RESPONSE OF SINGLE SPINAL MOTONEURONES TO TRANSCRANIAL MAGNETIC STIMULATION IN HEALTHY SUBJECTS AND PATIENTS WITH UPPER MOTOR NEURONE DISORDERS

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Abstract

The problem addressed by this study was: How does the human corticospinal tract influence the discharge of spinal motoneurones and what are the effects of neurological disease? The method employed was to study the firing probability of 78 tonically active single motor units of the upper limb following transcranial magnetic stimulation. This was performed in healthy subjects and in a group of patients with different upper motor neurone (UMN) disorders. The inducing current flowed in an anticlockwise direction through a circular coil which was positioned tangentially at the vertex.

Two peaks were produced in the peri-stimulus time histogram. The primary peak (PP) had an onset latency in healthy subjects ranging from 13 ms (deltoid and biceps) to 31 ms (first dorsal interosseous muscle) (FDI) and had a short duration of 4.6 ±1.7 ms (mean ± SD). PP frequently consisted of 1-3 sub-peaks, with a mean intermodal interval of 1.4 ms for FDI and 2.9 ms for forearm and upper arm muscles. This interval probably reflects the maximal rise time of one in a sequence of excitatory postsynaptic potentials (EPSPs) at the motoneurone. An increase either in the interval between the stimulus and the preceding voluntary discharge, or in the intensity of stimulation, raised the probability of discharges occurring within PP and influenced their latency.

The secondary peak (SP) had an onset latency in FDI ranging from 56-90 ms and a long duration of 20.9 ±12.0 ms. Evidence suggests that SP was caused by the rising phase of a late EPSP mediated via a pathway which included a peripheral afferent component.

When compared with healthy subjects, PP in UMN patients was found to be either normal, absent, delayed and dispersed (by up to 28 ms and 21 ms, respectively) or found to consist of sub-peaks with abnormally long inter-modal intervals. These findings suggest specific mechanisms including cortical inexcitability, variable degrees of slowing in the velocity of propagation in descending fibres, frequency dependent conduction block, delay between EPSPs caused by the operation of more than one pathway and ineffective spatial or temporal summation at the spinal motoneurone.

To my family

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Abbreviations Used in the Text

ADM	Abductor Digiti Minimi
AHP	After Hyperpolarization
CMAP	Compound Muscle Action Potential
CMC	Central Motor Conduction
CMN	Corticomotorneurone
CST	Corticospinal Tract
EDC	Extensor Digitorum Communis
EMG	Electromyogram
EPSP	Excitatory Postsynaptic Potential
FCU	Flexor Carpi Ulnaris
FDI	First Dorsal Interosseous Muscle
INTH	Interval Histogram
IPSP	Inhibitory Postsynaptic Potential
ISI	Interspike Interval
MND	Motor Neurone Disease
MS	Multiple Sclerosis
MUP	Motor Unit Potential
Non PP/SP-trial	Trial without a discharge at either the PP or SP latency
PP	Primary Peak
PP discharge	Discharge which occurs at the PP latency
PP-trial	Trial containing a discharge at the PP latency
PSTH	Peri-Stimulus Time Histogram
PT	Pyramidal Tract
PTN	Pyramidal Tract Neurone
SD	Standard Deviation
SP	Secondary Peak
SP-trial	Trial containing a discharge at the SP latency
T	Tesla (a magnetic field of 1T in magnitude has a flux density such that the force on a conductor 1 m long, placed perpendicular to the field and carrying a current of 1 A, is 1 Newton).
TA	Tibialis Anterior
TE	Transcranial Electrical
TES	Transcranial Electrical Stimulation
TM	Transcranial Magnetic
TMS	Transcranial Magnetic Stimulation
UMN	Upper Motor Neurone

CHAPTER 1

Introduction

Aims and Scope of the Enquiry

The problem addressed by this study was: How does the human corticospinal tract influence the discharge of spinal motoneurones and what are the effects of neurological disease?

The aim of the enquiry was to characterise the response of single human motoneurones to a corticospinal input and to identify abnormalities in disorders of the upper motor neurone (UMN) by comparison with the normative data. The specific objectives were to:

1) Estimate the expected time course of the rising phase of the excitatory postsynaptic potentials (EPSPs) underlying the peaks of firing probability in the peri-stimulus time histogram (PSTH), and test the effect of changes in the experimental parameters.

2) Describe the late changes in the PSTH and deduce their origin.

3) Test the expected mode of summation of the motoneurone's interspike membrane potential with the EPSP by studying the effect of factors which influence the probability and latency of evoking a discharge.

4) Examine the approach to the statistical comparison of interspike intervals and the implications for experimental design.

5) Identify neural mechanisms which contribute to the clinical effects of UMN lesions by performing parallel studies on a group of patients with neurological disease, using data from the above as the control.

My Contribution

I worked on this project as MRC Training Fellow, University Department of Clinical Neurology and William Carleton Gibson Junior Research Fellow, Green College, Oxford, from August 1988 until the date of submission. The work described in this study represents my contribution to the research of the Unit of Clinical Neurophysiology. This involved: 1) Designing and conducting the experiments, 2) Recruitment of patients and healthy subjects, 3) Clinical assessment of the patients, 4) Evaluation of the raw data, 5) Analysis of the data.

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In the Introduction, the background to this enquiry is developed by examining the descending pathways to spinal motoneurones and the postsynaptic potentials that are produced. This is followed by a discussion of the pathophysiology of the UMN syndrome and the principles of transcranial magnetic stimulation (TMS). The data and inferences concerning the healthy subjects and patients with UMN disorders are described in the Results and Discussion. The first section of the Introduction briefly reviews some of the historical developments in techniques that have been used for stimulation of the human brain.

Historical Developments

In the last decade, the development by Merton, Morton and others of modern methods for transcranial electrical stimulation (TES) (Gualtierotti and Paterson, 1954; Merton and Morton, 1980a,b; Rothwell et al., 1987) has laid the foundation for subsequent work utilising TMS. In 1985, Barker and colleagues reported the use of a pulse of magnetic field applied through the skull, which produced a twitch and a compound muscle action potential (CMAP) in forearm flexor muscles (Barker et al., 1985). The time varying magnetic field was induced by a large pulse of current (peak 4000 A at 110 μs) passed through a flat coil resting tangentially on the subjects' head over the motor area. The following year, Mills and Hess using needle electrodes during weak voluntary contractions demonstrated that the lowest threshold motor units (MUs) in human hand muscles could be selectively activated by the same technique (Hess and Mills, 1986a).

Stimulation of the human nervous system by a magnetic field was first reported, however, in 1896 (d'Arsonval, 1896). It was found that the retina

could be stimulated to produce the impression of light (phosphenes). These original experiments were later extended (Thompson, 1910 and Lovsund et al., 1980). Much larger stimuli were needed to exceed the threshold for peripheral nerve stimulation, and it was not until 1965 that Bickford and Flemming reported the production of muscle contractions using a 500 Hz sinusoidal magnetic field, peaking at 4 Tesla (T) and decaying to approximately 0.4 T after 20 ms (Bickford and Flemming, 1965). Interference from the magnetic stimulator, however, prevented the recording of a nerve or muscle action potential. Seventeen years later, Polson circumvented this problem and reported the production of a recordable muscle action potential (Polson et al., 1982).

Electrical stimulation of the human brain was first reported in the early Nineteenth Century. Following experiments by Fontana and Caldani in the late Eighteenth Century, in which convulsions had been produced by the application of electricity to the brain in frogs, it was Aldini (Galvani's nephew) who was the first to experiment with electroshock in man (Aldini, 1804). By experimenting on the heads of criminals obtained from the guillotine, he found that by passing an electric current through the ear and mouth he could evoke facial grimaces. The less the interval between decapitation and stimulation, the greater was the response. In living subjects, including himself, he used voltaic piles to produce a stimulating current applied to both ears, or one ear and the mouth, or on the forehead and nose.

Seventy years later, after further animal work by Fritsch, Hitzig and Ferrier, Dr Robert Bartholow, who was the Professor of Materia Medica and Therapeutics and of Clinical Medicine in Ohio, reported his findings on intracranial electrical stimulation of the human brain (Bartholow, 1874). His

subject was a 30 year old inpatient at the Good Samaritan Hospital with a malignant ulcer of the scalp of two inches in diameter, which had eroded the vault in the mid parietal region. When the dura mater on the left of the midline was stimulated with an insulated electrode "distinct muscular contractions occurred in the right arm and leg. The arm was thrown out, the fingers extended, and the leg was projected forward. The muscles of the neck were thrown into action, and the head was strongly deflected to the right." Stimulation to the right of the midline deflected the head strongly to the left and produced contractions in the extensors of the left arm and leg. When the stimulating needle was passed into the left posterior lobe "muscular contraction of the right upper and lower extremities ensued, as in the preceding observations. Faint but visible contraction of the left orbicularis palpebrarum, and dilatation of the pupils, also ensued. Mary complained of a very strong and unpleasant feeling of tingling in both right extremities, especially in the right arm, which she seized with the opposite hand and rubbed vigorously." Stimulation of the right posterior lobe produced the same effects in the left arm and leg and in the right orbicularis palpebrarum and pupils. At autopsy it was found that the electrode on the left side had entered the upper parietal lobule of Ecker, the gyrus centralis posterior of Henle and the posterior parietal lobule of Turner, one inch from the longitudinal fissure, penetrating to a depth of one inch. On the right the needle had passed through the same structures but more posteriorly, one and a half inches from the longitudinal fissure and penetrating to a greater depth of one and a half inches. Sixty three years later, during electrical stimulation of the cortical surface for the localisation of electrogenic foci under local anaesthetic, movements were evoked in the human hand and elsewhere (Penfield and Boldery, 1937). Finger movements were among the most localized of responses in the study. The hand is also preferentially accessible with TMS. Responses were obtained by stimulation of points

extending 5.5 cm along the Fissure of Rolando, mostly within 1 cm of the fissure. Of the 102 recorded points, 77 were precentral and 25 were postcentral. Movement of all the fingers together was the most common response.

Recent studies have made further observations on the mechanisms of human motor control. The following sections of the Introduction develop the framework for the present study.

Descending Pathways

Organization of the Corticospinal Tract

The human corticospinal tract (CST) has 3 anatomical divisions: the crossed lateral CST, the uncrossed lateral CST and the anterior CST. There are approximately one million fibres in each human pyramid (De Myer, 1959; Lassek and Rasputin, 1939) of which 60-94% are myelinated (De Myer, 1959; Lassek, 1942). In area 4 there are 25,000-30,000 Betz cells (Campbell, 1905; Lassek, 1940) and it is therefore estimated that 3-4% of pyramidal fibres are the axons of Betz cells. The majority of fibres in the pyramid arise from other cells in the precentral gyrus (Brodal, 1981) and are known as pyramidal tract neurones (PTNs) (Phillips and Porter, 1977). In the monkey, area 6 (pre-motor cortex), areas 1, 2 and 3 (primary sensory cortex), and areas 5 and 7 of the parietal cortex, and the supplementary motor area, all contribute axons to the pyramids (see York, 1987).

PTNs are arbitrarily divided into two populations "large" rapidly conducting PTNs and "small" slowly conducting PTNs. The conduction velocity dividing these two populations is arbitrarily set at 20 m/sec in the cat (Phillips and Porter, 1977). In the monkey, the majority of PTNs conduct slowly with a mode of 8-12 m/sec and a minority of PTNs conduct rapidly with a mode of 50-55 m/sec (Humphrey and Corie, 1978). A proportion of the latter make monosynaptic corticomotoneuronal (CMN) connections with spinal motoneurones in the monkey, particularly in distal forelimb muscles (Bernhardt and Bohm, 1954). It is the rapidly conducting PTNs that are thought to transmit the earliest effects of TMS (see following section on Magnetic Stimulation of the Human Brain). In humans, it is estimated that 90% of pyramidal fibres range from 1-4 μm in diameter and only 1.7% are

11-22 μm in diameter (Lassek, 1942). The latter would correspond to the fast PTNs of the monkey.

Connections of Corticospinal Neurones

The connections of corticospinal neurones are widespread (see Asanuma, 1981). In addition to the inputs to motoneurones described above, there is a disynaptic inhibitory input via the Ia interneurone (see Inhibitory Processes, below) and synapses with gamma motor neurones, Renshaw cells, blood vessels (for vasodilation), and also collaterals to other cortical areas. In the baboon, 50% of gamma motoneurones receive monosynaptic excitation (in addition to an inhibitory input) from CS neurones (Grigg and Preston, 1971). Inputs to the PT itself include those from other cortical areas, the commissure and afferents from the periphery.

Anatomical evidence for the presence of direct CMN synapses was produced by silver staining of degenerated axons in monkeys (Kuypers, 1960; Liu and Chambers, 1964) and later in man (Schoen, 1969). Their functional importance probably relates to the provision for fractionation of movements particularly of the hand and fingers (Lawrence and Kuypers, 1968; Lawrence and Hopkins, 1976). In a combined histological and electrophysiological experiment in the monkey, Lawrence and colleagues have found evidence to suggest that each principle collateral of a CMN axon made only a very few direct CMN synapses - possibly only one. It was noted that this was consistent with the small amplitudes of minimal and unitary CMN EPSPs evoked in forelimb and hand spinal motoneurones by cortical stimulation (Lawrence et al., 1985). Seven CMN synapses were identified by light microscopy which were located on the dendrites of spinal motoneurones at 40 to 750 μm from the soma, ranging in size from 0.6x3.0 μm to 2.4x3.6 μm. They arose from the main collaterals of PTNs as they

arborised in a dominantly longitudinal fashion within lamina IX. Projections from the motor cortex (labelled with either wheatgerm, lecithin, horse radish peroxidase or ^{3}H proteins) were found to extend from the border of the IIIrd and IVth contralateral laminae to the motor nucleus in lamina IX. There was also a dense ipsilateral projection to lamina VIII and a minor projection to ipsilateral V and VI. Of these, all the labelled axons were myelinated. Synaptic contacts were found on the soma of recipient motoneurones, and small, medium and large proximal dendrites. Interestingly, some CST axons exhibited polymorphic or flattened vesicles, which have been associated with inhibitory transmitters (Uchizono, 1965). This raises the possibility of a direct inhibitory CMN connection.

In the monkey, surface and intracortical micro-stimulation has demonstrated a complex topographical arrangement with overlapping focal representations for agonists, synergists and antagonists (Jankowska et al., 1975b). This is further complicated by the existence of spatially separated multiple foci for a give muscle (Strick and Preston, 1978). Indeed, in relaxed finger extensor muscles, stimulation with a double coil positioned over the arm representation in the antero-posterior axis, single MUs could be activated from scalp fields that were elongated in the antero-posterior axis and which overlapped one another (Amassian et al., 1989). It was also thought possible to activate different MUs differentially by stimulation at different scalp sites. In the monkey, EPSPs in spinal motoneurones produced by surface cortical stimulation also decrease in size from an optimum position as the electrode is moved (Landgren et al., 1962b; Phillips, 1967). Using intra-axonal injection of horseradish peroxidase in the monkey, however, there is also evidence that a single CS axon terminates in up to four motor nuclei (Shinoda et al., 1981). Using spike-triggered averaging of the EMG, a PTN has been found to be statistically

related to several muscles (Fetz and Cheney, 1978). Such divergence of collaterals to different motoneurone pools has been confirmed using antidromic stimulation in the cat (Shinoda et al, 1986). Some direct CMN connections active during precision grip, however, may be restricted to a single motor nucleus, particularly within individual motor nuclei of the muscles that move the fingers (Muir and Lemon, 1983; Buys et al., 1986). This convergence is confirmed by the demonstration of incremental depolarisations up to a maximum of a few mV upon the recruitment of CMNs (Clough et al, 1968).

Other Descending Tracts

Spontaneous EPSPs generated by the rubrospinal tract of the rhesus monkey range from 0.2 to 0.6 mV in amplitude (Shapovalov et al., 1971) with compound EPSPs of less than 1.2 mV, suggesting a restricted convergence of different fibres from the red nucleus of a single motoneurone. Membrane depolarization measurements have indicated that terminals of rubrospinal fibres are probably located at similar distances from the soma as Ia terminals (Shapovalov and Kurchavyi, 1974). The relatively small size of compound EPSPs in the uncrossed reticulospinal pathway also indicates a relatively limited degree of convergence on spinal motoneurones in the cat (Shapovalov and Gurevich, 1970). The vestibulo-spinal tract has two uncrossed descending components: the lateral vestibular spinal tract originating from the lateral vestibular (Deiters) nucleus and the medial vestibulo-spinal pathway originating from the medial vestibular nucleus. The lateral system has a strong excitatory projection to motoneurones of the neck and hind limb extensor muscles, although an equivalently strong excitatory projection to the forearm muscles is lacking (Wilson and Yoshida, 1969). The lateral vestibulo-spinal tract

also makes monosynaptic and polysynaptic connections, which are mostly ipsilateral in the cat (Nyberg-Hansen and Mascitti, 1964).

It is likely that pathways other than the rapidly conducting component of the CST are activated by TMS. These may contribute to late phases of the CMAP (see section on Mechanism of TMS).

Postsynaptic Changes in the Spinal Motoneurone

The CST and other descending pathways initiate and control the discharge of motoneurones via the generation of postsynaptic potentials (PSPs). The nature of PSPs and the mode of their summation with the interspike membrane potential is therefore central to this enquiry.

Postsynaptic Potentials

PSPs are changes in the postsynaptic membrane secondary to the release of neurotransmitter. Excitatory postsynaptic potentials (EPSPs) depolarize the membrane potential towards threshold whereas inhibitory ones (IPSPs) polarize the membrane away from threshold. Compound PSPs are produced by the summation of unitary PSPs. A unitary CMN EPSP may be defined as that which results from the activation of a single CMN synapse (Phillips and Porter, 1977). In the monkey, fast conducting PTNs make excitatory CMN connections with spinal motoneurones (see above) (Bernhardt, Bohm and Peterson, 1953; Preston and Whitlock, 1960, 1961; Landgren, Phillips and Porter, 1962a). Brief surface anodal cortical stimuli therefore elicit EPSPs at monosynaptic latencies which are occasionally followed by brief IPSPs (Hearn et al., 1962; Landgren et al., 1962a). These IPSPs occur at di-synaptic latency and are transmitted by the Ia inhibitory interneurone (Jankowska et al., 1976).

Some of the general characteristics of PSPs (Hubbard, 1969) are as follows: Firstly, their amplitude is graded according to the number of pre-synaptic fibres that are activated and the amount of transmitter released at each synapse; secondly, their rise time is much faster than their decay time (the latter approximating to the time constant of the postsynaptic membrane); thirdly, at chemically mediated synapses, PSPs are generated

by ionic mechanisms which differ from those which generate action potentials (PSPs have equilibrium potentials which are related to the algebraic sum of the electromotive forces of the ions involved in their generation); and fourthly, PSPs are electrotonically conducted along dendrites, soma and axon according to the cable properties of the post-synaptic membrane, their site of origin, and their initial amplitude.

Surface electrical or intracortical micro-stimulation of the motor cortex in the monkey may evoke EPSPs in lumbar motoneurones ranging from 30 μV to 1.2 mV, with the majority falling below 200 μV (Porter and Hore, 1969; Asanuma et al., 1979). Following single pulse intracortical microstimulation in the vicinity of CMNs, the most commonly evoked postsynaptic changes in motoneurones serving small hand muscles are a combination of an EPSP and an IPSP, with a mean (+/-SD) 10-90% rise time for the EPSPs of 1.1 (+/-1.4) ms (Lemon et al., 1987).

Changes at the Postsynaptic Membrane

Experiments utilizing Ia EPSPs have demonstrated an increase in the permeability of the soma membrane to potassium, chloride and sodium ions, so that sodium moves into the cell down its electrochemical gradient. During an IPSP there is a local increase in permeability to potassium and chloride and the subsequent efflux of potassium and influx of chloride causes depolarization (see Hubbard, 1969).

In animal preparations, descending corticofugal projections probably release glutamate as an excitatory neurotransmitter. Whether glutamate or aspartate acts as the excitatory neurotransmitter at the connection between spinal motoneurones and their descending pathways, however, remains to be demonstrated (Oertel, 1989). Reticulospinal cells may be excited by

aspartate or glutamate and inhibited by glycine. Gamma-amino butyric acid (GABA) acts as a postsynaptic inhibitor of the vestibulospinal cells. Ia and Ib primary afferents probably release glutamate or aspartate as the excitatory neurotransmitter to motoneurones and the recurrent collaterals of motoneurones release acetylcholine for the excitation of Renshaw cells (see Brooks, 1986).

PSPs result from the summation of potential gradients produced by many synaptic currents occurring at different sites over the postsynaptic membrane. The resultant shift in membrane potential depends upon the size of each input (corresponding to the area beneath the PSP), its rate, its position and its polarity (Calvin, 1972). The rise time of CMN EPSPs is longer than that for Ia afferent fibres (Porter and Hore, 1969; Shapovalov, 1975). Spatial summation is dependent upon the area underneath PSPs as they reach the trigger zone following the electrotonic spread of synaptic currents from the actual input sites. The rising phase of a synaptic potential is determined by active factors (neurotransmitter action) and by passive properties of the membrane. During this phase, the membrane conductance depend on the number of sodium and potassium channels opened, which in turn depends on the concentration of neurotransmitter. The peak of a PSP occurs where the inward flow of synaptic current becomes balanced by the outward current flow through non synaptic channels. At this brief moment the membrane potential reaches a steady state. The subsequent decay of the PSP depends on passive membrane properties, the membrane time and space constants.

The membrane time constant is the product of the resistance and capacitance of the post-synaptic membrane. The longer this is, the longer the duration of the PSP will be, which increases the probability of overlap

with another PSP leading to temporal summation. The space constant (length constant) determines the distance that a potential difference can spread passively across an axon or dendrite, as shown by the rate of voltage change of a PSP over distance from its site of initiation. This affects the efficiency of electrotonic propagation. A long length constant therefore increases the probability for spatial summation from different regions of the post-synaptic membrane at the trigger zone. If a motoneurone is depolarised beyond its firing threshold then an action potential is generated from its somatic membrane or axon hillock. The latter has the lowest threshold for spike initiation. Ia EPSPs generated on distal portions of a motoneurones dendritic tree tend to be smaller in amplitude, with slower rise and decay times, when compared with EPSPs from synapses on the cell body (Rall et al., 1967). The increased resistance and membrane capacitance of distal dendrites attenuates EPSPs. This, therefore, also has an effect on the spatial and temporal summation of PSPs.

Temporal Facilitation of Corticomotoneuronal EPSPs

In the baboon, the amplitude of EPSPs in cervical motoneurones evoked by weak surface anodal stimulation of the cerebral cortex was found to be increased by the application of successive stimuli (Landgren et al., 1962a). This increase in the effectiveness of CMN activity with repetitive firing has been termed temporal facilitation. A similar increase in amplitude was also found with separate EPSPs evoked by single long current pulses. With a strong cortical stimulus generating repetitive firing, a proportion of later waves in the compound EPSP have also been found to be larger than the first wave (Kernell and Wu Chien-Ping, 1967b). With a triplet of stimuli in a few CMN fibres, the amplitude of the EPSP generated by the third volley could be facilitated by each of the preceding two volleys providing the intervals between the volleys were less than 50 ms (Muir and Porter, 1973).

Each corticospinal impulse could produce a facilitation of up to 200% and then decay with a time constant of about 10 ms. A train of impulses produced a total facilitation which approximated to a linear sum of the facilitation produced by each impulse in the train (Muir and Porter, 1973). The post-synaptic membrane potential, here influenced by preceding events, therefore influences the amplitude of EPSPs (see below). This temporal facilitation, however, is opposite to the effect that would be expected. The precise mechanism of this facilitation is unknown and it is unlikely that recruitment of CMN axons or the effect of local interneurones are responsible (see Phillips and Porter, 1977). Changes in the availability of neurotransmitter, however, may be one factor. Temporal facilitation may possibly play a role in the initiation of natural movement (Porter, 1970).

Effect of Membrane Potential on PSP Amplitude

Depolarization of the postsynaptic membrane from its resting potential of approximately -70 mV decreases the amplitude of a Ia EPSP and increases the amplitude of an IPSP (Combs et al., 1955a). Reversal occurs in the region of 0 mV for an EPSP (Finkel and Redman, 1983). Excitatory synaptic activity therefore tends to drive the membrane potential away from its resting level past threshold (about -59 mV at the axon hillock) in the direction of its reversal potential. If the post-synaptic membrane is then polarised, the IPSP amplitude decreases towards its reversal point in the region of about -60 mV. Beyond this, the IPSP is inverted but maintains its inhibitory effect if it is below the firing level for the post synaptic membrane (see Kandel and Schwartz, 1985). In tonically active spinal motoneurones, as for those in the present study, the membrane potential varies between successive discharges and therefore the size of a PSP from a given synaptic input may vary according to the timing of the input with respect to the preceding discharge of the cell. The shape of unitary Ia EPSPs,

however, may only change by a small amount over several mV of depolarization. Furthermore, the conductance near the end of an ISI approaches that at rest with a slowly firing tonically active cell (Baldissera and Gustafsson, 1974a). Under these conditions EPSPs near threshold should resemble those at rest.

Factors Controlling the Firing Threshold of a Motoneurone

The the numerous factors that control the firing threshold of a spinal motoneurone with chemical synaptic input drive has been reviewed by Burke (1981). They fall into 4 main categories: 1) factors due to the interaction between properties of the motoneurone and characteristics of synaptic terminals, which determine the polarity and amplitude of the PSP; 2) intrinsic motoneurone factors (eg. absolute voltage threshold for an action potential); 3) factors linked to the organisation of synaptic inputs, determined by the relative distribution of synaptic efficacy for each input and the pattern of activity within these inputs at any given time, and 4) the synaptic driving potential (interspike membrane trajectory), determined by the difference between the instantaneous transmembrane potential and the ionic equilibrium potential for the synaptic process. Interaction between all these factors will influence the probability of evoking a discharge in a spinal motoneurone following a magnetic stimulus.

The polarity of a PSP is determined by the nature of the pre-synaptic neurotransmitter, and the characteristics of the postsynaptic receptors and the transmembrane ionic balances. The instantaneous excitability of a spinal motoneurone is the momentary balance of excitatory and inhibitory factors. The change in firing probability of a spinal motoneurone in response to single or multiple descending volleys generated by a TM stimulus in the present study will therefore depend on the summed effects

of several factors. In addition to the effect of the continuous voluntary drive and the descending volley, these factors may include segmental inputs, which may be either excitatory (particularly the group Ia and II spindle fibres) or inhibitory (particularly the group Ib Golgi tendon organ fibres); supra segmental inputs including the CST, reticular and rubral systems; and the level of pre-synaptic inhibition of any of these inputs.

Interaction Between Synaptic Activity and Interspike Membrane Potential

The sequence of interspike changes in membrane potential in a repetitively firing spinal motoneurone is as follows: a delayed depolarisation is followed by the interspike membrane trajectory, consisting of an initial polarising scoop followed by a depolarising ramp (Calvin and Schwindt, 1971). In the primary range of the f-I curve, increasing current strength produces scoops of reduced depth. The gradient of the subsequent ramp, however, does not alter (Schwindt and Calvin, 1972). Adaptation of the firing rate in response to changes in injected current are therefore produced by changes in the length of the interspike interval (ISI) according to the depth of the initial scoop. In the secondary range of the f-I curve, the gradient of the ramp begins to increase and in cells with a tertiary range, there is a further change in the scoop (Schwindt, 1973).

During an ISI, synaptic inputs are integrated by the tonically active motoneurone and consequent changes are combined with the underlying membrane potential set by the interspike membrane trajectory. The integrative component of the motoneurone is the axon hillock, which has the lowest discharge threshold. This acts as the trigger zone, causing the cell to fire if the degree of excitation exceeds the inhibition by a critical minimum. Features of this interaction between PSPs and the membrane potential of a repetitively discharging spinal motoneurone have been

simulated by a computer model by Ashby and Zilm (1982a): It was assumed that the essentials of the interaction between EPSPs and a rhythmically discharging neurone could be modelled by constructing trajectories representing the effective "distance" of an EPSP from threshold at any time during the ISI. This "distance" would be governed by a number of factors: 1) the trajectory of the membrane potential during the interspike interval; 2) the threshold during the ISI [which falls during the early part of the ISI and rises during the latter half (Calvin, 1974)]; 3) the conductances responsible for the after-hyperpolarisation, which are maximal immediately after a spike (Baldissera and Gustafsson, 1970; Schwindt and Calvin, 1973; Baldissera and Gustafsson, 1974; Mauritz et al., 1974); 4) voltage dependent alterations in membrane conductance [although probably overwhelmed by the after-hyperpolarisation conductance immediately after a spike (Nelson and Frank, 1967; Baldissera and Gustafsson, 1974)]; and 5) variations in EPSP amplitude with changes in membrane potential (see section on Post Synaptic Potentials). This computer model was shown to produce a PSTH with a peak associated with the rising phase of the EPSP, the magnitude of which was related to the number of stimuli and to the proportion of the ISI that the EPSP was within reach of threshold (Ashby and Zilm 1982a). After the peak there was a period of reduced firing probability, the duration of which was proportional to the amplitude of the EPSP rather than to the duration of its falling phase. These are also some of the features of the PSTHs obtained in the present study (see Results). The model also provides a framework for the inference of changes in the membrane potential of target spinal motoneurones (see Discussion).

Repetitive Firing of Single Motoneurones

Three modes of repetitive firing have been distinguished in motoneurones driven by the injection of intracellular current (Calvin, 1974). The occasional

spike mode occurs when the input depolarisations cross threshold only occasionally, producing an ISI typically greater than 150 ms. In the rhythmic firing mode, the depolarising input would keep the membrane potential above threshold but for the effect of after-hyperpolarisation, which converts input current magnitude into the cell firing rate, producing a regular ISI of less than 150 ms, as in the motoneurones of the present study. In the regenerative firing mode, depolarising spike afterpotentials intercept the falling threshold producing extra discharges at an ISI less than 5 ms, sometimes in bursts which stop before the ISIs lengthen to greater than 5 ms. (Calvin also suggests that deafferentation may serve as a stimulus for the regenerative mode, analogous to denervation supersensitivity to transmitter substances, which may have implications in the neural mechanism of spasticity.) A large EPSP occurring during rhythmic firing, generated by TMS for example, may force a discharge spike to occur earlier than expected. A large IPSP, however, may cause a delay (Calvin, 1972).

The Upper Motor Neurone Syndrome

Clinical Features

The upper motor neurone (UMN) syndrome describes the clinical features that follow a central lesion that interrupts the descending projections to spinal motoneurones. The features of this syndrome are weakness in a characteristic distribution, postural changes, increased tendon reflexes, spasticity, extensor plantar responses and loss of some cutaneous reflexes. In the arms, weakness is most apparent in shoulder abduction and external rotation, elbow extension, forearm supination, wrist and digit extension and in the actions of the intrinsic hand muscles (cf. Colebatch and Gandevia, 1989, below). In the legs, weakness is generally most pronounced in hip flexion and abduction, knee flexion and foot dorsiflexion and eversion. The affected limbs often assume a posture in which the arm in internally rotated and adducted at the shoulder, the elbow flexed, the forearm pronated and fingers and wrist flexed. In the leg, the hip is adducted and extended, the knee extended and the foot plantar flexed and inverted. The spasticity may be variable in degree and does not always match the distribution of the weakness.

The distribution of physical signs may therefore depend on anatomical factors. Indeed, Kameyam et al. (1963) found that in those cases where hemiplegia was on the same side as a lesion in the internal capsule this was associated with a large proportion of uncrossed CST fibres. Hemiplegia was also less pronounced in those patients found to have more innervation by CST fibres at post mortem examination. In patients with a large proportion of fibres in the anterior CST it was noted that hemiplegia, hypereflexia and the extensor plantar response could appear separately on the opposite or same sides. Furthermore, in addition to a lesion that

effects the CST, other cortical and sub-cortical descending projections are almost invariably involved in this syndrome (Brodal, 1981).

The motor deficits caused by a PT lesion have been shown to include loss of independent digit movements (Kuypers, 1981); slowness in the initiation of movements (Hepp-Raymond et al., 1974); hypotonia and reduced tendon reflexes; loss of abdominal and cremasteric reflexes; extensor plantar reflexes and atrophy of the affected limbs (Tower, 1940). The loss of ability to make discrete movements may also affect proximal muscles (Kuypers, 1981). Pure PT lesions in non human primates do not, however, always lead to spasticity or increased tendon reflexes (see for example Phillips, 1973). In the human UMN syndrome it is possible, therefore, that loss of independent finger movements and the presence of extensor plantar responses are the result of damage to PTNs, in contrast to spasticity, hypereflexia and a portion of the weakness, which may possibly result from the interruption of descending fibre systems that do not pass through the pyramid. The minimal effects of a pure PT lesion in a primate may therefore include an extensor plantar response, impaired skilled fine finger movements, and a variable degree of weakness depending upon the chronicity of the lesion.

Impairment of Fine Finger Movements and the Extensor Plantar Response

As described above, impairment of fine finger movements and the development of extensor plantar responses are frequently used as clinical indicators of a lesion affecting the PT, which may occur as part of the UMN syndrome. Although present following surgical section of the human PT (Bucy et al., 1964) it has been argued by Nathan and Smith (1955) that the extensor plantar response may, however, be present with sparing of the lateral CST in the spinal cord and, conversely, absent with CST lesions.

The extensor plantar (Babinski) response consists of dorsiflexion of the first toe (and sometimes abduction or fanning of the other toes) in response to a noxious stimulus delivered to the ball or hollow of the foot. Landau (1974) has suggested that it is a hyperactive flexor response in which the extensor of the great toe is included in the radiation of normal reflex activity. From neurophysiological studies (Van Gign, 1975 and 1976) it has been suggested that the criteria for a pathological plantar response should include: 1 Dorsiflexion of the first toe only when it is the result of activity in extensor hallucis longus; 2 abnormal contraction of extensor hallucis longus indicated by synchronous EMG activity in other flexor muscles (e.g. hamstrings) and tensor fasciae latae; and 3 voluntary withdrawals should last longer than the pathological extensor plantar response, but voluntary retraction should be diminished or inconstant with repeated stimulation, whereas the dorsiflexion of the first toe remains constant.

Impairment of fine finger movements is a less controversial clinical sign of a lesion affecting the PT. Lesion studies lead Lawrence and Kuypers (1968) to suggest that "in the absence of the corticospinal pathways, the descending subcortical spinal pathways are capable of guiding the range of activity which includes independent limb movement in addition to total body-limb activity. The corticospinal pathways superimpose speed and agility upon the subcortical mechanisms and, in addition, provide the capacity for a high degree of fractionation of movements as exemplified by individual finger movements." Impairment of fine finger movements in the UMN syndrome may reflect an inability to fractionate movements due to an inability to control graded force and/or a lack of sensory-motor integration.

Spasticity

Spasticity has been defined as a velocity-dependent increase in the tonic stretch reflex (muscle tone) with exaggerated tendon jerks, resulting from hyper-excitability of the stretch reflexes, as one component of the UMN syndrome (Lance, 1980). It is particularly evident in the presence of capsular lacunes (Mohr, 1982) in contrast to lesions of the medullary pyramids which characteristically cause a hypotonic paresis. UMN patients with spasticity frequently exhibit a degree of rigidity (dystonia) in addition, characterised by an abnormally increased non velocity-dependent muscle activity (including stretch reflexes), suggesting concomitant damage to extrapyramidal structures in the cord, brainstem, basal ganglia or cortex. Such rigidity can cause contractures, which may therefore be absent in patients with lacunar infarcts (Fisher, 1982).

The mechanism of spasticity reflects changes in the excitability of central synapses, the direct cause of which is undetermined. Rushworth (1960) reported the abolition of the hyperactive stretch reflex in man with diluted Procaine injections near the intramuscular nerve, with preservation of voluntary activity. This was interpreted as a selective action of Procaine on the gamma motor fibres innervating the muscle spindle. The subsequent use of microneurography in spastic patients has failed, however, to provide evidence for increased muscle spindle activity or increased Ia discharge in response to muscle stretch (Burke, 1983).

Currently, a number of hypotheses involving central disinhibition have been described. These are: decreased presynaptic inhibition of Ia terminals within the cord (Delwaide, 1973) which may therefore be mediated via receptors for GABA on the Ia terminal; augmentation of the stretch reflex by decreased 1b inhibition; inhibition of group II fibres (which normally excite

flexor motoneurones and inhibit extensor motoneurones in the cat spinal cord), although there is indirect human evidence opposing this hypothesis (Pierrot-Deseilligny et al., 1985); recurrent Renshaw inhibition (Katz and Pierrot-Deseilligny, 1982) - also implicated in the development of clonus; reduced reciprocal Ia inhibition (McLellan, 1977 and Knutsson, 1985). It is also probable that a combination of mechanisms are involved. Brooks (1980) has suggested that spasticity resulting from spinal lesions in animal preparations involves an imbalance of descending influences, with normal fusimotor status, and a facilitation of activity mediated by the pontine, reticular and vestibulospinal tracts leading to exaggerated extensor reflexes. In contrast, spasticity of a cerebral origin may involve disinhibition of the lateral vestibulospinal tract with a consequent facilitation of arm flexor muscles and leg extensors.

Central Paresis

Using clinical methods, Freund (1985) has subdivided the mechanisms underlying UMN weakness into three categories: reduced output paresis, subtraction paresis, and proximal (pre-motor) paresis. This reflects the heterogeneity of the clinical patterns of central paresis, possibly representing variations in the degree to which different efferent pathways are affected. Reduced output paresis described those patients with a moderate or severe paresis where the residual function could be used properly. Alternating movements could be performed with restrained force and the EMG pattern of the involved muscles showed antagonistic activation. In contrast, subtraction paresis was characterised by a minor degree of weakness on clinical testing, but a marked reduction in muscle force whenever the affected muscles had to act in concert, as in walking. The normal EMG pattern of activation of antagonistic muscle groups during alternating movements was disturbed and replaced by co-activation. In the

absence of antagonistic inhibition, antagonistic muscles would co-contract. Subtraction paresis therefore results from a disturbance in the timing of muscle activation and was frequently noted to be present in patients with multiple sclerosis (MS). Reduced output paresis, however, was more often associated with damage to the pre-central gyrus. Proximal paresis, sometimes associated with small capsular infarcts or lesions of the pre-motor areas that spare the pre-central gyrus, particularly affected the shoulder muscles and was associated with a limb-kinetic apraxia contralateral to the side of lesion. This was only apparent for movements of the whole limb, but not isolated hand or finger movements. Electromyography characteristically revealed diminished and delayed pre-innervation of proximal muscles, implying a localised defect in temporal activation. Lesions anterior to the hand area of the pre-central gyrus have also been reported to produce similar abnormalities in distal muscles (Freund, 1987). Colebatch and Gandevia (1989) have also observed that in a mixed group of patients with UMN lesions, the shoulder muscles were relatively spared of weakness, in contrast to the wrist and finger flexors which were relatively severely affected on the side contralateral to the lesion. Ipsilateral muscles were also weakened when compared with normal controls.

Pathophysiological Changes in the Human UMN Syndrome

Several changes have been described in the spinal cord, peripheral nerves and muscles contralateral to the affected cerebral hemisphere, some of which are controversial, and all of which may potentially influence the findings of TMS in such patients.

The normal recruitment of MUs is changed in hemiparesis (Freund et al., 1973) producing difficulties in recruiting even low threshold MUs, and there

are difficulties in maintaining a steady discharge rate, both of which may contribute to the technical difficulties involved in recording from single MUs during TMS in patients. It has also been shown that the double discharges from MUs which characteristically occur when subjects try to maintain steady and constant contractions (Andreassen and Rosenfalck, 1979) are characteristically absent in the presence of an UMN lesion.

Possible degenerative changes in motoneurones (Charcot, 1879) and losses of MUs in affected muscles (McComas et al., 1973) have been reported. Morphological changes occurring in affected muscles have also been described, including type I fibre hypertrophy, type II fibre atrophy, fibre type grouping, grouped fibre atrophy and the appearance of angular fibres (Brooke and Engel, 1969; Edstrom, 1970; Chokroverty et al., 1976; Segura and Sahgal, 1981). In a study on the physiological properties of FDI MUs in stroke patients, Mayer and Young (1980) found a different effect on fast (F) and slow (S) twitch units. In stroke patients with an acute flaccid hemiplegia the contraction times of F units, but not S units, were increased without any change in twitch tensions. In contrast, in patients with chronic spastic hemiplegia the twitch times of F units remained longer but with larger twitch tensions amongst S units. It was suggested that these changes resulted from alterations in MU activity and excitability caused by the hemiplegia and that the increased firing of S units may have been due to the recruitment order (connectivity) of motoneurones. The proportion of fatiguable MUs may also increase. Increases in the mean MUP amplitude with the maintenance of near normal maximum twitch tensions; reductions in the amplitudes of maximum M-potentials and possible abnormalities in neuromuscular transmission have also been reported by McComas et al. (1973). In addition to these changes, it is possible that alterations in peripheral nerve conduction may occur. Motor conduction velocity has

been reported to be normal (Edstrom, 1970; Segura and Sahgal, 1981; Namba et al., 1971) or reduced (Panin et al., 1967; Shigeno, 1972). Prolongation of motor terminal latencies (McComas et al., 1973) and slowing of sensory conduction (Namba et al., 1971) has also been reported.

Some features of the UMN syndrome may also reflect remodelling of the central reflex pathways and the input connections to motoneurones (McCouch et al., 1958; Goldberger and Murray, 1978; Bernstein et al., 1978; Goldberger, 1980; Bernstein and Bernstein, 1980; Veraa and Grafstein, 1981). These changes may include the degeneration of presynaptic connections to motoneurones; denervation hypersensitivity in motoneurones, collateral sprouting; and the possible activation of other, previously marginal inputs.

The UMN patients in the present study exhibited varied clinical features including weakness, hypereflexia, spasticity, extensor plantar responses and impairment of fine finger movements. A specific objective of this enquiry was to determine some of the neural mechanisms underlying these clinical features by a functional study of the corticospinal input to single motoneurones. The following section examines the principles of TMS, which was used to provide the descending input.

Magnetic Stimulation of the Human Brain

Stimulation of the motor cortex is an established method for functional studies of the CST. This has been illustrated by much of the experimental data in the preceding sections, in which electrical stimulation was most frequently used for animal studies. Transcranial magnetic stimulation (TMS) has emerged as a method for human motor studies. In the following section the principles of TMS are examined in relation to the requirements of the present study.

Surface Recorded Compound Responses to TMS

The central motor conduction (CMC) time has been estimated to be in the region of 7 ms (Hess et al., 1987a). To obtain this figure, the mean conduction time from motor root to muscle of 13 ms obtained by electrical stimulation over the cervical spine (Mills and Murray, 1986) was subtracted from the cortex to muscle conduction time of 20 ms. The CMC time of 7 ms would include the synaptic delay at the motoneurone and conduction along a short intradural segment of the motor root. For a synaptic delay of 1.0 ms and a root conduction of 0.3 ms it was therefore estimated that the remaining central latency would be in the region of 5.7 ms.

The estimated CMAP onset latency in abductor digiti minimi (ADM) is 31 ±1 ms (mean ±SD) in term babies, which decreases to normal adult values in some infants by the age of 2 years (Eyre et al., 1989). The maturation of CMC time is probably associated with the development of central myelination, which has generally been completed by 2 years of age (Yakovlev and Lecours, 1967). The diameter of axons in the pyramidal tract (PT) reach adult sizes by 4 to 7 years of age (Nathan and Smith, 1955). In adults, no correlation has been found between age and either the latency

or CMC times measured from the CMAPs of ADM. When measured from either ADM or tibialis anterior, however, latencies and CMC times (from the motor cortex to the lumbar region) were highly correlated with height (Jarratt, 1986, Nai-Shin Chu, 1989).

In anaesthetised monkeys below the age of 6 months, the threshold for evoking responses in the first dorsal interosseous (FDI) muscle or extensor digitorum communis (EDC) muscle has been found to be higher than for adult monkeys (and no difference in the threshold for active muscles when compared to relaxed ones below the age of 6 months) (Flament et al., 1990). In some of the younger monkeys it was found that a response could be elicited from deltoid when there were none from either FDI or EDC. In both adult and infant monkeys the response latency in deltoid was longer than in EDC, which the authors suggest may be evidence for an indirect route of excitation for deltoid. These findings are consistent with the above study on human infants and with earlier studies in the monkey, which have demonstrated that direct corticomotoneuronal (CMN) connections are not established until 6-8 months postnatally and that the shoulder muscles received few CMN connections (Kuypers, 1962).

When anodal TES was compared with TMS in human subjects, it was found that the CMC time with the former was 1.4-2.7 ms shorter with TES (Hess et al., 1986b; Day et al., 1987). It was noted that this was consistent with the difference in latency between the direct (D) wave and first indirect (I) wave in the corticospinal tract (CST) generated by surface anodal stimulation in non-human primates, which is 1-2 ms (Hern et al., 1962; Kernell and Wu Chien-Ping, 1967a). It has therefore been postulated that the direct component of electrical excitation may be absent with TMS and that TMS indirectly excites cortical neurones by exciting presynaptic fibres

whilst TES activates them directly. In the monkey, however, TM stimuli generate a volley in the right longitudinal fasciculus at C7 with a latency equivalent to an antidromic volley from C7 to the cortex (1.4 ms) (Edgley et al., 1989a). The corticospinal origin of the magnetically induced volley was confirmed by its collision with volleys evoked from the pyramid before the TM stimulus. Similar experiments on anaesthetised monkeys have also produced late responses corresponding to I waves (Edgeley et al., 1989b). Increasing the intensity of the TM stimulus did not consistently shorten the latency of volleys recorded at the pyramid, although a consistent reduction was observed with a TE stimulus. The latency reduction observed with TE stimulation was consistent with activation of the corticospinal pathway at a level that was deep to the cortex, approaching the medullary pyramid.

When recording from ADM with surface electrodes, a reduction in CMAP onset latency of 1.5 ms could be obtained by a simultaneous voluntary contraction of a contralateral ADM or by the contraction of the ipsilateral FDI (Hess et al., 1987a). Low intensity TM stimulation has also been shown to produce a larger EMG response in FDI when the muscle was involved in a voluntary isometric abduction of the index finger than when it was used at the same level of activity during a simple grip. This difference was not observed for TES, and was only observed when the stimulus strength was set at a level to produce a sub-maximal response (Datta et al., 1989). The authors argued that the motor cortical cells may have been more excitable during the abduction manoeuvre, which is consistent with evidence from the monkey which has shown that motor cortical cells are more active during a relatively independent finger movement than they are during a power grip (Buys et al., 1986). (It was also noted that the long-latency excitatory component at about 60 ms following electrical stimulation of the digital nerves (E2) was larger when recorded during voluntary finger

abduction than during a power grip.) The peripheral response produced by stimulation of the motor cortex depends on the excitatory states at the cortical and segmental spinal levels (Smythe and Fetz, 1985). In the cat the size of a descending volley produced by electrical microstimulation also depends on limb posture and locomotion (Palmer, 1986). "Motor set" (Evarts et al., 1984) also effects the excitatory state of motoneurones. Responses to human TMS may therefore be expected to vary according to several factors, including alertness, pre-innervation, expectation, and passive limb position (Wiesendanger, 1988).

Which Descending Pathways Mediate the Response?

The impulses evoked by a TM stimulus must be transmitted via one or more descending spinal pathways. Transmission is very rapid, with a central component of 5.7 ms (Hess et al., 1987a). The fast components of the PT may possibly, therefore, conduct the short latency excitatory response in man, particularly the earliest excitatory response in single MUs (see below). In primates, the PT has monosynaptic and polysynaptic connections with spinal motoneurones (Preston and Whitlock, 1961; Landgren et al., 1962a; Phillips and Porter, 1964) and in the anaesthetised monkey, the descending volley evoked by an TM stimulus can be collided with volleys evoked from the pyramid (Edgeley et al., 1989a). CMC time is within normal limits in Parkinson's disease, a disorder of extrapyramidal pathways (Dick et al., 1984, Thompson et al., 1986).

Pathways other than the CST, however, may also be activated by the stimulus and contribute to late phases of the CMAP. Such pathways could include the reticulospinal, rubrospinal and cerebellospinal tracts. Slowly conducting corticospinal neurones may also contribute to late responses. Transmission of impulses via the rubrospinal tract in the cat following TES

has not been excluded (Levy et al., 1984) and selective spinal cord lesions in the rat have indicated that PT activation is not involved (Adamson at al., 1989). There are, however, only 150-200 large cells in the caudal part of the human red nucleus, and only a small monosynaptic projection to motoneurones supplying distal muscles in the monkey (see Henneman and Mendell, 1981).

Unitary Responses

The use of needle electrodes during weak voluntary contractions has demonstrated that low threshold MUs in human hand muscles can be selectively activated by TMS (Hess and Mills, 1986a). These MUs were found to have conduction times from cortex to muscle ranging from 22.4 to 32.1 ms. Stronger TM stimuli caused the same MU to discharge 1.5 ms earlier (Hess and Mills, 1987a). (Increasing the intensity of a TM stimulus did not, however, influence the latency of the CMAP in a relaxed muscle.) Peri-stimulus time histograms (PSTHs) have shown an initial increased firing probability in single human MUs in FDI at 25-35 ms following a TM stimulus (Mills, 1988). This peak was found to be multimodal, with intermodal sub-peak intervals of 1.4 to 1.8 ms. Multiple preferred firing latencies have also been found using TES (Zidar, 1987; Day et al., 1987b) and have been interpreted as evidence for the generation of multiple descending volleys generated by a single stimulus (see Discussion). Single fibre EMG recordings during TES demonstrated preferred firing latencies with relatively little latency variation of consecutive MU responses, consistent with monosynaptic transmission in the spinal cord (Zidar et al., 1987).

When comparing the sub-peaks obtained by TM and TE stimulation, anodal TE stimulation has been found to produce the earliest sub-peak (termed

P0), with later sub-peaks produced by TM or cathodal TE stimulation, or produced by higher intensities of anodal stimulation, that could be grouped into four time bands relative to P0 (Day et al., 1989). These were: 0.5 to 0.5 ms (P1), 1 to 2 ms (P2), 2.5 to 3.5 ms (P3) and 4 to 5.5 ms (P4). (P0, however, may not necessarily correspond to the D wave.) Recording from 17 FDI MUs of the right hand, it was also found that an anticlockwise inducing current (Day et al., 1990) always included a P1 peak and usually a P3 peak in the PSTH. (The direction of current flow is described when viewing the circular coil from above). A P0 peak was not seen at the stimulus intensities used. Eight of these 17 MUs were also subjected to stimulation with a clockwise inducing current (Day et al., 1990) during which a P3 peak was recruited first, followed by a P0 or P2 at higher intensities. A P1 peak was not seen. At high stimulus intensities double discharges were occasionally seen, at interdischarge intervals comparable to the interval between neighbouring sub-peaks.

Calancie et al (1987) studied the effect of TES on single MUs in forearm flexor and extensor muscles. It was shown that increasing the stimulus intensity or voluntary pre-activation shortened the discharge latency of MUs. Examination of the firing probability demonstrated that evoked discharges were unlikely when the interval between the stimulus and the preceding voluntary discharge was short, and that amongst trials in which the MU did not fire in the initial excitatory phase, analysis of serial inter-spike intervals (ISIs) suggested the operation of a suppression effect (the basis for these inferences, however, are scrutinised in the Discussion). A second phase of excitation, termed E2, at 70-90 ms following stimuli of relatively high intensity, was also described. It was suggested that this could have been caused by a gamma loop or a non-specific startle effect. Examination of human proximal arm muscles has also shown a second

peak of raised firing probability with both TM and TE stimulation, occurring 10-12 ms after the primary peak and lasting 10-15 ms. This was most pronounced with TM stimuli when recording in the pectoral muscle, but was not observed in FDI (Colbatch et al., 1988).

Other Responses to TMS

The human diaphragm can be activated by TES (Gandevia and Rothwell, 1987) and by TMS (Lindon and Nisnick, 1989). The facial nerve can also be stimulated intracranially with a TM stimulus (Schriefer et al., 1988). TMS of the left frontal cortex by a voice triggered stimulus has produced complex laryngeal electromyographic (EMG) responses on the right side (Amassian et al., 1987). Stimulation of the frontal cortex on the non dominant side also produced contralateral laryngeal responses at similar latencies. TM stimuli delivered over the vertex evoked EMG responses in the jaw closing and suprahyoid muscles during voluntary contraction. TMS has also been demonstrated to alter the function of cortical areas related to speech, the perception of language symbols, and vision (Cracco et al., 1989b; Maccabee et al., 1989). Changes in the EEG consistent with a transcallosal response following TM and TE stimulation of the frontal cortex have been produced (Cracco et al., 1989). Surface recorded CMAPs from upper and lower limb muscles have demonstrated that magnetic stimulation is possible over the cervical and lumbar spinal enlargements (Ugawa et al., 1989), with latencies compatible with those obtained with high voltage electrical stimulation of the spinal nerve roots. During paradoxical sleep, the amplitude of CMAPs to TMS increase, suggesting that the descending volleys may overcome the characteristic inhibition of spinal motoneurones during this cycle of sleep (Hess et al., 1987d).

Inhibitory Processes

Stimulation of the nervous system can produce direct and indirect inhibitory effects in humans and animals. In the peripheral nervous system, magnetic stimuli at a 100/s have been found to directly reduce the electrically evoked twitch tension and CMAP from frog gastrocnemius muscle with a magnetic coil encircling the sciatic nerve (Brain and Wali, 1989). 95% block appeared within approximately 5 minutes, following which recovery was obtained within 5-6 minutes. The authors suggested a mechanism involving the interruption of transmembrane ionic fluxes by the the magnetic field.

In non human primates, corticospinal neurones inhibit spinal motoneurones disynaptically following epicortical electrical stimulation (Preston and Whitlock, 1960; 1961; Landgren, Phillips and Porter, 1962a; Phillips and Porter, 1964). Conditioning stimulation of motor axons to antagonistic muscles produced these disynaptic IPSPs when evoked from either group Ia afferents or via corticospinal fibres (Jankowska et al., 1976). When inputs from these two sources arrived synchronously there was a mutual facilitation of the inhibition. Inhibition in the spinal cord evoked by electrical stimulation of the cortex is therefore mediated by the Ia interneurones. Jankowska and colleagues also demonstrated that the corticospinal projection to these interneurones was similar in magnitude and organisation to that which projects to the motoneurones. TES has been found to inhibit the H-reflex in soleus muscle (Cowan et al., 1986) and inhibitory effects on the firing probability of repetitively discharging single motoneurones in human triceps muscle following TMS have also been detected in PSTHs (Ashby et al., 1990).

When recording surface responses, a TM stimulus may produce a muscle twitch followed by a silent period in the EMG lasting up to 140 ms after the stimulus (Day et al., 1989a). The CMAP in relaxed FDI following a TM stimulus can also be abolished by simultaneous supramaximal electrical stimulation to the peripheral nerve at the wrist (Inghilleri et al., 1990). The authors demonstrated that the suppression was probably caused by a combination of motoneurone after hyperpolarisation and by Renshaw-mediated inhibition following antidromic invasion after the peripheral nerve shock.

In monkeys, cats and rabbits the background activity of cortical neurones evoked by iontophoresis of L-glutamate can be reduced for 100 to 300 ms by surface and intracortical electric stimuli (Krnjevic et al., 1966). Antidromic pyramidal stimulation (Phillips, 1959) was found to be less effective in producing motor cortical inhibition than direct cortical stimulation. In Man, brain processing can be temporarily interrupted by a TM stimulus (Day et al., 1989b). A human subject trained to flex the wrist in response to an auditory tone given shortly after a visual warning was found to experience a delay in the onset of this movement when a TM stimulus was delivered at or just before the expected time of onset of movement. The delay was up to 150 ms, depending upon the intensity of the TM stimulus. It was not thought to be entirely due to inhibitory mechanisms in the spinal cord since innate reflexes or a second TE stimulus could produce activity within this interval. When bilateral movements were studied, the delay was only exhibited in the arm contralateral to the stimulated side. It was concluded that some aspect of execution of the task had been delayed by the TM stimulus and possibly that movements had been delayed by inhibition of neurones to the command signals that would normally initiate motor programmes.

Afferent Input and the Response to TMS

The effect of cutaneous stimuli on the responses evoked by TM and TE stimuli have been studied (Day et al., 1988) using a transcranial stimulus preceded by a single cutaneous electrical conditioning stimulus at 3 times sensory threshold. The surface EMG response from FDI (during a steady contraction) in response to a TE stimulus was reduced when it coincided with the inhibitory phase of the cutaneous reflex and enlarged when it coincided with the facilitatory phase. The response to a TM stimulus was different, however, exhibiting a reduction during the initial facilitatory period of the cutaneous reflex. Enlargement of the response only occurred later at a delay of 5-10 ms with respect to both the cutaneous reflex and the effect on the response to an TE stimulus. A similar effect was found when recording from single MUs. This suggested that the size of responses to a TM stimulus do not follow changes in spinal cord excitability in contrast to responses evoked by a TE stimulus. It was also postulated that transynaptic stimulation of fast conducting PTNs by a TM stimulus may be responsible for this difference.

CMAPs to TMS can be facilitated by the application of rectangular mechanical pulses to the digit when delivered 7-16 ms before the TM stimulus (Claus et al., 1988b), consistent with facilitation via the monosynaptic afferent Ia input to motoneurones enabling spatial summation of Ia volleys and descending corticospinal impulses. The amplitude of CMAPs to TMS are not, however, attenuated by vibration of the muscle tendon (Claus et al., 1988a) (as would have been expected had there been a postsynaptic inhibitory effect) which has been interpreted as evidence in support of the occurrence of presynaptic inhibition during vibration (Eccles et al., 1962). The excitatory response to vibration (probably produced monosynaptically by synchronised Ia volleys) may,

however, be facilitated by TMS (Barker et al., 1986). Muscle stretch may also affect the size of the EMG response in forearm flexor muscles to TMS when the stimulus is superimposed on the latency appropriate for the long latency stretch reflex (Day et al., 1988). The mechanism for this may involve excitation of the motor cortex by stretch induced afferent inputs.

The Mechanism of Transcranial Magnetic Stimulation

When a conductor cuts or is cut by magnetic flux, an electromotive force (EMF) is induced. The direction of the induced EMF depends on the direction of the magnetic flux and on the direction in which the flux moves relative to the conductor (as predicted by Fleming's right hand rule). The direction of the induced EMF is such that it tends to set up an electric current opposing the motion or the change of flux responsible for inducing that EMF (Lenz's law). The magnitude of the EMF is proportional to the rate at which the conductor cuts or is cut by the magnetic flux (derived from Faraday's Law of Electromagnetic Induction). Its waveform will reflect the first derivative of the time varying magnetic field produced by the pulse of current in a stimulating coil and the direction of the eddy current responsible for nerve excitation will be opposite to that during the most rapid rise in inducing current.

Stimulation of Nerve Fibres

For direct electrical stimulation of the brain or a mixed nerve, a current flows between the anode and cathode along a path which depends on the impedance of the tissues underneath the electrodes. The density of current flow generally decreases with distance from the electrodes. When the electrical current is applied to a nerve fibre, the trans-membrane voltage changes exponentially with time. It therefore takes a finite time before the membrane capacitance is fully charged, depending upon the membrane time constant. The duration of the stimulus needs to be long enough relative to the membrane time constant in order to produce the required degree of depolarisation. Very short stimuli, as in TMS, may achieve this by the effect of a stronger induced current to compensate for the relatively short stimulus time. Firing threshold is ultimately reached when the neural

membrane is depolarised sufficiently for the inward sodium current to exceed the outward potassium current.

Electrical stimulation of multifibre preparations by external electrodes is achieved when the applied electric field runs parallel to the nerve fibres with an inward flow of current (producing hyperpolarisation) beneath the anode and an outward flow of current (producing depolarisation) beneath the cathode. The resulting action potential is then propagated along the axon. With uniform stimulation of an isolated nerve the largest fibres are preferentially excited because the magnitude of the depolarisation depends on membrane current density, and although in large fibres the current is distributed over a larger surface, the core resistance decreases in proportion to the square of the radius, and the surface area increases in direct proportion with the radius. [When human nerves are stimulated transcutaneously, however, this inverse relationship between excitability and axon size may not apply (McComas et al., 1971)]. In addition to excitation by the application of an external cathode, some excitable cells-in which sodium channels are partially inactivated at the resting membrane potential-may be excited by anode off (rebound) excitation. Hyper-polarisation of the neural membrane extinguishes this inactivation and the return of the membrane from the hyper-polarised to the resting state activates these sodium channels triggering an action potential.

Electrical stimulation of a peripheral nerve is therefore most efficient when the imposed direction of the current flow is aligned with the long axis of the nerve (Rushton, 1927). Similarly, using a circular magnetic coil to stimulate the median nerve, the induced voltages and CMAPs are largest when the induced electrical field are orientated parallel to the long axis of a metallic conductor or the median nerve, respectively (Maccabee et al., 1988).

Reversing such a field through 180° produced single fibre muscle action potentials that differed in latency by 0.1 ms. The earlier latency was produced by a clockwise current (flowing proximally nearest the nerve) which generated a distal virtual cathode. It could be estimated that the virtual cathode and anode were situated no more than 0.1 ms times the conduction velocity apart, i.e. 5-6 mm.

Transcranial Magnetic Stimulation

TM stimuli probably excite nervous tissue by inducing a loop of current which passes obliquely through axons by entering through one node of Ranvier and exiting through a different node placed at some distance from the entry node. The subsequent activation of sodium channels would initiate an action potential (Bickford et al., 1987). Such a model regards the node of Ranvier as a leaky capacitor that needs to be charged (Barker et al., 1987). Barker et al have determined that the time constant of this leaky capacitor is approximately 300 μs and that the current stimulus into the node of Ranvier should be shorter than this in order to optimise the excitation. The brevity of this current flow may explain why large motor fibres are preferentially stimulated (Geddes, 1987). In order to obtain this rapid flow of induced current, the coil is excited by the discharge of a capacitor. The energy stored in the capacitor is defined by $E = CV/2$ (C = Capacitance V = Voltage). The current induced in the tissue is proportional to the rate of change of the magnetic field, which itself is proportional to the current in the coil. It follows that the current produced in the tissue is proportional to the rate of change of current in the coil. It may be assumed that in order to exceed the threshold for excitation, a critical current density is required for a specific time. The current density at any time is equal to the voltage gradient in the tissue divided by the resistivity of the tissue. The resistivity is the ability of a material to oppose the flow of

current. For grey matter this is approximately 250 Ohm cm, that for white matter about 750 Ohm cm and the average for the brain as a whole is approximately 500 Ohm cm (Geddes, 1987). The resistivity of CSF is about 65 Ohm cm, and therefore, current will preferentially flow in the CSF.

A TM stimulus has been shown to evoke both D and I waves in the monkey (Edgeley et al., 1989a and b) and probably a series of I waves in human subjects (Mills, 1988, Day et al., 1989, Boniface et al., 1991). It has been argued that I waves are the result of indirect (transynaptic) activation (see above). In comparison, anodal electrical epicortical stimulation in the monkey produces direct excitation by physical spread of current, in contrast to cathodal stimulation which excites corticospinal neurones indirectly (transynaptically) (Phillips, 1987). It is possible, therefore, that a TM stimulus produces its effects in a way that incorporates the features of both cathodal and anodal stimulation.

The excitation of neurones by a stimulus applied to the brain is influenced by three major factors (Ranck, 1975; Phillips and Porter, 1977). These are: 1) the distance of the target cell from the site of stimulation and the electrical excitability of its membrane; 2) the orientation of the cell's process with respect to the lines of stimulating current - a voltage gradient parallel to the long axis of the cell is most effective, and 3) the direction of flow along the lines of stimulating current, which is most effective along the direction of orthodromic propagation of impulses in that particular cell.

The cells that are first to respond to a transcranial stimulus are therefore likely to be those nearest the highest density of stimulating current, on the convexity of the pre-central gyrus. In the baboon, PTNs are largely orientated perpendicular to the skull and this may also be the case in man.

The highest density of current induced by a circular stimulating coil is in a tangential plane in an annulus directly underneath the windings and may, therefore, not directly excite vertically orientated PTNs. It has been suggested that this may explain why there was no evidence of the production D waves with TMS, at least when using an anticlockwise inducing current (Day et al., 1989; 1990) in comparison with intracortical electrical stimulation, and that tangentially oriented cells in the cortex including PT axon collaterals, interneurones and afferent inputs may be preferentially stimulated. (The direction of current flow is described as viewed from above). The precise location of the site of action of TMS, however, is undetermined. It may, for example, act upon dendrites, presynaptic terminals, cell bodies, efferent axons, or a combination of cellular elements. Variations in the intensity of TMS may also have an effect: weak TM stimuli may excite presynaptic elements, with stronger stimuli exciting cell bodies or axons, or both (Mills et al., 1987).

Depth and Orientation of Inducing Currents

TMS produces electric fields via the effects of electromagnetic induction and also electrostatic fields produced by a charge distribution on the body surface in accordance with boundary conditions for the current and electric field. An important feature which differentiates TMS from TES is the ability of magnetic fields to pass unattenuated through all body structures, including the skull. Penetration is increased by increasing the diameter of the stimulating coil. The intensity of the magnetic fields decays exponentially with distance from the coil (Hess, Mills and Murray, 1987a). This decay, however, is at a slower rate than the equivalent decay of an electrical field (Barker et al., 1987). (Effective penetration of TM stimuli therefore occurs without the creation of large electric fields on the scalp, which may account for the advantage of TM over TE stimulation, in that it is

painless.) In an infinite medium using a coil of 100 mm in diameter, the maximal electrical field on the surface of the coil occurs on a circle with the diameter of approximately 90 mm (Barker et al., 1987). At a depth of approximately 20 mm this circle of maximal electrical field is approximately the same in diameter although the localization of maximal field to this hypothetical circle or loop becomes less localised as the distance is increased from the coil. At a distance of approximately 80 to 100 mm from the coil, the electrical field is no longer localised into a circular pattern. (In the real situation, however, these observations may be altered by the presence of a finite nonhomogeneous medium.) The hand area of the motor cortex is approximately 25 mm below the surface.

For obtaining responses for small hand muscles with a circular coil, the optimum position for the coil is directly over the vertex, when held tangentially with respect to the skull. The onset latency of the CMAP is unaffected by the position of the stimulating coil within a area of 6 x 6 cm over the vertex, and the same MU can be activated from coil positions up to 7 cm apart (Hess et al, 1987a). The direction of current flow in the coil determines which hemisphere is preferentially excited: an anticlockwise monophasic pulse of inducing current activates the left hemisphere and vice versa (Hess et al.,1987, Day et al., 1990). Multiphasic pulses from a circular coil, however, evoke a response that is not influenced by coil polarity (Claus et al., 1990). For TMS with a monophasic pulse of current in a double coil, the optimum stimulus for responses in the hand was a forward flowing inducing current, oriented at 50° to the saggital plane, which is approximately at right angles to the main axis of the central sulcus (Boniface et al., 1991b).

Using TES with a cathode at the vertex, Rothwell et al (1987a) found that the largest CMAPs (with the lowest stimulation threshold) were recorded in the human hand when the anode was positioned 7 cm lateral to the vertex on the interaural line. With bipolar stimulating electrodes similarly orientated in the coronal plane the threshold for the detection of corticospinal volleys with epidural electrodes over the posterior surface of the human spinal cord was found to be the same for vertex cathodal and vertex anodal TES (Burke et al., 1990). The production of D or I waves, however, might be dependent upon on the orientation and/or magnitude of the stimulus. For bipolar electrical stimulation of the cortical surface in the monkey, when the anode of the dipole was nearer than the cathode to a cell situated on the convexity of the pre-central gyrus, stimulation was found to be direct (Phillips and Porter, 1962). The bifocal threshold was higher than the focal anodal threshold, possibly because the fraction of the total current flowing outwards through a somatic or axonal membrane was smaller with bifocal stimulation. As the cathode of the dipole was brought nearer to the PTN, the direct response failed, suggesting that the directly responding component of the cell was depressed by the surface cathode. In this study, the cells that were most readily stimulated from the lip of the central fissure were thought to be located in its anterior wall. The orientation of their long axes at right angles to those PTNs of the convexity was thought to have accounted for the direct response produced by both surface anodal and surface cathodal stimuli.

Summary

The problem addressed by this study is: How does the human corticospinal tract influence the discharge of single spinal motoneurones and what are the effects of neurological disease? In developing the background to this problem, several points have been raised in the Introduction.

The preceding sections on Descending Pathways and The Upper Motor Neurone Syndrome have emphasized the physiological and clinical significance of corticospinal inputs in motor control. Further, it is evident from the discussion of Postsynaptic Changes in the Spinal Motoneurone that one method for studying these inputs at the level of the single motoneurone would be to examine changes in firing probability. This was the method employed in the present study, in which TMS was used to generate the descending input. The initial task was therefore to characterise the response to TMS of single human spinal motoneurones. Changes that occurred in UMN disorders could be determined by comparison with the normative data.

The Introduction also identifies certain fundamental areas to be explored. The time course of the rising phase of the PSPs would need to be estimated in healthy subjects to provide a control for UMN patients. Determining the influence of any changes in the experimental parameters would also be important. In addition, several factors would be expected to effect the probability and latency of evoking a discharge in a tonically active motoneurone, such as the intensity of stimulation and the interval between the stimulus and the preceding voluntary discharge. These factors could be used to test the mode of summation of the motoneurones' interspike membrane potential with the EPSP. Again, the normative data would also

provide a control for UMN patients. Finally, in addition to early changes in the firing probability of the index motoneurone there may be late effects, either excitatory of inhibitory, which may or may not be transmitted via the same central pathway. The features and origin of late changes in firing probability should also be studied.

The following section describes the methodology used in addressing these questions.

CHAPTER 2

Materials and Method

Materials

Magnetic Stimulator

A Novametrix Magstim 200 magnetic stimulator was used. This was used to drive a circular copper coil of 26 turns with an internal diameter of 9 cm and a mean diameter of 13 cm. The stimulator stored energy in a large capacitor and discharged it rapidly through the stimulating coil. The discharge current was in the region of 5,000-10,000 A. This produced a peak induced magnetic field strength of 1.5 T at the centre of the circular coil, decreasing to 1 T at a distance of 25 mm and 0.2 mT at a distance of 1 metre. The current induced in the plane of the coil, measured with a 2.5 cm single turn search coil, was found to peak at a point between the centre and the inner turns (Fig. 1). The time course of the magnetic field peaked at approximately 150 μs after which it fell exponentially towards zero, with a time constant of 340 μs, inducing a current with a time course which followed the first derivative of the magnetic field, peaking in the region of 80 μs (Hess et al., 1987a). The peak magnetic field varied linearly with the output of the stimulator, indicated as a percentage of its maximum output. The stimulator introduced a 10 ms delay between the trigger input and output which therefore delayed the delivery of the stimulus by an extra 10 ms with respect to the Digitimer pulse. There was an interlock switch on the coil which prevented inadvertent discharges. The coil itself was double insulated and housed in an ABS cover. The coil was prevented from overheating by two temperature probe and internal temperature sensor protected the stimulator from abnormal temperature rise. The stimulator conformed to the following safety standards: BS5274 Pt 1 (UK), IEC 601-1 (International) and VDEO 750 T1/MED GV (West Germany).

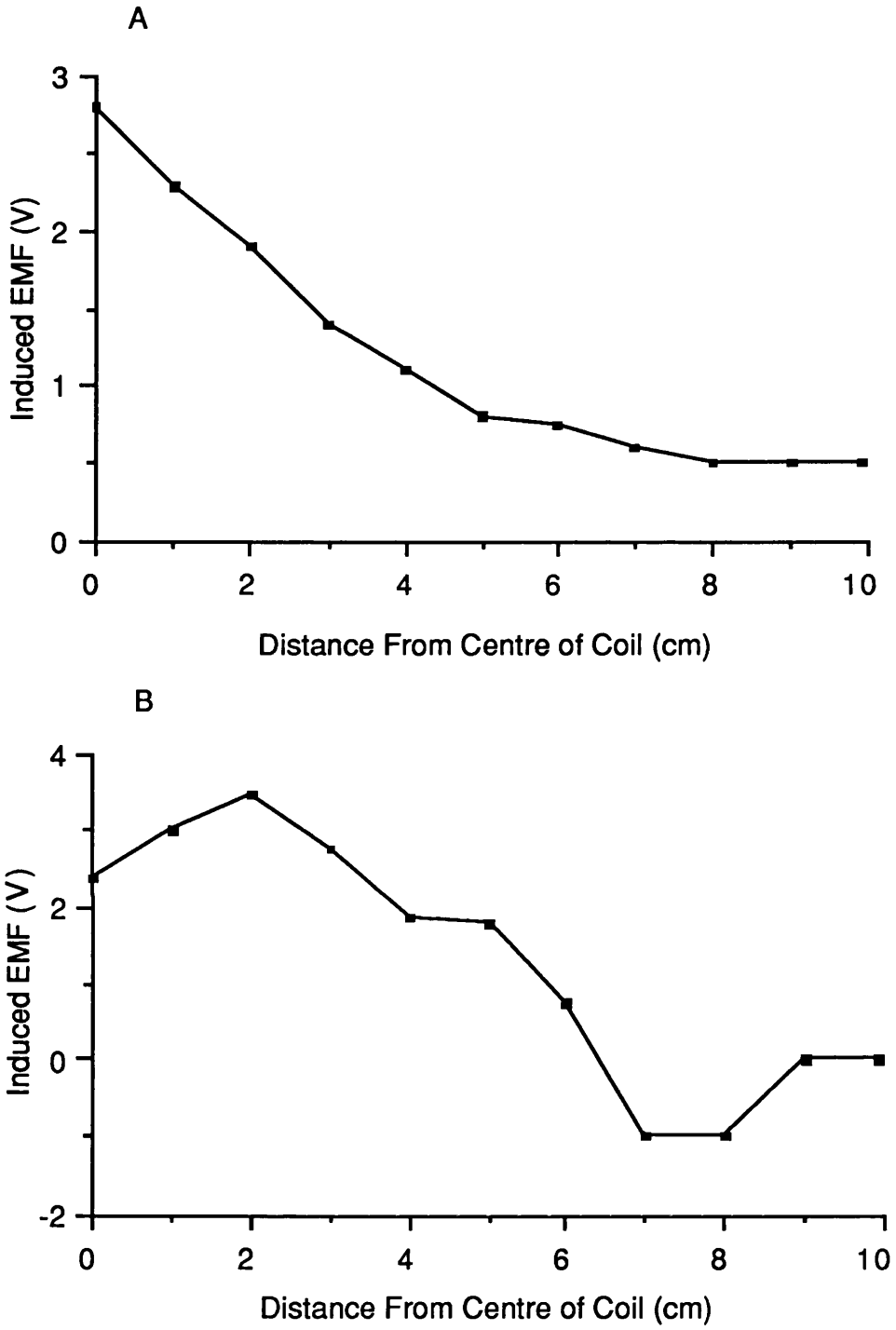


Fig. 1. EMF induced in a search coil (single turn, diameter 2.5 cm) at different points in space in relation to the circular Novametrix stimulation coil (26 turns, mean diameter 9 cm) at a stimulation intensity of 40% maximum. A: the induced EMF decreased with distance from the coil's centre when measured along a line perpendicular to the plane of the coil. B: in a plane that was parallel to the coil, with the search coil held 3 mm from its casing, the induced EMF peaked at 2 cm from the centre of the coil and then reversed polarity at between 5 and 6 cm.

Determining the Direction of Current Flow Through the Coil

The direction of current flow through the stimulating coil was determined using a concentric search coil and an oscilloscope. When the circular coil was viewed from above, this indicated an anticlockwise direction for recordings from the right arm and a clockwise direction for recordings from the left arm. The induced waveform was monophasic.

Concentric EMG Needle Electrodes

Concentric needle electrodes (Dantec 13L58) were used for studies on single MUs. These consisted of an insulated platinum wire inside a stainless steel cannula embedded in Araldite. Motor unit potentials (MUPs) were picked up by the tip of the platinum wire with the cannula acting as the indifferent electrode. They were 20 mm in length with a diameter of 0.3 mm. The configuration of the electrode tip was cut to an angle of 15 degrees with a platinum surface area of 0.015 mm^2. All needle electrodes were autoclaved between use.

First Dorsal Interosseous Muscle

A large quantity of data in this study was obtained from MUs in FDI. Human FDI consists of an estimated 120 MUs with approximately 300 muscle fibres per MU (Finestein et al., 1955). Using intramuscular microstimulation Young and Mayer (1981) have classified the MUs of human FDI according to their twitch contraction time (CT) and fatiguability. MUs fell into three distinct groups: 1) type S units (slow) with a CT greater than or equal to 70 ms, resistant to fatigue and with an intermediate to small twitch tension, which were the most numerous; 2) type FF units (fast-fatiguable) with CTs less than 70 ms, fatiguable and with large to small twitch tensions; 3) type FR units (fast fatigue-resistant) with CTs less than 70 ms, resistant to fatigue and with large to small twitch tensions. According to the size principle, small

spinal motoneurones with slower conducting axons are the first to be recruited. Low threshold MUs are of the type recorded in the present study. These MUs are, therefore, innervated by motoneurones of relatively small size, with high input resistances, low thresholds, relatively long periods of AHP, low degrees of accommodation and slow steady firing characteristics. Their axons are small in diameter with slow conduction velocities. At the neuromuscular junction, they have relatively small end plates and low end plate potential quantal content. Their muscle fibres are relatively small in diameter, with low levels of ATPase, glycogen and phosphorylase, but richly supplied with capillaries with relatively high levels of oxidative enzymes. With regard to their contractile characteristics, these MUs have relatively slow contraction times develop low tensions, but with a high resistance to fatigue (See Brown, 1984, for a review).

Human FDI is a bipennate muscle arising from the adjacent sides of the first two metacarpal bones, and is attached to the radial side of the proximal phalanx of the index finger and to the capsule of the adjoining metacarpo-phalangeal joint. It is inserted by fibres which run transversely in the extensor expansion with the lumbrical tendon to the distal phalanx. FDI, therefore, has little direct effect on the middle and distal phalanx of the index finger. Its chief actions are in producing abduction, flexion and rotation of the index finger. Its nerve supply is via the deep branch of the ulnar nerve, C8 and T1. Rarely, it has been found to be supplied exclusively by the median nerve (Sunderland, 1946).

Medlec MS6 and Mystro

The EMG signal was initially fed to a Medelec MS6 or Mystro for amplification, with a band pass of 32 Hz to 16 or 32 KHz. The signal was therefore passed through the pre-amplifier and amplifier (AA6 MK II for the

MS6). After amplification the signal was sent to the analogue to digital converter.

The 1401 Interface, CED and Select Software

The 1401 processor captured the EMG signal and fed it to the computer for later analysis. A multichannel signal averager for the 1401 was run on the Tandon computer. CED MRATE was also used for the generation of interval histograms or for occasional use in the construction of on-line PSTHs. The discharge times of the index MU were measured off line to the nearest 0.1 ms with software ('Select') written for this purpose in the Unit of Clinical Neurophysiology, Oxford.

The Digitimer Programmer 4030

The Digitimer provided a series of timed periods which could be changed or reset, providing an overall recycling function to enable repetition during an experiment. The signals were derived from a quartz crystal master oscillator operating at 2 MHz. A proportion of the signals were brought out to the matrix programming board which allowed the combination of pulse patterns necessary for triggering the magnetic stimulator. When necessary the Digitimer could be controlled by an external signal.

Computers

An IBM Compatible Tandon AT microcomputer was used for the storage and subsequent analysis of raw data. This had a 20 Megabyte hard disk and a Hercules graphics card. The majority of data analysis tasks were carried out on a Macintosh SE microcomputer, with a 20 Megabyte Hardisk. The measured discharge times of the stored data on the Tandon Computer were relayed in the form of ASCII files to the Macintosh with the Kermit programme via a null modem cable.

Window Discriminator

A World Precision Instruments Window Discriminator (Model 121) was used for spike triggered stimulation experiments (see Stimulation Protocols). The input signals were sorted according to their peak amplitude. A rectangular pulse was generated at the window output for each wave form peak that appeared within the window aperture. The lower level of the aperture was set with one control and the aperture size was set by the upper window level with respect to the lower level both outputs (either window or above). Signals of either polarity could be discriminated.

Vibrator

In some experiments, a LING V100 Vibrator driven by a LING 25E amplifier was used to deliver a rectangular pulse to the index finger. The mass of the moving system was 6.5 g and its peak thrust was 8.9 N at 1 KHz. It was driven from an external signal via an isolated BNC socket. The level of the output amplifier signal could be varied with a master gain control.

Safety Aspects of TMS

The passage of a prolonged magnetically induced current through tissue may potentially cause harm by producing neuronal hyperactivity or power dissipation (tissue heating) (Agnew and McLeary, 1987). The following calculations have been made, however, concerning the quantitative aspects brief exposure to such fields by TMS (Barker et al., 1987): with their TM stimulator, the induced current was estimated to peak at 0.25A with a maximal charge of approximately 50 μC per pulse. The latter is approximately 0.05% to 0.005% of the charges used in electroconvulsive therapy (Pippard and Ellam, 1981). With respect to energy dissipation, the average thermal power deposited in the brain would be less than 2 mW with a stimulus delivery rate of 1 every 3 seconds (as used in the present study). This is less than 0.01% of the heat produced in the brain by normal basal metabolism (Schmitt and Thews, 1983). For an average adult brain this is 300 times smaller than the maximum set by the American National Safety Standards, of 0.4 watts per kg (American National Safety Standards, 1982). For a TM stimulus peaking at 1.4 T at the surface of the brain, the total charge delivered per impulse (which is dependent on the product of current density, pulse duration and cross sectional area through which the current flows) has been calculated to be 5.5 μC for the scalp and 11 μC for the brain (Barker et al., 1988). The estimated brain charge density would be in the region of 0.5 $\mu C/cm^2$. No evidence of neural damage has been found with direct stimulation at a much higher charge density of 10 $\mu C/cm^2$ at a frequency of 50 Hz (Agnew and McLeary, 1987).

Metallic Objects

A time varying magnetic field peaking at 1.6 T would produce a peak field at the cortical surface of approximately 1.4 T (assuming a depth of 10 mm

below the stimulating coil) (Barker et al., 1988). A metallic implant, such as an aneurysm clip, would be repulsed and then attracted by this rapidly changing magnetic field. Such an object would be displaced if the net product of force and time imparted movement. The magnitude of the repulsive force is determined by 4 factors (Cadwell, 1989): 1) the force generated is proportional to the conductivity of the object; 2) the force is also approximately proportional to the object's cross sectional area in a plane perpendicular to the magnetic field, as more magnetic lines or flux are intercepted by large objects; 3) there is an inverse relationship between the force and the distance between the object and the coil, and 4) the force increases as the square of the inducing magnetic field. Using an MES-10 stimulator, Cadwell found that the mechanical energy imparted to aneurysm clips and metallic sutures was unmeasurable, when in direct contact with the stimulating coil at 100% stimulus intensity. Nevertheless, until such effects are studied in detail, the presence of intracranial metallic objects remains a contraindication to TMS (see Methods).

Seizures

In a series of patients with known epilepsy, Tassinari et al (1989) failed to show changes either in the frequency of seizures following TMS, or direct induction of seizure activity during stimulation. In animal studies, repetition frequencies of 50 Hz have been found to be the most effective in kindling epileptic seizures, irrespective of stimulus duration, and kindling could not be achieved at repetition frequencies of less than 10 Hz (Goddard et al., 1969). A stimulus frequency equivalent to 0.3 Hz was used in the present study. In another series of 14 patients with intractable focal epilepsy, however, TMS during pre-surgical evaluation produced 1 clinical observable focal complex seizure, without other side effects (Hufnagel et al., 1989). One 57 year old patient has also been reported to have suffered

his first seizure during TMS (Homberg and Netz, 1989). The patient had a large ischaemic scar, demonstrated by computerised tomography, following a mid cerebral artery infarction six months earlier. An electroencephalogram (EEG) before TMS showed a right hemispheric theta focus without evidence of epileptic activity. Approximately 30 s after the third stimulus the patient had a generalised tonic-clonic seizure, followed by 2 seizures 4 weeks later. A repeat EEG showed that the right hemispheric focus remained unchanged, without evidence of seizure activity. The authors suggest that TM stimulation should be avoided with large ischaemic scars. This has been extended in the present study to include all patients with mass lesions or a history of seizures.

Epileptic seizures have also been reported in two patients with MS (Kandler, 1990). In one patient, a 60 year old man had a single Jacksonian seizure four weeks after TMS during which he received 24 stimuli. The other patient, a 30 year old woman, had two grand mal seizures 3 weeks after TMS during which she had received 50 stimuli. This was, however, her fifth session of TMS; on all four earlier occasions she had received 40 stimuli at each session over a period of 6 months. It was noted that the prevalence of epilepsy in the general population is about 0.5%, and that in MS its prevalence is 1.1%-4.5% and that these two patients had been encountered among a series of 108 patients with MS who had received TMS at this centre. The temporal relationship between TMS and the onset of seizures makes a coincidence, however, an unlikely explanation for these findings.

During TMS in 15 patients suffering from medically intractable complex-partial seizures, subdural electrodes have shown focal activation in 3 patients suffering from several foci (Hufnagel, 1989). No epileptiform

potentials were induced outside epileptic foci which had been identified by electrocorticography. In one patient, a complex-partial seizure was induced and in all other patients, no clinical effects were reported by either the patients or the operator. In addition to the implication regarding the stimulation of patients with known epilepsy, therefore, TMS may possibly be of use for the localisation of epileptic foci before surgery.

Other Potential Physiological and Psychological Effects

Brief exposure to intense magnetic fields have not been shown to produce pathological changes (Adie, 1981; Elgers and Henneck, 1983). In animal studies, however, prolonged stimulation-induced neuronal hyperactivity has been shown to translocate potassium and calcium ions between extracellular and intracellular space (McLeary and Agnew, 1983). This was accompanied by morphological changes in the cortex at certain charge densities (Agnew, Ewen and McLeary, 1983). Furthermore, electrolyte shifts themselves may potentially produce cell damage (Griffiths et al., 1983; Rothman, 1985; Siesjo, 1981).

In a trial of 30 healthy subjects who received a mean of 38 TM stimuli, there was no significant change in their dominant EEG frequency or in their performance in a test battery of cognitive and motor tests (Bridgers and Delaney, 1989). Cognitive and motor function remained unaffected some months after the period of TM stimulation. There was, however, a slight but statistically significant decline in serum prolactin, which lacked correlation with the extent of stimulation, and an improvement in the oral word association test after stimulation. It was thought that the latter may be due to a practice effect and that similarly the change in prolactin level may not have related to TMS per se. In another study the secretion of pituitary

hormones was not shown to change over a period of up to 1.5 hours after TM stimuli (10-50 stimuli) (Merton et al., 1990).

In animal studies, TMS produced no change in the simultaneously recorded EEG, somatosensory evoked potentials, cerebral blood flow, blood pressure or heart rate, in anaesthetised cats (Eyre et al., 1988). Ventricular ectopic beats have been produced by direct stimulation through the chest wall in a dog (Silny, 1985; Bourland et al., 1989), although in rats (McRobbie and Foster, 1985) and rats sensitized with digitalis (Polson et al., 1982), no episodes of ventricular fibrillation were produced.

Exclusion Criteria

In accordance with the above evidence, some subjects were excluded from the present study (see Methods, overleaf).

Method

The discharge characteristics of 78 tonically active, low threshold single MUs were studied. These consisted of 34 MUs in FDI from 13 healthy subjects (aged 22 to 46 years) and 14 MUs in either deltoid, biceps, brachioradialis, flexor carpi ulnaris (FCU) or extensor digitorum communis (EDC) muscles of 3 of these subjects, and of 29 FDI MUs in 15 patients with UMN lesions (aged 35 to 72 years), 10 of whom had MS (aged 35 to 58 years). All studies were performed with the approval of the local ethical committee.

Patients with MS were classified according to the criteria proposed by Poser et al. (1983). The clinical details of the 10 MS patients are set out in Table 1. Impairment of fine finger movement was present in all MS patients except Patient A, but its interpretation was complicated by the presence of other neurological deficits affecting the hand.

Surface recorded CMAPs to TMS were also obtained from FDI in all subjects. Subjects were right handed. Informed consent was obtained and experiments were performed with the approval of the local ethics committee.

Exclusion Criteria

According to suggested exclusion criteria (see Barker et al., 1988 and preceding section), subjects with a past history of epileptic seizures, intracranial neurosurgery, or those fitted with cardiac pacemakers or cochlear implants were not recruited.

Table 1. Clinical Details of Patients with Multiple Sclerosis

Patient	Sex	Side Studied	Poser Classification*	Finger/Nose Ataxia	Tone increased at elbow or wrist	Exaggerated finger jerks	Upper limb reflexes	FDI strength (MRC)	Sensory abnormality
A	M	L	CPMS C1	+	-	-	E	4	-
B	F	R	CDMS A1	+	-	+	E	4	+[1]
C	M	L	CDMS A1	**	+	-	N	2	-
		R		**	-	-	N	4	-
D	M	R	LSDMS B2	+	-	-	N	5	-
E	F	L	CDMS A1	-	-	+	E	4	+[2]
		R		-	-	+	E	4	-
F	M	L	CDMS A2	-	-	-	N	4	+[3]
G	F	R	CDMS A1	-	-	-	N	5	+[4]
H	F	R	LSDMS B3	-	-	-	N	4	+[2]
I	F	R	CDMS A1	-	-	-	N	4	+[2]

* CDMS = Clinically definite MS, CPMS = Clinically probable MS, LSDMS = Laboratory supported definite MS.

** Proximal weakness and/or increased tone precluded testing

+ = present. - = absent. E = exaggerated. N = normal.

1 = Impaired light touch,vibration and joint position sense. 2 = Impaired light touch. 3 = Hyper-aesthesia.

4 = Impaired vibration sense.

Magnetic Stimulation

Circular Coil

The circular coil was held in place with a helmet, in a tangential plane to the scalp, with its centre over the vertex. This ensured that the coil position did not change during the course of an experiment. Up to 500 stimuli were delivered at one session. The inducing current when viewed from above flowed anticlockwise or clockwise for recordings from the right or left arms, respectively. In healthy subjects, recordings were made from the right arm only.

Stimulus Intensity

The intensity of stimulation used for healthy subjects ranged from 35% to 53% of the maximal output of the stimulator. In patients, the intensity of stimulation ranged from 50% to 73%. The stimulus intensity was set at the start of each experiment so that it was high enough to cause the MU to discharge at the primary peak (PP) latency (see below) without producing many compound MU discharges.

Stimulation protocols

Two stimulation protocols were used (Figs 2,3). In the first, stimuli were delivered regularly at once in 3.5-4s. Stimuli fell randomly in the on-going MU spike train which was confirmed by examining the distribution of the intervals between the stimulus and the previous voluntary discharge (see Results). In the second protocol, an MU spike was used to trigger the stimulator after a fixed delay of from 10 to 80 ms. The former are referred to as random stimulation experiments, the latter as spike triggered stimulation experiments.

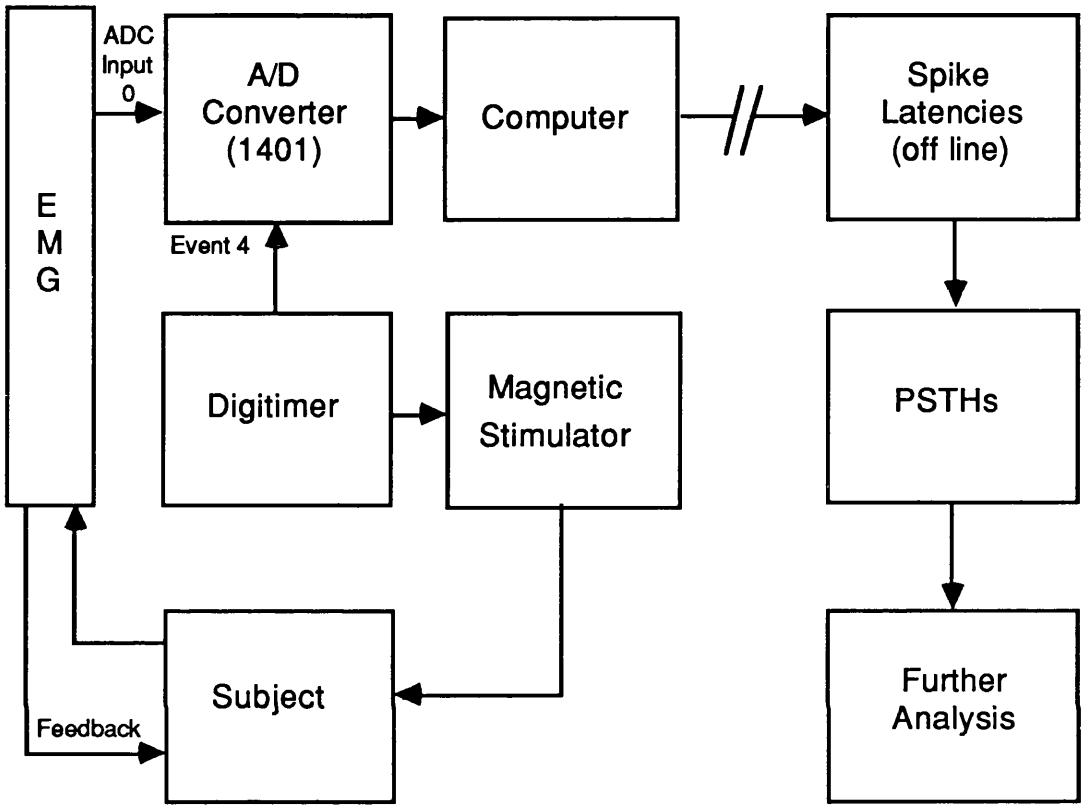


Fig. 2. Block diagram illustrating the arrangement for random stimulation experiments.

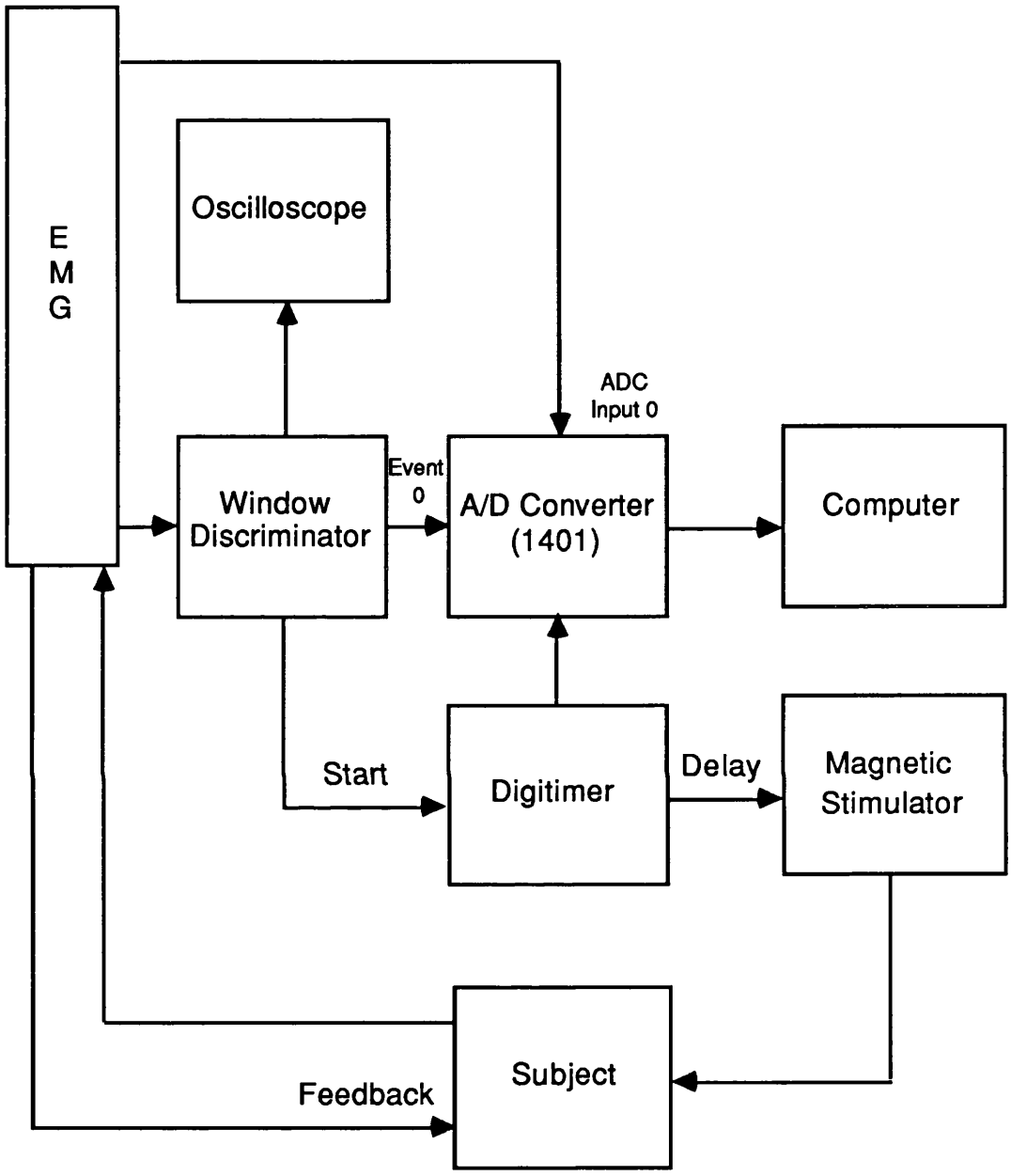


Fig. 3. Block diagram illustrating the arrangement for spike triggered stimulation experiments.

The stimulus intensity required to fire the MU, and that required to produce a just discernible surface recorded CMAP, both with FDI in the relaxed state, was also determined.

Recording

Subjects were seated, with their right arm supported in a comfortable position. For experiments on FDI, a horizontal platform supported the forearm, which was held in place by a series of clamps to prevent movement of other forearm muscles. Subjects were instructed to maintain a repetitive discharge from a single low threshold MU while making an isometric contraction against a resistance. Audiovisual feedback from the EMG was provided. The firing rate of the MU was not controlled.

The signal was amplified with a bandpass of 32 Hz to 16 or 32 kHz. The signal was then digitised at 10 kHz, producing a resolution of 0.1 ms (Cambridge Electronic Design 1401). Epochs of -250 to 250 ms relative to the stimulus were collected in random stimulation experiments, with a reduced pre-stimulus epoch of 50 ms for experiments involving increasing intensity of stimulation. In spike triggered experiments, the window discriminator was used to generate output pulses from the MU spikes, which then triggered the magnetic stimulator after a fixed delay. Epochs of -250 to 250ms relative to the triggering spike were digitised.

Spike Trains

The mean (+/- SD) pre-stimulus ISI for all MUs taken together was 115 (+/-22) ms for healthy subjects and 112 (+/-19) ms for the group of MS patients. In random stimulation experiments containing sufficient pre-stimulus data, there was found to be no significant serial dependency for ISIs in 15 out of

16 MUs ($p>0.05$, non-floating RHO) and significant serial dependency in only 1 MU.

Other Experiments

Peripheral Mechanical and TM Stimulation in the same MU

In 4 MUs from 3 healthy subjects, PSTHs were obtained during mechanical stimulation, in which the vibrator was used to deliver a rectangular pulse to the index finger. In 2 of these MUs (MU1-random stimulation experiment and MU2-spike triggered experiment) PSTHs were also obtained during TMS. The vibrator hammer was attached to the index finger at the level of the proximal interphalangeal joint with a circular connector. The arm was supported on a platform and was insulated with padding to prevent transmission of the mechanical stimulus to other parts of the forearm. The tap was delivered in a direction perpendicular to the long axis of the index finger, producing either a rapid adduction or abduction movement.

For the adduction stimulus, the pulse duration/pulse gain/number of trials/TM stimulus intensity (where used) was:

MU 1: 2 ms/0.75/1000/40%

MU 2: 2 ms/1.7/942/36%

MU 3: 1 ms/1.0/608

MU 4: 1 ms/1.0/1000.

For the abduction stimulus (MU 1) the same variables were: 2 ms/0.75/1000.

Because the direction of the abduction stimulus coincided with the direction of the movement required for the background activation of FDI, however, the abduction mode of peripheral stimulation made it difficult for the subject to maintain the activation.

Restriction of Finger Movement

In a random experiment on 1 MU, the index finger was fixed to prevent any movement, for 100 trials, followed by another 100 trials during which the finger was free to move.

Data Analysis

Evaluation of Raw Data

The discharge times of the index MU were measured off line to the nearest 0.1 ms, with 'Select' software (see Materials). Measurement off line had 5 advantages over electronic triggering on line:

1. Accurate identification of the same MU throughout the course of experiment.
2. The detection of compound discharges evoked by the TM stimulus.
3. Identification of the index MU within a compound discharge.
4. The stimulus artefact did not obscure the measurement of surrounding spikes.
5. Measurement off line allowed the subsequent sorting of the experimental trials into PSTHs or raster plots.

Defining the Features of the PSTH

Figure 4 illustrates the definition of peaks in the PSTH used in the present study. As in all random stimulation experiments, the stimulus is at time zero. The mean and SD of the bin count during the period before the stimulus was used for defining periods of increased firing probability. Such definitions therefore incorporated the pre-stimulus firing rate and the number of experimental trials. Bin widths of 1 ms or 0.2 ms were used. PP was considered to be present if filled bins intersected a line drawn at 2 SDs above

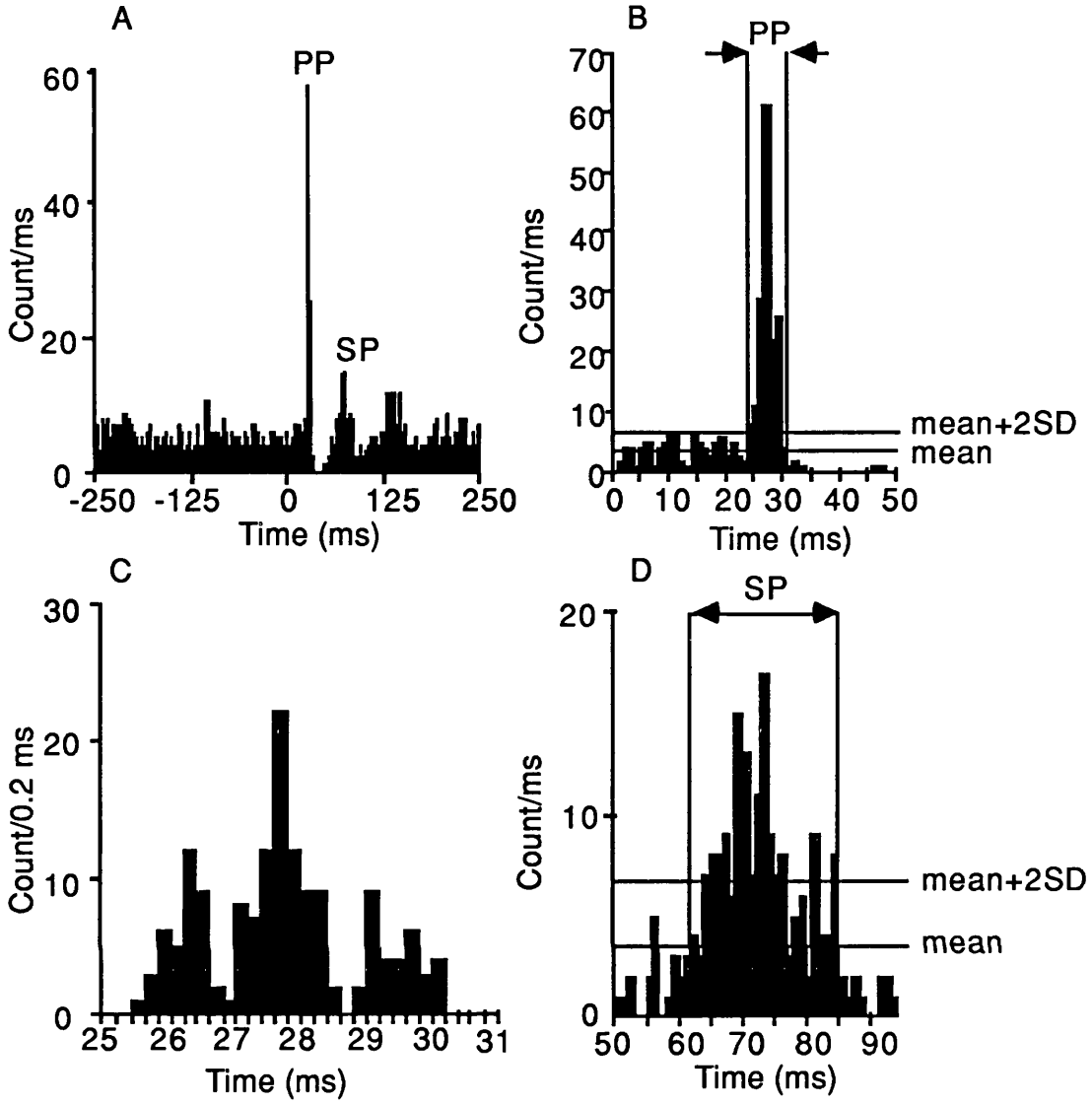


Fig. 4. Criteria for defining peaks in the PSTH (A) constructed with a bin width of 1 ms (500 trials). The stimulus is at time zero, as in all the illustrated PSTHs. The primary (PP) and secondary (SP) peaks are indicated. B: PP was considered to be present if filled bins intersected a line drawn at 2 SDs above the mean firing level calculated from the 250 ms before the stimulus. The latency and duration of PP were calculated from the intersection of the peak with the mean pre-stimulus firing level. C: PSTH of the same MU constructed with a bin width of 0.2 ms and showing 3 sub-peaks. All sub-peaks had a peak count of at least 10 times the mean pre-stimulus firing level. Intermodal intervals were calculated from the mid point times of the peak bin counts within each sub-peak. D: SP was considered to be present if filled bins intersected a line drawn at 2 SDs above the mean pre-stimulus firing level. The onset and offset of SP were then defined by the points at which the bin count fell below the mean pre-stimulus firing level for more than 2 consecutive bins.

the mean firing level calculated from the 250 ms before the stimulus (Fig. 4B) (bin width 1 ms). The latency and duration of PP were calculated from the intersection of the peak with the mean pre-stimulus firing level. All sub-peaks within PP had a peak count of at least 10 times the mean pre-stimulus firing level (Fig. 4C) (bin width 0.2 ms). A secondary peak (SP) was considered to be present if filled bins intersected a line drawn at 2 SDs above the mean pre-stimulus firing level (Fig. 4D) (bin width 1 ms). The onset and offset of SP were then defined by the points at which the bin count fell below the mean pre-stimulus firing level for more than 2 consecutive bins.

Sorting

In all experiments on single MUs, a series of trials could be sorted into those containing a discharge within PP (PP-trials) and those without (non-PP trials).

Alternatively, trials could be sorted into those containing a discharge within SP (SP- trials) and those without. Furthermore, because it was usually the case that if the MU fired in PP, it did not do so in SP, and vice versa, experiments could be sorted into three groups: PP-trials, SP-trials and non-PP/SP trials (Fig. 5).

Within each trial, individual ISIs could be sorted into those which occurred before the stimulus (referred to as pre-stimulus ISIs) or those which occurred during the application of a stimulus (referred to as peri-stimulus ISIs).

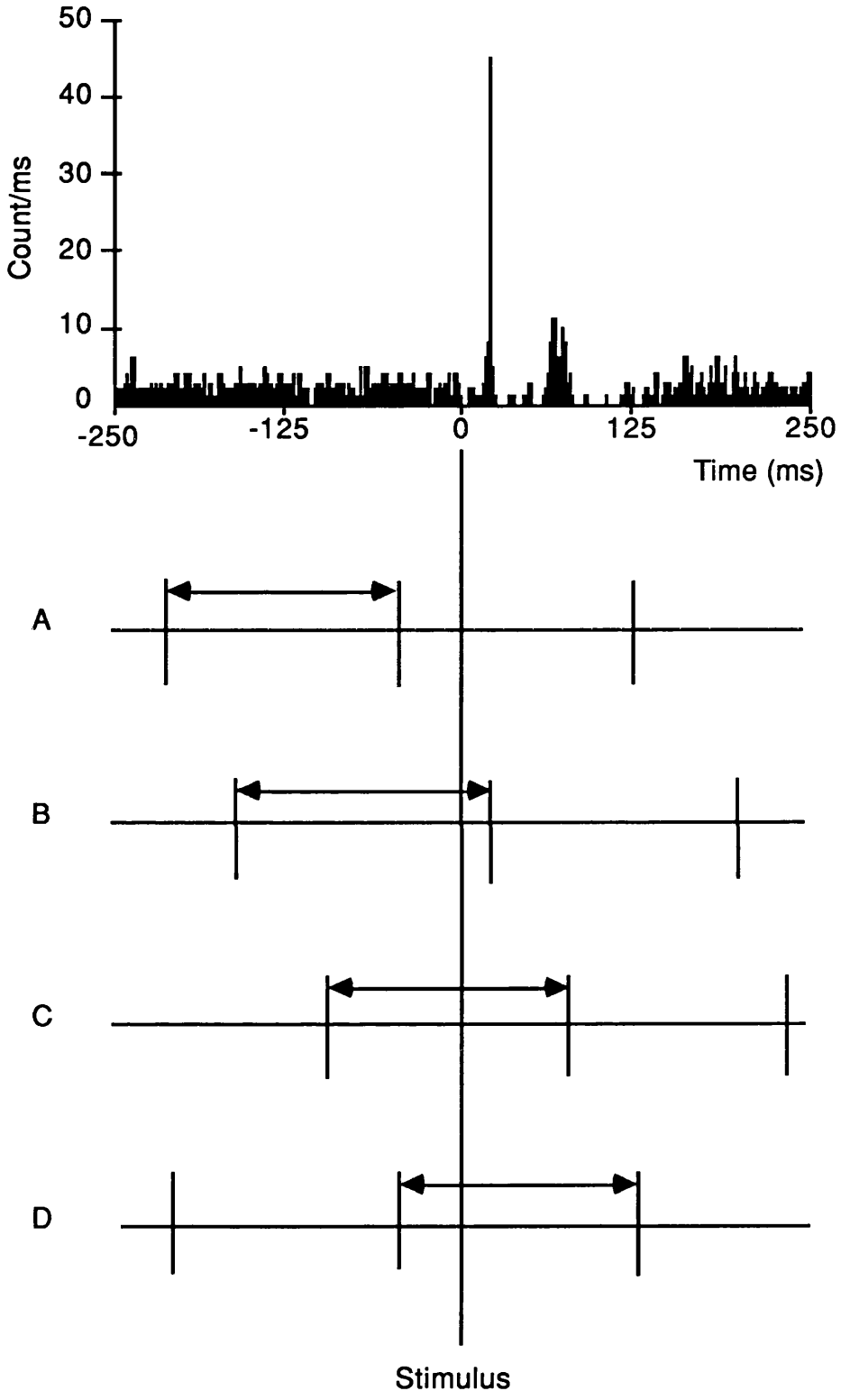


Fig. 5. Criteria for sorting ISIs. PSTH from a random stimulation experiment on a biceps MU (232 trials) with diagramatic representations of 4 spike trains (A-D) each aligned with the stimulus (vertical line) and the PSTH. A: pre-stimulus ISI. B,C,D: peri-stimulus ISIs (B: PP-trial, C: SP-trial, D: non PP/SP-trial).

Cross-Sorting

A process of cross-sorting could be applied to a pair of MUs that were simultaneously tonically active, and which both produced separate PSTHs with a significant PP at different latencies. PSTHs for an MU could therefore be sorted not only according to its own PP-trials (as above), but also according to the occurrence of PP-trials in the fellow MU (cross-sorting).

Student's t Test and Serial Changes in the ISI

The Student's t test was used to demonstrate principles and problems of determining the level of significance of observed serial changes in ISI (see Discussion). Its validity is strictly limited, particularly with reference to the presence of serial correlation between successive ISIs, as described in the Discussion. The data used was continuous, and normally distributed ($p < 0.05$, Kolmogorov-Smirnov one sample test). The t test is a robust test of significance, being relatively insensitive to violations of its mathematical assumptions (Boneau, 1960). Statistics were applied with the guidance of the Statistics Department, Oxford University.

CHAPTER 3

Results

Healthy Subjects

First Dorsal Interosseous Muscle:

Raw Data

Major Peaks in Firing Probability in the PSTH

Decrease in Firing Probability Following PP

Sub-peaks in the PP region of the PSTH

Double Discharges at the PP Latency

Simultaneous Recordings from 2 MUs

Factors Influencing the Probability of Evoking a PP Discharge

Other Arm Muscles

Serial Changes in the Interspike Interval

Other Experiments on FDI Relating to SP

Patients with Upper Motor Neurone Disorders

Cerebrovascular Disease

Motor Neurone Disease

Multiple Sclerosis

Peripheral Demyelination Combined with Prolonged

Central Motor Conduction Time

Group of Patients with Multiple Sclerosis

Healthy Subjects

First Dorsal Interosseous Muscle

Raw Data

Figures 6,7 and 8 show examples of raw data, consisting of MUPs from FDI MUs in 2 healthy subjects. In Fig. 7, several successive trials from a random stimulation experiment show the discharge of 2 recruited MUs, and in Fig. 8, discharges in the region of PP (see below) are shown.

Major Peaks in Firing Probability in the PSTH

Following the stimulus, at least 2 periods of increased firing probability were seen in PSTHs when constructed with a bin width of 1 ms. These peaks were identified by exceeding the mean pre-stimulus firing level, plus 2 SDs (Fig. 4, Methods).

Latency of the Primary and Secondary Peaks

All MUs showed an increased period of firing probability with an onset latency ranging from 20-31 ms after the stimulus. This was termed the primary peak (PP). This was followed by a period of reduced firing probability, which was itself followed by a second period of increased firing probability, at an onset latency ranging from 56-90 ms after stimulus, and with a peak latency varying from 64 to 100 ms (mean 81.5 ms). This was termed the secondary peak (SP). SP occurred in 10 of 26 FDI MUs according to the adopted definitions (Fig. 4). This late peak was found not to be due to a resumption of firing after PP discharges by the examination of sorted PSTHs. In the remaining 16 MUs, a second rise in firing

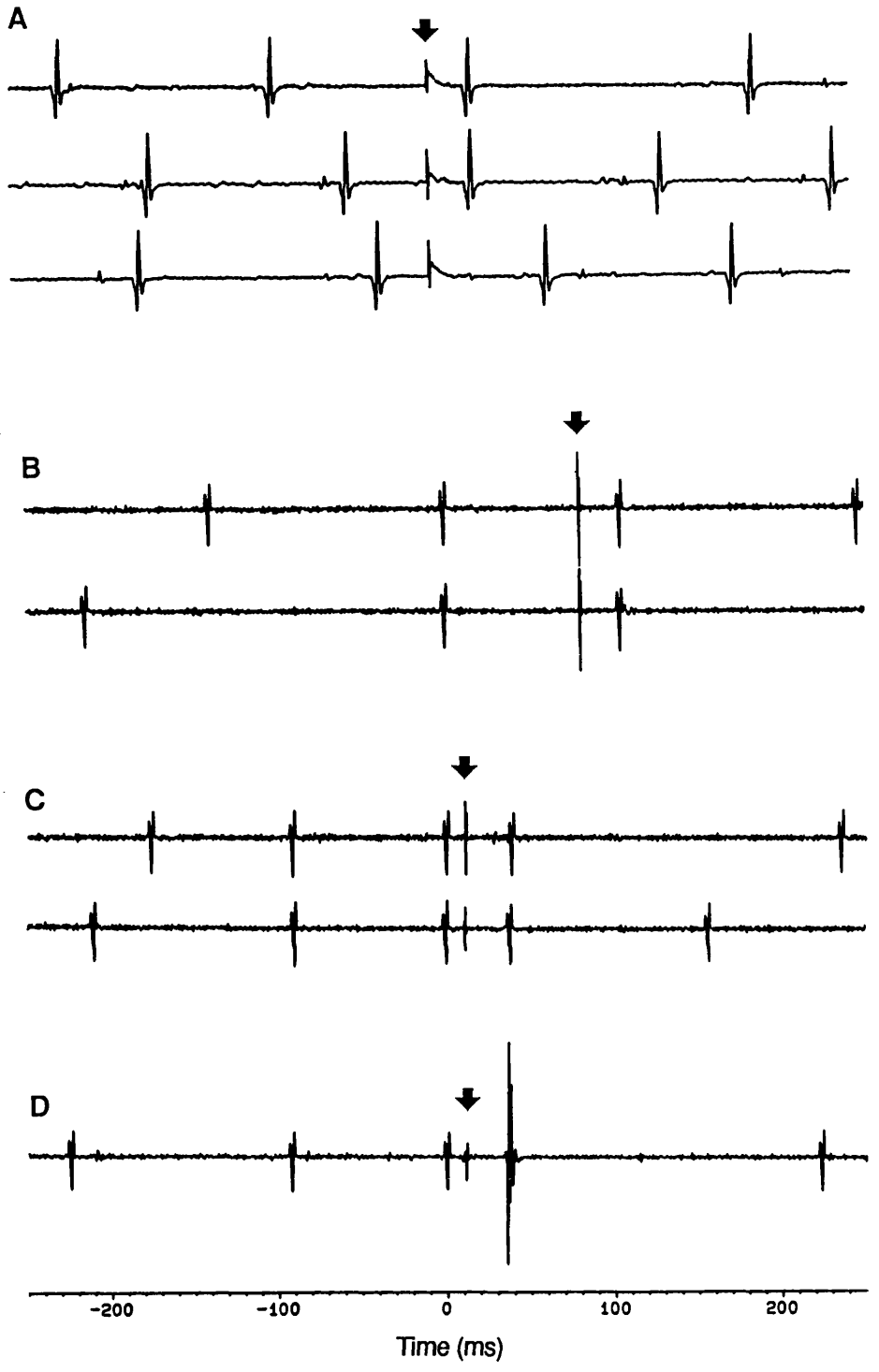


Fig. 6. EMG recordings from repetitively firing single FDI MUs. TM stimuli were delivered at times indicated by arrows. A: Random stimulation protocol in which the stimuli were applied randomly with respect to the on-going MU spike train. In the upper 2 records the MU fired at the PP latency; in the lower record at the SP latency. B: Spike triggered stimulation protocol in which a voluntarily occurring spike at time zero triggered the stimulator after a delay of 80 ms. The MU fired at the PP latency. C: Spike triggered trials in which the same MU as B triggered a stimulus at a delay of 10 ms. The MU again fired at the PP latency. D: The spike triggered stimulus caused a compound MU discharge.

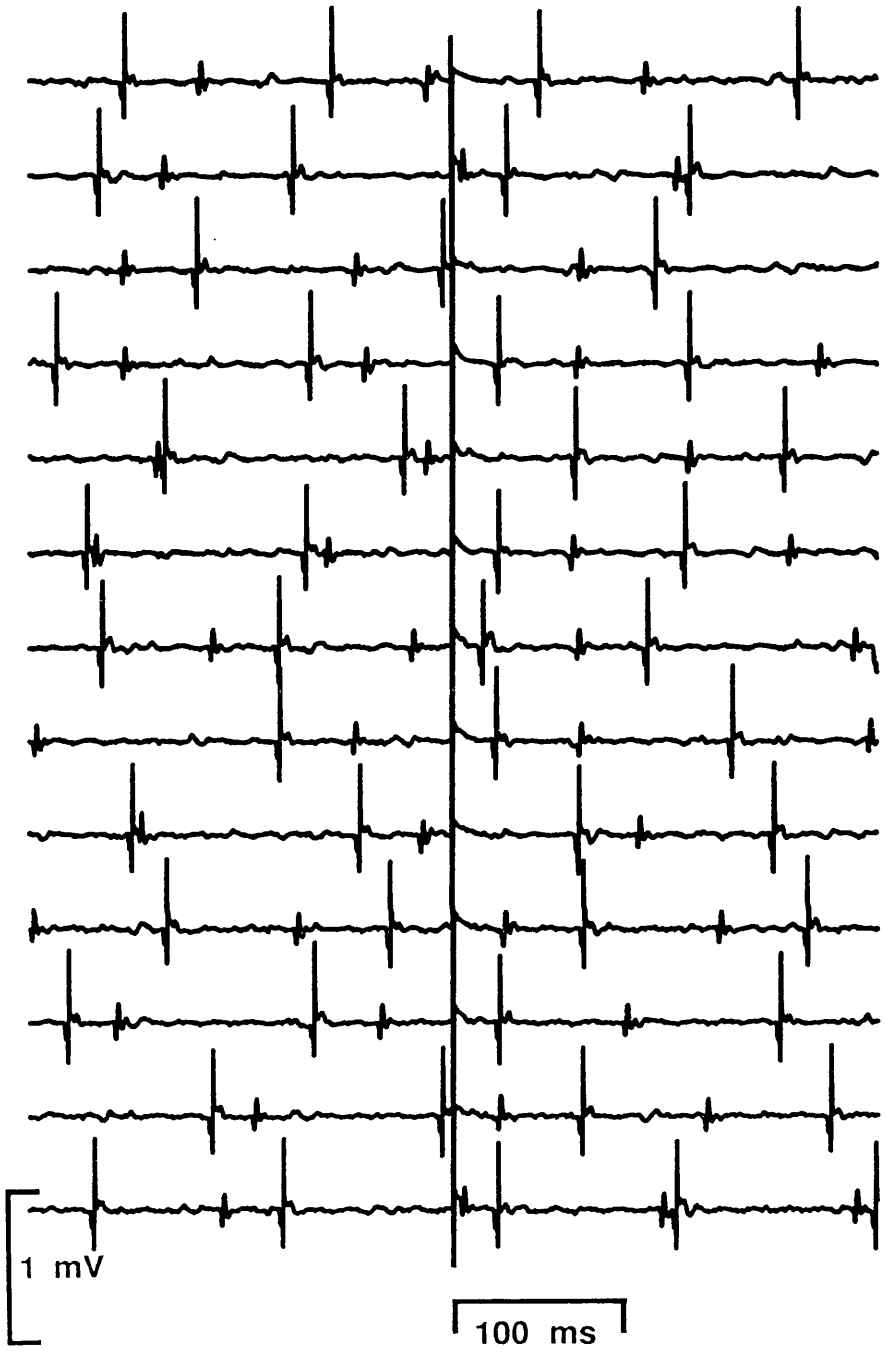


Fig. 7. EMG recordings from 2 FDI MUs in 13 trials, aligned with the stimulus (vertical line). In a proportion of trials, both the large and small MUPs occur at latencies of either 20 or 75 ms after the stimulus, corresponding to PP and SP, respectively. Neither MU fires at both latencies within the same trial.

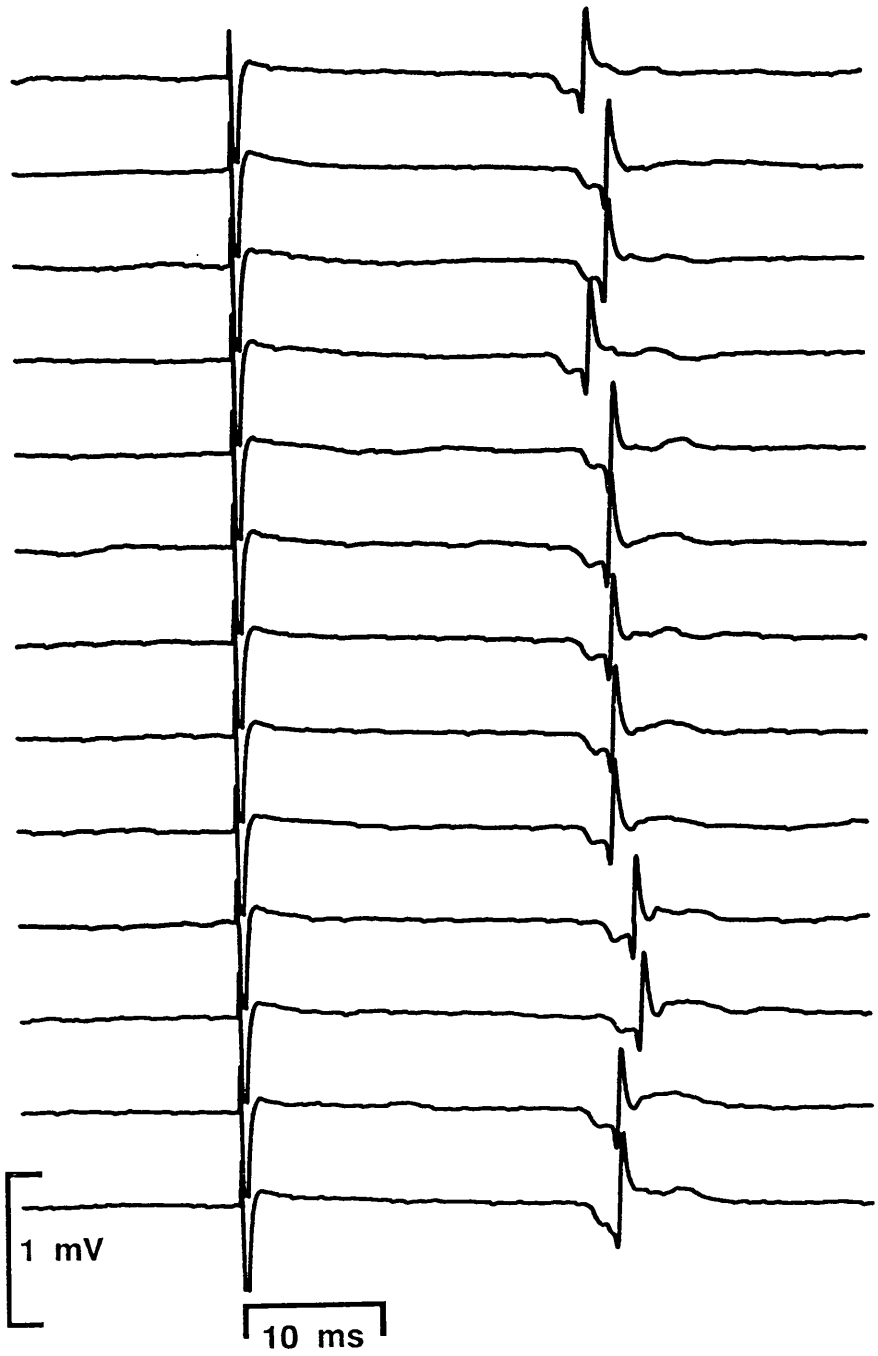


Fig. 8. EMG recordings from a single FDI MU in 13 trials each containing a discharge at the PP latency, aligned with the stimulus (vertical line). In each trial, the discharge occurs at one of three preferred latencies: early (trials 1, 4); intermediate (trials 2, 3, 5-9, 12, 13); and late (trials 10, 11) (trials numbered from above downwards).

probability was frequently seen peaking at about 70 ms after the stimulus, but was of insufficient height to meet the required definition for SP. (Of these 16 MUs, 7 were studied with spike triggered stimulation experiments. In the resulting PSTHs, the peak of increased firing probability that occurred due to a resumption of firing after PP may have overlapped SP and obscured its appearance.) PP and SP in PSTHs from other arm muscles are described below.

Duration of PP and SP

The duration of PP was shorter than that of SP. The duration of PP ranged from 2 - 9 ms with a mean (+/-SD) duration of 4.6 (+/-1.7) ms. The duration of SP ranged from 5 - 49 ms, with a mean (+/-SD) duration of 20.9 (+/-12.0) ms. SP was never found in isolation in a healthy subject. PP was always abruptly terminated by a period of zero firing probability and was always unimodal when constructed with a bin width of 1 ms.

The evolution of PP, SP and the intervening period of reduced firing probability as the number of trials is increased is seen in Fig. 9. During the course of the first 100 trials of a random stimulation experiment, the duration of PP was found to become progressively wider, until a maximum duration was reached, after which it remained constant (Fig. 10).

Decrease in Firing Probability Following PP

A period of decreased firing probability followed the offset of PP. The firing probability remained below the mean pre-stimulus firing level for a duration of 14-51 ms, with a mean (+/-SD) of 33.1 (+/-10.45) ms. In random stimulation experiments, its onset was defined as the offset of PP, and its offset was defined as the time at which the bin count first

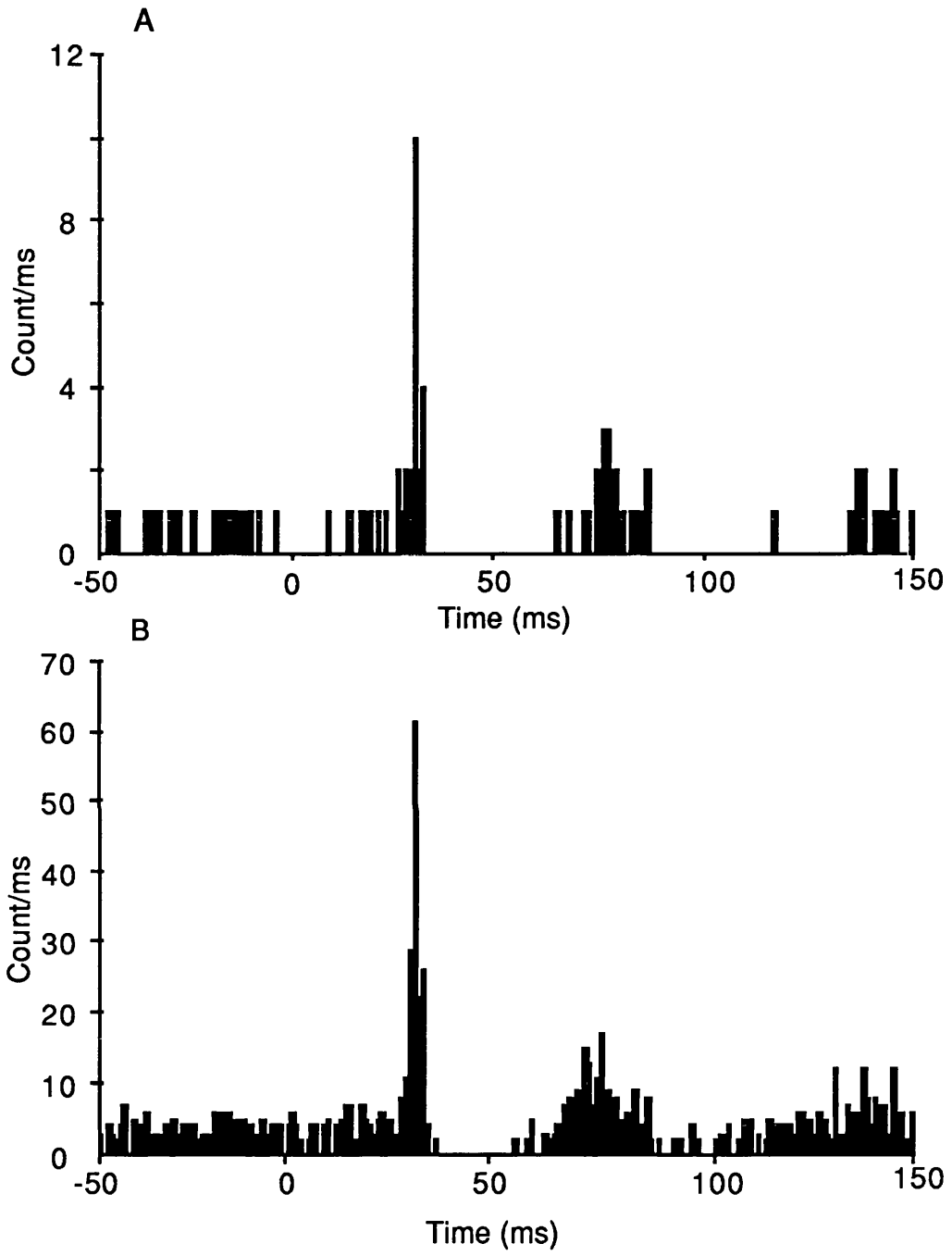


Fig. 9. 2 PSTHs from a random stimulation experiment, which consisted of a total of 500 trials, plotted at 50 trials (A) and 500 trials (B) to demonstrate the evolution of late features in the PSTH. PP and SP are evident at 50 trials, with periods of zero firing probability preceding and following SP. After 500 trials the onset latency of SP is earlier than at 50 trials although the peak latency of both PP and SP remained the same. A period of zero firing probability following PP seen at 50 trials persists at 500 trials, in contrast to the period following SP.

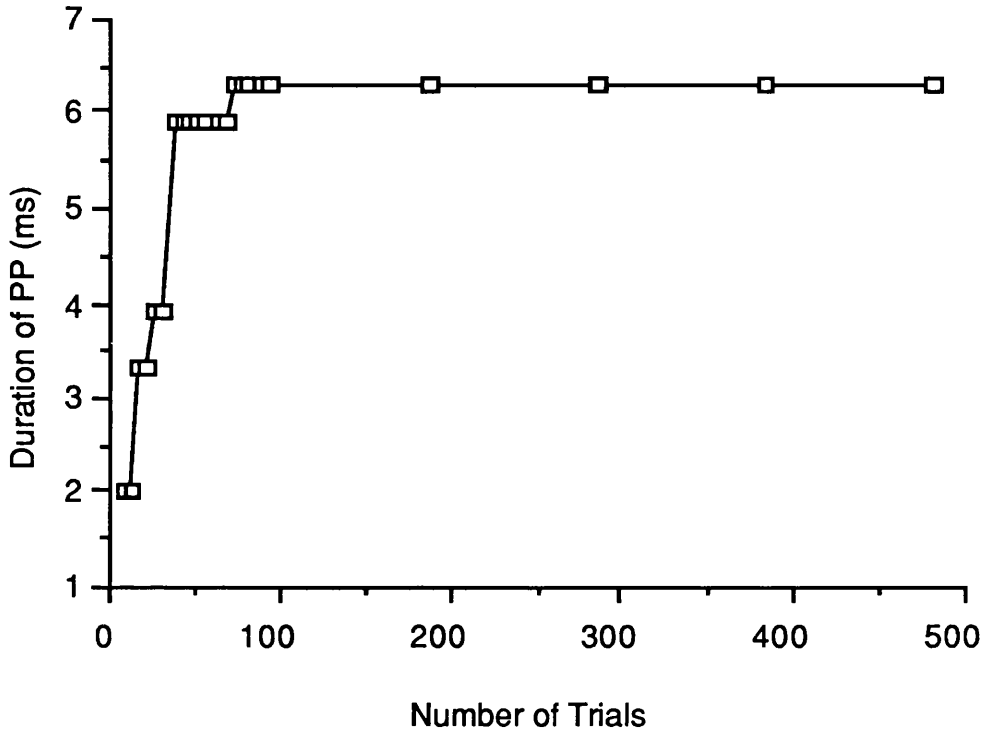


Fig. 10. The duration of PP (from the onset of the first sub-peak to the offset of the last sub-peak) plotted against the trial number for a random stimulation experiment on 1 FDI MU (500 trials). The duration of PP increased during the initial stages of this experiment until trial 73, when it reached a plateau.

exceeded the mean pre-stimulus bin count. The offset of the period of reduced firing probability shortly preceded, or occasionally coincided with, the onset of SP according to these criteria (see also Fig. 9). No correlation was found between the duration of this period and either the height of PP divided by the number of trials (Spearman Rho 0.23, $p > 0.05$, $n = 24$ pairs) or the proportion of PP-trials (Spearman Rho 0.15, $p > 0.05$, $n = 24$ pairs).

Sub-Peaks in the PP Region of the PSTH

In 17 of 26 FDI MUs, the PP region of the PSTH was found to consist of 2 or 3 sub-peaks, when plotted with a bin width of 0.2 ms (Figs 11A,C). Cumulative sums of the bin counts of such peaks were constructed (Fig. 11D). Of the remaining 9 MUs, only a single narrow peak was present within PP in 5 (Fig. 11B) and in 4 MUs, sub-peaks were not evident.

Intermodal intervals

The intermodal interval between neighbouring sub-peaks from the pooled data ranged from 0.6 to 2.4 ms, with a mean (±SD) of 1.4 (±0.4) ms (n=26). Between the first and second sub-peaks, the mean (±SD) intermodal interval was 1.3 (±0.4) ms (n=17). Between the second and third sub-peaks, the mean (±SD) intermodal interval was 1.4 (±0.4) ms (n=9). There was no significant difference between these 2 sets of intervals (Student's t test, $p>0.05$).

Height and Duration of Sub-peaks

The relative heights of sub-peaks from each MU were compared according to their rank order. The rank order was found to have no clear influence on peak height, in both random stimulation and spike triggered stimulation experiments. (A possible exception may apply to those PSTHs

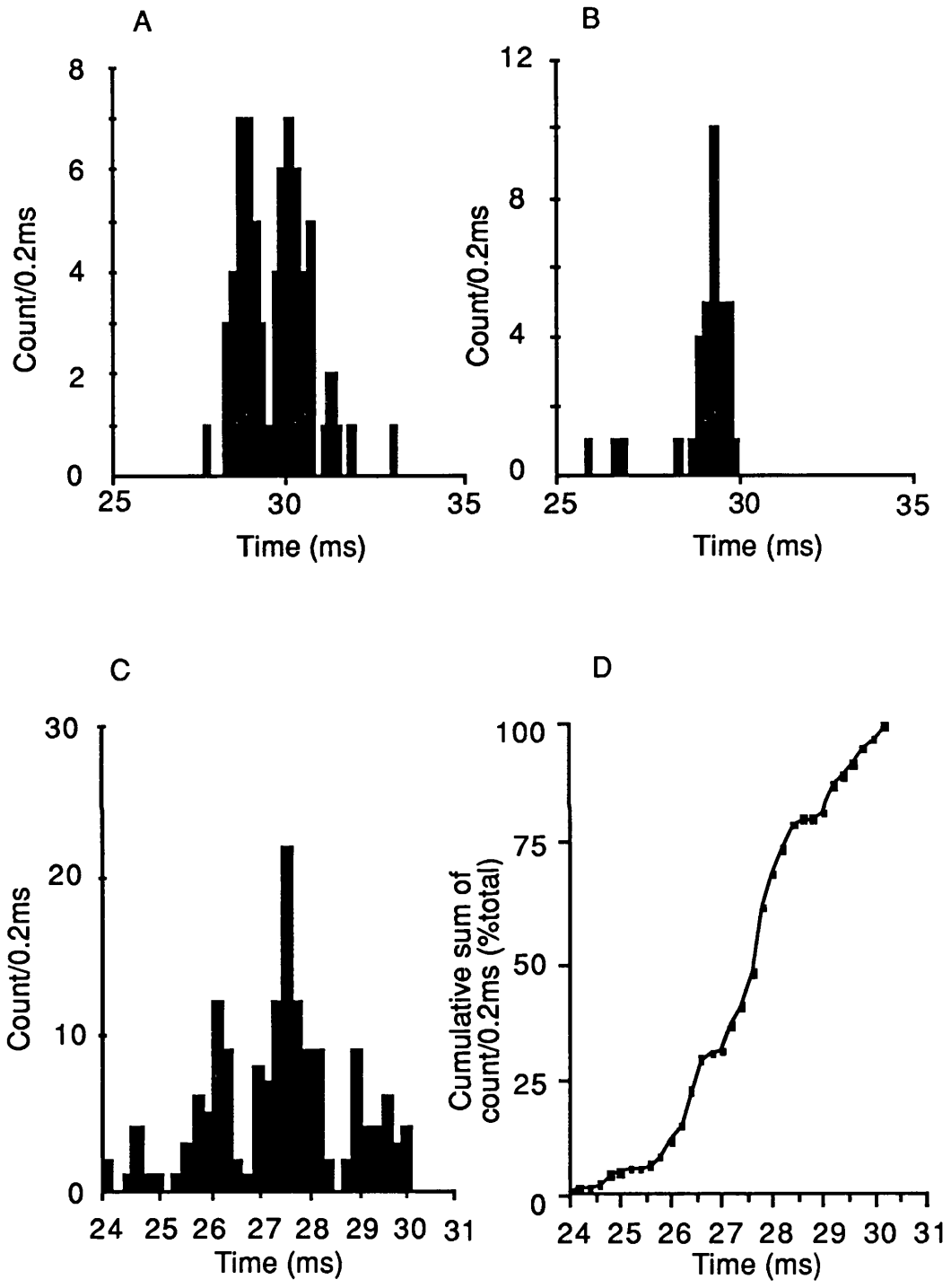


Fig. 11. PP region of 3 PSTHs from 3 MUs demonstrating sub-peaks (A, B, C) with the cumulative sum from C normalised to the total number of discharges within PP (D). Intermodal intervals: 1.4 ms (A), 1.4 and 1.4 ms (C). The positions of maximum slope on the cumulative sum correspond to the modes of each sub-peak from C and the inflections correspond to the minima between the sub-peaks.

with 3 sub-peaks, in which 2 spike triggered stimulation experiments produced sub-peaks of diminishing height at the progressively longer latencies, and in 6 random stimulation experiments which showed the opposite trend, with the third (longest latency) sub-peaks attaining the greatest height in 3 of the 6 MUs. The small sample numbers, however, precluded statistical testing for the significance of these differences.) There was also no obvious relationship between the duration of sub-peaks and their rank order.

Double Discharges at the PP latency

Clearly discernible double discharges of the MU under examination at the PP latency, without the superposition of other motor units discharging simultaneously in response to the stimulus, were found to be rare. At higher stimulus intensities double discharges may have been more frequent, but they could not be distinguished because of the evoked discharges of other MUs. Raw data showing a double discharge at the PP latency is shown in Fig. 12, with an inter-discharge interval of 3.9 ms. The interval between the 2 sub-peaks for this MU was 3.4 ms.

Simultaneous Recordings From 2 MUs

For 1 pair of MUs, cross-sorted PSTHs were constructed, sorting the PSTH for 1 MU into PP- or non PP-trials according to the presence or absence of a discharge at the PP latency in the other MU (see Methods). The peak latency for PP was 2 ms later for 1 MU when compared to the other, with no overlap. Cross-sorting demonstrated that, at the stimulus intensity used (42%), both MUs never fired at the PP latency within the same trial. This was confirmed by inspection of the raw data. In the cross-sorted PSTH for

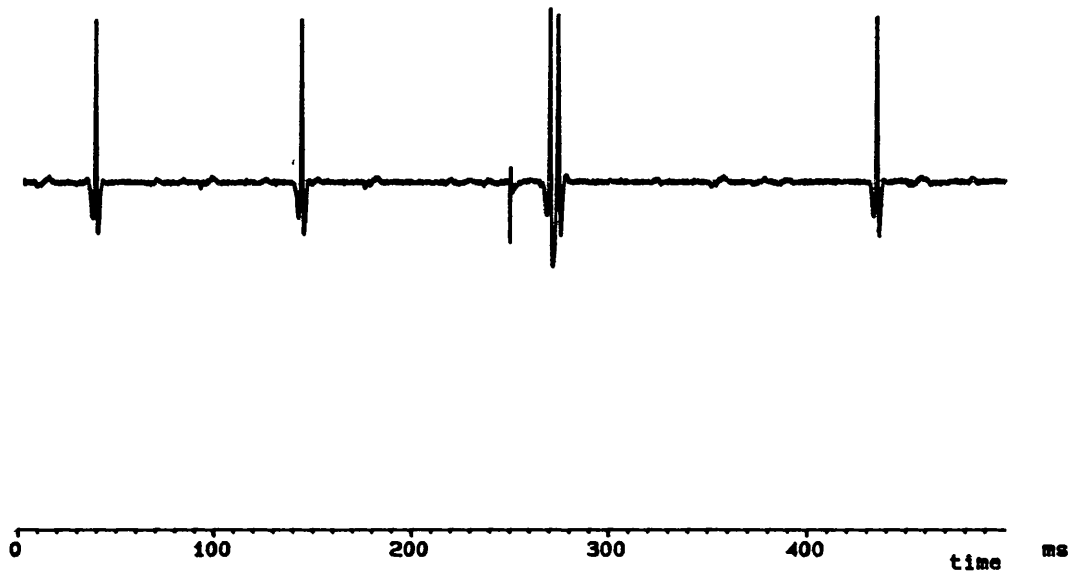


Fig. 12. EMG recording from an EDC MU showing a double discharge at the PP latency. The inter-discharge interval was 3.9 ms. (0.3 mV/cm, stimulus at 250 ms).

trials containing a discharge within PP (PP-trials) in the fellow MU, there was no obvious difference in the firing probability before the occurrence of PP (and indeed, before the application of the stimulus) when compared to earlier stages of the pre-stimulus period.

Factors Influencing the Probability of Evoking a PP Discharge

1. Intensity of Stimulation

Effect on PP

The effect of increasing the intensity of stimulation was studied in 7 MUs in random stimulation experiments. The trend in Fig. 13 shows an increase in the proportion of PP-trials with increasing relative stimulus intensity (i.e. stimulus intensity expressed as a percentage of maximum output divided by the intensity required to produce a just discernible CMAP). A similar trend is seen when plotted against stimulus intensity expressed simply as % maximum output.

With FDI relaxed, the stimulus intensity at which a just discernible CMAP recorded with surface electrodes over FDI could be obtained (CMAP threshold) ranged from 36 to 54 %.

Effect on latency of Sub-peaks

In 5 experiments (4 random stimulation, 1 spike triggered) PSTHs containing sub-peaks were produced for each MU at more than 2 stimulus intensities. Of these, the sub-peaks from 2 MUs did not show any obvious change in latency or form. Of the remaining 3, increasing the intensity of

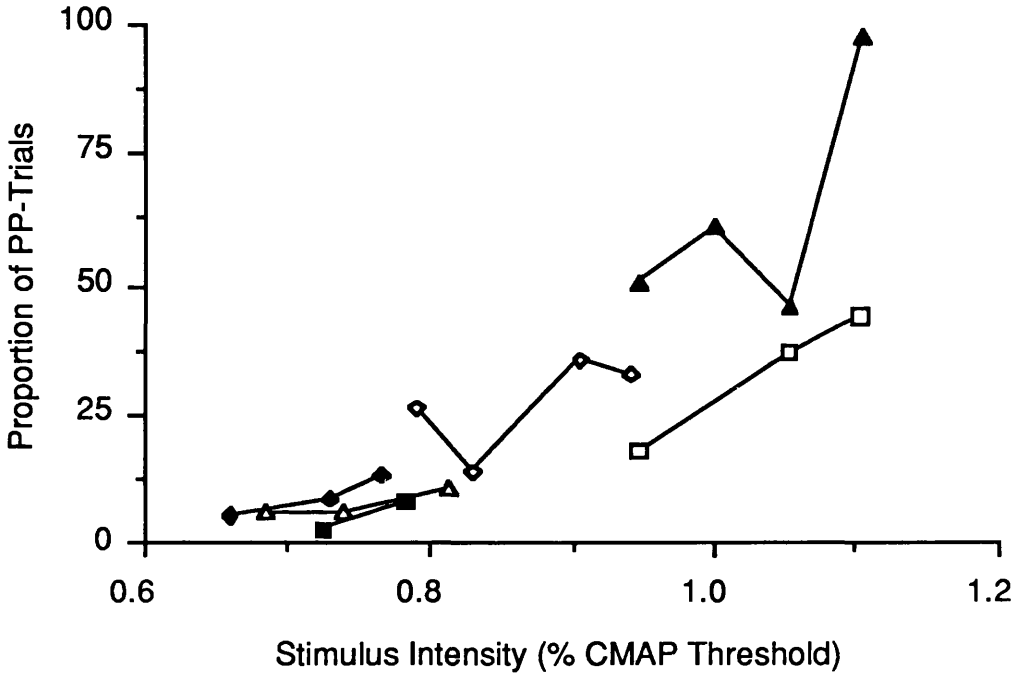


Fig 13. The proportion of PP-trials plotted against the stimulus intensity (expressed as a proportion of the stimulus intensity required to produce a just discernible surface recorded CMAP to TMS) for 6 FDI MUs in random stimulation experiments. Each point represents the proportion of PP-trials out of 100 experimental trials. There is a trend towards increasing proportion of PP-trials with increasing stimulus intensity.

stimulation produced sub-peaks that were earlier in latency in 2 MUs (Fig. 14) and longer in latency in 1 MU (Fig. 15).

In the 2 MUs where earlier sub-peaks were caused to appear, this had the effect of reducing the height of later sub-peaks and correspondingly increasing the height of earlier ones. If an earlier sub-peak was produced where one had not existed before, the interval between the new sub-peak and the following sub-peak was similar to the interval between sub-peaks at the lower intensity.

Effect on Slope of Cumulative Sum

In the above 4 MUs from random stimulation experiments, there was no obvious change in the slope of the normalised cumulative sums. This was true for both the whole cumulative sum, and for those segments of each sum that corresponded to individual sub-peaks.

2. Firing Rate

Instantaneous Firing Rate

In 5 MUs from random stimulation experiments, no significant difference was found between the pre-stimulus ISI for PP-trials (used as an index of instantaneous firing rate) and that for non PP-trials ($p>0.05$, Student's t test).

Mean Firing Rate

In random stimulation experiments on 2 MUs that were each made to discharge voluntarily at 2 different firing rates (see p 105) during a series of TM stimuli, there was no difference in the proportion of PP-trials at the 2 different mean firing rates ($p>0.05$, z test).

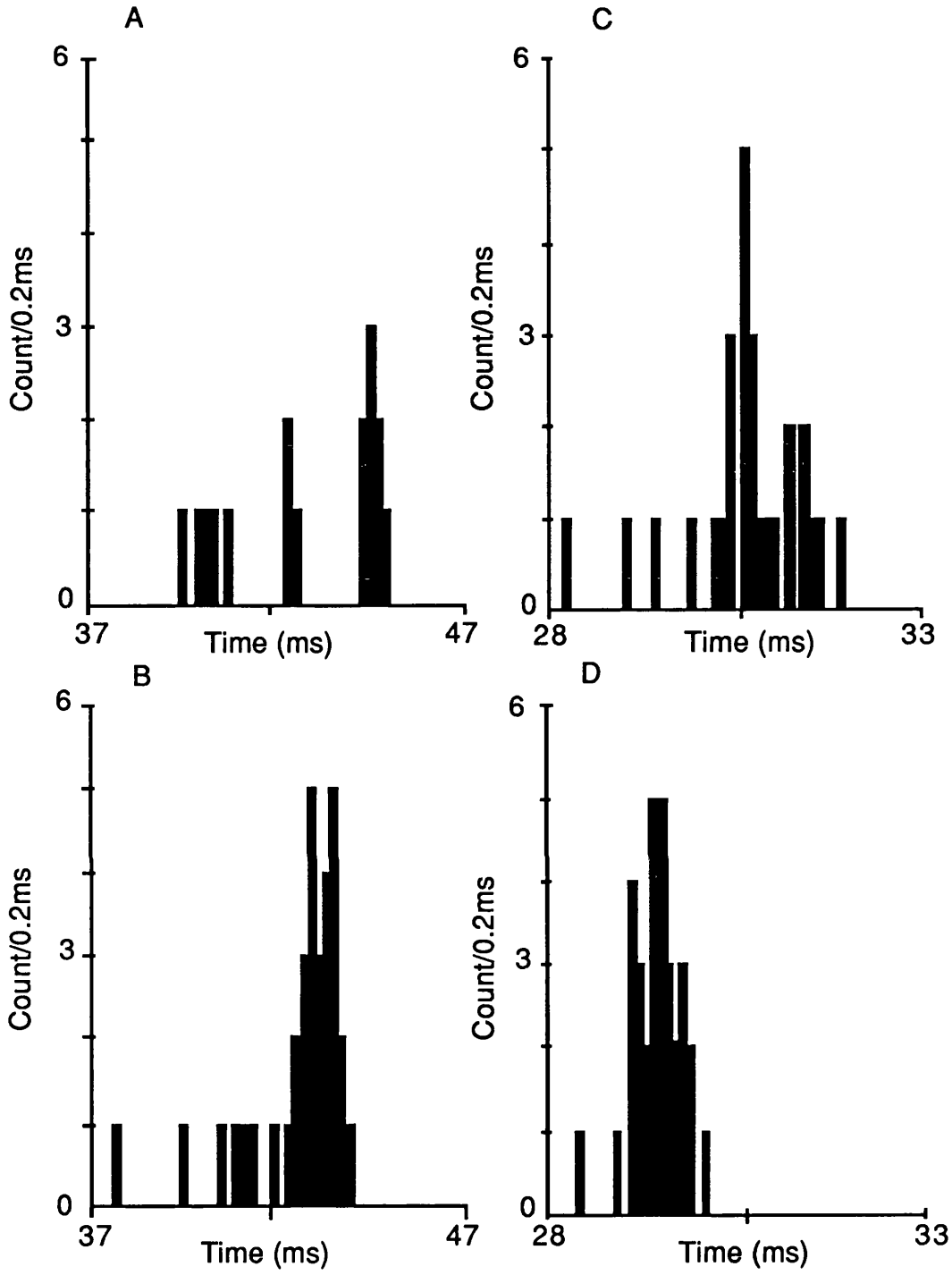


Fig. 14. PSTHs from random stimulation experiments on 2 FDI MUs in which stimuli at 2 intensities were given. The effect of increasing stimulus intensity upon sub-peaks is seen. A, B: PP region of the PSTH from a random stimulation experiment with a stimulus intensity of 42% (A, 80 trials) and 48% (B, 80 trials). C, D: results from another FDI MU at 42% (C, 93 trials) and 50% (D, 90 trials). On increasing the stimulus intensity, the principle sub-peaks evident at the lower stimulus intensity are replaced by new sub-peaks with modal latencies that are 1.3 and 0.9 ms earlier, respectively. This contrasts with the changes seen in Fig.15.

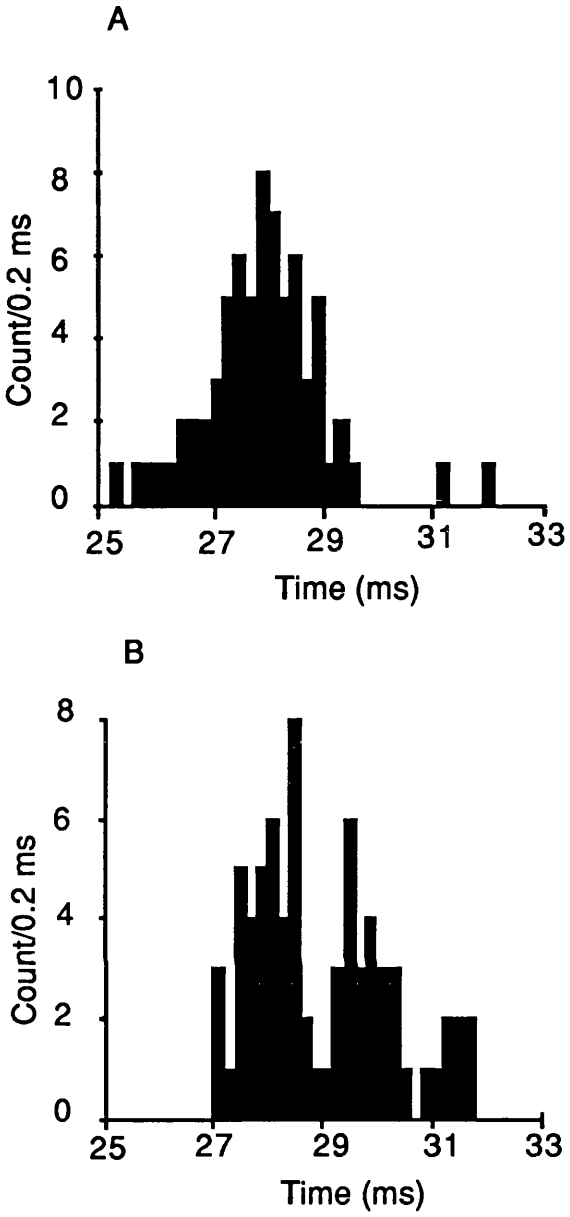


Fig. 15. PSTHs from a spike triggered stimulation experiment in which stimuli at 2 intensities were given 60 ms after a triggering MU spike. A: a stimulus intensity of 32% has produced a single peak with a modal latency of 28.0 ms (188 trials). B: a stimulus intensity of 42% has produced two additional sub-peaks at longer latencies, with intermodal intervals of 1.0 and 2.0 ms (99 trials). This contrasts with the changes seen in Fig. 14.

Mean pre-stimulus ISI/No. trials at low and high firing rates, respectively:	MU1 77.9 ms/88; 112.9 ms/96 MU2 96.3 ms/216; 114.8 ms/197
Proportion of PP-trials (%) at low and high firing rates, respectively:	MU1 0.52 and 0.45 MU2 0.41 and 0.39

3. Runs of PP-trials

It was noted that PP-trials sometimes occurred in a series of consecutive trials. This was termed a run of PP-trials when 4 or more PP-trials occurred in sequence (Fig. 16). To study this, the one sample runs test of randomness (Siegel and Castellan, 1988) was applied to an MU in a random stimulation experiment (500 trials). Of the 500 trials in this experiment, 31.7% were PP-trials, with 6 runs containing 4-6 consecutive PP-trials, of which 4 occurred in the first 100 trials. The order of the occurrence of PP and non-PP trials was found to be non random ($z > 1.96$, $p<0.05$).

Comparison of Interspike and Spike-Stimulus Intervals

In the first 100 trials of the above experiment, there were 4 runs of PP-trials. With regard to the instantaneous firing rate, no significant difference was found between the mean pre-stimulus ISI for runs of PP-trials (131.1 ms +/- 17.6 ms, n=15) and that for runs of non-PP trials (133.5, +/-24.8 ms, n=27) ($p>0.05$, unpaired Student's t test) (see Discussion for limits for these comparisons).

The mean interval from the stimulus to the preceding voluntary discharge (spike-stimulus interval) for runs of PP-trials (92.2 +/-18.3, n= 19) was significantly longer, however, than that for the runs of non PP-trials that occurred in the same 100 trials (55.5 +/- 41.9 ms, n=22, $p<0.001$) and also

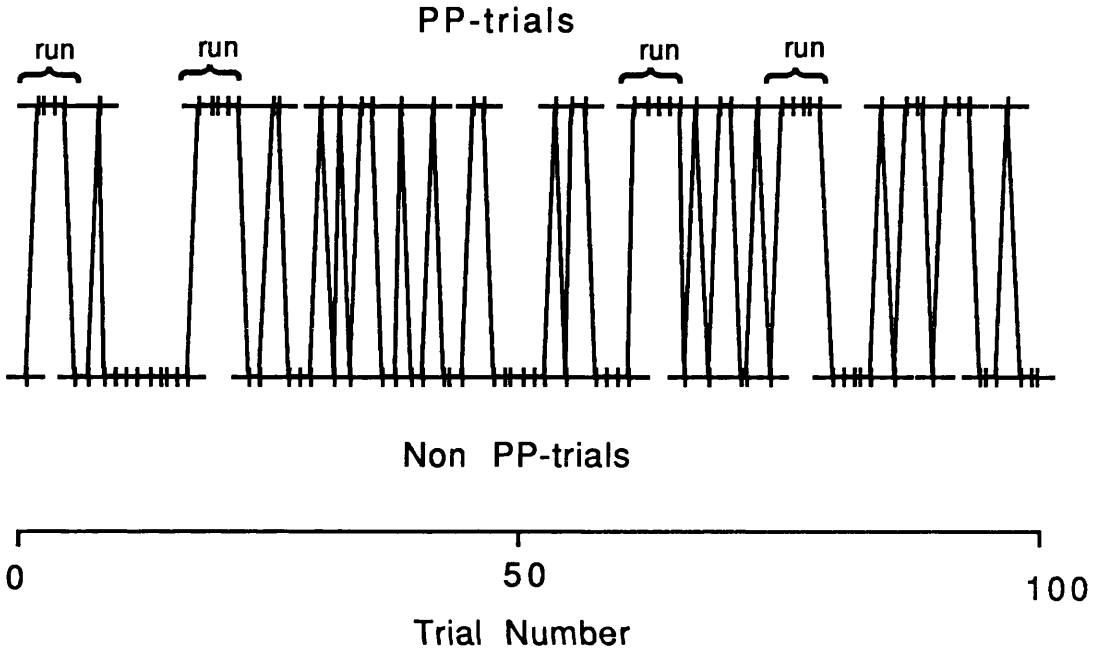


Fig. 16. The serial occurrence of PP-trials (top) and non PP-trials (bottom) in a sequence of 100 trials (from left to right) in a random stimulation experiment. Each cross (+) represents one trial. Four runs, each consisting of more than four consecutive PP-trials, are seen to occur (indicated by brackets).

significantly longer than that for a series of 22 successive trials taken from another part of the same experiment (61.2 +/- 39.6 ms, n=22, $p<0.003$).

Voluntary Activation

The total number of all the recruited MU spikes in each trial was used as an index of voluntary activation. Taking all the 6 runs of PP-trials that occurred in the total 500 trials of the experiment, no significant difference was found between the total number of all spikes counted in each trial during the runs of PP-trials, and that for the contiguous runs of trials that followed each of these runs of PP-trials ($p>0.1$, n=6, Willcoxon test).

Similarly, in the first 100 trials of the experiment, 4 runs of PP-trials and 4 runs of non-PP trials occurred. There was no significant difference in the number of all spikes for the 4 runs of PP-trials and the 4 runs of non-PP trials ($p>0.1$, n=4, Mann Whitney U test) (small sample sizes).

4. Spike-Stimulus Interval

Influence of Pre-stimulus Firing Probability

The occurrence of trials containing either a PP or SP discharge (PP- or SP-trials) and trials without a discharge at either the PP or SP latency (non PP/SP-trials) was strongly influenced by the spike-stimulus interval (Fig. 17).

There was a strong tendency for non-PP trials to contain discharges that occurred shortly before the onset of PP (Fig. 18). PP-trials and SP-trials were virtually mutually exclusive, with only 0-6.7% of trials (mean 4.1%) containing both a PP and an SP discharge in random stimulation experiments, except in 1 MU in which there were 30.4%.

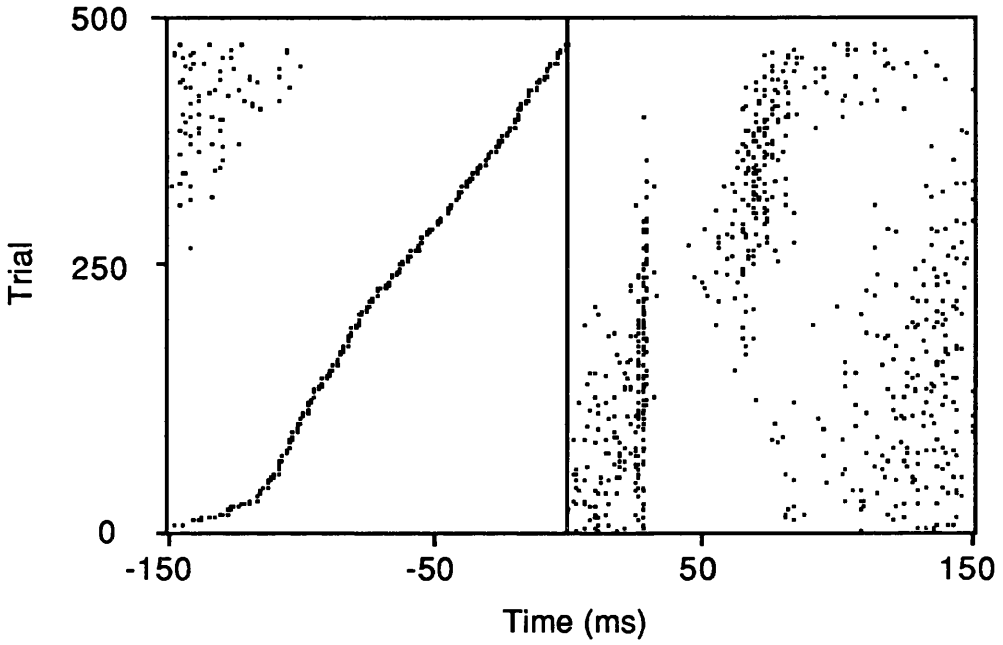


Fig. 17. Raster plot from a random stimulation experiment that has been sorted so that the spike-stimulus interval for each trial progressively decreases from the bottom of the figure (trial 0) to the top (trial 500). Each point represents one discharge. The stimulus is indicated by the central vertical line, and clusters of discharges are seen at the PP latency (peak 28 ms) and at the SP latency (peak 74 ms). Trials containing a discharge that occurred between the stimulus and the PP latency frequently have long spike-stimulus intervals. PP- trials frequently have intermediate spike-stimulus intervals, and SP-trials frequently have relatively short spike-stimulus intervals.

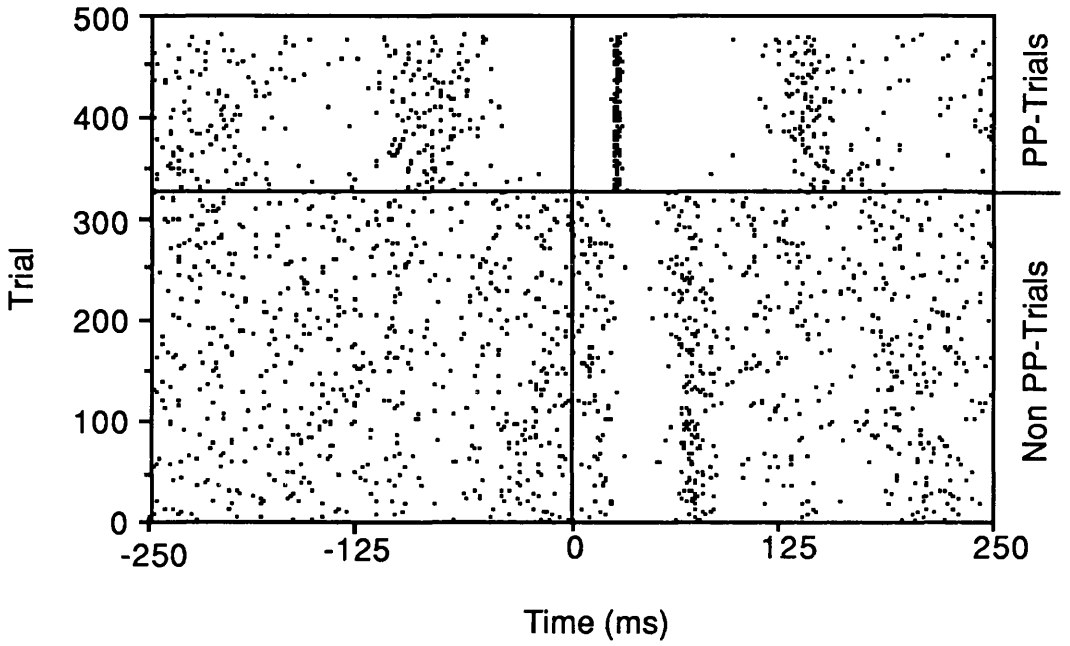


Fig. 18. Sorted raster plot from a random stimulation experiment (500 trials). The PP-trials and non PP-trials appear in the upper and lower panels, respectively.

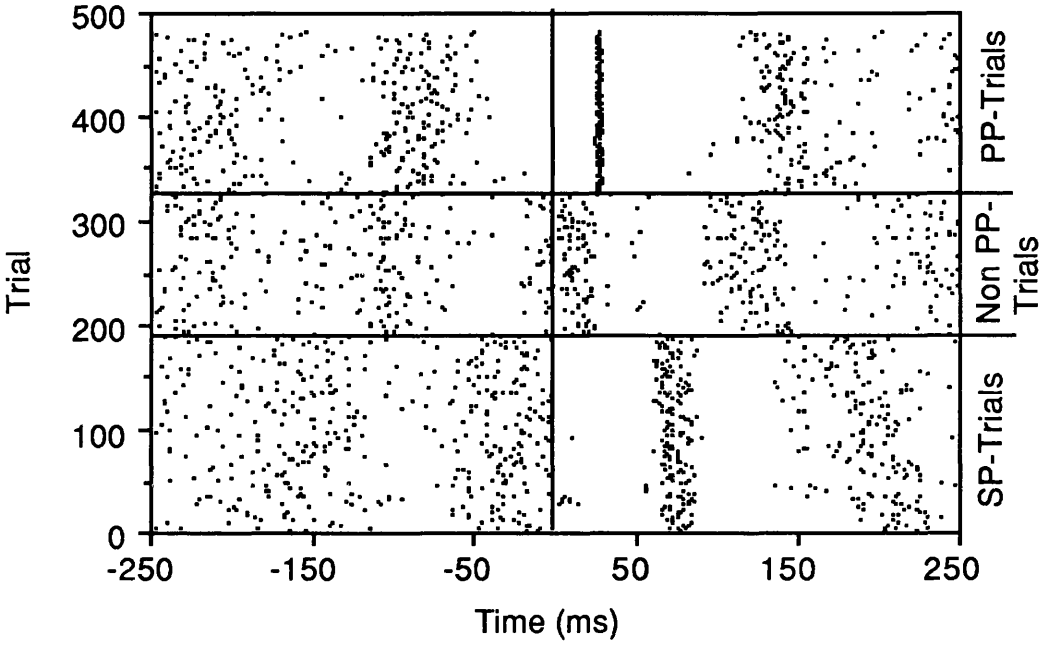


Fig. 19. Sorted raster plot, from the same random stimulation experiment illustrated in Fig. 18, in which PP-trials, SP-trials and non PP/SP-trials appear in the upper, middle and lower panels, respectively.

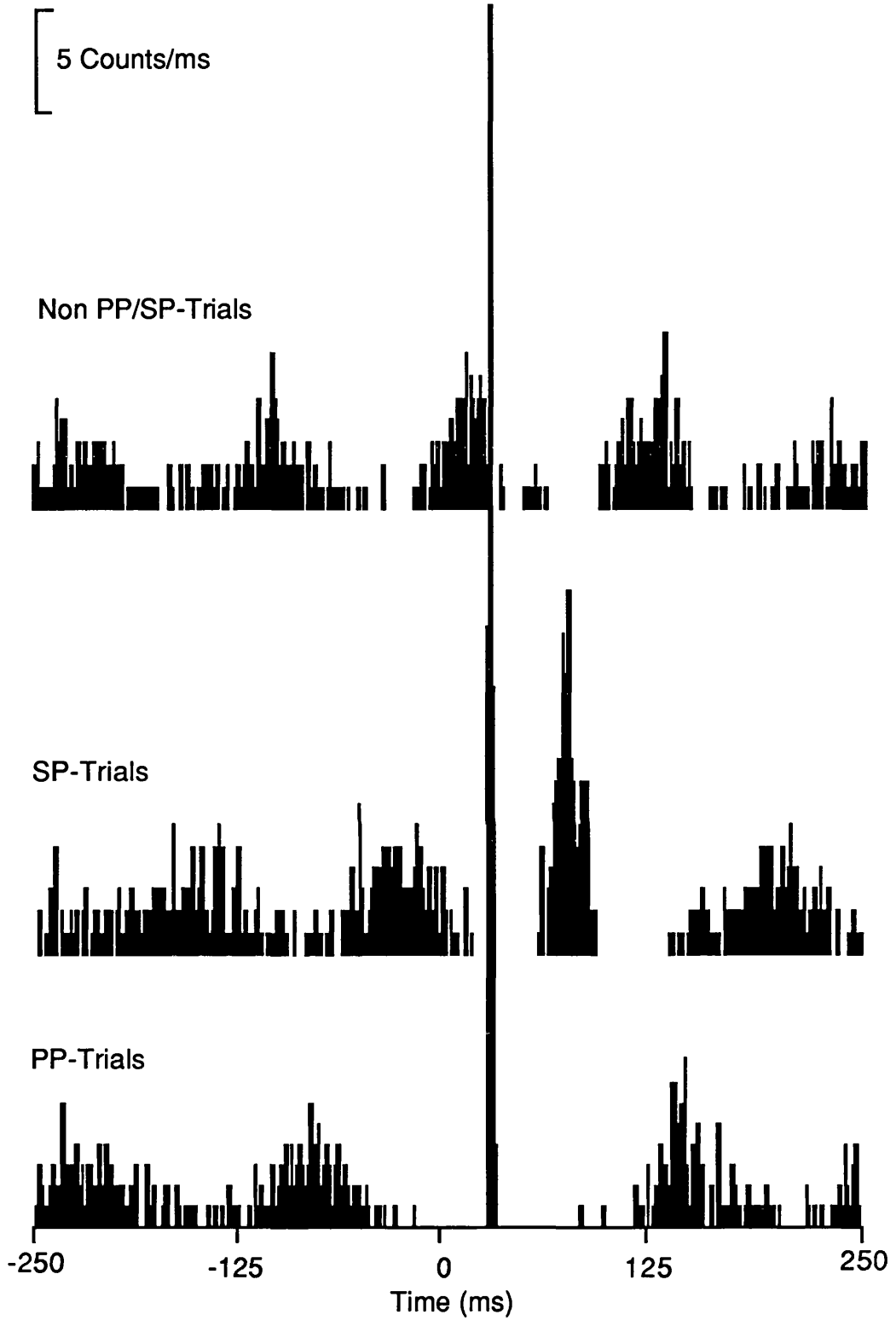


Fig. 20. Results from a random stimulation experiment (500 trials) sorted into 3 PSTHs showing the PP-trials (lower), the SP trials (middle) and the non-PP/SP trials (upper). Differences are seen in the pre-stimulus peaks in firing probability.

There was a strong tendency for non PP/SP-trials to contain discharges that occurred shortly before the onset of PP (Fig. 19). In contrast, SP-trials exhibited a cluster of discharges that occurred earlier than this group, but later than the cluster of discharges that preceded a PP discharge. In the PP-trials of the sorted PSTH in Fig. 20 the modal latency for the peak preceding PP in the PP-trials is -84 ms; the modal latency for the peak preceding SP in the SP-trials is -29 ms; and the modal latency for the peak which was followed by neither a PP or an SP discharge in the non PP/SP-trials was +12 ms.

In 4 MUs (with a mean ISI 121 ms), the mean interval between the corresponding modal latency for SP-trials and the modal latency for non PP/SP-trials was 41 ms .

5. Quantifying the Effect of the Spike-Stimulus Interval on the Probability of Evoking a PP Discharge

The distribution of the total sample of spike-stimulus intervals for MUs in random stimulation experiments was found to be relatively uniform (Fig. 21A). The spike-stimulus intervals for PP-trials, however, were not uniformly distributed (Fig. 21B). Intervals less than 40ms in this MU were not associated with discharges within PP. At intervals greater than 40ms, PP-trials occurred with increasing frequency at progressively longer intervals.

The proportion of PP-trials was found to increase with increasing spike-stimulus interval (Fig. 22) (This was plotted for spike-stimulus intervals that were less than the minimum ISI - see below). In 6 MUs from random stimulation experiments, the proportion of PP-trials was found to exceed 20% (horizontal line, Fig. 22A) at spike-stimulus intervals longer than 40ms,

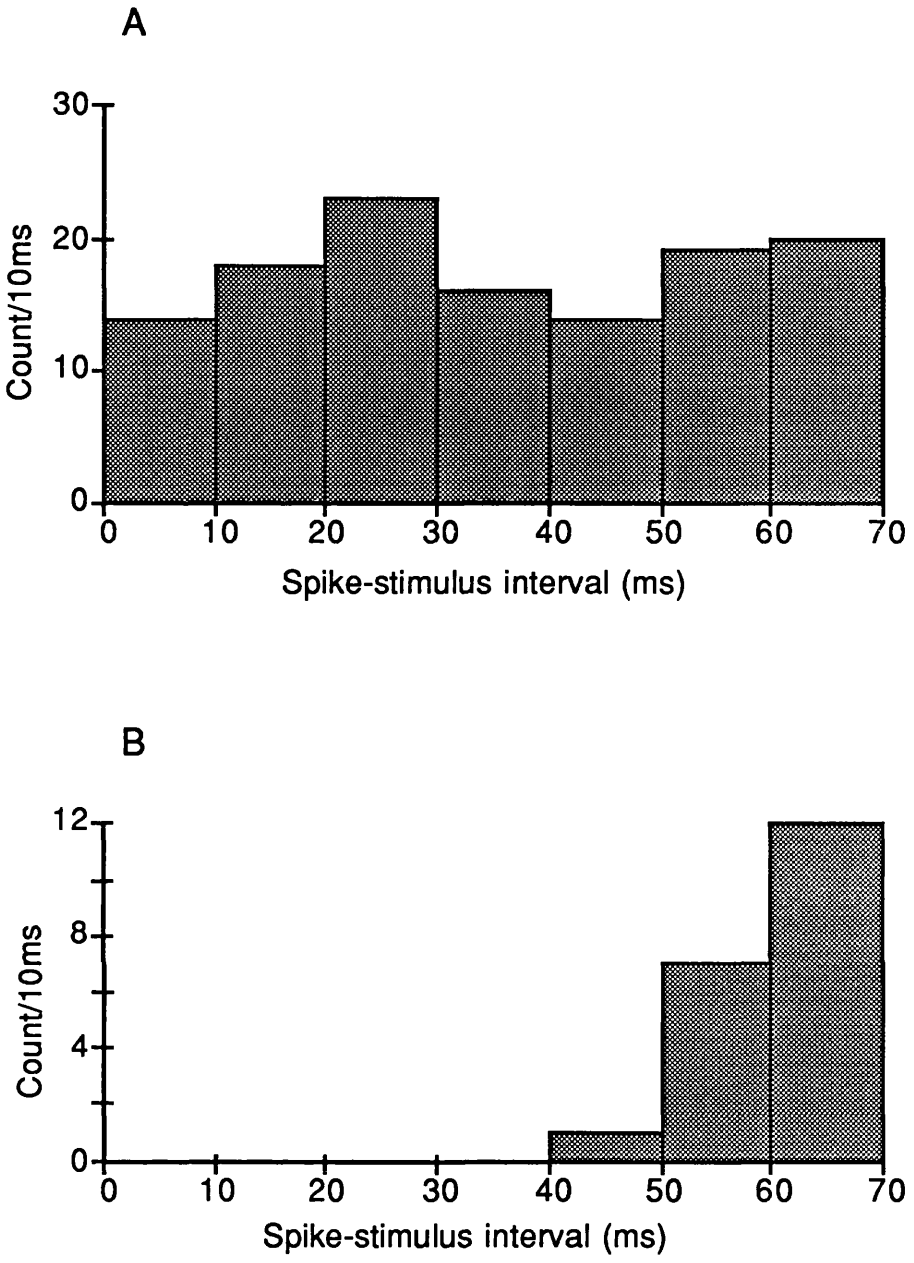


Fig. 21. The distribution of spike-stimulus intervals (interval between stimulus and preceding voluntary discharge) for 1 FDI MU in a random stimulation experiment. A: The distribution of the total sample of spike-stimulus intervals is relatively uniform. B: The spike-stimulus intervals for PP-trials are shown. Only intervals greater than 40 ms are associated with discharges within PP. The frequency of PP discharges increases with increasing spike-stimulus intervals.

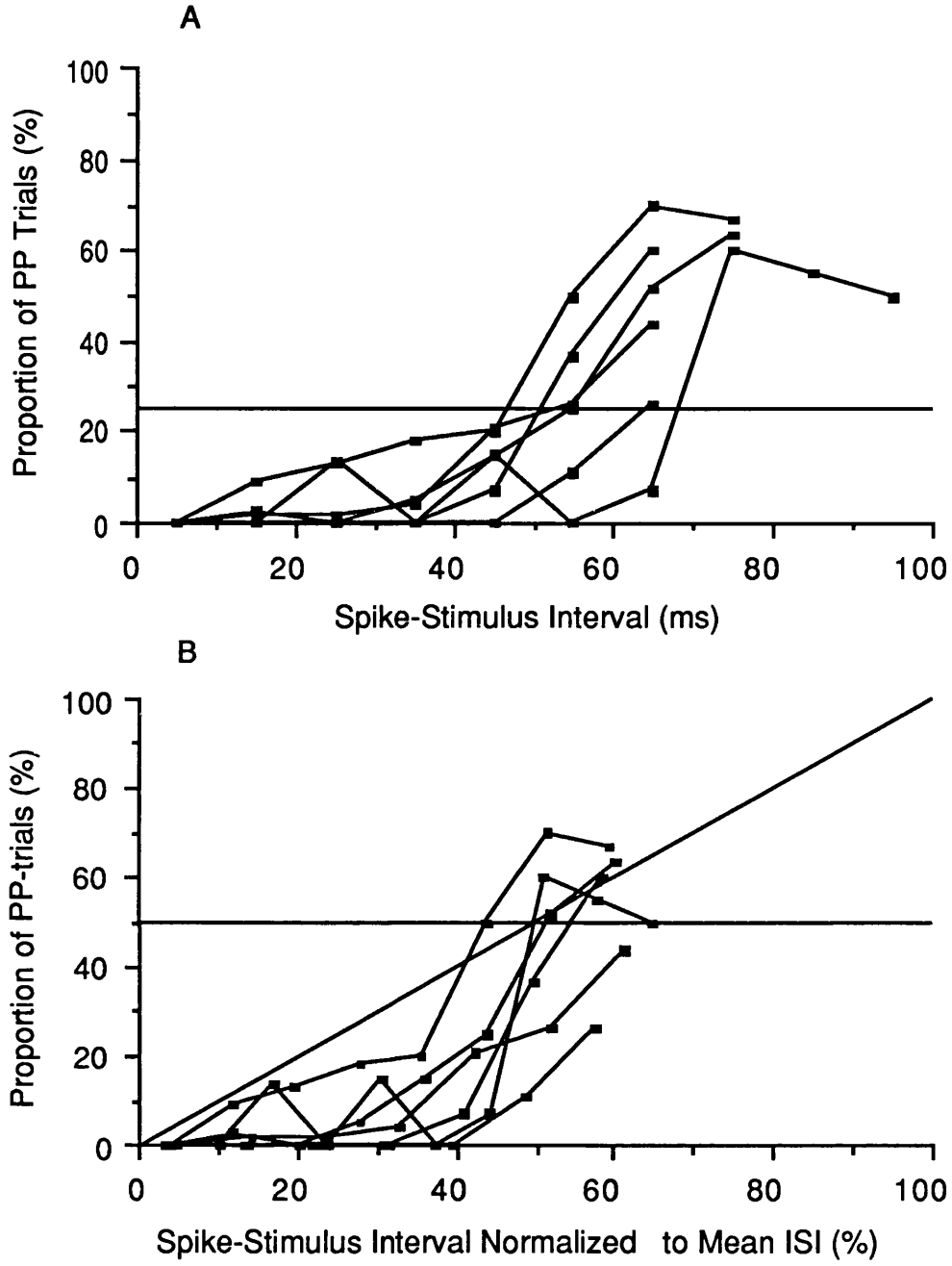


Fig. 22. The proportion of PP-trials plotted against spike-stimulus interval for 6 FDI MUs. A: The proportion of PP-trials exceeded 20% (horizontal line) at spike-stimulus intervals exceeding 40ms, after which there was an increase in the rate of rise of each curve. B: The same data normalised to the mean ISI shows that the proportion of PP-trials, in 4 out of the total 6 MUs, exceeded 50% at spike-stimulus intervals of 45-55% of their mean ISI. At spike-stimulus intervals shorter than this, the proportion of PP-trials remained below that which would have been expected if the excitability had increased linearly with time (oblique line).

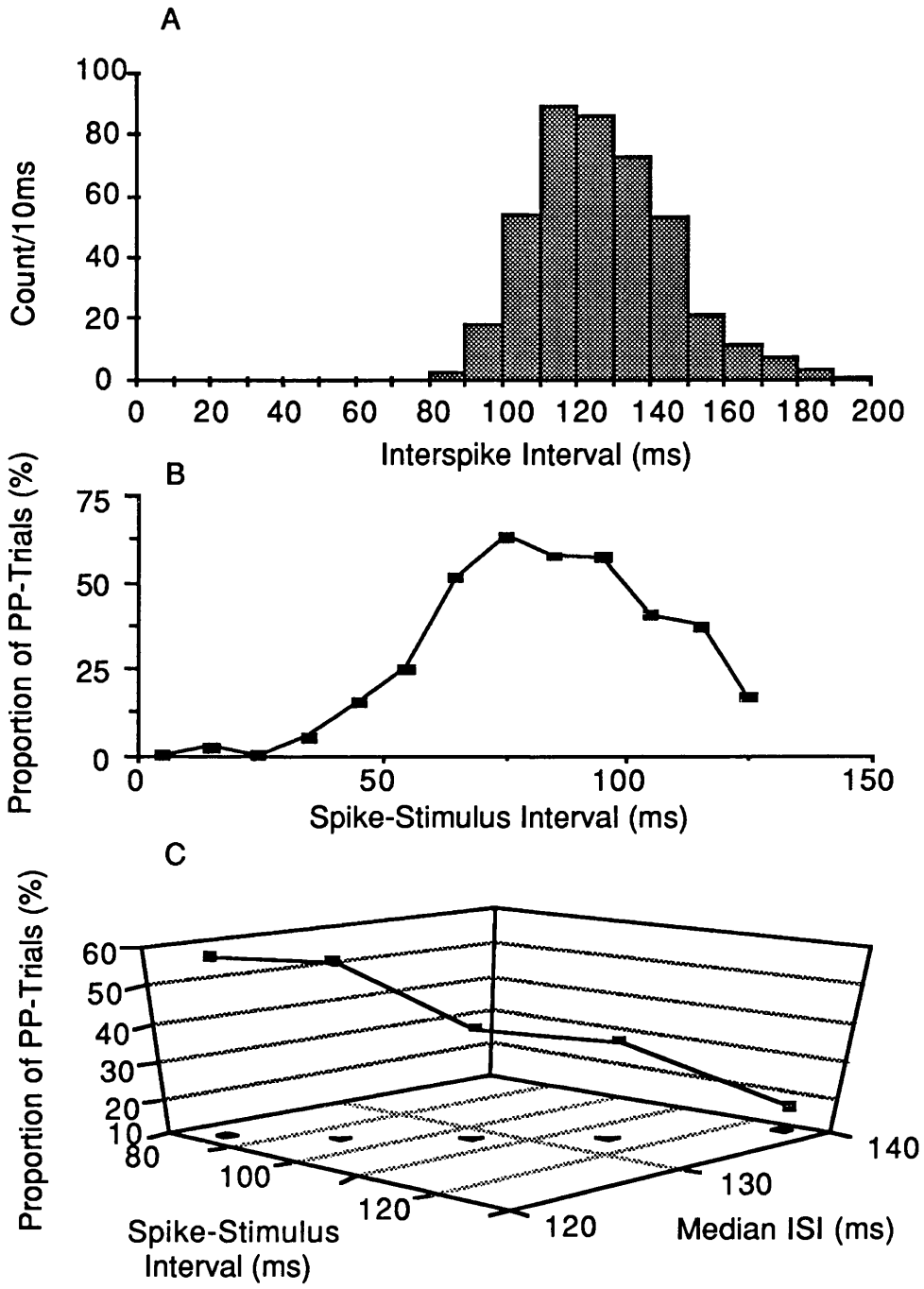


Fig. 23. Effect on the proportion of PP-trials of all spike-stimulus intervals in a random stimulation experiment. A: INTH of all pre-stimulus ISIs (500 trials). B: Proportion of PP-trials plotted against all spike-stimulus intervals in the experiment. The proportion of PP-trials increases to a maximum (as in Fig. 22) at a spike-stimulus interval corresponding to the minimum pre-stimulus ISI (80 ms for this MU) and then decreases. C: 3-dimensional plot for spike-stimulus intervals that were greater than the minimum ISI, with the proportion of PP-trials plotted against the median ISI and spike-stimulus interval. The decrease in the proportion of PP-trials at spike-stimulus intervals greater than the minimum pre-stimulus ISI, seen in B, is still evident despite lengthening of the median ISI (see Results and Discussion).

after which there was an increase in the rate of rise of each curve. The same data normalised to the MUs' mean ISI showed that the proportion of PP-trials, in 4 out of the total 6 MUs, exceeded 50% at spike-stimulus intervals of 45-55% of their mean ISI (Fig. 22B). At spike-stimulus intervals shorter than this, the proportion of PP-trials remained below that which would have been expected if the excitability had increased linearly with time (oblique line, Fig. 22B).

The proportion of PP-trials was found to reach its maximum at spike-stimulus intervals in the region of the the minimum ISI, after which it decreased (Fig. 23B). At spike-stimulus intervals that were less than the minimum pre-stimulus ISI (as shown in Fig. 22), all ISIs in the experiment were potentially sampled and the median of the sampled ISIs would correspond to the median of the interval histogram (INTH) (Fig. 23A). At spike-stimulus intervals that exceeded the minimum ISI, however, the MU may have sometimes discharged before the stimulus. Therefore the median of the sampled ISIs would exceed the median of the INTH. This change might be a factor in the decrease in the proportion of PP-trials at relatively long spike-stimulus intervals (Fig 23B). For 1 random stimulation experiment (500 trials) the proportion of PP-trials was therefore plotted at relatively long spike-stimulus intervals (ie. those which exceeded the minimum ISI) by calculating the median of that sample of ISIs that was longer than the corresponding spike-stimulus interval (Fig. 23C). The proportion of PP-trials was found to decrease at spike-stimulus intervals greater than the minimum ISI (as also seen in Fig. 23B) despite the accompanying increase in the duration of the median ISI.

In spike triggered stimulation experiments, the fixed delay from the triggering spike to the stimulus corresponds to the pre-stimulus interval

referred to above. Accordingly, the proportion of PP trials was also seen to increase, with increasing delay, as in the random stimulation experiments. In 1 MU, at a delay of 80 ms, the MU fired within PP approximately six times more frequently than it did at a delay of 10 ms (Fig. 24). It was also found that shortening the delay increased the latency of PP from 23-25 ms to 27-29 ms.

Effect of the Spike-Stimulus Interval on the Latency of PP Discharges

In 8 of 11 random stimulation experiments, the latency of PP discharges were negatively correlated with the spike-stimulus interval ($p < 0.05$, Spearman Rho). Fig. 39A (see Results for Group of Patients with MS) shows this trend towards increased latency of MU discharge within PP with decreasing spike-stimulus interval in a healthy subject .

For random stimulation experiments, the relationship between stimulus-spike interval (discharge latency) and spike-stimulus interval (the interval between the stimulus and the preceding voluntary discharge) was also plotted for simulated stimuli introduced during the pre-stimulus period (Fig. 25). This produced a distribution with an equal number of points about a line representing the median pre-stimulus ISI (Fig 25C). The equivalent graph for magnetic stimuli showed two clusters corresponding to SP and PP (Fig. 25B). In contrast to Fig. 25C, there was an unequal distribution in the number of points about the line representing the median pre-stimulus ISI. Within the cluster corresponding to SP, there was an increase in the number of points to the left of the median line and a decrease in the number of points to the right.

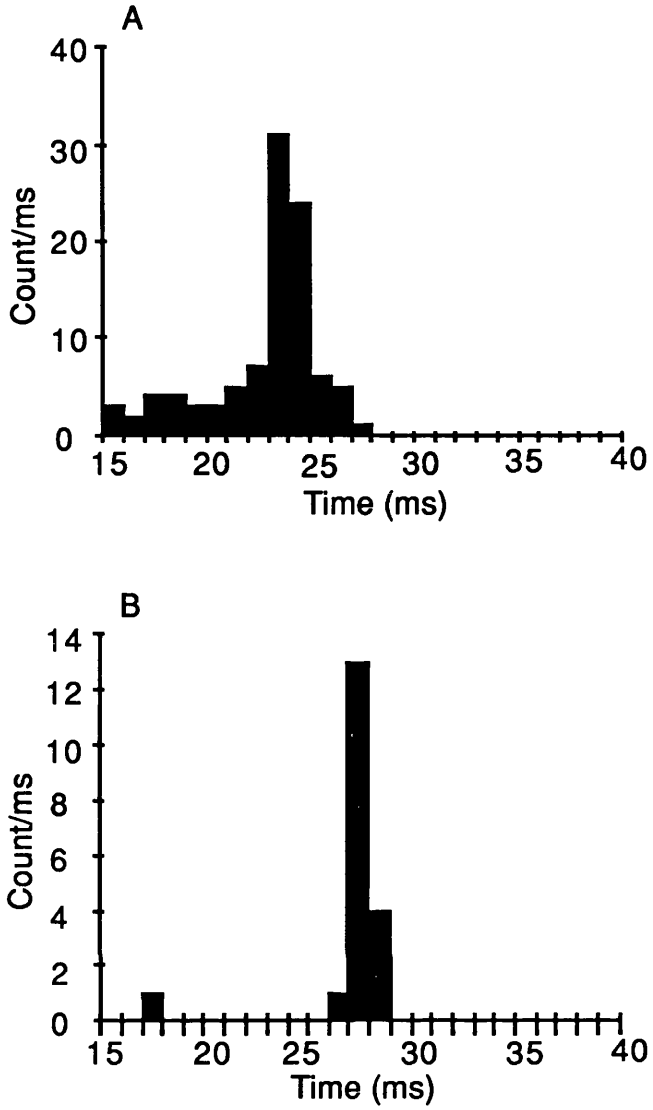


Fig. 24. PSTHs from a spike triggered experiment on an MU with stimuli given at 2 delays after the previous voluntary spike. A: Stimuli given at 80 ms after the previous voluntary spike produced a PP at a peak latency of 23.5 ms (125 trials). B: Identical stimuli given at a delay of 10 ms after the previous voluntary spike produced a PP at a latency of 27.5 ms (274 trials).

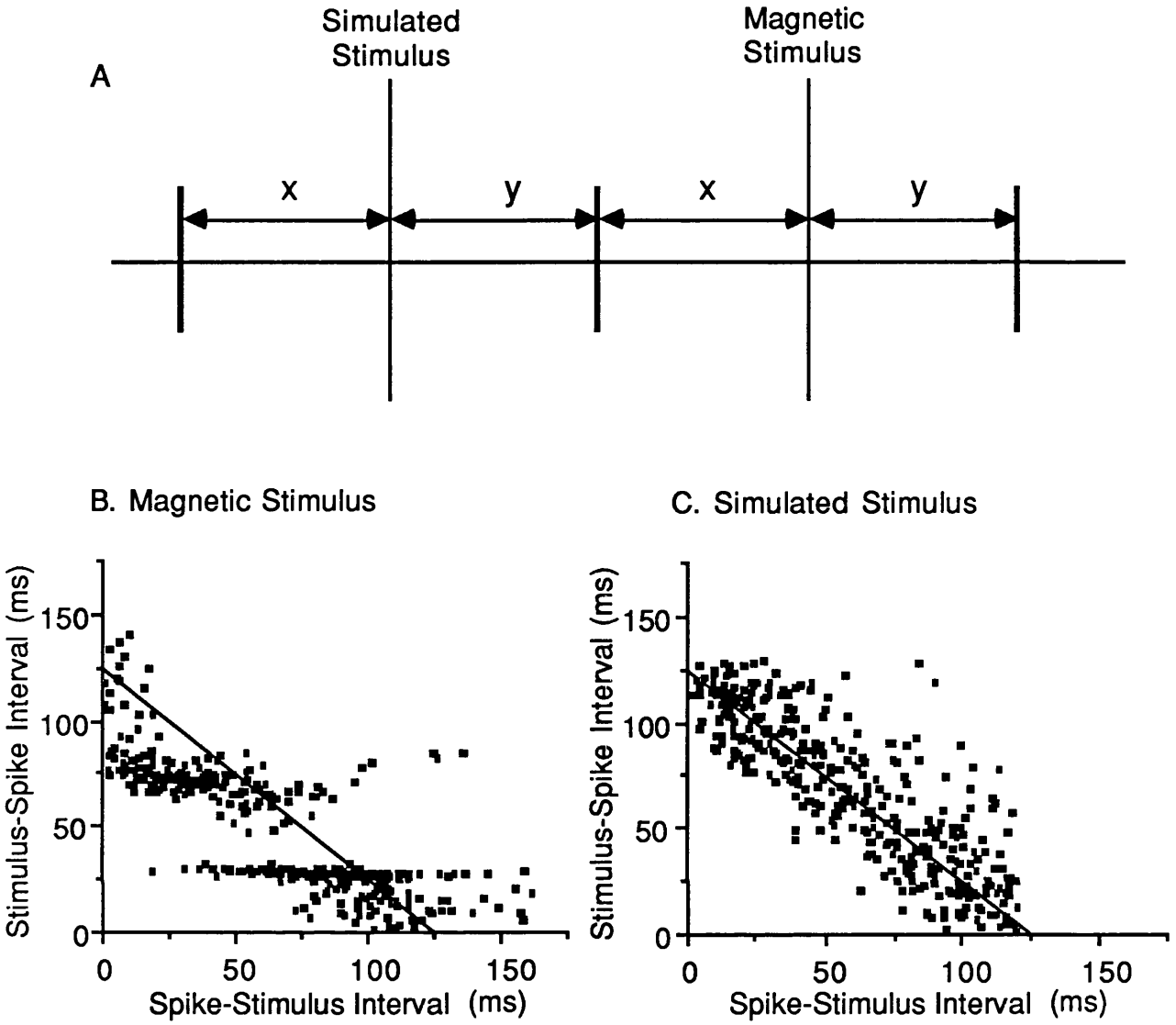


Fig. 25 The effect of magnetic stimuli on the duration of ISIs when compared with simulated control stimuli suggests that SP and PP discharges occur earlier than expected. A: Diagram of a train of 3 spikes with magnetic and simulated stimuli. x represents the spike-stimulus interval, y the stimulus-spike interval. In B and C the stimulus-spike interval (y) is plotted against the spike-stimulus interval (x) for data from a random stimulation experiment (n=414 trials). In B, magnetic stimuli have caused a clustering of points corresponding to PP and SP at stimulus-spike intervals in the region of 25 and 75 ms, respectively. As a control, C shows a similar graph plotted from the same experiment in which simulated stimuli were introduced during the period before the magnetic stimulus. The points occur in equal number about the oblique line which represents the median pre-stimulus ISI (124ms). The same line is also shown in B. In B, points to the left of the oblique line are therefore derived from ISIs that were shorter than the median pre-stimulus ISI. These are increased in number in the clusters that correspond to PP and SP when contrasted with C.

Other Arm Muscles

Latency and Duration of PP and SP

PSTHs from MUs in other arm muscles are seen in Fig. 26. The overall form of the PSTH was similar, with a PP of short duration in all MUs, followed in 10 of the 14 MUs examined by an SP. PP and SP were defined using the same criteria as for FDI MUs (see Fig. 4, Methods). SP was not detected in the 2 EDC MUs studied, and 1 of the biceps MUs. As for FDI, there was a strong tendency in any individual trial for MUs to fire either at the PP latency or at the SP latency, but not at both.

The onset latency for PP ranged from 13 ms (deltoid and biceps) to 29 ms (EDC). The duration of PP ranged from 2 ms (deltoid and brachioradialis) to 8 ms (EDC), with a mean (+/-SD) of 4.6 (+/-1.7) ms. The onset latency for SP ranged from 33 ms (deltoid) to 65 ms (FCU). The duration of SP ranged from 17-27 ms, with a mean (+/-SD) of 19.4 (+/-5.4) ms.

In 1 deltoid MU a late peak was present, but its height failed to fulfil the criteria for SP by only 1 count. In the same deltoid MU, an additional period of increased firing probability was present with an onset latency of 100 ms. This peak was found not to be due to a resumption of firing after a PP discharge by the examination of sorted PSTHs.

Sub-Peaks

PP was found to consist of 1 to 3 sub-peaks: 3 sub-peaks in 1 MU (from biceps); 2 sub-peaks in 5 MUs; 1 narrow peak in 6 MUs and no definite sub-peaks structure in 2 MUs (Fig. 27). The inter-modal interval between the first and second sub-peaks ranged from 1.4 ms (biceps) to 3.9 ms (brachioradialis). On grouping the data the mean (+/-SD) interval was 2.9

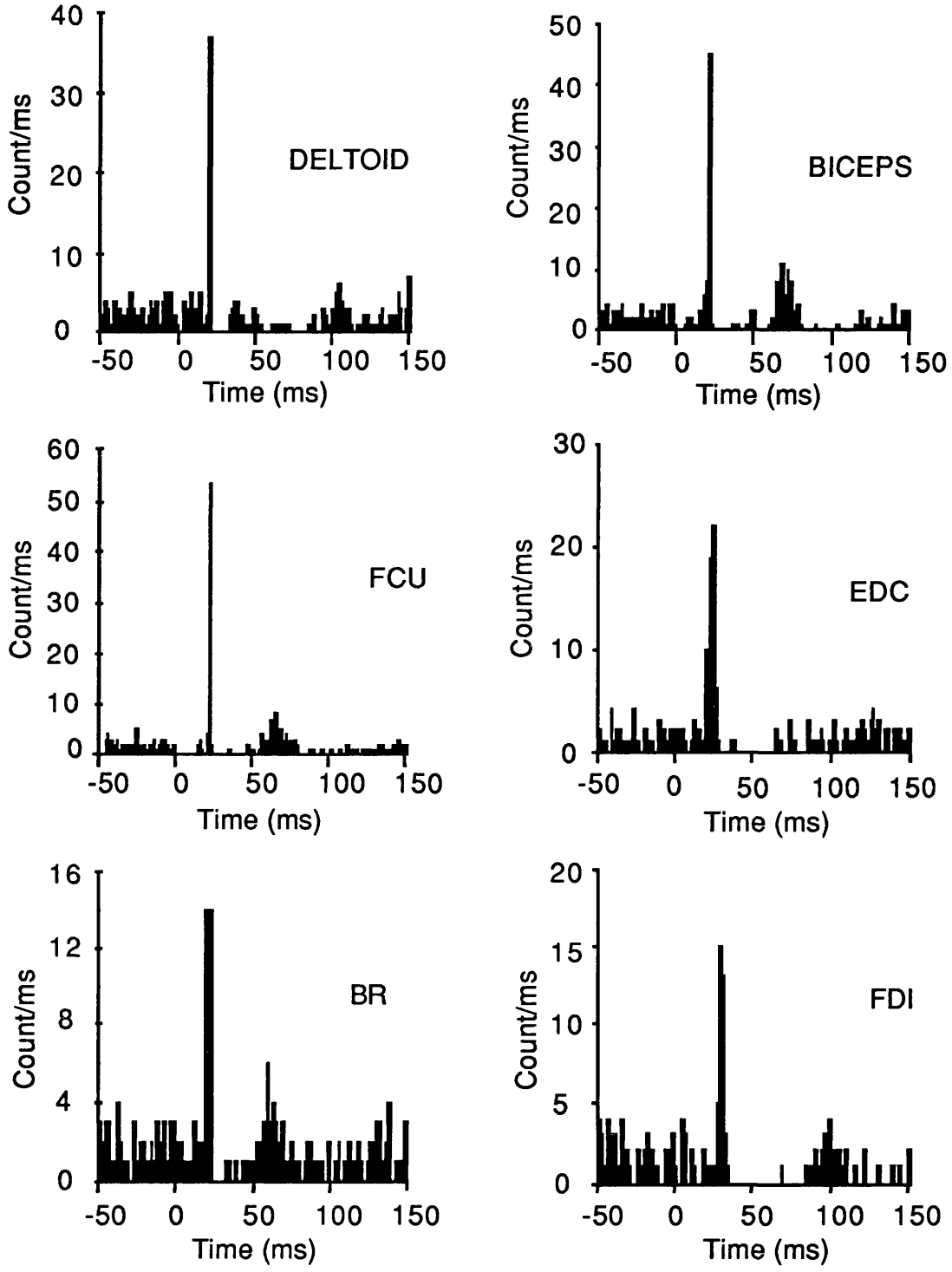


Fig. 26. PSTHs from 6 MUs in different upper limb muscles, from 3 subjects. PP is present in all PSTHs and SP in all but one (EDC). In deltoid there is also an additional late peak, with an onset latency of 100 ms. (Number of trials: deltoid 236, biceps 232, brachioradialis (BR) 146, FDI 168, FCU 184, EDC 137.) Brief periods of zero firing probability directly following the stimulus - also seen in some other PSTHs - are caused by stimulus artefact.

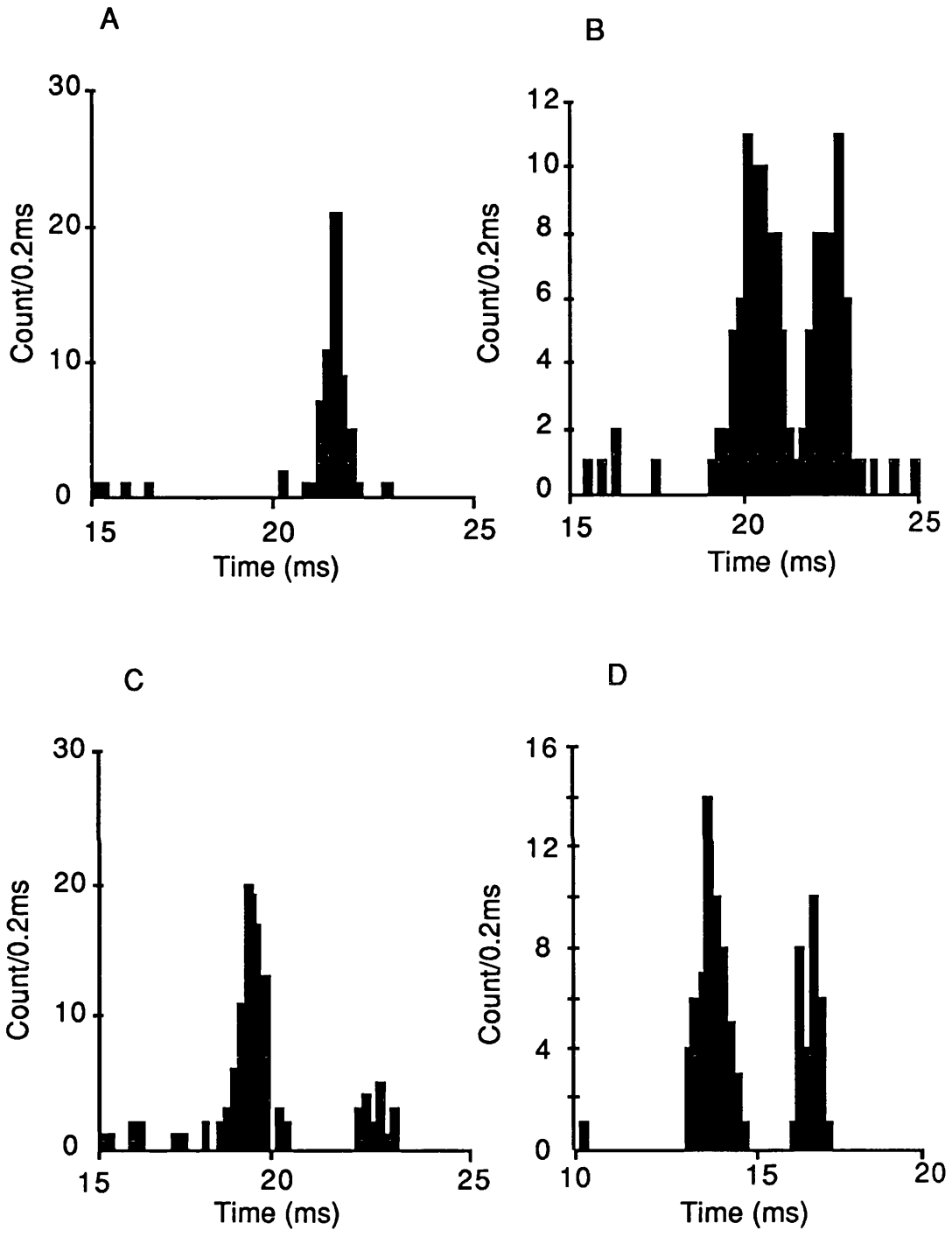


Fig. 27. PP region of PSTHs from 4 MUs in different arm muscles. A: single narrow peak from an FCU MU (184 trials). B: two sub-peaks from a biceps MU, with an inter-modal interval of 2.6 ms (205 trials). C: two sub-peaks from a brachioradialis MU, with an inter-modal interval of 3.6 ms (288 trials). D: two sub-peaks from a deltoid MU, with an inter-modal interval of 3.0 ms (183 trials). These intermodal intervals contrast with those seen in FDI MUs (Fig. 11).

(±0.9) ms (n=6). The single biceps MU that exhibited 3 sub-peaks had an inter-modal interval of 1.3 ms between the second and third sub-peaks.

The Latency of PP and SP with Respect to Peripheral Conduction Distance

The peak latency of PP and SP were both found to be significantly correlated with the distance from the spine of the C7 vertebra to the electrode site in the muscle ($p < 0.001$) (Fig. 28). The slope relating the peak latency of SP to distance (0.59 ms/cm) was found to be significantly greater than that relating the peak latency of PP to distance (0.19 ms/cm) ($p < 0.01$).

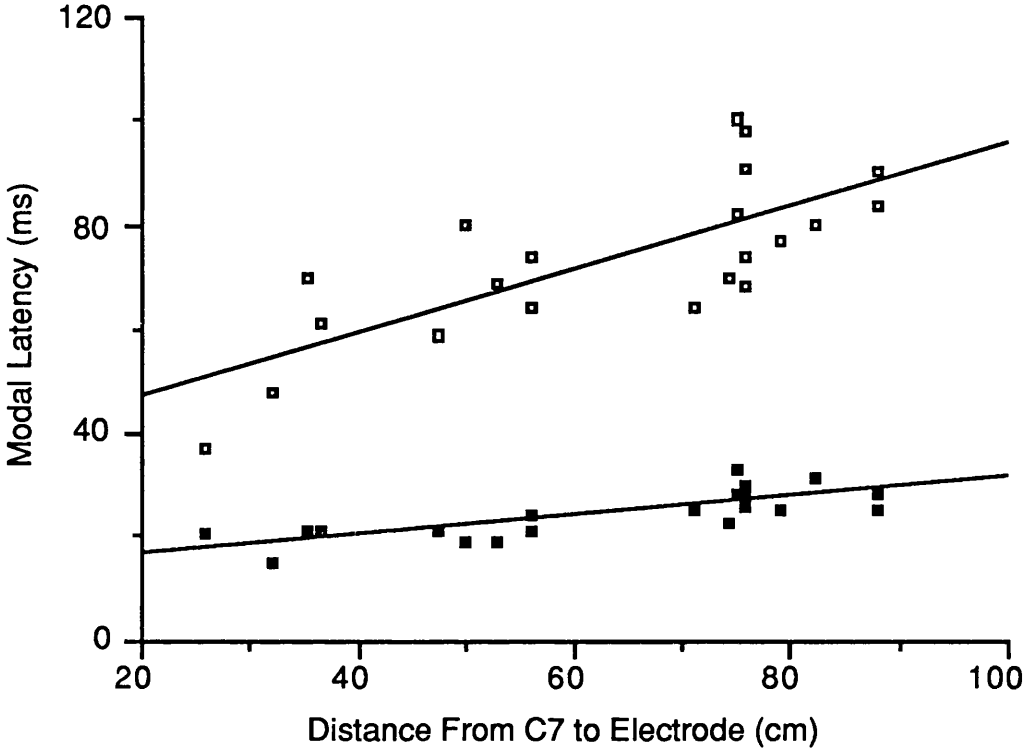


Fig. 28. The modal latencies of both PP (closed squares) and SP (open squares) are significantly correlated with the distance from the spine of the C7 vertebra to the electrode site ($p<0.001$). The slope relating SP to distance (0.59 ms/cm) is significantly greater than that relating PP to distance (0.19 ms/cm) ($p<0.001$). [The peak (modal) latency of SP was plotted rather than the onset latency as the latter is potentially altered by the height of PP (see Fig. 9). The peak latency probably corresponds to the point of maximum slope of the EPSP which, if motoneurones serving different arm muscles exhibit EPSPs with similar time courses, would occur at a constant interval after the onset of the EPSP.]

Serial Changes in Interspike Interval

These data are included in order to provide examples for the discussion of the principles that apply to comparison of ISIs (described for FDI MUs), leading to the development of new testing strategies. The limited validity of the paired Student's t test for the comparison of serial ISIs is qualified in the Discussion. (Only those results indicating a significant shortening of the mean peri-stimulus ISI when compared to the pre-stimulus ISI are described, except for those experiments where PP was absent, for reasons described in the Discussion.) This analysis is dependent upon the presence of any serial dependency (found not to be significant in most random stimulation experiments-see Methods) and its interpretation is dependent upon the effect of firing rate on the probability of evoking a PP discharge (see above).

Significant Shortening of the Peri-stimulus ISI

A significant shortening of the peri-stimulus ISI was defined by a comparison of the mean pre- and peri-stimulus ISIs with the paired Student's t test ($p < 0.05$):

1) PP- trials

In the PP-trials from 8 of 10 FDI MUs in random stimulation experiments, the mean peri-stimulus ISI was significantly shorter than the mean pre-stimulus ISI by 8.3-34.6 ms. In the PP trials from 13 of 14 MUs from other arm muscles in random stimulation experiments, the mean peri-stimulus ISI was also significantly shorter than the mean pre-stimulus ISI, by 10.1-37.4 ms.

2) SP-trials

In the SP trials from 2 of 10 FDI MUs in random stimulation experiments, the mean peri-stimulus ISI was significantly shorter than the mean peri-stimulus ISI by 12.1 and 17.4 ms. In the SP trials from 6 of 9 MUs from other arm muscles in random stimulation experiments, the mean peri-stimulus ISI was significantly shorter than the mean pre-stimulus ISI by 9.1-35.8 ms.

Serial Changes in the Absence of PP

By the deliberate use of low stimulus intensities (30-37%) in 8 MUs from 4 subjects in random stimulation experiments, no significant PP was produced in the PSTH or cusum. In 6 of these MUs the mean peri-stimulus ISI was found to be significantly longer than the mean pre-stimulus ISI by 6.5-40.1 ms ($p < 0.05$, paired Students' t test-see Discussion). In the remaining 2 MUs, the mean peri-stimulus ISI was also longer than the pre-stimulus ISI (by 6.3 and 6.5 ms, respectively) but the difference was not statistically significant.

Other Experiments on FDI Relating to SP

Peripheral Mechanical and TM Stimulation in the Same MU

In 4 MUs from 3 subjects, PSTHs were obtained during mechanical stimulation of the finger. In 2 of these MUs (MU1-random stimulation experiment and MU2-spike triggered experiment) PSTHs were also obtained during TMS.

Following mechanical stimulation (rectangular pulse, adduction movement) peaks similar to PP were evident in the PSTH, at peak latencies of 41 ms, 35 ms, 46 ms and 44 ms, with durations of 8 ms, 5 ms, 6 ms and 6 ms for MUs 1, 2, 3 and 4, respectively. A second peak similar to SP was also evident in the PSTH of MU 1 (peak latency 74 ms) and in MU 2 (peak latency 61 ms). The intermodal intervals between the first and second peaks were therefore 33 and 26 ms for MUs 1 and 2, respectively. No second peak was evident in the PSTHs from the remaining 2 MUs (MUs 3 and 4).

Following TMS in MUs 1 and 2, PP had a peak latency of 29 ms and 28 ms, and SP had a peak latency of 91 ms and 71 ms for MUs 1 and 2, respectively. The corresponding intermodal PP-SP intervals were therefore 62 and 43 ms.

In MU No. 1, mechanical stimulation (rectangular pulse, abduction movement) also produced a PSTH with a prominent decrease in firing probability starting at 57 ms, of 70 ms duration, that was not preceded by a prominent primary peak. In a repeat experiment on the same MU, a primary peak was produced with an onset latency of 44 ms and duration of 11 ms,

followed by a small secondary peak at an onset latency of 76 ms and duration of 14 ms (see Methods for technical considerations).

Finger Movement

In a random experiment on 1 MU, the index finger was fixed to prevent movement so that FDI remained isometric for 100 trials, followed by another 100 trials during which FDI was free to shorten. The proportion of SP-trials was 34.8% for isometric FDI and 32.3% when FDI was free to shorten.

Patients with Upper Motor Neurone Disorders

The results from single MU studies from FDI in 6 patients with UMN lesions of different origin are described in their clinical context, followed by the findings in a group of 9 patients with MS.

Cerebrovascular Disease

Patient 1 was a 64 year old company director who developed a severe left hemiparesis over the course of 24 hours, associated with neglect for left sided tactile and visual stimuli. A CT scan showed a small infarct in the posterior limb of the right internal capsule.

He was examined at intervals of 3 and 11 months following his stroke, at which times the power of the left FDI had partially recovered to 3 and 4- respectively (MRC scale). The stimulus intensity at which a just discernible response could be obtained with surface electrodes over the right FDI in a relaxed state was unobtainable (>90%) at 3 months, reducing to 74% at 11 months. The intensity required for the clinically unaffected left side was 40% at 3 months. Unitary recordings produced PSTHs from the affected side that lacked excitatory peaks at both 3 (Fig. 29A) and 11 months (stimulus intensity 70%), and a PP with an onset latency of 25 ms and a duration of 5 ms from an MU on the unaffected side (stimulus intensity 44%) at 3 months (Fig. 29B).

Motor Neurone Disease

Patient 2 was a 72 year old woman with MND, with an 18 month history of bilateral leg weakness. On physical examination she was found to have wasting and fasciculations in the small hand muscles, with moderate weakness in all limbs and a power of 4 in the right FDI (MRC scale). Tone

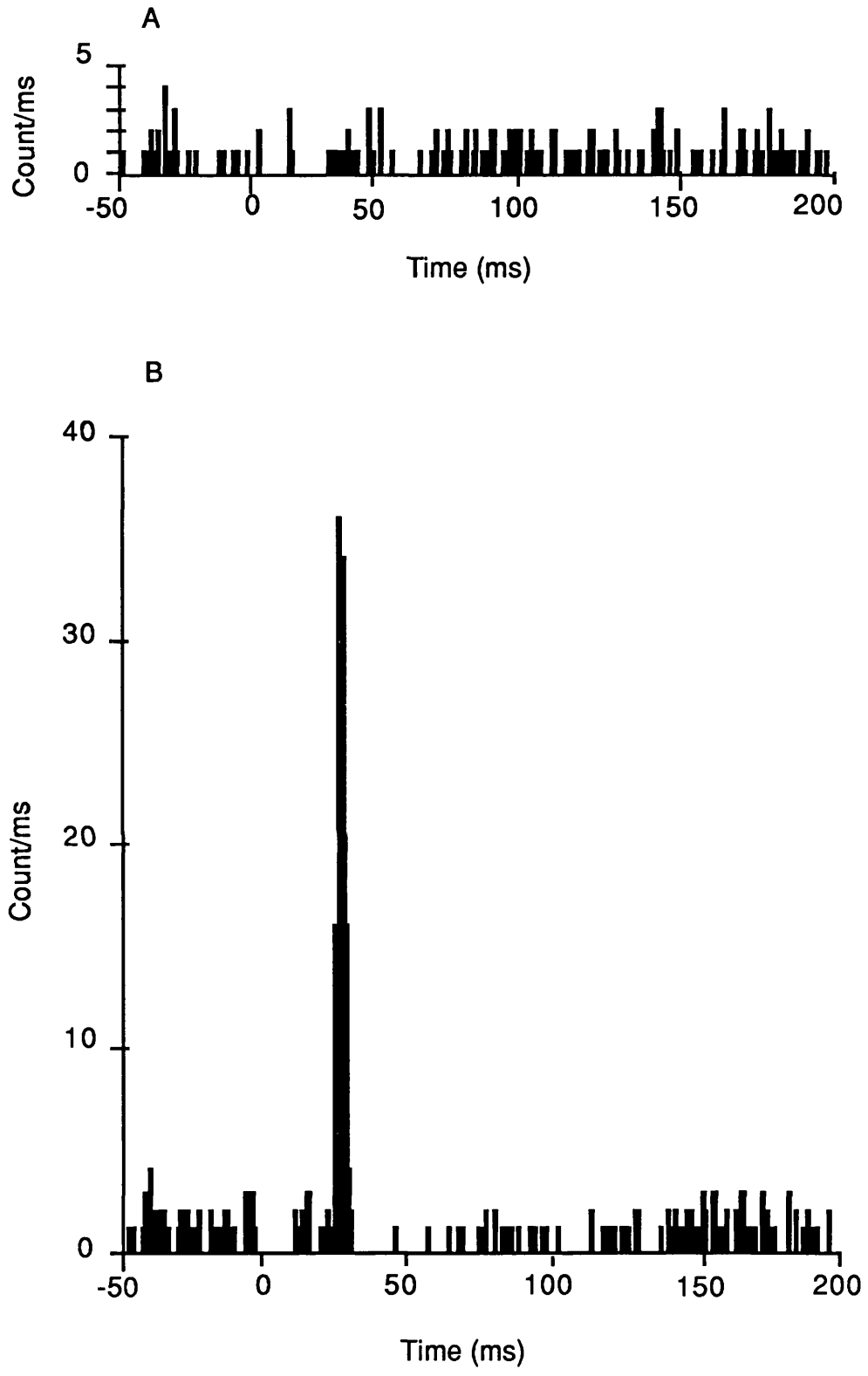


Fig. 29. PSTHs from random stimulation experiments on Patient 1, 3 months after a stroke. A: An FDI MU from the affected left side produced no excitatory peak (stimulus intensity 70%, 172 trials). B: An MU from the unaffected right side produced a PP with an onset latency of 25 ms and a duration of 5 ms (stimulus intensity 44%, 144 trials).

and reflexes were increased, with flexor plantar responses. There were no bulbar symptoms or sensory abnormalities.

On electromyography and nerve conduction studies (from the Unit of Clinical Neurophysiology, University Department of Clinical Neurology, Oxford) there was widespread chronic partial denervation with normal sensory action potentials.

Random stimulation in one MU produced a PP with an onset latency of 22 ms and a duration of 2 ms, comprising a single sub-peak. In a different MU, a spike triggered stimulation experiment with a spike-stimulus interval of 60 ms produced a PP at an onset latency of 23 ms with a duration of 6 ms (Fig. 30), comprising two sub-peaks with an inter-modal interval of 4.5 ms (Fig. 30D).

Patient 3 was a 65 year old man with MND, with an 18 month history of weight loss and weakness, principally affecting the right shoulder and both hands and also the legs. On physical examination he was found to have widespread wasting and weakness in the upper limbs (FDI power 3, MRC scale), mild leg weakness without obvious wasting and widespread fasciculation. There were no UMN signs. Electromyography and nerve conduction studies revealed fasciculation and evidence of chronic partial denervation in all four limbs.

Random stimulation in 1 MU produced a PP with an onset latency of 32 ms and a duration of 4 ms, which was closely followed by two further peaks (each of which exceeded the mean pre-stimulus firing rate plus 2 SDs) at intervals of 3 and 2 ms, respectively (PSTH bin width 1 ms). With a bin

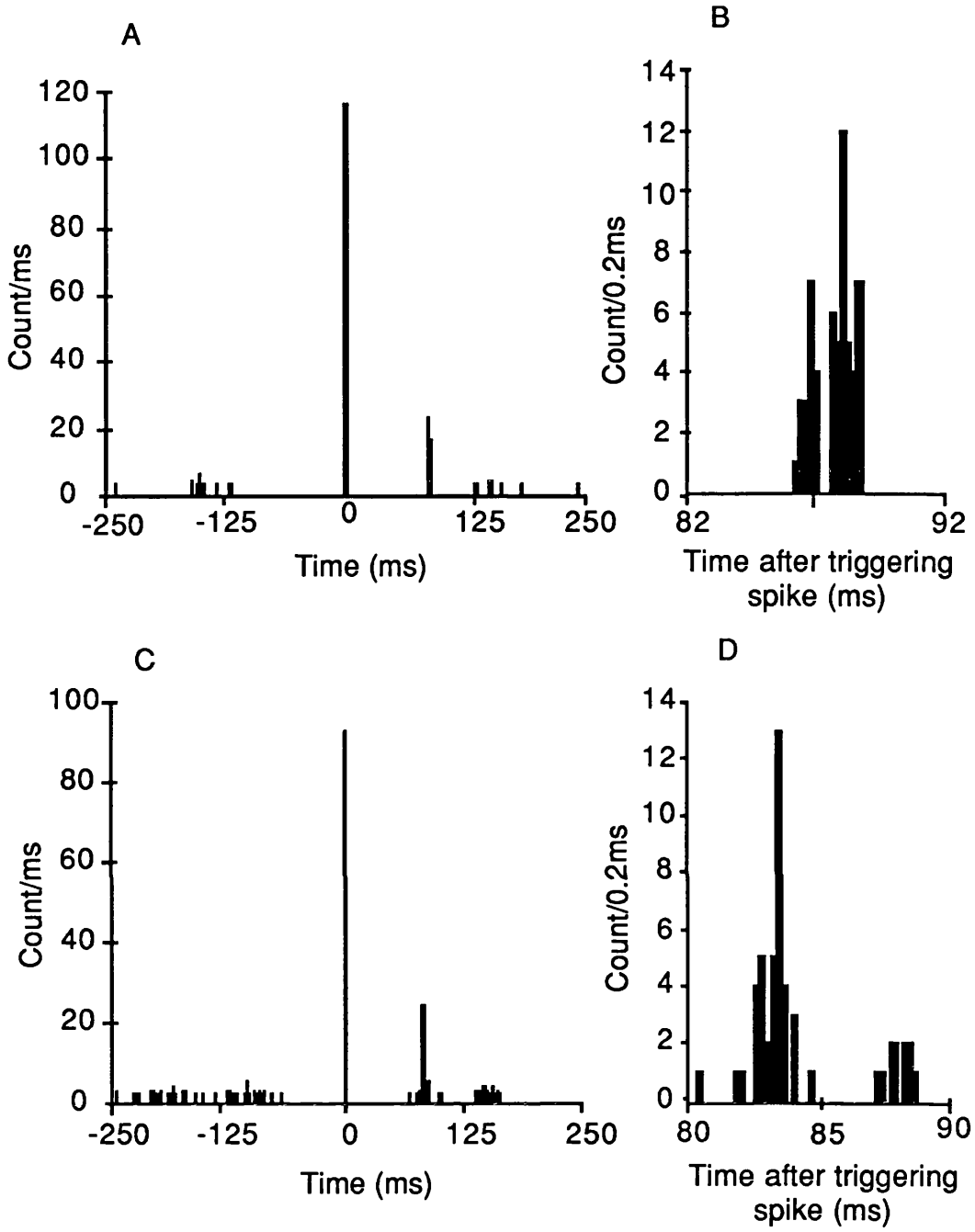


Fig. 30. PSTH from a spike triggered experiment on FDI in a healthy subject (A,B) and Patient 3 with MND (C,D). The triggering spike was at time zero, following which the stimulator was triggered after a delay of 60 ms in both subjects. A: PP has an onset latency of 86 ms after the triggering spike (26 ms after the stimulus) and a duration of 3 ms (179 trials). B: PP from A consists of 2 sub-peaks with an inter-modal interval of 1.2 ms. C: PP has an onset latency of 83 ms after the triggering spike (23 ms after the stimulus) and a duration of 6 ms (93 trials). D: PP from C consists of 2 sub-peaks with an inter-modal interval of 4.5 ms (contrast with B).

width of 0.2 ms, however, these peaks did not exhibit a clear sub-peak structure.

Patient 4 was a 54 year old man with MND, with a 2 year history of weakness in both legs and the left hand, generalised cramps and dysarthria. On physical examination there was evidence of mild weakness in both legs, the left arm and the right hand (FDI power 4, MRC scale) with hypereflexia of the left arm. Tone was not increased. There was generalised fasciculation which involved the tongue, the left side of which was wasted. There was marked wasting of the small hand muscles, particularly on the left, and of the right tibialis anterior. Electromyography and muscle biopsy (reported by the Department of Neuropathology, Oxford) showed evidence of denervation.

Random stimulation in 1 MU produced a PP with an onset latency of 29 ms and a duration of 3 ms, comprising one narrow peak when constructed with a bin width of 0.2 ms. It was also noted that MUs other than the index MU were also seen to discharge, at latencies ranging from 130-180 ms after the stimulus (Fig. 31). (At later stages of the same experiment MUs with a similar shape were seen to discharge voluntarily before the stimulus).

Multiple Sclerosis

Patient 5 (same as Patient C, below) was a 35 year old man with MS, with a 20 month history of progressive paraparesis, associated with an ataxic gait and urinary incontinence of recent onset. On physical examination he was found to have pale optic discs, nystagmus on left gaze, a marked spastic paraparesis with generalised hypereflexia and extensor plantar responses. Cerebellar signs were present in all limbs, but tone and power

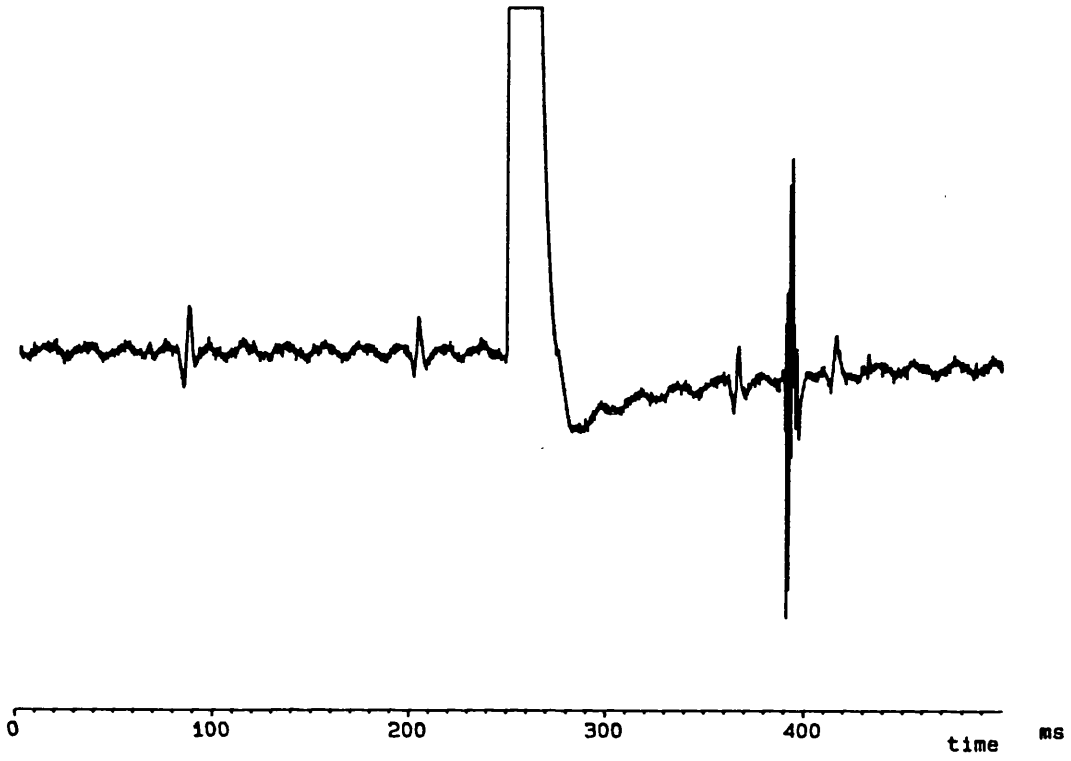


Fig. 31. EMG recording from Patient 4 with MND showing the discharge of the index MU (small MUP) before and after the stimulus, with a compound MUP at 140 ms after the stimulus. Discharges similar to the latter are not seen before the application of the stimulus. (0.1 mV/cm, stimulus time = 250 ms, stimulus artefact from 250-280 ms).

were normal in the arms. Vibration sense was absent in the legs and proprioception was impaired in the feet.

Three MUs from his right arm were studied, 1 from biceps and 2 from FDI. The biceps MU had a PP with an onset latency of 22 ms and a duration of 5 ms. In FDI, 1 MU showed no excitatory response and the other produced a PP with an onset latency of 33 ms with a duration of 16 ms. In the latter, the PP region of the PSTH was found to consist of one large peak followed by two much smaller peaks, each of which exceeded the mean pre-stimulus bin count plus 2 SDs, and each separated by periods of zero firing probability (Fig. 32A). The inter-modal intervals for these peaks were 8.5 and 7.5 ms, respectively. Within each peak there was no sub-peak structure evident when the PSTH was constructed with a bin width of 0.2 ms (Fig. 32B).

Peripheral Demyelination Combined with Prolonged Central Motor Conduction Time

Patient 6 was a 41 year old engineer who suffered from a chronic relapsing illness of 7 years duration. Symptoms included weakness, impaired balance, variable paraesthesiae and altered taste sensation. A sural nerve biopsy showed some loss of myelinated nerve fibres with axonal loss and demyelination on electron microscopy, and a muscle biopsy showed minor pathological changes suggesting denervation. He was treated with prednisolone and azathioprine. On physical examination at the time of the study, he was found to have proximal wasting of the arms without loss of power or fasciculation; weakness of hip flexion bilaterally; absent reflexes and flexor plantar responses. There was loss of pin prick and light touch sensation in a glove and stocking distribution, with impaired proprioception in the arms and legs and absent vibration sense below the hips. He had an

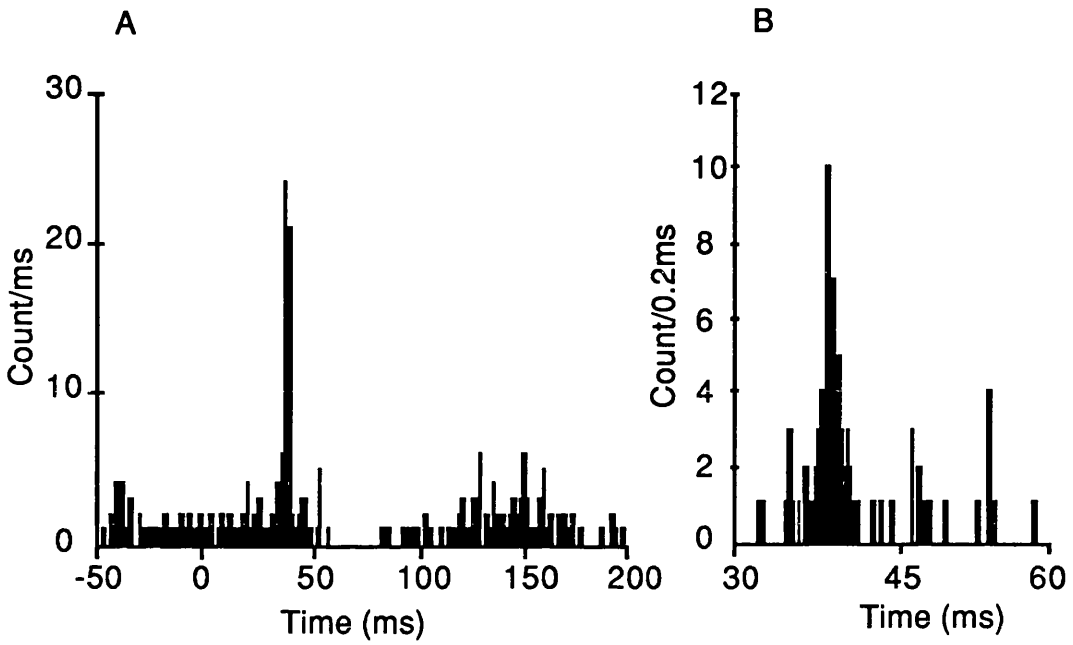


Fig. 32. PSTH from a random stimulation experiment on an FDI MU in Patient 5 with MS. A: PP has an onset latency of 35 ms and a duration of 20 ms (174 trials). B: The principal peak in PP is followed by 2 much smaller peaks at intervals of 8.5 and 7.5 ms. The initial wide peak lacks distinct sub-peaks.

ataxic gait with positive Rhombergism. On nerve conduction studies, the ulnar nerve conduction velocity was reduced to 39 m/sec, with absent median and sural sensory action potentials, confirming a diagnosis of chronic demyelinating peripheral neuropathy.

Using TMS with surface electrodes on the right FDI, the CMC time (Hess et al. 1987a) calculated from the latency from cortex to muscle and the F-wave latency, was found to be increased to 12 ms (mean+2.5SD = 8.3 ms for healthy subjects). This raised the possibility of an additional central lesion. PSTHs from 3 unitary recordings revealed PPs with onset latencies of 34, 36 and 39 ms. The latter 2 MUs displayed 2 sub-peaks with inter-modal intervals of 2.1 ms and 3.5 ms respectively (Fig. 33). The remaining MU, with a PP at an onset latency of 34 ms, displayed one narrow peak.

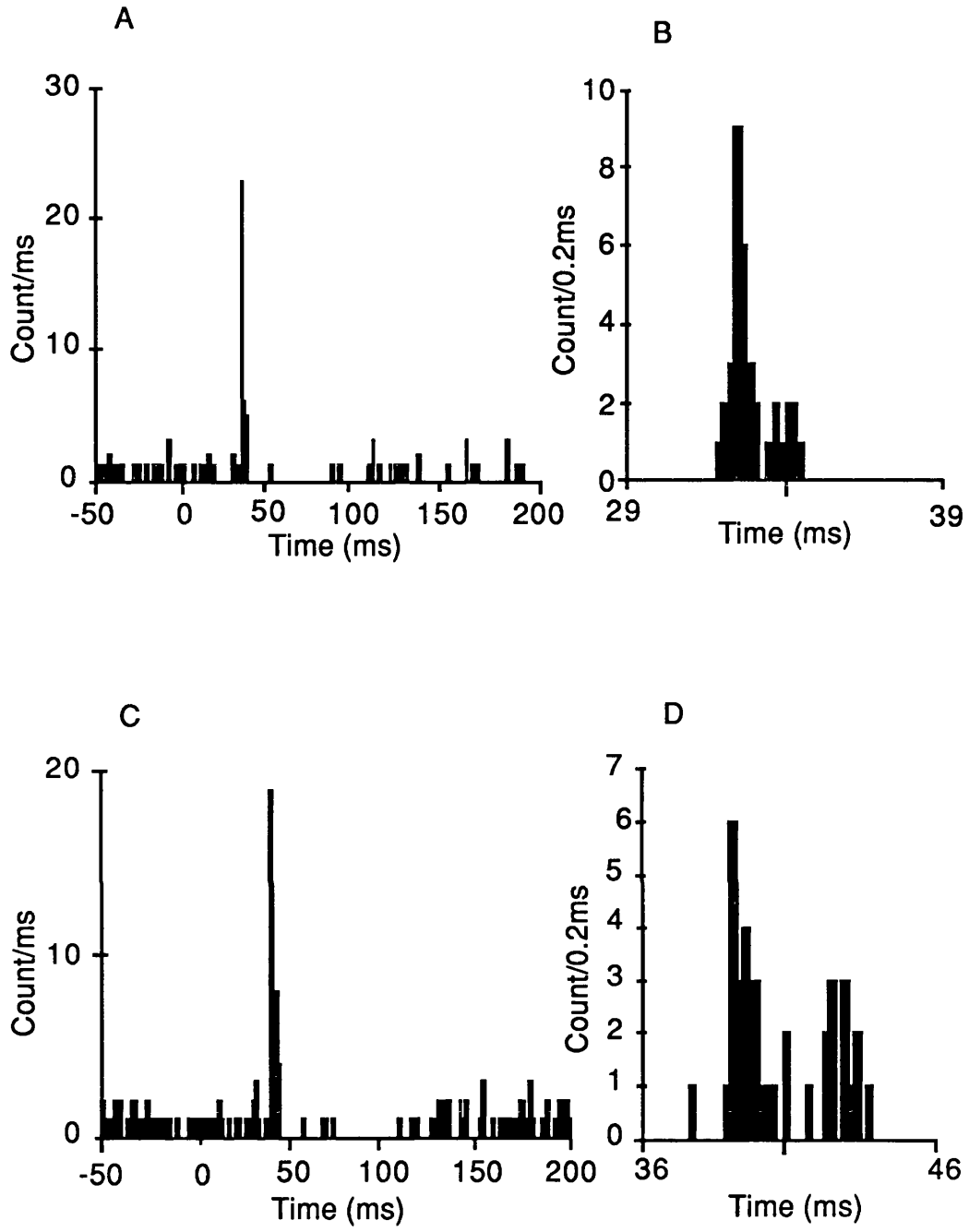


Fig. 33. Random stimulation PSTHs from an FDI MU in a healthy subject (A,B) and in Patient 4, with peripheral demyelination and prolonged CMC time (C,D). A: PP has an onset latency of 32 ms and a duration of 3 ms (68 trials). B: PP from A consists of 2 sub-peaks with an inter-modal interval of 1.6 ms. C: PP has an onset latency of 39 ms and a duration of 5 ms (84 trials). D: PP from C consists of 2 sub-peaks with an inter-modal interval of 3.5 ms (contrast with B).

Group of Patients with Multiple Sclerosis

In 19 of the 21 FDI MUs from the 10 MS patients (see Methods), the firing probability changed after the stimulus when compared with the mean pre-stimulus firing level. The features of these changes are summarised in Table 2.

Stimulus Intensity

With FDI relaxed, the stimulus intensity at which a just discernible compound muscle action potential recorded by surface electrodes could be obtained was frequently higher for patients with MS, ranging from 45 to 99%, when compared with a range of 36 to 54 % in healthy subjects. The patient (C) with a threshold of 45% for the right FDI had a threshold of >80% for the left FDI which was the clinically more severely affected side. Similarly, on studying single MUs in this patient, a stimulus intensity of 50 % produced a PSTH with a PP from the right side, whereas an intensity of 73 % produced no excitatory response on the left side. In another patient (E), the threshold intensity for surface responses was 90% on both sides which were equally affected clinically. A stimulus intensity of 72% produced no excitatory response in the PSTH for a MU in the right FDI (Fig. 34A), but the same intensity produced PSTHs from 2 MUs with late peaks in the left FDI.

The Primary Peak

With PSTHs constructed with a bin width of 0.2 ms, the intermodal interval between sub-peaks in 3 MUs was 2.6 ms (B1), 3.2 ms (B2), and 2.4 and 2.4 ms (I1) (see Fig. 35E). In the MUs from patients exhibiting such sub-peaks the intermodal interval was therefore equal to, or greater than, the

Table 2. Characteristics of Motor Units in Patients with Multiple Sclerosis

Patient-MU	Side studied	Stimulus Intensity (% max)	Surface Threshold (%max)	Primary peak Onset (ms)	Primary peak Duration (ms)	No. of Subpeaks	Inter-subpeak Interval (ms)
A-1	R	59	65	59	21	2	7
A-2	R	59	65	51	18	2	22
B-1	R	55	51	23	7	2	2.6
B-2	R	60	51	21	5	2	3.2
B-3	R	60	51	23	1	1	-
C-1	L	73	>80	Absent		-	-
C-2	R	50	45	33	16	2	8
D-1	R	67	70	35	20	3	8.5 and 7.5
E-1	L	72	90	69*	39	-	-
E-2	L	72	90	83*	15	-	-
E-3	R	72	90	Absent		-	-
F-1	L	52	67	27	2	1	-
F-2	L	52	67	26	4	1	-
G-1	L	70	NA	71*	27	-	-
G-2	L	70	NA	29	4	1	-
H-1	R	43	54	34	9	2	6
H-2	R	43	54	30	13	2	8
H-3	R	43	54	40	5	1	-
I-1	R	70	99	38	9	3	2.4 and 2.4
I-2	R	70	99	37	8	2	8
I-3	R	68	99	34	13	-	-

* Possibly secondary peak (see text). NA = not avaliable.

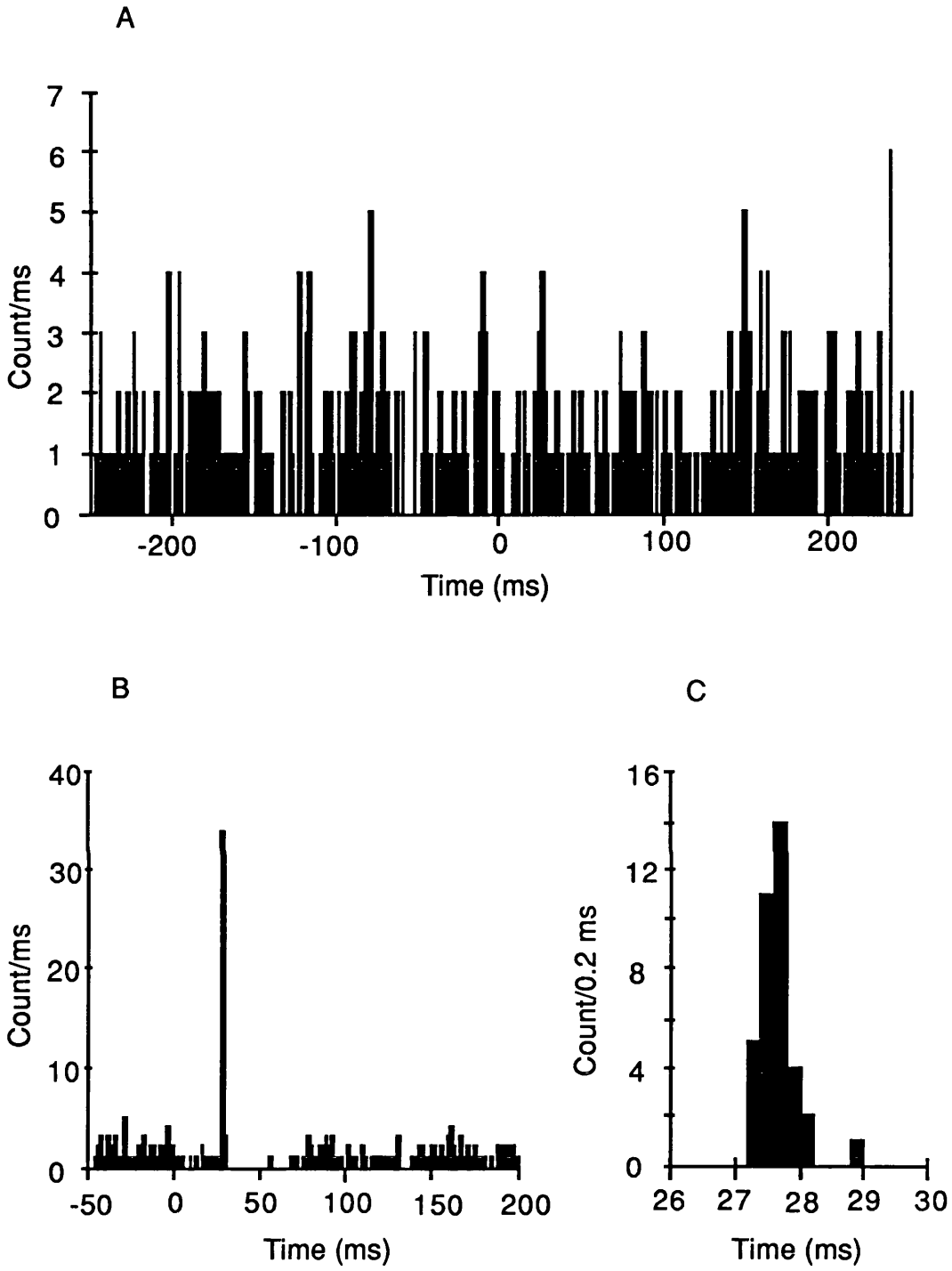


Fig. 34. PSTHs from 2 MS patients constructed with a bin width of 1 ms (A and B) and 0.2 ms (C) contrasting the features of an absent excitatory response with a normal response. A: PSTH plotted from -250 to +250 ms with respect to the stimulus, which was given at time zero. No rise in firing probability (confirmed with a cusum with limits of +/- 2 SD) is seen following the stimulus (MU E3, 120 trials, stimulus intensity 72 %). B: PP has an onset latency of 27 ms and a duration of 2 ms, comprising a single distinct sub-peak, as seen in C (MU F1, 95 trials, stimulus intensity 52 %).

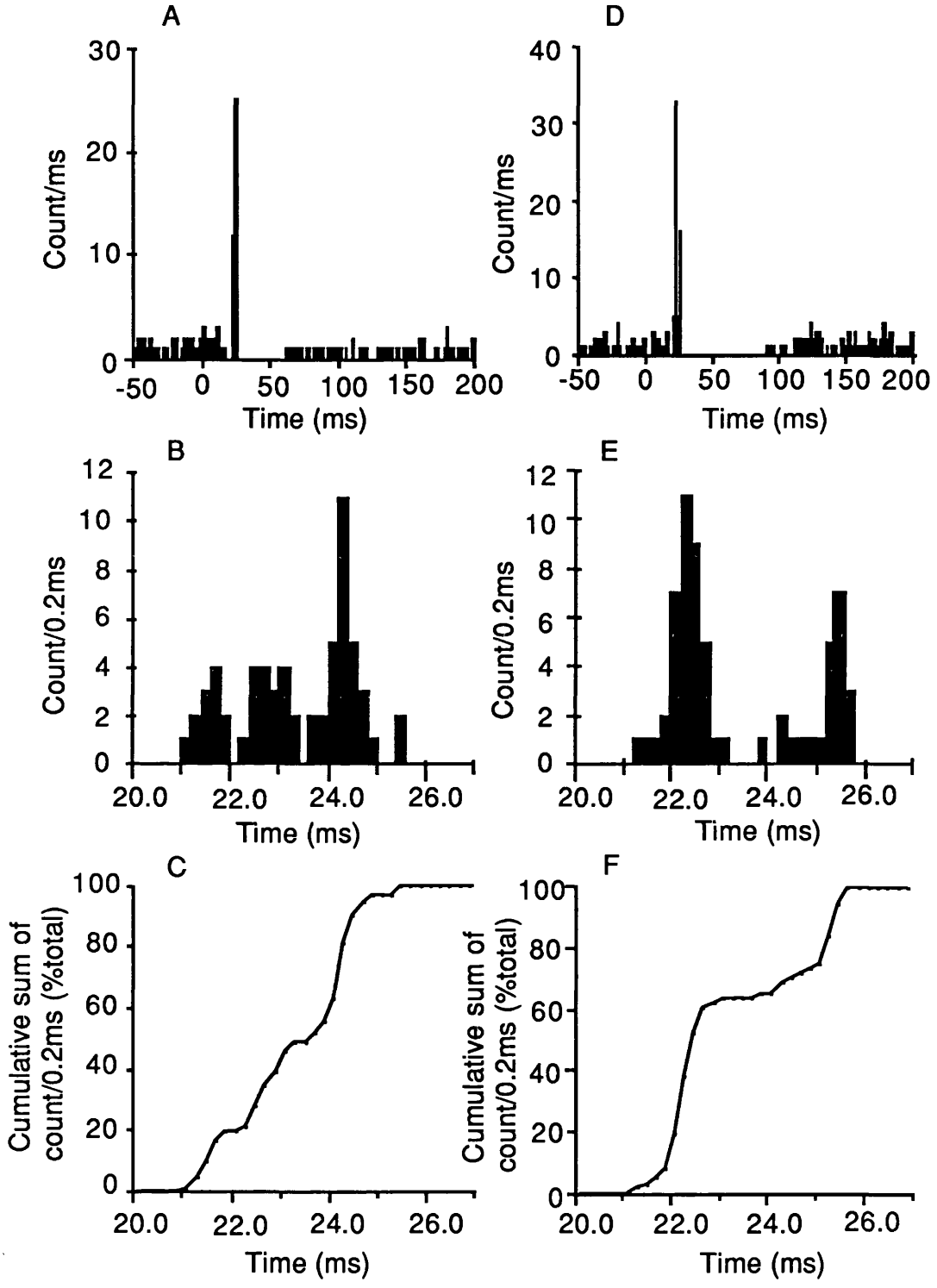


Fig. 35. Analysis of the discharge within PP in a healthy subject (A,B and C, 93 trials) and in a patient with MS (D,E and F, 120 trials). PSTHs constructed with a bin width of 1 ms (A and D) and 0.2 ms (B and E) are shown, with the corresponding cumulative sums normalised to the total number of discharges within PP (C and F). Three distinct sub-peaks are seen in B (with intermodal intervals of 1.0 and 1.6 ms) and 2 sub-peaks in E (with an intermodal interval of 3.2 ms). The contrasting shapes of the cumulative sums are evident in C and F.

maximum interval observed in a healthy subject (2.4 ms). The occurrence of other multiple peaks with prolonged intervals are described below.

In 9 MUs (A1,A2,C2,D1,H1,H3 and I1-3) the onset latency of those peaks which had the characteristic shape of PP was delayed by 2 to 28 ms with respect to the longest onset latency observed in healthy subjects (31 ms). In 5 of these 9 MUs, the duration of PP was increased by 4 to 12 ms, when compared with the PP of longest duration observed in a healthy subject (9 ms). In 6 of the same 9 MUs, plus 1 other (H2), the PP region of the PSTH, when constructed with a bin width of 1 ms, was found to consist of a principal component which was either preceded or followed by an additional one or two peaks of smaller height (Fig. 36 A,C,E). The height of each of these peaks exceeded the mean pre-stimulus firing level plus 2 standard deviations and the firing probability fell to zero between peaks. Intervals of 6-8.5 ms separated these successive peaks, with one interval of 22 ms (A2). This was not caused by multiple firings of the MU within the same trial, and was never found in healthy controls. These peaks had no discernible sub-peak structure when examined with 0.2 ms bins (see Fig. 36 B,D,F).

In 3 MUs (G1,E1,E2) a single peak of increased firing probability was found at latencies of between 69 and 83 ms and with durations of between 15 and 39 ms.

PPs of normal latency and duration, with single sub-peaks comparable to those found in healthy subjects, were found in 3 MUs (F1, F2 and G2) (Fig. 34B,C). By contrast, another MU (G1) from the same muscle in one of these patients had a PSTH containing a single peak of 27 ms duration with an onset latency of 71 ms. Of the 9 patients studied, this was the only example

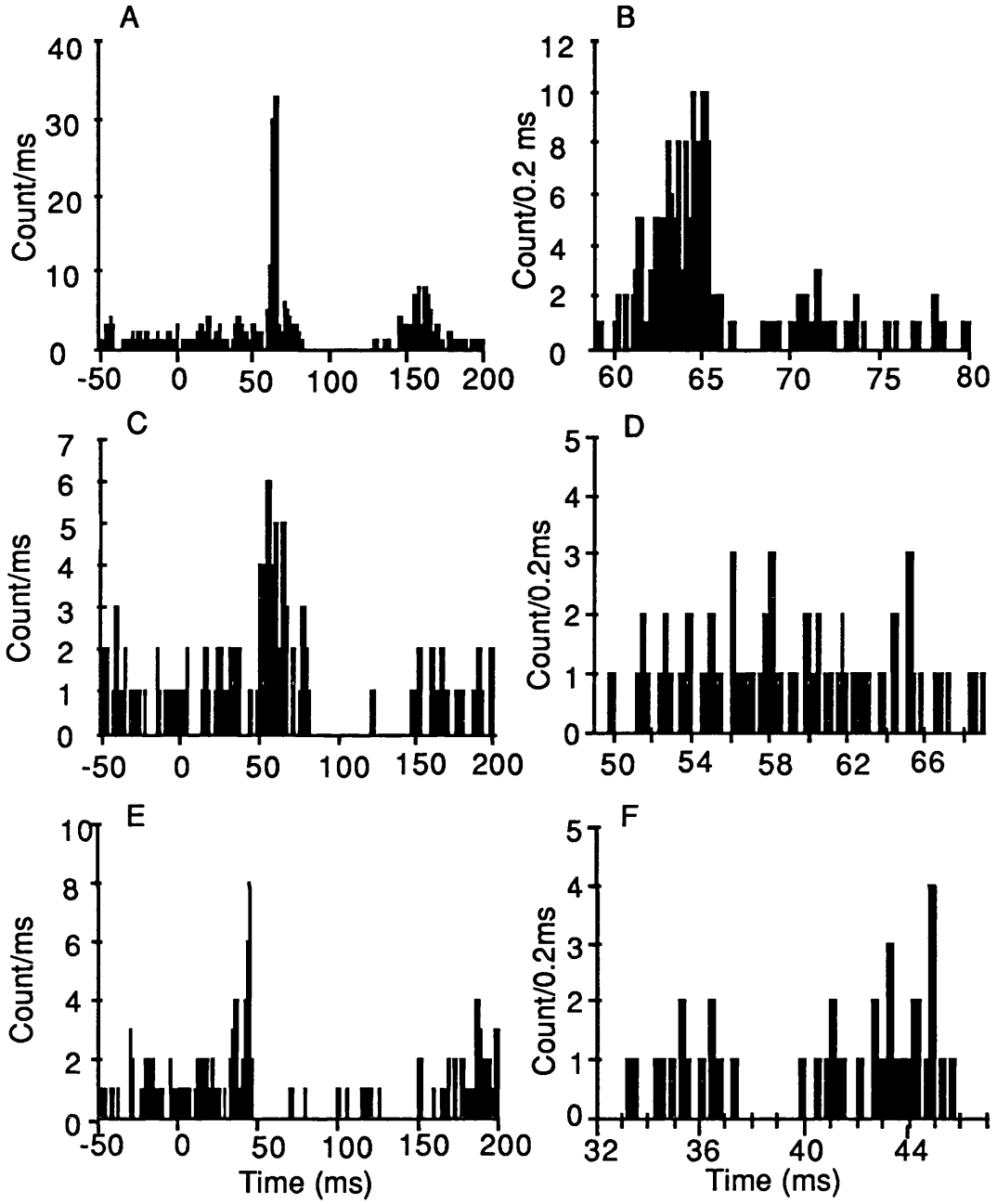


Fig. 36. PSTHs from 3 FDI MUs (A1, A2, C2) in 2 MS patients constructed with a bin width of 1 ms (A,C and E) and 0.2 ms (B,D and F) showing the features of PP. A: PP has an onset latency of 59 ms and a duration of 21 ms containing a principal peak followed by 2 much smaller peaks, each separated by periods of zero firing probability (as seen in C) and each exceeding the mean pre-stimulus firing level plus 2 SDs (MU A1, 221 trials). B: PP region from A, showing lack of distinct sub-peaks within the principal component of PP. C: PP has an onset latency of 51 ms and a duration of 18 ms, the principal component of which is followed by a peak of smaller height and duration (MU A2, 99 trials). D: PP region from C, showing lack of distinct sub-peaks. E: PP has an onset latency of 33 ms and a duration of 16 ms, comprising 2 peaks, each of which lacks distinct sub-peaks, as seen in F (MU C2, 70 trials).

where MUs exhibiting both normal and abnormal features were found in the same muscle.

Quantifying the Effect of Spike-Stimulus Interval on the Probability of Evoking a PP Discharge

The influence of the spike-stimulus interval on the probability of producing a PP discharge was studied in 9 MUs in the same way as for FDI MUs from healthy subjects (Figs 37, 38). The excitability displayed a similar change with time (Fig. 38B). It was initially relatively low and then showed a rise at longer spike-stimulus intervals. The variability in the time course of this change between different MUs was, however, greater than that found in healthy subjects. Also in contrast to controls, the proportion of PP trials in 8 of 9 MUs had already exceeded 20% at a spike-stimulus interval of 40 ms.

Effect of Spike-Stimulus Interval on the Latency of Discharge Within PP

The latency of the MU discharge within PP was in 7 of 10 MUs significantly correlated with the interval between the stimulus and the preceding voluntary discharge (Fig. 39B).

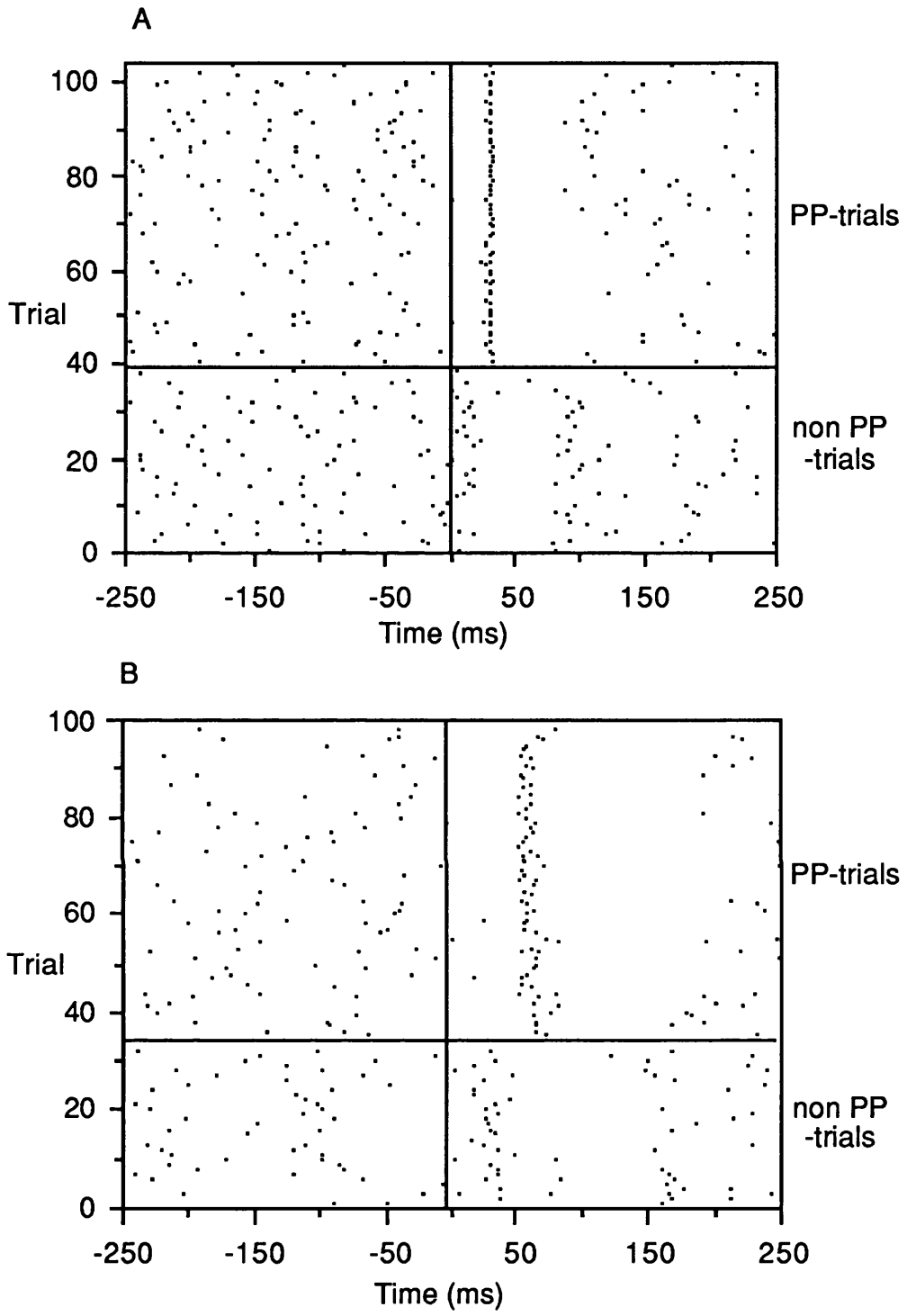


Fig. 37. Sorted raster plot of the discharges of a single MU from a healthy subject (A, 103 trials) and Patient A with MS (B, 99 trials, MU A2). Each point represents a single discharge. Stimuli were given at time zero. Trials have been separated into those with a discharge within PP (upper panel) and those without (lower panel). Differences in the firing times of spikes before the PP latency in the 2 panels are seen in both A and B. A broader cluster of PP discharges at a longer latency occurs in B when compared to A.

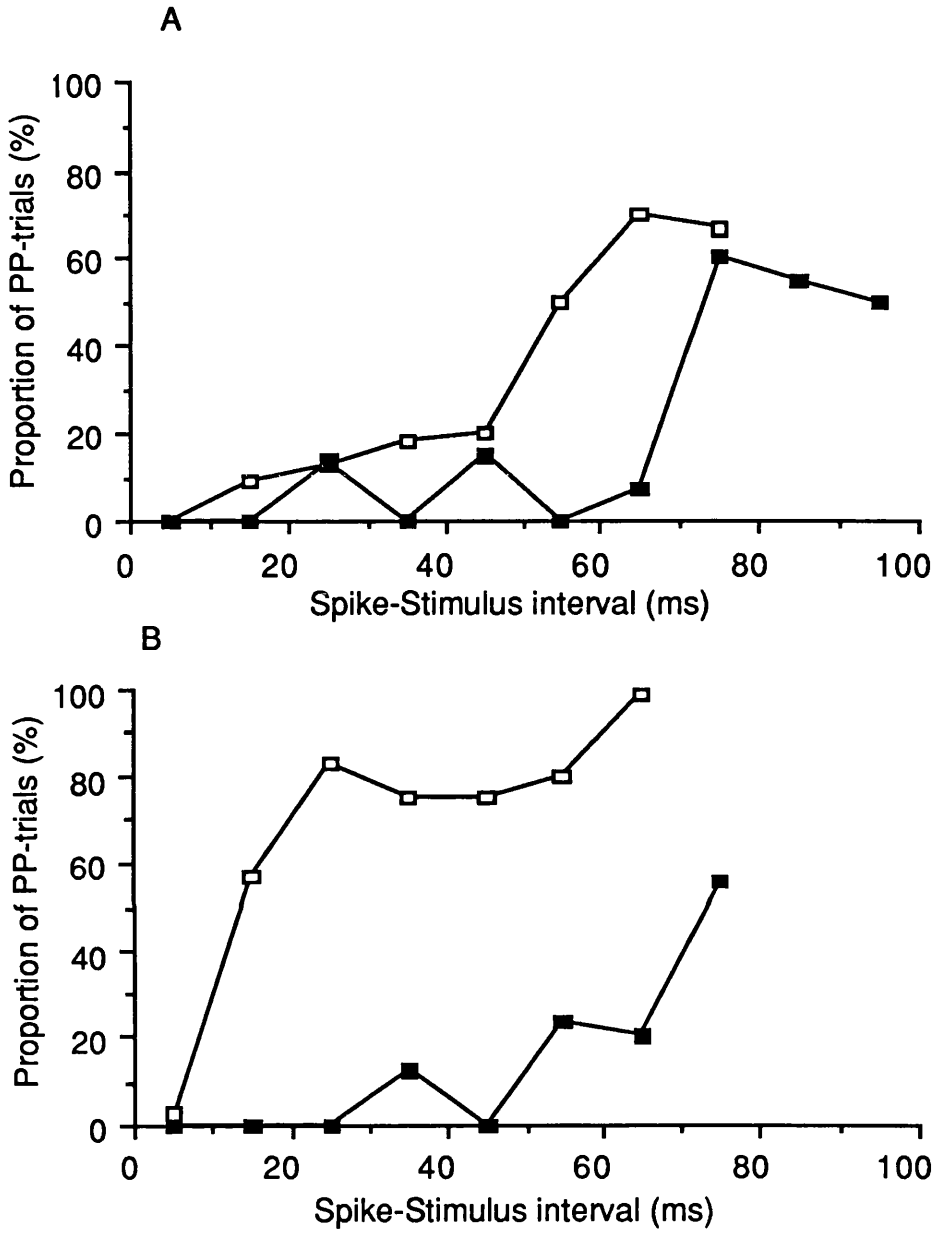


Fig. 38. Proportion of PP-trials plotted against the spike - stimulus interval for FDI MUs from a healthy subject (also seen in Fig. 22) and patients with MS. Each curve represents the minimum and the maximum rate of rise observed for each of the 2 subject groups (open and closed squares, respectively). A: 2 MUs from a healthy subject (filled squares: 168 trials, mean ISI 146.4 ms; open squares: 155 trials, mean ISI 124.9 ms). B: 2 MUs from patients with MS (B1-filled squares: 170 trials, mean ISI 143.6 ms; C2-open squares: 70 trials, mean ISI 103.8 ms). Spike-stimulus intervals greater than the minimum ISI for each MU are not shown, as explained in the Discussion. All MUs show a rise in excitability with time, with a greater variability between different MUs observed in MS patients.

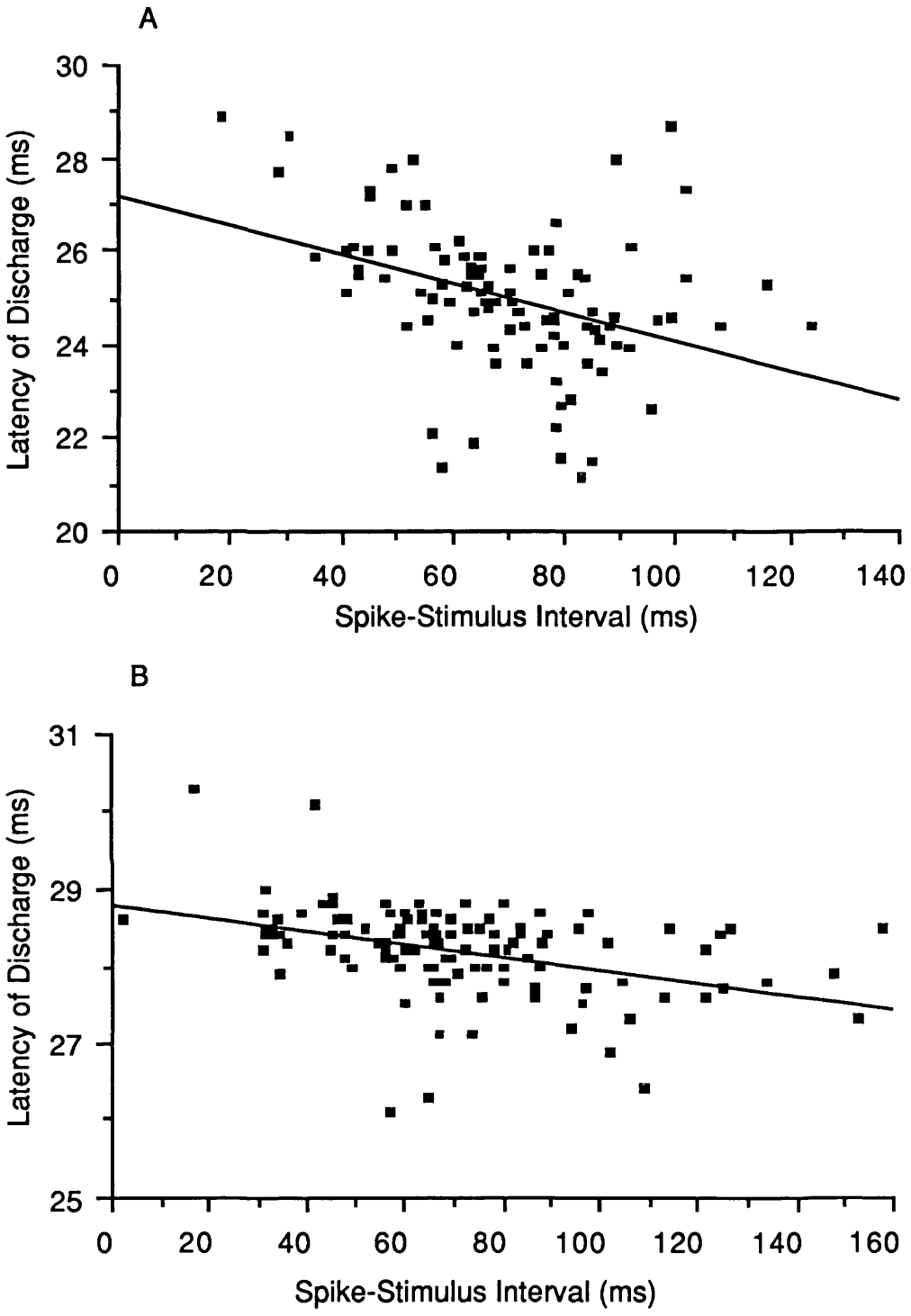


Fig. 39. The latency of discharge of a single MU within PP is negatively correlated with spike-stimulus interval. The longer the interval then the earlier is the discharge within PP. A: Healthy subject (correlation coefficient = 0.139, $p<0.001$, 492 trials, 96 PP-trials). B: MU F2 from an MS patient (correlation coefficient = 0.116, $p<0.001$, 276 trials, 118 PP-trials).

CHAPTER 4

Discussion

Scope of the Discussion

Inferences Made From Changes in Firing Probability

Healthy Subjects

Early Changes in Firing Probability

Late Changes in Firing Probability

Mechanism of Abnormalities in Patients with UMN Disorders

Summary

Scope of the Discussion

The discussion applies to that sub population of the motoneurone pool consisting of low threshold MUs, selectively studied by this technique. In addition, the short latency of the initial excitatory response to TMS suggests transmission via the fast conducting PTNs (see Introduction). Later effects (either excitatory or inhibitory) may provide information about slower conducting pathways.

Interpretation of the results is based upon the premise that a rise in firing probability in the PSTH is caused by either the rising phase of an EPSP, the decay of an IPSP, or by the combination of both processes. The principles underlying this interpretation are discussed in the next section.

Inferences Made From Changes in Firing Probability

The main tool for studying changes in firing probability in this study was the PSTH. A PSTH cross correlates the discharge times of the cell under study with respect to the time of the stimulus. Two neural effects are consequently manifested - the synaptic effects and the periodicity effects (Moore et al., 1970). The latter arise from repetitive firing and the former reveals the effect of PSPs. The statistical analysis and functional interpretation of neuronal spike data can therefore provide information on the mechanisms of spike production, features of the presynaptic input to the cell and the mechanisms by the which the latter is transformed into a postsynaptic output (Moore et al., 1966). This form of analysis assumes that neuronal processes involve a probabilistic element. Indeed, the activity of a motoneurone may be

described in terms of a stochastic point process by virtue of the random variations in ISIs and the indistinguishable nature of successive spikes.

An EPSP may cause a neurone to discharge only when its rising phase crosses threshold. By studying the change in firing probability that follows the application of a series of TM stimuli with PSTHs, therefore, information can be obtained about the time course of the rising phase of the underlying EPSP. In general, however, the rise time of a PSP may be over estimated from the analysis of a peak in the PSTH, particularly with a rapidly firing cell which spends proportionately more time near discharge thresholds. Furthermore, a slowly firing cell has an ISI proportionately greater than the refractory period so that the history of events immediately following a spike is less influential when the membrane potential next crosses its discharge threshold. In these conditions analysis of a peak in the PSTH is less able to detect the effects of past events. Cell refractoriness and AHP may, therefore, have less influence than the membrane trajectory and the summation of PSPs - particularly for low to moderate firing rates (Knox and Poppele, 1977). Furthermore, non linear summation near the reversal potential for IPSPs should have little effect. In contrast, any partial increase in active conductances near threshold may alter the relationship between the features of a peak in the PSTH and its underlying EPSP. The result would tend to increase the correlation for excitatory inputs and decrease it for inhibitory inputs (Knox and Poppele, 1977).

Inference of Synaptic Events

Moore and colleagues demonstrated that EPSPs in aplysia neurones broadly matched their PSTH peaks (Moore et al., 1970). In contrast, a neuronal model of the membrane potential that reached firing threshold by the integration of EPSPs and then resetting the membrane trajectory after

firing produced peaks in the PSTH resembling the derivatives of EPSP shapes (Knox, 1974). This has been confirmed for EPSPs in spontaneously firing neurones (Knox and Poppele, 1977) and small stretch evoked IPSPs in low levels of synaptic noise have been found to generate troughs in the PSTH which closely resemble the shape of the IPSP derivatives (Gustafsson and McCrae, 1984). For average common excitation potentials in inspiratory motoneurones, it has been suggested that a peak in the PSTH could be the summation of 2 linear terms, one proportional to the EPSP and the other proportional to its derivative (Kirkwood and Sears, 1978), consistent with findings in single fibre EPSPs (Kirkwood and Sears, 1982b). To test which of these hypotheses may apply to cat lumbar motoneurones, the shape of PSPs in the quiescent state was compared with peaks in the PSTH when obtained from electrical stimulation of peripheral nerve filaments during repetitive firing evoked by a depolarizing current (Fetz and Gustafsson, 1983). EPSPs ranging in amplitude from 0.15 to 3.1 mV were investigated. Their results suggested that simple EPSPs produced peaks in the PSTHs that resembled their temporal derivatives. The PSTH peak was also delayed by an average of 0.48 ms from the onset of the EPSP, and reached a maximum 0.29 ms before the summit of the EPSP. For synaptic inputs located on the soma, however, there would be theoretically no delay in the appearance of the peak (Knox, 1974). The height of the peak with respect to the background firing rate increased proportionately with EPSP amplitude and rising slope. The relationship between the temporal derivative of the peak and the EPSP shape, however, did not hold for complex EPSPs and large IPSPs: during complex EPSPs the second peak in the PSTH was often smaller than the first, and compound IPSPs produced primary troughs in the PSTH followed by a compensatory peak. It was concluded that their major observations were:

> "consistent with a motoneurone model in which a membrane potential ramp approaches a voltage threshold of spike initiation. Near threshold, EPSPs superimposed on the ramp advanced the occurrence of spikes to their rising phase, producing a correlogram peak resembling their temporal derivatives. Synaptic noise would increase the probability of sampling the peak of the EPSP, leading to wider correlogram peaks. IPSPs would delay the occurrence of spikes to their falling phase."

The post-stimulus firing probability of human MUs has been used to estimate the shape of the rising phase of Ia EPSPs, with the width of a peak in the PSTH providing an estimate of rise time. For Ia EPSPs evoked by electrical stimulation of afferent fibres estimates ranged from 2-5 ms in TA (Ashby and Zilm, 1982b) and for EPSPs evoked by tendon taps, a mean of 7.6 ms was obtained in quadriceps femoris (Noguchi et al., 1979). These figures exceed the duration of electrically evoked Ia EPSPs recorded intracellularly from cat spinal motoneurones of 1-1.5 ms (Coombs et al., 1955b). Reasons for this may have included temporal dispersal of the afferent volleys over the greater stimulation-recording distance in man, the use of mechanical tendon taps, synaptic noise, or the limits of resolutions set by the bin width of the PSTH (Ashby and Zilm, 1982b; Mau et al., 1984).

The shape of the rising phase of the initial portion of the action potential may also influence the shape of a peak in the PSTH. The more gradual upward deviation that precedes the abruptly rising phase of the spike (Schwindt and Calvin, 1972) may cause a finite and possibly variable delay between the crossing of the threshold and the actual initiation of the IS-spike at the soma (Gustafsson and McCrae, 1984). This potentially allows the shape of the trajectory in the region of the initiation of the spike to affect the exact latency of the IS-spike. The consequent temporal dispersion

would produce a widening of the peak-particularly for small, fast rising EPSPs.

Summarising the principles of the analysis of PSTHs (or correlograms) in the deduction of postsynaptic events, Mau et., al (1984) wrote:

> "When an EPSP is large relative to background synaptic noise the profile of the period of increased firing probability approximates to the first derivative of EPSP shape (provided that the slope of the combined EPSP membrane potential trajectory is always positive). Under these circumstances the width of the period of increased firing probability provides an estimate of the rise time of the underlying EPSP. The presence of synaptic noise comparable in amplitude to that of the EPSP (thus with small EPSPs) allows the falling phase of the EPSP to be sampled and represented in the PSTH profile. The peak becomes broader and the derivative of the EPSP no longer predicts the PSTH profile. IPSPs produce correlogram troughs that are longer than the initial rising phase of the IPSPs (Fetz and Gustafsson, 1983). Furthermore large IPSPs may result in a period of zero firing probability in the correlograms. Thus the correlogram produced by large IPSPs do not resemble any linear combination of the shapes of the IPSPs and/or their temporal derivatives. However, the profiles of correlograms resulting from small IPSPs, (and which do not contain any period of zero firing probability) may approximate the IPSP derivatives (Gustafsson and McCrea, 1984)."

Cumulative Sums

The cumulative sum derivative (cusum) of the PSTH (Ellaway, 1978) can be used to detect subtle changes in the mean level of counts in the PSTH that would otherwise be obscured by the variability in individual bin contents. Differences from the mean control level of counts in the PSTH are integrated in a cusum, in which facilitation will appear as a positive slope and inhibition as a negative slope. During repetitive firing, as in the present study, however, refractoriness may produce periodic fluctuations in the bin counts producing a periodicity which causes the cusum to deviate less from the horizontal line than predicted from a Poisson distribution of points (Davey et al., 1986). A regular spike train with a low coefficient of variation

of the distribution of ISIs produces a relatively flat cusum. Davey and colleagues have used the theory of stochastic point processes to derive an algorithm for calculating the best approximation of variance of the cusum. Significance limits set at 3 SDs of the cusum have been shown to provide a good fit for cusums derived from spike trains over a wide range of coefficients of variation (0.09-0.60). In the present study, cusums were used to confirm the absence of peaks from the PSTHs of some UMN patients.

Healthy Subjects

Early Changes in Firing Probability

The Mechanism Underlying PP

The rising phase of a compound EPSP would be expected to influence the firing probability of a rhythmically firing motoneurone in a manner that causes discharges to occur earlier than expected, producing a peak followed by a trough in the PSTH. This hypothesis is supported by the observed features of the PSTHs. That the rising phase of an EPSP underlies PP is also supported by the observation that the latency of discharge within PP is correlated with the spike-stimulus interval, so that long intervals produce relatively early discharges within PP, and vice versa. The decay of an IPSP may also produce such a correlation, but the duration of PP would not be as short as observed here.

If PP is caused by an EPSP, then the duration of PP will be related to its rise time (Knox, 1974 - see Introduction). In FDI, the mean (±SD) duration of PP was found to be 4.6 (±1.7) ms. This is within the maximum range for the rise time of compound EPSPs of 7.5 ms, in the forearm motoneurones of a baboon following electrical cortical stimulation (Kernell and Wu Chien-Ping, 1967b). The duration of PP, however, is influenced by the method used to measure it and the bin width of the PSTH. In the present study, which used a bin width of 1 ms and the intersection of the peak with the mean pre-stimulus firing level to determine onset and offset of PP, the duration of PP is probably overestimated. In addition, the duration of PP is influenced by the number of successive EPSPs produced in response to each of the

descending volleys and by the effects of noise (see below). One estimate for rise time is therefore to use the interval between neighbouring sub-peaks as a guide to the maximum rise time of one in the probable succession of EPSPs produced by a train of descending I waves (an approach that is explained below) which in the present study was 1.4 ±0.4 ms (mean ±SD).

These results compare with PSTHs obtained from TMS in other human studies (Mills, 1988; Day et al., 1989) and also in the awake monkey, from the responses of identified single MUs in the forearm to single pulse intracortical microstimulation in area IV (Palmer and Fetz, 1985). In the latter, forearm MUs were initially identified by post-spike facilitation of stimulus-triggered averages in the multiunit EMG during isometric wrist activity. PSTHs exhibited PPs with a mean (±SD) onset latency of 8.8 (±1.7) ms. The duration of the sub-peaks was 1.8 (±1.2) ms. Increasing the intensity of the intracortical stimulus produced larger PPs. The onset latencies of PP ranged from 6.5-11.2 ms, consistent with mediation via CMN projections (assuming a peripheral conduction time of 3-6 ms and a CMC time in the region of 3.5-5.2 ms - Lawrence, Porter and Redman, 1985). The mean duration of PP (1.8 ms) was close to the rise time of CMN EPSPs when evoked by electrical stimulation of the cortical surface (1.5 to 2.5 ms) (Landgren, Phillips and Porter, 1962a). The mean duration observed in the present study of 4.6 ms, however, is 2.5 times larger than the mean duration of PP in the above study, suggesting either that the compound EPSPs were of longer rise time or that there was more noise in the system (see Introduction) which includes the effects of variation in the ISI (see below).

Multi-modal Discharge Latencies in FDI and Other Arm Muscles

The multi-modal discharge of a spinal motoneurone in response to a TM stimulus, at intervals of 1.4 ± 1.7 ms in FDI (mean ± SD), is compatible with the arrival at the spinal motoneurone of a sequence of descending volleys. This hypothesis is consistent with recordings of the descending volleys produced by a single surface anodal electrical stimulus in the baboon (Kernell and Wu Chien-Ping, 1967a) and the corresponding sequence of PSPs produced in single forearm motoneurones (Kernell and Wu Chien-Ping, 1967b). Electrical stimulation of the motor cortex induces repetitive firing of corticospinal neurones in the cat (Patton and Amassian, 1954) and the monkey (Hern et al, 1962). There is initially a direct response by the corticospinal neurones (D wave) which is followed by repetitive discharges of the same fast corticospinal neurones (I waves). The interval between successive I waves is of the order of 1-2 ms (similar to the inter sub-peak interval observed here) in a sequence lasting up to 6.5-8.5 ms (Kernell and Wu Chien-Ping, 1967a). Multimodal discharge latencies following TMS (Mills, 1988; Day et al., 1989) and TES (Day et al., 1987) suggest similar intervals. Up to 4 I waves were recorded, by Kernell and Wu Chien-Ping, termed I1, I2, I3 and I4 according to their increase in latency. Sometimes a small fifth I wave was also seen. The interval between I2 and I3 was usually longer than the interval between I1 and I2 and the stimulus threshold of I1 was usually higher than the threshold of I2 and I3. Epidural recordings from the posterior surface of the human spinal cord during vertex anodal TES has also demonstrated a series of waves, at mean intervals of 1.0, 2.0, 3.1 and 3.8 ms after the initial D wave (Burke et al., 1990). The thresholds for I1 and I2 differed significantly from those for I3 and I4. Waves I1 or I2 usually had the lowest threshold.

Double discharges at the PP latency were found to occur infrequently. The inter-discharge interval sometimes approximated to the inter-modal sub-peak interval, consistent with post spike re-excitation by a subsequent descending volley (Day et al., 1989). Alternative mechanisms for the production of double discharges may involve the intermittent initiation of regenerative firing of the motoneurone (Calvin, 1974) by TMS via the effect of delayed depolarisation, or the combined effect of direct activation of the spinal motoneurone with subsequent indirect activation via collateral pathways with an increased number of synapses. Regardless of the mechanism, such events did not account for the appearance of sub-peaks in the PSTH.

Two inferences may be drawn from the observation that within the PP region of the PSTH there was no obvious relationship between the duration or height of sub-peaks and their latency. The absence of a sequential decrease in the duration of sub-peaks with increasing latency implies that within the sensitivity of this technique in providing an estimate of the rise time of EPSPs, no temporal facilitation (see Introduction) was demonstrated. Furthermore, the absence of a progressive increase in the duration of sub-peaks does not support the possibility that successive sub-peaks were produced by pathways involving progressively more synapses (Kirkwood and Sears, 1982 a, b). Similarly, no significant correlation was observed between the duration of PP and its latency with intracortical microstimulation in the monkey (Palmer and Fetz, 1985).

In 5 of the 6 MUs from upper limb muscles other than FDI that exhibited multiple sub-peaks, the inter-modal interval between the first and second sub-peaks was greater than the maximum inter-modal interval observed in FDI (2.4 ms). This might be attributed to the variable threshold for

successive descending volleys (Kernell and Wu, 1967a; Burke et al., 1990), also inferred from PSTHs in FDI MUs by Day et al (1989), but absent in FDI MUs in the present study. The intermediate (i.e. second) sub-peak in the 1 biceps MU examined that displayed an interval of 1.3 ms between each of 3 successive sub-peaks may have therefore represented the effect of an I wave with a higher threshold than the preceding and subsequent I waves (corresponding to the first and second sub-peaks respectively).

If the mode of each sub-peak represents the point of maximum gradient on the rising phase of each EPSP generated by a series of descending volleys, the above result may alternatively suggest that the interval between successive volleys arriving at FDI motoneurones is shorter than those intervals for motoneurones supplying other muscles of the upper limb. If the corticospinal connections to human spinal motoneurones serving more proximal arm muscles fire at a lower rate following a transcranial stimulus, this would imply that either their discharge was undetected by recordings from the human CST during surgery (Burke et al., 1990), or, that their discharge occurs at a harmonic of 2 or 4 times below the discharge frequency for connections to the motoneurones serving intrinsic hand muscles. This explanation would therefore seem unlikely.

With reference to studies in the monkey, it would also seem unlikely to attribute these shorter intermodal sub-peak intervals observed in FDI MUs, when compared to MUs from upper and lower arm muscles, to the operation of temporal facilitation of EPSPs (Landgren et al., 1962a). Such facilitation chiefly affects the amplitude of successive EPSPs, which would serve to increase the probability of evoking a PP discharge rather than influencing its latency. Shortening of the intermodal sub-peak interval might be expected to occur, however, if temporal facilitation were to

significantly increase the slope of the rising phase of successive EPSPs. Although small changes in the rise time of facilitated EPSPs have been found (Muir and Porter, 1973) this was not a consistent finding, and roughly equal numbers of cells showed a slight increase and a slight decrease in rise time. The mean half-widths for facilitated EPSPs was 13% greater than that for controls. In the present study it is also noted that progressive changes in the duration of successive sub-peaks, which would be consistent with temporal facilitation, was not found in FDI (see above).

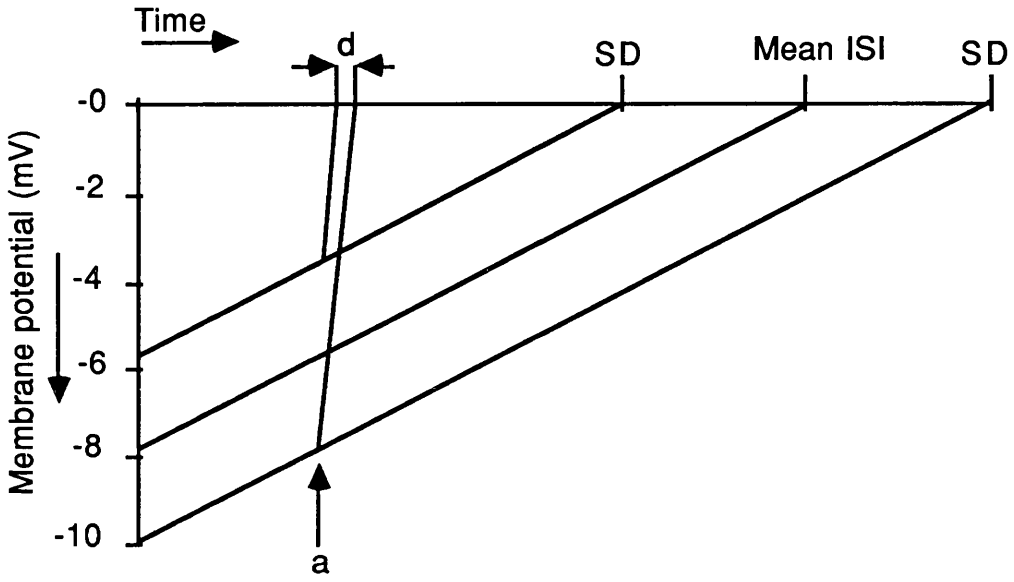
PPs were were readily obtainable in all the upper and forearm muscles tested at similar stimulus intensities, in contrast to the difference between proximal and distal muscles found with electrical stimulation of the cortical surface in the monkey. CMN EPSPs with the largest amplitude have been found in spinal motoneurones supplying intrinsic hand muscles (Fritz et al., 1985) and also EDC (Clough et al., 1968). The difference between these results and the results with TMS may, however, be due to the selection of low threshold MUs in the present study.

Temporal Dispersion of PP and its Sub-Peaks

The rapidly conducting corticospinal neurones, implicated by the relatively short CMC time with TMS, have a fairly limited range of conduction velocity in the monkey of between 50 and 70 ms. This small range would tend to synchronise the firings within a single sub-peak. In addition, there is evidence that the action potentials of PTNs may be synchronised by ionic and electrical interactions between neighbouring fibres (Schmied and Fetz 1987). Experimental variables, however, may be expected to have the opposite effect: Present results show that during the course of the first 100 trials of a random stimulation experiment, the duration of PP becomes progressively wider, until a maximum duration is reached, after which it

remains constant (Fig. 10). In contrast, the progressive increase in the duration of sub-peaks within PP progresses beyond the first 100 trials, with widening of individual sub-peaks still evident after 100 trials. (This did not necessarily effect, however, the slope of the corresponding normalised cumulative sum). It has been argued that the duration of each sub-peak reflects the rise time of the underlying EPSP. The variation in ISI during the experiment, however, may alter the point of take off for the EPSPs, and would be expected to increase the duration of sub-peaks beyond the rise time of the EPSP by dispersing the latency of evoked discharges. This is potentially an important effect, the magnitude of which can be estimated (Fig. 40). This is a simplified version of the interspike membrane trajectory for one ISI for a 10mV hyperpolarisation. Using data from one random stimulation experiment (500 trials), the mean ISI plus or minus 1 or 2 SDs predicts a dispersion effect (d) caused by the variation in take off point for the EPSP. Depending on various assumptions (a depolarisation of 10 mV between discharges, a compound EPSP of 7.5 ms duration, and a membrane trajectory involving a vertical scoop followed by a linear ramp), single sub-peaks or the whole PP in this experiment may be expected to be dispersed by the order of 0.9 and 3.5ms, respectively.

This temporal dispersion, however, does not affect the latency of the modal points of the sub-peaks, but it may affect their shape, and hence the shape of the cumulative sums. This effect is found to be constant, however, despite increasing the number of trials during the course of an experiment (Fig. 41). When each cumulative sum is normalised to the maximum number of discharges within PP as in Fig. 41C, the curves become superimposed. This particularly applies to the positions of maximum slope, corresponding to the modes of each sub-peak, as indicated by the arrows.



d (ms)

1.9	+/-1SD	} **Primary Peak**
3.5	+/-2SD	
0.5	+/-1SD	} **Sub-peak**
0.9	+/-2SD	

Fig. 40. Method for estimating the temporal dispersion in the latency of PP discharges produced by variation in the ISI, which alters the take off point (a) for the underlying EPSP. Diagramatic illustration of the interspike membrane trajectory, for a maximum hyperpolarisation of 10 mV and an EPSP with a maximum rise time of 7.5 ms. The mean ISI for this MU ±1 or 2 SDs indicated a maximum expected dispersion effect (d) affecting single sub-peaks, or the whole PP, of up to 0.9-3.5 ms, respectively.

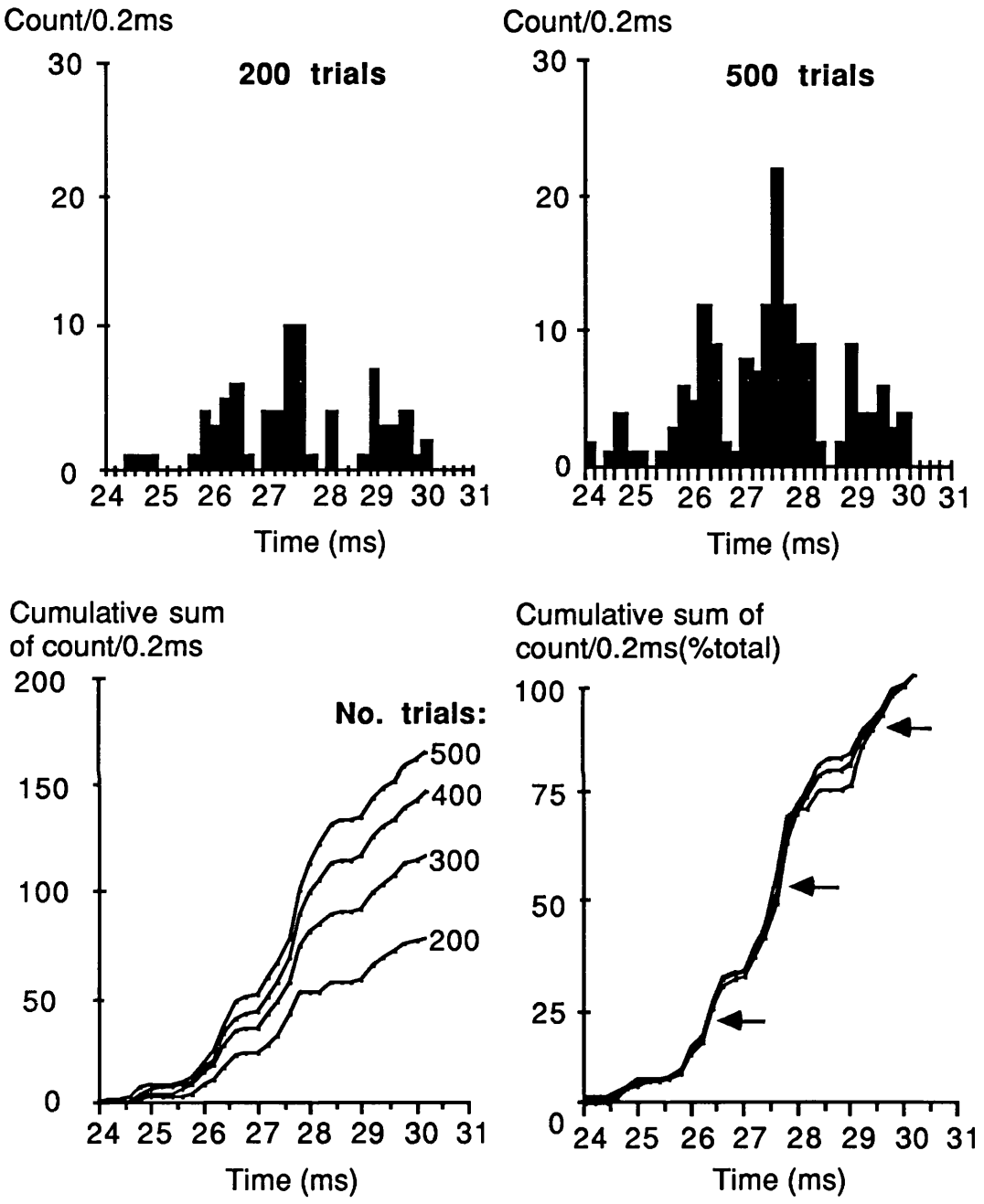


Fig. 41. The effect on sub-peak dispersion of increasing the number of trials. PSTHs of the PP region at 200 trials (A) and 500 trials (B) of a random stimulation experiment. The smaller height and duration of sub-peaks in A are reflected in the height and slope of the corresponding cumulative sums seen for 200, 300, 400, and 500 trials (C). D: the curves become superimposed when each cumulative sum from C is normalised to the maximum number of discharges within PP. This particularly applies to the positions of maximum slope, corresponding to the modes of each sub-peak, as indicated by the arrows.

Such temporal effects produced by noise have been studied directly in the cat, the details of which may be considered with reference to the findings of the present study. The demonstration that the shape of the histogram peak was related to the derivative of the synaptic potential by Fetz and Gustafsson (1983) (see Introduction), came from experiments which were performed with often large EPSPs under conditions of fairly low levels of synaptic noise. Both these factors may have a significant effect on the shape of the PP (Kirkwood, 1979). In their original study, Fetz and Gustafsson observed that smaller EPSPs (closer to the noise level) often produced histogram peaks that were significantly broader than the derivative. In order to examine further the possibility that EPSPs could be described by a combination of the derivative and the potential itself (Kirkwood and Sears, 1978, 1982b) Gustafsson and McCrae (1984) reported their study of small EPSPs and the influence of synaptic noise on the shape of the PP. EPSPs were evoked by brief triangular stretches of the triceps surae-plantaris muscles, during repetitive firing maintained by injection of depolarising current in cat spinal motoneurones. EPSPs ranged from 30 to 1040 μV with rise times ranging from 1.1-8.2 ms, with synaptic noise estimated to fluctuate from 1.5-3.5 mV (peak to peak), generated by keeping the muscle stretched to a near maximal degree. Other EPSPs were also recorded with a low level of synaptic noise. It was found that the height of the histogram peak with respect to the baseline firing rate increased in proportion to both the amplitude and rising slope of the EPSPs (see effect of stimulus intensity, below). The shape of the histogram peak and the subsequent trough in the PSTH was well described by a linear combination of the shape of the EPSP derivative and that of the EPSP itself. It was confirmed that large EPSPs (greater than 400 μV) in conditions of low background noise generated PPs resembling the derivative of the EPSPs. In addition, it was found that smaller EPSPs (100-300μV), in conditions of

high background noise (1.5-3.5 mV peak to peak) produced PPs that often deviated significantly from the derivative. This deviation could largely be accounted for by the addition to the derivative of a term proportional to the shape of the EPSP itself. More elaborate combinations than linear ones were not tested, and it appeared likely that a better match could be obtained using an alternative to the linear combination and it was shown that the shape of EPSPs could be obtained through leaky integration of the PSTH. One of the factors that would influence the parameters of such a circuit would include the slopes of the interspike voltage trajectories, as discussed above and illustrated in Fig. 40. It was pointed out that the relative EPSP component to the PSTH may be a function of the slope of the interspike voltage trajectory, with steeper slopes being less shadowed by the EPSP, producing smaller EPSP components.

Without such recourse to intracellular recording from spinal motoneurones the influence of temporal dispersion in the present study places the following simple limits on the study of EPSPs with the present results: firstly, the rise time of EPSPs would be less than the inter-modal sub-peak intervals (which were found to be 0.6 to 2.4 ms for FDI and 1.4 (biceps) to 3.9 ms (brachioradialis) in other arm muscles) and secondly, the maximum slope of the EPSP would be greater than the maximum slopes on the cumulative sums (Fig. 42).

Stimulus Intensity

The unitary excitatory contribution of a single CMN fibre is in the region of 100 μV or less in amplitude (Asanuma et al., 1979) which is similar to the amplitude of minimal EPSPs generated by graded stimulation of the cortex

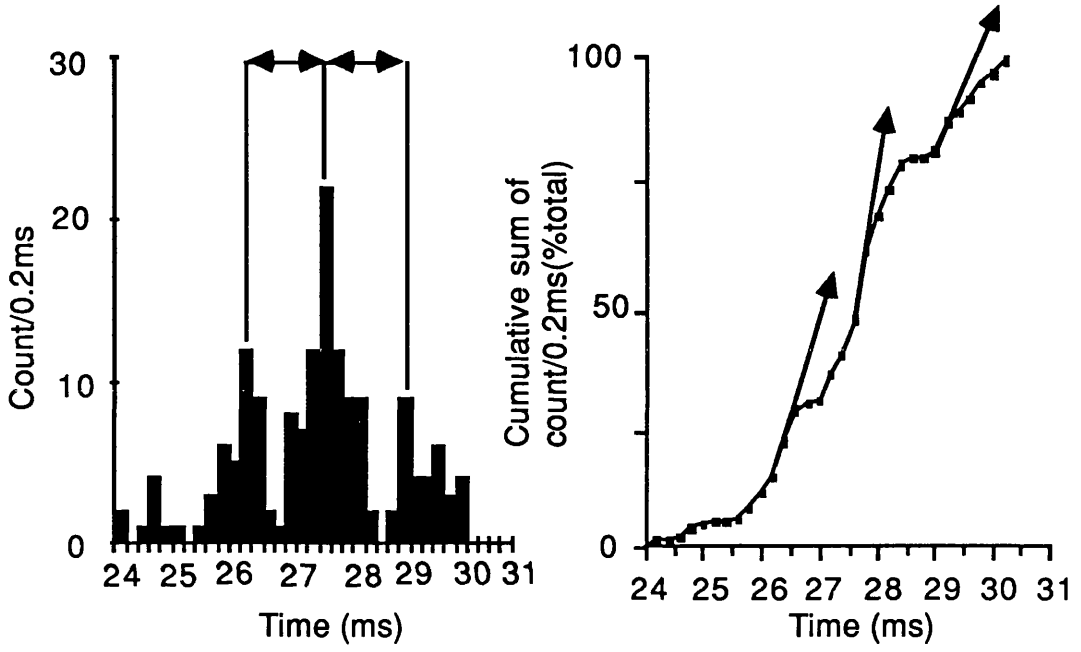


Fig. 42. Limits on the estimation of the expected shape of EPSPs conferred by temporal dispersion of sub-peaks. A: the rise time of EPSPs will be less than the intermodal sub-peak intervals. B: the maximum slope of each phase of the EPSP would be greater than the maximum slopes on the corresponding parts of the cumulative sum (arrows).

or the PT (Porter, 1985). When the current delivered to colonies of corticospinal neurones in the cortex of a monkey is increased using an exploring focal anode, a minimal EPSP may be evoked in a target motoneurone. As the current is increased further, the EPSP grows to a maximum amplitude indicating that more than one PTN projects to the target motoneurone (Phillips and Porter, 1964). The shape of the growth curve varies between different motoneurones. The amplitude of the EPSP could increase, for example, from zero to 0.5-2.0 mV for different motoneurones of the ulnar nerve in the monkey as the stimulus intensity was increased from 1 to 3 mA.

Increasing the intensity of TMS may similarly increase the amplitude and rise time of EPSPs by recruiting an increased number of PTNs in a colony (a colony is defined as all those PTNs making a monosynaptic connection with a single spinal motoneurone - Landgren et al., 1962b). This would increase the probability of motoneurone discharge, accounting for the observed increase in the proportion of PP-trials. The growth in the relative height of PP with increasing stimulus strength might be expected, therefore, to be proportional to the increase in the amplitude of the underlying EPSP. In cat spinal motoneurones, the primary peak maxima may increase with EPSP amplitude by 2.4% per μV relative to baseline (Fetz and Gustafsson, 1983). It was estimated that this would correspond to an estimated increase of 0.8% per μV for the mean height of the peaks. (A PP with a mean height of just 1% over the baseline in this experiment therefore, would correspond to an equivalent EPSP of 1.3 μV.) With single pulse intracortical stimuli the authors predicted an increase in EPSP amplitude with increasing stimulus intensity of 22.5 - 36.1 μV per μA. [This differs from the gradients found in the cervical motoneurones of the baboon with increasing stimulation, of 0.4 to 1.5 mV per mA (Phillips and Porter, 1964), a

difference which may be related to the more effective recruitment of corticospinal cells by intracortical stimulation (Stoney, Thompson and Asanuma, 1968)]. The height of PP obtained in the present study, however, and the observed time dependent changes in post-spike excitability, would suggest much larger EPSPs, of the order of mV (see below).

Effect of Stimulus Intensity on Sub-peaks

In 2 of 5 FDI MUs, the observation that increasing the intensity of stimulation produced new sub-peaks that were earlier in latency would suggest that increasing the stimulus intensity had increased the rate of rise of the underlying EPSPs so that the membrane potential was brought to threshold more swiftly. The absence of change in the slope of cumulative sums of the PP region, however, fails to support this hypothesis. It is more likely, therefore, that an increase in stimulus intensity produced an increase in the amplitude of EPSPs, which consequently brought the motoneurone to firing threshold on an earlier I wave (at the I2 latency rather than the I3 latency, for example). Reduction in firing latency may also have been augmented by any reduction in the latency of I waves: With increasing stimulus strength in the monkey, individual I waves were found to shorten in latency by 0.2-0.5 ms, although on some occasions the latencies remained constant (Kernell and Wu Chien-Ping, 1967a).

The finding that, in one MU in a spike triggered experiment additional later sub-peaks were produced by an increase in stimulus intensity may also be attributed to an increase in the amplitude of the EPSP, bringing the membrane potential to threshold during the course of relatively long ISIs - during which the membrane potential would follow a deeper scoop (hyperpolarisation) - with a consequent delay in the discharge time. It is also noted that this effect cannot be explained by any attendant decrease in

the minimum required spike-stimulus interval (a reduction in which was found to be associated with later firings within PP-see below) as this variable was made constant by spike triggered stimulation.

The present observations, all made with an anticlockwise inducing current, do not appear to support Phillip's (1986) alternative to the intra-cortical loop hypothesis of Jankowska et al. (1975a) for the repetitive discharge of corticospinal neurones in response to a single cortical stimulus. Phillips' hypothesis was described for epicortical anodal stimuli, but may also possibly apply to a clockwise inducing current (Day et al., 1989, Day et al., 1990). It was suggested by Phillips that a large thalamo-cortical volley produced in subcortical white matter synchronously with the D wave, produced a monosynaptic EPSP simultaneously in a large number of corticospinal neurones. Given that a cortical motor neurone may respond repetitively to a depolarising current at a frequency related to its intensity, and that the upper limit of this frequency (approximately 1000 impulses/sec) is set by the refractory period, the rising phase of this EPSP would produce a succession of I waves. In such circumstances, the strength of the stimulus may be expected to alter the rise time of the EPSP, which would then produce a discharge in the corticospinal neurone on crossing threshold at an interval which was greater than its refractory period. Alterations in the rise time of the EPSP with respect to the refractory period of the corticospinal neurone could produce varying combinations of successive I waves. Using data from Kernell and Wu Chien-Ping (1967a) it was postulated that on increasing the intensity of stimulation this may account for the appearance of an initial D wave, then accompanied by an I2 wave; an I2 and I3 wave; and subsequentley I1, I2, and I3 waves. With an anticlockwise inducing current (as also used in the present study) Day et al. (1989; 1990) observed sub-peaks designated P1 and P3 in the FDI of the

right hand, possibly caused by I1 and I3 respectively. Increasing the intensity of stimulation in the present study, however, never produced sub-peaks in healthy subjects with correspondingly long inter-modal intervals, as would be expected with the mechanism proposed above. (Unusually long intermodal sub-peak intervals in patients with MS, however, may possibly be caused by a different mechanism, as described below).

How can the Intensity of a TM Stimulus be Standardised?

The product of stimulus duration and amplitude, determined by the square root of the capacitor energy, is proportional to the stimulation capability of a magnetic stimulator. Subsequent current flow in the brain depends upon how much of the magnetic field is intercepted. The strength of a TM stimulus therefore depends on peak magnetic field and rise time, the coil geometry, the local anatomy and the neural threshold. In practice, standardisation of the stimulus requires the specification of the magnitude, rise time, duration, orientation and direction of the inducing current or applied magnetic field.

The heterogeneity of a volume conductor such as the brain precludes the calculation of local current density by dividing the local electric field by a specific resistivity, as can be done for a homogeneous medium. The resistivity has a wide spatial variation and is time-dependent. In addition, the current return paths are affected by the boundary geometry-for example, skull, CSF, falx cerebri or the bony foramina of the vertebral column.

Nervous tissue is excited according to the strength-duration curve, such that the shorter is the duration of the current pulse, the higher the magnitude of induced current required for excitation (Lapicque, 1909). In achieving excitation, it is necessary to exceed the required induced current density,

which is estimated to be in the region of 1.5 mA/cm^2 for long-duration pulses (Geddes and Bourland, 1990). For a short pulse of less than 1 ms, as used in TMS, the required current density will be much higher than this value. The ability of a stimulating coil to induce a current in a volume conductor can be evaluated in vitro by a bipolar electrode in a container of saline solution beneath which the excitation coil is placed (Geddes and Bourland, 1990). In the future, stimulus intensity in vivo might be standardised in terms of derived neural time constants (Barker et al., 1990): with surface electrodes over ADM, it was confirmed that threshold stimulation could be achieved with less stored energy within the stimulator (Novametrix-as used in this study) if a fast rise time was used. Modelling the nerve membrane as a resistor and capacitor in parallel, the rate of change of magnetic flux (proportional to the induced electric field) was detected and the effect of the time constant on the stored energy required to depolarise the nerve membrane was shown for different time constants. It was found that the time constant was about 150 μs for both ulnar nerve and the cortex. By integrating the induced electric field with the derived neural time constant (150 μs) a measure of the stimulus intensity could also be produced, in proportion to the voltage across the neural membrane. Its peak would take into account wave form, coil geometry and peak field, assuming that the body approximated to a homogeneous conducting medium. The symbol Et150 was suggested, in units of Vsec/m. Examples of 0.019Vsec/m and 0.015Vsec/m were quoted, when measured 12 mm from the surface of coils of 140 mm (as used in the present study) and 71 mm outside diameter, respectively.

Firing Rate

Within the limits tested, neither the duration of the pre-stimulus ISI nor the mean firing rate in random stimulation experiments was found to

significantly influence the probability of evoking a PP discharge. This has implications for the interpretation from serial changes in ISI of the advancement or delay of discharges following a stimulus (see below).

The further observation that PP-trials sometimes occurred in a non-random order suggests that the occurrence of runs of PP-trials (or non PP-trials) may be deterministic in origin (4 or more consecutive PP-trials was termed a run of PP-trials). Fortuitous changes in the instantaneous firing rate and an index of voluntary activation (provided by the total number of MU spikes from MUs recruited within the pick up of the electrode), however, were not found to be related to the occurrence of runs of PP-trials. The serial occurrence runs of favourably short (see below) spike-stimulus intervals, however, did seem to occur. This might be attributed to chance fluctuations in firing rate with respect to the interval between successive TM stimuli during the course experiment, combined with the effect of refractoriness in the motoneurone. The latter tends to produce a periodicity in the pattern of discharge equal to the mean ISI of the original spike train (Davey et al., 1986).

Interaction between MUs

Using the cross-sorting method in a pair of MUs which had PPs with different latencies, no evidence was found for changes in the firing probability before the stimulus that seemed to affect the probability of evoking a PP discharge in the fellow MU. It was found, however, that at the stimulus intensity used (42%), PP discharges never occurred in both MUs within the same trial. Inspection of the raw data confirmed that this finding could not be explained in terms of the rejection of compound responses at the PP latency. It is unlikely that such a finding could be explained by consistent differences in the membrane trajectory of each motoneurone.

Indeed the tonic activity of such motoneurones would be expected show a tendency towards synchronisation.

If the first descending volley arrived at both cell bodies simultaneously, then the 2 ms difference in the PP onset latency for these 2 MUs could either be due to differences in the time required for the motoneurones to be brought to threshold, or a difference in the peripheral conduction time. (These variables may be related to differences in the firing threshold and size of the two motoneurones.) The observation that PP discharges never occurred in both MUs within the same trial may be attributed to a short latency inhibitory mechanism, such as Renshaw cell inhibition, although this is unlikely given that the discharge of the MU with the longer PP latency was never associated with discharge of the other MU (this assumes that the difference in PP latency was due to differences in the time for the cell body to be brought to threshold so that one cell body consistently fired 2 ms after the other). An alternative possibility is the operation of a gating process - perhaps involving inhibition of PTNs projecting to one MU by the recurrent collaterals from PTNs projecting to another MU in the same muscle.

Effect of the Spike-Stimulus Interval

The excitability of rhythmically firing human spinal motoneurones was determined by relating the spike-stimulus interval to the probability of evoking a PP discharge. This will reflect the trajectory of the membrane potential, modified by the effects of changes in the membrane conductance responsible for hyperpolarisation, voltage dependent changes in the amplitude of EPSPs and variations in the firing threshold during the ISI. The observed inverse correlation between the duration of the spike-stimulus interval and the latency of PP discharges is also consistent with the summation of an EPSP with the ramp phase of the inter-spike membrane

trajectory, whereby the take-off point for the EPSP occurred at a greater distance from the firing threshold the shorter was the spike-stimulus interval. The discharge latency would be consequently longer with such short intervals.

The probability of a spinal motoneurone firing in response to a TM stimulus will depend upon the proximity of its membrane potential to firing threshold and its membrane conductance. The higher the conductance, the greater the difficulty in displacing the membrane potential towards firing threshold. Both these factors are a function of segmental inputs (excitatory inputs - group Ia and II spindle fibres); inhibitory segmental inputs (especially the group Ib Golgi tendon organ fibres); suprasegmental inputs (including corticospinal, reticulospinal, and rubrospinal tracts); and the level of presynaptic inhibition of any of these inputs. In random stimulation experiments, the intervals between stimuli and preceding voluntary discharges were shown to be randomly distributed over an interval ranging from zero up to the minimum ISI for that particular motoneurone. During this period, however, there was a low probability of evoking a PP discharge if the stimulus was delivered within the initial 30 ms following the preceding voluntary discharge. At later stages of the ISI, however, PP discharges occurred with progressively greater probability. In these experiments, EPSPs were effectively used to probe the excitability of the index motoneurone. Assuming a linear summation of the EPSP and the membrane potential, and that the former is extinguished by the subsequent spike (Brock et al., 1952; Coombs et al., 1955a), then the probability of evoking a discharge following the application of the TM stimulus depends upon the effective difference between the motoneurone's membrane potential and its firing threshold. The firing threshold of single spinal motoneurones in the cat falls during the early part of the ISI and rises

during the latter half (Calvin, 1974). Membrane conductances, responsible for AHP, are also subject to change during the ISI, being maximal immediately after a discharge and reducing thereafter (Shwindt and Calvin 1973; Mauritz et al 1974). The voltage dependent alterations in membrane conductance are probably overwhelmed by the AHP immediately after a discharge (Nelson and Frank, 1967; Baldissera and Gustaffson, 1974a). The amplitude of the EPSP may itself be subject to alterations during the course of the ISI (see Introduction). Membrane depolarisation reduces the amplitude of EPSPs, although there may be relatively little change in the sub-threshold region (Coombs et al., 1955b; Werman and Carlen, 1976; Edwards et al., 1976).

The above factors, in combination with the membrane trajectory, determine the overall excitability of the index motoneurone and hence the probability of evoking a discharge following TMS. The non-trajectory effects, however, become relatively small at late stages of the ISI. It may be argued, therefore, that the gradient of the late portion in the excitability curve obtained from these experiments may approximate to the gradient of the linear ramp phase of the underlying membrane trajectory (see also Ashby and Zilm, 1982a). One method of testing this would be to obtain an excitability curve at 2 or more stimulus intensities. The late phase of the curve, if reflecting the constant gradient of the ramp phase of the membrane trajectory, would be expected to remain constant.

By assuming, however, that the height above the excitability curve (solid arrow Fig. 43) indicates the difference between a cell's membrane potential and its firing threshold, the amplitude of the EPSPs associated with a given firing probability in a single spinal motoneurone can be estimated. A firing probability of 50% PP-trials was selected here in order to provide a notional

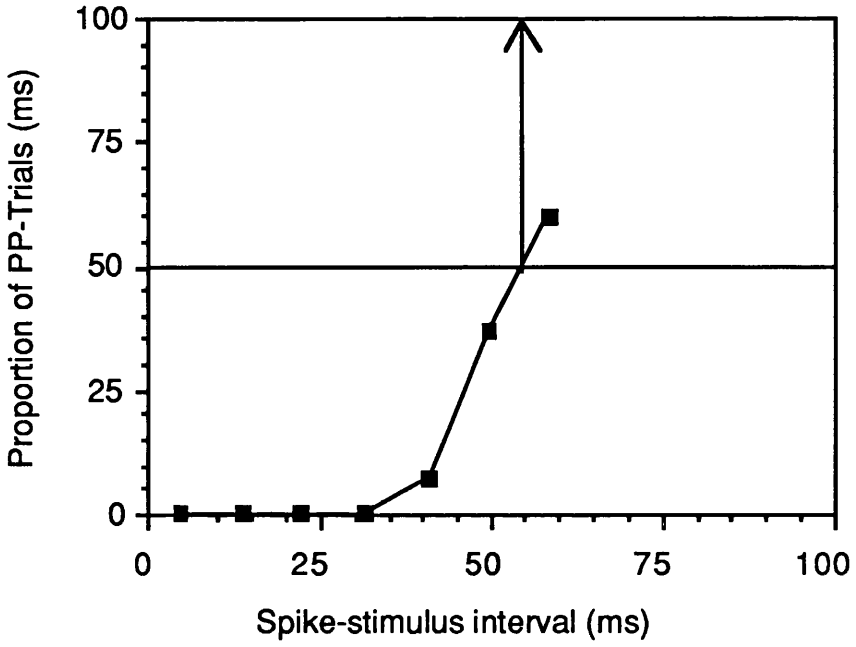


Fig. 43. Estimation of EPSP amplitude from the curve relating spike - stimulus interval to the proportion of PP-trials. If the height above the curve (solid arrow) indicates the difference between the cell's membrane potential and its firing threshold, then the amplitude of the EPSP associated with a given firing probability for this motoneurone can be estimated (50% PP-trials is selected here as a notional threshold). For a firing probability of 50% PP-trials, the corresponding spike-stimulus interval (54ms) was 49% of this cell's mean ISI, indicating an estimated EPSP amplitude of (100-49) = 51% of the maximum hyperpolarisation (eg. 10mV) = 5.1mV.

threshold value for these experiment (see below). For a firing probability of 50% PP-trials, the corresponding spike-stimulus interval (54ms, Fig. 43) was 49% of this cell's mean ISI, indicating an estimated EPSP amplitude of (100-49) = 51% of the maximum hyperpolarisation (eg. 10mV) = 5.1mV.

This is a relatively large EPSP and provides an indication of the effectiveness of a TM stimulus. Rather than the maximal EPSP amplitude, such a calculation would probably approximate to the median EPSP amplitude, with half the EPSPs exceeding this value and the other half falling below it. It has been estimated that compound EPSPs of 2-5 mV in amplitude produced by the activation of a whole colony of CMN cells (i.e. all contributing excitatory synapses to one spinal motoneurone) by the electrical stimulation of the exposed cortex in the non human primate, indicates the convergence of at least 50-100 CMN axons on to each spinal motoneurone (Phillips and Porter, 1977). Findings in the present study suggest that the amplitude of EPSPs may be of the same order of magnitude, indicating the convergence of a similar number of excitatory axons on to the motoneurone.

With electrically evoked Ia EPSPs in tonically firing human FDI MUs, it was found that only afferent volleys arriving 70 ms or later after a preceding spike were capable of evoking a discharge (Buller et al., 1980). Adopting assumptions similar to those above, it was estimated that EPSPs were of the order of 3 mV in amplitude.

Findings in the present study are also comparable to the maximal amplitude of EPSPs produced in intrinsic hand muscles following TMS in other studies (Day et al., 1989) in which a maximal amplitude of 5 mV for EPSPs following TMS was estimated from the maximal overall proportion of

PP-trials produced in an experiment, rather than by using a membrane trajectory model. This would therefore tend to produce larger estimates of EPSP amplitude by grouping all ISIs together, which would include those longer than the minimum ISI which would be expected to have deep scoops in membrane trajectory and hence require relatively large EPSPs to bring the cell to firing threshold.

The measurements of threshold expressed as a percentage of the maximum output of the stimulator, obtained from relaxed MUs, was found to be greater than that for voluntarily activated MUs. In addition to specific factors including intracranial flux density and the threshold of motor cortical cells, the minimum stimulus intensity required to initiate a PP discharge from a single relaxed MU should also reflect the resting membrane potential of the motoneurone. It may be inferred that, as expected, the latter is probably more polarised (negative) than the inter-spike membrane potential of the same cell when it is firing rhythmically.

Factors in the Analysis of Spike-Stimulus Intervals

In the analysis of this data in repetitively firing motoneurones there are several considerations:

1) The End Point of Each Curve

The proportion of PP-trials calculated for relatively long spike-stimulus intervals would apply only to correspondingly long ISIs. At spike-stimulus intervals greater than the minimum ISI, therefore, the data would be biased towards long ISIs. Consequently the end point for each curve was set at less than the minimum ISI (see Results).

At relatively long spike-stimulus intervals which exceed the minimum ISI this bias can be compensated for, however, by calculating the median ISI to which each pair of data points (spike-stimulus interval and proportion of PP-trials) corresponds. These 3 variables for a random stimulation experiment of 500 trials are shown in Fig. 23 (Results). The proportion of PP-trials was seen to decrease with increasing ISI (over a range of 120-140 ms) despite the increasing spike-stimulus interval. This may illustrate the importance of voluntary activation in enabling the stimulus to produce a PP discharge. The observed decrease in the proportion of PP-trials may also, however, have been due to the effect of trials with discharges that occur between the stimulus and the onset of PP [see 3) below] which tends to reduce the observed proportion of PP-trials. In addition to this influence on the gradient of the curve, the absolute value of each point for the proportion of PP trials on the vertical axis would be reduced by a constant relating to the small number of discharges that may occur at the PP latency by chance alone [see 3) below]. This would not, however, alter the gradient of the curve, but only its height. The natural constraints of this technique therefore preclude the direct assessment of the relative excitability of single motoneurones at long ISIs in this way.

2) CMC Time

Using surface electrodes in normal subjects, the estimated CMC time is 5.7 ms (Hess et al., 1987). Assuming an additional monosynaptic delay of 1 ms at the spinal motoneurone, the initial 6.7 ms following the application of a TM stimulus is occupied by the time take for a volley to descend from the stimulation site to the postsynaptic membrane of the motoneurone. At the threshold stimulus intensity for producing a just discernible CMAP, those MUs with the lowest firing threshold, similar to the MUs studied here, would probably be selectively recruited. This delay therefore probably occupies at

least the initial 6.7 ms of the curves in Figs 22, 23B, 37 (Results) and Fig. 43.

3) Influences on the Proportion of PP-Trials.

In addition to the excitability of the motoneurone, the proportion of PP-trials is influenced by two factors. Firstly, some discharges may have occurred within the PP region of the PSTH by chance. Compensation for this effect in the analysis would therefore have the effect of reducing the proportion of PP-trials for each motoneurone by a constant amount, thus slightly reducing the height of each excitability curve with respect to the vertical axis. Secondly, the effect of trials with discharges that occur in the interval between the stimulus and the PP latency would have the effect of reducing the apparent proportion of PP-trials for the final 20-22 ms of each curve (the effect terminating at the minimum ISI). Compensation for this effect in the analysis would involve increasing the proportion of PP-trials for the final two data points in each curve by an appropriate amount according to the number of such trials when expressed as a proportion of the total number of trials in the experiment. This, in turn, would have the effect of increasing the gradient of the final stages of each curve. Despite this, however, the final stages of each curve are steeper than the initial stages, indicating real time dependent changes in excitability, the physiological mechanisms of which are described above. Notable exceptions are 2 MUs (Fig. 22, Results) in which it was also found that the highest proportion of trials containing discharges between the stimulus and the PP latency had also occurred (26 and 35 %). The effect of such trials may therefore explain why the final stages of these two curves exhibit a negative slope.

Threshold for Activating Single MUs

The above discussion demonstrates the difficulty in interpreting the absolute values of excitability in terms of the underlying membrane potential and of the amplitude of EPSPs. The data is clearest in its raw form as shown in the excitability curve of Fig. 43. In this example, no PP-trials occur at spike-stimulus intervals less than 30 ms. This principle can be extended to obtaining a notional threshold, as used above, which may be defined as the spike-stimulus interval (normalised to the mean ISI) corresponding to 50% of PP-trials. This would be the interval at which delivery of stimuli had a 50% chance of evoking a PP discharge.

Late Changes in Firing Probability

Late Changes in Firing Probability Produced by Peripheral Stimuli

Late changes in firing probability have been reported to include periods of decreased and/or increased firing probability. In their computer model of a rhythmically discharging neurone (Ashby and Zilm, 1982a), the period of decreased firing probability following a peak in the PSTH was found to be proportional to the amplitude of the EPSP and not to the duration of its falling phase. (It was noted, however, that for real motoneurones this period may be affected by late EPSPs or IPSPs evoked by the stimulus.) In contrast, periods of increased firing probability may be caused by a periodicity effect (Moore et al., 1970) where the stimulus has entrained the rhythmically firing motoneurone to produce periodic fluctuations of impulse density occurring at multiples of the mean ISI (Ashby and Labelle, 1977). Additional causes of late peaks in the PSTHs of motoneurones (Ashby and Zilm, 1982b) may potentially include monosynaptic Ia EPSPs; polysynaptic Ia EPSPs (Watt et al., 1976); IPSPs (Fetz et al., 1979); Ib inhibitory activity (Watt et al., 1976) or artifactual effects produced by the bimodal reflex latency in human spinal motoneurones (Trontelj, 1973).

In a further study, brief mechanical pulses applied to the belly of FDI produced a short latency peak in the PSTH from both the first and second dorsal interosseous muscles (Buller et al., 1980). This was followed by a period of decreased firing probability lasting 80 to 100 ms. At a latency of about 70 ms there was a second increase in firing probability superimposed on this period of reduced firing probability. When a tap was delivered to the tip of the nail of the index finger, a similar pattern resulted, with a peak that started at 39 ms after the tap in one example and reaching a maximum 7 ms later, with a subsequent period of decreased firing

probability with a minimum at about 58 ms, terminated by a more prolonged period of increased firing probability with a maximum at 76 ms. There was a subsequent prolonged period of reduced firing probability with superimposed slow fluctuations of firing which return to the control level at about 300 ms after the stimulus (Stevens and Usherwood, 1976; Stevens et al., 1976). The first peak in the PSTH was interpreted as a monosynaptic spinal excitatory response and the second period of excitation as a supraspinal mechanism (Caccia et al., 1973; Godaux and Desmedt, 1975). Late events in the PSTH therefore included periods of both increased and decreased firing probability. The period of decreased firing probability may also have been influenced by the inhibitory effects of an afferent volley (Ashby and Labelle, 1977) or summation of the AHP secondary to the earlier occurrence of the spike produced by the EPSP (Ito and Oshima, 1962).

Late changes in firing probability were also observed in the present study, in response to TMS. These findings will now be discussed.

The Origin of SP

A rise in firing probability, such as SP, may be due to the rising phase of an EPSP (Knox and Poppele, 1977) or to the decay of an inhibitory postsynaptic potential (IPSP) (Fetz and Gustafsson, 1983) or a combination of both. EPSPs and IPSPs are both detected in spinal motoneurones of the monkey following surface and intracortical electrical stimulation of the motor cortex (see Introduction). The effect on the ISI of magnetic stimuli when compared with simulated control stimuli suggests that SP and PP discharges occur earlier than expected (Fig. 25, Results). The relationship

between stimulus-spike interval (discharge latency) and spike-stimulus interval (interval between the stimulus and the preceding voluntary discharge) for simulated stimuli that were introduced during the pre-stimulus period produced a distribution with an equal number of points about a line representing the median pre-stimulus ISI (Fig. 25C). Any points to the left of the line were derived from ISIs that were shorter than the median pre-stimulus ISI and vice versa. The equivalent graph for magnetic stimuli, however, shows two clusters corresponding to SP and PP (Fig. 25B). In contrast to the control, there was an unequal distribution in the number of points about the median pre-stimulus ISI. Within the cluster corresponding to SP, there was an increase in the number of points to the left of the median line and a decrease in the number of points to the right, suggesting an active change in the expected latency of SP discharges in response to magnetic stimuli, rather than a passive redistribution of data points. These findings are consistent with the operation of the rising phase of an EPSP.

The relatively long duration of SP suggests the operation of an EPSP with a correspondingly long rise time. This would indicate temporal dispersion of the excitatory inputs to the spinal motoneurone, which may be due either to the time course of the event that generates SP (asynchronous recruitment of cortical neurones or muscle spindle acceleration, for example) or transmission via a group of fibres with varying conduction velocities. Such effects may have also been augmented by the variability of the excitability of the motoneurone at relatively early stages of the ISI (where the putative EPSP for SP would characteristically summate with the membrane trajectory) under the influence of complex changes in the membrane conductance responsible for hyperpolarisation (Schwindt and Calvin, 1973). The timing of such an EPSP with respect to the membrane trajectory

and its inferred rise time suggest how the interval histogram (INTH) may influence the shape of SP and also the shape of PP (Fig. 44).

The Decrease in Firing Probability After PP

The lack of correlation found between the duration of the period of reduced firing probability that follows PP and either the proportion of PP-trials, or the height of PP divided by number of trials, suggests that this period is not only the result of the simple decrease in firing probability produced by the excitatory effects of an EPSP, which advances the latency of discharges occurring within PP. Computer modelling of a simple EPSP predicts such a correlation (Ashby and Zilm, 1982a), which suggests that other mechanisms - for example, the operation of an IPSP or another EPSP - may contribute to the duration of this period of reduced firing probability.

Pathways Mediating SP

Possible pathways for SP include a reflex input from the periphery (from the evoked discharge of MUs other than the index MU) transmitted via a segmental or suprasegmental pathway; an input from another corticospinal pathway which either conducts more slowly than the pathway mediating PP and/or involves more synapses, or, collateral activation of gamma motoneurones by the initial descending volley(s) and subsequent firing of the motoneurone via Ia inputs.

If a peripheral afferent component in the pathway for SP is postulated, then SP might be comparable to the secondary rise in firing probability that occurred in FDI MUs in response to mechanical pulses applied to the index finger (or to the belly of the muscle) in this and other studies (Stevens et al., 1976; Buller et al., 1980). The interval between PP and SP would then correspond to the conduction time from muscle - to spinal cord - to muscle.

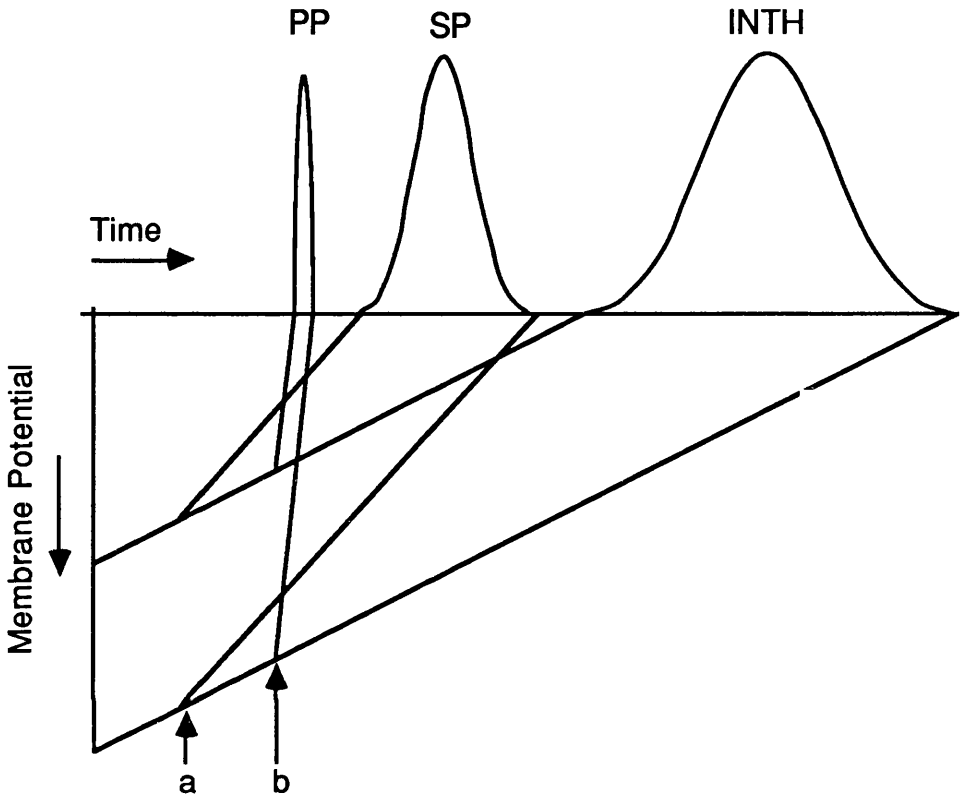


Fig. 44. Factors influencing the shape of PP and SP. Diagramatic representation of the interspike membrane trajectory for a rhythmically firing motoneurone over a range of ISIs (as for Fig. 40) with onset times for EPSPs underlying PP and SP represented by arrows at a and b, respectively. The shape of the interval histogram (INTH) probably influences the shape of SP and PP. See Discussion for assumptions regarding the rise time of EPSPs. Peaks are not drawn to scale.

This is consistent with the gradient of the line relating SP peak latency to distance from the cord, which is almost 3 times that relating PP peak latency to distance (Fig. 28, Results). [This assumes that the nerve fibres involved conduct at approximately the same velocity to both proximal and distal muscles and does not take into account any small differences in central motor conduction time to muscles innervated from different spinal segments. The peak (modal) latency of SP was plotted rather than the onset latency as the latter is potentially altered by the height of PP (see Fig. 9, Results). The peak latency probably corresponds to the point of maximum slope of the EPSP which, if motoneurones serving different arm muscles exhibit EPSPs with similar time courses, would occur at a constant interval after the onset of the EPSP]. Further, in the 2 MUs that received both TMS and peripheral mechanical stimulation, the intermodal PP-SP interval (43 and 62 ms) was 8-21 ms longer than the modal latency of the first peak that occurred in response to peripheral mechanical stimulation in the same MUs (35 and 41 ms) and 10-36 ms longer than the interval between the first and second peaks (33 and 26 ms) that occurred in response to peripheral mechanical stimulation. If the PP-SP intervals had been shorter than the above data obtained from mechanical stimulation in the same MU, this would not have supported a peripheral afferent component in the pathway for SP.

Further studies may enable SP to be attributed to either excitation of Ia afferents from the agonist muscle by gamma motoneurone discharge; excitation of Ib afferents from a contraction of the antagonist; or defacilitation of Ib afferents from the agonist. Whatever the mechanism, gross movement may not be required, however, as suggested by the random stimulation experiment on FDI in which clamping of the finger to prevent movement had no obvious effect on the production of SP.

Whether SP is produced by the operation of a slowly conducting descending pathway and/or by a peripheral mechanism, remains to be determined. A future challenge is to provide further evidence for the pathway or pathways mediating SP.

Statistical Comparison of Interspike Intervals : Implications for Future Studies

Validity of Test Strategy

The development of an approach to this problem will now be discussed, using the Student's t test to demonstrate certain principles, and concluding with an experiment designed to circumvent potential problems.

The nature of random stimulation experiments creates certain conditions for the application of statistical methods for comparing pre- and peri-stimulus ISIs. It is not possible to draw inferences regarding the significance of apparent excitation or suppression of repetitively firing single MUs, however, without statistical analysis. In their study on TES, for example, Calancie et al (1987) used only a small number of trials (20), comparing pre- and peri-stimulus ISIs without recourse to statistics, which therefore failed to provide evidence of the significance of apparent serial changes.

Limited Validity for Paired Comparisons of Serial ISIs

The first limitation for the application of statistical methods for comparing pre- and peri-stimulus ISIs in random stimulation experiments is the presence of length bias sampling in the selection of peri-stimulus ISIs. This dictates that peri-stimulus ISIs will tend to be longer than the mean ISI, due to the higher probability of the stimulus falling within a long ISI than within a short ISI. This therefore produces a bias towards long peri-stimulus ISIs, in a way that is theoretically predictable from the INTH (if necessary, therefore, this bias could be corrected by weighting the frequency of peri-stimulus ISIs according to the INTH). The consistent finding that the mean peri-stimulus ISI was longer than the pre-stimulus ISI, from experiments in which PP had been lacking (see Results), may therefore indicate either the operation of

length biased sampling, or the operation of a physiological process that delayed the appearance of the next spike, or a combination of the two processes.

A second difficulty would have arisen from serial dependency of ISIs (see Introduction) in the presence of which the use of a t test for the comparison of pre- and peri-stimulus ISIs (Tuckwell, 1989) would not be valid. Serial correlation may exist between successive ISIs in a repetitively firing MU, with a coefficient that is negative, usually about -0.2 for most human MUs (Andreassen and Rosenfalck, 1980). Short and long ISIs therefore tend to alternate. (In spasticity the serial correlation coefficient becomes positive, so that a long ISI is usually followed by another long ISI and a short ISI by a short). The parameters, SD and serial correlation coefficient, however, are sensitive to trends in firing frequency. To reduce the effects of these trends a floating mean interval (Andreassen and Rosenfalck, 1980) averaged over 19 ISIs, can be used to produce the floating serial correlation coefficient (FRHO). In the present study, which analyses epochs of 500 ms rather than a long series of ISIs, a non-floating RHO was therefore used.

In random stimulation experiments, however, only 1 out of 16 MUs had a significant degree of correlation between the pre-stimulus ISI and the preceding ISI (RHO = -0.26, $p<0.01$). The remaining 15 MUs showed no significant serial correlation when measured with the non floating correlation coefficient. The lack of serial correlation may possibly have been a consequence of the experimental method, in which subjects were asked to recruit a low threshold MU and maintain its activity without controlling its firing rate.

An EPSP would be expected to advance the occurrence of discharges and the decay of an IPSP would be expected to produce a delay (Fetz and Gustafsson, 1983). If a Student's t test was used, however, a result indicating that the population mean for the peri-stimulus ISI sample was significantly longer than that for the pre-stimulus ISI sample may be due to the operation of length bias sampling, rather than the occurrence of discharges following the stimulus at times later than expected. In the converse situation, however, in which the mean peri-stimulus ISI was found to be shorter than expected, this might infer a genuine change, as it is opposite to that which would be expected to result from length bias sampling. In random stimulation experiments this was found, for example, in 8 of 10 MUs from FDI and 13 of 14 MUs from other arm muscles for PP-trials, and 2 of 10 MUs from FDI and 6 of 9 MUs from other arm muscles for SP-trials (see also Fig. 25, Results).

Interpretation of Apparent Shortening of the Peri-Stimulus ISI

Apparent shortening of the peri-stimulus ISI, however, has two interpretations. The underlying mechanism may involve either :

a) bringing the membrane potential of the motoneurone to firing threshold by an EPSP, hence actively advancing the occurrence of the subsequent discharge from its expected time, or

b) a passive selection of relatively short peri-stimulus ISIs into the category of PP- or SP-trials due to the inherently smaller difference between the membrane potential of the motoneurone and its firing threshold during the course of a short ISI.

Random stimulation experiments in which the proportion of PP-trials was found not to differ significantly when the MU was fired at 2 different rates provide evidence in support of active advancement of PP discharges (a),

rather than passive selection of relatively short peri-stimulus ISIs (b). (Supporting evidence against the passive selection of short ISIs is evident from experiments using Ia afferent excitation volleys in MUs from human TA (Ashby and Zilm, 1982b) and triceps muscle (Kudina, 1988) in which the proportion of trials containing a discharge within the primary peak was decreased by an increase in the MU firing rate).

Testing the Adequacy of Definitions used for Sorting

The first change in firing probability following the stimulus is the onset of PP, before which there is no apparent change. This corresponds to the minimum conduction interval from brain to muscle. The first descending volley reaches the motoneurone pool at a point in time during this interval, before which the firing probability of the motoneurone is not affected. The change in firing probability, manifested by PP, is then further delayed by the peripheral conduction time. For the purpose of comparison of serial ISIs, therefore, those peri-stimulus ISIs terminated by a discharge occurring after the stimulus but before the onset of PP should really be treated in the analysis as pre-"stimulus" ISIs, as these terminal discharges occur before the arrival at the motoneurone of the first descending volley. This discrepancy between the theoretical criteria for sorting ISIs into pre-stimulus/peri-stimulus sample groups and the method used in practice for this study, however, would tend to reduce the significance of any difference observed between pre- and peri-stimulus ISIs by the contamination of the latter with the former. Moreover, random stimulation experiments (n=3) it was found that peri-stimulus ISIs terminated by a discharge occurring between the stimulus and the onset of PP did not significantly differ from those pre-stimulus ISIs terminated by discharges occurring within an equivalent time period directly before the stimulus (paired Student's t test). In conclusion it would appear acceptable to sort trials into pre- and peri-

stimulus sample groups. For the purpose of detecting relatively small changes in the serial ISIs, however, it can be argued that trials should be sorted into pre- and peri-PP sample groups according to the onset latency of PP (see below).

Design of Future Experiments

In order to remove length bias sampling and the potential problems of serial correlation, spike triggered stimulation experiments could be performed, with stimulus-trials randomly alternating with no stimulus-trials (thus avoiding the comparison of serial ISIs), at a fixed spike-stimulus delay which is less than the minimum ISI (thus removing length bias sampling errors). Such a spike-stimulus delay would ensure that ISIs of all lengths are sampled. Trials could be grouped into pre- and peri-PP trials according to the onset latency of PP (see above). Statistical comparisons would then be made between peri-PP ISIs from trials in which a stimulus was delivered and peri-PP ISIs from trials in which no stimulus was delivered (unpaired Student's t test). This could be done for the whole experiment or for PP-, SP- or non PP/SP-trials.

If SP is caused by the rising phase of an EPSP then discharges occurring at the latency of this peak should occur earlier than expected (Fetz and Gustafsson, 1983). The operation of an IPSP (Hern et al., 1962; Landgren et al., 1962a - see Introduction) might also be revealed by the appearance in non PP/SP-trials of peri-PP ISIs that were longer than expected.

Mechanism of Abnormalities in Patients with Upper Motor Neurone Disorders

The interpretation and discussion of findings in patients with UMN disorders is based on a comparison with the results for healthy subjects and their mechanistic implications. Each of the first three general categories of abnormality affecting PP, discussed below for single MUs (absent responses, delayed responses and changes in sub-peaks), was detected in more than one disease group. Correlations are made between these findings and the changes in CMAPs that have been reported to occur in the same disorders (MS, MND and cerebrovascular disease). Specific changes are then discussed with respect to single MUs the group of 9 patients with MS, with particular reference to relevant studies on primates and cats.

Absent Unitary Responses

Absent responses, despite using stimulus intensities which caused an excitatory response in healthy subjects, were found in patients with MS (Fig. 34A), MND and stroke. This could be due to a number of mechanisms: reduced excitability of cortical structures, degeneration of corticospinal fibres, ineffective spatial or temporal summation at the motoneurone or intrinsic inexcitability of motoneurones. Evidence for the operation of some of these processes can also be inferred from some of the other findings in UMN patients in the present study (see below).

Absent or Delayed CMAPs in Cerebrovascular Disease

Absent responses in single MUs can be compared with some studies on CMAPs in response to transcranial stimulation. In a series of 20 stroke

patients, CMAPs recorded from biceps and thenar muscles evoked by TES over the undamaged hemisphere were normal in most patients (18 out of 20) and in 2 patients there was a slight increase in the CMC time (Berardelli at al., 1987). TES over the damaged hemisphere produced no response in 15 patients; in 2 the CMAPs were absent in one muscle; and in the remaining 3 the CMAPs were delayed in one or both muscles. In contrast, cervical stimulation produced CMAPs that were normal in 18 patients and delayed in the thenar muscle of 2 patients. In a similar study, it was noted that in most patients who were capable of some limb movement, inter side latency delays were no greater than 8 ms (Thompson et al., 1987). In the baboon, the train of I waves may continue for 6.5-8.5 ms (Kernell and Wu Chien-Ping, 1967a) which was taken by the authors as the upper limit of delay in CMC time, values in excess of which would indicate unequivocal slowing of central conduction. Delays in CMC time less than this may have conceivably been due to a failure of temporal summation. In stroke patients, the CMAPs were small in size and short in duration, consistent with a reduction in the number of descending fibres and/or the number of descending volleys as in the present study. (CMC time can be estimated in two ways, according to the method used for estimating peripheral conduction time. The latter can be estimated from the F wave latency or by electrical stimulation over the vertebral column, which excites motor roots.) The mean normal CMC time plus 2.5 SD is 8.3 ms (Hess et al., 1987b). MacDonell and colleagues, also using TES, showed that delayed CMAPs were not found in any of the patients studied with cortical infarcts (CMAPs were either normal or absent) but only in patients with subcortical lesions. Some patients with subcortical lesions had an absent CMAP to TES, as in Patient 1 from the present study, who had a subcortical lesion with an absent unitary response to TMS.

Delayed Unitary Responses

The delayed responses in single MUs, which were found in MS (Fig. 36) and MND, could be attributed to slowed velocity of propagation in corticospinal fibres. It is likely, however, that individual corticospinal fibres would be slowed to different extents and the consequent desynchronisation of the corticospinal volley could account for the observed widening and loss of sub-peak structure in PP. On some occasions this dispersion might be so great as to prevent the motoneurone reaching threshold, leading to clinical weakness. Peripheral demyelination may also have contributed to the delay in the onset of PP in Patient 6 (Fig. 33). (In contrast to this, however, PSTHs with normal features were also found in MUs in patients with MS (see below) and MND.)

The observation in 1 MND patient that MUs other than those voluntarily recruited before the application of a stimulus may discharge at a long latency (130-180 ms) following the stimulus, suggests a reversal of the normal recruitment order of activated MUs. In normal subjects, activation of single MUs lowers their threshold to activation by a TM stimulus (Hess and Mills, 1986a). It is possible that this late excitatory response corresponded to SP, which would suggest that in this patient MUs could show greater sensitivity to the mechanism underlying SP than to PP. In the future, it is suggested that studies are made on SP in patients with UMN lesions, particularly those with prominent hypereflexia or spasticity.

Absent or Delayed CMAPs in MND

Relatively high stimulus intensities were required for the activation of single MUs in patients with MND and MS in the present study. In order to obtain CMAPs in MND, high stimulus intensities were also required when

compared to controls, using TM (Ingram and Swash, 1987) and TE (Berardelli et al., 1987) stimulation. CMC time was also increased in a proportion of these patients. Prolonged CMC time or the absence of response to brain stimulation were found to be more frequent than low amplitude responses without prolonged CMC time with TMS (Schriefer et al., 1989). It was also found that subclinical UMN involvement could be revealed with TMS but that the patterns of abnormality were not specific to MND, also occurring, for example, in MS. In MND, loss of descending inputs would be expected to cause a decrease in the amplitude of CMAPs. Drop out of larger, faster conducting axons and a reduction in the number of descending impulses arriving at the motoneurone may contribute to the delay in CMC time. It was also suggested by Schriefer et al that unusually large delays in CMC time (of around three times the normal mean), which were always accompanied by a reduction in the CMAP amplitude, could be attributed to secondary demyelination of degenerating fibres, or transmission via other oligosynaptic pathways. These mechanisms may also contribute to corresponding changes in single MUs.

Changes in the Sub-Peaks

Sub-peaks that were present but separated by abnormally large intervals (Figs 30,35) as found in patients with MS and MND, may indicate absence of an intervening sub-peak. In such circumstances the corresponding descending volley would either have been absent from source, due to a cortical abnormality, or blocked in its descent, possibly in a frequency dependent manner (McDonald and Sears, 1970) (see below). This may not necessarily be an absolute phenomenon, as in Fig. 35, where there was a small rise in firing probability between 2 clear sub-peaks.

Changes in the CMAP in MS

Delay in the onset of PP was found in MUs in patients with MS (see below). CMC time was also found to be prolonged in MS using TE (Cowan et al., 1984) and TM (Hess et al., 1987c) stimulation. In the study using TMS, the amplitude of CMAPs were at least 18% of those obtained from stimuli at the wrist. Prolonged CMC time was found to be correlated with brisk finger flexor jerks. In the absence of neurological signs in the arms, CMC time was never-the-less prolonged in 10-20% of patients. In the detection of subclinical lesions, it was noted that weakness of a hand muscle was often associated (79%) with an abnormality of central motor conduction, whereas in the presence of normal strength, there was a 50% probability of an abnormality. An increase in the mean amplitude and persistence of the F response, suggesting an increase in motoneurone excitability, has been found in MS patients with increased CMC time and in MS patients with normal CMC time but with clinical evidence of UMN disorder (Smith et al., 1989). Possible changes in the relative excitability of single MUs were also found in the present study (see below). Using TES, it was noted that the greatest inter side conduction delays were seen in patients with MS (Thompson et al., 1987). Where the delay was greater than 8.5 ms (see above) it was likely that there had been slowing in the central motor pathways. The presence of small prolonged CMAPs may have indicated temporal dispersion of the descending volley, evidence for which was obtained in the present study from single MUs (see below).

Mechanism of Abnormalities in Single Motor Units in Patients with MS

What are the Electrophysiological Effects of UMN Lesions in Animal Studies ?

Impairment of discrete movements may reflect damage to the direct CMN connections that normally allow for direct selection of motoneurones in motor tasks. Single electrical stimuli applied to the cortical surface of monkeys with partial pyramid sections failed to evoke synchronised short latency EMG responses in contralateral distal muscles, although more intense trains of stimuli evoked longer latency asynchronous EMG responses in the same muscles (Felix and Wiesendanger, 1971). In the absence of direct CMN connections, longer temporal summation times may be required to recruit motoneurones. Slowness in the initiation of movements may also reflect an inability to maintain MU discharge during motor tasks (Wiesendanger, 1973b) in addition to a reduction in the numbers of MUs that can be recruited. Parallel changes in surface recorded CMAPs to TES occur in UMN patients, as described above.

In a study on anaesthetised adult monkeys, 3-6 months after hemisection of the thoracic cord at the T8-T10 level, surface anodal shocks applied to the hind limb area of the motor cortex provoked EPSPs in gastrocnemius motoneurones on the control side and on the hemisected side (Aoki and Mori, 1979). On the control side, monosynaptic EPSPs with a latency of 5-7 ms and exhibiting temporal facilitation were produced by single shocks delivered to the contralateral motor area. On the hemisected side, stronger stimulus intensities were required, producing EPSPs that were smaller in amplitude and rarely large enough to produce spike discharges. The latencies were longer, from 6-15 ms and the late EPSPs were polysynaptic.

Temporal facilitation was less pronounced than on the control side. In most of the motoneurones tested (10 out of 14), ipsilateral cortical stimulation also produced EPSPs, with the same or slightly stronger stimulus intensities. The same experimental animals, when examined in the acute period several weeks after hemisection, produced neither flick movements nor surface recorded EMGs of the affected limb in response to motor cortical stimulation. In the chronic stage, however, voluntary movements were evident in the affected hind limbs. It was suggested that crossed or uncrossed corticospinal fibres may have made new functional connections with motoneurones several months after hemisection, involving collateral sprouting from descending fibres, not just confined to that from segmental Ia fibres (Aoki and Mori, 1978).

Chronic transection of the cord at L5 in the cat increases the mean amplitude of Ia EPSPs in motoneurones (Nelson et al., 1979, Nelson and Mendell, 1979). Ia EPSPs with particularly large mean amplitudes (greater than 1 mV) had input resistance values and time constants that were within normal limits, or only slightly higher, suggesting that changes had been initiated at the synapse itself (pre- or post-synaptically) rather than resulting from a normal synaptic current flowing across a larger input resistance. The enlargement of EPSP amplitudes only occurred in motoneurones relatively close to the lesion, also suggesting a mechanism that would involve denervation of motoneurones by elimination of their interneuronal input. EPSPs with brief rise times exhibited the largest increase in amplitude, suggesting that terminals on the most proximal portion of the motoneurone were most affected (Nelson and Mendell, 1979). In the quadriceps motoneurones of monkeys that were chronically hemisected at the T8 level, enhancement of the ipsilateral knee jerk and quadriceps monosynaptic reflex was associated with a slight enlargement of small (0.1-1.1 mV) Ia

EPSPs, with no change in the resting membrane potential (Aoki and Mori, 1978). EPSPs on the hemisected side were found to have a faster rise time and slower decay time than those on the control side. This increase in synaptic efficacy was interpreted as evidence to suggest collateral sprouting from Ia fibres.

The duration of the peak in the PSTH obtained from single human MUs in the quadriceps femoris muscle following triangular wave form patella tendon taps has been found to be significantly longer in spastic patients (Noguchi et al., 1979). The duration for normal subjects was 7.6 +/- 1.3 ms (mean +/- SD) and for spastic subjects was 9.0 +/- 1.8 ms. It was noted that this would be consistent with the hypothesis that Ia axons may sprout following a UMN lesion to form synaptic contacts on distal portions of the dendrites of motoneurones, producing Ia EPSPs of increased rise time. Indirect evidence in chronic stroke patients has also suggested an increase in the amplitude of common EPSPs relative to synaptic noise (Bremner et al., 1989).

In extrapolating from some of these experiments to the changes that may take place in patients with UMN lesions, however, the interval between the lesion and the time of recording must be considered. Although there is some similarity in the changes that occur in Ia EPSP properties produced by acute and chronic spinal transection, the mechanisms underlying acute changes may differ from those underlying the chronic changes. Acute changes do not generally depend on the level of transection and may involve humoral mechanisms, whereas the chronic changes appear to require motoneurone denervation and might be caused by collateral sprouting and/or denervation supersensitivity (Mendell, 1984).

Minimum Effective Stimulus Intensity

A common difference between MS patients and healthy controls in the present study was an elevation in the threshold intensity both at which surface recorded responses and at which unitary responses could be detected. In one patient the difference in threshold between the two sides corresponded to the severity of the UMN signs. Furthermore, in 2 MUs, it was found that despite using a stimulus intensity well above that which produced a PP in healthy subjects, no response in the PSTH could be detected. One possibility for these findings is that the cerebral neurones usually activated by such stimuli have high thresholds for activation because of demyelination of fibres. Alternatively, the number of effective corticospinal inputs to the motoneurone could be depleted, or the excitability of the motoneurone reduced.

Effect of the Spike-Stimulus Interval

Despite abnormalities in their PSTH (see below), some motoneurones in MS patients displayed behaviour that was similar to that observed in healthy subjects. Specifically, the probability of evoking a PP discharge was increased by an increase in the spike-stimulus interval. This would suggest that the normal intrinsic processes governing excitability operated in these motoneurones. The observation that in 8 out of 9 MUs PP discharges could be induced at an earlier stage of the ISI than in healthy subjects, however, may reflect an alteration in the time course of the motoneurone membrane trajectory, or the production of larger EPSPs. The later may have resulted either from the required use of higher stimulus intensities for the production of PPs in MS patients, from hyper-excitability of presynaptic elements or from changes in the postsynaptic membrane.

Delay and Increased Duration of PP

It was found that in some MUs in patients with MS, the onset of PP was delayed by up to 28 ms when compared with healthy subjects. In these MUs, PP was frequently of longer duration than in healthy subjects. The delay could be due to demyelination and therefore slowed velocity of propagation in corticospinal fibres. This is consistent with the known physiological effects of central demyelination which include decreased conduction velocity in demyelinated nerve fibres (McDonald, 1963) reflecting the increased time required to depolarise the axon membrane to the excitation threshold. It is probable that different corticospinal fibres which project to a single motoneurone are variably demyelinated, with propagation velocities that are variably reduced. A combination of these factors may therefore have caused the late arrival and the increased duration of excitatory effects observed in these motoneurones. The absence of discernible sub-peaks in some MUs would also indicate the desynchronisation of descending impulses by varying degrees of demyelination between different corticospinal fibres. Desynchronisation would lead to less effective temporal summation of EPSPs at the motoneurone which, in some cases, might never reach firing threshold. This would be expected to cause, or contribute to, the development of clinical weakness.

In a series of patients with UMN lesions including MS, MND and stroke, studied with TES, the greatest delay in the onset of the CMAP was found in patients with MS (Thompson et al., 1987). This delay was also associated with an increased duration of the response in MS, but not in MND. The authors' suggestion that a delay in the onset of CMAPs may be caused by slowing of conduction in the largest diameter fibres of the pyramidal tract, or

dispersion or reduction in size of the descending volley is supported by the present findings in single MUs.

Alteration in Sub-Peaks

In some MUs it was found that, although PP was of normal latency and duration, the sub-peak structure was disturbed such that the interval between adjacent sub-peaks was prolonged by up to 0.8 ms when compared to the longest inter sub-peak interval found in a healthy subject. Multiple peaks in the PP region of the PSTH at longer inter-peak intervals were associated with PPs of delayed onset and increased duration (see below). It has been argued that sub-peaks represent the EPSPs caused by the successive arrival of corticospinal impulses at the motoneurone. The prolongation of intervals between neighbouring sub-peaks in patients suggests that a sub-peak may be missing. It is possible to attribute this to the inability of partially demyelinated central fibres to conduct all of the corticospinal impulses in the high frequency train produced by the stimulus. Demyelination causes an increase in the capacitance and resistance of the demyelinated internode, which, in the extreme case, precludes depolarisation of the axon membrane to threshold, blocking the propagation of an impulse (Lafontaine et al., 1982). This refractory period of transmission is also increased, which lengthens the interval at which two successive impulses may be transmitted (McDonald and Sears, 1970). The relative refractoriness decreases the amount of inward current generated at each node during the propagation of the second of two closely spaced impulses, and therefore the peak current may fall short of that required to bring the membrane to threshold for the second impulse because of demyelination of the nerve fibre. The second impulse is consequently blocked (Rasminsky and Sears, 1972). Such conduction block may become frequency dependent when the demyelinated nerve

fibre is stimulated repetitively at intervals greater than the refractory period of transmission.

Frequency dependent conduction block has been demonstrated in the cat posterior column where, with frequencies of from 290-410/s, alternate impulses failed to cross a demyelinated lesion (McDonald and Sears 1970). Human corticospinal fibres, which may conduct trains up to 700/s according to the inter sub-peak intervals in the present study, may be subject to the same phenomenon. Alternatively, missing sub-peaks could be attributed to changes in the differential threshold for successive I waves, as observed in the intact monkey and in humans (Kernell and Wu Chien-Ping, 1967a, Day et al.,1989). Evidence for differential thresholds was not observed, however, in the current experiments on healthy subjects.

Other Alterations in the Shape of PP

The presence of more than one peak in the PP region of PSTHs from 5 of the 21 MUs, at intervals of 6 to 8.5 ms (or 22 ms in 1 MU), frequently associated with PPs of delayed onset, prolonged duration and absent sub-peaks, could indicate an even greater degree of frequency dependent conduction block (inspection of the raw data confirmed that they were not caused by double discharges). These peaks may also, however, represent the operation of more than one physiological pathway (possibly caused by the use of high stimulus intensities) or the same pathway that had become partially demyelinated producing a discrete division into fibre groups with different conduction velocities. The sprouting of corticospinal neurones on to partially denervated spinal motoneurones could produce new pathways involving a different number of synapses. Axonal sprouting in the spinal cord may follow partial deafferentation (Liu and Chambers, 1958). Morphological reorganization of the spinal cord rostral to the site of

hemisection in the monkey and rat has also been demonstrated, with similar changes in a patient with an abscess of the spinal cord (Bernstein and Bernstein, 1973). Pathological loss of effective excitatory synapses, and/or axonal sprouting to form new synapses may have contributed to changes in the time course of the EPSP underlying PP. Alternatively, the period of reduced firing probability occurring between the peaks could represent an inhibitory process acting during the course of a temporally dispersed PP, or, such multiple peaks may have been produced by a change in the mechanism that is thought to produce repetitive corticospinal cell firing in response to a single stimulus (Jankowska et al., 1975; Phillips, 1987).

SUMMARY

1. The problem addressed by this study was: How does the human corticospinal tract influence the discharge of spinal motoneurones and what are the effects of neurological disease? The aim was to characterise the response of single human motoneurones to a corticospinal input and to identify abnormalities in UMN disorders by comparison with the normative data. The specific objectives were to: estimate the expected time course of the rising phase of the EPSPs underlying the peaks of firing probability in the PSTH and test the effect of changes in the experimental parameters; describe the features of late responses and deduce their origin; test the expected mode of summation of the motoneurone's interspike membrane potential with the EPSP by studying the the effect of factors which influence the probability and latency of evoking a discharge; examine the approach to the statistical comparison of ISIs and the implications for experimental design; identify neural mechanisms which contribute to the clinical effects of UMN lesions by performing parallel studies on a group of patients with neurological disease, using data from the above as the control.

2. The method was to study the firing probability of 78 repetitively firing single MUs from FDI and other muscles of the upper limb following TMS. This was performed in healthy subjects and in a group of patients with different UMN disorders. The inducing current flowed in an anticlockwise direction through a circular coil which was positioned tangentially at the vertex.

3. Two peaks were produced in the PSTH. The primary peak (PP) had an onset latency ranging from 13 ms (deltoid and biceps) to 31 ms (FDI) and had a short duration of 4.6 ±1.7 ms (mean ± SD). The second, termed the secondary peak (SP), which was present in more than half MUs studied, had an onset latency in FDI ranging from 56-90 ms, was smaller in height,

and longer in duration (20.9 ±12.0 ms in FDI) than PP. The modal latency of both PP and SP was linearly related to peripheral conduction distance. All the MUs studied were of low threshold and discussion is limited to this sub-population of the motoneurone pool.

4. Both peaks in firing probability are thought to be caused by the rising phase of an EPSP, the rise times of which, it has been argued, would approximate to the duration of the respective peaks. PP frequently consisted of 1-3 sub-peaks, with a mean intermodal interval of 1.4 ms for FDI and 2.9 ms for forearm and upper arm muscles. This interval may reflect the maximal rise time of one in a sequence of EPSPs at the motoneurone, the expected shape of which were estimated with a cumulative sum. The experimental variables affecting these data include the total number of trials and variation in the ISI during the course of the experiment. The mean intermodal sub-peak interval for MUs in arm muscles other than FDI tended to be longer than 1.4 ms, which suggests a difference in the thresholds for successive I waves not seen in FDI in the present study.

5. PP and SP were also found in the PSTHs of single MUs from deltoid, biceps, brachioradialis and FCU. The onset latency of PP and SP was shorter in more proximal muscles, consistent with a shorter peripheral conduction distance. A late peak with an onset latency of 100 ms was also observed in deltoid. This late peak was not due to the resumption of firing after PP discharges.

6. The effect of increasing the stimulus intensity was to increase the probability of evoking a discharge at the PP latency. This was also associated with reduction in the onset latency of PP, and changes in the

latency of sub-peaks. Findings suggest enlargement of the amplitude of the compound EPSP by an increase in stimulus strength. Changes in mean or instantaneous firing rate, however, were not found to influence the probability of evoking a discharge.

7. The spike-stimulus interval influenced the probability of evoking a discharge. Spike-stimulus intervals of less than 30 ms in healthy subjects were seldom associated with discharges at the PP latency. At longer spike-stimulus intervals, discharges at the PP latency occurred with increasing frequency. The spike-stimulus interval was also negatively correlated with discharge latencies within PP. These findings are consistent with the expected course of the interspike membrane trajectory and the summation of EPSPs to cause discharges in a proportion of trials, depending on the difference between the cell's membrane potential and its firing threshold.

8. The serial occurrence of discharges at the PP latency in runs of consecutive trials can be non-random. This was not attributable to measurable variables other than favourably short spike-stimulus intervals.

9. The statistical comparison of serial pre- and peri-stimulus ISIs in random stimulation experiments is potentially dependent upon serial dependency and length bias sampling. Within limits, its use suggests the occurrence of discharges within PP and SP at times that are earlier than expected, consistent with the operation of an EPSP. In order to remove length bias sampling and the potential problems of serial dependency, future spike triggered stimulation experiments are described, with stimulus-trials that alternate randomly with non stimulus-trials. Statistical comparisons can be made between the duration of ISIs from the former and latter, in order to test for the expected changes caused by the operation of either EPSPs or

IPSPs. This can be applied to healthy subjects and patients with neurological disease.

10. Evidence suggests that SP was caused by the rising phase of a late EPSP mediated via a pathway that included a peripheral afferent component. In principle therefore, SP might be initiated via a segmental or long loop reflex, or by gamma motoneurone discharge and subsequent MN firing via Ia afferents. An additional central component in the pathway for SP, such as a slowly conducting descending pathway, is not excluded by present results.

11. The most common difference between patients with UMN lesions and healthy controls was an elevation in the threshold intensity both at which surface recorded responses and at which PP could be detected. When present, PP was found to be either normal, absent, delayed and temporally dispersed (by up to 28 ms and 21 ms, respectively) or having sub-peaks separated by abnormally long intervals.

12. Absent responses in UMN patients may indicate cortical inexcitability, degeneration of descending fibres, ineffective spatial or temporal summation at motoneurones or intrinsic excitability of motoneurones. Delay and temporal dispersion suggests variable degrees of slowing in the velocity of propagation in descending fibres causing a compound EPSP that was late in arrival and increased in duration.

13. Sub-peaks that were present but separated by abnormally large intervals may indicate the absence of the intervening sub-peak (suggesting that the corresponding descending volley could have been absent from source, due to a cortical abnormality, or blocked in its descent, possibly in a

frequency dependent manner). Alternatively, a delay between volleys may have been caused by the operation of a pathway involving additional synapses. These mechanisms may underlie some of the clinical features of UMN disorders.

14. Despite the above abnormalities in their PSTH, some spinal motoneurones in MS patients displayed behaviour that was similar to that observed in healthy subjects. Specifically, the probability of evoking a PP discharge was increased by an increase in the spike-stimulus interval. This suggests that the normal intrinsic processes governing excitability operated in these motoneurones. The observation that PP discharges could be evoked at an earlier stage of the ISI than in healthy subjects, however, may reflect an alteration in the time course of the motoneurone membrane trajectory, or the production of larger EPSPs. The later may have resulted either from the required use of higher stimulus intensities for the production of PPs in MS patients, from hyper-excitability of presynaptic elements or from changes in the postsynaptic membrane.

15. Future work arising from this thesis will include:

a) further study of the pathway that mediates SP (see 10, above)

b) experiments designed to test for the expected changes in ISI that would be caused by the operation of an EPSP or an IPSP (as in 9, above)

c) abnormalities of SP occurring in the presence of UMN lesions

d) study of the association between different abnormalities in the unitary response to TMS and the specific physical signs of an UMN lesion.

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