

**CROSSED REFLEXES OF THE HUMAN LOWER LIMBS
A STUDY USING THE HOFFMANN REFLEX**

BY

PAMELA EVANS

DEPARTMENT OF PHYSIOLOGY
UNIVERSITY COLLEGE LONDON

A thesis submitted for the degree of Doctor of Philosophy,
University of London
2001

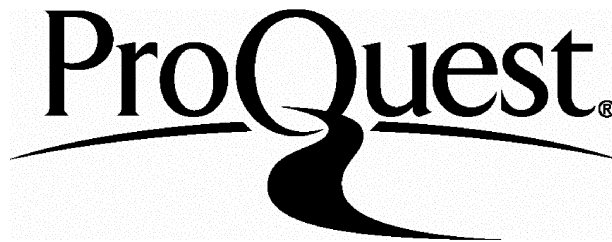
ProQuest Number: U642964

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest U642964

Published by ProQuest LLC(2015). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code.
Microform Edition © ProQuest LLC.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

ABSTRACT

Knowledge of the mechanisms involved in the modulation of activity in spinal pathways has increased considerably in recent years. The way in which spinal afferent activity interacts with pattern generator networks and descending control is of particular interest.

During locomotion the activity of the two lower limbs is coordinated and it would be anticipated that proprioceptive afferents from one limb might contribute to coordination of the two limbs. The purpose of the present study was to examine possible communication between the limbs. Connections between afferents in the peripheral nerves of one lower limb and the motoneurons of muscles in the opposing limb were investigated. Conditioning electrical stimuli were applied to peripheral nerves in humans at rest and changes in the H-reflexes of muscles in the contralateral limb were monitored. Four pathways have been examined, including pathways between afferents of the tibial nerve and the femoral nerve and the contralateral motoneurone pools of soleus and quadriceps. Crossed inhibitory connections have been identified in three of the four pathways and in two of these the earliest component of the inhibition has been shown to be mediated by group I afferents. A late crossed facilitation has been demonstrated, which could be explained by activity in group II afferents.

In relation to the use of the H-reflex as a tool for inferring synaptic inputs to motoneurons, a study was made of the variability of the soleus H-reflex. A decrease in variability with increasing size was identified. The underlying mechanisms which may contribute to the variability changes were examined. A further comparative study was made of the behaviour of the H-reflex of gastrocnemius.

ACKNOWLEDGEMENTS

I should like to thank Philip Harrison, my supervisor, for his good-humoured support and for giving me such a great opportunity to study human motor control.

I should like to thank all my present and former colleagues, especially Evelyn Coggins, for making it possible for me to commence the part-time PhD and Ann Thomson for ensuring that I could complete it. I should also like to thank all the students who have carried out experiments with me or who have acted as subjects. A special word of thanks to Caroline Alexander, who during the last few years has often shared the laboratory with me. Her good nature and wicked sense of humour make her the perfect working companion, both on a day to day basis and during life's crises.

I want to thank all my friends for knowing when not to ask me if the thesis was finished, but also for knowing when to take me out for a drink.

I want to thank my parents - my father for quietly being a very good father and my mother, whose somewhat chaotic good humour I hope I have inherited, but who sadly did not live to witness the completion of this thesis.

Finally, I want to thank especially my husband and three daughters who, in recent years, have turned "long suffering" into an art form.

CONTENTS

	Page
Title page	1
Abstract	2
Acknowledgements	3
Contents	4
List of figures and tables	8
General Introduction	14
 Chapter one: HISTORICAL INTRODUCTION TO CROSSED REFLEXES	 16
1.1 Work from animal experiments	16
1.2 Crossed and bilateral effects observed in humans	36
1.3 Background of the present study	42
 Chapter two: INTRODUCTION TO THE HOFFMANN REFLEX	 44
 Chapter three: A STUDY OF THE INHERENT VARIABILITY OF THE HOFFMANN REFLEX	 53
3.1 Introduction	53
a) The sensitivity of the H-reflex to conditioning stimuli	53
b) The variability of the H-reflex	56
3.2 Method	51
a) Experimental Set-up	60
b) Stimulation	60
c) Recording	61
3.3 Results	63
 3.4 Discussion	 72
a) Changes in the relationship between the stimulating electrode and the nerve trunk	75
b) The response of the Ia afferent fibres to electrical stimulation	76

c) The response of motor axons to electrical stimulation	88
d) Fluctuations in central excitability	
e) The response of the motoneurone pool to the Ia afferent volley	89
f) The effect of changes in presynaptic inhibition	103
g) The effect of the electrical activation of Ib and cutaneous afferents	104
3.5 Conclusion	106

Chapter four: A COMPARATIVE STUDY OF THE BEHAVIOUR OF THE H-REFLEXES AND THE M WAVES OF GASTROCNEMIUS AND SOLEUS

109

4.1 Introduction	109
4.2 Method	111
a) Stimulation	111
b) Recording	112
4.3 Results	112
4.4 Discussion	122
4.5 Conclusion	127

Chapter five: THE INVESTIGATION OF PATHWAYS BETWEEN AFFERENTS OF THE POSTERIOR TIBIAL NERVE AND THE CONTRALATERAL SOLEUS MOTONEURONE POOL

129

5.1 Method	131
a) Stimulation	132
b) Recording	134
c) Analysis	134
5.3 Results	138
5.4 Discussion	141
a) Conduction Distances	143
b) Conduction Velocity	147
c) Electrical Stimulation of Human Afferents	149
d) Duration of Effects	151
e) Analysis of the Observed Crossed Effects	156

Chapter six: THE INVESTIGATION OF PATHWAYS BETWEEN AFFERENTS OF THE FEMORAL NERVE AND THE CONTRALATERAL QUADRICEPS MOTONEURONE POOL	161
6.1 Method	161
a) Stimulation	162
b) Recording	163
c) Analysis	164
6.2 Results	168
6.3 Discussion	171
 Chapter seven: THE INVESTIGATION OF PATHWAYS BETWEEN AFFERENTS OF THE FEMORAL NERVE AND THE CONTRALATERAL SOLEUS MOTONEURONE POOL	 175
7.1 Method	175
a) Stimulation	176
b) Recording	178
c) Analysis	178
7.2 Results	179
7.3 Discussion	181
 Chapter eight: THE INVESTIGATION OF PATHWAYS BETWEEN AFFERENTS OF THE DEEP PERONEAL NERVE AND THE CONTRA- LATERAL SOLEUS MOTONEURONE POOL	 187
8.1 Method	187
a) Stimulation	188
b) Recording	190
c) Analysis	190
8.2 Results	191
8.3 Discussion	193

Chapter nine : FURTHER CROSSED EXPERIMENTS	196
9.1 Examination of the effect of varying the intensity of a conditioning stimulus applied to the contralateral tibial nerve upon the soleus H-reflex	196
9.2 The effect of conditioning stimulation applied at three times the motor threshold intensity to the contralateral tibial nerve upon the soleus H-reflex at the 55ms condition / test interval	199
Chapter ten : DISCUSSION OF THE RESULTS FROM THE CROSSED EXPERIMENTS	200
REFERENCES	221
APPENDIX I	
II	

LIST OF FIGURES AND TABLES

- 3.1** Consecutive M and H responses superimposed upon one another - the greater variability of the H-reflex can be seen clearly p.57
- 3.2** Amplitudes of one hundred consecutive M waves and H-reflexes plotted against time - the scatter of the H-reflexes is obviously greater than that of the M waves p.58
- 3.3** Recordings of several consecutive H-reflex responses at three different levels of stimulus intensity from one subject p.64
- 3.4a** Standard deviation of the H-reflex plotted against stimulus intensity in two subjects p.65
- 3.4b** Standard deviation of the H-reflex expressed as a percentage of the mean amplitude (coefficient of variation) plotted against stimulus intensity p.65
- 3.5** Variability of the Soleus H-reflex in relation to the intensity of electrical stimulation p.66
- 3.6** Variability of the Soleus H-reflex in relation to its amplitude p.68

- 3.7** Variability of the Soleus H-reflex in relation to its amplitude
- results from one subject p.69
- 3.8** Distribution of afferent fibre diameters from Soleus in the
cat based upon the work of Lloyd and Chang (1948) p.78
- 3.9** Distribution of Ia fibre diameters from Soleus in the cat
based on the work of Hunt (1954) p.78
- 3.10** A line graph to represent the plot by Jack (1978) showing
the relationship between electrical excitability and conduction
velocity in afferent fibres of soleus in the cat p.81
- 3.11** Graph to demonstrate the predicted relationship between the
conduction velocity of a nerve fibre and its threshold to
electrical stimulation p.82
- 3.12** The predicted relationship between conduction velocity and
threshold to electrical stimulation of fibres in the Ia afferent
range p.84
- 3.13** The predicted additional percentage of Ia afferent fibres
reaching threshold in relation to increases in stimulus
intensity p.85
- 3.14** Predicted total percentage of Ia afferent fibres reaching
threshold with increasing stimulus intensity p.86

- 3.15** A simplified plan of typical distribution of soleus motor units - based upon the histograms of Bagust (1974) p.94
- 3.16** Model of number of soleus motoneurones reaching threshold in relation to stimulus intensity p.97
- 3.17** Plot to show the relationship between individual Ia EPSP amplitudes and contraction strength based upon the work of Harrison. (1981) p.98
- 4.1** H-reflexes and M waves from soleus and gastrocnemius recorded simultaneously in response to graded stimulation of the posterior tibial nerve p.113
- 4.2** Recruitment curves of gastocnemius and soleus H-reflexes recorded from one subject on three separate occasions p.114
- 4.3** Simultaneous recordings from gastrocnemius and soleus at different levels of stimulus intensity - subject 1 p.116
- 4.4** Simultaneous recordings from gastrocnemius and soleus at different levels of intensity of stimulation subject 3 p.117
- 4.5** The amplitudes of the soleus H reflex plotted against the amplitudes of the gastrocnemius M wave in two subjects p.118
- 4.6** Recruitment curves of gastrocnemius and soleus recorded from one subject on three separate occasions p.120

4.7	Simultaneous recordings of soleus and gastrocnemius in response to stimulation of the tibial nerve in the popliteal fossa	p.121
4.8	Simultaneous recordings from soleus and gastrocnemius in response to the stimulation of the tibial nerve, showing the similarity of the shapes of the M wave and the H-reflex response recorded over gastrocnemius	p.123
5.1	The effect of conditioning by stimulation of the contralateral tibial nerve upon the soleus H-reflex - examples of recordings	p.135
5.2	Amplitudes of a typical run of consecutive M waves recorded during a conditioning experiment	p.137
5.3	The effect of a conditioning stimulus applied to the contralateral tibial nerve upon the soleus H-reflex - results from individuals	p.139
5.4	The effect of conditioning by electrical stimulation of the posterior tibial nerve upon the contralateral soleus H-reflex - pooled results	p.142
Table 1	Peripheral nerve pathway measurements	p.144
Table 2	Estimated nerve conduction distances and times	p.146

- 6.1** The effect of conditioning by stimulation of the contralateral femoral nerve upon the quadriceps H-reflex - examples of recordings p.165
- 6.2** An M wave and an H-reflex recorded from Quadriceps - unrectified and full wave rectified p.166
- 6.3** The effect of a conditioning stimulus applied to the contralateral femoral nerve upon the quadriceps H-reflex p.169
- 6.4** The effect of a conditioning stimulus applied to the contralateral femoral nerve upon the quadriceps H-reflex pooled results - using two measurement strategies p.170
- 7.1** The effect of conditioning by stimulation of the contralateral femoral nerve upon the soleus H-reflex - examples of recordings p.177
- 7.2** The effect of a conditioning stimulus applied to the contralateral femoral nerve upon the soleus H-reflex - individual results p.180
- 7.3** The effect of conditioning of the soleus H-reflex from stimulation of the contralateral femoral nerve - pooled results p.182
- 8.1** Simultaneous recordings from tibialis anterior and peroneus longus illustrating the preferential stimulation of the deep peroneal nerve p.189

- 8.2** The effect of a conditioning stimulus applied to the contralateral deep peroneal nerve of the soleus H-reflex - individual results
p.192
- 8.3** The effect of conditioning by stimulation of the contralateral deep peroneal nerve upon the soleus H-reflex - pooled results
p.194
- 9.1** The effect of varying the intensity of a conditioning stimulus applied to the contralateral tibial nerve upon the soleus H-reflex - at 5ms and 15ms condition / test intervals
p.198

GENERAL INTRODUCTION

The manner in which the central nervous system controls the coordination of the weightbearing limbs - in order to allow stability, mobility and flexibility of movement - has long been of interest to scientists studying the control of movement. Early investigations concentrated upon demonstrating reflexes which produced activity in the contralateral as well as the ipsilateral limbs and upon identifying differences in the activity produced in each limb by the same stimulus.

At the time of these early studies it was perceived that the reflexes themselves were probably responsible for such limb activities as the alternating “stroke” and “return” phases of the cycle of locomotion, in that complex movements were thought to be built up from chains of simple reflexes.

Modern perceptions of the control of such inherent rhythmical movements are very different. The evidence for the presence of networks of neurones, so wired together as to generate and maintain the rhythmical activity required for locomotion is overwhelming and has been shown in invertebrates and vertebrates alike. However, much of the recent work examining the behaviour of these pattern generator networks has shown that they are far more flexible than was first imagined. It seems likely that all vertebral pattern generator networks have close two-way relationships both with descending tracts from the higher centres and also with the spinal pathways which mediate sensory input and motor activity. The system as it is now envisaged functions in a way which involves modulation of pattern generator activity by descending information and by spinal reflex activity

and the modulation of spinal reflex activity by pattern generator activity as well as by descending influences.

The present search for crossed pathways in the human spinal cord and the attempts to identify neurones which may be involved in these pathways should be seen, therefore, as a step towards a better understanding of the lumbar interneurones which mediate interlimb coordination in humans and which are likely to be closely linked to the central pattern generator networks for locomotion.

It is also important to emphasise that the necessary use of such indirect methods of detection as the Hoffmann reflex means that finding the pathways depends not only upon the potential density of the connections between the neurones involved, but also upon the level of excitability of those neurones at the time of the experiments. Selective modulation of the excitability of commissural interneurones involved in different crossed pathways is presumably an important method by which the central nervous system controls the diverse but coordinated activities of the two lower limbs. The present study was restricted to the examination of crossed pathways in subjects at rest in relaxed, supported sitting and the results will therefore reflect the excitability of the mediating interneurones in these particular experimental conditions.

I.0 HISTORICAL INTRODUCTION TO CROSSED REFLEXES

1.1 WORK FROM ANIMAL EXPERIMENTS

When Sherrington described the “Reflex of Crossed Extension” in 1910 he perceived it as being accessory to, rather than part of, the ipsilateral flexion reflex. This was because, although he had shown that the flexion response to the appropriate stimulus was “a matter of practical certainty” in his studies of the cat, the crossed response “has a variability which indicates that it is adjunct to rather than part and parcel of the reflex of the stimulated limb itself”. He observed that the crossed extension reflex had a wider receptive field than the flexion reflex and that its threshold, in relation to that of the flexion reflex, varied considerably under different circumstances. He also noted that “with more natural stimuli and especially such as are more suited to the proprioceptors” it was possible sometimes to observe crossed flexion accompanying ipsilateral extension. One other pertinent observation made at the time was that if the stimulus was maintained, in the spinal animal the crossed reflex usually began as extension, but then became an alternating flexion and extension.

In his extensive descriptions of spinal reflexes in the cat Graham Brown (1912) also observed on occasions “irregular” contralateral flexion reflexes in spinal and decerebrate animals, but, unlike Sherrington, he did not find them accompanying ipsilateral extension. He found that on occasions faradic-type electrical stimulation of the long saphenous nerve would induce a phase of relaxation of the contralateral extensor (gastrocnemius) muscle prior to its contraction. With a stronger intensity of stimulation a contralateral flexor (anterior tibial) contraction was sometimes observed

during the extensor relaxation phase and sometimes even appeared as the dominant contralateral response.

By the time Perl carried out his studies of crossed reflexes in the 1950s the advances in electrophysiology of the intervening years had meant that he could then record motoneurone discharges from contralateral ventral roots instead of simply observing reflex changes in muscle length. He was also able to examine contralateral effects upon ipsilateral reflexes. So it became possible to estimate the delay between the stimulus time and any changes in motoneurone discharge and this in turn could be used, along with measurements of conduction distance, to relate minimal afferent conduction times to observed effects.

Using spinal (3-6 hours post-transection) and, occasionally, decerebrate cats, Perl's preliminary findings in 1957 were that faradic stimulation of low threshold cutaneous afferents, conducting at 40 metres or more per second, produced a short latency facilitation of contralateral flexors at approximately the same latency as the ipsilateral flexor facilitation which had been demonstrated by Lloyd (1943) from similarly large cutaneous afferents. Such a volley produced very little effect upon contralateral extensors, although occasionally signs of a slight inhibition were detected. But volleys including activity in smaller myelinated fibres with conduction velocities between 10 and 40 metres per second produced a prolonged inhibition in the contralateral flexors, following the initial facilitation and a prolonged facilitation of the contralateral extensors at a similar latency. Perl indicated that the timing and duration of these later effects made it likely that they were associated with the crossed extensor reflex.

Perl (1958) looked next at the crossed effects of muscle afferents and here he also found changes occurring at different latencies, associated with the activation of different diameter afferents. However, stimulation of the largest diameter muscle afferents produced some crossed effects at much shorter latencies than those observed from cutaneous afferents. A volley in the largest afferents from the quadriceps or hamstring muscles produced a very rapid inhibition followed immediately by an excitation of the motoneurons of the corresponding muscle of the opposite hindlimb. Both effects occurred within 5ms and could be dissociated by changes in stimulus intensity which produced differences in the proportions of the different diameter group I fibres which were activated. These group I effects applied both to flexors and extensors. When the stimulation also activated group II afferents (conducting at less than 70 metres per second) the response in contralateral extensor muscles changed very little, but in the contralateral flexor motoneurons an inhibition appeared which tended to outlast and to reduce the size of the group I-induced facilitation. Group III activity tended to be linked with a long duration and relatively long lasting facilitation of contralateral flexors and extensors.

Finally Perl (1959) examined the crossed effects of muscle stretch in order to attempt to clarify the relative contributions of the known large diameter muscle afferents to the changes in contralateral motoneurone excitability which he had earlier observed. He used both monosynaptic reflexes and the firing rates of individual motoneurons to monitor the contralateral effects of different levels of stretch tension and also of contraction. Perl concluded from the results that the very short latency crossed inhibition observed initially in response to the lowest levels of electrical stimulation of muscle afferents was the contralateral, (reciprocal) effect of the activity of the group one spindle (Ia) afferents. The subsequent facilitation was also a

contralateral reciprocal effect and was considered to be produced by the activity of the tendon organ (Ib) afferents.

In the 1960s Holmqvist published the results of a series of experiments in which she sought to clarify the contributions made by different afferents to crossed reflex actions. When she examined the contralateral effects of low threshold muscle afferents Holmqvist was unable to demonstrate group I effects in acute spinal cats, but she showed effects from repetitive stimulation in chronic preparations. She attempted to change the proportion of fast and slow components of the group I fibres activated in the hope of finding separate crossed effects attributable to the spindle afferents and to the tendon organ afferents (as Perl had shown); but she was unable to detect dual crossed effects. Holmqvist's findings showed frequent excitation of extensors from contralateral group I extensor afferents; variable effects from flexors to extensors and from flexor and extensor afferents to contralateral flexors. She noted particularly pronounced effects involving the quadriceps muscles; both those involving quadriceps afferents and those affecting quadriceps motoneurons (Holmqvist 1961a & b).

Using mainly acute spinal cats she, too, found pronounced crossed effects when she came to look at the effects of activity in higher threshold muscle afferents of a variety of peripheral nerves. However Holmqvist found that, unlike the ipsilateral effects, the contralateral effects from these afferents were distinctly variable. She listed the four variations found as : -

1. facilitation to extensor and inhibition to flexor nuclei
2. facilitation to both flexor and extensor nuclei
3. inhibition to extensor and facilitation to flexor nuclei
4. inhibition to both extensor and flexor nuclei

of which those involving excitation to the extensors were the most common (Holmqvist 1960,1961b).

Reversal of reflex effects was already well-recognised in certain ipsilateral reflexes (Graham Brown 1912 for example) and the explanation of alternative interneuronal pathways from the same afferents seemed to offer a modern view of a more flexible system of spinal networks. However the fact that the crossed actions often involved reciprocal effects, but sometimes did not, led Holmqvist to envisage “a more labile relationship between these interneuronal pathways than on the ipsilateral side” - probably a not-surprising result of the longer interneuronal chains.

Holmqvist also found that these same crossed actions from high threshold muscle afferents were observed in response to stimulation of those cutaneous afferents and high threshold joint afferents also classified in the category of flexor reflex afferents (FRAs).

Her work on the supraspinal control of crossed reflex activity (Holmqvist 1961b) demonstrated that with different brainstem lesions different crossed activities seemed to be dominant; apparently released from descending inhibitory control. These findings provided a possible explanation for the variety of crossed effects seen in response to volleys in FRAs in different preparations and this confirmed her belief in the differential nature of the supraspinal control of facilitation and of inhibition to flexors and extensors.

Finally, Holmqvist's work examining differences in the crossed FRA effects to motoneurons of slow and fast extensor muscles was published posthumously under her married name (Bosemark 1966). The study showed that although there was a facilitation of the monosynaptic reflex of the fast muscle (medial gastrocnemius) at a latency of around 5 milliseconds (ms) and a later smaller depolarisation (after 30ms) no

motoneurones discharged as a direct result of this crossed excitation from cutaneous and muscle nerves. However although the monosynaptic reflex of the slow muscle (soleus) generally showed a smaller facilitation, which occurred only at the longer latency (30ms or more), the motoneurones discharged repeatedly in response to this crossed excitation.

Around this time Rosenberg began to use intracellular recordings to examine the responses of several groups of hindlimb extensor motoneurones to stimulation of the ipsilateral and the contralateral sural and posterior tibial nerves. This allowed the direct measurement of the size, shape and timing of postsynaptic potentials (PSPs) in these neurones from unanaesthetised decerebrated or low spinal cats. In his reports of the work, published in 1966 and 1970, Rosenberg described how, in anaesthetised cats, cord dorsum recordings were used to observe thresholds to stimulation in many single afferent fibres and, along with calculations of their conduction velocities, these were used to plot graphs of the relationship between fibre conduction velocity (and estimated diameter) and excitation thresholds in the sural and posterior tibial nerves. Eccles, Eccles and Lundberg (1960) had produced a similar plot for muscle afferents, but the range of fibre sizes was somewhat different from those in cutaneous nerves (Hunt and McIntyre 1960).

Rosenberg then used these graphs in his analysis of the responses produced by ipsilateral and contralateral stimulation of cutaneous nerves in 94 hindlimb extensor motoneurones in 23 cats. He found that in general the motoneurones of the muscles of the ankle and the foot rather than those of the knee responded to stimulation of the sural, the posterior tibial and the saphenous nerves. He observed most commonly the typical pattern of inhibitory postsynaptic potentials (IPSPs) in ipsilateral and excitatory postsynaptic potentials (EPSPs) in contralateral extensor motoneurones in

the decerebrate and spinal animals. The contralaterally-produced EPSPs sometimes only occurred after repetitive stimulation and were generally longer-lasting than the ipsilaterally-produced IPSPs. The former were generally detected at around three times the threshold for activating the afferent nerve while the latter generally appeared at around two times that threshold. Some cells showed EPSPs in response to ipsilateral as well as contralateral stimulation and a few cells showed bilateral inhibitory responses (though this last pattern was never seen in the spinal preparations). Rosenberg indicated that these observations of some variation in the intracellular crossed effects could be seen as further evidence in support of Holmqvist's view of alternative pathways available for supraspinal selection.

When he examined the effect of anaesthesia upon these responses he found similar IPSPs in the extensor motoneurons following ipsilateral stimulation, but the EPSPs following contralateral stimulation were not observed and appeared to be depressed in these preparations. These findings seemed to indicate that certain cells mediating the crossed effects were particularly vulnerable to the effects of the anaesthetic.

Rosenberg also used graded stimulation and latencies of responses - along with the threshold/diameter relationship plots - to show the range of afferent diameters associated with the ipsilateral and crossed actions. He found that the largest afferents in the cutaneous nerves (approximately seventy per cent of the alpha fibres) did not produce contralateral or ipsilateral effects and that the range of diameters of the afferents producing contralateral EPSPs was similar to the range producing ipsilateral IPSPs, although the former range was somewhat narrower and included slightly smaller diameter myelinated fibres. He suggested that the function of the largest cutaneous afferents, with ineffective connections to the

motoneurones, might be more concerned with the transmission of innocuous information from touch receptors to the higher centres for interpretation.

He was also able to estimate central transmission times; comparing the delays in motoneurones whose ipsilaterally-induced IPSPs had similar thresholds to their contralaterally-induced EPSPs (and would therefore be mediated by afferents conducting at the same velocities). He found that in a large majority of these motoneurones the contralateral effects had a latency which was between 1ms and 5ms longer than the ipsilateral effects. He therefore came to the same conclusion as Holmqvist - that the crossed pathways were longer than the ipsilateral pathways by at least one interneurone.

Jankowska and her colleagues found that the effects of the stimulation of FRAs were profoundly altered by the injection of L-DOPA into the spinal cord of unanaesthetised acute spinal cats, (L-DOPA is believed to act by allowing the release of noradrenaline from a descending influence, probably the reticulospinal pathways). The short latency effects normally seen in response to the stimulation of high threshold muscle and cutaneous afferents in this preparation were inhibited and replaced by longer-lasting, long-latency EPSPs in the ipsilateral flexor and the contralateral extensor motoneurones of both fast and slow muscles (Jankowska, Jukes, Lund and Lundberg 1967a). By combining ipsilateral and contralateral stimulations they demonstrated that each of the pathways inhibited the transmission in the other pathway, thus revealing an ipsilateral/contralateral reciprocal organisation. They also showed that this reciprocal inhibitory effect was likely to be a post-synaptic effect produced via spinal interneurones and not the result of presynaptic control. The authors suggested a possible organisation involving mutually - inhibiting last order interneurones

transmitting activity from ipsilateral and contralateral FRAs, which they perceived as being “eminently suited to subserve rhythmic alternating movements” of which stepping is a prime example.

In addition they observed a primary afferent depolarisation (PAD) in Ia afferent terminals which also appeared (presumably as a result of the release of a descending inhibitory influence) following the injection of L-DOPA. Moreover they pointed out that ipsilateral and contralateral reciprocal arrangements could be disturbed by Ia reciprocal inhibition; so that the additional control of Ia terminals by FRAs would make functional sense.

In an accompanying paper (Jankowska et al. 1967b) the same team identified interneurons in Rexed's lamina VII whose discharge patterns were of a latency and duration to indicate that they mediated the long lasting effects from FRAs to motoneurons and primary afferents. These interneurons consisted of three types - some excited by ipsilateral FRAs, some by contralateral FRAs and some by both. Like the responses produced in motoneurons these interneuronal discharges were only present after the administration of L-DOPA and could be inhibited by continued activity in the same FRAs.

Recording from motoneurons in the cat hindlimb Jankowska also demonstrated connections between propriospinal neurons and interneurons interposed in the ipsilateral and contralateral FRA reflex pathways (Jankowska, Lundberg and Stuart 1973) and suggested that these neurons might reasonably be concerned with the co-ordination between fore and hind limbs.

Continuing the search for possible sources of control of the spinal pathways from FRAs Bruggencate and Lundberg recorded motoneurone discharges in response to FRA stimulation while conditioning with impulses in vestibulospinal fibres (Bruggencate and Lundberg 1974). They found that impulses in the vestibulospinal fibres produced facilitation in pathways mediating crossed excitation to extensor motoneurones and crossed inhibition to flexor motoneurones from FRAs, indicating convergence upon common interneurones. Reversed conditioning revealed that volleys in the spinal pathways facilitated vestibulospinal effects upon contralateral motoneurones. This was consistent for excitation of extensor motoneurones; but a more mixed picture was revealed in relation to flexors. In knee flexor motoneurones, volleys in contralateral FRAs produced parallel facilitation of the IPSPs from vestibulospinal fibres and from Ia interneurones, indicating that here the vestibulospinal influence was via Ia inhibitory interneurones. But in ankle flexors the vestibulospinal evoked IPSPs were facilitated by FRA activity via last order interneurones not shared with Ia afferents and in flexors of the toes the dominant vestibulospinal effect was excitatory and this was again facilitated by contralateral FRA stimulation.

The authors postulated that although there was no reason to believe that vestibulospinal control would not take place independently of FRA activity, nevertheless it was reasonable to assume a functional significance for the integration of impulses in the vestibulospinal tract and contralateral afferents. They offered a hypothetical model in which FRA activity might “reinforce and prolong movements evoked from higher centres” by the convergence of information from large receptive fields onto interneurones interposed in both pathways. Such a model would also allow the flexible integration of the alternative FRA pathways sometimes seen (such as bilateral excitation of extensors), which are assumed to be mutually

inhibitory, so that descending activation would be channeled into the already active pathway. Such a model would include the patterns of lower limb co-ordination commonly used which do not include the reciprocal activity of locomotion - such as bilateral weightbearing in standing.

The relationship between crossed reflex pathways and Ia (reciprocal inhibitory) interneurons was further explored by Hultborn and colleagues (Hultborn, Illert and Santini 1976) using direct recording from interneurons. They found that volleys in a mixture of contralateral high threshold afferents, all of which could be categorised as FRAs, produced similar effects. The predominant contralateral effect was an excitation in extensor-coupled Ia interneurons (those inhibiting the antagonistic flexors) which indicated that FRAs evoked parallel actions upon contralateral extensor motoneurons and coupled Ia interneurons. A similar parallel effect had been observed from ipsilateral FRAs upon flexor motoneurons and coupled Ia interneurons (Fu, Jankowska and Lundberg 1975).

Thus by the end of the 1970s, the work on crossed pathways from afferents recognised as coming under the umbrella term of “flexor reflex afferents” had yielded, with many generally concurring results, some detailed information concerning the most common pattern of effects; the alternative patterns available; an interlimb reciprocal arrangement; the convergence of descending influences upon interneurons interposed in the crossed pathways; the convergence, in some instances, of the crossed pathways upon interneurons in certain ipsilateral reflexes; a presynaptic effect upon Ia afferents and some variability in the quality and quantity of these crossed connections between afferents from different peripheral nerves and the motoneurons of different contralateral muscles examined.

In contrast to the emerging FRA picture the information concerning crossed pathways from group I afferents was sparse. The studies of Perl and Holmqvist were eventually re-examined by Baxendale and Rosenberg (1975,1976,1977), who suggested that the conflicting results might be due to an inability to truly distinguish between Ia and Ib afferents. The use of graded increases in intensity of stimulation to discriminate between the electrical thresholds of the two types of afferents would not separate them when there was a large overlap in the distributions of thresholds between the two populations - as was often the case. They therefore used recently developed techniques which allowed "unambiguous selective activation" to examine separately the crossed effects from the activation of Ia and Ib muscle afferents from soleus in decerebrate cats.

In their study of Ia crossed effects (Baxendale and Rosenberg 1976) they used low amplitude, high frequency vibration which Brown, Engberg and Matthews (1976) had shown would produce a pure Ia discharge and they recorded the effects on a number of contralateral flexor and extensor muscles in two ways. Baxendale and Rosenberg measured length changes in muscles isotonically loaded with weights and they looked at changes in the averaged monosynaptic ventral root potentials. They noted that the crossed reflex effects were difficult to produce, but when they did occur they were consistent, showing a crossed inhibitory effect upon both flexors and extensors, (though it was most difficult to elicit in the flexors).

When they came to look for crossed effects from Ib afferents, Baxendale and Rosenberg (1977) selectively activated these by raising the threshold of the Ia afferents to electrical stimulation with prolonged high frequency low amplitude vibration of the muscle tendon, as had been described by Coppin, Jack and McLennan (1970). Having identified the group I threshold of the soleus muscle nerve before the period of vibration, they then stimulated at

1.2 to 1.3 times threshold during the ten minutes after the vibration stopped (when the Ia threshold remained raised). As the stimulus intensity was below that which would activate group II afferents, the authors felt secure in interpreting the contralateral effects seen as being mediated by a pathway from Ib afferents. Using weighted muscles again, they recorded changes in muscle length in the contralateral soleus and tibialis anterior muscles and found, in the case of both muscles, that whenever there was a crossed effect it was excitatory. Once again they acknowledged that these crossed reflexes were often difficult to elicit, but they were seen more easily in soleus than in tibialis anterior.

With the advance of techniques of electrophysiology and morphology it had become possible to stimulate and record intracellularly, as well as extracellularly, not only from motoneurons, but also from interneurons. This made it possible to examine effects and identify connections between cells more directly and led to the emphasis upon the study of the behaviour of last-order interneurons especially. As inputs from many sources might converge on these cells en route to the motoneurons, they were naturally regarded as a logical starting point in the search for greater knowledge of the complex spinal circuitry of mammals.

Harrison and Zytnicki (1984) used electrical stimulation of afferent fibres from a number of different hindlimb muscle nerves and recorded intracellularly from contralateral motoneurons in anaesthetised acute low spinal cats. Afferent volleys were monitored in the cord dorsum. Effects in response to up to 1.5 times threshold were registered as group I effects; from 2 to 5 times threshold as group II effects and above 10 times threshold as group III effects.

Volleys in group II afferents from quadriceps produced the well-documented response of EPSPs in contralateral extensor and IPSPs in contralateral flexor motoneurons. Volleys in group III afferents also produced these effects, but in this case they were seen in response to stimulation of all the muscle nerves activated. Only occasionally did they detect any motoneurone responses to volleys in contralateral group I afferents. But effects in response to the latter were detected in ipsilateral reflexes. Ia reciprocal inhibition was facilitated and so were Ib effects (both inhibition of agonists and excitation of antagonists) in response to contralateral group I stimulation. The authors interpreted these findings as an indication of the convergence onto common interneurons of crossed group I pathways and the ipsilateral reflex pathways concerned. Similar convergence onto reflex pathways from ipsilateral primary afferents had already been documented (Harrison et al. 1983, Lundberg et al. 1977).

In a subsequent study Harrison and Zytnicki, working with Jankowska, (Harrison et al. 1986) decided to investigate the connections of a group of interneurons located in Rexed's lamina VIII of the cat lumbosacral spinal cord, in the light of the fact that neurons located here had been known to have axons which crossed the spinal cord. In the first series of experiments using retrograde labelling by transneuronal transport of horseradish peroxidase conjugated with wheatgerm agglutinin (WGA-HRP) from contralateral motoneurons, they located a group of contralaterally projecting interneurons in lamina VIII (and a narrow, adjacent strip of lamina VII) stretching from lumbar segment four to the first sacral segment.

In the second series of experiments they looked at what they considered to be a group of corresponding interneurons in lamina VIII of the sixth lumbar spinal segment in anaesthetised spinal cats. They were able to

demonstrate contralateral projections from many of these cells to motoneurons, this time by antidromically activating them from weak stimulation of the contralateral motoneurons. Using intracellular and extracellular recordings from these cells they observed that although they were most powerfully activated by high threshold cutaneous and muscle afferents (group II and above) and from stimulation of the spinal cord at thoracic level (indicating influence by descending pathways), a substantial proportion of them did respond to activation by group I afferents. The majority of these were affected by ipsilateral group I input, some responded to contralateral group I input, but cells rarely responded to both. Sometimes group I input produced EPSPs (at mono, di- or trisynaptic latencies ipsilaterally and di- or trisynaptic latencies contralaterally) and sometimes the group I input produced IPSPs (at latencies that indicated pathways with just one additional interneurone). A large number of cells showed both EPSPs and IPSPs. EPSPs from ipsilateral and contralateral inputs were most readily evoked from quadriceps and then from hamstring afferents. IPSPs were produced predominantly from extensor afferents. Sometimes it appeared that the greatest group I effect was produced at what was considered to be the Ia threshold and sometimes at the Ib threshold.

The distinct, though relatively weak, group I inputs which the authors found in lamina VIII interneurons (many of which appeared to be projecting monosynaptically to contralateral motoneurons) was in keeping with the previous studies which had found weak group I contralateral connections. The authors indicated that these connections could reasonably indicate that the neurons concerned were interposed in both crossed reflexes and crossed descending actions. In the light of the weak group I inputs and the stronger inputs from higher threshold afferents they proposed that both the excitatory and the inhibitory group I actions might act functionally to gate transmission of more powerful inputs through the

lamina VIII interneurones. On a cautionary note Harrison and his colleagues (Harrison et al. 1986) indicated that although their labelling experiments had shown commissural cells only in lamina VIII, there had been some doubts cast upon the universal effects of the method of labelling - for instance it was possible that only inhibitory and not excitatory commissural interneurones had been labelled. Certainly the presence of other commissural cells could not be ruled out.

The results from these studies were indicative once more of strong contralateral group II pathways. There appeared to be interesting similarities between the comparative strengths of crossed effects of group II afferents from different muscles found by Harrison and Zytnicki (1984) and the comparative strengths of inputs of group II afferents from different muscles on to interneurones found in the midlumbar segments of the spinal cord (Edgley and Jankowska 1987a&b). Edgley and his colleagues (Arya, Bajwa and Edgley 1991) decided to extend the work on crossed effects specifically from group II afferents by comparing the effects from a range of different muscles.

They activated group II muscle afferents by electrical stimulation and recorded intracellularly from contralateral motoneurones in anaesthetised cats whose spinal cords were either intact or transected. When the spinal cord was intact, they found that the predominant contralateral effect in all the motoneurones was a short latency inhibition, whereas in the spinal cats the group II stimulation produced the classic pattern of EPSPs in extensor motoneurones and IPSPs in flexor motoneurones. In both preparations afferents from certain muscles (quadriceps especially and then tibialis anterior, extensor digitorum longus and flexor digitorum longus) were found to have a more powerful effect on the contralateral motoneurones than afferents from other muscles and indeed afferents from

gastrocnemius-soleus and the hamstrings generally had minimal effects, under these experimental conditions.

The IPSPs seen in the animals with intact spinal cords were of a similar latency to the ipsilateral effects produced by stimulation of group II afferents and mediated by a pathway containing a single midlumbar interneurone. The authors speculated that, if these contralateral IPSPs seen in the intact animals were also mediated by a pathway ascending to the midlumbar region and then descending to the lower lumbar and sacral segments which contained the hindlimb motoneurones, then their latency would also only allow for one interneurone. It wasn't possible to draw any such conclusions about the longer latency EPSPs and IPSPs seen in the spinal animals, as the delays involved would allow for a number of different possible pathways.

The finding of different crossed effects with different preparations was quite in keeping with previous studies (Holmqvist 1960, 1961b, Rosenberg 1966, 1970 and Jankowska et al. 1967a) and Edgley and his colleagues considered the functional implications of the widespread inhibition seen with the spinal cord intact. The excitation of contralateral extensors seen in spinal animals would be appropriate during the alternate limb activity of walking, but would not be useful, for instance, during standing and during locomotory activities involving bilateral flexion and extension, such as bounding. Thus it was easy to imagine the need for various crossed pathways, modulated to allow the domination of different ones in different functional situations.

Running concurrently with these investigations of crossed activity were many other studies looking at the connections of accessible interneurones, particularly last-order interneurones and those which mediated activity

from specific afferents. Midlumbar interneurons were first investigated because they appeared to receive a major input from group II afferents. The pattern of convergence found from different afferents on to them and their projection on to hindlimb motoneurons (Edgley and Jankowska 1987b, Cavallari, Edgley and Jankowska 1987, Harrison, Jami and Jankowska 1988) had already led to speculation about the role of these interneurons in the use of the stretch of ipsilateral proximal extensors and the hip joint position as signals for the switch between extensor and flexor activity during the cycle of locomotion.

Edgley and Jankowska (1987b) had reviewed a number of other findings concerning interneurons in the midlumbar segments, which included the discovery of rhythmic activity in the midlumbar neurons themselves; the fact that the fourth lumbar segment had to be intact in order to produce rhythmic locomotor activity in awake chronic spinal cats and the fact that midlumbar cells contributed to the modulation of “centrally-initiated movements”. Phase-dependent reflex modulation (an example of interaction between the central pattern generators and spinal reflex pathways) had also been demonstrated in pathways from a number of different afferents to the hindlimbs and it had been shown by Rossignol and Gauthier (1980) that intact dorsal roots from the fourth and fifth lumbar segments were critical to some of these modulations.

Edgley and Jankowska (1987b) had pointed out that the effects reviewed might not all be attributable to only one population of midlumbar interneurons, but they identified seven characteristics which neurons with an involvement in such modulation of locomotor activity should be expected to share. Of these only one - that they should be influenced by information concerning muscle length and joint position from the

contralateral limb - had not been demonstrated in relation to the midlumbar interneurones.

Consequently Bajwa, Edgley and Harrison (1991) investigated the response of midlumbar interneurones to activity in contralateral afferents. Using intracellular recordings in cats with intact spinal cords, they found that a large majority of the cells did indeed respond to stimulation of contralateral as well as ipsilateral group II afferents, though rarely to stimulation of contralateral group I afferents. They also found a remarkable similarity between the ipsilateral and the contralateral group II influences upon individual cells. Bilateral excitation was the most common effect observed, although IPSPs were produced from both sides in a substantial number of cells and over half of the cells which responded to ipsilateral input with an EPSP/IPSP sequence responded similarly to contralateral input. The latencies of the contralateral effects indicated a disynaptic pathway for the earliest EPSPs. As the afferents concerned are not believed to cross the spinal cord, this would indicate the presence of a single commissural interneurone between the afferent and the midlumbar cell. Consequently the crossed IPSPs appeared to be the result of a trisynaptic pathway.

This work once again highlighted the particularly powerful crossed effects produced from the group II afferents from quadriceps and sartorius, the relatively strong effects produced from tibialis anterior, extensor digitorum longus and flexor digitorum longus, and the weak or absent crossed group II effects seen from gastrocnemius-soleus.

The work of Bajwa, Edgley and Harrison confirmed the effects of activity in contralateral group II afferents upon midlumbar interneurones and thus established midlumbar neurones as having all the qualities required of

neurones with a role in the switch from extensor to flexor activity during locomotion and an intimate relationship with the spinal pattern generator network.

The similarities observed between the effects produced in these cells by ipsilateral and by contralateral group II activity emphasised the existence of some form of close coupling between the midlumbar neurones on each side of the spinal cord, evident in these animals with intact spinal cords. The similar effects could be the result of collateral axons from the ipsilateral midlumbar cells projecting to their contralateral equivalent cells while simultaneously projecting to motoneurones. This would be expected to produce bilateral symmetrical activity - well-recognised as one of the patterns of ipsilateral and contralateral activity seen in certain experimental preparations and known to be necessary for certain functions of the lower limbs. If this particular coupling arrangement exists then it would be necessary for modulation of the group II input on to these cells or modulation of the activity of these cells themselves to occur when reciprocal, alternating activity of the limbs is required as in walking. In spinal animals this reciprocal activity is evident in the crossed extension pattern seen in response to group II activation and would indicate a powerful change in the dominant modulatory influence upon the group II actions on the midlumbar interneurones.

Most recently Aggelopoulos and Edgley (1994) have demonstrated that transmission of crossed effects from quadriceps afferents depends upon the integrity of the fifth lumbar segment. So regardless of which particular coordinating actions of the lower limbs may be dominant at any particular moment, the body of evidence at present available points to the close links between ipsilateral and contralateral lower limb activity which appear to involve pathways into and out of the mid-lumbar or caudal segments of the

spinal cord. Located in the mid-lumbar region are interneurons on to which information is converging from many sources, but upon which group II afferents from different muscles have marked ipsilateral and contralateral effects and group I afferents have weak effects. It would appear that some of these populations of primarily group II-activated interneurons project to ipsilateral motoneurons and some may project to contralateral motoneurons directly or via the contralateral midlumbar interneurons.

1.2 CROSSED AND BILATERAL EFFECTS OBSERVED IN HUMANS

Since the 1970s there has been a rapid expansion of the field of human motor control studies. This development has been assisted by, and at times even prompted by, the availability of an increasingly sophisticated range of investigative techniques. The design of some of these studies and the interpretation of results have revealed signs of much closer links between the interests of human and animal physiologists in this field.

Studies in humans involving the monitoring of the effects of conditioning stimulation of a variety of peripheral nerves upon ongoing EMG activity or upon Hoffmann reflexes (H-reflexes) have revealed links between certain types of afferent neurones and the motoneurone pools of specific muscles (see for instance Pierrot-Deseilligny, Morin, Bergego and Tankov 1981, Meunier, Pierrot-Deseilligny and Simonetta 1993, 1994, Marque et al. 1996, Simonetta-Moreau et al. 1999). Also, from such studies, investigations have developed into the way in which activity in different afferent neurones is integrated in spinal reflex pathways (for example Bergego, Pierrot-Deseilligny and Mazieres 1981, Pierrot-Deseilligny, Katz and Morin 1981). Such work echoes the animal studies which have

provided and continue to provide increasing insights into the role of reflexes in movement.

In particular there has been a growing interest in the similarities and differences between quadrupeds and humans in the way in which afferent input and spinal pattern generating networks interact during locomotion (see Jankowska and Edgley 1993, Dietz 1996, McCrea 1998 and Zehr and Stein 1999 for reviews discussing relevant experimental evidence). Interlimb coordination is therefore of central interest and a number of observations have been made specifically concerning contralateral and bilateral reflex effects in humans.

In 1972 Masland used test H-reflexes from gastrocnemius and looked at the recovery curves recorded following contralateral as well as ipsilateral conditioning stimulation of the posterior tibial nerve. He found that a contralateral conditioning stimulus just sufficient to evoke an H-reflex on the same side appeared to have no effect upon the test H-reflex on the other side. However a contralateral conditioning stimulus sufficient to evoke a maximum H-reflex on that same side produced an early, small, long duration inhibition and a facilitation occurring at a latency of approximately 100ms. This response mirrored that seen with a weaker ipsilateral conditioning stimulus. However, the use of a maximum H-reflex as the test reflex would have made the detection of both facilitation and inhibition difficult under these circumstances (see discussion below in chapter 3).

Robinson, McIlwain and Hayes (1979) showed a long-lasting facilitation of the soleus H-reflex between 50 and 250ms, peaking at 150ms; but this time the conditioning stimulus to the contralateral tibial nerve was below threshold for evoking the H-reflex on the same side and the test reflex used

was 20% of the maximum H-reflex. The recovery curve illustrated in their study showed a substantial reflex facilitation. They offered two possible explanations for the long lasting facilitation. They considered that it might be mediated via a long-loop reflex involving supraspinal centres or that it might be due to the effects of activity in cutaneous afferents (no comment was made upon the quoted suggestions of earlier authors, such as Gassel, [1970] that group III afferents might be involved, despite the fact that subliminal stimuli were used).

Delwaide, Crenna and Fleron (1981) examined the effect upon the ipsilateral and contralateral soleus H-reflex and tibialis anterior H-reflex or F wave of conditioning by cutaneous nerve stimulation. In response to stimulation of the sural nerve both the ipsilateral and contralateral recovery curves showed two separate marked periods of facilitation occurring at similar latencies. The contralateral soleus reflex facilitations began at 70ms and at 140ms. At a stimulation intensity of 2.5 times the threshold for perception a small, early inhibition was also seen - at around 25ms. This latter response was replaced by an early small facilitation when the contralateral conditioning stimulus intensity was increased to 4.5 times threshold for perception - a level at which the stimulus was painful.

Koceja and Kamen (1992) examined the effect of conditioning using (1) a contralateral tibial nerve stimulation sufficient to produce a soleus H-reflex 50% of maximum amplitude and (2) a contralateral tendon tap to the achilles tendon upon an ipsilateral soleus H-reflex (also 50% of the maximum reflex size). Their results indicated a long-latency, long-lasting facilitation of the H-reflex (commencing at around 40ms) in response to the contralateral electrical stimulation and a long-lasting inhibition in response to the tendon tap. The authors speculated that the facilitation was probably

the result of activity in cutaneous afferents and the inhibition was the result of presynaptic inhibition.

Bergego, Pierrot-Deseilligny and Mazieres (1981) reported that stimulation applied to the sole of the foot facilitated the transmission of the effects of Ib activation in the contralateral limb. Stimulation of the medial gastrocnemius nerve at 0.8 times the motor threshold produced a short latency facilitation of the quadriceps, considered to be the result of Ia afferent activity, followed by a depression identified by the authors as Ib-mediated. Pierrot-Deseilligny and his colleagues had, in a previous paper (Pierrot-Deseilligny et al. 1981), indicated the evidence for their belief in the Ib effects, while acknowledging that knowledge of the convergence of Ia as well as Ib afferents upon so-called Ib interneurons made it impossible to be confident that the effects were due to Ib afferents in isolation. The facilitation seen as a result of stimulation to the sole of the contralateral foot applied to both excitatory and inhibitory Ib effects. These results were later mirrored by the findings of Harrison and Zytnicki (1984) in cats, described above. The observations led to some interesting speculation about how signals from the sole of one foot might be used to modulate reflex activity in the contralateral limb during locomotion.

Delwaide and Pepin (1991) identified a short latency modulation of Ia reciprocal inhibition in the upper limb in humans in response to a contralateral conditioning stimulation. They used the reduction in the flexor carpi radialis H-reflex produced by weak stimulation of the ipsilateral radial nerve as the test reciprocal inhibition. Stimulation of the contralateral radial nerve at just below the threshold of the M wave produced a (16.5%) decrease in the size of the Ia-mediated inhibition and stimulation of the contralateral median nerve at a similar intensity produced a small (8.6%) increase in the size of the inhibition. Stimulation

of purely cutaneous branches of the contralateral nerves did not result in any changes in the ipsilateral reciprocal inhibition. Once again these findings were in keeping with those of Harrison and Zytnicki (1984) in the cat hindlimb. Delwaide and Pepin (1991) considered the observed effects to be the result of activity in Ia fibres and they proposed a possible spinal circuit (which involved excitatory commissural interneurons in lamina VIII) which could account for their findings. In their proposal the commissural interneurons were activated by group I ipsilateral afferents and in turn projected directly to contralateral Ia interneurons.

Tax, Duysens, Trippei and Deitz (1990) recorded responses in EMG activity of biceps femoris to contralateral sural nerve stimulation during different phases of walking. Reflex responses were recorded at a latency of 80ms and were much more pronounced in the stance phase, despite lower levels of spontaneous EMG activity in biceps femoris. This latency was similar to earlier-recorded ipsilateral responses during the swing phase (at 57-85 ms) and to responses during the stance phase in response to contralateral mechanical perturbations (at around 70ms). The results of the study, showing consistent changes in the size of the crossed response in relation to different phases in the gait cycle, indicated to the authors that the crossed pathways were being modulated at premotoneuronal level, perhaps by central pattern generator mechanisms.

Other human studies which have highlighted crossed responses are those which have examined the effect of perturbations upon the EMG activity of ipsilateral and contralateral muscles in standing or during walking. In particular recording from humans balancing on moveable platforms, Dietz and Berger (1982) observed bilateral increases in the EMG activity of tibialis anterior in response to either unilateral tibial nerve stimulation or to a brisk anterior tilt of the support of one foot. Both of these stimuli

produced a plantar flexor contraction and unilateral foot displacement. Symmetrical bursts of muscle activity occurred in tibialis anterior around 55ms to 70ms after the foot plantar flexion.

The authors considered that the short latency of this bilateral response indicated that it was mediated at spinal level and they argued that presumably this was because brainstem-mediated postural responses would be too slow in such a precarious balancing task. They demonstrated that the symmetrical activity seen was not present in situations where only one foot was in contact with a supporting surface and argued that probably the spinal coordinating mechanisms would be specific to different postural motor sets. This work has been followed by a large number of studies by Dietz and his colleagues in which interlimb responses to perturbation have been examined under different circumstances.

For example, following further observations of reactions to perturbations (Dietz, Quintern and Berger 1984a & 1984b), they recorded cerebral potentials in response to tibial nerve stimulation during stance and during gait (Dietz, Quintern and Berger 1985). They found that during gait the early (43ms) response disappeared and was replaced by a response at a longer latency (63ms). A similar response was observed in stance when group I afferents were subjected to ischaemic block. The authors postulated that, during gait, signals in group I afferents are blocked (and partially blocked during stance) and balance responses are mediated by group II inputs.

They also found that under different stance conditions interlimb coactivation in response to perturbation was centrally coordinated, producing different, but complementary, responses in each limb in order to restore balance (Dietz, Horstmann and Berger 1989). They related these

coordinated strategies to the evidence from animal experiments of different output patterns from group II activated INs in the midlumbar area of the spinal cord.

A recent study by Corna, Galante, Grasso, Nardone and Schiepatti (1996) showed a very early (SLR) and a medium latency (MLR) bilateral response in the EMG activity of several muscles to unilateral perturbations of a supporting platform in standing humans. The early response, being at around 40ms, was considered to be produced by group I activation. But the SLR was not seen in the contralateral limb when this limb was on firm ground instead of on the supporting platform, whereas the MLR was present, although its size and latency had changed. The authors considered the MLR to be the coordinated postural response, probably mediated by group II activity.

1.3 BACKGROUND OF THE PRESENT STUDY

In the light of the detailed results from animal work and the increasing, but mixed collection of observations from human subjects, it was decided that a search for further evidence of early crossed reflexes should be instigated, using electrical stimulation in awake humans at rest.

An earlier investigation by Harrison and Mehra (personal communication) into crossed effects in the human lumbar spine revealed no significant early effects upon quadriceps activity (monitored by surface EMG) as a result of electrical stimulation of the contralateral quadriceps. However it was expected that the Hoffmann reflex would allow a more sensitive examination of changes in motoneurone pool excitability in response to electrical stimulation. Thus in the present study the effect of conditioning

by stimulation of a peripheral nerve in the contralateral limb was examined over a 50ms interval.

Preliminary experiments were carried out using stimulation (at two times the motor threshold) of the posterior tibial nerve to condition the contralateral gastrocnemius H-reflex. These revealed a small inhibition, of the mean H-reflex amplitude, which was maximal at the 10ms condition/test interval. A facilitation was also observed - but at a longer latency (40ms and longer) - when the intensity of the conditioning stimulus was increased to three times the threshold of the motor response (see appendix I). Methodological problems resulted in a number of investigations related to the use of the H-reflex in general and the gastrocnemius M wave and H-reflex in particular and details of these are given in chapters 3 and 4.

It was decided that, using electrical stimulation of a peripheral nerve and monitoring changes in a contralateral H-reflex, several different pathways would be examined for evidence of crossed effects from large diameter afferent fibres. The evidence from animal studies indicated that different populations of commissural interneurons might mediate more powerful crossed effects from some muscle afferents than from others (Harrison and Zytnicki 1984). The pathways investigated were:

- (I) The effects of conditioning of the soleus H-reflex by a stimulus applied to the contralateral posterior tibial nerve.
- (II) The effects of conditioning of the quadriceps H-reflex by a stimulus applied to the contralateral femoral nerve
- (III) The effects of conditioning of the soleus H-reflex by a stimulus applied to the contralateral femoral nerve
- (IV) The effects of conditioning of the soleus H-reflex by a stimulus applied to the deep peroneal nerve.

2.0 INTRODUCTION TO THE HOFFMANN REFLEX

An early and a late muscle response to the submaximal electrical stimulation of mixed peripheral nerves was first recorded in 1912 by Piper (Piper 1912) but it was in 1918 that Paul Hoffmann observed that the later response to stimulation of the tibial nerve in the popliteal fossa consisted of a compound muscle action potential which resembled the reflex response to a quick stretch of the calf muscle (the tendon jerk) and that it occurred at a similar latency (Hoffmann 1918). In the light of his observations he postulated that the responses to both the electrical and the mechanical stimulation involved the same reflex pathway and that they must be mediated by rapidly conducting afferents with a minimal central delay.

It was not until the 1940s that Lloyd (1943) demonstrated in cats that the stretch reflex consisted of a monosynaptic pathway involving activity in group I muscle afferents. But, following this identification, Magladery and McDougal in 1950 confirmed the nature of the pathway in humans during their detailed studies of the responses to electrical stimulation using surface electrodes to record muscle action potentials.

They called the early response the M (motor) wave and described how its latency was directly related to the length of the motor nerve pathway between the point of stimulation and the muscle in which the response was observed, indicating that it was the result of direct electrical stimulation of the motor fibres. By stimulating the peripheral nerves at more than one point they were able to use the differences in the latency of the M responses from the different points to estimate the conduction velocities of the fastest-conducting motor axons in the nerve.

They confirmed Hoffmann's findings that submaximal electrical stimulation of the tibial nerve produced a late response whose characteristics were similar to "the potential changes accompanying stretch reflexes" and they called this the H wave, after Hoffmann. By progressively increasing the stimulus strength they identified the rapid growth of the H wave at relatively low intensities and its subsequent gradual diminution (as the M wave grew) at higher intensities, extending Hoffmann's observations.

They also used the changes in the relative latencies of the M and the H responses with different stimulation sites to compare the estimated conduction velocities of the afferent and efferent fibres. While acknowledging a limited level of accuracy and considerable variation in the estimates from subject to subject, a consistent finding was that the estimated afferent conduction velocities were always approximately one and a half times that of the estimated efferent conduction velocities. In using their observations to confirm that the H response was mediated by rapidly conducting muscle afferents in a pathway which was the counterpart of the two-neurone stretch reflex of the cat (identified by Lloyd, 1943), they pointed out the much slower conduction velocities which they had observed in humans compared with the well-documented conduction velocities of the large sensory and motor axons of cats. Although these assessments of human conduction velocities agreed with the results of previous work by Dawson and Scott (1949), at the time the authors were unsure about the large discrepancy between the cat and the human figures. They offered a tentative explanation relating to the much longer conducting distances of human nerves and the fact that their estimates of conduction velocities in distal sections of both motor and sensory nerves indicated that these were decreased in relation to those in proximal sections.

Magladery and McDougal (1950) also discriminated between the H wave, (seen by Hoffmann in the posterior tibial and quadriceps muscles) and another late response, (seen by Hoffmann in the intrinsic muscles of the hand) which they called the F wave. They clearly documented ways in which the behaviour of F waves differed from that of H waves:- they usually occurred at relatively high intensities of stimulation; they did not diminish with increasing intensity and they appeared irregularly, that is they did not appear with every shock. Although the authors mentioned the possibility of F waves resulting from impulses travelling antidromically in the motor axons (the explanation upon which modern authors are now generally agreed) their tests indicated that the conduction velocities of nerves mediating the F wave were not the same as those mediating the M wave and so they favoured the explanation that the F wave was another reflex response, but one involving slower conducting afferent fibres.

The work of Magladery and McDougal had confirmed the existence of an electrically-induced response in humans, with all the hallmarks of a monosynaptic reflex, which was relatively easily accessible and which was therefore, potentially, a most valuable tool for the study of motoneurone behaviour in humans. There then followed a number of classic studies in which various aspects of the methods of eliciting this H-reflex were explored and the problems of interpretation of the results were examined.

In 1955, in his doctoral thesis, Paillard also confirmed those characteristics of the H-reflex which indicated both its mediation by the largest afferent fibres and its monosynaptic pathway. He emphasised its value in human studies and indicated how systematic comparisons of the behaviour of the H-reflex with that of the stretch-induced reflex could distinguish between central excitability changes and those at the level of the peripheral receptor (Paillard 1959). He went on to highlight a number of important concerns

in relation to the experimental conditions of eliciting the H-reflex and he emphasised the importance of the positioning and stability of the subject during the experimental process; the maintenance of the relationship between the peripheral nerve and the stimulating electrodes and the importance of the location of the recording electrodes.

Hugon (1973) continued the examination of methodological aspects of the use of the soleus H-reflex and he looked at the behaviour of each of the different components of the reflex over a range of stimulus intensities, using different methods of surface recording and found them to remain roughly proportional to one another during bipolar recordings. He also noted that at its maximum the M wave should represent the excitation of all the motor fibres. Therefore its amplitude could be used in comparison with that of the H response to indicate roughly what percentage of the motoneurone pool was reflexly activated - provided that one could be confident that the M wave represented activity only in soleus and that it was not contaminated by that in gastrocnemius. This served to emphasise the importance of the positioning of both the recording and the stimulating electrodes. Finally Hugon produced a practical protocol for H-reflex testing which was both realistic and also took into account his and previous authors' findings.

During the Brussels International EMG Congress of 1971 the use of the Hoffmann reflex was thoroughly explored and following this a paper was prepared containing input from many authorities in the field offering important suggestions concerning standardisation of methods used to elicit the Hoffmann reflex and the tendon reflex (Brunia et al. 1973). Although accepting a number of possible alternate strategies they highlighted, with explanations, optimum set-ups. For instance, they indicated the fact that for long experiments subjects would be more comfortable in supported sitting

than in prone and emphasised once again, as had Paillard and Hugon, the importance of finding an optimum position for the stimulating electrodes with which a large H-reflex could be elicited at a low intensity of stimulation without the presence of an M wave.

The relationship between the amplitudes of the H-reflex and the M wave and the intensity of stimulation - the recruitment curves - was soon recognised as reflecting the differential nature of the distribution of axon diameters (and thus thresholds of electrical excitability) of the two populations of nerve fibres concerned (Magladery et al. 1951, Paillard 1955). The fact that the largest primary afferents in muscles such as soleus are of a larger diameter than the largest alpha motoneurone axons accounts for the appearance of the Ia-mediated reflex at lower intensities of stimulation.

Once discovered, the H-reflex became an increasingly popular tool to monitor changes in motoneurone pool excitability, although aspects of the relationship between the H-reflex and the M wave, which affect the function of the reflex as a tool, have only gradually become clear. Some authors (for example Funase, Imanaka and Nishihira 1994) have identified changes in the H-reflex threshold in relation to that of the M wave and changes in the maximum amplitude of the H-reflex compared with the maximum M wave amplitude as being more sensitive measures of changes in the excitability of the motoneurons on to which the Ia afferents synapse. Certain aspects of the relationship between the M wave and the H-reflex which have implications for the choice of an optimal experimental procedure are examined later.

The discovery of the presynaptic inhibition of muscle spindle afferents by Frank and Fuortes in 1957; its investigation in cats (e.g. Eccles, Schmidt

and Willis 1962) and the much later emergence of evidence for the mechanism in humans (Morin, Pierrot-Deseilligny and Hultborn 1984, Hultborn, Meunier, Morin and Pierrot-Deseilligny 1987) had a major effect upon the interpretation of the results of H-reflex studies. Changes in the excitability of the monosynaptic reflex arc could no longer be assumed to be the consequence of changes in the post-synaptic properties of motoneurons. A tonic inhibition of afferents, which can be increased or decreased during movement to allow selective control of sensory input, now appears to be an important phenomenon in motor control and can have a profound effect upon the motoneurone output in response to the activation of Ia afferents.

Another of the most important modern developments in the history of the H-reflex has been the careful examination of the likely nature of the afferent volley involved and its implications for the interpretation of H-reflex studies. In 1983 Burke, Gandevia and McKeon used microelectrodes to record from the tibial nerve in the popliteal fossa during electrical stimulation and during tendon percussion. They also used post stimulus time histograms (PSTHs) to examine the EPSPs produced in soleus motoneurons as a result of the two forms of stimuli and extended this aspect of the work in their subsequent study (Burke et al. 1984). The authors demonstrated that the difference in the dispersion of the two volleys (first described by Gassel and Diamantopoulos in 1966) was one of a number of factors indicating differences between the two reflexes. The tendon percussion resulted in a widely dispersed response. By the time the effect of the tendon tap reached the receptors in the muscle belly it had become a series of vibrations which caused the spindle receptors to discharge several times. Some mechanoreceptors in other muscles and cutaneous receptors throughout the limb were also seen to be activated by the wave of vibration. Thus it was clear that the dispersed volley, lasting 25

to 30 milliseconds, was probably mediated by other afferents as well the Ia afferents.

The volley in response to electrical stimulation was much more discrete, but even so the difference in conduction velocities between the fastest and the slowest Ia fibres would mean that the arrival of the volley at the motoneurone pool could be spread over as much as ten milliseconds. On top of this, Jankowska, McCrea and Mackel (1981) had already demonstrated in the cat that Ia afferents made oligosynaptic as well as monosynaptic connections with homonymous motoneurons. And although the volley would be likely to be more selective than that produced by the mechanical stimulus, responses were sometimes observed in Ia afferents from intrinsic foot muscles and cutaneous foot afferents.

Another means by which the electrically-induced volley in soleus Ia afferents would be likely to be contaminated in the H-reflex is by activity in Ib afferents. It has been assumed that in humans the electrical thresholds between Ia and Ib afferents are much too close to make it possible to separately activate them and that the action potentials in the fastest Ib fibres could arrive at the motoneurone pool as little as one millisecond behind those in the fastest Ia fibres (Pierrot-Deseilligny, Morin, Bergego and Tankov 1981). Therefore the H-reflex might well represent the combined effects upon the motoneurone pool of Ia excitation and Ib inhibition.

The contribution of volleys in oligosynaptic pathways to both the tendon jerk and the H-reflex could only be significant if the average rise time of the composite EPSPs produced in the motoneurons by the two reflexes was of a sufficient duration before the cell discharged to allow time for the volleys to have an effect. In their second study Burke and his colleagues looked at rise times of EPSPs in individual cells and at the time course of

the reflexly-induced changes in excitability of the motoneurone pool and were able to demonstrate that, although the mechanically induced changes were of sufficiently long duration that the likelihood of substantial oligosynaptic contributions could not be denied, even the electrically-induced excitability changes were of a duration which could allow for a contribution from volleys in group Ib afferents.

The major thrust of the authors' arguments in relation to their findings was that in both of these reflexes the Ia contribution is likely to be contaminated by volleys in other pathways and that this contamination is quite different in the two reflexes; that the activity in these other "contaminating" pathways may well represent a significant part of the reflex, especially in the case of the tendon jerk; that in each of the reflexes the pathways thus involved would contain interneurons of different populations whose excitability could be selectively controlled and that as a result the different reflexes could often be modulated differently and should no longer be seen simplistically as representing the same reflex pathway, differentiated only by the exclusion of the muscle spindle from the H-reflex arc.

In relation to the use of the H-reflex in the present study, a number of methodological and theoretical concerns emerged. These problems led to related investigations, which are described in detail in chapters 3 and 4 and are considered, where appropriate, in the discussions of the results of the "crossed" experiments.

Since the early 1970s the use of the H-reflex has expanded enormously and it is now used in a wide variety of studies. Ways of eliciting the reflex in a greater number of muscles have emerged and comparisons between the latency and the behaviour of the reflex in normal subjects and in subjects

with neurological conditions has revealed information concerning some of the pathological changes occurring.

In normal subjects it has been used to reveal modulatory changes in the excitability of the reflex arc during movement which are independent of changes in muscle activity and therefore signal selective supraspinal or spinal control. Conditioning of the H-reflex by the electrical stimulation of other peripheral nerves has turned out to be a fruitful method of identifying spinal pathways linking cutaneous afferents and the afferents from certain muscles to the motoneurons of other muscles. Such methods have been used especially by Pierrot-Deseilligny and his colleagues (e.g. Pierrot-Deseilligny et al. 1981, Hultborn et al. 1987 and Meunier, Pierrot-Deseilligny and Simonetta 1993) to investigate the pattern of projections from different afferents in the human limbs and the control of some of the identified pathways during movement. In the present study a similar approach was adopted in using the H-reflex to look for evidence of crossed reflexes in humans.

3.0 A STUDY OF THE INHERENT VARIABILITY OF THE HOFFMANN REFLEX

3.1 INTRODUCTION

Preliminary trials of the crossed experiments using a provisional experimental procedure revealed that changes induced in the H-reflex as a result of contralateral conditioning were likely to be relatively small and that the variability of the reflex was relatively large.

Such observations threw into focus some important properties of the soleus H-reflex as a measurement tool. When attempting to detect statistically significant changes due to a conditioning effect, both the possible size of the conditioning effect and the variability of the reflex due to randomly occurring influences must be considered, as well as the ability of the H-reflex to reflect accurately changes in the reflex arc in terms of changes in its amplitude.

3.1.a THE SENSITIVITY OF THE H-REFLEX TO CONDITIONING STIMULI

The larger the size of a conditioning effect, the easier it will be to detect it and Crone, Hultborn, Mazieres, Morin, Nielsen and Pierrot-Deseilligny (1990) have shown that the H-reflex exhibits a sensitivity to facilitation or inhibition which changes with its size. The findings of these researchers - that the effect of conditioning stimuli is greatest when the H-reflex is between one third and two thirds of its maximum size - is of relevance to the present study. The authors also highlighted another important factor affecting the detection of a conditioning effect. That is, that under certain

circumstances the H-reflex amplitude does not represent all the motoneurones which have been reflexly activated.

As described above in chapter two, when the amplitude of the soleus H-reflex is plotted as a function of the intensity of the electrical stimulus, a recruitment curve is produced. The specific features of the recruitment curve are the increases in amplitude of the reflex with increasing stimulus intensity (the rising phase of the recruitment curve) and the decreases in the reflex amplitude with increasing intensity of stimulation which occur at higher intensities (the falling phase). The reflex usually reaches its maximum amplitude at the intensity at which the M wave appears, or just above this level. During the falling phase of the recruitment curve, in the presence of the M wave, the reflex decreases in size because some of the larger reflexly recruited motoneurones have axons which are now being activated directly in the M wave. As a result of the collision effect, the discharges from these motoneurones are occluded and will not reach the muscle. Therefore they cannot contribute to the reflex response recorded in the EMG. In other words the recorded reflex amplitude will not be an accurate representation of the numbers of reflexly activated motoneurones.

When the motoneurone pool is subject to an inhibitory effect, if this effect is distributed evenly to all motoneurones, then the motoneurones most likely to be derecruited as a result of the inhibition are those which have been the last to reach threshold in response to the reflex input. But as these may not contribute to the recorded reflex response, because of the collision effect, then any inhibition of these particular motoneurones will not be detectable.

The effect of an excitatory conditioning input upon the reflex response of the motoneurone pool could also be partially hidden in the presence of a

direct motor response. Those motoneurons which have not quite reached threshold as a result of the unconditioned reflex volley would be the most likely to be reflexly recruited under the influence of an evenly-distributed excitatory conditioning input. These would be relatively large cells, because the smaller ones would already have been reflexly recruited at lower stimulus intensities. In the presence of an M wave the axons of these larger motoneurons would probably already be being stimulated directly. If this is the case, then their discharge will not contribute to the reflex response recorded at the muscle and their reflex recruitment will go undetected.

For these reasons the use of H-reflexes of the descending limb (falling phase) of the recruitment curve does not allow changes in motoneuron excitability to be accurately assessed. Large conditioning effects could still cause recruitment or derecruitment of a sufficient number of those motoneurons not affected by the collision effect to produce detectable changes in the reflex amplitude, but the size of the conditioning effect would not be measurable. Small conditioning effects may make no impact upon the reflex amplitude.

To ensure maximum sensitivity of the H-reflex to conditioning effects, experimental procedure should involve the following :- the use of reflexes of the rising phase of the recruitment curve; the use of reflexes of an amplitude between one third and two thirds of the maximum amplitude and the use of reflexes of a similar size (in relation to the maximum H-reflex amplitude) in different trials.

3.1.b THE VARIABILITY OF THE H-REFLEX

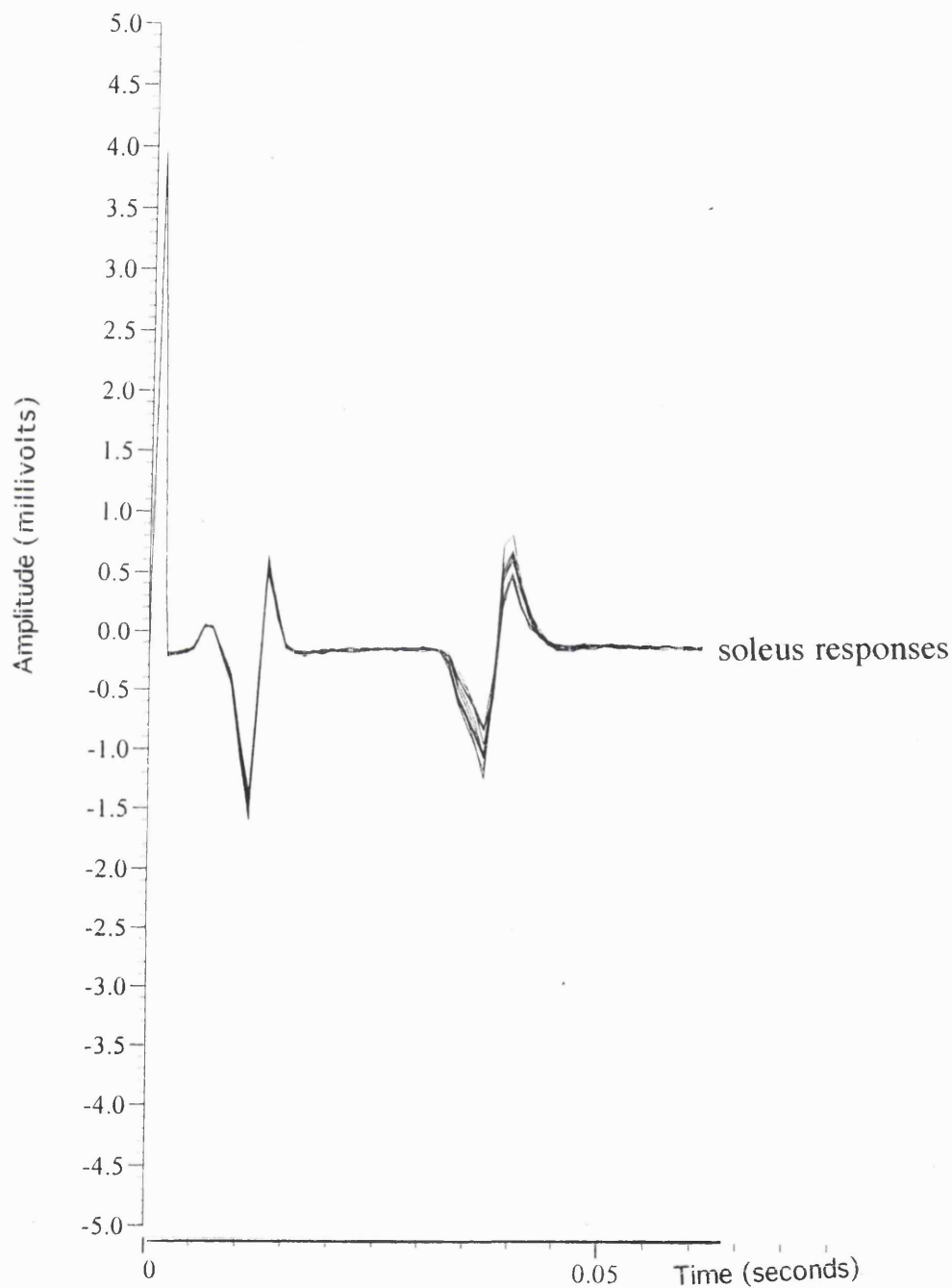
When H-reflex and M wave responses to repeated stimulation are compared it can be seen that the M wave amplitude usually remains relatively unchanging while the size of the H-reflex often changes visibly from one reflex to the next. In figure 3.1 a number of consecutive responses have been superimposed and the greater variability of the soleus H-reflex can clearly be seen. Similarly, in figure 3.2 the peak to peak amplitudes of one hundred consecutive soleus H-reflexes and those of M waves of similar size have been plotted against time and the scatter of the individual H-reflexes is obviously greater.

The large variability of the H-reflex has long been recognised (Simon 1962, Hugon 1973, Gottlieb & Argarwal 1978, Crone & Neilson 1989). In the course of the wide use of the H-reflex, emphasis has often been placed on stabilising the stimulation arrangement so that external factors contributing to the variability are kept to a minimum (for example Paillard 1955, Simon 1962, Hugon 1973).

Hugon (1973) observed that the number of tests required to identify the mean value and the standard deviation of the reflex varied according to the variability of the reflex in each subject. He identified twenty measurements as being usually sufficient to achieve this. However he did not consider the size of any facilitation or inhibition of the reflex which could be detected under these conditions.

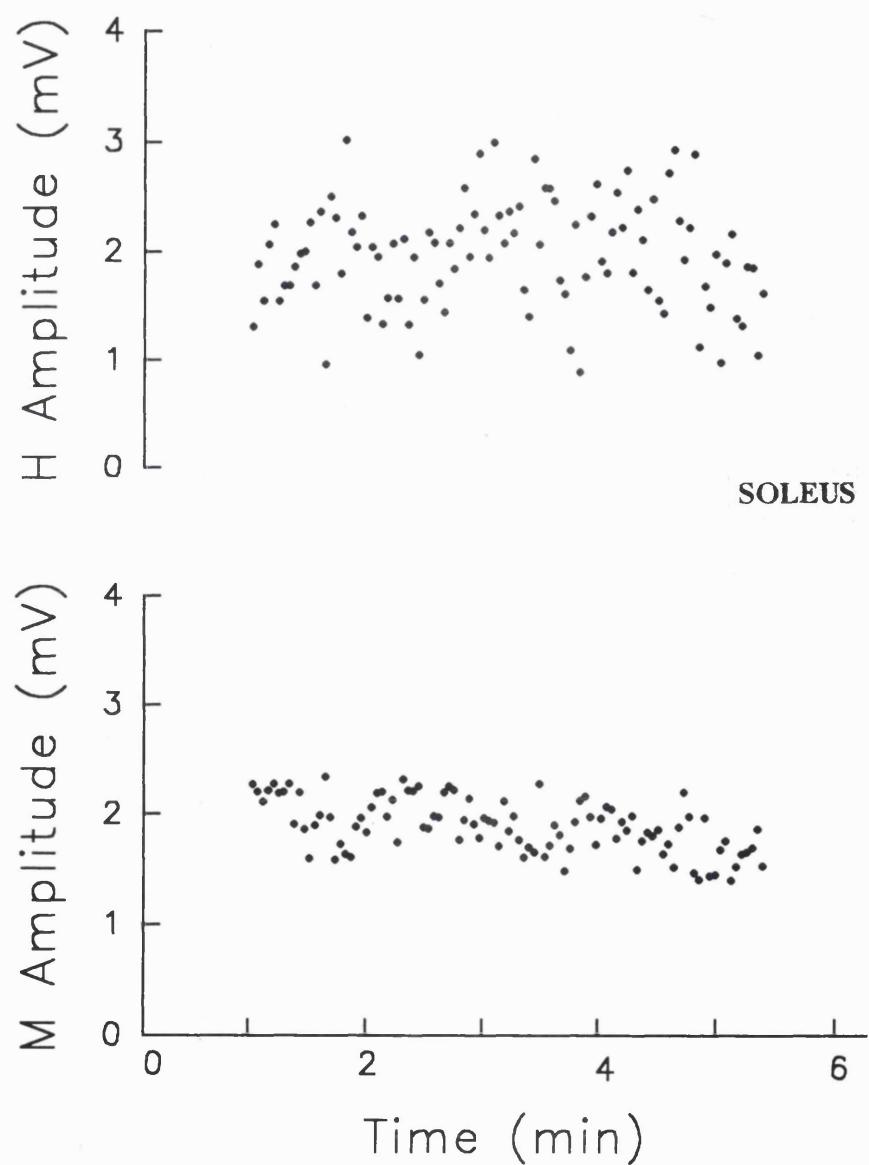
Veale, Rees and Mark (1973) used samples of between twenty and fifty H-reflexes in their study of Renshaw cell activity. On examination of the H-reflex literature, many researchers have followed the guidelines of Hugon and compared groups of twenty responses. In their study of the effects of

FIGURE 3.1 CONSECUTIVE M AND H RESPONSES SUPERIMPOSED UPON ONE ANOTHER - THE GREATER VARIABILITY OF THE H-REFLEX CAN BE SEEN CLEARLY



M and H responses in soleus produced by electrical stimulation of the tibial nerve at twice the threshold intensity of the M wave. This intensity was chosen to produce M and H responses of a similar size. Stimulus applied at 0 seconds

FIGURE 3.2 AMPLITUDES OF ONE HUNDRED CONSECUTIVE M WAVES AND H-REFLEXES PLOTTED AGAINST TIME - THE SCATTER OF THE INDIVIDUAL H-REFLEXES IS OBVIOUSLY GREATER THAN THAT OF THE M WAVES



the Jendrassik manoeuvre upon the H-reflex and the tendon reflex by Bussel, Morin and Pierrot-Deseilligny (1978) there is an example of an occasion when a sample size of twenty was not sufficient to detect a small change. The authors noted that a sustained handgrip had a less marked effect upon the H-reflex than that of a brisk grip. They pointed out that, because of the small conditioning effect, they would need to collect more reflexes from those subjects who exhibited a relatively high H-reflex variability, but they were constrained by the duration of the period of ischaemia within which they could collect data. As a result they were only able to study the effect in those subjects in whom the reflex variability was low.

It had become clear that monitoring changes induced by conditioning stimulation of contralateral nerves over a suitable range of time intervals involved long experiments. In order to be able to choose a collection rate which would allow the detection of significant changes against the background noise, a study was made of the variability of the reflex over a range of reflex amplitudes in seven subjects.

3.2 METHOD

Eight experiments were carried out on seven normal subjects aged between twenty and forty seven years, who gave their informed consent. In each experiment between forty five and one hundred individual soleus H-reflex responses were recorded at each of a series of stimulus intensity levels, selected in random order, between that intensity which produced a threshold H-reflex and that which produced a maximum M wave.

3.2.a EXPERIMENTAL SET-UP

During stimulation and recording each subject was seated in a high-backed chair with the target lower limb supported at the thigh and at the foot so that the limb was maintained with the knee resting at an angle of approximately fifty degrees from full extension and the ankle at an angle of approximately ten degrees of plantar flexion. Care was taken to ensure that the subject was able to remain comfortably at rest in the supported sitting position throughout the period of data collection. The subject was asked to relax and to avoid unnecessary movement. The surface electromyographic (EMG) recording was used to check that the soleus muscle was relaxed.

3.2.b STIMULATION

Electrical stimuli were delivered using a constant current high voltage stimulator (Digitimer D57), which was triggered externally by a programmable timer (Digitimer Programmer D4030) and this was also used to trigger the recording of the electromyographic response of the soleus muscle to each stimulus.

Square wave pulses of one millisecond duration were used in order to preferentially elicit the reflex response (Paillard 1955, Hugon 1973, Veale et al. 1973 and Deschuytere et al. 1983).

The interstimulus interval was 2.636 or 3.636 seconds in six of the eight experiments. In two experiments longer intervals were used - 5.136 and 8.636 seconds.

The cathode was a one centimetre diameter, saline-moistened, lint-covered ball electrode with a short "stalk" which pierced and was secured to a rubber strap. On the posterior surface of the knee the cathode position was adjusted to a point over the tibial nerve where it was possible to obtain an H response without the presence of an M response and then the strap was secured in position. The anode was a five by three centimetre flat, saline moistened, lint-covered metal electrode which was placed on the anterior surface of the thigh just proximal to the base of the patella and secured with a rubber strap or a crepe bandage.

3.2.c RECORDING

Hugon (1973) recommended placement of the electrodes over the mid-dorsal tendinous region, but used more lateral or medial positions on occasions. Brunia et al. also recommended a position on the "longitudinal axis of the calf", distal to the insertion of gastrocnemius, but they suggested the side of the calf "where the soleus muscle is not covered by the gastrocnemius tendon" as another acceptable location. Mineva et al. (1993) recorded from many sites over the soleus muscle area while comparing compound muscle potentials of H and T-reflexes. They found that the amplitudes of monopolarly recorded responses were generally smaller the further the electrodes were from the midline, but the largest monopolar

and bipolar responses were obtained when electrodes were placed more than three centimetres below the insertion of gastrocnemius.

In the present study, when simultaneous recordings were made from the midline and from either side, the midline responses were usually slightly larger, but the responses behaved similarly. In view of the fact that one of the most important considerations was to try to obtain a response from soleus uncontaminated by that from gastrocnemius and as good responses were obtained from recording directly over the soleus muscle fibres on either side of the tendon, this was the area which was chosen. Two blue sensor disposable adhesive recording electrodes were placed on the cleaned skin over the body of the soleus muscle on either side of the central tendinous area, about four centimetres apart and well (approximately five to six centimetres) below the visible boundaries of the gastrocnemius muscle. The third (reference) electrode was placed on the skin over the antero-medial surface of the tibia, a muscle-free area.

The bipolar surface electromyographic recordings were differentially amplified (usually five hundred or one thousand times) so that the amplitudes of the displayed reflex responses were clearly visible at all intensities of stimulation. The amplified signal was filtered with a low bandpass of 30 Hz and a high bandpass of 500 Hz. The signal was fed into an analogue digital converter (C.E.D.1401) which was connected to a personal computer (IBM 486 DX). The running programme - Signal Averager version 5.43 - allowed on-line display and storage of the filtered and amplified signal. The sample rate used was 1000 Hz and a sweep length of 200 ms was displayed, which was synchronised with the delivery of a stimulus.

The recordings from each experiment were analysed at a later date. The amplitudes of the individual H-reflex and M wave responses were measured from peak to peak (Brunia et al. 1973, Gottlieb & Agarwal 1971,1978, Hultborn et al. 1987, Nadeau and Vanden-Abeelee 1988) and the amplitudes of all the responses for each intensity level were stored in separate files. Examples of recordings of several superimposed responses at a number of stimulus intensity levels from one subject are illustrated in figure 3.3. The mean, the standard deviation and the coefficient of variation were calculated for the sample from each intensity level for each subject.

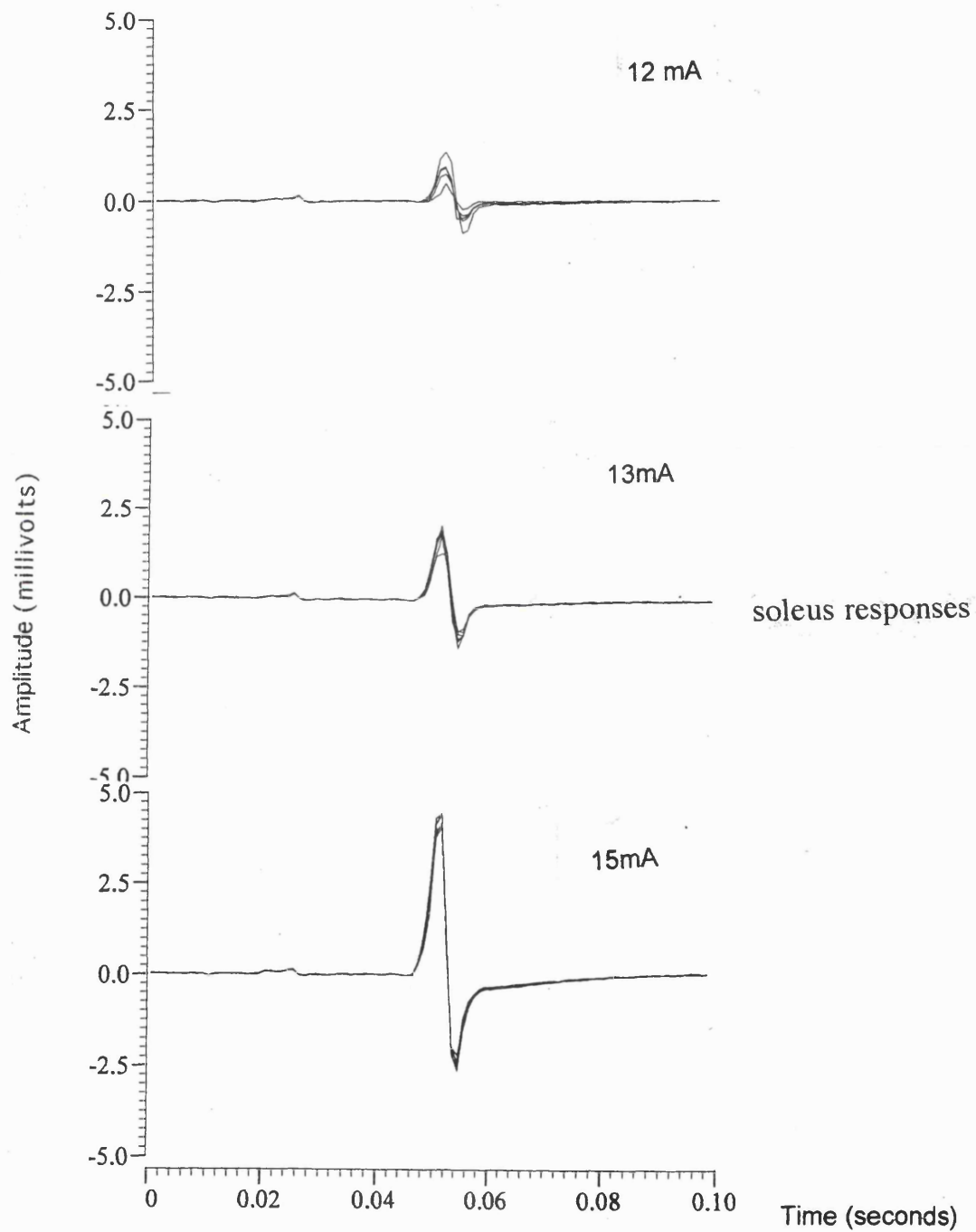
3.3 RESULTS

The results from all seven subjects showed a similar pattern, but in one of these subjects it was not possible to produce an H-reflex without the presence of an M wave. Two experiments were carried on this subject and in both cases the H-reflex increased in size with increasing stimulus intensity in the presence of a slow-growing M wave. (Similar observations have been made by Magladery and McDougal, 1950 and Ishikawa et al. 1966).

In all seven subjects, the standard deviation of the H-reflex increased at first as the mean reflex amplitude increased and it soon reached a plateau while the reflex amplitude was still increasing. This is illustrated in the plots from two subjects in figure 3.4 a.

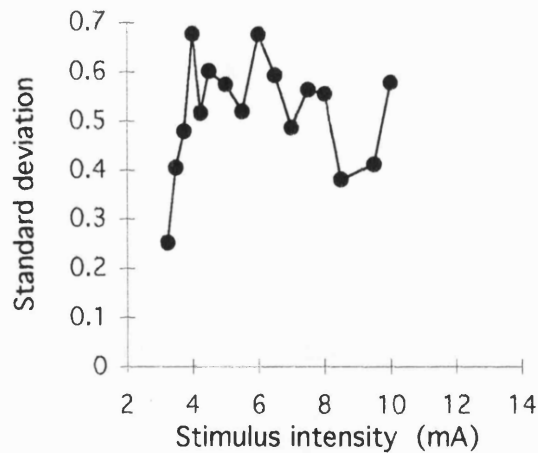
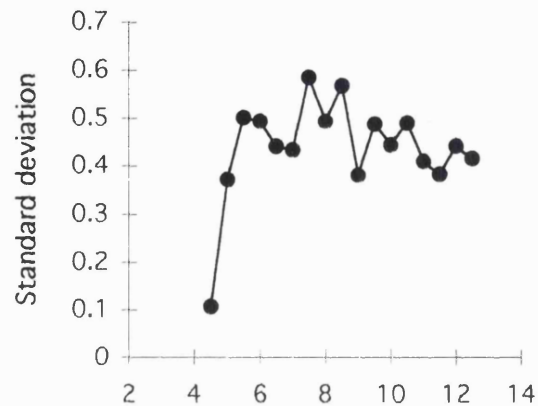
However, when the standard deviation was expressed as a percentage of the arithmetic mean amplitude (the coefficient of variation) it clearly decreased as the reflex grew with increasing intensity of stimulation during the rising phase of the recruitment curve (see figure 3.4 b). The separate results from the seven subjects are displayed in figure 3.5 and it is clear that, although

FIGURE 3.3 RECORDINGS OF H-REFLEX RESPONSES AT THREE DIFFERENT LEVELS OF STIMULUS INTENSITY FROM ONE SUBJECT. SEVERAL CONSECUTIVE RESPONSES ARE SUPERIMPOSED AT EACH LEVEL OF STIMULUS INTENSITY



Responses in soleus produced by stimulation of the tibial nerve. M threshold intensity was 18mA
Stimulus applied at 0.01s

FIGURE 3.4 (a)
STANDARD DEVIATION OF THE H-REFLEX PLOTTED
AGAINST STIMULUS INTENSITY IN TWO SUBJECTS



(b)
STANDARD DEVIATION OF THE H-REFLEX EXPRESSED AS A PERCENTAGE OF
THE MEAN AMPLITUDE PLOTTED AGAINST STIMULUS INTENSITY IN THE
SAME TWO SUBJECTS

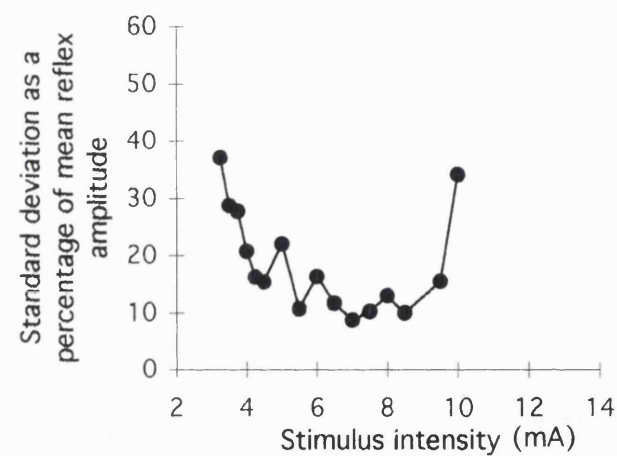
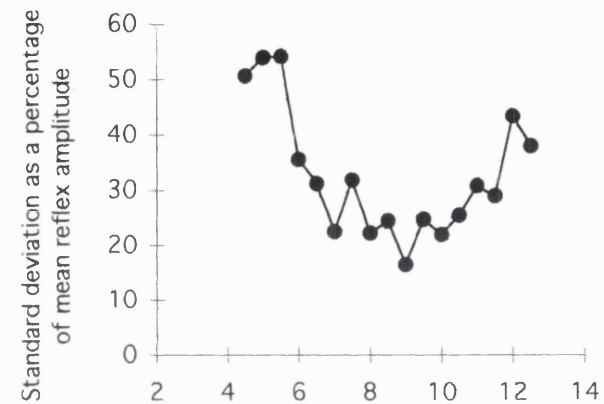
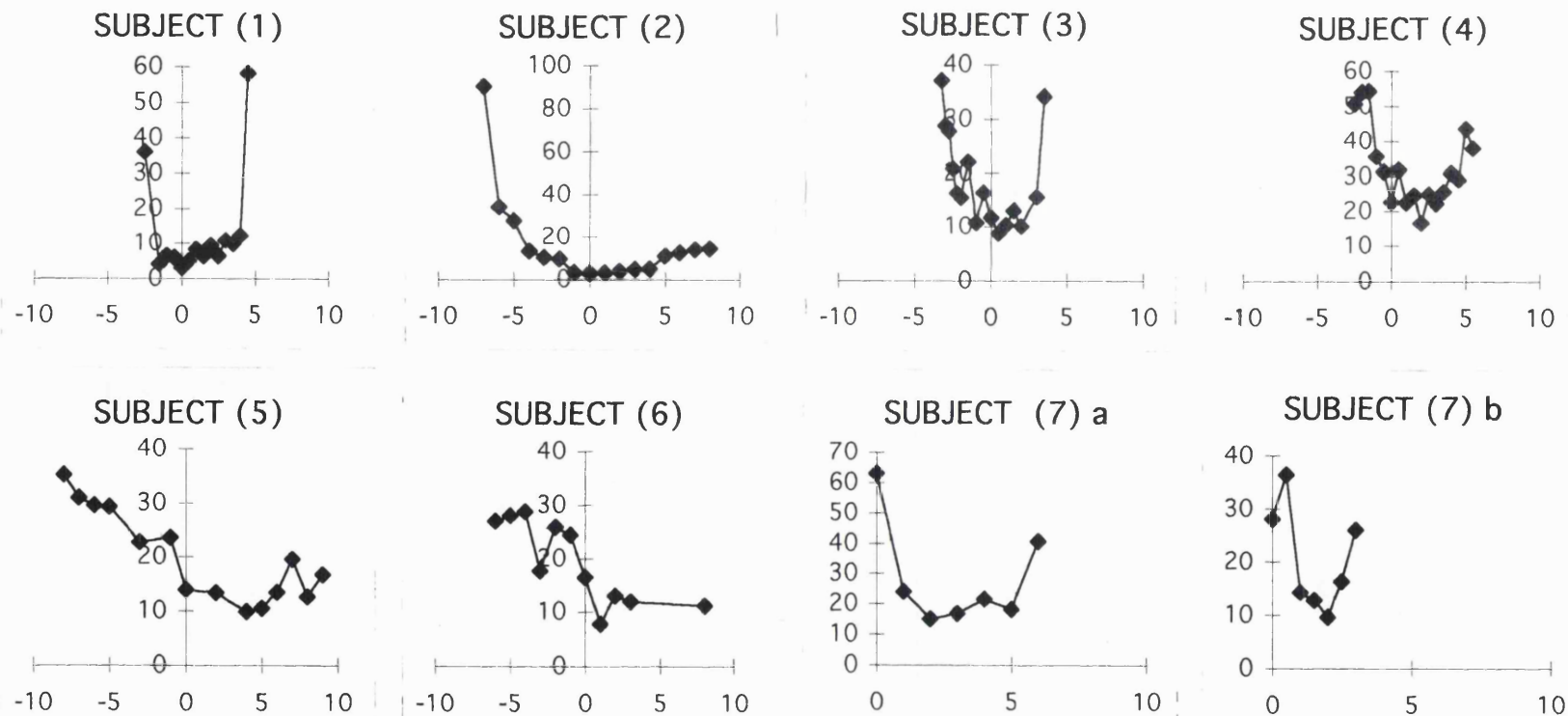


FIGURE 3.5 VARIABILITY OF THE SOLEUS H-REFLEX IN RELATION TO THE INTENSITY OF ELECTRICAL STIMULATION

Results from seven subjects. For each graph, the abscissa has been offset so that 0 mA represents the threshold of the M response. Ordinate : standard deviation expressed as a percentage of mean reflex amplitude. Each point represents the results from a run of 45, 50 or 100 reflex responses. Two sets of results from subject 7. In both cases it was not possible to elicit the reflex without an M wave.



interindividual differences are large, there is a remarkably consistent pattern. With increasing intensity of stimulation, as the reflex grows towards a maximum amplitude around the M threshold intensity, a reduction in the variability of the reflex occurs in every subject.

The inverse relationship between the variability of the reflex and its size emerges clearly when the coefficient of variation is plotted against the amplitude of the reflex as in figure 3.6. In this figure the plots from two of the subjects are illustrated, but the relationship was clear in all seven subjects.

(The expression of variability in terms of the coefficient of variation is particularly useful because it allows the scatter of measures in a sample to be identified immediately in terms of the mean of the sample and in the case of the H-reflex most conditioning effects are also expressed as a percentage of the mean test reflex size.)

No detectable differences were seen in the results from the experiments in which longer interstimulus intervals were used. In figure 3.7 results are displayed from the two experiments using subject seven. The interstimulus interval used was 8.136 seconds in one experiment and 2.636 seconds in the other. The plots of variability of the H-reflex against reflex amplitude show a similar pattern.

FIGURE 3.6 VARIABILITY OF THE SOLEUS H-REFLEX IN RELATION TO ITS AMPLITUDE

VARIABILITY OF THE H-REFLEX IS EXPRESSED AS THE COEFFICIENT OF VARIATION (STANDARD DEVIATION AS A PERCENTAGE OF THE MEAN). THIS IS PLOTTED AGAINST THE AMPLITUDE OF THE H-REFLEX EXPRESSED AS A PERCENTAGE OF THE MAXIMUM REFLEX AMPLITUDE. PLOTS FROM TWO SUBJECTS EACH DATA POINT REPRESENTS THE MEAN OF ONE HUNDRED REFLEXES A RELATIONSHIP BETWEEN THE VARIABILITY OF THE REFLEX AND ITS SIZE IS CLEARLY ILLUSTRATED

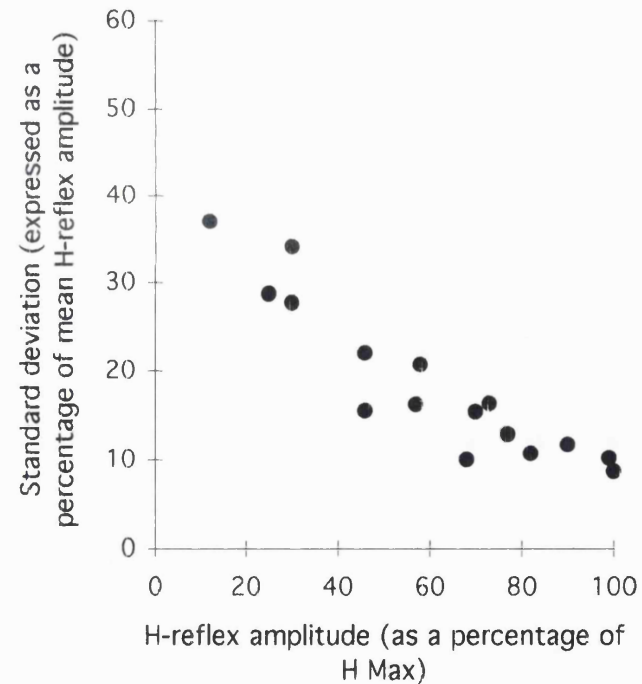
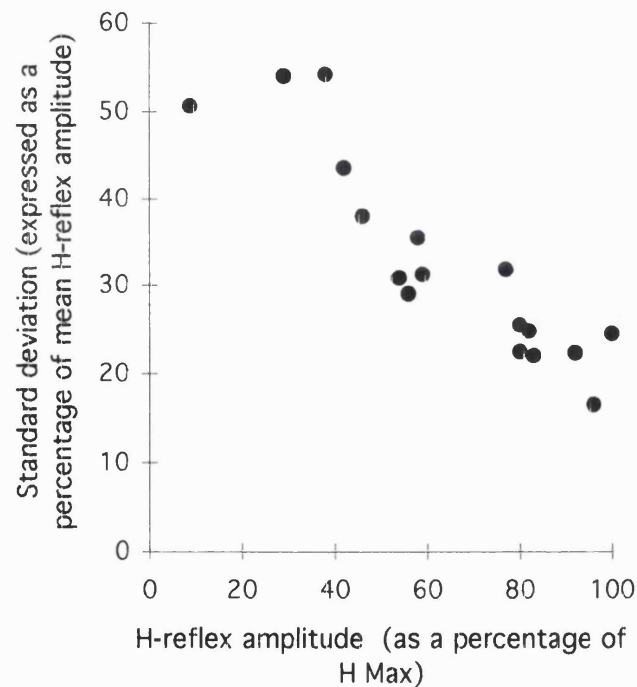
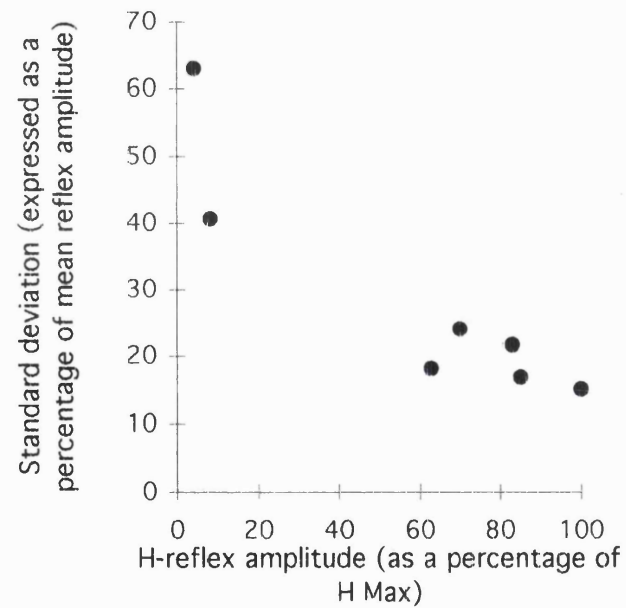


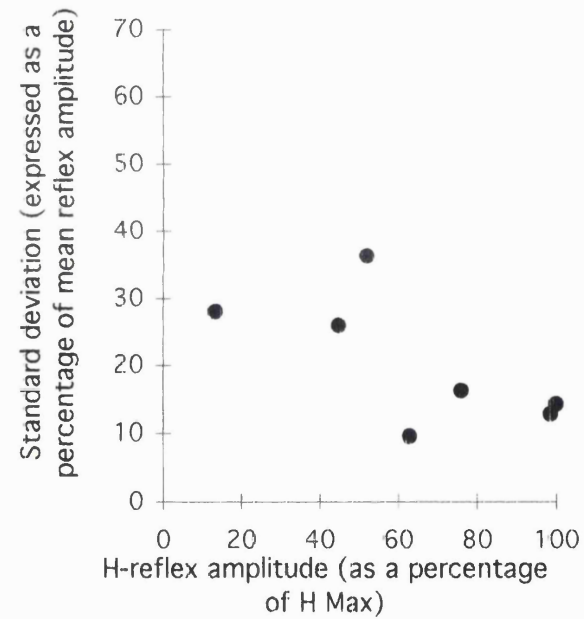
FIGURE 3.7 VARIABILITY OF THE SOLEUS H-REFLEX IN RELATION TO ITS AMPLITUDE - RESULTS FROM ONE SUBJECT

RESULTS FROM TWO EXPERIMENTS PERFORMED UPON THE SAME SUBJECT, BUT USING DIFFERENT INTERSTIMULUS INTERVALS - 8.136 seconds (a) AND 2.636 seconds (b)

BOTH SETS OF RESULTS SHOW DECREASING H-REFLEX VARIABILITY WITH INCREASING REFLEX AMPLITUDE



(a)



(b)

Although the degree of variability of the reflex was quite different in different individuals, the consistency of the variability / amplitude relationship between individuals was such that useful general ratios could be drawn from it. H-reflexes with a mean amplitude of thirty per cent of the maximum have a variability of at least twice that of reflexes with a mean amplitude of eighty per cent of the maximum and approximately one and a half times that of reflexes with a mean amplitude of sixty per cent of the maximum.

It was possible to use the results from the present study to estimate sample sizes needed in order to have an eighty per cent chance of detecting small changes in the reflex size due to conditioning stimuli, if they exist. The estimated magnitude of the conditioning effect is calculated using the coefficient of variation as the measure of inherent variability in a typical equation, here taken from Keppel et al. (1992).

Estimated magnitude of conditioning effect $\hat{\omega}_A^2$

= $\frac{\text{variability due to conditioning}}{\text{total variability (variability due to conditioning + inherent variability)}}$

$$= \hat{\omega}_A^2 = \frac{\hat{\sigma}_A^2}{\hat{\sigma}_A^2 + \hat{\sigma}_{S/A}^2}$$

(where $\hat{\sigma}^2$ represents variance and $\hat{\sigma}_A^2$ represents estimated population variance due to conditioning and $\hat{\sigma}_{S/A}^2$ represents estimated inherent population variance).

The estimated conditioning effect could then be used to estimate the sample size required.

$$\text{Estimated sample size} = \phi^2 \left(\frac{1 - \hat{\omega}_A^2}{\hat{\omega}_A^2} \right)$$

with the value of ϕ available in a power functions table.

Calculations were made in relation to detecting five per cent and ten per cent changes in mean reflex amplitude. Examples were taken from one subject with a relatively high H-reflex variability and from one subject with a relatively low H-reflex variability, using reflexes with mean amplitudes of approximately thirty, sixty and eighty per cent of the maximum in each case.

In the subject with relatively high H-reflex variability :-

For reflexes of amplitude (of maximum H amplitude)	30%	60%	80%
Coefficient of variation was	47%	27%	16%
Estimated sample size to detect a 5% conditioning effect (power function 0.8)	400	120	42
Estimated sample size to detect a 10% conditioning effect (power function 0.8)	90	30	11

In the subject with relatively low H-reflex variability :-

For reflexes of amplitude of maximum H amplitude	30%	60%	80%
---	-----	-----	-----

Coefficient of variation was	27%	17%	12%
------------------------------	-----	-----	-----

Estimated sample size to detect a 5% conditioning effect (power function 0.8)	120	46	25
--	-----	----	----

Estimated sample size to detect a 10% conditioning effect (power function 0.8)	30	12	6
---	----	----	---

Using this, in all the crossed experiments, where small changes were expected, samples of between 30 and 50 reflexes were used, in order to detect changes as small as and smaller than 10% of the mean.

3.4 DISCUSSION

When surface electrodes are used to electrically stimulate peripheral nerves in human experiments, there are both similarities and differences between the responses elicited under these conditions and those produced in animal experiments, where the peripheral nerve can be placed directly upon the stimulating electrodes.

In most experiments upon anaesthetised animals the current flows directly from the cathode onto the outer covering of the nerve trunk with which it is in contact and the contact between the electrode and the nerve can be

kept relatively stable. The nerve trunks of most animals used in electrophysiological experiments are relatively small. Therefore most of the axons within the nerve are very close to the electrode and are generally activated in order of their threshold to electrical stimulation.

In human experiments, even when the cathode is placed at an optimal point for stimulation - where the peripheral nerve lies close beneath the surface - the current has to penetrate the skin and a variety of subcutaneous tissues before it reaches the nerve trunk. In addition, mixed peripheral nerves in humans have large diameters. This means that considerably more current will be needed to cause those axons which are at the greatest distance from the electrode to reach threshold than that current needed to activate similar axons which are lying more adjacent to the electrode. These factors will be considered in more detail below, when the underlying causes of variability of the H-reflex and the possible causes of its changing variability with size are examined.

The most important factors can be divided into two groups - peripheral factors and central factors. These are listed and then examined in more detail below.

The major peripheral sources of variability are

(1) Changes in the relationship between the stimulating electrode and the nerve trunk - these could result in moment to moment differences in the current reaching the nerve fibres.

(2) The range and distribution of axon diameters (and therefore electrical excitability) of the primary afferents in the tibial nerve. This would affect the numbers of Ia fibres which would be brought close to the threshold of

electrical activation and might or might not fire in response to the stimulus. The numbers of axons “straddling” the threshold at any particular intensity of stimulation would contribute to the reflex variability at that intensity.

(3) The range and distribution of electrical excitability of the motor axons in the tibial nerve - the numbers close to threshold would contribute to the M wave variability and could affect the H-reflex.

The major central sources of variability are : -

(4) Fluctuations in central excitability affecting the excitability of the soleus motoneurone pool - this could contribute to differences in the numbers of motoneurons (MNs) activated in response to each stimulus.

(5) The pattern of recruitment of the soleus MNs in response to excitation by Ia afferents - the numbers of MNs which are on the edge of activation in response to the composite Ia volley. These may or may not fire in response to the volley and therefore could contribute to fluctuations in the reflex size.

(6) Changes in presynaptic inhibition of the Ia afferents - if the levels of presynaptic inhibition of these afferents fluctuated or changed with different stimulus intensities, then this could also contribute to changes in the reflex variability.

(7) The consequences of Ib afferent and large diameter cutaneous afferent activity upon motoneurone recruitment.

Of these seven major sources of variability, which are the ones whose contribution could change with increasing intensity of stimulation? Such

sources might cause the differences in variability related to reflex size which were observed in the present study.

3.4a CHANGES IN THE RELATIONSHIP BETWEEN THE STIMULATING ELECTRODE AND THE NERVE TRUNK (source 1)

The distance between the stimulating electrode on the skin surface and the nerve trunk will vary between individuals, as will the resistance to current flow of the tissues lying between them. This probably accounts for the differences in the intensity of stimulation required to produce an H-reflex in different individuals.

Changes which occur between the stimulating electrode and the skin and between the subcutaneous tissue and the nerve trunk may well make a substantial contribution towards H-reflex variability. Under normal circumstances tissues slide in relation to one another during movement, as muscles shorten and lengthen. Even small movements of the lower limb under study are likely to move the electrode against the skin and, even if this relationship is maintained, the subcutaneous tissues such as muscle and fat are likely to move in relation to the skin and to the nerve trunk. Movement of tissue layers across one another can result in changes in tissue depth and resistance and these changes will cause changes in the current which reaches the nerve trunk. This source is likely to be a major cause of the different degrees of underlying variability in different individuals. It can be demonstrated that improvements in technique, such as improved fixation of the stimulating electrodes and of the limb itself and improved support of the subject, will yield a lower general variability of the H-reflex.

3.4b THE PATTERN OF RESPONSE OF THE Ia AFFERENT FIBRES IN THE TIBIAL NERVE TO ELECTRICAL STIMULATION (source 2)

The above source of variability should not change systematically with stimulus intensity. However, at higher stimulus intensity levels, larger numbers of fibres are activated by the current. The numbers of fibres brought just to the threshold of activation (and therefore potentially to activation or non-activation during minor fluctuations of current) by the current-induced changes in membrane potential would become a smaller and smaller proportion of the total number of activated fibres as the intensity of stimulation increased. This factor could contribute to the decrease in the coefficient of variation with increasing reflex size. The numbers of fibres likely to be close to their threshold of activation at different levels of stimulus intensity will depend upon the range and distribution of Ia afferent fibres sizes within the nerve trunk.

In order to create a simple visual model to predict the proportion of the Ia fibres in the tibial nerve which would reach threshold with each equal step increase in stimulus intensity (from the threshold of the largest fibres), evidence was collected from a number of different sources and combined to model this effect.

Distribution of Ia afferent diameters

The frequency of distribution of Ia afferents of different diameters within the total population from the soleus muscle would have an effect upon the way in which the population responded to electrical excitation. If there was an equal distribution of Ia afferent axons of different diameters in the tibial nerve trunk and if the threshold to electrical activation of a fibre was directly proportional to its diameter, then one would expect that the

numbers of axons likely to be newly activated with each equal step increase in stimulus intensity to be the same. Thus at any intensity the effect of fluctuations in the current or fluctuations in excitability of individual fibres (leading to activation or non-activation of those fibres straddling threshold) would be similar. Under such circumstances the reflex variability from this source would be the same at all levels of stimulus intensity.

No direct evidence is available of the range and the frequency of distribution of diameters of the primary afferents fibres from soleus in man. Indirect evidence of the estimated range of conduction velocities of human Ia afferents has come from studies measuring latencies and duration of Ia effects involving pathways of measured distance (for example Burke et al. 1984, Hultborn et al. 1987). Detailed evidence, however, is available from cat studies. Evidence of primary afferent projections in man point to qualitative similarities with those of the cat, but some quantitative differences perhaps reflecting phylogenetic adaptation in relation to the demands of bipedal stance and locomotion (see discussion in Morin et al. 1981). Under these circumstances it seems reasonable to assume a similar distribution of fibres in human peripheral nerves, although within a different range of conduction velocities.

Information concerning the distribution of afferent diameters in the nerve to soleus in the cat was published initially in 1948. As a result of histological studies, Lloyd and Chang (1948) produced frequency / distribution histograms of fibres of different diameters. Figure 3.8 is based upon the trimodal shape of the histogram of these authors, which indicated group I, II and III distribution peaks and showed a relatively normal distribution of the group I afferents. However, the group I peak included both Ia and Ib fibres. But Hunt (1954) later identified separately the distribution of axons from type A (Ia) and type B (Ib) receptors in the

FIGURE 3.8 DISTRIBUTION OF AFFERENT FIBRE DIAMETERS FROM SOLEUS IN THE CAT BASED UPON THE WORK OF LLOYD AND CHANG (1948)

The group one fibres form the right-hand section of the trimodal distribution.

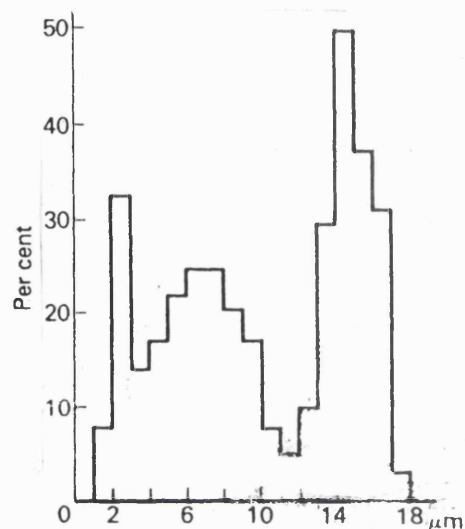
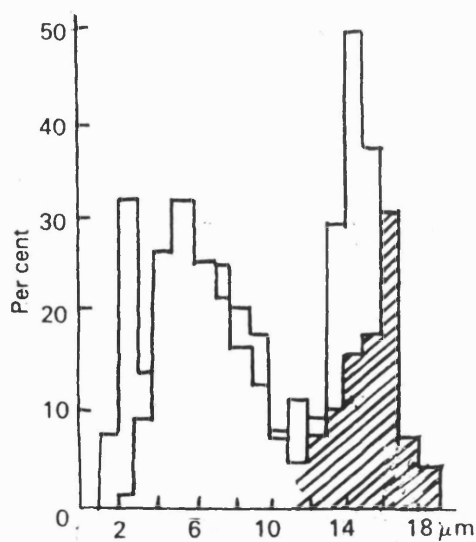


FIGURE 3.9 DISTRIBUTION OF Ia FIBRE DIAMETERS FROM SOLEUS IN THE CAT BASED UPON THE WORK OF HUNT (1954)

The distribution of Ia fibre diameters identified by Hunt are superimposed upon Lloyd and Chang's distribution chart. It can be seen that the distribution fits well into that section of the group I area containing fibres with the largest diameters.



hatched area = Ia distribution

nerve to soleus and showed that the combination of the two distributions in the group I range fitted quite well with Lloyd and Chang's original distribution chart. Figure 3.9 shows Hunt's distribution of Ia afferent fibre diameters as a hatched area superimposed upon the chart of Lloyd and Chang.

The pattern of distribution of fibre diameters which is indicated by the evidence described above could certainly lead to changes in the H-reflex variability at different stimulus intensity levels. This would result from the differences in the numbers of fibres which would be close to threshold at the different stimulus levels. But this distribution needs to be considered in conjunction with evidence of the relationship between fibre diameter and threshold to electrical activation.

The relationship between fibre diameter and threshold to stimulation

Hursh (1939) first showed a direct linear relationship between fibre diameter and conduction velocity for cat medullated nerve fibres and indicated that to calculate the velocity from the diameter a conversion factor of six was required. The Hursh ratio has since been shown to be inexact by a number of authors (see for example Boyd & Davey 1968 and Mathews 1972) when used in relation to smaller fibres, but for fibres in the primary afferent range the linear relationship is accepted.

Experimental evidence, initially from Eccles and Lundberg (1959), had demonstrated a non-linear relationship between the threshold to electrical stimulation and the conduction velocity of a fibre. Later, Ellaway, Murphy and Tripathi (1982) examined electrical thresholds of individual afferent fibres over a wide range of conduction velocities, with a particular interest in the thresholds of group II and group III muscle fibres, in the nerve to

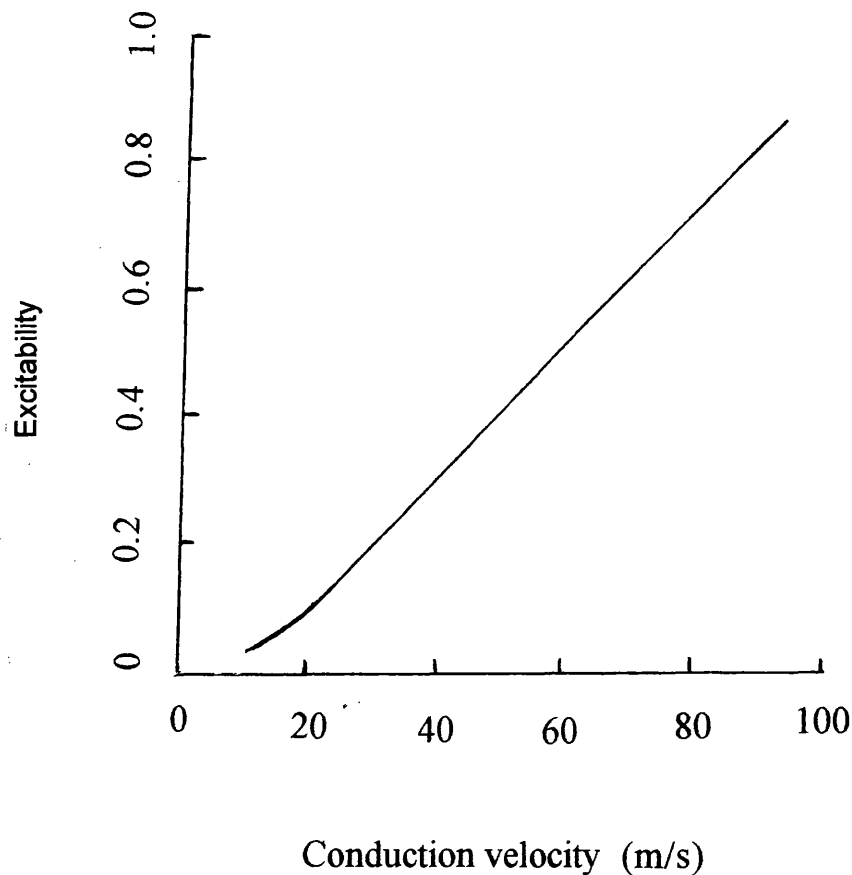
the medial gastrocnemius muscle of the cat. They produced a plot of electrical threshold against conduction velocity from their findings which closely resembles the relationship seen by Eccles and Lundberg but in which the non-linear quality is even more marked.

A direct linear relationship between electrical excitability and conduction velocity (and therefore fibre diameter) was illustrated in the cat by Jack (1978). Figure 3.10 is a line graph based upon Jack's original plot.

In order to predict the proportion of the Ia fibres in the tibial nerve which would reach threshold at different levels of stimulus intensity a plot was created which demonstrated the relationship between conduction velocity and threshold. The threshold to electrical stimulation is the reciprocal of electrical excitability. In order to obtain approximate values from experimental work, values for electrical excitability and conduction velocity were taken from the line graph of Jack's linear relationship plot in figure 3.10. A value of one was taken as the threshold of the largest of the primary afferents and then the rest of the predicted threshold values used were calculated as the reciprocals of the excitability values taken from the line graph.

The relationship which emerges from this conversion (figure 3.11) is similar to the ones demonstrated by the evidence of Eccles and Lundberg (1959) and Ellaway, Murphy and Tripathi (1982) in which axons with higher conduction velocities have activation thresholds which are clustered closely together in the flatter section of the curve, but axons with lower conduction velocities have thresholds which are more widely spread, in the sharply rising section of the curve.

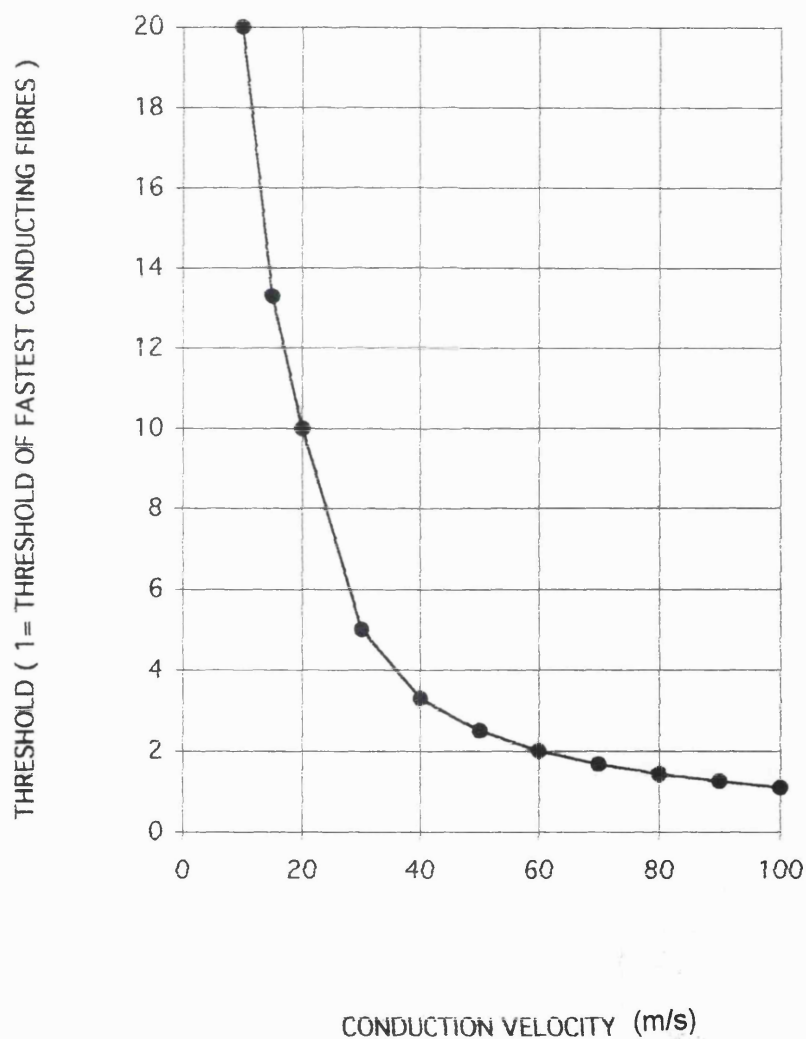
FIGURE 3.10 A LINE GRAPH TO REPRESENT THE PLOT BY JACK (1978) SHOWING THE RELATIONSHIP BETWEEN ELECTRICAL EXCITABILITY AND CONDUCTION VELOCITY IN AFFERENT FIBRES OF SOLEUS IN THE CAT



Ordinate: Excitability = the reciprocal of the threshold to electrical stimulation.
1 = the excitability of the largest afferent fibres in the nerve trunk

FIGURE 3.11 GRAPH TO DEMONSTRATE THE PREDICTED RELATIONSHIP BETWEEN THE CONDUCTION VELOCITY OF A NERVE FIBRE AND ITS THRESHOLD TO ELECTRICAL STIMULATION

Values for conduction velocity and electrical excitability were taken from the line graph (figure 3.10) based upon Jack's plot (1978). The excitability values were then converted to their reciprocal, threshold to electrical excitability. It can be seen that the difference in threshold per unit change in conduction velocity is much smaller in the larger, faster conducting fibres.



The graphs from the experimental results of Eccles and Lundberg (1959) and of Ellaway, Murphy and Tripathi (1982) as well as the simple theoretical model of predicted thresholds produced using Jack's results, all demonstrate this relationship over a wide range of conduction velocities. If another plot is produced, again using the values from Jack's graph - but this time just using that section of the relationship involving the range of conduction velocities of group I afferents - then a similar relationship emerges (see figure 3.12). But here the greater rate of increase in threshold per unit change in conduction velocity that occurs with fibres of lower conduction velocity is less pronounced than when the whole range of afferent fibres is included, though it is still distinct.

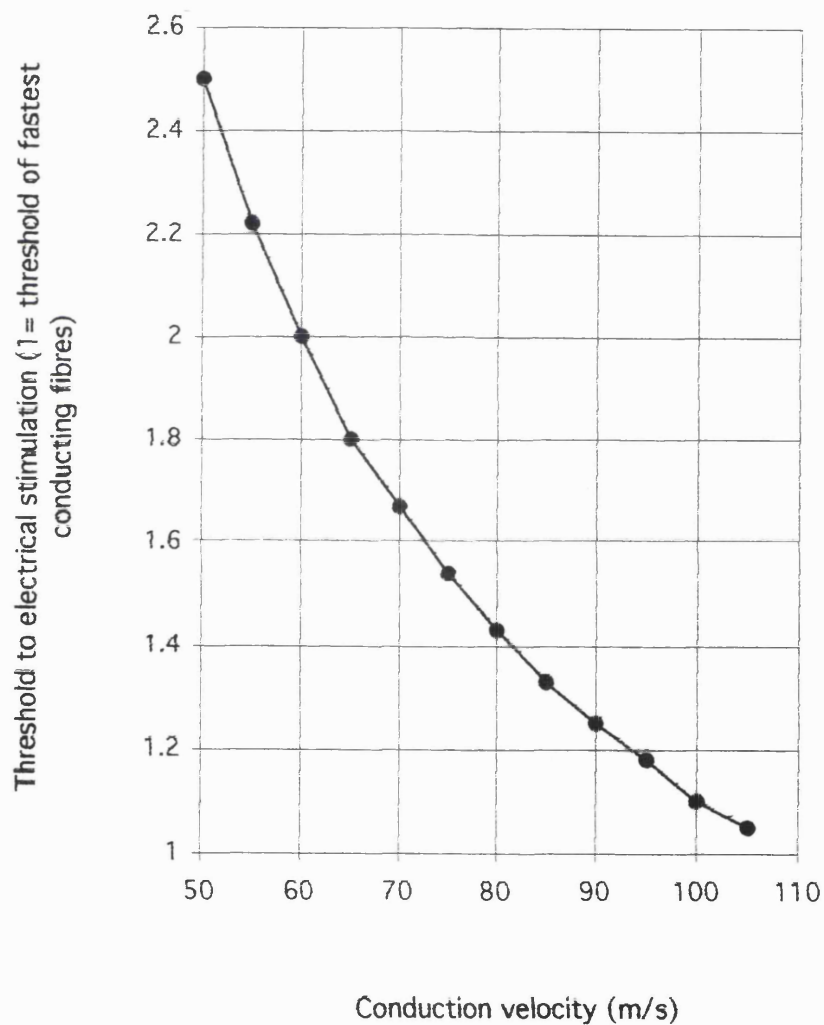
The pattern of response of Ia fibres to electrical stimulation

Subsequently, by substituting axon diameter values for the equivalent conduction velocity values, it was possible to use the constructed plot (figure 3.12) to obtain approximate values for the predicted threshold of activation for each of the major axon diameter size divisions within the Ia range. This information was then combined with the frequency / distribution values from the chart of Hunt (1954) to create a graph predicting the proportion of the Ia fibres which would reach activation threshold at each increase in stimulus intensity, relative to the threshold of the largest fibres.

The predicted values are displayed as a line graph showing the additional percentage of fibres recruited at each unit increase in stimulus intensity (figure 3.13) and as a bar chart showing the total percentage of fibres activated at each stimulus intensity level (figure 3.14).

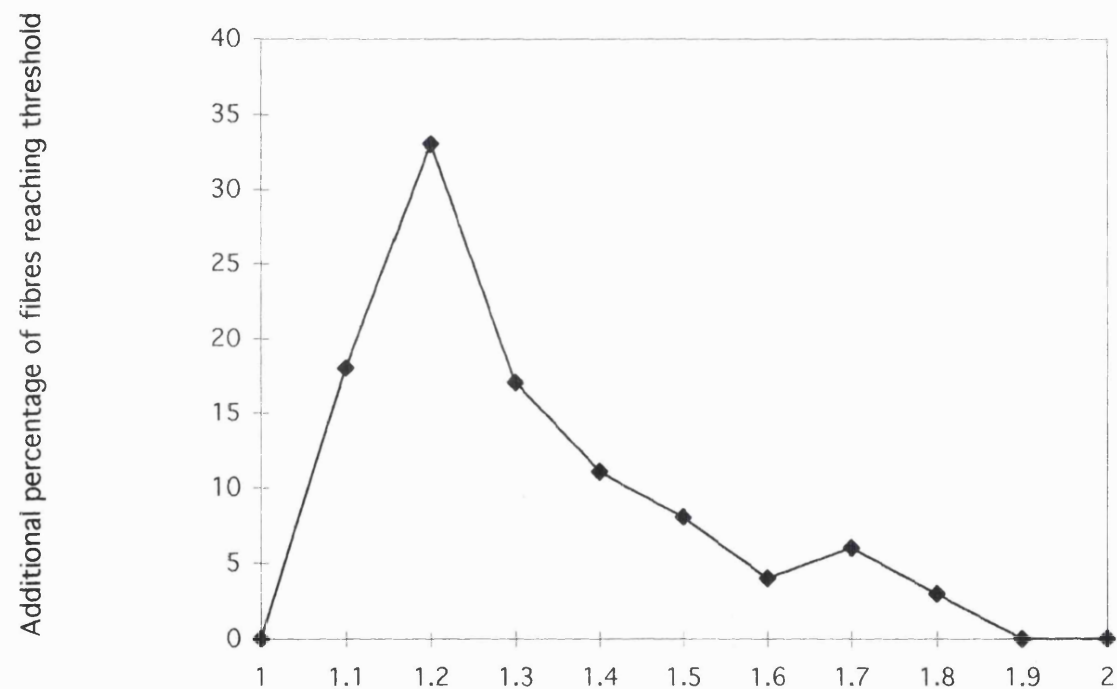
FIGURE 3.12 THE PREDICTED RELATIONSHIP BETWEEN CONDUCTION VELOCITY AND THRESHOLD TO ELECTRICAL STIMULATION OF FIBRES IN THE Ia AFFERENT RANGE

This graph shows a similar pattern to that in figure 3.11. The values for conduction velocity and threshold were again taken from the line graph (figure 3.10) based upon Jack's plot (1978), but the range of fibre conduction velocities is more restricted in this case.



**FIGURE 3.13 PREDICTED ADDITIONAL PERCENTAGE OF Ia
AFFERENTS REACHING THRESHOLD IN RELATION TO
INCREASES IN STIMULUS INTENSITY**

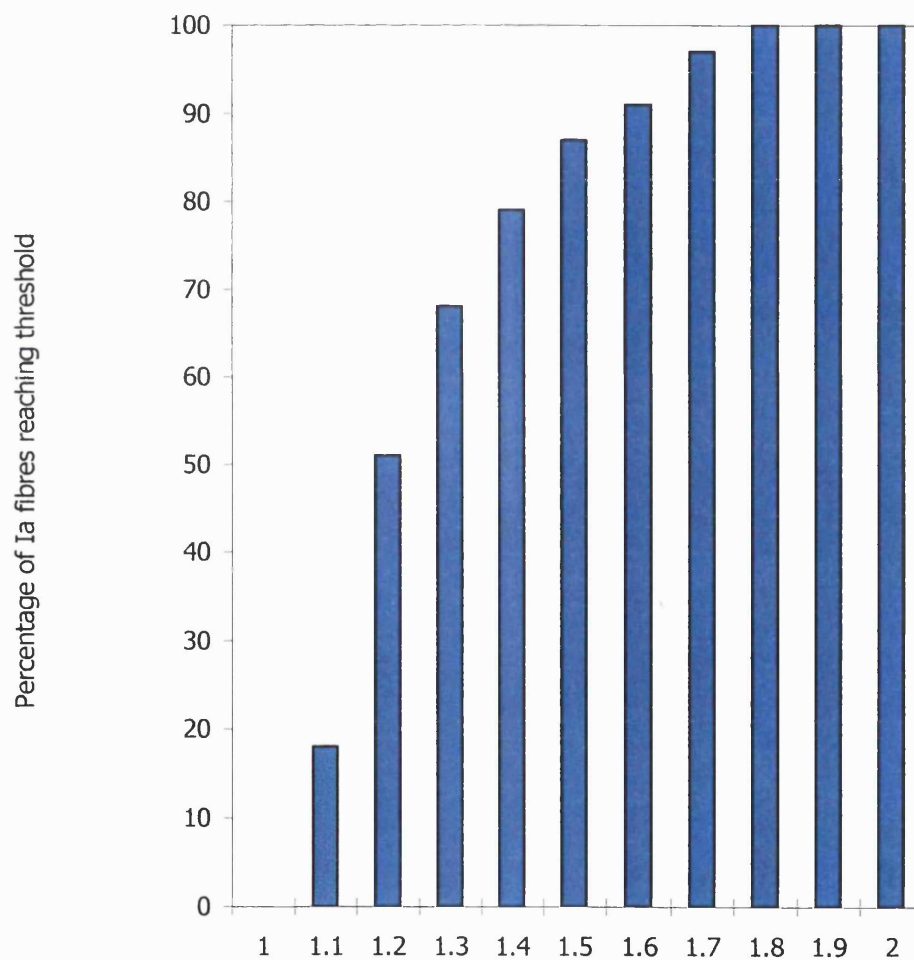
Graph created using values transposed from figures 3.9 & 3.12



Stimulus intensity in relation to the threshold (1) of the largest fibres

FIGURE 3.14 PREDICTED TOTAL PERCENTAGE OF Ia AFFERENT FIBRES REACHING THRESHOLD WITH INCREASING STIMULUS INTENSITY

Graph created using values transposed from figures 3.9 & 3.12



Stimulus intensity in relation to the threshold of the largest fibres (1)

It is clear from both charts that at the lower intensities of stimulation (when the proportion of fibres activated is less than seventy per cent) a much larger proportion of the population of fibres will be additionally activated for each step increase in stimulus intensity than is the case when the great majority of the fibres are already activated (when increases in stimulus intensity only recruit a small percentage of extra fibres).

This model of the pattern of recruitment of Ia fibres in response to electrical stimulation has obvious consequences as a source of H-reflex variability. At the lower intensities many more fibres will be reaching or be close to reaching activation threshold and therefore there are likely to be bigger differences in the numbers of Ia afferent fibres activated or not activated as a result of small fluctuations in the intensity of current reaching them.

The evidence used above is drawn from experiments on the cat nervous system. It seems reasonable to assume that the model produced above is likely to be relevant to the human nervous system too. The model is in keeping with the findings of the present study, which showed much greater H-reflex variability in smaller reflexes, produced at lower stimulus intensities. But, it is important to consider the differences between the direct stimulation of the nerve trunk in the cat and the indirect mode of stimulation of the much larger peripheral nerve in human experiments. Because of the size of the human nerve trunk, some fibres in it will be at a greater distance from the stimulating electrode than others. Therefore the likelihood of activation of each fibre will depend upon its distance from the electrode as well as upon its intrinsic excitability. This point was clearly illustrated by the study of Gracies, Pierrot-Deseilligny and Robain (1994) in which they found evidence of the continued recruitment of Ia fibres with intensities of electrical stimulation of up to four times the threshold of the

motor response (the equivalent of seven times the calculated threshold of the Ia fibres). The study emphasised the fact that there will be considerable overlap between the recruitment by electrical stimulation of fibres of different diameter. Smaller diameter fibres lying close to the surface may well be activated before larger diameter, but more distant, fibres. The major implication of this is that the intensity of stimulation alone cannot be used to identify the separate effects produced by different groups of fibres.

This situation may obscure to some extent (but not entirely) the pattern of Ia recruitment described above. Those fibres that are equidistant from the current source will still be recruited in order of their threshold of activation; but there will be overlap of recruitment between fibres of different diameter lying at different distances from the electrode. If fibres of different type and diameter are distributed relatively evenly throughout the nerve trunk, then overall, at any intensity of stimulation, more of the relatively larger diameter fibres will be recruited than the relatively smaller ones. It is clear that order of recruitment is not completely obscured or it would not be possible to produce the H-reflex response without an M wave.

The predicted pattern of recruitment of Ia fibres revealed is likely to be a contributory factor to the changes in variability observed with reflex size.

3.4c THE PATTERN OF RESPONSE OF THE MOTOR AXONS IN THE TIBIAL NERVE TO ELECTRICAL STIMULATION (source 3)

The pattern of distribution of motor axons of different diameter (and thus of different threshold to electrical excitation) could certainly contribute to any changes in the variability of the M wave with size. (The combined effects upon variability of [i] the distribution frequency, and [ii] the

relationship between fibre diameter and threshold, are discussed in detail above in relation to Ia afferents). But this factor would only affect the variability of the H-reflex when the M wave was present - that is, at the top of the H-reflex recruitment curve and in its descending limb. However, in that situation, when other potential sources of variability may have stabilised, it could be the major cause of variability changes.

3.4d FLUCTUATIONS IN CENTRAL EXCITABILITY (source 4)

Fluctuations in central excitability are likely to vary with time and such things as mood and comfort and one would expect large differences between subjects. However, efforts made to ensure adequate support and comfort of subjects during the data collection should assist their ability to relax. This and instructions given concerning avoidance of movement should reduce avoidable changes in levels of excitability, both generally and in the soleus motoneurone pool in particular. But fluctuations in membrane potential are inevitable and if they are not related to specific muscle activity, then they should have similar effects at different stimulus intensities (unless a general increase in excitability of the motoneurone pool revealed a characteristic of the distribution of motoneurone recruitment thresholds identified as a possible source in 5). Such fluctuations would not contribute to M wave variability.

3.4e THE RESPONSE OF THE MOTONEURONE POOL TO THE Ia AFFERENT VOLLEY (source 5)

When predicting the pattern of response of the soleus motoneurones to the electrically-induced Ia volley and its repercussions for changes in the behaviour of the H-reflex with size, several points need to be examined. The distribution of motoneurone sizes within the pool, the difference in

magnitude of the EPSPs produced by Ia afferents in motoneurones of different sizes and the difference in magnitude of EPSPs produced by different diameter Ia afferents will all affect the pattern of recruitment. In addition the consequences of fluctuations in quantal release of the transmitter at synapses and the relationship between a motoneurone's size and its contribution to the population muscle potential, as expressed by the surface EMG recording of the H-reflex, also need to be considered.

Again, most of the detailed knowledge of the soleus motoneurone pool and its behaviour comes from cat experiments, although some evidence is available from human work using less direct methods.

Motor unit recruitment

Eccles, Eccles and Lundberg (1957) in their early intracellular recordings first observed that EPSPs produced by Ia afferents were largest in small motoneurones. Henneman (1957) postulated that the excitability of motoneurones depended upon their size and subsequently Henneman and his colleagues (Henneman, Somjen and Carpenter 1965a, 1965b), following investigations of motoneurone responses to stretch and then to a variety of stimuli, interpreted this principle of recruitment in terms of its functional value. Such a pattern of recruitment ensured the constant usage of fatigue-resistant small motor units and reserved those units which could produce large tensions, but which were quick to fatigue, for occasional use when large forces were needed.

It had long been established that axon diameter was directly (though not linearly) related to cell body size (Cajal, 1909) and that axon diameter and conduction velocity were directly related (Hursh, 1939). Having demonstrated a linear relationship between the maximum tetanic tension

produced by a motor unit and the conduction velocity of its motor axon (in the cat soleus muscle and the smaller units of medial gastrocnemius, McPhedran et al. 1965 and Wuerker et al. 1965), it was believed that the motoneurone cell body size in some way determined the maximum contraction force of the unit. Such a relationship meant that another valuable functional consequence of the size principle was that at all underlying force levels the recruitment of additional motor units produced a proportionate, and therefore appropriate, increment of force.

In the light of what appeared to be a consistent recruitment order the size-related differences in excitability of motoneurons were considered to be due, probably, to size-related differences in their intrinsic membrane properties, in particular the greater input resistance of smaller cells. And this explanation gained greater plausibility when Burke (1968) demonstrated a linear relationship between input resistance and the amplitude of Ia EPSPs in motor units of the cat triceps surae.

However it soon became clear that, despite its tempting logic, the growing body of evidence did not entirely support the size principle. Burke (1968) himself had identified the fact that motoneurone size did not always directly relate to the contraction force of the motor unit. In particular, fast twitch units with very different twitch tensions often had similar-sized cell bodies (Cullheim and Kellerth 1978). Harrison and Taylor (1981) showed that the correlation between conduction velocity and Ia EPSP amplitude did not hold across the whole spectrum of motor units of a heterogenous muscle such as the medial gastrocnemius,

In 1979 Burke acknowledged the importance of extrinsic as well as intrinsic factors in determining motoneurone excitability. Burke, Rymer and Walsh (1976) Dum and Kennedy (1980), Harrison, Taylor and

Chandler (1980) and Harrison and Taylor (1981) all found evidence of a relationship between Ia EPSP amplitudes and motor unit contraction force and the latter authors showed that this relationship existed across the whole spectrum of motor units. Harrison (1981) showed that the relationship was inverse and non-linear and he examined the potential importance of the bias of the powerful Ia input in preserving the functionally optimal recruitment order. So that, regardless of other intrinsic factors affecting motoneurone excitability, it could be shown that the Ia afferent presynaptic organisation alone could ensure the recruitment of motor units in order of their contraction strength even in the presence of substantial excitation from other sources whose presynaptic input was distributed uniformly to all motoneurons in the pool.

Much of the evidence examined above arose from studies in the cat of the motoneurone pools of muscles which contain a wide range of motor unit types. In the present study it is the effect of the Ia volley upon the motoneurone pool of soleus which is under examination. In the cat the motor units of soleus are relatively homogenous, because soleus is a muscle consisting almost exclusively of small, slow-twitch, fatigue-resistant units. The range of unit contraction forces and motoneurone conduction velocities is much smaller than in heterogeneous muscles.

In humans, the available evidence for motor unit distribution comes indirectly from studies of the range of muscle fibre type in soleus. An autopsy study examining distribution of muscle fibre size in thirty six human muscles found that the soleus muscles examined had between 86% and 89% type one muscle fibres (Johnson et al. 1973). More recently, a histochemical study revealed that human soleus muscles studied consisted of approximately 80% type one fibres; the rest of the fibres being type two fatigue-resistant fibres, with no fast glycolytic fibres (Harridge et al.

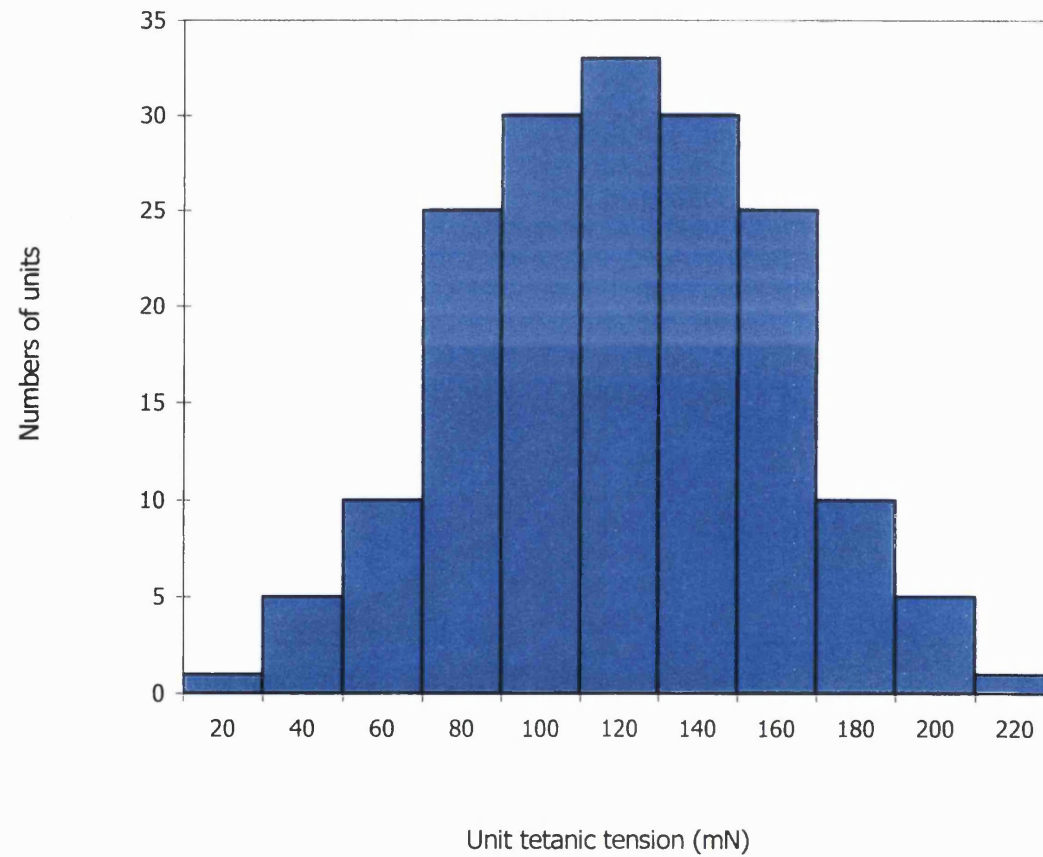
1996). These studies indicate that the human soleus muscle is predominantly a slow muscle, with a motoneurone pool of predominantly small units. It appears to exhibit similar characteristics to the soleus muscle of the cat.

The distribution of soleus motor units by tetanic force

McPhedran et al. (1965) had identified a relationship between motor unit tension and conduction velocity in soleus, as had been shown consistently to be the case for slow twitch units of other muscles. Bagust (1974) extended and confirmed these findings for soleus and he produced histograms showing ranges of motoneurone conduction velocities and motor unit tetanic tensions of units examined in his experiments. Bagust's frequency / distribution graphs for each of these motor unit characteristics revealed relatively normal distribution curves, with most of the units lying within a narrow range of tetanic tensions (70-160 mN) or conduction velocities (55-75 m/s). A simplified normal distribution bar chart, based upon the distribution of the cat soleus motor units found by Bagust (1974) and representing one hundred and seventy five imaginary soleus motor units was constructed (see figure 3.15). Although the range of motor unit sizes used was based upon actual values for the cat, the assumption was made that the pattern of distribution was likely to be similar in humans, although the actual values would be different.

Using the values from this chart it was possible to predict a pattern of motoneurone recruitment in relation to step increases in stimulation intensity. For instance in response to a very weak Ia afferent volley only the few motoneurons with the very smallest unit tensions and the lowest conduction velocities would receive sufficiently large composite Ia EPSPs

**FIGURE 3.15 A SIMPLIFIED PLAN OF TYPICAL
DISTRIBUTION OF SOLEUS MOTOR UNITS- BASED UPON
THE HISTOGRAMS OF BAGUST (1974)**



to reach threshold. However, with a slightly stronger volley the relatively large numbers of units with tetanic tensions around 70mN would reach threshold and the amplitude of the reflex would begin to climb sharply for each small increase in stimulus intensity (and therefore afferent volley strength).

Within the middle range in which many units of a similar size are distributed, the reflex amplitude would continue to increase sharply with each increment of stimulus intensity because, with each increment, many extra cells would reach threshold. The numbers reaching threshold with each increment would probably be relatively similar within the densely distributed middle range (although greatest in the area of the central peak) and therefore the variability would be consistently high, as many cells close to threshold would be affected by any small fluctuations in the current reaching them with each applied stimulus.

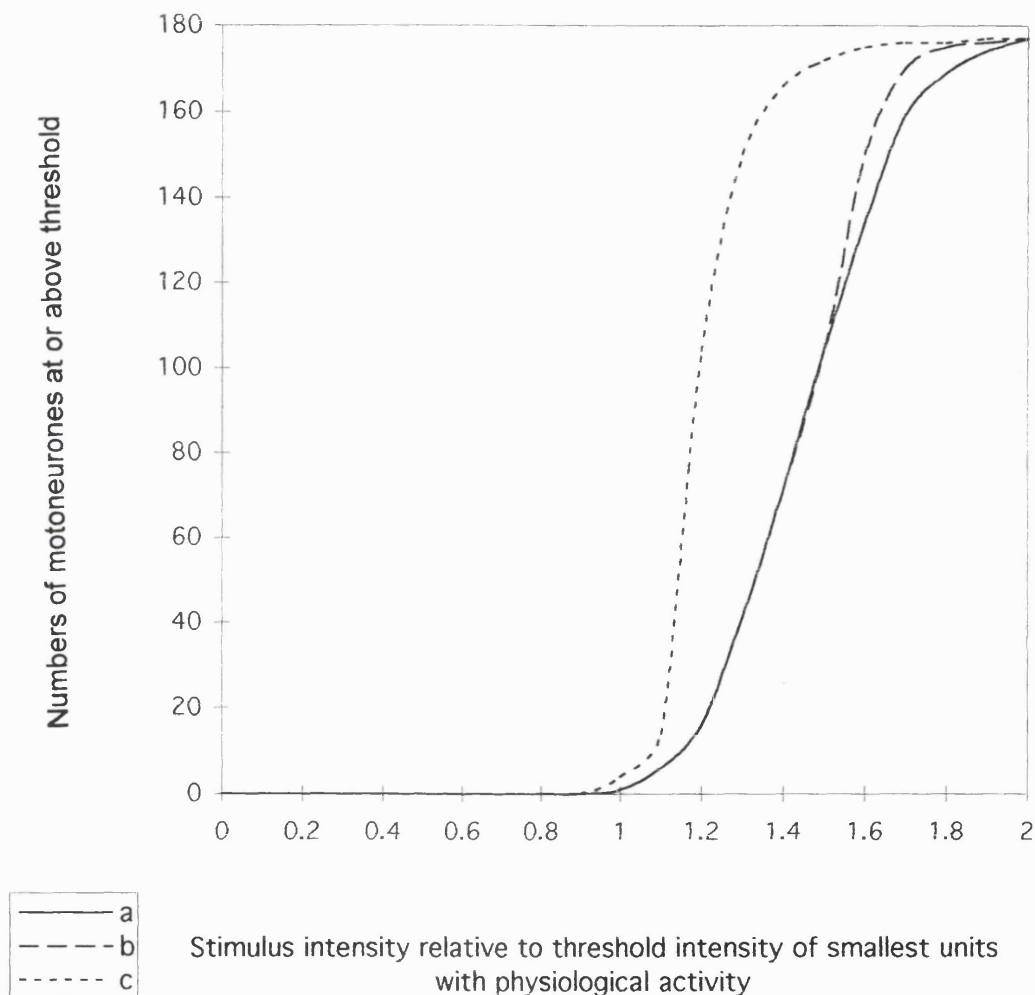
Because of the homogeneity of the soleus units, the differences in size of the units close to threshold at different points on the rising recruitment slope would be relatively similar, but the underlying reflex amplitude itself would be increasing. Therefore when expressing the variability in relation to the mean amplitude (the coefficient of variation) this would be decreasing at consecutively higher points on the recruitment curve.

As the stimulus intensity continued to increase, most of the units in the densely-distributed middle portion of the normal distribution curve would have been recruited. After the motor units in the central peak of distribution had been recruited, slightly fewer units would be reaching threshold for each incremental rise in stimulus intensity. However the numbers of larger units in the middle range recruited per unit increase in intensity would still be substantial, but the steepness of the slope of

recruitment would begin to decrease at this point, decreasing the variability too. When all the units in the middle range had been recruited the numbers of units reaching threshold for each incremental rise in stimulus intensity would drop sharply. This would be visible in a decrease in the steepness of the curve of recruitment and would lead to a decrease in variability. It is likely that in many subjects the largest motor units would not be involved in the H-reflex because often the maximum H-reflex amplitude is around 50% of the maximum M wave amplitude, although there are considerable differences between individuals.

The values from the simplified model of figure 3.15 were transposed to create a graph to illustrate the pattern of motoneurone recruitment described above in the 175 motor units of the bar chart. In order to produce the pattern shown by the solid line plot [a] in figure 3.16 a number of assumptions have been made which allow concentration upon the perceived major influences on variability. Firstly, it is assumed that the non-linear properties of the inverse relationship between Ia EPSP magnitude and motor unit force (see figure 3.17, based upon the findings of Harrison, 1981) would probably have little effect upon the shape of the recruitment curve and the variability at different stimulus levels, because almost all of the soleus units fit into the range of units with tetanic tensions below 20gm.wt. or 200mN of force in the cat. This means that they all lie in a portion of the curve where the relationship is relatively linear and the difference in the Ia EPSP magnitude for any unit of difference in motor unit force would be similar throughout this range. Therefore each additional increment in stimulus intensity would cause the additional activation of motoneurones included in a proportional increment of unit tetanic tension. However the consequences of the non-linear relationship, should they be present, are discussed below.

FIGURE 3.16 MODEL OF NUMBER OF SOLEUS MOTONEURONES REACHING THRESHOLD IN RELATION TO STIMULUS INTENSITY



Reflex activation of soleus motoneurons in response to electrical stimulation of the tibial nerve

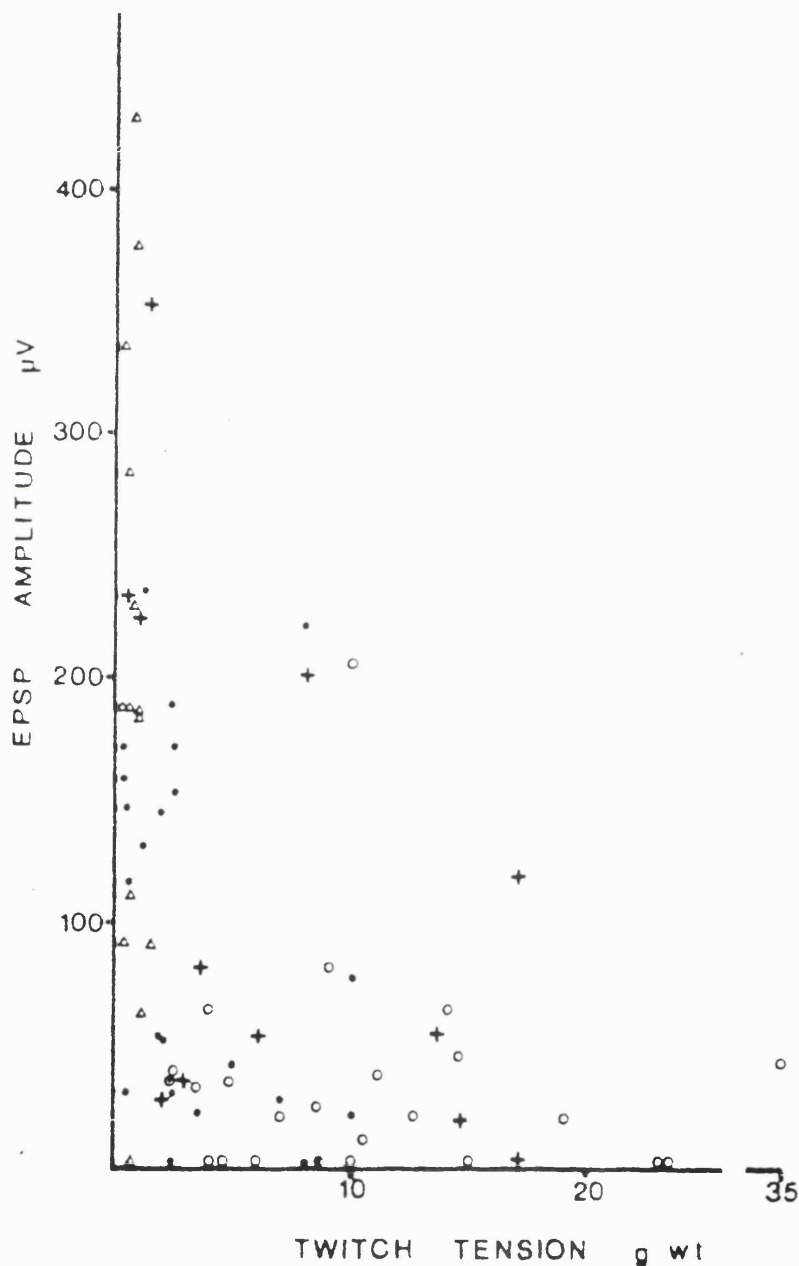
a = predicted recruitment of MNs if equal numbers of additional Ia afferents were **activated at** each incremental increase in stimulus intensity using MU distribution values from fig. 3.15

b = speculative view of consequences of the effect of non-linear aspects of relationship between EPSP amplitude and MU force upon a.

c = predicted recruitment curve of MNs if values of predicted numbers of Ia afferents reaching threshold with incremental steps in stimulus intensity are transposed from fig.3.14.

FIGURE 3.17 PLOT TO SHOW THE RELATIONSHIP BETWEEN INDIVIDUAL Ia EPSP AMPLITUDES AND CONTRACTION STRENGTH OF MOTOR UNITS BASED UPON THE WORK OF HARRISON (1981)

The results illustrated were taken from motor units of the cat gastrocnemius muscle. There is an inverse relationship between the two, but in the work of Harrison (1981) it is described as a hyperbolic rather than a linear relationship.



Data points from type S units indicated by Δ ; type FR units by \bullet ; type FF units by \circ ; type F(INT) units by \odot ; unclassified units by $+$.

Secondly, for the purposes of producing the basic hypothetical relationship represented by the solid line plot [a] in figure 3.16 (which can then be adjusted in the light of additional important effects) it is assumed that each increment of stimulus intensity would activate an equal number of additional Ia fibres of similar diameter. Then if one increment were to activate all the motoneurons of units within the range 0 to 20 mN unit tetanic tension, the next increment would activate all those within the range 20 to 40 and so on. One represented the recruitment threshold of the smallest motor units and two the threshold at which almost all the motor units would be recruited.

If the non-linear nature of the relationship between EPSP amplitude and motor unit tetanic tension were to have an effect, it would be the result of the fact that there tend to be smaller differences between the EPSPs produced in larger motoneurons in relation to differences in their unit forces (Harrison 1981) and this would cause the larger units in the range to have thresholds rather closer to one another than one would expect if the relationship was linear. Thus towards the top of the recruitment curve the slope might be steeper and the variability slightly higher (the broken line [b] in figure 3.16 represents the sort of change that one might expect).

Finally, the changing nature of the electrically-induced Ia volley with increasing stimulus intensity certainly would have an added effect upon the shape of the recruitment curve and the variability of the H-reflex. Two aspects of this need to be considered. At the lowest stimulus intensities the volley would consist of activity only in the largest of the Ia fibres. Mendell and Henneman (1971) and Harrison (1981) demonstrated that the magnitude of Ia EPSPs to all motoneurons generally correlated with the afferent conduction velocity. Therefore at low stimulus intensities the

effect upon the motoneurone pool for each active Ia fibre would tend to be greater than at higher intensities.

The other, most important factor that would change the curve of motor unit recruitment is the relationship illustrated in figures 3.13 and 3.14. At the lowest intensities many more of the largest fibres in the Ia range would be additionally activated per unit increase in stimulus intensity compared with the number of smaller fibres activated per unit increase at higher intensities (see above in section 3.4d for explanation).

These two factors would greatly increase the number of motoneurones reaching threshold for each incremental increase in stimulus intensity at low stimulus intensities. This would result in a further increase in the steepness of the rise of the H-reflex recruitment curve. It can be seen from figure 3.14 that, at 1.2 times the electrical threshold of the largest Ia fibres, 50% of the Ia fibres would already have reached threshold. But in the motor unit recruitment curve [a], which was based upon an equal increase in Ia activation for each incremental rise in stimulus intensity, at 1.2 times the threshold of the largest Ia fibres it was predicted that only 20% of the Ia fibres would have reached threshold. If the values from the Ia recruitment pattern of figure 3.14 are transposed into the motoneurone recruitment curve plot of figure 3.16, then the new plot is illustrated by the broken line curve [c]. As a result, the majority of the motoneurones (those occupying the densely populated middle section of the unit tetanic tension distribution curve) would all have reached threshold at a lower intensity of stimulation than if the Ia afferents were equally distributed in relation to diameter and were recruited in similar numbers with each added increment of stimulus intensity. So the plateau at the top of the recruitment curve would now be flatter and longer-lasting. The very large numbers of small motor units which would be recruited at low intensity levels would account

for the rapid early growth of the reflex and the very high variability of the smaller reflexes.

Fluctuations in synaptic potentials

Fluctuations in the synaptic potentials produced by individual Ia afferents in motoneurones have long been recognised and studies of the nature of these fluctuations have revealed that the EPSPs produced by a fibre are the result of transmission occurring in an all-or-none manner at each of the several synaptic boutons with which the Ia fibre makes contact with the motoneurone (Redman and Walmsley 1981,1983). The EPSPs fluctuate by discrete amplitudes which reflect the sum of the transmission at all of the boutons in the connection and the probability of failure of transmission may be different at each bouton (Walmsley, Edwards and Tracey 1987). Transmission may always occur at some boutons but, for instance, on only one of three occasions in another.

Such fluctuations are an obvious potential source of variability, but they are only likely to contribute significantly at the very lowest levels of stimulus intensity, when just a few Ia fibres are activated and no motoneurones are recruited. As soon as a substantial number of fibres are activated then fluctuations in the amplitudes of EPSPs produced by each of them will tend to cancel one another out and simply add to the background noise level. Indeed the mean variability of the composite Ia EPSPs is very small. Fluctuations in synaptic potentials are therefore unlikely to contribute to the H-reflex variability reported here.

Motor unit activity and EMG

Changes in the peak to peak amplitude of the H-reflex are taken to represent evidence of changes in the number of active motor units, but it is important to know the relationship between the twitch or tetanic tension of a motor unit and its contribution to the reflex as measured by the total surface EMG. The analysis above has considered the reflex behaviour in terms of the numbers of units activated and their contribution to the total reflex as represented by their unit forces.

Milner-Brown and Stein (1975) observed that although there was a significant tendency for units with larger forces to contribute a greater voltage to the surface EMG, the unit contribution made to the EMG increased less rapidly in relation to its size than did its contribution to the total force generated by the muscle. In fact the relationship between the contribution of a unit to the peak to peak EMG was shown to increase as the square root of the unit force. The EMG relationship is further complicated (and therefore made less orderly than the force relationship) by the effect of overlap of unit potential changes and by the fact that a unit's contribution will depend upon the location of its muscle fibres in relation to the surface electrode (Basmajian and De Luca 1985, Turker 1993).

This less steep relationship is nevertheless a linear one and because of the relative homogeneity of the soleus motoneurone pool the difference between the unit contributions to the total EMG and to the total force are unlikely to change the patterns influencing the variability of the reflex as discussed above.

3.4f THE EFFECT OF CHANGES IN PRESYNAPTIC INHIBITION (source 6)_

The classic forms of presynaptic inhibition of Ia afferent terminals are produced via GABAergic synapses. Increased presynaptic inhibition leads to a reduction in the size of the EPSPs produced in MNs by Ia afferents and this would reduce both the size of the H-reflex for any given stimulus intensity and that part of the reflex variability associated with MN recruitment. But this form of presynaptic inhibition is known to be produced by activation of group I afferents from flexor, not extensor muscles and from some descending fibres (Rudomin 1990). Thus, during the electrical activation of the posterior tibial nerve - a nerve to the extensor muscles - it is unlikely to play an important role.

Although the reflex contraction of the soleus muscle could lead to activation of Ia afferents in the stretched muscles of the flexor compartment, (the anterior tibial muscles), any presynaptic inhibition induced would have a maximal duration of approximately 500ms and therefore its effect would have disappeared long before the arrival of the volley produced by the next electrical stimulus,

But another form of presynaptic inhibition has recently been identified as the mechanism which underlies post-activation depression of the H-reflex (Hultborn et al. 1996). This long-recognised, long-lasting reduction in the reflex amplitude could make a contribution to changes in variability if the interstimulus interval used is less than eight seconds, the duration required for the reflex to return to its original size. Hultborn and his colleagues have demonstrated that this depression in transmission is confined to the Ia terminals which have just been activated. Depression of the EPSPs produced in MNs by these Ia terminals would mean that, for any given

number of Ia fibres repeatedly activated, the numbers of MNs recruited would be reduced and this would be paralleled by a reduction in the reflex size.

At short interstimulus intervals, (less than four seconds), when the degree of post-activation depression would be substantial, there would be a reduction in reflex variability associated with fluctuations in the number of MNs recruited, because fewer motoneurons would be recruited at all intensities of stimulation. But this would be balanced by the relative reduction in reflex size. The reflex amplitude would be reduced at every point on the recruitment curve, although the pattern of recruitment would not change. Therefore presynaptic mechanisms probably do not play a major part in the changes in variability seen with changing reflex size. The similar results obtained in the present study from the subject from whom recordings were taken using both a long and a short interstimulus interval are in keeping with this conclusion.

3.4g THE EFFECT OF THE ELECTRICAL ACTIVATION OF Ib AFFERENTS AND CUTANEOUS AFFERENTS (source 7)

The possibility that the H-reflex could be contaminated by afferent activity other than of the Ia afferents was examined in detail by Burke, Gandevia and McKeon (1983,1984). As a result of the similar thresholds to electrical stimulation and the similar range of conduction velocities of the two populations of afferents, it is likely that Ib afferents would be activated along with the Ia fibres. Volleys in Ib afferents could lead to the production of IPSPs in soleus motoneurons via Ib interneurons. These could certainly reach the motoneurone pool during the rising phase of the Ia population EPSP and therefore could play a part in the characteristic behaviour of the H-reflex. An increasing Ib population IPSP (as the

intensity of stimulation was increased) would reduce the numbers of motoneurones recruited by the Ia population volley and therefore reduce the rate of recruitment per unit increase in intensity. At higher intensities, where the Ib effects would be greater, this could contribute to the reduced variability by reducing the numbers of motoneurones brought close to threshold.

However, in the light of what is known of the density of the Ia connections to individual MNs and the divergence of Ib effects (Harrison and Riddell 1991) it is reasonable to assume that any Ib effect is likely to be a relatively minor one. It should be acknowledged, though, that if there were differences in the numbers of Ib afferents electrically activated with each reflex, as a result of fluctuations in the current intensity, then this could make a small contribution towards the reflex variability. But variability from such a source would depend upon the distribution of Ib fibre diameters and would be expected to increase at higher intensities.

Large diameter cutaneous afferents could possibly be activated by an electrode in the popliteal fossa and could reach the spinal cord within two point five milliseconds of the Ia volley (Burke et al.1983). In view of their likely polysynaptic intraspinal pathway, they would be unlikely to exert an effect upon the motoneurone pool early enough to affect the reflex discharge. But short latency cutaneous pathways have been demonstrated in the cat lower limb (Fleshman et al. 1988) and Pierrot-Deseilligny and his colleagues (Pierrot-Deseilligny, Bergego, Katz and Morin 1981) have demonstrated an early postsynaptic inhibition of Ib interneurones in pathways from lower limb muscles in man. It is clear that the integration of fast cutaneous reflexes with other reflex pathways plays an important role during normal movement

CONCLUSION

This study has identified a change in the variability of the H-reflex with size. A number of factors probably contribute to the reduction of the coefficient of variation of the reflex with increasing reflex amplitude. The main causes of the general, underlying variability of the reflex are likely to be both central and peripheral - moment to moment changes in central excitability and fluctuations in the current reaching the nerve trunk (as a result of the indirect method of stimulation). The changes in variability with size can be explained mainly in terms of the effect of these fluctuations in current upon the electrical activation of the Ia afferent fibres at different stimulus intensities and upon the consequent recruitment of motoneurones at different stimulus intensities.

The examination of evidence in the present study of the distribution frequency and the electrical thresholds of Ia fibres from the cat soleus has produced a prediction of the pattern of electrical activation of the soleus Ia afferent fibres at different stimulus intensities, as revealed in figure 3.13. Despite the inherent differences in the methods of electrical stimulation between animal and human experiments, it is believed that this pattern of activation is relevant in human H-reflex experiments as well as in animal work. The pattern of activation of Ia afferents and the consequent pattern of recruitment of motoneurones at different stimulus intensities (as discussed above and illustrated in figure 3.16), would account for the classic shape of the H-reflex recruitment curve, with the steeply rising early and middle phases. It is also likely to underlie the changes in variability with size observed in this study and it could explain the non-linear relationship between the size of the H-reflex and the size of conditioning effects (as described by Crone et al.1990) as well.

When using the H-reflex as a tool to detect changes in excitability of the MN pool, knowledge of its variability can be used to estimate the numbers of test and conditioned reflexes which should be collected in order that an effect can be detectable. When one is attempting to detect small changes, then reducing the noise of background variations becomes particularly important. The findings of this study indicate that the variability decreases as the reflex increases in size and that the choice of size of the test H-reflex used could make a big difference to the number of reflexes needed in a sample in order to be able to detect a small conditioning effect.

However these findings need to be considered in the light of the study of Crone et al. (1990) in which it was found that the effects of conditioning stimuli from a variety of sources were greatest when the test H-reflexes used were approximately one third of the maximum M wave amplitude. The authors demonstrated that the size of a conditioning effect followed a pattern; growing larger as the reflex size was increased from very small, reaching a plateau in the middle range and steadily decreasing in very large reflexes. They discussed the causes of this pattern and focused upon changes in the numbers of motoneurons whose reflex recruitment would be affected by conditioning effects with H-reflexes of different sizes.

The choice of an optimum size of H-reflex should therefore be made in the light of the two studies. Crone et al. (1990) found, as have other authors (for example Taborikova and Sax 1968, Hugon 1973) and as was seen in the present study, that in most subjects the maximum H-reflex amplitude was around 50% of the maximum M wave amplitude. This would indicate that reflexes of the size recommended by Crone et al. (1990) - 30% of maximum M wave amplitude - would be approximately 60% of the maximum H-reflex size. At this level the variability of the reflex is about two thirds of its value when the H-reflex is 30% of its maximum size.

Although the variability is further reduced in larger reflexes, so is the size of any conditioning effect. Reflexes of around 60% of the maximum amplitude would seem to give the best chance of detection of conditioning effects with the appropriate sample size.

4.0 A COMPARATIVE STUDY OF THE BEHAVIOUR OF THE H-REFLEXES AND THE M WAVES OF GASTROCNEMIUS AND SOLEUS

4.1 INTRODUCTION

In the preliminary series of experiments that led up to the collection of the main body of data presented in this study, surface recording electrodes were situated over the body of the gastrocnemius muscle and reflexes were collected in the presence of a large M wave. It transpired that both of these factors, together with other factors, complicated efforts to accurately measure and interpret the findings of these preliminary experiments. Consequently, it was deemed necessary to analyse the behaviour of the H-reflexes and M waves of both gastrocnemius and soleus.

The existence of a separate gastrocnemius H-reflex at rest remains controversial (Hugon 1973, Brunia et al. 1973, Schieppati 1987, Hultborn & Neilsen 1995). Many authors refer non-specifically to the use of the gastrocnemius / soleus H-reflex. Hugon (1973) believed that the H-reflex responses recorded at rest over gastrocnemius (with higher amplification) were actually the responses of soleus, detected through the muscle bulk of gastrocnemius. Brunia and his fellow authors considered that in a relaxed subject “the H-response practically involves only the soleus muscle whereas the direct M response involves both” unless the recording electrodes were placed so as to selectively pick up the response of soleus. On the other hand other authors have appeared to describe H-reflexes specifically in gastrocnemius. For instance Deschuytere and colleagues discuss the increased latency of H-reflexes of medial gastrocnemius with chronic compression of the first sacral nerve root (Deschuytere et al.1983) and

Nadeau and Vanden-Abee (1988) examined differences between maximal H- and M-responses of left and right soleus and gastrocnemius muscles.

If the H-reflex observed in the preliminary experiments was from gastrocnemius, then as it was recorded in the presence of a large M wave, the size of the H-reflex could conceivably be reduced by virtue of antidromic action potentials in motor axons colliding with orthodromic ones and cancelling one another out. Therefore the reflex amplitude might represent the activity of some reflexly activated motor units, but only those whose axons were not also being activated directly to produce the M wave. Quantifying changes due to conditioning in such reflexes is not possible, (see discussion above in chapter 3).

In the case of the soleus H-reflex, the amplitude of the reflex usually appears to peak around the threshold intensity of the M wave, sometimes at a slightly lower intensity and sometimes at a slightly higher one (Taborikova and Sax 1968, Hugon 1973 and see figure 4.1 in the present study). The presence of a large reflex response recorded over gastrocnemius in the presence of the large M wave indicated that the behaviour of the responses from soleus and gastrocnemius might be quite different.

However the presence of the M wave was of particular interest. The M wave itself can be a valuable tool in identifying peripheral causes of variability. In response to these observations a series of experiments were performed to examine more closely the behaviour of the M and H responses recorded over soleus and over gastrocnemius by comparing their recruitment curves.

4.2 METHOD

Simultaneous recordings were made of the M wave and H-reflex recruitment curves of gastrocnemius and soleus on two or more occasions in seven subjects who gave their informed consent. The experimental set-up was similar to that described in chapter 3 above.

4.2a STIMULATION

Details of the stimulating apparatus are given above in chapter 3. The cathode was a lint-covered metal electrode approximately 1cm in diameter. This was moistened and placed over the posterior tibial nerve in the popliteal fossa. The anode, a larger, flat, lint-covered electrode, was placed on the anterior aspect of the thigh just above the base of the patella.

Square wave pulses of 1ms duration were used and when a stable soleus H-reflex was obtained in the absence of a soleus M wave the cathode was secured with a strap. This is considered to be the method by which the soleus H-reflex is optimally elicited (Magladery et al. 1951, Paillard 1955, Hugon 1973). It is also the method used by most authors. Although the aim was to examine any gastrocnemius responses as well, it was in the context of the normal use of the soleus reflex that this was of particular interest. Stimuli were delivered with an interpulse interval of 7.836 seconds.

The intensity which produced the first visible response was noted and the intensity required to produce maximal M waves from both muscles was also noted. The range between these two intensities was divided into an appropriate number of equal steps - usually consisting of 0.5mA or 1mA - and ten recordings were collected for each step increase in intensity between the two ends of the scale.

4.2b RECORDING

Two disposable surface electrodes (details given in chapter 3) were placed on the cleaned skin over the body of the soleus muscle 3 or 4 cm apart on either side of the central tendinous area, at least 6cm below the visible boundaries of the gastrocnemius muscle. The other set of recording electrodes were placed 3 or 4 cm apart on the skin over the medial gastrocnemius muscle. Reference electrodes were placed on the skin over the antero-medial surface of the tibia.

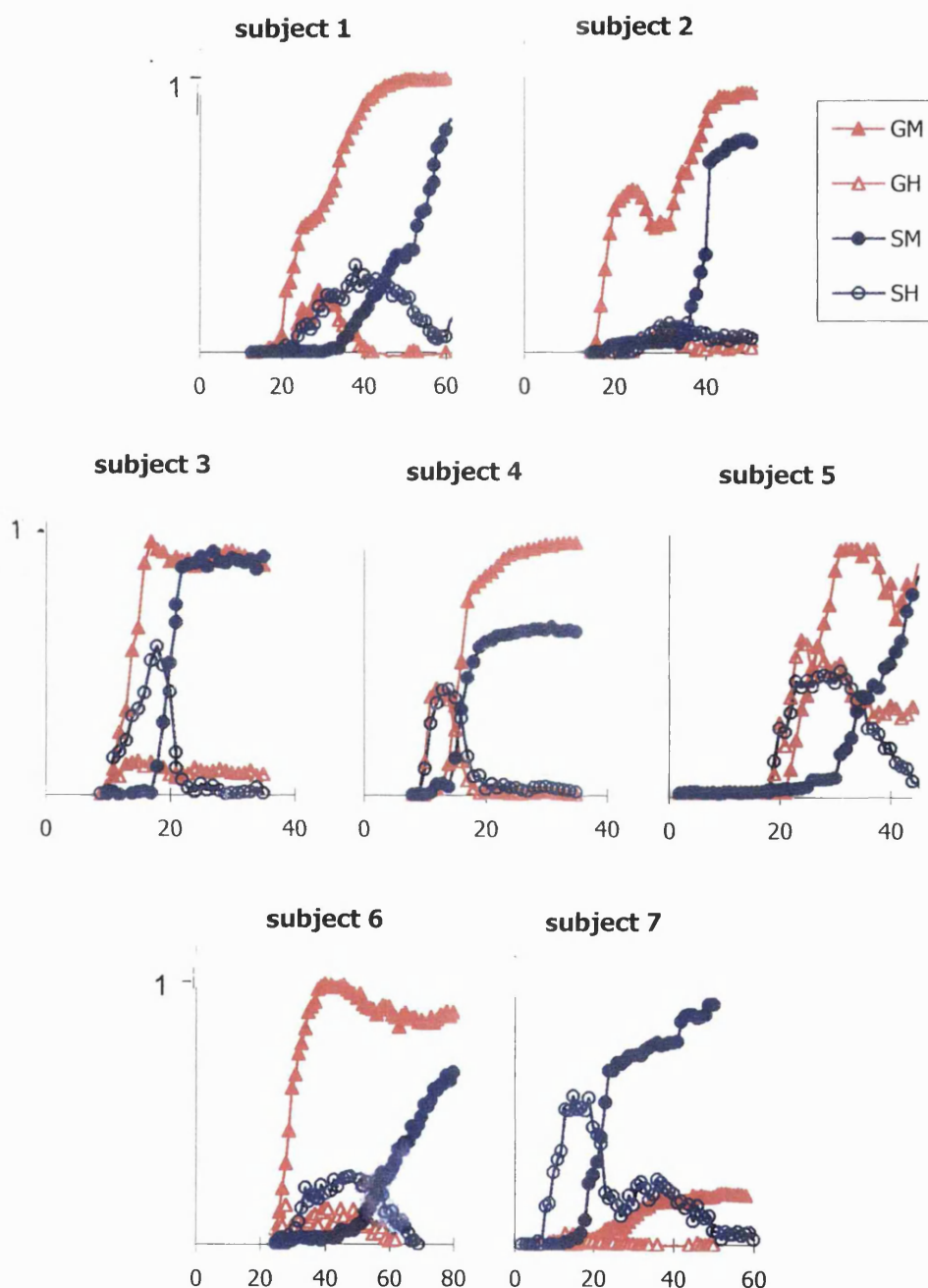
The bipolar surface electromyographic recordings were differentially amplified (usually between 500 and 2000 times) so that the amplitudes of the displayed reflex responses were clearly visible at all intensities of stimulation. The responses recorded over soleus and over gastrocnemius were always amplified equally in each individual. The amplified signals were filtered with a low bandpass of 30 Hz and a high bandpass of 500 Hz and stored as described above in chapter 3. At a later date, the peak to peak amplitudes of the ten recordings of the four responses were measured and the means were calculated. Plots were then made to show the relationship between the mean amplitudes of the M and H responses from each muscle and the intensity of the applied stimulus.

4.3 RESULTS

Figure 4.1 shows examples of dual recruitment curves from each of the seven subjects. There is a considerable degree of variability between the individual subjects, but repeated recruitment curves from the same subject were remarkably consistent. For example figure 4.2 shows three separate

FIGURE 4.1 H-REFLEXES AND M WAVES RECORDED FROM SOLEUS AND GASTROCNEMIUS SIMULTANEOUSLY IN RESPONSE TO GRADED STIMULATION OF THE TIBIAL NERVE

Examples of results from each of the seven subjects.
For each subject all responses were amplified equally



For each graph, abscissa : Intensity of electrical stimulation (mA)

Ordinate : Amplitude of responses scaled in relation to the maximum M wave amplitude, which has been given a value of 1.

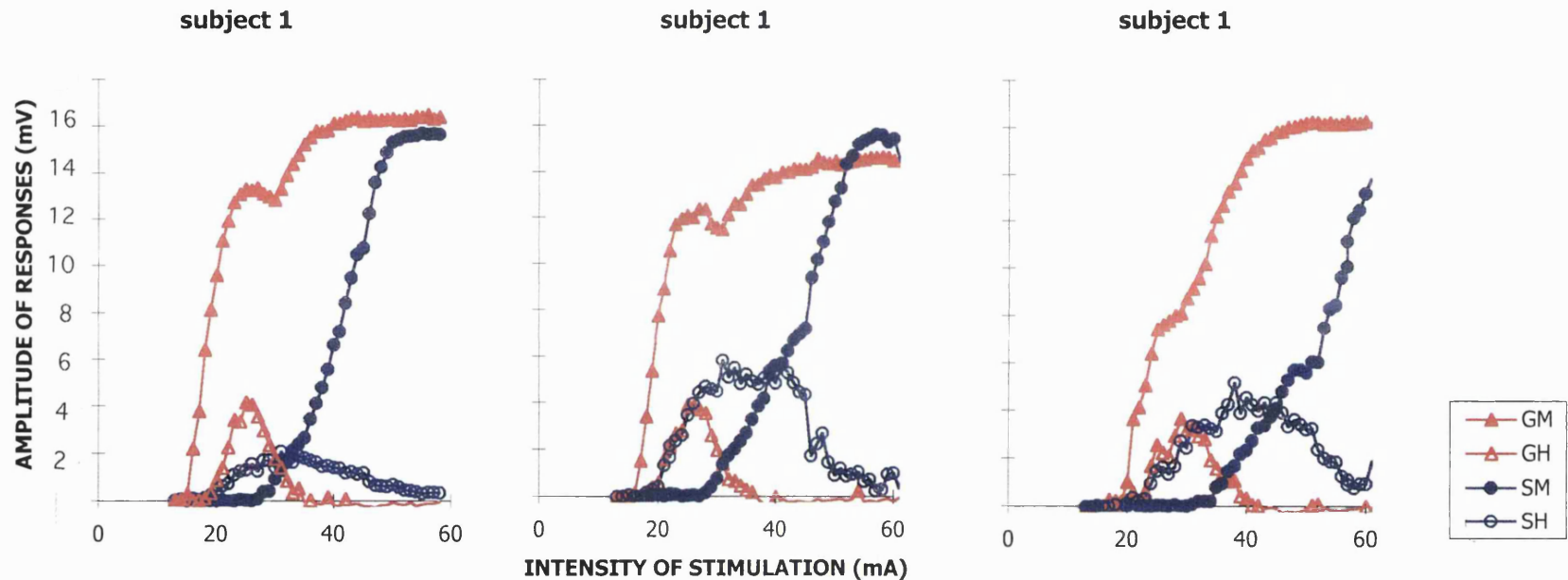
GM=gastrocnemius M wave GH=gastrocnemius H-reflex SM=soleus M wave SH=soleus H-reflex

In subjects 2 and 5 the GM is seen to decrease briefly with increasing intensity. This was seen occasionally in the presence of an increasing SM. It is believed to result from signals from the two muscles cancelling one another out, as the GM was seen to change shape.

FIGURE 4.2 RECRUITMENT CURVES OF GASTROCNEMIUS AND SOLEUS RECORDED FROM ONE SUBJECT ON THREE SEPARATE OCCASIONS

All responses were equally amplified.

The reflex response recorded over gastrocnemius (GH) can be seen to decrease in size and disappear in the presence of the soleus H-reflex



GM = gastrocnemius M wave GH = gastrocnemius H-reflex SM = soleus M wave SH = soleus H-reflex

The brief decrease in amplitude of the gastrocnemius M wave with increasing intensity of stimulation was confirmed when the area under the curve of the rectified responses was measured in addition to peak to peak measurements. As the other responses were unaffected and the M wave showed a changing shape, the decrease is thought to result from activity detected from the soleus M wave cancelling out some of the gastrocnemius activity.

recordings from subject one. Two of these were collected on the same day and one a week later.

In six of the seven subjects a short latency motor response (M wave) was recorded from gastrocnemius. This response appeared at a lower intensity of stimulation than the intensity at which the soleus M wave appeared. (In subject seven the M wave response from gastrocnemius remained extremely small and did not appear at a lower intensity than the soleus M wave). In subjects 2 and 5 in figure 4.1 the gastrocnemius M wave was seen to decrease in amplitude temporarily in the presence of an increasing soleus M wave. This response was uncommon, although it is seen in results from two of the subjects here. The reasons for this decline are not clear, but a possible explanation is that the signal recorded over gastrocnemius is contaminated by activity in the underlying soleus and some opposing signals may cancel one another out. The gastrocnemius M wave can be seen in figures 4.3 and 4.4, which show examples of individual simultaneous recordings from gastrocnemius and soleus at different levels of intensity of stimulation in two different subjects.

In subjects one to six, at low intensities of stimulation, the soleus H-reflex increased in size with increasing intensity, in the presence of the M wave recorded over gastrocnemius. This can be observed in figure 4.5 in which soleus H-reflex amplitudes were plotted against gastrocnemius M wave amplitudes in two subjects.

Five of the seven subjects showed a second response recorded from the surface electrodes over gastrocnemius, at a latency consistent with a reflex response. This response grew in amplitude in the presence of the gastrocnemius M wave, although in two of the five (subjects 3 and 6) it remained very small. The behaviour of the reflex response recorded from

FIGURE 4.3 SIMULTANEOUS RECORDINGS FROM GASTROCNEMIUS AND SOLEUS AT DIFFERENT LEVELS OF STIMULUS INTENSITY

Subject 1

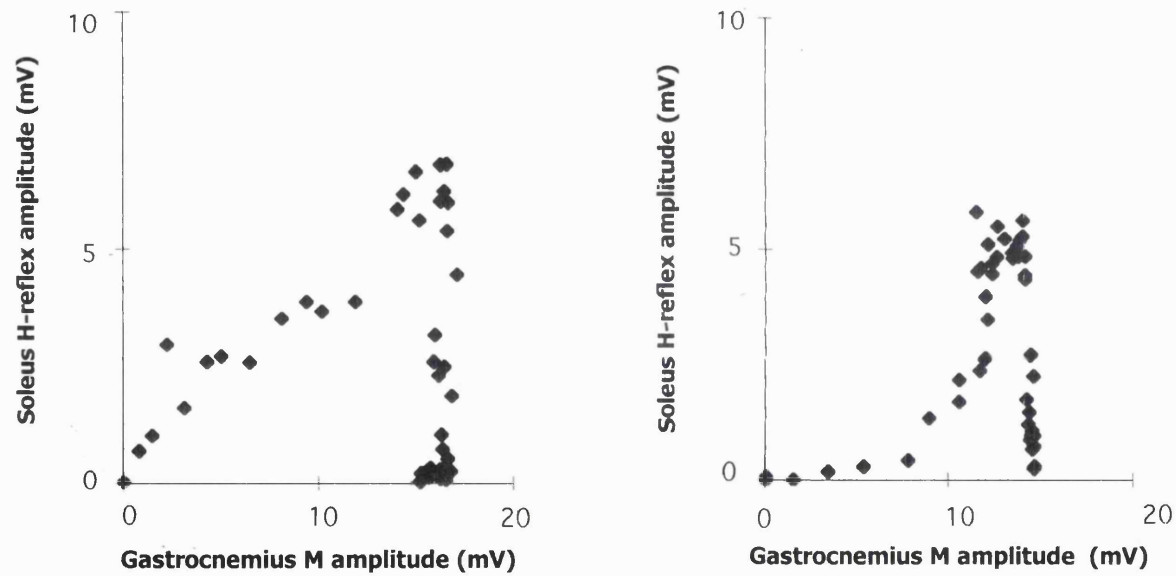


**FIGURE 4.4 SIMULTANEOUS RECORDINGS FROM GASTROCNEMIUS AND SOLEUS AT DIFFERENT LEVELS
OF INTENSITY OF STIMULATION**

Subject 3



FIGURE 4.5 THE AMPLITUDES OF THE SOLEUS H REFLEX ARE PLOTTED AGAINST THE AMPLITUDES OF THE GASTROCNEMIUS M WAVE IN TWO SUBJECTS



The plots illustrate the way in which both responses increase in amplitude at low intensities of stimulation and the M wave then peaks, maintaining a high amplitude while the H-reflex decreases in size.

gastrocnemius was in this respect quite different from the behaviour of the H-reflex of soleus, which usually decreases in amplitude soon after the appearance of the soleus M wave.

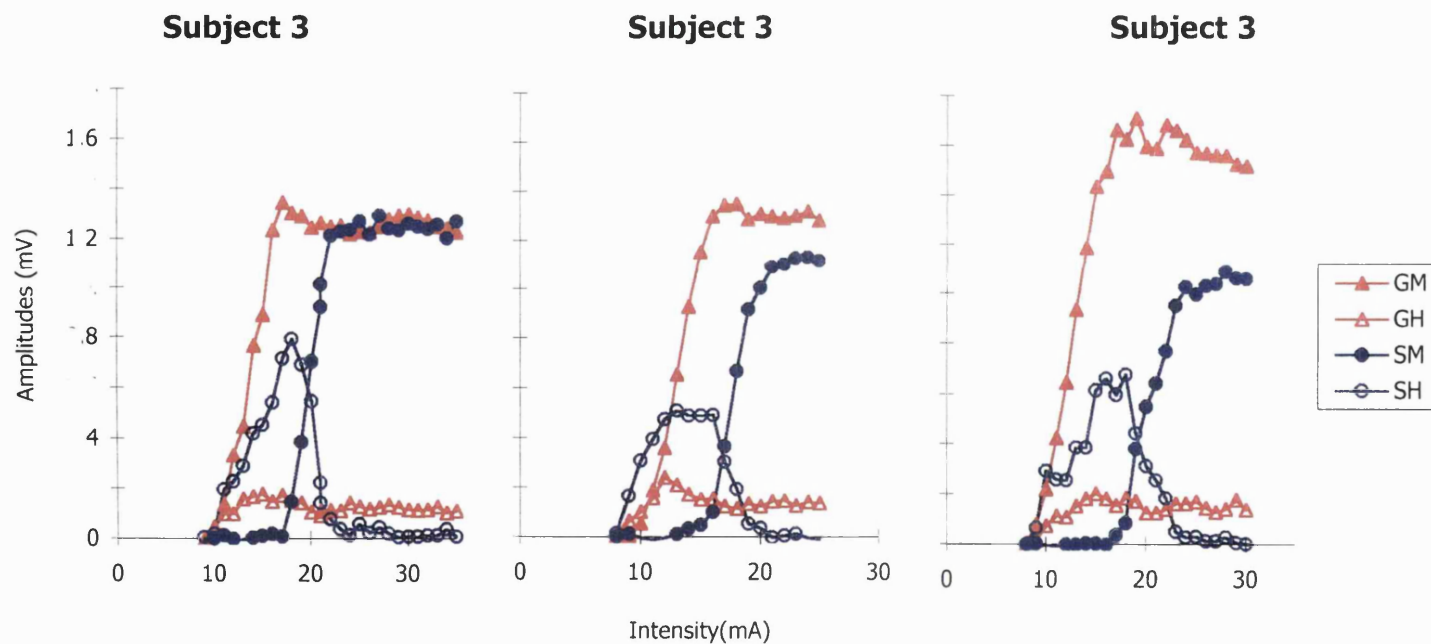
The reflex response recorded from gastrocnemius usually appeared at an intensity of stimulation similar to that of the threshold of the soleus H-reflex. But it did not behave exactly in parallel with the soleus reflex. For instance, subject 3 in figure 4.6 exhibited a gastrocnemius H-reflex which did not disappear with increasing intensity, even in the presence of what appears to be a maximal gastrocnemius M wave. At high intensities it is possible that the persistent long latency response could be an F wave, caused by invasion of the largest motoneurons by action potentials travelling antidromically in the motor axons from the stimulation site. It can be seen from the simultaneous recordings from soleus that, in contrast, the soleus H-reflex did gradually disappear. This pattern can also be seen in the dual recruitment curve of subject 5 in figure 4.1. This meant that there were occasions when the gastrocnemius reflex appeared in the absence of the soleus H-reflex.

In other subjects, also, the reflex response recorded from gastrocnemius behaved differently from that recorded over soleus; but in the case of subject 1 (see figure 4.2) the gastrocnemius H-reflex began to diminish quite sharply as the stimulation intensity increased, while the soleus H-reflex continued to reduce in size gradually with increasing intensity. This is consistent in all three recordings from this subject.

When individual recordings of the responses were examined, a number of further observations were made. Firstly, in every subject, both the M wave and the reflex response from gastrocnemius always appeared at a shorter latency than the equivalent responses of soleus (see figure 4.7 for instance).

FIGURE 4.6 RECRUITMENT CURVES OF GASTROCNEMIUS AND SOLEUS RECORDED FROM ONE SUBJECT ON THREE SEPARATE OCCASIONS

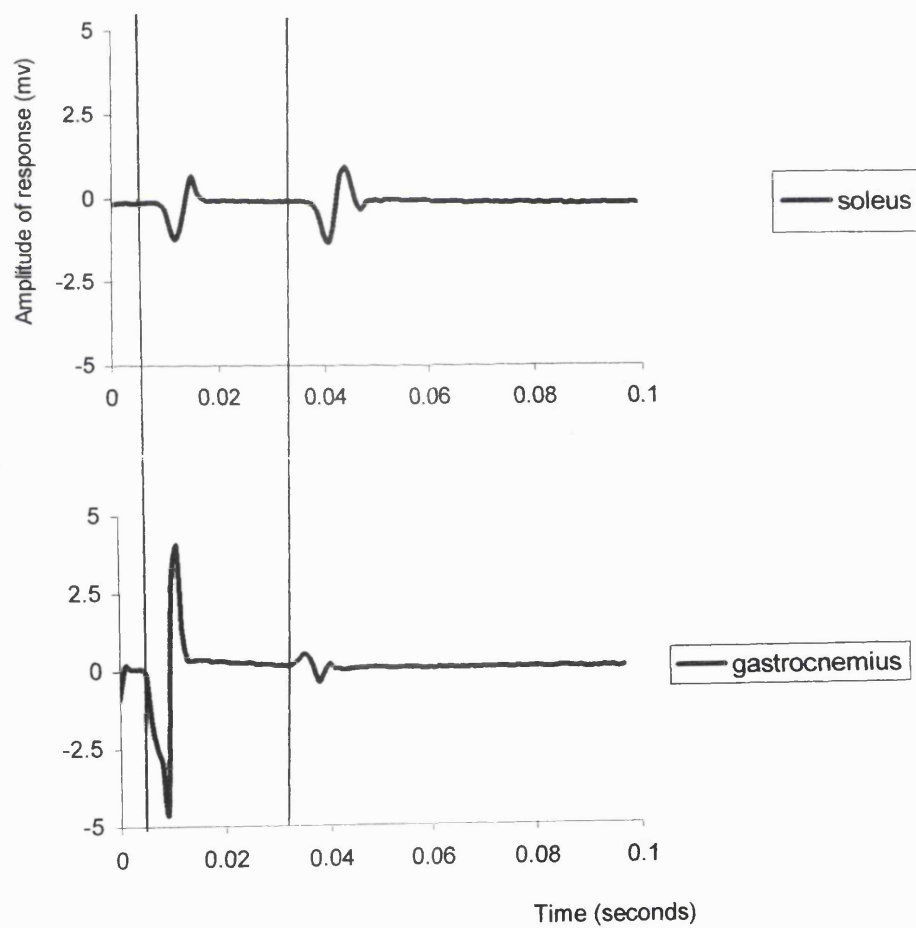
Three recordings of gastrocnemius and soleus recruitment curves taken from the same subject. All responses were equally amplified. In all cases it can be seen that the M wave recorded from gastrocnemius (GM) appears at a much lower intensity of stimulation than the soleus M wave (SM). The soleus H-reflex continues to grow in amplitude with increasing intensity in the presence of the gastrocnemius M wave. A reflex response recorded from gastrocnemius can be seen at intensities above that at which the soleus H-reflex has disappeared.



GM = gastrocnemius M wave GH = H-reflex recorded from gastrocnemius SM = soleus M wave SH = H-reflex recorded from soleus

FIGURE 4.7 SIMULTANEOUS RECORDINGS OF SOLEUS AND GASTROCNEMIUS IN RESPONSE TO STIMULATION OF THE TIBIAL NERVE IN THE POPLITEAL FOSSA

The responses were equally amplified. The cursors highlight the difference in latency between the gastrocnemius responses and those from soleus.



Measurements were made of the latencies of the gastrocnemius and the soleus M waves from ten sets of recordings from four subjects. The measurements were taken from responses of similar size. The difference between the latencies was then calculated. Similar measurements were then taken of the latencies of the reflex responses from gastrocnemius and soleus. The mean difference between the latencies of the M waves of the two muscles was 3.45ms (SD 1.11). The mean difference between the reflex responses from the two muscles was 3.66ms (SD 0.97).

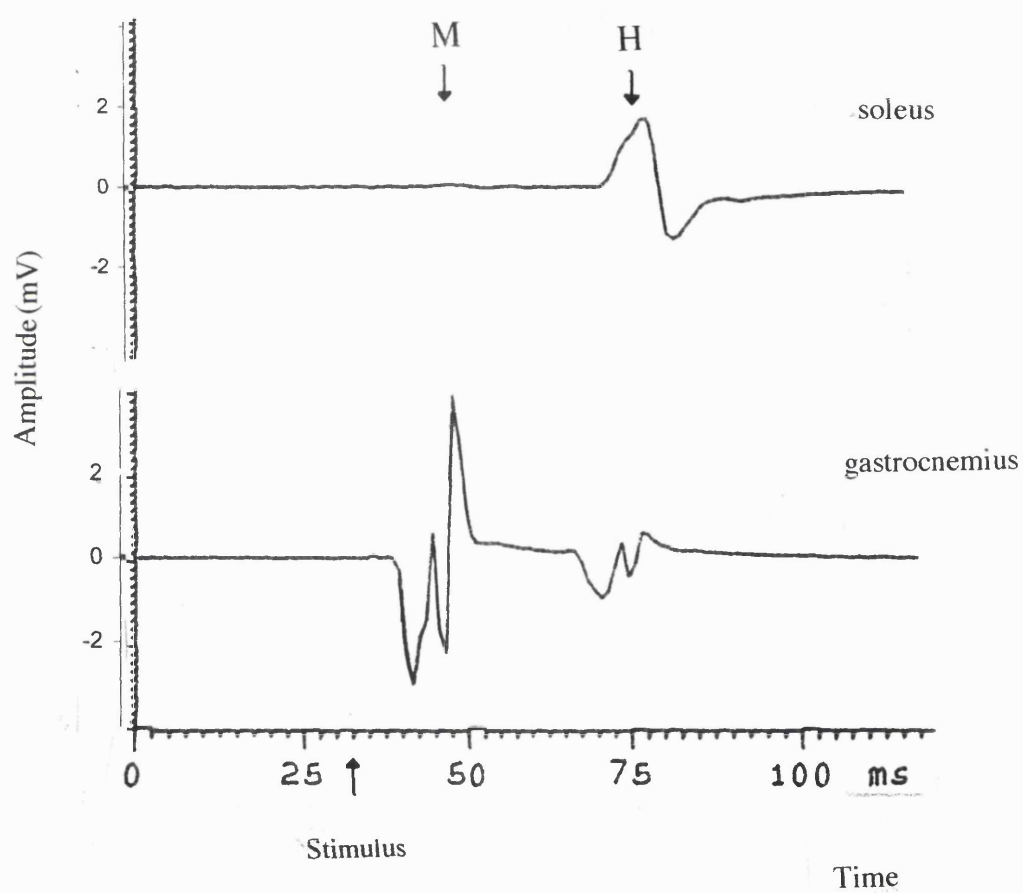
Secondly, the shape of the reflex response from gastrocnemius was often similar to the shape of the gastrocnemius M wave, but quite different from the shape of the soleus H-reflex (see figure 4.8).

In summary, all of the observations indicate that gastrocnemius has a reflex distinct from the soleus H-reflex, in contrast to the reports of Hugon (1973), Brunia et al. (1973) and others.

4.4 DISCUSSION

The results of this study support the conclusion that the differences seen between the behaviour of the M and the H responses of soleus and the M and H responses of gastrocnemius can be explained by the fact that they reflect the different characteristics of the two muscles. Soleus consists almost entirely of small motor units and therefore the diameters of its motor axons are generally small, whereas gastrocnemius is a more mixed muscle with a much wider distribution of motor axon diameters (Burke 1978). These differences will affect their relative responses to electrical stimulation, where the largest fibres have the lowest thresholds to activation.

FIGURE 4.8 SIMULTANEOUS RECORDINGS FROM SOLEUS AND GASTROCNEMIUS IN RESPONSE TO STIMULATION OF THE TIBIAL NERVE SHOWING THE SIMILARITY OF THE SHAPES OF THE M WAVE AND THE REFLEX RESPONSE RECORDED OVER GASTROCNEMIUS



The appearance of a gastrocnemius M wave at an intensity of stimulation similar to the threshold intensity of the soleus H-reflex might be predicted if some of the large gastrocnemius motoneurons have axons whose diameters are similar to those of the large Ia afferents. In fact, the stimulating arrangements in the present study are those used most commonly to ensure optimal stimulation of the Ia afferent fibres from soleus, but the branches to soleus and gastrocnemius usually lie in very close proximity in the middle of the popliteal fossa. When Pierrot-Deseilligny and his colleagues particularly wish to separately stimulate the branches, they stimulate the medial gastrocnemius nerve more distally and medially and the lateral soleal nerve on the posterior surface of the calf (see for instance Meunier et al. 1993).

With the soleus H-reflex, as the intensity of stimulation is gradually increased, in most individuals there is a substantial reflex activation of the soleus motoneurons by activity in Ia afferents before the motor axons begin to respond directly to the stimulation. Indeed, in some individuals the reflex appears to have peaked in size before the M wave appears (see subjects 4 and 5 in figure 4.1 and Taborikova and Sax 1968, for example). This reflects the large difference between the diameters of the Ia afferent fibres and the largest soleus motor axons. In gastrocnemius, however, there are motor units with large motor axons and, in most of the subjects in the present study, some of these appear to be large enough to be activated and produce an M wave at an intensity the same as or lower than that at which Ia afferent activity leads to the production of an H-reflex in soleus.

These observations concerning the gastrocnemius M wave are in keeping with what is known of the detailed distribution of motor axon sizes of gastrocnemius in the cat (Cullheim and Kellerth 1978) and of muscle fibre types in human gastrocnemius (Polgar et al. 1973). At high intensities, in

the presence of a large soleus M wave, the gastrocnemius M wave response may sometimes be contaminated by electrical activity in soleus. The evidence for this is that, as described earlier, occasionally the gastrocnemius response was seen to change in shape (and sometimes to decrease and then increase in amplitude after seeming to peak) in the presence of a large soleus M wave (see footnote to figure 4.1).

In relation to the reflex response from gastrocnemius that was observed in five subjects; this was seen to grow in amplitude with increasing intensity of stimulation in the presence of the gastrocnemius M wave. It has been argued that this finding could be explained by the fact that the response observed was, in fact, the H-reflex from soleus detected by the electrodes over gastrocnemius. This reflex would not be affected by any collision effect from action potentials in gastrocnemius motor axons. However, from what follows it is clear that these observations are due at least in part to the presence of a separate gastrocnemius reflex.

The range of motoneurone sizes in the gastrocnemius pool is sufficiently large that smaller motoneurones could continue to be reflexly recruited in the presence of a large M wave, because the M wave represents the direct activation of only the largest of the motor axons. In contrast to the situation with the predominantly small motor units of soleus, the collision effect would only come into play when gastrocnemius motoneurones in the middle range were activated both directly and reflexly and at this point the M wave could already be large. Thus the differences in the behaviour of the reflex responses from the two muscles could be accounted for in this way.

In relation to the consistently shorter latency of the reflex from gastrocnemius compared to that of the soleus H-reflex; if the electrodes

over gastrocnemius were picking up only the reflex response from soleus, then one might expect the response to have a similar latency to the response recorded from soleus. The recording electrodes over gastrocnemius were more proximal, but the motor end plates of soleus, where the axons enter the muscle, are situated approximately half way along the length of each fibre (Hugon 1973). The fibres of soleus are short and it is thought that the electrical response of the muscle fibres propagates out towards the ends and that it is almost simultaneous among the fibres (Hugon 1973). Indeed Mineva and colleagues (1993) found that the latencies of the soleus H-reflexes they elicited were independent of the site of the recording electrodes (even though the sites were as much as 16cm apart). The differences which the authors observed were in the durations of the responses, not in their latencies.

In view of these observations, if the reflex response recorded over gastrocnemius was actually that of soleus, then it is unlikely that it would be observed earlier than that detected by electrodes over soleus itself.

What factors could explain some of the inter-individual differences seen in the behaviour of the responses recorded from gastrocnemius? These would appear to be due to three factors. The first factor is that although the stimulating and recording conditions would have been similar for each subject, the actual arrangement of the fibres in the peripheral nerve trunk and the dividing branches would be slightly different in each individual.

The second factor is that although certain factors affecting central excitability levels could be controlled by ensuring relaxation of the individual as a whole and of the muscles concerned in particular, the level of central excitability from numerous other sources could be slightly

different in each subject. This could affect how easily the motoneurons were recruited by the afferent input.

The third factor is the possibility that the individual differences seen also reflect the fact that the exact distribution of motor axon diameters of gastrocnemius (in relation to the distribution of the Ia diameters) can be somewhat different in different individuals. In the light of the consistency of the responses seen in repeated recordings from the same individuals, the third explanation seems plausible.

For example, in the case of the two subjects who displayed a gastrocnemius H-reflex which did not completely disappear, then it is possible that only the largest gastrocnemius motor axons would need to be activated to produce a large M response and that some of the smallest motor axons might not be accessible to a current intensity high enough to activate them directly without considerable discomfort, especially if the stimulating arrangement favoured the branch supplying soleus. The gastrocnemius M wave might therefore not reach a true maximum size. If, however, the electrodes over gastrocnemius were detecting a soleus H-reflex, then this would mean that these electrodes were detecting activity in some soleus fibres while the electrodes recording directly over soleus itself were no longer detecting any activity because all the reflexly recruited motor axons were affected by the collision effect.

4.5 CONCLUSION

These findings indicate that the presence of an H-reflex of gastrocnemius can be demonstrated in some subjects at rest. The differences in the behaviour of the responses from gastrocnemius compared to those of soleus

may be explained in terms of the different distributions of Ia afferent and motor axon fibre diameters in the two muscles.

In some subjects, in whom a substantial gastrocnemius reflex can be demonstrated (three of the seven subjects in the present study would be suitable) it should be possible to select and make experimental use of a reflex which can be facilitated or inhibited in the presence of an M wave.

Finally, and perhaps most importantly, the presence of a gastrocnemius M wave at low intensities of stimulation in most subjects means that recording from soleus and gastrocnemius simultaneously during H-reflex experiments would allow the use of an optimum-sized soleus H-reflex for conditioning purposes while the stability of the stimulation arrangement is monitored with the gastrocnemius M wave.

5.0 THE INVESTIGATION OF PATHWAYS BETWEEN AFFERENTS OF THE POSTERIOR TIBIAL NERVE AND THE CONTRALATERAL SOLEUS MOTONEURONE POOL

INTRODUCTION

Human studies involving crossed effects have often identified changes at long latencies. The consistency of the results from perturbation studies indicating coordinated, bilateral reflex responses at around 55-70ms is persuasive (Deitz et al. 1984a &b, 1989, Corna et al.1996). Such a latency fits well with the evidence from animal studies indicating group II-mediated whole limb responses and powerful crossed group II effects under some circumstances (Holmqvist 1960,1961b,Rosenberg 1970, Jankowska et al.1967b, Harrison and Zytnicki 1984, Harrison et al 1986).

However the results from experiments using electrical stimulation to condition contralateral H-reflexes are more mixed. The differences in the intensity of stimulation used and the size of the test H-reflexes used has made comparison difficult. Long latency facilitation has often been observed, but its character has been quite different between studies (Robinson et al. 1979, Delwaide et al 1981, Koceja and Kamen 1992). These differences could perhaps be accounted for in terms of the different mixtures of fibres which were being activated under the different experimental conditions.

Comparisons could be drawn between the late facilitations seen in some of the stimulation experiments and the responses observed in the perturbation studies, but such comparisons need to take into account the fact that the latencies of the responses to perturbation relate to the total timing required to see a reflex response, whereas in the conditioning experiments, the

intervals examined relate to the differences in the arrival times of the effects of the conditioning volleys and the test volley at the test motoneurone pool.

At very short latencies there have been occasional observations relating to a small early inhibition (Delwaide et al.1981, Koceja and Kamen 1992), but the focus of these particular studies has been upon the later, larger effects.

The emphasis of the present study was to monitor closely the early period following conditioning stimulation in which crossed effects from group I and group II fibres could appear. In the light of the evidence from animal studies and from observations such as those from Corna et al. (1996), the hypothesis was that we should detect changes related to crossed group II activity, but that the effects might be quite different in supported sitting from those seen in standing. Because electrical stimulation activates the largest diameter fibres preferentially, then large numbers group I fibres would be activated. As a result it might be possible to detect weak group I crossed effects should they be present.

The first series of experiments in the search for evidence of early crossed reflexes involved the study of crossed effects which were mediated by afferents in the posterior tibial nerve of one lower limb and influenced the soleus motoneurone pool of the contralateral lower limb. The experimental procedure involved using the H-reflex to identify any changes in the excitability of the soleus motoneurone pool which occurred as a result of conditioning by the electrical stimulation of the contralateral posterior tibial nerve.

5.1 METHOD

A series of preliminary experiments were performed applying stimuli to the posterior tibial nerve and recording changes in the contralateral gastrocnemius H-reflex. Using a conditioning stimulus intensity of two times the threshold of the motor axons the results revealed a small early inhibition, which was most pronounced at a condition/test interval of 10ms. When a stimulus intensity of three times the motor threshold was used the short latency inhibition was followed by a longer latency facilitation, at a latency of 50ms (see appendix I for illustrations of these preliminary results).

Eight detailed experiments were then carried out on seven healthy subjects, all of whom gave their informed consent. During each experiment the interval between the application of the conditioning stimulus and the application of the stimulus producing the test H-reflex (the test stimulus) was regularly changed, so that a series of unconditioned and conditioned reflexes was collected for each of a range of condition/test (C/T) intervals. The number of conditioned and unconditioned reflexes collected in each series was varied between experiments, but was always between 60 and 120. The effect of conditioning could then be examined over a total time span of fifty milliseconds. Between ten and fifty milliseconds the intervals were varied by five milliseconds, but between zero and ten milliseconds the intervals were varied by two or three milliseconds. The order in which the C/T intervals were presented was varied randomly.

The subjects were seated in a high-backed chair with both lower limbs supported at the thigh and the foot so as to maintain the stability of the stimulating and recording set-up. Both knees were usually held at an angle of approximately forty degrees of flexion from full extension, but the

angle varied somewhat between individuals, depending upon the angle at which an optimum response was produced. The ankle joints were maintained in approximately ten degrees of plantarflexion.

Before an experiment was commenced the comfort of the subject was checked and they were asked to relax and avoid unnecessary movement. Surface EMG recordings were used to check that the muscles under examination were relaxed. During long experiments the subject was given the opportunity to move and resume a more comfortable position from time to time between data collection.

5.1a STIMULATION

The stimulating equipment used was similar to that described above in chapter 3, but in the crossed experiments two Digitimer D57 constant current stimulators were used. The second stimulator, which was generally used to produce the conditioning stimulus, had been adapted by the manufacturers to give an increased output and as a result it included a feature which restricted the longest pulse duration to 0.5ms. Thus this was the pulse duration used for the conditioning stimuli.

On the lower limb in which the test H-reflex was produced the stimulating arrangements were as described above in chapter 3. Once a stable reflex was produced (which was present at low stimulus intensity levels, in the absence of an M response) the stimulating electrode (the cathode) in the popliteal fossa was secured with the perforated rubber strap. The stimulus was adjusted to give an H-reflex which was around 60% of the maximum H-reflex amplitude (Evans et al. 1995 and chapter 3 above).

For each experiment the stimulus used to produce the test reflex was of 1ms duration. The test reflexes were elicited at regular intervals. The interval chosen varied between experiments but was always between 4 and 8 seconds. The choice was a compromise between the desire to minimise the effects of the phenomenon of post-stimulus depression by using a long interstimulus interval (see chapter 3 above for details) and the need to collect reflexes over a substantial number of condition/test intervals in the time available. The long interstimulus intervals combined with the collection of a large numbers of reflexes for each condition/test interval resulted in long experiments and the subject's time and patience were always important factors in the choice of procedure.

On the contralateral side, the stimulating electrodes were placed in similar positions to those on the test side, in preparation for the production of the stimuli which would be used to condition the test reflex. Once the optimum position of the cathode was established the electrode was secured and the stimulus intensity was increased, usually to two times the intensity at which the M wave started to appear, but in two of the eight experiments it was increased to three times the threshold of the M wave.

For each run of data collection, once the condition/test interval had been selected, this interval was fed into the programmer which acted as the external trigger. The programmer then introduced the appropriate delay before the activation of the test stimulus. This allowed the conditioning stimulus to be introduced at the correct interval in advance of the test stimulus.

During each collection the stimulator producing the conditioning stimulus was turned on and off intermittently in a non-uniform manner, so that

small numbers (between 2 and 7) of conditioned and unconditioned reflexes were nonsystematically interleaved (Meunier et al.1984).

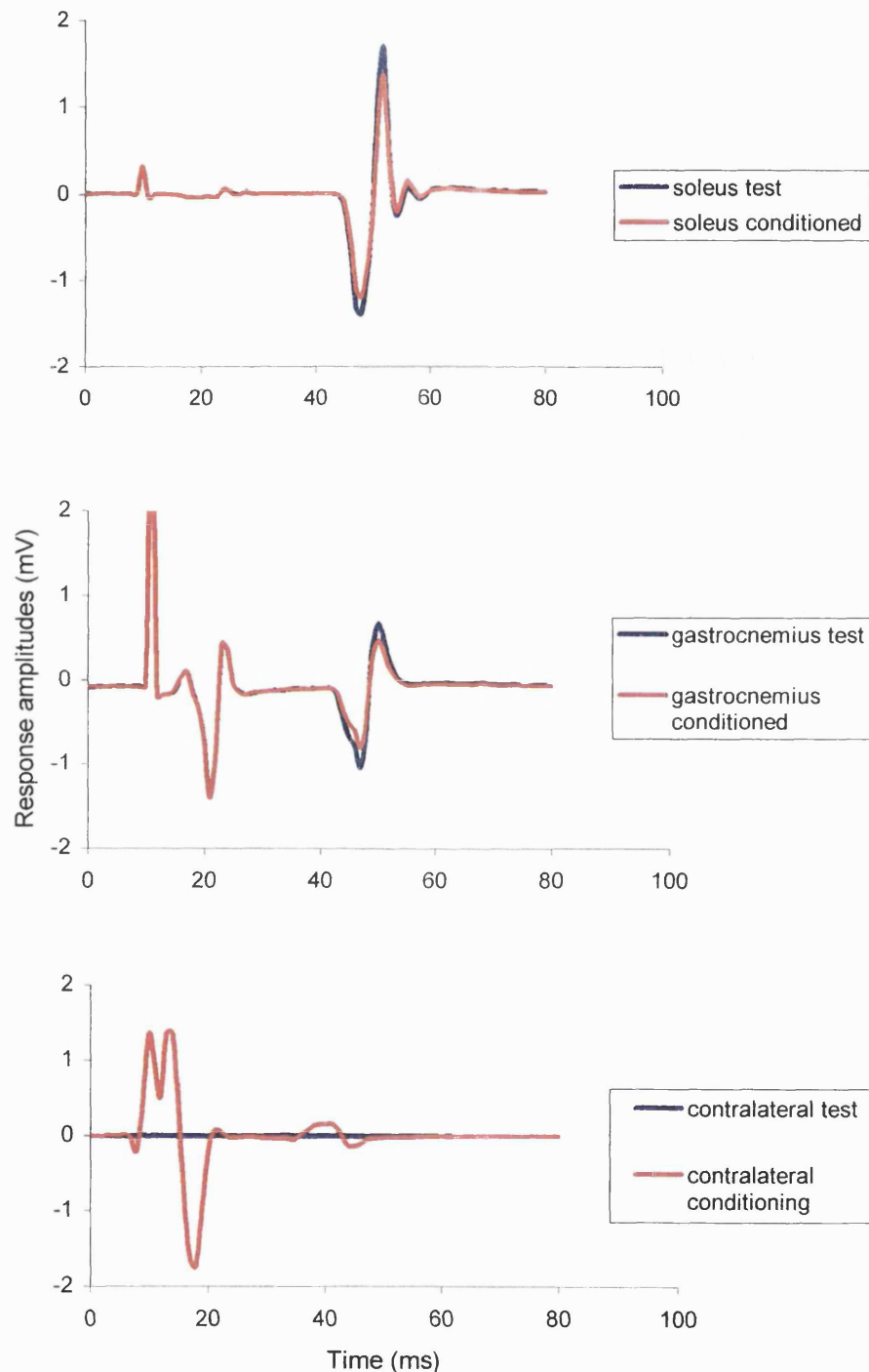
5.1b RECORDING

On each lower limb two disposable adhesive recording electrodes were placed over the body of the soleus muscle (as described above in chapter 3) and a reference electrode was placed over the anteromedial surface of the tibia. Two electrodes were also placed over the gastrocnemius muscle bulk so that recordings of the gastrocnemius M wave could be used to monitor the stability of the stimulus presentation. Sampling of sweeps of 200ms duration of the filtered (bandpass 30 - 500 Hz) and amplified surface EMG recordings (see chapter 3 above for details and apparatus) was synchronised with the timing of the delivery of the conditioning stimulus, both in its presence and in its absence.

5.1c ANALYSIS

For each experiment the recordings from each condition/test interval were stored to be measured and analysed at a later date. An example of a recording using a condition/test interval of 10ms is illustrated in figure 5.1. The figure shows the average of the conditioned responses in red superimposed upon the average of the test (unconditioned) responses in blue from the soleus muscle in the top trace and from gastrocnemius in the middle trace. The lower trace shows the average of the recordings from the contralateral soleus muscle during application of the conditioning stimulus in red and during non-application in blue. The soleus H-reflex amplitude can be seen to be reduced by conditioning. The peak to peak amplitudes of the individual H-reflexes and M waves were later measured and stored separately in four files - one containing the test reflexes, one

FIGURE 5.1 THE THE EFFECT OF CONDITIONING BY STIMULATION OF THE CONTRALATERAL TIBIAL NERVE UPON THE SOLEUS H-REFLEX - EXAMPLES OF RECORDINGS



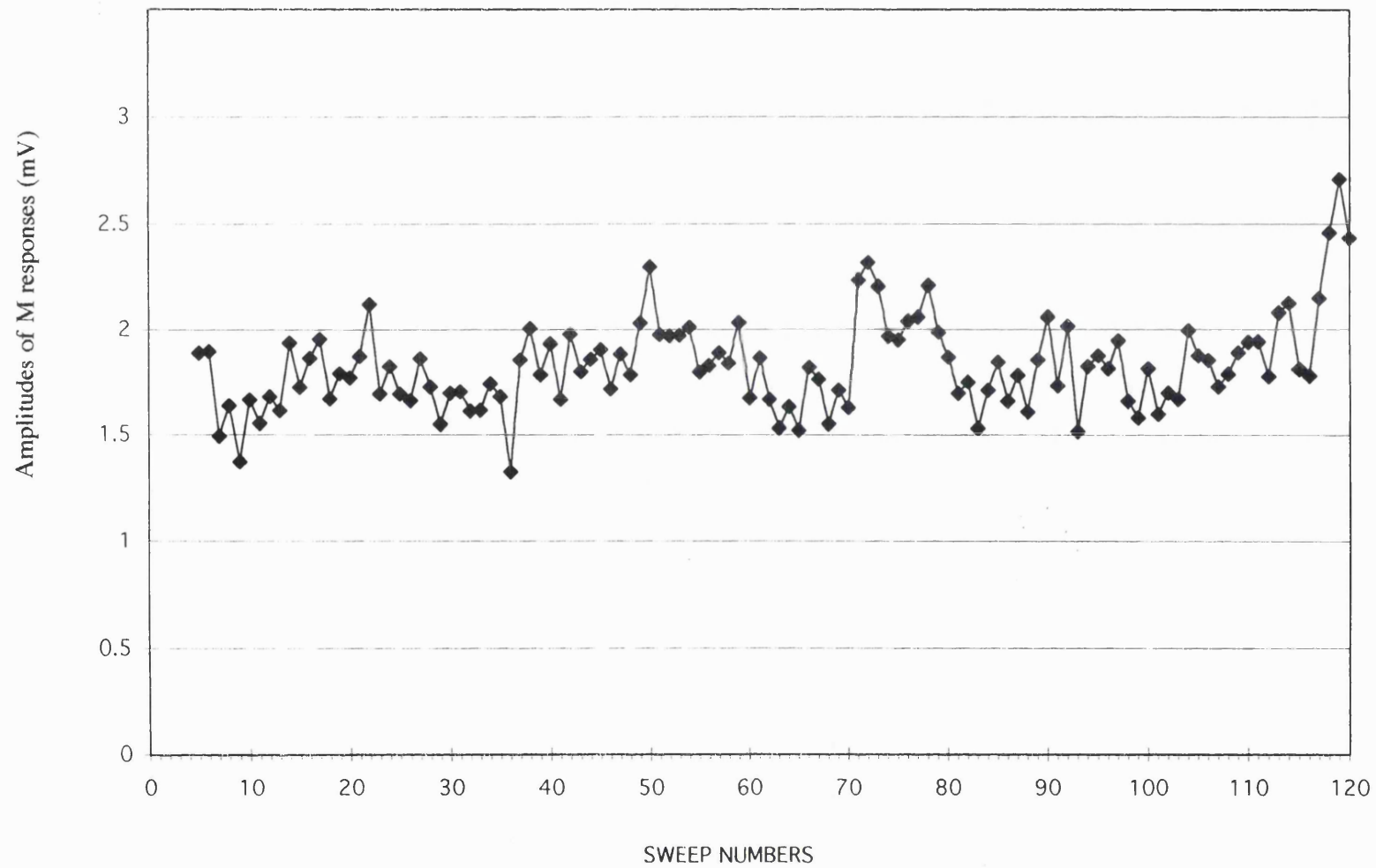
Averages of responses from simultaneous recordings from left soleus muscle (top trace) left gastrocnemius muscle (middle trace) and the right soleus muscle (lower trace) Traces represent approximately 40 test (blue) and 40 conditioned (red) responses. The contralateral stimulus was applied at 0ms, the test stimulus at 10ms. The M wave of gastrocnemius was used to monitor peripheral stability. The soleus H-reflex is inhibited

containing the conditioned reflexes, one containing the test M responses and one containing the conditioned M responses. These measurements were converted to a Microsoft Excel file for analysis.

The mean amplitude, the standard deviation and the standard error of the mean were calculated for each of the two groups of M responses and the amplitudes of the individual responses in each group were plotted as in figure 5.2 to allow visual assessment of the details of the variability of the M responses. Occasionally there were obvious outliers, lying more than two standard deviations from the mean, probably resulting from subject movement. These were removed along with their matched H-reflexes, but no more than two from any run of sixty or more sweeps. Rarely, where the M variability was unacceptably large, then the results from that run were discarded.

Descriptive statistics were used to check samples of test reflexes for acceptable levels of skewness and kurtosis in order to assess whether the samples of measurements were likely to be those from a normally distributed population. For each condition / test interval sample used the mean amplitude, the standard deviation and the standard error of the mean were calculated for each of the paired groups of unconditioned and conditioned H-reflexes and a two-tailed Student's t-test was used to identify significant differences.

**FIGURE 5.2 AMPLITUDES OF A TYPICAL RUN OF CONSECUTIVE
M WAVE AMPLITUDES RECORDED DURING A CROSSED
CONDITIONING EXPERIMENT**



5.3 RESULTS

In order to compare the results from the individual experiments, in each experiment, for each of the condition/test intervals used, the mean amplitude of the sample of conditioned H-reflexes was expressed as a percentage of the mean amplitude of the sample of test H-reflexes. The results from each subject were then plotted individually in figure 5.3. There was considerable variation between subjects, particularly at the later C/T intervals, but a pattern of a consistent early inhibition with a slow return to the baseline amplitude emerged.

At the 0 ms C/T interval the mean conditioned reflexes from all the experiments were close to the value of the mean test reflexes and displayed no evidence of a conditioning effect.

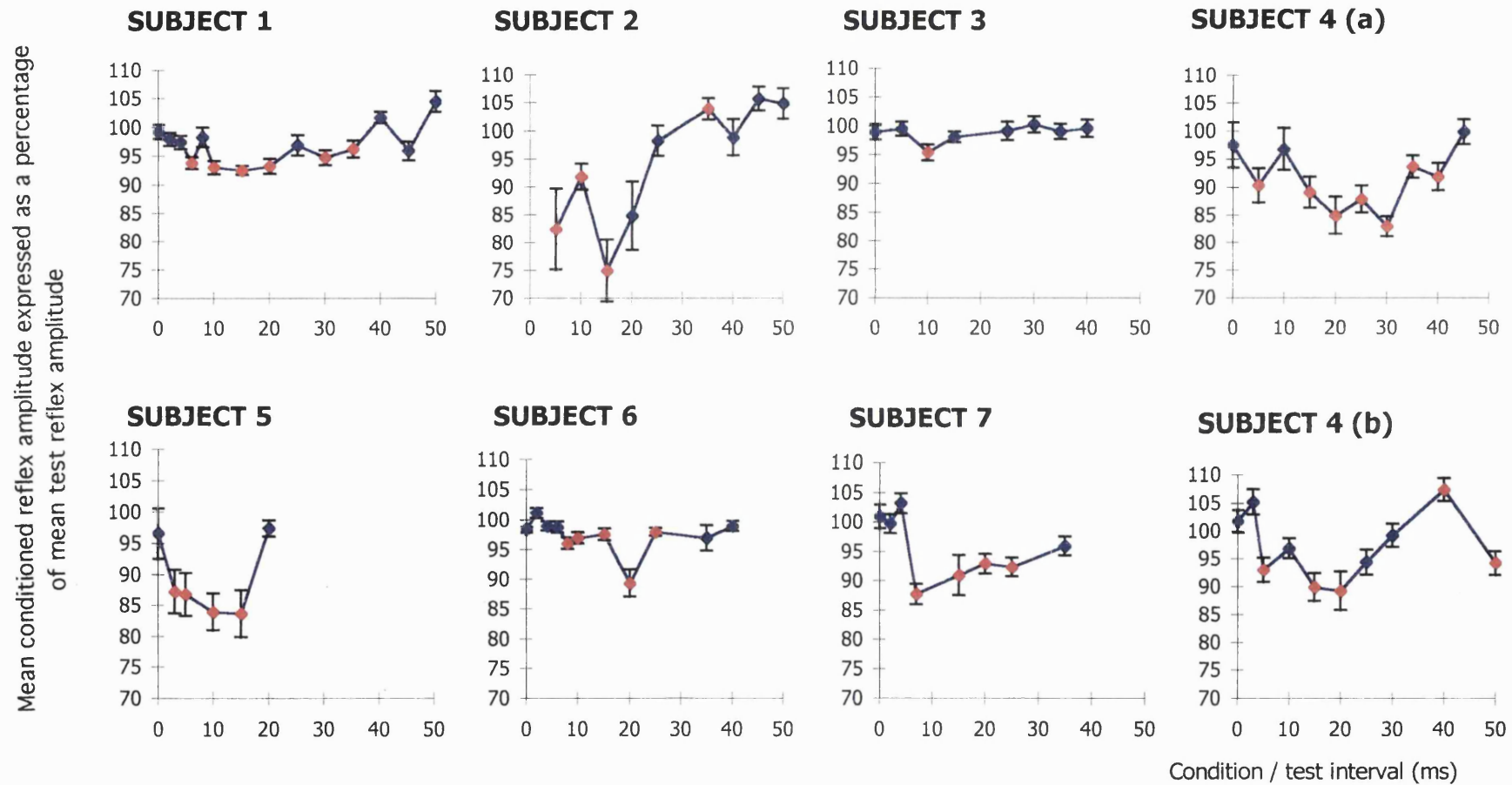
At the 3 ms C/T interval, in one of the five experiments in which this interval was used the mean conditioned reflex was significantly smaller than the mean test reflex ($p < 0.05$).

At the 5 ms C/T interval in five of the eight experiments the mean conditioned reflex was significantly smaller ($p < 0.05$) than the mean test reflex.

The 10 ms C/T interval was used in seven of the eight experiments. In five of the experiments the mean conditioned reflex was significantly smaller than the mean test reflex ($p < 0.05$).

At the 15 ms C/T interval, in seven of the eight experiments, the reduction in the size of the mean conditioned reflex was significant ($p < 0.05$).

FIGURE 5.3 THE EFFECT OF A CONDITIONING STIMULUS APPLIED TO THE CONTRALATERAL TIBIAL NERVE UPON THE SOLEUS H-REFLEX



Results from individual subjects. For each condition / test interval 30-50 conditioned reflexes and 30-50 test reflexes were collected, Therefore each point represents the results from 60-100 reflexes. Error bars = SEM. Red points represent statistically significant differences.

The 20 ms C/T interval was used in seven experiments and five of these showed a significant reduction ($p<0.05$) in the amplitude of the conditioned reflex.

The 25 ms C/T interval was also used in seven experiments and the conditioned reflex was significantly smaller ($p<0.05$) in three of these.

The 30 ms C/T interval was used in four experiments and in two of these the conditioned reflex was significantly smaller ($p<0.05$).

Of the six experiments in which the 40 ms C/T interval was used, in four experiments the mean conditioned and test reflexes were almost identical, in one experiment the mean conditioned reflex was significantly smaller ($p<0.05$) and in one experiment it was significantly larger ($p<0.05$).

In three experiments the 45 ms C/T interval was used and in each case no significant difference was seen between the means of the two groups of reflexes.

In the three experiments in which the 50 ms C/T interval was used the mean conditioned reflex amplitude was almost identical to that of the mean test reflex in two experiments and was significantly smaller ($p<0.05$) in one.

In two experiments reflexes were collected at a C/T interval of 55ms. In one of these experiments the mean conditioned and test reflexes were almost identical and in the other experiment the mean conditioned reflex was significantly larger ($p<0.05$). Finally, in the one experiment which used the 60 ms C/T interval the mean conditioned reflex was significantly larger ($p<0.05$) than the mean test reflex.

The results from the individual experiments were pooled for each C/T interval which had been used in at least four experiments. The two groups of original values - the mean test H-reflex amplitudes and the mean conditioned H-reflex amplitudes from each experiment - were analysed. As normal distribution of these values could not be confirmed, the Wilcoxon's Signed Rank test was used to analyse the paired data. The reduction in size of the conditioned reflex was found to be statistically significant in the pooled results at the condition/test intervals of 5ms ($p < 0.05$), 10ms ($p < 0.01$), 15 ms ($p < 0.01$), and 20 ms ($p < 0.05$).

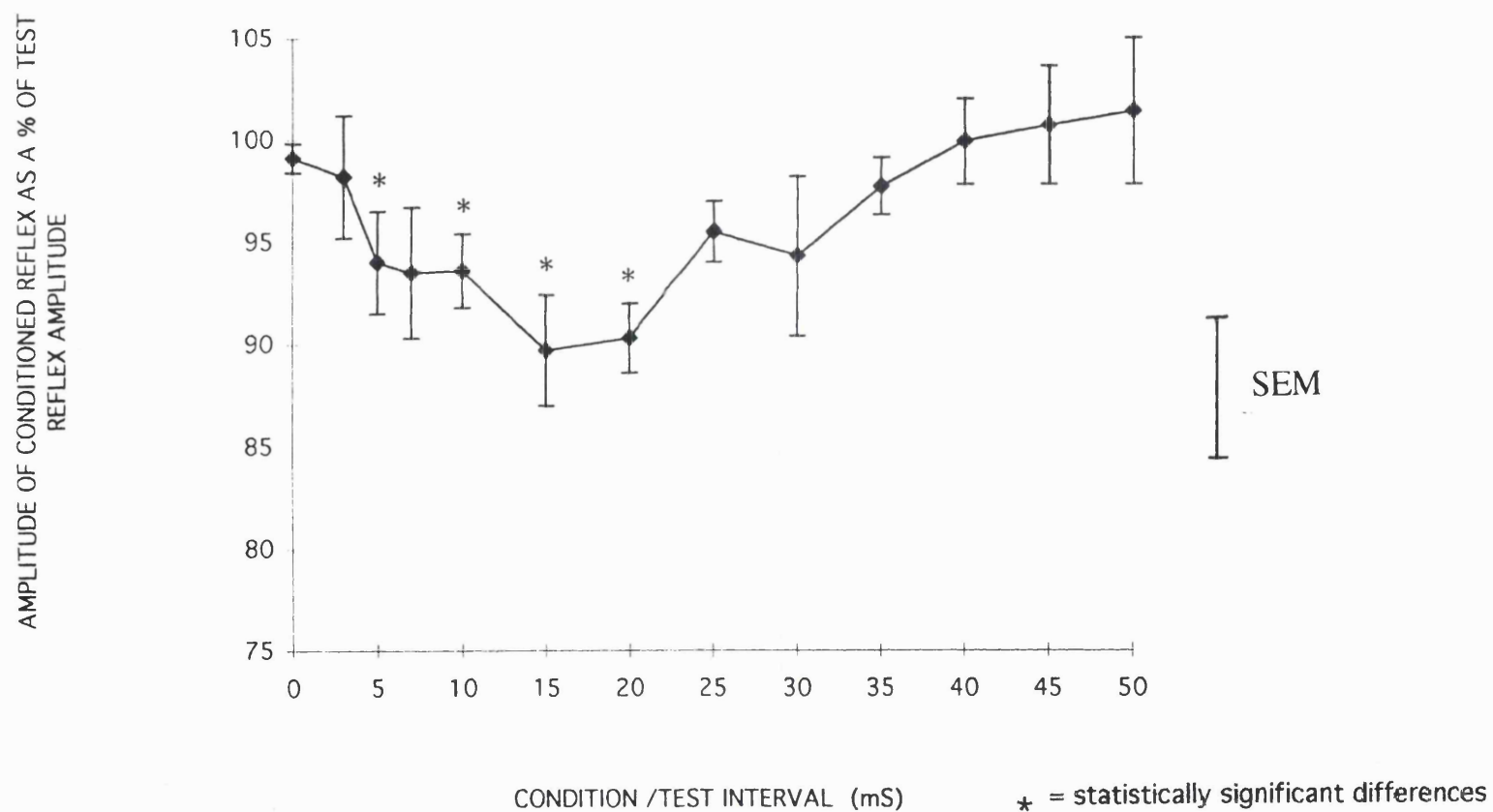
The mean amplitudes of the conditioned reflexes from the pooled results were expressed as a percentage of the mean amplitudes of the pooled test reflexes and these were plotted against the C/T intervals. The pattern of inhibition over time can be seen in figure 5.4.

5.4 DISCUSSION

The results of this section of the thesis show that conditioning stimulation of the contralateral tibial nerve produce inhibition of the soleus H-reflex. In order to interpret the observed crossed effects and predict the conduction velocities of the neurones which may be mediating them, it is necessary to be able to (a) compare the likely conduction distance of the contralateral pathway with that of the ipsilateral pathway of the soleus H-reflex, (b) examine the available evidence concerning the conduction velocities of different afferents in the human peripheral nerves used in the present experiments (c) consider the possible range of afferents that might be activated by the intensity of stimulation used to produce the conditioning volley (d) and consider the likely duration of changes in excitability of the motoneurone pool elicited both by the test stimulus and by activity in a range of contralateral afferents.

Figure 5.4 THE EFFECT OF CONDITIONING BY ELECTRICAL STIMULATION OF THE POSTERIOR TIBIAL NERVE UPON THE CONTRALATERAL SOLEUS H-REFLEX

GROUPED DATA



5.4a CONDUCTION DISTANCES

Opportunities to make accurate, direct measurements of the length of peripheral nerve pathways are obviously restricted in human studies. Estimates made from surface measurements are of limited value when a proportion of the passage of the nerve is not close to the surface and runs in the inaccessible posterior wall of the abdominal cavity.

The approach taken in this study was to take direct measurements from one cadaver and then to reconstruct the examined nerve routes in seven skeletons of different heights and measure these. The direct measurements and the measurements of the reconstructed paths were compared with measurements made by Hultborn, Meunier, Morin and Pierrot-Deseilligny (1987). For this series of experiments the distance measured was from the site of stimulation of the posterior tibial nerve in the popliteal fossa to the first sacral spinal segment, where soleus motoneurons would be located, (this corresponds to the level of the upper border of the first lumbar vertebra). Also direct measurements were taken from all the specimens of the distance from the greater trochanter of the femur to the lateral malleolus of the fibula. This measurement indicated the length of the lower limbs and could be used as a guide to compare the relative heights of the cadaver and the skeletons.

The individual measurements are listed in Table I. The mean length of the pathway from the popliteal fossa to the first lumbar vertebra was 70 ± 4.4 centimetres and the mean distance from the greater trochanter to the lateral malleolus was 78 ± 4.4 centimetres. Analysis of the paired values from each specimen indicated a positive correlation between the two distances measured ($r=0.71$). The direct measurement of the nerve from the cadaver fell well within the range of the estimated values, but towards the lower

TABLE I PERIPHERAL NERVE PATHWAY MEASUREMENTS

	greater trochanter to lateral malleolus (cm)	T12/L1 vertebral joint to popliteal fossa (cm)	base of femoral Δ to T12 (cm)	neck of fibula to popliteal fossa (cm)
cadaver	73	62	25.5	6
skeleton 1	72	69	26	
skeleton 2	82	73	27	
skeleton 3	81	74	28.5	
skeleton 4	77	71	26	
skeleton 5	81	67	25	
skeleton 6	84	76	27	
skeleton 7	76	70	25	
mean	78	70	26	

femoral Δ = base of femoral triangle, point of stimulation of femoral nerve

end of the range. However, it can be seen from Table I that the measured leg length of the cadaver also lay towards the lower end of this range; therefore it was reasonable to assume that the cadaver was of a relatively short height and its position in the range was due to this and not to a systematic error in the method used to estimate the nerve lengths in the skeletons.

Hultborn and his colleagues (Hultborn et al. 1987) gave a value of 66cm for the same nerve pathway from the popliteal fossa to the soleus motoneurone pool. They did not indicate the height of the subject used and acknowledged the limited accuracy of their estimate, but the closeness of their value to the mean obtained in this study would appear to substantiate the present measurements. A difference of 4cm would lead to a difference in the latency of the H-reflex of no more than 1ms.

The difference in the length of the ipsilateral and contralateral pathways to the soleus motoneurone pool can only be estimated from current knowledge, which indicates that proprioceptive afferents do not cross the midline of the spinal cord. Contralateral projections of some primary afferents, believed to be cutaneous, have been found in some species of mammals, but were rarely seen in the lumbo-sacral region (Culberson et al. 1979). Consequently the shortest possible crossed pathway must contain at least one extra commissural interneurone which projects directly to the motoneurone pool and would therefore include one extra synapse. Longer pathways might include propriospinal neurones projecting, for instance, several segments upwards to a population of interneurones, the equivalent of the midlumbar interneurones of the cat - where integration of interlimb activity may occur - and then back again to the soleus motoneurone pool. In this case more time would be required for the intraspinal journey and probably for yet another synapse at least.

TABLE II ESTIMATED NERVE CONDUCTION DISTANCES AND TIMES

PERIPHERAL NERVE PATHWAY AND TYPE OF AFFERENT NEURONE	ESTIMATED CONDUCTION VELOCITY (m/s)	ESTIMATED TRAVELLING DISTANCE (mm)	MINIMAL ESTIMATED TRAVELLING TIME (ms) (including 1 synapse at MN pool)
IPSILATERAL POSTERIOR TIBIAL NERVE FROM KNEE TO SOLEUS MNs		700	
FASTEST GROUP Is	64		12
CONTRALATERAL POSTERIOR TIBIAL NERVE FROM KNEE TO SOLEUS MNs		700	+ crossing spinal cord & 1 extra synapse
FASTEST GROUP Is	64		14
FASTEST GROUP IIs (& SLOWEST Is)	40		20
SLOWEST GROUP IIs	12		60
IPSILATERAL FEMORAL NERVE FROM FEMORAL Δ TO QUADRICEPS MNs		270	
FASTEST GROUP I	64		5.5
CONTRALATERAL FEMORAL NERVE FEMORAL Δ TO QUADRICEPS MNs		270	+ crossing spinal cord & 1 extra synapse
FASTEST GROUP I	64		7.5
FASTEST GROUP II (& SLOWEST I)	40		9.5
SLOWEST GROUP II	12		24.5
CONTRALATERAL FEMORAL NERVE FROM FEMORAL Δ TO SOLEUS MNs		290	+ crossing spinal cord & 1 extra synapse
FASTEST GROUP I	64		8
FASTEST GROUP II (& SLOWEST I)	40		10
SLOWEST GROUP II	12		26
CONTRALATERAL COMMON PERONEAL NERVE FROM NECK OF FIBULA TO SOLEUS MNs		760	+ crossing spinal cord & 1 extra synapse
FASTEST GROUP I	64		14.5
FASTEST GROUP II (& SLOWEST I)	40		22
SLOWEST GROUP II	12		65

5.4b CONDUCTION VELOCITY

A number of authors have calculated the conduction velocity of afferents in the human posterior tibial nerve. Magladery and McDougal (1950), using two different stimulation sites in the popliteal fossa and examining the changes in the latencies of the M and H responses in four individuals, described the highest conduction velocity of the afferents producing the H-reflex as 60 metres per second (m/s), but they acknowledged limitations in their method and considerable variability in their results.

In 1983 Burke, Gandevia and McKeon used microelectrodes to record directly from the posterior tibial nerve and they too produced H-reflexes using two different stimulation sites in four individuals and used the differences in distance and latency to calculate conduction velocity. The fastest group one afferents were found to conduct at between 62 and 67 metres per second and the slowest at 36 to 45 m/s, although the latter figures were perceived as an underestimate, because they were calculated from measurements of the end rather than the onset of the potentials concerned.

Hultborn et al. (1987) used post-stimulus time histograms of the firing of individual soleus motor units to identify the latencies of facilitation following stimulation of soleus Ia fibres at two different sites. They calculated that the fastest fibres conducted at 64 m/s. Hultborn and his colleagues also made similar calculations for the femoral nerve during the same series of experiments. The values above have been used as guidelines in the present study.

Far less information is available concerning human group II afferent conduction times, but in a recent study by Nardone and Schieppati (1998)

the latencies of soleus M waves and H-reflexes were used along with the latencies of the short and medium latency responses (SLR and MLR) to perturbation in soleus and flexor digitorum brevis (FDB) to calculate estimated group II conduction velocities. They estimated the mean conduction velocity of fibres mediating the FDB MLR was 21.4m/s. They were also able to estimate the central delay involved in the ipsilateral group II pathway from FDB, which they calculated to be 6.7ms compared to the estimated central delay of the group I pathway which was 1.4ms.

These figures were compared to the estimates of fastest and slowest rates made by comparing relative rates of conduction between the groups using the detailed study of afferents in the cat by Jack (1978). Jack classified muscle afferents in hindlimb cat nerves according to their relationship with the fastest fibres in that nerve, because there was considerable variation between individual cats and between individual nerves. Group II afferents were those with a conduction velocity of less than 65% of that of the fastest fibres. Such a comparison would yield fastest conduction velocity of group II afferents to be around 40m/s and the slowest around 12m/s. The direct estimates of Nardone and Shieppati (1998) fit comfortably within this range, although the 21.4 m/s value is lower than the comparison with Jack's might have led one to expect for the fastest group II fibres. However the values are for afferents from a small foot muscle and may not represent the very fastest group II fibres, but this is the most direct estimate available and could point to previous overestimates.

Simonetta-Moreau, Marque, Marchand-Pauvert and Pierrot-Deseilligny (1999) also made estimates of maximum group II conduction velocities, from perceived ipsilateral group II excitatory effects in the human lower limb. Their estimates were close to Table II which was constructed simply to consider minimum possible arrival times using the peripheral nerve

pathway distances described above. The ranges of conduction velocities of the group I afferents in the human posterior tibial nerve was taken to be from 64 m/s to 40 m/s. The range of conduction velocities of group II afferents was taken to be between 40 m/s and 12 m/s. Because it is the arrival of conditioning volleys at the motoneurone pool that can cause changes in excitability, it is the differences in the conduction distance and conduction times of the afferent pathways which are important. An extra 2 ms was added to the travelling time for all contralateral pathways, as the estimated minimum time required to traverse the spinal cord and to include one extra synapse in the pathway. This delay time was extended for group II fibres in the discussions in the light of the findings of Nardone and Schieppati (1998).

5.4c ELECTRICAL STIMULATION OF HUMAN AFFERENTS

In animal experiments the relationships between the thresholds to electrical stimulation of the different populations of afferent fibres in peripheral nerves have been extensively studied. The similarities in the range of conduction velocities and thresholds of the Ia and Ib afferent populations in cats are well-documented (e.g. Eccles & Lundberg 1959, Matthews 1972, Jack 1978) and led to the development of methods designed to block or change the threshold of one population in order to allow selective activation (e.g. Coppin, Jack & MacLennan 1970).

It has been suggested that in some cat peripheral nerves it is possible to separate group I stimulation from that of group II fibres (see Jack 1978). In humans the Ia and Ib fibre populations have thresholds and distributions of conduction velocities which cannot be separated by selective electrical stimulation (Pierrot-Deseilligny, Morin, Bergego & Tankov 1981).

The threshold to activation which can be most easily and most reliably recorded in humans is the threshold of the largest motor axons (MT). This is probably most accurately identified by recording the intensity at which the first visible component appears above or below the baseline surface EMG at the latency of the M wave. Hultborn et al. (1987) have shown that the threshold of human Ia afferents of soleus is approximately 0.6 times that of the motor axons (which explains why it is routinely possible to obtain an H-reflex response without producing an M wave).

In the cat group II fibres are activated at intensities of between 2.5 and 5 times the Ia threshold intensity (Eccles and Lundberg 1959, Edgley and Jankowska 1987). If the relative relationship of the thresholds between the fibres is the same in humans as it is in cats, then for the group II fibres to be activated by intensities of between 2.5 and 5 times the Ia afferents threshold intensity, intensities of between 1.5 and 3 times the threshold of the motor response would be needed.

Because of the large circumference of human peripheral nerve trunks, fibres will respond to low intensity percutaneous stimulation not only because they have certain thresholds to excitation, but also in relation to where they lie in the nerve trunk. Fibres which do not lie in that section of the trunk which is adjacent to the surface electrode may require higher stimulus intensities than would be predicted in order to be activated. Gracies et al.(1994) found that Ia afferents were still being activated at 4 times MT. A major implication of such findings is that there may be greater overlap of effects from different fibres. Maximal activation of each group is likely to occur when large numbers of higher threshold fibres have already been recruited.

5.4d DURATION OF EFFECTS

The identification of afferents which could be mediating the crossed effects requires not only the calculation of the latency of the earliest possible influence of each group of afferents, but also the potential duration of effects produced by the whole population of fibres in each group.

Duration of individual EPSPs

The duration of individual group Ia EPSPs in cat motoneurones has been well-documented. In comparison with an action potential the EPSP rise time and the longer-lasting decay are extended, due to the capacitance of the cell membrane. Scott and Mendell (1976) recorded rise times of between 0.3 and 3 milliseconds in homonymous motoneurones of the cat lower limb, with the average rise time being around one millisecond. The total duration, including the decay time, was between 5 and 10 milliseconds; but in all the examples illustrated the voltage had returned close to the baseline after 5 milliseconds (ms).

Duration of composite EPSPs

One might expect that the duration of a composite Ia EPSP (produced in a motoneurone in response to activation of all or many of the Ia fibres in the nerve concerned) would be likely to be of a longer duration than individual EPSPs, because of the dispersion of the volley reaching the motoneurone. The degree of dispersion of the volley (due to the activation of fibres with different conduction velocities) will depend upon the length of the pathway. In cats, the high conduction velocities and short conduction distances mean that an electrically-induced Ia volley arriving at the motoneurones will still be relatively synchronous. But volleys in human

peripheral nerves will be more widely dispersed and in the present set of experiments the pathway of the conditioning volley - from the popliteal fossa to the soleus motoneurone pool - is a relatively long one. If the slowest human Ia afferent fibres are conducting at 40 m/s, then impulses travelling from the popliteal fossa will reach the soleus motoneurones approximately 7 ms later than impulses in the fastest fibres.

Ashby and Labelle (1977) had found rise times of composite Ia EPSPs in humans of 3.6 ms using H-reflexes in single soleus motoneurones. But Fetz and Gustaffson (1980, 1983) used a cumulative probability plot (the "cusum" of Ellaway, 1978) and demonstrated that the intracellularly recorded duration of the peak of increased firing probability of individual cat motoneurones could be used to give an accurate indirect measure of the rise time of composite postsynaptic potentials. This relationship, specifically with the rise time, could be demonstrated for composite EPSPs because they were large relative to the membrane noise and therefore the motoneurones would only discharge during the rising phase of the EPSP.

Using a similar method to interpret the post-stimulus time histograms (PSTHs) of the discharge patterns of single human soleus motoneurones Burke, Gandevia and McKeon (1983) found composite EPSPs in eight motoneurones with a mean rise time of 1.9 ms (range 1-3 ms) in response to electrical stimulation of the tibial nerve in the popliteal fossa. In a further study (Burke et al. 1984) the authors used the same method to examine composite EPSPs in forty motoneurones and found a mean rise time of 2.4 (\pm 1.4 S.D.) ms.

Miles, Turker and Le (1989), using a stimulation paradigm which controlled the prestimulus firing frequency of motor units and allowed a more direct estimate of the contour of the rising phase, found that the rise

times of composite Ia EPSPs in human soleus motoneurons were consistently 2 ms or less and hence appeared very similar to those measured in animals.

These estimated composite EPSP rise times of around 2 ms are shorter than one might anticipate, but this is probably due to a number of factors. Firstly, the motoneuron firing rate would not change until some time after the beginning of the composite rise time (Burke et al. 1984) and, at the low intensities of stimulation usually used, it is likely that only towards the peak of the rise time would the voltage change be sufficient to recruit some of the motoneurons. This would be especially relevant in the present experiments where the motoneuron pool is in a relaxed state. This may mean that the methods used above would not detect the earliest part of the rising phase. Secondly, although the calculated dispersion is of a long duration, the pattern of distribution of fibre diameters, the distribution of their thresholds and the greater effect of larger Ia afferents upon the motoneuron pool (as discussed above in chapter 3) would result in a conditioning afferent volley in which the slower fibres would be relatively small in number and in effect. Thirdly, the composite EPSP producing the increase in firing rate could be counteracted by a subsequent composite IPSP from activated Ib afferents with similar thresholds and conduction velocities. Pierrot-Deseilligny, Morin, Bergego & Tankov (1981) have shown that activity in the fastest Ib afferents can arrive at the motoneurons within 1 ms of the fastest Ia fibres.

The duration of the changes in excitability of the motoneuron pool

When the response of the motoneuron pool as a whole to an electrically induced volley is examined, then it becomes clear that in humans the dispersion of the afferent volley, combining with differences in the

responses of the individual motoneurons, may produce an effect with an extended duration.

Stimulating in the popliteal fossa, Burke et al. (1984) used conditioning stimuli just below threshold to condition soleus test H-reflexes which were just above threshold. They compared the peak to peak amplitudes of thirty to forty pairs of reflexes at small step intervals between C/T intervals of -5 and 30ms in order to detect the duration of the change of excitability. The rising phase lasted less than five milliseconds, but the exact duration could not be established because of the refractoriness of the responding afferent fibres at very short C/T intervals. However the onset of the rising phase was shown to be significant at negative conditioning intervals of -3ms. This phenomenon is presumably the result of the effects of that part of the rising phase of the composite EPSP which is normally subthreshold for motoneurone firing. The increase in excitability was shown to decline over 15 to 20ms from the peak of the rising phase.

The shape and duration of the subthreshold changes in excitability of the motoneurone pool is in keeping with what one would predict. Taking into account the dispersion of the composite volley and its characteristics (as described above in relation to Ia effects upon individual motoneurons and in detail in chapter 3) one would expect a steeply-rising increase. This would occur in response to activity in large numbers of the fastest and even larger numbers of the middle range of fibres in the early part of the volley. Although their arrival would not be synchronous in humans, all the fibres conducting between 65m/s and 51 m/s, for example, would arrive within 3ms of one another. The effect of the arrival of the smaller numbers of slower fibres - further spread in time by the differences in the responses of different sized motoneurons in the pool - would result in the slow decay of the excitation as described by Burke and his colleagues (1984).

This slow decay is an important characteristic of the effect of a conditioning stimulus upon the motoneurone pool as a whole. It can contribute to a change in the size of a test reflex by bringing more of the motoneurons to a level of excitability from which they can be brought to threshold by the test volley. But it contrasts with the effect of a test stimulus, where only those early parts of the afferent volley which arrive in time to contribute to the rise time will have an effect upon motoneurone firing.

The effects related to interposed interneurons

The shape and duration of individual EPSPs and IPSPs from interneurons interposed in the crossed pathways from group I and group II afferents will contribute in a similar way to the shape and duration of the excitability changes which a volley beginning in the contralateral group I or group II fibres would have upon the soleus motoneurone pool. The identity of such interneurons is unknown, as are the characteristics of any post-synaptic potentials which they may produce in the motoneurons.

For the purposes of simplicity a number of assumptions have been made in relation to the effects of contralateral conditioning. Firstly, it is assumed that the individual EPSPs and IPSPs produced in the motoneurons by interneurons are produced by activating similar changes in the motoneurone membrane permeability and that they will therefore have a relatively similar shape and duration to those produced by Ia afferents. (IPSPs and EPSPs produced in cat motoneurons via interneurons interposed in pathways from cutaneous afferents, for example, illustrated in Rosenberg [1970] were of a similar shape and duration). Secondly, it is assumed that the dispersion of the volley reaching the first and subsequent synapses could be echoed in the pattern of activation of the populations of

interposed interneurons. Thirdly, it is assumed that the interneurons themselves, in view of their short conduction pathways, will not contribute further to the dispersion of the volleys which activate them. Finally, it is acknowledged that at each synapse in the pathway there will be an attenuation of the initial volley.

5.4e ANALYSIS OF THE OBSERVED CROSSED EFFECTS

In table II the estimated afferent conduction time of 12ms for the test soleus H-reflex represents the time at which the test afferent volley is expected to reach the motoneurone pool. This corresponds to zero on the C/T interval scale in figure 5.4. However the reflex response of the motoneurons may occur several milliseconds after this, towards the peak of the rise time (Burke et al.1984). This could mean that contralateral volleys arriving 2 or 3ms after the test volley might conceivably influence the motoneurone pool sufficiently to affect the size of the reflex response. The conduction times of volleys travelling in the different afferent fibres in the contralateral pathway presented in table 2 have also been calculated in terms of their arrival at the motoneurone pool.

An early significant decrease in size occurs at the 5ms C/T interval in figure 5.4. It can be seen from Table 2 that the shortest possible delay time for the arrival of a contralateral group I volley would be 2ms longer than the delay time for the ipsilateral volley. Therefore when the conditioning volley commences 5ms before the test volley, it could arrive 3ms ahead and so this could clearly be a group I effect. Such an effect would be expected to peak around 5ms after it began.

The shortest possible extra time which a contralateral group II volley would need to reach the motoneurons would be 8ms; so this initial

inhibition would appear to occur too early to be the result of group II activation. But if one considers the possible “pre-firing” effect, Burke and his colleagues (1984) found evidence that Ia conditioning produced motoneurone pool excitability changes as early as 3ms before the reflex firing of the motoneurons. So it could be argued that a 40m/s group II volley, beginning 5ms before the test volley, would arrive as the motoneurons were beginning to fire in response to the test volley which had arrived 3ms earlier. However to have an influence at such a late point (which could only affect the last motoneurons to be recruited) it would need to be immediately very powerful. Several things make such an effect unlikely. Firstly, Table II represents the minimum possible theoretical delay times and the results from Nardone and Schieppati (1998) would indicate a much longer central delay and possibly a slower maximum conduction velocity for the group II fibres.

Secondly, the effect observed by Burke et al. (1984) 3ms before an increase in the motoneurone firing rate, was in response to conditioning by a homonymous group I volley in a monosynaptic pathway. This would be a potent excitatory input, little affected by dispersion or synaptic attenuation, whereas a volley from contralateral group II afferents would be more dispersed and would arrive at the motoneurone pool via at least two but probably more synapses. It is unlikely that it would immediately have such a measurable effect. Finally, if this inhibition represented the earliest part of a group two input then it should be followed by a sharp decline in the conditioned reflex size as the influence of the rest of the volley took effect, but this is not the case.

The next visible component in figure 5.4 is a plateau between the C/T intervals of 5 and 10ms. However this is quite likely to be an anomaly resulting from the pooling of the individual results. If the early inhibition

is the result of crossed group I input then such a plateau could represent the end of the effect of the composite IPSP rise time upon the motoneurone pool and the beginning of the slow decay of this effect. The early inhibition could be a crossed Ia effect and the plateau could be due to a shadowing Ib composite EPSP. Any Ib crossed effects might be excitatory or inhibitory, but Perl (1959) documented excitatory crossed effects from Ib afferents and Baxendale and Rosenberg (1977) also observed crossed Ib excitatory effects, particularly upon soleus. The effect of activity from large diameter cutaneous afferents could also modulate these early effects.

Between the C/T intervals of 10 and 15ms there is a continuing decline in the amplitude of the conditioned reflex. If the conditioning stimulus activated fibres 15ms before the test stimulus was applied, then in theory a volley in contralateral group II fibres conducting at 40m/s would reach the soleus motoneurone pool at the earliest 2ms before the test volley (allowing for a 7ms central delay). Therefore a group II composite IPSP from the very fastest fibres could influence the motoneurone pool at this point.

Despite the kink in the graph at the 25 and 30ms C/T intervals, the shape of the rest of the plot is in keeping with a gradual increase in reflex amplitude due to a slow decay of the inhibitory effects; with the reflex returning to its unconditioned size by a C/T interval of 40ms. If the decay from the peak of a composite Ia-induced change of motoneurone pool excitability has a duration of 15 to 20ms, the slow return to baseline might seem rather extended here. One would expect a longer decay from a group II volley, which would be much more dispersed, but then the return to baseline by the 40ms C/T interval might appear rather early in this light. However the slow increase in reflex amplitude could also result from the decay of more than one inhibitory source from fast-conducting fibres - such as a mixed group I effect or a combination of group I and cutaneous inhibition.

When the separate plots from each of the individual subjects in figure 5.2 are examined, the large intersubject variability can clearly be seen. But closer examination reveals that the differences arise mainly at the longer C/T intervals. In almost all of the subjects a form of early inhibition is shown; but differences occur later, particularly in the rate at which the reflex returns to its control size. A number of possible explanations are offered here.

Firstly, it is likely that some of the variability is the result of limitations in the accuracy of the experimental method, resulting in large random differences - particularly at the longer C/T intervals where, generally, measurements from fewer subjects were available. Secondly, the long experiments meant that some of the recordings were collected after long periods of restricted activity on the part of the subject and fatigue and/or restlessness in theory could have affected the excitability of neurones interposed in the conditioning pathways, which anyway would normally be subject to a variety of descending influences. Thirdly, and perhaps most importantly, the percutaneous method of stimulating human peripheral nerve trunks means that the fibres activated will depend as much on their orientation in the nerve trunk in relation to the stimulating electrode as on their threshold to stimulation (Gracies et al. 1994 and as discussed above). The exact proportion of fibres activated could be quite different from subject to subject and it is the proportion of fibres close to their threshold with a two times motor threshold stimulation which will vary most. These are the very fibres whose activation would have most influence at the longer C/T intervals, where the amount of dispersion of the afferent volley could affect decay times and long latency effects.

In conclusion, it is argued that the earliest component of the observed crossed inhibition of the soleus H-reflex is mediated by group I afferents

and the second inhibitory component, if it is not an anomaly, could be mediated by other, slightly smaller fibres, such as Ib or cutaneous afferents or possibly by large group II afferents.

6.0 THE INVESTIGATION OF PATHWAYS BETWEEN AFFERENTS OF THE FEMORAL NERVE AND THE CONTRALATERAL QUADRICEPS MOTONEURONE POOL

Animal experiments have shown that the midlumbar commissural interneurons are excited especially by afferents from the quadriceps, the proximal extensor muscles of the lower limbs (Harrison and Zytnicki 1984, Arya et al. 1991). It seems likely that afferent signals from these large extensor muscles during length changes in locomotion could play an important part in the switch from extensor to flexor activity. In the light of these observations the possible effect of activity in afferent fibres of the femoral nerve upon the contralateral quadriceps motoneurone pool was examined. The experimental procedure consisted of using the H-reflex of quadriceps to monitor changes in excitability of the quadriceps motoneurone pool in relation to electrical stimulation of the contralateral femoral nerve.

6.1 METHOD

Ten experiments were performed on nine healthy volunteers, all of whom gave their informed consent. During each experiment the effects of conditioning were monitored, as before, over a time span of fifty milliseconds, by collecting samples of conditioned and test reflexes for each of a range of randomly presented C/T intervals from 0 to 50ms.

The subjects were supported on a plinth with a moveable back support which allowed them to rest in a reclined position with additional support for the head. The angle at the hip joint was approximately 120 degrees (Guiheneuc & Ginet 1974) and the knees were fully extended or held in

approximately 10 degrees of flexion with a small roll or sandbag. But the exact posture for optimal stimulation and recording varied between individuals. The subjects were usually (see below) asked to relax and to avoid movement during each run of data collection.

6.1a STIMULATION

The apparatus used for stimulation was identical to that described above. The test stimulus was a 1ms duration and the conditioning stimulus a 0.5ms duration rectangular pulse. Stimulation of the femoral nerve was achieved by placing the cathode on the skin over the centre of the base of the femoral triangle (where the femoral nerve is close to the surface), just below where it passes under the inguinal ligament. The cathode was a 1.5cm diameter lint-covered metal hemisphere which was saline-soaked and held in place with either a bandage wrapped around the top of the thigh or by placing a sandbag over the electrode. The anode was a 6cm by 8cm flat rectangle of metal, also covered with lint, with a waterproof covering on the outer surface and placed either under the buttock (Hultborn et al.1987) or attached below the anterior superior iliac spine (Aiello et al.1983).

On the test side a moderate-sized quadriceps H-reflex - which could be both increased and decreased in amplitude - was used, usually in the presence of a small M wave. In two subjects a quadriceps H-reflex could not be obtained at rest and the subjects, therefore, performed a low level contraction during the stimulation and recording, during which an H-reflex could be elicited. They were given visual feedback to allow them to maintain a steady EMG output and periods of rest were interspersed with the periods of collection to prevent fatigue. In two of the other eight experiments the very small M wave was not consistently measurable at all the C/T intervals and in one experiment a small H-reflex could be obtained

only in the presence of a larger M wave. On the contralateral side an identical method of stimulation was used. Once an acceptable H-reflex and M wave were obtained, the cathode position was secured and the threshold for the motor response was found. Then the intensity of stimulation was increased to two times the M threshold in nine experiments and to three times the M threshold for one experiment. The test stimuli were introduced with an interstimulus interval of between 7 and 9 seconds.

As in the previous series of experiments, for each randomly presented condition/test (C/T) interval, this interval was fed into the programmer which acted as the external trigger. The programmer then introduced the appropriate delay before the activation of the test stimulus. This allowed the conditioning stimulus to be introduced at the correct interval in advance of the test stimulus. A sample of between fifty and one hundred interleaved conditioned and test (unconditioned) reflexes were collected. The C/T intervals chosen for this series of experiments were 0, 3, 5 and intervals of every further 5 ms up to 50ms.

6.1b RECORDING

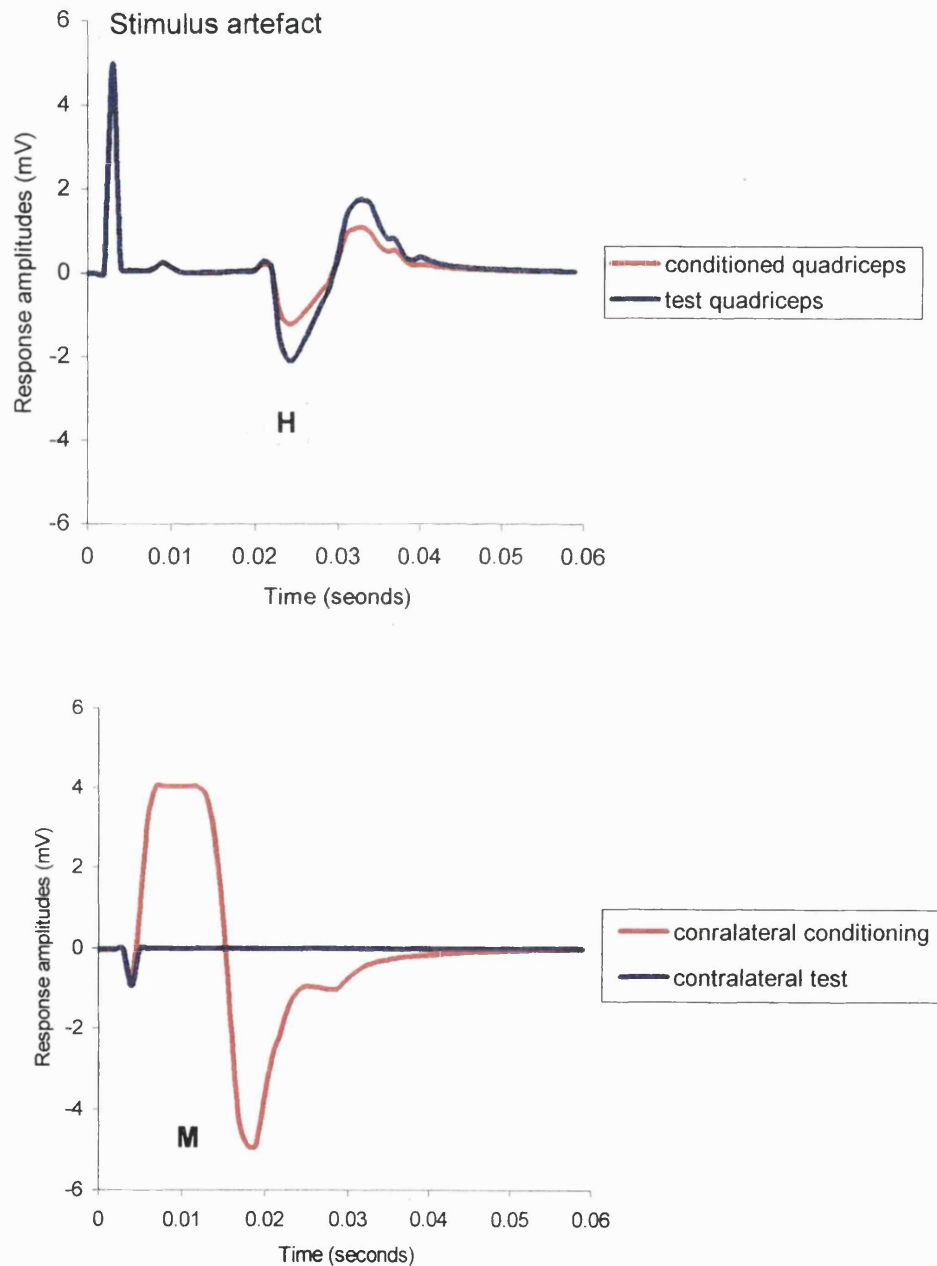
On each lower limb two disposable adhesive electrodes were positioned on the skin overlying the rectus femoris muscle. The distal electrode was placed 8cm proximal to the base of the patella (Forget et al.1989) and the second approximately 5cm more proximal. The third, (reference) electrode was positioned over the anteromedial surface of the tibia. As described earlier, the surface EMG was filtered with a low bandpass of 30Hz and a high bandpass of 500 Hz. The signal was amplified - usually between 500 and 2000 times - and displayed in 200ms sweeps, synchronised to commence with the conditioning stimulus, and stored for later analysis.

6.1c ANALYSIS

The quadriceps H-reflex appears on average between 14.5 (e.g. Guiheneuc and Ginet 1974 using rectus femoris) and 18 milliseconds (Mongia 1972 using vastus medialis) depending upon the muscle from which the recording is taken and the position of the recording electrodes. Consequently the time interval between the M wave and the H-reflex is much shorter than that between the two responses of soleus. The interval was found to be on average around 14ms for vastus medialis (Aiello et al. 1983) but around 9.5ms for rectus femoris (Guiheneuc and Ginet 1974).

Both the quadriceps responses tend to have a longer duration than those of soleus. This would be expected in the light of the much greater size of the proximal muscle and thus the increased distance of some of the activated muscle fibres from the recording electrode, also the larger range of motoneurone sizes in rectus femoris would result in an extended duration of the rise time of increased excitability over the motoneurone pool. As a result the quadriceps H-reflex can sometimes occur before the EMG has returned to baseline following the M wave response and the earliest section of the reflex may be superimposed upon the latest part of the M wave. In the present study with most subjects a very small M wave was used. Figure 6.1 shows superimposed recordings of the H-reflex and a threshold M wave from the rectus femoris on the test side in the top trace and from the contralateral rectus femoris on the conditioning side in the lower trace. In some subjects the M wave was larger and the extended responses consisted of a number of phases, as in figure 6.2, in which the original recorded response is shown in the top trace and the full wave rectified response is shown in the lower trace. When recordings such as those in 6.2 were used, the subject was asked to contract the quadriceps before and after the run of data collection, so that the activity-enhanced H-reflex could be more

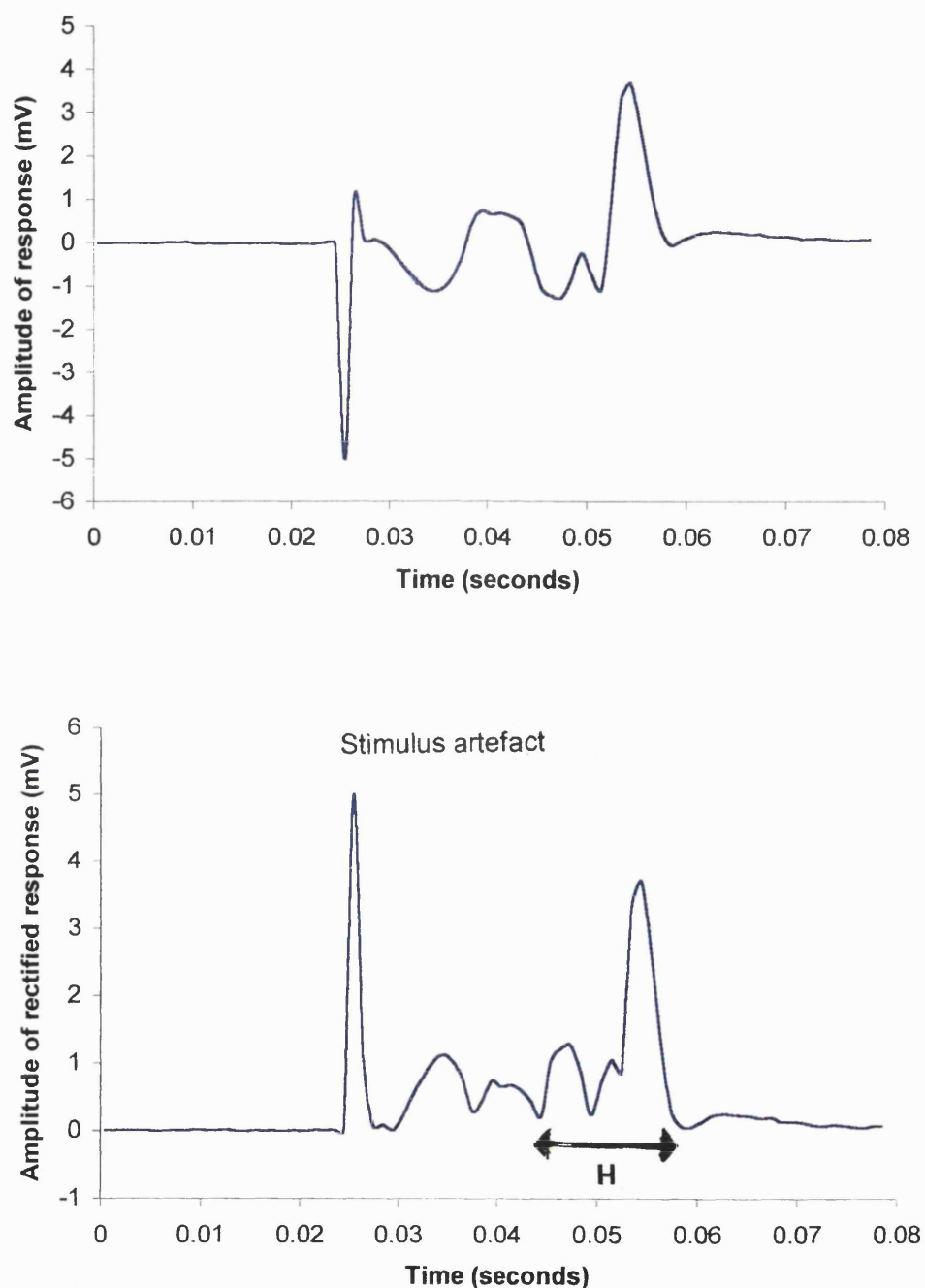
FIGURE 6.1 THE EFFECT OF CONDITIONING BY STIMULATION OF THE CONTRALATERAL FEMORAL NERVE UPON THE QUADRICEPS H-REFLEX - EXAMPLES OF RECORDINGS



Averages of responses from simultaneous recordings from left rectus femoris - test side - top trace) and right rectus femoris (conditioning side - lower trace). Traces represent approximately 40 test responses (blue) and approximately 40 conditioned responses (red). In this case the test M wave was very small.

Conditioning stimulus at zero. Test stimulus at 3ms
Intensity of conditioning stimulus = 2x M threshold

FIGURE 6.2 AN M WAVE AND AN H-REFLEX RECORDED FROM QUADRICEPS - UNRECTIFIED AND RECTIFIED



Top trace - unrectified response of quadriceps

Lower trace - rectified response

The M-wave and the H-reflex of quadriceps are very close together and occasionally overlap. To ensure the magnitude of the reflex response was correctly measured, both the peak to peak amplitudes of the original traces and the area under the curve of the rectified response were measured.

securely identified. Peak to peak measurements were taken of the identified reflexes, but in order to ensure that the whole reflex response was used, all the reflexes were later remeasured. The responses were full wave rectified and the area under the curve was measured for each response. For each experiment the peak to peak amplitudes and the area under the rectified curve of the individual H-reflexes from each C/T interval were measured from the stored recordings. The measurements were transferred to microsoft excel files for analysis.

As described above, for each C/T interval the amplitudes of the individual M waves were plotted against time to check the stability of the stimulating arrangement. Where the variability of the M wave was unacceptably large the results were discarded. The means and the variability were calculated for all four samples. The two-tailed unpaired Student's t-test was used to compare the samples of test and conditioned H-reflex amplitudes of 50 to 100 reflexes and the test and conditioned M wave amplitude samples. Provided the mean M waves from the test and conditioned samples matched, the sample was used.

In the two experiments where the M waves were too small to be reliably measured, the amplitudes of the two samples of H-reflexes for each C/T interval were plotted against time and the two individual reflexes with the greatest amplitude and the two with the lowest amplitude were removed from each sample. This method was originally suggested by Hugon (1973) reduce the effect of outliers. With the very large samples collected it was assumed that this would not affect the underlying pattern of distribution of the individual measurements. They were then compared as above.

In the two experiments in which the subjects maintained an underlying quadriceps contraction, both the M wave amplitudes and the H-reflex

amplitudes were so variable that it was impossible to detect any conditioning-induced changes against the background noise. Consequently these two experiments have not been used in the analysis of the results.

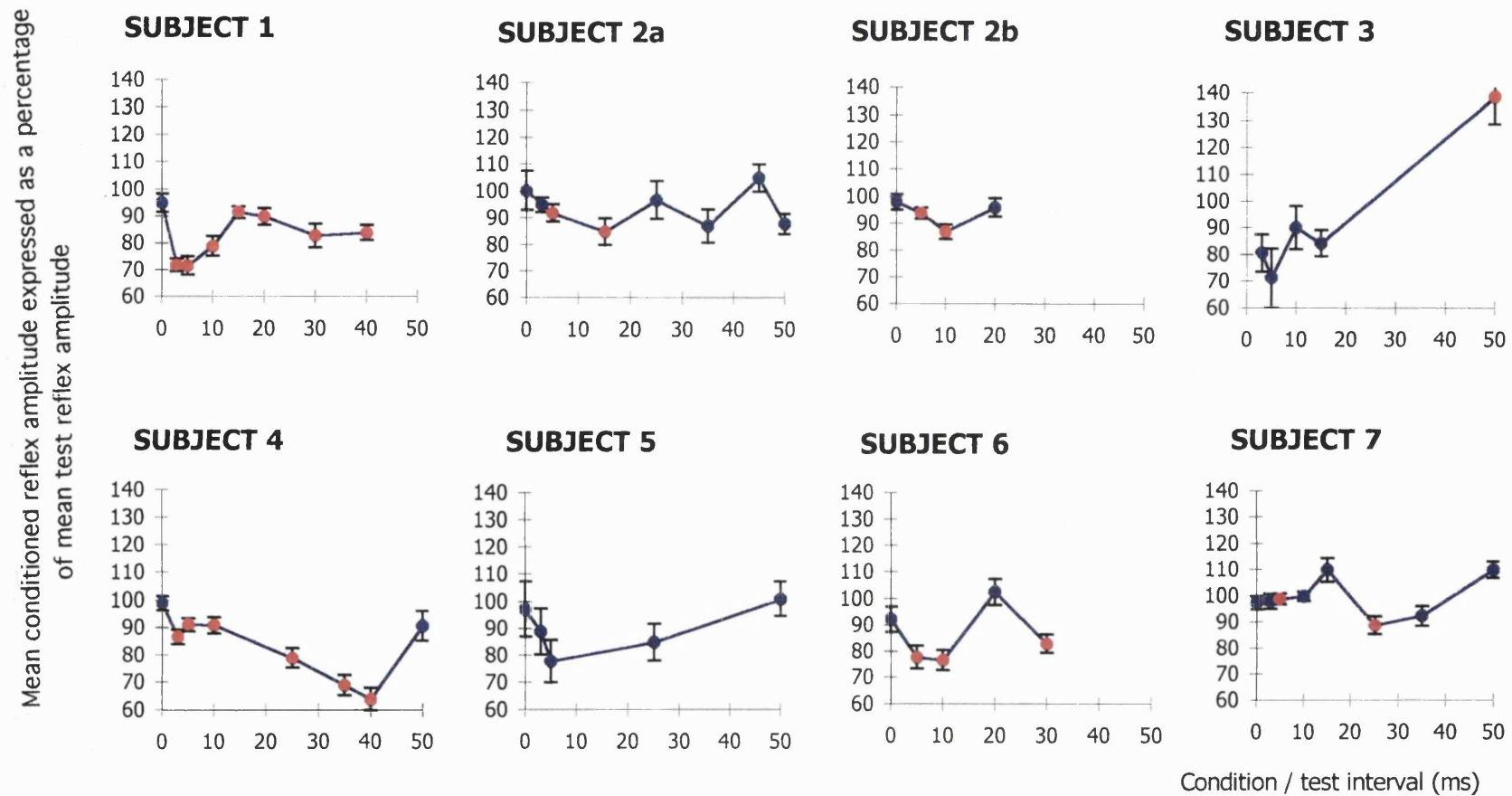
6.2 RESULTS

In all the results from the individual experiments, for each C/T interval the mean conditioned reflex amplitude was expressed as a percentage of the mean test reflex amplitude so that the results from all eight experiments could be compared and pooled. As in the previous series of experiments the peak to peak amplitude results from the individual experiments were then plotted individually in figure 6.3. Once again there was considerable variation between subjects, but in seven of the eight experiments there was a visible early inhibition.

For each of the C/T intervals in which responses had been collected from sufficient numbers of subjects the original mean reflex amplitudes from the test reflex and conditioned reflex samples were collected from each experiment and analysed using the Wilcoxon's Signed Rank test. A statistically significant inhibition ($p < 0.05$) was found at 3 and 5ms.

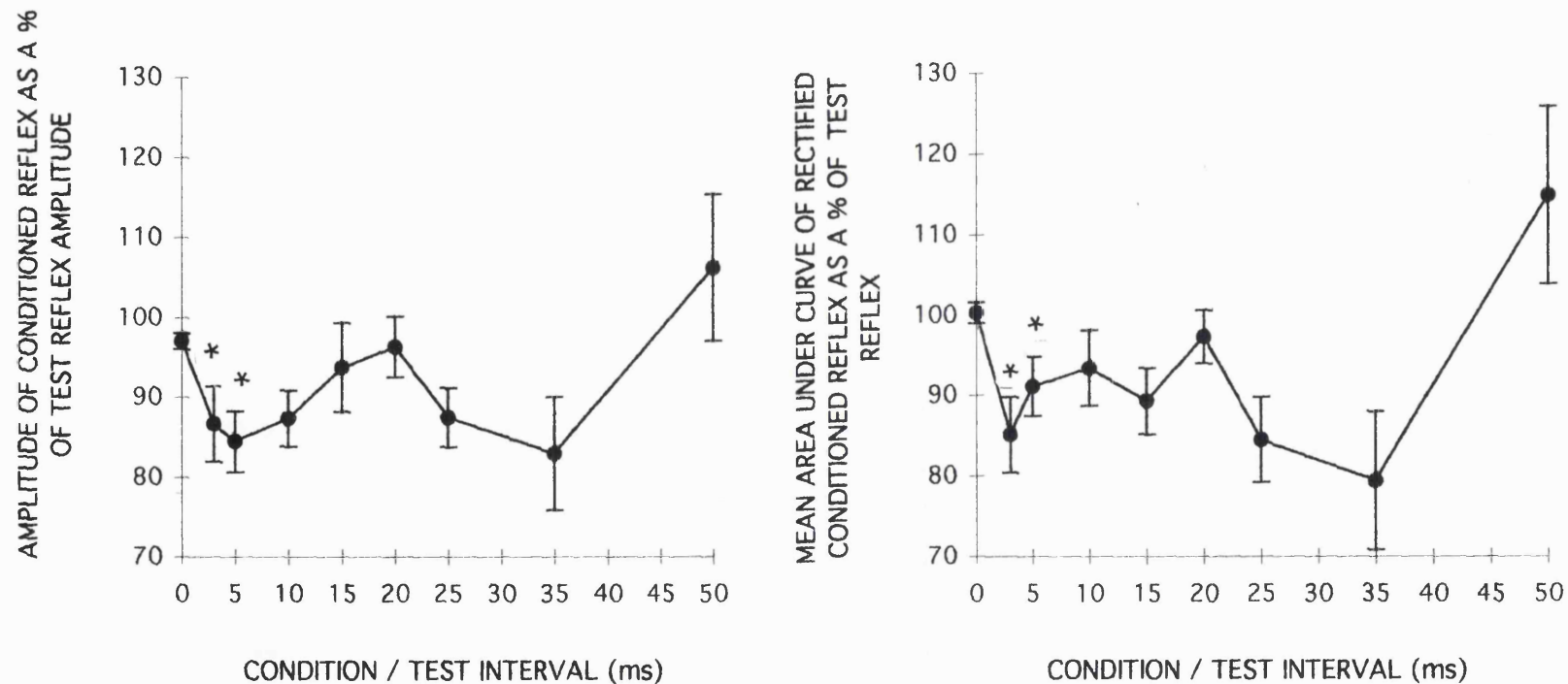
The mean amplitudes of the conditioned reflexes from the pooled results were also expressed as a percentage of the mean amplitudes of the pooled test reflexes and these were plotted against the C/T intervals concerned in figure 6.4. In this figure the pooled results from the area measurements are also illustrated and both plots show an early inhibition, followed by a return towards baseline and then what could be a further inhibition.

FIGURE 6.3 THE EFFECT OF A CONDITIONING STIMULUS APPLIED TO THE CONTRALATERAL FEMORAL NERVE UPON THE QUADRICEPS H-REFLEX



Results from individual subjects. For each condition / test interval 25 - 50 test and 25 -50 conditioned reflexes were collected from each subject. Error bars = SEM. Red points represent statistically significant differences.

FIGURE 6.4 THE EFFECT OF A CONDITIONING STIMULUS APPLIED TO THE CONTRALATERAL FEMORAL NERVE UPON THE QUADRICEPS H-REFLEX - USING TWO DIFFERENT MEASUREMENT STRATEGIES



Pooled mean amplitudes from all subjects.

Error bars = SEM

* = statistically significant differences

6.3 DISCUSSION

In order to interpret the observed crossed effects use will be made of the combination of evidence concerning conduction distances and human afferent conduction velocities discussed above in chapter 5 and used to calculate the estimated minimal travelling times in table II. Reference will also be made to the information concerning the duration of effects and the electrical activation of human nerve fibres, also discussed in detail in chapter 5.

ANALYSIS OF THE OBSERVED CROSSED EFFECTS

The estimated minimum afferent conduction time for the quadriceps H-reflex from table II is 5.5ms. This represents the arrival time of the earliest part of the Ia volley at the quadriceps motoneurone pool. Table II also indicates that a group I volley from the contralateral femoral nerve stimulation site would take a minimum of 7.5ms to reach the motoneurones.

At the C/T interval of 0ms, when the ipsilateral and contralateral sites would be stimulated simultaneously, the pooled results from the peak to peak measurements in figure 6.4 show a small (3%) inhibition, however this is not apparent in the pooled results from the measured areas. By the 3ms C/T interval there is a distinct and significant decrease in the size of the reflex. Action potentials in the largest group I fibres, resulting from stimulation of the contralateral femoral nerve 3ms before the application of the test stimulus, could arrive at the motoneurone pool 1ms before and action potentials in the slowest contralateral group I fibres would arrive 1ms after the arrival of the earliest part of the test volley.

However the arrival of a relatively synchronous group I volley would be almost complete at this interval and this would seem to be the most likely cause of the observed inhibition at this interval.

At the 5ms C/T interval the inhibition has been maintained. This could be due to the effect of the rise time of IPSPs produced by action potentials in the slowest-conducting group I fibres. With a 5ms delay in the activation of the test stimulus compared with the activation of the conditioning stimulus, these last action potentials would arrive 1ms before the test volley, and their effect upon the motoneurone pool would peak 1 to 2 ms after the arrival of the test volley (before the peak of the composite Ia test volley).

With the short afferent conduction distance in this pathway, action potentials travelling in the fastest group II fibres, conducting at around 40 m/s, could in theory arrive 1ms before the test volley. But such a scenario would not allow for the likely central delay identified by Nardone and Schieppati (1998) at around 7ms for an ipsilateral pathway.

At the 10ms interval the inhibition is still statistically significant, but it appears to have peaked. The slowest component of a contralateral group I volley, activated 10ms before the test volley, would have arrived at the motoneurone pool 5 or 6ms before the test Ia volley (if the predicted pathway is assumed to contain 1 or 2 extra synapses). Under these circumstances the contralateral group one effect, (which would probably be peaking by the arrival of the slowest part of the volley), would be diminishing at this interval.

Between the 10ms and 20ms C/T intervals there is steadily reducing inhibition, the time sequence of which would be appropriate for a rather slow decay of the group I effect, but it could also be the result of a

combination of effects from more than one type of fast conducting fibre, such as a short lasting Ia inhibition combined with a slower reduction mediated by cutaneous afferents.

At the 25ms C/T interval there is a further reduction in the conditioned reflex size. A volley which had been activated in group II afferents (conducting at 21 m/s and with a central delay of approximately 7ms, as estimated by Nardone and Schieppati 1998) 20ms before the test volley would arrive at the motoneurone pool approximately simultaneously with the test volley and could therefore contribute to this apparent late inhibition, appearing after the 20ms C/T interval. It could also be due to a longer-lasting effect, such as presynaptic inhibition. However the character of this late effect cannot be properly examined because at 30, 40 and 45ms C/T intervals there are too few measurements; but the mean amplitude at the 35 ms C/T interval confirms this.

Finally, at the 50ms C/T interval, the reflex has returned to the test size and, indeed, may be subject to a facilitation. Three of the five individual results at this interval show a substantial increase in the reflex size, although only one increase is statistically significant and the pooled results are not.

As in the previous series of experiments the individual results from the later C/T intervals vary considerably. The problem of interpretation is increased in the present series by the small number of individual results at these late intervals, but it is reasonable to assume, as discussed in detail above in Chapter 5, that much of this variability is due to inevitable differences in the exact proportion of fibres activated in each subject by the conditioning stimulus.

In conclusion, the early inhibition of the reflex is consistent with mediation by activity in contralateral group I fibres and there may be a contribution from group II activity or a presynaptic effect producing a possible later inhibition.

7.0 THE INVESTIGATION OF PATHWAYS BETWEEN AFFERENTS OF THE FEMORAL NERVE AND THE CONTRALATERAL SOLEUS MOTONEURONE POOL

The previous two chapters dealt with sets of experiments exploring possible connections between contralateral homonymous muscles. The present chapter sets out to examine contralateral connections between heteronymous physiological extensors. The experimental procedure consisted of using the H-reflex of soleus to monitor changes in excitability of the soleus motoneurone pool in relation to electrical stimulation of the contralateral femoral nerve.

7.1 METHOD

Seven experiments were performed on seven healthy volunteers, all of whom gave their informed consent. In this particular set of experiments the conditioning afferent conduction pathway was considerably shorter than the afferent pathway of the test reflex. Hence volleys in group I afferents activated by the contralateral conditioning stimulus could reach the soleus motoneurone pool 4ms before the test volley. Therefore the effects of conditioning were monitored over a timespan of sixty milliseconds by collecting samples of conditioned and test reflexes for each of a range of randomly presented C/T intervals from -10 to 50ms.

The subject reclined on a plinth which gave head and trunk support. One of the lower limbs was supported with the knee in semiflexion and the ankle held in a position close to plantigrade, optimal for the evocation of the soleus H-reflex (as described in detail above in chapter 4). The other lower limb was supported in an extended position (as described in detail in

chapter 5) in order to stimulate the femoral nerve. The subject's comfort was assured before the experiment commenced and it was checked regularly. If necessary the subject was given the opportunity to move and reposition between periods of data collection.

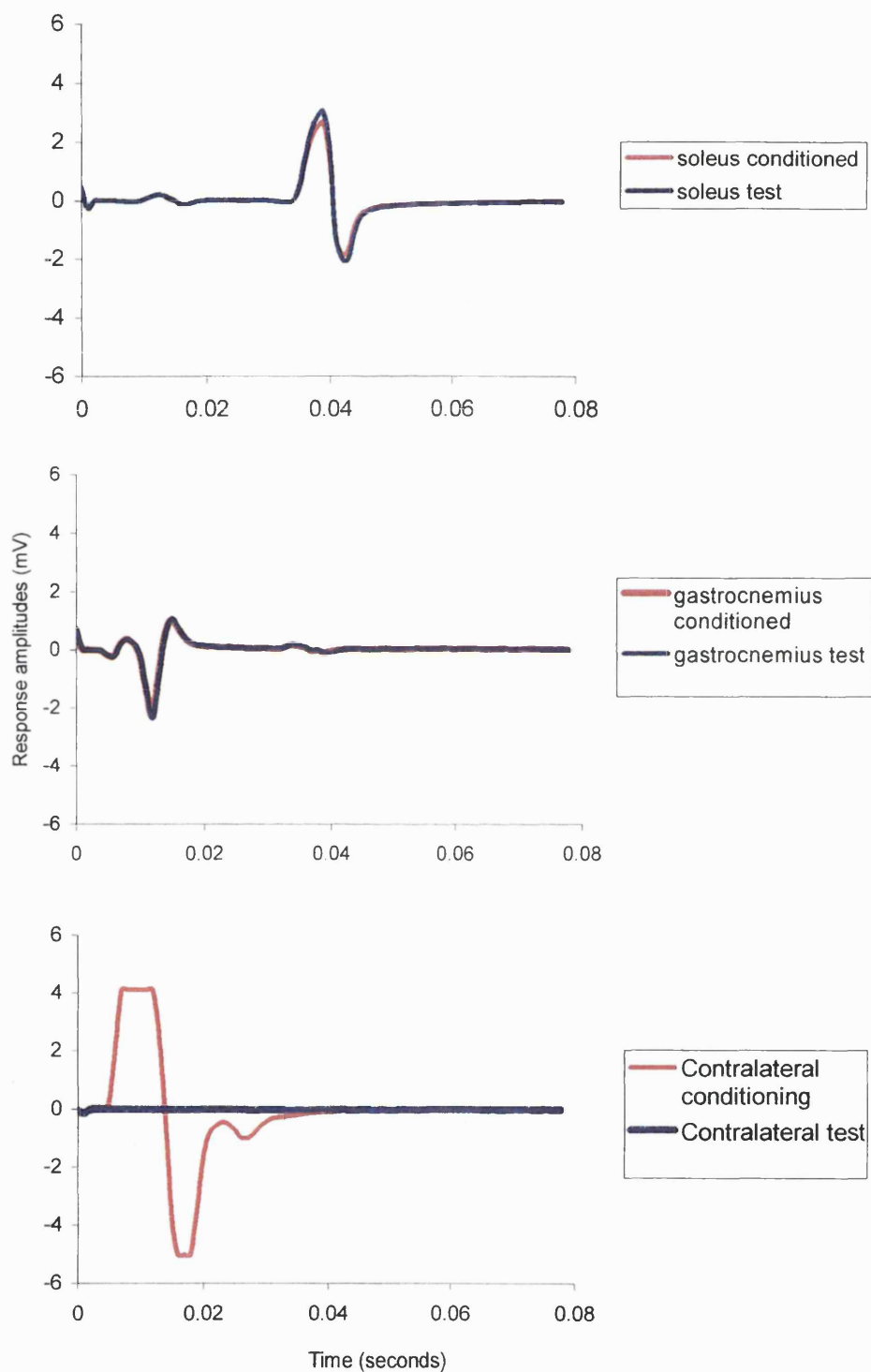
7.1a STIMULATION

The method of stimulation of the posterior tibial nerve on the test side and the femoral nerve on the conditioning side was as described in detail in chapters 4 and 6. On the test side a submaximal soleus H-reflex was used, usually in the absence of a soleus M wave. However, comparative studies of the responses of soleus and gastrocnemius (described above in chapter 4) had revealed that in most subjects it was possible to elicit an M wave from gastrocnemius well below the threshold of the soleus M. This allows the use of an optimal H-reflex size (around 60% of maximum H-reflex amplitude) whilst still monitoring peripheral changes and it reduces the problems of variability associated with using very small soleus M waves. So the gastrocnemius M wave was used to monitor the constancy of the stimulus. An example of simultaneous recordings from soleus and gastrocnemius on the test side and from the contralateral rectus femoris is shown in figure 7.1.

On the contralateral side, once suitable quadriceps M and H responses were obtained with a stable stimulating set-up, the threshold of the M wave was found and the intensity of the stimulation was increased, for the purposes of conditioning, to two times this threshold in six of the subjects and to three times the threshold in one subject.

The test stimuli were delivered every 5.636 seconds and for each C/T interval the conditioning stimuli were delivered intermittently, in a non-

FIGURE 7.1 THE EFFECT OF CONDITIONING BY STIMULATION OF THE CONTRALATERAL FEMORAL NERVE UPON THE SOLEUS H-REFLEX - EXAMPLES OF RECORDINGS



Averages of responses from simultaneous recordings from left soleus muscle (top trace) left gastrocnemius (middle trace) and right rectus femoris (lower trace) Traces represent approximately 30 test (blue) and 30 conditioned (red) responses. C/T interval = 0ms

uniform manner, at the preset interval, so that test and conditioned reflexes were interleaved. Sixty reflexes were collected at each C/T interval.

7.1b RECORDING

On the test side two adhesive recording electrodes were placed on the skin overlying the soleus muscle and a reference electrode was attached over the anteromedial surface of the tibia as described in the experiment on H-reflex variability in chapter 3. Additionally, two recording electrodes were placed over gastrocnemius with a reference electrode again over the anteromedial surface of the tibia. On the conditioning side two recording electrodes were attached over rectus femoris, proximal to the base of the patella (as described earlier) and a reference electrode was placed over the surface of the tibia.

Surface EMG recordings from all three muscles were filtered (low band pass 30Hz, high band pass 500Hz), amplified (usually between 500 and 2000 times) and fed into the C.E.D. 1401 to be displayed on-line and stored as three separate channels.

7.1c ANALYSIS

For each C/T interval of each experiment the peak to peak measurements of the individual M and H responses were measured from the stored recordings and transferred to microsoft excel files for analysis.

The amplitudes of the M waves were plotted as described earlier. The means and variability of the two groups of M responses for each C/T interval were compared to ensure similarity of the two groups and provided they matched the samples of conditioned and unconditioned

reflexes were compared, as before, using the two-tailed unpaired Student's t-test.

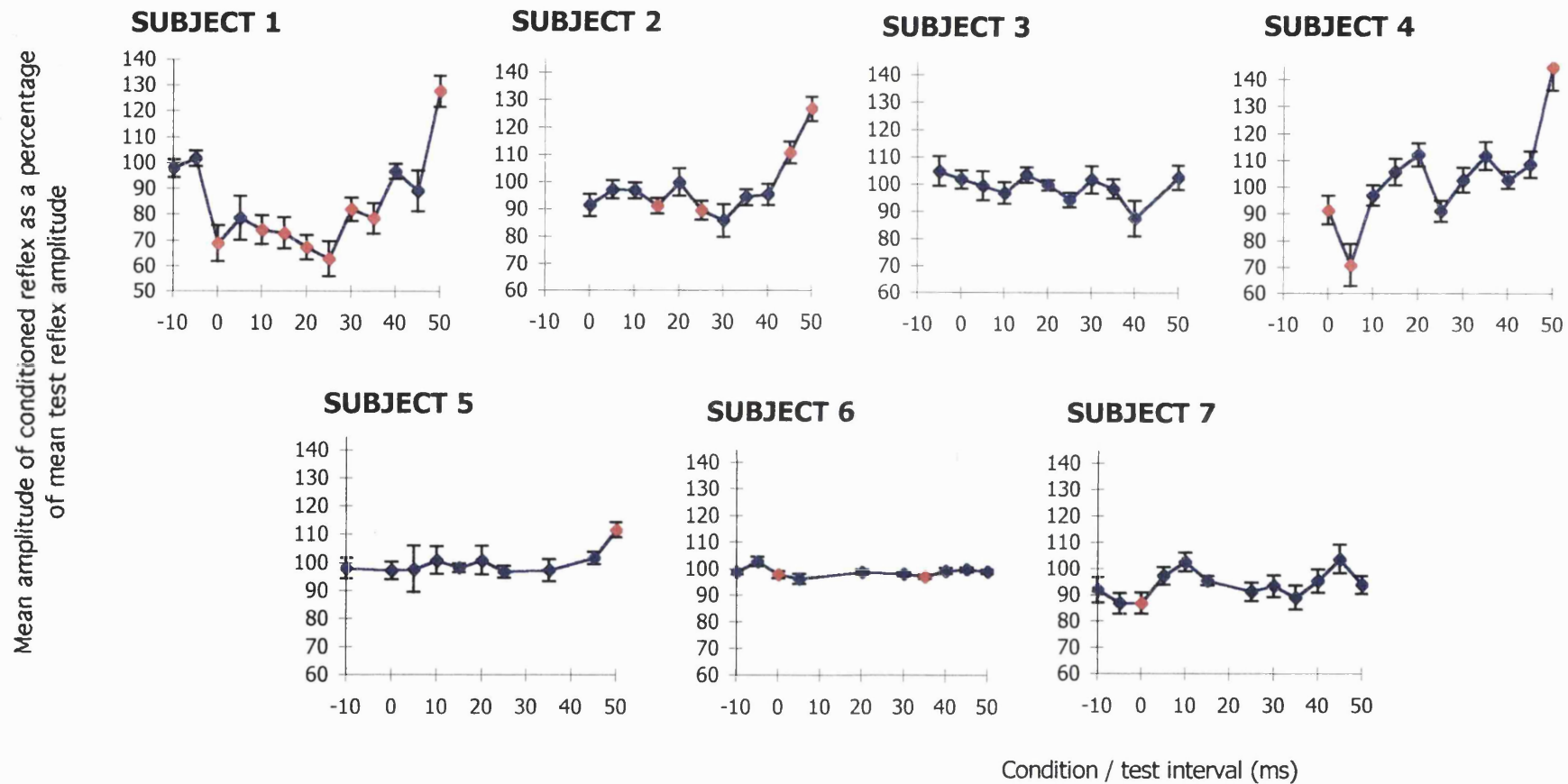
7.2 RESULTS

As described in the earlier series of experiments, the mean conditioned reflex amplitude from each C/T interval was expressed as a percentage of the equivalent mean test reflex amplitude, so that the results from the individual experiments could be pooled and compared. The percentage reflex amplitudes from each of the experiments were plotted against the C/T intervals, separately, as illustrated in figure 7.2.

Examination of figure 7.2 reveals that in this series of experiments three of the seven subjects show very little evidence of any effects of conditioning stimulation and two subjects show a pronounced and long-lasting inhibition.

In six of the seven experiments the 0ms C/T interval was used twice. Of the thirteen pairs of matched mean amplitudes, five showed a statistically significant inhibition of the conditioned reflex ($p < 0.05$, all significant changes referred to below will be at the $p < 0.05$ level). When two samples were taken the value shown in the plots is the average of the two sample means.

FIGURE 7.2 THE EFFECT OF A CONDITIONING STIMULUS APPLIED TO THE CONTRALATERAL FEMORAL NERVE UPON THE SOLEUS H-REFLEX



Results from individual subjects . For each condition test / interval 25 - 50 test and 25-50 conditioned reflexes were collected from each subject. Error bars = SEM. Red points represent statistically significant differences.

For each of the C/T intervals the original mean reflex amplitudes from the test reflex and conditioned reflex samples were collected from each experiment and analysed using the Wilcoxon's Signed Rank test. A statistically significant inhibition ($p < 0.05$) was found at 0ms.

The mean amplitudes of the conditioned reflexes from the pooled results were also expressed as a percentage of the mean amplitudes of the pooled test reflexes and these were plotted against the C/T intervals in figure 7.3. Where more than one sample had been measured at the 0ms C/T interval, the average of the two results was used in the plot. Figure 7.3 shows an early inhibition, followed by a return towards baseline, then a possible further inhibition and finally a late facilitation.

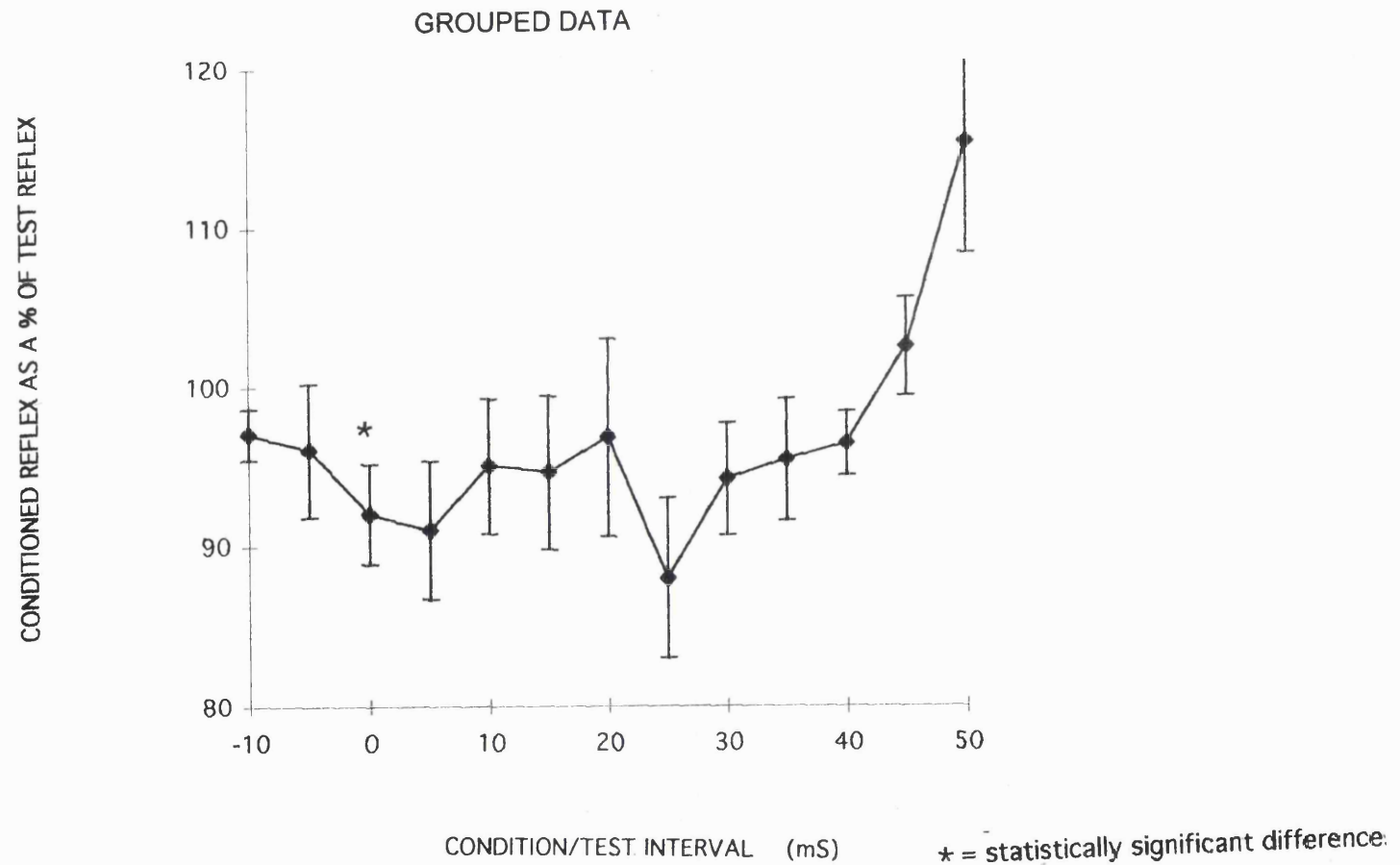
DISCUSSION

In order to interpret the observed crossed effects, the evidence concerning conduction distances and human afferent conduction velocities discussed above in chapter 5 and the estimated travelling times in table II (page 149) will be used, as well as the information concerning the duration of effects and the electrical activation of human nerve fibres, also discussed in detail in chapter 5.

ANALYSIS OF THE OBSERVED CROSSED EFFECTS

The main difference between this series of experiments and those described earlier is that in the present study the afferent conduction distance of the conditioning volley is considerably shorter than the afferent conduction distance of the test volley.

Figure 7.3 THE EFFECT OF CONDITIONING OF THE
SOLEUS H REFLEX FROM STIMULATION OF THE
CONTRALATERAL FEMORAL NERVE



In each series of experiments an accurate estimate of the actual distance travelled by the conditioning volley is important for the prediction of the arrival times of action potentials travelling in different diameter fibres at the appropriate motoneurone pool. This is because of the effect of distance upon the degree of dispersion of the volley in fibres conducting at different velocities. But in the present series of experiments, the accurate assessment of the difference between the two distances is also of great importance, so that the arrival times at the soleus motoneurone pool of action potentials in the different fibres of the conditioning pathway can be predicted in relation to the arrival there of Ia activity in the longer test pathway.

On examination of table II (page 149) it can be seen that the estimated distance (made from the series of measurements described earlier and illustrated in table I) from the stimulation site in the popliteal fossa to the ipsilateral soleus motoneurone pool is 700mm and the estimated distance from the stimulation site in the femoral triangle to the soleus motoneurone pool is 290mm (plus distance to traverse the spinal cord to reach the contralateral pool). The former measurement is very close to (40mm greater than) the estimate of Hultborn et al. (1987) of the same pathway and the latter measurement is just 20mm more than the estimate of that pathway by the same authors. Discounting, at present, the extra distance required to cross the cord, the estimated difference in length in the present study between the two afferent pathways is 410mm - just 20mm more than that estimated by Hultborn and his colleagues. This gives some confidence in the relative accuracy of the present estimate. The extra time to traverse the cord and to cross the minimum of one extra synapse are included in the estimate of the total travelling time required in table II.

Table II shows that the Ia test volley is expected to reach the soleus MNs 12ms after delivery of the stimulus. Action potentials in the fastest

conducting contralateral fibres are expected to arrive 8ms after delivery of the conditioning stimulus. So that if the two stimuli were delivered at the same time, the conditioning volley in the fastest contralateral fibres would arrive at the motoneurone pool 4ms ahead of the test volley. So if the delivery of the conditioning stimulus is postponed for 10ms, then the conditioning primary afferent volley will arrive around 6ms after the test volley - by which time the test volley will have begun to cause the motoneurons to fire. The small reduction in size of the mean conditioned reflex seen at this interval could be due to conditioning.

If the conditioning stimulus is delivered 5ms after the test stimulus, (-5ms C/T interval) then activity in the fastest contralateral primary afferents could arrive at the soleus MNs just 1ms behind the earliest part of the test volley and could have an effect during the period of rising excitability of the motoneurone pool which the test volley has caused and which will result in motoneurone firing

There are a number of possible explanations for the absence of a significant effect at this interval. Firstly, it could be that a crossed connection between primary afferents from quadriceps and soleus MNs is either absent or weak. Secondly, the one extra synapse could be an underestimate and more synapses would mean that the conditioning volley would arrive later in relation to the test volley. Thirdly, the margin for error in the measurements of the two afferent distances could be such that the true afferent travelling time was up to 1ms longer than the estimated time. If either the second or the third (or both) scenarios were true then the conditioning volley could arrive just too late to have a measurable effect at this interval.

When the test and conditioning stimuli are delivered at the same time, action potentials in the fastest-conducting contralateral afferents could arrive 4ms before the earliest of the test action potentials and could certainly be responsible for the small, but statistically significant inhibition seen in figure 7.3. However, at this 0ms C/T interval action potentials in the fastest conducting (40m/sec) contralateral group II fibres could, in theory, also arrive at the MNs ahead of the test volley - as early as 2ms before. But if the Nardone and Schieppati (1998) estimate is used, with a conduction velocity of 21.4 m/s and a central delay of at least 7 ms, the contralateral group II volley would arrive around 8ms after the ipsilateral test volley and so are unlikely to contribute to it.

The inhibition, visible at the 25ms C/T interval - and appearing to extend until the 40ms interval - would appear to be caused by either the effect of activity in contralateral group II fibres conducting at 20m/sec or less with a long central delay, or as the result of a slow, presynaptic mechanism activated by larger diameter afferents. The latter mechanism will be discussed below in relation to the crossed effects from all the experiments.

Finally, at the 50ms C/T interval, there is a pronounced facilitation which, although statistical significance could not be demonstrated in the pooled results, was significant in four of the seven individual results. This late facilitation is echoed in results from some subjects throughout the different series of experiments on crossed effects. The lack of statistical significance here could be due partly to the small numbers of samples taken. This late facilitation is unlikely to be the effect of activity in very small contralateral fibres, in view of the stimulus intensity used. It could represent group II activity with a long central delay or be due to a presynaptic mechanism.

The interpretation of the effects seen in this series of experiments is made with some caution. The effects seen are smaller than those observed in the two studies, described in chapters 5 and 6 and point to weaker crossed effects here from group I and possibly group II fibres. The effects are small despite the fact that the use of the gastrocnemius M wave here allowed the use of a smaller reflex, optimum conditions for revealing the effect of conditioning stimulation (Crone et al. 1990).

It is likely that the small, but significant inhibition seen at the 0ms C/T interval is the result of group I activity. A group II contribution cannot be completely excluded, because in theory a group II conditioning volley, activated at the same time as the test volley, could arrive at the motoneurone pool 2ms before the test volley. But in the light of the evidence indicating a longer central delay for group II effects, the contribution of group II fibres seems unlikely.

8.0 THE INVESTIGATION OF PATHWAYS BETWEEN AFFERENTS OF THE DEEP PERONEAL NERVE AND THE CONTRALATERAL SOLEUS MOTONEURONE POOL

Having investigated crossed effects between homonymous muscles and heteronymous extensor muscles, it seemed possible that crossed effects between antagonist muscles might present a different pattern. Tibialis anterior is a physiological flexor, however it is also an important postural muscle and the cocontractions of soleus and tibialis anterior play a major role in balance in standing.

The experimental procedure consisted of using the H-reflex of soleus to monitor changes in excitability of the soleus motoneurone pool following electrical stimulation of the contralateral deep peroneal nerve.

8.1 METHOD

Eight experiments were performed on eight healthy volunteers, all of whom gave their informed consent. During each experiment mixed samples of test and conditioned reflexes were collected for each of a range of condition/test intervals.

The subjects rested in reclined sitting with both lower limbs in a semiflexed position, supported proximally at the thigh (with the knee in approximately 70 degrees of flexion) and distally at the foot (with the ankle dorsiflexed at approximately 90 degrees).

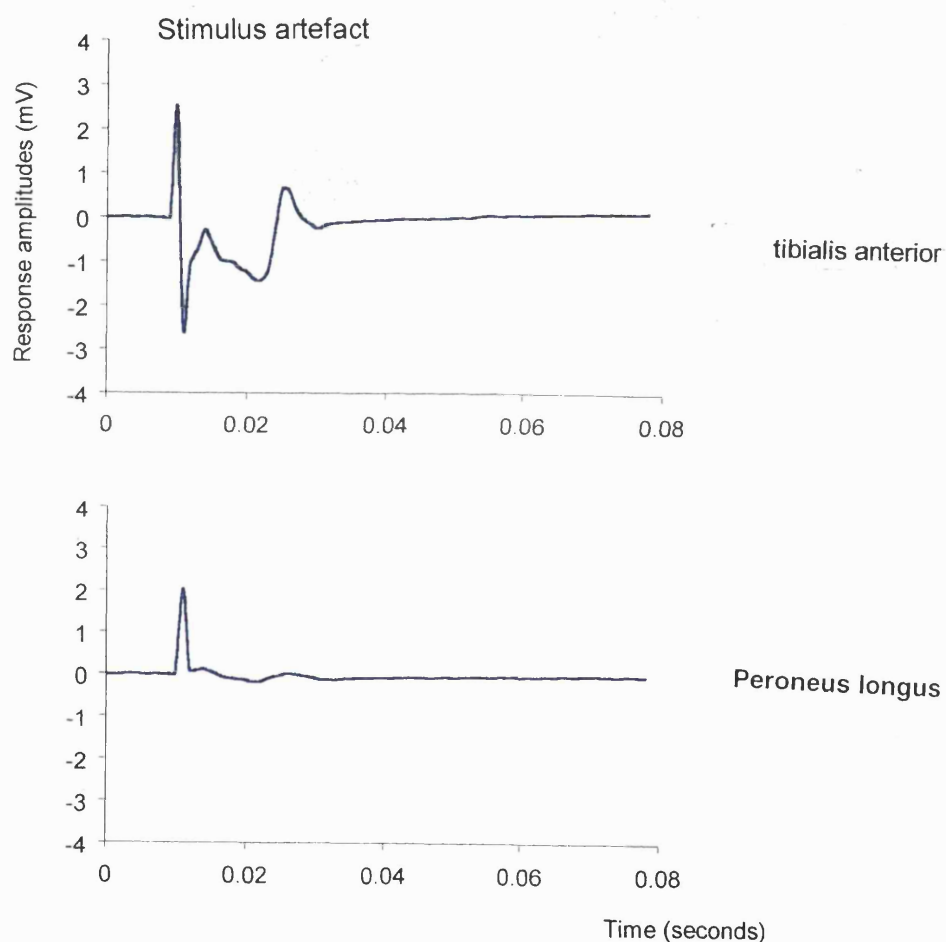
8.1a STIMULATION

The apparatus used for stimulation was as described in chapter 3 . As before, the test stimulus was a 1ms duration rectangular pulse and the conditioning stimulus was a 0.5ms rectangular pulse. Stimulation of the tibial nerve, and the use of an H-reflex approximately 60% of H Max on the test side, were as described previously. The test stimuli were introduced with an interstimulus interval of 3.636 seconds.

Stimulation of the deep peroneal nerve was usually achieved by placing a bipolar electrode horizontally over the skin with the cathode just anterior to the head of the fibula, where the nerve emerges from winding around the neck of the fibula, and divides into the deep and superficial branches. This position favoured stimulation of the deep branch supplying the anterior tibial muscles rather than the superficial branch to the peronei (Pierrot-Deseilligny, Morin, Bergego & Tankov 1981). This was tested on two subjects. Recordings were made from both muscle groups in order to ensure that the deep branch was being preferentially stimulated. The M response in the peronei was absent in one and appeared at a much higher threshold than the anterior tibial response in the other. Figure 8.1 shows an example of simultaneous recordings from the tibialis anterior and peroneus longus during the delivery of a conditioning stimulus at twice the intensity of the motor threshold. It can be seen that there is just a small disturbance of the baseline in the recording from peroneus longus.

The electrode consisted of two lint-covered 1cm diameter heads held in a small moulded plastic frame. This was secured in place with a bandage when the optimal position for stimulation had been found. The intensity of stimulation for conditioning was adjusted to two times the threshold

FIGURE 8.1 SIMULTANEOUS RECORDINGS FROM TIBIALIS ANTERIOR AND PERONEUS LONGUS ILLUSTRATING THE PREFERENTIAL STIMULATION OF THE DEEP PERONEAL NERVE



Simultaneous recordings taken from tibialis anterior (top trace) and peroneus longus (lower trace) in response to stimulation of the deep peroneal nerve at the neck of the fibula. All responses equally amplified. Stimulus given at 0ms. Stimulus artefact is from contralateral test stimulus delivered at 10ms. In the presence of a large response in tibialis anterior there is only a small change in the baseline in the recording from peroneus longus

intensity of the M response in six of the eight experiments. In the other two experiments, in one the conditioning stimulus intensity was three times the M threshold and in the other it was four times the M threshold.

For each separate sample of reflexes the C/T interval was randomly selected and the appropriate delay was then introduced between the conditioning and the test stimuli and between 70 and 100 interleaved conditioned and unconditioned reflexes were collected.

8.1b RECORDING

The recording arrangement on the test side was as described above. On the conditioning side two disposable adhesive electrodes were positioned on the skin overlying the body of the tibialis anterior muscle just lateral to the upper part of the anterior border of the tibia. The accuracy of the electrode positioning could be checked by palpating this superficial muscle and feeling it contract when the foot was dorsiflexed and inverted. The reference electrode was positioned on the skin overlying the lower half of the anteromedial surface of the tibia.

Surface EMG recordings from both sides were filtered (low band pass 30Hz, high band pass 500Hz), amplified (usually between 500 and 2000 times) and fed into the analogue digital converter (C.E.D. 1401) and displayed on-line and stored for later analysis.

8.1c ANALYSIS

Peak to peak measurements of the individual M and H responses were measured from the stored recordings and these were transferred to microsoft excel files for analysis. The M and H responses were divided into

conditioned and unconditioned samples and the two groups of M waves were compared using the two-tailed unpaired Student's t-test. Provided that the means and the variability of the two groups were similar then the two groups of corresponding H-reflexes were compared using the same test.

8.2 RESULTS

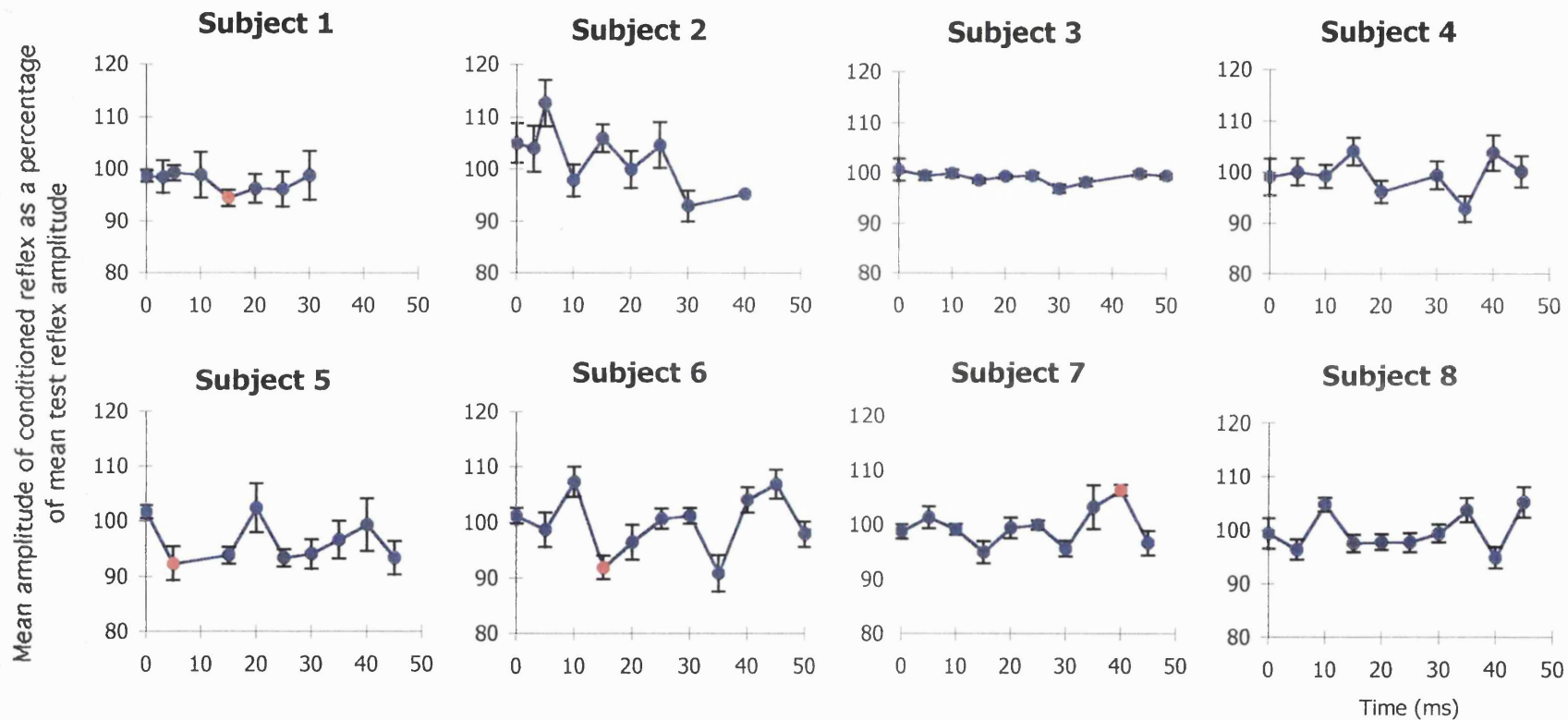
As before, in each experiment, for each C/T interval, the mean conditioned reflex amplitude was expressed as a percentage of the mean test reflex amplitude so that the results from the individual experiments could be compared. The percentage reflex amplitudes from each of the experiments were plotted against the C/T intervals in figure 8.2.

It can be seen from figure 8.2 that crossed effects from afferents in the common peroneal nerve appear to be very small under these experimental conditions. A small inhibition may be present between the 15ms and 35ms C/T intervals.

At the 0ms interval all the mean conditioned reflex amplitudes were similar to the amplitudes of their paired mean test amplitudes. At the 5ms C/T interval the mean conditioned reflex from only one of the eight subjects showed a decrease in amplitude which was significant ($p < 0.05$). Of the seven experiments in which the 10ms C/T interval was used, there were no significant differences.

At the 15ms C/T interval in two of the eight subjects the mean conditioned reflex was significantly smaller - one of these was the experiment with the conditioning intensity at 4 times the M threshold. But at the 20ms C/T interval there were no significant differences, nor at the 25ms C/T interval. Later intervals revealed no pattern of changes.

FIGURE 8.2 THE EFFECT OF A CONDITIONING STIMULUS APPLIED TO THE CONTRALATERAL DEEP PERONEAL NERVE UPON THE SOLEUS H-REFLEX



Results from individual subjects. For each condition / test interval 25-50 test and 25-50 conditioned reflexes were collected from each subject. Error bars = SEM. Red points represent significant differences.

When the individual results were pooled and analysed as described in the previous experiments, no significant differences between the test and the conditioned reflexes were revealed at any of the C/T intervals. The mean percentage changes for each C/T interval were calculated from the pooled results and plotted in figure 8.3).

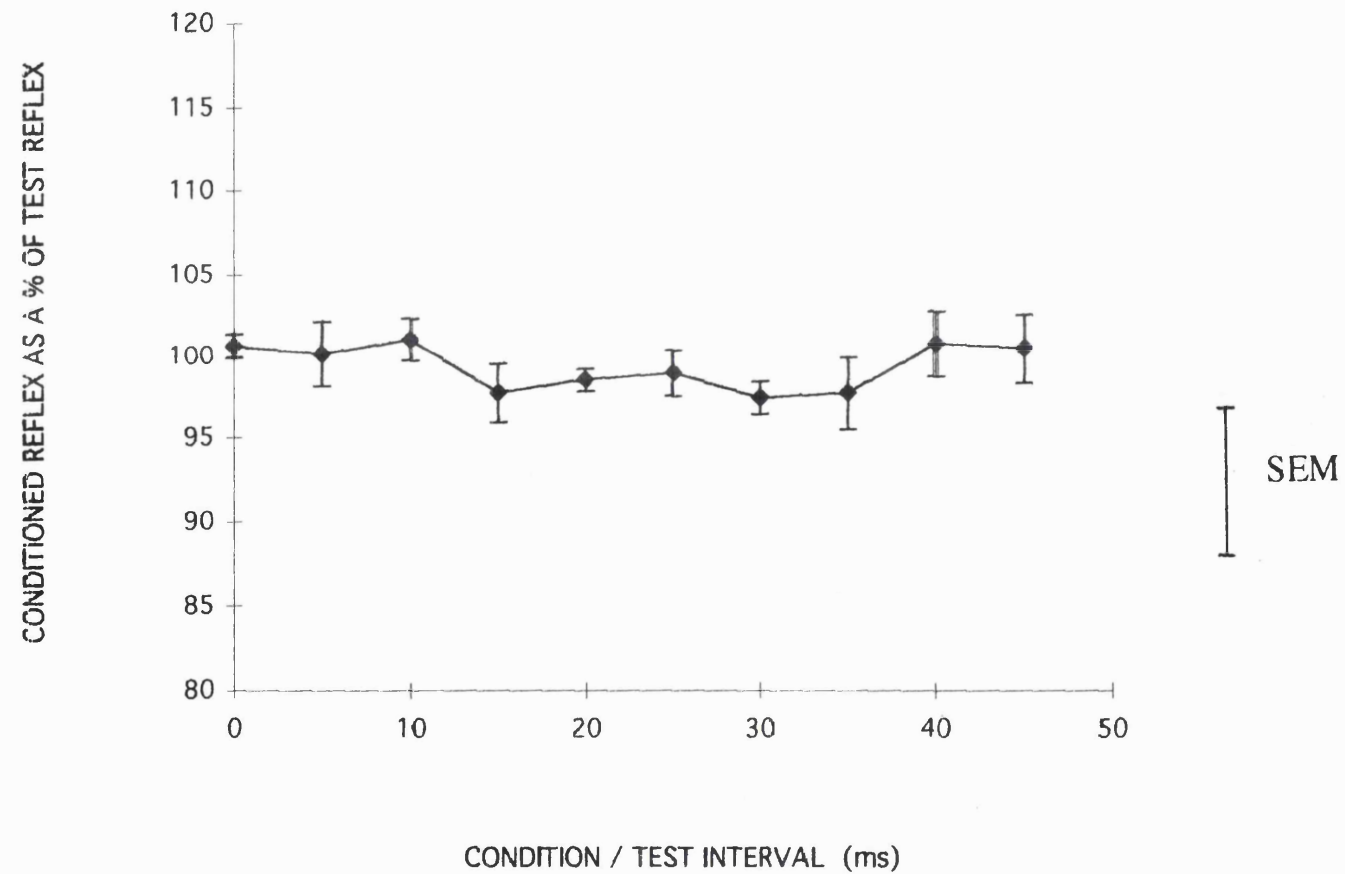
In the light of the effects observed on the application of a conditioning stimulation to the other peripheral nerves a further experiment was carried out on two subjects to examine the possibility of a weak crossed effect. The two C/T intervals of 15ms and 40ms were used and the contralateral conditioning stimulus was administered at intensities of 1, 1.5, 2, 2.5 and 3 times the M threshold. 60 sweeps were collected at each C/T interval. In the first subject, at the 15ms C/T interval, the mean amplitude of the conditioned reflex was significantly smaller than that of the mean test reflex at conditioning intensities of 2.5 and 3 times the M threshold. At the 40ms C/T interval no significant changes were seen at any intensity levels. In the second subject at the 15ms interval inhibitions were measured at 2 and 3 times the M threshold, but they were not statistically significant and no changes were seen at the 40ms interval. In summary, the results from this set of experiments do not reveal any significant changes in the H-reflex due to crossed effects.

8.3 DISCUSSION

The distance between the stimulation site at the neck of the fibula and the stimulation site in the popliteal fossa had been measured as 60mm (see table I). It can be seen from table II that, if the test and conditioning stimuli were applied at the same time, the action potentials produced in the largest diameter primary afferents of the contralateral deep peroneal nerve as a

**FIGURE 8.3 THE EFFECT OF CONDITIONING STIMULATION
APPLIED TO THE CONTRALATERAL DEEP PERONEAL NERVE
UPON THE SOLEUS H-REFLEX**

GROUPED DATA



result of the conditioning stimulation would arrive at the soleus motoneurone pool about 2.5ms after the test volley. Action potentials in large secondary afferents in the contralateral deep peroneal nerve conducting at 40m/s with a brief central delay could in theory arrive 10ms after the test volley. The effects of activity in group II fibres conducting at 21m/s (Nardone and Schieppati1998) with a central delay of at least 7ms should reach the motoneurone pool approximately 30ms after the test volley.

It follows that any possible group I effect could, therefore, be detectable at the 5ms C/T interval. Correspondingly, an inhibition at a C/T interval of 15ms would indicate the peak of a small group I effect or it could indicate a group II effect if fast conducting group II fibres had been activated. The significant reduction in the reflex size at that interval in the subject who received stimulation at four times the motor threshold might indicate that a group II effect simply required a higher intensity of stimulation in order to be detected, but the mixed results from the two subjects described above, in which the intensity of stimulation was varied, lend credence to the idea that if crossed inhibitory effects do exist in this particular pathway, they are weaker than those seen in the previous chapters.

9.0 FURTHER CROSSED EXPERIMENTS

The results from the four sets of crossed experiments left a number of unresolved questions. Attempts were made to try to clarify two issues experimentally. The first was the question of whether fast group II fibres contribute to a later component of the early inhibition, which was seen in three of the four sets of experiments. The second question was whether the late excitation, seen consistently, but with relatively small numbers in two of the four sets of experimental results (and in the preliminary experiments carried out using intensities of stimulation at three times the motor threshold) could be confirmed with repeated observations at a long conduction / test interval.

9.1 EXAMINATION OF THE EFFECT OF VARYING THE INTENSITY OF A CONDITIONING STIMULUS APPLIED TO THE TIBIAL NERVE UPON THE CONTRALATERAL SOLEUS H-REFLEX

The results from the series of experiments described in chapter 5 had revealed an inhibition of the soleus H-reflex which appeared to reach a maximum at the 15ms condition / test (C/T) interval, when activity in group II fibres could be contributing to the pattern seen. The effect of conditioning using a range of stimulus intensities was examined in four subjects who gave their informed consent. The conditioning was applied at the 15ms C/T interval and at the 5ms C/T interval. The experimental method was as described in chapter 5.

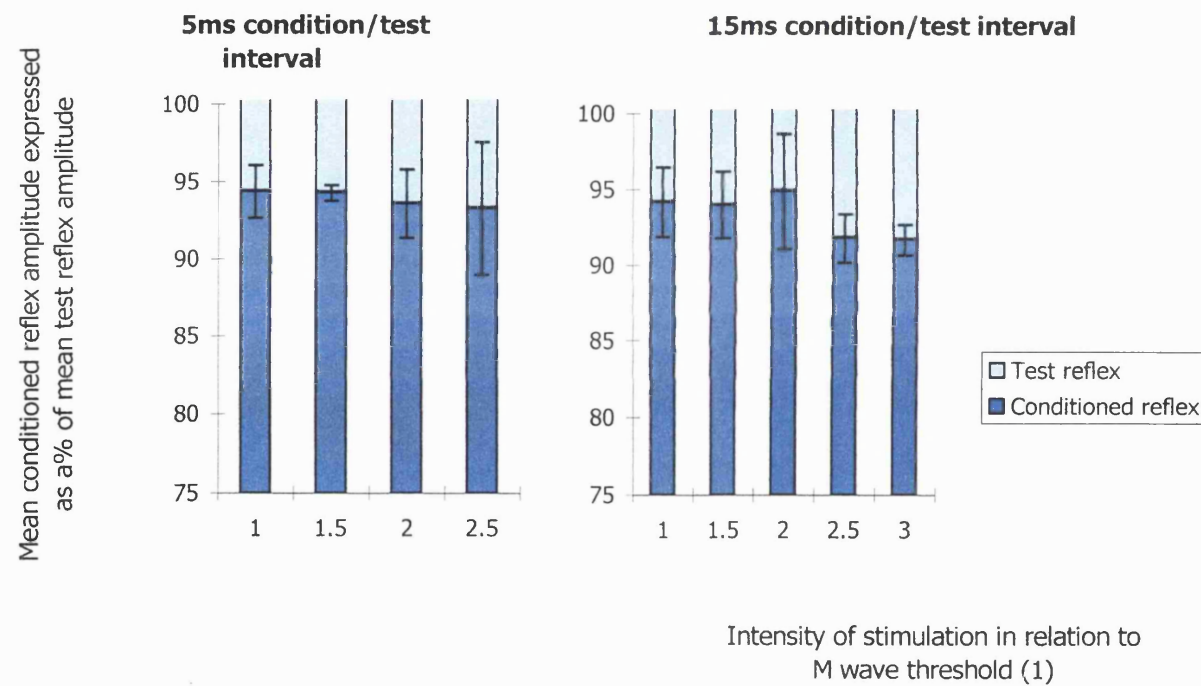
At each stimulus intensity samples of 100 reflexes (in which conditioned and unconditioned reflexes were interleaved) were collected. The stimulus intensities used were 1, 1.5, 2 and 2.5 times the motor threshold intensity at 5ms and 1, 1.5, 2, 2.5 and 3 times the motor threshold intensity at 15ms.

The collected samples were stored and then measured and analysed as described above. The mean conditioned reflex amplitudes were expressed as a percentage of the mean test reflex amplitudes in order to allow comparison between subjects. The pooled results for the two C/T intervals are shown in a bar chart in figure 9.1. At both intervals there is a small but distinct reduction in the reflex size when the conditioning intensity is at motor threshold. This was shown to be statistically significant at the 5ms interval ($p < 0.05$) using Wilcoxon's Signed Rank test. At both intervals there appears to be a slight increase in the inhibition at the higher stimulus intensities. This is particularly the case at the 15ms interval, but this change is very small and it does not appear to be increasing.

These results do not indicate an effect from group II fibres. Such small changes could simply be the result of random differences or the recruitment of slightly more distant group I fibres in the nerve trunk with increased stimulus intensity (Gracies et al. 1994) and this must be the case at the 5ms interval, which is too early for group II fibres to have an effect.

FIGURE 9.1 THE EFFECT OF VARYING THE INTENSITY OF A CONDITIONING STIMULUS APPLIED TO THE CONTRALATERAL TIBIAL NERVE UPON THE SOLEUS H-REFLEX

The effect was examined at two condition / test intervals



Error bars = SEM

9.2 THE EFFECT OF CONDITIONING STIMULATION APPLIED AT THREE TIMES THE MOTOR THRESHOLD INTENSITY TO THE CONTRALATERAL TIBIAL NERVE UPON THE SOLEUS H-REFLEX AT A CONDITION / TEST INTERVAL OF 55ms.

The experimental method was as described in chapter 5. Experiments were performed on eight subjects who gave their informed consent. A contralateral conditioning stimulus intensity of 3 times the motor threshold was used and 14 samples of 100 interleaved conditioned and unconditioned reflexes were collected at the 55ms conditioned/test interval from the eight subjects.

For each sample the individual peak to peak reflex amplitudes were measured and the conditioned and unconditioned samples were compared using the Student's t-test. In eight of the fourteen samples the mean conditioned reflex amplitude was significantly larger than the mean test reflex amplitude ($p < 0.05$). For each pair, the mean conditioned reflex amplitude was expressed as a percentage of the mean test reflex amplitude. The pooled results were analysed using Wilcoxon's Paired Rank test and the differences between the pairs were significant ($p < 0.01$).

These results confirm the somewhat scattered but repeated observations, noted during the crossed experiments, of a late facilitation of the test reflex around the 50ms C/T interval. This is likely to be the result of activity in contralateral group II fibres, some of which were activated at twice the motor threshold during the initial experiments. By increasing the intensity of stimulation more larger diameter fibres were probably activated and a repeatable pattern has emerged.

10.0 DISCUSSION OF THE RESULTS FROM THE FOUR SETS OF CROSSED EXPERIMENTS

EARLY EFFECTS

The most striking and consistent observation from the crossed studies is that of a short-latency crossed inhibitory effect upon the H-reflex of the extensor motoneurone pools. This is evident in three of the four pathways investigated.

Conditioning of the soleus H-reflex by stimulation of the contralateral tibial nerve

From the plot of the results from this first series of experiments (figure 5.4), in which the soleus H-reflex was conditioned by stimulation of the posterior tibial nerve, the biphasic shape of the decrease in size of the reflex (where the inhibition seen is statistically significant at 5, 10, 15 and 20ms, $p < 0.05$) could indicate two separate inhibitory components and the slow return of the reflex to its test level could be in keeping with a decaying effect.

For this pathway the evidence for a group I-mediated inhibition is firm. The appearance of the inhibition at such a short latency virtually excludes the contribution of other, slower-conducting fibres to the early changes. The plot indicates the appearance of a subsequent further drop in the reflex size after the 10ms condition/test interval, at a latency at which the effect from a group I volley, for instance, should no longer be peaking. However the inhibition does appear to increase as far as the 15ms C/T interval and

the results from several of the subjects show an inhibition which extends to around 20ms.

At the 15ms C/T interval, theoretically, effects from activity in the fastest-conducting group II fibres could be expected to appear. The intensity of the conditioning stimulus to the tibial nerve was usually at 2 times the threshold of the largest motor axons. This intensity would be more than 3 times the threshold intensity of the Ia afferents (- see the discussion in chapter 5). Therefore a substantial proportion of the group II afferents, which have a threshold of approximately 2.5 times the Ia threshold, could be activated. However the further experiments, described in chapter 9, in which changes at the 15ms C/T interval were examined, revealed no further significant reduction in the reflex size with increasing stimulus intensity. These results indicate that it is unlikely that group II afferents are mediating the later component of the early inhibitory effect.

The rather slow return of the reflex amplitude to the unconditioned size could indicate a combination of effects. A further inhibitory influence, from slightly slower-conducting fibres - other group I fibres or cutaneous afferents with diameters equivalent to the group I range, superimposed upon the group I mediated early changes, could extend the inhibition and produce the pattern seen.

At the later intervals, one subject showed very significantly increased reflexes at 55 and 60ms C/T intervals (these are not shown in the plot as too few samples were collected at these intervals). Of the two subjects who received conditioning stimulation at three times the motor threshold, one showed a significant reflex facilitation at the 35ms C/T interval, with two further facilitations (which were close to significance levels) at the 45ms

and 50ms intervals. The second subject showed a significant reflex facilitation at the 40ms C/T interval.

The results of the later experiments, using a 3 times motor threshold conditioning intensity, described in chapter 9, confirm the presence of a facilitation, significant at the 55ms C/T interval. It is possible that the excitatory influence responsible may have an earlier effect, but this could be masked sometimes at earlier intervals by concurrent inhibition.

Conditioning of the quadriceps H-reflex by stimulation of the contralateral femoral nerve

The appearance of the inhibition in this set of experiments (see figure 6.4) is in keeping with the shorter afferent conduction pathway involved. Once again, the appearance of the effect at such a short condition/test interval (significant at 3 and 5ms C/T intervals, [$p < 0.05$]) indicates that it must be being produced by group I activity.

The appearance of a very small reduction in the reflex amplitude at the 0ms interval, in the pooled results of the peak to peak measurements, is not present at the 0ms interval in the other plot of the pooled results in figure 6.4 (when the area under the rectified reflex curve was measured). In theory a contralateral group I volley could arrive at the motoneurone pool in time to influence the rising level of excitation from the test volley.

The inhibition which develops from the 3ms C/T interval onwards is larger, develops more rapidly and appears to decay more rapidly than that seen in the first set of experiments. The afferent pathway here is much shorter and in theory the leading edge of a group II-induced volley being conducted at 40m/s could reach the motoneurone pool within 2ms of

activity in the fastest group I afferents (provided that a similarly short central pathway was involved). Therefore, if group II-mediated crossed activity were to be contributing to this group I-initiated inhibition, its effects could commence during the “rise time” of the population IPSP produced in the motoneurone pool by the contralateral group I activity. The two volleys would not be sufficiently separated in time to allow identification of the different contributions.

The results from the later experiments, in chapter 9, carried out to examine a group II input to the early inhibition (in the pathway between the contralateral tibial nerve and the soleus motoneurone pool), make a group II contribution here unlikely. The estimates of Nardone and Schieppati (1998) and Simonetta-Moreau et al. (1999) also point to the likelihood of a longer central delay and possibly a slower group II maximum conduction velocity than the theoretical minimum anticipated here (Nardone and Schieppati 1998).

The differences in the timing of the appearance of the inhibition and the duration of its effects in these two sets of experiments could all be accounted for by the different afferent conduction pathways used in the two sets of experiments. The degree of dispersion which will occur in a volley carried by a population of afferent fibres with a range of conducting velocities will depend upon the length of the pathway to the spinal cord. Therefore the arrival of action potentials at the spinal cord in the first series of experiments will be much more dispersed than in this second series. The slower development of the inhibition in the first set of experiments would be consistent with an effect produced by such a dispersed volley.

The return of the H-reflex amplitude to its test level in this series of experiments could also reflect several concurrent influences and it is possible that both muscle afferents and cutaneous afferents contribute.

The large inhibition at the 35ms C/T interval is not significant and may be unduly influenced by a very large inhibition seen in one subject (hence the large standard error). The numbers of samples available were small between the 30 and 45ms C/T intervals and the effect of pooling may have produced an artificial pattern. In the results from the individual subjects (figure 6.3), a pattern of a much slower return of the reflex amplitude to the baseline level is visible in several of the subjects.

At the 50ms C/T interval the individual results remain very variable, but two subjects display facilitated reflexes. It seems likely that both inhibitory and excitatory influences are present.

Conditioning of the soleus H-reflex by stimulation of the contralateral femoral nerve

In this the third set of experiments, in the pooled results (seen in figure 7.3) an inhibition is visible which has a rather similar shape, in terms of its development over time, to that seen in the first set of experiments, but the inhibition here is smaller than in the other two sets of experiments. The shift of the curve to the left - in relation to the condition/test intervals at which the effects appear - is due to the fact that the afferent conduction distance of the conditioning volley is much shorter than that of the test volley (see chapter 7 for an explanation of the time-scale difference). As a result, the one early point at which the inhibition is statistically significant ($p < 0.05$) is at the 0ms condition/test interval. At this interval the effects could in theory be mediated by group I or group II activity or both.

The fact that the slope of the development of the inhibition in this set of experiments, where conditioning is via the femoral nerve, is similar to that seen in the first set of experiments, where the conditioning was via the tibial nerve, is interesting. One might have expected to see a more rapidly developing reduction in the reflex size, rather like that seen in the previous set of experiments, where conditioning was also via the femoral nerve. These differences may represent inaccuracies in the experimental method, but another explanation may be that the intensity of stimulation which activated the contralateral group I fibres in this set of experiments and in the previous set was effectively lower than that used in the first experiment, where the posterior tibial nerve was used. Because of the different relationships between the largest motor axons and the largest afferents in the two different nerves, the Ia threshold is considerably lower than the motor threshold in the tibial nerve, but the discrepancy is not so great in the femoral nerve. A rather smaller volley, from this lower intensity of stimulation, might take longer to have an impact (via the interposing interneurons) upon the motoneurone pool. This could possibly account for the slower development of the effects in this set of experiments. Why then were the crossed effects upon the quadriceps H-reflex from stimulation of the femoral nerve more rapidly-developing at the quadriceps motoneurone pool? A more powerful link between the proximal muscles of the two limbs than that between the distal muscles, which would not be unexpected in terms of interlimb activity (Harrison & Zytnicki, 1984 and Arya, Bajwa & Edgley, 1991), could explain this.

The significant facilitation at the 50ms C/T interval in four of the subjects here adds weight to the evidence of a late excitatory influence produced by activity in fibres in the group II range of diameters.

Conditioning of the soleus H-reflex by stimulation of the contralateral deep peroneal nerve _

The results from this final set of experiments show no significant changes in the test reflex at any of the condition/test intervals as a result of conditioning stimuli applied to the contralateral nerve. The indications are that the crossed effects are absent or weaker in this pathway. There could be some significance in the fact that the pathway investigated in this case was between the contralateral equivalent of antagonist muscles.

The evidence for the existence of group I crossed effects

The observation of an inhibitory crossed effect as a result of activity in group I afferents is in keeping with some results from the animal studies (Perl 1958,1959, Holmqvist 1961a &b). The work of Perl (1958,1959), on acute spinal cats, indicated different crossed effects from Ia and from Ib activity, with inhibition of contralateral extensors produced by Ia activation. Baxendale and Rosenberg (1976) also identified a weak, group Ia-induced effect upon contralateral muscles in decerebrate cats, which was always inhibitory, in both flexors and extensors. They, too, identified a weak, excitatory, Ib-mediated effect, especially upon the soleus muscle (Baxendale and Rosenberg 1977).

Harrison and Zytnicki (1984) had noted some occasional crossed effects upon motoneurons from group I activity in spinal cats and Harrison, Jankowska and Zytnicki (1986) showed that a population of midlumbar interneurons in lamina VIII and the border of lamina VII - some of which had been found to project to contralateral motoneurons - received some group I input as well as a major input from group II afferents. The function of the group I input identified might be simply to contribute to various possible patterns of afferent input during particular movements. Different active patterns of input from the numerous sources shown to converge upon these commissural neurons could in themselves lead to the activation of a particular pattern of output (as originally suggested by Holmqvist in 1961 and later enlarged upon by Arya, Bajwa and Edgley in 1991; see Schomburg 1990, Dietz 1992 and Corna et al.1996 for discussions).

When Delwaide and Pepin (1991) demonstrated that stimulation of the median nerve could reduce reciprocal inhibition of the flexor carpi radialis

muscle in the contralateral upper limb they were echoing the results of Harrison and Zytnicki (1984) in the cat. Delwaide and Pepin (1991) believed that the contralateral influence seen was mediated by Ia afferents, because they were not seen when purely cutaneous branches of the nerve were activated. The effects revealed by the latter authors were at a very short latency and of a relatively modest size and in this sense they resembled the effects seen in the present study.

The inhibition seen in the present study would fit most easily with the observations upon crossed effects from Ia activity, but the ipsilateral group I effects described by Pierrot-Deseilligny and his colleagues (see for instance Pierrot-Deseilligny et al. 1981) and the crossed effects upon Ia inhibition described by Delwaide and Pepin (1991) are more discrete, more short-lived. The timescale of the effects in the present study is different. The onset is precise but the effects are extended. Such a pattern could be explained by a combination of effects from more than one source, with the later components resulting from the effects of a spread of slower conducting afferents or the development of presynaptic inhibition.

In their EMG studies of the effects of perturbation, Corna and his colleagues (Corna et al. 1996) demonstrated that the SLR responses, at latencies indicating group I mediation, were only present bilaterally when afferent input was bilateral. They interpreted their observations in terms of a system in which commissural neurones capable of mediating group I crossed excitatory effects required bilateral input in order to reach threshold. The electrically -induced volleys reaching the spinal cord would be of a quite different composition from the volleys produced by perturbation, but a large group I volley producing a consistent but small effect could be explained by the level of excitability of such commissural neurones.

The evidence for the existence of group II crossed effects

A contribution from group II activity to the effects observed in the present study would be consistent with the large numbers of observations concerning group II crossed effects from cat studies (Holmqvist, 1960 & 1961a, Rosenberg, 1970, Harrison & Zytnicki, 1984, Harrison et al., 1986 and Arya, Bajwa & Edgley, 1991) and from human balance work (Dietz & Berger, 1982, Dietz et al, 1984 a & b, Dietz et al. 1989, Corna et al. 1986).

In human balance studies the earliest bilateral EMG response to perturbation, demonstrated by Dietz & Berger (1982), was at a latency of 55ms. This early activity, which appeared bilaterally and was present even with unilateral perturbations (provided that both limbs were performing a supporting function) has been interpreted as group II-mediated activity. Using estimates made during the present study and evidence from other authors it is possible to make a reasonably accurate estimate of the minimal latency which would be required for a group II mediated effect in the balance experiments.

The pathway from the stretched soleus muscle to the L1 vertebra and back to the muscle would involve at least 700mm x 2 (popliteal fossa to L1 and back, see Table 1) + 400mm (ankle to popliteal fossa and back to the muscle - see Pierrot-Deseilligny, Morin, Bergego & Tankov 1981) = 1800mm. Burke, Gandevia and McKeon in 1983 measured the average latency of the reflex response to a tendon tap of the achilles tendon as 36.4ms. Even allowing for extra synapses and the extra intraspinal travelling time which would be needed if the commissural pathway were to involve the equivalent of the contralaterally projecting midlumbar interneurons identified in the cat (Harrison, Jankowska & Zytnicki 1986),

the almost 20ms of extra time seen in the human balance experiments would be difficult to account for if the response was mediated by group I afferents.

Action potentials travelling at 64 m/s in the largest Ia afferents would take 12ms (popliteal fossa to L1 motoneurones, including 1 synapse - see Table 2) + 3.5ms (soleus to popliteal fossa) = 15.5ms to reach the motoneurone pool. Burke, Gandevia and McKeon (1983) also recorded the earliest latency of the first change in the afferent firing rate following a tendon tap as approximately 4ms. If this activation time and the afferent travelling time above are subtracted from the reflex latency of 36.4ms then what remains - 16.9ms - is the efferent conduction time.

A volley travelling at 40m/s in the largest group II afferents would take, on average, 26.3ms to reach the L1 spinal segment from the ankle. Add to this the receptor activation time and the efferent conduction time, plus 3ms to account for traversing the spinal cord (and a minimum of 2 synapses in the pathway) and the minimal latency of a group II contralateral reflex response would be around 50ms. This estimated time does not allow for any extra intraspinal journey time, delays at any extra synapses or any delays related to the extra time needed by each extra neurone involved in the reflex pathway to reach threshold.

The above estimate would, therefore, be in line with the favoured interpretation of the early bilateral effects seen from around 55ms in several human balance experiments (e.g Dietz & Berger 1982,1984) as a group II-mediated response probably involving interneurones which are under supraspinal control and which receive converging signals from a variety of afferents. In fact more recent studies by Dietz (e.g Dietz et al.1989) and those by Schieppati and his colleagues (Nardone et al.1990,

Corna et al. 1996) have given values for the latency of the bilateral MLR responses to perturbation in soleus or tibialis anterior as around 70ms. Taking into account the estimated central delay produced by Nardone and Schieppati (1998) and their maximum estimated conduction velocity of the group II fibres from flexor digitorum brevis, a reflex response mediated by such fibres would have a minimal latency of around 75 ms. The late excitation observed in the present study appears at around 50ms, which, if it is mediated by group II fibres, seems rather late - particularly in the sets of experiments in which the stimulation of the femoral nerve was used and the afferent pathway was relatively short. However, Schieppati and his colleagues (Corna et al. 1996) have demonstrated that with unilateral perturbations the supposedly group II mediated MLRs are further delayed compared to those seen with bilateral perturbations. They highlighted two possible causes - the likely longer central delay of crossed pathways and the greater time taken to bring the commissural neurones to threshold with only unilateral input.

Recent work presented by Pierrot-Deseilligny and his colleagues (Marchand-Pauvert et al.1999) has shown potent bilateral enhancement of the cortical excitation of lower limb motoneurones in humans as a result of peripheral afferent stimulation. These observations were in keeping with interactions occurring at the level of midlumbar interneurones and they reinforce the current view that rostrally-located lumbar interneurones are not simply cells which relay afferent and descending signals, but are centres in which the integration of incoming signals results in the selection of muscle synergies controlling both lower limbs (McCrea 1992, Jankowska & Edgley 1993).

The evidence for the existence of crossed effects from cutaneous afferents

The findings of Edgley and Wallace (1989) indicated a crossed excitatory effect from cutaneous afferents with fibre diameters equivalent to the group I diameter range, contrary to the earlier findings of Rosenberg (1970), who had observed that the largest cutaneous afferents did not produce effects in contralateral motoneurons, but appeared to convey innocuous signals, relating to body contacts, to the higher centres (see description of Rosenberg's findings in chapter I).

Pierrot-Deseilligny and his colleagues (Bergego et al. 1981) demonstrated what appeared to be the facilitation of Ib pathways from ankle extensors to the quadriceps muscle, using a contralateral stimulus to the sole of the foot at 0.8 times the motor threshold intensity and Delwaide et al. (1981) showed that stimulation of the sural nerve at 2.5 times the threshold for perception produced a small early contralateral inhibition and an excitation at 70ms.

Burke et al. (1983) found the average conduction time of the fastest cutaneous afferents in the sural nerve in humans was around 50ms. This would indicate that volleys in the group I cutaneous afferents would not be the first portion of the group I activity to reach the spinal cord following stimulation, but they could be responsible for the crossed effects seen in the present study.

Interpretation of the effects seen in the present study in the light of the evidence described above

Bajwa, Edgley and Harrison (1992) found that many midlumbar interneurons were activated by both ipsilateral and contralateral afferents. The most effective afferents were from similar sources in both cases. Such a pattern suggested strong bilateral interaction (such as had been observed indirectly in the human experiments of Dietz et al. 1989) and the latencies of the responses indicated the involvement of a single commissural neurone for the production of crossed EPSPs and the inclusion of one extra interneuron in the pathway producing IPSPs.

The inhibitory crossed effect seen in the present study from group I activity would be in keeping with the widespread inhibitory effects observed in anaesthetised cats with intact spinal cords (Arya, Bajwa and Edgley 1991, see also Jankowska & Edgley 1993) and the latencies of the effects seen here could fit the pathways they described. The crossed extension effect and the group I-induced crossed excitation were seen only in spinal animals (Harrison, Jankowska and Zytnicki 1986, Arya, Bajwa and Edgley 1991).

It is of course difficult to make direct comparisons between observations from awake humans at rest and observations from anaesthetised cats, even if both have intact spinal cords! For instance Rosenberg (1970) noted the depression of crossed effects with anaesthesia. However, together they do indicate the existence of crossed inhibitory pathways. Their importance and their functional significance remain to be explored, although it is clear that inhibition of contralateral extensors would be appropriate when the limb concerned was performing the swing phase of walking and bilateral

inhibition of extensors would be required, for instance, during the flexion stage of jumping and in many postural sets in which the lower limbs were flexed.

The results from the balance experiments described above could be said to reinforce the findings of powerful group II projections, made during the animal studies, and to add ammunition to the current belief that important functional crossed reflexes are mediated by group II activity. In this light the present observations - of some crossed effects mediated entirely by group I afferents - are surprising.

However a number of factors need to be considered. The small inhibitory crossed effects upon extensor motoneurons shown in the present study could be in keeping with a relatively minor but significant input from group I afferents onto commissural neurones involved in interlimb coordination.

The effects observed here are all relatively small and in the light of both the observations of projections to cat interneurons which could be involved in contralateral pathways and the less direct evidence from the human experiments, one might have expected to see far greater group II effects. A number of points need to be considered in relation to this. For instance, if the present observed effects involve the equivalent of the lamina VIII midlumbar interneurons studied in the cat then several factors could contribute to the pattern seen.

Firstly, the consistently small effects would be in keeping with a relatively depressed activity level of the commissural interneurons involved. This could make sense in terms of the lack of a need for descending control of interlimb coordination in relaxed sitting.

Secondly, stimulation of certain brainstem sources of descending control have revealed the possibility of selective inhibition of group II activity upon midlumbar interneurons (Noga et al.1992), so that the dominance of this input could be reduced under certain circumstances. In standing, the integrating interneurons would be most efficiently activated by group II input, perhaps aided by some group I input. Thus in standing the pattern of convergence of group II inputs, possibly from several muscles and joints from both limbs, integrating with descending output, would lead to an appropriate bilateral response to maintain posture. However in the present study the subjects were at rest in supported sitting and in this particular postural set a different input pattern might dominate, (possibly with presynaptic inhibition of group II activity). Given increased intensity of stimulation, as in the final experiment using an intensity of stimulation of 3 times motor threshold, the commissural neurones could receive sufficient group II excitation to reach threshold and, even in relaxed sitting with reduced descending facilitation, a crossed excitation is observed.

Thirdly, the group I effects might appear relatively large because at the usual conditioning intensity used here (twice the threshold of the motor response) a much larger percentage of group I afferents would be activated than group II afferents. It is also important to note that at an intensity of stimulation of two times motor threshold fewer group II fibres may be activated in the femoral nerve than in the tibial nerve, because of the different relationships between the diameters of the motor axons and the afferent fibres in the two nerves.

Fourthly, reduction of presynaptic inhibition of Ia terminals could also increase the effectiveness of the group I input. Indeed a lack of presynaptic inhibition at rest in sitting could be an important contributing factor to the

detection of the crossed I effect in the present study. In contrast, an increase in presynaptic inhibition could minimise the contribution of group I input in situations where this would be counterproductive and indeed this has been shown to occur during locomotion (Dietz, Quintern & Berger 1985, Capaday & Stein 1986,1987) and during balancing (Dietz & Berger 1984).

Finally, it is possible that the group I inputs to the interneurons concerned in lower limb coordination are relatively greater in humans than in cats, perhaps as a direct result of the development of bipedal stance and locomotion. Although the Ia activity is often inhibited presynaptically (partially in standing and considerably in walking), the need for selective control of a potentially substantial input under different circumstances would seem to have functional value (such as the phase-dependant control described during walking by Tax et al. in 1990 and the changing input to dynamic fusimotor neurones during running and jumping observed by Prochazka in 1989).

Unlike the early effects, the changes within the 20ms to 40ms C/T interval range in all four sets of experiments displayed such great interindividual variability that no consistent underlying trend could be detected and interpreted in the light of current knowledge. However certain points relating to this variability should be examined.

Firstly, at some of the longer C/T intervals, there were observations available from only a small number of experiments. If experiments had become very extended and a decision had been made to cut them short to avoid fatigue or discomfort of the subject, then some collections at the longer C/T intervals had been sacrificed in preference to those at the shorter C/T intervals.

A second factor which makes small effects of higher threshold afferent activity difficult to detect against background variability is that with long conduction distances the slower the rate of conduction the greater will be the dispersion of the volley. For instance action potentials being conducted at 16m/s, travelling over an afferent conduction distance of 700mm, will arrive 10ms before action potentials being conducted at 13m/s and 24ms before action potentials being conducted at 10m/s. Therefore one may be looking for long, slow changes rather than sharp, well-defined ones

Perhaps the most important factor pertaining to the inter-individual variability is that, however careful were the attempts to standardise the positioning of the electrodes and the intensity of stimulation, the exact combination of afferents activated would be different in each individual (as indicated above in the discussions of the separate studies). Such differences would have less of an effect upon the group I activation, because substantial numbers of fibres from this group would always be activated at the intensity level used.

However, in the case of group II afferents, some of these might or might not be activated. This would depend upon whether their orientation within the nerve trunk in relation to the stimulating electrode allowed the fibre to receive enough current for the cell membrane to reach threshold potential. Therefore the numbers of these fibres stimulated could be very different in each individual experiment.

A possible second inhibition is visible in each of the pooled results plots from the two sets of experiments using the femoral nerve to condition. However this inhibition has not been shown to be statistically significant. It occurs at an equivalent latency in both sets, when the difference in the

length of the test afferent pathway in relation to the conditioning afferent pathway in the one set is taken into account. It could feasibly be due to the recruitment in some subjects of smaller group II fibres whose contribution, by changing the input pattern reaching the lumbar interneurons, had resulted in a different output pattern.

The later or extended inhibition could be due to slow modulatory effects, such as changes in presynaptic inhibition, initiated by the activation of certain group I or group II fibres. Indeed slow, long-lasting effects have been regularly reported during the history of crossed experiments (see for example Jankowska et al. 1967a).

Classically, presynaptic-inhibition of Ia afferents in cats (Rudomin 1990) and in humans (e.g. Hultborn, Meunier, Morin & Pierrot-Deseilligny 1987, Aimonetti et al. Paris 1999) develops with a latency of approximately 20 to 50ms and lasts up to 500ms. In the light of the known pre-synaptic interactions of various afferents - for example, the reduction of group I-mediated pre-synaptic inhibition of Ia terminals by cutaneous afferents - this could be yet another factor contributing to the large intersubject variability seen at the longer C/T intervals in the present study. Differences in the mixture of afferents electrically activated in each subject would obviously have an effect upon such interactions.

In the light of the late excitation observed in some subjects in all four sets of experiments and in the experiments in chapter 9 with a higher stimulus intensity, the individual differences described above could complicate a pattern which, in any case, may represent an inhibition and a concurrent excitation at the longer C/T intervals.

The late excitation, although later than might have been anticipated from the perturbation experiments, is in keeping with the well-recognised pattern of a group II-induced crossed excitation. The latency measurements of 70 - 80ms in the perturbation experiments relate to the total reflex time and to a situation in which commissural interneurons receive input from bilateral volleys. The particular conditions in the present study - the relatively depressed levels of the commissural interneurons in relaxed sitting and the unilateral input - could account for the time required to see an effect and the higher levels of intensity required.

CONCLUSION

The results from three sets of experiments in the present study have demonstrated an inhibitory crossed effect upon the H-reflexes of lower limb extensor muscles in humans. The results from two of these sets of experiments show that the earliest component of the crossed inhibition is mediated by group I afferents.

The late facilitation, observed in some subjects and confirmed in the later experiments is probably mediated by group II fibres.

In the light of the evidence from Delwaide and Pepin (1991) and Harrison and Zytnicki (1984) it is possible that the early crossed inhibition seen in the present study is mediated through Ia interneurons. It would be interesting to try to demonstrate a crossed effect upon reciprocal inhibition in the lower limbs. Reciprocal inhibition between soleus and tibialis anterior is often difficult to demonstrate, but using that between quadriceps and the hamstrings might be more practical.

As each of the three peripheral nerves used in the application of the conditioning stimuli were mixed nerves, then the possible contribution to the crossed effects of cutaneous afferents, with diameters equivalent to group I and group II fibres, cannot be excluded. A useful study might be to isolate and stimulate purely cutaneous branches of these nerves and compare the crossed effects at relevant condition/test intervals.

In relation to the late facilitation, if this is a response which represents the crossed pathway used by the coordinated group II balance responses, then examining such responses in patients with motor dysfunction might be useful. It appears increasingly likely that group II activated interneurons play an important part in the coordination of balance reactions during standing and walking (see for instance Jankowska and Edgley 1993). If the descending control of the commissural group II activated interneurons is disturbed in stroke patients, then the response may be absent or changed. Monitoring these responses during recovery might indicate how important the recovery of control of this pathway is to the return of good function.

REFERENCES

Aggelopoulos NC, Edgley SA, (1994) 'Segmental localisation of the relays mediating crossed group II inhibition of hindlimb motoneurons in the spinal cord of the anaesthetised cat'. *Journal of Physiology Proceedings* 475: 40 (P)

Aiello I, Serra G, Migliore A, Tugnoli V, Roccella P, Cristofori MC, Manca M, (1983) 'Diagnostic use of H-reflex from vastus medialis muscle'. *Electromyography and Clinical Neurophysiology* 23: 159-166

Aimonetti JM, Vedel JP, Schmied A, Pagni S, (1999) 'Task-dependent efficiency of Ia presynaptic inhibition in humans'. Presented at the common meeting of the Societe des Neurosciences and the British Neuroscience Association in Paris.

Arya T, Bajwa S, Edgley SA, (1991) 'Crossed reflex actions from group I muscle afferents in the lumbar spinal cord of the anaesthetized cat'. *Journal of Physiology* 444: 117-131

Ashby P, Labelle K, (1977) 'Effects of extensor and flexor group I afferent volleys on the excitability of individual soleus motoneurons in man'. *Journal of Neurology, Neurosurgery and Psychiatry* 40: 910-919

Bagust J, (1974) 'Relationships between motor nerve conduction velocities and motor unit contraction characteristics in a slow twitch muscle of the cat'. *Journal of Physiology* 238: 269-278

Bajwa S, Edgley SA, Harrison PJ, (1992) 'Crossed actions on group II activated interneurons in the midlumbar segments of the cat spinal cord'. *Journal of Physiology* 455: 205-217

Basmajian JV, De Luca CJ, (1985) Muscles Alive. Their functions revealed by electromyography. 5th Edition. Williams & Wilkins. Baltimore U.S.A.

Baxendale RH, Rosenberg JR, (1975) 'Crossed reflex actions of group I muscle afferents'. *Journal of Physiology* 251: 53D

Baxendale RH, Rosenberg JR, (1976) 'Crossed reflexes evoked by selective activation of muscle spindle primary endings in the decerebrate cat'. *Brain Research* 115: 324-327

Baxendale RH, Rosenberg JR, (1977) 'Crossed reflexes evoked by selective activation of tendon organ afferent axons in the decerebrate cat'. *Brain Research* 127: 323-326

Bergego C, Pierrot-Deseilligny E, Mazières L, (1981) 'Facilitation of transmission in Ib pathways by cutaneous afferents from the contralateral foot sole in man.' *Neuroscience Letters* 27: 297-301

Bosemark B, (1966) 'Some aspects of the crossed extensor reflex in relation to motoneurons supplying fast and slow contracting muscles'. in *Muscular Afferents and Motor Control*, Nobel Symposium I, Ed. Granit R 261-268

Boyd IA, Davey MR, (1968) Composition of peripheral nerves. Livingstone. Edinburgh.

Brock LG, Eccles JC, Rall W, (1951) 'Experimental investigations on the afferent fibres in muscle nerves'. *Proceedings of the Royal Society B* 138: 453-475

Bruggencate G, Ten, Lundberg A, (1974) 'Facilitatory interaction in transmission to motoneurons from vestibulospinal fibres and contralateral primary afferents'. *Experimental Brain Research* 19: 248-270.

Brown MC, Engberg I, Matthews PBC, (1976) 'The relative sensitivity to vibration of muscle receptors of the cat'. *Journal of Physiology* 192: 773-800

Brunia CHM, Hugon M, Delwaide P, Pierrot-Deseilligny E, Desmedt JE, (1973) 'A discussion of the methodology of the triceps surae T- and H-reflexes'. *New Developments in Electromyography and Clinical Neurophysiology* 3: 773-780

Burke D, (1983) 'Critical examination of the case for or against fusimotor involvement in disorders of muscle tone'. *Motor Control Mechanisms in Health and Disease*, Ed. Desmedt JE

Burke D, Gandevia SC, McKeon B, (1983) 'The afferent volleys responsible for spinal proprioceptive reflexes in man'. *Journal of Physiology* 339: 535-552

Burke D, Gandevia SC, McKeon B, (1984) 'Monosynaptic and oligosynaptic contributions to human ankle jerk and H-reflex'. *Journal of Neurophysiology* 52: 435-448

Burke RE, (1968) 'Group Ia synaptic input to fast and slow twitch motor units of cat triceps surae'. *Journal of Physiology* 196: 605-630

Burke RE (1978) 'Motor units: physiological / histochemical profile, neural connectivity and functional specialisations'. *American Zoology* 18:127-134

Burke RE, Rymer WZ, Walsh JV, (1976) 'Relative strength of synaptic input from short latency pathways to motor units of defined type in cat medial gastrocnemius'. *Journal of Neurophysiology* 39: 447-458

Cajal, (1909) 'Histologie du systeme nerveux de l'homme et des vertebres'. Paris Maloine, Vol 1

Cavallari P, Edgley SA, Jankowska E, (1987) 'Post-synaptic actions of midlumbar interneurons on motoneurons of hind-limb muscles in the cat'. *Journal of Physiology* 389: 675-689

Coppin CML, Jack JJB, McLennan CR, (1970) 'A method for the selective activation of tendon organ afferent fibres from the cat soleus muscle'. *Journal of Physiology* 219: 20P

Corna S, Galante M, Grasso M, Nardone A, Schieppati M, (1996) 'Unilateral displacement of lower limb evokes bilateral EMG responses in leg and foot muscles in standing humans'. *Experimental Brain Research* 109: 83-91

Creed RS, Denny-Brown D, Eccles JC, Liddell EGT, Sherrington CS, (1972) Reflex Activity of the Spinal Cord. Oxford

Crone C, Hultborn H, Mazieres L, Morin C, Nielsen J, Pierrot-Deseilligny E, (1990) 'Sensitivity of monosynaptic test reflexes to facilitation and inhibition as a function of the test reflex size : a study in man and the cat'. *Experimental Brain Research* 81: 35-45

Crone C, Hultborn H, Jespersen B, Nielsen J, (1987) 'Reciprocal Ia inhibition between ankle flexors and extensors in man'. *Journal of Physiology* 389: 163-185

Crone C, Nielsen J, (1989) 'Methodological implications of the post activation depression of the soleus H-reflex in man'. *Experimental Brain Research* 78: 28-32

Culberson JL, Haines DE, Kimmel DL, Brown PB, (1979) 'Contralateral projection of primary afferent fibers to mammalian spinal cord'. *Experimental Neurology* 64: 83-97

Cullheim S, Kellerth J-O, (1978) 'A morphological study of the axons and recurrent axon collaterals of cat alpha motoneurons supplying different functional types of muscle unit'. *Journal of Physiology* 281: 301-313

Dawson GD, Scott, (1949) 'Recording of nerve action potentials through the skin in man'. *Journal of Neurology, Neurosurgery and Psychiatry* 12: 259

Delaguna TG, Orlovsky GN, Pavlova GA, (1983) 'The capacity for generation of rhythmic oscillations is distributed in the lumbosacral spinal cord of the cat'. *Experimental Brain Research* 53: 81-90

Delwaide PJ, Crenna P, Fleron MH, (1981) 'Cutaneous nerve stimulation and motoneuronal excitability : I, soleus and tibialis anterior excitability after ipsilateral and contralateral sural nerve stimulation'. *Journal of Neurology, Neurosurgery and Psychiatry* 44: 699-707

Delwaide PJ, Pepin JL (1991) 'The influence of contralateral primary afferents on Ia inhibitory interneurons in humans'. *Journal of Physiology* 439:161-179

Deschuytere J, DeKeyser C, Daschuttere M, Rosselle N, (1983) 'H-reflexes in muscles of the lower and upper limbs in man : Identification and clinical significance'. *Advances in Neurology* 39 *Motor Control Mechanisms in Health and Disease* 951-960

Dietz V, (1992) 'Human neuronal control of automatic functional movements: Interaction between central programs and afferent input'. *Physiological Review* 72: 33-69

Dietz V, Berger W, (1982) 'Spinal coordination of bilateral leg muscle activity during balancing'. *Experimental Brain Research* 47: 172-176

Dietz V, Horstmann GA, Berger W, (1989) 'Interlimb coordination of leg-muscle activation during perturbation of stance in humans'. *Journal of Neurophysiology* 62, 3

Dietz V, Berger W, (1984) 'Interlimb coordination of posture in patients with spastic paresis. Impaired function of spinal reflexes'. *Brain* 107: 965-978

Dietz V, Quintern J, Berger W, (1984a) 'Corrective reactions to stumbling in man : functional significance of spinal and transcortical reflexes'. *Neuroscience Letters* 44: 131-135

Dietz V, Quintern J, Berger W, (1984b) 'Cerebral evoked potentials associated with compensatory reactions following stance and gait perturbation'. *Neuroscience Letters* 50: 181-186

Dietz V, Quintern J, Berger W, (1985) 'Afferent control of human stance and gait: evidence for blocking of group I afferents during gait'. *Experimental Brain Research* 61: 153-163

Dum RP, Kennedy TT, (1980) 'Synaptic organisation of defined motor unit types in cat tibialis anterior'. *Journal of Neurophysiology* 43: 1631-1644

Eccles JC, Eccles RM, Lundberg A, (1957) 'The convergence of monosynaptic excitatory afferents on to many different species of alpha motoneurones'. *Journal of Physiology* 137: 22-50

Eccles JC, Eccles RM, Lundberg A, (1960) 'Types of neurone in and around the intermediate nucleus of the lumbosacral cord'. *Journal of Physiology* 154: 89-114

Eccles RM, Lundberg A, (1959) 'Synaptic actions in motoneurones by afferents which may evoke the flexion reflex'. *Acta Ital Biol* 97: 199-221

Eccles JC, Schmidt RF, Willis WD, (1962) 'Presynaptic inhibition of the spinal monosynaptic reflex pathway'. *Journal of Physiology* 161: 282-297

Edgley SA, (1991) 'Mammalian spinal interneuronal circuitry and locomotion'. from Locomotor Neural Mechanisms in Arthropods and Vertebrates. Ed. Armstrong DM, Bush BMH, Manchester University Press

Edgley SA, Jankowska E, (1987a) 'Field potentials evoked by group II muscle afferents in the midlumbar segments of the cat spinal cord'. *Journal of Physiology* 385: 393-413

Edgley SA, Jankowska E, (1987b) 'An interneuronal relay for the group I and II muscle afferents in the mid lumbar segments of the cat spinal cord'. *Journal of Physiology* 389: 647-674

Edgley SA, Wallace NA, (1989) 'A short-latency crossed pathway from cutaneous afferents to rat hindlimb motoneurons'. *Journal of Physiology* 411: 469-480

Ellaway PH, (1978) 'Cumulative sum technique and its application to the analysis of peristimulus time histograms'. *Electroencephalography and Clinical Neurophysiology* 45: 302-304

Ellaway PH, Murphy PR, Tripathi A, (1982) 'Closely coupled excitation of gamma-motoneurons by group III muscle afferents with low mechanical threshold in the cat'. *Journal of Physiology* 331: 481-498

Evans P, Willars C, Harrison PJ, (1995) 'The inherent variability of the soleus H-reflex in man'. *Journal of Physiology Proceedings*, 487(P)

Fetz EE, Gustafsson BG, (1980) 'Relations between shapes of postsynaptic potentials and cross corelogram peaks in cat motoneurons'. *Society of Neuroscience Abstract* 6: 715

Fetz EE, Gustafsson B, (1983) 'Relation between shapes of post-synaptic potentials and changes in firing probability of cat motoneurons'. *Journal of Physiology* 341: 387-410

Fleshman JW, Rudomin P, Burke RE, (1988) 'Supraspinal control of a short latency cutaneous pathway to hindlimb motoneurons'. *Experimental Brain Research* 69:449-459

Forget R, Pantieri R, Pierrot-Deseilligny E, Shindo M, Tanaka R, (1989) 'Facilitation of quadriceps motoneurons by group I afferents from pretibial flexors in man'. *Experimental Brain Research* 78: 10-20

Frank K, Fuortes MGF, (1957) 'Presynaptic and postsynaptic inhibition of monosynaptic reflexes'. *Federation Proc.* 16:39-40

Fu TC, Jankowska E, Lundberg A, (1975) 'Reciprocal Ia inhibition during the late reflexes evoked from the flexor reflex afferents after DOPA'. *Brain Research* 85: 99-102

Funase K, Imanaka K, Nishihira Y, (1994) 'The excitability of the soleus motoneurone pool revealed by the developmental slope of the H-reflex as reflex gain'. *Electromyography and Clinical Neurophysiology* 34:477-481

Gassel MM, (1970) 'A critical review of evidence concerning long-loop reflexes excited by muscle afferents in man'. *Journal of Neurology, Neurosurgery and Psychiatry* 33: 358-362

Gassel MM, Diamantopoulos E, (1966) 'Mechanically and electrically elicited monosynaptic reflexes in man'. *Journal of Applied Physiology* 21: 1053-1058

Gottlieb GL, Agarwal GC, (1978) 'Stretch and H-reflexes during phasic voluntary contractions of the human soleus muscle'. *Electroencephalography and Clinical Neurophysiology* 44: 553-561

Gracies JM, Pierrot-Deseilligny E, Robain G, (1994) 'Evidence for further recruitment of group I fibres with high stimulus intensities when using surface electrodes in man'. *Electroencephalography and Clinical Neurophysiology* 93: 353-357

Graham-Brown T, (1912) 'Studies in the Physiology of the Nervous System: VII. Irregular Reactions.' *Quarterly Journal of Exper. Physiol* 5: 267-286

Grillner S, Zangger P, (1979) 'On the central generation of locomotion in the low spinal cat'. *Experimental Brain Research* 34: 241 - 261

Guiheneuc P, Ginet J, (1974) 'Etude du reflexe de Hoffmann obtenu au niveau du muscle quadriceps de sujets humains normaux'. *Electroencephalography and Clinical Neurophysiology* 36: 225-231

Harridge SDR, Bottinelli R, Canepari M, Pellegrino MA, Reggiani C, Esbjornsson M, Saltin B, (1996) 'Whole muscle and single fibre contractile properties and myosin heavy chain isoforms in humans' *European Journal of Physiology* 432: 913-920

Harrison PJ, (1981) 'Presynaptic organisation of inputs to cat spinal motoneurons'. Ph. D. Thesis.

Harrison PJ (1983) 'The relationship between the distribution of motor unit mechanical properties and the forces due to recruitment and to rate coding for the generation of muscle force'. *Brain Research* 264: 311-315

Harrison PJ, Jankowska E, Johannisson T, (1983) 'Shared reflex pathways of group I afferents of different cat hind-limb muscles'. *Journal of Physiology* 338: 113-127

Harrison PJ, Jankowska E, Zytnicki D, (1986) 'Lamina VIII interneurons interposed in crossed reflex pathways in the cat'. *Journal of Physiology* 371: 147-166

Harrison PJ, Jami L, Jankowska E, (1988) 'Further evidence for synaptic actions of muscle spindle secondaries in the middle lumbar segments of the cat spinal cord'. *Journal of Physiology* 389: 647-674

Harrison PJ, Riddell JS, (1991) 'How many motoneurons are inhibited by a single interneurone?'. In *Locomotor neural mechanisms in arthropods and vertebrates*. Editors Armstrong DM, Bush BMH. Manchester University Press

Harrison PJ, Taylor A, (1981) 'Individual excitatory post-synaptic potentials due to muscle spindle Ia afferents in cat triceps surae motoneurons'. *Journal of Physiology* 312: 455-470

Harrison PJ, Taylor A, Chandler RB, (1980) 'The significance of motor unit size relative to the Ia excitation for the gain of the stretch reflex'. *Adv. Physiol Sci Vol. 1*

Harrison PJ, Zytnicki D, (1984) 'Crossed actions of group I muscle afferents in the cat'. *Journal of Physiology* 356: 263-273

Henneman E, (1957) 'Relation of size of neurones and their susceptibility to discharge'. *Science* 126: 1345-1347

Henneman E, Somjen G, Carpenter DO, (1965a) 'Functional significance of cell size in spinal motoneurons.' *Journal of Neurophysiology* 28: 560-590

Henneman E, Somjen G, Carpenter DO, (1965b) 'Excitability and inhibibility of motoneurons of different sizes'. *Journal of Neurophysiology* 28: 599-620

Hoffmann P, (1918) 'Über die Beziehungen der Sehnenreflexe zur willkürlichen Bewegung und zum Tonus'. *Z. Biol*

Holmqvist B, (1960) 'Crossed reflex actions evoked by high threshold muscle afferents'. *Experientia* 16/10: 459-460

Holmqvist B, (1961a) 'Crossed spinal reflex actions evoked by volleys in somatic afferents'. *Acta Physiol. Scandinavia* 52: Suppl 181

Holmqvist B, (1961b) 'Crossed reflex actions by low threshold muscle afferents'. *Experientia* 17: 88

Hugon M, (1973) 'Methodology of the Hoffmann reflex in man'. *New Developments in Electromyography and Clinical Neurophysiology* 3: 277-

Hugon M, Paillard J, (1955) 'Depression durable du reflexe achilleen, consecutif a l'allongement du triceps sural, chez l'Homme'. *J.Physiologie* 47,1:193-196

Hultborn H, Illert M, Nielsen J, Paul A, Ballegaard M, Wiese H, (1996) 'On the mechanism of the post-activation depression of the H-reflex in human subjects'. *Experimental Brain Research* 108: 450-462

Hultborn, Illert, Santini, (1976) 'Convergence on interneurons mediating the reciprocal Ia inhibition of motoneurons.' *Acta Physiol. Scand.* 96: 351-367

Hultborn H, Meunier S, Morin C, Pierrot-Deseilligny E, (1987) 'Assessing changes in presynaptic inhibition of Ia Fibres: A study in man and the cat'. *Journal of Physiology* 389: 729-756

Hultborn H, Nielson JB, (1995) 'H-reflexes and F-responses are not equally sensitive to changes in motoneuronal excitability'. *Muscle and Nerve* 18: 1471-1474

Hunt C.C, (1954) 'Relation of function to diameter in afferent fibres of muscle nerves'. *Journal of General Physiology* 38: 117-131

Hunt, McIntyre, (1960) 'Properties of cutaneous touch receptors in cat'. *Journal of Physiology* 153: 88-98

Hursh, (1939) 'Conduction velocity and diameter of nerve fibres'. *American Journal of Physiology* 127: 131-139

Jack JJB, (1978) 'Some methods for selective activation of muscle afferent fibres'. Studies in Neurophysiology, ed Porter, 155-176, Cambridge University Press

Jankowska E, Edgley S, (1993) 'Interactions between pathways controlling posture and gait at the level of spinal interneurons in the cat'. *Progress in Brain Research* 97:

Jankowska E, Jukes MGM, Lund S, Lundberg A, (1967a) 'The effect of DOPA on the spinal cord 5. Reciprocal organisation of pathways transmitting excitatory action to alpha motoneurons of flexors and extensors'. *Acta Physiol. Scand.* 70: 369-388

Jankowska E, Jukes MGM, Lund S, Lundberg A, (1967b) 'The effect of DOPA on the spinal cord 6. Half-centre organisation of the flexor interneurons transmitting effects from reflex afferents'. *Acta Physiol. Scand.* 70: 389-402

Jankowska E, Lundberg A, Stuart D, (1973) 'Propriospinal control of last order interneurons of spinal reflex pathways'. *Brain Research* 535: 227-231

Janowska E, McCrea D, Mackel R, (1981) 'Oligosynaptic excitation of motoneurons by impulses in group Ia muscle spindle afferents in the cat'. *Journal of Physiology* 316: 411-425

Jankowska E, Noga B, (1990) 'Contralaterally projecting lamina VIII interneurons in the middle lumbar segments in the cat'. *Brain Research* 535: 327-330

Jankowska E, Riddell JS, (1994) 'Interneurons in pathways from group II muscle afferents in sacral segments of the feline spinal cord'. *Journal of Physiology* 475.3: 455-468

Johnson MA, Polgar J, Weightman D, Appleton D, (1973) 'Reference data on the distribution of fibre types in 36 human muscles: An autopsy study'. *Neurological Science* 18,1: 111-129

Keppel G, Saufley Jr JH, Tokunaga H, (1992) Introduction to design and analysis. WH Freeman and Co. New York

Koceja DM, Kamen G (1992) 'Contralateral influences on triceps surae motoneuron excitability'. *Electroencephalography and clinical Neurophysiology* 85: 177-182

Lloyd DPC, (1943) 'Conduction and synaptic transmission of the reflex response to stretch in spinal cats'. *Journal of Neurophysiology* 6: 317-326

Lloyd DPC, Chang HT, (1948) 'Afferent fibres in muscle nerves'. *Journal of Neurophysiology* 11: 199-208

Magladery JW, McDougal Jr DB, (1950) 'Electrophysiological studies of nerve and reflex activity in normal man. I. Identification of certain reflexes in the electromyogram and the conduction velocity of peripheral nerve fibres'. Bulletin of The Johns Hopkins Hospital 86 Baltimore

Magladery JW, Porter WE, Park AM, Teasdall RD, (1951) 'Electrophysiological studies of nerve and reflex activity in normal man. IV. The two-neurone reflex and identification of certain action potentials from spinal roots and cord'. Bulletin of The Johns Hopkins Hospital 88 Baltimore

Marchand-Pauvert V, Simonetta-Moreau M, Pierrot-Deseilligny E, (1999) 'Cortical control of spinal pathways mediating group II excitation to human motoneurons'. Presented at the joint meeting of the Societe des Neurosciences and the British Neuroscience Society in Paris

Marque P, Pierrot-Deseilligny E, Simonetta-Moreau M, (1996) 'Evidence for excitation of the human lower limb motoneurons by group II muscle afferents'. Experimental Brain Research 109:357-360

Masland WS, (1972) 'Facilitation during the H-reflex recovery cycle'. Archives of Neurology 26: 313-319

Matthews PBC, (1972) Mammalian Muscle Receptors and their Actions. Edward Arnold Ltd. London (Monographs of the Physiological Society)

McCrea DA, (1992) 'Can sense be made of spinal interneuron circuits?'. Behavioural and Brain Sciences 15: 633-643

McPhedran AM, Wuerker RB & Henneman E, (1965) 'Properties of motor units in a homogenous red muscle (soleus) of the cat'. Journal of Neurophysiology 28: 71-84

Mendell LM, Henneman E, (1971) 'Terminals of single Ia fibres: location, density and distribution within a pool of 300 homonymous motoneurons'. Journal of Neurophysiology 34: 171-187

Meunier S, Pierrot-Deseilligny E, Simonetta M, (1993) 'Pattern of monosynaptic heteronymous Ia connections in the lower limb'. Experimental Brain Research 96: 534-544

Meunier S, Pierrot-Deseilligny E, Simonetta-Moreau M, (1994) 'Pattern of heteronymous recurrent inhibition in the human lower limb'. *Experimental Brain Research* 102: 149-159

Miles TS, Turker KS, Le TH, (1989) 'Ia reflexes and EPSPs in human soleus motoneurons'. *Experimental Brain Research* 77: 628-636

Milner-Brown HS, Stein RB, (1975) 'The relation between the surface electromyogram and muscle force'. *Journal of Physiology* 246: 549-569

Mineva A, Dushanova J, Gerilovsky L, (1993) 'Similarity in shape, timing and amplitude of H- and T-reflex potentials concurrently recorded along the broad skin area over soleus muscle'. *Electromyography and Clinical Neurophysiology* 33: 235-245.

Mongia SK, (1972) 'H-reflex from quadriceps and gastrocnemius muscles'. *Electromyography and Clinical Neurophysiology* 12: 179-190

Morin C, Pierrot-Deseilligny E, Hultborn H, (1984) 'Evidence for presynaptic inhibition of muscle spindle Ia afferents in man'. *Neuroscience Letters* 44: 137-142

Nadeau M, Vanden-Abee J, (1988) Maximal H- and M-responses of the right and left gastrocnemius lateralis and soleus muscles '. *Electromyography and clinical Neurophysiology* 28: 307-311

Nardone A, Corra T, Schieppati M, (1990) 'Different activations of the soleus and gastrocnemii muscles in response to various types of stance perturbation in man'. *Experimental Brain Research* 80: 323-332

Nardone A, Schieppati M, (1998) Medium-latency response to muscle stretch in human lower limb: estimation of conduction velocity of group II fibres and central delay'. *Neuroscience Letters* 249:29-32

Noga BR, Bras H, Jankowska E, (1992) 'Transmission from group II muscle afferents is depressed by stimulation of locus coeruleus/subcoeruleus, Kolliker-Fuse and raphe nuclei in the cat'. *Experimental Brain Research* 88: 502-516

Paillard J, (1955) 'Reflexes et regulations d'origine proprioceptive chez l'homme'. PhD Thesis, Arnette: Paris

Paillard J, (1959) 'Functional organization of afferent innervation of muscle studied in man by monosynaptic testing'. *American Journal of Physical Medicine* 38: 239-247.

Perl ER, (1957) 'Crossed reflexes of cutaneous origin'. *American Journal of Physiology* 188: 609-615

Perl ER, (1958) 'Crossed reflex effects evoked by activity in myelinated afferent fibres of muscle'. *Journal of Neurophysiology* 21: 101-112

Perl ER, (1959) 'Effects of muscle stretch on excitability of contralateral motoneurons.' *Journal of Physiology* 145: 193-203

Pierrot-Deseilligny E, Bergego C, Mazieres L, (1983) 'Reflex control of bipedal gait in man' in Motor Control Mechanisms in Health and Disease. Ed. Desmedt JE, Raven Press, New York 1983

Pierrot-Deseilligny E, Morin C, Bergego C, Tankov N, (1981) 'Pattern of group I fibre projections from ankle flexors and extensor muscles in man'. *Experimental Brain Research* 42: 337-350

Piper H, (1912) 'Die Aktionsstome menschlicher Muskeln. Die Metodik der Untersuchung am Seitengalvanometer und die Prinzipien der Stromkurvenanalyse. Typenunterschiede der Willkurkontraktion.' *Z. biol. tech. Methode*. 3: 52

Prochazka A, (1989) 'Sensorimotor gain control: a basic strategy of motor systems?'. *Prog. Neurobiol* 33: 281-307

Proske U, Waite PME, (1976) 'Properties of types of motor units in medial gastrocnemius muscle of the cat'. *Brain Research* 67: 89-101

Redman S, Walmsley B, (1981) 'The synaptic basis of the monosynaptic stretch reflex'. *Trends in Neuroscience* 4: 248-251

Redman S, Walmsley B, (1983) 'Amplitude fluctuations in synaptic potentials evoked in cat spinal motoneurons at identified group Ia synapses'. *Journal of Physiology* 343: 135-145

Robinson KL, McIlwain JS, Hayes KC, (1979) 'Effects of H-reflex conditioning upon the contralateral alpha motoneuron pool'. *Electroencephalography and Clinical Neurophysiology* 46: 65-71

Rosenberg ME, (1966) 'Responses of extensor motoneurons to stimulation of ipsilateral and contralateral cutaneous nerves'. *Journal of Physiology* 185: 21-22P

Rosenberg ME, (1970) 'Synaptic connexions of alpha extensor motoneurons with ipsilateral and contralateral cutaneous nerves'. *Journal of Physiology* 207: 231-255

Rossignol S, Gauthier L, (1980) 'An analysis of mechanisms controlling the reversal of crossed spinal reflexes'. *Brain Research* 182: 31-45

Rudomin P, (1990) 'Presynaptic inhibition of muscle spindle and tendon organ afferents in the mammalian spinal cord'. *Trends in Neuroscience* 13, No. 12

Schieppati M, (1987) 'The Hoffmann reflex: A means of assessing spinal reflex excitability and its descending control in man'. *Progress in Neurobiology* 28: 345-376

Schomburg ED, (1990) 'Spinal sensorimotor systems and their supraspinal control'. *Neuroscience Research* 7: 265-340

Scott JG, Mendell LM, (1976) 'Individual EPSPs produced by single triceps surae Ia afferent fibers in homonymous and heteronymous motoneurons'. *Journal of Neurophysiology* 39, 4: 679-692

Sherrington CS, (1910) 'Flexion - reflex of the limb, crossed extension reflex and reflex stepping and standing'. *Journal of Physiology* 40: 28-121

Simon JN, (1962) 'Dispositif de contention des electrodes de stimulation pour l'etude du reflexe de Hoffmann chez l'homme'. *Electroenceph. and clinical Neurophysiology* 22: 174-176

Simonetta-Moreau M, Marque P, Marchand-Pauvert V, Pierrot-Deseilligny E, (1999) 'The pattern of excitation of human lower limb motoneurons by probable group II afferents'. *Journal of Physiology* 517: 287-300

Spaulding SJ, Hayes KC, Harburn KL, (1987) 'Periodicity in the Hoffmann reflex recovery curve'. *Experimental Neurology* 98: 13-25

Taborikova H, Sax DS, (1968) 'Motoneurone Pool and the H-reflex'. *Journal of Neurology, Neurosurgery and Psychiatry* 31:354-361

Tax AAM, Duysens J, Trippei M, Dietz V, (1990) 'Gating of contralateral reflex responses in biceps femoris during human locomotion'. 107-110 In Disorders of posture and gait. Ed. Brandt T, Paulus W, Bles W, Dieterich M, Krafczyk S, Straube A. Publ. Georg Thieme Verlag Stuttgart-New York

Turker KS, (1993) 'Electromyography. Some methodological problems and issues'. *Physical Therapy* 73: 698-710

Veale JL, Rees S, Mark RF, (1973) 'Renshaw cell activity in normal and spastic man'. *New Developments in Electromyography and Clinical Neurophysiology* Ed.Desmedt JE 3: 523-537

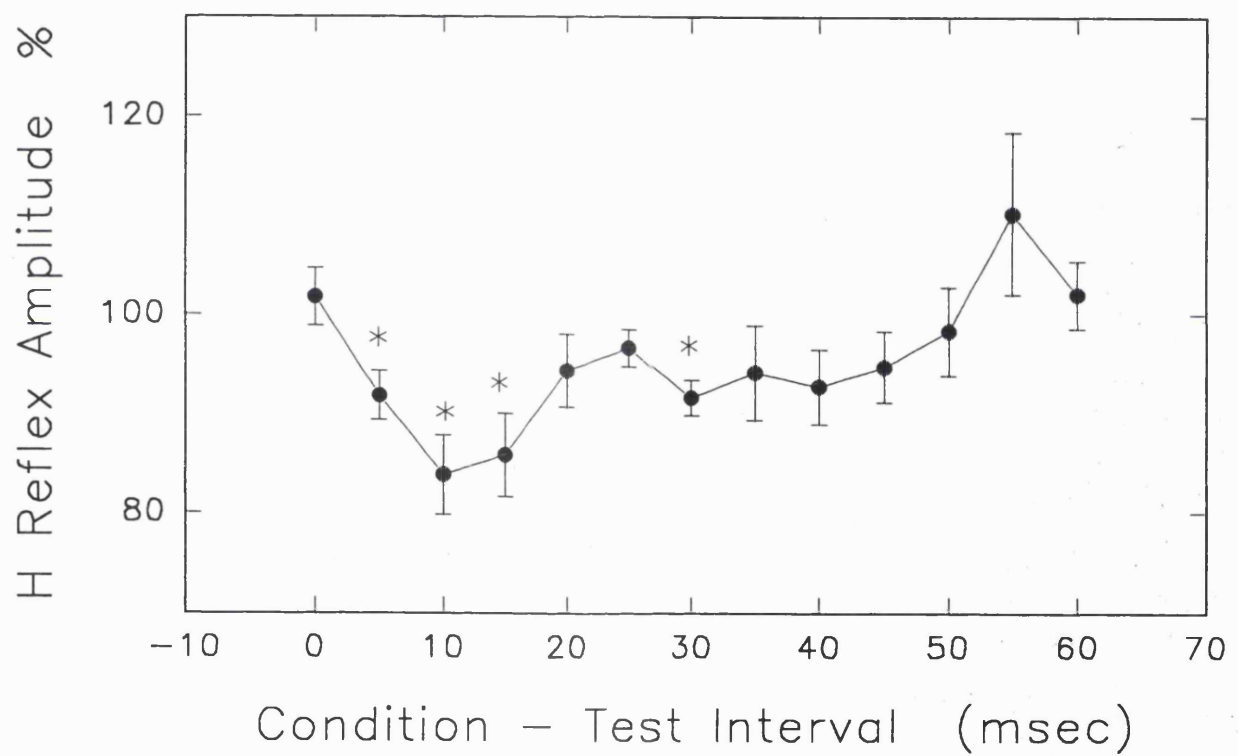
Walmsley B, Edwards FR, Tracey DJ, (1987) 'The probabilistic nature of synaptic transmission at a mammalian excitatory central synapse'. *Journal of Neuroscience* 7, 4: 1037-1046

Williams LRT, Sullivan SJ, Seaborne DE, Morelli M, (1992) 'Reliability of individual differences for H-reflex recordings'. *Electromyography and clinical Neurophysiology* 32: 43-49

Wuerker RB, McPhedran AM, Henneman E, (1965) 'Properties of motor units in a heterogonous pale muscle (M. gastrocnemius) of the cat'. *Journal of Neurophysiology* 28: 85-99

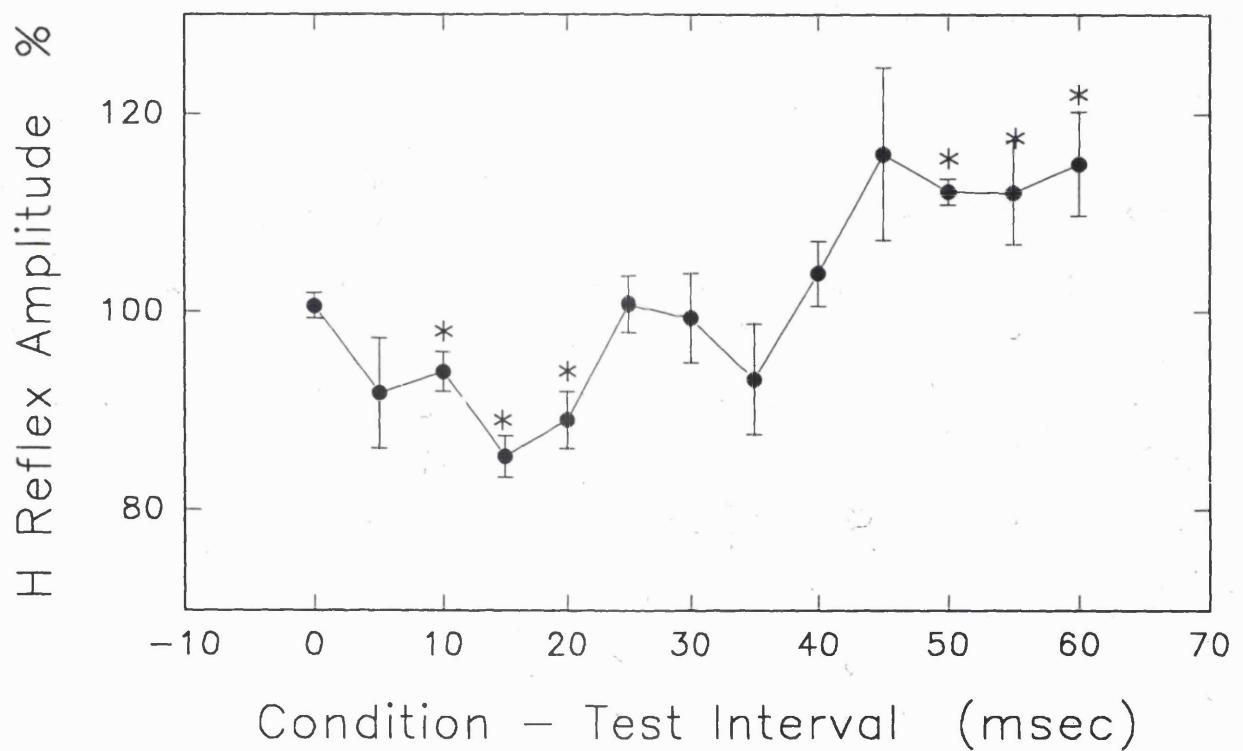
Appendix I

Conditioning stimulus: 2T



EXAMPLES OF SOME RESULTS FROM THE PRELIMINARY
CROSSED EXPERIMENTS

Conditioning stimulus: 3T



EXAMPLES OF SOME RESULTS FROM THE PRELIMINARY
CROSSED EXPERIMENTS

Appendix II

Crossed proprioceptive reflexes in the human leg

P.J. Harrison, P. Evans, S. Greenslade and R. McCabe

*Department of Physiology, University College London,
Gower Street, London WC1E 6BT*

It is to be expected that crossed reflex actions are important in the co-ordination of the two legs during normal stepping and standing. However, despite a number of recent animal studies (Harrison & Zytnicki, 1984; Harrison *et al.* 1986; Bajwa *et al.* 1992) there is a remarkable lack of information regarding crossed reflexes in man. In the present investigation, following approval by the local ethical committee, crossed reflex actions were observed in healthy human subjects by observing the modulation of the ipsilaterally evoked H reflex from the gastrocnemius or from the soleus muscle, in response to stimulation of the tibial nerve contralaterally.

Stimulating and recording electrodes were sited so as to record H reflexes both ipsilaterally and contralaterally. The ipsilateral reflex was then observed while varying the stimulus strength to the contralateral electrodes and while varying the interval with which the contralateral (conditioning) stimulus preceded the ipsilateral (test) stimulus. The stimulation sequences were varied to allow responses evoked by the test stimulus preceded by a conditioning stimulus to be interleaved with responses evoked by the test stimulus alone. The two sets of data were then averaged separately for analysis. Successful recordings were obtained from six voluntary subjects.

Conditioning stimulation at two times nerve threshold produced an inhibition of the gastrocnemius H reflex. This inhibition was significant with a conditioning-test interval as short as 5 ms ($P = 0.02$, *t* test) and was maximal with an interval of 10 ms ($P = 0.001$). Conditioning stimulation at three times threshold produced, in addition to the inhibition, a significant facilitation of the H reflex, albeit at longer conditioning-test intervals (40 ms and greater) (cf. Kocaja & Ramen, 1992).

From these results it is evident that crossed reflexes are operative in normal awake individuals. At present it is uncertain whether the short latency inhibition is due to group I or to group II afferent fibres. The later facilitatory response is presumably due to the recruitment of smaller diameter fibres, possibly of group II or group III afferent origin.

Supported by the MRC.

REFERENCES

- Bajwa, S., Edgley, S.A. & Harrison, P.J. (1992). *J. Physiol.* **455**, 205–217.
- Harrison, P.J., Jankowska, E. & Zytnicki, D. (1986). *J. Physiol.* **371**, 147–166.
- Harrison, P.J. & Zytnicki, D. (1984). *J. Physiol.* **356**, 263–273.
- Kocaja, D.M. & Ramen, G. (1992). *EEG & Clin. Neurophys.* **85**, 177–182.

The inherent variability of the soleus H reflex in man

P. Evans, C. Willars and P.J. Harrison

Department of Physiology, University College London, Gower Street, London WC1E 6BT

The H reflex is commonly used as a tool for inferring synaptic inputs to motoneurons. However, the H reflex is subject to some inherent variability which sets a limit on the usefulness of the technique. The aim of the present experiments is to assess this variability.

H reflexes were recorded from the soleus muscle with surface electrodes, while stimulating with a monopolar electrode placed in the popliteal fossa overlying the tibial nerve. The stimulus strength was varied over a range that allowed the H reflex recruitment curve to be plotted. Thus as the stimulus strength is increased, the H reflex amplitude increases up to the threshold for activation of motor axons (the ascending limb of the recruitment curve). Further increases in stimulus strength lead to a decline in H reflex amplitude while the M response amplitude increases (the descending limb of the recruitment curve). At each stimulus strength, a sequence of up to 200 responses was recorded, digitized and stored for off-line analysis.

While the M response remained relatively constant, the H reflex clearly varied. This variability was observed to change systematically with changing stimulus strength. Thus during the ascending limb of the recruitment curve, as the stimulus strength was increased (causing the H reflex amplitude to increase) the variability of the H reflex amplitude also increased. When the variability was expressed in relation to the average H reflex amplitude (standard deviation as a percentage of the mean), this variability was at a minimum when the H reflex amplitude was at a maximum. This suggests that when using the modulation of the amplitude of the H reflex to assess the efficacy of synaptic inputs to motoneurons, a greater degree of precision will be achieved when the stimulus strength is adjusted to produce a maximum amplitude H reflex. This finding should be considered in relation to the findings of Crone *et al.* (1990), who described that the largest modulation of the H reflex occurs when the test H reflex amplitude is about one-third of the maximum M response amplitude. At this amplitude, the inherent variability of the H reflex may mask any underlying reflex modulation of the H reflex evoked by a conditioning stimulus. Clearly therefore, in studies using the H reflex to assess the efficacy of putative synaptic inputs to motoneurons, there will be a trade-off in the choice of the test H reflex amplitude in achieving the maximum absolute reflex modulation with the minimum inherent variation of the test reflex.

REFERENCE

- Crone, C., Hultborn, H., Mazieres, L., Morin, C., Nielsen, J. & Pierrot-Deseilligny, E. (1990). *Exp. Brain Res.* 81, 35–45.

P.078.19

CROSSED REFLEXES EVOKED FROM A VARIETY OF
PERIPHERAL NERVES IN HUMANS

Evans, P. & Harrison, P.J .

Department of Physiology, University College London, Gower Street,
London WC1E 6BT, U.K.

Studies in cats have located a group of interneurons in lamina VIII of the midlumbar and lower lumbar segments of the spinal cord which project to contralateral motoneurons (Harrison, Jankowska & Zytnicki, 1986, *J.Physiol* 371, 147-166; Jankowska & Noga, 1990, *Brain Res* 535, 327-330). In addition, there are a group of midlumbar interneurons which project to ipsilateral motoneurons yet receive input from contralateral afferents (Bajwa, Edgley & Harrison, 1992, *J.Physiol* 455, 205-217). Such interneurons are likely to mediate crossed reflexes from group I and group II afferents and are expected to be involved in interlimb coordination. Indeed, observations of crossed reflexes in cats indicate that these reflexes can be accounted for on the basis of the pattern and strength of the input to these interneurons alone (Arya, Bajwa & Edgley, 1991, 444, 117-131).

In order to relate these findings to the control of human movement, we have investigated crossed reflex pathways in humans from a variety of peripheral nerves. H reflexes of soleus and quadriceps were conditioned by electrical stimulation of various contralateral nerves. In most cases an early inhibition was seen, which, allowing for differences in the conduction delay due to differences in the conduction path, would indicate consistent crossed inhibition from a group I source. Longer latency actions were sometimes observed which may be attributed to the action of group II fibres.

H reflex; crossed reflex, muscle afferents

Abstract 84.9

**CROSSED INHIBITION BETWEEN QUADRICEPS MUSCLES
IN THE HUMAN LOWER LIMB**

**EVANS, P., HARRISON, P.J*, ROBERTS, A., Dept. of Physiology,
University College London, Gower Street, London WC1E 6BT, U.K.**

Crossed reflex actions are likely to be integral to the coordination of the lower limbs during standing and walking. Studies in cats have revealed midlumbar and lower lumbar interneurons which project to contralateral motoneurons and midlumbar interneurons which receive bilateral input from group II afferents (see Bajwa et al. 1992 J.Physiol. 455, 205-217).

In the light of these findings we have been investigating crossed reflexes in humans. Using electrical stimulation of a number of peripheral nerves we have previously looked at the effects of conditioning stimuli upon the contralateral H reflex of soleus. Most recently we have examined the effects of conditioning the H reflex of a more proximal muscle, quadriceps, via stimulation of the contralateral femoral nerve. Preliminary studies reveal a powerful inhibition at a latency which can only be explained by a pathway involving fast conducting afferents in the group I range. This finding is in keeping with results from other crossed reflexes we have examined in humans including those between the posterior tibial muscles (Harrison et al. 1994 J.Physiol 479, 28P), but is in contrast to a number of animal studies in which strong group II crossed effects have been revealed, but in which the group I crossed effects have been weak or absent.