculature in the vicinity of lower cervical spine would also have occurred with the end of range motion, muscle spindles would have also been stimulated (Bolton & Holland, 1998). Due to the high-speed application of the thrust, the velocity sensitive primary muscle spindles (group Ia) were ten times more likely to be activated than secondary receptors (group II) (Rothwell, 1994). It was also highly probably that subjects were not completely relaxed during the manipulative thrust. The group Ib muscle GTO receptors, which are known to be extremely sensitive to muscle contraction, are therefore likely to have been stimulated (Pearson & Gordon, 2000). These conclusions concur with animal studies, where activation of group I and II muscle afferents occurred with forces replicating spinal manipulation (Pickar & Wheeler, 2001).

4.9.1.3 Cutaneous Afferents

Cutaneous receptors around the lower cervical spine would also have been stimulated by the mechanical pressure applied to the skin with both interventions (Burke et al., 1988). However, because the positioning intervention did not change motoneuron excitability, it is unlikely that cutaneous receptors contributed to the manipulation effect, to any great extent. This finding concurs with Murphy et al. (1995), who found that anaesthetised skin had no influence on the effect of sacroiliac joint manipulation.

4.9.1.4 Nociceptive Afferents

In symptomatic subjects, pain was experienced on end range neck rotation. Thus, nociceptive afferents would have been stimulation with the end range manipulative thrust. Nociceptive input therefore into the CNS with manipulation is likely to account for the significant difference motoneuron excitability observed between normal and symptomatic subjects.
Nociceptive afferents in pain sensitive tissue in the lower cervical spine would have been activated with manipulation of the painful segment (Basbaum & Jessell, 2000). Further, nociceptive input from deep structures, such as joints and muscle, have been shown to be more effective at triggering central neurons (Wall & Wolf 1984). Manipulation of the pathological segment was likely to trigger thinly myelinated A-delta afferents (group III) and the slower unmyelinated C-fibres (group IV) (Grigg, Schaible, & Schmidt, 1986). Manipulation would have also led to non-noxious activation of mechanoreceptors within pathological and non-pathological paraspinal tissue, due to the abnormal central processing by sensitised neurons (central sensitisation).

4.9.2 Spinal Pathways

The excitatory mechanism of spinal manipulation on motor output may have been mediated at a spinal cord level in the CNS. There is some evidence in animal studies that passive-movement-induced motor responses are of spinal cord origin. The motor responses associated with high velocity cervical motion, such that manipulation would produce, still occurred after transection of the spinal cord (Bilotto, Schor, Uchino, & Wilson, 1982; Ezure, 1983; Ezure, Schor, & Wilson, 1983).

4.9.2.1 Ia-Afferent Pathway

Stimulation of the Ia-pathway may be responsible for the excitatory effect of manipulation on the motor system, observed in the current study. It has been well documented that group Ia-afferents have strong, excitatory, monosynaptic connections to homonymous motoneurons and synergistic motoneurons (Renshaw, 1940; Llyod, 1946). Further, the importance of the excitatory Ia-
pathway has been highlighted in animal studies, with the ability of individual Ia-af-ferents to excite all homonymous spinal motoneurons (Pearson & Gordon 2000).

4.9.2.2 Group II Afferent Pathway

Group II afferents may have been activated by sustained stretch immediately before the manipulative thrust was applied, but are less likely to have contributed to excitatory effect for the following reasons. Group II afferents have been found to have relatively weak monosynaptic connections to homonymous motoneurons (Pearson & Gordon, 2000). Group II afferents form excitatory disynaptic connections with group II interneurons, which also receive convergent afferent information from joint, cutaneous and group II proprioceptors (Lundberg, Malmgren, & Schomburg, 1987a, 1987b). The group II interneuron, however, mainly forms inhibitory connections with motoneurons (Pearson & Gordon, 2000).

4.9.2.3 Ib-Afferent Pathway

In the current study, group Ib (GTO) afferents would have been activated by muscle contraction of both cervical spine muscle and the forearm flexor muscles. Contraction of the forearm flexor muscles would, however, have had an inhibitory effect on the C6/7 spinal motoneuron pool (Pearson & Gordon, 2000). The only mechanism that would lead to an excitation on motoneurons would be contraction of antagonist muscles (Eccles, Eccles, & Lundberg, 1957). Group Ib muscle afferents are therefore, not as likely to add to the excitatory effect on motoneurons with spinal manipulation.

4.9.2.4 Propriospinal Pathways

Short polysynaptic spinal pathways may also be activated with neck manipulation. Neck afferents have been linked to limb motoneurons via
polysynaptic spinal pathways (Kenins, Kikillus, & Schomburg, 1978; Nakajima, Maeda, Ishii, & Miyazaki, 1981). In the cat, a population of interneurons have been identified, from C4 – C8, which could increase flexor tone of the ipsilateral limb with head rotation to the contralateral side (Ezure, 1983; Ezure et al., 1983). In the human cervical spinal, propriospinal neurones are also known to connect spinal segments within the cervical enlargement (Rothwell 1994). This indicates that segmental afferent stimulation, such that manipulation would activate, could activate a number of motoneuron pools.

In short, there is evidence that a spinal mechanism is operating with manipulation. Large diameter afferents are the most likely to be responsible, especially input from muscle proprioceptors. It is unlikely however, that the mechanism for the effect of manipulation is purely spinal. Manipulation has been shown to have a neurophysiological effect up to 60 seconds (Disman et al., 2002; Disman & Bulbulian 2000), whereas an exclusively spinal reflex mechanism would have occurred within milliseconds (Rothwell, 1994). Afferent input into the CNS from spinal manipulation is more likely, therefore, to modulate motor output at a supraspinal level. Moreover, previous H-reflex experiments have demonstrated that manipulation reduced α-motoneuron excitability (Dishman & Bulbulian, 2000; Murphy et al. 1995), whereas, in the current study, manipulation was observed to significantly increase motoneuron excitability, as measured by the TMS method. On this basis, the present study gives some supportive evidence for a cortical mechanism for the effect of manipulation.
4.9.3 **Cortical Pathways**

In the current study, the excitatory effect of neck manipulation may have been mediated via pathways that traverse the motor cortex. Recent TMS investigations in humans, have also shown that afferent stimulation can modulate motoneuron excitability, which is at least in part, due to changes at a cortical level (Baldissera & Leocani, 1995; Day et al., 1991; Deuschl et al., 1991; Palmer & Ashby, 1992). One comprehensive study established that the motor cortex was activated with afferent stimulation, without any significant change to peripheral, spinal or brainstem activity (Kaelin–Lang et al., 2002).

A well-documented example of such a cortical pathway is the long-loop component of the stretch reflex. This transcortical pathway involves activation the motor cortex through stimulation of Ia-muscle afferents. This pathway is likely to project to the cortex via the dorsal column and relays in the thalamus (Lemon, 1979; Lemon & Porter, 1976a, 1976b; Palmer & Ashby, 1992). However, there is some evidence to suggest that afferent stimulation may directly activate corticospinal cells that project directly onto spinal motoneurons (Asanuma & Arissian, 1984; Baldissera & Leocani 1995; Cheney & Fetz, 1984).

Further evidence for a cortical pathway comes from the fact that the sensorimotor cortex has well organised sensory input, motor output connections in animals (Rosen & Asanuma, 1972) and humans (Terao et al., 1995). In other words, for a given muscle, sensory information for that muscle is received in close anatomical relation to that muscle’s output.
In the current study, the mechanism underlying manipulation had a time course of up to 60 seconds. That is, manipulation induces changes in excitability in the CNS that outlasts the synaptic interactions of reflex pathways. This would indicate the mechanism for the effect of manipulation may have a supraspinal origin, through polysynaptic pathways (McKay, et al., 2002; Ridding et al., 2001; Stefan et al., 2002). For example, excitation of cortical motoneurons, in the current study, is more likely to occur through polysynaptic pathways via thalamic-cortical circuits (Asanuma, Larsen, & Zarzecki, 1979) or connections from the somatosensory cortex (Asanuma, 1981; Kaneko, Caria, & Asanuma, 1994). Thalamic nuclei, such as the ventrolateral pars oralis or the ventrolateral pars caudalis, relay afferent information to the motor cortex. The latter is known to have diffuse links to the primary motor cortex (Asanuma, Larsen, & Yumiya, 1980).

4.9.3.1 Brainstem and Accessory Pathways

Brainstem pathways could also play a role in the modulation of motor output, activated by spinal manipulation (Rekling et al., 2000; Basbaum & Jessell, 2000). Excitatory effects on spinal motoneurons have been observed through multiple accessory serotonergic and noradrenergic pathways via the brainstem (White et al., 1996). In the current study, the time frame for the observed effect of manipulation is suggestive of multiple accessory pathways. On this basis, it is possible that manipulation could have exerted the observed facilitatory effect on motoneurons through brainstem pathways.

Recent evidence suggests that SMT exerts a co-ordinated effect on the nociceptive, sympathetic and motor systems (Wright, 1995). There is some evidence that noxious stimulation, activating group III afferents, may evoke an
analgesic response involving the descending inhibitory system of the PAG matter (Basbaum & Jessell, 2000; Gozariu, Bragard, Willer & Le Bars 1997; Fields & Basbaum, 1994). Furthermore, stimulation of descending inhibitory pathways, originating from the PAG is known to have an analgesic response (Fields & Basbaum, 1994). The faster conducting myelinated (group III) fibres ascend to higher centres via the spinothalamic tract. At the level of the mid-brain, collateral pathways projecting to the area of the PAG matter have been identified (Basbaum & Jessell, 2000). In contrast, the slower conducting unmyelinated (group IV) fibres do not influence the PAG matter (Bowsher, 1991). Hence, it may be possible that manipulation activates group III afferents whilst blocking the transmission of group IV fibres, through descending inhibitory pathways (Bowsher, 1991; Melzack, 1991).

In addition, there is some evidence that noradrenaline and serotonin receptors mediated the analgesic effect of joint mobilisation (Skyba et al., 2003). Serotonergic and adrenergic pathways originate from the PAG area and are also known to enhance excitatory inputs onto spinal motoneurons (White et al., 1996).

Further study would be required to ascertain whether a brainstem pathway mediated the excitatory effect of manipulation. To date there has been no research investigating that effect of manipulation on brainstem pathways, using the TMS technique.
Chapter Five     Conclusions

The current study demonstrated that in normal subjects, cervical spine manipulation had a significant effect on cortical motoneurons in human subjects performing active movement. The neural mechanism underlying the effect of manipulation could have been mediated spinally, supraspinally or both. The precise level or levels at which the neural mechanism originated cannot be determined from this study.

What can be determined is that in normal subjects, manipulation has a significant excitatory effect on cortical neurons projecting to forearm flexors at the onset of a voluntary contraction. In comparison, manipulation, which does not result in joint cavitation, has an inhibitory effect in symptomatic subjects performing a contraction under the same conditions. This suggests that manipulation of the painful zygapophyseal joint, whose central neurons are likely to be sensitised by tissue injury, may result in inhibition of cortical activity. In contrast, if the manipulation is associated with cavitation there is some indication that cortical neuronal activity is facilitated.

5.1.1 Clinical Implications

The outcome of this work shows that spinal manipulation is not purely mechanical but is capable of inducing complex neurophysiological modulation to influence motor function. The excitatory effect of manipulation on motoneurons may explain the clinically observed increase in the range of motion and improvement in motor control. It is probable that the underlying mechanism for the effect of manipulation involves the summation of sensory afferents activated by various spinal and paraspinal tissues as a consequence
of the high velocity thrust on spinal and/or cortical neurons which then result in excitation.

It is likely that I-a muscle afferents play a major role in mediating the effect of manipulation through the cortex, via the transcortical pathway. The excitatory effect of this transcortical pathway activated by muscle afferents has been demonstrated in many human studies (Baldissera & Leocani, 1995; Kaelin-Lang et al., 2002). The effect of manipulation is also likely to be evoked by joint afferents, however, the supraspinal pathway is not as yet clear.

The exact mechanism underlying the effect of manipulation on motor output cannot be ascertained by this study. Nevertheless, it could be postulated that the excitatory effect of manipulation on motoneuron activity may influence motor unit activation. This could lead to improved motor output unit recruitment and co-ordination during movement. Previous theories on the impact of pain on motor function, proposed that abnormal processing of sensory input by central neurons already sensitised by tissue injury, lead to changes in muscle recruitment patterns. This explained the excitation of spinal α-motoneurons seen in experimental studies and in the clinically observed muscle spasm. Accordingly, it was hypothesised that the inhibitory effect of manipulation on spinal α-motoneurons, reduced muscle spasm by altering the abnormal recruitment of motor unit and hence broke the pain-spasm-pain cycle.

Recent evidence contradicts these suppositions as cortical and spinal motoneuron activity has been demonstrated to be depressed by nociceptive input (Le Pera et al., 2001; Farina et al., 2001; Stevenson et al., 2003). Further,
the current study provides evidence to suggest that manipulation of the painful segment, when joint cavitation does not occur, may induce motor depression in a similar manner to that of nociceptive stimulation. Thus, manipulation of the painful joint may worsen the symptoms, due to activation of noxious and innocuous input. This is not the case, however, when manipulation results in joint cavitation, as excitation of motoneuron activity was observed.

The findings of this study have implications for the clinical rationale of when to apply spinal manipulation, although, caution is required interpreting the conclusions on symptomatic subjects because of the small subject sample. The results would suggest that when treating painful joints, the use of techniques that do not increase pain may be more beneficial, for example oscillatory mobilisation techniques that respect pain and not activate nociceptors. Accordingly, manipulation may be more appropriate in the treatment of joint stiffness when pain is not a concern. Further, manipulation of a zygapophyseal joint that is segmentally related to a distal injured site, may improve motor function of the muscles within that segmental supply.

On the other hand, there was evidence to suggest that manipulation of the painful joint that results in joint cavitation may be assumed to have more favourable effects on motor activity. Thus, cavitation may confirm that the therapeutic effect of the manipulation has been elicited.

5.1.2 Further Research

A larger study comprising of approximately 20 symptomatic subjects would be required to ascertain whether the observed effects of manipulation on symptomatic subjects are, indeed, significant. Further exploration of the duration of the effect of manipulation would also be useful, as a small latent
The effect of manipulation was observed after 15 minutes in normal subjects. Moreover, valuable information would also be gained by measuring the clinical effects of manipulation along with changes in cortical motoneuron excitability. For example, measuring cervical spine range of movement before and after manipulation. This was not measured in normal subjects in the present study, as little change in range of cervical movement was expected.

This study also highlighted the fact that a wash-out period greater than three days is required to minimise learnt effects with the TMS method. Any future research using the TMS technique with a cross over experimental design would need to take this into consideration.

Exploration of the effect of manipulation on motoneurons out of the segmental nerve supply would also be clinically valuable. From this it could be determined if the neurophysiological effect of manipulating an adjacent non-painful spinal joint is still mediated to the motoneuron supplying the injured area.

The precise level or levels at which the mechanism underlying the neural effect of manipulation originates could be explored further with transmastoid magnetic stimulation of the brainstem. This would be important in determining the effect that the descending brainstem pathways have on motor output, and add to the accumulating evidence of the descending inhibitory mechanism.

The current research gives some indication of the importance of cavitation associated with manipulation. To demonstrate a significant effect, however, further evaluation would be required using a larger number of subjects. In the
present study approximately 66% of neck manipulations resulted in cavitation. An optimal number of 60 subjects would be required in order to have statistically sound numbers in both the cavitation and non-cavitation groups.

Lastly, assessing the effect of the other SMT techniques, such as mobilisation and traction, using TMS method, would also ascertain whether joint cavitation is necessary to mediate the effects of manipulation in symptomatic subjects.
References


Appendix I

Participant Information Sheet

The Effect of Neck Manipulation on Brain Activity

You are invited to take part in this study by: Researchers Marian Simmonds, Masters of Health Science student (School of Physiotherapy) and Dr Andrea Vujnovich (Head of School of Physiotherapy).

The purpose of the study is to ascertain the neurophysiological mechanism for the improvement in movement after neck manipulation.

The study will take place in the Neurophysiology Laboratory, Center for Physical Rehabilitation Research at Auckland University of Technology (AUT), Akoranga campus, Akoranga Drive, Northcote. The data collected will be used for thesis as part of a master’s degree.

What happens in the study? The participant will be seated in a reclining chair. A snug fitting cap will be placed on the participant’s head. Three small electrodes will be placed on the participant’s right forearm. A machine (Transcranial Magnetic Stimulator (TMS)) will stimulate neurons in the participant’s brain by using an alternating magnetic current, which is painless and non-invasive. This machine will measure activity in the area of the brain that controls movement while the participants make a small wrist movement.

There will be two separate trials, Trial A and Trial B. Trial A involves positioning of the lower neck with manipulation. Trial B involves positioning of the lower neck without manipulation. Brain activity will be measured five times during each trial.

The participant will need to allow up to one hour in the laboratory on two days. All neck pain participants are involved only in Trial A. Therefore these participants will need to allow 45 minutes of time in the laboratory for one day only.

What is manipulation? Manipulation is putting your neck in a certain position and applying a quick, controlled, local thrust, which usually produces a click or popping sound in the joint. The joint manipulated is in the lower neck (6th and 7th vertebra).

What are the benefits and risks involved? After treatment with manipulation you should have less pain, feel more flexible and comfortable in your movements. Relief can be dramatic and long lasting. Occasionally some people may have temporary soreness.
In rare circumstances manipulation treatment has produced unfortunate complications including stroke, leading to temporary or permanent disability. However, the risk from neck manipulation is considerably lower than that of having a general anaesthetic, or from taking anti-inflammatory drugs. The mechanism by which the mishap occurs is not fully understood but usually thought to be caused by damage to the vertebral artery.

**Before the trial begins** the participant will be asked questions to find out if there has been any real or possible damage to the nerves or blood vessels closely associated with the spinal column of your neck or if there are any other reasons why manipulation is not indicated. The physiotherapist will also assess the function of the participant’s neck to see whether there is any problem with the blood flow in the vertebral arteries. The tests do not give an absolute indication of risk but they do provide the best available assurance that it is safe to manipulate your neck. **The physiotherapist, with post graduate experience in manipulation, is available at all times should any complications arise** (ph 025 272 5746).

How was a person chosen to be asked to be part of the study? The study is seeking two groups of volunteers.

<table>
<thead>
<tr>
<th>Group 1: Normal</th>
<th>Group 2: Neck pain</th>
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<tr>
<td>- Healthy volunteers who have had no neck and/or shoulder pain in the past 6 months.</td>
<td>- Volunteers who have had a recent episode of neck pain with no history of trauma or surgery to the neck.</td>
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<td>- No evidence of referred arm pain.</td>
<td>- No evidence of referred arm pain.</td>
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<td>- An improving state of symptoms.</td>
<td>- An improving state of symptoms.</td>
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<td>- The physiotherapist has assessed your neck and deems it appropriate for manipulation of the C6/C7 level on the right side.</td>
<td>- The physiotherapist has assessed your neck and deems it appropriate for manipulation of the C6/C7 level on the right side.</td>
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Both groups of volunteers:
- **Must not have** any neurological condition, diabetes, peripheral vascular disease, hemophilia, epilepsy, implanted electronic devices or metal implants in the brain.
- **Must not be taking** medications that may affect brain excitability, steroids, anticoagulants (such as warfarin), or strong painkillers.
- **Must not have** any signs or symptoms of: radiculopathy, upper cervical spine dysfunction or vertebrobasilar insufficiency (such as a history of dizziness, seeing double, difficulty swallowing, difficulty speaking or drop attacks).

**Compensation:** In the unlikely event of a physical injury as a result of your participation in this study, you will be covered by the accident compensation legislation within its limitations. If you have any questions about ACC please feel free to ask the researcher for more information before you agree to take part in this trial.

**How is my privacy protected?** All participants will be identified only by a code number. All data is kept in locked filing cabinets in the Centre for Physical
Rehabilitation Research, or on computer file with coded access only. No material which could personally identify you will be used in any reports of this study.

**Costs of Participating:** There will be no costs involved except your time. If you have travelled to get to the laboratory, petrol vouchers are available.

**Opportunity to consider invitation:** The participant will be given sufficient time to consider whether he/she wishes to partake in the study. The participant may withdraw from the study at any time without being disadvantaged and no reason has to be given for withdrawing from the study.

If you have any queries or concerns about your rights as a participant in this study you may wish to contact a Health and Disability advocate on (0800) 555 050 Northland to Franklin.

Please feel free to contact the researcher if you have any questions about the study.

(Acknowledgment: parts of this information sheet have been adapted from the Australian Physiotherapy Association, Patient Information - Cervical Manipulation, handout).

**Participant Concerns** - Any concerns regarding the nature of this project should be notified in the first instance to the Project Supervisor, Dr Andrea Vujnovich, andrea.vujnovich@aut.ac.nz, 917 9685. Concerns regarding the conduct of the research should be notified to the Executive Secretary, AUTEC, Madeline Banda, madeline.banda@aut.ac.nz, 917 9999 ext 8044.

Approved by the Auckland Ethics Committee on 28th August 2002.
Appendix II

Consent to Participation in Research

Title of Project: The Effect of Spinal Manipulation on Excitability of the Motor Cortex

Project Supervisor: Dr Andrea Vujnovich

Researcher: Marian Simmonds

- I have read and understood the information provided about this research project.
- I have had an opportunity to ask questions and to have them answered.
- I have had time to consider.
- My participation in this study is confidential and no material, which could identify me, will be used in any reports on this study.
- I understand the compensation provisions for this study.
- I understand that I may withdraw myself or any information that I have provided for this project at any time prior to completion of data collection, without being disadvantaged in any way. If I withdraw, I understand that all relevant tapes and transcripts, or parts thereof, will be destroyed.
- I agree to take part in this research.

Participant signature: ....................................................

Participant name:

Date:

Project Supervisor Contact Details: Dr Andrea Vujnovich School of Physiotherapy, Faculty of Health Studies, AUT. Ph: 917 9685. E-mail: andrea.vujnovich@aut.ac.nz

Approved by the Auckland University of Technology Ethics Committee on 28th August 2002.
Appendix III

Subjective Examination

History

Pain: Resting VAS: I I I I I I I I I I

0 5 10

Aggs.: Ease: 24Hr Beh.:

Sleep:
1st am:
Day:
EOD:

PHx: TRHx

Activity Level:

Medical History:

Medications:

Contraindications to manipulation:

VBI:

SSSSNIPRDs

Site Nature
Stage Irritability
Severity Progress/Rate
Stability Regular or Irregular

Provisional Diagnosis:
**Objective Examination**

Asymmetry:

ROM Cxsp:  

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<tr>
<th>VAS (0-10) end of range:</th>
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Neurological:

- Sensation
- Strength

Neural tension

Palpation:

- Ligament testing

Tissue Texture:

Tissue Tenderness:

VBI end range rotation:

Left                Mid                Right

**SSSSNIPRDs**
Appendix IV

Data Sheet - Normal Subjects

Order of Trial: _______________________
Date: ___________________                               Time:______________________
Name: ___________________________________   DOB_______________________
Gender:_____________       Handedness_________________

VBI tests day 1: ________________________________

Contraindications to manipulation:   Y     N

Cervical R) SB ROM before experiment 1: ______________After:_____________

Date 2: _______________________                          Time 2:______________________

VBI tests day 2: __________________________________

Contraindications to manipulation:   Y     N

Cervical R) SB ROM before experiment 2: ______________After:_____________

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<tr>
<th>MagStim</th>
<th>Experiment 1</th>
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<td>Threshold + 20%</td>
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Data Sheet - Symptomatic Subjects

Date: _______________________                               Time: ______________________
Name: ___________________________________  DOB_______________________
Gender:_____________      Handedness _________________
VBI tests: _______________________________
Contraindications to manipulation:    Y      N
Cervical R) SB ROM before manipulation: _________________After:_____________
Resting VAS before manipulation: After manipulation:

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VAS End of Range before manipulation: After manipulation:

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MagStim

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<td>Threshold + 20%</td>
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Site                Nature
Stage               Irritability
Severity            Progress/Rate
Stability           Regular or Irregular

Diagnosis: