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THE UNIVERSITY OF ALBERTA

RECRUITMENT OF NORMALLY INNERVATED AND REINNERVATED MUSCLES AND MOTOR UNITS

BY

© CHRISTINE KAYE THOMAS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF Ph.D.

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Recruitment of normally innervated and reinnervated muscles and motor units

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ABSTRACT

Spike triggered averaging was used to study the recruitment order and twitch tension of motor units in normal human muscles during isometric contractions in different directions, during functional joint movements and in reinnervated human hand muscles after complete section and resuture of the ulnar and median nerves.

Linear correlations were found between twitch tension and threshold force of first dorsal interosseous or abductor pollicis brevis motor units for each contraction direction. Rank order of motor unit recruitment during different directions of contraction was correlated but not identical. Therefore, an orderly pattern of recruitment according to increasing twitch size adequately described the function of these muscles for all directions of contraction. Recruitment order was largely preserved in these muscles during functional joint movements involving the repetitive opening and closing of scissors. The recruitment reversals observed were usually between pairs of units with similar thresholds.

Human hand muscles were shown to be reinnervated both appropriately by some of their original motor axons (activated only by voluntary contraction of the muscle in which they were recorded) and inappropriately by motor axons that had previously innervated other muscles with different functions (activated by voluntary contraction of muscles innervated by the same nerve). Following ulnar or above-

elbow median nerve sections, there was no correlation between motor unit size (twitch amplitude) and recruitment threshold. This absence of orderly recruitment was attributed to misdirection of motor axons during regeneration. Following median nerve section at wrist level, where the reinnervated muscles have more synergistic actions, orderly recruitment appeared to be re-established. Thus, the size principle of motor unit recruitment could be re-established after nerve section in humans, if motor axons innervate their original muscles or ones with closely synergistic functions.

In young cats, motor axons were misdirected to muscles with antagonistic functions by cross-union of the common peroneal and tibial nerves in one hindlimb. These nerves normally innervate all distal flexor and extensor muscles respectively. During locomotion, the cross-reinnervated flexor muscles only fired during extension of the limb. The cross-reinnervated extensor muscles were active in the extensor and sometimes in the flexor phase of locomotion when the nerve cross was less than complete. The inappropriate timing of muscle activity during locomotion demonstrated that the muscles were primarily activated according to the innervating nerves. In acute experiments 18-24 months after the initial surgery, the success of the nerve crosses was determined by measuring the compound action potentials generated at the lumbar 6, lumbar 7 and sacral 1 spinal roots in response to the stimulation of the

control and crossed medial gastrocnemius, lateral gastrocnemius-soleus and common peroneal nerves. Dissection and stimulation of ventral root filaments innervating the cross-reinnervated extensor muscles showed that muscle unit force and contractile speed were directly correlated with the size of the innervating nerve. These results indicate that peripheral reorganization of nerve and muscle properties according to size occurred, even when motoneurons were misdirected to antagonistic muscles and produced inappropriate movement patterns.

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LIST OF ABBREVIATIONS

AC	alternating current
Ag/AgCl	silver/silver chloride
AHP	after hyperpolarization potential
APB	abductor pollicis brevis
CFL	critical firing level
Co.	company
CP	common peroneal
CT	contraction time
DC	direct current
E	extensor
Ec	crossed extensor
EMG	electromyogram
EPSP	excitatory postsynaptic potential
F	flexor
Fc	crossed flexor
FDI	first dorsal interosseous
FDL	flexor digitorum longus
FF	fast fatiguable
FG	fast twitch glycolytic
FI	fast fatigue intermediate
FOG	fast twitch oxidative glycolytic
FR	fast fatigue resistant
Hz	hertz
IPSP	inhibitory postsynaptic potential
IR	innervation ratio

kHz	kilohertz
kΩ	kilohm
LG	lateral gastrocnemius
LGS	lateral gastrocnemius-soleus
L6	lumbar spinal root 6
L7	lumbar spinal root 7
MG	medial gastrocnemius
μA	microamp
μV	microvolt
mN	milli Newton
msec	millisecond
mV	millivolt
N	newton
nC	nanocoulomb
N. J.	New Jersey
pC	picocoulomb
PPSP	population postsynaptic potential
PSP	postsynaptic potential
Q	charge
R	resistance
RIPSP	recurrent inhibitory postsynaptic potential
S	slow
S.D.	standard deviation
S.E.	standard error
SO	slow twitch oxidative
S1	sacral spinal root 1
ST	semitendinosus

TA	tibialis anterior
TP	tibialis posterior
V	volts
VL	vastus lateralis

CHAPTER 1

INTRODUCTION

The progressive recruitment of motoneurons and the modulation of their firing rates are two processes involved in the gradation of muscle force (Adrian and Bronk, 1929; Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932; Denny-Brown, 1929). In 1965, it was proposed that the recruitment of motoneurons occurred in order of increasing cell size (Henneman, Somjen and Carpenter, 1965a). Since then, the applicability of this size principle has been examined in various muscles, species and during different recruitment conditions, both before and after nerve injury. Some evidence has been provided for the biophysical basis of orderly motoneuron recruitment and for the organization of the afferent input to the motoneuron pool. Other studies have examined how the excitability of the motoneurons relates to the force output of the innervated muscle fibers. Yet, some aspects of the size principle are controversial and recruitment has been proposed to occur by alternative processes. These include: a) recruitment by motoneuron type, but randomly within a type (Fleshman, Munson, Sypert and Friedman, 1981) b) recruitment in a force dependent way by a systematic gradation of intrinsic motoneuron properties which are independent of cell size (Gustafsson and Pinter, 1985).

In this thesis, questions relating to some of the apparent exceptions to the orderly recruitment of normally innervated and reinnervated motor units (a motoneuron and all the muscle fibers it innervates; Liddell and Sherrington, 1925) are examined. As an introduction to this, several areas of related literature are reviewed briefly. These are:

a) the size principle proposed to explain motoneuron excitability.

b) the evidence for orderly recruitment by graded intrinsic motoneuron properties and for recruitment by motor unit type but randomly within a type.

c) the organization of afferent input to the motoneuron pool and how it relates to motoneuron excitability.

d) the recruitment order of motor units in human muscles.

1.1 The size principle

The size principle was proposed to explain the inverse relationship between alpha motoneuron size and excitability in cat hindlimb muscles (Henneman et al., 1965a). It implied that the recruitment of smaller motoneurons was followed by the progressive recruitment of larger motoneurons. This orderly recruitment in flexor and extensor motoneurons occurred irrespective of whether the excitatory stimuli arose physiologically or electrically, via one or several synapses, or from ipsilateral, contralateral (Henneman, 1957; Henneman, Somjen and Carpenter, 1965b), or supraspinal

sources (Somjen, Carpenter and Henneman, 1965). In these papers, action potentials were recorded extracellularly from small groups of cut ventral root filaments. The amplitudes of the action potentials were proportional to the reflex threshold of the units. However, they were also assumed to be proportional to axon conduction velocity or diameter (Gasser, 1941) and a direct index of cell size. With cut ventral roots, the parent muscles of the responsive motor axons could not be determined directly. Presumably then, orderly motoneuron recruitment was demonstrated amongst those fibers of the combined triceps surae motor pools belonging to one ventral root. Therefore, it was unclear as to whether this orderly recruitment applied to the motoneurons of a single motor pool (the motoneurons innervating one muscle; Creed et al., 1932) or several pools.

With respect to efferent properties, the slower the conduction velocity of the motor axons to the medial gastrocnemius (MG) or soleus muscles, the smaller the tension developed by the innervated muscle fibers (McPhedran, Wuerker and Henneman, 1965; Wuerker, McPhedran and Henneman, 1965). In the MG muscle, the relationship between motor axon conduction velocity and motor unit tetanic tension was neither linear nor simple. Histochemically, soleus muscles consisted of one fiber type while MG muscles consisted of three fiber types. The functional properties of the motor units were proposed to

depend on motoneuron size. This then determined the excitability and usage of these motor units and in turn, specified the fiber type required (Henneman and Olson, 1965). From the percentage, mean diameter and cross-sectional area of the different muscle fiber types measured, it was suggested that nerve fiber diameter was related to the number of innervated muscle fibers, but innervation ratios were not determined. Therefore, the relationship between motor axon size and motor unit size was also unclear.

In relation to afferent input, it was suggested tentatively, that alpha motoneurons may receive input which was equal in density but unequal in effect. Stimulation of group 1a afferent fibers evoked the largest aggregate excitatory postsynaptic potentials (EPSPs) in cat motoneurons that had the slowest conducting motor axons (Eccles, Eccles and Lundberg, 1957). Assuming that these differences inversely reflected cell size, Henneman and colleagues proposed that the variations in excitability of different sized cells resulted from differences in cell input resistances.

In summary, orderly motoneuron recruitment had been demonstrated and differences in input resistances were proposed to explain the relationship between cell size and excitability. There was a non-linear relationship between motoneuron excitability and the force output of the muscle

unit. How the afferent input to the motoneuron pool was organized to allow orderly motoneuron recruitment needed to be examined as did the application of this principle to other species and recruitment conditions.

1.2 Recruitment by increasing motoneuron size

Intracellular injection of tracer substances like horseradish peroxidase or procion yellow has allowed detailed visualization of the dendritic and axonal morphology. Motoneuron size has been described in terms of mean soma diameter, soma surface area, the surface area of the soma and dendritic membrane and the combined dendritic trunk parameter (Burke, Dum, Fleshman, Glenn, Lev-Tov, O'Donovan and Pinter, 1982; Cullheim, 1978; Kernell, 1966; Ulfhake and Kellerth, 1982, 1983; Zwaagstra and Kernell, 1981). These anatomical measures of cell size have often been correlated with physiological indices of cell size such as axon conduction velocity and motoneuron input resistance in both invertebrates and vertebrates. For example, in the swimmeret system in the lobster, soma diamater and conduction velocity were positively correlated (Davis, 1971). In alpha motoneurons innervating the cat hindlimb muscles, motor axon conduction velocity covaried with mean soma diameter and the diameter of the motor axon in the white matter or the initial segment (Cullheim, 1978). The soma surface area and the cross-sectional area of all of the dendritic stems were approximately proportional (Kernell,

1966; Ulfhake and Kellerth, 1983). These cells with small somas had on average, fewer and thinner stem dendrites, thinner and slower conducting axons, greater input resistances and longer after-hyperpolarization (AHP) durations than large cells. Cells with higher input resistances were proposed to be more excitable because they generated larger amplitude EPSPs for a given amount of synaptic current (Barrett and Crill, 1974; Kernell, 1966).

Thus, conduction velocity was directly related to axon diameter and motoneuron size as proposed originally (Henneman et al., 1965a). Later, the critical firing level (CFL) of a motoneuron was defined quantitatively for the plantaris motor pool of the cat. After a conditioning tetanus, the plantaris muscle nerve was stimulated. The maximum monosynaptic reflex response on the proximal end of the cut ventral root was compared to the antidromic response on the distal end of the cut ventral root. The CFL (the % of the maximum output of the pool at which a single ventral root filament ceased to fire) was directly related to the extracellularly recorded nerve action potential, motor axon diameter or conduction velocity and so cell size. Motoneuron recruitment was strictly ordered by size in unit pairs with critical firing level differences that were greater than 2.5%. If the pair had closer critical firing levels, recruitment was on average, 86% ordered. Generally then, a motoneuron responded only if all the lower ranking cells in the pool discharged with it. Therefore, motoneuron

Recruitment was shown to be ordered by increasing cell size within a single motor pool (Clamann, Gillies, Skinner and Henneman, 1974; Clamann and Henneman, 1976; Henneman, Clamann, Gillies and Skinner, 1974). This rank order of motoneuron recruitment was unaltered by inhibitory inputs that were predominantly from postsynaptic, mixed, recurrent, presynaptic, non-muscular or supraspinal sources (Clamann, Gillies and Henneman, 1974).

Similarly, a correlation was found between motoneuron size and the order of recruitment in the soleus, MG, tibialis anterior (TA) and plantaris muscles of the cat. Motoneuron size was judged by the conduction velocity of the motor axon (Bawa, Binder, Ruenzel and Henneman, 1984; Clamann, Ngai, Kukulka and Goldberg, 1983). A few recruitment reversals (3%) were observed in MG and soleus unit pairs that had similar thresholds.

However, was the size of motoneurons related directly to their input resistance? Stein and Bertoldi (1981) pointed out that if input resistance was linearly related to other measures of motoneuron size such as conduction velocity, then all motoneurons would have the same membrane properties and shape. Re-evaluation of the relationship between input resistance and conduction velocity, suggested that larger motoneurons were not just larger samples of small cells because the line giving the best fit to the data was steeper than expected. Experimental confirmation of these

suggestions (Kernell and Zwaagstra, 1981), indicated that the input conductance (inverse of input resistance) of motoneurons with large diameter axons was unexpectedly high. Their cell bodies were not larger than normal. Thus, the lower than average specific membrane resistance in larger cells, also appeared to be important in making these cells less excitable. As differences in motoneuron size were not directly reflected by differences in motoneuron input resistance, both size and input resistivity (an intrinsic membrane property) could be important in relative motoneuron excitability.

1.3 Motoneuron recruitment by a systematic gradation of intrinsic membrane properties

Recently, comparisons have been made between the variations in motoneuron size, intrinsic membrane properties and the excitability of lumbar alpha motoneurons of the cat (Gustafsson and Pinter, 1984a, 1984b, 1985). There were strong positive correlations between intrinsic membrane properties such as the input resistance, membrane time constant (a measure of specific membrane resistivity) and AHP duration. Calculations of total cell capacitance agreed well with former anatomical measurements of cell surface area or size (Ulfhake and Kellerth, 1982). However, for a given cell surface area, the entire range of values for input resistance, time constant or AHP duration could be represented. An alternative measure of cell size, axon

conduction velocity, was also correlated weakly to the measured membrane properties. Therefore, the variation in intrinsic membrane properties appeared to be relatively independent of motoneuron cell size (Gustafsson and Pinter, 1984a).

Other observations indicated that differences in intrinsic membrane properties may influence the threshold for spike initiation and so motoneuron discharge. Motoneurons with fast conducting axons accommodated during ramp current stimulation by increasing their recruitment threshold (Burke and Nelson, 1971). In the cat MG muscle, differences in motoneuron excitability (as measured by motoneuron rheobase) were independent of cell size (or axon conduction velocity) (Fleshman, et al., 1981).

To assess whether motoneuron size or intrinsic membrane properties were better matched to motoneuron excitability, Gustafsson and Pinter (1984b, 1985) measured the range of motoneuron rheobases, input resistances and sizes. The range of rheobase values exceeded that of the input resistances but was even wider than the range of the cell capacitances (sizes). When rheobase values were normalized to be independent of cell size (rheobase current / total cell capacitance), the range of excitabilities was reduced. However, the distribution was skewed towards high values and very similar to the distribution typically recorded for motor unit tetanic tensions. In addition, the relationships between motoneuron membrane properties were very similar to

the relationships between motor unit properties. Plots of normalized rheobase versus AHP duration and tetanic tension versus contraction time followed very similar distributions. These results provided strong but indirect evidence that differences in intrinsic membrane properties complete the functional link between motoneuron excitability and motor unit tension development. Thus, these authors suggested an alternative to the orderly recruitment of motoneurons by increasing cell size. That is, the higher specific resistivity and different geometrical structure of more excitable cells primarily determined their input resistance and hence responsiveness to synaptic current. They proposed that a systematic gradation of intrinsic motoneuron properties which was independent of cell size, could allow recruitment in a force-dependent way under conditions of constant synaptic density or efficacy (Gustafsson and Pinter, 1985).

1.4 Motoneuron or motor unit types

Motor units of animal muscles are often classified into physiological types according to their force output in response to repetitive stimulation of the motoneuron or motor axon. Motor units are designated as slow (S) or fast (F) using criteria such as contraction time, "sag" in tension during unfused tetani or a cumulative force index. Fast units are further subdivided into fast, fatigue resistant (FR); fast, intermediate fatiguable units (FI) and

fast, fatiguable (FF) by their fatigue characteristics. (Burke, 1967; Burke, Levine, Tsairis and Zajač, 1973; Kernell, Eerbeck and Verhey, 1983). The same physiological classification of motor units can be determined from the motoneuron to input resistance ratio. The accuracy of the prediction is increased further when AHP half decay time is used to separate fast and slow units (Zengel, Reid, Sypert and Munson, 1985). Typically, force output is ordered such that S < FR < FF units although overlap between values of different types usually occurs. Therefore, within a muscle, there is a range of force produced by different motor units. The distribution for the force output of units is usually skewed towards high forces (Stuart and Enoka, 1984).

1.5 Force output of different unit types

Motor unit force output depends on three factors. These include the innervation ratio (IR, the number of muscle fibers per unit), the average cross-sectional area of the constituent muscle fibers and their specific tension (Burke, Levine, Salcman and Tsairis, 1974). Combinations of physiological and histochemical techniques have been used to determine how each of these factors contributes to the force output. Repetitive and relatively long term stimulation of a single motoneuron or motor axon can presumably deplete its muscle fibers of glycogen (Burke et al., 1973; Edström and Kugelberg, 1968; Kugelberg and Edström, 1968). The muscle fibers belonging to a motor unit can be stained

histochemically for relative activities of mitochondrial oxidative enzymes and enzymes related to anaerobic glycolysis (Peter, Barnard, Edgerton, Gillespie and Stempel, 1972) and/or for myofibrillar ATPase reactivity patterns (Brooke and Kaiser, 1970).

The resulting two classification systems are almost interchangeable. Therefore, slow twitch, oxidative (SO) or type I fibers depend almost exclusively on aerobic metabolism. Fast twitch, oxidative and glycolytic (FOG) or type IIA fibers show capacity for both aerobic and glycolytic metabolism while fast twitch, glycolytic (FG) or type IIB fibers primarily exhibit anaerobic metabolism. A fourth type, IIAB, show properties intermediate between types IIA and IIB. These fibers are thought to contribute to S, FR, FI and FF motor unit types (Burke et al., 1973). From serial sections, the muscle fibers of the unit are reconstructed in 3-dimensional form and IRs determined. However, failure of serial sections to overlap can make it difficult to identify all the muscle fibers belonging to a unit, especially in muscles with complex internal architecture (Burke, 1981).

IRs are usually studied in cat hindlimb muscles in relation to motor unit type. Discussion will be restricted to these even though IRs are also species dependent. In MG, tibialis posterior (TP) and flexor digitorum longus (FDL), there is a tendency for S and FR units to have similar IRs.

These are lower than FF IRs (Burke, 1981; Burke and Tsairis, 1973; McDonagh, Binder, Reinking and Stuart, 1980a). In TA, the IR of S units is somewhat lower than that of FR and FF units (Bodine, Roy, Eldred and Edgerton, 1985). As unit tensions increase such that S < FR < FF units, there is some discrepancy as to whether IRs follow a similar trend. However, it is clear that motor units with larger, faster conducting axons (FR, FF units) do not necessarily innervate more muscle fibers than those with smaller, slower conducting axons (S units), as originally proposed (Henneman and Olson, 1965).

The mean cross-sectional area of muscle fibers typically increases in order of type I, IIA, IIB although overlap can occur. The greatest differences are usually between IIA and IIB fibers (McDonagh, Binder, Reinking and Stuart, 1980b). Equating histochemical and physiological measures, the increases in cat muscle fiber size and unit tetanic tension are in the same direction. However, these interpretations are complicated by differences in IRs and by possible changes in fiber area and oxidative enzyme contents with use (Stuart and Enoka, 1984).

The specific tension of a muscle fiber can be calculated by dividing the unit force output by the total muscle fiber cross-sectional area. In the cat MG, FDL, TP and TA muscles, the specific tension of S units tends to be lower than that of FR or FF units (Bodine et al., 1985; Burke, 1981; Burke and Tsairis, 1973; McDonagh et al., 1980a).

Therefore, providing that the low specific tensions of S units is correct, the small force output of these units may result more from the low specific tension than from their smaller IRs or fiber areas. In comparison, differences in fiber size or IRs may explain the different tension outputs of FF and FR units. In the cat TA muscle, fiber size differences were favored.

1.6 The relationship between unit type and motoneuron size

In type identified cat MG motoneurons, the mean diameter of the soma, motor axon or stem dendrites progressively increased in the order S < FR < FF units (Burke et al., 1982; Ulfhake and Kellerth, 1982). However, the range of each measured parameter overlapped between different unit types so cell body size was not related strictly to motor unit type.

Physiological measures of motoneuron size yield similar results. In the motor unit populations of the cat flexor carpi radialis muscle or various hindlimb muscles, the mean motor axon conduction velocity (motoneuron size) of S units was significantly slower than that of all fast unit types. Significant increases in the mean tetanic tension occurred in the order S < FR < FF. In the whole muscle populations, there were relatively weak positive correlations between motor axon conduction velocity and unit tetanic tension (Botterman, Iwamoto and Gonyea, 1985; reviewed in Stuart and Enoka, 1984). This could result from the fact that motor

axons have a much narrower range of conduction velocities than unit tetanic tensions. It implies that axon conduction velocity (motoneuron size) is a poor indicator of muscle unit tetanic tension (muscle unit size). The relationship between axon conduction velocity and unit tetanic tension was generally not significant within motor unit types. This emphasized again some dissociation between motoneuron size and motor unit type.

1.7 Recruitment by motor unit type

One index of motoneuron excitability is the amplitude of the Ia EPSP evoked in a motoneuron in response to its stimulation. EPSP amplitude will depend on the number and location of the synaptic terminals on the motoneuron and its intrinsic membrane properties (Stuart and Enoka, 1984). In cat motoneurons innervating different hindlimb muscles, Ia EPSP amplitude was ordered such that S > FR > FF units. The reverse trend, S < FR < FF, was found for unit tetanic tension. For the whole population of motor units in a muscle, Ia EPSP amplitude was inversely related to unit tetanic tension. This suggested that some factor closely related to contraction strength strongly influenced Ia EPSP amplitude and excitability (Burke, Rymer and Walsh, 1976; Dum and Kennedy, 1980b; Fleshman, Munson and Sypert, 1981; Harrison and Taylor, 1981). Was it cell size? In these studies, motor axon conduction velocity (cell size) of S and FR units was well correlated to EPSP amplitude. FF units had

unduly small amplitude Ia EPSPs in comparison to their conduction velocities suggesting a difference in the presynaptic organization to these units. However, after removing the effects of an intrinsic membrane property, input resistance, the differences in Ia EPSP amplitudes of unit types remained. As Ia EPSP amplitude was negatively correlated to unit tension for the whole population, Ia synaptic organization and motoneuron excitability appeared more closely related to motor unit tetanic tension than to motoneuron size. These results prompted the proposal of recruitment by motor unit type. As Ia EPSP amplitude and unit tetanic tension was not correlated within unit types, recruitment was proposed to be random within a type (Fleshman et al., 1981).

In 1985, recruitment in the cat plantaris muscle was shown to be ordered strictly by increasing contraction strength (unit tetanic tension). As tetanic tension was correlated to unit type, recruitment was expected to occur by motor unit type as well (Zajac and Faden, 1985). These data argued strongly against the proposal of recruitment by type but random recruitment within a type. In this study, recruitment by increasing axonal conduction velocity held for S,S or S,F pairs of motor axons but was random for F,F pairs because all fast units had similar conduction velocities. As size and type were correlated for the most part, recruitment by either regime could not occur

independently. However, even if recruitment by motoneuron size does not occur at higher force levels, as is suggested by these studies, it is important to remember that considering recruitment alone tends to oversimplify the control of muscle force generation. The activation history of the motoneuron and rate coding, especially at higher force levels, also have important influences on the force generated within a muscle (Burke, Rudomin and Zajac, 1976; Kernell et al., 1983).

1.8 The organization of the afferent input to the motoneuron pool

Studies relating the distribution of the afferent input to the motoneuron pool have concentrated on group Ia or II afferents to alpha motoneurons because the connections are monosynaptic and clearly defined.

At the light microscopic level, functionally identified group Ia afferent contacts to triceps surae motoneurons in the cat have been observed purely next to the soma, purely on the distal dendrites or a combination of the two (Burke, Walmsley and Hodgson, 1979). A minority (9%) of boutons from different Ia afferents contact the soma or proximal dendrites (Brown and Fyffe, 1981).

More information relating the distribution of group Ia or II afferents to motoneurons has been gathered from physiological studies. By stretching a muscle or stimulating its nerve, the afferent impulses of single fibers can be

averaged in continuity from dorsal root filaments, followed by the corresponding EPSPs evoked in identified motoneurons. These techniques have shown that Ia afferents from the cat MG muscle send terminals to an average of 93% of the motoneurons supplying that muscle but to only 65% of the motoneurons innervating its synergist, lateral gastrocnemius (LG) (Mendell and Henneman, 1968, 1971). Connectivity averaged 96% to S and FR cells and 87% to FF cells (Fleshman et al., 1981). A complete projection was predicted and has been shown to occur shortly after spinal cord transection (Nelson, Collatos, Niechaj and Mendell, 1979).

Within the cat MG motor pool, group II afferents connected monosynaptically to an average of 48% of their homonymous motoneurons. Therefore, they projected to fewer homonymous motoneurons than group I afferents (93%; Munson, Sypert, Zengel, Lofton and Fleshman, 1982).

These group Ia and II projections may be better explained by physical proximity than by species relations. When comparing the potentials evoked by a single group Ia or II afferent in one or adjacent ventral root(s), the amplitudes of the postsynaptic response of the population of fibers (PPSP) were largest when the entry level of the afferent and the recording level coincided. They were even larger if the conduction velocity of the afferent was large. Similarly, the size of the afferent fiber may govern in part, the distribution of active terminals on the motoneuron surface. The rate of rise of the PPSPs showed weak negative

correlations with the afferent conduction velocity. This suggested that the larger the afferent, the closer were its terminals to the cell body (Lüscher, Ruenzel, Fetz and Henneman, 1979; Lüscher, Ruenzel and Henneman, 1980).

Monosynaptic input from single afferents to homonymous motoneurons was also topographically weighted within the homonymous MG pool of the cat. Larger amplitude EPSPs were produced in large motoneurons (high rheobase) when the afferent and innervated motor unit were located close together in the muscle. Small motoneurons (low rheobase) received more equal input from Ia afferents located throughout the whole muscle (Lucas and Binder, 1984).

However on a cell to cell level, connectivity does not appear to be determined strictly. Averaging different Ia inputs to many motoneurons within a pool showed that the probability of a functional connection with a homonymous motoneuron was greater: a) the faster the conduction velocity or size of the afferent and b) the shorter the longitudinal distance between the entry point of the Ia afferent and the location of the motoneuron. Motoneuron size may also influence the probability of connections from group Ia afferents. When both the Ia afferent and the motoneuron were large, as determined by the afferent and motor axon conduction velocities, functional projection was complete (Clamann, Henneman, Lüscher and Mathis, 1985). Overall, Ia connectivity to the motoneuron pool appeared to be

probabilistic despite the ordered output of the pool (Henneman, 1985). Perhaps if all afferent inputs to the pool were considered, it may follow a more organized pattern.

1.8.1 Inputs organized for orderly motoneuron recruitment

Activation of Ia afferents by stimulation of the respective muscle nerves or by stretch of either the MG, LG, soleus, TA or extensor digitorum longus muscles of the cat evoked composite EPSPs in the respective homonymous motoneurons. The amplitudes decreased with input resistance or in the order S > FR > FF motor units (Burke et al., 1976; Dum and Kennedy, 1980b; Fleshman et al., 1981; Harrison and Taylor, 1981). This pattern is consistent with smaller or S cells being more excitable.

The amplitude of disynaptic IPSPs evoked in MG or TA motoneurons increased in the order S > FR > FF cells (Burke, Jankowska and Ten Bruggenate, 1970; Dum and Kennedy, 1980b). If there were the same number of contacts to cells of different sizes, one would expect this pattern of inhibition to be consistent with orderly motoneuron recruitment by size.

1.8.2 Inputs projecting equally to all motoneurons

Within the MG pool, the amplitudes of the EPSPs evoked by stimulation of group II afferents were unrelated to afferent conduction velocity, motoneuron rheobase or input resistance, motor unit tetanic tension or type. The projection was therefore similar to all cells whereas group

I afferents excite small or S cells more than large or fast (FR, FF) cells. (Munson, et al., 1982).

Both the vestibulospinal and ipsilateral medial longitudinal fasciculus tracts descend from the ipsilateral ventral quadrant. Stimulation of either tract produced monosynaptic EPSPs in type identified motoneurons with amplitudes that were unrelated to motor unit type (Burke et al., 1976; Dum and Kennedy, 1980b).

The amplitude of monosynaptic EPSPs evoked in cat MG, LG and soleus motoneurons can also be reduced via presynaptic inhibition by applying trains of conditioning stimuli to the posterior biceps femoris and semitendinosus nerves. In all motor unit types, there was a similar average percentage decrease in EPSP amplitude, although absolute decreases were largest in S cells and smallest in FF cells (Zengel, Reid, Sypert and Munson, 1983).

1.8.3 Inputs which are inconsistent with orderly motoneuron recruitment

In type identified cat triceps surae alpha motoneurons, motor axon diameter in the white matter and the number of axon collateral swellings (interpreted as synaptic terminals) which originated from motor axons, increased in order of soleus S < MG S < FR < FF units (Cullheim and Kellerth, 1978). Physiologically, antidromic stimulation of a heteronymous muscle nerve produced, via Renshaw cells, recurrent IPSPs (RIPSPs) in MG motoneurons. RIPSP amplitude

was greatest in S cells and smallest in FF cells (S > FR > FF) even after removing the effects of different input resistances. In view of the anatomical data, FF motoneurons were proposed to provide more excitation to the Renshaw pool yet receive the smallest effects (Friedman, Sypert, Munson and Fleshman, 1981).

Activation of cutaneous afferents in the sural and saphenous nerves by skin pinch or electrical stimulation were shown to evoke polysynaptic PSPs in MG or TA motoneurons. These potentials were composed of mixed excitatory and inhibitory components. The effects were primarily excitatory to fast twitch motor units and inhibitory to slow twitch motor units suggesting that the synaptic organization was qualitatively different to different motor unit types (Burke, 1967; Dum and Kennedy, 1980b; Kanda, Burke and Walmsley, 1977). Similarly, repetitive stimulation of the red nucleus caused predominantly IPSPs in slow twitch motor units and mixed or EPSPs in fast twitch motor units (Burke, 1967, 1970; Pinter, Burke, O'Donovan and Dum, 1982; Illert, Lundberg and Tanaka, 1976). Experiments involving these inputs have been proposed to show the potential for altered motoneuron recruitment. However, recruitment of motor axons was primarily ordered in the first deep lumbrical muscle of the cat's foot with either mechanical stimulation produced by pinching the plantar cushion of the foot or electrical stimulation of the

motor cortex. A minority of exceptions were common in pairs of units, although these reversals varied in an unpredictable way from time to time in the same cat and from cat to cat (Kernell and Sjoholm, 1975). Similar observations were observed in other cat muscles (Wyman, Waldron and Wachtel, 1974). In addition, the firing order of motoneurons depends on the balance of all the synaptic input to the motoneuron. Even though fractionation of inputs can change the critical firing level of a motoneuron (Clamann et al., 1983) it is unlikely to occur naturally.

Similarly, stimulation of extensor muscle nerves such as the quadriceps, flexor hallicus longus or FDL at group I afferent strength evoked PSPs in MG motoneurons. These potentials were mediated via oligosynaptic group Ib inputs and produced a pattern of PSPs similar to those evoked by sural nerve stimulation. That is, mainly inhibitory effects in S motoneurons and excitatory effects in F motoneurons (Powers and Binder, 1985a, 1985b).

1.9 Transmission failure and posttetanic effects

From the functional connectivity of group Ia and II afferents to homonymous motoneurons, a hypothesis relating to transmission failure and its relief at branch points has been proposed to relate the organization of the afferent input to motoneuron excitability (Luscher, Ruenzel and Henneman, 1979). Larger diameter Ia afferents produced EPSPs with a wide range of amplitudes. Smaller Ia afferents

produced small amplitude EPSPs. These data suggested that only large diameter afferents had enough synapses on a motoneuron to produce a large EPSP (Henneman, Luscher and Mathis, 1984; Clamann et al., 1985). In comparison, others have found Ia EPSP amplitude to be unrelated to afferent conduction velocity or size (Lucas, Cope and Binder, 1984). In the study showing correlations, the large amplitude EPSPs were evoked in the smaller motoneurons. It was proposed that the invasion of Ia terminals was a graded process that was normally more complete in small cells because of their fewer branch points. This produced larger EPSPs in these small cells making them more excitable.

Active and inactive synapses to motoneurons probably exist. Stretch activated Ia afferents evoked different shaped EPSPs in homonymous motoneurons when the membrane potential was changed from one level to another (Henneman et al., 1984). After tetanizing the MG muscle nerve in cats, the greatest potentiation of the averaged monosynaptic composite EPSPs occurred in the largest motoneurons. During tetanization, more synapses to large than small cells were assumed to be available for activation. This resulted in more post-tetanic potentiation (PTP) in large cells. The differential distribution of PTP to different motoneurons has been found by others (Lev-Tov, Pinter and Burke, 1983) and was proposed to result from a net increase in neurotransmitter release from a set of fully activated Ia terminals on a given motoneuron. However, these authors

point out that it is impossible to distinguish increased transmitter release from failure of action potentials to reach terminals when recording only from the postsynaptic cell. Depression or enhancement of Ia EPSP amplitude depended also on the frequency, duration or activation history of the conditioning tetanus.

1.10 Summary

The motoneurons within a motor pool vary in terms of their excitability, size, intrinsic motoneuron properties, motor unit type, force output and synaptic input. Motoneuron size as measured anatomically or by axon conduction velocity or cell capacitance is correlated to some extent, with all these other properties. Cell body size is smallest for S units and largest for FF units but overlap occurs between the three unit types. Therefore, there is some dissociation in the relationship between cell body size and motor unit type. The input resistance of large motoneurons is unexpectedly low and deviates from the linear relationship found between input resistance and cell size for smaller motoneurons. The force output of S and FR units is well matched to motoneuron size but that of FF units is larger than expected from the conduction velocity of these units. Similarly, the amplitude of the Ia EPSP evoked in S and FR motoneurons correlates well with axon conduction velocity. FF units have unduly small amplitude Ia EPSPs in comparison to their conduction velocities. This suggests a difference

in the presynaptic organization to these units or in the intrinsic membrane properties of the motoneurons.

Afferent input systems to the motoneuron pool are either graded to match the excitability of the motoneurons, project equally to all motoneurons or primarily excite fast. (FR, FF) motoneurons and inhibit S motoneurons. That motoneuron recruitment is predominantly ordered suggests that it is strongly influenced by the input from monosynaptic Ia afferents or other inputs that are correlated with cell size. From the group Ia and II connections to homonymous motoneurons, afferent input was proposed to be organized for size ordered motoneuron recruitment according to a transmission failure and relief hypothesis. It proposes that there is a graded invasion of the synaptic terminals to motoneurons which is more complete in smaller cells because there are fewer branch points in the terminations to these cells. This results in the smaller cells being more excitable.

Although a minority of reversals of motoneuron recruitment seem to occur in most recruitment studies, their occurrence is often unsystematic, between units with similar thresholds and may be attributed to tissue damage during nerve dissection. Thus, recruitment has been shown to be ordered for the most part, by motoneuron size and strictly ordered by increasing contraction strength (tetanic tension). Tetanic tension was correlated with motor unit

type so recruitment was expected to occur by type as well. These results argue strongly against random recruitment of motor units within unit types. Strong but indirect evidence has been provided for orderly recruitment by graded intrinsic motoneuron properties.

1.11 The order of motor unit recruitment in human muscles

Using spiked triggered averaging, Milner-Brown, Stein and Yemm (1973b) extended these ideas of orderly recruitment by demonstrating that the threshold forces at which motor units in the first dorsal interosseous (FDI) muscle of humans were recruited during voluntary isometric contractions were linearly correlated with the twitch tension of the motor units. A weaker correlation was found between the surface EMG and threshold force of recruitment (Milner-Brown and Stein, 1975). Numerous studies have since confirmed the orderly recruitment of motor units in a variety of muscles for isometric contractions (Büdingen and Freund, 1976; Freund, Büdingen and Dietz, 1975; Goldberg and Derfler, 1977; Monster and Chan, 1977; Tanji and Kato, 1973; Yemm, 1977) slow and fast ramp contractions, ballistic contractions (Desmedt and Godaux, 1977, 1978), contractions elicited via the stretch reflex (Calancie and Bawa, 1986) and in patients with neuromuscular disorders such as amyotrophic lateral sclerosis, polymyositis and following pressure block of a nerve (Milner-Brown, Stein, Lee and Brown, 1981). Recruitment by motor unit type has not been

tested in human subjects because of the difficulties in physiologically typing motor units which are voluntarily activated.

Like animal studies, some human studies indicate that the recruitment order of motor units can be altered. The orderly recruitment of motor units has been shown to be disrupted during some fast twitch or ballistic type movements (Grimby and Hannerz, 1974; 1976), during the early phase of a non-facilitated stretch reflex (Hannerz, 1973), when proprioceptive afferents from the active muscles are blocked (Grimby and Hannerz, 1968; Hannerz and Grimby, 1979), when cutaneous afferents are stimulated electrically (Datta and Stephens, 1981; Garnett and Stephens, 1980; 1981) or by tactile stimulation (Kanda and Desmedt, 1983), when voluntary contraction of tibialis anterior is superimposed over reciprocal inhibition induced by the tonic vibration reflex evoked in the soleus (Bawa, 1981), when the soleus muscle is subjected to postural oscillation during normal quiet standing (Mori, 1973) and when conscious oscillations are introduced to a voluntary contraction of leg muscles (Cremer, Gregor and Edgerton, 1983).

Two other situations in which recruitment reversals have occurred in humans are particularly interesting because they involve activation of motor units in relatively normal movement patterns. When a muscle produced movement in more than one direction or contracted as a synergist rather than as a prime mover some examples of recruitment reversals

between pairs of motor units have been demonstrated (Desmedt and Godaux, 1981; ter Haar Romemy, Denier van der Gon and Gielen, 1982, 1984; Thomas, Schmidt and Hambrecht, 1978). The results of these studies suggest that there could be a distinctly different order for recruiting motor units during muscle contractions in different directions. This possibility was assessed in this thesis by examining the recruitment order of motor units in the FDI and abductor pollicis brevis (APB) muscles during different directions of isometric contraction.

Variable and orderly motor unit recruitment has also been reported during some functional tasks (Eriksson Stålberg and Antoni, 1984; Grimby, 1984; Mariani, Maton and Bouisset, 1980; McClean, 1984; Smith Zimmerman and Abbas, 1981; Sussman, Macneilage and Powers, 1977). In these studies, recruitment of motor units has been examined using criteria such as spike amplitude, displacement of a body part and discharge pattern. Using any one of these criteria to identify a given motor unit by size has been shown to have serious drawbacks (Hoffman and Luschei, 1980; Monster and Chan, 1977; Olson, Carpenter and Henneman, 1968) and may explain the inconsistent results of these studies. A more direct assessment of recruitment order within a muscle according to motor unit size during a functional task is required and was accomplished in this thesis.

Another apparent exception to the orderly pattern of

recruitment in humans, is the random recruitment of motor units in reinnervated muscle after complete severance and resuture of the ulnar nerve (Milner-Brown, Stein and Lee, 1974). Yet, when the nerve to a single muscle is cut and resutured in the cat, orderly relationships between motor unit properties were re-established over a period of months (Gordon and Stein, 1982). One important difference between the animal and human situations is that, at the wrist, the ulnar nerve branches extensively to innervate many intrinsic hand muscles. Consequently, the regenerating motor axons could reinnervate their original muscles or inappropriate ones with different functions.

In studies of regeneration in rat muscles, motor axons appear to show no specificity to innervate their original muscles (Gillespie, Gordon and Murphy, 1986; Miledi and Stefani, 1969). Misdirection of motor axons does appear to occur after facial nerve injuries involving regeneration and has been proposed as one explanation for the mass movements of facial muscles after such injuries (Ford and Woodhall, 1938; Freuh, 1983; Kimura, Rodnitzky and Okawara, 1975). Similar suggestions have been assumed to explain the poor motor control observed clinically after peripheral nerve section and repair (Esslen, 1960; Ford and Woodhall, 1938). This proposal has been addressed in this thesis, by studying the pattern of motor axon reinnervation and motor unit recruitment after complete section and resuture of the ulnar or median nerve at different levels in the human forearm.

Even though there is no suitable test for the return of voluntary function in animals, it is possible to test directly how misdirection of motor axons from many motor pools to foreign muscles affects the relationships between nerve and muscle properties and their function. In this thesis, two common nerves containing axons to many hindlimb muscles were cross-united in young cats. The axons from many motor pools were therefore misdirected to foreign muscles. The function of the cross-reinnervated muscles was examined during locomotion. In acute experiments, the reorganization of the relationships between motor unit properties in the reinnervated extensor muscles was examined.

To summarize, three apparent exceptions to orderly motor unit recruitment in humans are investigated in this thesis. These include motor unit recruitment order during voluntary isometric contractions in different directions, during functional joint movements and in reinnervated muscles after complete section and resuture of a peripheral nerve. As disorderly motor unit recruitment in reinnervated human hand muscles may result from misdirection of motor axons to foreign muscles, the effects of misdirecting motor axons to inappropriate muscles on muscle function and motor unit properties was tested directly in cats after cross-union of two common nerves in one hindlimb.

1.12 Specific aims

The purpose of this thesis was to investigate:

1) the order for recruiting motor units in the first dorsal interosseous (FDI) and abductor pollicis brevis (APB) muscles of normal human subjects during isometric voluntary contractions in different directions.

2) the order of motor unit recruitment in the FDI and APB muscles of normal human subjects during functional joint movements.

3) the pattern of motor unit innervation and recruitment in reinnervated human hand muscles after severance and resuture of either the ulnar or median nerve at different levels in the forearm.

4) the function of cross-reinnervated hindlimb muscles of the cat during locomotion. Motor axons were deliberately misdirected to antagonistic muscles by cross-union of the common nerves innervating all of the flexor (common peroneal; CP) and extensor (tibial) muscles of the distal hindlimb of the cat.

5) the peripheral reorganization of the motor units in cross-reinnervated triceps surae muscles of the cat hindlimb.

CHAPTER 2

MOTOR UNIT RECRUITMENT IN HUMAN FDI AND APB MUSCLES DURING ISOMETRIC CONTRACTIONS IN DIFFERENT DIRECTIONS

The combined contraction of a number of muscles in different synergistic patterns can produce net movement in different directions. Some human studies have demonstrated that the recruitment threshold or the level of force at which a motor unit just begins to fire repetitively is influenced by the direction of contraction (Desmedt and Godaux, 1981; ter Haar Romeny et al., 1982; 1984; Thomas et al., 1978). For example, during flexion as compared to abduction of the first dorsal interosseous (FDI) muscle, alteration of recruitment threshold occurred in 8% of the motor unit pairs (Desmedt and Godaux, 1981). As many as 50% of the motor unit pairs studied in the abductor pollicis brevis (APB) muscle showed altered recruitment thresholds with different directions of contraction (Thomas et al., 1978). These results suggested the potential for reversals in rank order of recruitment and that there could be a distinctly different order for recruiting motor units during contractions in different directions. Alternatively, is there only a certain amount of variability in the recruitment thresholds for different directions of contraction? If a unit is activated preferentially in a contraction in one direction, are its muscle fibers so organized that it contributes more force to this

contraction? The present study was designed to answer these questions by studying the recruitment order and the twitch tension of human FDI and APB motor units in different directions of contraction.

2.1 METHODS

Spike triggered averaging (Stein, French, Mannard and Yemm, 1972) was used to examine the relationship between the twitch tension amplitude and recruitment threshold of 144 motor units in the FDI muscle of 4 volunteers during three different contractions: abduction of the index finger, flexion of the index finger and adduction of the thumb coupled with flexion of the index finger, hereafter referred to as adduction (Figure 2.1). The methods were repeated to obtain data from 48 motor units in the APB muscle of 2 volunteers during isometric abduction and opposition contractions of the thumb.

2.1.1 Muscle actions

The FDI muscle acts alone during abduction of the index finger, acts as a synergist with the flexor digitorum longus muscle during flexion of the index finger, and cooperates with the adductor pollicis and flexor digitorum muscles during flexion of the index finger coupled with adduction of the thumb (Netter, 1969). Our observations confirm that, despite the attachment of this muscle to the first metacarpal, it does not contribute to adduction of the thumb without flexion of the index finger to produce a pinch

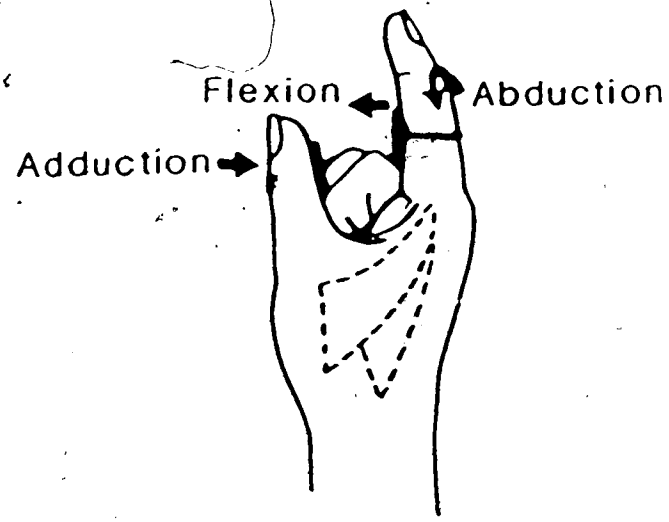

Figure 2.1 Schematic diagram of the hand position used for recording from the FDI muscle. The thumb and index finger were held in a comfortable open-handed position while the lateral three fingers gripped a pistol-like handle. The dashed lines indicate the approximate location of the FDI muscle between the first and second metacarpals. The custom built force transducers mounted at right angles to each other were positioned against the anterior and lateral aspects of the proximal interphalangeal joint of the index finger to respond to forces during abduction and flexion. The Grass strain gauge was positioned against the interphalangeal joint of the thumb to respond to adduction of the thumb. The position of the force transducers is indicated by solid bars while the direction of contraction is indicated by arrows.

between the thumb and index finger (Netter, 1969; see Chapter 4). It should be noted that a natural pinch would involve flexion at a number of joints of the index finger and adduction of the thumb. The APB muscle acts with abductor pollicis longus during abduction and with opponens pollicis during opposition.

2.1.2 Motor Unit Recording

Selective recordings of single motor unit potentials were obtained by using pairs of fine wire bipolar electrodes similar to those described by Basmajian and Stecko (1962). Two insulated tungsten or stainless steel wires (25 μm) were inserted into a disposable 27 gauge needle. Each wire had its tip bared by 0.1 mm and was bent to form a 1.5-2.0 mm hook. All these electrodes were gas sterilized before use.

2.1.3 Surface EMG Recording

Surface EMG activity was recorded using either Beckman mini-potential electrodes or Grass Ag/AgCl electrodes positioned over the midline of the muscle 1-3 cm apart. A saline saturated plate electrode was strapped to the wrist as a ground. All unit and surface EMG activity was amplified, filtered (10-10,000 Hz) and recorded on FM tape (Honeywell 5600 C).

2.1.4 Tension Recording

FDI muscle. To minimize unwanted movement, the subject rested the lower forearm in a frame and had the arm strapped

down at the wrist. Subjects had their hands positioned as illustrated in Figure 2.1. This procedure kept the muscle at a relatively constant and reproducible length, but did not completely eliminate contributions of other hand muscles to the measured forces (see spike triggered averaging, p. 38). Abduction and flexion forces were measured by two custom built force transducers firmly mounted at right angles to each other. These transducers were constructed of aluminium bars 5 cm x 1 cm x 1 cm narrowed to 0.35 cm in the center where four strain gauges were attached. Each transducer had a minimum sensitivity of 1 mN and a maximum allowable force of 300 N. A Grass FT03 strain gauge with a maximum sensitivity range of 1 mN - 20 N and a displacement of 0.5 mm/N was used to measure the adduction forces.

APB muscle. The dorsum of the hand rested against a wooden block. The hand was held in place by a strap across the palm. The custom-built force transducers described above were positioned on a clamp stand so as to register the action of the APB muscle. One transducer registered the abduction contraction while the other transducer the opposition contraction.

All outputs from the strain gauges were both A.C (bandwidth 0.1 - 100 Hz) and D.C. (bandwidth 0 - 50 Hz) coupled from the bridge amplifiers. D.C. forces were used to measure recruitment thresholds and were recorded on magnetic tape while A.C. forces were used for spike triggered averaging of the twitch forces.

2.1.5 Force threshold

The force required to voluntarily recruit the unit and fire it repetitively during the early part of each contraction was read from a calibrated digital voltmeter connected to the output of the appropriate force transducer.

2.1.6 Spike Triggered Averaging

Twitch tensions and contraction times of individual motor units were obtained by using the spike potential of each motor unit to trigger a PDP 11/34 computer programmed to average the forces and the EMG associated in time with the potential of that unit. If the impulses of other motor units occur randomly relative to those of the unit being studied, averaging will extract the tension and EMG changes produced by the unit from the overall force and EMG fluctuations in the whole muscle. The method depends on:

a) the accurate identification and discrimination of the motor unit potentials. The characteristic shapes of single motor unit potentials were observed to change with changes in muscle position. Also, the spikes would often decrease in amplitude when they first began to fire but with repetitive firing, their amplitude became constant. Averaging was started after the unit had been firing for several seconds, and the unit potentials had become stable. To ensure that the same unit was being recorded from during each contraction, the unit potentials were monitored from both

electrodes as the direction of force was gradually changed.

Using an electronic window discriminator (Bak Electronics Model Dis 1) units were selected which exceeded a threshold voltage and passed through a window that could be set at a variable delay position and with a variable aperture. An acceptance pulse was then generated to trigger the computer averaging program. The discriminated potentials were also used to trigger a storage oscilloscope and then displayed after passing through an appropriate delay line. The waveforms of the accepted spikes should superimpose. Any failure to do this, revealed inaccurate discrimination. With changes in the direction of exerted force, different units were often accepted by the window discriminator. Therefore, slight adjustments in the settings of the window discriminator were essential to maintain accuracy of the triggering during the different contractions.

b) the asynchronous activity of the motor units. If other motor unit activity is time locked to the activity of the discriminated motor unit potential, the force and EMG contributions from these units will be averaged and distort the twitch profile of the discriminated unit. Synchronized firing of motor units could be observed by monitoring the unit potentials from both needle electrodes on a storage oscilloscope, but was best identified by comparing the averaged unrectified and rectified EMG (Milner-Brown, Stein and Yemm, 1973a). If the discharge of several units tended to be synchronized, the averaged rectified EMG would be

broader and larger than expected from the unrectified EMG and the ongoing unit activity. Thus, even though the absolute value of the contraction force may be contaminated to some extent by forces from other muscles, the motor units were confined to the muscle being recorded from. The averaged motor unit twitch tensions will not be affected appreciably by contractions of other muscles unless the activity of these synergistic motor units is time locked to the unit being averaged. Data showing synchronization by this method were excluded from further analysis. Some synchronization of FDI motor unit activity has been observed with cross-correlation histogram techniques (Datta, Fleming, Hortbagyi and Stephens, 1985). However, it was unclear from this study how much this would contribute to the force generated by spike triggered averaging.

c) the ability of subjects to activate the unit at a low steady frequency. During averaging, the twitch amplitude and rise time is underestimated if partial fusion of the twitches occurs. Distortion of the twitch profile during averaging depends mainly on the contraction time of the motor unit being studied and an inability to record spike trains of low enough frequency to avoid partial twitch fusion (Andreasson and Bar-on, 1983; Calancie and Bawa, 1986). At a given firing frequency, the longer the contraction time, the greater the twitch fusion and resulting distortion. Consequently, the effect is greatest

on slow contracting units which typically generate small twitch tensions. To minimize distortion of the twitch profile, the subjects were asked to maintain the motor unit firing frequency at a low steady rate (8-10 Hz) by listening to the sound produced when the spike trains were fed to a loudspeaker and by observing the instantaneous rate displayed on a storage oscilloscope from an interspike frequency converter (Bak Electronics). Since there were no marked differences in the firing frequency or the contraction time of a unit in each of the contraction directions, it is unlikely that nonlinear addition of twitches was a significant factor in accounting for the different twitch tensions generated in the different directions of contraction.

2.1.7 Procedure

All of these experiments were conducted with the informed consent of the subjects. For each recording session, two electrodes were inserted 0.5-1.0 cm into the belly of the FDI or APB muscle approximately 0.5-1.0 cm apart. Because multiple insertions were necessary, the site of the insertion was varied so as to obtain a widespread sample of the population of motor units and to minimize neuromuscular damage. The subjects contracted their FDI or APB muscle in any of the contraction directions while the window discriminator was adjusted to identify one unit. When a unit was triggering accurately two averages of each unit

were completed for each direction of contraction:

a) 20 sweeps to average the shape of the unit potentials.

b) 500 sweeps to average the twitch tension of the motor unit and the associated unrectified and rectified EMG.

With higher threshold units or units which were difficult to activate repetitively, it was sometimes necessary for the subject to pause during the averaging when the force threshold increased due to probable fatigue of the unit. However, because the threshold force for each unit was relatively consistent during the beginning of each contraction, it was used as a further criterion of accurate unit identification when the subject resumed contraction following a pause.

Various units were subsequently identified and averaged during the different contractions. Each subject was free to choose the order of the contractions for the averaging of each unit but this varied from unit to unit in order to randomize any effects of fatigue on the measured force.

Approximately 10% of all units initially selected for study could not be accurately identified and discriminated during the different contractions because of technical problems. Either the triggering was unreliable or small changes in potential shape made unit identification difficult in one or two directions. Sometimes during an average, a unit would stop firing without any perception of fatigue or obvious drop in tension as the fraction of the total tension being generated by one unit would be very

small. That same unit would often resume firing some time later. With practice, the subjects became better at activating the units in the different directions even though it still often required a gradual transition from one contraction to another. No evidence was found among the units for a separate group of units which were preferentially activated in only one or two directions.

2.1.8 Analysis of Data

By recording the motor unit potentials and acceptance pulses on FM tape, the accuracy of the unit discrimination could be checked, when necessary, by retriggering from the recorded signal. If the retriggered acceptance pulses did not match the recorded acceptance pulses, the data were eliminated from further analysis.

Graphs of twitch tension as a function of threshold force or contraction time were plotted on logarithmic scales from the pooled data of each subject for each of the different contraction directions. Similarly, the twitch tension and threshold force data for each contraction direction were plotted against data for other contraction directions. All data were fitted with straight lines according to a least mean squares criterion (Sokolnikoff and Redheffer, 1958). Since the errors were comparable on the x- and y-axes, the line chosen to fit the data was one with a slope which represented the geometric mean between the lines and which would minimize deviations in the x- and the y-axes (see

Figure 2.2B and details in Results).

Although it is possible that single motor units were recorded from more than once with repeated experiments, this has not unduly biased the results. Data obtained from only one recording session showed the same trends as the pooled data.

In order to compare the recruitment order of the motor units during each contraction, the threshold data were ranked from the smallest to the largest threshold unit. The rank orders for the different contraction directions were plotted against each other.

Data from all subjects and the FDI and APB muscles followed similar trends. As most data were obtained from two subjects in the FDI and APB muscles, these data appear in the figures. Some data from each muscle serve as control data in Chapter 4.

2.2 RESULTS

Figure 2.2A shows spike triggered averages of the force generated by a FDI single motor unit when the subject activated the unit during the three different contractions. Note that the time course of the contraction is similar in all three contractions, but the magnitude of the force is greater in the abduction contraction in which the muscle is the prime mover than in the flexion or adduction contractions in which it acts as a synergist (Figure 2.2A).

Figure 2.2B shows a number of units from one subject in

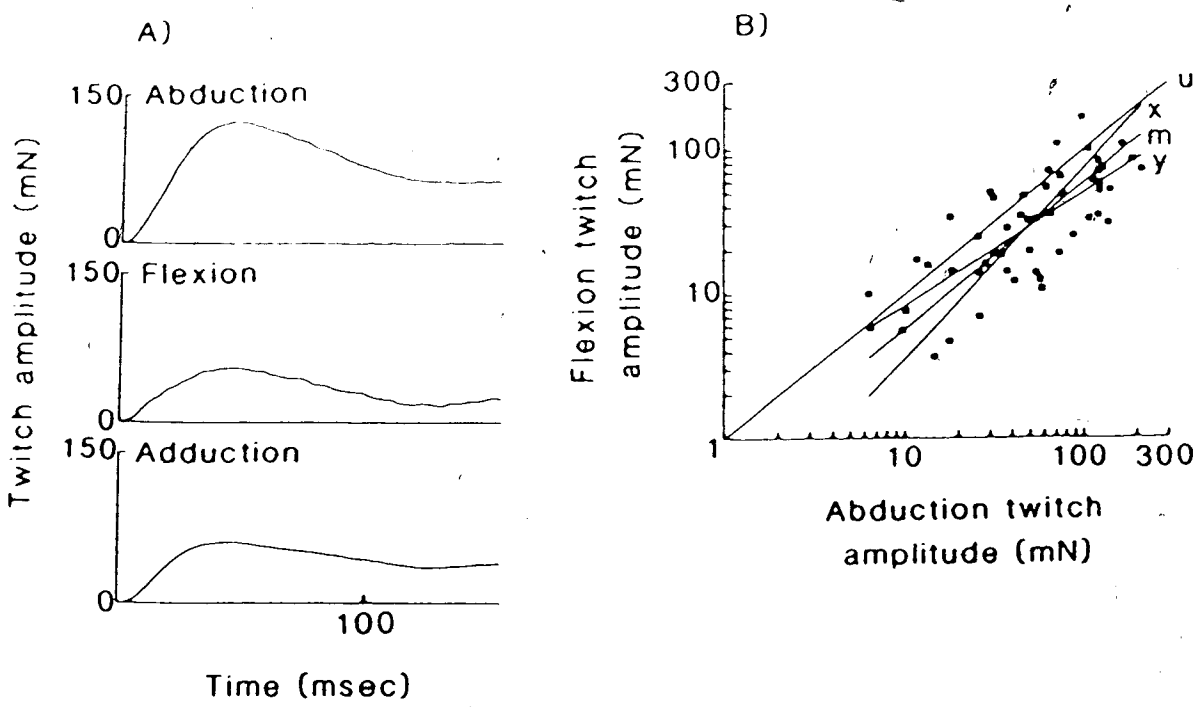

Figure 2.2 Twitch profiles (A) of a FDI single motor unit generated by spike triggered averaging during the contractions of abduction, flexion and adduction. In B, flexion twitch tension is plotted against abduction twitch tension for one subject. Twitch amplitudes were generally greater during abduction than during flexion as the majority of points fell below the line of unit slope (u). Also drawn are lines that minimize the deviations in the x or y directions and the line whose slope is the geometric mean (m) of these two. As comparable errors occur in both the x and y directions, the "m" line was considered the line of best fit and was plotted in subsequent figures when the relationships were significantly different from zero at the 5% level of confidence.

which spike triggered force averages were generated during the abduction and flexion contractions. Four lines are shown. The line labelled "u" is one of unit slope which would be followed if units generated the same force during both contractions. Since the data mainly fall below the line, this confirms for the whole population of motor units that forces were generally greater during the abduction contraction than the flexion contraction.

The other three lines all pass through the geometric mean of the data, which had values of 47 mN for abduction tension and 28 mN for flexion tension. The corresponding arithmetic means for this subject were 64 mN and 40 mN. With either measure, the flexion tensions of the motor units were approximately 60 % of the abduction tensions. The lines minimizing the deviations in the x- and y- directions are plotted. Errors will of course occur in both directions so the most appropriate line was the one labelled "m" because its slope is the geometric mean of the x and y lines. This is approximately the line one would tend to fit by eye and is used throughout this paper.

Figure 2.3 illustrates the significant positive correlations found between twitch tension amplitude and recruitment threshold force for abduction, flexion and adduction of the FDI muscle and abduction and opposition of the APB muscle. The slopes of these relationships were similar for all three directions of contraction of the FDI muscle (0.94 $\pm$ 0.10, for abduction; 0.88 $\pm$ 0.13, for

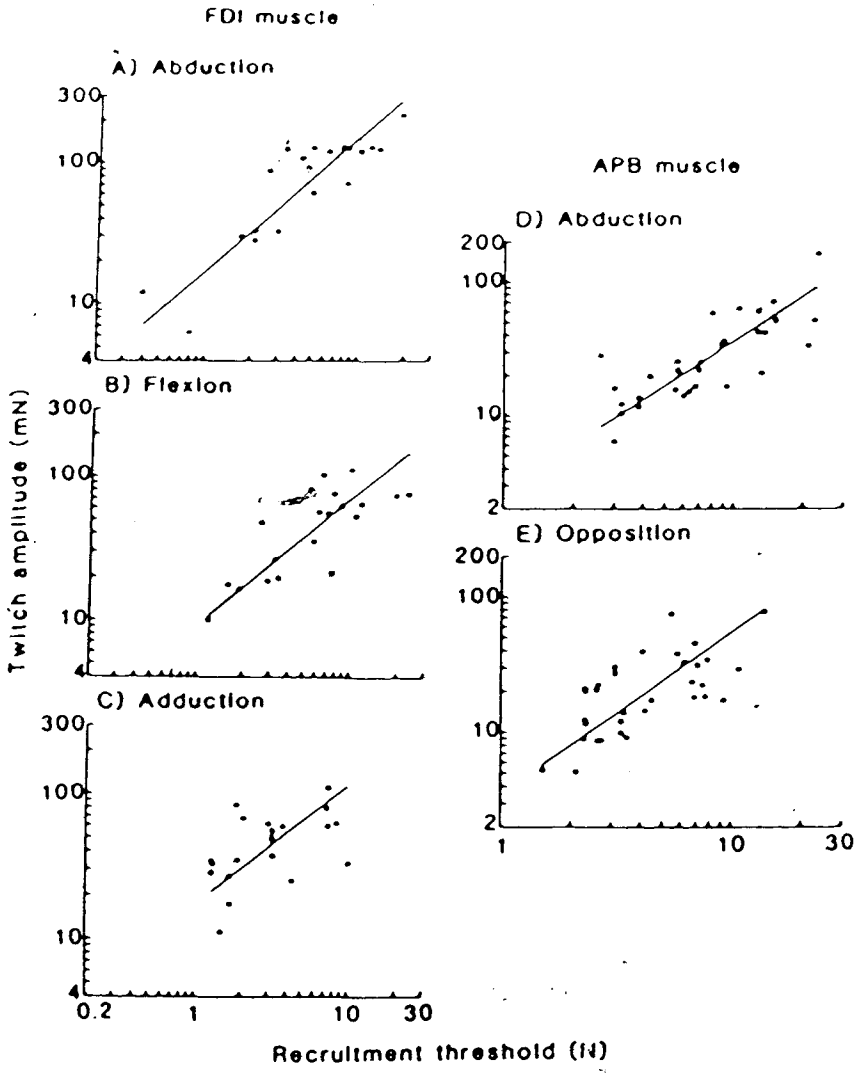

Figure 2.3 Twitch tensions of motor units from two recording sessions from one subject plotted as a function of the recruitment threshold of the units during the contractions of abduction (A), flexion (B) and adduction (C) of the FDI muscle and abduction (D) and opposition (E) of the APB muscle.

flexion; 0.82 ± 0.16, for adduction, mean ± S.E.) and the two contraction directions of the APB muscle (1.09 ± 0.13 for abduction; 1.19 ± 0.16 for opposition). These data show that motor units which produce large twitch tensions are recruited at higher force thresholds than motor units which generate small twitch tensions. Thus motor unit recruitment appears to be ordered by increasing twitch amplitude (size) in the FDI and APB muscles irrespective of the direction of the isometric contraction.

For each muscle, there were negative correlations between contractile speed and unit twitch tension in each contraction direction (Figure 2.4). The relationships were significant but weak. Units with slow contraction times therefore tended to generate small twitch tensions while those with fast contraction times generated larger twitch tensions.

In the FDI muscle, motor units were recruited up to 78 % of the maximal voluntary abduction force (54-78% for different subjects) which is greater than the relative motor unit thresholds reported by Milner-Brown et al., (1973b) but similar to those reported by Desmedt and Godaux (1981) and Young and Mayer (1981). The force thresholds at which motor units were recruited during flexion (49-64%) and adduction (14-45%) relative to maximum voluntary contraction were lower than those obtained during abduction. These results were expected as other muscles were likely to contribute to the maximum force that can be generated in both flexion and

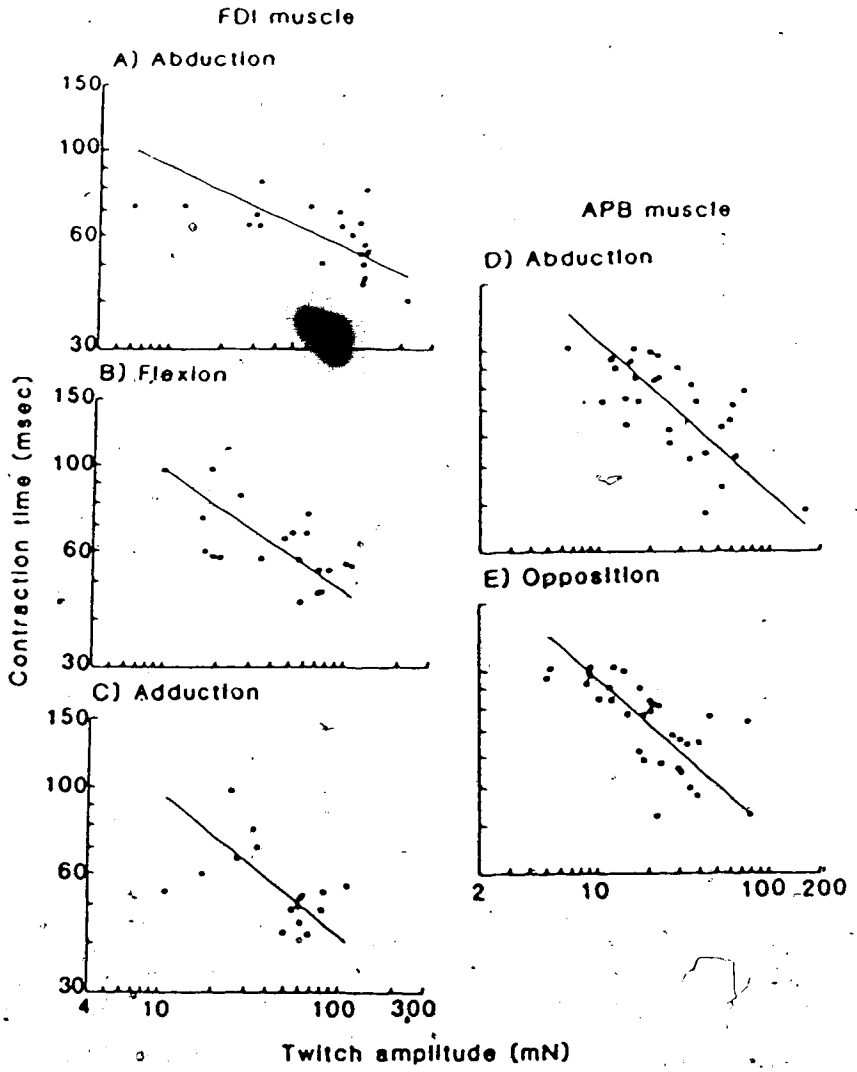

Figure 2.4. Contraction times of motor units from two recording sessions from one subject plotted as a function of the twitch tension generated during the contractions of abduction (A), flexion (B) and adduction (C) of the FDI muscle and abduction (D) and opposition (E) of the APB muscle.

adduction. In the APB muscle, these values ranged from 51-69% for abduction and 26-29% for opposition.

In terms of absolute values, the FDI flexion recruitment thresholds were generally greater than those in abduction as most points in Figure 2.5A lie above the line drawn at 45° to the axes. However, the reverse trend occurred for FDI units in adduction and abduction contractions (Figure 2.5B). In the APB muscle (Figure 2.5C), opposition thresholds were generally lower than abduction thresholds.

Figure 2.6 correlates by increasing recruitment threshold, the rank order of recruitment of motor units during the different contraction directions for each muscle of one subject. There was a significant positive correlation between the abduction and flexion or adduction ranks in the FDI muscle and between abduction and opposition thresholds in the APB muscle. Therefore, low threshold units in abduction were likely to be low threshold units in flexion, adduction or opposition respectively. The rank order correlation coefficients for the data presented in Figure 2.6 were 0.97, 0.88 and 0.88 for flexion, adduction and opposition versus abduction respectively. In the lower ranked units, the data were more scattered for adduction and opposition. Thus, although the recruitment order in abduction is similar to that in flexion, adduction or opposition, it was not necessarily identical.

Frequency distributions for the FDI twitch tensions and recruitment thresholds of all units from all subjects are

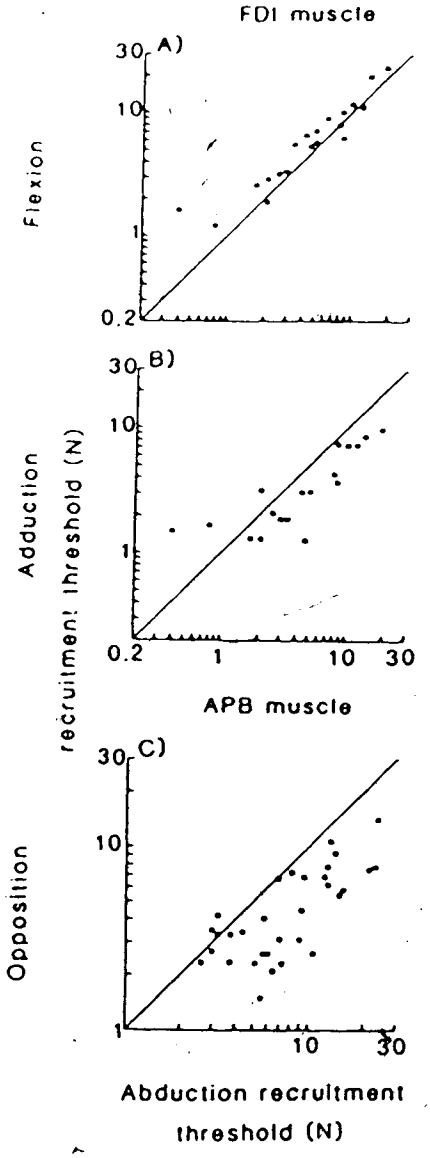

Figure 2.5. Flexion (A) and adduction (B) thresholds plotted against abduction threshold for the FDI muscle of one subject. In C, opposition thresholds from the APB muscle are plotted against abduction thresholds. Units with the same threshold for recruitment during both contractions lie on the line drawn at unit slope.

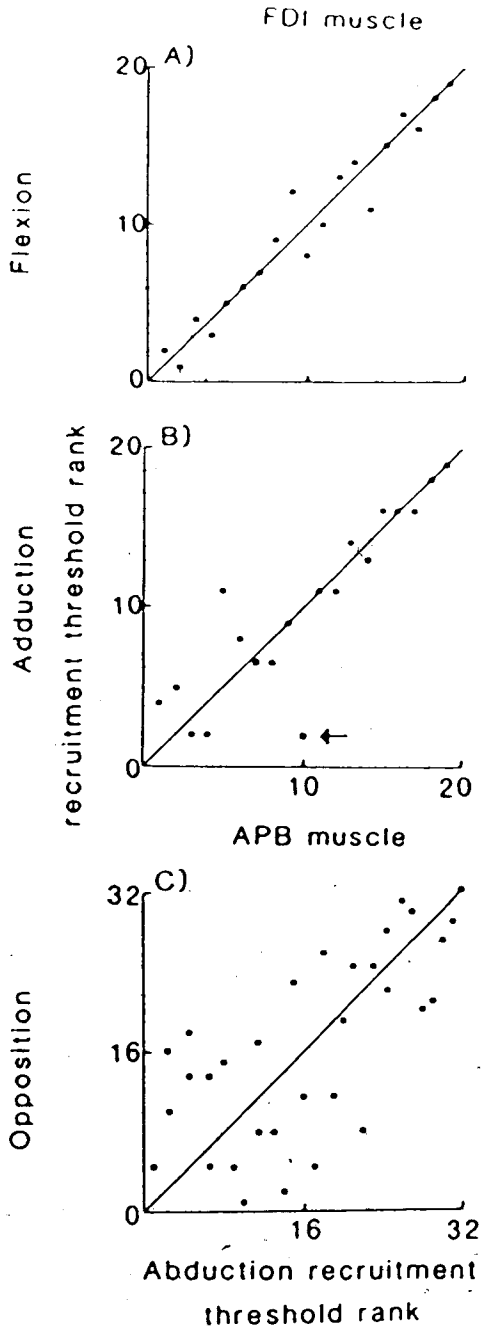

Figure 2.6. Unit rank in flexion (A), adduction (B) for the FDI muscle and opposition (C) for the APB muscle plotted against unit rank in abduction for the respective muscles of one subject. Units lying on the line drawn at unit slope have the same rank during both contractions and would presumably be recruited in the same order during each contraction.

plotted in Figures 2.7A and 2.7C. The distribution is skewed for each different direction of contraction and is similar to that reported by Milner-Brown et al., (1973b) for abduction. That is, most of the units studied had small twitch tensions (<30 mN) and low recruitment thresholds (<4 N). In contrast, the frequency histogram for unit contraction time (Figure 2.7B) shows a fairly symmetrical distribution. In the APB muscle of each subject, there were similar skewed distributions for twitch tension and recruitment threshold. The contraction time distribution was unimodal.

2.3 DISCUSSION

The recruitment of motor units in the FDI and APB muscles has been shown to be ordered according to increasing twitch amplitude (size) for each of the different directions of contraction. The units could not be physiologically classified by type, but recruitment by size cannot be distinguished from recruitment by type, because these two variables are correlated (Zajac and Faden, 1985). Nonetheless, these data extend the previous findings for the FDI muscle during isometric abduction contractions (Milner-Brown et al., 1973b; Desmedt and Godaux 1977; 1978) by suggesting that the orderly recruitment of motor units describes the function of the FDI muscle irrespective of whether this muscle acts alone as in abduction, as a synergist with flexor digitorum longus as in flexion, or

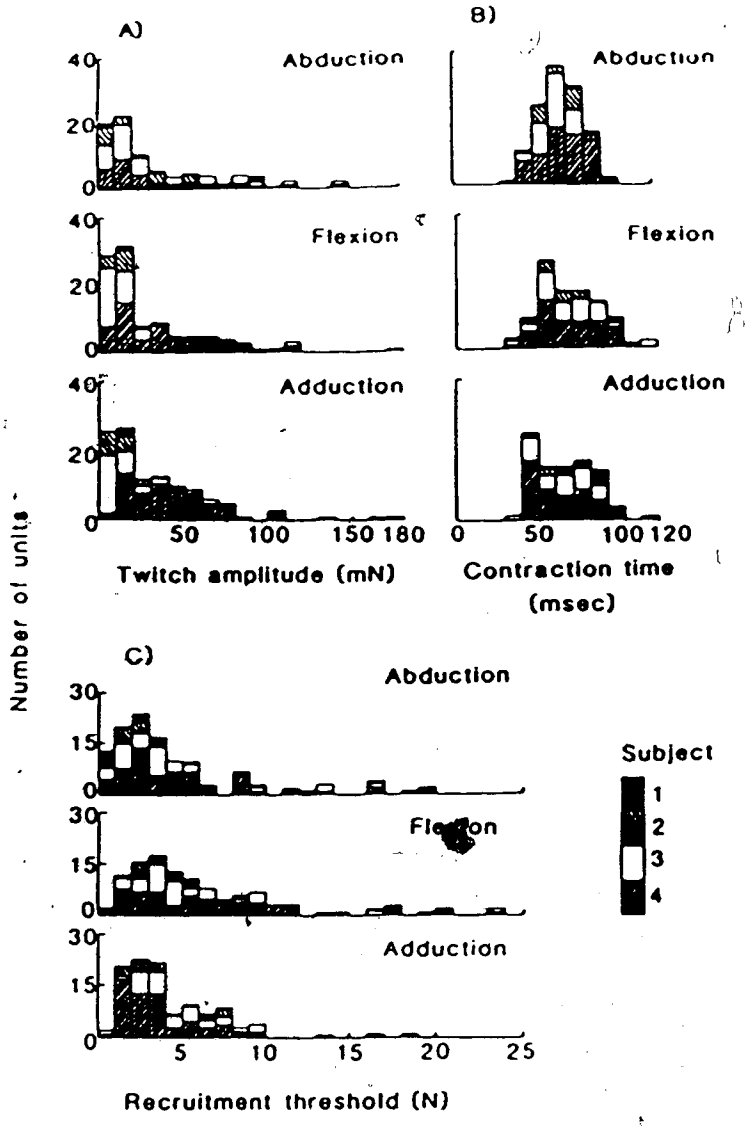

Figure 2.7. Histograms showing the distribution of motor units for the FDI muscles of all subjects with respect to their twitch tension (A), contraction time (B) and threshold (C) during the contractions of abduction, flexion and adduction.

cooperates during adduction of the thumb. The same conclusion can be drawn for the APB muscle during isometric abduction and opposition of the thumb.

Although the data do not show a marked alteration of motor unit recruitment in response to contractions in different directions (cf. Desmedt and Godaux, 1981; ter Haar Romeny et al., 1982; 1984; Thomas et al., 1978) the slight scatter in the FDI data (Figure 2.6A) would perhaps account for the small percentage (8%) of recruitment alterations with flexion as compared to abduction reported by Desmedt and Godaux (1981). The potential for rank reversals with different directions of contraction of the FDI muscle is more obvious from inspection of Figure 2.6B. For example, the unit marked with an arrow would be recruited second during adduction and tenth during abduction. Note, however, that there is a significant positive correlation in the rankings for all three directions of contraction. Therefore, the data indicates a very similar but not identical rank order of motor unit recruitment in the three directions of contraction. Similarly, the greater scatter in the APB data (Figure 2.6C) could reflect the variations in recruitment threshold during different directions of contraction in 50% of the APB motor units studied by Thomas et al., (1978). The motor units in that study were recruited up to forces of 5 N which is a relatively low value. These units could therefore represent those lower threshold units which showed the greatest scatter in our data.

In the FDI or APB muscles, no clear evidence was found to support selective activation of motor units for different tasks. Different groups of motoneurons in the cat sartorius muscle do function in different phases of locomotion (Loeb, Pratt and Marks, 1984). This suggests that task groups of motoneurons may only apply to muscles with wide insertions such that some fibers can act to produce different joint movements. If more than one task group was involved in these different muscle contractions reported here, then one would expect a large proportion of the identified motor units to be easily activated in one or two of the directions of contraction and either difficult (have a higher threshold) or impossible to activate in the other directions. The data showed no such preferential activation of motor units because each unit contributed tension in each of the different directions. One might argue that the 10% of motor units that could not be recorded from in different contractions represented those units which were preferentially activated in one direction or another. However, technical problems associated with accurate unit identification and discrimination could in the majority of cases account for the inability to obtain data from these units (see Methods).

Obviously, some of the differences between the rank order of recruitment of a given motor unit during each contraction may be due to errors inherent in the recording technique

Varying amounts of activity in synergistic muscles during contraction of the FDI or APB muscles may account for the differences in the thresholds of a particular unit with different directions of isometric contraction. In terms of force generation, the range of motor unit twitch tensions recorded from all of our subjects during abduction of the FDI muscle was within the ranges of values previously reported for that muscle (Milner-Brown et al., 1973b; Stephens and Usherwood, 1977; Young and Mayer, 1981). Each motor unit contributed approximately the same proportion of total force in the different contraction directions of the FDI muscle although the absolute values were generally greatest during the prime action of the muscle (abduction) as compared to flexion or adduction. Motor units in the APB muscle developed similar tension in both contraction directions. Presumably, the biomechanical organization of the muscle fibers allows force to be generated most effectively during the prime action of the muscle. Thus, with respect to the FDI muscle, flexion of the index finger and adduction of the thumb are likely to be more effectively accomplished by the flexor digitorum and adductor pollicis muscles respectively.

In relation to contraction time, the data from all subjects from either muscle did not clearly separate into two populations. In FDI, 70 msec has been used to separate fast from slow twitch motor units (Sica and McComas, 1971; Young and Mayer, 1981). Therefore, less than half our motor

units would be considered slow twitch. Histochemically (Johnson, Polgar, Weightman and Appleton, 1973), the FDI and APB muscles have been estimated to contain an average of 57% and 63% type I or slow twitch muscle fibers respectively. However, as innervation ratios have not been determined for units in these muscles, interpretation of physiological and histochemical data is complicated. Those cases in which subjects had to pause during averaging were indicative of recording from fatiguable motor units, as reported previously (Stephens and Usherwood, 1977; Young and Mayer, 1981). Although a similar number of impulses was required as that to test fatigue (Burke, 1971), the rate of unit firing was much lower in this study. The effects of fatigue should therefore be less marked. The distributions for twitch tension, threshold and contraction time of motor units in the FDI and APB muscles were similar to those previously reported for the FDI muscle during abduction contractions (Milner-Brown et al., 1973b). Overall, as the FDI data closely parallel the results of others, it was considered representative of the population of motor units in that muscle keeping in mind the mechanical limitations of recording from intact muscle (Calancie and Bawa, 1986). Therefore, measurement error alone does not account for the changes in rank order seen in the data.

In conclusion, the data show that recruitment by twitch amplitude (size) can provide a general description of motor

unit recruitment in the FDI or APB muscles during different directions of contraction. However, training (Duchâteau and Hainaut, 1981) and other factors may introduce some variability in the recruitment order of a motor unit during a given task.

CHAPTER 3

HUMAN MOTOR UNIT RECRUITMENT DURING ISOMETRIC CONTRACTIONS AND A FUNCTIONAL TASK

During functional tasks such as locomotion (Grimby, 1984), forearm flexion movements (Mariani et al., 1980), different lip displacements (McClean, 1984) or speech production (Sussman et al., 1977) motor unit recruitment has been described as orderly. Variability in motor unit recruitment has been described in different jaw movements or syllable repetition (Eriksson et al., 1984; Smith et al., 1981). In these studies, recruitment of motor units has been examined using several different criteria. These include: a) spike amplitude (Eriksson et al., 1984; Smith et al., 1981; Sussman et al., 1977), b) lip displacement (McClean, 1984) or c) discharge pattern (Grimby, 1984; Mariani et al., 1980). However, using any one of these criteria to identify a given motor unit by size has serious drawbacks. For example, spike amplitude varies with the distance of the muscle fibers from the recording electrode (Olson et al., 1968). The force generated in a given direction with different lip displacements can vary widely (Hoffman and Luschei, 1980). Near recruitment threshold, the discharge pattern depends on the speed of contraction and relaxation (Monster and Chan, 1977). Therefore, a more direct demonstration of motor unit recruitment order according to size is required within a muscle during dynamic movements.

The purpose of the present study was to establish whether the apparent potential for rank reversals in recruitment order of motor units during isometric contractions of the FDI and APB muscles as reported in Chapter 2 carried over to functional joint movements.

3.1 METHODS

To assess the potential for altered motor unit recruitment, the recruitment order of pairs of motor unit in the FDI and APB muscles of 2 subjects was determined. The order was based on the relative time at which each unit of the pair fired during repeated scissors movements. To identify the size of these motor units and therefore their expected recruitment order during scissors movements, the recruitment threshold of each unit during an isometric contraction was compared to its twitch tension determined by spike triggered averaging.

3.1.1 EMG activity

Both fine wire (see Chapter 2) and bipolar needle electrodes constructed according to the method of Bawa and Tatton (1979) were used to detect motor unit potentials during the repetitive opening and closing of the scissors. The recording and amplification of all EMG activity was identical to that outlined in Chapter 2 except that the potentials detected by bipolar needle electrodes were filtered with a bandwidth of 300-10,000 Hz.

3.1.2 Scissors

To record the scissors movements, a 0-10 kΩ potentiometer was attached to the axis of a 15 cm pair of high quality steel scissors. The output of the potentiometer was recorded on magnetic tape for later analysis and displayed on a pen recorder to visually check that the repeated and unresisted opening and closing of the scissors was consistent in rhythm and amplitude.

3.1.3 Procedure

For each recording session, two fine wire electrodes or a single bipolar needle electrode was inserted (0.5-2.0 cm deep) into the belly of either the FDI or APB muscle of one of the subjects. The pair of fine wire electrodes were placed 0.5-1.0 cm apart. The actual site of insertion varied from day to day for each subject in order to sample a range of motor units from the population.

Initially the subject would repeatedly open and close the scissors so that motor units could be identified and accurately discriminated. The window discriminator was set to accurately and reliably trigger off just one of the motor units. All motor unit activity and the acceptance pulse from the window discriminator were then recorded on magnetic tape for up to two minutes of scissors movements. These data were later analyzed to determine the recruitment order of the unit pairs during the scissors movements. Each unit was then monitored during an isometric contraction and averaged for:

a) 500 sweeps to determine the twitch tension, contraction time and associated EMG of the unit.

b) 20 sweeps to obtain the shape of the spike potential.

A Grass FT03 force transducer was used to monitor the force from both muscles. Abduction or flexion of the index finger was used during the isometric contraction of the FDI muscle while abduction of the thumb was used for the APB muscle. Other motor units were isolated by adjusting the settings on the window discriminator. With a needle electrode, other units could also be recorded by repositioning or reinserting the needle in the muscle.

3.1.4 Analysis

For the isometric contraction data for both muscles, graphs of twitch tension as a function of threshold force or contraction time were plotted on logarithmic scales. They were fitted with straight lines to minimize the deviations according to a least mean squares criterion as described in Chapter 2.

To ensure that the same motor unit was discriminated during an isometric contraction and during the scissors movements, the shape of each previously identified unit was reaveraged from the taped scissors data. It was compared to that recorded during an isometric contraction.

On a pen recorder, a permanent record was made of the:

a) analog output of the scissors.

b) motor unit activity accompanying the repeated scissors

movements.

c) acceptance pulse from the window discriminator.

The actual recruitment order of the motor unit pairs during repeated opening and closing of the scissors could be determined from the temporal relationships of the acceptance pulses.

The time between the recruitment of each pair of motor units studied was then determined in up to 30 (range 5-30) repetitive scissors movements. In each scissor movement, the analysis of the firing interval was limited to:

a) unit activity that was phasic and so confined to either the opening or closing of the scissors (APB and FDI muscles, respectively).

b) the first occurrence of each spike (see Figure 3.2).

The taped data of the scissors movements and the motor unit activity were replayed at 1/16th the recording speed to a digitizing storage oscilloscope (Tektronix, 5D10). The record of motor unit discharge during each scissors movement was stored in turn. After the pair of motor units were visually identified by shape and amplitude, a cursor was positioned on the same prominent peak of each of the two potentials. The time interval between them was then recorded. The interval between spikes (in msec) was assigned a positive value when the units were recruited in order of increasing threshold, and a negative value when recruited in the reverse order. If the interval exceeded an arbitrarily defined value of 100 msec, the unit recruitment

order was only designated by sign. For each pair of potentials, the overall mean and standard deviation of the time intervals which were less than 100 msec were calculated for all the scissors movements analyzed, as well as the mean interval for those movements in which recruitment was ordered. In some cases, the activity of 3 simultaneously active single motor units could be reliably followed during scissors movements. For these units, pair-wise comparisons were made as above.

3.2 RESULTS

Figure 3.1 shows the recruitment threshold and twitch tension amplitude of the motor units identified in the FDI and APB muscles of one subject. Those units that were identified during scissors movements are plotted with closed symbols. These units had recruitment thresholds that were approximately 50% of the maximum recruitment threshold recorded from the population of motor units studied. Even so, there were significant positive correlations between twitch tension amplitude and recruitment threshold of just those identified in scissors movements or all the motor units during isometric contractions. These data confirm the findings that the recruitment of these motor units is ordered by twitch size during isometric contractions (see Chapter 2).

Figure 3.2 illustrates the actual discharge of 2 single units identified in the FDI muscle during a single cycle of

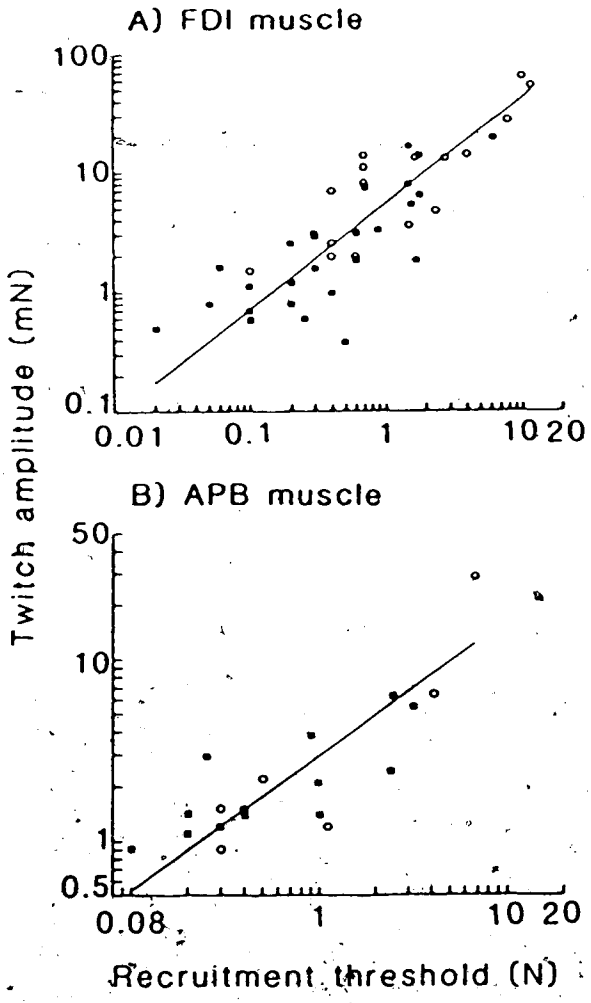

Figure 3.1 Twitch tension amplitude of FDI (A) and APB (B) motor units of one subject plotted as a function of the recruitment threshold of the units during isometric contractions. Those units also identified during scissors movements were plotted with closed symbols. Regression lines minimizing the deviations in both directions were drawn because the relationships were significantly different from zero at the 5% level of confidence.

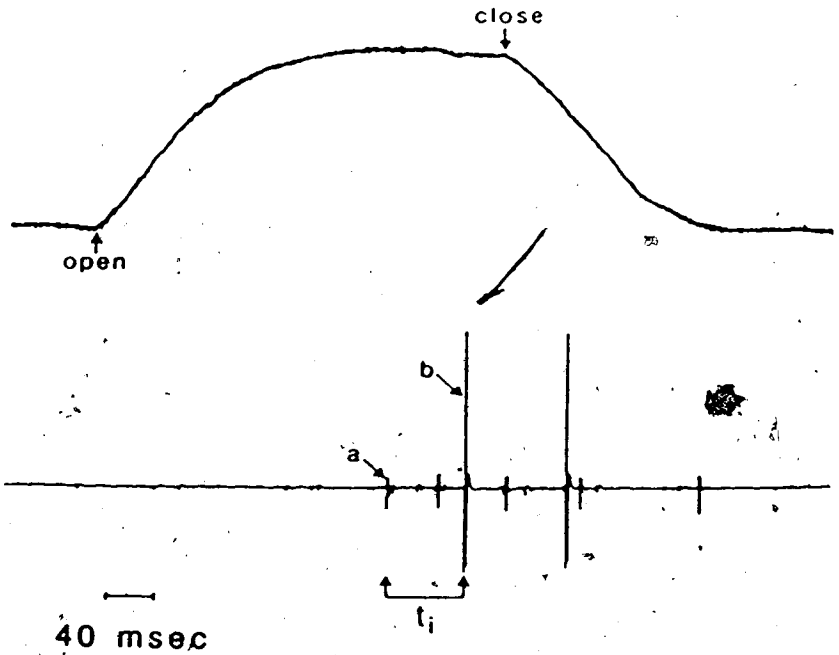

Figure 3.2 Discharge of two motor units identified in the FDI muscle during one opening and closing of the scissors. The lower threshold unit (1.6N) labelled "a" fired 66 msec before the higher threshold unit (6.3 N) labelled "b". The firing interval was therefore assigned a positive value. If the recruitment order had been reversed, the firing interval would have been assigned a negative value.

opening and closing the scissors. The recruitment threshold of unit "a" during an isometric contraction was 1.6 N, while unit "b" had a threshold of 6.3 N. The interval between the first occurrence of each spike (t_i) was 66 msec. Since the actual recruitment order of this pair was consistent with their respective thresholds measured during an isometric contraction, the interval was assigned a positive value. If the recruitment order had been reversed, the interval would have been assigned a negative value. Motor unit discharge during each scissors movement was analyzed in this way. Note that both units fire just before and during the closing of the scissors. Similar data were obtained from the APB muscle of each subject except that these motor units tended to fire well before the onset of scissors opening, and typically fired more times during a movement cycle than units from the FDI muscle. Presumably, the motoneurons are excited simply by the subject holding the scissors. As this requires some opposition of the thumb, the units fire well before the actual movement begins.

Figure 3.3 shows the variability in the data. It summarizes the overall firing interval ($\pm$ s.d.) between the recruitment of each pair of motor units in the FDI and APB muscles during the scissors task for one subject. The unit pairs were plotted, from top to bottom, in order of decreasing percentage of reversals. In those cases where no reversals were observed and the majority of intervals did not exceed 100 msec, the pairs were plotted in order of

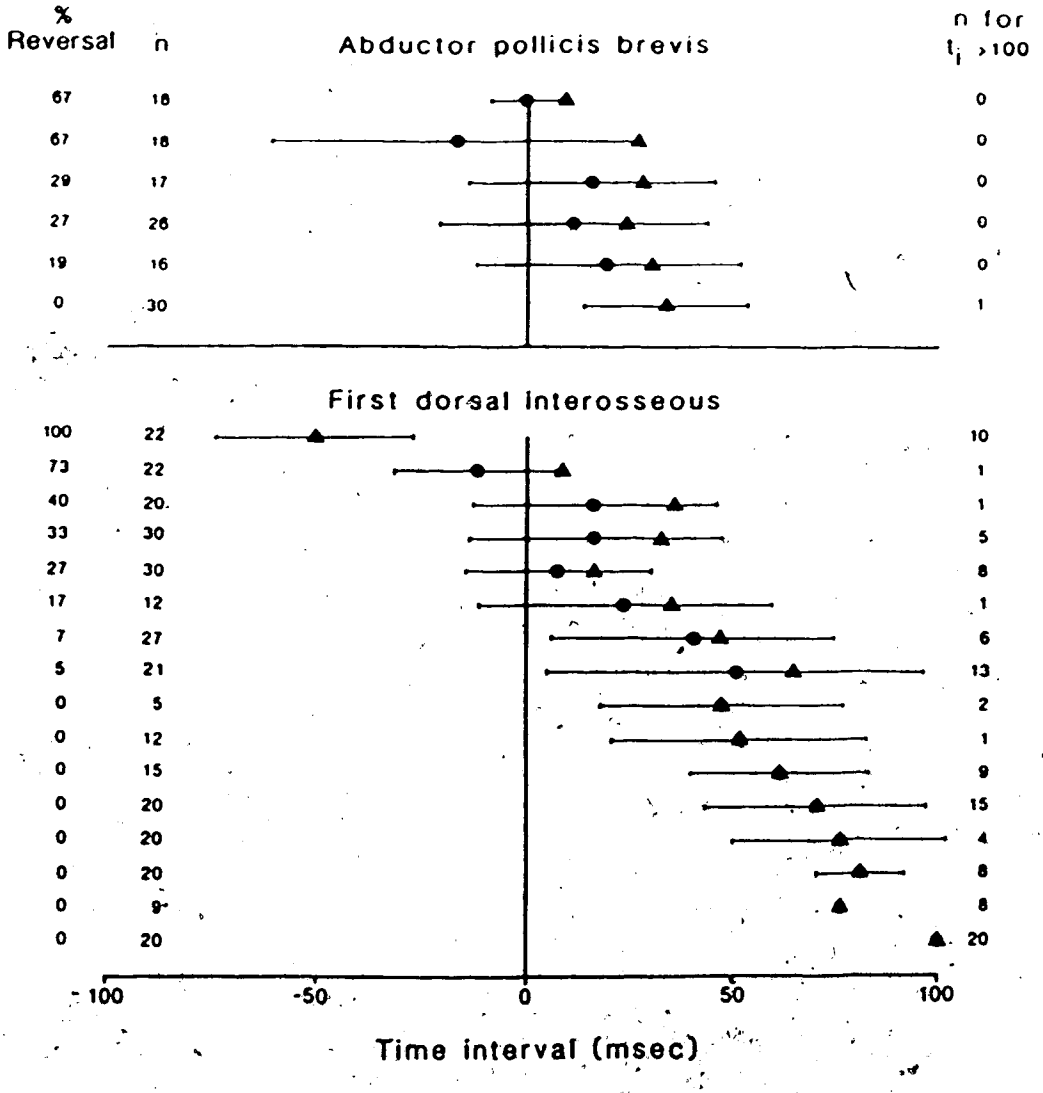

Figure 3.3 Plot of the mean (± s.d.) time interval between the first occurrence of all motor unit pairs recorded from the FDI and APB muscles of one subject. Up to 30 (range 5-30) scissors movements contributed to the overall mean (± s.d.) firing interval (●). The triangles (▲), represent the mean interval for all the scissors movements in which the unit pairs fired in order of increasing threshold. Listed to the left of the figure are the numbers of scissors movements analyzed and the overall percentage of recruitment reversals observed. At the right are the numbers of analyzed scissors movements in which the time interval between spikes exceeded 100 msec. In these, the recruitment order was only designated by sign.

increasing sample size. Plotted at the bottom, were those pairs where the majority of intervals exceeded 100 msec. The overall mean interval between the recruitment of the units is represented by the circles and the triangles represent the mean of the units that fired in order of increasing threshold. Thus, for those pairs in which recruitment was completely ordered, the overall and ordered mean firing intervals were the same. Only the standard deviation limits of the overall mean were plotted. For most unit pairs, these values were large. Differences in the velocity of the scissors movements may account for this large variability in the intervals between the recruitment of a unit pair (Desmedt and Godaux, 1981; Tanji and Kato, 1973), even though the subjects attempted to keep the scissors movements consistent in rhythm and amplitude. The percentage of recruitment reversals ranged from 0-100% and 0-67% in the FDI and APB muscles respectively as listed on the left of the graph. However, it should be noted that several motor unit pairs were always recruited in order of increasing threshold and only two unit pairs in each muscle showed recruitment reversals in more than 50% of the analyzed scissors movements.

Figure 3.4 shows the percentage of recruitment reversals recorded in all pairs of motor units during scissors movements from the two subjects. All motor unit recruitment thresholds were normalized to the highest threshold recorded for each muscle in each subject. Ten pairs of FDI motor

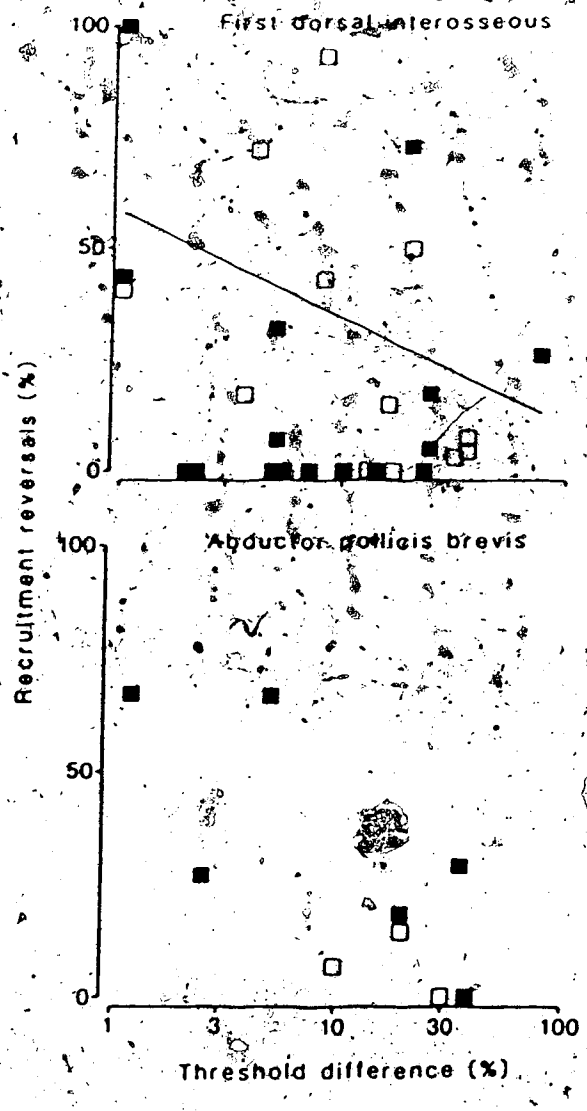

Figure 3.4 Percentage of recruitment reversals in all motor unit pairs in the FDI (A) and APB (B) muscles plotted against the threshold difference (% of maximum threshold of units identified during scissor movements). The different symbols represent data from different subjects. Up to 30 (range 5-30) scissors movements were analyzed for each pair of motor units. A regression line minimizing the deviations in the x and y directions was drawn only for those motor units in each muscle in which recruitment reversals occurred when the relationships were significant at the 5% level of confidence.

units (36%) with threshold differences ranging from 2-32% were always recruited in order of increasing threshold. In 14 unit pairs (50%), recruitment reversals occurred but the incidence was 50% or less. Therefore, only 4 unit pairs (14%) showed greater than 50% recruitment reversals. In the APB muscle, 2 unit pairs (22%, threshold differences 30-39%) always showed ordered recruitment, 5 unit pairs (56%) showed 50% or fewer recruitment reversals, leaving only 2 unit pairs (22%) with greater than 50% recruitment reversals. The motor unit recruitment during repeated scissors movements was therefore predominantly ordered by twitch size in both the FDI and APB muscles. In Figure 3.4, lines minimizing the deviations in both directions were fitted only to data from unit pairs that showed recruitment reversals. The occurrence was generally greater between unit pairs that had similar thresholds. A significant negative relationship was found in the FDI muscle. In the APB muscle, the sample was small. The correlation was negative but not significant.

3.3 DISCUSSION

The ordering of motor unit recruitment during isometric contractions has been shown here to be preserved, for the most part, during repeated scissors movements in both the FDI and APB muscles. For easier reading of the text, the data from the FDI muscle is now presented first. The APB data follows in brackets.

What could account for the observed alteration in the

"expected" recruitment order of the motor unit pair in 24% (26%) of the scissors movements recorded? Recruitment reversals observed between pairs of units in which: a) the firing intervals were 5 msec or less and/or b) the thresholds were within 5% of each other, could fall within experimental error.

During the scissors movements, five percent (8%) of the observed reversals occurred within 5 msec of each other. Fluctuations in the velocity of the scissors movements (Desmedt and Godaux, 1981; Tanji and Kato, 1973) or changing the load by some movement of the hand or wrist could account for differences in the time of recruitment of both units relative to the scissors movement. However, these changes would be expected to affect both units of the pair in the same way, and would not alter the recruitment order. An exception would be the case in which the axon of the higher threshold motor unit conducted impulses at a sufficiently fast rate that its action potential preceeded that of the low threshold unit, thus appearing to be recruited earlier at the motor unit level. Assuming a minimum conduction distance of 0.5 m from the motoneuron pool to the electrode recording site and a conduction velocity of 45 m/s, at least some of the "reversals" occurring within 5 msec of each other could simply be attributed to differences in axonal conduction velocities, and not to mechanisms occurring at the soma or dendrite level.

In unit pairs that showed recruitment reversals, the

occurrence of these reversals was generally greater when the difference in threshold between the pair of units was small. The recruitment threshold of a unit tended to show small fluctuations with slight changes in hand position, speed of movement or with time. Threshold differences which were 5% or more of the maximum recorded for units identified during scissors movements could be confidently distinguished. Therefore 13% (20%) of the recruitment reversals observed between unit pairs with threshold differences of less than 5% of maximum may have resulted from experimental error. In addition, the movement of the scissors on the fingers could influence the size ordered recruitment of motor units as variations in tactile activity of the fingers can alter recruitment order (Kanda and Desmedt, 1983).

Taking the extreme case, the observed reversals that occurred between pairs of units with similar thresholds and small firing intervals could be pooled. In this case, 15% (21%) of the reversals seen in this study could be accounted for. It is unlikely that the remaining 9% (5%) of reversals were just the random changes that may occur between units of very similar size. In these data were 2 FDI motor unit pairs that showed greater than 50% recruitment reversals. These 2 pairs alone contributed to 6% of the observed reversals recorded in that muscle. Thus, the remaining 3% (5%) of reversals were scattered amongst the other motor unit pairs.

This data could therefore support previous human studies

that have monitored predominantly "orderly" motor unit activity during dynamic movements (Grimby, 1984; Mariani et al., 1984; Sussman et al., 1977). However, these studies have used spike amplitude, lip displacement or firing pattern as recruitment threshold criteria, none of which can reliably indicate recruitment by motor unit size. The measurement of recruitment threshold and twitch tension for each unit in this study allows a better determination of the extent of the reversals of motor unit recruitment by size during repetitive movements. Scatter in data recorded during isometric contractions may be a good reflection of the potential for these recruitment reversals, especially between low threshold units, which usually have closer thresholds.

In conclusion, there is a strong relationship between motor unit size (twitch amplitude) and recruitment order during isometric contractions which is largely preserved during a functional task. Recruitment reversals during repeated dynamic movements were most common between units with similar thresholds and occurred unpredictably in subject to subject during different recording sessions.

CHAPTER 4

PATTERNS OF INNERVATION AND MOTOR UNIT RECRUITMENT IN REINNERVATED HUMAN HAND MUSCLES AFTER COMPLETE ULNAR AND MEDIAN NERVE SEVERANCE AND RESUTURE

If we want to tie a shoelace, write a letter, eat or play a musical instrument, we are confronted with the need to control our hand muscles precisely. Our ability to co-ordinate these and many other fine movements is generally assumed to result from direct corticospinal connections to the motoneurons innervating the hand muscles (Lawrence and Kuypers, 1965; Clough, Kernell and Phillips, 1968) and from an orderly recruitment of motoneurons from small to large within each spinal motor pool (Henneman et al., 1965a; Milner-Brown et al., 1973b).

One notable exception to this recruitment pattern occurred after resuture of a completely severed ulnar nerve in the forearm. Normal motor unit twitch amplitudes were re-established but the threshold force for recruitment of these reinnervated motor units remained abnormal. In addition to a disordered recruitment of motor units, these individuals showed poor motor co-ordination for fine movements (Milner-Brown et al., 1974). In these patients, the axons had been severed and so disconnected from a pathway to their peripheral end organs. As the ulnar nerve contains axons which innervate several intrinsic hand muscles, many muscles were denervated. During regeneration, the axons had to grow

across the suture line and along the endoneural tubes to innervate the denervated muscle fibers in the periphery (Sunderland, 1978). These axons had opportunities to reinnervate their original or foreign muscles. Therefore, the disordered recruitment of reinnervated motor units could have resulted from misdirection of motor axons to foreign muscles with different functions and/or from changes in the activity of the motoneurons.

Studies on animals have shown that severed motor axons do not reinnervate their original muscles specifically (Gillespie et al., 1986). Motor axons also reinnervate muscle fibers irrespective of their original type. This is reflected by the altered distribution of the muscle fibers of reinnervated muscle units. They tend to be grouped together, rather that scattered throughout the muscle in the normal mosaic pattern (Dubowitz, 1967; Karpati and Engel, 1968). As most animal muscles are heterogeneous (Ariano, Armstrong and Edgerton, 1973), these newly formed muscle units must contain fibers with different histochemical composition and contractile properties. However, reinnervated motor units become like normal motor units with time, in that their muscle fibers show homogeneous histochemical composition and contractile properties (Kugelberg, Edström and Abbruzze, 1970). These changes must have involved transformation of many muscle fibers.

Similarly, regenerating human motor axons are unlikely to

grow along their original endoneural tubes, although the pattern of motor innervation in reinnervated human muscles has not been demonstrated. If reinnervation is specific, a change in the activity of the motoneurons could alter the threshold forces for voluntary recruitment of the motor units and thereby produce disorderly recruitment within the reinnervated muscles. Alternatively, non-specific reinnervation of muscles could produce disorderly motor unit recruitment because of misdirection of motor axons from different motor pools. Even though orderly relationships between motor unit properties may be re-established in motor units appropriately reinnervated by their original motor axons, these relationships may be obscured by the data from misdirected motor units.

Thus, the purpose of the present study was to determine the pattern of innervation and recruitment of motor units in reinnervated human hand muscles after complete severance and resuture of the ulnar and median nerve at different levels.

4.1 METHODS

4.1.1 Clinical assessments

Clinical assessments and experiments were all completed with the informed consent of the patients. All patients (2 female, 11 male; 16-59 years) had accidentally severed the ulnar (6 patients) or median (7 patients) nerve in the past 5 years. Surgical reports confirmed that the nerves had been completely sectioned and surgically repaired. All nerve

sections were at wrist level except for two above-elbow median nerve sections.

The injured nerve and that of the control nerve in the other arm were each electrically stimulated at two levels to measure axonal conduction velocity. A bipolar needle electrode was inserted percutaneously into one or two reinnervated muscles in turn. The interference pattern was examined for the presence or absence of normal and polyphasic motor unit potenials. Muscle bulk and strength, together with general motor and sensory function were assessed clinically.

4.1.2 Recruitment of reinnervated motor units

Spike triggered averaging was used to record the twitch tension, contraction time, unrectified and rectifed EMG of the reinnervated motor units in relation to their recruitment threshold, as described earlier (see Chapter 2). However, in these experiments, motor unit potentials were recorded with a Disa bipolar needle electrode inserted percutaneously into the FDI or abductor digiti minimi (ADM) muscle of the ulnar nerve patients and into the APB or opponens pollicis (OP) muscle of median nerve patients (Figure 4.1). Surface EMG activity was recorded with two Beckman surface electrodes positioned on the belly of the respective muscles.

To measure the force of voluntary muscle contraction from the FDI and ADM muscles of ulnar nerve patients, a force

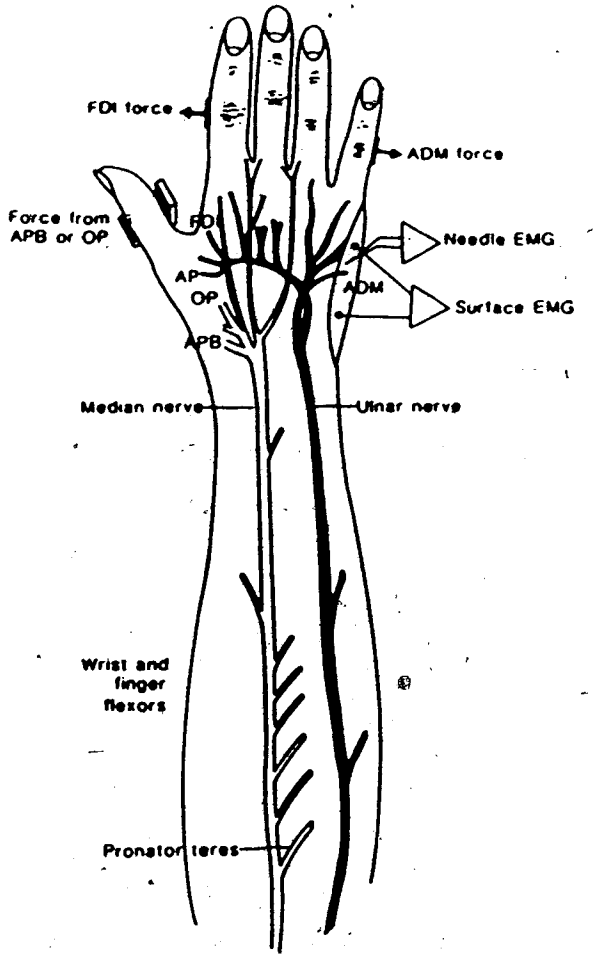

Figure 4.1. Schematic diagram of the path of the ulnar and median nerves below the elbow joint. As each nerve travels down the forearm and into the hand, it innervates muscles with separate motor pools and different functions. The recording set up is only illustrated for the ADM muscle. However, EMG from single motor units was recorded with bipolar needle electrodes from the FDI or the ADM muscle after ulnar nerve section and the APB or OP muscle after median nerve section. Surface EMG was recorded from two electrodes positioned on the belly of the respective muscles. Force was recorded from the different muscles at the positions illustrated by the solid bars although only one or two transducers were used for each patient. The FDI, ADM, AP and other interossei and lumbrical muscles were all contracted in turn to determine the original motor pool of ulnar-reinnervated motor units whereas the APB, OP, PT and wrist and finger flexors were activated for motor pool identification of median-reinnervated motor units.

transducer (Grass FT-03) was positioned against the proximal interphalangeal joint of the index and little finger respectively. For the median nerve patients, two strain gauges mounted at right angles (for details, see Chapter 2) were positioned against the anterior and medial aspects of the thumb interphalangeal joint to record force from the APB and OP muscles respectively.

4.1.3 Identification of the original single motor unit spinal pool

After averaging the reinnervated motor unit parameters, the patient attempted to activate the same motor unit by voluntarily contracting different ulnar-innervated muscles in ulnar nerve sections (FDI, ADM, adductor pollicis (AP), other interossei and lumbricals) or different median-innervated muscles in median nerve sections (APB, OP, wrist and finger flexors, pronator teres (PT); Figure 4.1).

Normally innervated motor units were activated only by contraction of the muscle being recorded from. Reinnervated motor units that showed the same behavior were considered to be appropriately innervated by motor axons from their original motor pool. Those reinnervated motor units that could also be activated by the contraction of other ulnar- or median-innervated muscles after the respective nerve sections, were considered to be reinnervated by motor axons from foreign motor pools. The original motor pool for these motor units was determined objectively from the muscle

action which resulted in the recruitment of the motor unit in the recording muscle at the lowest level of force and rectified EMG. The patients confirmed subjectively that the motor unit was activated with the least effort by the contraction of that muscle.

Errors in this technique could arise when the respective muscles have closely synergistic functions. For example, in the APB muscle, motor units can be activated by abduction or opposition contractions of the thumb (see Chapter 2). Following median nerve sections, these motor units were identified only as being of thenar muscle origin. Their actions could be separated clearly from those of the pronators and wrist and finger flexors. Similarly, the ulnar innervated FDI and AP muscles can act as synergists. However, as FDI motor units do not co-operate in adduction of the thumb without flexion of the index finger (see Chapter 2) motor units could be identified in these muscles by different muscle actions.

4.1.4 Procedure

In turn, different motor unit potentials were discriminated by having the subject exert various levels of voluntary force and/or by repositioning the needle electrode in the muscle. The mechanical properties of each motor unit were averaged and the motor unit pool identified as outlined above. One to three recording sessions of 1-2 hours were completed for each patient on different days.

4.1.5 Analysis

From the data from each patient, graphs of the twitch tension amplitude, contraction time and recruitment threshold were plotted on logarithmic coordinates. Data were also pooled and plotted similarly for ulnar nerve and median nerve sections. Regression lines were fitted according to a least mean squares criterion (Sokolinoff and Redheffer, 1958). These data were compared to those obtained from subjects with normally innervated hand muscles.

The numbers of motor units from the different identified spinal motor pools in the reinnervated muscles were pooled separately for all ulnar and above-elbow median nerve section patients. A calculation was made of the contribution (%) of each muscle to the newly formed spinal motor pools after these different nerve sections.

4.2 RESULTS

4.2.1 Clinical assessments

The injured nerves typically had lower conduction velocities than those recorded from the control nerves in the contralateral arm. The evoked compound muscle action potentials were smaller in amplitude and more dispersed than normal. In the reinnervated muscles, there was generally increased EMG activity with needle insertion and a reduced interference pattern composed of both normal and polyphasic motor unit potentials. Muscle strength and bulk were

sometimes less than normal. Most patients had difficulty in isolating finger movements and described changes in their ability to complete fine motor skills such as fastening buttons and picking up small objects. Usually, tolerance to cold was reduced and tactile sensation was impaired.

In all patients, the motor and sensory deficits were specifically confined to the typical distribution of the nerve that had been injured. This suggested that there were no variations from the normal innervation of individual muscles although this does occur occasionally (Sunderland, 1978).

4.2.2 Innervation of reinnervated muscles

The ADM, FDI and APB muscles were reinnervated appropriately by some of their original motor axons and inappropriately by motor axons that had previously innervated different muscles with different functions. Typical examples of the EMG patterns of reinnervated motor units are illustrated in Figure 4.2 for the ADM muscle. The second trace in Figure 4.2A shows that surface EMG activity was always recorded in the ADM muscle when the ADM, FDI or AP muscles were activated. As normal subjects can activate the ADM muscle independently of FDI or AP, this suggests that many motor axons had been misdirected from other ulnar innervated muscles to the ADM muscle. Figure 4.2A also shows an example of the typical EMG pattern of a normally directed motor unit. The motor unit fired during contraction

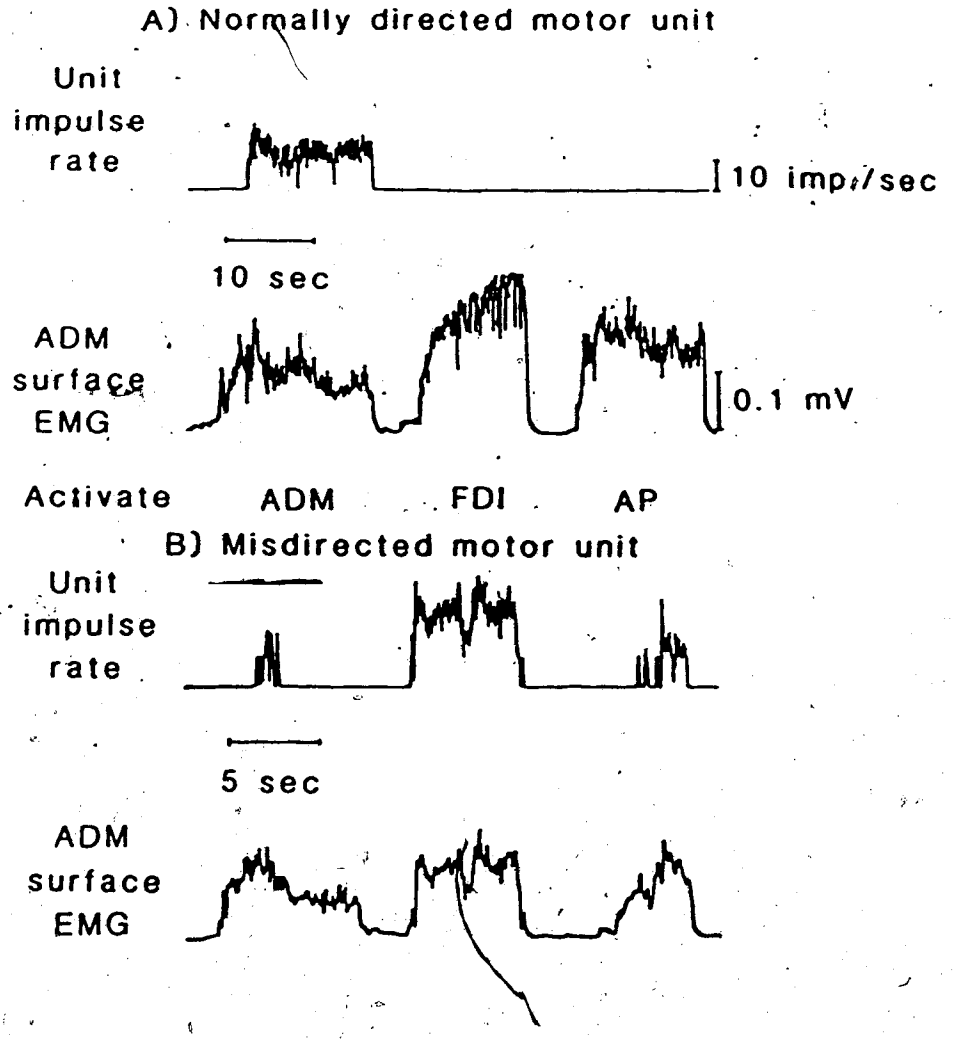

Figure 4.2. Unit impulse rate and rectified surface EMG activity of a normally directed (A) and misdirected (B) motor unit recorded in a reinnervated ADM muscle. EMG activity was recorded from the ADM muscle during contraction of either the ADM, FDI or AP muscles suggesting misdirection of many motor axons to that muscle. A normally directed unit (A) was activated only by the contraction of the ADM muscle. The motor axon had therefore appropriately reinnervated its original muscle. The misdirected motor unit (B), was activated most easily and at the lowest level of ADM rectified EMG by contraction of the FDI muscle so presumably belonged to the original FDI motor pool.

of the ADM muscle but was silent during contraction of other ulnar reinnervated muscles such as the FDI or AP. The motor axon had therefore appropriately reinnervated its original muscle. In comparison, misdirected motor axons from foreign motor pools formed motor units which could be activated by voluntary contraction of other muscles innervated by the same nerve (Figure 4.2B). This motor unit was recruited at the lowest level of ADM rectified EMG by contraction of the FDI muscle. Higher levels of ADM rectified EMG had to be reached before the motor unit was recruited by the action of that muscle or the AP muscle. Therefore, the motor unit was presumably in the spinal motor pool of the FDI muscle prior to the nerve section, but grew back to reinnervate muscle fibers in ADM.

Table 4.1 shows the origin of the motor axons in the reinnervated muscles after ulnar and above-elbow median nerve section and resuture. After complete ulnar nerve section at wrist level, only 34% of the motor axons reinnervating the ADM muscle were from its original motor pool. The remaining motor axons had been misdirected from the original FDI (31%), AP (18%) and other interossei and lumbricals (17%) motor pools. Similarly, 39% of the motor axons reinnervating the FDI muscle were from the original FDI motor pool, whereas 11, 22 and 28% were from the ADM, AP and other interossei and lumbrical motor pools respectively. After median nerve section above the elbow, 48% of the reinnervated APB muscle motor pool was formed by original

Table 4.1 Origin of motor axons in reinnervated muscles after complete ulnar or median nerve section and resuture.

A) Ulnar nerve sections at wrist level

Muscle	Motor units activated most easily by contraction of:				
	FDI	ADM	AP	Others	Total
ADM	15 (31%)	16 (34%)	9 (18%)	8 (17%)	48
FDI	22 (39%)	6 (11%)	12 (22%)	16 (28%)	56
Total	37 (36%)	22 (21%)	21 (20%)	24 (23%)	104

B) Median nerve sections above the elbow

Muscle	Motor units activated most easily by contraction of:				
	Thenar	PT	Flexors	Others	Total
APB	20 (48%)	2 (5%)	14 (33%)	6 (14%)	42

The numbers represent data from 6 ulnar and 2 above-elbow median nerve section patients.

thenar (APB, OP, flexor pollicis brevis) motor axons. Five, 33 and 14% of the motor axons were from the PT, wrist and finger flexor or other motor pools respectively. Thus, these data clearly show that misdirection of motor axons occurs during reinnervation. However, this may not be an entirely random process because in both the FDI and ADM muscles, there was some tendency to record from a higher percentage of normally directed motor units than motor units from any one foreign motor pool.

4.2.3 Size relationships in reinnervated muscles

Figure 4.3 shows a typical example of the significant positive correlation found between the size of the motor unit (twitch amplitude) and the voluntary force at which the motor unit fired repetitively in a normally innervated FDI muscle (Figure 4.3A). Following ulnar nerve section at wrist level, the range of reinnervated motor unit twitch tensions and recruitment thresholds was comparable to normal values. However, the correlation between these two variables was not significant in an individual reinnervated muscle (Figure 4.3B) or when data were pooled from several reinnervated muscles (Figure 4.3C). For normally directed motor units (■) in the FDI muscle, these correlations were positive but only significant in one muscle studied. Data recorded from reinnervated ADM muscles showed similar trends.

In a normally innervated APB muscle, significant positive correlations were also found between twitch tension

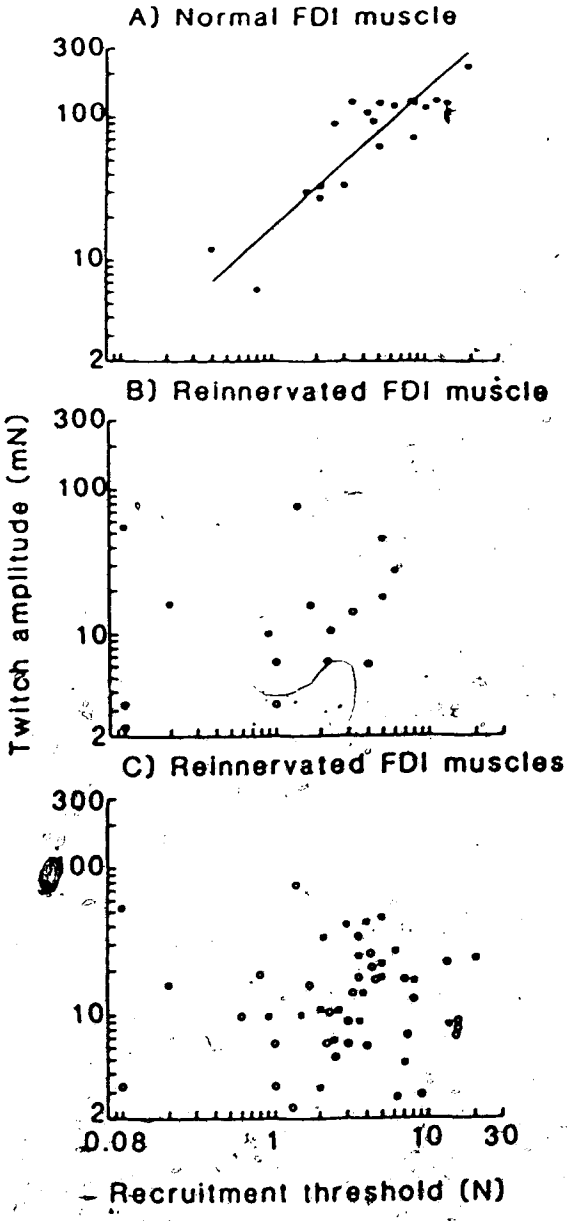

Figure 4.3. Motor unit twitch amplitude plotted against the threshold force of recruitment in a normally innervated FDI muscle (A), one (B) and four (C) reinnervated FDI muscles after ulnar nerve section and resuture at wrist level. Different symbols represent normally directed (■) and misdirected (○) motor units in all graphs. Regression lines minimizing the deviations in the x and y directions are drawn in these and subsequent figures only when the relationships were significantly different from zero at the 5% level of confidence.

amplitude and recruitment threshold (Figure 4.4A). After median nerve section and resuture at the elbow, these relationships were not re-established in a reinnervated muscle (Figure 4.4C) or when the data from two muscles were pooled (Figure 4.4E). In normally directed APB motor units (■), these relationships were positive but not significant. In comparison, when the median nerve was sectioned and resutured at wrist level, the muscles were reinnervated by motor axons from motor pools with closely synergistic actions. Orderly relationships between recruitment threshold and twitch amplitude were re-established in a reinnervated muscle (Figure 4.4B) or when data from several reinnervated muscles were pooled (Figure 4.4D).

Figure 4.5 shows that there were significant negative correlations between motor unit contraction time and twitch amplitude in normally innervated FDI and APB muscles (Figure 4.5A, 4.5B). Following ulnar or median nerve section and resuture, these correlations were weak (Figure 4.5C) or absent (Figure 4.5D) in reinnervated muscles. The range of contraction times recorded in reinnervated muscles was similar to those recorded in the respective normal muscles. However, in all reinnervated muscles, the mean motor unit contraction time was faster than normal, but the differences were not statistically significant. As the patients sometimes found it difficult to activate a single motor unit at a low steady frequency, the erratic discharge pattern could have resulted in an underestimation of some of the

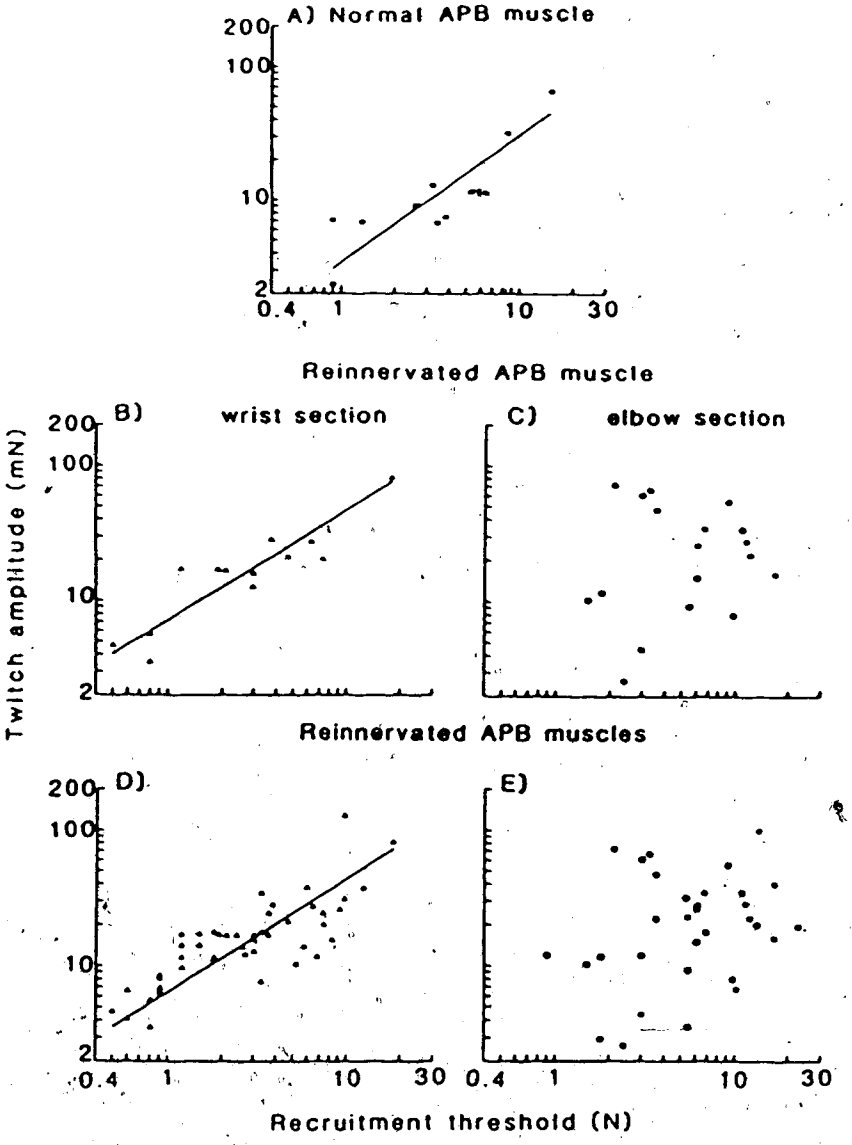

Figure 4.4. Motor unit twitch amplitude versus recruitment threshold in a normally innervated APB muscle (A). Data are plotted from one (B, C) and several (D, E) reinnervated APB muscles after median nerve section at the wrist and above the elbow respectively. Normally directed motor units (■) are represented by a different symbol to reinnervated motor units with motor axons of undetermined origin (▲) and misdirected reinnervated motor units (o).

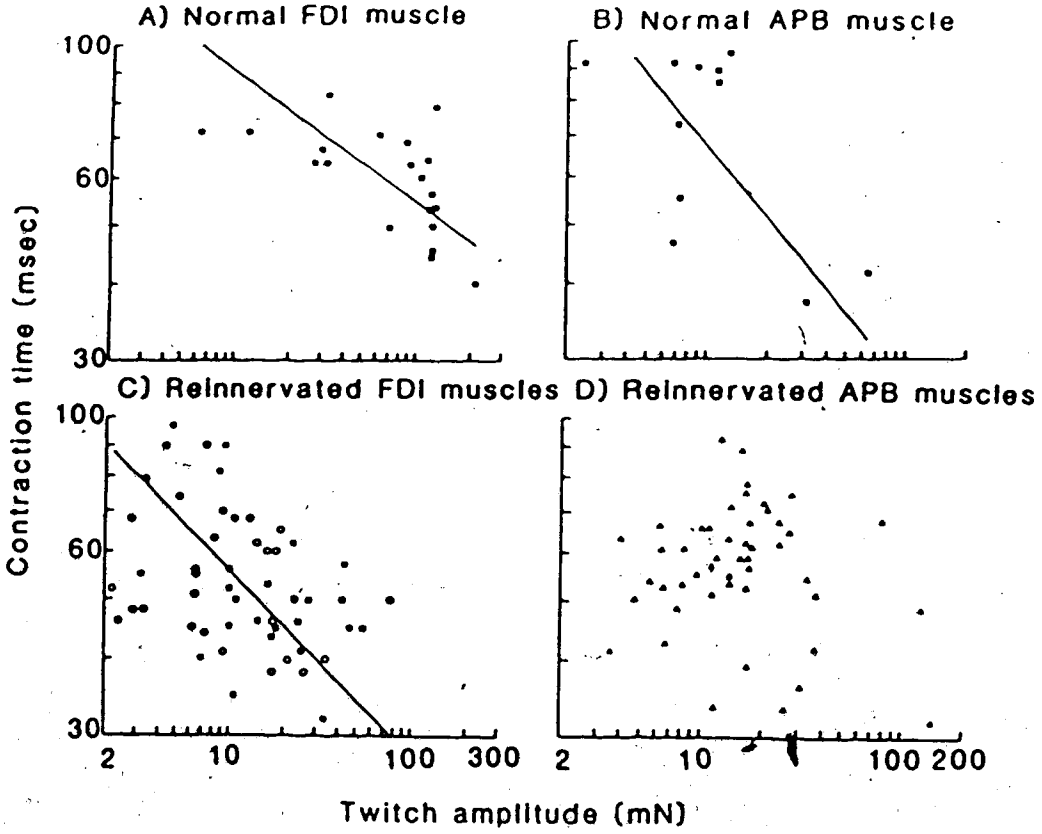

Figure 4.5. Motor unit contraction time plotted as a funqtion of twitch amplitude in a normally innervated FDI (A) and APB (C) muscle. Data are plotted for reinnervated FDI (B) and APB (D) muscles after ulnar and above elbow-median nerve severance and repair respectively.

motor unit rise times.

4.3 DISCUSSION

After complete ulnar or median nerve severance and resuture in humans, regenerating motor axons were shown here, either to reinnervate their original muscles or to be misdirected to foreign muscles. Only when motor axons reinnervate muscles with closely synergistic functions (median nerve at wrist), the orderly size relationships between motor unit properties are re-established (Figure 4.4B, 4.4D).

4.3.1 Innervation of reinnervated muscles

The poor motor co-ordination for fine movements observed clinically after peripheral nerve section and repair and the mass movements of facial muscles after facial nerve injury have sometimes been assumed to result from misdirection of regenerating motor axons (Esslen, 1960; Ford and Woodhall, 1938; Freuh, 1983; Kimura et al., 1983). In the present study, this assumption was substantiated for peripheral nerve sections by identifying the original spinal motor pool of reinnervated motor units following ulnar and median nerve section. In the reinnervated FDI, ADM and APB muscles, two patterns of motor unit activity were recorded. Some motor units could be activated only by the action of the muscle in which they were recorded so they functioned normally. Presumably, the motor axons of these motor units reinnervated their original muscle. Other motor units could

be activated best by contracting muscles innervated by the same nerve. These motor units were presumably formed by the motor axons being misdirected to reinnervate a foreign muscle. It was not tested to see if one axon innervated several muscles, but this is unlikely since axon sprouting usually occurs over very short distances (Slack and Pockett, 1981). Similarly, the location of the cell body in the spinal cord was presumably unaltered. Following peripheral nerve section and resuture in animals, the location of the cell bodies remain intact and the motor axons exit from the appropriate ventral roots (Brushank and Mesulam, 1980).

Alternative explanations could be offered for the abnormal motor unit activity observed here. These include: a) ephaptic transmission b) alterations in the synaptic connections in higher brain centers c) motor axons splitting to innervate different muscles. Impulses can be generated ectopically in chronically injured nerve fibers and possibly from hyperexcitability of the membrane (Rasminsky, 1983). Although this process may contribute to the motor unit activity recorded here, it seems unlikely that the impedance matching between the axons would be consistently appropriate to evoke transmission from local currents.

Monkeys (Brinkman, Porter and Norman, 1983) and cats have been observed to perform well co-ordinated goal-directed movements after cross-union of large common nerves in the forearm. With deliberate cross-union, such re-education may

result from compensation by the action of synergistic muscles. In cats, the ability to perform co-ordinated voluntary movements may also result from sprouting of corticorubral neurons onto the proximal portion of the red nucleus (Tsukahara, Fujito, Oda and Maeda, 1982). However in man, the red nucleus and the rubrospinal tract is less well developed (Carpenter, 1981). If synaptic alterations in these or other higher centers such as the corticospinal tract did occur in man after complete nerve section and repair, they do not appear to result in well co-ordinated finger movements. The patients in the present study reported difficulties in controlling their hand muscles during some functional tasks.

Examples of axon splitting have been reported in the extraocular and lumbrical muscles of the cat (Crandall, Goldberg, Wilson and McClung, 1981; Emonet-Dénand, LaPorte and Proske, 1971). This phenomenon is rare in human nerve regeneration. In some patients who have sectioned the ulnar (2/18, 11 %) or facial (1/50, 4 %) nerve, synchronous activity of some motor units was observed in different muscles reinnervated by the same nerve (Esslen, 1960). Asynchronous motor unit discharges were much more common in all muscles examined, implying simple misdirection of the axon to a foreign muscle, as is proposed here.

From the present data, reinnervation of human hand muscles is clearly not specific. Similar findings have been reported in rat muscles when motor axons were provided with

a choice of muscles to reinnervate (Gillespie et al., 1986). However, as more normally directed motor units than misdirected motor units from any one foreign pool were recorded in the ADM and FDI muscles after ulnar nerve section, this would suggest that reinnervation is not an entirely random process either. The ADM or FDI muscle can be activated by different actions to that of the AP muscle so units activated by these muscle actions are unlikely to be mistaken with other motor pools. Alternatively, the tendency for more normally directed motor units to be recorded in these muscles may result from the careful rematching and resuture of the severed fascicles during surgery. This may provide the regenerating axons with a better opportunity to regrow along their original endoneural tubes. Similarly, if the nerve section is quite close to the point of entry of the individual nerves into the muscles, there will be fewer branch points at which axons could be misdirected. The actual randomness of the reinnervation observed here could be substantiated by anatomical information on the numbers of motor axons in the ulnar nerve at the wrist and the respective muscle nerves, but this information is not currently available to our knowledge.

4.3.2 Size relationships in reinnervated muscles

Following ulnar and above-elbow median nerve section and resuture, the regenerating axons came from spinal pools with different functions. In the reinnervated FDI, ADM and APB

muscles, motor unit recruitment was disorderly. The ulnar nerve data confirm the previous findings of Milner-Brown et al., (1974). Furthermore, the data show that the lack of orderly motor unit recruitment by size after these complete nerve sections, results from misdirection of motor axons from foreign motor pools.

In contrast, after median nerve section and resuture at the wrist, motor axons from synergistic motor pools reinnervated the denervated muscle fibers. Despite presumed misdirection of motor axons from foreign motor pools, orderly size relationships between motor unit properties were found in the APB muscles. Thus, orderly motor unit recruitment can be re-established after complete nerve section and resuture in humans, if the reinnervated muscles have closely synergistic functions. One factor favouring recovery after this injury may be the proximity of the section to the denervated muscle fibers. Near the level at which a muscle nerve leaves the main nerve trunk to innervate a muscle, the fibers of that individual nerve are sharply localized (Sunderland, 1978). With good fascicular repair after a clean cut, misdirection of axons may be reduced.

The relationships found between recruitment threshold and twitch amplitude in normally directed motor units did show some tendency for normal reorganization and orderly recruitment. However, even though the size relationships for

one muscle or one motor pool may be restored, these original pools were dispersed between different muscles after reinnervation because of misdirection of regenerating motor axons. Thus, recruitment in any one muscle would depend on the recruitment of motor units from several pools with different functions. This was reflected by the difficulties the patients had in isolating individual finger movements. Typically, all muscles innervated by the same nerve would contract to some extent. These inappropriate movements may result from orderly recruitment of motor units by size within the original motor pools.

Data from animal studies support the proposal that misdirection of motor axons rather than a change in the activity of the motoneurons may explain disorderly motor unit recruitment and poor muscle control after complete nerve section and repair in humans. Full recovery of motoneuron properties and synaptic contacts onto axotomized motoneurons occurs following muscle reinnervation (Sumner and Sutherland, 1973). When motoneurons reinnervate their former muscles, the physiological relationships between nerve and muscle properties also returned to normal. Thus, the tension and contractile speed of the muscle units were directly related to the amplitude of the extracellularly recorded action potential as found in normally innervated muscles (Gordon and Stein, 1982). Self-reinnervated muscles function appropriately during locomotion. In comparison, cross-reinnervated cat muscles typically fired according to

the pattern expected of the innervating motor pool during locomotion (Luff and Webb, 1985; O'Donovan, Pinter, Dum and Burke, 1985; see Chapter 5). Therefore, these data would tend to suggest that motor units are recruited at a level of force that is appropriate within their original motor pool as is proposed here.

The impaired sensory function of the patients in the present study probably results from aberrant sensory reinnervation. Even though sensory axons reinnervate their end organs specifically, they are unlikely to reinnervate their original mechanoceptor. Misguided afferent fibers have been proposed to explain the perceptual mislocations of tactile stimuli following ulnar and median nerve section in humans (Hallin, Wiesenfeld and Lindblom, 1981; Mackel, Kunesch, Waldhor and Struppler, 1983) and the abnormal response characteristics of muscle afferents after cross-reinnervation of the cat soleus muscle (Gregory, Luff and Proske, 1982). Thus, even though the afferent reponds to an appropriate mechanoceptor, the localization of the sensory stimulus may be interpreted incorrectly. This mismatching of sensory connections may be distorted further by misdirection of the motor axons to foreign muscles.

In conclusion, after complete ulnar or median nerve section and resuture in humans, the reinnervation of hand muscles was shown to be abnormal physiologically. Motor axons reinnervated their original or foreign muscles. Even

so, the reinnervated muscles served some useful function during power or gripping movements. Finely co-ordinated movement sequences were impaired, in part due to misdirection of motor axons and in part to impaired tactile sensation. Thus, the data in this study not only emphasise the need for largely preserving the pattern of nerve and muscle connections for size ordered motor unit recruitment but also show the importance of this pattern of recruitment for control of fine movements.

CHAPTER 5

INNERVATION AND FUNCTION OF CAT HINDLIMB MUSCLES AFTER CROSS-UNION OF THE TIBIAL AND PERONEAL NERVES

In humans, complete section of large nerves such as the ulnar or median involves the denervation of many muscles from different motor pools. During regeneration, some motor axons are misdirected to foreign muscles. This situation represents an extreme test of the rematching of nerve and muscle properties during reinnervation for the restoration of muscle function. In cats, a similar extreme test of the peripheral reorganization of nerve and muscle properties after reinnervation would follow cross-union of large common nerves. These nerves contain many axons from several motor pools so the denervated muscle fibers would become reinnervated by motoneurons which typically support different muscle functions. Unless there could be some rematching of central neural activity to peripheral nerve and muscle properties, the reinnervated muscle would function inappropriately and generate the activity pattern appropriate to the innervating nerve.

Earlier studies have assessed how cross-reinnervation of muscles influences their function. When the nerve to a flexor and extensor muscle in the forelimb or hindlimb of the rat was cross-united, there was full reversal of flexor and extensor movements of the elbow and ankle joints respectively. These functional reversals were observed to

persist with little compensation in stereotyped movements like locomotion (Sperry, 1941; 1942). However, no EMG activity was recorded during movement to support the behavioral observations and the completeness of the nerve cross was not assessed.

Studies involving cross-reinnervation of two forelimb muscles of rats (Cohen, 1978), two hindlimb muscles of cats (Luff and Webb, 1985) or numerous muscles in the monkey forearm (Tsukahara et al., 1982) have avoided these problems. In these studies, the cross-reinnervated muscles were primarily activated according to the innervating nerve. Yet, some modification to the walking pattern was proposed to have occurred, although interpretation of data was complicated by impure crosses. In contrast, pure cross-reinnervation of cat soleus and FDL muscles produced muscle activation patterns during locomotion which were always consistent with those expected for the innervating motor pool rather than the innervated muscle (O'Donovan et al., 1985).

In goal-directed movements, both monkeys and cats were able to co-ordinate their movements accurately despite their crossed forelimb nerves (Brinkman et al., 1983; Sperry, 1947; Tsukahara et al., 1982). In monkeys, EMG activity was not recorded to support these observations of appropriate muscle function. The ability of cats to perform co-ordinated voluntary movements may result from sprouting of

corticorubral neurons onto the proximal portion of red nucleus neurons (Tsukahara et al., 1982).

The purpose of the present study was to investigate how the cross-union of large nerve trunks affects muscle function during locomotion. The common flexor and extensor nerves which supply the distal hindlimb were cross-united in young cats. Therefore, motoneurons from many different motor pools were deliberately misdirected to muscles with antagonistic functions. It was considered that cross-reinnervating all major flexor and extensor muscles of the distal hindlimb might provide a more potent stimulus for central adaptation than crossing single muscle nerves. Also, young cats 2-6 months of age might have greater plasticity than adult cats. The function of the cross-reinnervated muscles was examined during locomotion. The alternating swing and stance phases of locomotion result from spinally generated patterns of activity in the nerves to the flexor and extensor muscles of the limbs (Shik and Orlovsky, 1976; Grillner, 1981). During one step cycle, the extensor muscles typically fire when the limb is on the ground (stance). The flexor muscles show a double burst pattern with one short burst when the limb is off the ground (swing) and another during the stance phase (Grillner, 1975).

5.1 METHODS

The seven 2-6 month old cats used in this study were given injections of penicillin one day prior and immediately

after surgery. During surgery, the animals were anesthetized with sodium pentabarbitol and maintained on this anaesthetic at a depth that withdrawal reflexes could not be elicited. Under fully aseptic conditions, the tibial and peroneal nerves to the left hindlimb were exposed in the popliteal fossa, cut 5-10 mm below the sciatic branch point and surgically cross-united using fine sutures (8-0 silk).

Normally the peroneal nerve innervates all of the flexor muscles while the tibial nerve innervates all of the extensor muscles of the distal hindlimb. In the present study, the common peroneal (CP) nerve is used as an example of a flexor nerve and the medial gastrocnemius (MG) and lateral gastrocnemius-soleus (LGS) nerves as examples of extensor nerves. Following cross-reinnervation, nerve impulses were recorded distal to the nerve suture. The nerve distal to the suture line on the CP nerve is referred to as the crossed CP nerve (and contains primarily extensor axons) while the nerves distal to the suture line on the MG and LGS nerves are referred to as the crossed MG and crossed LGS nerves (and contain primarily flexor axons; Figure 5.1).

5.1.1 Recording EMG activity during locomotion

The animals were trained to walk on a motor driven treadmill. An appropriate speed was determined for each animal during training. At regular intervals 10-18 months after surgery, EMG activity was recorded during locomotion using stainless steel wire electrodes (9 strand, teflon

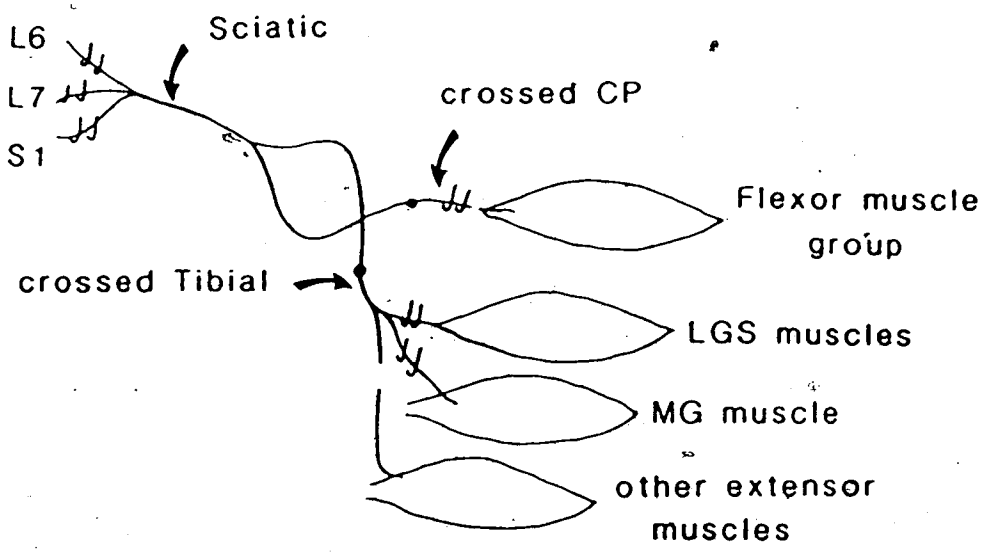

Figure 5.1. The CP nerve normally innervates all of the distal flexor muscles while the tibial nerve normally innervates all of the distal extensor muscles of the hindlimb. Following a complete cross union, the innervation of flexor and extensor muscles would be reversed. Compound action potentials were recorded on the L6, L7 and S1 ventral and dorsal roots in response to stimulation of the normal and crossed MG, LGS and CP nerves. At the time of recording, the nerves to flexors and other extensor muscles were cut (broken lines) to minimize EMG artifacts.

coated cables with a total diameter of 0.2 mm; Bergen Wire Rope Co., Bergen, N.J.). The tips of the wires were bared of insulation and bent back to form a hook then inserted into the vastus lateralis (VL, knee extensor) and semitendinosus (ST, knee flexor) or lateral gastrocnemius (LG, ankle extensor) and tibialis anterior (TA, ankle flexor) muscles of the normally innervated and reinnervated hindlimbs of each cat. During the insertion of three electrodes per muscle, the animals were maintained under a volatile anaesthetic (Flurothane). The free ends of the three electrodes were braided and led to an amplifier by means of wire spring connectors. Two of the three electrodes provided for bipolar recording while the third served as an earth. The long lengths of wire external to the muscle enabled the wires to move freely with the limb movements during each step cycle while the electrodes remained inserted within the muscle because of the hooks at their ends.

The unrectified EMG activity was recorded on tape while rectified and filtered EMG (30 Hz Paynter filter) was monitored on an oscilloscope, a pen recorder, and superimposed on a video image of the cat walking on the treadmill at a comfortable speed. Then, the movements of each hindlimb during the step cycles were matched with the corresponding EMG activity recorded on tape by playing back the signals recorded on the video tape recorder.

5.1.2. Acute experiments

Acute experiments were completed 18-24 months after the initial surgery. In these, the success of the motor and sensory nerve crosses was determined by adapting the method of charge distributions developed by Hoffer, Stein, and Gordon (1979). The peripheral organization of the cross-reinnervated motor units in the extensor muscles was also studied (see Chapter 6). Under sodium pentabarbitol anesthesia, a laminectomy was performed from S1 to L6. Apart from the control and crossed MG, LGS and CP nerves, all nerves to both hindlimbs were cut. The intact nerves were dissected free of other tissue over a distance of 10-15 mm in the popliteal fossa. For the experiments described in Chapter 6, the individual tendons of the muscles being recorded (MG or LG and soleus) were separated. Bipolar electrodes were stitched to the fascias of these muscles to record EMG activity. The cat was then placed in a stereotaxic frame and fixed there by clamps on either side of the head and at the hip, knee and ankle joints. Each control nerve was placed as far distally as possible on a separate pair of electrodes for electrical stimulation. The ipsilateral L6, L7, and S1 ventral and dorsal roots were cut sequentially and close to their entry point into the spinal cord. They were placed on an array of six electrodes to measure:

a) the impedance of each root (with respect to the cut end of the root) using a 10 kHz sinusoidal signal.

b) the average of 20 compound action potential responses to

supramaximal stimulation of the control MG, LGS and CP nerves (Figures 5.2A).

Data were similarly recorded from the experimental side of the cross-reinnervated cats (Fig. 5.2B) and from one side of two control cats.

To compare potentials with different time courses on roots of different sizes, a computation was made of the electrical charge contributed by each nerve to each root (Hoffer et al., 1979). The area of the compound action potential was computed by a method equivalent to integrating the current over time. That is, the area in mV.ms was divided by the impedance of the root in kΩ to give the electrical charge generated by the control and crossed MG, LGS, and CP nerves at the L6, L7, and S1 ventral and dorsal roots in units of μA.ms = nC. This gives the charge (Q) as a measure of the electrical contribution of a nerve to a root. The charge from different roots can be summed and compared.

Determination of success of nerve regeneration. If regeneration is unsuccessful, sensory fibers may atrophy. Motor nerve fiber diameters atrophy initially and then stabilize (Hoffer et al., 1979). The voltage (V) generated at the dorsal and ventral spinal roots by stimulating these nerves will depend on the number of nerve fibers contributing to the potentials, their cross-sectional area, the dispersion of the potentials and the electrical resistance (R) of the root. Thus, an indication of the

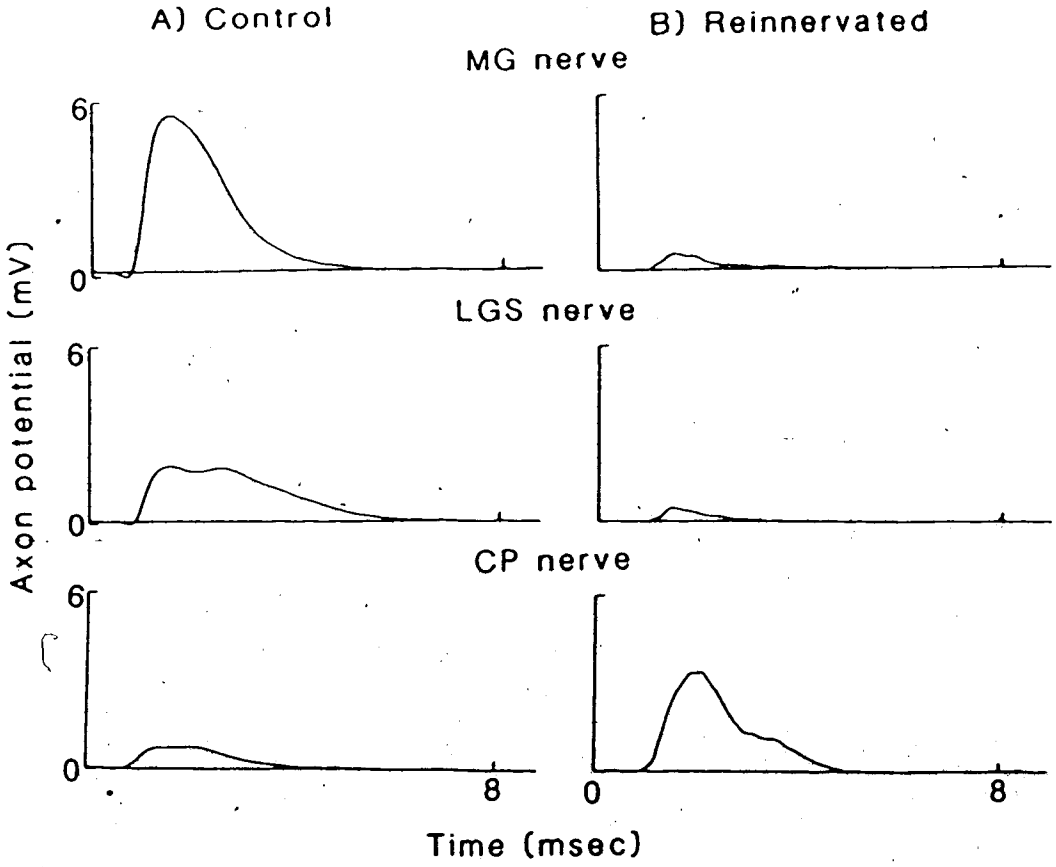

Figure 5.2. Examples of compound action potentials recorded on the S1 ventral root in response to stimulation of the control MG, LGS and CP nerves (A) and crossed MG, LGS and CP nerves (B).

success of the nerve regeneration was obtained by comparing the difference in the total charge generated at the ventral and dorsal roots (L6, L7, S1) in response to supramaximal electrical stimulation of the control and crossed MG, LGS and CP nerves. A further indication of the success of nerve regeneration was obtained by measuring the tetanic tension that could be developed in the control MG, LG and soleus muscles, compared to that developed in the corresponding reinnervated muscles. Whole muscle forces were recorded in response to electrical stimulation of the appropriate muscle nerves. In turn, the tendon of each muscle was attached to a force transducer (Grass FT10). Isometric muscle twitches were recorded at different muscle lengths to find the length at which maximal tension was produced. The tetanic response of the muscle was measured at this length in response to 100 Hz stimulation at 2-3 times threshold.

Determination of the completeness of the nerve cross. The short length of the nerves crossed in the young cats made it essential to check the completeness of the cross-reinnervation. To determine the completeness of the nerve cross, a comparison was made between how the charge generated by the control and crossed MG, LGS and CP nerves was distributed between the ventral and dorsal roots. A large proportion of the charge produced from stimulation of the MG, LGS and CP nerves was generated at the L7 ventral and dorsal roots. The remaining charge from these nerves was predominantly generated at either the L6 or the S1 ventral

and dorsal roots. To determine whether this remaining charge contribution tended to be more lumbar or sacral, L7 was used as a neutral zone and the fraction of the charge generated at L6 was arbitrarily assigned a weighting of -1 and the fraction of the charge generated at S1 a weighting of +1. The weighted charge contributions to the L6 and S1 roots were summed. The mean root entry of the nerve relative to L7 could then be determined. Thus, a negative root entry value indicated more charge generated at the L6 root than the S1 root while a positive root entry value indicated more charge generated at the S1 root than the L6 root.

The mean root entry values for control nerves were compared to those determined for crossed nerves to establish the completeness of the motor and sensory nerve crosses. To determine how far a crossed extensor nerve (MG or LGS) had been reinnervated by a flexor the following percentage was calculated:

a) crossed MG or LGS completeness (%) $= \frac{Ec - E}{F - E} \times 100$

where F - E is the difference in mean root entry of control flexor and extensor nerves and Ec is the mean root entry for the crossed extensor nerve. If Ec = F, the computed value is 100% (complete cross), whereas if Ec = E the value is 0 (no cross). Similarly, for a crossed flexor nerve (CP) reinnervated by an extensor:

b) crossed CP completeness (%) $= \frac{Fc - F}{E - F} \times 100$

where Fc is the mean root entry of the crossed flexor nerve.

5.2 RESULTS

Figure 5.1 illustrates the method used to measure the compound action potentials on the L6, L7, and S1 ventral and dorsal roots following stimulation of the normal and crossed MG, LGS and CP nerves. Shown in Figure 5.2 are typical examples of the compound action potentials measured on the control (A) and reinnervated (B) S1 ventral root of one cat. Much larger potentials were generated on the control S1 ventral root by stimulation of the MG or LGS nerves than the CP nerve. Following reinnervation the situation tended to be reversed with larger potentials on the S1 ventral root from stimulation of the crossed CP nerve than the crossed MG or LGS nerves.

5.2.1 Success of nerve regeneration

Table 5.1 outlines the total charge generated at the ventral and dorsal roots (L6, L7, S1) by the control and crossed MG, LGS and CP nerves. These mean ($\pm$ S.E.) charge distributions were obtained by pooling data from all animals. The mean charge generated by each crossed nerve at the dorsal and ventral roots was less than the mean charge generated by the corresponding control nerve. Therefore, fewer than normal motor and sensory axons innervated these muscles or alternatively, their axons remained somewhat atrophic. Another indication of the success of the regeneration was given by measuring the tension developed in

Table 5.1. Total charge generated on dorsal and ventral roots by stimulation of the control and crossed nerves.

	Dorsal roots		Ventral roots	
	Total charge (pC)			
Nerve	control	reinnervated	control	reinnervated
MG	81 ± 11	76 ± 18	160 ± 37	141 ± 19
LGS	101 ± 9	81 ± 10	229 ± 39	136 ± 22
CP	864 ± 55	541 ± 97	659 ± 81	639 ± 122

Each value represents the mean and standard error for 6 cats.

the reinnervated extensor muscles. The mean tetanic tension developed in the reinnervated MG, LG and soleus muscles was 91, 68 and 92 % of that developed in the corresponding normally innervated muscles.

5.2.2 Completeness of the nerve cross

As there was substantial charge generated by the MG, LGS and CP nerves at both the ventral and dorsal L7 roots, the effects of cross-reinnervation were most clearly reflected by changes in the charge distributions to the L6 and S1 roots. Data from 6 cats were pooled to produce mean charge distributions. In Figure 5.3, the mean charge (± S.E.) generated by the control and crossed MG, LGS and CP nerves at only the L6 and S1 ventral and dorsal roots is expressed as a percentage of the total charge generated by each of these nerves. MG and LGS nerves contributed substantial charge to the S1 ventral and dorsal roots while the charge from the CP nerve was greater at the L6 ventral and dorsal roots. Following reinnervation, the opposite trends were observed. Major contributions of charge from the crossed MG and LGS nerves were generated at the L6 ventral and dorsal roots and from the crossed CP nerves at the S1 ventral and dorsal roots.

Similar trends were indicated by the overall mean root entries which can be obtained from the difference between the S1 (weighted by +1) and L6 (weighted by -1) mean root entries. These were positive for the MG, LGS and crossed CP

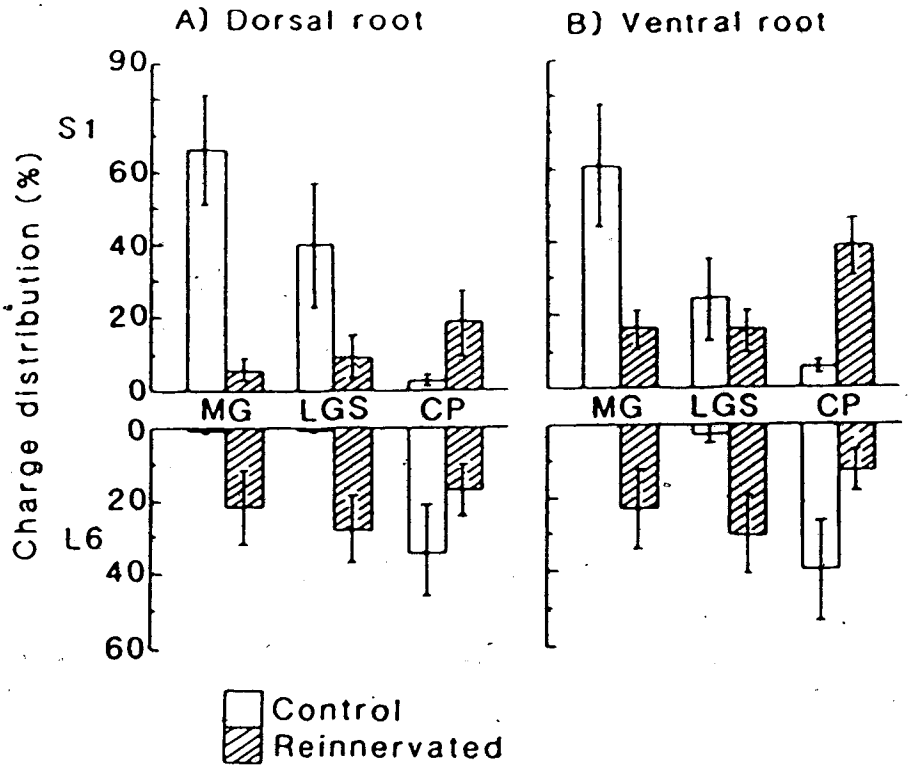

Figure 5.3. Mean (± S.E.) charge distributions of control MG, LGS and CP nerves and crossed MG, LGS and CP nerves on the S1 and L6 dorsal (A) and ventral roots (B). The means represent data from 6 cats. Mean root entries can be obtained from the difference between the S1 (weighted by +1) and L6 (weighted by -1) mean root entries.

nerves (0.66, 0.40, 0 for sensory nerves; 0.60, 0.22, 0.26 for motor nerves) but were negative for the CP and crossed MG and LGS nerves (-0.32, -0.16, -0.19 for sensory nerves; -0.35, -0.07, -0.14 for motor nerves). As described in the Methods, values of +1, 0 and -1 would indicate root entry of the axons centered about S1, L7 and L6 roots respectively.

As these mean root entry values reflect the distribution of charge at the ventral and dorsal roots, the completeness of the motor and sensory nerve crosses was assessed from them. Table 5.2 shows that the completeness of the motor nerve crosses ranged from 65-96% for the crossed CP nerve, and from 41-99% and 39-73% for the crossed MG and LGS nerves respectively. The success of the sensory nerve crosses was generally better than the motor nerve crosses for the crossed MG and LGS nerves but poorer than the motor nerve cross for the crossed CP nerve. Values ranged from 76-94%, 67-97% and 25-52% for the MG, LGS and CP nerves respectively.

It should be noted that the tibial nerve supplies branches to the MG, LG and soleus muscles as well as to some foot muscles. The tibial nerve branch to the foot normally contributes substantial amounts of charge to both the dorsal and ventral roots (Hoffer et al., 1979). As the calculations of nerve cross completeness only included the charge contributions from the MG and LGS nerves, the estimate for the completeness of the sensory CP nerves is artificially

Table 5.2. Completeness of motor and sensory cross-reinnervation.

Cat	Dorsal roots			Ventral roots		
	MG	LGS	CP	MG	LGS	CP
1	88	72	52	75	54	88
2	84	78	38	57	39	65
3	94	90	25	99	68	96
4	78	67	52	75	62	81
5	85	89	30	60	73	81
6	76	97	26	41	57	90
Range	76-94	67-97	25-52	41-99	39-73	65-96
Mean	84.2	82.2	37.2	67.8	58.8	83.5
S.E.	2.7	4.8	5.1	8.1	4.9	4.4

Values for 6 cats are given in % together with the range, mean and standard error of the sample. These were calculated from equations a) and b) (see Methods).

low, while that for the motor CP cross is artificially high (see Discussion).

5.2.3 EMG activity during locomotion

Illustrated in Figures 5.4 and 5.5, are the patterns of EMG activity recorded in the reinnervated and control flexor and extensor hindlimb muscles during locomotion. In each figure, the upward and and downward pointing arrows below trace A correspond to the beginnings of the swing and stance phases in the reinnervated hindlimb respectively. During each step cycle, some control flexor muscles gave a single burst which increased during swing and gradually declined during the early stance phase (Figure 5.4D). Other control flexor muscles tended to show a double burst pattern with a short burst during the swing phase and another early in the stance phase (Figure 5.5C). Control extensor muscles alternated with the control flexor muscles in the same hindlimb and began to fire just prior to stance. Their activity increased and was usually maintained throughout the stance phase of the step cycle (Figures 5.4G; 5.5D). However, when normal animals are walking, flexor and extensor activity in one hindlimb is approximately 180° out of phase with flexor and extensor activity in the other hindlimb.

In Figure 5.4 all EMG activity was recorded from muscles ipsilateral to the nerve-cross surgery. The cross-reinnervated flexor (TA) and control extensor (VL) muscles

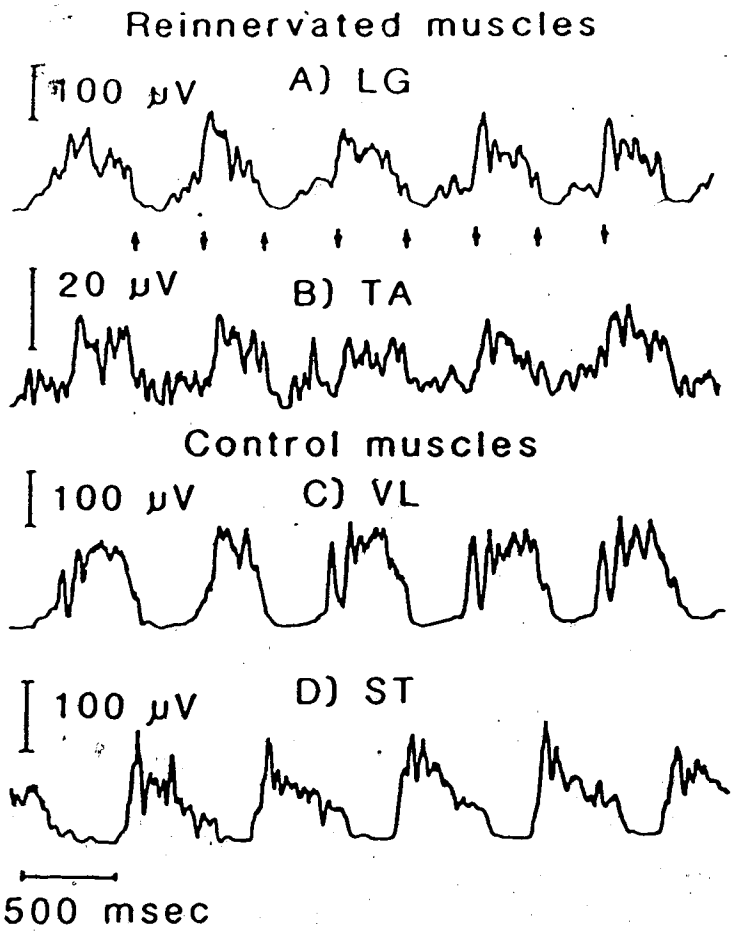

Figure 5.4. Rectified and filtered EMG activity recorded from cross-reinnervated ankle extensor (A) and flexor (B) muscles and control knee extensor (C) and flexor muscles (D) muscles during locomotion. All EMG activity was recorded from muscles ipsilateral to the nerve-cross surgery. Upward and downward pointing arrows below trace A correspond to the beginnings of the swing and stance phases in that leg.

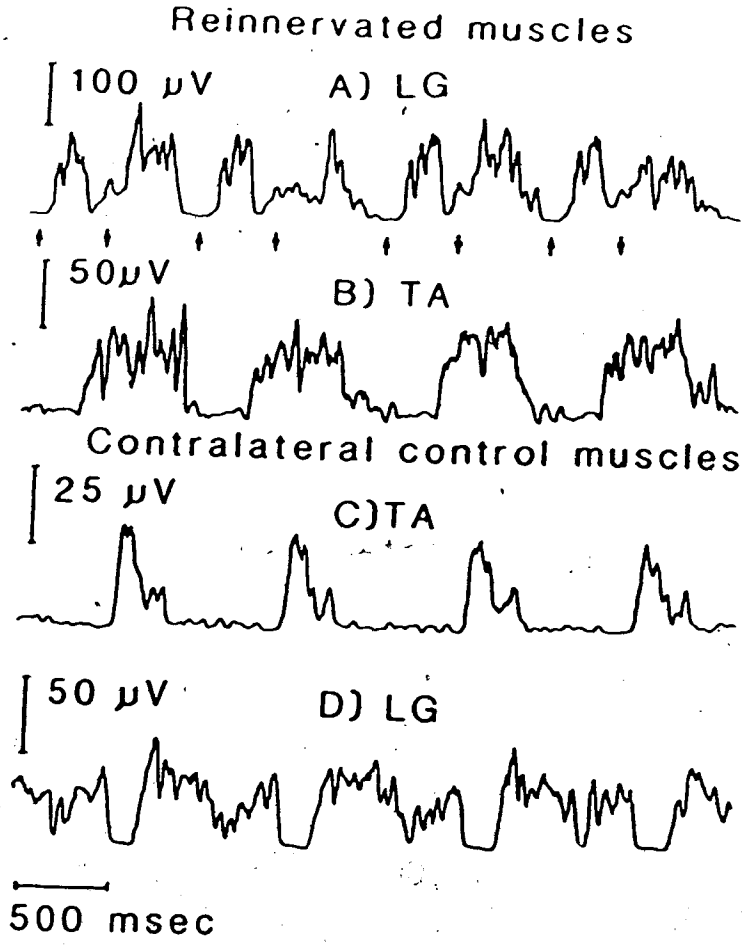

Figure 5.5. Rectified and filtered EMG activity recorded from cross-reinnervated extensor (A) and flexor (B) muscles and contralateral control flexor (C) and extensor (D) muscles during locomotion. The upward pointing arrows below trace A represent foot lift (beginning of swing phase) in the reinnervated leg while the downward pointing arrows indicate foot fall (beginning of the stance phase) in the same leg.

both fired mainly in the stance phase rather than in alternate phases. Similarly, the cross-reinnervated extensor (LG) showed some activity in the swing phase when the control flexor (ST) muscle was active. The cross-reinnervated muscles were therefore functioning inappropriately and according to a pattern which was intermediate between typical flexor and extensor EMG activity. In addition, a larger burst in the cross-reinnervated LG also occurred during stance. Since this cross was only 62% complete for the LG, the additional EMG activity could have arisen from the 38% of fibers which were not cross-reinnervated, rather than genuine functional compensation (see Discussion).

Figure 5.5 shows the same tendency for inappropriate timing of cross-reinnervated flexor and extensor muscle activity but in reinnervated and contralateral control muscles of a different cat. The cross-reinnervated flexor muscle (TA) is essentially in phase with the contralateral control TA muscle. Activity in the reinnervated flexor muscle begins shortly before foot contact and continues throughout stance. This is the typical pattern for an ankle extensor (see above and Halbertsma, 1983) although the duration of the contralateral extensor EMG is longer. Similarly, a burst occurs in the cross-reinnervated extensor (LG) in phase with its contralateral control extensor muscle (cf. Figures 5.5A and 5.5D). This burst in the cross-reinnervated extensor is in the swing phase as expected for

an ankle flexor. An additional burst of EMG activity was present in the cross-reinnervated extensor muscle during the stance phase (Figure 5.5A) which was comparable in amplitude to that present during the swing phase. As the motor nerve cross for the LG was only 68% complete, this activity could have resulted from unsuccessful cross-reinnervation rather than from genuine functional compensation (see Discussion).

The data shown in Figures 5.4 and 5.5 were typical of that recorded from all animals. The most common variation was in the relative amounts of stance related activity in the crossed LG (cf. Figures 5.4A and 5.5A), which was largest in those animals in which the cross was least complete.

5.3 DISCUSSION

In the present study the nerve innervating all of the distal flexor muscles was cross-united with the nerve innervating all of the distal extensor muscles in one hindlimb of young cats. To assess if genuine functional compensation during locomotion resulted from alterations in central neural connections following reinnervation, the success of the nerve crosses had to be determined.

5.3.1 Factors determining the completeness of the cross

The data indicated that the motor nerve cross was generally better for the crossed CP nerve than for the crossed MG and LGS nerves (Table 5.2). These results were

expected because there are more than twice as many motor axons in the tibial nerve than in the CP nerve (Hoffer et al., 1979). However, these data for the crossed CP nerves were artificially high because only the charge contributions from the MG and LGS branches of the tibial nerve were included in the cross purity calculations. This results in the mean root entry value for control extensor nerves being less positive or tending more towards lumbar root entry. Overall, the denominator in the cross-purity calculation is smaller, making the cross-purity appear more successful. As the mean root entries for control MG and LGS nerves in the present study were similar to those reported by Hoffer et al., (1979), the present cross purity calculations could be adjusted by using the complete tibial charge contributions from Hoffer et al., (1979). This resulted in the mean completeness of the present motor CP cross being reduced from 83.5 to 69.5%.

The completeness of the sensory cross was high in the crossed MG and LGS nerves. The least success was found for the sensory component of the crossed CP nerve. Part of this may reflect the tendency for there to be fewer sensory axons in the tibial nerve than in the CP nerve (Hoffer et al., 1979). The absence of the tibial charge contribution to the foot in the purity calculations will also result in an artificially low value for the completeness of the sensory components in the crossed CP nerves. After the appropriate adjustment for the complete tibial charge contribution using

the data of Hoffer et al., (1979), the completeness of the sensory components in the crossed CP nerve was substantially increased.

Differences between motor and sensory reinnervation will also influence the success of the nerve cross. Motoneurons reinnervate muscle fibers exclusively and non-specifically (Miledi and Stefani, 1969; Gillespie et al., 1986; see Chapter 4, 6). In comparison, sensory nerve fibers only make connections with appropriate target organs. Those sensory fibers that are unsuccessful in reinnervating end organs, will atrophy (Davis, Gordon, Hoffer, Jhamandas and Stein, 1980; Mackel et al., 1983). Thus, motor reinnervation was expected to be better than sensory reinnervation. Our data show this for the crossed CP nerves. The discrepancy for the crossed MG and LGS nerves are probably explained by the proportions of sensory and motor axons in the CP nerve and the exclusion of the complete tibial nerve charge contribution from the purity calculation as described above.

5.3.2 EMG patterns during locomotion

Cross-reinnervated extensor and flexor patterns of muscle activity were inappropriate in relation to the timing of control flexor and extensor muscle activation patterns in both ipsilateral and contralateral legs. EMG activity in the cross-reinnervated flexor was in phase with ipsilateral control extensor and contralateral control flexor EMG activity. Some reinnervated extensor EMG activity was in

phase with ipsilateral control flexor and contralateral control extensor EMG activity. These cross-reinnervated extensor and flexor muscles were largely reinnervated by flexor and extensor motoneurons respectively. Therefore, these data indicate that the muscles were activated according to the normal patterns of activity of the antagonist nerves that now supplied them. Similar results were reported by Tsukahara et al., (1982) following cross reinnervation of flexor and extensor forelimb nerves of cats and by others following cross-union of only two muscle nerves (Cohen, 1978; Luff and Webb, 1985; O'Donovan et al., 1985).

As well as the reversed EMG activity during locomotion, additional EMG activity occurred in the cross-reinnervated extensor muscles during the stance phase of locomotion (see Figure 5,4, 5.5; Tsukahara et al., 1982). In terms of movement patterns, this was reflected by incomplete movement reversals. Some cats initially placed the foot dorsum on the ground, but quickly turned the foot over to take weight on the pad during the stance phase. The stance phase was shorter than normal with more weight bearing occurring in the contralateral limb. Several cats maintained an extended ankle joint during locomotion which, combined with shortening of the stance phase, prevented the ankle joint from collapsing onto the ground. In order to bring the leg through to the next flexion phase, the hip joint had to be

lifted higher than usual during the relatively longer swing phase. Such compensation was necessary to maintain balance during locomotion.

Also, when the limb hits the ground, flexor reflex afferents will be excited which will now be directed to excite the crossed extensor muscles. If the muscle is stretched under the weight of the body, stretch reflexes may also be elicited in these muscles.

Finally, since the motor nerve crosses were less than complete, some of the additional EMG activity during locomotion could be due to extensor nerve axons which returned to their original muscles. Additional inappropriate EMG has been recorded in other studies where the nerve cross was impure (Cohen, 1978; Luff and Webb, 1985; O'Donovan et al., 1985) but was absent following pure nerve crosses (O'Donovan et al., 1985). For all these reasons, there was no compelling evidence for genuine modification of central neural connections, as suggested by some previous authors (Cohen, 1978; Luff and Webb, 1985; Tsukahara et al., 1982).

In conclusion, the data show that, even when cross-reinnervation was completed in young cats and when the success of the motor nerve cross was relatively high, a spinally generated behavior like locomotion was still dominated by the original, and now inappropriate flexor and extensor EMG patterns. The spinal synaptic connections were not greatly altered even one to two years after surgery. Although the cats attempted functionally to compensate their

movement patterns during locomotion, their walking was neither adept nor smooth.

CHAPTER 6

PERIPHERAL REORGANIZATION OF MOTOR UNIT PROPERTIES FOLLOWING CROSS-REINNERVATION OF ANTAGONISTIC MUSCLES IN THE CAT HINDLIMB

Cross-union of two common nerves in the cat demonstrated that the function of the cross-reinnervated muscles changed towards that of the innervating nerves (see Chapter 5). In terms of muscle properties, other studies have shown that the cross-union of the nerves to the slow soleus and primarily fast twitch FDL muscle of cats, resulted in the contractile speed of the reinnervated muscles changing towards that of the foreign innervating nerve. These changes were more complete in the cross-reinnervated FDL muscle than the cross-reinnervated soleus (Buller, Eccles and Eccles, 1960). At the motor unit level, the cross-reinnervated FDL units had physiological, histochemical, biochemical and immunocytochemical profiles typical of self-reinnervated or normal soleus motor units. Thus, the majority of units were physiologically classified as slow. Although grouped together, the constituent muscle fibers were all type I and all cross-reacted with light chains of slow twitch myosin. Staining was high for oxidative enzymes but low for glycolytic enzymes or myosin ATPase activity (Bagust, Lewis and Westerman, 1981; Chan, Edgerton, Goslow, Kurata, Rasmussen and Spector, 1982; Dum, O'Donovan, Toop and Burke, 1985a; Gauthier, Burke, Lowey and Hobbs, 1983). Similar

physiological results have been reported following cross-reinnervation of the cat LG muscle by MG motoneurons (Foehring, Sypert and Munson, 1984).

In comparison, soleus muscles cross-reinnervated by FDL axons appeared far more resistant to change and much more attention has been focused on them. The majority of muscle units were physiologically slow, although muscle unit contractile speed increased towards that of the innervating nerve. A minority of FR and FF motor units were found in the soleus muscle reinnervated by MG axons (Foehring et al., 1984). A few FR but no FF units were found in the soleus muscle reinnervated by FDL axons even though normal FDL motor axons typically innervate approximately 25% FF units. Motor unit size appeared to be re-established or became larger, as reflected by measurements of motor unit tetanic tension. However, in one study the fast twitch units developed the smallest tensions which is abnormal (Lewis, Rowlison and Webb, 1982). Histochemically, the ATPase reactivity and oxidative enzyme staining of muscle fibers was appropriate for type I or IIA fibers (Chan et al., 1982; Dum, O'Donovan, Toop, Tsairis, Pinter and Burke, 1985b). Myosin was altered but was unlike that of normal FDL or soleus. The fibers reacted strongly against the light chain of slow twitch myosin and one light chain of fast twitch myosin (Gauthier et al., 1983).

In all these studies, individual muscle nerves were

severed and cross-united so that the muscles were cross-reinnervated by axons from a single motor pool. In humans, complete severance of large nerve trunks containing axons that innervate many muscles is far more common than severance of individual muscle nerves. The denervation of many muscles with different functions presents a complex reinnervation situation (see Chapter 4). Disorderly recruitment of reinnervated human motor units after complete section and resuture of either the ulnar or median nerve in the forearm was shown to result from the misdirection of motor axons to foreign muscles with different functions (see Chapter 4). In that study, the relationships between nerve and muscle properties was inferred indirectly from the level of force required to recruit motor units and the twitch tension they generated during voluntary isometric contractions. In cats, a similar extreme test of muscle function after reinnervation was attempted by the cross-union of large common nerves in the distal hindlimb (see Chapter 5). In this chapter, the peripheral reorganization of those reinnervated motor units is examined directly by recording and correlating the relationships between nerve and muscle properties. For appropriate muscle function, the nerve and muscle properties within individual reinnervated motor units and across the new motor unit populations of the muscles would have to be rematched to the motoneuron following reinnervation.

6.1 METHODS

Following the surgery and acute experiments described in Chapter 5, the peripheral organization of cross-reinnervated motor units in the MG muscle of three cats and in the LG and soleus muscles of three other cats was studied. After the cat was positioned in the stereotaxic frame, the individual tendons of the muscles being recorded (MG or LG and soleus) were attached to a separate force transducer (Grass FT03 or FT10) to record muscle tension. Whole muscle forces were recorded in response to electrical stimulation of the appropriate muscle nerves. EMG activity was recorded using bipolar electrodes stitched to the fascia of these muscles (see Chapter 5).

The intact nerves were then placed over five electrodes spaced 1-2 mm apart. Triphasic action potentials were recorded differentially between the central electrode and the adjacent electrode on either side of it. To further reduce contamination of the signal by EMG the two outer electrodes were grounded. The cut S1, L7 and L6 ventral roots were then split into fine filaments to isolate single motor units. The firing of a motor unit was considered all-or-none when variation of the strength of the single shocks delivered to the filament produced no grading of the nerve or EMG potential and twitch tension. The single nerve filament was then repetitively stimulated and, the evoked neural (0.1-10 kHz) and EMG potentials (3-10 kHz) averaged together with muscle force as follows:

a) 30 responses to 1 Hz stimulation to measure twitch tension and contraction time (CT). Contraction time was measured from the peak of the unit EMG to the time of peak tension.

b) 2-5 responses to trains of 20 pulses at an interval corresponding to 40% of the CT to estimate tetanic tension.

c) a few trains of 25 stimuli at 100 Hz to produce posttetanic potentiation, but not to fatigue the muscle.

d) 20-30 potentiated twitch responses to 1 Hz stimulation.

e) stimulation for 800 msec with an interstimulus interval equal to 125% of contraction time, to test for the "sag" property (Burke et al., 1973). The unfused tetanus was considered to "sag" if it began to decline during the period of stimulation rather than increasing or reaching a steady level.

f) stimulation at 40 Hz for 330 msec at 1 sec intervals for 2 minutes. The fatigue index was determined from the ratio of peak tension at 2 minutes to initial peak tension.

At the end of the experiment, the sciatic nerve was exposed to determine the distance between the central stimulating and recording electrodes. Axon conduction velocity was calculated by dividing the conduction distance by the contraction time.

6.1.1 Assessment of axon size and motor unit size

The size relationships between the nerve and muscle

variables in control and reinnervated triceps surae muscles were determined, as illustrated for the MG muscle in Figure 6.1. The peak to peak amplitude of the average triphasic potentials recorded on the muscle nerve was used as an indicator of axon size, while the size of the motor unit was determined by tetanic tension.

The relationships between the amplitude of the extracellularly recorded action potential, muscle unit force and contractile speed of the normally innervated and reinnervated muscles were each plotted on logarithmic scales and fitted with straight lines to minimize deviations in the both directions according to a least mean squares criterion (Sokolnikoff and Redheffer, 1958). The best fitting lines were drawn only when the slopes of the lines were significantly different from zero at the 5% confidence level.

As the motor unit force was directly correlated with axon potential amplitude (Figure 6.2A, C, E) and negatively correlated with the contraction time of the motor units (Figure 6.3A, C, E) in normally innervated MG, LG and soleus muscles, the slopes of the lines were used to determine the extent to which normal nerve and muscle relationships returned after reinnervation. It should be noted that the correlation was weak for one LG muscle (Figure 6.2C). The data for only one soleus muscle is shown because the other muscle samples were small (Figures 6.2E, 6.3E).

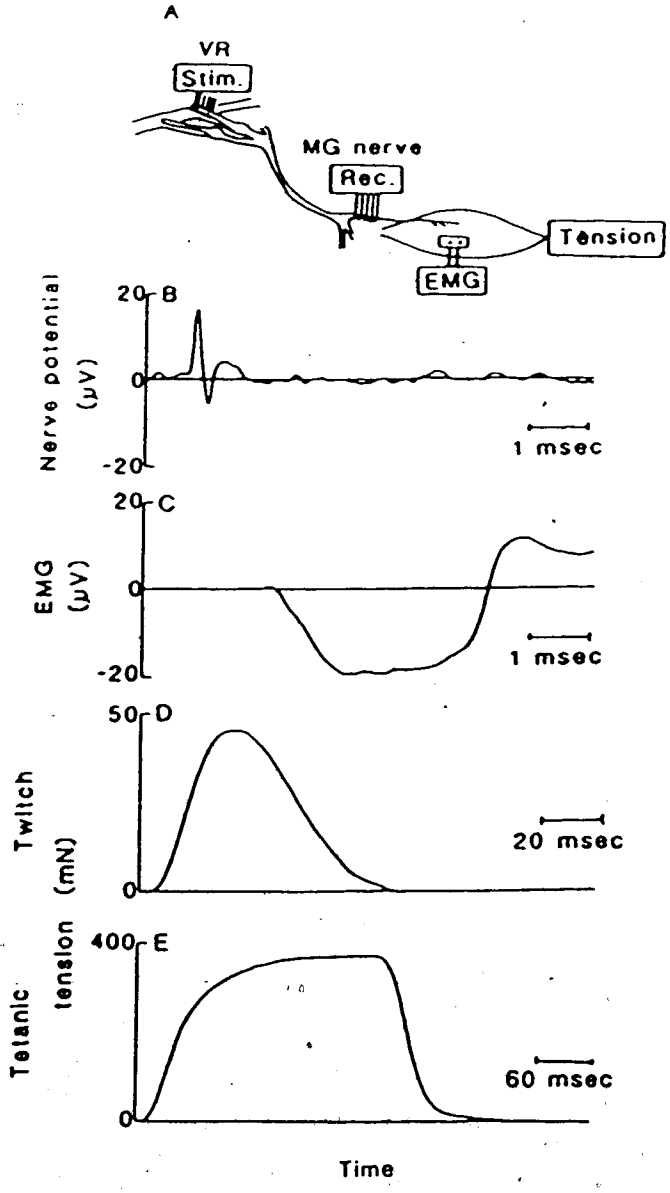

Figure 6.1. Schematic diagram (A) of the method used to stimulate a single nerve axon to the MG muscle and to record an all-or-none action potential on the MG nerve (B), on the EMG electrodes (C), and an isometric twitch (D) or tetanic (E) contraction. Each trace represents the average of 30 stimuli (5 stimuli for tetanic tension) and has a time scale appropriate for the time course of the signal illustrated. Data were recorded similarly following stimulation of single axons to either the LG or soleus muscles.

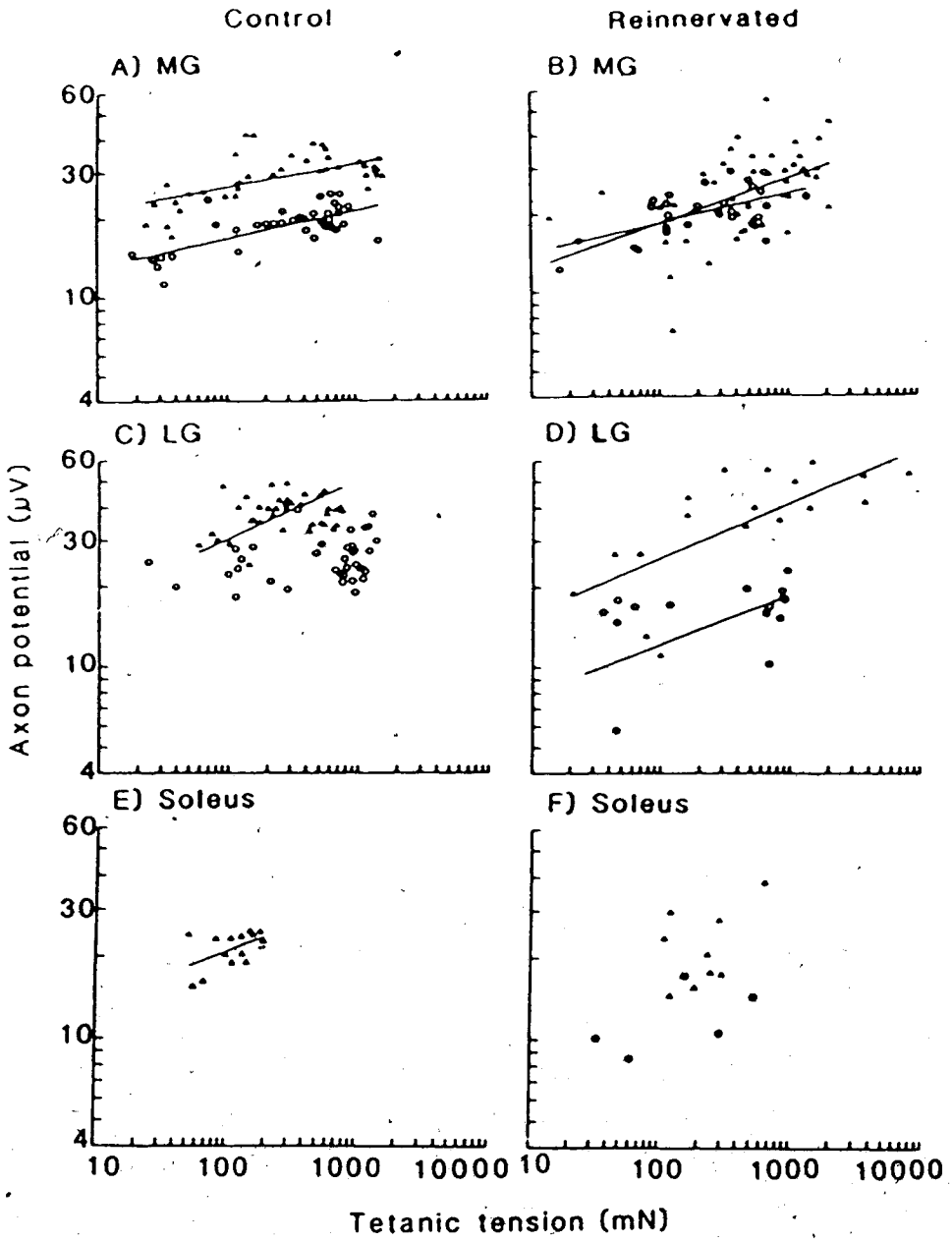

Figure 6.2. Axon potential amplitude recorded on the muscle nerve plotted as a function of tetanic tension for single motor units in two normally innervated MG (A) and LG (C) muscles, one soleus muscle (E) and two reinnervated MG (B), LG (D) and soleus (F) muscles. Data from different muscles are plotted using different symbols. All regression lines drawn in these and subsequent plots have slopes which are significantly different from zero (at the 5% level of probability) on the logarithmic scales used.

6.2 RESULTS

6.2.1 Motor unit properties following reinnervation

The positive correlation found between axon potential amplitude and muscle unit force in normally innervated muscles, was re-established in reinnervated muscles. In Figure 6.2, typical examples of these relationships from different muscles are represented by different symbols. The slopes of the regression lines were 0.10 ± 0.02 and 0.08 ± 0.02 (mean ± S.E.; Figure 6.2A) for the normally innervated MG muscles, 0.11 ± 0.03 and 0.19 ± 0.04 (Figure 6.2B) for reinnervated MG muscles, 0.06 ± 0.03 and 0.05 ± 0.03 (Figure 6.2C) for normally innervated LG muscles, 0.21 ± 0.05 and 0.19 ± 0.08 (Figure 6.2D) for reinnervated LG muscles, 0.19 ± 0.09 for a normally innervated soleus muscle and 0.24 ± 0.19 and 0.14 ± 0.11 for reinnervated soleus muscles. The slope of the relationship for the LG muscle represented by the open symbols (o) and each reinnervated soleus muscle was not significant. Demonstrating significant relationships depends in part, on the range of measured values and the sample size. For example, the range of axon potentials for the LG muscle was similar at all tetanic tensions so overall the relationship was positive as expected but weak. For both control and reinnervated soleus muscles, the sample sizes were small. If the data from the reinnervated soleus muscles were pooled, the slope of the relationship was 0.24 ± 0.11 and significant.

The slopes of the relationships were steeper than normal in one reinnervated MG and each LG muscle because of the wider range of axon potentials in these muscles. In addition, there was some variation in the slopes of the relationships for different reinnervated muscles. This was not indicative of differences in recovery, as similar variation was seen in control muscles.

Although the mean axon potential varied in different nerves, the overall axon potential mean for all control nerves was very similar to that found for all regenerated nerves. In all normally innervated muscles and all but one reinnervated LG muscle, a similar range of motor unit sizes was observed.

Figure 6.3 shows examples of the negative relationship typically found between contraction time and tetanic tension in normally innervated and reinnervated motor units of the MG, LG and soleus muscles. The slopes of the best fitting lines were -0.19 ± 0.03 and -0.21 ± 0.04 (Figure 6.3A) in the normally innervated MG muscles, -0.24 ± 0.03; -0.10 ± 0.05 in the normally innervated LG muscles (Figure 6.3C) and -0.29 ± 0.11 for the normally innervated soleus muscle (Figure 6.3E). Generally, the motor unit contraction times were slower in soleus than the MG or LG muscles. Following reinnervation, the range of contraction times for all muscles was similar and comparable to that observed in the normally innervated LG muscles. The more homogeneous

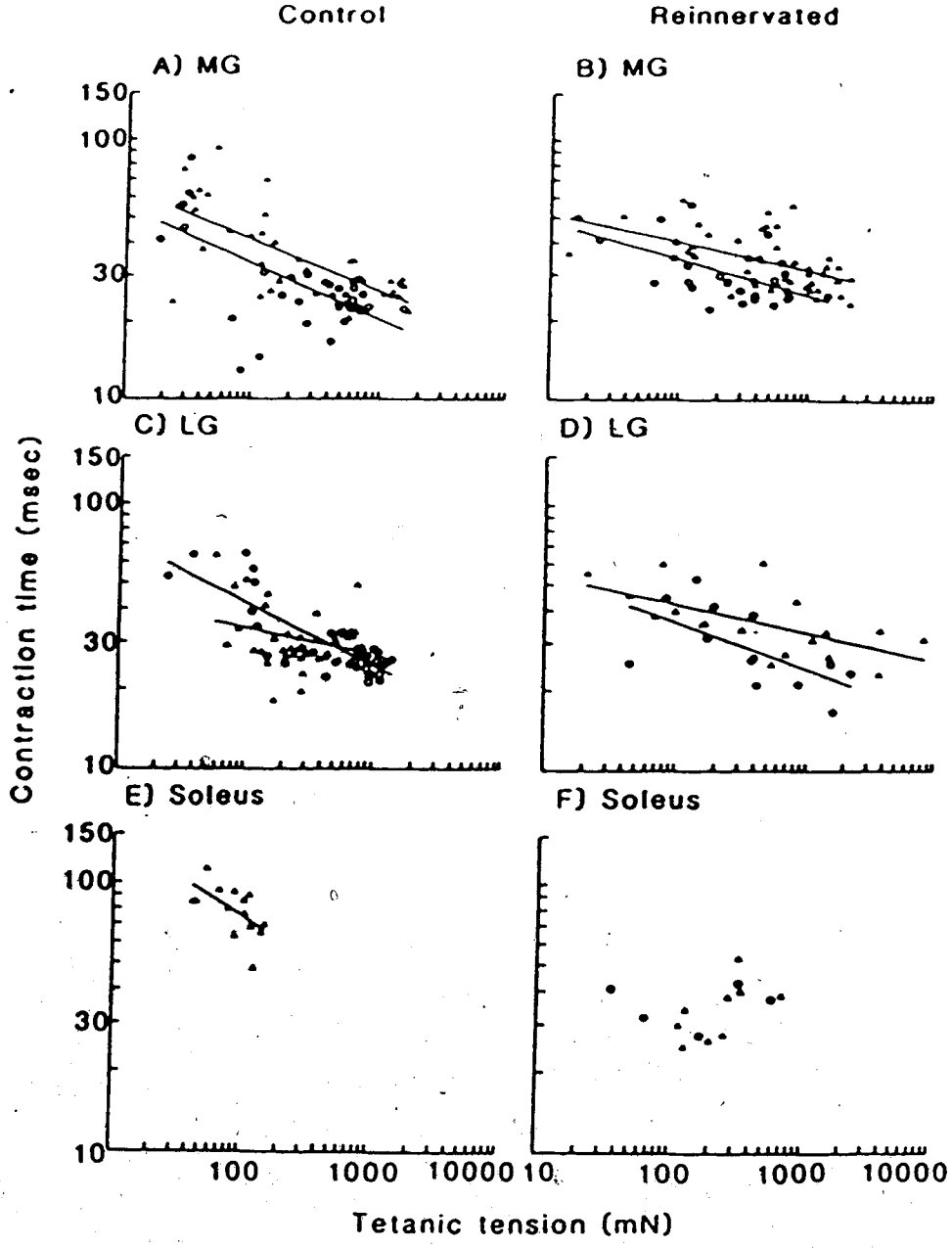

Figure 6.3. Contraction time plotted as a function of tetanic tension for single motor units in two normally innervated MG (A) and LG (C) muscles, one soleus muscle (E) and two reinnervated MG (B), LG (D) and soleus (F) muscles. Different symbols represent data from different muscles.

contraction times of the reinnervated MG motor units resulted in contractile speed and force output relationships with shallower than normal slopes in these muscles (-0.10 ±0.03; -0.13 ± 0.04; Figure 6.3B). Slopes for the reinnervated LG muscles were similar to normal (-0.11 ± 0.03; -0.17 ± 0.07; Figure 6.3D). For reinnervated soleus muscles the samples sizes were small and the relationships were not significant.

6.2.2 Alternative measures of axon size and motor unit size

In normally innervated muscles there was a strong positive correlation between the extracellularly recorded axon potential amplitude and axonal conduction velocity (Figure 6.4A). Because the tibial and peroneal nerves were cross-united just below the sciatic branch point in these experiments, the axonal conduction velocity could not be measured above the suture line. Therefore, following reinnervation the conduction velocities were slower than normal, presumably due to conduction delays across the suture line. The correlation between axon potential amplitude and conduction velocity was therefore weaker (Figure 6.4B). Because of these complications, axon potential amplitude was considered the better measure of axon size.

Figure 6.5 compares the positive relationship between peak to peak EMG amplitude and tetanic tension in one normally innervated and one reinnervated MG muscle. The same

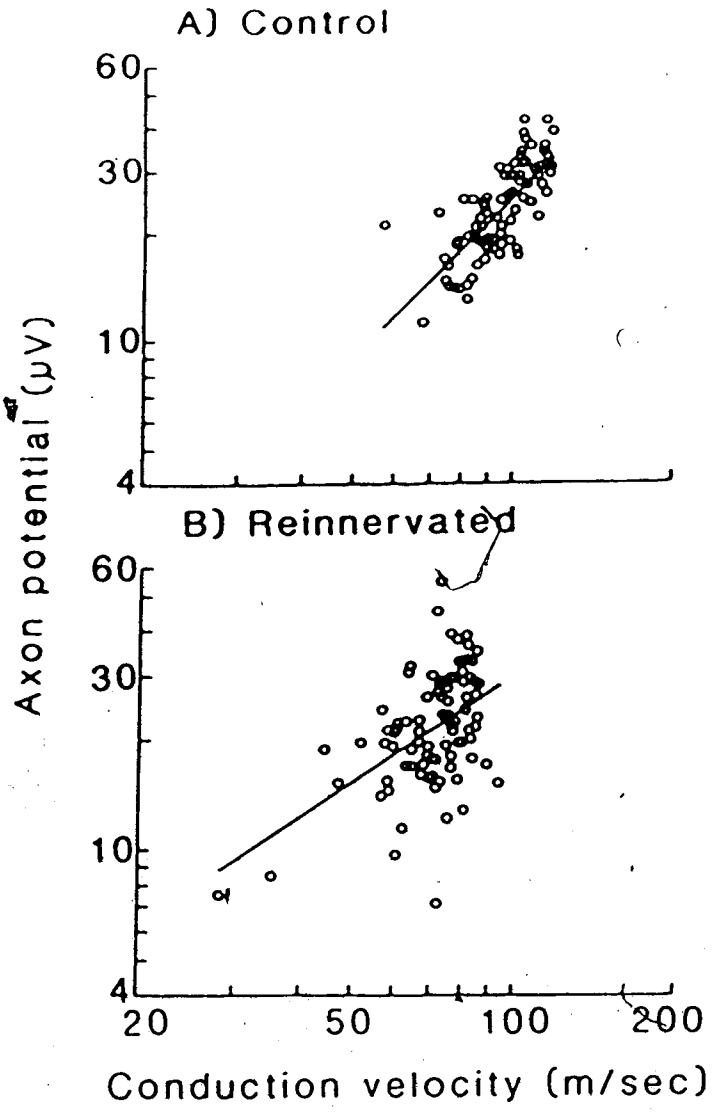

Figure 6.4. Extracellularly recorded axon potential amplitude plotted as a function of conduction velocity for all normally innervated (A) and reinnervated (B) MG muscles.

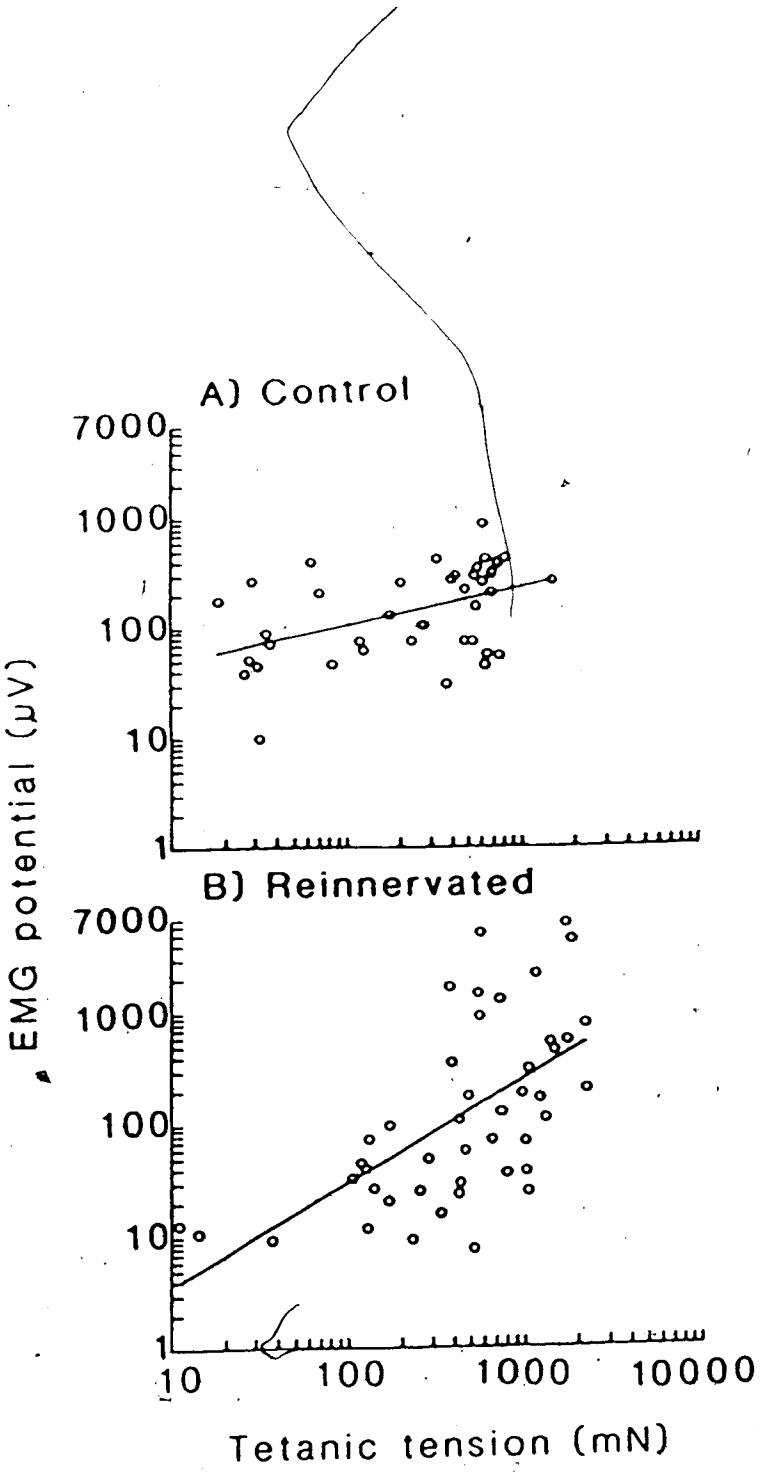

Figure 6.5. Peak to peak EMG amplitude plotted as a function of tetanic tension for one normally innervated (A) and one reinnervated (B) MG muscle.

trend was found in normal LG and soleus muscles and re-established following reinnervation. However, as EMG amplitude also depends on the distance of the muscle fibers from the surface electrodes, tetanic tension was considered to be more indicative of motor unit size.

Twitch tension provides another measure of motor unit size. As the motor unit response was more reliable with tetanic stimulation of the motor axon and the range of motor unit tetanic tension values was wider than that recorded for twitch tension, tetanic tension was considered the better measure of motor unit size.

6.2.3 Classification of motor units

In Figure 6.6 all the normally innervated and reinnervated motor units from different MG, LG and soleus muscles are compared according to their fatiguability during tetanic contractions and their contractile speed. Those motor units which showed "sag" during unfused tetani are represented by a different symbol from those motor units which showed no sag. All of the normally innervated and all but 15 reinnervated motor units could be classified as either S, FR, FI, or FF types by utilizing their "sag" property and fatigue characteristics. The 15 reinnervated motor units which were unclassified, fatigued despite the absence of "sag".

An alternative way to classify each motor unit was to utilize contractile speed and fatigue characteristics. Those

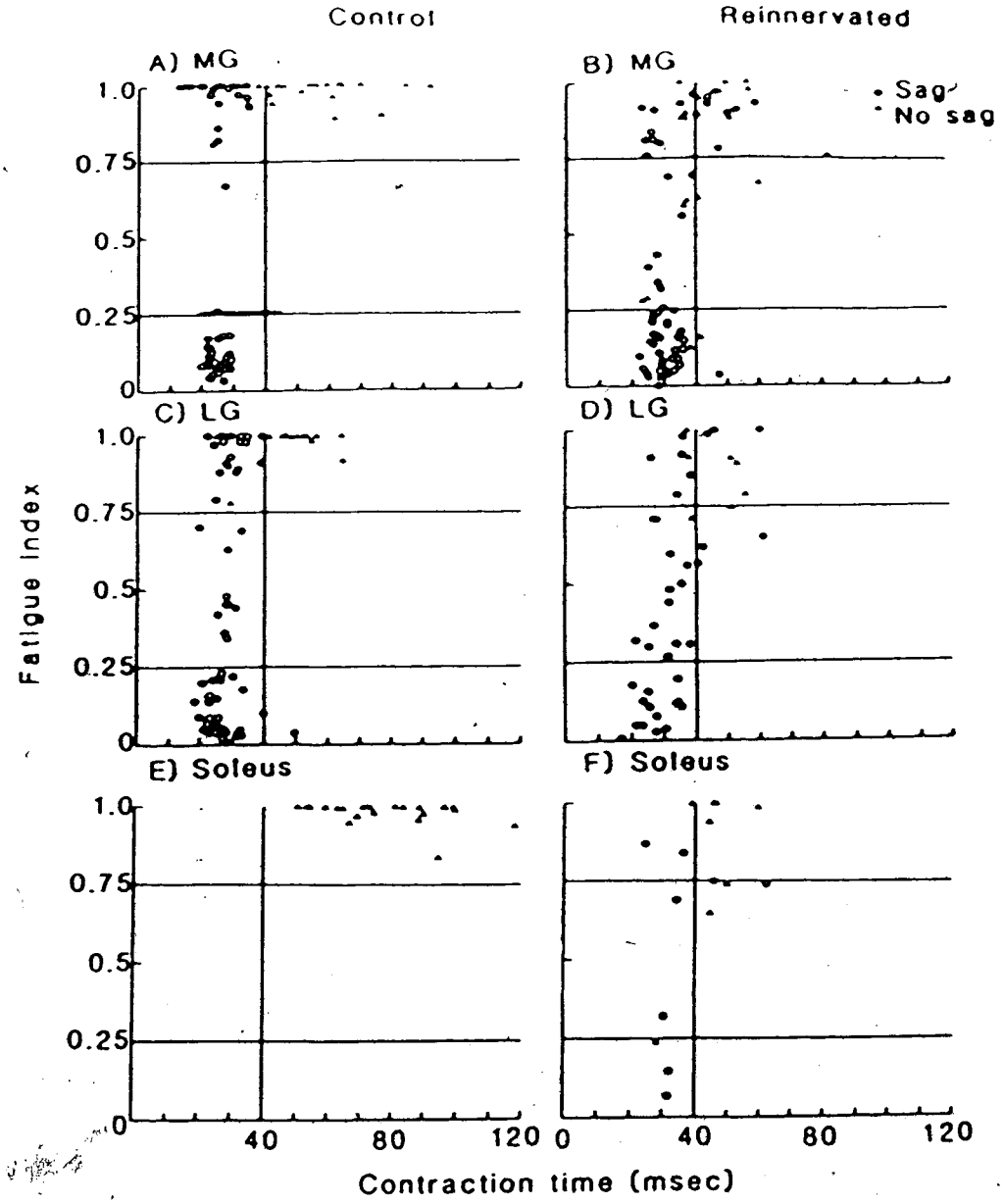

Figure 6.6. Fatigue index of normally innervated motor units from all MG, LG and soleus (A, C, E) muscles and reinnervated motor units from all MG, LG and soleus (B, D, F) muscles plotted as a function of their contraction time and "sag" property. Motor units were separated into fast or slow by the presence and absence of "sag" or by the vertical line drawn at a contraction time of 40 msec. Those motor units with a fatigue index above the horizontal line drawn at 0.75 were considered to be fatigue resistant while those below the horizontal line at 0.25 were fatiguable. Motor units between these two fatigue index limits showed intermediate fatigue characteristics.

motor units with a contraction time of less than 40 msec were classified as fast while those with a contraction time of 40 msec or greater were classified as slow. These criteria produced two main differences. One normally innervated and 9 reinnervated motor units were unclassifiable because they fatigued yet had relatively slow contraction times. Secondly, 7 normally innervated and 7 reinnervated motor units, which were classified as FR units, were previously classified as slow motor units by their sag properties.

Irrespective of the classification scheme used, the distribution of the motor units in the reinnervated muscles was strikingly similar and much like the distribution of motor units in the control LG muscles. These results were indicative of a marked change in the contractile properties of reinnervated soleus units. Each of the four motor unit types was found in reinnervated soleus muscles, whereas normally innervated soleus units are all of the slow type. Following reinnervation, there was also an increase in the numbers of motor units showing intermediate fatiguability in all of the different muscles.

Figure 6.7 shows comparisons of the mean axon potential ($\pm$ S.E.) and tetanic tensions ($\pm$ S.E.) for different unit types found in MG, LG, and soleus muscles of all control and reinnervated cats. The motor units were classified by their fatigue characteristics and contractile speed. The mean axon

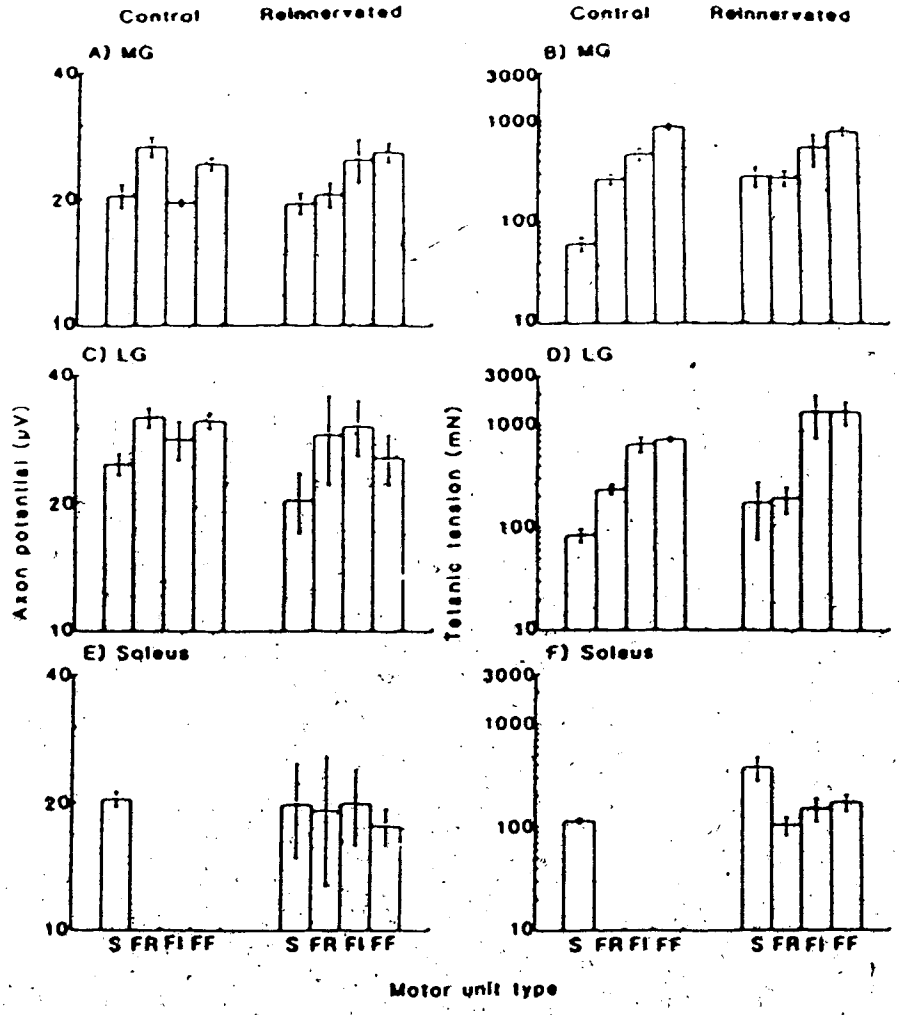

Figure 6.7. Mean (+ S.E.) axon potentials and tetanic tensions for S, FR, FI , and FF motor unit types in all MG, LG, and soleus muscles. Figures represent data from 2 normally innervated and 3 reinnervated MG muscles, and 4 normally innervated and 3 reinnervated LG or soleus muscles. Units were classified by contractile speed (S > 40 msec; F < 40 msec) and fatigue properties during unfused tetani (fatigue index: S, FR > 0.75; 0.25 < FI < 0.75; FF < 0.25).

potential for control and for reinnervated slow units in the MG, and LG muscles was generally less than that found for fast units. Soleus units were normally all of the slow type. Following reinnervation, axon potential means were similar for the slow and fast soleus units. With respect to tetanic tensions, there was some overlap between unit types in normally innervated MG and LG muscles but the mean force of slow motor units was generally less than the mean force of fast units. Following reinnervation, overlap of tetanic tensions between the different unit types was considerable. For soleus, the sample sizes were small. Following reinnervation, the range of force for all unit types was similar, although the mean force generated by slow units was actually larger than that generated by fast units.

6.3 DISCUSSION

In the present study the flexor and extensor nerves of the distal hindlimb of cats were surgically cross-united to study the extent of peripheral reorganization of nerve and muscle properties. Because these common nerves contained axons from many different motor pools with antagonistic functions, these experiments represented an extreme test of the rematching of size relationships between nerve and muscle.

Significant positive correlations were re-established between axon potential amplitude and tetanic tension in individual reinnervated MG and LG muscles but only in

reinnervated soleus muscles when data from two muscles were pooled. The slopes of the best fitting lines were generally comparable to those found in normally innervated muscles. Thus, these findings verify that reorganization of nerve and muscle properties according to size occurred after reinnervation, as previously shown following reinnervation of the triceps surae muscle group of cats by their own nerves (Gordon and Stein, 1982). Furthermore, these results demonstrate the remarkable potential for such reorganization since these extensor muscles were largely reinnervated by flexor motoneurons (see Chapter 5).

The firing pattern of the innervating nerve presumably had a strong influence on the rematching of nerve and muscle properties in reinnervated motor units. Earlier studies have shown that predominantly fast twitch muscles become slow contracting when activated with a slow firing pattern of nerve activity (Buller et al., 1960). Also, if denervated or cross-reinnervated muscles are stimulated with their former activation patterns, they retain their typical contractile properties (Lømo, Westgard, and Dahl, 1974, Salmons and Sreter, 1976).

Following reinnervation of the MG and LG muscles, the significant inverse relationships between contraction time and force output were re-established. These relationships were weaker than normal in the reinnervated MG muscles because of the much narrower range of contraction times in

these reinnervated motor units. Significant relationships were not re-established in reinnervated soleus muscles but the sample sizes were small. In this study, the reinnervated MG, LG and soleus muscles were largely innervated by flexor motoneurons. The units tended to have faster contraction times, similar to the normal motor units found in flexor muscles (Dum and Kennedy, 1980a). These results are consistent with the idea that the flexor motoneurons determine the contractile speed in the muscles they supply.

Using sag and fatigue characteristics, all of the normally innervated motor units from MG and LG muscles could be classified as S, FR, FI, or FF, while soleus motor units were always S. Following reinnervation, all of the different motor unit types were found in each of the different muscles studied. Thus, the contractile properties of the muscle fibers can be influenced by their innervating motoneuron (Buller et al., 1960), since a flexor muscle like TA contains all four motor unit types (Dum and Kennedy, 1980a). These data also indicate that motoneurons show little or no preference for their former type of muscle fiber and can innervate muscle fibers of all types.

In contrast, two other studies in which soleus muscles were cross-reinnervated by FDL axons reported that all or the majority of motor units were classified physiologically as S. The remaining were FR. The muscle fibers were histochemical type I or IIA (Chan et al., 1983; Dum et al., 1985b). The physiological differences may reflect the

differences between the nerve crosses. Cross-uniting the common nerve to all flexor muscles of the distal hindlimb as opposed to one flexor muscle nerve may result in a larger proportion of FF and FI motor axons from which to sample. A few FF units were recorded in soleus muscles cross-reinnervated by MG motoneurons (Foehring et al., 1984). In addition, 15 reinnervated motor units in the present study were unclassifiable using "sag" and fatigue criteria because they fatigued, yet showed no sag during unfused tetani. These motor units with properties intermediate between the normal unit types were found in all of the triceps surae muscles. Therefore, the motoneuron does not appear to be the exclusive influence involved in determining cross-reinnervated muscle properties as suggested by others (Chan et al., 1982; Dum et al., 1985b; Foehring et al., 1984).

Alternatively, fast motor units can be separated from slow motor units by using a contraction time of 40 msec instead of the "sag" property. Seven motor units previously classified as S by their sag property, were now classified as FR units. However, this different classification scheme would only explain the different proportions of S to FR units in the present and other studies. Using these criteria, 1 normally innervated and 9 reinnervated motor units in this study had muscle properties intermediate between the normal unit types.

Regardless of how motor units were classified, there was

a notable increase in the number of the reinnervated motor units in all muscles with intermediate fatigue characteristics. These data were consistent with demonstrations at the muscle level of incomplete conversions of contractile, biochemical and histochemical muscle properties following cross-reinnervation (Jolesz and Sreter, 1981). Similarly, soleus muscle fibers cross-reinnervated by FDL motor axons showed immunocytochemical reactions that were intermediate between those of normal soleus and FDL muscle fibers (Gauthier et el., 1983). Similar incomplete changes may account for the intermediate changes in motor unit contractile properties observed in the present study. One way in which such incomplete conversion of muscle properties is reflected physiologically at the single motor unit level may be in terms of intermediate fatiguability. A further indication of these intermediate motor unit properties was reflected by the greater overlap in tetanic tensions of different reinnervated unit types.

In conclusion, the data in the present study show that there was peripheral organization of nerve and muscle properties according to size in the reinnervated motor units and muscles. Recovery of motoneuron properties and synaptic contacts onto axotomized motoneurons also occurs after muscle reinnervation (Sumner and Sutherland, 1973). Even so, the patterns of muscle activity are determined largely by the motor nerves and the fine control of movement results from the orderly recruitment of motor units in any one motor

pool (Henneman et al., 1965a). However, following reinnervation, the motoneurons innervating any one of the triceps surae muscles came from many different motor pools with antagonistic functions. Thus, although they may be activated in order of increasing size or excitability, they may be activated for quite inappropriate movements. An indication of inappropriate muscle function was demonstrated by abnormal movement patterns during locomotion (see Chapter 5).

CHAPTER 7

GENERAL DISCUSSION

Henneman first proposed that the susceptibility of motoneurons to discharge was a function of their cell size (Henneman, 1957; Henneman et al., 1965a). Since then, there have been numerous animal and human experiments which suggest, that for an overall excitatory input to a motoneuron pool, there is a characteristic order of motoneuron recruitment (Henneman et al., 1974; Milner-Brown et al., 1973b). Recruitment of motor units has been shown to occur by size or type (Zajac and Faden, 1985) and may occur in a force dependent way by a systematic gradation of intrinsic motoneuron properties (Gustafsson and Pinter, 1985). Until human motor units can be classified by physiological type or axon conduction velocities readily measured, their recruitment can only be described in terms of motor unit size. Overall, recruitment order is influenced by motoneuron size and geometry, intrinsic motoneuron properties, as well as the strength and connectivity patterns of the synaptic inputs. How these parameters combine to determine the functional threshold of a motoneuron is still be determined. Similarly, the functional importance of active and inactive synapses in relation to motoneuron excitability is just beginning to be explored as are the effects of different activation patterns on the operation of the afferent connections.

From the present study, it is clear that exceptions to orderly motor unit recruitment in human subjects during contractions of a muscle in different directions or during functional joint movements are only apparent. When the changes in recruitment threshold during different directions of contraction of small hand muscles are viewed in relation to the whole population of motor units within a muscle, recruitment by increasing twitch size adequately describes the function of these muscles for all contraction directions. Similarly, motor unit recruitment during isometric contractions is a good reflection of the actual recruitment order of motor units in these muscles during functional joint movements. For the size principle of orderly motoneuron recruitment to be the common pattern for activating motoneurons in so many different types of movements, suggests that the overall organization of different motor pools is very similar. To appreciate the special functions of muscles, the motor unit properties of the whole muscle must be viewed in relation to the actions and properties of other muscles.

A unique opportunity to study the importance of the normal organization of nerve and muscle properties for orderly motor unit recruitment and voluntary muscle function, occurs after accidental severance of a large human nerve containing axons that innervate a number of muscles.

When complete nerve section and repair in the human

forearm or the cat hindlimb involves the accidental or deliberate misdirection of motor axons to foreign muscles, there is a strong tendency for muscle unit properties to reorganize in a functionally meaningful way. That orderly size relationships between nerve and muscle properties were also re-established in cross-reinnervated cat hindlimb muscles, suggests that similar reorganization may occur in reinnervated human motor units if nerve recordings could be easily made.

One interesting question which arises from these findings is how the nerve and muscle properties within a motor unit are rematched after reinnervation. Since the classic cross-reinnervation experiments of Buller et al., (1960), it has been clear that alpha motoneurons exert powerful control over the contractile properties of the muscle fibers they innervate. Even though the territory of reinnervated motor units has been shown to be altered (Dubowitz, 1967; Kaparti and Engel, 1968), the motor unit histochemical properties become appropriate to the innervating motoneuron (Dum et al., 1985a). Similarly, the physiological properties of reinnervated motor units were shown here to change towards those of the innervating motor pools after cross-reinnervation of large common nerves and by others after cross-reinnervation of single muscle nerves of the cat hindlimb (Chan et al., 1982; Dum et al., 1985a). This provides convincing evidence that many muscle fibers are converted as a result of their new innervation. However, if

this "motoneuron specificity" was complete, all muscle fibers would be converted following cross-reinnervation. Cat soleus muscle fibers appear resistant to change in some cross-reinnervation experiments (Chan et al., 1982; Dum et al., 1985b) suggesting some residual role for intrinsic muscle properties.

The pattern or the amount of neural activity may be important in respecifying muscle properties (Pette, Smith, Staudte and Vbrova, 1973; Eerbeck, Kernell and Verhey, 1984). When denervated or cross-reinnervated muscles are stimulated with their former activation patterns, they retain their typical contractile properties (Lømo et al., 1974; Salmons and Sreter, 1976). However in these studies, all motor units in a muscle have had the same pattern of activity imposed on them. The activity of individual motoneurons is presumably influenced strongly by their relative position within the excitability hierarchy of the motor pool. Therefore it is difficult to assess how the typical activity patterns of individual motoneurons affect their muscle properties, especially when the range of responses of the population of motor units in a muscle is viewed.

After muscle reinnervation, full recovery of motoneuron and synaptic contacts occurs as well as appropriate reorganization of motor unit properties. The mechanisms that operate normally to organize these relationships and link

them to the excitability of the motoneurons within each pool still appear to act after complete nerve section and repair. Yet, after such as injury, there was no evidence to suggest that the destination of the motor axon altered the motoneuron activity patterns during voluntary movements or in a spinally generated behavior such as locomotion. Each motor pool still seemed to operate as a functional unit in that motoneurons were recruited into action in an orderly manner even though the pool was anatomically dispersed in the periphery by misdirection of axons.

When the lack of specificity between nerve and muscle connections following reinnervation is compared to the precise pattern of connectivity formed during development, it would seem that the mechanisms controlling these pattern formations during development are now lost. Admittedly, the developmental environment is very different from that following nerve section and repair, but findings in this area could have important implications following nerve injury. With current biochemical and immunocytochemical techniques, it would seem possible that the cellular and molecular nature of the developmental cues which constrain and direct axon outgrowth could be defined. In the adult, these developmental cues could be important. Neural cell adhesion molecules have been found on muscle fibers of patients with denervating diseases. These types of molecules are also transiently expressed on embryonic myotubes (Cashman, Covault, Wollman and Sanes, 1985). Alternatively,

such cues may be too weak to be effective, not activated in response to injury or simply inappropriate.

To conclude, it would seem that the size principle of motor unit recruitment can be re-established after nerve section in humans, if motor axons innervate their original muscles or ones with closely synergistic functions. However, substantial misdirection of motor axons occurs despite careful resuture of fascicles within the nerve. This obscures the orderly pattern of recruitment for one muscle or one motor pool. Recruitment order in any one reinnervated muscle will depend on the activation of motor units from different motor pools which formerly executed different movements. Therefore, it is clear that misdirection of motor axons during reinnervation is a clinical as well as a physiological problem, despite the impressive reorganization of motor unit properties after reinnervation of muscles with synergistic functions that was demonstrated here.

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