MODULATION OF IA INPUT: MOTONEURONE OUTPUT RELATIONSHIP

MODULATION OF THE IA INPUT: MOTONEURONE OUTPUT RELATIONSHIP OF HUMAN FLEXOR CARPI RADIALIS DURING MUSCLE CONTRACTION

By

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ABSTRACT

A novel method has been developed to determine the quantitative relationship between the percentage of Ia fibres stimulated synchronously, and the percentage of human flexor carpi radialis (FCR) motoneurons (MNs) discharged reflexly. The method assumes a normal distribution of Ia fibre thresholds to electrical stimulation. Among the 11 healthy subjects tested during relaxation, there were considerable differences in the reflex excitability of the FCR MNs to quantitative Ia fibre inputs. The Ia fibre input-FCR MN output curves were either initially steeply-rising, initially slowly-rising, or initially and latterly steeply-rising. When the results were averaged, however, the curve for the 11 subjects in the relaxed state appeared to be fairly linear throughout the entire range of the Ia fibre inputs, and a mean of 82% of the Ia fibres discharged approximately 20% of the MNs.

Regardless of the variability in the shape of individual input-output curves during relaxation, potentiation of the FCR MN output was observed during weak wrist flexion in 10 of the 11 subjects over the full range of the Ia fibre inputs. In contrast, a depression of the MN output was exhibited in all 8 subjects who weakly contracted the extensors over the full range of the Ia fibre inputs. The changes in the Ia input-MN output relationship in going from rest to voluntary contractions of wrist muscles are thought to reflect modulation by presynpatic inhibition of the Ia terminals. With very large Ia inputs during wrist extension, however, there is a steep rise in the input-output curve, which could indicate a decrease in presynaptic inhibition of Ia terminals in the FCR muscle. The

modulation of the input-output relationship observed in the present study is consistent

with the task-dependent differences of reflex excitability observed by Stein et al. (1988).

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LIST OF ABBREVIATIONS

ECR	Extensor Carpi Radialis Muscle
FCR	Flexor Carpi Radialis Muscle
H-reflex	Hoffmann-Reflex
MU	Motor Unit
MN	Motoneuron
SO	Slow Twitch Oxidative
FOG	Fast Twitch Oxidative
FG	Fast Twitch Glycolytic
S	Slow Twitch
FR	Fast Twitch, Fatigue Resistant
FF	Fast Twitch, Fatigable
MVC	Maximal Voluntary Contraction
AHP	Afterhyperpolarization
EPSP	Excitatory Postsynaptic Potential
GTO	Golgi Tendon Organ
LLR	Long-Latency Response
SLR	Short-Latency Response
PAD	Primary Afferent Depolarization
RC	Renshaw Cell
M-wave	Motor Response
M_{max}	Maximal M-wave
H_{max}	Maximal H-reflex
EMG	Electromyographic Activity
A/D	Analog to Digital Converter

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CHAPTER 1

INTRODUCTION

1.1   Introduction

Changes in reflex excitability accompanying various muscle contractions have

been studied extensively in our laboratory and by other research groups (Hultborn et al.

1979, 1987; McComas et al. 1979; Nardone et al. 1989; Pierrot-Deseilligny et al. 1999;

Romano et al. 1987; Upton et al. 1971). Investigations on the task dependence of spinal

reflexes has led to a better understanding of the involvement of the spinal cord and the

motor cortex during various motor tasks ranging from postural maintenance to voluntary

manipulation of objects by hand (Abbruzzese et al. 1994; Burke et al. 1993; Stein et al.

1988).

Excitability in the human spinal cord can be studied in terms of the changes in the

amplitudes of spinal reflex responses in going from the resting state to the specific motor

task. The spinal reflex arc (monosynaptic or oligosynaptic) not only can be excited by

muscle stretching, for example, in the tendon jerk, but also by electrical stimulation of the

muscle afferents – Hoffmann-reflex (H-reflex). H-reflex amplitudes have been shown to

change between the resting state and during muscle contraction (Aymard et al. 2001;

Burke et al. 1989; Hultborn et al. 1987; Romano et al. 1987; Pierrot-Deseilligny et al.

1999).

When the plantar flexors were voluntarily activated, facilitation of the H-reflexes

of the soleus muscle was observed with increased plantar flexion torque up to 50% of

maximal voluntary muscle activation (Butler et al. 1993). An earlier study by Romano and Schieppati (1987) compared active shortening with active lengthening of the plantar flexors and found that the H-reflex of the soleus muscle was depressed during active lengthening as compared to active shortening. As well, the H-reflex was found to increase from passive to active in both shortening and lengthening contractions. H-reflex facilitation with voluntary contraction has also been demonstrated theoretically by Kernell and Hultborn (1990). Furthermore, Hultborn and colleagues have shown that the H-reflex of the flexor carpi radialis (FCR) muscle was strongly depressed by passive wrist extension, during which the muscle was lengthened (Hultborn et al. 1996). Recently, researchers have also examined the changes in FCR H-reflexes at the onset of voluntary wrist flexion and extension, and for the tonic wrist flexion (Aymard et al. 1995; Aymard et al. 2001).

One method of measuring the changes in reflex excitability for each condition requires not only controlling the strength and timing of the voluntary contraction, but also ensuring that the amplitude of the test H-reflex was kept constant for both resting and contraction states (Crone et al. 1990; Hultborn et al. 1987). These early experiments were performed by adjusting the size of test H-reflex, and by measuring the change in the size of H-reflex produced by the conditioning stimuli to monitor the spinal cord modulation. They determined the relative change in motoneuron (MN) responses but provided no knowledge of the changes in sensory fibre (Ia) inputs.

The research presented in this thesis attempts to better understand the input-output properties of the human spinal reflex and its role during muscle contraction in a normal

person. Specifically, estimations were made of the percentage of Ia fibres that have to be synchronously excited for a given percentage of MNs to discharge. The evaluation of these parameters was based on the assumption of the normal distribution of Ia fibre thresholds to electrical stimulation.

In the past, most H-reflex research has concentrated on the soleus muscle since it is a postural muscle with a large number of stretch receptors. In the present study, we instead focused on the FCR muscle, since not only does this muscle control flexion and abduction of the hand at the wrist, but it can also act as a synergist for muscles producing finger movements. We evaluated the input-output relationship of the human spinal reflex in the resting state and during voluntary wrist lengthening and shortening contractions using a novel method of estimation. Therefore, the percentages of Ia fibres excited and FCR MNs discharging during each condition could be simply derived from the experimental data without the complexity of experiments involved in adjusting the sizes of the test reflexes.

1.2   Summary of Chapters

The thesis aimed to explore the quantitative aspects of spinal cord modulation, and the multiple peripheral and spinal mechanisms associated with voluntary FCR muscle contractions. The overall goal was to obtain a more precise model of cortical, spinal, and peripheral control of the FCR muscle.

Chapter 2 gives an overview of the activation of motor units and the monosynaptic spinal reflex arc. Possible contributions of spinal neuronal systems to

muscle contractions are then examined, including the fusimotor, presynaptic, recurrent inhibition, and descending systems. In Chapter 3 the experimental methods to quantify the input-output properties of the spinal pathway in response to electrical stimulation are described in detail. Chapter 4 presents the experimental results of modulation of the spinal reflexes during voluntary muscle contractions. In Chapter 5, a motor control model to describe the spinal reflex modulation in human FCR muscle is proposed, that attempts to incorporate different mechanisms and interactive aspects of the motor control during voluntary muscle contractions. Chapter 6 presents the conclusions resulting from this research and proposes new possible directions of investigation.

CHAPTER 2

BACKGROUND

2.1   Introduction

This work has as one of its principal aims the study of peripheral and central mechanisms pertinent to the neural control of an upper limb muscle. In this chapter, a general overview is given of the multiple spinal mechanisms relevant for the control of the output of motoneuron (MN) pools according to the demands of the motor task. Particular attention will be paid to the aspects of the spinal action of sensory afferent (Ia) fibres during voluntary muscle contractions. Finally, a short review of the current knowledge about the supraspinal control of muscles will be presented.

2.2   Functional Considerations of Motor Units

2.2.1   Motor unit

The smallest functional unit of the neuromuscular system is the motor unit (MU). A MU is defined as an anterior horn cell, a cell body of an alpha-motoneuron (α-MN) in the ventral horn of the spinal cord, its axon that exits the spinal cord, and all of the muscle fibres innervated by the axon (Lidell et al. 1925; Burke et al. 1975), as illustrated in Figure 1. As it enters the muscle, the axon branches up to several hundred times. Each branch of the axon terminates on a single muscle fibre (Figure 2).
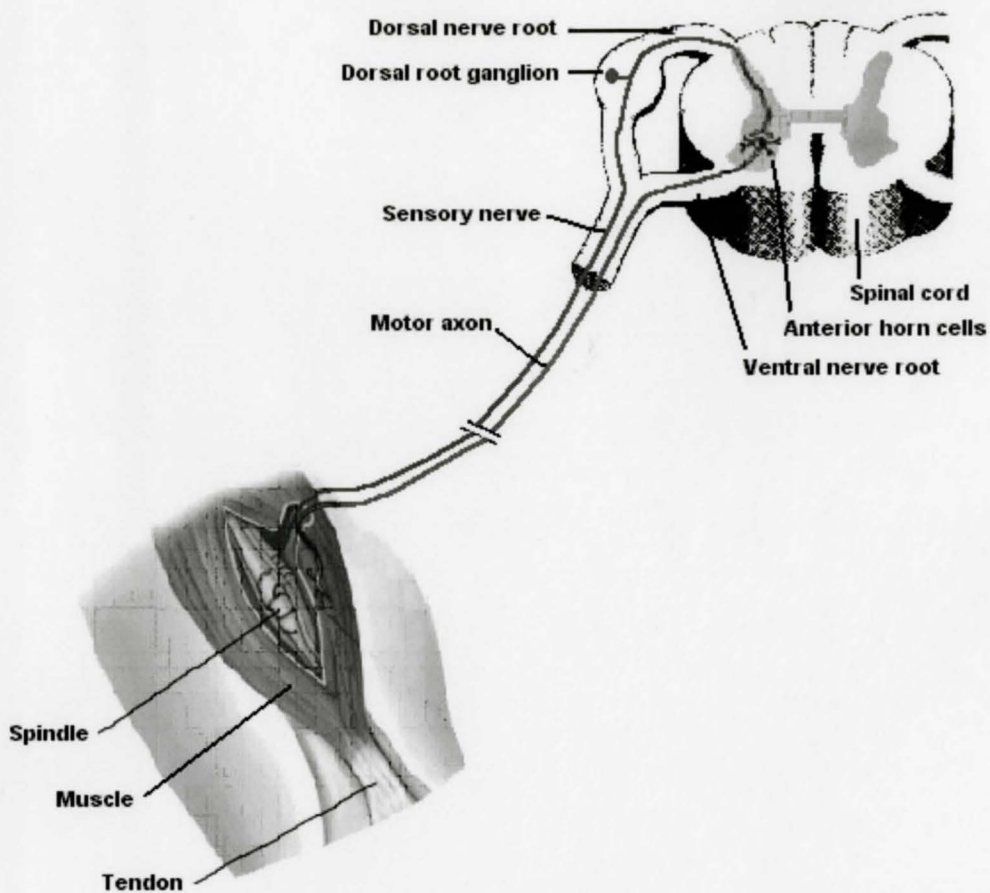
Figure 1. The motor unit consists of one anterior horn cell, its axon and terminal branchings, and all the extrafusal muscle fibres it innervates (modified from Kandel et al. 2000).
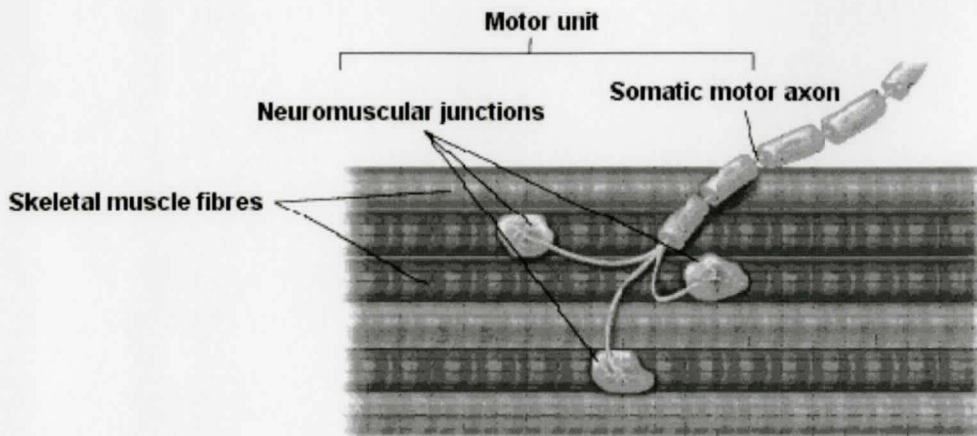
Figure 2. The activation of the motor unit consists of the depolarization passing from the anterior horn soma to the axon and spreading via the neuromuscular junction along each muscle fibre (modified from Kandel et al. 2000).

Several investigators have attempted to determine the number of muscle fibres innervated by one axon (Feinstein et al. 1975). This information has been calculated by counting the number of muscle fibres in the muscle and dividing that number by the number of α-MN supplying the muscle. The innervation ratios differ from muscle to muscle, and these differences are functionally significant. Small muscles that produce precise movements tend to have a low innervation ratio and larger muscles that produce gross movements have high innervation ratios.

Innervation ratios are one of the means in understanding the function of MUs during human movement. Another approach is to think about the characteristics of muscle fibres of a given MU The MUs and muscle fibres can be classified on the basis of their mechanical, metabolic, and histochemical properties. There are several methods of classifying muscle fibres. One method based on the histochemical staining reaction for myosin ATPase identifies three major fibre types: type I, IIA, and IIB (Brooke et al.

1970). Another system based on the metabolic and contractile properties classifies MUs

as S (slow twitch) containing SO (slow twitch oxidative) muscle fibres, FR (fast twitch,

fatigue resistant) containing FOG (fast twitch oxidative) muscle fibres, and FF (fast

twitch, fatigable) containing FG (fast twitch glycolytic) muscle fibres (Peter et al. 1972;

Burke 1981). The characteristics of muscle fibres are summarized in Table 1.

Table 1. Characteristics of muscle fibre types

Fibre Type	Type I	Type IIA	Type IIB
Contraction Time	Slow	Fast	Very fast
Colour	Red	Midway	White
Size of Motoneuron	Small	Large	Very large
Resistance to Fatigue	High	Intermediate	Low
Activity Used for	Aerobic	Long term anaerobic	Short term anaerobic
Force Production	Low	High	Very high
Mitochondrial Density	High	High	Low
Capillary Density	High	Intermediate	Low
Myoglobin Content	High	Intermediate	Low
Oxidative Capacity	High	High	Low
Glycolytic Capacity	Low	High	High
Major Storage Fuel	Triglycerides	Creatine phosphate, glycogen	Creatine phosphate, glycogen

2.2.2   Factors influencing motor unit activation

A muscle is capable of adjusting its tension output to meet the demands of various types of tasks by the interaction of two mechanisms: recruitment (varying the number of active MUs), and rate coding (varying the firing rate of active MUs). In both small and large muscles, the recruitment mechanism plays a major role at low force levels (Kukulka et al. 1981). The S MUs have long twitch durations, and relatively low axonal conduction velocities. These units are preferentially active during weak sustained contractions (Kernell et al. 1982a, b; Spielmann et al. 1993). At high force levels, regulation is achieved mainly by variation in the firing rate of already active MUs. Faster contracting, more fatigable MUs come into play during stronger and faster contractions.

In most motor functions, there seems to be an orderly recruitment of MUs. According to Henneman's size principle, the neurons with slowly conducting axons innervating slowly contracting fatigue-resistant muscle fibers appear to be recruited before neurons with rapidly conducting axons innervating rapidly contracting fatigable muscle fibers (Henneman et al. 1965a, b). As the force level increases, larger and faster MUs are recruited up to 50% - 80% maximal voluntary contraction (MVC) depending on the muscle (DeLuca et al. 1982; Kukulka et al. 1981; Milner-Brown et al. 1973a, b) after which the additional increase in force is accomplished with increased firing rate of the active units (Deluca et al. 1982; Milner-Brown et al. 1973a, b).

2.3   Introduction to Sensory and Motor Neurons

2.3.1   Excitation of a motor neuron

All input and output signals are brief electrical pulses (~1 ms) called action potentials, which are generated by the activation of the nerve fibres in response to any stimulus above nerve cell membrane threshold. The information content in these signals is their time of arrival at the dendrites, but not in their shape or amplitude or duration. Therefore these signals are very similar to the digital control systems with all the impulses having the same shape, amplitude, and duration but different time of arrival. The dendrites projecting from the cell body of a neuron receive inputs from many other nerve fibres and pass them on to its cell body. The cell body integrates all the inputs. The inputs can be summated spatially when inputs from multiple neurons arrive at the dendrites simultaneously, and temporally when inputs from a few neurons arrive at a high pulse frequency to produce an excitatory postsynaptic potential (EPSP) in the neuron. When the algebraic sum of the inputs – EPSP – exceeds the nerve cell membrane threshold, an action potential is generated at the axon hillock which travels down the motor axon to excite the muscle fibres. As shown in Figure 3, the excitatory and inhibitory inputs can come from corticospinal centers and sensory structures in the body. Other neurons in the nervous system, such as those in the brain or spinal cord, perform a similar function, except that the generated action potential is sent to other neurons rather than the muscle fibres.
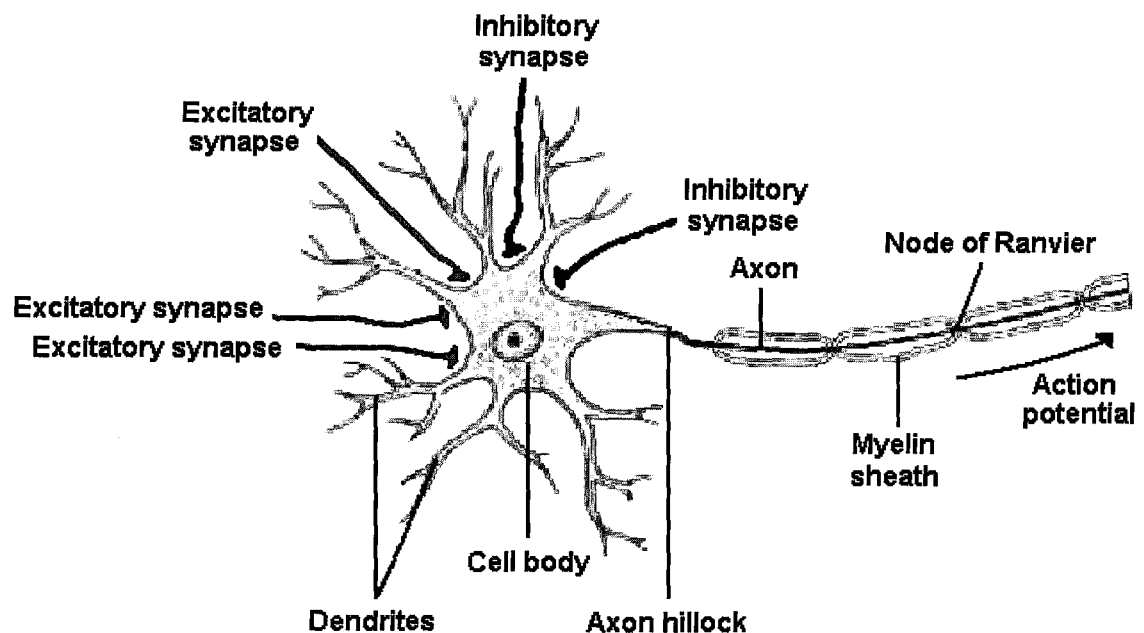
Figure 3. The structure of a motor neuron with a cell body, many dendrites, and the axon leaving at the other end to form a motor endplate of a muscle or towards the dendrites of the next neuron.

Input neuron signals to either the dendrites or sometimes the cell body of an output neuron through a connection called synapse. At the end of the input nerve axon sending the action potential to the synapse, specialized chemicals called neurotransmitters are released into the synapse. The neurotransmitters travel across to the end of the dendrite or cell body of the output neuron, influencing the output neuron to either increase (excite) or decrease (inhibit) briefly the cell membrane potential.

2.3.2   Muscle spindle Ia afferent fibre input

Muscle contains two important mechanoreceptors which transduce mechanical stimuli into nerve impulse activity. The muscle spindles sense changes in muscle length

while the Golgi tendon organs (GTOs) sense changes in tension that the muscle exerts on the tendon.

Muscle spindles are located in parallel with the extrafusal muscle fibres, as illustrated in Figure 1. They undergo the same length changes as the rest of the muscle. Therefore their afferents are very important for signaling the central nervous system about the limb position and movement. Within the muscle spindles, there are two types of fibres: nuclear bag and nuclear chain fibres (Figure 4A). Large, primary Ia afferents (~17 μm) originate from both the nuclear bag and nuclear chain fibres. Smaller, secondary II afferents (~8 μm) originate only from the nuclear chain fibres. Ia afferents from the nuclear bag fibres signal velocity. They adapt quickly after stretch and become silent when the position is constant. Both Ia and II afferents from a nuclear chain fibre are sensitive to the muscle length and fire in proportion to the fibre's length.

Muscle stretch or experimental electrical stimulation of group Ia afferents from the primary muscle spindle endings causes the firing of the spindle afferents, which is followed by a reflex contraction of the muscle. Inputs from the group Ia afferent fibres, shown in Figure 4A, have been suggested to primarily modulate the MU discharge rates, and thereby the reflex contractions. Muscle spindle Ia afferents are highly sensitive to the velocity of stretch (phasic stretch), and small muscle fibre length changes (tonic stretch). Inputs from the muscle spindle II afferents, shown in Figure 4A, also contribute to the facilitation of reflex action in response to changes in the muscle length. However, in the experimental situation, the small diameter of II afferent fibres precludes their

excitation by relatively low amplitude electrical pulses. Only Ia afferent fibres can be excited because large fibres are electrically stimulated more easily than small fibres.

It should be noted that the reflex contraction is not as simple as it first appears. γ-MNs innervate the intrafusal muscle fibres and can change the sensitivity of the sensory fibre endings to stretch. The afferent volley resulting from muscle stretch also involves the large, group Ib afferent fibres (~16 μm) from the Golgi tendon organs, shown in Figure 4B. It may be impossible to restrict the stimulus to only afferents from the muscle spindles being tested because GTOs are very sensitive to muscle stretch (Burke et al. 1983). In the case of voluntary isometric contractions, the size of the EPSP is limited by postsynaptic inhibition from the group Ib afferent fibres (Pierrot-Deseilligny et al. 1981). The Ib afferents fire in response to both active and passive tension, but the threshold for detecting the passive tension is very high. It is assumed that the changes in the discharge pattern of GTOs might reflect the changes in the activation and recruitment of MUs during voluntary contractions, but the extent of interaction of GTOs in the electrical stimulation of muscle afferents and voluntary muscle contractions still remains unclear.

A.

B.
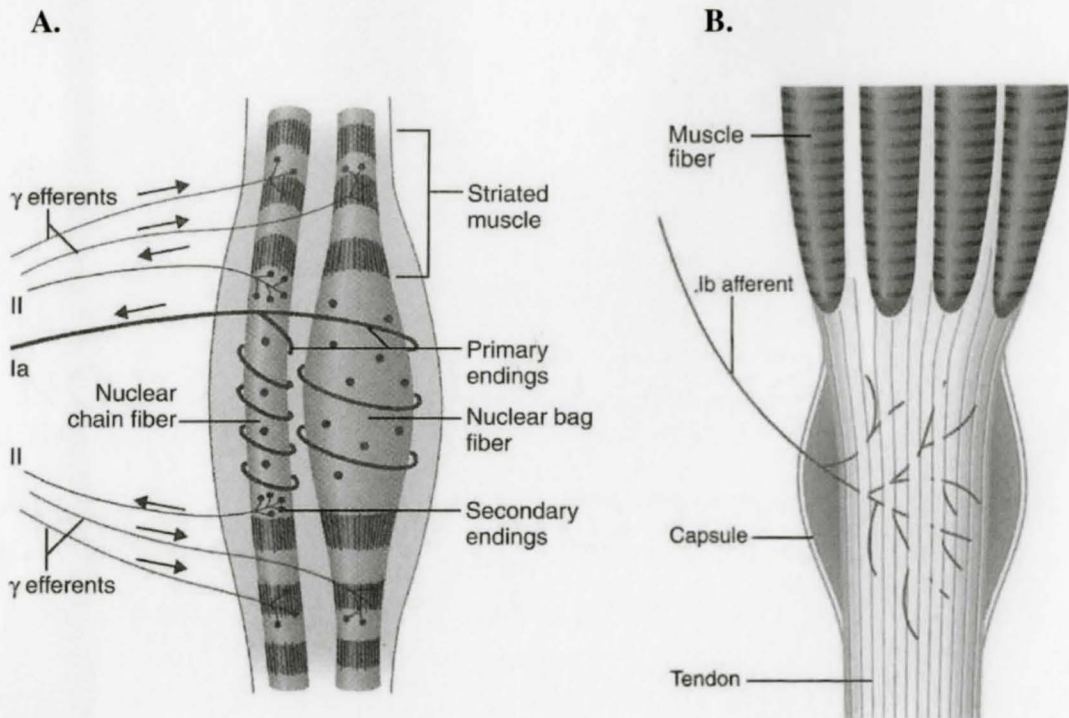
Figure 4. **A.** Sensory organs of a muscle spindle. **B.** Golgi tendon organs (adapted from Kandel et al. 2000).

2.3.3   Electrical stimulation of motor nerves

The peripheral nervous system is composed of nerves, each nerve containing hundreds to thousands of nerve fibres or axons. These are a combination of α- and γ-motor axons, as well as sensory nerve fibres from muscle spindles and other sensory organs. To excite the nerve fibres, a depolarizing pulse is applied to the nerve by either the surface or needle electrodes. The vast majority of diagnostic or experimental stimulations are by surface electrodes because of the relative ease of application and lessened discomfort to the subject. One disadvantage of surface electrodes is that the electrodes are not selective to a specific area. With surface stimulation of a motor nerve,

the excitation of an individual axon is determined not only by the amplitude of the

stimulating pulse but also by the axon diameter and its proximity to the stimulating

electrode. Given equal electrode-axon distances, large, myelinated axons are stimulated

much more easily than smaller, myelinated axons. Consequently large diameter α-motor

nerve fibres (~14 μm) and muscle spindle Ia afferent fibres (~17 μm) are primarily

activated during electrical stimulation rather than the smaller γ-motor nerve fibres (~5

μm) and II afferent fibres (~8 μm).

2.4   Monosynaptic Spinal Reflex

A monosynaptic or polysynaptic spinal reflex can be elicited by muscle stretch

(the tendon jerk), and by electrical stimulation of muscle afferent fibres. Some reflexes

are monosynaptic (one synapse), but most reflexes are polysynaptic involving more than

one synapse in the spinal cord. The monosynpatic reflex arc is shown in Figure 1. It

consists of an Ia afferent fibre from the muscle spindle entering the spinal cord and

forming a synapse on a motor neuron of a muscle. Since there is a delay in neural

transmission at the synapses, the more synapses that are encountered in a reflex pathway,

the more time that is required to effect a reflex.   Furthermore the size of the EPSP

underlying the stretch reflex is determined by a combination of the temporal summation

of individual spindle afferents and spatial summation of multiple spindle afferents

innervating a single MN. However, in the experimental test situation where the Ia

afferent fibres are electrically stimulated, spatial summation mainly determines the size

of EPSP of each individual MNs. The H-reflex is therefore a measure of the number of

MNs that have been excited. Contrary to the stretch reflex, the procedure for evoking the H-reflex bypasses the muscle spindle, and thus the H-reflex amplitude can give an estimation of α-MN excitability (Spencer et al. 1984).

2.5   Central Processing of Motor Unit Discharges

2.5.1   Introduction

Voluntary contraction is initiated by the higher center in the brain, but moment-to-moment adjustment of the MU discharges requires a feedback mechanism. Therefore the regulation of MU discharges at the spinal level depends on the peripheral feedback, i.e., muscle length (Nardone et al. 1989), where MN recruitment and firing rates during voluntary muscle contraction are additionally regulated by a reflex originating in response to the length changes in the muscle.

Indeed there are numerous possible processes at the spinal level, that could contribute to the modulation in MU recruitment and firing rate during voluntary muscle contraction. They include the reflex effects exerted by the large- and small-diameter afferent fibres on α- and γ-MNs and their presynaptic modulation, recurrent inhibition (see below), as well as other neuromodulatory influences acting on MNs and on the interneuronal spinal network. Of course, the higher brain center at the supraspinal level sends the control information through the descending pathways (including the corticospinal pathways) as shown in Figure 5. These pathways are the feedforward component of muscular control and are influenced by afferent feedback.

The temporal behavior of these multiple processes may also vary during voluntary muscle contraction. It was suggested by Gandevia (2001) that, in the first few seconds of a strong sustained contraction, the firing rate of MNs was driven down, possibly supported by the inhibitory reflex effects exerted by Ib afferents from the Golgi tendon organs in the contracting muscle, the recurrent inhibition exerted by the firing MNs onto themselves, and the "muscle spindle disfacilitation" resulting from the mechanical unloading of the muscle spindles during muscle contractions (see below). In summary, there must be a significant amount of motor programming involved in coordinating the contracting actions of the various muscles crossing the joints. What we know of the motor programming circuitry is described below.
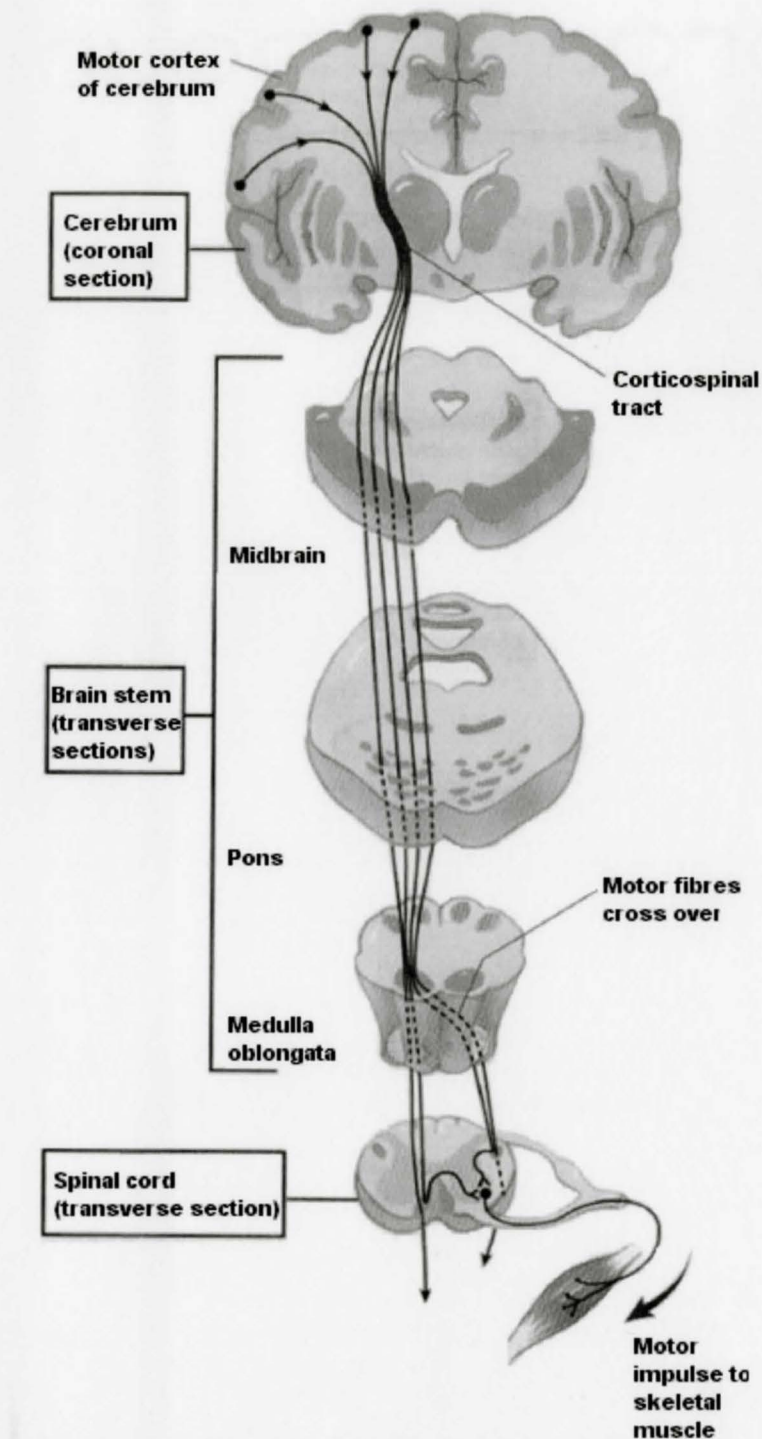
Figure 5. Motor fibres of the corticospinal tract begin in cerebral cortex, and descend in the spinal cord. There, they synapse with neurons whose fibres lead to the motor neurons that supply skeletal muscles (adapted from Shier et al. 2002).

2.5.2   Possible role of the intrinsic motoneuron properties

The final central nervous system structure that is activated prior to the muscles, or rather evoking their contraction, is the α-MN. The properties of MNs can be described in terms of their mean firing rate and variability during different types of voluntary contractions. MNs have been found to modulate their firing rate during different types of voluntary contractions. Low firing rates are generated during periods of low voluntary contraction. As the subject produces greater force, the mean firing rate of a MN increases. Even during constant force contraction, the firing rate of MNs fluctuates. Therefore the firing rate of each MN is best characterized by its mean and variance. The important factors determining the firing rate and variability include the levels of feedforward and feedback controls, afterhyperpolarization (AHP) – the transient drop in cell membrane potential below the resting level following the action potential, and electrical noise in the membrane due to spontaneous synaptic bombardment.

MNs innervating the upper limb muscles have been found to be relatively fast firing compared to those of the lower limb (Kudina 1999). During slow voluntary ramp increases in the force, MNs first fire sporadically, then fire rhythmically at a low rate. The minimal rhythmic firing rate depends on the muscle and shows considerable inter-subject variability. As the subject increases the effort, there is a corresponding increase in the force and mean firing rate. Kudina (1999) suggested that the low firing rates may be generated by the intrinsic properties of the MNs during the periods of low voluntary contractions. The relationship between the voluntary force and mean firing rate has been explored by Monster and Chan in 1977 for the extensor digitorum communis muscle

during finger extension (Binder et al. 1996). One interesting feature of their data was that the slope of the relationship between the mean firing rate and force was steeper for high threshold compared to low threshold MNs. This feature can possibly be explained by the fact that the low level contractions can be smoothly controlled by small increments in the firing rate of the low threshold MNs. At higher levels of effort, larger, faster twitch MNs will be necessitated to increase their firing rate to produce large increments in the force.

Along with the general decrease in mean firing rate with decreases in force, there is a concomitant change from a lower to a higher variability in the firing rate of individual MNs. It has been suggested that at low firing rates, the high variability is due to the fact that the action potentials are being triggered directly by spontaneously firing cells (synaptic noise) rather than after an occurrence of an action potential (membrane's rapid depolarization). The excitation of the peripheral afferent sources, such as the muscle afferent nerves and cutaneous afferent nerves, may also have a strong effect on the firing behaviour of MNs discharging with relatively low mean firing rates. Therefore the rate of excitation of MNs is not merely the summation of all excitatory and inhibitory inputs but is also determined by the motor nerve cell potential properties at the given time.

2.5.3   Neural control of mechanical responses of the muscle

Romano and colleagues' hypothesis that changes in the reflex excitability during muscle shortening and lengthening contractions appears appropriate in matching the nervous command to the mechanical responses of the muscle (Romano et al. 1987). A

volley of nervous impulses that reaches a muscle is created in the central nervous system and is modified by the sensory feedback information. The feedback information is required for adjusting the reflex pathway to fit to the task at hand. Therefore the reflex response has a crucial role in the force regulation during voluntary activity.

The capacity of a muscle to produce force is dependent on the muscle's basic components. Each muscle is composed of a great number of muscle fibres. A muscle fibre is composed of many tightly packed myofibrils. The basic functional unit of the myofibril is a sarcomere. Many sarcomeres pack together in series, and contain actin and myosin filaments overlapping with each other. The interaction of these filaments generates a force that is transmitted to tendons through serial and lateral force transmission (Monti et al. 1999).

Force developed by a muscle varies with its length. The isometric force-length relationship, obtained during the maximal activation at different muscle lengths, shows that the greatest force can be produced at lengths in the middle of the range, where the overlap between actin and myosin filaments is maximal (Figure 6).
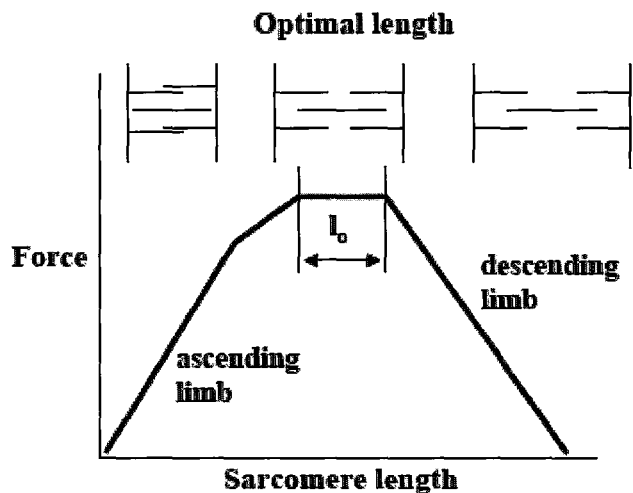
Figure 6. Force-length relationship of an intact, active human muscle. l_0 denotes the optimal sarcomere length where the force developed is maximum (modified from Zajac 1989).

During a muscle shortening contraction, less force is developed due to the in-series elastic properties of sarcomeres. In response to the decrease in force on the muscle spindles, and thereby the decreased muscle spindle firings (Ia and II afferents), the efficacy of connections between Ia fibre inputs to MNs is consequently increased to recruit more MUs. The additional MUs are necessary to produce more forceful muscle contraction to overcome the mechanical disadvantages. The lengthening contractions can be interpreted according to the same reasoning. When the muscle is under lengthening contraction, a greater force of muscle is developed owing to its increase in the length of muscle spindles. Stretch of the muscle spindles increases both the firings of Ia and II afferents in the spindles. In turn, the muscle spindle activity generates a reflex response which decreases the efficacy of Ia afferents and therefore compensates for the perturbation (Romano et al. 1987). The modulation of central effect of the Ia fibre inputs

during muscle shortening and lengthening contractions contributes to the rapid

compensation for deviations from the expected load.

2.5.4 Changes in short-latency and long-latency stretch reflexes

A short-latency stretch reflex (SLR) is a monosynaptic activation of MNs by the

Ia afferents. When a muscle is suddenly perturbed, the SLR responds. This response

cannot be voluntarily controlled. However, a later response can be voluntarily controlled.

This second pathway begins with the Ia afferents, travels up to the spinal cord, reaches

the thalamus, then the somatosensory and motor cortex, and then travels down to the

spinal cord, resulting in a compensating activity in the MNs (Figure 7). Unlike the short-

latency reflex, its activity is programmable by the brain. Therefore this long-latency

reflex (LLR) is believed to have a cortical origin.
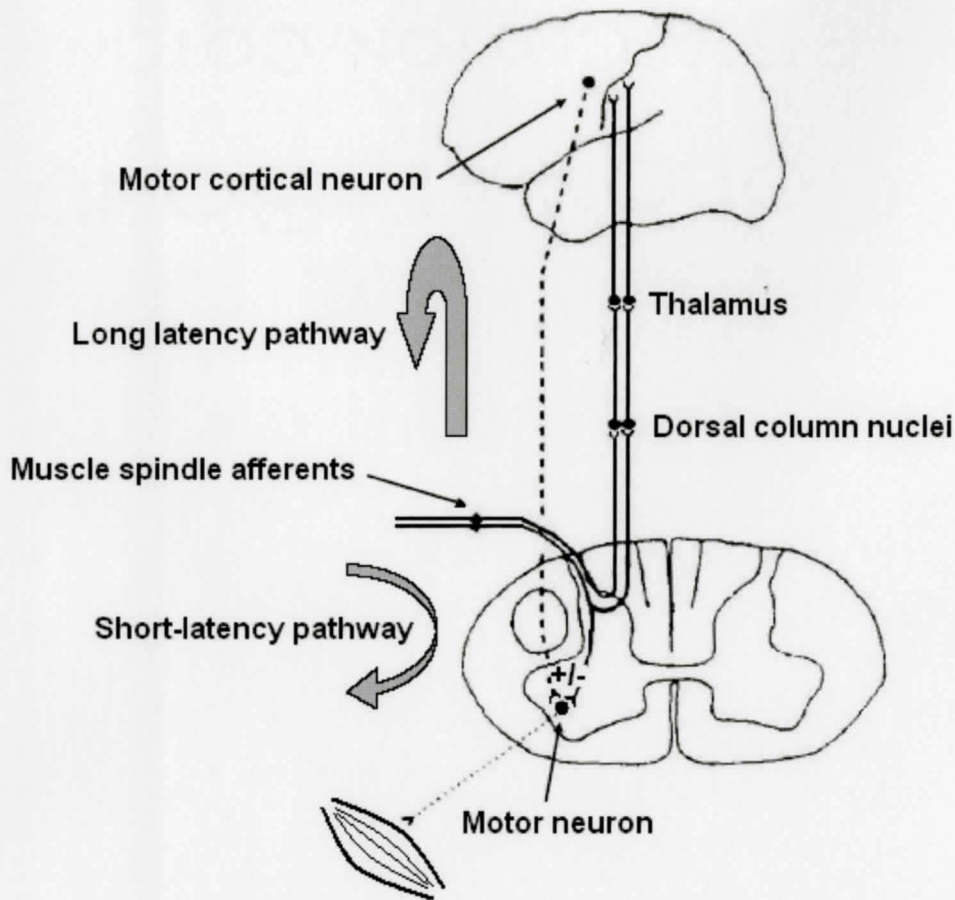
Figure 7. Stretch reflex has both the short-latency and long-latency components. **+/-** represents excitatory or inhibitory synapse with the motor neuron.

It has been shown that a MU can be recruited with the lowest stimulus during the LLR period. If the stimulus strength or the excitability of the MN pool were increased, the MU would respond during the SLR period as well (Calancie et al. 1990). The change in H reflex amplitude can be explained by a modulation in the transmission along neural elements between the nerve stimulation and the MNs responding in the H-reflex during muscle contractions. It is presumably due to the modulation in presynaptic inhibition (see below) of the Ia terminals induced by different motor tasks of the subjects or by the interaction of interneurons in the oligosynaptic pathways. As a consequence, the reflex

effects might be mediated, at least partly, by inhibitory interneurons, such as neurons

mediating presynaptic inhibition or recurrent inhibition. Relevant data concerning the

changes in presynaptic and recurrent inhibitory systems during muscle contractions will

be briefly described.

2.5.5   The role of presynaptic inhibition

In vertebrates, the terminals of Ia afferent nerves in the spinal cord experience a

depolarization called primary afferent depolarization (PAD), which is inhibitory rather

than excitatory in its effect (see below). The PAD reduces the effectiveness of any

afferent inputs coming through the depolarized terminals causing presynaptic inhibition,

shown in Figure 8A. Unlike the postsynaptic integration of all synaptic inputs,

presynaptic inhibition allows the selective control of synaptic inputs of individual axonal

branches (Rudomin 1999; Rudomin et al. 1999). It is important to note that the

presynaptic interneurons are controlled by many modulating influences from other

afferents and tracts descending from supraspinal structures (Jankowska 1992; Rudomin et

al. 1999). In human subjects, radial nerve stimulation produced presynaptic inhibition of

H-reflexes in the FCR. This inhibition can be reduced by cutaneous inputs from dorsal or

palmer surface of fingers (Pierrot-Deseilligny 1996).

It is now well known that PAD, associated with presynaptic inhibition, is

produced by $GABA_A$-ergic interneurons that make axon-axon synapses with the

intraspinal terminals of afferent fibres (Figure 8B). Following the release of GABA by

spinal interneurons and the activation of $GABA_A$ receptors in afferent terminals, there is

an efflux of chloride ions that produces PAD and reduces transmitter release from these afferent terminals (Rudomin 1999). These GABA-ergic interneurons can simultaneously exert the presynaptic inhibition on primary afferents and also postsynaptic inhibition of MNs through direct synaptic connections on the dendrites (Jankowska 1992). Although most terminals from muscle afferents receive presynaptic inhibition, the effects can vary depending upon the location of the terminal, in the ventral horn near the MN or through descending projections from motor centres (Lomeli et al. 1998).

A.

B.
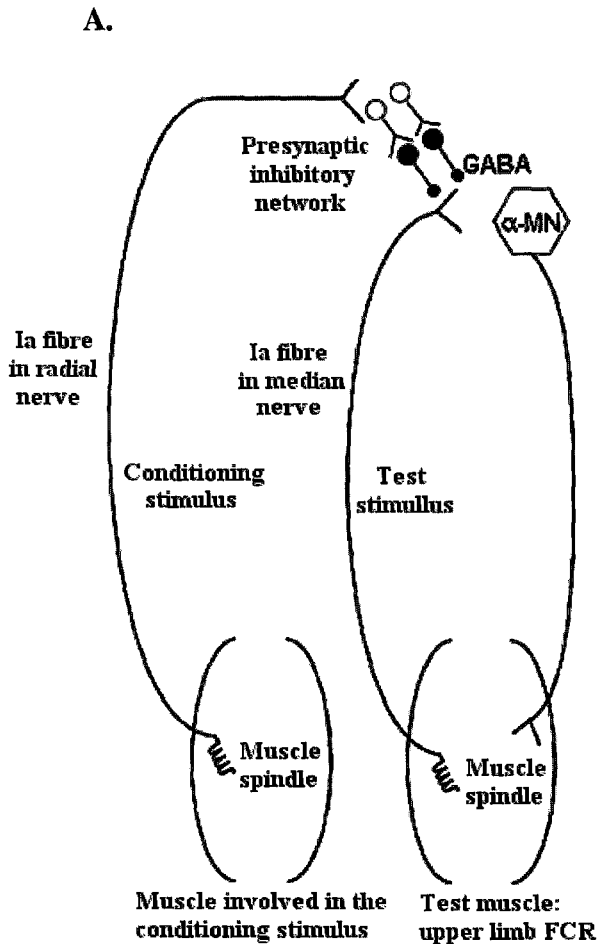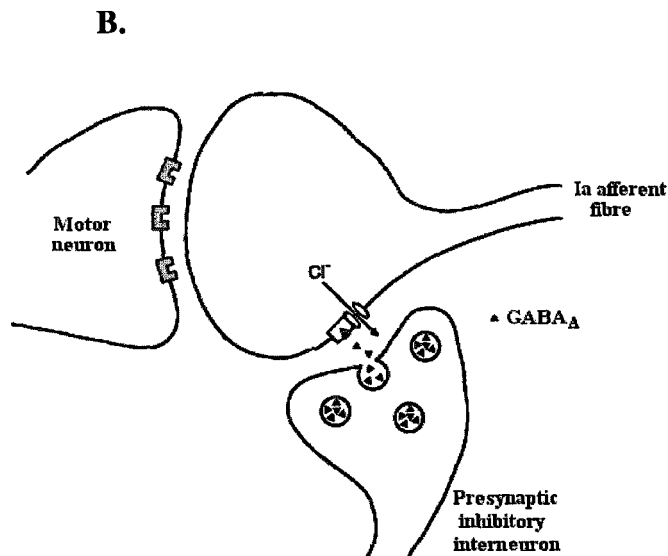
Figure 8. **A.** Interneurons make axon-axon inhibitory synapses (GABA$_A$-ergic) with the endings of Ia fibres. **B.** The hypothetical arrangement of a synapse made by a group Ia afferent fibre on a motor neuron and the axon-axon contact made by a GABA$_A$-ergic interneuron. Release of GABA$_A$ causes an efflux of chloride ions (Cl$^-$) from the Ia ending, resulting in a depolarization.

Rossi et al. (1999) examined the effectiveness of the Ia monosynaptic volleys, and have proposed that, during voluntary contractions in humans, decreased presynaptic inhibition allows Ia activity to contribute to the excitation of voluntarily activated MNs, while increased presynaptic inhibition prevents the activation of MNs not involved in the contraction, thus increasing motor selectivity. The strength of presynaptic inhibition is highly dependent upon the task to be performed, with for example progressively diminished presynaptic inhibition of the soleus spindle afferents during the transition from stance to swing phase with increasing walking speeds (Capaday et al. 1986). Similar mechanism could be expected to operate during muscle shortening and lengthening contractions.

This so called "classical" type of presynaptic mechanisms should be distinguished from the homosynaptic depression. Homosynaptic depression is a post-activation depression related to the mechanism of changed (reduced) probability of transmitter release at the previously active Ia terminals (Hultborn et al. 1996). At frequencies of stimulation below 100 Hz, transmitter depletion and depression of EPSPs seem to occur (Curtis et al. 1960). This might provide a self-regulating mechanism for counteracting excessive Ia synaptic drive to MNs. Thus, while Ia impulses evoke a powerful monosynaptic excitatory action on the MNs, at the same time they can give rise to presynaptic events that lead to the long-lasting depression of the reflex.

2.5.6   The role of recurrent inhibition

Renshaw cells (RCs) receive their main excitatory input from α-MNs and inhibit α-MNs, and other RCs. They exert the classical autogenic inhibition of homonymous MNs – an inhibition that occurs in the same muscle where the GTO is stimulated, shown in Figure 9 (Ellaway 1971). Their main purpose has been suggested to be a regulation of reflex gain at the motoneuronal level (Hultborn et al. 1979). In humans, both the extensor carpi radialis (ECR) and FCR experience this homonymous recurrent inhibition from their own RCs. Even though ECR and FCR are antagonists, they have been shown to receive inhibition from each other's RCs (Aymard et al. 1997). Although RC inhibition may limit the MN discharge rate in cocontractions when the two muscles act as the synergists, their effects are complex. Their activity might be modulated by

descending motor pathways, local interneurons and by afferent feedback (Windhorst 1996).
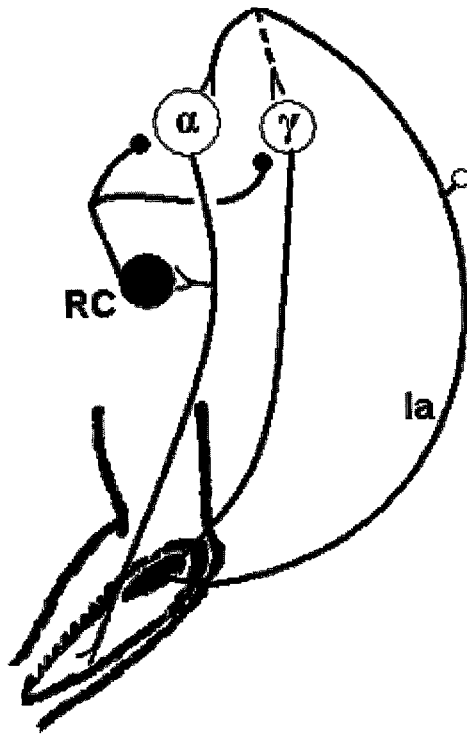
Figure 9. The connections of Ia afferents and Renshaw cells (RC). The Renshaw cells are excited by the homonymous motor neurons and project back by the same motor neurons.

2.5.7 Propriospinal neurons and supraspinal control

Propriospinal neurons, along with their connections, may be the likely candidates for the mediation of spinal reflexes. That way, propriospinal connections may support the integration of descending and peripheral inputs during different movements. These spinal connections may involve multiple interneurons. Cells that give origin to the long descending propriospinal tract are found in deep layers of the spinal cord, especially in

laminae VII and VIII. Cells of shorter propriospinal fibres have more extensive distribution, occupying both dorsal and ventral horns, with the exception of laminae IX (Menetrey et al. 1985). Propriospinal pathways connecting the cervical and lumbar enlargements have been of particular interest for a very long time because of their potentially important role in forelimb coordination in cats. It is suggested that they could also be relevant for target-reaching movements to retrieve food, target-reaching is mediated by the propriospinal pathway. Based on the experimental observations, a similar propriospinal system might also be involved in the improvements of voluntary motor function in the hemiplegic patients (Pierrot-Deseilligny 1996).

The cortical and subcortical areas of the brain coordinate the muscles which generate the force required for different movements. The original cortical sensory map organization studies suggested a discrete neuronal representation of each body part in the primary motor cortex. Later studies have shown multiple cortical sites in the hand/wrist area of the cortex for finger movement, depending on the type of movement. Georgopoulos et al. (1999) have proposed separate subpopulations of neurons coded for the direction of movement, being called the "neuronal population vector". The population vector may provide a tool for decoding and monitoring activity controlling the fingers over time, across different tasks, and through different cortical areas.

CHAPTER 3

METHODS

3.1   Introduction

As described in Chapter 2, there are many complex mechanisms involved in the control of skeletal muscles. In this thesis, we concentrated on the role of muscle spindles and hence Ia fibre afferents play in the control system. More specifically, we want to study how the reflex pathway is modulated by the descending control information from supraspinal centers. To that end, we selected the FCR muscle in the forearm as the test muscle. It is innervated by the median nerve which can be stimulated using surface electrodes at the cubital fosssa (elbow) to give both motor response (M-wave) and H-reflex, shown in Figure 10. As well it has been studied by other researchers interested in the reflex control. This Chapter describes the experimental methodology used to determine how the feedback control system is modulated by descending supraspinal inputs.

3.2   Subject Setup

Experiments were carried out on 11 healthy subjects (7 men and 4 women), aged 22-60 years, all of whom had given written informed consent to the experimental procedures, which had been approved by the university ethics committee and conformed with the guidelines in the Declaration of Helsinki. The subjects were seated comfortably

in a chair. The elbow was semi-flexed (110°). The examined forearm was laid semi-supine on the table, and supported by a sandbag.

Before placing the electrodes on the skin, the skin was thoroughly cleaned with alcohol to assure good adhesion and to remove any surface lipids. Electrode cream was also rubbed in at the stimulating and recording sites to lower skin impedance. Before rubbing in the electrode cream, skin should be dry to avoid the contamination from alcohol. Furthermore the skin was allowed to dry thoroughly before applying the electrodes. The experimental setup is shown in Figure 11.

3.3   Stimulation

Electrical pulses were delivered through bipolar surface electrodes (0.9 cm diameter silver plates 3.0 cm apart, cathode proximal) connected to a Model D57 Constant-Current Stimulator (Digitimer Ltd., Welwyn-Garden City, UK). The electrical stimulator was controlled through a Digitimer D4030 Trigger Generator (Medical Systems Corp., Great Neck, NY, USA). The width of the stimulating pulse was set to 0.2 ms, since long pulses are more effective in exciting Ia fibres (Burke et al. 1984). The pulses were applied to the median nerve, as shown in Figure 11, on the anterior aspect of the arm at approximately 1 cm above elbow level at a site where the threshold for eliciting the FCR H-reflex was lowest. The cathode was place proximally to avoid the theoretical possibility of anodal block. Each stimulus can excite α-MNs directly which result in the M-wave and/or sensory afferents which travel proximally to the spinal cord, exciting α-MNs reflexively.
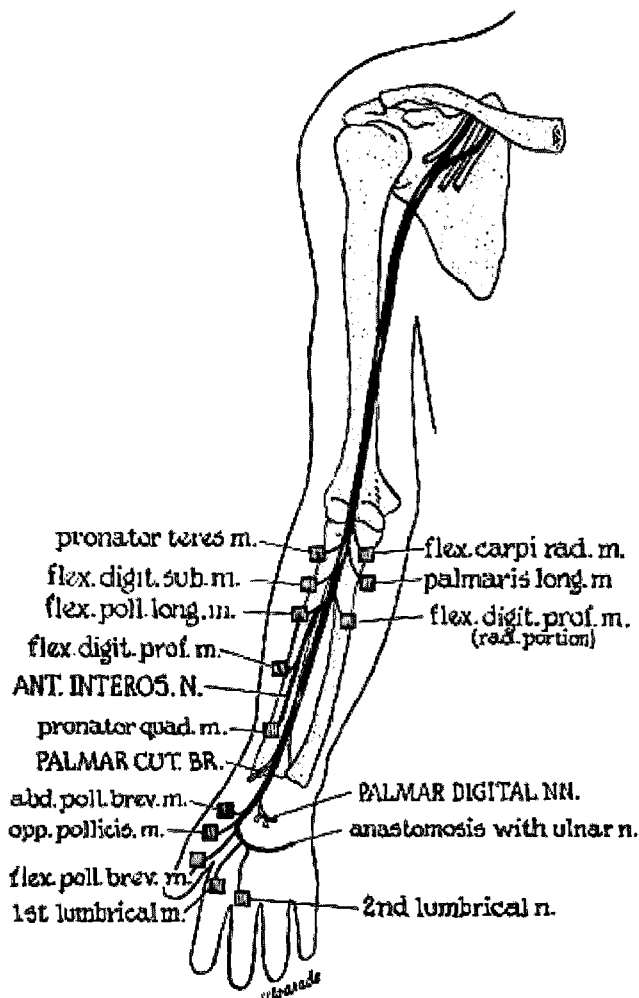
Figure 10. Branches of the median nerve (adapted from Jenkin et al. 2002).

3.4   Data Acquisition and Analysis

As shown in Figure 11, two halves of an adhesive EKG electrodes (Q-trace Gold

5500; Kendall Ltd., Chicopee, MA, USA) were secured to the skin 2 cm apart over the

FCR muscle belly with the long axis transverse to the fibre direction, in the proximal

forearm to record the evoked muscle responses. A ground EKG electrode was attached

on the anterior-medial aspect of the elbow. It was placed between the stimulating and recording electrodes.

The signals were differentially amplified to remove 60 Hz noise, and band-pass filtered between 10 Hz and 1 kHz, using a Model 1700 4-Channel Differential AC Amplifier (A-M Systems Inc., Everett, WA, USA). This model contains four independent amplifiers so that several channels can be recorded simultaneously. The default gain of the amplifier was set at 1000 for H-reflex and M-wave. This gain was reduced to 100 for large signals to avoid electronic saturation.

The output from the amplifier was displayed on a Hewlett-Packard Model 141B storage oscilloscope and was simultaneously fed through an analog-to-digital converter (A/D) at a sampling rate of 4 kHz (National Instruments PCI-6024E data acquisition computer board). To initiate data acquisition, the board accepted an analog trigger signal at the PFI0/TRIG 1 pin on the I/O connector. Triggering of data collection was initiated 50 ms prior to the stimulus, and 100 to 150 ms of data were collected. The data were collected, analyzed, and stored using a custom written program (Labview, National Instruments Corp., Austin, TX, USA). This program estimates the phase and amplitude of the 60 Hz noise present in the 50 ms pre-stimulus period, constructs a coherent 60 Hz signal and subtracts this from the original signal. The result is a clean signal which is free from 60 Hz noise without altering the frequency content of the responses even when the surface recorded M-waves and H-reflexes contain 60 Hz noise components. The peak-to-peak amplitudes of the processed and filtered H-reflexes response and M-waves,

were measured and recorded automatically as microvolts using the data acquisition

program written in Labview (Figure 12).
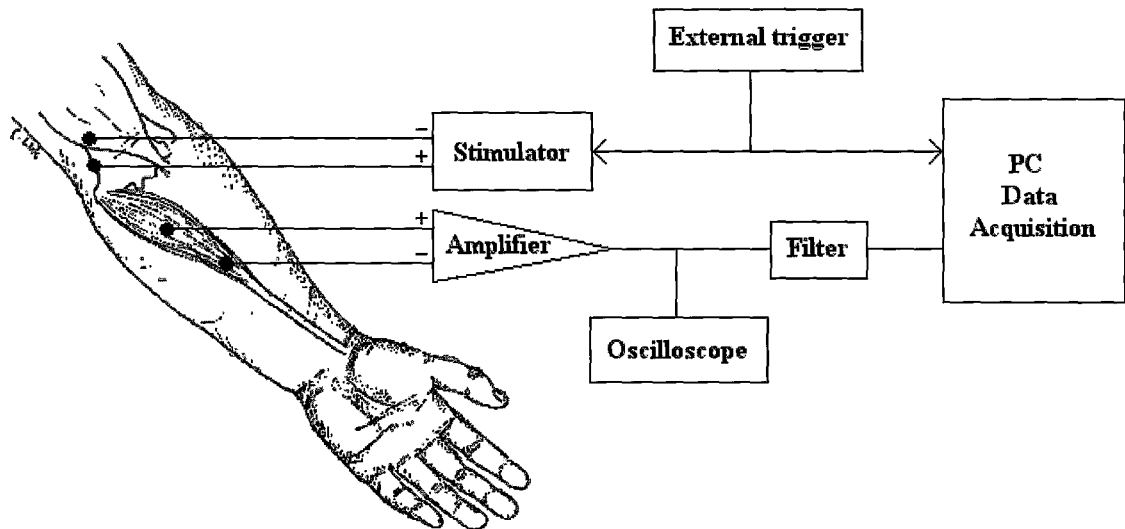
Figure 11. Experimental setup for flexor carpi radialis muscle of the forearm.
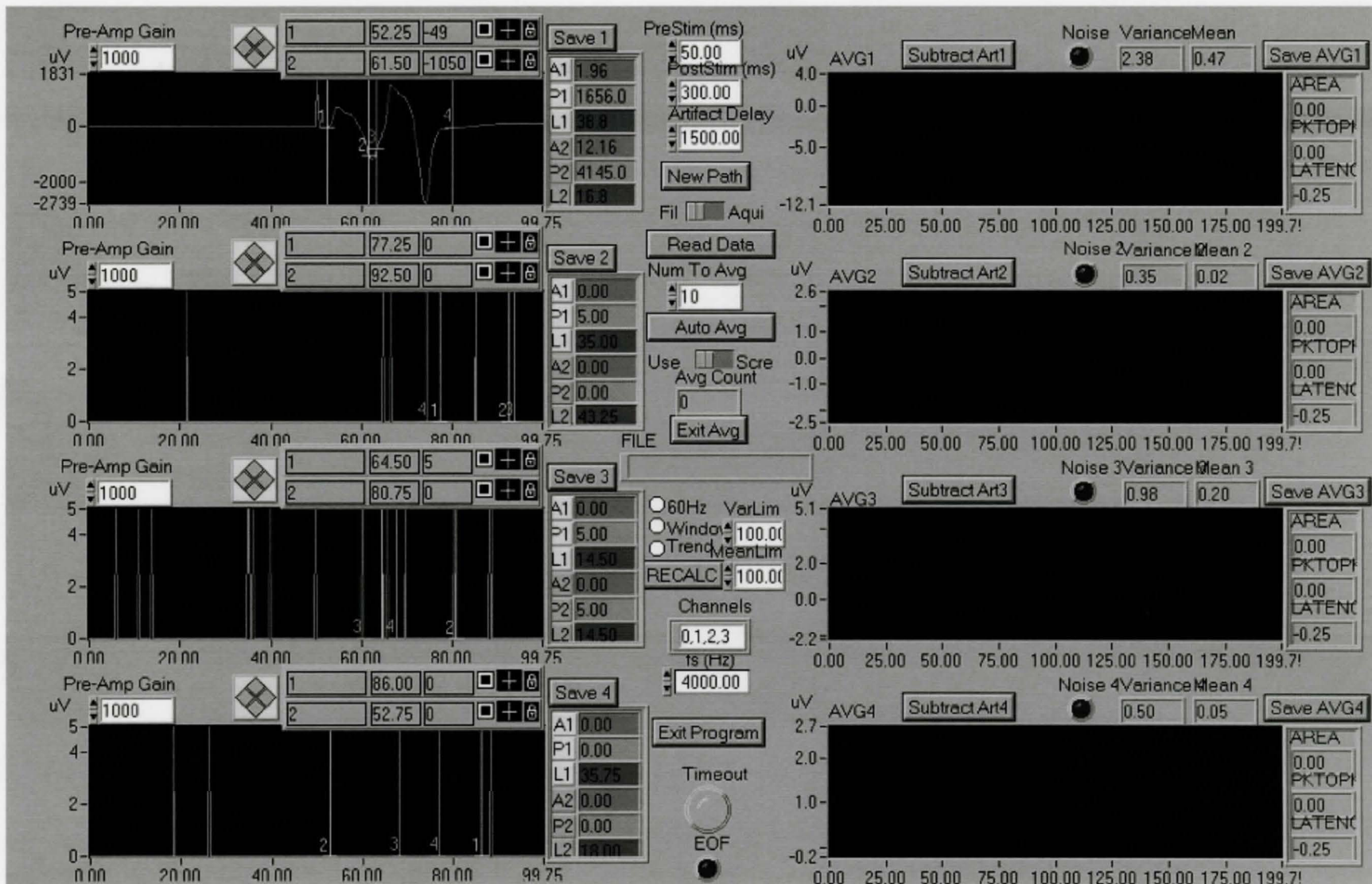
Figure 12. Front panel of data acquisition program written in Labview. The M- and H-reflex responses for 100 ms data are shown in the top left window with time cursors. P1 and P2 are the peak-to-peak amplitudes of M-waves and H-reflexes respectively

3.5   Procedures

3.5.1   General procedure

Subjects were tested in two separate trial blocks, with a different type of voluntary contraction being tested in each. The first trial block compared the states of relaxation and attempted wrist flexion, whereas the second block compared the states of relaxation and attempted wrist extension. The subjects were first instructed to perform approximately 5-10% voluntary MVC that represented the weak isometric contraction level. To ensure a steady contraction, the subjects maintained a constant effort against the resistance provided by the experimenter. We experimented earlier with soleus muscle control using a force feedback signal display to the subject, but this more complex control scheme resulted in much greater variability in H-reflex responses. We attributed this to the much greater concentration required to track the target force level resulting in varying modulation of the supraspinal inputs. Subsequently, the maximal M-wave (M_{max}) was determined by imposing 0.5 mA increments in current until a maximal motor response was obtained. The current was then raised by 1-5 mA to ensure that the amplitude did not increase further.

The experimental procedure to determine the threshold of the most excitable Ia fibres required the subject to flex the wrist weakly and steadily while the electric stimulus was decreased in steps of 0.2 mA until the smallest possible H reflex was observed (probably a single motor unit). This method, which depended on the summation of Ia and descending voluntary inputs in the MNs, was previously employed by Burke et al. (1984) and Shindo el al. (1994) because threshold for the H-reflex was reached at a lower

stimulus intensity in the forearm flexor muscles during a voluntary contraction than in relaxation. The stimulus intensity was then increased in steps of 0.2-1.0 mA until the maximal H-reflex (H_{max}) had been found, allowing for collision with the antidromic motor volleys (Magladery et al. 1951). This intensity was that of the highest-threshold Ia fibres (see below).

In each trial block, the subject's median nerve was activated at the elbow level with current intensities randomly selected in steps of 0.2-1.0 mA between the lowest and highest Ia fibre thresholds. In each trial, the chosen electrical stimulus was delivered when the wrist was either in the control (resting state), or conditioned (instructed voluntary contractions) situations. At the completion of each contraction the subject was asked to relax with the maintained wrist position. The order of the required action types (relaxation or contraction) in each trial was randomly alternated to eliminate any anticipatory effects. There were 20 to 30 trials in each action type. There was also a 3-5 min rest period between the trial blocks to reduce the effects of fatigue. After the trial blocks were completed, M_{max} was again measured. To ensure the stimulating and recording conditions did not change throughout the experiment, pre- and post-M_{max} were compared. There was no difference (less than $\pm 5\%$) between the two values.

In either the state of relaxation or isometric wrist extension contraction, a trial was rejected from further analysis if there was any electromyographic (EMG) activity in the 50 ms observation period prior to stimulus onset. Isometric wrist flexion contraction trials were also rejected if the pre-stimulus EMG activity was excessive. To ensure that the stimulation of the Ia fibres was not affected by changes in muscle / electrode

configuration, the H-reflex amplitudes were only accepted for further analysis when the M-wave amplitudes at the same stimulus intensity in the control and conditioned situations did not differ by more than 5%.

Throughout the experiment, subjects were asked not to look at the storage oscilloscope and the computer screen because the induced mental state might interfere with the subject's state of relaxation.

In each trial of both the control and conditioned situations, peak-to-peak amplitudes for both the H- and M-potentials were measured and displayed online. The H-reflex amplitudes were then normalized to the M_{max} so as to represent the percentage of the MN population discharging for the associated stimulus intensity. Expressing the H-reflex amplitude as a percentage of M_{max} ensured that any alteration in the size of the H-reflex was primarily due to the effect of the variable in the experiment (voluntary muscle contraction), allowing the assessment of reflex excitability in the same conditions. The percentage of Ia fibre population stimulated was estimated by the threshold method (see below). The percentage of MN population discharging reflexly was plotted against the percentage of the Ia fibre population stimulated. Inspection of the MN output versus Ia fibre input curves clearly revealed the input-output properties of the spinal pathway, thereby the reflex excitability in the control and conditioned situations. With the larger stimuli, a portion of the reflexly-elicited motor responses would have been blocked by collision with the antidromic motor volleys shown in Figures 13A-D (Magladery et al. 1951; Pierrot-Deseilligny et al. 1999). As a result of this, the H-reflex was also expressed as a percentage of the difference between the M_{max} and M-response at the associated

stimulus intensity. A correction of the Ia input- MN output curves was made to account

for any collision between the reflexly-elicited and antidromic motor responses.
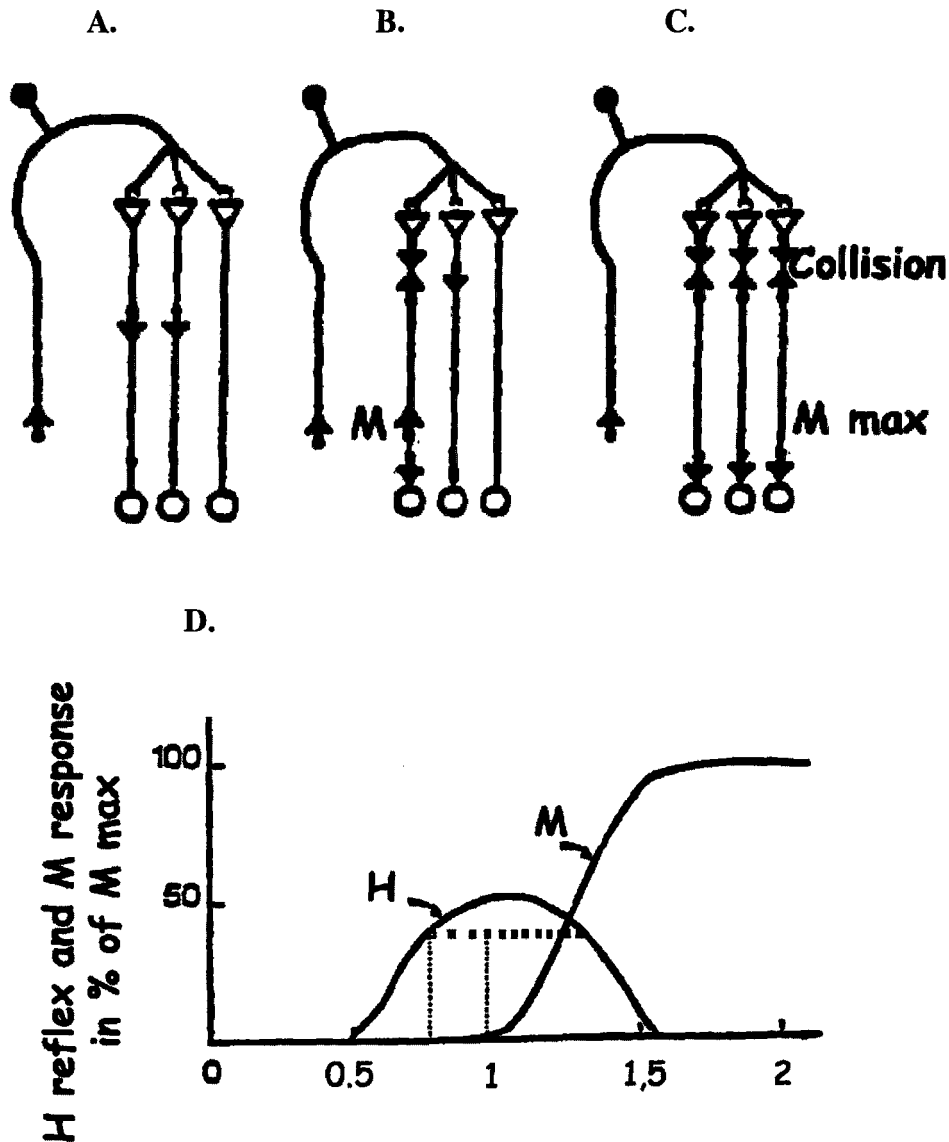
Figure 13. **A.** The stimulus activates only Ia fibres and results in some motor axons in a H-reflex. **B.** The stimulus is increased and results in a direct motor response and H-reflex. **C.** The stimulus is supramaximal for all motor axons and results only in a maximum motor response, the reflex response being blocked by a collision with the antidromic motor volley. **D.** The amplitude of motor and reflex responses, expressed as a

percentage of maximal motor response, is plotted against the intensity of electrical stimulation of posterior tibial nerve (expressed in the multiples of motor threshold). The dotted line shows that the rate of increase in reflex response amplitude begins to decline at the stimulus intensities below 1 x motor threshold (adapted from Pierrot-Deseilligny et al. 1999).

3.5.2   Threshold method for Ia input-motoneuron output relationship

As stated above, the threshold method was used to determine the percentage of Ia

fibres which would have been excited at any given stimulus intensity. Assuming a

normal distribution of the Ia fibre-thresholds about the mean, a cumulative sigmoid curve

was established based on the two defined thresholds of the least and most excitable Ia

fibres (Figure 14). The midpoint between the two thresholds was assumed to be the

intensity required to excite 50% of the Ia fibre population, with the thresholds for the

least and most excitable Ia fibres corresponding to 3 standard deviations from either side

of the mean. The equally-spaced points for 1, 2, and 3 standard deviations on either side

of the mean on the sigmoid curve corresponded to 0, 2, 16, 50, 84, 98, and 100% of the Ia

fibre population. From this curve, it was possible to estimate the percentage of Ia fibres

which must have been excited when a stimulus of any given intensity was delivered.
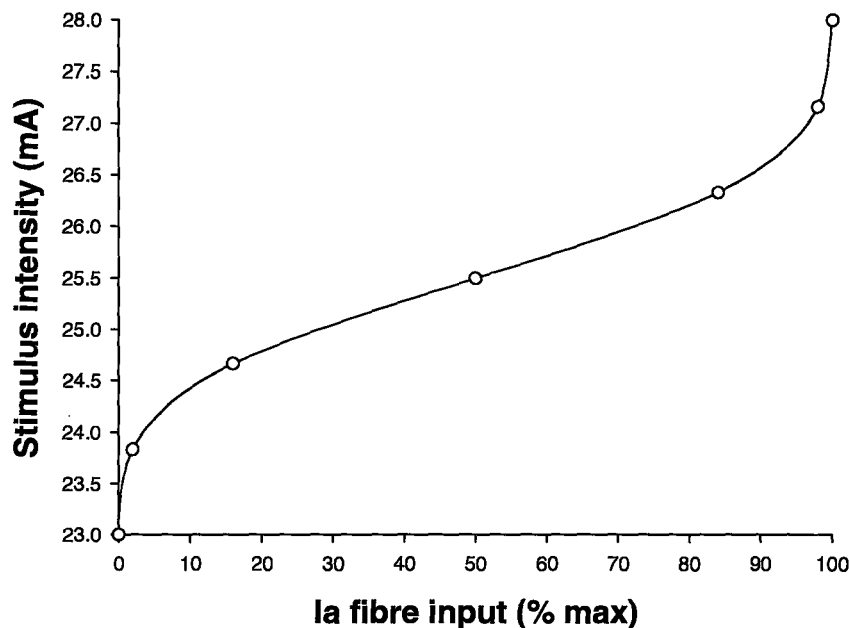
Figure 14. An example of a cumulative sigmoid curve of stimulated Ia fibres drawn between the lowest and highest fibre thresholds in a 24-year old man.

For any intermediate stimulus intensity, the percentage of Ia fibres stimulated was determined by referring to the cumulative sigmoid curve of a particular subject (Figure 14). Given the percentage of Ia fibres activated at a given stimulus intensity, the corresponding percentage of MN pool responding was determined. This was done by calculating the percentages of MN pool for the different stimulus intensities employed in the experiments, and relating this to the percentage of Ia fibre inputs at each of the corresponding stimulus intensities. An example is given in Table 2, which displayed the results during relaxation in a 24-year old man.

Table 2. Calculated results for a 24-year old man during relaxation, with the H-reflex expressed as a percentage of the M_{max}

Stimulus strength (mA)	H-reflexes (mV)	% Ia fibre input	% Motoneuron output
23.0	0	0	0
23.5	0.07	0.7	0.80
24.0	0.04	3.9	0.46
24.2	0.07	6.0	0.80
24.5	0.15	11.6	1.72
25.0	0.31	28.0	3.56
25.2	0.47	36.0	5.40
25.5	0.37	50.0	4.25
26.0	0.52	72.0	5.97
26.2	0.52	79.5	5.97
26.5	0.95	88.5	10.91
27.0	0.94	96.5	10.79
27.2	0.95	98.0	10.91
27.5	0.79	99.0	9.07
28.0	0.93	100.0	10.68

3.5.3   Thresholds of cutaneous nerve fibres

In order to provide additional evidence of the normal distribution of Ia fibre thresholds to electrical stimulation, electrical potentials of the cutaneous nerve fibres in response to electrical stimulation of the peripheral nerves were measured. The digital nerves are the terminal branches of the ulnar and median nerves and innervate the lateral aspects of each finger (Gould 1991). Antidromic impulse volleys were recorded from the

terminal digital nerves of the middle finger following median nerve stimulation at the wrist. An adhesive EKG electrode was cut into four strips and two strips were secured circumferentially to the skin over the distal phalanx of middle finger. The first electrode was placed at the most radial aspect, while the second electrode was placed on the most ulnar aspect. A ground EKG electrode was attached in the middle of the palm.

Median nerve stimulation at the wrist was performed using bipolar stimulating electrodes with a stimulus pulse width of 0.2 ms. The stimulus strength was increased until a maximal response was recorded from the finger. The potentials was amplified, digitized at a sampling rate of 20 kHz, and filtered. They were then averaged online 5-20 times using the averaging program written in Labview. Averaging was necessary to reduce noise contamination of the relatively small digital nerve potentials. The averaged nerve potential, expressed as a percentage of the maximal nerve potential, was plotted against stimulus strength to give the cumulative distribution of nerve fibre thresholds to electrical stimulation (Figure 16B).

3.5.4   Median nerve stimulation

For a further evidence of the distribution of fibre thresholds to electrical stimulation, motor potentials of the FCR muscle in response to electrical stimulation were measured, using the same stimulus arrangement as for the Ia fibres. The upper limb motor axons were excited, and the activity from the FCR muscle was recorded in an antidromic manner by means of two bisected EKG electrodes over the FCR muscle belly (see above). The stimulus strength was increased until a M_{max} was observed. Having

recorded the M_{max} at rest, the peak-to-peak amplitude of M-wave, expressed as a

percentage of M_{max} was plotted against the given stimulus strength to approximate the

upper limb motor fibre thresholds to electrical stimulation (Figure 16A).

CHAPTER 4

RESULTS

4.1   Introduction

The H-reflex is the electrical equivalent of a monosynaptic stretch reflex, and was

elicited by stimulating the Ia fibers of the median nerve. Such stimulation was

accomplished by using slow (less than 0.3 Hz), long-duration (0.2 ms) pulses of gradually

increasing intensities. The pulse volley traveled along the Ia fibers, passed the dorsal root

ganglion, and excited FCR anterior horn cells whose α-motor axons activated the FCR

muscle. The M-wave was obtained by stimulating the α-motor fibres of the median nerve

directly and recording from the FCR muscle, using the same stimulating and recording

electrodes as for the H-reflex with one electrode over the endplate zone of the FCR

muscle.

The muscle response had a fairly simple waveform, with an initial negative

deflection (up) followed by a positive wave (down). It is generally characterized by its

amplitude, duration, latency, and shape. The peak-to-peak amplitude was measured from

the maximum of the negative peak to the maximum of the positive peak of the muscle

response and expressed in microvolts. The latency was measured from the onset of the

stimulus artifact to the point of takeoff from the baseline and was measured in

milliseconds. The amplitudes of the muscle responses depended on the numbers and

sizes of FCR MUs being activated. The responses indicated a fairly synchronous

discharge of the FCR MUs. It was noted that the motor response changed in relationship

to the point of nerve stimulation. The more proximally the median nerve was stimulated, the lower the amplitude and the longer the duration of motor responses were seen. These effects were due to the temporal dispersion of FCR MUs activated.

In view of the longer conducting pathway, the latency of the H-reflex was greater than that of the M-wave. Typically, the H-reflex was first seen at low stimulus strength without any M-wave preceding it (Figure 15A). As the stimulation strength was increased, the direct M-wave appeared (Figure 15B). With further increases in stimulus strengths, the M-wave became larger and H-reflex decreased in amplitude (Figure 15C). When the M-wave became maximal, the H-reflex nearly disappeared (Figure 15D).

A.
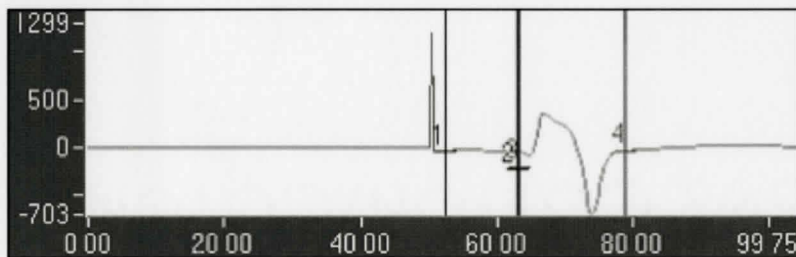
B.
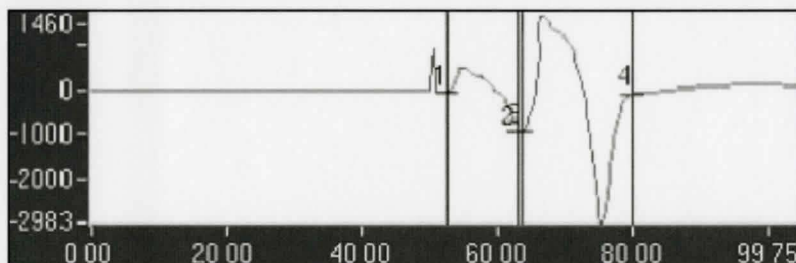
C.
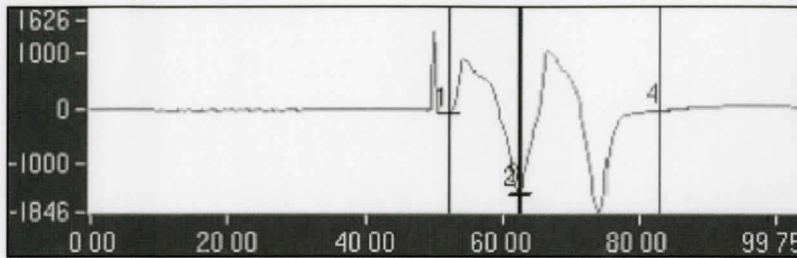
D.
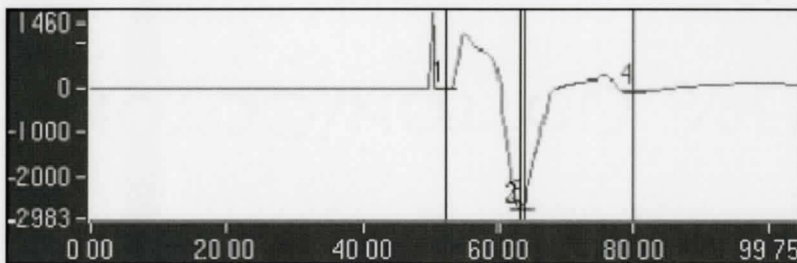
Figure 15. **A.** At the reflex threshold, H-reflex appears. **B.** At the motor threshold, M-wave appears with increase in the reflex amplitude. **C.** With a further increase in the stimulus strength, amplitude of the M-wave increases while the reflex amplitude decreases. **D.** When the M-wave approaches maximal, H-reflex begins to disappear.

4.2   Assumption of Threshold Method

The threshold method assumes that the stimulation thresholds of a functionally homogenous population of Ia fibres are distributed normally about the mean. Single or compound Ia fibre potentials cannot be measured directly following stimulation of a peripheral nerve since the responses can include α-motor or other fibre potentials. However other populations of fibres such as cutaneous sensory or α-motor fibres can be studied separately It was therefore necessary to study the motor and digital nerve threshold potentials using the same stimulus arrangement as for the Ia fibres. Measurements of the α-motor potentials, as represented by the M-wave, were performed

in 11 subjects, whereas the digital nerve threshold potentials were measured in 3 subjects using the sensory nerve action potentials. In all the 11 subjects but 2, the shape of the relation between the FCR motor potentials and current intensities was sigmoid, and approximately normal as shown by the fitted normal cumulative density function. In the antidromic sensory nerve potential testing, the distribution of the nerve potentials against the stimulus intensity was characterized as approximately normal, displaying a roughly sigmoid curve. Examples of the results obtained are shown in Figures 16A-B.

A.
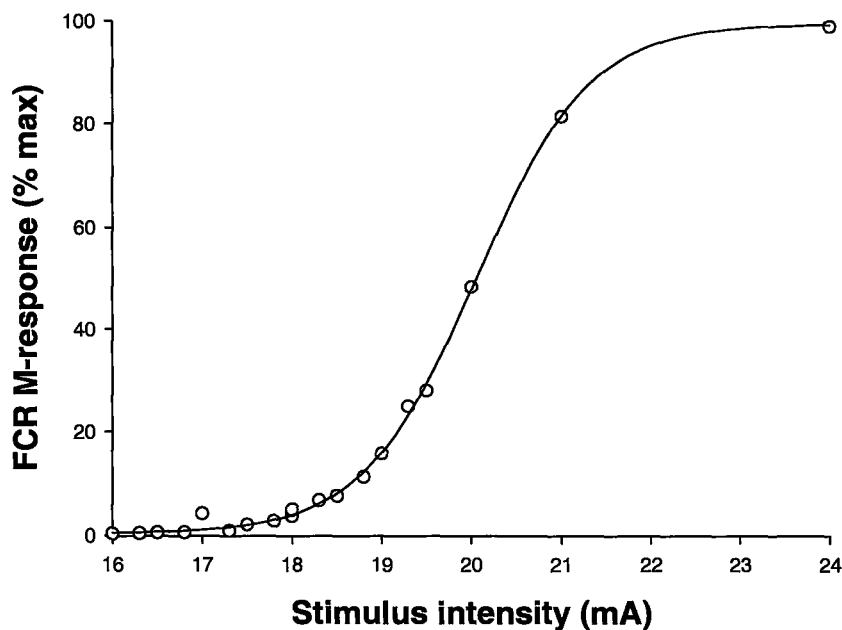
B.
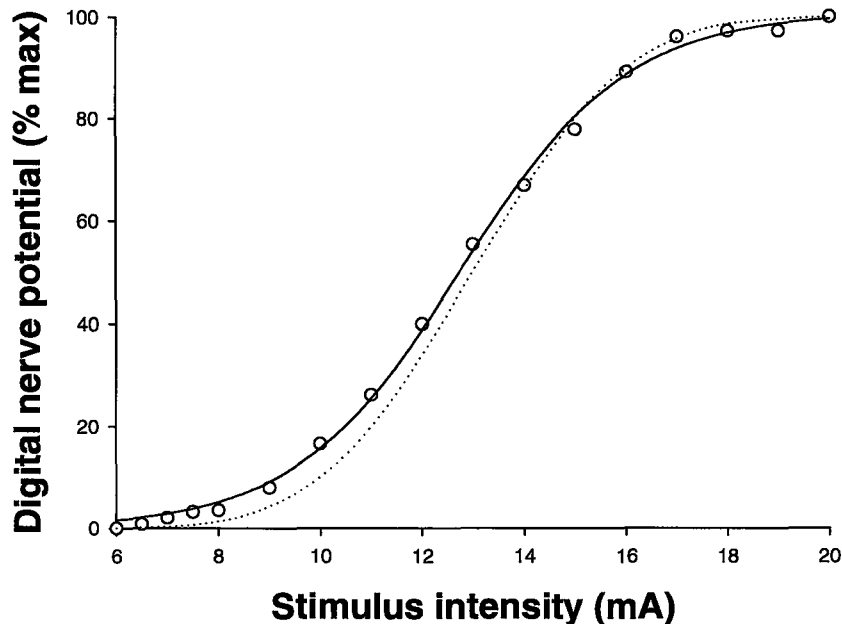
Figure 16. **A.** An example of an approximately normal distribution of peak-to-peak amplitudes of the flexor carpi radialis direct motor potentials to electrical stimulation in a 28-year old man. **B.** The solid line (with open circles) shows an example of an approximately normal distribution of peak-to-peak amplitudes of the digital nerve potentials to electrical stimulation in a 19-year old man. The dotted line shows the fitted cumulative density function for a normal distribution.

4.3   Recruitment Curve

The recruitment curve, as shown in Figure 17, summarizes the variations of H-reflex and direct M-responses with stimulus intensities. At each stimulus intensity, the muscle responses were expressed as a percentage of M_{max}. Typically, in the first part, amplitude of the H-reflex increased with increasing stimulus strength from the reflex threshold. At a stronger stimulus strength when the motor threshold was reached, a small direct M-potential usually appeared. Peak-to-peak amplitudes of the H-reflex and M-

response were strongly related, so that a decrease in reflex sizes occurred at the steepest

part of the relation between M-response and stimulus intensities. The reflex size became

very small when the M-response started to saturate at the high end of stimulus strength.
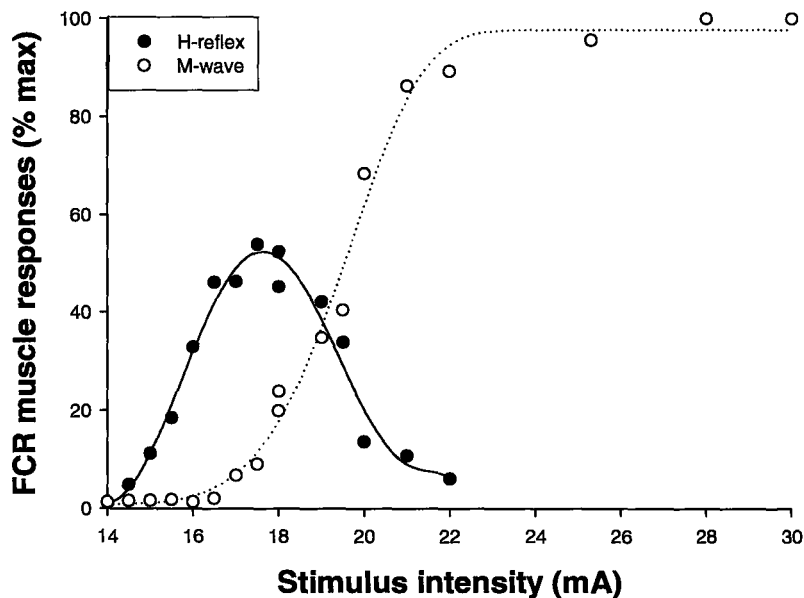
Figure 17. Representative example of the peak-to-peak amplitudes of H-reflex and FCR
motor responses (expressed as a percentage of maximal motor response) versus the
electrical stimulus intensity delivered to the median nerve at the elbow level in a 26-year
old woman.

4.4   Intersubject and Intrasubject Variability

Based on the data obtained in the studies, peak-to-peak amplitudes of the reflex

elicited at the same stimulus strength varied widely in normal subjects. Even reflex sizes

varied in the same subject for identical stimulus strength, indicating a spontaneous

fluctuation of the excitability of the MN pool. The range of stimulus intensities over

which the Ia fibres in the FCR muscle were activated also varied in normal subjects (Table 3). A narrow stimulus strength range suggests a similar excitability of Ia fibers from the FCR muscle, subsequently possibly indicating the presence of a more homogeneous pool of Ia fibers from the FCR muscle in the median nerve. However a less homogeneous pool of Ia fibres could also be stimulated by a narrow stimulus strength range if all the fibres are concentrated in one area of the median nerve.

Table 3. Stimulus strength range over which FCR Ia fibres are activated in 11 subjects

Subject	Stimulus strength range (mA)	Difference (mA)
Subject No. 1	20.0 - 35.0	15.0
Subject No. 2	15.0 - 19.5	4.5
Subject No. 3	12.5 - 16.0	4.0
Subject No. 4	14.0 - 24.0	10.0
Subject No. 5	19.0 - 24.0	5.0
Subject No. 6	16.5 - 20.0	3.5
Subject No. 7	15.0 - 20.0	5.0
Subject No. 8	17.0 - 27.0	10.0
Subject No. 9	15.0 - 19.0	4.0
Subject No. 10	14.0 - 18.0	4.0
Subject No. 11	23.0 - 28.0	5.0

Two methods of normalization were employed to reduce the intersubject and intrasubject variability: (i) The H-reflex potential was expressed as a percentage of the amplitude of M_{max} or the difference between M_{max} and M-response at the associated stimulus intensity to determine the value of MN output at the associated stimulus

intensity; (ii) The threshold method was used to estimate the percentage of Ia fibre input

at a given stimulus strength (see section 3.5.2). The percentage of MN output was plotted

against the percentage of Ia fibre input. These methods allowed us to determine the

input-output relationship of the FCR muscle at the spinal level, and minimize the

intersubject and intrasubject variability.

4.5   Statistics

The dependent variable chosen to differentiate task dependent changes

(lengthening vs. shortening actions) in the spinal reflex excitability was the FCR MN

output. To evaluate the MN output, amplitude of the H-reflex was normalized to either

the amplitude of M_{max} or the difference between the amplitude of M_{max} and that of the M-

wave evoked by a given stimulus strength. For all analyses, the independent measure

was the percentage of Ia fibre input. Paired-sample t-test was used to statistically

compare the individual and mean ratio differences among the test conditions on each

dependent measure. The statistics for all the investigations were performed with

commercially available software, SigmaPlot (Chicago, IL).

4.6   Evaluation of Motoneuron Output

The measurements of the peak-to-peak amplitudes of H-reflexes in both the

control and conditioned situations can be simply expressed as a percentage of the M_{max},

corresponding to the percentage of responding MNs in the available pool, as shown in

Tables 4A, 5A, 6A, and 7A. These tables show the H-reflex expressed as a percentage of

M_{max}, subsequently the fraction of total MUs in the muscle activated by the H-reflex. 11 subjects were tested for the effects of flexion contractions, whereas only 8 subjects were tested for the extension contractions.

If the H-reflex was evoked together with a M-response, the MN output would not be evaluated correctly by expressing as a percentage of M_{max} because of the collision effect between an orthodromic H-reflex discharge and an antidromic motor volley within the MN axons. Therefore the MN output in each situation was calculated to include the effects of collision using Equation (1), as shown in Tables 4B, 5B, 6B, and 7B.

$$\% \text{ MN output} = \frac{\text{H-reflex amplitude}}{M_{max} - M_{recorded}} \text{ X } 100\% \tag{1}$$

The mean and corrected mean MN output values for all subjects in Tables 4-7 were compared, as shown in Tables 8-11. As expected, the differences were < 6% at the lower stimulus levels where only a few MNs are activated directly. The correction becomes more prominent for higher stimulus levels when direct stimulation of the MNs is more common (Figure 17). The differences were compared statistically using a paired two-tailed t-test. As expected, the differences were found to be statistically insignificant ($p < 0.05$). No differences in the mean MN output values were found between the two methods in each situation (paired t-test: $p < 0.05$) because normalization was employed to minimize intersubject variability. Hence, the collision effects could be ignored except at the high stimulus levels. In general, only the H: M_{max} ratios were employed as a measure of spinal cord modulation during voluntary muscle contractions in the following analysis.

Table 4A. Ia fibre input- motoneuron output relationship for the FCR muscles during relaxation of the first trial block, with the H-reflex expressed as a percentage of M_{max}

Subject		% Ia fibre input									
		10	20	30	40	50	60	70	80	90	100
Subject No. 1		3.0	6.9	13.0	21.3	30.0	38.8	46.0	51.0	54.5	54.5
Subject No. 2		2.0	4.2	6.0	8.0	9.8	11.6	13.4	15.0	16.7	17.9
Subject No. 3		3.8	7.0	10.0	11.8	13.0	15.0	18.8	26.3	40.0	63.8
Subject No. 4		5.4	8.6	10.0	10.0	9.7	9.3	9.7	11.4	15.7	23.3
Subject No. 5	% Motoneuron output	4.9	7.8	12.2	16.2	20.1	23.4	25.7	26.9	27.0	27.0
Subject No. 6		0.5	5.0	11.3	17.5	22.8	27.5	31.5	35.5	39.8	43
Subject No. 7		0	0	0	0	0	0	0.3	1.1	4.0	13.5
Subject No. 8		2.3	3.3	3.9	4.0	5.1	7.8	11.9	18.8	28.9	42.5
Subject No. 9		0	1.2	1.6	1.6	1.6	2.0	2.5	3.6	5.2	8.5
Subject No. 10		2.2	3.4	3.8	3.9	3.9	4.3	5.1	6.4	8.3	11.0
Subject No. 11		4.5	7.0	8.8	9.6	10.7	12.0	14.3	17.9	22.0	27.6

Table 4B. Ia fibre input- motoneuron output relationship for the FCR muscles during relaxation of the first trial block, with the H-reflex corrected for collision effects

Subject	% Ia fibre input									
	10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Subject No. 1	3.0	6.9	13.0	21.3	30.0	38.8	47.0	54.0	58.0	60.0
Subject No. 2	2.0	4.2	6.0	8.0	10.8	13.6	16.4	19.2	22.4	25.6
Subject No. 3	3.8	7.0	10.0	11.8	13.0	15.0	18.8	26.3	41.5	67.5
Subject No. 4	5.5	8.6	10.0	10.0	9.7	9.3	9.7	11.4	16.0	24.0
Subject No. 5	4.2	7.8	11.8	16.2	21.0	24.7	26.0	26.2	27.2	34.0
Subject No. 6	0.5	5.0	11.3	17.6	23.5	28.4	32.9	36.9	41.8	49.0
Subject No. 7	0	0	0	0	0	0	0	0	1.2	18.2
Subject No. 8	2.3	3.3	3.9	4.0	5.1	7.8	11.9	19.2	30.0	45.0
Subject No. 9	0	1.2	1.6	1.6	1.6	2.0	2.5	3.6	5.2	8.5
Subject No. 10	2.2	3.4	3.8	4.0	4.0	4.3	5.0	6.4	8.8	12.4
Subject No. 11	4.5	7.0	9.0	10.0	11.0	12.0	14.0	17.8	23.0	31.0

(The y-axis label for the table above reads: % Motoneuron output)

Table 5A. Ia fibre input- motoneuron output relationship for the FCR muscles during relaxation of the second trial block, with the H-reflex expressed as a percentage of M_{max}

Subject	% Ia fibre input									
	10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Subject No. 1	1.0	1.4	2.1	2.3	4.2	4.9	7.4	9.4	11.6	14.0
Subject No. 2	9.6	16.8	22.1	25.7	28.3	29.6	30.2	30.6	30.9	31.5
Subject No. 3	0	0	0	0	0	0.6	1.7	3.8	7.7	15.2
Subject No. 4	0	2.9	3.4	4.8	5.5	5.6	5.6	5.9	8.2	14.0
Subject No. 5	4.0	5.0	6.5	9.0	12.6	17.0	22.2	28.5	35.9	43.8
Subject No. 6	2.4	4.0	5.6	7.0	8.9	11.0	14.4	19.0	25.0	32.8
Subject No. 7	1.2	2.0	2.5	2.8	3.2	4.0	5.3	7.2	9.8	13.4
Subject No. 8	4.0	6.8	9.0	10.4	11.4	12.2	13.0	14.0	15.6	17.6

(The y-axis label for the table above reads: % Motoneuron output)

Table 5B. Ia fibre input- motoneuron output relationship for the FCR muscles during relaxation of the second trial block, with the H-reflex corrected for collision effects

Subject		% Ia fibre input									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Subject No. 1		1.0	1.4	2.0	2.3	4.6	6.6	9.0	11.8	15.0	18.8
Subject No. 2	% Motoneuron output	10.9	19.0	24.5	28.1	30.3	31.5	32.2	33.0	34.5	36.2
Subject No. 3		0	0	0	0	0	0.6	1.7	3.8	7.7	15.2
Subject No. 4		0	2.9	3.4	4.8	5.5	5.6	5.6	5.9	8.2	14.0
Subject No. 5		4.0	5.0	6.5	9.0	12.6	17.8	23.2	30.0	38.0	46.5
Subject No. 6		2.4	4.0	5.6	7.0	8.9	11.0	14.4	19.0	26.0	35.0
Subject No. 7		1.2	2.0	2.5	2.8	3.2	4.0	5.0	6.8	9.8	14.2
Subject No. 8		4.2	7.1	9.0	10.4	11.2	12.0	13.0	14.5	16.8	20.0

Table 6A. Ia fibre input- motoneuron output relationship for the FCR muscles during wrist flexion contraction (first trial block), with the H-reflex expressed as a percentage of M_{max}

Subject		% Ia fibre input									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Subject No. 1		18.0	25.0	31.0	35.8	39.5	42.6	47.0	53.5	58.0	60.0
Subject No. 2		5.5	10.0	13.6	16.6	18.8	20.0	20.1	20.1	20.1	20.1
Subject No. 3	% Motoneuron output	0	0	2.5	8.0	15.0	22.3	28.0	32.8	35.0	35.0
Subject No. 4		22.1	28.4	32.1	34.3	35.0	36.6	39.0	42.4	48.6	57.6
Subject No. 5		14.0	17.3	20.0	25.2	31.8	37.0	39.0	39.0	42.8	50.0
Subject No. 6		19.0	22.0	24.0	33.0	45.5	56.0	61.2	63.0	63.0	63.0
Subject No. 7		6.0	10.0	12.8	13.5	13.5	14.0	16.0	20.2	28.0	39.0
Subject No. 8		0.5	5.0	12.2	21.9	32.2	42.5	51.3	57.9	60.9	60.9
Subject No. 9		2.2	4.3	6.0	7.4	8.3	8.4	8.8	10.0	13.6	21.6
Subject No. 10		7.6	9.0	9.2	9.2	9.4	10.0	11.2	12.8	14.4	15.4
Subject No. 11		19.1	25.0	27.6	28.9	30.1	32.5	35.8	39.2	41.5	41.5

Table 6B. Ia fibre input- motoneuron output relationship for the FCR muscles during wrist flexion contraction (first trial block), with the H-reflex corrected for collision effects

Subject		**% Ia fibre input**									
		10	20	30	40	50	60	70	80	90	100
Subject No. 1		19.0	27.0	33.0	37.2	41.0	44.0	47.8	52.0	58.0	66.0
Subject No. 2		6.6	11.1	14.8	18.1	20.4	22.8	24.2	24.9	25.2	25.2
Subject No. 3		0	0	2.5	8.0	15.0	22.3	28.0	32.8	35.0	35.0
Subject No. 4		23.2	30.0	34.0	36.0	37.6	38.4	41.0	46.0	54.0	65.6
Subject No. 5	% Motoneuron output	14.0	18.1	21.0	25.0	31.0	37.0	40.8	41.8	41.8	45.0
Subject No. 6		20.0	23.2	27.3	37.0	50.0	62.5	70.0	70.0	70.0	76.0
Subject No. 7		6.0	10.0	12.8	14.0	14.2	15.0	16.9	21.0	29.0	41.5
Subject No. 8		0.8	5.5	13.2	23.0	33.5	44.0	53.5	60.5	64.0	64.0
Subject No. 9		2.0	4.3	6.4	7.7	8.4	8.4	8.7	10.0	14.0	22.0
Subject No. 10		7.6	9.0	9.4	9.4	9.4	10.0	11.2	12.8	14.8	16.8
Subject No. 11		19.9	25.6	28.0	29.0	30.9	33.8	37.8	41.8	44.0	44.0

Table 7A. Ia fibre input- motoneuron output relationship for the FCR muscles during wrist extension contraction (second trial block), with the H-reflex expressed as a percentage of M_{max}

Subject		% Ia fibre input									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Subject No. 1		1.0	1.4	1.9	2.3	2.8	3.5	4.6	6.0	8.1	10.6
Subject No. 2		3.6	6.4	9.0	11.1	12.6	13.9	14.5	14.8	14.8	14.8
Subject No. 3		0	0	0	0	0.2	0.4	0.8	1.2	2.1	3.8
Subject No. 4	% Motoneuron output	1.9	3.0	3.4	3.4	3.4	3.4	3.4	3.8	5.0	6.9
Subject No. 5		2.3	4.9	6.1	6.5	6.0	4.9	4.2	6.2	13.2	27.5
Subject No. 6		1.2	2.3	3.2	4.0	5.0	5.0	5.2	5.3	5.3	5.3
Subject No. 7		0	0.2	0.6	1.0	1.2	1.4	1.8	2.0	3.0	5.0
Subject No. 8		2.2	3.1	3.1	3.1	3.1	3.1	3.8	5.6	8.6	13.2

Table 7B. Ia fibre input- motoneuron output relationship for the FCR muscles during wrist extension contraction (second trial block), with the H-reflex corrected for collision effects

Subject		% Ia fibre input									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Subject No. 1		1.2	2.0	2.4	2.6	2.9	3.6	4.8	7.0	10.0	14.4
Subject No. 2		3.6	7.0	9.8	12.0	13.8	15.0	16.0	16.2	16.2	16.2
Subject No. 3		0	0	0	0	0.2	0.4	0.8	1.2	2.1	3.8
Subject No. 4	% Motoneuron output	1.9	3.0	3.4	3.4	3.4	3.4	3.4	3.8	5.0	6.85
Subject No. 5		2.3	4.9	6.1	6.5	6.0	4.9	4.2	6.2	14.0	29.0
Subject No. 6		1.0	2.0	3.2	4.0	5.0	5.6	5.9	6.0	6.0	6.0
Subject No. 7		0	0.2	0.6	1.0	1.2	1.4	1.8	2.0	3.0	4.8
Subject No. 8		2.4	3.0	3.0	3.0	3.0	3.0	3.8	5.8	9.2	14.7

Table 8. Mean and corrected mean (± S.E.M.) data for the Ia fibre input- MN output for the FCR muscles during relaxation of the first trial block

| | | % Ia fibre input | | | | | | | | | |
		10	20	30	40	50	60	70	80	90	100
% Motoneuron output	**Mean**	2.6	4.9	7.3	9.4	11.5	13.8	16.3	19.4	23.8	30.2
	± S.E.M.	0.6	0.9	1.4	2.1	2.8	3.6	4.1	4.5	4.9	5.5
	Corrected mean	2.5	4.9	7.3	9.5	11.8	14.2	16.7	20.1	25.0	34.1
	± S.E.M.	0.6	0.8	1.3	2.1	2.9	3.6	4.2	4.7	5.2	5.8
	Difference	0.1	0.0	0.0	0.1	0.3	0.4	0.4	0.7	1.2	3.9

Table 9. Mean and corrected mean (± S.E.M.) data for the Ia fibre input- MN output for the FCR muscles during relaxation of the second trial block

| | | % Ia fibre input | | | | | | | | | |
		10	20	30	40	50	60	70	80	90	100
% Motoneuron output	**Mean**	2.8	4.9	6.4	7.8	9.3	10.6	12.5	14.8	18.1	22.8
	± S.E.M.	1.1	1.9	2.5	2.8	3.1	3.3	3.4	3.6	3.9	4.1
	Corrected mean	2.9	5.2	6.7	8.1	9.5	11.1	13.0	15.6	19.5	25.0
	± S.E.M.	1.3	2.1	2.7	3.1	3.3	3.5	3.6	3.9	4.2	4.4
	Difference	0.1	0.3	0.3	0.3	0.2	0.5	0.5	0.8	1.4	2.2

Table 10. Mean and corrected mean (± S.E.M.) data for the Ia fibre input- MN output for the FCR muscles during voluntary wrist flexion contraction

		% Ia fibre input									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
% Motoneuron output	**Mean**	10.4	14.2	17.4	21.3	25.4	29.3	32.5	35.5	38.7	42.2
	± S.E.M.	2.5	2.9	3.1	3.3	3.9	4.6	5.2	5.5	5.5	5.3
	Corrected mean	10.8	14.9	18.4	22.3	26.5	30.7	34.5	37.6	40.9	45.6
	± S.E.M.	2.6	3.1	3.3	3.5	4.2	5.0	5.7	5.8	5.8	6.0
	Difference	0.4	0.7	1.0	1.0	1.1	1.4	2.0	2.1	2.2	3.4

Table 11. Mean and corrected mean (± S.E.M.) data for the Ia fibre input- MN output for the FCR muscles during voluntary wrist extension contraction

		% Ia fibre input									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
% Motoneuron output	**Mean**	1.5	2.7	3.4	3.9	4.3	4.5	4.8	5.6	7.5	10.9
	± S.E.M.	0.4	0.8	1.0	1.2	1.4	1.5	1.5	1.5	1.6	2.8
	Corrected mean	1.6	2.8	3.6	4.1	4.4	4.7	5.1	6.0	8.2	12.0
	± S.E.M.	0.4	0.8	1.1	1.3	1.5	1.6	1.7	1.6	1.8	3.0
	Difference	0.1	0.1	0.2	0.2	0.1	0.2	0.3	0.4	0.7	1.1

4.7   Intersubject Control Variability

Inspection of the scatter plots of MN output versus Ia fibre input curves clearly revealed that the relation between the two variables had three distinct shapes among the subjects in the control situations. The various shapes of the curves presented in Figure 18 demonstrated the issue of variability of the Ia fibre input- MN output relationship obtained in the resting state of the first trial block (wrist flexion protocol) in which 11 subjects were tested, as shown in Tables 4A-B. The individual relaxation curves were either (i) initially steeply-rising ('convex'), indicating a substantial fraction of low-threshold MNs (1 subject); (ii) initially slowly-rising ('concave'), indicating that most MNs appeared to form a high-threshold population (5 subjects); or (iii) initially and latterly steeply-rising ('complex'), indicating the presence of low-threshold and separate high-threshold MN pools (4 subjects).
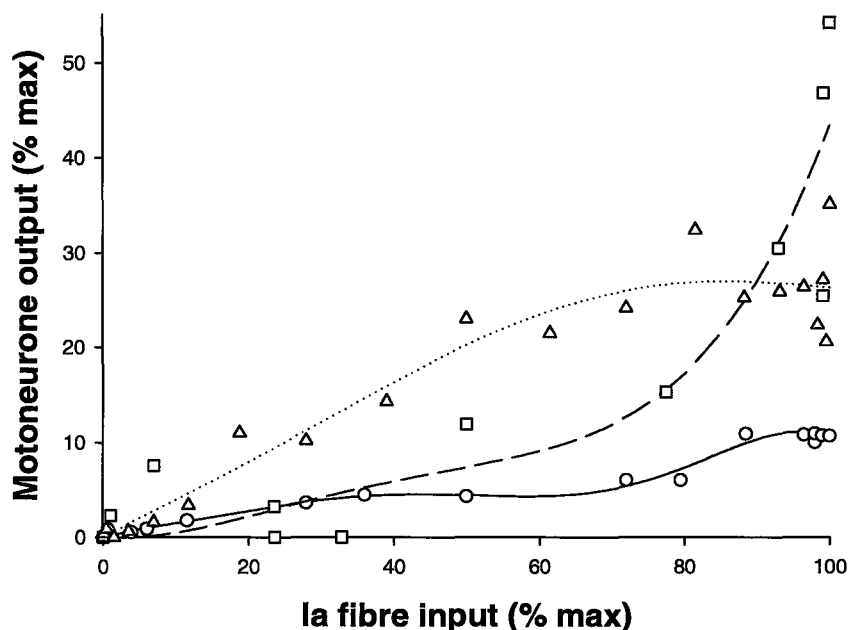
Figure 18. Typical forms of the Ia fibre input- MN output relationship of the FCR muscles obtained in the control (relaxed) situation, addressing the issue of variability of the relationship. (Δ) represents a 'convex'shape, with an initially steeply-rising segment; (□) represents a 'concave' shape, with an initially slowly-rising segment; (o) represents a 'complex' shape, with both the initially and latterly steeply-rising segments.

Among the 11 subjects, 8 subjects were tested in the second trial block (wrist extension protocol), as shown in Tables 5A-B. The relaxation curves in 7 of the 8 subjects were similar in shape with the ones in the first trial block, with little changes observed. In one subject, however, the results could not be classified. The relaxation curve with a steeply-rising initial segment only in the first trial block appeared to include a steeply-rising latter segment in the second trial block, as shown in Figure 19. It suggests the presence of an additional subpopulation of high-threshold MNs in the latter case.
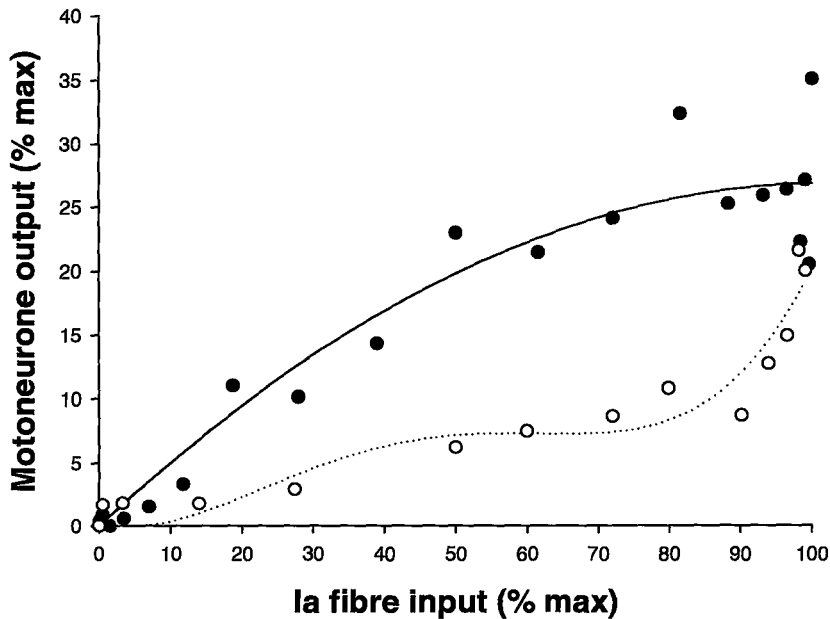
Figure 19. The Ia fibre input- MN output relationship obtained in the resting states of the first trial block and the second trial block in the same subject (60-year old man). (●) represents the Ia fibre input- MN output relationship in the relaxed state of the first trial block; (○) represents the Ia fibre input- MN output relationship in the relaxed state of the second trial block.

Comparison of the two extreme cases during the resting state in Figure 20 indicates that 70% of the Ia fibres were required to elicit 0.3% of the MNs in one case, and 46% of the MNs were discharged with the same number of Ia fibres activated in the other case. The value of MN output discharging at the same level of Ia fibre input was essentially different for all the subjects. The high MN output value in the hyper-reflexive subject was due to numerous possible built-in mechanisms acting at the spinal level, which could contribute to the significant spatial synaptic effects on MNs. In the hypo-reflexive subject, it appeared that failure to elicit a reflex response, even when half of the Ia fibre population was activated, was probably due to high sensitivity to various

inhibitory mechanisms that would reduce the efficiency of the synaptic effects on MNs,

or very little spatial spread of the Ia afferent terminals in the MN pool.
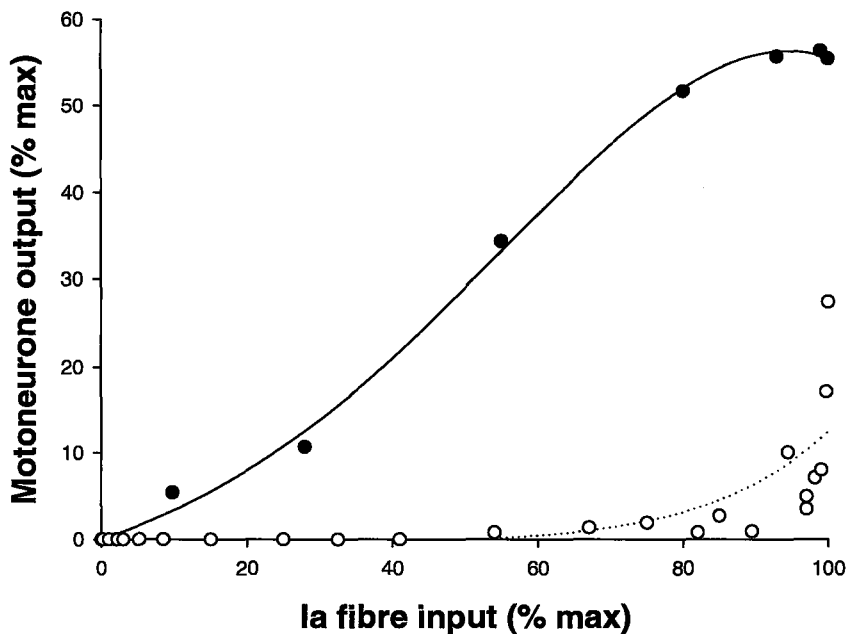
Figure 20. Ia fibre input- MN output relationships of the flexor carpi radialis muscles in two subjects during relaxation. (●) represents the Ia fibre input- MN output relationship in a 25-year old woman; (o) represents the Ia fibre input- MN output relationship in a 24-year old man.

The inter-stimulus interval in the experiments involving electrical stimuli varied

between 3 and 10 s. There was also a 3-5 min rest period between the trial blocks. These

intervals and the rest period were used to minimize the effect of fatigue from the

repetitive electrical stimulation. In addition, the stimulus strengths delivered to the

subject, and the order of required action types performed by the subject at the same

stimulus strength were pseudorandom and equi-probable to eliminate any anticipatory

effects. Regular alteration might produce erroneous results. This prevented the subject from voluntarily altering the reflex responses.

The subjects were requested to avoid visually monitoring that might interfere with their state of mental relaxation. By the same reasoning, the mental task of matching the target line to the display of their actual force exerted on an oscilloscope was eliminated from the experiments. The induced influence from the descending pathway might facilitate unexpected changes in the spinal cord excitability. An additional level of complexity was added when the duration of the studies was too long. The point to note was the significant inconsistency of reflex amplitudes when the subject had to perform a prolonged experiment.

4.8   Mean Input-Output Relationship in the Resting State

The relaxation results presented in this section were obtained from the 11 subjects in the resting state of the first trial block (wrist flexion protocol), and the 8 subjects during relaxation of the second trial block (wrist extension protocol) in whom their values of MN output were averaged. The mean input-output relationship appears to be more linear as shown in Figures 21-22. The combination of the various forms of the curves (Figure 18) led to a more linear relationship with a shallow slope, such that 82% of the Ia fibre input was required to be stimulated synchronously in order to discharge approximately 20% of the MN output (Figure 21). In general, the amplitude of the MN output increased linearly with increasing Ia fibre input, however the steepness of the input-output relationship was higher when a large percentage of Ia fibre population was

activated. This was probably because the decline in the inhibitory feedback which often

appears at high Ia fibre input contributed to an increased efficiency of synaptic effects on

MNs. Another reason could be that the Ia fibres have their terminal branches on a large

number of MNs but that it requires the input of a large number of Ia fibre single pulses to

depolarize these MNs past the thresholds.
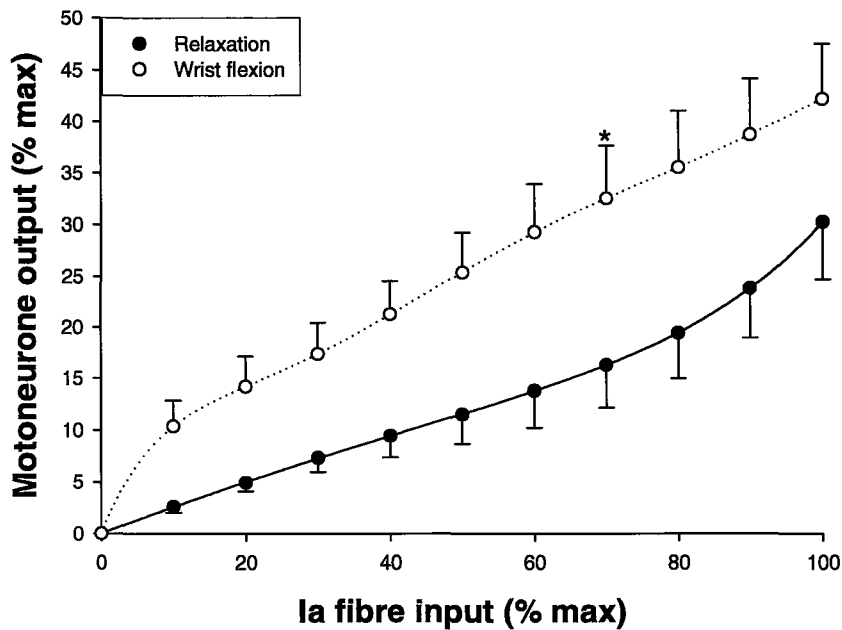
Figure 21. Averaged data (n=11) obtained in the control and conditioned situations of the
first trial block. * indicates the largest increase of the mean motoneuron output values
during wrist flexion compared to those in the relaxed state.
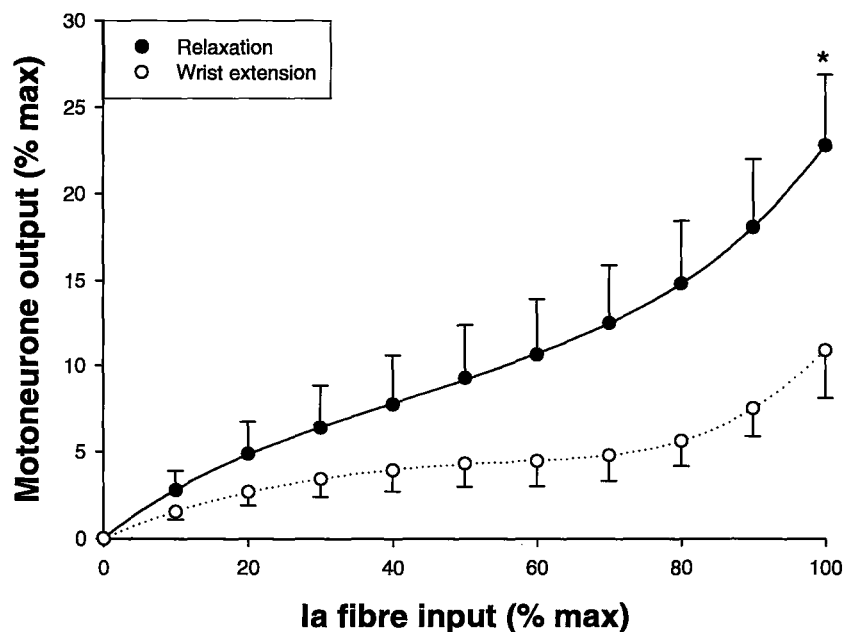
Figure 22. Averaged data (n=8) obtained in the control and conditioned situations of the second trial block. * indicates the largest decrease of the mean motoneuron output values during wrist extension compared to the ones in relaxed state.

The mean values of MN output over the full range of Ia fibre input during the resting state were calculated for each trial block (Tables 8-9). There was no statistical difference (paired t-test: $p < 0.01$) between the two mean input-output relaxation curves. The differences between the two sets of the MN output mean values ranged from 0% to 7.4% (Table 12). The mean relaxation curves in the two trial blocks were compared to exclude the possibility that the observed changes in the reflex excitability were influenced by factors other than the voluntary contractions of wrist muscles.

Table 12. Mean (± S.E.M.) data for the Ia fibre input- MN output for the FCR muscles in the control situations of the two trial blocks, with the H-reflex expressed as a percentage of M_{max}

		% Ia fibre input									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
% Motoneuron output	**Relaxed mean (flexion)**	2.6	4.9	7.3	9.4	11.5	13.8	16.3	19.4	23.8	30.2
	± S.E.M.	0.6	0.9	1.4	2.1	2.8	3.6	4.1	4.5	4.9	5.5
	Relaxed mean (extension)	2.8	4.9	6.4	7.8	9.3	10.6	12.5	14.8	18.1	22.8
	± S.E.M.	1.1	1.9	2.5	2.8	3.1	3.3	3.4	3.6	3.9	4.1
	Difference	0.2	0.0	0.9	1.6	2.2	3.2	3.8	4.6	5.7	7.4

The following section is divided into two parts. In the first part we describe the quantitative relationship between the Ia fibre input and FCR MN output in the relaxed state and voluntary contraction of the wrist flexor at 5-10% MVC. In the second part, the relationship between the Ia fibre input and FCR MN output in the relaxed state and voluntary contraction of the wrist extensor at 5-10% MVC is presented. The input-output relationship reflects a quantitative change in the reflex excitability as a function of the requirements of two different types of voluntary wrist movements.

4.9   Voluntary Wrist Flexion

In general, the H-reflex potentials in the FCR muscles were increased during the

wrist flexion contractions than during relaxation at the same stimulus strength, whereas

the M-wave did not change significantly, as seen in Figures 23A-B.

A.
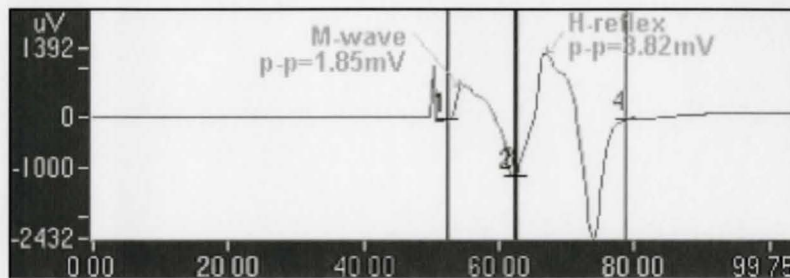
B.
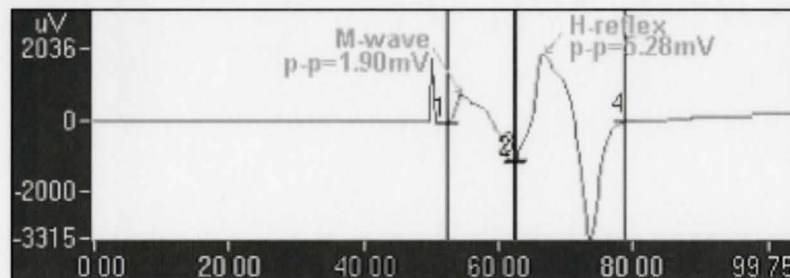
Figure 23. Recordings and measurements of the muscle responses during relaxation and
voluntary contractions of the wrist flexor in a 24-year old man using Labview **A.** The
upper waveform represents the raw muscle signals during relaxation. **B.** The lower
waveform represents the raw muscle signals during voluntary contractions of the wrist
flexor.

Figure 21 presents the mean values of MN output calculated using the results of

11 subjects tested in the control and conditioned situations of the first trial block. The

averaged Ia fibre input- MN output curves in each situation appeared to be more linear

than the individual curves in each subject. During wrist flexion contraction, an increase

of the MN output was present throughout the entire range of Ia fibre inputs, from the threshold of the most excitable Ia fibres to that of the least excitable Ia fibres. All mean MN outputs were significantly larger (paired t-test: $p < 0.001$) compared with those obtained at rest, ranging from the increase of 7.8% with 10% of Ia fibres activated, to 16.2% with 70% of Ia fibres activated (Table 13).

In Figure 21, the means were plotted with their standard error bars rather than the more common standard deviation. This was necessary because of the large standard deviation bars resulting from the different control strategies exhibited by the 11 subjects as discussed earlier. The relative maximum facilitatory effect of the mean MN output with wrist flexion was approximately 298.6% when 10% of the Ia fibres were synchronously activated. The relative effect was expressed as a percentage of the control reflex amplitude. For comparably small amplitudes of the control reflexes, the relative facilitatory effects on the H-reflexes were highly significant.

Table 13. Mean ($\pm$ S.E.M.) data for the Ia fibre input- MN output for the FCR muscles in the control and conditioned situations of the first trial block, with the H-reflex expressed as a percentage of M_{max}. All absolute differences are statistically significant ($p < 0.001$).

		% Ia fibre input									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
% Motoneuron output	**Mean (relaxation)**	2.6	4.9	7.3	9.4	11.5	13.8	16.3	19.4	23.8	30.2
	$\pm$ S.E.M.	0.6	0.9	1.4	2.1	2.8	3.6	4.1	4.5	4.9	5.5
	Mean (wrist flexion)	10.4	14.2	17.4	21.3	25.4	29.3	32.5	35.5	38.7	42.2
	$\pm$ S.E.M.	2.5	2.9	3.1	3.3	3.9	4.6	5.2	5.5	5.5	5.3
	Absolute difference	7.8	9.3	10.1	11.9	13.9	15.5	16.2	16.1	14.9	12.0
	Relative difference	298.6	189.8	138.4	126.6	120.9	112.3	99.4	83.0	62.6	39.7

In all the subjects but one, facilitation of the H-reflex was observed over the full range of Ia fibre inputs during wrist flexion. This subject, however, exhibited a depression of the H-reflex at the high-threshold end of the input- output curve.

Figure 24 illustrates a typical example of facilitation of the H-reflex with voluntary contraction of the wrist flexor in a subject. The peak-to-peak amplitudes of M-waves were statistically similar between the relaxation and voluntary muscle contractions (less than $\pm$ 5%). This allows us to conclude that there was no significant change in the median nerve stimulation geometry between the two motor tasks. The FCR M-responses between the two motor tasks in this subject are illustrated in Figure 25. In this figure, many of the M-wave responses during relaxation were identical to the responses during

wrist flexion contraction, especially at high stimulus intensities, therefore were hidden in Figure 25. With the similarity of the M-responses between the two tasks at the same stimulus strength, the MN outputs were significantly higher (paired t-test: $p < 0.001$) at all Ia fibre input levels during wrist flexion than during relaxation. These results would indicate that the increase in the excitability of the spinal MN pool or recruitment gain of the MNs was related to the voluntary contractions of the wrist flexors. The MN output values increased in the range between 4.4% and 6.4% during wrist flexion, with the control reflex amplitudes ranging from 2.2% to 11.0% (Table 14).

The threshold of the most excitable Ia fibres in this subject was lowered by 1.0 mA in the resting state of the second trial block compared to that of the first trial block. This was probably related to an increase in the electrogenic sodium pump activity resulting from the repetitive stimulation. In any case, it was clear that the change of reflex threshold was relatively small. We confirmed the stability of the conditions of stimulation and recording by measuring the M_{max} again after the two trial blocks were completed. In this subject, it was found that the M_{max} before and after the experiment were similar (less than $\pm$ 5%). Although the data from another subject did have a wide variation, there were no statistical differences in the M_{max} between the two tasks in the same subject, affirming the stability of the conditions in the subjects investigated.
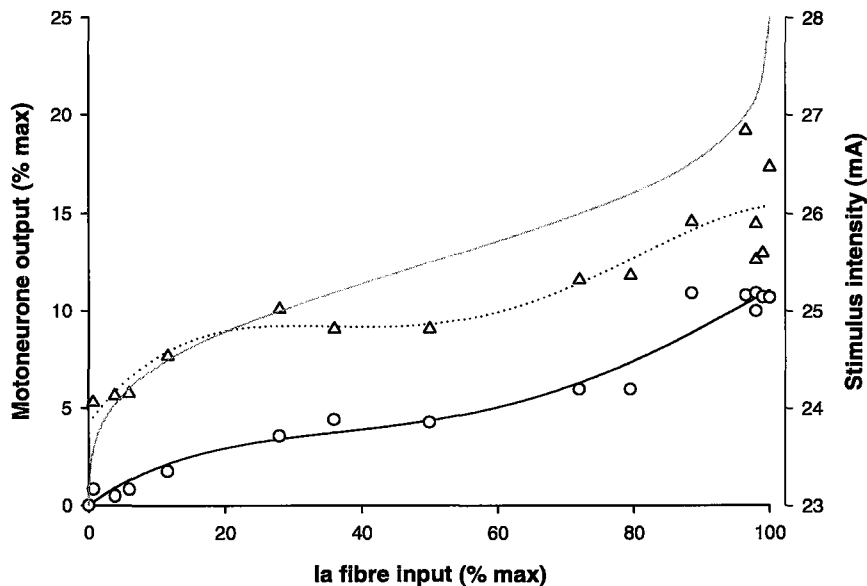
Figure 24. Representative example of potentiation of the Ia fibre input- motoneuron output relationship in a 24-year old man. (o) represents the Ia fibre input- motoneuron output relationship in the relaxed state, with the H-reflex expressed as a percentage of the maximal motor response; (Δ) represents the Ia fibre input- motoneuron output relationship during the wrist flexion; (–) represents a normal distribution of Ia fibre thresholds between the threshold of the most excitable Ia fibres to that of the least excitable Ia fibres.

Figure 25. Peak-to-peak amplitudes of the flexor carpi radialis direct motor potentials to electrical stimulation in a 24-year old man. (●) represents the flexor carpi radialis motor responses, expressed as a percentage of maximal motor response, during the resting state in the first trial block; (o) represents the flexor carpi radialis motor responses, expressed as a percentage of maximal motor response, during voluntary muscle contractions in the first trial block.

Table 14. H-reflexes for the Ia fibre input- MN output for the FCR muscles in the control and conditioned situations of the first trial block in a 24-year old man, with the H-reflex expressed as a percentage of M_{max} respectively. All absolute differences are statistically significant ($p < 0.001$).

		10	20	30	40	50	60	70	80	90	100
% Motoneuron output	**Relaxation**	2.2	3.4	3.8	3.9	3.9	4.3	5.1	6.4	8.3	11.0
	Wrist flexion	7.6	9.0	9.2	9.2	9.4	10.0	11.2	12.8	14.4	15.4
	Absolute	5.4	5.6	5.4	5.3	5.5	5.7	6.1	6.4	6.1	4.4
	Relative	245.5	164.7	142.1	135.9	141.0	132.6	119.6	100.0	73.5	40.0

*(Table header: **% Ia fibre input**)*

4.10   Voluntary Wrist Extension

The H-reflex potential amplitudes were decreased during the wrist extension contractions of the FCR muscles from those during relaxation at the same stimulus intensity, whereas the M-waves did not change significantly, as seen in Figures 26A-B.

A.
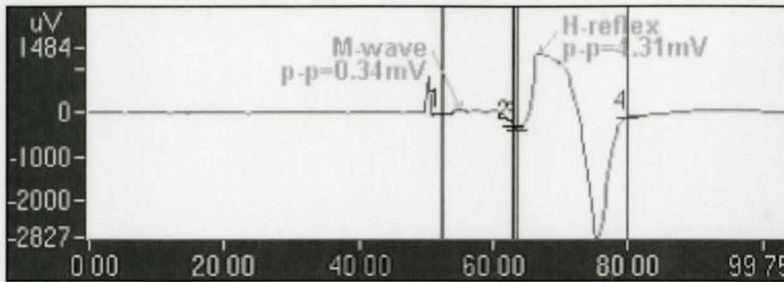
B.
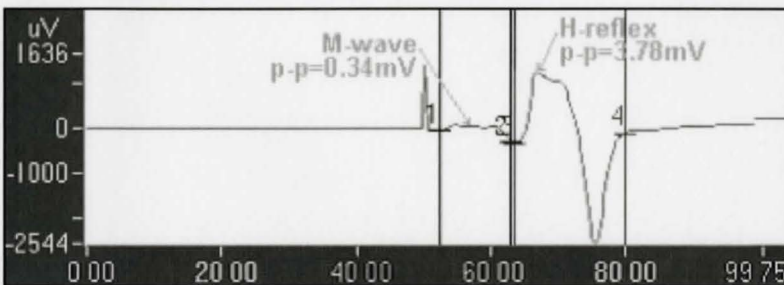
Figure 26. Recordings and measurements of the muscle responses during relaxation and voluntary contractions of the wrist extensor in a 28-year old man using Labview. **A.** The upper waveform represents the raw muscle signals during relaxation. **B.** The lower waveform represents the raw muscle signals during voluntary contractions of the wrist extensor at the same stimulus level.

The MN outputs of all 8 subjects during relaxation and voluntary wrist extension contractions were averaged and compared as shown in Table 15 and Figure 22. During wrist extension contractions, the MN outputs decreased from the level corresponding to the resting state over the full range of Ia fibre inputs. A steep rise in the mean input-output curve was appeared during both the relaxation and wrist extension at the high-threshold values of Ia fibres. This is readily apparent in Figure 22. The mean MN outputs were significantly smaller (paired t-test: $p < 0.001$) compared with those obtained at rest at all levels of Ia fibre input, ranging from a reduction of 1.3% with 10% of Ia

77

fibres activated, to 11.9%, with all the Ia fibres activated (Table 15). In Figure 22, the

means were plotted with their standard error bars rather than the more common standard

deviation. This was necessary because of the large standard deviation bars resulting from

the different control strategies exhibited by the 8 subjects as discussed earlier. The

relative maximum inhibitory effect of the mean MN output was approximately 62.1%

when 80% of Ia fibres were activated. In contrast to the results for wrist flexion

contractions, the relative differences were roughly uniform over the entire range of Ia

fibre inputs.

Table 15. Mean ($\pm$ S.E.M.) data for the Ia fibre input- MN output for the FCR muscles in the control and conditioned situations of the second trial block, with the H-reflex expressed as a percentage of M_{max}. All absolute differences are statistically significant ($p < 0.001$).

		% Ia fibre input									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
% Motoneuron output	**Mean (relaxation)**	2.8	4.9	6.4	7.8	9.3	10.6	12.5	14.8	18.1	22.8
	$\pm$ S.E.M.	1.1	1.9	2.5	2.8	3.1	3.3	3.4	3.6	3.9	4.1
	Mean (wrist extension)	1.5	2.7	3.4	3.9	4.3	4.5	4.8	5.6	7.5	10.9
	$\pm$ S.E.M.	0.4	0.8	1.0	1.2	1.4	1.5	1.5	1.5	1.6	2.8
	Absolute difference	-1.3	-2.2	-3.0	-3.9	-5.0	-6.1	-7.7	-9.2	-10.6	-11.9
	Relative difference	-46.4	-44.9	-46.9	-50.0	-53.8	-57.5	-61.6	-62.1	-58.6	-52.2

In all 8 subjects, an inhibition of the H-reflex was observed over the full range of

Ia fibre inputs. An example of an inhibition of the H-reflex with voluntary contraction of

the wrist extensor in a subject is shown in Figure 27. There were no significant

differences of the FCR direct M-responses between the contractions of the wrist extensor

and relaxation in this subject (less than $\pm$ 5%), as shown in Figure 28. The results

confirm that the stimulating and recording conditions were similar between the two tasks.

With the similarity of the M-responses, the MN outputs were significantly lower during

wrist extension than during relaxation at the same Ia fibre input level (paired t-test: $p <$

0.001). The reflex threshold differed by 0.5 mA between the resting states of the two

trial blocks. Table 16 shows the quantified data for this subject. For this subject, the

absolute and relative difference between the two states increased with synchronous

activation of the Ia fibres. The MN outputs decreased in the range between 0% and

11.4%, with the control reflex amplitudes ranging from 0% to 15.2%.

Figure 27. Representative example of depression of the Ia fibre input- motoneuron output relationship in a 25-year old man. (o) represents the Ia fibre input- MN output relationship in the relaxed state, with the H-reflex expressed as a percentage of the maximal motor response; (∆) represents the Ia fibre input- motoneuron output relationship during wrist extension; (–) represents a normal distribution of Ia fibre thresholds between the threshold of the most excitable Ia fibres to that of the least excitable Ia fibres.
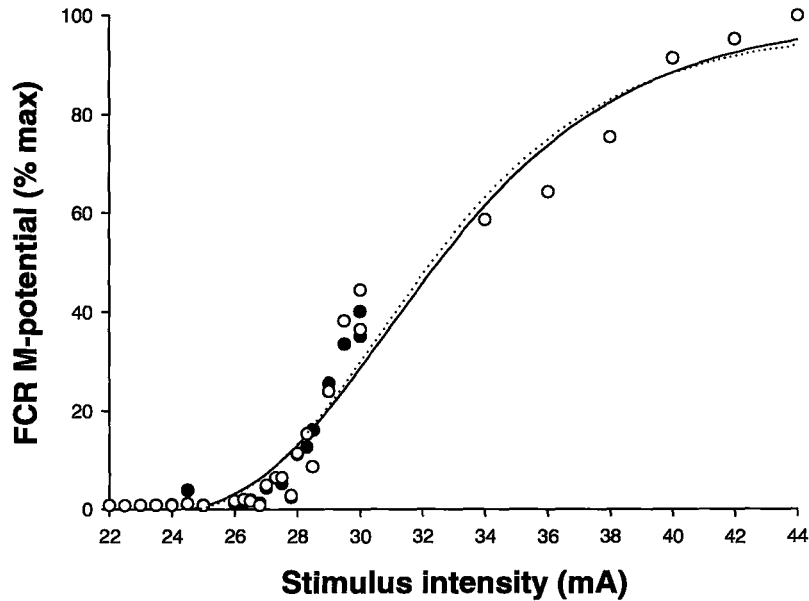
Figure 28. Peak-to-peak amplitudes of the flexor carpi radialis direct motor potentials to electrical stimulation in a 25-year old man. (●) represents the flexor carpi radialis motor responses, expressed as a percentage of maximal motor response, during the resting state in the first trial block; (o) represents the flexor carpi radialis motor responses, expressed as a percentage of maximal motor response, during the voluntary muscle contractions in the second trial block.

Table 16. H-reflexes for the Ia fibre input- MN output for the FCR muscles in the control and conditioned situations of the second trial block in a 25-year old man, with the H-reflex expressed as a percentage of M_{max}. All absolute differences are statistically significant ($p < 0.001$).

						% Ia fibre input				
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
% Motoneuron output	**Relaxation**	0.0	0.0	0.0	0.0	0.0	0.6	1.7	3.8	7.7	15.2
	Wrist extension	0.0	0.0	0.0	0.0	0.2	0.4	0.8	1.2	2.1	3.8
	Absolute	0.0	0.0	0.0	0.0	0.2	-0.2	-0.9	-2.6	-5.6	-11.4
	Relative	----	----	----	----	----	-33.3	-52.9	-68.4	-72.7	-75.0

With either voluntary muscle contractions of the wrist flexors or wrist extensors, the slope of the mean input-output curves changed over a range of Ia fibre inputs, as shown in Figures 21-22. One salient feature of the curves, which occurred during relaxation and more prominently during wrist extension, was a steep slope at the high end of the Ia fibre thresholds. At the larger Ia fibre inputs, a small addition of synchronous activation of Ia fibres contributed to a larger increase of MN discharges. During wrist extension, the steepening of the relation peaked at about 90-100% of Ia fibre inputs. This steep slope attained with high Ia fibre inputs was probably due to a decline in the negative feedback mechanism when the activation of Ia fibres was very high.

4.11   Flexor Carpi Radialis Motor Unit Number Estimation

The number of MUs in a human FCR muscle was estimated using a manual counting system based on the assumption that each increment in the M-response is produced by the excitation of an additional MU. The incremental M-responses were elicited by the graded electrical stimulation of the median nerves. The stimulus intensity was slowly increased from the subthreshold level until a small, all-or-none M-response was evoked. The stability of this response was established by observing the almost identical response a few times. The intensity was slowly increased until the response increased in an incremental fashion. This process was repeated for a total of 10-15 increments. After 10-15 increments were obtained, a M_{max} was evoked. The averaged amplitude of the single MU action potential was calculated by dividing the number of increments into the peak-to-peak amplitude of the corresponding incremental M-

response. This value was divided into the peak-to-peak amplitude of the M_{max} to yield the MU number (McComas et al. 1971, 1991). The incremental stimulation technique was performed in 2 subjects. The mean number of FCR MUs obtained was 134 ± 6. It was known that the number of FCR MNs is the same as the number of MUs, this implies that the mean number of MNs in a human FCR muscle is in the range between 128 and 140.

CHAPTER 5

DISCUSSION

5.1   Methodological Implications

Early experiments by some researchers were performed by adjusting the reflex amplitudes in the control situations to obtain comparable effects on the monosynaptic reflexes of the soleus and quadriceps muscles in various conditioned situations (Crone et al. 1990; Hultborn et al. 1987). The procedure becomes less valid because the amount of facilitation or inhibition is determined independent of the control reflex amplitudes. Though the relative changes in reflex sizes can be determined using this procedure, it provides no knowledge of the changes in the Ia fibre inputs involved.

The present study uses a novel method – "the threshold method" – to determine the reflex excitability of the FCR muscles to the Ia fibre inputs (De Bruin et al. 2005). An assumption of a normal distribution of the Ia fibre stimulation thresholds about the mean was employed to characterize the Ia fibre input- MN output relationship of the FCR muscles. This supposition was supported by evidence of the roughly normal distribution of digital nerve and FCR M-potentials to electrical stimulations of various intensities (Figures 16A-B).

De Bruin et al. (2005), in their study of the reflex properties of the soleus muscle, also found that the assumption of a normal distribution of Ia fibre thresholds is reasonable. An obvious question in the experimental procedure and analysis is whether we are stimulating the Ia afferent fibres only. Another group of sensory fibres, the Ib

fibres, originating from sensors in the tendon, are also present in the median nerve. Since they have similar diameters to the Ia fibres, they could also be stimulated by the same amplitude pulses as for the Ia fibres. Their input to the MNs through interneurons is inhibitory, and this could affect the Ia fibre input- MN output relationship. In experiments using supramaximal stimulation of the Ia fibres in the soleus muscles, more subjects appeared to exhibit a satisfactory separation of the thresholds of the Ia and Ib fibres. In a few others, a depression in the MN excitability at very high stimulus intensities was observed which could be attributed to the activation of Ib afferent fibres. However the effect of Ib contamination is likely to be negligible, occurring at stimulus intensities well beyond the testing stimulus intensity range used in the experiment. In addition, as shown by Figure 29, the peak-to-peak amplitude of the H-reflex in a single FCR MU is little affected by the longer latency Ib inhibitory input. This experiment demonstrated that contribution of the Ib inhibitory pathways to changes in the FCR H-reflex can be insignificant. Finally, even though the Ib fibres could be excited by the same pulse that stimulates the Ia fibres, the effective input threshold from the inhibitory interneuron is probably much higher than the input threshold to the monosynaptic Ia connections. Altogether, assumption of the normal distribution of Ia fibre thresholds is considered to be valid.
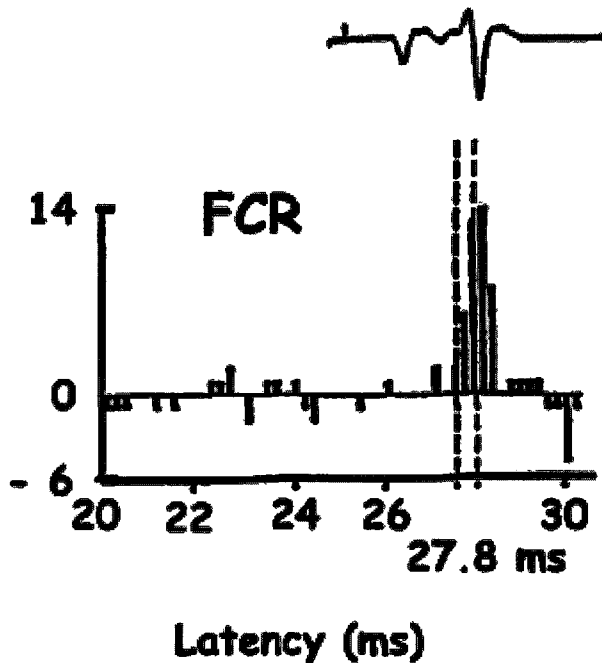
Figure 29. Peak of the monosynaptic Ia excitation in a single flexor carpi radialis unit following median nerve stimulation (0.2 ms bin width). Since during its first 0.6 ms, the Ia excitation is not contaminated by Ib inhibitory effect, the first three bins of the peak (between the two dashed lines) only depend on the size of the monosynaptic Ia excitatory postsynaptic potentials (adapted from Pierrot-Deseilligny et al. 1999).

It has been found in the present study that reflex excitability was significantly modulated positively or negatively with voluntary wrist flexion and extension contractions respectively, although variability of the H-reflexes was exhibited between subjects and even in the same subject under the same experimental conditions. Despite this variability, a model of neuronal control of the FCR muscle may give us an insight into the recruitment pattern of FCR muscles and feedback mechanisms in voluntary muscle contractions.

5.2   Recruitment Gain in the Motoneuronal Pool

A concept of MN recruitment, introduced by Kernell and Hultborn (1990), would be a good model used for the theoretical analysis of the input-output relationship within a FCR MN pool in the two situations (at rest and during voluntary FCR contractions). Although the relation between the afferent and supraspinal inputs to a MN pool is complex and involves several mechanisms, the model helps to generally illustrate the effects of voluntary muscle contractions on the recruitment gains.

The different recruitment gains are thought to be produced by the differences in synaptic distribution. The recruitment gain is defined as the slope of the input-output relation, as produced by a net inhibition as well as a net excitation (Figure 30). An increase in the recruitment gain is equivalent to an effective increase in the MNs that can be excited by that level of Ia fibre input. In contrast, a decrease in the recruitment gain is equivalent to a smaller spatial summation of afferent inputs to the MN pool. During voluntary contractions, the recruitment gain becomes significantly increased and a compression of the functional thresholds in the MN pool is hypothesized. A downward shift of the reflex thresholds was observed during voluntary contractions in the present study (Figures 21-22) in contrast to an upward shift of thresholds demonstrated by Pierrot-Deseilligny et al. (1999) in Figure 30. In the case of theoretical analysis, the slope of input-output relation is assumed to be linear for simplicity's sake. This is not unreasonable since the curves of Figures 21-22 can be approximated by straight lines.
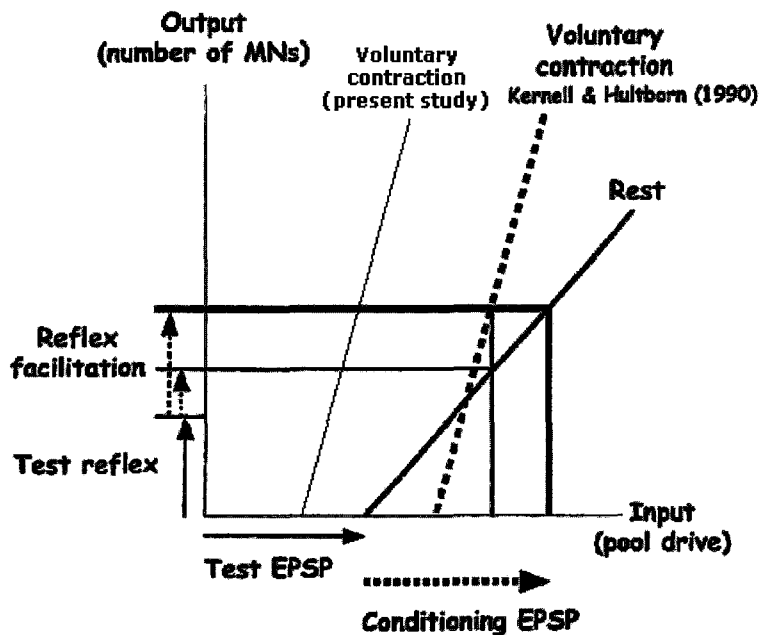
Figure 30. The input-output relation in the motoneuronal pool is represented at rest (thick continuous line) and during voluntary contraction (thick interrupted line). The thin continuous line represents a hypothetical input-output relation during voluntary contraction in the present study of flexor carpi radialis muscle (adapted from Pierrot-Deseilligny et al. 1999).

When electric stimuli were delivered to a voluntarily activated FCR muscle of a healthy subject, changes in the recruitment gains have been observed (Figures 21-22). The recruitment gain during wrist flexion contraction can result from a decline in the effectiveness of negative feedback paths or an increase in the background MN excitatory inputs, producing a reflex with high and positive feedback gains. On the other hand, an opposite effect was exhibited during wrist extension. However we expect to see a non-linearity in the spatial summation of Ia fibre inputs to FCR MN pool (Figures 21-22). The nonlinear increase in activation of the MN pool during voluntary contractions may result from a synaptic input system with an uneven distribution that leads to a differential change of recruitment thresholds for the MN population (Kernell et al. 1990). It has been

commonly agreed that it would be of importance in normal physiology to regulate the

output through an adjustment of the recruitment gain. Depending on initial load of the

muscle, an error signal from the afferent fibres produces MN correctional responses to

regulate the ongoing level of efferent activity. A simplified model of reflex control is

shown in Figure 31.
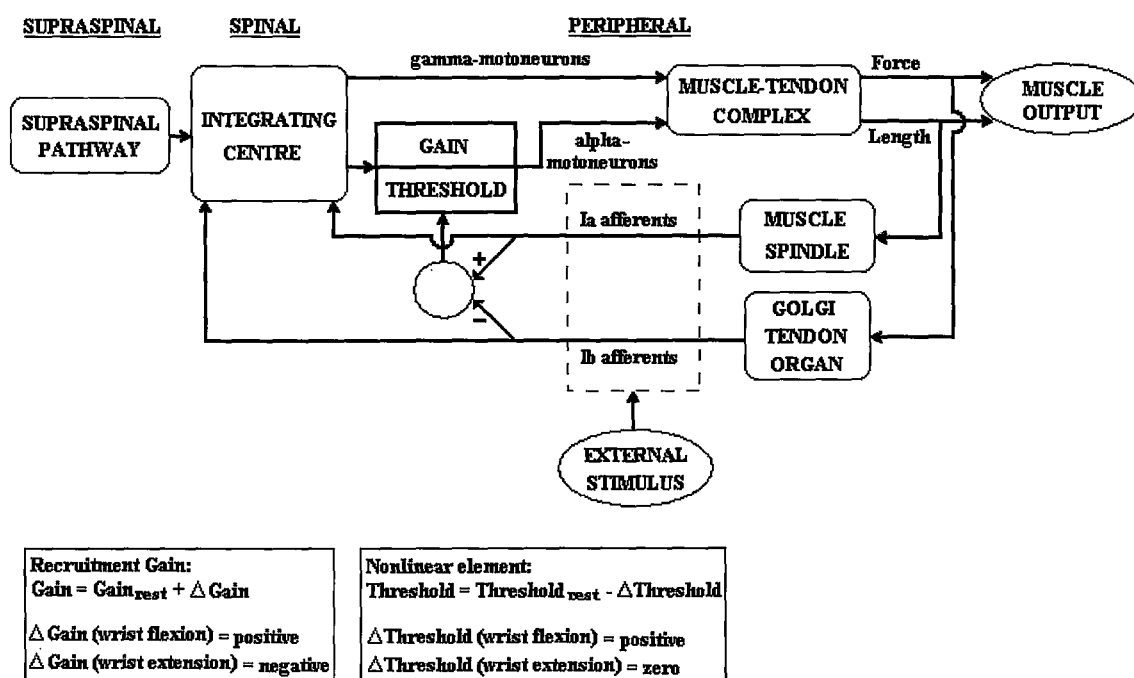
Figure 31. Steps of a reflex control system illustrating a feedback loop to produce correctional responses.

5.3   Shapes of Input-Output Relationship During Relaxation

The findings during relaxation showed the characteristics of three major forms of

the Ia fibre input- MN output relationship, thus indicating variability of the H-reflexes

among different subjects (Figure 18). The forms of the relationship were either initially

steeply-rising, initially slowly-rising, or initially and latterly steeply-rising. The three major forms of the Ia fibre input- MN output relationship have an important implication on the reflex organization of FCR MN pools. The convex shape of the input-output relationship (initially steeply-rising) is most likely due to a substantial fraction of the FCR MN population having a low threshold, while the concave shape (initially slow-rising) indicates that, in those subjects, most FCR MNs have high thresholds. Perhaps the sigmoid shape, with steeply-rising initial and latter segments in the input-output curve, could be explained by the presence of separate populations of low- and high-threshold MNs (De Bruin et al. 2005).

Interestingly, while a noticeable reflex response was evoked following an electric stimulus in one healthy subject, no reflexes were obtained in another healthy subject. In the two extreme cases, the percentage of MNs activated with 70% of Ia fibres was 46% in the subject with brisk reflexes, while in contrast, 0.3% of MNs were discharged at the same level of Ia-afferent activation in the other subject with no tendon jerks (Figure 20). Such obvious differences between subjects are not surprising due to a considerable variation of the spinal reflexes from one healthy individual to another. Generally it is easy to obtain reflexes in most subjects, however, it can be difficult or impossible to elicit them in a few others. In the literature concerning the simple reflex function, the brisk reflexes are ascribed to the synchronous activity of Ia afferents, whereas insignificant synaptic effects on MNs, regulated through the asynchronous discharges of Ia afferents, lead to the absent reflexes (Stein 1974).

The averaged percentage of the MNs discharged reflexly was a nearly linear and increasing function of the percentage of Ia fibres activated synchronously. The nearly linear form of the mean input-output relationship is a combination of variable non-linear shapes of the input-output relationship in all subjects. Therefore, although linear models can describe the mean input-output relationship, they cannot predict the responses of each individual. A linearly increasing input-output curve would indicate a recruitment of constant fractions of MNs with increasing MU potentials (Henneman 1957). This follows that MN population has a linear cumulative distribution of reflex firing thresholds.

Since the mean input-output curve is nonlinear at the high end, it must reflect that high-threshold Ia fibres recruit an increasing fraction of MNs or larger MNs. This, of course, can also be explained by the concept of the subliminal fringe (Denny-Brown et al. 1928). This concept describes how each individual Ia fibre branches has synapses on many MNs, with the MN territories for each Ia fibre are overlapping. Many Ia fibres may be providing excitatory input to many MNs but few of those MNs are depolarized past threshold. When a sufficient number of the Ia fibres with higher stimulus thresholds are excited, many of these subthreshold excited MNs are now depolarized past threshold resulting in a larger H-reflex. As well, monosynaptic reflexes depend on additional modulating influences arising from the properties of the neural circuitry. Therefore the mean input-output relationship represents a balance between excitatory and inhibitory components of the corticospinal volleys, afferent inputs, and depression of presynaptic inhibition of late-recruited MNs. De Bruin et al. (2005) also suggests that presynaptic

inhibitory mechanisms may contribute to the reflex excitability observed in the lower limb. Previous studies of voluntary muscle relaxation in FCR muscles further confirmed a possible key role of the modulation of the presynaptic inhibition in muscle relaxation (Buccolieri et al. 2003).

In the following sections, spinal reflex actions of the afferent fibres reaching MNs during voluntary wrist flexion and extension, as well as the effects of input (presynaptic inhibition) and output stage (recurrent inhibition) on their modulation will be briefly discussed.

5.4   Changes in Fusimotor Activity During Shortening and Lengthening Muscle Contractions

In our experiments, the voluntary contraction was kept low (5-10% of the MVC) and the muscle was maintained in a nearly isometric state. Furthermore the experimental Ia fibre input to the MN pool was a single synchronous pulse of a number of Ia (and perhaps Ib) fibres. However the voluntary contractions during the experiments may have resulted in small shortening or lengthening of the muscle, hence muscle spindles can alter their background activity and input to the control system. A brief discussion of the effects of active shortening or lengthening contractions on the muscle-spindle control system (fusimotor system) is therefore warranted.

Fusimotor activity may be altered in different motor tasks, partly due to variations in the descending drive and partly due to the activation of afferent fibres and their reflex pathways in various conditions. The decline of the afferent inflow from muscle spindles when the muscle is voluntarily shortened could contribute to a decrease in MN

excitability. Schieppati et al. (1985) and Nardone et al. (1985) demonstrated as expected that during voluntary shortening contractions of the soleus muscle, muscle spindles' afferent discharge rates decrease. In contrast, lengthening contractions of the muscle pulls on the muscle spindles and increases their discharge rates, thus possibly enhancing MN excitability.

Wrist flexion, or equivalently a small shortening contraction, unloads the muscle fibres, resulting in the slackness of muscle-spindle stretch receptors. Thus, fewer spindle discharges occur and consequently feedback from the stretch receptors becomes less or even absent. To prevent this, muscle spindles are activated to maintain tension on the stretch receptors. It is suggested that enhanced influences of fusimotor drives consequently causes an increase in muscle spindle outflow through α-γ coactivation (Vallbo 1971).

Literature also suggested that muscle spindle afferent inflow increased in response to muscle lengthening (Romano et al. 1987; Gregory et al. 1988). During wrist extension, or equivalently a small lengthening contraction, muscle spindle discharges are higher. A compensation for the higher discharges from spindle endings is associated with a reduced responsiveness of the fusimotor drives. It is suggested that correction of the fusimotor activity may restrict the muscle from developing excessively high forces. All information received from the muscle spindle sensory endings is relevant because the precise adjustment of MN activity requires continuous information about the changes in muscle state in order to alter the contribution of various pathways in producing an optimal tension of the muscle spindles and force output of the muscle fibres.

5.5   Mechanism Involved at the Motoneuron Input Stage – Presynaptic Inhibitory System During Muscle Contractions

In further work, possible contributions of other selected spinal neuronal systems to muscle contractions will be explored, including the activity of interneuronal networks and their influence on the presynaptic inhibition and recurrent inhibition systems.

Following electrical stimulation of the FCR Ia fibres during voluntary wrist flexion, mean amplitudes of the MN output were increased from 2.6 ± 0.6 to 10.4 ± 2.5 with 10% of Ia fibre input activated, and from 30.2 ± 5.5 to 42.2 ± 5.3 with 100% of Ia fibre population stimulated (mean $\pm$ SEM), as shown in Table 13. On the contrary, during voluntary wrist extension, mean amplitudes of the MN output were reduced from 2.8 ± 1.1 to 1.5 ± 0.4 at 10% at Ia fibre input level, and from 22.8 ± 4.1 to 10.9 ± 2.8 at 100% of input level, as shown in Table 15.

The potential contribution of presynaptic inhibition to the observed modulation in H-reflexes during voluntary contractions has been investigated in many previous studies. As described by Schieppati et al. (1985), changes in the amplitude of H-reflex during voluntary contraction were associated with presynaptic mechanisms. They proposed that changes in presynaptic inhibition was induced by peripheral feedback from the muscle spindles, and caused a difference in the efficacy of excitatory connections between Ia afferents to MNs. It has been concluded through indirect measurement that presynaptic inhibition was actively modulated in matching the reflex excitability to deviations in the mechanical properties of soleus muscle.

Studies in the spinal control of upper limb movements suggest that the most likely cause of reflex modulation during voluntary contraction is attributed to mechanisms at

the presynaptic level. Facilitation of the reflex excitability during voluntary wrist flexion could likely be caused by a decrease in presynaptic inhibition. It is also possible that the increase in the reflex size is the result of an increased synchronization of MN discharges. During FCR voluntary contraction, the duration of H-reflex was often shorter compared with those evoked at rest (Burke et al. 1989). A briefer duration of H-reflex suggests that the increase in reflex excitability is a consequence of more synchronous discharges of MNs. However the present results showed no consistent change in reflex duration with voluntary contraction.

In 2001, Aymard et al. investigated the changes in presynaptic inhibition of Ia terminals directed to FCR MNs in normal human subjects at rest and during voluntary wrist flexion and wrist extension. Two approaches were used. First, radial-induced D1 inhibition of the FCR H-reflex assessed the excitability of PAD interneurons controlling presynaptic inhibition of the homonymous Ia afferents (Berardelli et al. 1987). The greater the excitability of PAD interneurons, the greater the presynaptic inhibition of primary Ia volleys of the H-reflex. FCR H-reflexes were conditioned by the stimulation of the radial nerve, which accompanied the stimuli to the median nerve. Second, heteronymous monosynaptic Ia facilitation induced in the FCR H reflex assessed the ongoing presynaptic inhibition of Ia terminals (Hultborn et al. 1987).

Depression of the D1 inhibition and heteronymous Ia facilitation of the FCR H-reflex were observed more prominently at the onset of and during tonic voluntary wrist flexion. The depression of D1 inhibition and heteronymous Ia facilitation were not observed during tonic voluntary wrist extension but their effects were detected at the

onset of voluntary wrist extension. These findings suggest that presynaptic inhibition is decreased on Ia terminals projecting to MNs at the onset of and during tonic voluntary shortening contractions, and not changed during tonic voluntary lengthening contraction. The decrease in presynaptic inhibition raises the possibility of involvement of other mechanisms in the inhibition of H-reflexes at the onset of lengthening contraction, for example, as caused by changes in the AHP and setting of the recurrent Renshaw inhibition. However, our study was intended to investigate the modulation of FCR H-reflex during sustained contractions (5-10% of the MVC). It is therefore hypothesized that the presynaptic mechanism is primarily responsible for modulation of the recruitment gain in FCR MN pool in response to any Ia fibre input during voluntary wrist flexion and extension.

Meunier et al. (1998), using transcranial magnetic stimulation, investigated the cortical-induced facilitation of presynaptic inhibition of the FCR Ia terminals (i.e., depression of the FCR H-reflex) during voluntary contractions. To explain H-reflex facilitation during voluntary contractions, Iles (1996) proposed that cortical depression of presynaptic inhibition could occur through interneurons in the flexor reflex afferent pathways activated by cutaneous afferents. The effect of cutaneous depression of presynaptic inhibition was suggested as dominant over the facilitation of PAD interneurons by cortical stimulation to support the facilitatory effect observed in the voluntarily-activated FCR muscles. Other evidence has suggested that an excitatory drive generated in the propriospinal interneurons could account for the FCR reflex facilitation during wrist flexion (Burke et al. 1992). Therefore the alteration of reflex

responses during voluntary contraction of wrist muscles is predominately determined by

the changes in presynaptic inhibition. Presynaptic inhibitory effects could be mediated

by interneurons, or through the direct influence of muscle spindle afferents, though

potential contribution from supraspinal pathways, including cortical pathways could not

be excluded (Malmgren et al. 1988). As a conclusion, the interaction between sensory

information from muscle spindle afferents and presynaptic pathways could occur through

spinal or descending pathways. The various pathways could facilitate or depress the

presynaptic pathways, as illustrated in Figures 32A-B.

A.
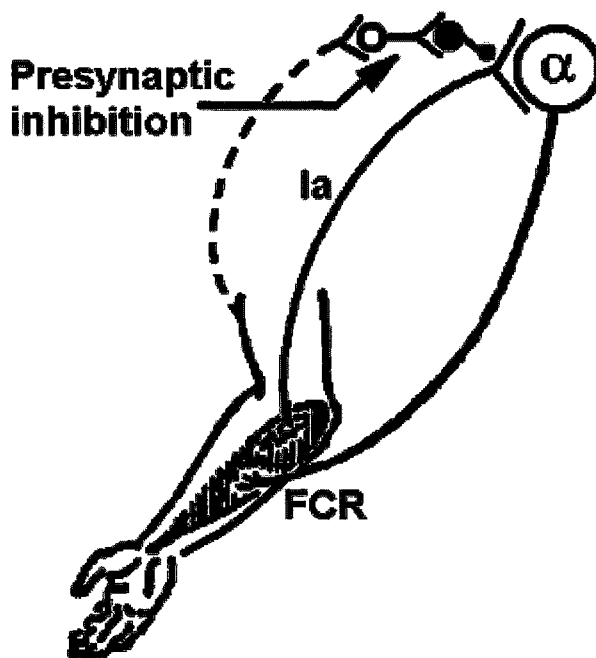
B.
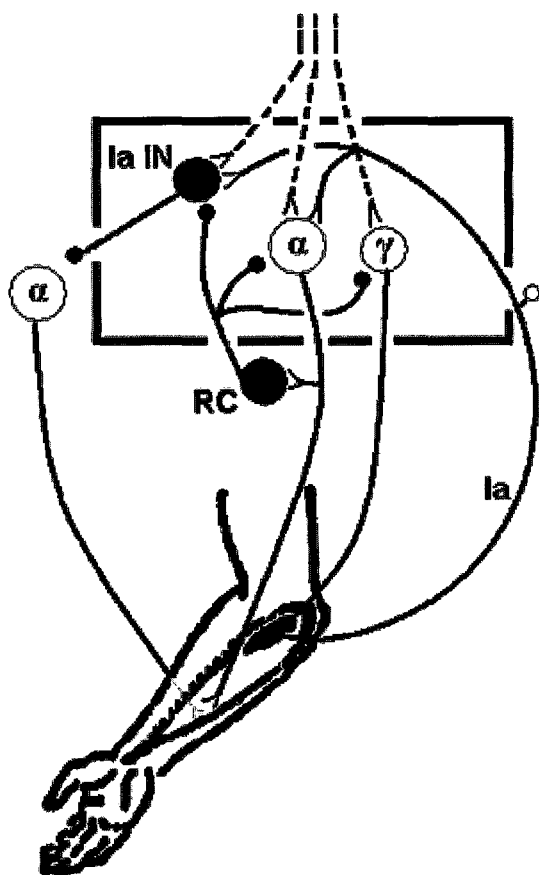
Figure 32. The projection of Ia afferents monosynaptically or polysynaptically to the alpha-motoneurons in the central nervous sytem. **A.** Presynaptic inhibition of Ia terminals, accomplished by primary afferent depolarization interneurons, modulates the output of motoneuronal pools. **B.** Interneurons (**Ia IN**) bring together signals from various peripheral and descending pathways. The Ia afferents synapse with the interneurons. The interneurons have inhibitory synapses with alpha-motoneurons. The dotted lines represent inputs from the descending tracts. Renshaw cells (**RC**) are excited by alpha-motoneurons and project back to the alpha- and gamma-motoneurons, and interneurones (modified from Rothwell 1987).

It was demonstrated in the present study that the activation of high-threshold Ia afferents arising from the FCR produced greater modulation during voluntary contraction of wrist muscles. During voluntary wrist extension, reflex depression was the greatest

when 100% of Ia fibres were activated synchronously (Table 15). Reflex potentiation

was also found to be greater during voluntary wrist flexion with large Ia fibre inputs

involved (Table 13). The best explanation for these observations is that large MNs with

are the most sensitive reflexly to excitation and inhibition (Pierrot-Deseilligny et al.

1999). The high-threshold Ia afferents activate large MNs innervating fast MUs in the H-

reflex. As a result, reflex responses evoked by high-threshold Ia afferent volleys are

under the greatest influence of excitation and inhibition.

5.6   Mechanism Involved at the Motoneuron Output Stage – Recurrent Inhibition System During Muscle Contractions

Modulation of recurrent inhibition by voluntary contractions seems to be as prone

to changes as presynaptic inhibition. Renshaw cells receive their main excitatory input

from motor axon collaterals (Figure 32B), but the efficiency of these synaptic

connections could be altered by the descending drives (Hultborn et al. 1979). This makes

recurrent inhibition highly related to the motor task and dependent on a fine balance

between the excitation from the MNs and the modulatory signals from descending and

segmental afferent sources.

During a steady weak voluntary contraction of the soleus muscles (5 -10% of the

MVC), the stimulation increased recurrent inhibition to homonymous MNs, presumably

because of the predominant effect of increased excitatory drive, which recruits new MNs

that could potentially excite Renshaw cells (Hultborn et al. 1979). Increased excitability

of the Renshaw cells during muscle discharges may serve to limit the muscle from

developing excessively strong force.

The present results demonstrate that voluntary flexion evoked MN facilitation which might be induced by a reduction in the recurrent inhibition. However evidence of its effect is not directly available. To the best of our knowledge, the alterations in presynaptic inhibition are well in accordance with our findings, suggesting significant influences on reflex excitability during voluntary contraction of wrist muscles. Likely presynaptic mechanisms predominate over postsynaptic mechanisms when it comes to counteracting the potential effects of afferent inflow to MNs from muscle spindles in voluntarily-contracting conditions. These could modulate the gain in the negative feedback branch and consequently the MN outputs. Therefore inhibitory effect of presynaptic origin from the voluntarily-contracting wrist extensor muscles reduces the activation of FCR spinal reflexes, whereas the depression of its effect from the wrist flexor muscles facilitates the spinal reflexes, contributing to a fine regulation of muscle activity.

Another possible mechanism underlying reflex depression could be the post-activation depression, described by Hultborn et al. (1996). It was supposed that homosynaptic depression might contribute to the reduction of reflex amplitude during passive dorsiflexion of the ankle joint. The post-activation depression, which is due to a reduced transmitter release from previously activated Ia fibres, interferes with the reflexes during movements. In experiments on decerebrate cats, Hultborn et al. (1996) showed that a similar long-lasting depression of the triceps surae reflexes was evoked by previous conditioning stimulation of triceps surae group Ia afferents. However, FCR reflex depression described in the wrist extension most probably results from a

mechanism other than the homosynaptic depression because the activation of low-threshold muscle afferents was not enhanced.

5.7   Mechanism Involved in Increase of Slope with Large Ia Fibre Inputs

One salient feature of the Ia fibre input- MN output curve is the appearance of a steep rise in the curve at the high-threshold end during relaxation, and particularly during wrist extension (Figure 19). The steeply-rising segment is likely to represent a higher excitation or sensitization of high-threshold afferent fibres, with effects on the spinal premotoneuronal network involved in the modulation of MN output. Modulating influences arising from the properties of the spinal circuitry have not been investigated in our study and the evidences concerning the mechanisms are thus indirect. Characteristics of the recruitment of MNs and presynaptic inhibitory mechanism are of primary interest to influence the steepness of the input-output relationship (Heckman 1994). As shown by Capaday et al. (1987) and Heckman (1994), presynaptic inhibition had a more profound effect on the recruitment gain. The factor which is likely to contribute to the nonlinearly increasing MN recruitment is a depression of presynaptic inhibitory mechanism at the spinal level. Such decline in the presynaptic inhibition may overlap with an increased synchronization of the high-threshold muscle afferents, and their interaction can thus be attributed to higher activation of the MNs with large Ia fibre inputs stimulated.

5.8 Estimations of Distribution of Ia Terminals on Motoneurons

It has been pointed out that the precise distribution of the Ia afferent terminals within a pool of MNs has never been established. Therefore, it would be useful to estimate the number of Ia fibres converging on terminals of a single MN to characterize the spatial extent of the terminal branchings on the surface of individual MNs. In brief, the number of Ia fibres involved in the active synapses with individual FCR MNs during different movements of wrist muscles will be examined.

In the present study of human FCR muscle, it is possible to estimate the proportions of Ia fibres stimulated by referring to the number of fibres required to elicit a threshold response, equivalent to bringing 1% of the MN population to the firing level. When the results for all the subjects during relaxation of the first trial were pooled, it was estimated that, on average, synchronous activation of 4% of the Ia fibres was required to fire 1% of the MNs in the pool. During relaxation of the second trial, 1% of the MNs was estimated to be excited, on average, by synchronous activation of 6% of the Ia fibres. Since the amount of Ia activation to evoke a threshold response is different between the two relaxation trials, these two values are averaged. The mean activation of Ia fibres required to evoke a threshold response at rest is 5%. Using anatomical techniques, the human FCR has been found to contain approximately 129 muscle spindles, and therefore 129 Ia fibres in the human FCR muscles (Von Hoyer, 1963). In addition, we have estimated a mean value of 134 ± 6 for the size of FCR MN pool. Thus, in unselected subjects at threshold, there are about an average of 6.5 Ia fibres projecting to approximately 1.3 MNs in the FCR muscles.

In marked contrast, the number of Ia fibres that would fire a given number of MNs decreases dramatically from one subject to another. For example, a subject with brisk reflexes exhibits a discharge of 42% of the FCR MNs with 66% of the Ia fibres synchronously activated, that is, 85.1 Ia fibres discharged approximately 56.3 MNs. On the other hand, a discharge of 1% of the MNs with 66% of activation of the Ia fibres is found in a subject with no tendon reflexes, that is, the same number of Ia fibres discharged only roughly 1.3 MNs.

The threshold method enables us to quantify the contraction-induced inhibition and excitation in terms of changes in the Ia fibre numbers. For example, when 50% of the Ia fibres are activated and the wrist is flexed, the mean reflex increases from $11.5 \pm 2.8\%$ of the MN pool to $25.4 \pm 3.9\%$. During relaxation, however, 25.4% of the MNs would be fired by approximately 92% of the Ia fibres. Therefore the effect of the facilitation is equivalent to the gain of excitatory input of 42% of the Ia fibre population (i.e., 92%-50%). In contrast, the mean reflex reduces from $18.1 \pm 3.9\%$ of the MN pool to $7.5 \pm 1.6\%$ when 90% of the Ia fibres are stimulated and the wrist is extended. As observed during the resting state, 7.5% of the MNs would be fired by approximately 38% of the Ia fibres. Therefore the corresponding loss of excitatory input is 52% of the Ia fibre population (i.e., 90%-38%) from the effect of inhibition.

CHAPTER 6

CONCLUSION

6.1   Summary

Our motivation for doing this study was to determine the input-output properties of the H-reflex in a human upper limb. It would be useful to estimate the percentage of Ia fibres that have to be synchronously excited to discharge a given percentage of MNs and to characterize changes in the recruitment gain in various types of natural motor tasks. Task-dependent differences were reflected in changes in the input-output relationship. The results clearly showed that the percentage of MN discharges was statistically different between relaxation and voluntary contraction of wrist muscles (5-10% MVC) at the same level of Ia fibre input. The activation of muscle afferent fibres during contraction evokes competing excitatory and inhibitory influences on the MN pool. The facilitatory or inhibitory effects, which were seen going from rest to voluntary wrist flexion or extension respectively, are likely to be influenced more by modulation in the presynaptic inhibition, as suggested by the current knowledge of mechanisms involved in the control of voluntary movements. The inhibitory reflex effects were counteracted during wrist flexion, whereas its effects were promoted to reduce MU firing rate during wrist extension. However modulation of the MN excitability, homosynaptic depression, level of γ-driven Ia activity, Renshaw cell inhibition, and supraspinal centres may also play a role in the control of FCR muscles.

The present results are based on the threshold method in which the firing thresholds of Ia fibres were assumed to be distributed normally. The nature of the input-output relationship, and especially the change in steepness which occurs at the high level of Ia fibre input, are important considerations. The linear relationship implies that the amount of additional activation of a H-reflex is either constant, or relatively so. More importantly, the increase in steepness of the relationship with large Ia fibre input means that greater amount of activation occurs when high-threshold Ia afferents (large Ia fibre inputs) are excited. Excitation of the high-threshold afferent fibres may provoke an increased sensitization on the spinal premotoneuronal network involved in the modulation of MN output.

It follows that adjustment of the amplitude of test reflex response is not a valid procedure for ensuring that the amount of facilitation or inhibition be independent of the test response amplitude. Clearly, measuring the entire input-output relationship in different tasks overcomes the problem, and has the potential to increase the understanding of motor control of the wrist movements. While it has been suggested that reflex modulation plays a critical role in normal motor functions, the exact mechanisms underlying this modulation are largely unknown. Understanding how the spinal reflexes are modulated in response to different mechanical requirements of wrist movements and which mechanisms are likely to contribute to these modulations provides insights into the motor control strategies of the FCR muscles.

6.2   Future Work

The biomechanics of the forearm joint, recruitment patterns of forearm muscles, interneurons and spinal cord circuits mediating the output of MNs, and the supraspinal control of forearm muscles have been the major challenges in understanding the motor control of upper limb movement. To our knowledge, this study is the first work that tries to determine the task-dependent modulation in input-output relationship of the FCR muscles by explicitly quantifying the percentage of activation of Ia afferents required for a given percentage of MN discharges. Thus, the input-output relationship between different motor tasks helps characterize the motor control model of human FCR muscles. We tested our method with voluntary movement of the wrist muscles, and the results were promising.

The limitation of this work is that the assumption of the threshold method has not been tested using the supramaximal stimulation of FCR sensory nerve fibres, though it was satisfactorily supported by results of the stimulation of digital sensory and FCR motor nerve fibres. In addition, evidence of the limitation on the size of H-reflex in quadriceps muscles from activated Ib afferents was found by Marchand-Pauvert et al. (2002). This limits us to only addressing the effects of Ia afferents on MN outputs. To investigate the influence of Ib afferents on the FCR reflex discharges, the technique of supramaximal stimulation of FCR sensory nerve fibres can be used. Alternatively, the time dependence of the H-reflex peaks examined using post-stimulus time histograms of single MUs in FCR muscles could be used. Indeed, using supramaximal stimulation, it was found that the suppression of MN outputs in the soleus muscle does not occur in

most subjects, or at most occurs at the very high stimulus strength (De Bruin et al. 2005). If Ib inhibition does not impose a limitation on the size of the H-reflex, the assumption that the size of H-reflex is determined by the activation of Ia afferents would be valid.

Although the threshold method has been used to determine the Ia fibre input- MN output relationship of the FCR and soleus muscles, it will be interesting to design new experiments addressing the validity of this method in other limb muscles and changes in input-output relationship between different normal motor tasks, different levels of contractions in the same task, or different changes in the muscle length produced by dynamic movements of the joint relative to a neutral position. Despite the observed changes in input-output relationship with different movements of wrist muscles in this study, it would be useful to determine several typical parameters of input-output relationship as a measure to characterize the task-dependent changes in the input-output relationship. Those parameters should manifest themselves in different limb muscles during normal motor activity.

The exact effects of cutaneous inputs on spinal reflex modulation during voluntary wrist movements are not yet established, although available evidence identifies its possible contribution. To examine the contribution of cutaneous signals on spinal reflex excitability, electrical stimulation can be applied to the superficial cutaneous nerves, or simply mechanical stimulation to the skin of the palm and fingertips can be applied, and the changes of FCR H-reflexes are investigated between different wrist movements or different wrist angles tested. This allows a comparison to be made of the

amount of facilitation/inhibition of H-reflexes brought about by the activation of

cutaneous afferents.

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APPENDIX – Consent Form

McMaster University

SCHOOL OF REHABILITATION SCIENCE	1400 Main St., W.	Phone. 905-525-9140
FACULTY OF HEALTH SCIENCES	IAHS, Room 403	Ext. 27802 or 27821
	Hamilton. ON L8S 1C7	Fax: 905-524-0069
	http://www.fhs.mcmaster.ca/rehab	

CONSENT FORM

Project: Synaptic Connectivity and Plasticity
Funded By: National Sciences and Research Council
Principal Investigator: Dr. Victoria Galea

You are invited to participate in a study of how muscles that control the ankle and wrist communicate with the brain and spinal cord. Understanding how this happens in a healthy central nervous system will then allow more understanding of the consequences of injury, disease and drugs on the parts of our body that allow us to move. We are specifically interested in the changes we find following periods of imposed immobilization such as would occur when wearing a cast. You were selected as a possible participant in the study because you have told us that you are healthy, have never had surgery performed on your wrists or ankles and are not pregnant.

If you decide to participate you will be asked to attend one session whereby surface electrodes will be placed on the skin over a muscle that controls the ankle and, in some cases, also over a muscle controlling the wrist. Since these are recording electrodes you will not feel any discomfort. One way of determining the activation of muscle is to use small surface electrical shocks over the nerves supplying that muscle. The intensity of the shock is for the most part very low, however it is necessary to keep increasing the intensity until there is no further increase in the muscle response. The stimulation will give you an unfamiliar sensation; it is like a brief muscle spasm, may be accompanied by a prickling of the skin and at high intensities may feel uncomfortable. The stimuli will never be sufficiently strong to cause injury or tissue damage however you must let the researcher know right away if you experience discomfort so that the stimulation can be stopped immediately. These procedures do not cause any damage however you may experience a reaction to the tape holding the electrodes on the skin. This reaction occurs rarely and takes the form of a rash and itching sensation. If this does occur, experiments will be stopped, the electrodes will be removed and the skin washed with soap and water. The rash, if present, should disappear within a day or so.

As we have discussed, you will receive compensation for your participation, dependent on the time the experiment takes.

Any information that is obtained in connection with this study and that can be identified with you will remain confidential. Results will be reported in scientific publications without identification.

If you have any questions we expect you to ask us. If you have any additional questions later feel free to call Dr Victoria Galea, (905) 525-9140, #22189

You will be given a copy of this form to keep.

You are making a decision whether or not to participate.

Your signature indicates that you have decided to participate, having read and understood the information provided above.

Your Name:

Your Signature: Date: Time:

Address:

Telephone:

Signature of Investigator:

Signature of Witness: