INTRINSIC ORGANIZATION OF A MOTONEURON POOL IN THE ADULT RAT

by

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ABSTRACT

It is becoming increasingly clear from physiological and histochemical observations that many skeletal muscles are not used in an all-or-none fashion, but rather that some parts of the muscle are more active in one movement than another. Such a "functional compartmentalization" could have an appropriate anatomical correlate; for example, within the pool of motoneurons supplying an individual muscle, subsets of motoneurons could be spatially grouped according to the location of their peripheral muscle fields. For a number of technical reasons, this possibility is difficult to investigate in the typical skeletal muscle. The cutaneous trunci muscle (CTM) of the rat, however, is ideally suited for such an investigation. This vast thin sheet of muscle is inserted into the deep surface of back and flank skin and is reflexly activated by nociceptive information from the overlying skin. A punctate stimulus evokes a localized contraction of the CTM in the immediate vicinity of the stimulus: the reflex activation of this muscle is therefore organized behaviorally into functional compartments. The simplest expectation would be that the peripheral nerves that drive the CTM reflex are segmentally organized. While this is true for the sensory nerves, I find that it is not the case for the motor ones. I have used electrophysiological and histochemical techniques to examine the pattern of motor innervation of the CTM and retrograde tracers to study its motoneuron pool. Interestingly, all the CTM motoneurons are located in the

cervical spinal cord, several segments rostral to even the most rostral sensory input that activates the CTM. My findings indicate that there is a spatial organization within the CTM motoneuron pool; this organization corresponds to the pattern of motor nerve innervation of the muscle, and moreover, seems appropriate for the compartmentalized nature of the reflex activation of the muscle by cutaneous sensory nerves.

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SECTION I

INTRODUCTION AND OVERALL OBJECTIVE

There are a number of indications that the development of an embryo proceeds according to defined spatial coordinates (Wolpert, 1971). Indeed, in some lower vertebrates (e.g. Amphibia) it has been shown that even prior to fertilization the ovum exhibits a marked polarity that appears to determine subsequent developmental pathways (Spemann, 1938). Other spatial features have been described in early organogenesis, in that certain embryonic regions or "fields" are not visibly marked, but are destined to give rise to specific organs such as eyes, nose, or ears (Balinsky, 1981). Of special interest to the neurobiologist are the processes by which target regions or organs become appropriately connected to the nervous system; these are still largely unknown. However, in the histogenesis of the vertebrate spinal cord there are a number of recognizable patterns that can be described in terms of orderly spatial and temporal gradients (Nornes and Das, 1974), that in recent studies appear to be correlated with the sequence of synapse formation in simple reflex circuits (Sims and Vaughn, 1979; Sims, Vaughn and Wimer, 1981). Neuronal projection patterns have been best studied in the sensory nervous system, specifically the projections from sensory receptors in the periphery to higher brain centres. Investigations of the rodent mystacial vibrissal system, for example, have revealed a structure-function relationship such that the relative order of the mystacial hairs in

each of the five rows of whiskers is preserved in the pattern of their connexions to both the thalamic nuclei and to the barrel fields of the somatosensory cortex (Woolsey and Van der Loos, 1970). In the motor system, while there have been a number of studies describing the gross topographic projections of pools of motoneurons to the periphery (e.g. Romanes, 1964; Hollyday, 1980), very little is known about the intrinsic organization of these motor pools. One of the difficulties in analyzing motor projections is that the spatial characteristics of the target become obscured due to torsion and migration of the developing muscle mass away from its origin (Sullivan, 1962; Grim, 1971). However in the adult there are indications that some parts of the same muscle may be used differentially in certain movements, that is, there is a functional compartmentalization of certain skeletal muscles (Herring, Grimm and Grimm, 1979). These observations suggest the possibility that there may be an anatomical basis for the inferred behavioral compartmentalization of the muscle: one question that arises is - could there be an underlying spatial organization of the reflex circuitry? This is a difficult possibility to examine in the typical skeletal muscle for a number of technical reasons. However, we have described the reflex activation of a thin cutaneous muscle which appears ideally suited for an investigation of this kind (Nixon, Theriault, Jackson and Diamond, 1984). This muscle, the cutaneous trunci muscle (CTM) of the rat, is reflexly activated by nociceptive

stimuli to the overlying skin; the important characteristic of this is that a punctate stimulus evokes a localized contraction of the CTM: the muscle is therefore behaviorally compartmentalized. Since the CTM is a thin flat sheet of muscle with its sensory and motor nerves anatomically separate, the possibility was presented for an investigation of the question raised above. The principal objective of this study therefore was to examine, in the CTM preparation, whether the motoneuron pool driving this muscle has within it an intrinsic spatial organization which corresponds to the behaviorally compartmentalized use of the muscle.

SECTION II

BACKGROUND

A. PATTERNS OF NEURONAL CONNECTIVITY IN THE ADULT NERVOUS SYSTEM

As a result of technological advances in fixation, microtomy and staining techniques in the early 19th century, it soon became clear that the constancy of form and structure characterizing the external features of the nervous system extended to the fine internal structure as well (Williams and Warwick, 1975). During the same era, physiologists studying the overt behavior of the nervous system came to the conclusion that many of the visible expressions of nervous activity appeared as highly predictable, stereotyped patterns of behavior, known as reflexes (Liddell and Sherrington, 1925). These two lines of investigation, morphological and behavioral, promoted interest in the possibility that a structure-function relationship may exist, wherein the overt behavior of the nervous system would have a distinct and characterizable anatomical substrate. More recent modern neuroanatomical and neurophysiological investigations have established the general principle that neurons processing similar kinds of information tend to be clustered together spatially (Brodal, 1981), and furthermore, that these neurons or groups of neurons are connected to one another and to their target organs in the periphery in often strikingly invariant patterns of neuronal circuitry, characteristic of all individuals in any one species from

insects (Macagno, Lopresti, and Levinthal, 1973) to vertebrates (Jacobson, 1978). In fact, there was no evidence of random connectivity within the nervous system (but see Wall, 1977). These observations have given rise to one of the most fascinating central issues in contemporary neurobiology: how do the observed precise patterns of neuronal connectivity become established during development?

Despite a tremendous amount of interest in defining the mechanisms that play a role in the establishment of orderly central and peripheral connections, a general understanding of the process remains elusive. Many hypotheses have been put forward, ranging from a connectivity that is entirely or largely genetically programmed at one extreme (see Hughes, 1968a, b) to random outgrowth and "functional validation" at the other (Weiss, 1947). There are a number of alternative explanations that incorporate some limited degree of prespecification (i.e., genetic programming) coupled with an influence of environmental cues, that together establish the correct projection patterns and synaptic connectivity (see reviews by Landmesser, 1981; Hollyday, 1983). A great deal of attention has been focussed on the extent to which <u>spatial</u> relationships among neurons and their targets during development determine the subsequent adult patterns of neuronal circuitry. Most experimental investigations have dealt with the visual (Horder and Martin, 1978) or somatosensory (Woolsey and Van der Loos, 1970; Nornes, Hart and

Carry, 1980a, b) systems; however, the same kind of questions are now beginning to be investigated in the motor system (Sims and Vaughn, 1979; Hollyday, 1981). The objective of this thesis was to characterize some of the patterns of connectivity in a particularly favorable skeletal muscle (the CTM reflex) including a study of the spatial organization inherent in the motor system; and from the results, it was hoped that an understanding of how such an organization might facilitate the establishment of the neuronal circuitry in the reflex, particularly that which underlies the unusually well-defined local sign seen in the reflex activation of the CTM.

In this section of the thesis some of the physiological and anatomical evidence regarding the specificity of connectivity in the nervous system, and especially how this might be dependent upon spatial factors, will be reviewed with particular (but not exclusive) emphasis on the neuromuscular system.

1. Central Nervous System (CNS)

One of the fundamental features of organization of the CNS is the preservation of body space within the orderly arrays of afferent and efferent projections (Brodal, 1981). For example, in the dorsal funiculus of the spinal cord (a large myelinated tract of axons representing a central, ascending projection of large diameter primary afferent fibres), a somatotopic map of the body is preserved such that afferent information dealing with the arm is anatomically

separate from that of the leg. In addition to separation of fibres on the basis of spatial (i.e., body) origin, CNS tracts are further anatomically sub-divided on the basis of modality, e.g., in the dorsal funiculus fibres carrying touch information are located more centrally (towards the central canal) than are fibres for pressure sensation, the latter being located nearest to the pial surface of the spinal cord (Warwick and Williams, 1975; Brodal, 1981). A subsequent relay centre for fibres in the dorsal funiculus is the thalamus, which in turn projects, in a characteristic somatotopic pattern, the sensory information from the periphery to the somatosensory area of the cortex, SmI (located on the post-central gyrus).

The pyramidal cells of SmI are also arranged in a correspondingly somatotopic fashion, giving rise to the sensory "homunculus", wherein the representation of different body parts is mapped onto discrete cortical areas. The early microelectrode studies of Penfield and co-workers revealed that stimulation of punctate locations in SmI reproducibly gave rise to specific sensation in a corresponding part of the body (Penfield and Rasmussen, 1937). They also demonstrated that stimulation of points on the precentral gyrus, the motor cortex, consistently evoked discrete movements in specific and predictable body areas (Penfield and Rasmussen, 1937; Penfield, 1950). Thus a somatotopic pattern within the <u>efferent</u> projections of the motor cortex is also evident.

Beginning with a "mirror-image" motor homunculus encoded in the spatial distribution of the pyramidal cells in the precentral gyrus, the efferent projection of the motor cortex is also somatotopically organized. Most recently, retrograde labeling techniques have shown that the cells of origin of the corticospinal tract, a more or less direct projection to the spinal cord, maintain this topographic specificity in their projection to the various interneuronal pools of the spinal cord (Warwick and Williams, 1975; Schwindt, 1981; Brodal, 1981). The red nucleus in the brainstem also receives a somatotopically arranged projection from the motor cortex, and in turn gives rise to an equally organized tract of fibres (the rubrospinal tract) which terminates in specific areas of the spinal cord (Schwindt, 1981). While it is clear that the corticospinal tract (and to a lesser degree the rubrospinal tract) play a major role in the central control and initiation of skilled, voluntary, discrete and rapid movements of the hand and fingers, anatomical and physiological data point to a very complex organization of the motor cortex (Brodal, 1981; Schwindt, 1981). Although the general pattern is well known, there is no agreement as to how individual muscles, or parts of muscles, may be represented in the motor areas of the cortex. To further complicate matters, sharp distinctions cannot be made between "sensory" and "motor" areas (see Brodal, 1981), although the association connections between the pre- and post-central gyrii are arranged in a strict somatotopic fashion so that the arm region

of the sensory cortex projects to the arm region of the motor cortex (Jones and Powell, 1968, 1969).

The composite picture that emerges from these studies is that functionally and anatomically, the CNS exhibits a high degree of spatial ordering, based upon a map of the body surface, in both the projections and the internal organization of groups of neurons allotted to a particular body area. A comparatively detailed account is not yet available for the peripheral nervous system, and very few investigations for example have been published dealing with the individual motoneuron pools. It is of interest to know whether these groups of neurons, each directly subserving a small portion of body space, also have a detectable organization based either upon the peripheral locations of their targets or upon their own intrinsic properties. Are DRG cells and motoneurons as finely organized in their projections to the periphery as are the central connections to which they give rise or from which they receive synaptic input?

2. Peripheral Nervous System (PNS)

This section of the Background will be concerned with the extent to which spatial order is maintained in the PNS, specifically in the motor system. One of the distinctive characteristics of the organization of the nervous system is the columnar arrangement of motoneurons in the brainstem and spinal cord (Sidman, 1970). The classical studies of Romanes (1941, 1946, 1951) on toluidine blue or silver-stained preparations of the mammalian spinal cord have shown

that in the adult, motoneurons are arranged in longitudinal (rostro-caudal) columns (seen in transverse sections in Lamina IX of Rexed (1952)), and that in the brachial and lumbar enlargements of the spinal cord there exist two spatially distinct columns of motoneurons: one located laterally in the ventral horn, the other medially. Using anatomical tracing techniques in the rabbit, Romanes (1941) provided evidence that the lateral motor column (LMC) innervates limb musculature while the medial motor column (MMC) provides innervation to axial (or thoracic) musculature. In addition, more cranial segments of the LMC were found to innervate proximal muscles (e.g., thigh or forearm) while caudal portions of the LMC supplied distal musculature (e.g., ankle or wrist). The studies of Romanes and others have been confirmed and extended to include virtually all vertebrate species, from frogs to gorillas (see reviews by Elliott, 1944; Romanes, 1964; Hughes, 1968). Thus on a gross topographic level, the relative spatial positions of the motor cell columns in the spinal cord are highly and characteristically predictive of the peripheral locations of the muscles they innervate.

At a more refined level, the patterns of connectivity within a single motor cell column also reveal target specificity. The early physiological studies of spinal reflexes in the dog (Sherrington, 1906) indicated that each individual muscle in the hindlimb likely received its innervation from a particular segment of the lumbar or sacral spinal cord. Considerable experimental evidence has since

accumulated to corroborate Sherrington's prediction, to show that an individual limb muscle is indeed innervated by restricted group or pool of motoneurons, located in a stereotyped position within the LMC (Romanes, 1964; Landmesser, 1978a; Hollyday, 1980). While the early investigations of motor pool locations relied largely upon the histological detection of retrograde degeneration, current methodologies capitalize on the ability of motoneurons to retrogradely transport tracer molecules such as horseradish peroxidase (HRP) following intramuscular injection (e.g. Kristensson and Olsson, 1971) or direct application of the tracer to the central ends of cut muscle nerves (e.g. Olsson and Kristensson,, 1979). More sophisticated techniques combine direct physiological identification of single motoneurons (i.e., by stimulating muscle nerves and recording antidromic action potentials with intracellular microelectrodes) with direct anatomical identification of the cell using an intracellular injection of tracer and subsequent histological visualization (Cullheim and Kellerth, 1976; Burke, Dum, Fleshman, Glenn, Lev-Tov, O'Donovan and Pinter, 1982). Such techniques have permitted even more refined analyses than ever before, and have stimulated interest in the possibility that individual motor pools may have a demonstrable, intrinsic organization. In summary, the unanimous conclusion from the studies presented above is that there are highly specific and stereotyped patterns of spatial connectivity between the spinal cord position of

motoneuron pools and the muscles they innervate.

B. COMPARTMENTALIZATION OF SKELETAL MUSCLES

There are a number of reports in the literature indicating that single skeletal muscles may be compartmentalized - histochemically, functionally, or both. On the basis of this evidence it is not unreasonable to consider that such a compartmentalization of skeletal muscle may be a further indication of spatial ordering in the peripheral motor system. One objective of this thesis was to see whether the organization of the motor innervation of the cutaneous trunci muscle (CTM) corresponded in some fashion to the pattern of its reflex activation, which suggests that small discrete muscle compartments must exist. It will be useful first to describe some of the current terminology and concepts.

1. Classification of Muscles, Motoneurons and Motor Units

Two main categories of vertebrate striated muscle have been described: tonic and twitch. Tonic muscle fibres typically have multiple innervation and are not capable of generating a propagated muscle action potential; instead transmitter release at tonic motor endplates results in a graded depolarization and thus in a graded, or local, depolarization of the muscle fibre (Burke and Ginsborg, 1956). Tonic muscle fibres are found predominantly in invertebrates and lower vertebrates; there are very few examples of tonic muscles in higher vertebrates (Hess, 1970). In contrast twitch muscle fibres are found in higher vertebrates and are characterized by single

(focal) innervation and by all-or-none propagated action potentials resulting in a rapid contraction of the whole muscle fibre (hence the name "twitch"). Currently, there are three main types of twitch muscle fibres, described on the basis of mechanical, structural, histochemical and electrophysiological parameters. Mammalian skeletal muscle is composed mainly of the twitch types, and as the preparation studied in this thesis is a mammal, further discussion will be devoted to twitch muscle.

As described above (Section II, A2) an individual skeletal muscle is innervated by a specific pool of motoneurons located in a stereotyped position in the spinal cord. Each motoneuron pool is related not only spatially, but quantitatively, to its muscle, i.e. a characteristic number of motoneurons is allotted to each particular muscle (e.g. Hollyday, 1980). The number of muscle fibres innervated by one motoneuron (the "motor unit", see below) varies greatly depending on the muscle and the species. For example, the innervation ratio (obtained usually by estimating the number of muscle fibres in the whole muscle and then dividing this value by the number of motoneurons supplying that muscle) in a tiny middle ear muscle the stapedius, ranges from 2 to 3 muscle fibres per motor axon (Shaw and Baker, 1983), while in the human medial gastrocnemius muscle the corresponding value has been estimated as close to 1000 (Burke, 1978). There are a number of inherent technical difficulties in measuring innervation ratios, but one general principle that

emerges from all these studies is that muscles used for vernier movements (i.e. finely controlled and graded contractions) have very low innervation ratios, while those used for relatively coarse and powerful movements (e.g. gastrocnemius) have high innervation ratios.

In order to allow a quantitative description of muscle contraction, Liddell and Sherrington (1925) introduced the term "motor unit", referring to the motoneuron axon and all the muscle fibres innervated by it. This term is more precise than "innervation ratio" and is used preferentially in the literature. A more useful definition of the motor unit suggested by Burke (1967) also includes the motoneuron soma, while the term "muscle unit" refers only to the muscle fibres innervated by a single motoneuron. Sherrington's concept of the motor unit has proven to be a cornerstone in the current thinking about the organization of motor systems and the neural control of movement, in that it forms the "quantum unit of muscle action" (Burke, 1981) and that selective and synchronized activation of individual motor units by the CNS underlies the entire repetoire of muscular action.

Two types of motoneurons have been described, initially on the basis of the diameter and conduction velocity of the axons and later on the basis of functional properties of the muscle fibres they supplied (Erlanger and Gasser, 1937; Sherrington, 1906; Leksell, 1945; Kuffler, Hunt and Quilliam, 1951): alpha (α) and gamma (γ) motoneurons. The large diameter (10-15 μm) rapidly-conducting (100

m/s) myelinated axons of alpha motoneurons are responsible for the innervation of the majority of extrafusal skeletal muscle fibres, and thus are responsible for the execution of muscular contraction. The smaller (6-8 μm) slower-conducting (50 m/s) gamma fibres provide the motor innervation to the muscle fibres of the spindles, i.e. to the intrafusal fibres (see review by Matthews, 1981) and function in the reflex regulation of muscle length and tension. As this thesis is specifically concerned with extrafusal muscle and thus with alpha motoneurons, further discussion will be restricted to the literature pertaining to alpha motoneurons.

Despite the suggestion from Ranvier's (1874) work that the motoneurons supplying fast and slow muscles may be differentiated on a similar basis (i.e. that there exist fast and slow motoneurons), such a possibility could not be investigated until several decades later, mainly because of technical limitations. Liddell and Sherrington (1925) recognized that individual motor units within a single muscle could be brought into action sequentially over time (the concept of recruitment); however, it was not until the advent of intracellular microelectrode recording techniques that it became clear that all motoneurons within a single pool were <u>not</u> functionally equivalent. The analysis of intracellular recordings of cat motoneurons supplying fore- and hindlimb muscles by Eccles, Eccles, and Lundberg (1957, 1958) firmly established the existence of two types of alpha motoneurons. On the basis of characteristic spike

potentials, duration of after-hyperpolarization and axonal conduction velocity, Eccles et al. (1957) distinguished slow twitch ("tonic") and fast twitch ("phasic") motoneurons. In addition, using measurements of isometric twitch contraction times in cat gastrocnemius and soleus muscles, they were able to correlate the fast and slow motoneuron types with predominantly fast-pale and slow-red twitch muscle respectively. In summary then, by the late 1950's two major types of twitch skeletal muscle had been described on the basis of anatomical and functional characteristics. In addition, on the basis of mainly physiological evidence, two major types of twitch motoneurons, fast and slow, were described and associated with the two muscle types.

The development of muscle fibre histochemistry in the 1960's (Padykula, 1952; see Romanul, 1964 for review) permitted the description of several distinct muscle fibre types. Mainly on the basis of histochemical profiles, several classification schemes have been proposed (see reviews by Close, 1972; Burke, 1981), however, interspecies and intermuscle variation have hindered the establishment of a universally-accepted nomenclature. Currently there are two classification systems that appear to have gained general recognition: that of Peters et al. (1972), based upon ATPase reactivity patterns and metabolic enzymes, and that of Burke, Levine, Tsairis and Zajac (1973), which is based on the mechanical and physiological properties of the motor units and has the additional

merit of correlating these properties with histochemical profiles. For the purposes of the present discussion only a simplified review of the major muscle fibre types will be presented. Mainly on the basis of myosin ATPase reactivity patterns and isometric contraction speeds, three major groups of twitch fibres have been distinguished: fast glycolytic (FG, or fast fatiguable (FF) in the terminology of Burke et al., 1973), slow oxidative (SO or S), and an intermediate fibre type, fast oxidative glycolytic (FOG, or fatigue resistant, FR). Although this basic tripartite division appears to have gained general acceptance (Burke, 1981; Henneman and Mendel 1981), there have been an increasing number of observations that rather than falling into distinct categories, muscle fibre types appear to form a continuum (Botterman et al., 1982). FG muscle fibres are responsible for the fastest, most rapid and powerful contractions; the fibres are large, contain few mitochondria and a large amount of glycogen, an indication of their mainly anaerobic metabolism. By the same token, FG fibres are quickly fatigued and thus unable to maintain a prolonged tetanic contraction. SO muscle fibres are small to intermediate in cross-sectional area, and contain many mitochondria and oxidative enzymes. Because of their aerobic metabolism, SO fibres are extremely fatigue-resistant although they are not able to develop much tension. FOG muscle fibres are intermediate between FG and SO types, and are characteristically capable of rapid and sustained contraction. In general, it appears that most vertebrate

muscles contain a heterogenous population of muscle fibres, and in fact it is quite unusual to find a histochemically homogenous muscle (the cat soleus is one of the few examples; Burke, 1978).

1a. Relationship of Motoneurons and Muscle Units

Several general principles have been established concerning the relationship of motoneurons and their muscle units which bear upon the major objective of this thesis, which was to see whether there is a particular pattern or architecture to a motoneuron pool that could relate, for example, to its pattern of innervation of the target muscle. Implicit in the early work of Sherrington and his colleagues (Liddell and Sherrington, 1925) was the assumption that all the muscle fibres in one motor unit responded in an all-or-none fashion, in contrast to the graded responses of which a whole muscle is capable. Although technical difficulties associated with the isolation of a single motor unit have prevented a direct investigation of this question, the weight of experimental evidence indicates that in the behaving mammal, each action potential in a motor axon reliably activates all the fibres in its muscle unit (see review by Burke, 1981). That all the muscle fibres in one motor unit are consistently co-activated is of obvious functional importance in the production and control of reliable mechanical action.

Another important characteristic of motor units concerns the extent to which there is uniformity of all fibres within one muscle unit with respect to their mechanical and histochemical properties.

Edstrom and Kugelberg (1968) presented the first detailed account of the twitch tension, fatiguability, metabolic enzyme profile, and spatial distribution of single motor units in mammalian muscle (the rat tibialis anterior). Later studies by Burke and colleagues (1971, 1973, 1974) also examined the correlation between the physiology and histochemistry of a large number of fibres in single motor units in the cat triceps surae muscle group (which consists of the soleus, medial and lateral gastrocnemii). These types of experiments have revealed that with very few exceptions (e.g. see Botterman, Iwamoto and Conyea, 1983), all the fibres belonging to one motor unit share identical metabolic and mechanical properties. Although the etiology of such mechanical and histochemical uniformity is currently not known, one possible advantage of this kind of arrangement may be the facilitation of a safe and smooth production of mechanical force.

The spatial distribution of muscle fibres constituting one unit within the total muscle volume has been found to be quite variable depending on the species, muscle and fibre type (Henneman and Olson, 1965; Edstrom and Kugelberg, 1968; Kugelberg, 1973; Burke and Tsairis, 1973; Burke, Levine, Salcman and Tsairis, 1974; Botterman, Hamm, Reinking and Stuart, 1983a, b). The general rules governing what proportion of the total muscle volume may be occupied by a single muscle unit is still an unresolved and currently very topical issue. This question has an obvious bearing on the functional use of the muscle: widely distributed motor units would

not likely predispose a muscle to a compartmentalized type of behavior, whereas the activation of motor units which are confined to a relatively small region of the muscle would result in the contraction of discrete muscle sub-volumes. It might be expected that muscles used for manipulation would tend to have the latter type of organization, i.e. compartmentalized motor units, while those muscles responsible for posture or for the execution of rapid ballistic movements would not. (For further discussion of the signifigance of histochemical and and functional compartmentalization of skeletal muscles see below, Section II, B2 and 3).

Another general principal which has emerged from the study of motoneurons and their muscle units is the relationship of the conduction velocity of the motor axon, the size of the cell body and the motor unit type. The physiological studies of Eccles, Eccles and Lundberg (1958) demonstrated that alpha motoneurons with fast conduction velocities innervated fast-twitch motor units while those with slow conduction velocities drove slow-twitch motor units. Based on measurements of the maximum tetantic tension developed by a muscle unit and the conduction velocity of its motor axon, McPhedran, Wuerker and Henneman (1965; Wuerker, McPhedran and Henneman, 1965) postulated that motor unit size is directly proportional to conduction velocity and hence to the diameter of the motor axon. Prompted by the early neuroanatomical studies of Cajal (1909), Henneman and co-workers (Henneman, Somjen and Carpenter, 1965a, b;

Somjen, Carpenter and Henneman, 1965) further investigated the functional implication of such a relationship. Assuming that the large action potentials they recorded extracellularly from cat lumbar ventral root filaments derived from large motoneurons, and the small impulses originated from small motoneurons, they proposed the "size principle", wherein the excitability and inhibitability of a motoneuron was a function of its soma size (small neurons were most easily excited by various types of reflex input and were least susceptible to inhibition; the converse was proposed for the excitability and inhibitability of large motoneurons). It was a number of years however before evidence was established for a direct relationship between cell body size, axon diameter and conduction velocity in alpha motoneurons (Barrett and Crill, 1971; Cullheim, 1978), and another few years before the relationship between cell size and motor unit type was properly investigated (see Burke, 1981).

From the point of view of this thesis it is important to note that although the apparent size of a motoneuron could well be correlated with certain physiological and mechanical properties of its muscle unit, there is no known spatial organization of motoneurons within motoneuron pools based on soma size. Intracellular labeling studies using Procion red or yellow (Bryan, Trevino and Willis, 1972) or HRP (Burke, Strick, Kanda and Walmsley, 1977) revealed that alpha and gamma, as well as small and large alpha motoneurons supplying cat hindlimb muscles were intermingled

throughout the length of their respective motoneuron pools.

While the size principle of motor unit recruitment (i.e. the smaller, more easily excited motoneurons are brought to threshold sooner than larger motoneurons) has generally been substantiated in similar experimental conditions; the almost unavoidable corollary of these findings - that large motoneurons drive fast-twitch motor units and small motoneurons drive slow-twitch units - has recently come under close scrutiny as a result of a number of elegant studies using direct anatomical measurements of functionally identified motoneurons (see Burke, 1981 for a review of the literature). Despite the great interest in determining whether there exist anatomical differences by which motoneurons may be characterized according to the fibre type of the muscle unit they supply, no satisfactory evidence has been presented that clearly associates fibre type and motoneuron morphology at the light or electron microscope level. When the average somal diameters of physiologically-identified slow-twitch and fast-twitch motoneurons are compared (Burke et al. 1977, 1982; Ulfake and Kellerth, 1982) considerable overlap in the distributions is apparent and although the mean soma size of "slow" motoneurons tends to seem somewhat smaller than that of "fast", there is no signifigant difference.

Ultrastructural studies of physiologically-identified motoneurons provide only suggestive evidence that synaptic input may differ systematically between slow and fast motoneuron types

(Conradi, Kellerth, Berthold and Hammarberg, 1979; Kellerth, Berthold and Conradi, 1979). The experimental procedure in these studies essentially consisted of analyzing at 6 μm intervals, the type and distribution of synaptic boutons on the soma and proximal portions of the primary dendrites of four functionally-identified cat motoneurons (two FR, two S types) that were intracellularly labeled with Procion yellow. Attempts to distinguish fast and slow motoneurons on the basis of cytophotometric measurements of different oxidative enzymes (Penny, Kukums, Eadie and Tyrer, 1975) have proven singularly unsuccesful. Currently, the only way to distinguish motoneuron types is on the basis of certain electrophysiological characteristics, most of which are ascertained following intracellular microelectrode penetrations. The studies of Burke et al. (1982) provide the only direct evidence that the cell membrane resistivity of slow motoneurons is systematically higher than that of fast motoneuron types.

To summarize, several general principles have been established which characterize the relationship of motoneurons and their muscle units. It is generally accepted that all the fibres of one unit are activated in response to a motor axon spike and that the histochemical and mechanical profiles of all the muscle fibres constituting one muscle unit are homogeneous. Additionally, slow-twitch units tend to develop small tensions and are innervated by slowly-conducting axons; the converse is true of fast-twitch

units. The size principle (which has recently been critically re-evaluated; e.g. Clamann, Ngai, Kukula and Goldberg, 1983; Pinter, Curtis and Hosko, 1983) states that motor units are recruited in order of size, from small to large, by an increasing excitatory drive to the motoneuron pool, while on the other hand, increasing inhibition causes units to fall silent in the reverse order of their size (large units are most easily inhibited). Presently there is no firm evidence by which motoneuron morphology (at the light or EM level) may be correlated with the fibre type of its muscle unit. However, combined physiological and anatomical studies do indicate that fast and slow motoneurons may be reliably distinguished on the basis of their input resistance; fast type motoneurons having a significantly lower input resistance than slow type motoneurons, indicating that they are probably larger, or (cf. Burke et al., 1982) that the membrane resistivity of fast motoneurons is lower than that of slow. In addition, fast motoneurons appear to have a faster axonal conduction velocity than do slow type motoneurons (Henneman et al., 1965; Burke et al., 1981).

2. Histochemical Compartmentalization

A large number of studies have shown that the different muscle fibre types are not randomly distributed throughout the muscle volume, but rather that similar fibre types appear spatially grouped, giving the muscle a histochemically compartmentalized appearance when viewed in cross-section (see Botterman, Binder and Stuart, 1978) In

fact, it appears to be a general rule that the majority of mammalian skeletal muscles show distinctive slow-oxidative and fast-glycolytic fibre compartments, although exceptions do exist (e.g. cat semitendinosus (Bodine, Roy and Meadows, 1982); cat soleus (Burke et al., 1974); and a number of cat neck muscles (Richmond and Abrahams, 1975)). While a great number of muscles have been examined in a variety of species including cat, rat, mouse, dog, rabbit, pig, guinea pig and human, the most comprehensive studies have been on the fore- and hindlimb muscles of cats and rats, notably the flexor carpi radialis (Gonyea and Ericson, 1977; Galvas and Gonyea, 1980), the plantaris (English, 1980), and the triceps surae muscles (Yellin, 1969; Burke and Tsairis, 1973; Burke et al., 1977; English, 1980). A fairly typical pattern of fibre-type distribution in mammalian muscles consists of a superficial layer of white or fast-twitch fibres and a deeper core of red or slow-twitch muscle fibres. Examples of this type of histochemical compartmentalization are found in cat and rat medial gastrocnemius (Yellin, 1969; Burke et al., 1977; English, 1980), and tibialis anterior (Gordon and Phillips, 1953; Edstrom and Kugelberg, 1968), as well as in cat flexor carpi radialis (FCR) (Gonyea and Ericson, 1977), cat neck muscles (including complexus, rectus capitis major and splenius; Richmond and Abrahams, 1975), and in rat medial pterygoid, temporalis and masseter (see Botterman et al., 1978).

Although the functional interpretation as well as the

developmental origin of this fibre type compartmentalization are not yet known, several reasonable assumptions may be made. As described above (Section II, B1) muscle fibre types are classified on the basis of their histochemical and mechanical properties, and with these properties in mind it is not unreasonable to assume that a histochemical compartmentalization of fibre types may reflect a functional specialization of certain parts of individual muscles. This point of view has recently received favorable attention (Botterman et al., 1978; English, 1980; Galvas and Conyea, 1980), and will be discussed in greater detail below (Section II, B2). Another suggestion regarding the functional meaning of histochemical compartments in skeletal muscle focuses on metabolic concerns. Burke (1981) points out that a core of slow-oxidative fibres (i.e. an area of high metabolism and high resting blood flow) surrounded by a layer of fast-twitch fibres (i.e. with a lower metabolism and fewer vascular demands) could well minimize conductive heat loss, which in small mammals may be a significant problem. These two interpretations are not necessarily mutually exclusive, but as will be shown below, several reports on the physiological behavior of certain muscles favor a functional interpretation rather than a metabolic one.

3. Functional Compartmentalization

One objective of this thesis was to describe some of the electromyographic characteristics of the reflex activation of the

cutaneous trunci muscle (CTM) in the rat. This flat sheet of skeletal muscle shows a behaviorally compartmentalized pattern of contractions, in that a localized sensory stimulus of the overlying skin evokes a very localized reflex response of the CTM (Nixon, Theriault, Jackson and Diamond, 1984; and see Results below).

The results of the present thesis show that the behavioral compartmentalization seen in the reflex activation of the CTM has an interesting underlying anatomical basis. It was therefore of relevance to determine whether there are reports of other mammalian muscles that also demonstrate patterns of regional contractions; if so, the present findings concerning the anatomical organization of the CTM motoneuron pool could possibly bear upon the organization of motor circuitry in general. The evidence for organization of synaptic input to motoneuron pools will be dealt with later (Section II, C); here we are concerned with the <u>behavioral</u> evidence that reflex responses may be evoked in restricted portions of whole muscles.

The early observations of Cohen (1953, 1954) on the stretch reflex in cat quadriceps indicated that individual strips of the muscle were capable of responding independently to localized stretches applied to different regions of the muscle. This localization of proprioceptive reflexes has also been demonstrated to occur in other muscles (Pilotto, Schor, Uchino and Wilson, 1981; Brink, Jinnai and Wilson, 1981; Cameron et al., 1981; Fritz, Ilert

and Saggau, 1981; Botterman et al., 1983a). The implication of these studies is that in the normally active animal, muscles which are subjected to a variety of specific pulls and torques need not respond as a unit, but rather are able to produce selective regional contractions. Of the few studies which have directly addressed this issue, the best evidence comes from a study of a masticatory muscle in the pig, the masseter (Herring, Grimm and Grimm, 1979). Using correlative electrophysiological, histochemical and morphological techniques these authors demonstrated unequivocally that patterns of EMG activity in the normally active muscle demonstrated regional differences which were directly related to the chewing load requirements. A histochemical analysis of the muscle revealed that posterior portions of the masseter contained predominantly fast-twitch fibre types, while the more anterior compartments were mainly slow-twitch. Although no analysis of motor unit territories was presented, Herring et al. were able to demonstrate that fast and slow muscle fibre compartments displayed a functional specialization, in that the fast glycolytic compartment of the muscle was active during movements of crushing or the power stroke, while the slow-oxidative fibres were active throughout the masticatory contraction. The results from this study therefore provide the strongest evidence that histochemical compartmentalization in a skeletal muscle is directly correlated with normal functional, rather than metabolic, activity in the muscle.

The question of functional compartmentalization in skeletal muscle has also been investigated indirectly, by an analysis of muscle sub-volumes defined by electrical stimulation of either ventral roots or rootlets of small intramuscular nerve branches. Sherrington's (1906) investigations of the ventral root innervation of hindlimb muscles in the dog revealed an apparent segmental progression in the motor innervation of single long thigh muscles. These observations were later confirmed by Markee and Lowenbach (1945) who used electrophysiological techniques to demonstrate that in muscles which receive efferent innervation from more than one ventral root (i.e. rectus femorus), electrical stimulation of the more cranial root produced contraction in a proximal part of the muscle while stimulation of the more caudal root evoked EMG activity in a more distal portion of the same muscle. While it has been a long-standing clinical observation that muscles with a plurisegmental somitic origin like the diaphragm or pectoralis receive a progressive segmental motor innervation (Williams and Warwick, 1975), it is of interest from a developmental point of view to note that the same pattern of progressive cranio-caudal ventral root innervation may be observed in limb musculature.

These observations, combined with Cohen's study of the localization of the stretch reflex, led Swett, Eldred and Buchwald (1970) to propose that some plan of somatotopic specificity must exist between the muscle and its sensory and motor innervation. In

their investigations of the cat medial gastrocnemius, Swett et al. showed that electrical stimulation of ventral root filaments in a craniocaudal sequence evoked overlapping zones of contraction in a proximal to distal sequence in the muscle and also noted a small but consistent post-fixation in the level of sensory outflow to the medial and lateral gastrocnemii as well as to the soleus (i.e. the motor outflow to these muscles always originated cranial to the level of sensory outflow for each muscle). In an earlier examination of the sensory innervation to the gastrocnemius and soleus, Swett and Eldred (1959) had described a much less precise somatotopic afferent innervation of the muscle. A number of subsequent investigations of other skeletal muscles (e.g. Brown and Booth, 1983) have appeared and the results indicate that a segmental progression in ventral root innervation is not uncommon, although not without exception (e.g. foot musculature, Sherrington, 1906; Romanes, 1964).

More recently, the area of a muscle innervated by small intramuscular nerve branches has been investigated (see present Results also). Electrophysiological recordings (English and Letbetter, 1982; Bodine, Roy, Meadows, Zernicke, Sacks, Fournier and Edgerton 1982; Botterman, Hamm, Reinking and Stuart, 1983a, b) and glycogen depletion studies (Letbetter, 1974; English, 1980) in cat hindlimb muscles reveal that each intramuscular nerve branch supplies a distinct muscle sub-volume. Additionally, it has been shown that individual nerve branches supply different histochemical compartments

in the cat FCR, and form distinct motor end-plate bands that are correlated with the fibre type compartment (Galvas and Gonyea, 1980). These types of experiments indicate that skeletal muscles may be functionally compartmentalized, at least under certain experimental conditions. While in some cases this experimentally-evoked compartmentalization does reflect how the muscle is used behaviorally (i.e. the pig masseter and the rat CTM), there are exceptions where a muscle which has distinct nerve branches innervating different muscle sub-volumes is found to contract as one unit (i.e. the cat semitendinosus, Bodine et al., 1982; Botterman et al., 1982b).

To summarize this section, it appears that a large number of skeletal muscles show distinct fibre type compartmentalization, which in a few cases has been directly correlated with functional activity in the muscle. These types of experiments support the idea that in normal behavioral activity, skeletal muscles may demonstrate patterns of regional contractions rather than ones involving the whole muscle.

4. Relationship of Structure and Function

One aspect of investigations into motor system organization that has quite recently come under close scrutiny is the disparity between the results determined in experiments involving decerebrate or spinalized animals and the patterns of muscular activity displayed by the awake, normally behaving animal. There are several inherent difficulties in extrapolating these experimental results to the movement of normally active animals where for one thing, movement is

not the result of isolated muscles but rather is the combined output of many muscle groups responsive to a variety of load requirements over several joints (Clamann, 1981; Botterman et al., 1983b). Furthermore, with respect to compartmentalization, hindlimb muscles do not exhibit the range of functional complexity or the number of behavioral tasks performed by forelimb muscles (Conyea and Ericson, 1977), and thus may not be expected to show a very high degree of functional compartmentalization. These considerations stress the need for analyzing muscle function in normally behaving animals and in muscle groups which are used for fine movements or manipulations rather than for posture or ballistic movements.

An additional consideration in the analysis of motor systems is the role of the internal architecture of the muscle with respect to muscle function. Theoretically, a muscle consists of a number of muscle fibres which are arranged in a geometric pattern between two simple or complex connective tissue surfaces. The simplest arrangement is, of course, a parallel fibre arrangement where muscle contraction develops force along one vector. While many of the general principles of muscle physiology have been established using strap-like parallel fibred muscles, this type of arrangement does not characterize most muscles (Gans and Bock, 1965). In fact, many skeletal muscles are characterized by complex internal aponeuroses where the muscle fibres lie in various parallel but staggered arrangements (e.g. gastrocnemius, masseter). These pinnate muscles

(pinnation refers to the orientation of the long axis of the muscle fibre with respect to the direction of exerted force or induced motion; Gans and Bock, 1965) are obviously suited to develop force along more than one vector. It stands to reason then, that there should be a functional correlate of this anatomy, i.e. that selective regional contractions may be evoked according to segmental or supraspinal commands. The paucity of reports in the literature which deal with functional compartmentalization in single muscles may stem in part to the difficulties of matching experimental conditions to the way in which the nervous system activates motor units selectively. The following section will briefly examine some of the literature which deals with the afferent control of movement and what implications this may have for the internal organization of motoneuron pools.

C. EVIDENCE FOR AN INTERNAL ORGANIZATION OF MOTONEURON POOLS

The pattern of regional contractions seen within the CTM that can be produced by its reflex activation indicates that out of the whole motoneuron pool supplying the muscle, small subpopulations of motoneurons can be selectively activated. The major objective of this thesis was to see whether an anatomical organization of the CTM pool could be revealed that related to the reflex behaviour of the muscle. Although the division of muscles into discrete subvolumes by their major motor nerves has been recently demonstrated (see Section II, B), only one other report (an abstract) apart from this thesis

has appeared identifying the relative (anatomical) spinal cord locations of motoneurons supplying different muscle sub-volumes (in the cat medial gastrocnemius; Weeks and English, 1982).

There is, however, an extensive literature dealing with the functional organization of synaptic input to motoneuron pools. In both animals and humans there is a great deal of physiological evidence to indicate that synaptic input to motoneurons from segmental and supraspinal sources is topographically and functionally segregated (see reviews by Botterman, Binder and Stuart, 1978; Burke, 1981; Clamann, 1981; Henneman and Mendell, 1981; Schwindt, 1981). However, while many of the conclusions drawn about patterns of synaptic input to motoneuron pools have typically been based on intracellular recordings from cat lumbosacral motoneurons, the difficulties of obtaining precisely quantifiable measurements under these recording conditions has not been given much attention. Many of the variables affecting motoneuron excitability, and thus the measurement of postsynaptic potentials, are difficult to control rigorously from animal to animal and from cell to cell (e.g. the level of anaesthesia). As noted by Burke (1981) the most stringent conditions require the evaluation of evoked postsynaptic potentials in type-identified motoneurons by functionally-identified afferent inputs. Obviously these conditions can be met only in certain mono- or di-synaptic systems which project directly or through a well-defined set of second order interneurons to motoneuron pools.

Since polysynaptic pathways themselves are not well understood (see Schwindt, 1981), attempts at quantification of these input systems are fraught with even greater difficulties. Despite these methodological drawbacks, certain general principles have emerged in the study of synaptic input systems to motoneuron pools. For the purposes of the present discussion, they may be most conveniently divided into monosynaptic (i.e. largely segmental) and polysynaptic (predominantly supraspinal) pathways.

C 1. Organization of Monosynaptic Input

The origin of monosynaptic input to motoneuron pools derives predominantly from spindle, or Ia, afferents (Eccles et al.,1957) with a minor proportion coming from Golgi tendon organs (GTOs; Kirkwood and Sears, 1975) and from a few supraspinal sources (e.g. the vestibulospinal tract; Schwindt, 1981). The most thoroughly studied of these systems is the Ia projection, where it has been shown that reflex activation of the spindle results in direct excitatory post synaptic potentials (EPSPs) in motoneurons supplying the same muscle (homonymous motoneurons) as well as in motoneurons innervating synergist muscles (heteronymous motoneurons; Eccles et al., 1957; Burke, 1977; Botterman et al.,1983a). Intracellular investigations of cat triceps surae motoneurons indicate that Ia input is strongest in homonymous motoneurons, in terms of EPSP amplitude (Burke, 1981) and in the extent of Ia projections, where a single Ia afferent may synapse with 65-94% of its homonymous

motoneurons (Mendell and Henneman, 1968; Scott and Mendell, 1976). Since the motoneuron pools of triceps surae muscles overlap and intermingle within the same spinal cord segments, the observed preferential projection of spindle afferents to their own motor pool must be based on more than simple geometry. The term "species specificity" was proposed to describe this differential synaptic connectivity of Ia afferents (Eccles et al., 1957; Scott and Mendell, 1976). The observation that spindle afferents provide excitatory input to motoneurons driving synergist, but not to antagonist, muscles (Eccles et al., 1957; 1958) is indicative of a further functional organization of synaptic input to motoneuron pools. As pointed out by Botterman et al. (1982b) observations that Ia connectivity is related to muscle synergy stress the need for analyzing muscular activity in terms of <u>movement</u> rather than (or at least in addition to) individual muscles.

A further level of specificity in Ia projection patterns can be seen in the intramuscular localization of the stretch reflex, first demonstrated by Cohen (1953; 1954) and later confirmed and extended by other investigators (Brink, Jinnai and Wilson, 1981; Bilotto, Schor, Uchino and Wilson, 1981; Fritz, Ilert and Saggau, 1981; Botterman et al., 1983a, b). In a recent physiological investigation Botterman et al. (1983a) demonstrated a clear topographic organization of the biceps femoris motor pool. This cat hindlimb muscle consists of three intramuscular compartments in

parallel with each other and each supplied by separate nerve branches (English and Letbetter, 1981). Intracellular recordings from the biceps motor nucleus revealed that the more cranially-located motoneurons supplied the anterior muscle compartment while progressively more caudal motoneurons supplied middle and posterior compartments (Botterman et al., 1983a). These authors examined the pattern of Ia EPSPs in biceps motoneurons and found that motoneurons received the strongest Ia input from spindles located within the same compartment. These studies provide the best evidence to date of the localization of monosynaptic input within individual motoneuron pools.

The observation that muscle receptors (both spindles and GTOs; Cameron, Botterman, Reinking and Stuart, 1981) preferentially drive those motoneurons innervating muscle fibres in the immediate vicinity of the receptor led to questions about the anatomical basis of this specificity. Was this apparent topographic organization of synaptic input a purely central phenomenon, or were the dorsal root fibres spatially organized in their projections (e.g. as had been demonstrated for the pattern of ventral root innervation of certain skeletal muscles; see Section II, B,2)? Although a large number of reports indicate that there is a definite <u>functional</u> localization of Ia and Ib input within motoneuron pools, there appears to be very little relationship between the peripheral location of a spindle or GTO in the muscle and the relative order of entrance of their

afferent fibres into the spinal cord (Swett and Eldred, 1959). In the light of these findings then, it would appear that the somatotopic organization of monosynaptic (Ia and Ib) connectivity in motoneuron pools is most likely determined by factors other than the spatial order of centrally-projecting dorsal root fibres.

It is important to note that not all skeletal muscles demonstrate a clear topographic organization of their motor pools. The most thoroughly investigated example of this is the cat semitendinosus, a hindlimb muscle which is divided into distal and proximal compartments by a connective tissue band, with each compartment supplied by a different nerve branch (Bodine et al., 1982). Although the internal architecture of this muscle suggests the two compartments may be independently activated, EMG analysis reveals that the muscle tends to function as one unit during locomotion (Murphy, Bodine and Roy, 1981). Intracellular analysis of the semitendinosus motor pool reveals a homogenous distribution of Ia connectivity (Botterman et al., 1982b), while the motoneuron pool itself does not show any internal (spatial) organization corresponding to the two muscle compartments (Bodine et al., 1982). As pointed out by Botterman et al. (1982b), the in-series arrangement of the two compartments almost requires the muscle to function as a unit for the effective development of force, therefore the lack of topographic organization in the motor nucleus is not surprising.

Another characteristic of the functional organization of

motoneuron pools derives from the observation that spindles and GTOs are found predominantly in slow-twitch muscles or in slow-twitch compartments of heterogenous muscles (e.g. Richmond and Abrahams, 1975a,b; Botterman et al., 1978). These observations have lead to numerous investigations of the functional association between muscle receptors and motor units (reviewed in Cameron et al., 1978; Burke, 1981; Henneman and Mendell, 1981). One clear principle that emerges from these studies is the obvious one: that there is a preferential projection of Ia and Ib afferents according to motor unit type, slow-type motoneurons receiving a much stronger projection than fast-type motoneurons in the same pool (Eccles et al., 1957; Burke, 1968). In fact, this pattern of synaptic efficacy (where slow motoneurons are preferentially driven by muscle receptor input) characterizes a number of other di- and polysynaptic projection systems to motoneuron pools (see below). Interestingly, investigations of monosynaptic input from supraspinal sources do not show any correlation with motor unit type (Burke, Pymer and Walsh, 1976).

To summarize, investigations of the monosynaptic projection patterns of Ia and Ib afferents reveal the existence of both functional and spatial organization of this input to individual motoneuron pools, where the topographic specificity between muscle receptors and motor units is preserved within the central (spinal cord) circuitry. There is a further segregration of monosynaptic

input to motor pools that is clearly related to motor unit type.

2. Organization of Polysynaptic Input

Polysynaptic input to motoneuron pools originates from several projection systems including muscle receptors (i.e. spindles, GTOs and joint receptors), large and small diameter cutaneous afferents, and a number of brainstem and cortical pathways (i.e. rubrospinal, vestibulospinal and corticospinal). These projections (which by definition involve one or more interneurons) do not exert their influence on motoneurons which are otherwise electrically quiescent, but are frequently found to facilitate or inhibit other currently active input systems which synapse onto tonically-active motoneurons (Burke, 1981; Jankowska and Lundberg, 1981; Schwindt, 1981), thereby making quantitative measurements of synaptic input exceedingly difficult. There is, however, substantial evidence indicating that rather than having a "private" set of interneurons for each input system, that a limited number of interneuron pools are shared (e.g. Harrison, Jankowska and Johannisson, 1983; Jankowska and McCrea, 1983). It will be most useful to discuss these projection systems in categories, according to the similarity of effects exerted by them on motoneuron pools.

On a very simple level motoneuron pools may be subdivided into groups on the basis of function. Flexor motoneurons innervate muscle groups whose mechanical action at a joint results in flexion, i.e. of the limb; these motor pools are located most laterally in the

ventral horn of the spinal cord. Extensor motoneuron pools exert the opposite mechanical action at a joint, i.e. extension of the limb, and are found more medially within the ventral horn (Landmesser, 1978b; Hollyday, 1980). A number of polysynaptic pathways have been shown to provide primarily excitatory input to flexor motoneuron pools and inhibitory input to extensor motor pools. The converse has been found for other polysynaptic projection systems.

Electrical stimulation of cutaneous nerves in cat hindlimb, at intensities sufficient to activate the small diameter fibres (e.g. nociceptive afferents) results in EPSPs in flexor motoneurons (Eccles and Lundberg, 1959). In studies of the projection patterns of GTOs (Harrison et al.,1983), and of the corticospinal and rubrospinal tracts (Brodal, 1981; Schwindt, 1981; Hongo, Jankowska and Lundberg, 1969a,b) it was concluded that these systems provide mainly excitatory input to flexor motoneuron pools and inhibitory input to extensor pools. The vestibulospinal tract appears to provide the opposite pattern of input to motoneurons, where flexors are inhibited and extensors are excited (Schwindt, 1981). These studies show that a number of projection systems to spinal cord motoneurons preferentially drive pools of motoneurons that are functionally and spatially distinct.

Within individual flexor and extensor motoneuron pools there is a further segregation of polysynaptic input, which appears to be strongly correlated with motor unit type; this pattern parallels the

one described for monosynaptic projections where slow-type motoneurons receive a stronger input than do fast-type motoneurons. Examples of this include the disynaptic projections of Ia and Ib afferents (Burke, Jankowska and ten Bruggencate, 1970; Burke, Pymer and Walsh, 1976) as well as the polysynaptic projections of small diameter cutaneous afferents (Burke et al., 1970) onto spinal motoneurons. The opposite recruitment order (i.e. fast motoneurons activated before slow motoneurons) is characteristically seen in response to input from large diameter cutaneous afferents and from the rubrospinal tract. Burke et al. (1970) have shown that electrical stimulation of the red nucleus or of the sural nerve in cats, most reliably evoked EPSPs in fast type motoneurons while often none could be recorded in the type-identified slow motoneurons. Although the slow-to-fast recruitment order of motoneurons has, in the past, been assumed to be a rigidly fixed feature of motor control (Henneman and Mendell, 1981), there is increasing evidence that this is not the case. While the studies of Burke and colleagues described above provide the most direct evidence, a number of indirect studies also support the idea that fast motoneurons may be recruited before slow in a variety of conditions (see Clamann et al., 1983; Pinter et al., 1983. Desmedt and Godaux (1981) have given evidence that motor units in the human interosseus muscle are recruited in order of slow to fast during voluntary abduction of the index finger. However, when the muscle acts as a synergist rather than as main mover, a certain

percentage of these units reversed their recruitment order so that fast ones were active before slow. Such studies lend support to the idea that motor commands are patterned in terms of movement, rather than in terms of individual muscles. The extensive projection patterns of spindle and GTO afferents to synergist motor pools (described in Section II, C,1) suggest a morphological basis for this at the segmental level. Interestingly, a recent report by Humphrey and Reed (1983) demonstrates the existence of two discrete locations within the wrist area of the motor cortex in primates which appear to preferentially evoke flexor or extensor activity respectively in the wrist and proximal arm muscles. Such studies support the idea that motor circuitry is not rigidly wired such that small slow-twitch motor units are inevitably brought to action first and large fast-twitch units secondarily, but rather that the nervous system is able to select out the appropriate type (and combination) of motor units required to execute the immediate motor task.

To conclude this section, polysynaptic projection systems appear to be organized , on one level, in terms of the flexor or extensor properties of motoneuron pools. On a more refined level, the synaptic efficacy of these input systems shows a strong correlation with motor unit type. The general conclusion which may be drawn from all the studies reviewed in this section strongly supports the idea that motoneuron pools within the spinal cord are functionally and anatomically organized according to the movement requirements of the

animal.

D. HOW ARE PATTERNS OF MOTOR CONNECTIVITY ESTABLISHED DURING DEVELOPMENT?

One objective of the thesis studies was to investigate whether there was any architecture or pattern to the association between CTM motoneurons and their target muscle that could contribute to our understanding of the way in which the nervous system develops. From the literature reviewed above, it is clear that the adult motor system is characterized by specific and stereotypic patterns of synaptic connectivity, a number of which have distinct spatial correlates. One question that arises is, how does this circuitry become established during development? Is there, for example, an initial random outgrowth of axons followed by a subsequent pruning of incorrect or redundant contacts? While there exists a vast literature on the development of the nervous system (a review of which is beyond the scope and requirements of this thesis), in this section I have chosen to focus mainly on the evidence that a largely intrinsic (i.e. genetic) developmental program is responsible for the establishment of the primary cytoarchitecture of the nervous system, and that only later in development do extrinsic, or environmental, influences shape the final patterns of neuronal circuitry.

1. Intrinsic Factors During Neurogenesis.

The embryonic development of the nervous system is characterized by a number of orderly spatial and temporal gradients

in the origin, migration and settling patterns of neuroblasts (Hamburger, 1948; Fujita, 1964; Nornes and Das, 1974; Hollyday and Hamburger, 1977; Holley, Wimer and Vaughn, 1982, a, b, c). Tritiated (^{3}H-) thymidine studies (e.g. Fujita, 1964) have demonstrated that the process of neurogenesis begins in the most rostral aspect of the embryonic spinal cord (or neural tube) and proceeds in the caudal direction. (Interestingly, a similar maturational gradient along the proximo-distal axis of the limb bud appears to exist for the process of myogenesis (Bonner and Adams, 1983; Rutz, Haney and Hauschka, 1982)). Within a given transverse segment of the spinal cord, cells originate in an orderly gradient along the ventral to dorsal axis with large, medium and small neurons (roughly corresponding to motoneurons, intermediate gray, and substantia gelatinosa neurons respectively) generated sequentially in groups with some temporal overlap (Nornes and Das, 1974; Sims and Vaughn, 1979). It is of interest to note that these three embryonic populations of neurons show characteristic distinctive migration and settling patterns. In regions which develop from the ventral portion of the neural tube, the cells migrate out and organize themselves into longitudinal columns, i.e. the motoneuron columns of the spinal cord and brainstem (Romanes, 1964; Fidman, 1970; Nornes and Das, 1974). Neurons which develop from the dorsal region of the neural tube display an active migration through their predecessors and settle in a laminar pattern, i.e. the dorsal horn of the spinal cord, tectum, cerebellum, and

cortex (Sidman, 1970; Nornes and Das, 1974). Neurons which develop from intermediate portions of the neural tube (the interneurons of the intermediate gray matter) settle medially and dorsally in a diffuse pattern upon the columns of motoneurons and subsequently upon their predecessors (Nornes and Das, 1974; Sims and Vaughn, 1979).

A number of classical and elegant studies have shown that the early development of the nervous system proceeds according to a largely intrinsic timetable. The most thorough and painstaking analyses of this question have been made by Landmesser and Pilar (1974a, b, c) in the chick ciliary ganglion where electrophysiological, light- and electron-microscopic techniques were used to characterize the normal pattern of cell death in this system and the abnormal patterns of development in ganglia surgically disconnected from their target organs. Landmesser and Pilar showed that ganglion cells lacking a target organ differentiated in normal numbers and sent axons out to the periphery; additionally characteristic synapses were formed with them by preganglionic axons. However at Stages 35-36 when normal ganglion cells have formed synaptic connexions with their target organs, the ganglion cells deprived of a periphery underwent massive cell death. Less comprehensive investigations in amphibian (Hughes, 1968; Hamburger, 1977) and mammalian (McClennan and Hendry, 1981) embryos provide additional support for the idea of an early independence and a later dependence for survival of neurons upon their target organs. The

above investigations suggest that the unfolding of a rigidly-specified developmental program is responsible for the establishment of the primary cytoarchitecture upon which the adult nervous system is built. That excised and transplanted pieces of neural tube will go on to form recognizable brain and spinal cord structures either in culture or in host tissues (Balinsky, 1981; Jacobson, 1978) provides additional support for this interpretation.

^{3}H-thymidine studies in birds (Hollyday and Hamburger, 1977) and mammals (Nornes and Das, 1974) have clearly shown that the single continuous longitudinal column of motoneurons, first laid down during neurogenesis, later segregates into two large clusters (as seen in transverse section in the brachial and lumbar segments), the visceral and somatic motor cell columns. The somatic, or lateral motor cell column (LMC) subsequently subdivides into lateral and medial columns, which in the mammal most clearly reflect a functional organization: the lateral motor column supplies the innervation to predominantly extensor muscles; the medial column drives flexor muscle groups (Romanes, 1964; Szekely and Czeh, 1967). Behavioral and EMG analysis of locomotor activity in normally active chicks show that muscles which are co-activated during the various phases of the step cycle have motor pools located in similar topologic positions along the longitudinal extent of the LMC (Jacobson and Hollyday, 1982a, b). In addition to an apparent structure-function relationship of motor nuclei, silver-stained preparations of cat spinal cord reveal that

motoneurons within a single cluster tend to have similarly oriented dendrites, ~~with~~ neighbouring clusters displaying different orientations (Sprague and Ha, 1964). Longitudinally-oriented dendrite bundles have been described in light and electron micrograph studies (Sterling and Kuypers, 1967; Scheibel and Scheibel, 1970; Schoenen, 1982); the speculation has been put forth that these bundles may provide the basis for common presynaptic inputs. The results of this thesis indicate that within a single motoneuron pool distinct dendritic bundles exist; this may possibly reflect the different functional activities (and thus the different presynaptic inputs) of the motoneuron sub-columns elaborating these dendrites. The studies described above therefore seem to indicate that the spatial organization of the nervous system laid down during early embryogenesis has a functional correlate in the adult.

The onset of synapse formation within the spinal cord also occurs according to a remarkably invariant timetable, along predictable spatial gradients. Among the first observable responses of bird and mammal (including human) embryos to environmental stimuli is the withdrawal of one or both forelimbs in response to mechanical or electrical stimuli applied to one forelimb (Windle and Baxter, 1936; Windle and Fitzgerald, 1937). Recent correlative autoradiographic, EM and behavioral studies of the forelimb reflex in rodents confirm that the onset of synaptogenesis in this simple reflex circuit appears to be determined largely by the sequence in

which component neurons are generated, i.e. synaptogenesis began first in the ventral horn neuropil between motoneurons and interneurons, and later was observed in the dorsal neuropil between interneurons and dorsal root ganglia projections (Vaughn and Grieshaber, 1972; Sims and Vaughn, 1979). Furthermore, the precocious, intermediate and late development of reflex traits characteristic of three different species of mice was shown to have a genetically-associated variability (Vaughn, Hendrickson, Chernow, Grieshaber and Wimer, 1975; Sims, Vaughn and Wimer, 1981).

Electrophysiological investigations of the development of coordinated motor output in the hindlimb of chick embryos indicates that the neural circuitry, i.e. the motor programs, appropriate for alternating agonist-antagonist muscle activation are established within the spinal cord without detectable synaptic input from either afferent or descending sources (Narayanan and Malloy, 1974; Bekoff, Stein and Hamburger, 1975; Oppenheim, 1975; Bekoff, 1976). Observations of the patterns of embryonic motility in chick embryos following surgical transposition of brachial and lumbosacral spinal cord segments have further indicated that the different motor programs for walking or flying are intrinsic to specific spinal cord segments very early on in embryogenesis (Straznicky, 1963; Szekely, 1968; Narayanan and Hamburger, 1971; Hamburger, 1977). The results of electrophysiological investigations of the sequential input-output maturation of the kitten motor cortex (Bruce and Tatton, 1980)

provide further support for the concept that the motor system matures in advance of, and likely without significant input from the sensory system. It has further been shown that sensory neurons appear to have an unexpected dependency on motoneurons in establishing their peripheral connexions: by removing the motoneuron-containing regions of the neural tube in chick embryos (prior to outgrowth of motor axons), Landmesser, O'Donovan and Honig (1982) found that, while the muscles were present, the sensory nerve bundles usually supplying them were absent. These studies strongly indicate that the establishment of the basic patterns of motor circuitry follows a rather rigid and predictable developmental program, which appears to be correlated with distinct spatial characteristics of the motoneurons themselves and takes place without much peripheral or central input.

There is, however, a large body of experimental evidence dealing with the outgrowth patterns of motoneurons, showing that despite an initial specific projection pattern, motoneurons are capable of responding to an altered periphery (e.g. Landmesser, 1978a, b; Lance-Jones and Landmesser, 1980a, b; Whitelaw and Hollyday, 1983a, b, c). Such studies provide information about the mechanisms by which motor axons reach their appropriate targets. The remainder of the Background therefore will be concerned with how environmental cues play a role in the progressive development of the adult motor pool map.

2. Role of Environmental Cues

The electrophysiological and morphological evidence reviewed above indicates that much of the organization displayed in adult motor projection patterns derives largely from spatial and temporal gradients laid down during early neurogenesis. These observations have given rise to a number of hypotheses, contending either that the entire pattern of motor circuitry may be accounted for solely by spatial (or "morphogenetic") features (Horder, 1978), or alternatively by largely temporal-maturational factors (Bennett, Davey and Uebel, 1980). While it is clear from in vivo and in vitro experiments that growing axons display patterns of fasciculation and an active responsiveness to the mechanical substrate over which they grow (Harrison, 1910; Speidel, 1933; Weiss, 1934; Letourneau, 1975a, b), it is equally apparent, particularly from limb grafts and spinal cord deletion or reversal experiments in chick embryos (Hamburger, 1934; Hollyday, Hamburger and Farris, 1977; Morris, 1978; Lance Jones and Landmesser, 1981b; Hollyday, 1981; Whitelaw and Hollyday, 1983a, b, c), that motor axons are capable of recognizing and responding to specific cues in the limb bud independently of spatial and temporal factors.

Investigations of the patterns of innervation of supernumerary limbs in chick embryos suggest that a number of developmental mechanisms is likely to influence the pattern of motor projections in the normal embryo. While the basic topographic

organization of motor pools in the LMC remained unchanged despite the addition of a supernumerary leg, one consistent observation was that laterally-located motoneurons drove muscles derived from the DMM and medially-positioned motoneurons provided the innervation to muscles originating from the VMM (Hollyday, Hamburger and Farris, 1977; Morris, 1978; Hollyday, 1981; Whitelaw and Hollyday, 1983b). These results demonstrate a selectivity on the part of motoneurons for at least one characteristic of the novel target tissue, a preference also evident in the normal development of limb innervation (e.g. Landmesser, 1978a). Furthermore, it was found that both the type of plexus and the detailed pattern of nerve branching were determined by the type of limb (i.e. by the target itself) and were not intrinsic to the segmental nerves (Morris, 1978; Hollyday, 1981). These results provide support for the idea that specific environmental cues present in the limb bud provide pathway selection and guidance for the growing motor axons. Early innervation studies in normal chick embryos suggest that from the outset motor axons grow to the region in the limb bud where their target muscle will eventually differentiate (Landmesser, 1978a; Lance-Jones and Landmesser, 1981a); this evidence is incompatible with the idea that axons explore the entire limb in search of suitable targets.

A very thoughtful and elegant series of experiments by Whitelaw and Hollyday (1983a, b, c) involving deletions, duplications and rotations of partial or complete limb buds in chick embryos

throws further light on this issue. In experiments where limb segments (i.e. calf, thigh or foot) were surgically deleted, ^{3}H-thymidine autoradiography showed a selective loss of the motor columns normally supplying the deleted segment. These findings do not support the concept that the timing or spatial arrangement of motor axons in the limb bud is uniquely responsible for the motor projection patterns (e.g. Horder, 1978; Bennett, Davey and Uebel, 1980), but rather imply a "matching up" of motoneurons and their targets. In serial and parallel duplications of leg segments motoneurons were found to terminate in a particular location (i.e. a specific segment) rather than at a specific muscle (Whitelaw and Hollyday, 1983b). The implication of these experiments is that under the imposed (experimental) conditions, the target selectivity expressed by motoneurons is under some kind of control over the proximo-distal growth. The last series of experiments (Whitelaw and Hollyday, 1983c), involving limb bud rotations about the dorso-ventral axis provides evidence that the selectivity of motoneurons for dorsally or ventrally derived muscle is most likely determined at the level of the plexus proximal to the limb bud.

One conclusion that may be drawn from these experimental results is that environmental factors (e.g. limb bud mesenchyme) play a large role in restricting the "developmental potential" (Balinsky, 1981) of the motoneuron, specifically in terms of its outgrowth and its final phenotypic expression (e.g. flexor or extensor motoneuron).

At the base of the limb bud, motor axons choose a pathway leading to the DMM or the VMM, the choice determined apparently on the basis of the position (and thus on the level of maturation) of the parent cell bodies in the spinal cord. It is interesting to speculate that such may mechanisms depend upon "positional information" (Wolpert, 1969, 1971) encoded in the motoneurons, related to the major axes of the body. The fibre guidance referred to here is obviously correlated with the antero-posterior and medio-lateral axes of the ventral horn and of the limb (the antero-posterior axis of Xenopus limb is derived from the embryonic dorso-ventral axis, which is distorted by development (Lamb, 1976); it is, therefore, a possibility that this may also be the case for higher vertebrate development.

From the literature reviewed in this section it is tempting to speculate upon a developmental sequence leading to the establishment of the adult patterns of motor connectivity: the basic cytoarchitecture of the spinal cord is laid down very early in neurogenesis according to intrinsic spatial and temporal factors, without substantial input from peripheral or descending sources. Since the initial projection patterns of motor axons to their final target sites appear to be quite specific, there must necessarily be some matching up of neurons and their targets, based possibly on positional information encoded in both ectodermal (i.e. spinal cord) and mesodermal (i.e. muscle) derivates. The organizing principles which ensure there is a selective matching up of motoneuron pools and

their target muscle most likely involve intrinsic, maturational and environmental cues.

SECTION III

RATIONALE

Spatial organization clearly plays an extensive role in the development of the nervous system. While it is comparatively easy to analyze the development of spatial patterning in a system where the embryonic 'nearest neighbour' relationship of cells and/or their axons are retained (i.e., as in the retino-tectal projection), the question becomes extremely difficult in a system where the positional axes of the embryonic tissue have become obscured, for example as in the torsion and migration of muscle masses away from their origin. Moreover the complex internal architecture of most skeletal muscles constitutes a formidable experimental obstacle to the understanding of spatial relationships between motoneurons and their target fields in the muscle, and the patterns of reflexly-evoked contractions in the adult muscle, measured under a variety of experimental conditions, do not always offer a clear picture of the underlying arrangement of motor connectivity. The questions addressed in this thesis arose from observations of the reflex behavior of the CTM, a thin sheet of cutaneous skeletal muscle in the rat which displays a distinctly compartmentalized pattern of reflex activation. While the CTM is reflexly activated by segmental sensory inputs from the skin in the dorsal thoracic and lumbar trunk, and the local sign is segmentally expressed, all the motor output originates from a circumscribed region of the cervical spinal cord, exiting the

brachial plexus to innervate the muscle. It is not immediately apparent, therefore, how the precise somatotopic connectivity seen in the local sign character of the reflex may be established. One possibility is that there may be an underlying anatomical organization of the motor circuitry which corresponds to the observed behavioral pattern of muscular contractions, for example that the motoneurons would be grouped in the spinal cord according to the position of their terminal fields in the muscle. Since the CTM is essentially a flat, two-dimensional sheet of muscle, and the sensory nerves that drive the reflex are anatomically separate from its motor nerves, the possibility was presented for an electrophysiological and anatomical investigation of the organization of this reflex. The unique characteristic of the CTM preparation was the opportunity it gave for an examination of the pattern of motor circuitry at the level of a single motoneuron pool. The results of such an investigation may bear on the general question of how the specific patterns of connectivity between neurons and their targets become established during development.

SECTION IV

GENERAL STRATEGY OF THE INVESTIGATION

It was decided first to examine the behavior of the CIM in response to its physiological activation. Following this, the distribution of the motor nerves was studied, and finally the organization of the cells of origin of these motor nerves. The results from all of these approaches, it was hoped, would suggest at least a partial answer to the original question, whether or not the motoneuron organization in the spinal cord was a spatial one that related to the reflex activation of the muscle. This strategy gave rise to the separate investigations which, for convenience, can be stated as Specific Aims. These are listed below:

1. What is the pattern of reflex activation of the CIM?

2. What is the pattern of motor innervation of the muscle?

a) Is there a segmental innervation of the CIM by the ventral roots?

b) Is there a segmental innervation of the CIM by the major motor nerves of the CIM?

3. Does the CIM motoneuron pool have an intrinsic spatial organization?

a) Is there a gross organization that corresponds to the "dermatomal" pattern of sensory activation of the muscle?

b) Is there an organization of the motoneuron pool corresponding to a division of the muscle by its motor nerves?

c) Is there an organization of the pool that corresponds to the behaviorally compartmentalized use of the muscle?

SECTION V

MATERIALS AND METHODS

1) Animals

A total of 250 adult female Wistar rats were used in this study. The animals weighed between 200-275 grams, and were obtained from Woodlyn Farms, Guelph, Ontario. The rats were housed in groups of 2-3 per cage on wood-chip bedding and were provided with food and water ad libitum. Following surgery each animal was housed individually.

2) Anaesthesia

All animals were anaesthetized with sodium pentobarbitol (45 mg/kg) for both operations and perfusions. In experiments lasting longer than one hour a femoral cannula was inserted and the level of surgical anaesthesia maintained by infusion of a 10% sodium pentobarbitol solution in 0.9% saline. Surgical anaesthesia was determined by the lack of a reflex response to a tail pinch. Core temperature was maintained with a rectal thermistor probeat 37 $\pm$ 1°C by a controlled circulating water pad placed beneath the animal. In experiments where the reflected muscle preparation was used (see below Section V, 3) a heat lamp was also used to maintain body temperature.

3) Reflex Activation of the Cutaneous Trunci Muscle (CTM)

When the animal had reached the level of surgical

anaesthesia, the hairy skin covering one half of the back and flanks was shaved in order to expose the skin surface covering one side of the CTM. The location of the CTM is shown in Figure 1. Experiments were usually performed on the left side, although essentially similar results were obtained when the right side of the animal was investigated. The length of the animal was measured from the occipital protuberance to the base of the tail, and felt-tipped pen marks were made at centimeter (cm) intervals along the dorsal midline (see Figures 2 and 3). These marks provided reference points for the analysis of experimentally-evoked responses.

i) Behavioral Assessment. Reflex activation of the CTM in 10 rats was produced by stimulating back or flank skin with light pinches using rat-toothed forceps, with focal heat application using a thermistor-controlled metal-tipped probe at 55°C, or by application of small pieces of ice. The area of skin puckering in response to the stimulus was noted visually and recorded photographically (see Figure 2), or delineated with felt-tipped pen marks directly on the skin.

ii) Electrophysiological Assessment. To investigate the electromyographic (EMG) characteristics of a sensory nerve-evoked reflex response in the CTM, a "reflected muscle preparation" was developed. In order to map the left side of the animal, a longitudinal midline incision was made through the skin of the back with a razor blade, approximately one centimeter to the right of the dorsal midline, extending from the nape of the neck to the base of

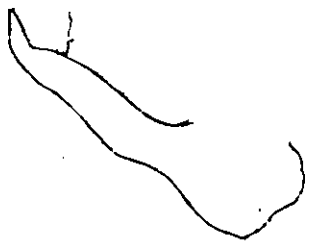
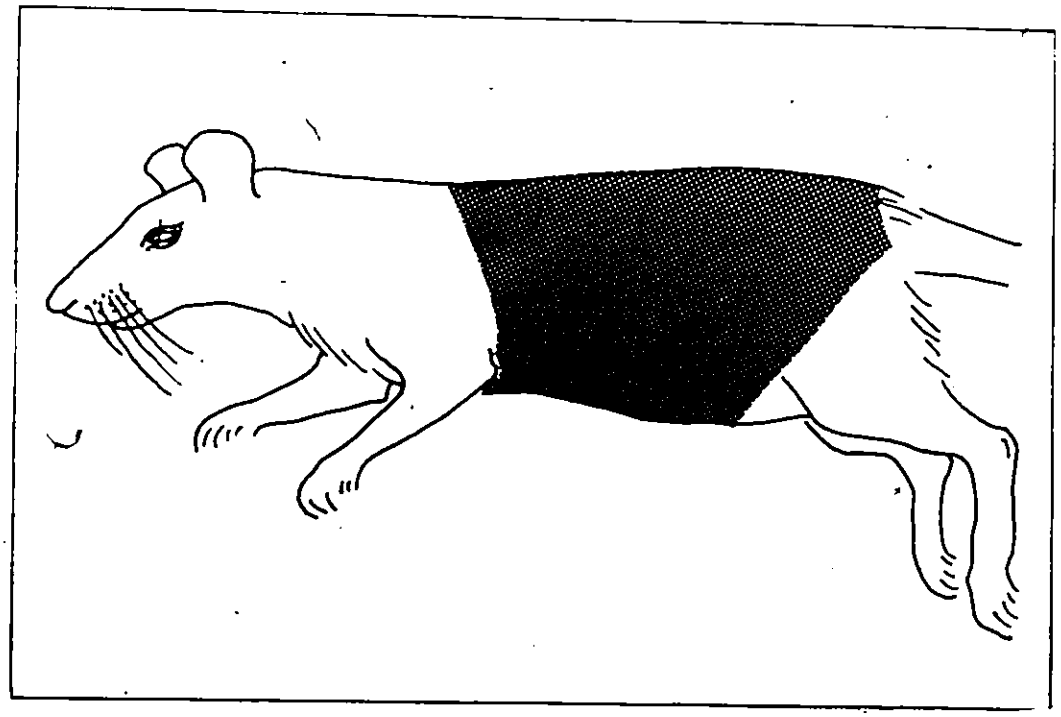


Figure 1 The cutaneous trunci muscle (CTM).
1A The distribution of the CTM.
1B A transverse section of the skin showing the location of the CTM in a 14 day old rat. Note that many hair follicles are visible in the dermis. Deep to the CTM is loose connective and blood vesels.

A



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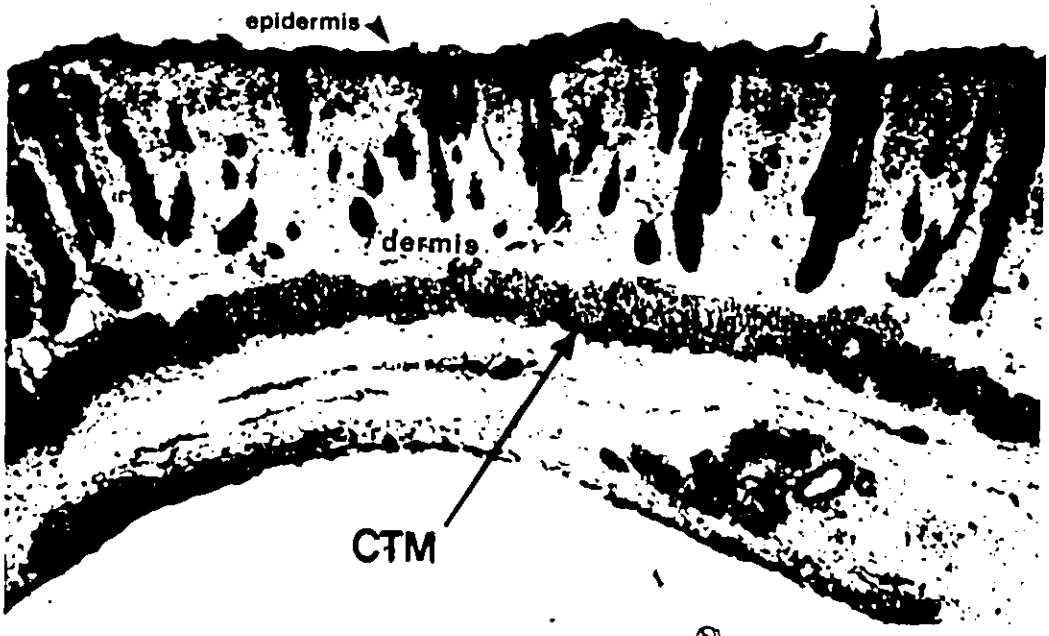
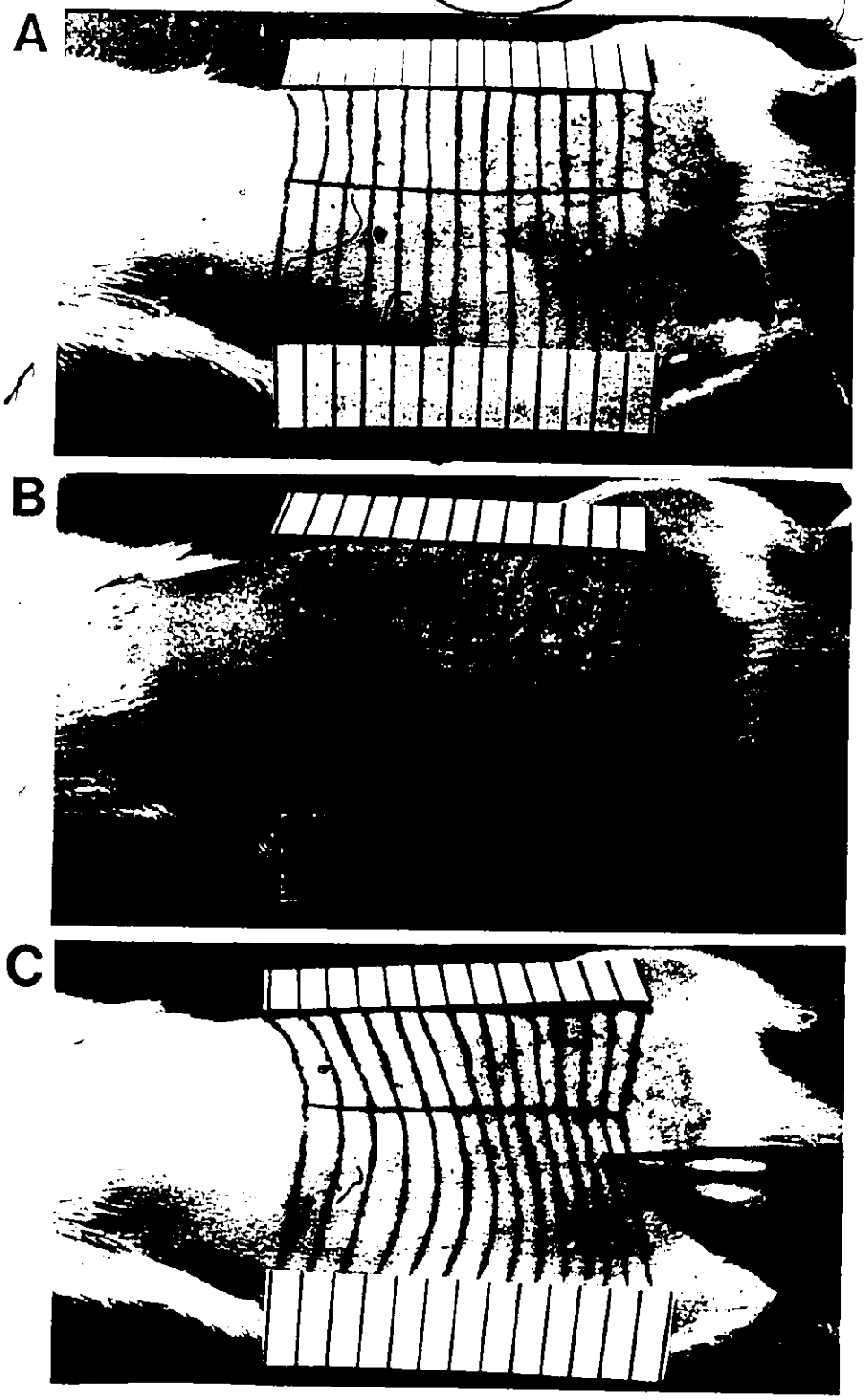


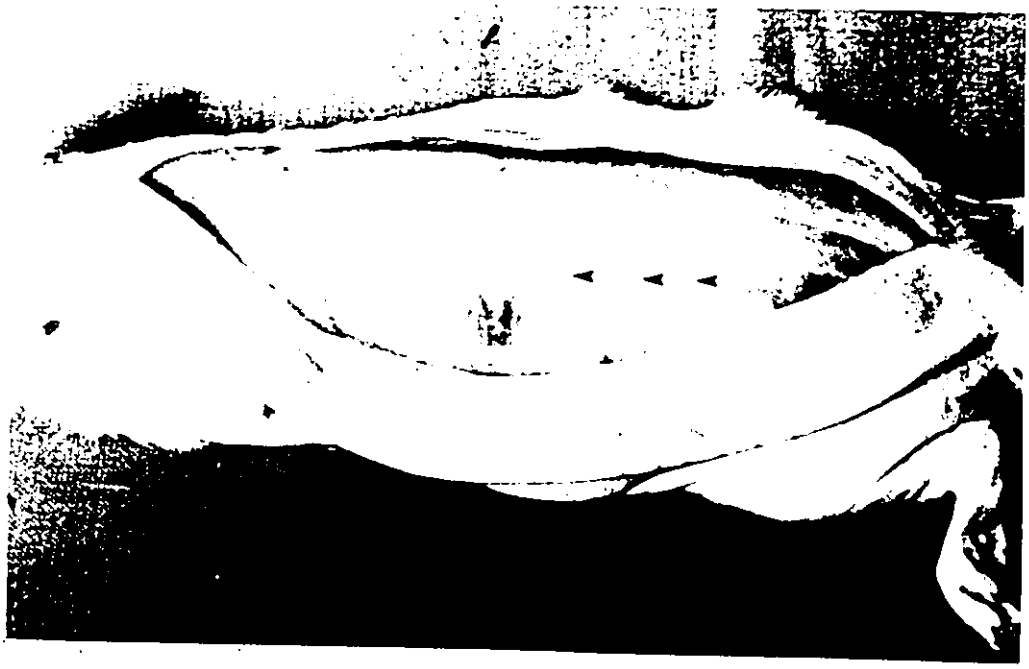
the tail. When the free edge of the skin flap was retracted laterally from the midline, the dorsal cutaneous nerves (DCNs) were revealed (see Figure 3A); these nerve bundles emerge from the body wall on the surface of the latissimus dorsi muscle for 2-3 cm before they enter the skin to supply the afferent innervation of the back (see Figure 3B). The lateral and ventral cutaneous nerves (LCNs and VCNs) follow a similar pattern to enter the skin and provide the sensory innervation to the lateral and ventral skin respectively. Several millimeters of one or more of these cutaneous nerves were dissected out, ligated and cut some millimeters from the skin. Two transverse incisions were then made through the thickness of the skin on the left side, the anterior one extending from the longitudinal incision laterally behind the blade of the scapula to the elbow, and the posterior one extending from the most caudal extent of the longitudinal incision laterally down the most caudal portion of the flank. The remaining cutaneous nerves, and the connective tissue holding the skin (and CTM) to the body wall, were severed and the skin reflected outwards from the body wall, exposing the surface of the CTM and its motor innervation arising from the brachial plexus (see Figure 4). This procedure results in the reflected muscle preparation. The segmental cutaneous nerves could then be electrically stimulated on bipolar platinum electrodes and the location of the resultant EMG activity in the CTM mapped with bipolar platinum electrodes (see Figure 5). The electrodes were placed on the

Figure 2 Localized response of the CTM to a skin pinch.
2A shows only 1 cm lines drawn on the shaved back skin of a rat.
2B shows an anteriorly-placed forceps pinch.
2C shows a posteriorly-placed forceps pinch.
The horizontal line indicates the approximate location of the dorsal midline. Note that the focus of contraction (where the lines are closest together) in 2B and 2C lies 1-2 cm rostral to the forceps tips and that it is bilaterally distributed.


A
B
C

3A The location of several DCNs indicated by arrowheads.
3B Typical locations of several low-threshold mechanosensory DCN fields (only alternate ones were mapped).

A



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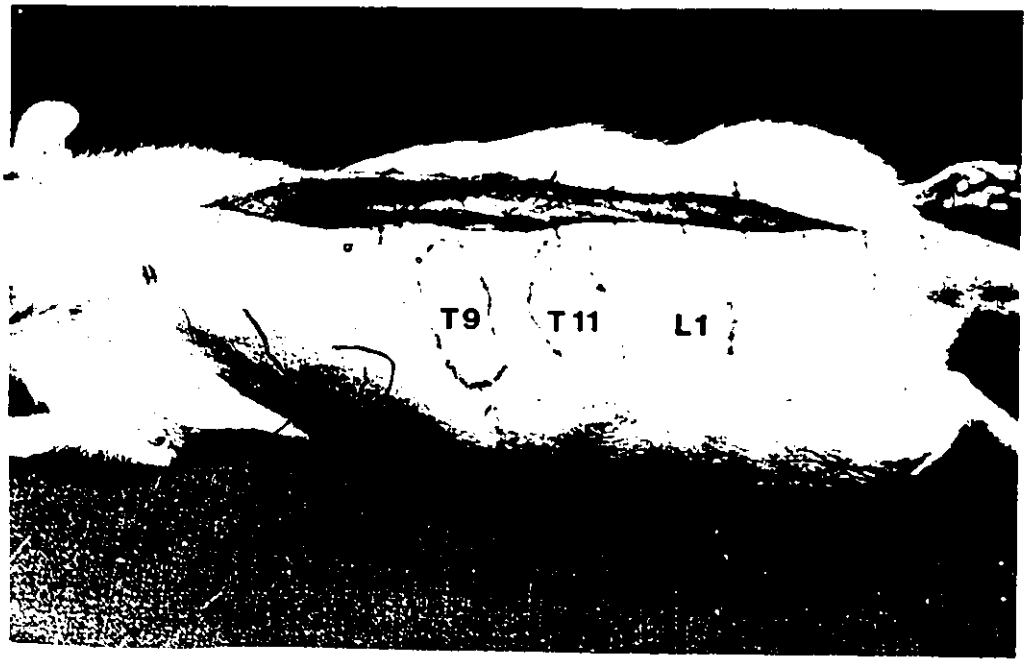


surface of the muscle, so that their tips were in a plane at right angles to the longtitudinal axis of the muscle fibres (see Figure 5A); the distance between the electrode tips was approximately 2 mm, while the distance between each of the electrodes was 1 cm. The EMG signals were then fed into a Grass P-15 amplifier and displayed on a Textronix D13 oscilloscope screen and either photographed or recorded on a Sangamo 3500 tape recorder for later analysis.

4) The Muscle

i) Muscle Fibre Typing. Under surgical anaesthesia four animals were shaved and the CTM reflected outwards as described above. Selected areas of the muscle were cut out with a razor blade, rapidly frozen in isopentane cooled to -180°C with liquid nitrogen and then cut on a cryostat into 10-14 μm transverse serial sections. The left medial gastrocnemius and soleus of the rats were also removed and prepared in the same manner. The method of Padykula and Herman (1955) for the analysis of muscle fibre type in rat muscle was used to evaluate the myosin-ATPase characteristics of the CTM and medial gastrocnemius (see Figure 11). In this procedure an alkali pre-incubation causes fast-twitch glycolytic fibres to stain darkly while slow-twitch oxidative fibres appear very pale.

ii) Motor Endplate Distribution. A preliminary examination was made of the the pattern of motor endplate distribution in the CTM. A reflected muscle preparation was used in 4 animals; the exposed surface of the muscle was dissected free of most of the overlying

connective tissue and then cut with a razor blade into 5x5 cm pieces which were then pinned out in a dish. Rows of endplates were visualized following the whole-mount cholinesterase method of Ginsborg and MacKay (1960). In this procedure acetylthiocholine, present in the incubation medium, is hydrolyzed by the muscle ATPase enzyme in the presence of copper sulphate. This results in the formation of a copper-thiocholine complex, and following an incubation with ammonium sulfide, a black precipitate is visualized at the motor endplates (see Figure 6).

In a second series of experiments the Toop method (1976) was used for demonstrating motor endplates in 60 μm cross-sections of skeletal muscle of three rats. In this procedure motor endplates are visualized following a silver-cholinesterase reaction (see Figure 8).

5) Pattern of Ventral Root Innervation

In six rats a cervical laminectomy was performed and the ventral roots of segments C6 to T1 were exposed. The selected root (one per animal) was cut centrally and the distal ends drawn into a bipolar suction electrode. Kugelberg and Edstrom (1968, 1970) have shown that contractions of rat fast-twitch glycolytic muscle, induced by repetitive stimulation of its motor nerve, produce striking changes in the glycogen content of these active muscle fibres. Their procedure was followed in these series of experiments. Square wave electrical stimuli were applied at 40 Hz (1.0 V amplitude, 0.1 ms duration) to the selected ventral root for 30 secs on, 30 secs off,

for 10-15 minutes. The muscle was quickly cut with a razor blade into pieces (see Figure 12 for a diagram of the areas of muscle analyzed), rapidly frozen in isopentane cooled with liquid nitrogen, and then processed for conventional Periodic Acid Schiff (PAS) histochemistry (Pease, 1968). Muscle fibres depleted of glycogen did not stain up in this procedure, but those that were unstimulated retained their glycogen content and stained darkly (see Figure 13).

6) Pattern of Motor Nerve Innervation of the CTM

i) EMG Analysis of the Major Motor Nerve Fields. The motor nerves of the CTM exit the brachial plexus in three to seven characteristic bundles which are easily accessible for 5-6 mm before they penetrate the epimysium of the CTM (see Figure 4). In order to map the individual motor nerve fields, a reflected muscle preparation was set up in 23 animals. Each of the major motor nerve bundles (and in many cases several of their subsequent ramifications) were individually stimulated on bipolar platinum electrodes with square wave stimuli of 0.2 to 1.0 V amplitude and 0.2 ms duration (which was 2-3 times the threshold value, i.e. 2-3 times that just adequate to cause a detectable response in the muscle), at 0.5 Hz. The evoked EMG activity was then mapped with handheld bipolar platinum electrodes (the distance between electrode tips was 2 mm), and the border between active and inactive muscle fibres was marked directly on the muscle surface with a fine felt-tipped pen. Subsequently, a thin clear acetate sheet was gently placed on the surface of the CTM and

Figure 4 The reflected muscle preparation.
4A Dorsal view of the preparation.
4B Diagrammatic representation of the preparation.

A

B

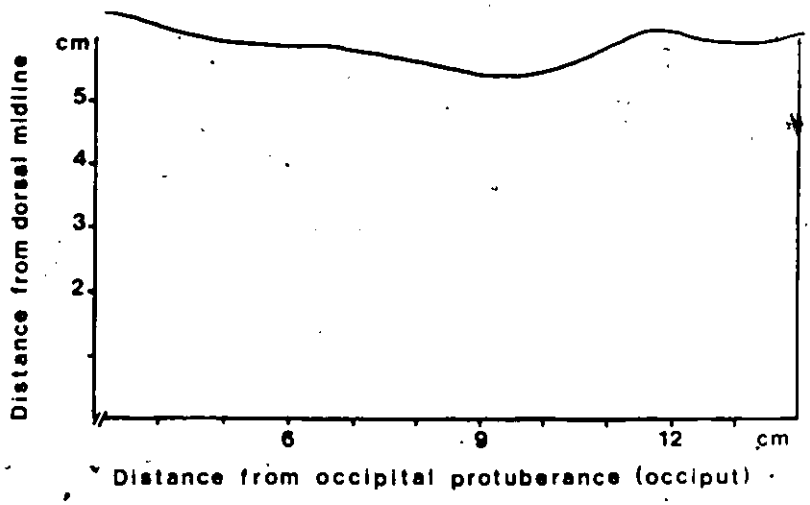
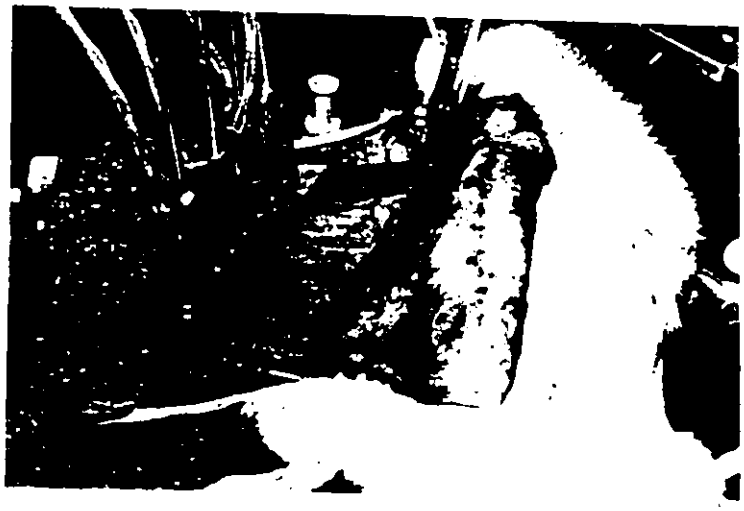

cm
5
4
3
2
Distance from dorsal midline
6
9
12
cm
Distance from occipital protuberance (occiput)

Figure 5 Local sign in the CTM reflex.

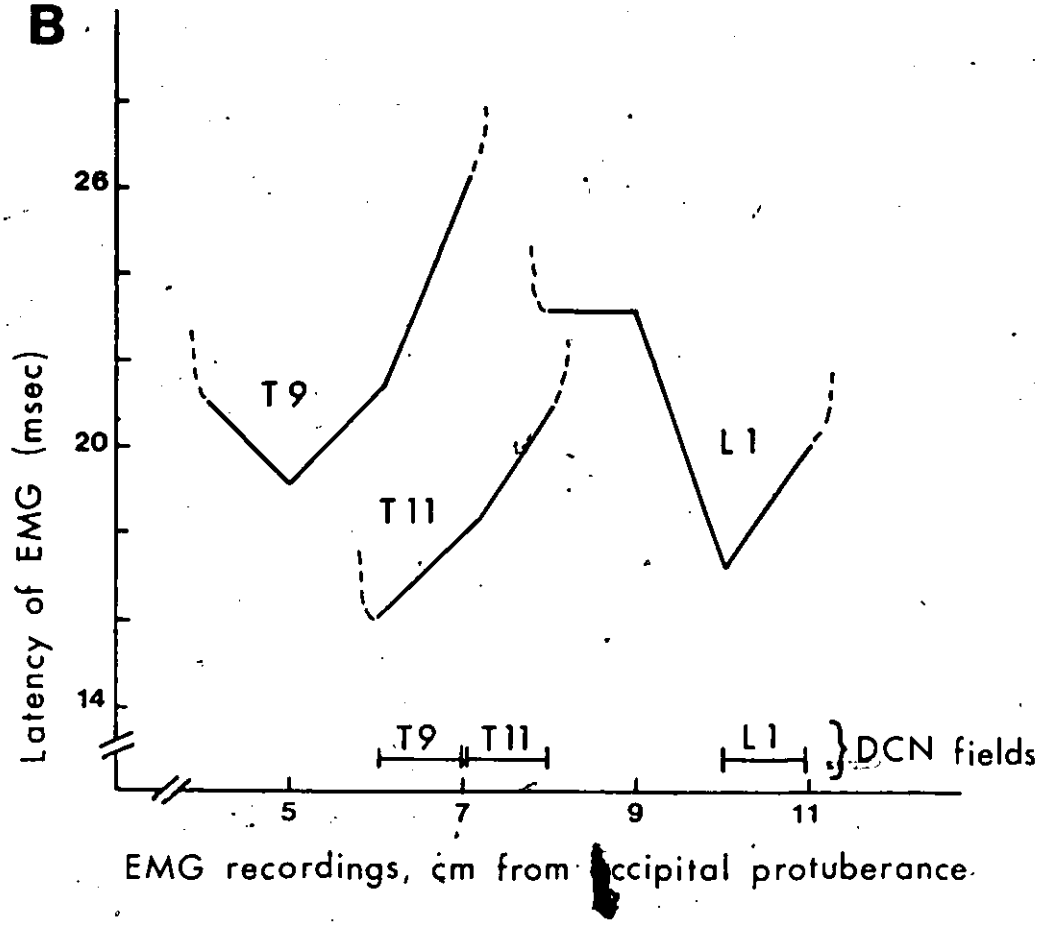
5A shows the experimental set-up used to map evoked electromyographic (EMG) activity in the reflected muscle preparation.

5B shows the latency of the EMG reflex response to electrical stimulation of the DCNs indicated; the approximate extents of the respective dermatomes are drawn just above the abcissa. The broken lines indicate the borders between active muscle and the adjacent region in which no EMG activity was detected. The shortest latency EMG for each DCN is located 0.5 to 2.0 cm rostral to the centre of the corresponding DCN field.

A



B


26
20
14
Latency of EMG (msec)
T9
T11
L1
T9
T11
L1
DCN fields
5
7
9
11
EMG recordings, cm from occipital protuberance

the dotted outline of the field was traced onto the acetate sheet. This map was then transferred to paper and area measurements were made with the use of a visual digitizer, the Bit Pad One (Summagraphics, Laboratory Computer Systems Inc., Cambridge, Mass.).

In three animals finely graded electrical stimuli were applied to a selected motor nerve and the area of EMG activity in the muscle was mapped as described above. By using a very small electrical stimulus it was hoped that only one or a few motor axons would be activated and thus the resulting area of EMG activity would indicate the size of a motor unit (see Results, Section VI D,4).

ii) EMG Analysis of Motor Nerve Twigs.

The CTM is a very thin sheet of muscle (averaging about 10 muscle fibres in thickness; see Results, Section VI A,1), and consequently many of the major nerve bundles and their subsequent ramifications can be visualized throughout the length of the muscle. The question arose as to whether these ramifications would divide up the muscle field of their parent motor nerve into some recognizable pattern. For these experiments (done on ten animals), a reflected muscle preparation was set up and 2-4 mm of small motor nerve bundles ("nerve twigs") were carefully dissected free from most of the surrounding connective tissue and muscle fibres. The location of the nerve twig was measured in terms of the distance from the occipital protuberance (x value) and the distance from the dorsal midline (y value). The x,y values of each of the nerve twig experiments are

shown in Figure 34. The dissected length of uncut nerve was raised onto bipolar platinum electrodes and electrically stimulated (0.4 to 0.6 V amplitude, 0.1 ms duration, 0.5 Hz and the area of active muscle was mapped as described in Section V, 6i. An example of such a map is shown in Figure 34. In two cases the identity of the parent motor nerve was established by electrically stimulating the twig and noting which of the major motor nerves conveyed the recorded antidromic action potential (see Figure 35).

iii) Glycogen Depletion Studies. In another series of experiments, done on 10 animals, a motor nerve field was mapped as described above, and then the selected nerve was stimulated electrically so as to cause tetanic contraction of the muscle field (0.2 - 1.0 V amplitude; 0.1 ms duration; 40 Hz; 30 sec on, 30 sec off); the location of the border between active and inactive muscle fibres was marked directly on the muscle surface with a felt-tipped pen. Subsequently the tissue was processed for PAS histochemistry as detailed in Section V, 5. After the tissue was cut on the cryostat, a diamond knife was used to make a scratch on the slide directly above the location of the still-visible pen mark. In this manner it was possible to correlate the histochemically- and electrophysiologically-determined field borders.

7) Location of the CIM Motoneuron Pool

In this series of experiments (done on twenty animals) a small area (2x5 cm) of hairy skin extending laterally from the dorsal

midline to the elbow was shaved. A small incision was made laterally behind the blade of the scapula to expose the CIM motor nerves as they exit the brachial plexus. When carefully made, this incision did not damage the muscle fibres of the CIM nor its nerve supply. The forelimb was extended, rotated anteriorly and taped down in this position to facilitate viewing and dissection of the motor nerves. Several millimeters of the CIM motor nerves were dissected free of the overlying connective tissue anterior to the site where they penetrate the muscle fascia. A small molded paraffin wax boat containing 25-50% horseradish peroxidase (HRP) solution (HRP Type VI, Sigma Chemical Co., St. Louis, Missouri) in 0.9% saline was placed beneath the nerves which were then cut. The central ends were placed in the HRP solution and a petroleum jelly seal was placed around the rim of the boat to prevent leakage of the HRP solution. After 15-20 minutes the boat was removed and the area gently swabbed with a saline-moistened Q-tip to remove any excess HRP adhering to the cut nerves. The incision was sutured and the animal left to recover from the anaesthetic. Eighteen to thirty-six hours later the rat was deeply anaesthetized and perfused transcardially with 250 ml of heparinized phosphate-buffered saline followed by 500 ml of fixative (2.5% gluteraldehyde and 1% paraformaldehyde). The cervical and thoracic spinal cord (C5 to T2) was dissected out and the ventral root entry zones marked with razor cuts and/or India ink. A modification of the Mesulam (1978) HRP technique, which uses

tetramethyl benzidine as the chromogen, was used to examine the location and size of the CTM motoneuron pool. Either transverse, sagittal or horizontal 40 μm sections of the spinal cord were taken and reacted for HRP histochemistry. Sections were mounted on gelatin chrome-alum coated slides and counter-stained with 1% neutral red or 0.25% thionin before being cover-slipped and analyzed for the presence of HRP-containing profiles. In cases where HRP leakage was apparent or suspected, or where spinal cord sections were damaged or lost during the histological procedures, those animals were not included for analysis in this study.

8) CTM Motor Nerves

In two animals the entire left CTM motor nerves were excised and pinned in a dish. The nerves were then fixed with 2% gluteraldehyde in phosphate buffer, washed and post-fixed in 1% OsO_4. Following a second wash in acetate buffer the nerves were block stained with 5% aqueous uranyl acetate and then embedded in Spurr resin. Semi-thin sections were cut on a Cambridge-Huxley ultramicrotome and stained with toluidine blue. The sections were viewed with a Zeiss photomicroscope, photographed, and myelinated axon counts were made for each nerve bundle.

9) HRP Injections Into the Muscle

i) Multiple Injections.

In 16 animals a strip of dorsal skin was shaved, the distance from the occipital protuberance marked in centimeter intervals along

the dorsal midline skin, and a longtitudinal incision made through the thickness of the back skin. At the selected location (e.g. 13 cm from the occipital protuberance) the connective tissue holding the skin to the body wall was blunt-dissected away so as to expose the surface of the CTM. Care was taken not to damage the segmental cutaneous nerves or the muscle surface. A total of 4-50 µl (usually 10 µl) of 25-50% HRP solution was injected directly into the CTM along a single transverse axis in 5 separate injections. The incision was sutured and the animal left to recover from the anaesthetic. Twenty-four to forty-eight hours later the rat was deeply anaesthetized and perfused transcardially; the spinal cord was removed and reacted for HRP histochemistry as described above in Sectiion V, 7.

ii) Single HRP Injections. In 32 rats the same surgical procedure was followed as described above for multiple HRP injections. However in these experiments only one 2 µl injection of HRP solution was made directly into the muscle. The exact x,y values of each site (see Section V, 6ii for an explanation of the x,y values) were noted and are presented in diagrammatic form in Figure 30. After injection of the HRP solution, the incision was sutured and the animal left to recover from the anaesthetic. After an appropriate survival time (i.e. 24-48 hours), the spinal cord was processed for HRP histochemistry as detailed in Section V, 7.

10) Location of Motoneuron Somata Corresponding to:

i) Individual Motor Nerves. The motor nerves to the CIM exit the brachial plexus in several characteristic bundles (refer to Section V, 6i and to Figure 16) which are easily accessible for experimental manipulation. In forty-six animals the location, within the CIM motor pool itself, of the cell bodies whose axons constitute one of the major CIM motor nerves was analyzed using the retrograde transport of HRP by cut axons. The selected motor nerve was gently dissected clear of connective tissue, cut distally and the central end dipped into a solution of 25-50% HRP solution for 15-20 minutes. The same procedure for HRP histochemistry was followed as described in Section V, 7 and the location of HRP-labelled profiles was examined in either transverse or horizontal 40 µm sections of the spinal cord.

ii) Nerve Twigs. Following the mapping of a motor nerve twig (see Section V, 6i for the protocol), the twig was cut distally and the central end placed in 25-50% HRP solution. After an appropriate survival time the spinal cord was reacted for HRP histochemistry.

11) HRP Diffusion Studies and Motoneuron Labelling

In four animals a reflected muscle preparation was set up and the borders of the selected motor nerve field were determined by EMG recordings. All the other motor nerves were then ligated centrally (i.e. near to their origin from the brachial plexus) with silk suture thread, cut close to the knot, and several mm of each of these nerves were excised immediately distal to the knot. This procedure ensured that retrograde transport of HRP to the spinal cord could occur only

in the selected (intact) motor nerve. A single 2 µl injection of 50% HRP solution was then made directly into the muscle at a distance of either 1.0 cm (n=2), 1.5 cm (n=1), or 2.0 cm (n=1) from the electrophysiologically determined border of the motor nerve field. Following an appropriate survival time the spinal cord was analyzed for HRP-labelled cells.

SECTION VI

RESULTS

A) THE CUTANEOUS TRUNCI MUSCLE (CTM)

1) Gross Anatomy

The CTM of the rat is a thin sheet of skeletal muscle that underlies back and flank skin. It takes its origin bilaterally on the lateral aspect of the humerus (from the ridge of the greater tubercule) and inserts beneath the dermis of the skin posterior to the axilla all the way along the back and flanks to the base of the tail (see Figure 2). The ventral distribution of the muscle was not studied in detail; however histochemical observations (see Methods Section V, 5) and behavioral testing (e.g., pinches to the belly area in an attempt to elicit any obvious reflex contractions of the CTM) indicate that the CTM muscle fibres from the left and right sides of the animal do not extend all the way to the ventral midline, i.e. there appears to be several millimeters of belly skin devoid of CTM muscle fibres. These observations agree with those of Langworthy (1925) who made an anatomical study of this muscle in several rodents and carnivores.

The CTM is quite thin, consisting of 10-15 muscle fibres in cross-section (see Figure 11); however as the muscle fans out ventrally this thickness is reduced to 1-3 muscle fibres. The muscle fibres themselves run rostrocaudally as seen in Figure 6. When one of the CTM motor nerves is electrically stimulated at just-threshold

intensity and the portion of responding muscle is mapped, the smallest areas of EMG activity measure 1-1.5 cm in maximum rostrocaudal length (these preliminary motor unit studies are described more fully in Results Section VI, D, 1). These results suggest that the CTM muscle fibres in the adult rat are likely to be less than 2 cm in length. By using the dissecting microscope to focus up and down through the whole-mount preparation (described in Section VI, A, 3), and based on the histochemical observations described below, the muscle fibres did not appear as regular rows in which all the fibres are parallel and of the same length, but rather there are staggered segments of rows; the fibres of adjacent such segments interdigitate with each other in the longitudinal axis without any obvious precision of origin and insertion. A proposed pattern of pinnation for the CTM is drawn in Figure 7, which includes for comparison, a diagram representing the pattern of pinnation of the soleus and semitendinosus based upon descriptions in the literature (e.g. Gans and Bock, 1965). In cross-section, it appears that the CTM muscle fibres attain a diameter of 25 μm or greater (no correction for shrinkage has been made; see Figure 11).

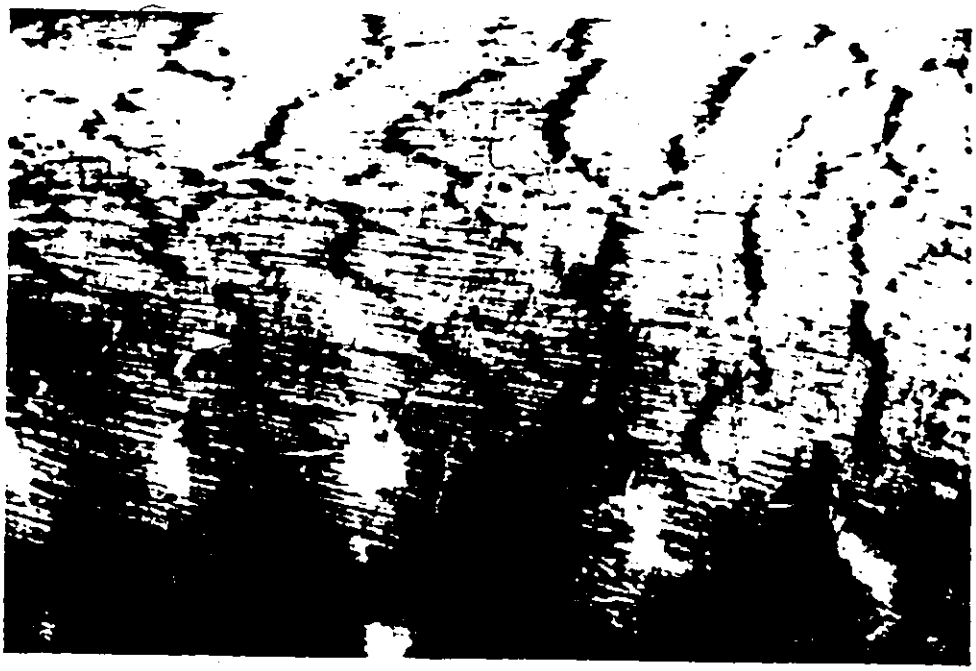
3) Motor Endplate Zones

A whole mount preparation stained for acetylcholinesterase (AChE) was used to examine the pattern of motor endplate distribution in the CTM of four animals. Staggered rows of endplates were found oriented at right angles to the long axis of the muscle fibres (see

Figure 6 Whole mount of the CTM showing rows of acetylcholinesterase (AChE)-stained motor endplates (a few are indicated by arrowheads).

Figure 7 Proposed pattern of pinnation of the region of the CTM asterisked in Figure 6. Compare with the known patterns of pinnation of the soleus (7B) and the semitendinosus (7C). Refer to text, Section VI, A 3 for further explanation.

6



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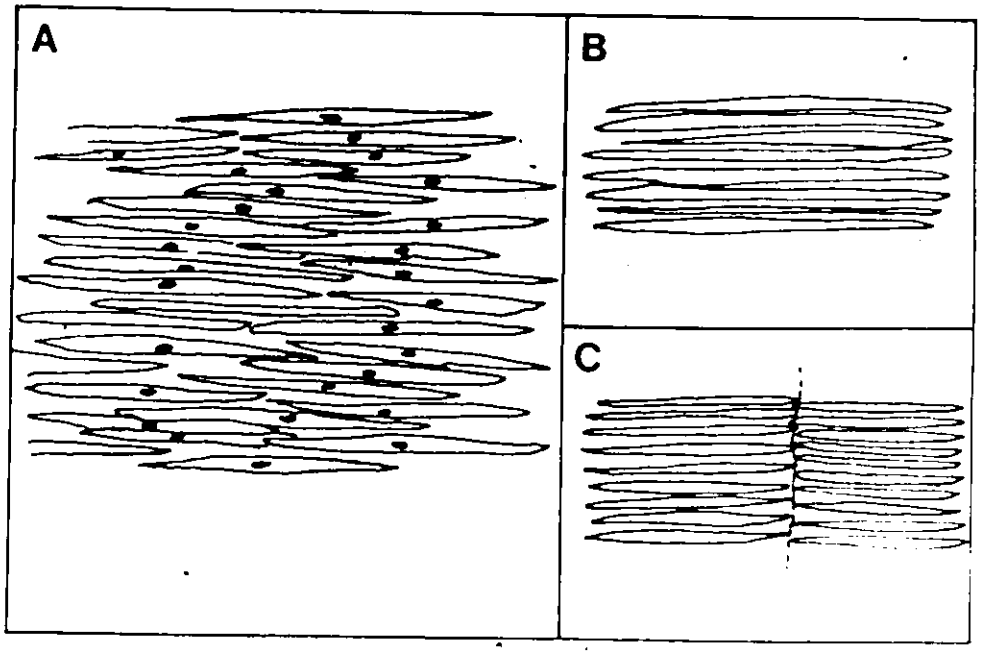
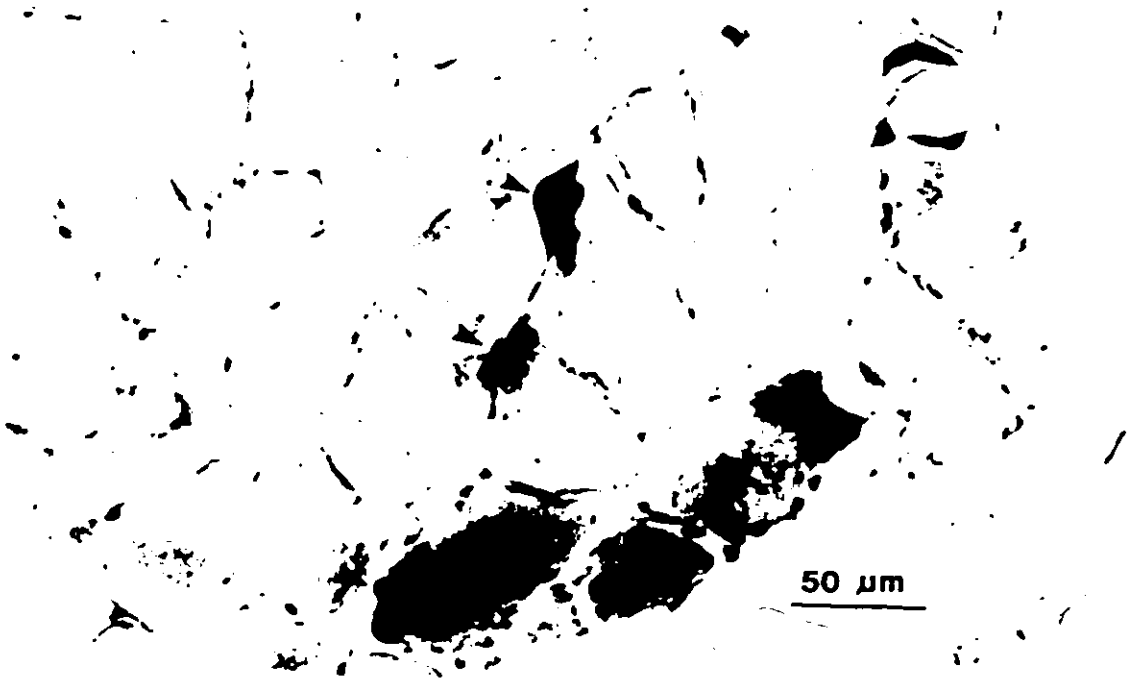


Figure 8. A 60 μm transverse section of the CTM showing Toop silver-stained motor endplates (arrowheads).

Figure 9 A 60 μm transverse section of the CTM showing a muscle spindle (Toop silver stain), indicated by arrowhead.

8



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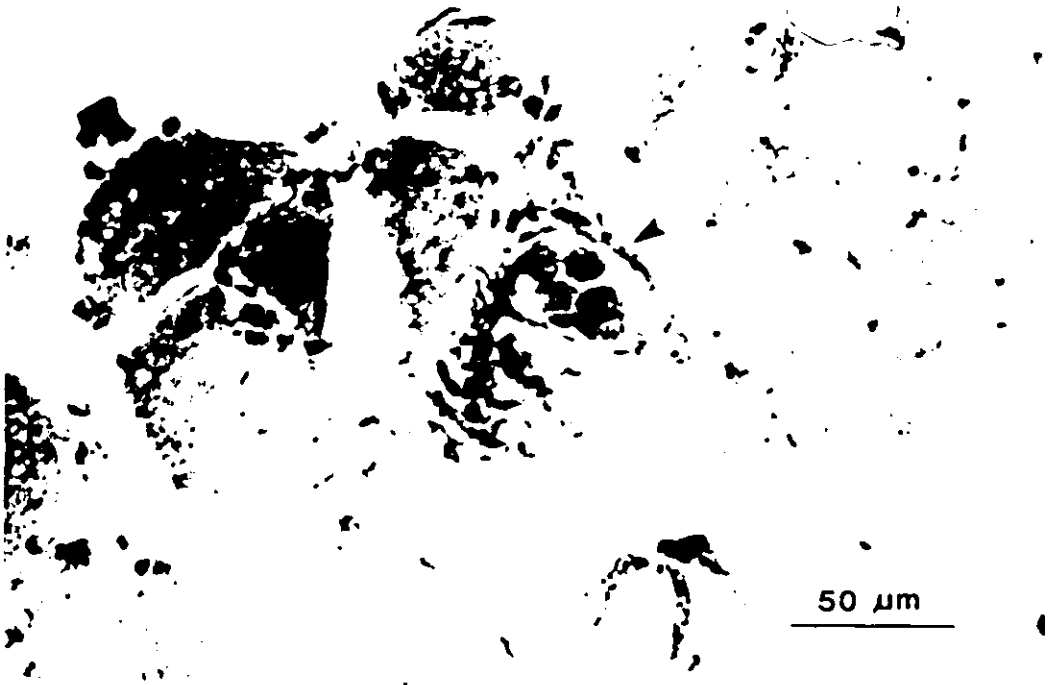
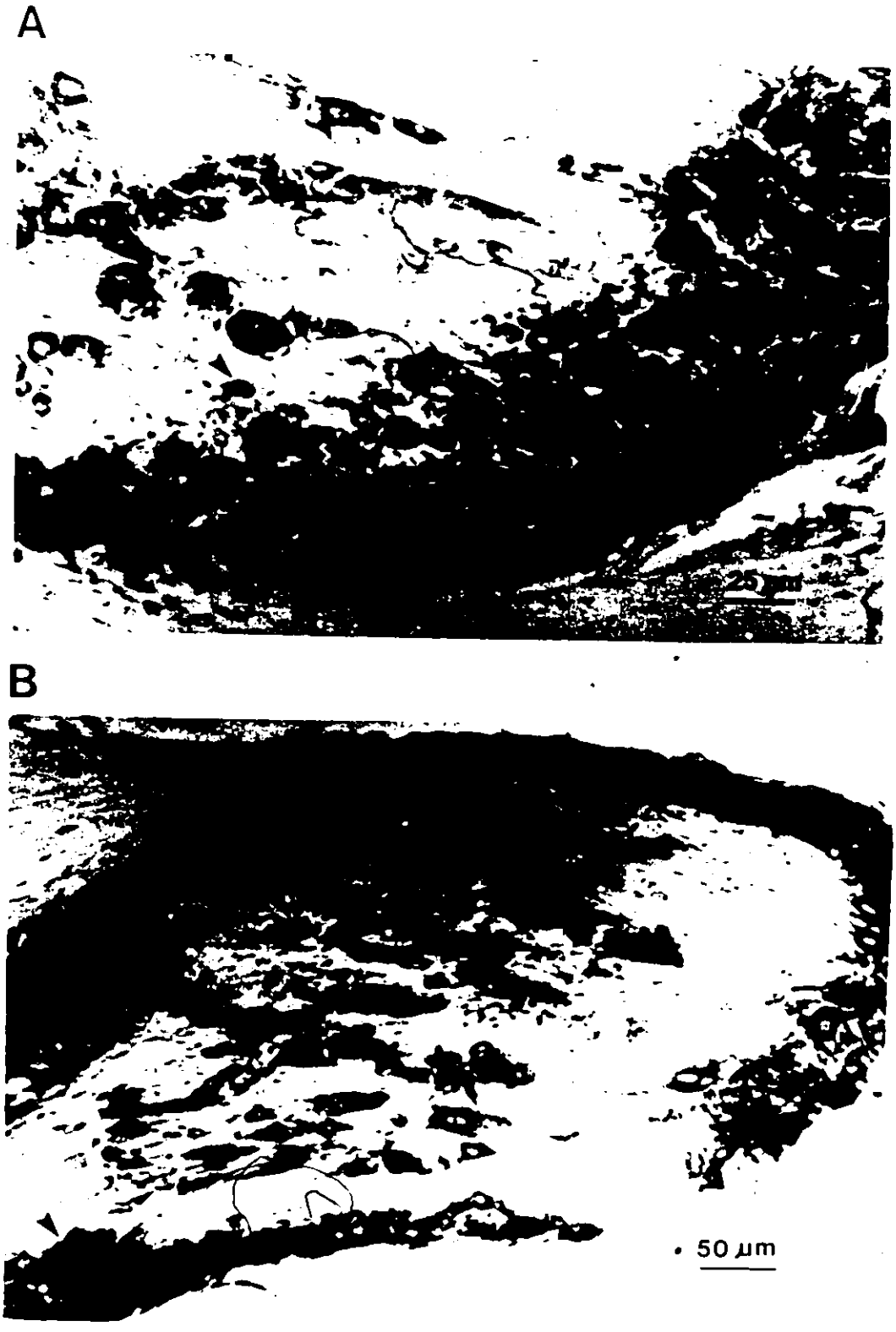


Figure 6). This pattern was observed in all four cases and there was no obvious difference from one location in the muscle to another. The distance between rows of endplates was usually about 6mm, but this varied from 4 to 8 mm. If, as suggested above, the individual muscle fibres are about 1.5-2.0 cm in length, these figures raise the possibility of multiple innervation of single muscle fibres. However, when examined closely with the dissecting microscope, no two rows of endplates appeared in register with one another (see Figures 6 and 7). This observation suggests that consecutive rows of endplates were not located on the same muscle fibres. If indeed each endplate is centrally located on a single muscle fibre, the average length of the fibre would be somewhat greater than a centimeter (6 mm + 6 mm). These observations are in agreement with the electrophysiological estimates of motor unit size described in detail in Results, Section VI, 4, indicating that the CIM muscle fibres are likely to be 1.5-2.0 cm in length in an adult rat of about 250 gm.

3) Preliminary Studies of Muscle Fibre Type

Histological studies in the early 1900's showed that most mammalian muscles are made up of at least two kinds of muscle fibres (see review by Close, 1972), while more recent physiological and histochemical work has revealed three principal types of skeletal muscle fibres (fast, slow, and intermediate) that differ in dynamic properties, the activities of glycolytic and oxidative enzymes, myoglobin content and certain properties of myosin (Close, 1972;

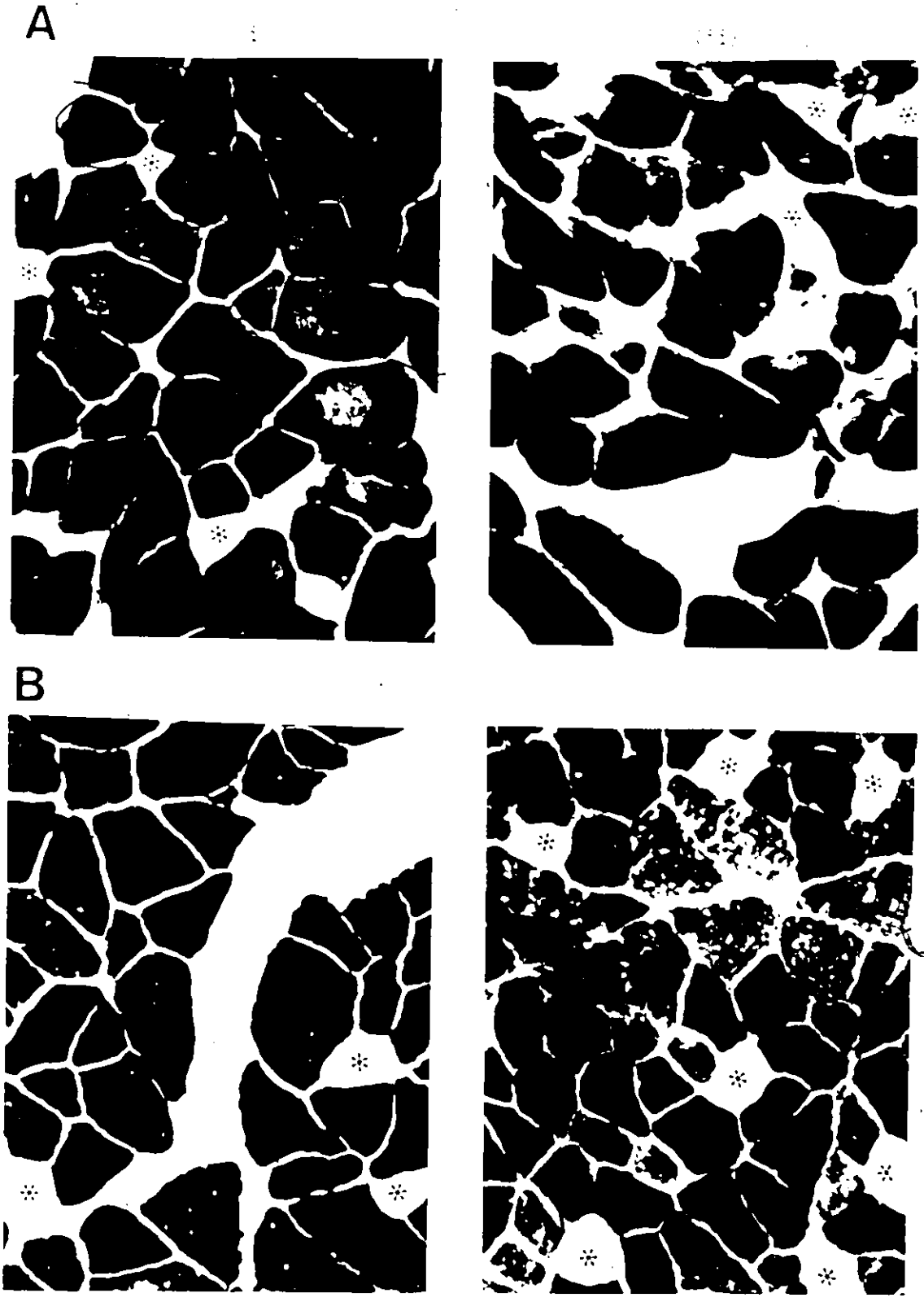
Figure 10 Dorsal root ganglion (DRG) cells labeled by retrograde transport of horseradish peroxidase (HRP) in the CIM nerves (10A) and in the pectoralis minor nerves (10B); some are indicated by arrowheads.


A
B
50 µm

myoglobin content and certain properties of myosin (Close, 1972; Buchthal and Schmalbruch, 1980). Skeletal muscles containing a histochemically uniform population of slow fibres are classified as slow-twitch muscles, e.g., the cat soleus (Burke and Tsairis, 1973), while those muscles containing a mixture of all three fibre types, with a preponderance of fast and/or intermediate fibre types have been classified as fast-twitch muscles e.g. the rat medial gastrocnemius (Edgerton and Simpson, 1969). An unequivocal identification of most fibres can be achieved by staining serial sections for myosin ATPase, phosphorylase, and oxidative enzymes (Stein and Padykula, 1962; Yellin and Guth,1970). Such a study of the CIM, however, was beyond the scope of this thesis. Therefore only a rudimentary analysis was made of the CIM fibre type by comparing two histochemical characteristics of the CIM with those of a fast-twitch muscle (the rat medial gastrocnemius) and a predominantly slow-twitch muscle (the rat soleus). In one series of experiments 10-14 μm sections of the CIM and medial gastrocnemius were taken from four rats and stained in parallel for myosin ATPase according to the method of Stein and Padykula (1955). When sections from each of these muscles were compared, the CIM closely resembled the gastrocnemius in that both light and dark staining muscle fibres were present in the sections (see Figure 11).

A second series of experiments, involving glycogen depletion studies (refer to Methods Section V, 5) indicates that the CIM muscle

Figure 11 Myosin ATPase stain of two rat muscles.
A CTM
B medial gastrocnemius
In the first column (i), an alkali-stable reaction shows up slow-twitch fibres (marked with asterisks). The next column (ii) shows the results from an acid-stable reaction. In this procedure pale-staining fibres indicate an intermediate fibre type. As can be seen, both muscles contain a small number of intermediate fibre types. The fast-twitch fibres (which stain darkly in both the acid- and alkali-stable reactions) are found to make up the majority of fibres in the gastrocnemius and also in the CTM, indicating a classification of fast-twitch muscle for both of them. The present results are in agreement with previous findings.


A
B

fibres use a predominantly glycolytic metabolism; these results are consistent with a classification of predominantly fast-twitch glycolytic fibre type for the CTM. Figure 13 shows CTM muscle fibres staining positively for glycogen, using conventional PAS histochemistry.

4) Summary Statement of Findings

The CTM is a thin sheet of skeletal muscle underlying back and flank skin. Its muscle fibres appear to be quite short (between 1.5- 2 cm) and run rostrocaudally, originating and inserting in a non-uniform pattern beneath the dermis of the skin. Staggered rows of endplates are oriented at right angles to the longtitudinal axis of the muscle fibres, i.e. staggered short rows of endplates (each row measuring approximately 2-6 mm in length) run dorsoventrally while the muscle fibres run rostrocaudally. Measurements of the distance between rows of endplates along the rostrocaudal axis, in conjunction with EMG estimates of motor unit size suggest that the muscle fibres are singly-innervated. Preliminary studies of fibre typing in the CTM are consistent with a classification of fast-twitch, glycolytic fibre type.

B) REFLEX ACTIVATION OF THE CTM

Although not in itself one of the major objectives of this investigation, it was important to know the way in which the CTM is normally used in life, especially reflexly. Conceivably this would have a character that might make the organization of the CTM

Figure 12 Areas of the CTM sampled for glycogen depletion studies (described in Results, Section VI C and D).

Figure 13 Periodic Acid Schiff (PAS) stain of unstimulated (13A) and tetanically stimulated (13B) CTM.

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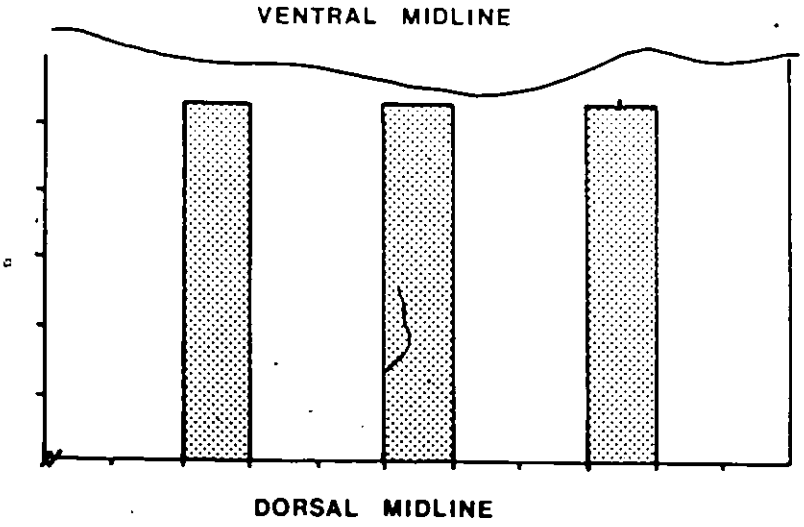
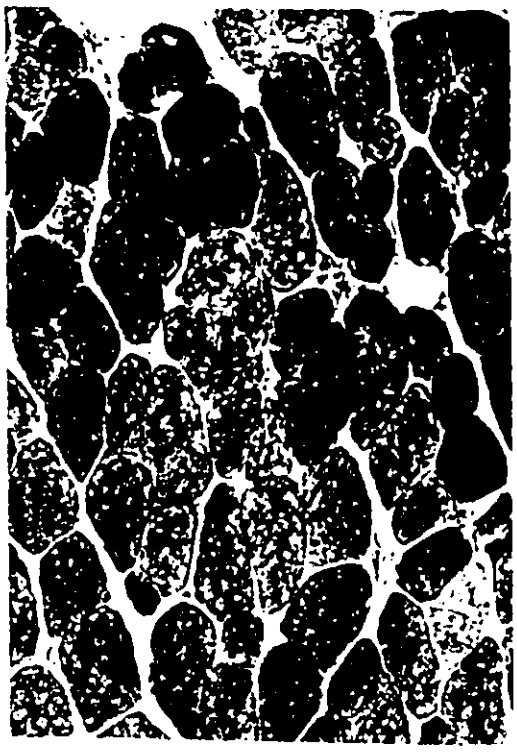


13A



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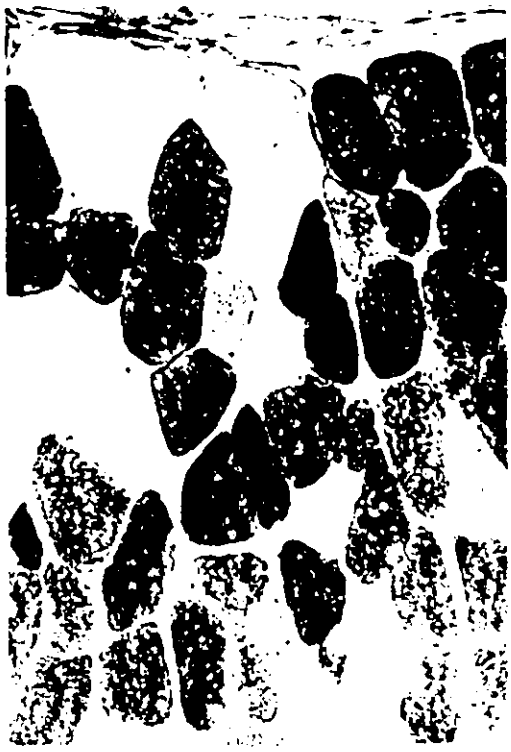


motoneuron pool and its relationship to the motor innervation of the muscle understandable. Therefore certain characteristics of the reflex activation of the CTM will be presented here, but only briefly (the work is fully described in Nixon, Theriault, Jackson and Diamond, 1984).

1) Behavioral Analysis

If the skin of a rat's back is lightly pinched, a localized contraction of the underlying CTM can be observed in the immediate vicinity of the pinch (see Figure 2). If the stimulus is moved anteriorly or posteriorly, the focus of the contraction also moves anteriorly or posteriorly (Figure 2). The reflex activation of the CTM therefore exhibits "local sign", a term coined by Sherrington (1947) to describe the orienting of a reflex movement towards the site of the sensory stimulation. These observations imply that the CTM is functionally compartmentalized. While the simplest explanation of the underlying reflex circuitry is that both the sensory and motor nerves are segmental, this is the case only for the sensory nerves. We have shown that the reflex contraction of this muscle is elicited by nociceptive information (mechanical and thermal) carried in the dorsal cutaneous nerves (DCNs); moreover, only the Aδ and C fibres are effective, and not the large low-threshold (touch sensitive) Aα fibres (Nixon et al., 1984). The DCNs are segmental, and those which can activate the CTM extend from the fourth thoracic segment (T4) to the fourth lumbar segment (L4) of

the spinal cord (see Figure 4 for the location of the DCNs). If two or more of these DCNs are cut, an area of sensory denervation in the back skin results, so that when this area of skin is pinched, no reflex contraction of the CTM is evoked. These results also demonstrate that a skin stimulus adequate to activate the muscle does not do so directly, but reflexly. The motor nerves to the CTM are unaffected by cutting the DCNs, since they exit the brachial plexus to innervate the muscle (Hebel and Stromberg, 1976; and see below); thus the sensory and motor nerves of this reflex are anatomically separate.

2) Electrophysiological Analysis

For these experiments, the area of skin supplied by a DCN was first determined; this was done by recording from the nerve while brushing the skin very lightly with a fine (1-2 bristles) handheld brush. The area of skin from which impulses were evoked in the DCN is designated as the low-threshold mechanosensory field of that nerve, and in separate experiments it has been shown that this field is only slightly smaller than the high-threshold (nociceptive) field of the same nerve (see Nixon, Doucette, Jackson and Diamond, 1984), and Figure 3B for a typical example of three DCN fields). The borders of each field were marked directly onto the shaved skin surface with a felt-tipped pen and the distance in centimeters from the occipital protuberance to the centre of the low-threshold field noted. When a DCN was electrically stimulated and the pattern of EMG activity in

the muscle analyzed, the shortest latency EMG activity was found to be located 1-2 cm rostral to the centre of the low-threshold field of that DCN. These results provide a quantitative measure of the local sign in the CTM reflex (see Figure 5).

3) Muscle Spindles

From the results described above, it appears that the CTM has evolved especially to respond to nociceptive stimulation of the overlying skin. In addition, the anatomical location of this skeletal muscle suggests that it contributes very little to the maintainence of posture and balance. It was of interest, therefore, to determine if there was a signifigant population of muscle spindles within the CTM. Evidence for the presence of muscle spindles was looked for by recording an afferent discharge evoked in the motor nerves during a stretch applied to the muscle. The CTM of three animals was examined in this fashion and at no time could an afferent discharge be recorded from the CTM motor nerves, or fine filaments of these, during a stretch applied to several different locations in the muscle. The apparent absence or paucity of muscle spindles in the CTM was also examined histochemically. A thorough examination of Toop silver-stained cross-sections of various areas of the CTM in three animals revealed only one example of a spindle. In five cases where the CTM motor nerves were soaked in HRP solution for motoneuron studies (see Methods Section V,7) the dorsal root ganglia (DRGs) were also examined for labeled cells. It was usual to find no labelled

cells in the DRGs; however on two occasions one large and a few small DRG cells were found containing HRP reaction product. Figure 10A shows an example of these labelled DRG cells. The absence of HRP labeling in CTM ganglia was not due to problems with the HRP technique, for when the pectoralis minor nerves were cut and soaked in HRP solution (n=5) several large DRG cells containing HRP could regularly be found (see Figure 10B). Therefore, from the combined electrophysiological and histochemical results described in this section it appears that the CTM has very few spindles.

4) Summary Statement of Findings

We have previously shown that a punctate nociceptive stimulus to the skin elicits a localized reflex contraction of the underlying CTM (Nixon et al., 1984). From electrophysiological and histochemical investigations spindles do not appear to be present in numbers large enough to provide a major Ia (spindle) reflex drive to the CTM. The cutaneous input that does effectively drive the CTM reflex enters the spinal cord segmentally in the dorsal cutaneous nerves extending from T4 to L4. This vast sheet of muscle is therefore functionally compartmentalized in terms of its reflex activation. While the sensory information that evokes this reflex is segmentally organized, and the pattern of contraction in the muscle is also segmental, the motor nerves to the CTM originate from the brachial plexus (see below). From these physiological and anatomical observations a number of questions emerge, of which the major one

addressed here is: how is this reflex organized? In particular, could there be an anatomical correlate to the observed behavioral use of the muscle which extends to the underlying reflex circuitry?

C) IS THERE A PATTERN OF VENTRAL ROOT INNERVATION?

The location of the ventral roots of origin for the CTM motor nerves was described as C8 and T1 by Hebel and Stromberg (1976). However, using retrograde transport of HRP by the CTM motor nerves (see below, Results, Section VI, E) I was able to localize the CTM motoneuron pool from caudal C6 to rostral T1, inclusive. The ventral roots of origin for the CTM motor nerves are therefore most likely to be C6, C7, C8 and T1. Since, as shown above, the muscle is functionally compartmentalized in terms of its reflex behaviour, the question was asked: do the vental roots partition up the CTM in a manner corresponding to the segmental (or "dermatomal") pattern of the sensory activation of the muscle? This question is presented diagrammatically in Figure 14.

In six animals only one of the four ventral roots (C6, C7, C8 or T1) was electrically stimulated so as to cause tetanic contraction of that area of the muscle innervated by the selected ventral root. Pieces of muscle were then processed for PAS histochemistry (see Figure 12 for the sampling protocol) and the distribution of glycogen-depleted fibres analyzed. The results are detailed in Appendix I and are shown in a composite diagram in Figure 15. There were two major technical difficulties involved in these experiments.

Figure 14 Hypothetical pattern of ventral root partitioning of the CTM (indicated above), viewed relative to the sensory fields of the DCNs in the overlying skin (indicated below). The orientation of the CTM is that shown in Figure 4B.

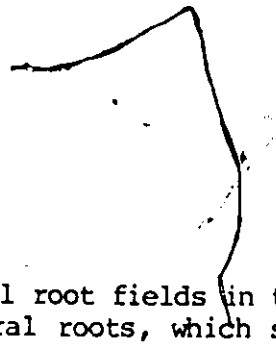
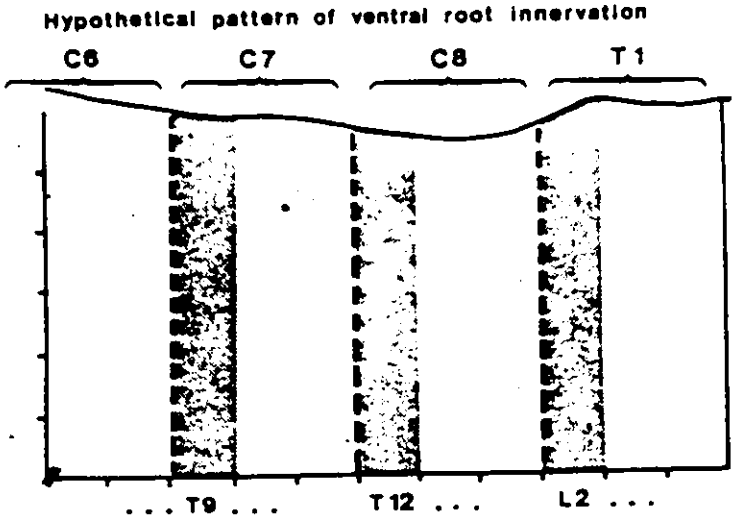


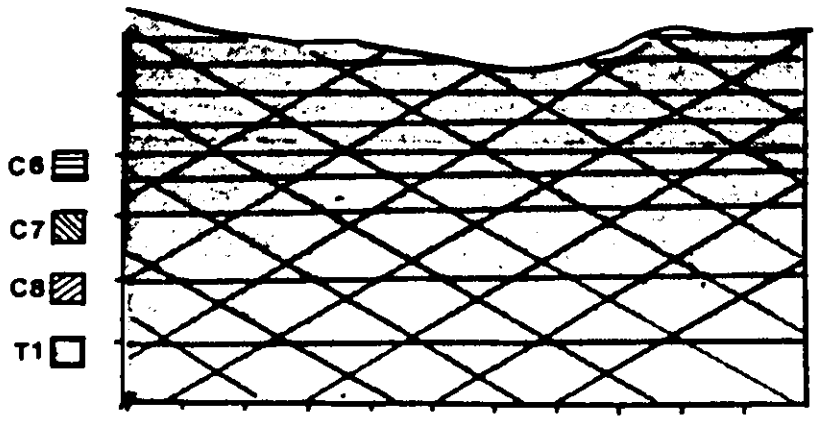
Figure 15 Observed pattern of the ventral root fields in the CTM. Note that in contrast to the other ventral roots, which supplied fibres all over the muscle, the C6 field shown was found in one of two animals, the other animal having the same C6 root distribution in the muscle as for the other ventral roots. See text (Section VI, C) for further explanation. The T1 field is shown by stippling, the C8 and C7 fields by diagnol lines, and the C6 field by horizontal lines (the closer together the lines, the more dense the innervation by C6).

14



Sensory (skin) fields of the DCNs

15



Observed pattern of ventral root innervation

The intervertebral arteries in the cervical spinal cord run quite dorsally in the transverse spinous processes of the vertebrae and thus complicate the surgery necessary to expose the ventral roots of C6 to T1. In addition, continued minor bleeding and exudation of cerebrospinal fluid following exposure of the spinal cord for electrical stimulation of the ventral roots may have facilitated stimulus spread from the suction electrode. However, the preliminary findings suggest that the ventral roots do not obviously divide up the muscle into discrete territories, but rather it appears that each ventral root supplies muscle fibres all over the CTM. In one animal the C6 ventral root appearded to innervate more densely a muscle territory confined to a longitudinal strip of ventral musculature as well as innervating muscle fibres located all over the CTM (see Figure 15). For the reasons enumerated above, however, (especially the possibility of stimulus spread from the suction electrode) the experiments reported in this section of the thesis are not very satisfying; technical improvements and a larger number of animals are required before a more definitive statement can be made concerning a possible pattern of ventral root innervation in the CTM. However, the results do suggest that a clear pattern of the sort indicated in Figure 14 is unlikely.

D) IS THERE A PATTERN OF MOTOR NERVE INNERVATION?

Since the motor innervation of the CTM arises from one discrete nerve plexus, it was posible to examine whether the major motor

nerves had definable territories in the muscle, as has been previously demonstrated for the cat lateral gastrocnemius and plantaris (English and Letbetter, 1982). The CTM motor nerves were dissected out in a large number of rats (250); they were found to exit the brachial plexus and course along the anterior surface of the muscle in several discrete bundles before penetrating the epimysium to innervate the CTM. Usually seven major bundles of motor nerves could be identified which fasciculated into a relatively constant recognizable pattern (see Figure 16 for a diagrammatic representation). These motor nerves therefore are easily accessible for 5-6 mm before they penetrate the CTM, and consequently experimental manipulation of individual nerve bundles was found to be possible without extensive damage to the nerves, the underlying CTM, or to the surrounding axillary musculature.

1) EMG Analysis of Motor Nerve Fields

A reflected muscle preparation (see Figure 4) was used to examine the pattern of motor nerve innervation of the CTM. In 28 animals one or more of the major motor nerves was electrically stimulated and the resulting area of EMG activity mapped. Each motor nerve was found to drive a longitudinal (rostro-caudal) band of muscle. This observation was constant for all animals and all motor nerves examined. Three main groups of motor nerves have been designated as dorsal, lateral, and ventral motor nerves according to the area of the CTM they were found to innervate. The general

Figure 16 Location of the three major muscle fields of the three major motor nerves, obtained from EMG and histochemical studies.

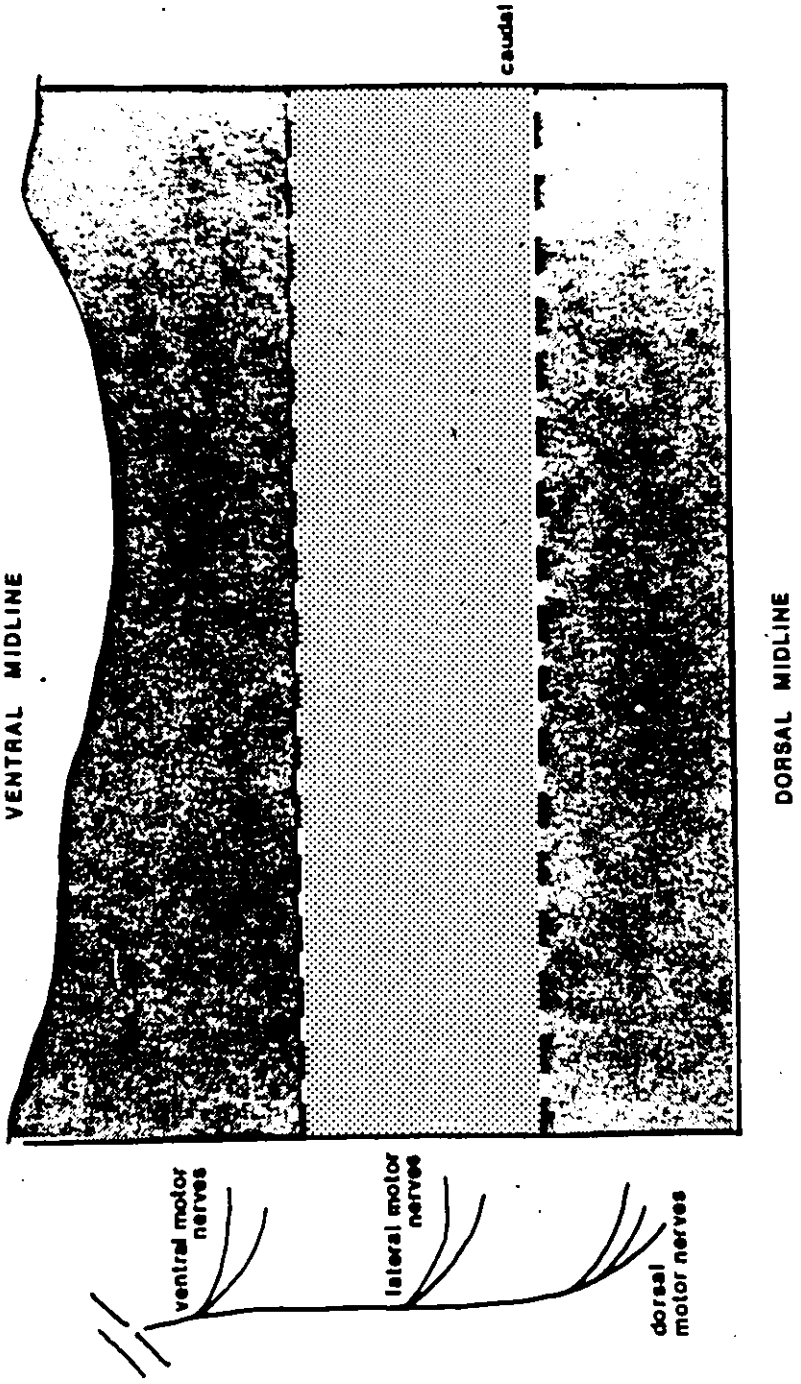
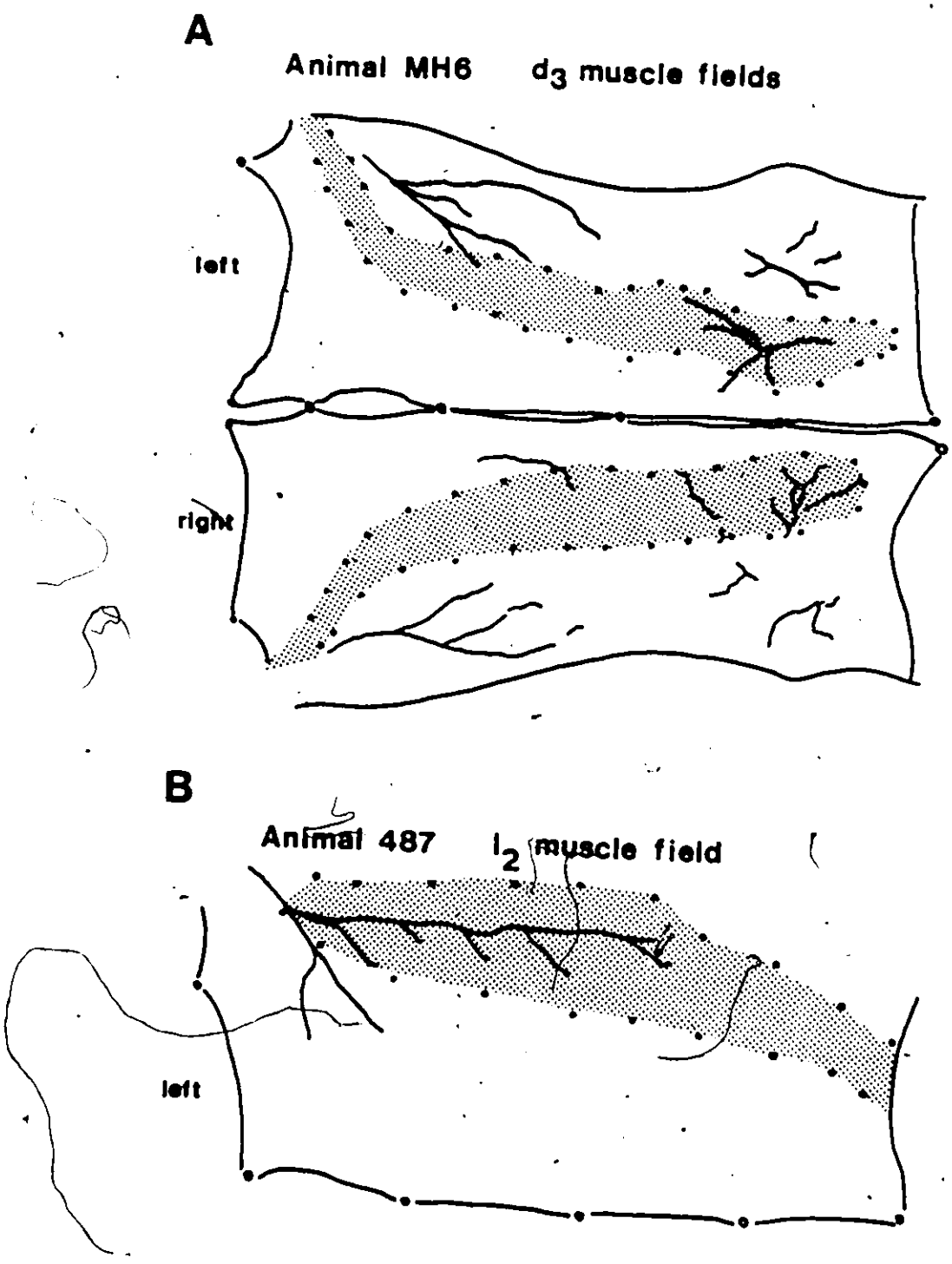

caudal
VENTRAL MIDLINE
DORSAL MIDLINE
ventral motor nerves
lateral motor nerves
dorsal motor nerves

Figure 17 Individual motor nerve fields.
17A Typical example of the left and right third dorsal motor nerve (d_3) fields in one animal.
17B Typical example of the left second lateral motor nerve (l_2) field.


A
Animal MH6 d_3 muscle fields
left
right
B
Animal 487 l_2 muscle field
left

locations of the muscle fields for each of the three major motor nerve groups are diagrammed in Figure 16 .

In all cases examined the borders of the muscle fields were found to be relatively smooth, with no jagged or irregular edges (see Figure 17). Two of the seven major motor nerves were selected for a detailed examination of the representative characteristics of their muscle fields. The full findings are provided in Appendix II and III, summarized in Tables I, II and diagrammed in Figure 17. The muscle field of the third dorsal motor nerve (d_3) was examined in greater detail than that of the second lateral motor nerve (l_2); however, as will be seen below, the results are essentially similar. The area and the location of the muscle field were remarkably constant from animal to animal and from side to side. This is shown in Table I and Figure 17. The area of the left d_3 muscle field was measured to be 11.7 $\pm$ 0.5 cm^2 (SEM, n=14), and the right area totalled 11.1 $\pm$ 0.4 cm^2 (SEM, n=14). The width of each field (dorso-ventral distance) at 6 cm from the occipital protuberance was 1.26 $\pm$ 0.5 cm (SEM, n=14) on the left, and 1.30 $\pm$ 0.6 cm (SEM, n=14) on the right side of the animal. The locations of the d_3 muscle fields were predictable with respect to each other: at 6 cm from the occipital protuberance the dorsal borders of the left and right fields were separated by a distance of 3.2 $\pm$ 0.2 cm (SEM, n=7), while at 12 cm they were separated by a distance 1.1 $\pm$ 0.1 cm (SEM, n=7). Results from a less detailed examination of the left l_2 muscle field

TABLE I DORSAL MOTOR NERVE (d_3) FIELD AREAS

Animal #	Left d_3 Area (cm^2)	Right d_3 Area (cm^2)
265	11.3	9.3
266	9.3	9.8
MH2	11.7	11.0
282	10.7	11.3
283	13.3	8.7
289	9.4	10.7
294	10.2	11.6
MH1	14.2	12.0
288	12.2	10.8
MH8	15.0	10.5
MH4	12.2	15.1
MH5	10.3	10.6
MH6	10.8	11.3
MH7	13.5	13.4

(n=14)

Left d_3 area = 11.7 $\pm$ 0.5 cm^2 (SEM)

Right d_3 area = 11.1 $\pm$ 0.4 cm^2 (SEM)

TABLE II LATERAL MOTOR NERVE (l_2) FIELD AREAS

Animal #	Left l_2 Area (cm^2)
196	19.8
487	17.6
488	15.8
499	24.8
500	22.6
501	24.3
504	16.3

(n=7)

Left l_2 area = 20.2 ± 1.4 cm^2 (SEM)

TABLE III OVERLAP BETWEEN ADJACENT MOTOR NERVE FIELDS

Animal #	First Field	Overlapped By	% Area Overlap
196	d_2	d_3	58%
"	d_3	d_2	59
"	d_3	l_1	53
"	l_1	d_3	42
195	d_1	d_2	43
"	d_2	d_1	56
484	d_2	d_3	50
"	d_3	d_2	49
504	l_1	l_2	42
"	l_2	l_1	54

(n=10)

Average overlap = 50.6 $\pm$ 2.1% (SEM)

in seven animals revealed an essentially similar pattern to that of the d_3 muscle field. As shown in Table II, the l_2 field measured 20.2 $\pm$ 6.4 cm^2 (SEM, n=7) in total area, and extended for 2.5 $\pm$ 0.3 cm (SEM, n=7) in width at 6 cm from the occipital protuberance.

In a separate series of experiments the amount of overlap between the muscle fields of adjacent motor nerves was examined. As shown in Table III there was a considerable degree of such overlap as assessed electrophysiologically (50.6 $\pm$ 2.1% (SEM) overlap of the total area of each individual field; range 42-59%, n=10). However results from EMG maps of the most dorsal of the dorsal motor nerve (d_1) suggest that this muscle field (in 3/5 cases examined) was not entirely shared with the other (ipsilateral) dorsal motor nerves. In this respect d_1 appears to have a muscle field that is territorially exclusive. The histochemical results below confirm this interpretation.

2. Histochemical Analysis of Motor Nerve Fields

In six animals one of the major motor nerves was electrically stimulated as described above (Methods Section V, 5) so as to cause tetanic contraction of its muscle field, and then pieces of muscle were processed for PAS histochemistry (see Figure 12). The results from these experiments are shown in Table IV and a representative photograph of a glycogen-depleted field (a cross-section of the CTM) is presented in Figure 13B. In all of the cases examined except for the d_1 muscle field the histochemical borders were not sharply

defined so that glycogen-depleted and glycogen-filled muscle fibres coexisted throughout the extent of the field. These results are in agreement with the EMG studies described above where the average overlap between adjacent muscle fields was approximately 50%. In the case of the d_1 muscle field, the dorsal-most border (i.e. at the midline) was very clearly defined (refer to Figure 18). These results, in conjunction with the EMG studies described above indicate that the d_1 muscle field is unique in having its own muscle territory.

3) Correlation of Motor Nerve Field Borders By the Two Techniques

In three animals one of the major motor nerves was electrically stimulated and its muscle field mapped as described above (Section V, 6i). The same nerve was then stimulated so as to cause tetanic contraction of the area of the CTM it supplied and the tissue processed for PAS histochemistry (see Section V, 6iii for the methodology and Figure 12 for the protocol). The results from these studies were essentially similar for all three animals. An example of the dorsal motor nerve field shows a very good correlation between the borders as determined electrophysiologically (Figure 18A) and histochemically (Figure 18B).

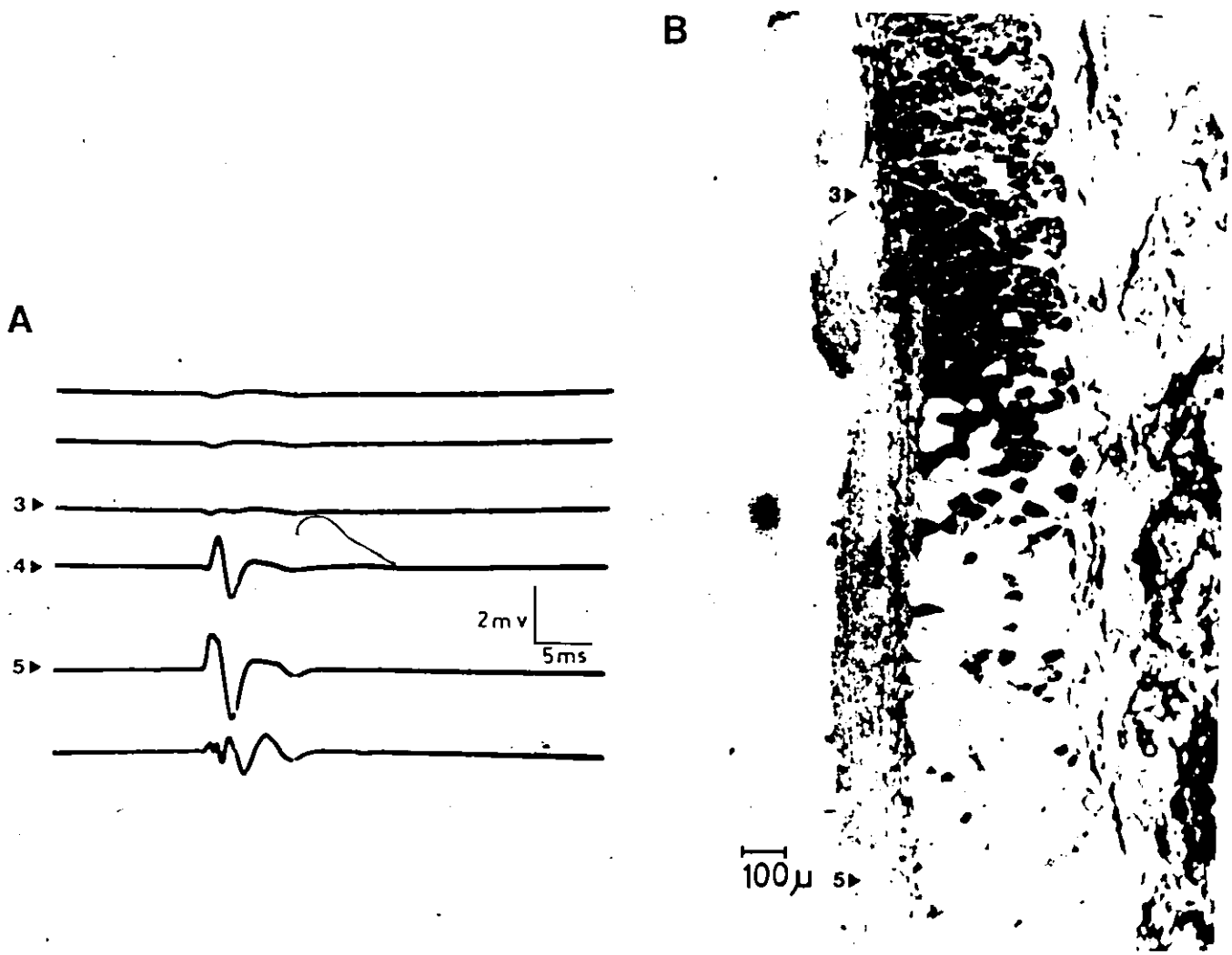
4) Preliminary Studies of Motor Unit Size

As described earlier, the reflex activation of the CTM is organized into functional compartments, and from visual observations these appear to be less than 2 cm^2. Theoretically, if this is a

Figure 18 Correlation of the EMG and PAS muscle field borders of the d_1 motor nerve.

A shows the EMG activity recorded at six locations (each 2 mm apart) on the surface of the CIM.

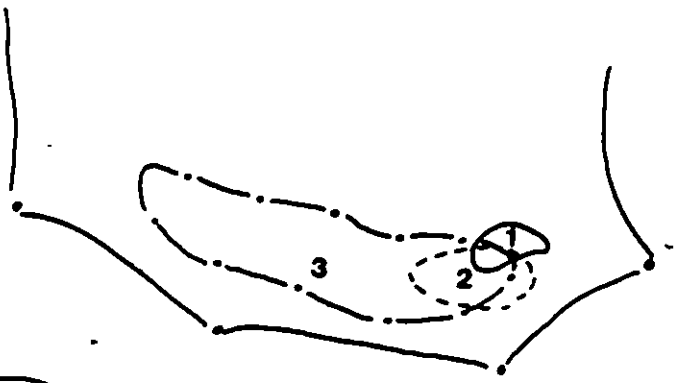
B shows PAS stain of the tetanically-stimulated d_1 field. Numbered arrowheads show the locations of the EMG electrodes for the correspondingly numbered records in A. Pale fibres are glycogen-depleted.


A
3
4
2mv
5ms
5
B
3
100μ
5

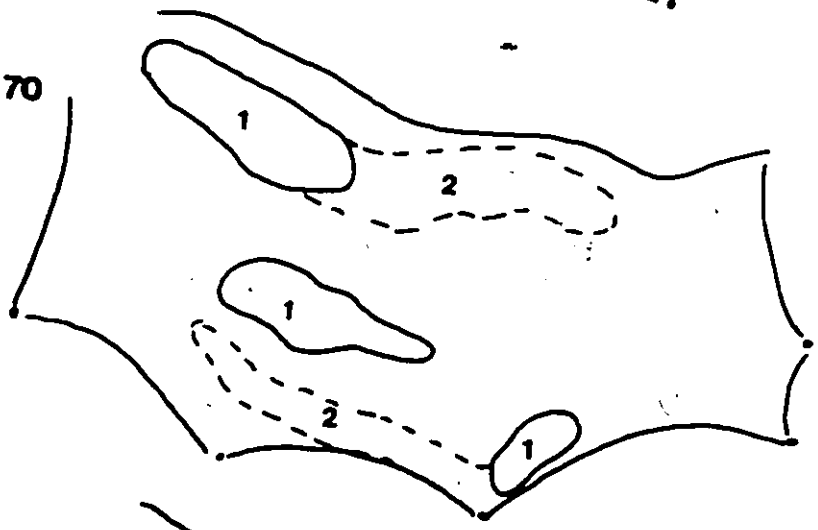
fast-twitch, focally-innervated muscle, the smallest motor unit size should not exceed the smallest behaviorally-defined compartment. Therefore it was of interest to determine the approximate size of a CTM motor unit; a few preliminary examinations were made of this point. In three animals finely graded electrical stimuli were applied to a selected motor nerve and the area of EMG activity in the muscle was mapped. By using finely-graded electrical stimuli on the nerve it was hoped that only one or a few motor axons would be activated and thus the resulting area of activity in the muscle would give an indication of the motor unit size. The results from these three animals are shown in Figures 19, 20, Table V, and are detailed in Appendix V. With the smallest effective stimulus applied to the nerve the area of EMG activity measured approximately 1.2 x 0.8 cm. In two out of three cases the electrical stimulus to the nerve evoked two or more separate foci of EMG activity, suggesting that more than one motor axon was being activated. When the stimulus intensity was incremented the size of the EMG fields also increased and new foci of EMG activity could be recorded along the longtitudinal (rostrocaudal) axis of the motor nerve field. The areas of the evoked EMG activity are plotted along a single ordinate in Figure 20 and an examination of the distribution of these areas suggests a step-wise increment in size. The smallest unit area measures approximately 1 cm^2 while the remaining areas appear on the plot as multiples of this value, i.e. 3 cm^2, 4 cm^2, 9 cm^2, and so on. One interpretation of these data is

Figure 19 Motor unit sizes determined electromyographically in three animals. Finely graded electrical stimuli were applied to selected (different) motor nerves; the resulting areas of EMG activity are shown. Numbers refer to the order in which each successive increment in response appeared.

Animal 63



Animal 70



Animal 71

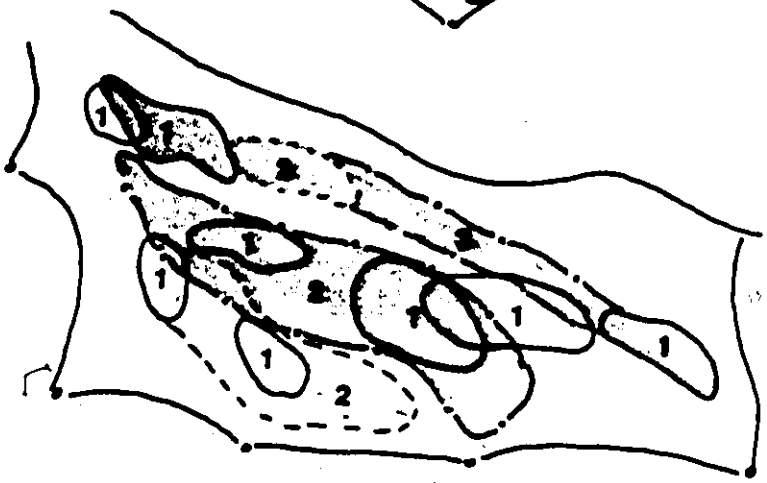


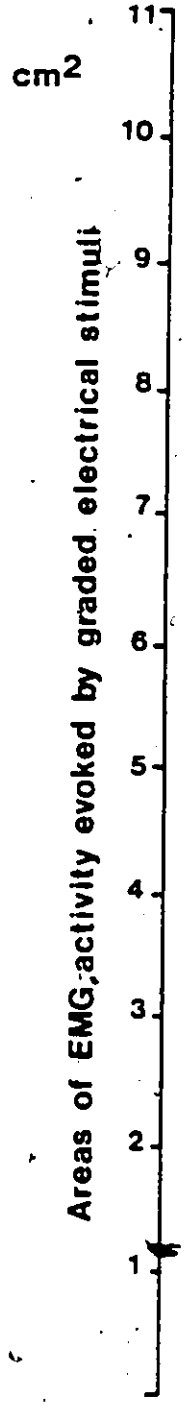
TABLE IV MOTOR UNIT AREAS

Animal #	Motor Nerve Stimulated	Area of Evoked EMG Activity (cm^2) Following:		
		Stimulus 1	Stimulus 2	Stimulus 3
63	dorsal	0.5	1.5	7.0
70	lateral	4.4	-	9.1
"	dorsal	2.6	-	
"	dorsal	0.9	3.8	
71	lateral	0.4	-	-
"	"	2.3		
71	lateral	1.0	1.4	6.9
"	"	1.1		
71	dorsal	0.9	-	-
"	"	2.5	10.4	
71	dorsal	0.8		
"	"	0.9	6.7	

(n=14)

Note that in these experiments, no distinction was made between subdivisions of the major motor nerves.

Figure 20 Areas of EMG activity evoked by progressively increasing the intensity of stimulation (same data as 19) plotted along a single ordinate.


Areas of EMG activity evoked by graded electrical stimuli
cm2
11
10
9
8
7
6
5
4
3
2
1

that the smallest area of EMG activity evoked by an electrical stimulus to the motor nerve represents the area of one motor unit, and that with increments in stimulus intensity more motor axons are activated thus increasing the area of EMG activity in a step-wise manner, corresponding to the total number of motor units activated in the motor nerve field.

5) Summary Statement of Findings

It would not be unreasonable to expect a territorial pattern of ventral root innervation in the CTM as there are other examples in the literature (Markee and Lowenbach, 1945; Swett, Eldred and Buchwald, 1970). The physiological studies of Swett et al. have shown a proximo-distal pattern of innervation in the cat medial gastrocnemius according to the rostro-caudal location of the contributing ventral roots. Another study reported by Brown and Booth (1983) suggests there may be a pattern of ventral root innervation in the rat gluteus maximus muscle. However, as explained earlier, the present results do not suggest that the CTM is divided up into clearly definable regions each supplied by a single ventral root. The study of the major motor nerve fields was more rewarding; these fields run rostrocaudally, extending for the entire length of the muscle, i.e. at right angles to the main axis of the sensory dermatomes of the DCNs. Nevertheless, the results described in this section demonstrate that although there is a functional division of the CTM according to the peripheral motor nerve territories in the

muscle, this pattern of motor innervation does not parallel the behavioral use of the muscle. We have shown that the CTM is organized into functional compartments of 2 cm^2 or less on the basis of its reflex activation (see above, Section V, B, and Nixon et al., 1984). Preliminary studies of motor unit size and muscle fibre length indicate that the rostrocaudal extent of a motor unit correlates well with the rostrocaudal extent of a behavioral compartment. However, in the dorso-ventral axis, the smallest motor unit is considerably wider than the behavioral compartment. This suggests that more than one motor unit constitutes a behaviorally-defined reflex compartment.

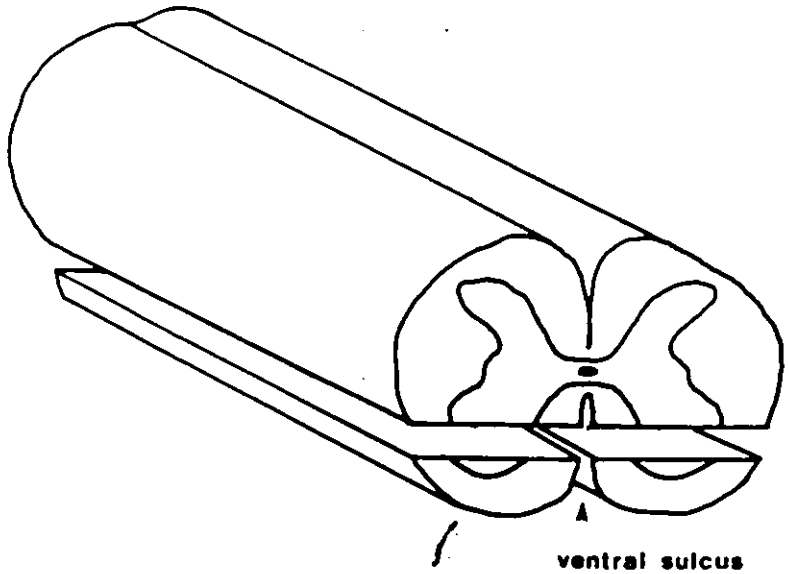
From a consideration of these results, the question then arises: could the CTM motoneuron pool itself be anatomically organized in a pattern appropriate either to the sensory or to the motor pattern of activation of the muscle?

E) THE CTM MOTONEURON POOL

1) Location and Distribution of the Cells

The retrograde axonal transport of HRP by motoneurons was initially described by Kristensson and Olsson (1971), and this technique has in recent years become a standard tool for neurobiologists studying the organization of the nervous system. In the present study, the central ends of cut CTM motor nerves were soaked in a solution of 25-50% HRP and the spinal cord later analyzed (as described in Methods, Section V, 7) for the presence of retrogradely-labeled cells. The results are detailed in Appendix IV

Figure 21 A 40 μm horizontal section of the spinal cord on the left side showing a portion of the entire CIM motoneuron pool retrogradely labeled with horseradish peroxidase (HRP). Inset indicates the plane of section.


ventral sulcus

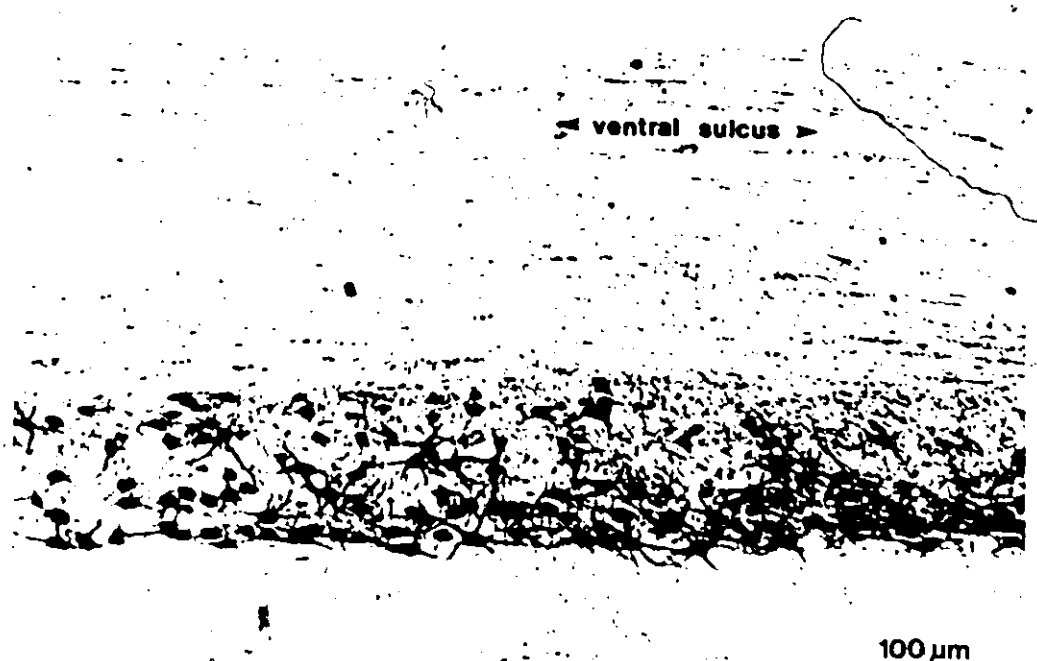
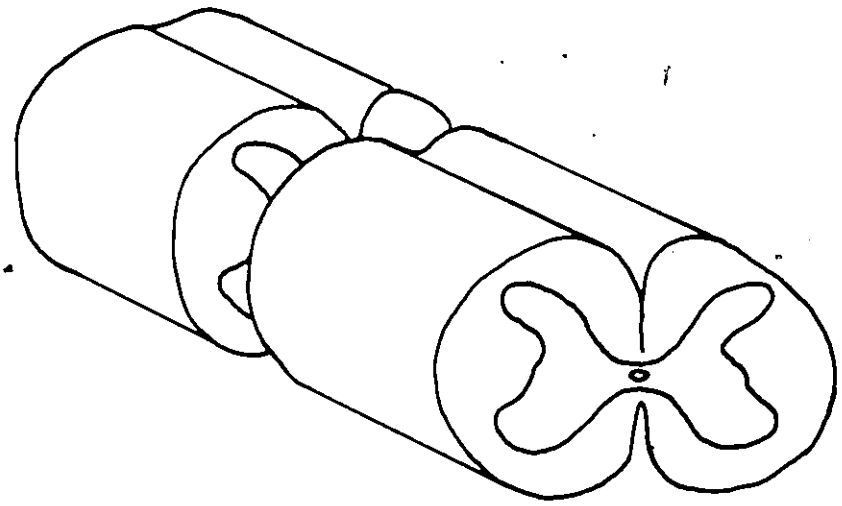
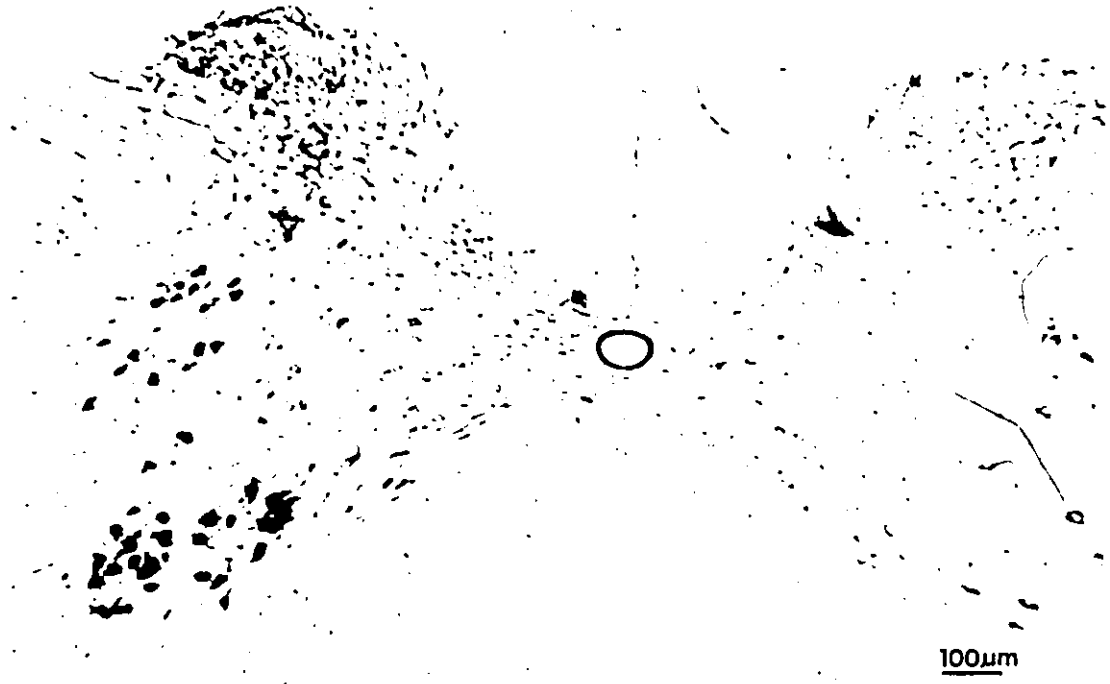

ventral sulcus
100 µm

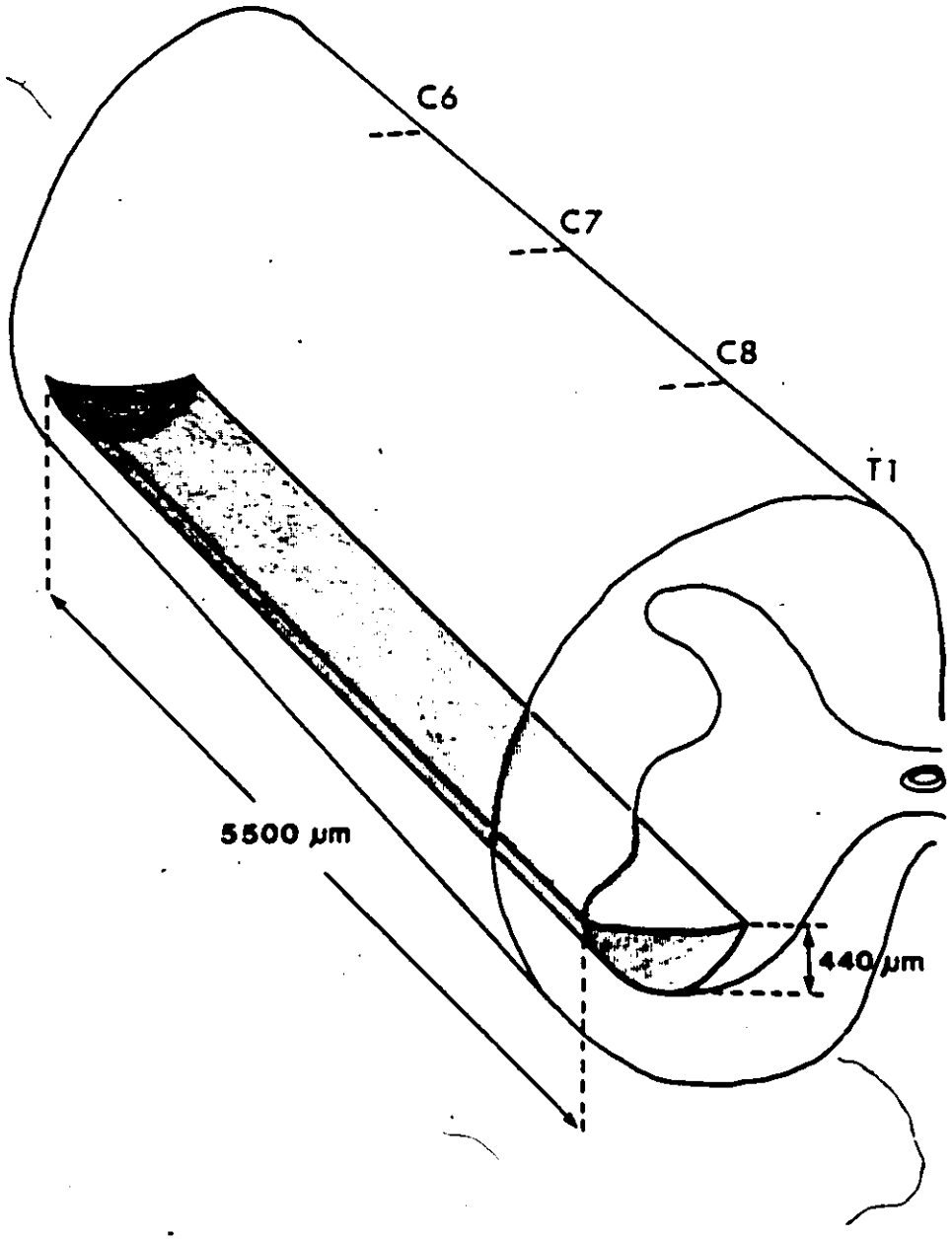
Figure 22 A 40 μm transverse section of the cervical spinal cord showing a portion of the entire CIM motoneuron pool, on the left side, retrogradely labeled with HRP. Inset shows plane of section.




100µm

and are presented in Figures 21, 22, 23 and 24. In all eighteen animals examined, the location and distribution of HRP-filled cells were similar: the cells extended in a column from the caudal end of C6 to the rostral portion of T1. In approximately one quarter of the cases (4/18) no labeled cells were found at the level of C6. This observation may reflect a real distribution of the CTM motoneurons or it may result from experimental error in marking the ventral root entry zones when preparing the spinal cord for histochemistry (the razor blade cuts used to mark the ventral root entry zones were not always perfectly straight and so some error in determining the beginning and end of a spinal cord segment may be expected.) All the labeled cells appeared to be motoneurons, judging by their location (all in the ventral horn in Lamina IX of Rexed (1964)),and by their distinctive morphology. Most of the cells were in the size range of alpha motoneurons (see below for a more detailed description) and the smaller HRP-labelled cells appeared similar to those described by Strick et al. (1976) as gamma, or fusimotor, motoneurons. Evidence that a transneuronal transfer of HRP may occur has been extremely limited in the literature despite the extensive use of this enzyme for mapping neuronal projections. In a recent report by Hongo et al.(1981) transneuronal passage of HRP in an <u>anterograde</u> direction from afferent fibres to spinal interneurons was observed following electrical stimulation of the afferents. In the experiments reported here no evidence for the transneuronal passage of HRP could be found.

Figure 23 Diagrammatic representation of the location and distribution of the entire (left) CTM motoneuron pool (not accurately to scale).


C6
C7
C8
T1
5500 µm
440 µm

When viewed under moderately high power (e.g. 40x objective) in the light microscope a granular reaction product was always present (see Figure 22), indicating the vesicular uptake and transport of the enzyme by axotomized motoneurons (see Kristensson and Olsson, 1979). The labeled CIM motoneurons were found at the most ventrolateral edge of the ventral horn, and in transverse section were clustered in a crescentic group of cells (see Figure 22B). The CIM motoneuron pool extended for approximately 5200 μm in the rostrocaudal direction (refer to Figure 21 for a horizontal section through the CIM motoneuron pool). In the dorso-ventral axis (i.e. the depth of the pool) the labeled cells occupied approximately 440 μm of the ventral gray matter. The location and dimensions of the pool are diagrammed in Figure 23. The majority of the motoneuron population was located in C8 (64%), while C7 contained 20%, T1 contained 15%, and C6 contained only 1% of the population. These results are tabulated in Figure 24 and Table V.

2) Number and Size of Motoneuron Somata

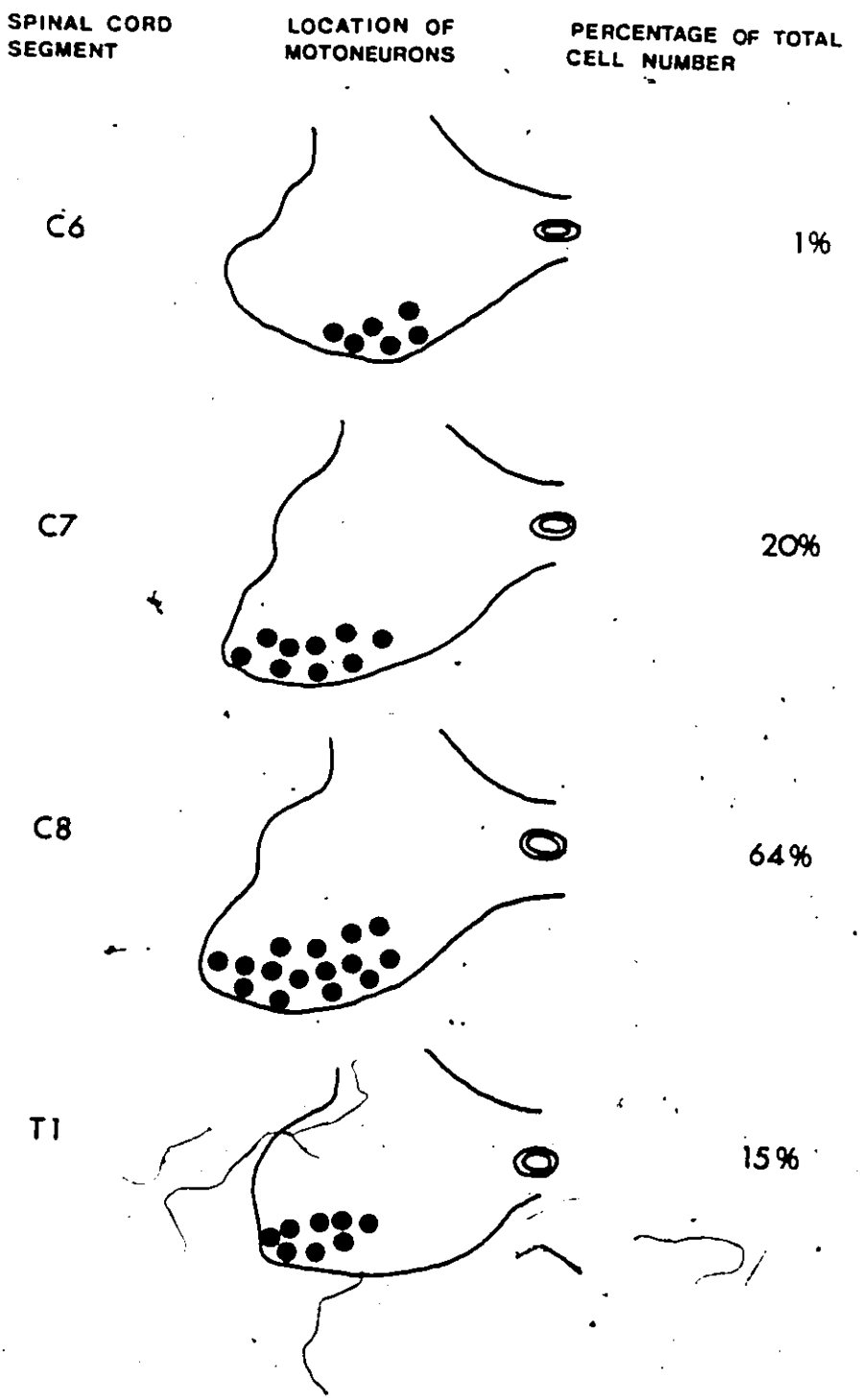
The entire number of retrogradely labeled motoneuron somata in the CIM pool of eighteen animals was counted. So that each cell would be counted only once, the presence of a nucleolus in a labeled profile was required before it was counted as a cell. The nucleolus was readily apparent as a clear central area within all the HRP-labeled motoneurons except for the smallest cells which were few in number and were characteristically more densely filled with

TABLE V SEGMENTAL DISTRIBUTION OF CTM MOTONEURONS

Animal #	Total # Motoneurons	Cells in C6 # (% of Total)	Cells in C7 # (% of Total)	Cells in C8 # (% of Tptal)	Cells in T1 # (% of Total)
447	1094	11 (1)	142 (13)	766 (70)	175 (16)
451	1234	12 (1)	333 (27)	703 (57)	186 (15)
	-	$\bar{x}$ = 1%	$\bar{x}$ = 20%	$\bar{x}$ = 64%	$\bar{x}$ = 15%

(n=2)

Figure 24 Segmental distribution of the CIM motoneuron pool. (No motoneurons were ever observed in C5 or T2).


SPINAL CORD SEGMENT
LOCATION OF MOTONEURONS
PERCENTAGE OF TOTAL CELL NUMBER
C6
1%
C7
20%
C8
64%
T1
15%

reaction product than the larger motoneurons. This observation is in agreement with that of Strick et al. (1976) who described several morphological characteristics of cat medial gastrocnemius motoneurons retrogradely labeled with HRP. Interestingly, they commented that the most heavily stained cells were almost without exception the gamma, or spindle, motoneurons. In the present experiments, counts of HRP-stained somata revealed a very large population of CTM motoneurons: 1183 $\pm$ 33 (SEM) motoneurons per side. Although most experiments were performed on the left side (16/18), a comparison of cell counts on the right showed similar numbers (see Appendix III).

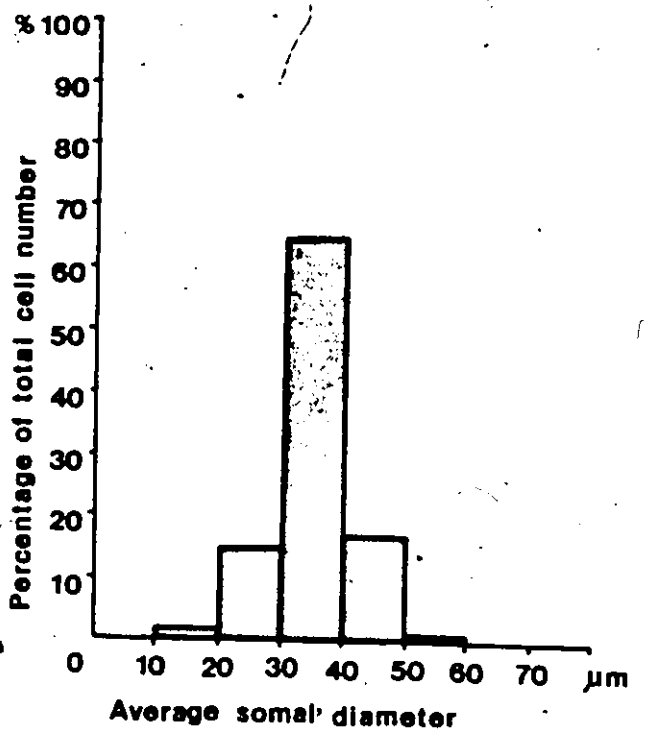
In one animal a photographic serial reconstruction of the entire motoneuron pool was prepared and the average somal diameter was calculated. Each 40 µm horizontal section of the spinal cord which contained HRP-labeled motoneurons was photographed in parts beginning at the caudal end of the section. Usually each spinal cord section could be contained in 4-5 serial photographs. The photographs were then cut and trimmed, and each individual spinal cord section was thus reconstructed photographically. The number of composite photos reconstructed for this animal was eleven, corresponding to the number of spinal cord sections containing HRP-labeled cells. The maximum and minumum diameters of each of the 1070 motoneurons were measured and then averaged to give an estimate of the average somal diameter for each cell. No correction was made for shrinkage. The results are presented in Figure 25 and Table VI. As can be seen in

Figure 25 Distribution of cell size in the entire (left) CTM motoneuron pool of one animal. The same data are presented in the Table VI and in the histogram.

TABLE VI AVERAGE DIAMETER OF CTM MOTONEURONS IN ANIMAL #360

Average Somal Diameter (μm)	#. of Cells	% of Total
1-9	0	0
10-19	23	2
20-29	158	15
30-39	699	65
40-49	178	17
50-59	12	1
60-69	0	0

(n=1070)
Average somal diameter = approx. 40 μm



this histogram the average somal diameter of the CTM motoneurons is quite small, most of the cells (65%) averaging 30-40 μm in diameter. These results agree with a report by Baulac and Meininger (1981) who compared the cellular volumes of 250 CTM motoneurons with 262 pectoralis major motoneurons and found the CTM cells to have a unimodal distribution of "small to medium sized" cells (the CTM motoneurons measured 12,000 - 24,000 μm^3, while the pectoralis motoneurons measured 24,000 - 36,000 μm^3).

Axon counts of the CTM motor nerves of two animals agreed with the HRP studies. The left motor nerves were excised, fixed and prepared for electronmicroscopy (see Methods Section V, 8). Semi-thin sections of thesenerves were photographed and the numbers of myelinated axons were counted. The diameter of a random sample of 150 axons was measured; the results are presented in Table VII and a representative photograph is shown in Figure 26. The CTM motor nerves contain approximately 1142 myelinated axons (total counts from two animals were 1160 and 1124). These counts agree well with the number of motoneuron somata determined by HRP studies. Most of the axons appear to be of similar diameter, approximately 12 μm (11.9 $\pm$ 0.2 μm (SEM)).

3) Columnar Organization of Axons and Dendrites

In horizontal sections taken through the ventral white matter in the cervical spinal cord, it was noticed that short rows of HRP-labeled axons were present (see Figure 27). These orderly rows of

Figure 26 CIM motor axons.

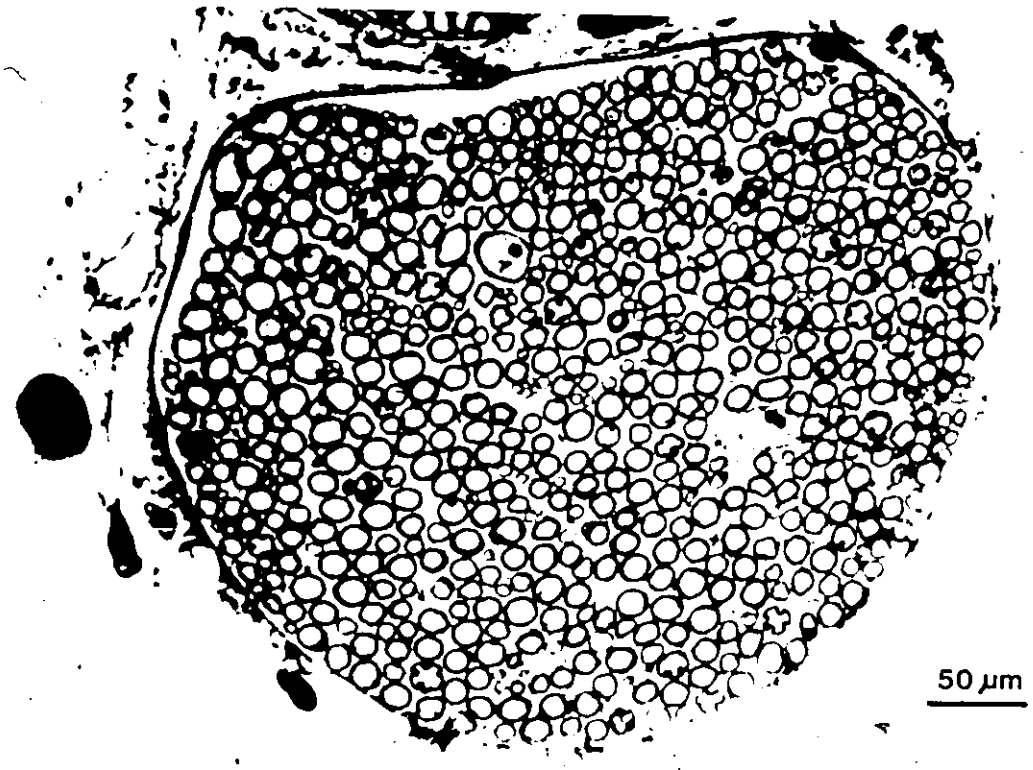
26A Table VII shows the distribution of the diameter of 150 randomly selected myelinated axons in a CIM motor nerve.

26B Photomicrograph of a single CIM motor nerve bundle (l_2, see Figure 16). Transverse 0.5 μm plastic section of OsO_4 stained nerve.

TABLE VII (ON MYELINATED AXON DIAMETERS IN ACPAL =4 d.

Myelinated Axon Diameter (μm)	= % Axons	% Total
6-7	4	3
8-9	11	7
10-11	31	20
12-13	64	43
14-15	28	19
16-17	12	8
·17	0	0

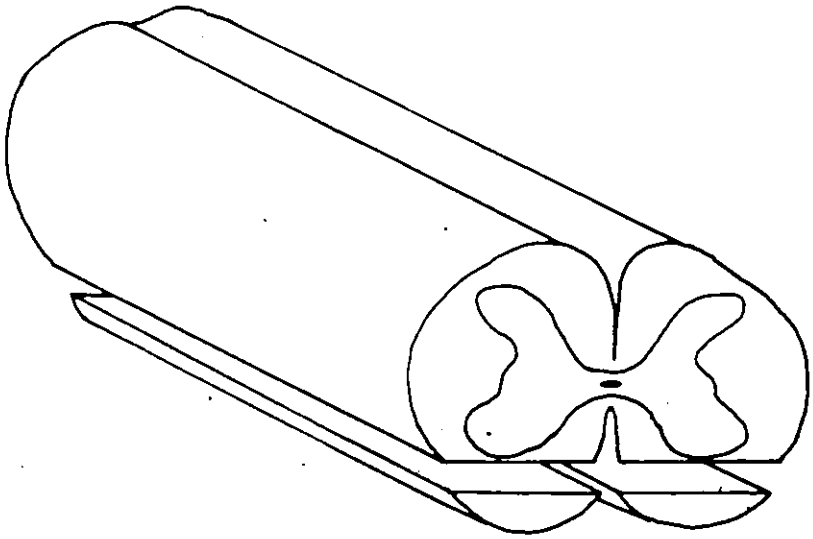
(n=150)
Average axonal diameter = 11.9 ± 0.2 m (SEM)



motor axons were detectable within 40 μm of the HRP-stained motoneuron somata. In transverse section (Figure 28) the axons are seen to emerge from the ventral gray matter and course directly through the white matter to the ventral roots of exit without undulating or changing direction. The appearance of short rows of labeled axons seen in horizontal section is therefore not due to a random or haphazard pathway of exit by the CIM motoneurons, but rather appears to reflect a very early (with respect to the location of the parent cell bodies) ordering of the projection pattern of the CIM motoneurons. These rows of axons were observed regularly in experiments (n>30) where the motor nerves were cut and soaked in HRP solution. A relatively short survival time (18-24 hours) and a concentrated HRP solution (40-50%) seemed to favour the staining of these motor axons. Whether the rows of axons retain their neighbour relationships in the peripheral nerves has not yet been investigated. The findings described above indicate that, in addition to the columnar organization of the CIM motoneuron pool, the axons of these cell bodies are also organized in a columnar fashion at the level of the cell bodies or at least very shortly after they enter the ventral white matter.

Another regular observation made on horizontal sections of the CIM motoneuron pool concerned the orientation of the major dendritic branches of the CIM motoneurons. The larger dendrites were seen to run in a longtitudinal (rostro-caudal) direction, in effect

Figure 27 Horizontal section through the ventral white matter showing rows of HRP-labeled motor axons. Inset shows plane of section. Shaded area of white matter in the drawing represents the region shown in the photograph.



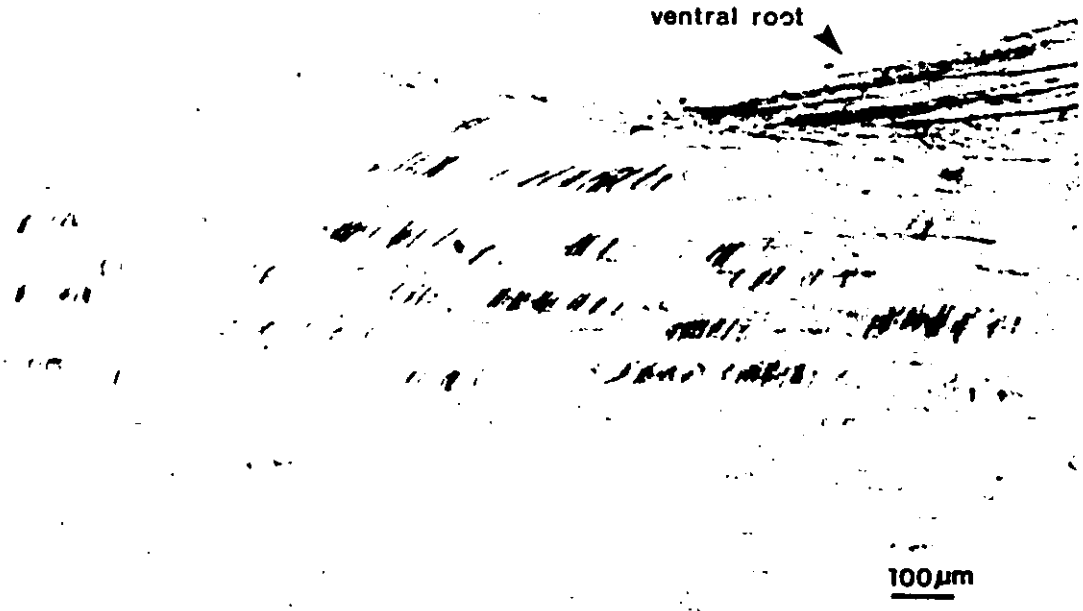
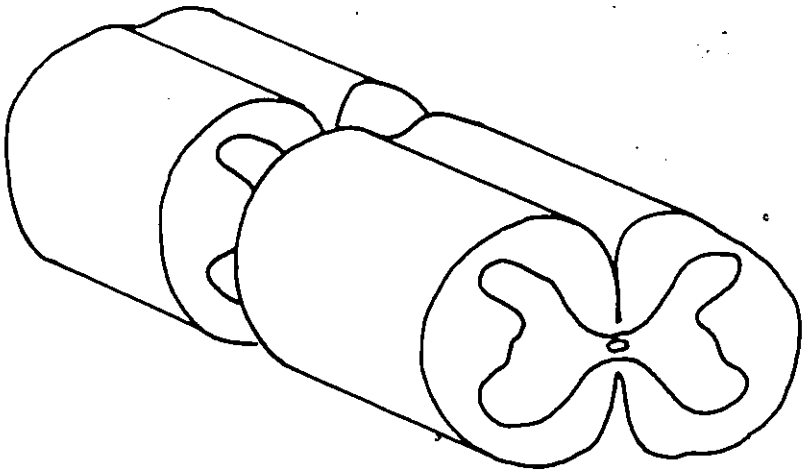

ventral root
100μm

Figure 28 Transverse 40 μm section of the cervical spinal cord showing HRP-labeled motoneuron somata and their axons (a few bundles are indicated by arrowheads). Note the course of the motor axons through the ventral white matter; compare with Figure 27. Inset shows plane of section.



50 µm

parallel to the columnar organization of the pool itself (see Figure 21). These results agree with a report published by Scheibel and Scheibel (1970) who described a longtitudinal pattern of dendritic organization in the lateral motor column of rat cervical spinal cord as seen in Golgi-Cox preparations.

4) Summary Statement of Findings

The CIM motoneuron pool is distributed throughout the ventrolateral gray matter between caudal C6 and rostralT1. The cells are relatively small (30-40 μm in diameter) and numerous (1183 ± 33 (SEM)). The axons of the CIM motoneurons are organized in short rows or columns as they exit the ventral white matter; additionally, the major dendritic branches of these motoneurons are also arranged in a columnar pattern, extending largely within the confines of the CIM motoneuron pool.

F) HRP INJECTIONS INTO THE MUSCLE: LABELING AND DIFFUSION STUDIES

1) Multiple Injections

In order to investigate the possibility that the CIM motoneuron pool may have an intrinsic anatomical organization corresponding to the behavioral use of the muscle, the following series of experiments were conducted. A dorsal midline incision was made and several μl of 25-50 % HRP solution was injected along a into the muscle, i.e. into the area of the muscle that would be reflexly activated by sensory input from the overlying skin. In the early experiments where 50 μl of HRP was injected (n=6) the labeled cells

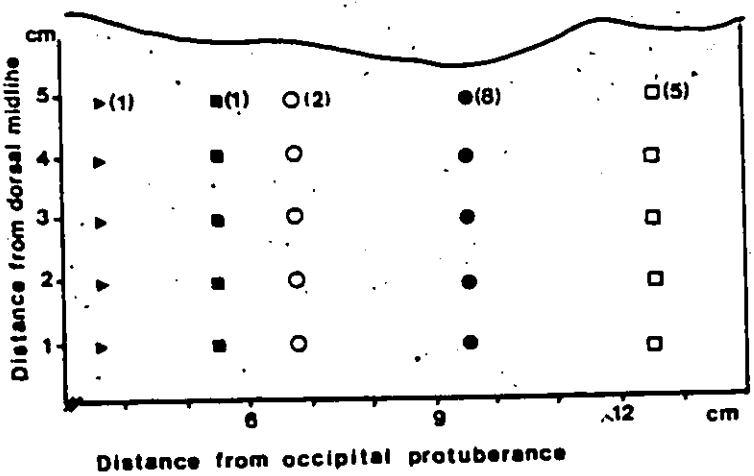
were found distributed throughout the length and depth of the CTM motoneuron pool (refer to Appendix V for the tabulated results). This distribution of cells was found regardless of where the HRP injection was placed, and no distinctive organization of motoneurons could be associated with the "dermatomal" location of the muscle field. In an effort to restrict the area of muscle exposed to the HRP solution, the remainder of the injections were restricted to a total of 20 µl (n=3) or 10 µl or less (n=7). However, motoneurons that were labeled in these experiments were still distributed apparently randomly throughout the extent of the CTM motoneuron pool. HRP-stained cells were found from C6 to T1 and were positioned in both medio-lateral and dorso-ventral locations within the confines of the CTM motoneuron pool. These results then failed to reveal any spatial organization of the CTM motoneuron pool that could be related to the "dermatomal" pattern of reflex activation of the muscle.

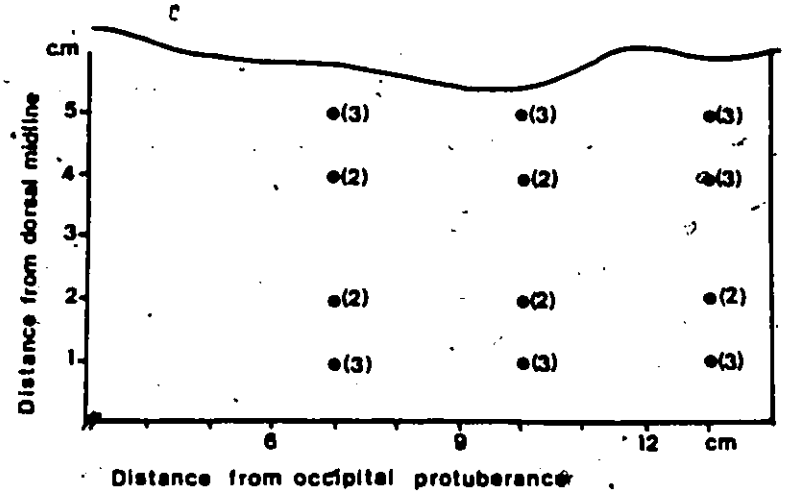
2) Single Injections

The objective of these experiments (done on 32 animals) was to label motoneurons that drive a small area of muscle, and ideally a single behaviorally-defined reflex compartment. One single 2 µl injection of 40 % HRP per animal was placed directly into a selected area of the muscle (see Figure 30 for a composite diagram of the injection sites) and after an appropriate survival time the spinal cord was analyzed for the distribution and location of labeled cells. The results are detailed in Appendix VI and are summarized in Figure

Figure 29 Composite diagram showing the location of the sites of multiple HRP injections into the CIM. Like symbols refer to the placement of the five injections that constituted a single experiment; the numbers in parentheses indicate the number of individual experiments done at that location in the CIM.

Figure 30 Composite diagram showing the location of the sites of single HRP injections into the muscle. Each symbol indicates the site of a single injection of HRP that constituted one experiment; the numbers in parentheses refer to the number of individual experiments done at that location in the CIM.


cm
Distance from dorsal midline
5
4
3
2
1
▶(1)
■(1)
○(2)
●(8)
□(5)
6
9
12
cm
Distance from occipital protuberance


cm
Distance from dorsal midline
5
4
3
2
1
●(3)
●(3)
●(3)
●(2)
●(2)
●(3)
●(2)
●(2)
●(2)
●(3)
●(3)
●(3)
6
9
12
cm
Distance from occipital protuberance

30 and Table VII. Injections that were placed within 1-2 cm of the dorsal midline tended to label motoneurons situated medially within the CTM pool (11/16 rats). The labeled cells were not restricted to a dorsal or a ventral location within the pool but were found in both positions. When the HRP injections were placed more ventrally in the muscle (4-5 cm from the dorsal midline), labeled motoneurons were located toward the lateral edge of the CTM pool (16/16 rats) and tended to be more dorsally positioned (10/16 animals). The results from these experiments indicate that dorsal muscle is innervated by medially-located motoneurons and that more ventrally-positioned muscle is driven by motoneurons located laterally within the CTM pool.

3) Diffusion Studies

An anatomical organization of the CTM motoneuron pool corresponding to the pattern of its reflex activation (i.e. dermatomal) was expected to be revealed by the localized injections of HRP into the muscle. As described above, no obvious organization of the CTM motoneuron pool was found. However, if there was considerable diffusion of the enzyme away from the injection site, any genuine dermatomal organization of the motoneurons could have been obscured. Therefore the experiments in this section were done to investigate this possibility. In four rats a single 2 µl injection of 40% HRP was placed at a distance of either 1.0 cm (n=2), 1.5 cm (n=1) or 2.0 cm (n=1) outside the border of a motor nerve field determined

electrophysiologically. All the motor nerves to the CIM were cut and the central ends ligated, except for the one nerve for which the EMG map was made. The results are shown in Table IX and Appendix VII. In only one case HRP-labeled cells were found. In this animal the injection was made 1.0 cm from the dorsal border of the l_1 muscle field. These results suggest that a 2 µl injection of HRP solution diffuses away from the site over a total dorso-ventral distance of approximately 2 cm. The results described in Section VI, F 2 agree well with this diffusion estimate. While the experiments in this section indicate the probable extent of dorso-ventral diffusion they do not provide a good estimate of HRP diffusion in the rostrocaudal axis, and it is this axis which is the more important one from the point of view of defining a "dermatomal" organization of the motoneuron pool.

4) Summary Statement of Findings

The results from experiments where multiple injections of HRP were placed into the muscle area lying beneath a single dermatome do not reveal any spatial organization of the CIM motoneuron pool that could be easily related to the reflex activation of the muscle by its sensory nerves. In contrast, however, single injections into the CIM suggest that a columnar group of medially-placed motoneurons drives dorsally-located muscle, while the ventral muscle receives its innervation from a column of more laterally-placed motoneurons. This point is considered in greater detail in the final Discussion.

TABLE IX HRP DIFFUSION STUDIES

Animal #	Distance from Border of Field	HRP Labeled Cells
528	1.0 cm	12
529	1.0 cm	none
530	1.5 cm	none
531	2.0 cm	none

(n=4)

G) INDIVIDUAL MOTOR NERVES: LOCATION OF SOMATA WITHIN THE CTM POOL

In this series of experiments, done on 48 animals, the possibility that the CTM motoneuron pool is anatomically organized according to the peripheral motor nerves was investigated. In each animal, one of the seven major motor nerve bundles was cut and soaked in 25-50% HRP solution and the spinal cord processed for HRP histochemistry as described in Section V, 7. The results are presented in detail in Appendices VIII, IX and X, and are summarized in Figures 31, 32 and 33.

1) Dorsal Motor Nerves

The location of the cell bodies which contribute motor axons to the dorsal motor nerves was examined in 22 animals. The results are presented in Appendix VIII and Figures 30, 32 and 33. When all three dorsal motor nerves (d_1, d_2, d_3) were cut and soaked in HRP solution (two animals), about 600 motoneurons were found distributed throughout the length of the parent CTM pool, i.e. from C6 to T1. These motoneurons were not randomly distributed, but showed up as a column of cells located somewhat dorsally and medially within the CTM pool (see Figure 32A). In the remainder of the animals (n=20) the location of motoneuron somata for each individual motor nerve was determined. While d_1 and d_2 motoneurons did not appear to be restricted to any particular segmental level of this "dorsal motor nerve column", in six out of ten cases examined, the d_3 motoneurons

were clustered more caudally within the column, i.e. in C8 and T1. The area of muscle innervated by d_3 was confirmed electrophysiologically before the nerve was soaked in HRP solution in 5 animals. The size and location of these d_3 fields were essentially the same as those determined in the EMG study of the major motor nerve fields (Results, Section VI, D; compare Figures 32A and 17A).

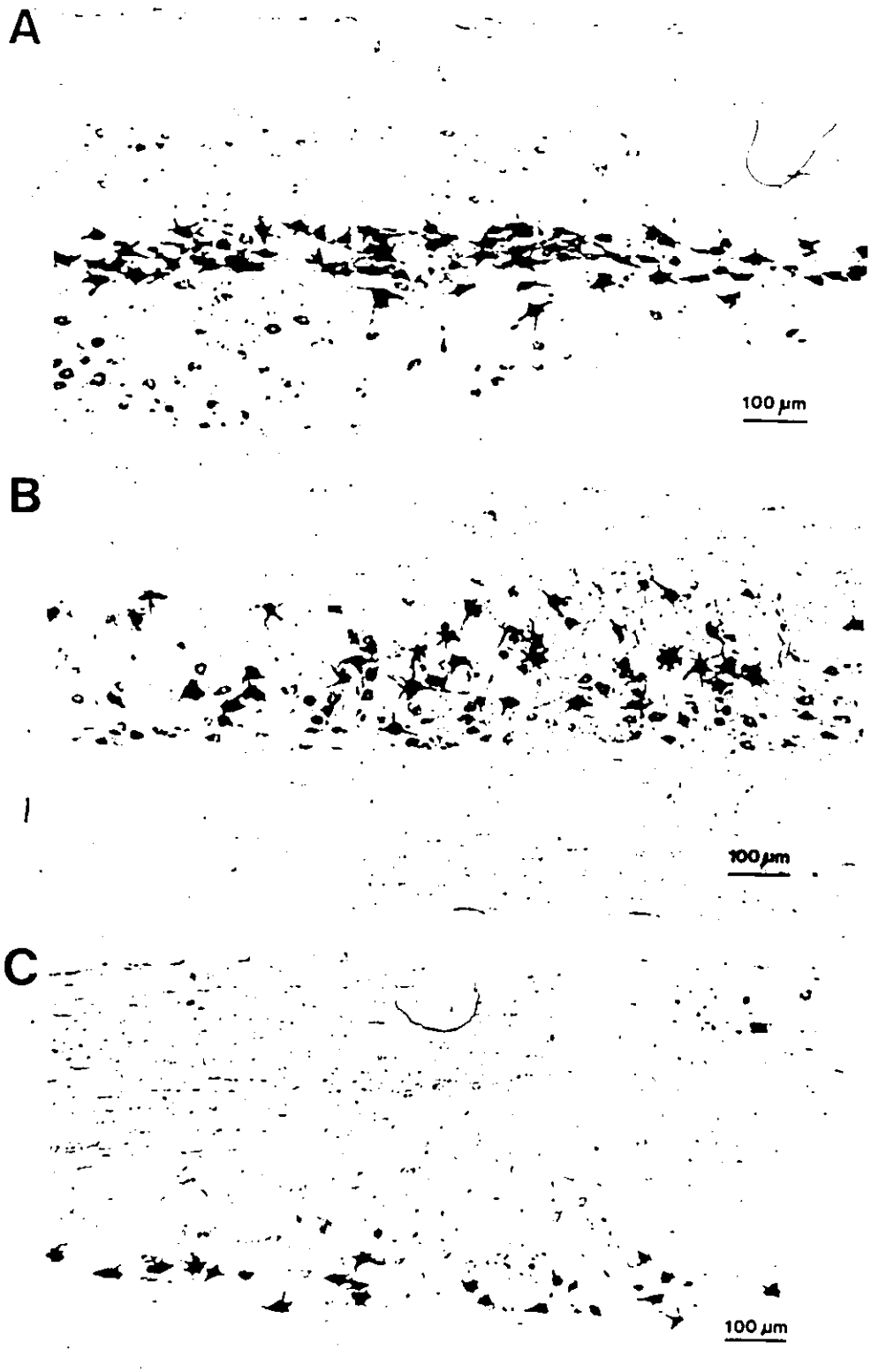
The relationship between the region of muscle innervated by the dorsal motor nerves and the location of their cell bodies within the CTM pool is diagrammatically presented in Figure 33. In summary, the dorsal motor nerves derive from a column of motoneurons positioned medially within the CTM pool and extending for approximately the entire dorso-ventral depth (400 μm) and length (5000 μm) of this pool in the ventral gray matter; the most ventral of the dorsal motor nerves (d_3) however, often derives from the most caudal segmental levels of this dorsal motor nerve column.

2) Lateral Motor Nerves

In 19 animals the location and distribution of the motoneuron somata whose axons constitute the lateral motor nerves was similarly determined. The results are detailed in Appendix IX and summarized in Figures 31, 32 and 33. In six animals two of the lateral motor nerves, l_1 and l_2 were labeled with HRP; the stained motoneurons numbered about 400 and formed a column of cells situated laterally and ventrally within the motor pool, extending for the entire length of the pool (5200 μm). In four other animals l_1 only was labeled; in

Figure 31 Photomicrographs of three horizontal 40 µm sections through the region of the CIM motoneuron pool following retrograde transport of HRP in the

31A the dorsal motor nerves
31B the lateral motor nerves
31C the ventral motor nerves.


A
100 µm
B
100 µm
C
100 µm

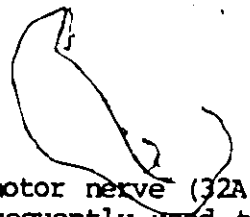
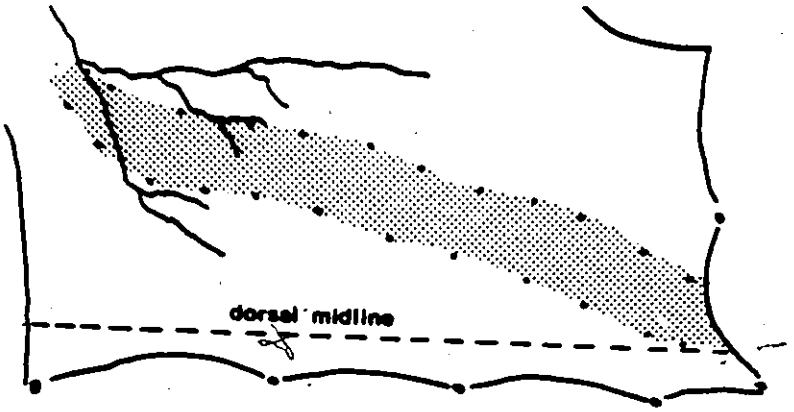


Figure 32 Muscle fields of the dorsal motor nerve (32A) and the lateral motor nerve (32B) that were subsequently used to provide the HRP-labeled motoneurons that were shown in Figure 31.

A

Animal 502 d_3 muscle field



B

Animal 488 l_2 muscle field

three of these cases motoneurons were found restricted to a caudal segmental level (i.e., C8 and T1), although they remained laterally and ventrally located within the CIM motor pool. When the location of motoneuron somata contributing to the l_2 motor nerve was examined (n=9), HRP-stained cells were found over the entire length of the CIM pool, i.e., C6 to T1. The l_2 motoneurons were also very laterally (9 out of 9 cases) and ventrally (7/9) positioned. The area and location of the l_2 muscle field in 5 of these animals was determined electrophysiologically before the nerve was exposed to HRP; as can be seen in Appendix III the values obtained agree well with those determined earlier for the lateral motor nerve fields (Section VI, D; compare Figures 32B and 17B).

To summarize, the lateral motor nerves originate from a column of motoneurons (the "lateral motor nerve column") which extends the full length of the CIM motor pool and is located laterally and ventrally within the pool; the most dorsal of the lateral motor nerves often originates from the most caudal segmental level of this lateral motor nerve column.

3) Ventral Motor Nerves

When similar experiments were performed on the ventral (v_1 and v_2) motor nerves in five animals, another columnar arrangement of cells was revealed, the "ventral motor nerve column". The results are tabulated in Appendix X and presented in Figures 31 and 33. This narrow column contained about 200 cells and extended for the full

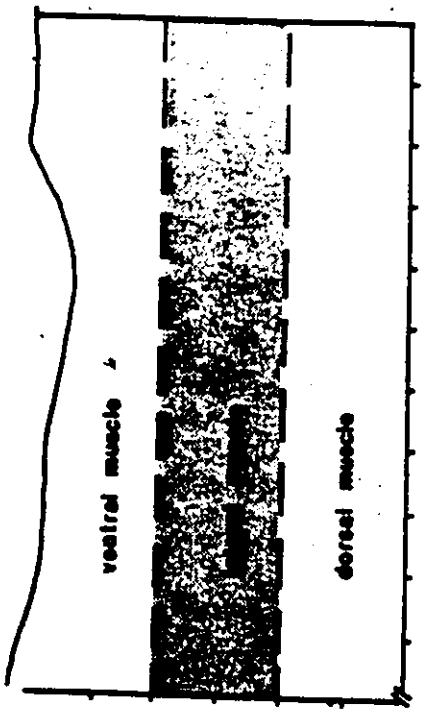
length of the pool, i.e. from C6 to T1, but while it was located on the lateral edge of the ventral horn, it appeared to be more dorsally positioned than the lateral motor nerve column. In two animals v_1 alone was labeled with HRP in order to discover whether there was a further spatial organization of motoneurons in the ventral motor nerve column, The results from these experiments did not show any such organization; motoneurons were labeled throughout the full length of the pool.

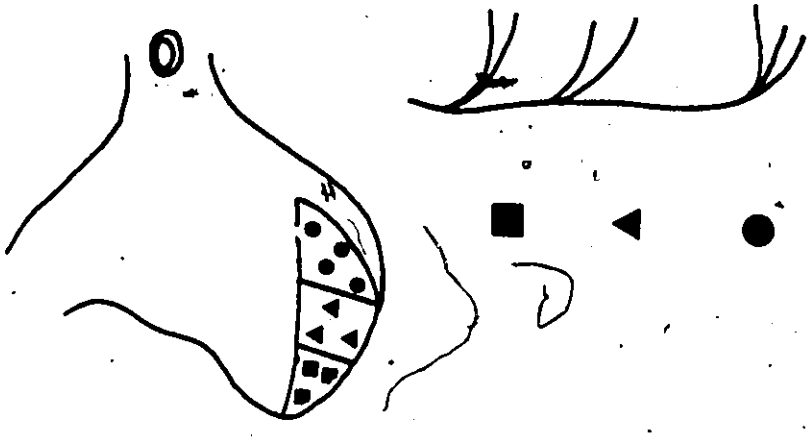
In summary, the ventral motor nerves derive from a laterally and dorsally positioned column of motoneurons, extending the entire length of the parent CTM motoneuron pool.

4) Summary Statement

The results from the above experiments show that the CTM motoneuron pool is divided up spatially on the basis of the peripheral motor nerves and thus has an intrinsic organization that can be related to the disposition of their muscle fields. Within the parent motoneuron pool are narrower columns of cells whose mediolateral position in the pool correlates with the dorso-ventral location of their muscle fields. Dorsal motor nerves originate from a medial column of motoneurons, while lateral and ventral motor nerves derive from progressively more laterally located columns. The distinction between the lateral and ventral motor nerve columns is not entirely clear-cut however, and the resolution of the technique has not excluded the possibility that these two major motor nerves

Figure 33 Composite diagram relating the spatial locations of the major motor nerve fields and the corresponding spinal cord positions of the motoneurons supplying those fields.


ventral muscle
dorsal muscle



originate from essentially the same column.

H) LOCATION WITHIN THE CIM POOL OF THE MOTONEURONS SUPPLYING SMALL EMG COMPARTMENTS

1) Motor Nerve Twigs

The CIM is reflexly activated in "compartments" (see Results, Section VI, B); this means that a localized sensory input selects out only a certain sub-population of CIM motoneurons, specifically those motoneurons that activate muscle fibres in the immediate vicinity of the site of sensory stimulation. The motor nerve fields of the CIM run longitituдinally; therefore each motor nerve field must contain many of these small "reflex compartments". These observations lead to the question: could there be a further level of spatial organization within the CIM pool, in that each motor nerve "sub-column" is fractionated into small populations of motoneurons, each serving a reflex compartment spatially organized within the CIM motoneuron pool? One way to investigate this possibility is to try to isloate the nerve isolate branch supplying a reflex compartment and then to label it with HRP in order to identify the motoneurons. Since the CIM is so thin in cross-section, it was possible to dissect out quite small motor nerve bundles or "twigs" and map their fields; however, it seemed unlikely that these would be small enough to supply individual reflex compartments. Nevertheless the experiment seemed worthwhile as a first attempt. One immediate problem was to locate nerve twigs in the ventral and caudal portions of the CIM; in these

areas, the muscle thins out considerably and the fine nerve bundles are difficult to resolve against the connective tissue background of the associated dermis. As a result, in nine out of ten animals the twigs that were dissected out were located between 6 and 10 cm from the occipital protuberance (Figure 34 shows a diagram of the locations of all the twigs examined). In each experiment the twig was first electrically stimulated and the active EMG area mapped as described in Section V, 10 ii. Following this procedure the twig was then cut at its most distal point and the central end dipped into HRP solution. The spinal cord was processed for HRP histochemistry after an appropriate survival time (see Section V, 7 for protocol). The results are given in detail in Appendix XI and are presented in summary in Figures 35. As can be seen from Figure 35, the area of muscle innervated by a twig generally depended upon the rostro-caudal location of the twig in the muscle: the more rostral the twig, the greater the length, and the area, of its muscle field. There was however, a tendency for a rostral twig to label more motoneurons than a caudal twig (compare animals #410 and 491). In 7 out of 8 animals the motoneurons backfilled from the twigs were found throughout the entire length of the CTM pool. From these experiments then, it is not possible to determine whether the rostro-caudal location of the twig muscle field is spatially represented in the CTM pool. However these results agree with those described for the HRP studies of the major motor nerves (Results, Section VI, G) in that dorsally-located

Figure 34A Table VIII shows the areas of the muscle fields supplied by the identified motor nerve twigs that were subsequently used to provide the HRP-labeled motoneurons photographed in Figure 35.

34B Composite diagram of the locations of the motor nerve twigs.

TABLE VIII MOTOR NERVE TWIGS: MUSCLE FIELD AREAS

Animal #	x,y Location of Twig (cm)	Area of Muscle Field (cm^2)	Identity of Motor Nerve
409	6;1.5	8.0	-
410	6;2.0	9.9	-
409	7;4.5	9.1	-
410	8;3.0	8.7	-
490	9;3.0	9.1	d_3
404	10;1.0	7.1	-
405	10;1.0	4.7	-
403	10;2.0	5.7	-
504	10;4.0	4.7	-
491	11;1.5	5.3	d_2

(n=10)

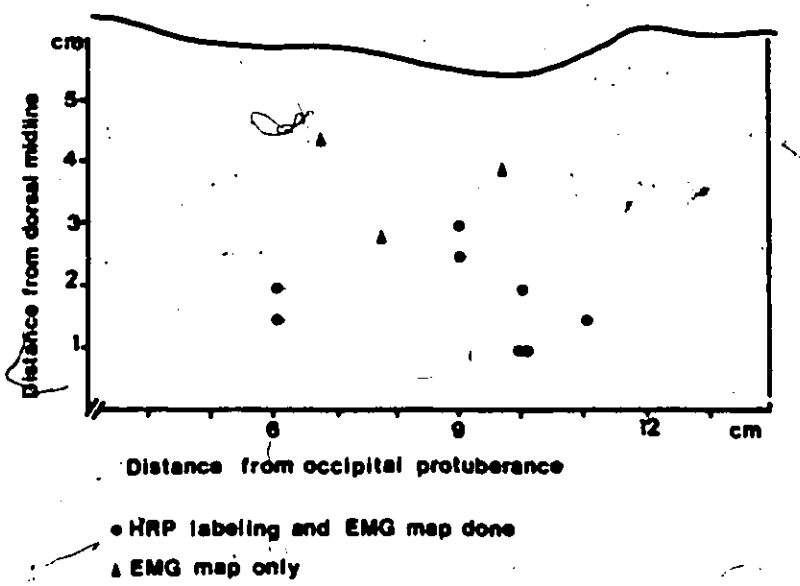
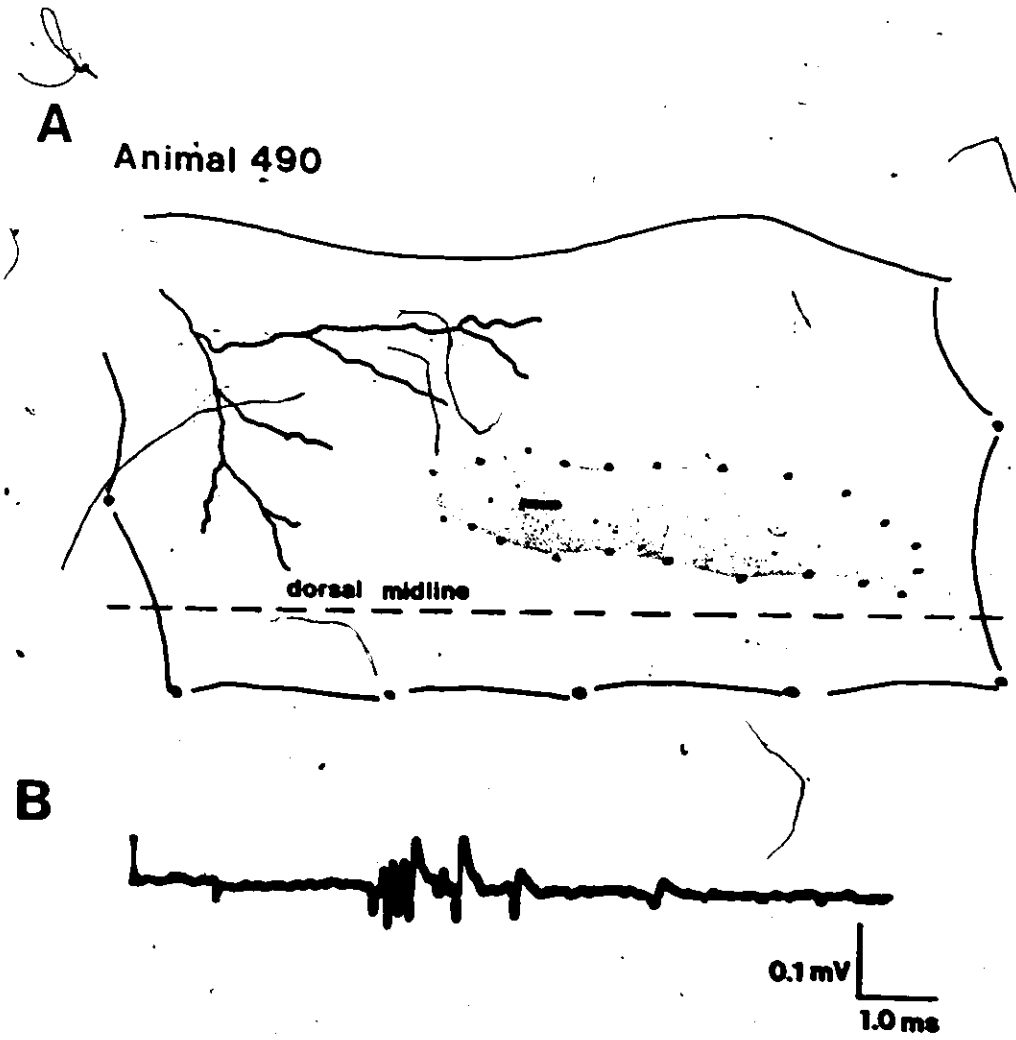


Figure 35A EMG defined muscle field of one motor nerve twig.

35B Antidromic action potential evoked in the d_2 motor nerve following electrical stimulation of the twig used in 35A.

35C Photomicrograph of a 40 µm horizontal section showing some of the motoneurons labeled following retrograde transport of HRP in the motor nerve twig shown in 35A. Five labeled motoneurons are indicated by arrowheads.


A
Animal 490
dorsal midline
B
0.1 mV
1.0 ms
C
200 µm

twigs supplied dorsal muscle, and had cell bodies located medially in the CIM pool, while ventral twigs had muscle fields situated in the ventral portion of the CIM and labeled motoneurons along the lateral edge of the CIM motoneuron pool.

2) Summary Statement of Findings

The objective of these experiments was to label motoneurons that drive a small area of muscle, ideally a single reflex compartment and to determine, if possible, whether or not they occurred as a discrete group, appropriately located within the CIM pool. While it was possible to label motoneurons driving small EMG compartments, it turned out not to be possible to discover any further spatial organization of these cells within the parent motor pool. This however might well be a question of resolution; if the finest nerve twigs could have been dissected out for examination, their muscle fields would likely approximate the size of a reflex compartment and by virtue of the small number of axons in the twig, the location of their somata in the CIM pool might well reveal a finer spatial organization than previously observed. The results of the present experiments by no means disallowed this possibility. Thus the CIM motoneuron pool may have an intrinsic organization wherein motoneurons have a spatial distribution that corresponds to the behaviorally compartmentalized use of the muscle. More refined experiments are required to resolve this question.

SECTION VII DISCUSSION

1. STATEMENT OF MAJOR FINDINGS

The results of this thesis provide evidence that the intrinsic organization of a motoneuron pool relates directly to the spatial coordinates of its target tissue, the muscle. Histochemical and electrophysiological investigations of the muscle territories of the major motor nerves have shown that the CTM is divided up into distinct major longtitudinal muscle fields, and that each of the major motor nerves derives from a longtitudinal sub-column of motoneurons within the CTM motor nucleus. How could such an arrangement become established? While the results of this thesis do not provide direct evidence about the development of a structure-function relationship, other reports in the literature may provide some clues.

The columnar arrangement of motoneuron somata occurs very early in development, most likely without any afferent or descending input (Bekoff, 1976). Studies of cerebellar Purkinje cells (Pakic, 1972) indicate that, at least in these neurons, the initial formation and elaboration of dendrites is determined by intrinsic factors; in other neurons the final shape and morphological specializations (e.g. spines) are largely dependent on local interactions with afferent input (Jacobson, 1978). With regard to the development of the CTM circuitry, it seems quite likely that the subcolumns of motoneurons

and their dendrites could be established prior to the arrival of impulses along afferent pathways (the CTM pool is located considerably rostral to the segmental origin of the afferent projections and would therefore be more mature).

Autoradiographic tracing studies of the development of the intersegmental system in the chick spinal cord (Nornes, Hart and Carry, 1980a, b) have shown that the interneuronal projection system develops relatively early in embryogenesis (by day 6 the interneuronal projections from lumbar segments extend to the brachial levels) and further, that the onset of embryonic motility correlates well with the development of the intersegmental system. They also demonstrated that axons from an individual spinal segment layer upon those of more rostral (i.e. older) segments, maintaining a parallel arrangement with their nearest neighbours and thus preserving a topographic order in the tract, effectively delivering an orderly set of inputs to the target area. The implication from these studies is that the subcolumnar arrangement of groups of CTM motoneurons may provide a major neuroanatomical substrate for establishment of at least the initial afferent (i.e., interneuronal) circuitry in the CTM reflex. How then would the information conveyed by the incoming fibres eventually gain access to the appropriate sub-population of motoneurons in the CTM pool?

From the results of the twig and direct muscle injections, it is not clear that motoneurons driving a reflex compartment may occupy

discrete regions of the "parent" CTM motoneuron pool. Instead it appears as though they may be dispersed throughout their sub-column. If this is indeed the case, then the basis of their functional relationship must be determined by factors other than a primarily spatial one. For example, the selective stabilization of the appropriate synaptic circuitry (see Changeux and Danchin, 1976) may play a role in the development of local sign in the CTM reflex. In other words, perhaps the initial formation of synapses is nonspecific; later, with the conduction of impulses through the spinal cord that are associated with the successful dislodging of an irritant stimulus from the skin, for example, afferent input from particular levels of the spinal cord may come to drive most effectively those motoneurons innervating muscle fibres located at the respective levels. Alternatively, perhaps some "positional" feature of the peripheral terminals (i.e., in the skin and muscle) may become encoded in the cell-surface molecules of the sensory and motor neurons and may thus influence the subsequent patterns of synaptic connectivity. This point of view implies that "positional information" from the skin and/or muscle directs the formation of central circuitry. Miner (1956) presented the first experimental evidence that misdirected wiping reflexes occurred in frogs following skin graft rotations; the interpretation of these results was that information encoded in the skin influenced the central connexions of cutaneous afferents. Despite numerous reinvestigations of this

phenomenon (see Frank and Westerman, 1982), the issue still remains controversial.

2. QUESTIONS ARISING FROM THE REFLEX ACTIVATION OF THE CTM

The major findings reported in this thesis arose from observations of the pattern of reflex behavior exhibited by the CTM. We have previously shown that a punctate nociceptive stimulus to the overlying back or flank skin of the rat elicits a localized contraction of the underlying muscle (Nixon, Theriault, Jackson and Diamond, 1984). The sensory nerves that drive the CTM reflex originate from the thoracic and lumbar segments of the spinal cord, and the local sign is segmentally expressed. Therefore, the simplest expectation would be that the motor output to the muscle is also segmentally organized, as in the case of the intercostal muscles, where sensory and motor axons supplying a strip of intercostal musculature derive from the same thoracic segment (Kirkwood and Sears, 1975). Interestingly, this is not the case for the CTM: the CTM motoneuron pool is located in the cervical spinal cord and all the motor nerves exit from the brachial plexus. Despite the anatomical separation of the sensory and motor components of this reflex, the local sign character of the reflex response implies that the nociceptive input from any one sensory dermatome has a preferred access to that fraction of the motoneuron pool which supplies the muscle underlying that region of the skin. Thus, there must be a

sort of "matching" between groups of primary sensory neurons, interneurons, and motoneurons which relates to the body location of the sensory endings in the skin and of the muscle fibres of the CTM. It has been shown in the visual (Jacobson, 1978) and somatosensory systems (Woolsey and Van der Loos, 1970) that the afferent projections from peripheral sensory receptors to the cortex are organized in a precise somatotopic map. This is also true for a number of efferent (corticospinal) projection systems; a similar organization, however, has not been described for the projections of individual pools to their muscles. As stated above, the major objective of this thesis was to gain a further understanding of this issue.

Physiological investigations of this reflex revealed a very strong cutaneous sensory drive of the CTM and lead to questions about the contribution of the more conventional source of afferent input to skeletal muscles, that of the spindle. Histochemical and electrophysiological results described in this study reveal that the CTM is essentially devoid of spindles. This negative finding supports the idea that the muscle is not used in postural control; by comparison, those muscles important in balance and equilibrium, such as the vertebral musculature, have been shown to have an extremely high density of muscle spindles (Richmond And Abrahams, 1975b). The preliminary histochemical studies of the CTM indicate a predominantly fast-twitch muscle fibre classification, a finding which is also

compatible with a lack of spindles (as fully described in the Background, spindles are found almost exclusively among slow-twitch fibres). Interestingly, the homologous muscle in the chicken, the dorsocutaneous latissimus dorsi (Bates, 1948; Crim, 1971), has also been reported to contain few, if any spindles (Bourgeois and Toutant, 1982).

Another question concerns the functional role of the CTM in the animal; since it is apparently not involved in posture or balance, what is the muscle used for? The evidence presented in this thesis and in Nixon et al. (1984) on the reflex activation of the CTM by nociceptive cutaneous afferents strongly suggests that at least one function of this muscle is to dislodge irritant stimuli, such as fleas or insects. The CTM is also present in ungulates and it is a common observation that when a horsefly bites the back skin of a horse or cow, the skin is seen to ripple in the immediate vicinity of the insect. Langworthy (1924, 1925) speculates that in armadillos the CTM functions to tense up the plates, in hedgehogs to help roll the animal into a ball, and in porcupines to straighten up the quills in response to predators. The CTM in rats is most likely also used to tense up the loose skin of the abdomen and thorax of the female during mating (author's observations). In this context, it has been noted that the ventral extent of the CTM in many species is divided into several portions, one of which encompasses the prepuce of the penis in males and the perineum in females, with fibres inserting

around the anus and base of the tail (see Langworthy, 1924 for review). Although a similar distribution of the ventral CTM was not described in rodents, it seems reasonable to consider that this muscle could play some role in the mating process.

2. PATTERN OF MOTOR INNERVATION OF THE MUSCLE

A major portion of this thesis was concerned with a description of the patterns of motor innervation in the CTM. These studies incorporated electrophysiological and/or histochemical analyses of the muscle fields of the ventral roots, the major motor nerves and many of their ramifications, as well as small motor nerve bundles or twigs. It has become apparent from the results of many experimental investigations over the last few years that both the ventral roots and the intramuscular nerve branches supplying a muscle have electrophysiologically and histochemically definable territories within the muscle; in other words, the motor innervation of skeletal muscle is not randomly dispersed but rather exhibits characteristic spatial patterns (see review in the Background, Section II, P).

i) Ventral Roots. Investigations of several different hindlimb muscles in a number of species (Sherrington, 1906; Markee and Lowenbach, 1945; Swett et al., 1970) indicate that there is a predictable relationship between the segmental level of a ventral root and the location of its muscle territory, such that if more than one ventral root supplies an individual muscle, the more cranial root

is found to innervate proximal musculature while caudal roots innervate progressively more distal portions of the muscle. The results of the present thesis, however, do not shed light on this relationship; in a small number of experiments it was not possible to resolve a distinct pattern of ventral root innervation in the CTM. This negative finding may indicate there are no definable ventral root territories in this muscle, i.e. that each of the four roots, which are likely to contribute motor innervation to the CTM, supplies muscle fibres all over the extent of the muscle. Alternatively, the small number of experiments (1-2 per root) and the technical difficulties associated with the electrical stimulation of cervical ventral roots (described more fully in Results, Section VII, C) may have obscured a true pattern of ventral root innervation in the CTM.

It is interesting to note that a relationship between the rostro-caudal sequence of ventral roots and the proximo-distal one of their territories in individual muscles also exists on another level, i.e. within the brachial and lumbar enlargements of the spinal cord, the more rostral motor pools are found to innervate proximal limb musculature, while more caudally-positioned motor pools (and hence their ventral roots of origin) provide the innervation more distal limb musculature (see Romanes, 1964 for review). It is tempting to speculate that this kind of spatial relationship arises, at least in part, from a matching up of the temporal sequence of development in the spinal cord (i.e. the rostrocaudal gradient of neurogenesis;

Norres and Das, 1974) and a comparable temporal sequence of myogenesis within the limb bud (see review by Hauschka and Pitz, 1983). While comparatively little is known about the myogenic gradient within single muscles, it does not seem unreasonable to suggest that the pattern of ventral root innervation described for individual muscles may become established through similar maturational factors.

ii) Motor Nerves. The concept that intramuscular nerve branches supply discrete muscle sub-volumes has received much experimental support over the last ten years (Ledbetter, 1974; English, 1980; Calvas and Conyea, 1980; English and Ledbetter, 1982; Bodine et al., 1982; Botterman et al., 1982a, b). The results of this thesis confirm and extend the previous findings. Whereas the majority of former investigations reported on either the histochemical or the electrophysiological characteristics of the sub-division of a muscle by its major motor nerve branches, the present study provides information about both of these parameters of individual muscle sub-volumes, and has in addition correlated the borders of individual muscle fields using these two techniques. The results clearly demonstrate that the CTM motor nerves divide up the muscle into characteristic longitudinal (rostro-caudal) territories. In other skeletal muscles such as the pig masseter (Herring, Grimm and Grimm, 1979), the cat semitendinosus (Bodine et al., 1982), and the cat medial and lateral gastrocnemii (Swett, Eldred and Buchwald, 1970;

English, 1980), discrete muscle sub-volumes are often delimited by internal aponeuroses. This however, is not the case for the CTM: there appear to be no definable connective tissue boundaries which serve to divide up the muscle into distinct sub-volumes. In this respect it is particularly intriguing that the borders of the major motor nerve fields are smooth and regular (see Figure 17). These observations give rise to several questions, one of which is: how does a motor nerve field become established? The work of Landmesser (1978b) on the early motoneuron projection patterns to normal chick hindlimb muscles suggests that even prior to cleavage of the primary muscle masses into individual muscles, there is a very definite regionalization in the projection of spinal nerves, such that they appear to have "chosen" the appropriate territory. Studies by Smith and Hollyday (1983) extend these findings to the early organization of motor nuclei in the rat thoracic spinal cord. Other work by Whitelaw and Hollyday (1983a) on patterns of motor innervation in the chick hindlimb following deletions of limb segments suggest that motoneurons display an intrinsic selectivity for their termination sites. Whether similar regional selectivity may be extrapolated to the projection of a single motoneuron pool to a single muscle has not yet been demonstrated; however on the basis of findings to be discussed in the next section, it does not seem an impossibility.

Other questions which arise from the analysis of the CTM muscle sub-volumes relate to function. In addition to the smooth

borders, quantitative measurements of the major CTM motor nerve fields reveal a remarkably constant size and characteristic location for each field, from side to side and from animal to animal. While there are no similar studies in the literature available for comparison, it is likely that the CTM is not unique in this respect and that if these techniques had been applied to other muscles (e.g. the gastrocnemii) similar relationships would most likely obtain. What then could be the functional significance of distinctive muscle subvolumes? On one level, it has been most clearly shown in the pig masseter that the anatomical (and histochemical) compartmentalization does reflect a functional division: during normal masticatory activity, selective contractions of the anterior and posterior compartments allow the muscle to perform a number of different functional activities (Herring, Grimm and Grimm, 1979); although not measured, these selective contractions were presumably reflexly-modulated i.e. by afferent spindle discharges, resulting from different chewing load requirements. The results of the present thesis on the patterns of motor innervation in the CTM support this point of view, and further, in conjunction with our previous report (Nixon, Theriault, Jackson and Diamond, 1984) provide clear evidence that localized contractions are indeed reflexly-evoked.

While each motor nerve field in the CTM contains many 'reflex compartments', the major axis of the field (i.e. longitudinal) is more or less at right angles to the segmental pattern of the sensory

nerve fields that "drive" the muscle. Since the CTM motor nerves originate from the cervical spinal cord, it is most likely that the myogenic contribution to this muscle derives from the brachial somites (Bates, 1948; Beresford, 1979). If the muscle were then to grow away from its origin in a predominantly caudal direction, as does the bulk of pectoral musculature (Sullivan, 1962; Grimm, 1971; Beresford, 1979), the development of longitudinal muscle fields is not difficult to envision. In thinking about the functional purpose of this arrangement, it is significant to recall that the bilateral expression of local sign in the CTM reflex is largely confined to the dorsal skin, rather than to the flanks of the animal. In this context, it is worth noting that the dorsal muscle fields appear smaller and more precise than the lateral or ventral motor nerve fields (compare Figures 17A and B). To return to the matter of the 'smoothness' of the borders of the muscle fields, perhaps this characteristic could arise as a function of afferent input? It is well known that the elimination of polyneuronal innervation in skeletal muscles occurs shortly after birth (Redfern, 1970), during the same period when ascending and descending (i.e. corticospinal, propriospinal) tracts are becoming established (Gilbert and Stelzner, 1979; Schreyer and Jones, 1983). However, it is not well understood what role, if any, afferent input plays in the elimination of polyneuronal innervation and the subsequent establishment of motor nerve fields. With respect to the CTM, perhaps the afferent input to

its motoneuron pool contributes to the shaping the muscle field borders, particularly in the dorsal portion where the muscle fields appear smaller and more precise, possibly reflecting the finer localization required in the bilateral expression of local sign.

iii) Motor Unit Territories. Early investigations of the spatial distribution of single motor unit territories were largely confined to cat triceps surae muscles (e.g. Burke and Tsairis, 1973). On the basis of these studies, the concept arose that individual motor units were widely distributed throughout the muscle volume. More recently, however this point of view has undergone considerable revision, the major objection being that this arrangement may not be typical of most muscles (see review in Background, Section II, B). The demonstration of circumscribed motor units in functionally or histochemically compartmentalized muscle is currently an unresolved and very topical issue in this field. Preliminary investigations of motor unit territories in the CTM throw some light on this issue. Estimates of motor unit size and location were obtained by using finely graded electrical stimuli to branches of CTM motor nerves and simultaneously recording the area(s) of EMG activity in the muscle. The active EMG areas measured by this technique were assumed to reflect the muscle territory innervated by one or a few motor axons. The minimum areas were always smaller than the area of a reflexly-evoked compartment, an observation consistent with the interpretation that a reflex compartment contains one or more

motor-units. Also in keeping with this hypothesis are estimates of CTM muscle fibre length; AChE staining suggests that CTM muscle fibres are quite short, the length corresponding well to measurements of the longitudinal axis of 'motor unit' territories (approximately 1.0 to 2.0 cm). These findings, while far from conclusive, are consistent with the hypothesis that relatively few CTM motor units are contained within a 'reflex compartment'. Unequivocal evidence for this hypothesis would be obtained by tetanically stimulating a single motoneuron (or its axon; i.e. by an intracellular microelectrode) and then analyzing the muscle for the location and distribution of glycogen-depleted muscle fibres. Such an experiment would also provide conclusive evidence about the length of single muscle fibres.

4. ORGANIZATION OF THE CTM MOTONEURON POOL

i) Characteristics of the Entire CTM Pool

The major portion of this thesis was devoted to an extensive characterization of the motoneuron pool supplying the motor innervation to the CTM. On the basis of retrograde HRP labeling, the CTM motor pool was localized to the ventrolateral region of the cervical spinal cord, extending from the caudal end of C6 to the rostral edge of T1, a distance of approximately 5 mm. The position occupied by the CTM pool in the spinal cord is characteristic of motoneurons providing innervation to the pectoral musculature in the

rat (Baulac and Meininger, 1981); this observation is consistent with the presumed embryonic (i.e. somitic) origin of the CTM (Grim, 1[illegible]; Beresford, 1979). The rostro-caudal extent of the CTM pool (2 to 3 segments) is typical of most motor nuclei (Romanes, 1964); however, what is most unusual about the CTM pool is the large number of motoneurons constituting it: approximately 1150 cells. There is little doubt this is a correct estimate of the size of the CTM motor nucleus. Transneuronal labeling by HRP has never been reported to occur in a retrograde fashion (i.e. from motoneurons to interneurons) despite the extensive use of this molecule over the past ten years in the study of nervous system connectivity. Furthermore, the short interval between incubation and perfusion (18-24 hrs) would allow little time for transneuronal passage (this time period was chosen for counting somata because only a limited labeling of dendrites occurred). As noted in the Results (Section VI, F, 1) profiles were counted as cells only when a nucleolus was visible; additionally each cell demonstrated the typical granular reaction product of retrogradely-transported HRP (injection or transneuronal passage of HRP results in a diffuse cellular label; see Honge et al. 1982). The possibility of leakage to other muscles may also be eliminated as an explanation for labeling so many cells, for the incision site was always carefully monitored and at the end of each incubation, the cut nerve end was gently swabbed of HRP and was often sealed in a paraffin wax cuff. In several cases where leakage of HRP solution

was apparent the animal was processed for HRP histochemistry and the location of cell bodies noted. In all cases the pale-staining ectopic motoneurons could be localized to the pectoralis major or minor motor pools (Paulac and Meininger, 1981), muscles which are anatomically adjacent to the CTM. Corroborative evidence for the number of cells in the CTM pool was obtained from axon counts of CTM motor nerves; the counts were in complete agreement. The evidence thus far discussed is entirely compatible with the view that the CTM pool is unusually large (estimates of the rat pectoralis major pool equal 250 cells (Paulac and Meininger, 1981), while the cat medial gastrocnemius pool contains about 300 cells (Burke and Tsairis, 1973)). What then is the purpose of allotting such a large number of motoneurons to a relatively small muscle? One way of approaching this question is by relating it to motor unit size. The classical understanding is that fine reflex control of a muscle is effected by a small motor unit size (Erodal, 1981) and therefore, proportionally more motoneurons would be allotted to a muscle used for 'vernier' movements. The physiological analysis of reflex behavior in the CTM (this thesis; Nixon, Theriault, Jackson and Diamond, 1984) clearly shows a very localized pattern of reflex contractions, in conjunction with a small motor unit size. These observations are consistent with the demonstration that the CTM motor pool contains a large number of motoneurons.

Another observation made in this study of the CTM motor

nucleus concerns the average cell size. Measurements of the average soma diameter of 1070 CTM motoneurons reveals a unimodal size distribution with a peak at 30-40 um. A similar distribution was found for axonal diameter, such that the average CTM motor axon is 12 um in diameter. Both these measurements, indicating that CTM motoneurons are small, are internally consistent. It has been previously shown that the size of soma and axon are directly related (Barrett and Crill, 1971; Cullheim, 1978). Why CTM motoneurons should be at the small end of the scale, however, is not immediately apparent. Despite the popular contention that small motoneurons innervate slow-twitch muscle fibres, there is no statistically significant relationship between motoneuron size and motor unit type (Burke et al., 1982). Further argument against this concept is provided by the preliminary electrophysiological and histochemical characterizations of the CTM as a fast-twitch glycolytic muscle. Although no studies have directly addressed the issue, one line of current thinking about the functional significance of cell size is that there may be a metabolic constraint, such that small cells can support only a limited amount of target tissue (Henneman and Mendell, 1981). The apparently small motor unit size in the CTM is consistent with such a hypothesis.

During the processing of horizontal sections of the spinal cord, it was often noted that CTM motoneurons seemed to display a distinctive longitudinal organization of their major dendritic

processes. Scheibel and Scheibel (1970) have previously described a predominant rostro-caudal bundling of dendritic processes in Golgi-Cox preparations of rat cervical spinal cord. Their observations have been repeatedly confirmed (e.g. Scheenen, 1982), although recent intracellular HRP labeling of cat cervical motoneurons indicate that not all cervical motoneuron pools may be characterized solely on the basis of longitudinal dendritic projections (Keirstead and Rose, 1982). It has been proposed that the spatial overlap of the dendritic projections of motoneurons may provide one mechanism by which these motoneurons receive similar synaptic inputs (Scheibel and Scheibel, 1970). This turns out to be a very attractive proposition in the case of the CTM where a definite functional role can be ascribed to subcolumns of CTM motoneurons - particularly in reference to the bilateral expression of local sign.

Another interesting observation in HRP-labeled spinal cord relates to the CTM motor axons. In horizontal sections through the ventral white matter it was regularly observed that short rows of HRP-stained axons were present (see Figure 27). While no other similar reports exist in the literature with which to compare the present findings, there is a vast number of studies dealing with the spatial ordering of axons in the optic nerve (e.g. Bunt and Horder, 1983). What developmental mechanisms are responsible for this relationship is a hotly disputed issue (see Horder and Martin (1978) for the 'morphogenetic versus chemospecificity' point of view);

however it is clear that ganglion cell axons in the optic nerve of lower vertebrates retain a "nearest neighbour" relationship based on their point of origin in the retina. In the light of the present findings, and those of Landmesser (1978a) and Smith and Holyday (1983) which show that projection pattern of spinal nerves and the location of the cell bodies giving rise to them display an initial selectivity for target sites, it is tempting to speculate that within a single motoneuron pool, motor axons destined for a particular target region in the muscle arrive at their destination using certain 'nearest neighbour' cues. In order to test this possibility in the CTM it would be necessary to trace the short rows of axons seen in the ventral white matter through the peripheral nerves to their termination sites in the CTM. One way to go about this would be to inject HRP into a small defined area of the CTM and follow the position of the labeled axons up the spinal nerves and into the white matter of the cord.

ii) Subdivisions of the CTM Pool Corresponding to Muscle Fields

The most significant finding reported in this thesis concerns the location of motoneuron somata which contribute axons to the individual motor nerves. Retrograde transport of HRP in the major CTM motor nerves revealed a sub-columnar organization of the CTM motor nucleus, wherein the most medial sub-column of CTM motoneurons was found to send motor axons out to the dorsal motor nerves, thus innervating the dorsal strip of musculature. The lateral and ventral

motor nerves were similarly found to derive from progressively more lateral subcolumns of motoneurons. These results constitute the first demonstration in the literature of the spatial location of motoneuron subpopulations supplying innervation to individual muscle compartments that have been electrophysiologically and histochemically characterized. Thus the CTM pool has an intrinsic organization related to the peripheral fields of the major motor nerves.

Since each motor nerve field contains many 'reflex compartments', each motoneuron sub-column likely contains smaller groups of motoneurons that drive such compartments. The obvious question is 'are these groups of motoneurons spatially resolvable?' There is no a priori reason why motoneurons innervating a particular reflex compartment in the muscle should be clustered together in the spinal cord. A number of alternative possibilities exist which could equally well account for (and very likely do play some role in) the establishment of specific patterns of motor circuitry, as seen in the local sign character of the CTM reflex. For example, motoneurons innervating the same reflex compartment could be scattered homogenously through out their particular motoneuron sub-column, and the appropriate circuitry could become established on the basis of dendritic projections. In order to gain an understanding of which of these two simple hypotheses might be most likely in the CTM, several different experimental strategies were employed to attempt to label a

'reflex compartment'. These are discussed in the next section.

iii) Are There Subgroups of Motoneurons Organized According to Reflex Compartments?

The results from direct intramuscular injections of HRP and from the HRP-labeling of tiny intramuscular motor nerve branches suggested that smaller subcolumns of motoneurons might exist. These types of experiments always resulted in the labeling of a smaller number of motoneurons predictably located in medial to lateral positions in the cord depending on the intramuscular site, and generally organized in short segments of columns. For technical reasons it was not possible to limit the HRP labeling to a 'reflex compartment' and thus to resolve a distinct spatial organization among the motoneurons corresponding to such a compartment. The diffusion studies indicated that even with the smallest HRP injection used in this study, the area of muscle likely to be labeled would be at least 2 to 4 cm^2. The motor nerve twig studies suffered from a comparable problem: it was not possible to dissect out the finest motor nerve branches and so the territory innervated by a twig was several times that estimated for a reflex compartment. When the locations of the stained motoneurons were then compared, for example from a rostral versus caudal position in the muscle, it was very difficult to determine whether the rostro-caudal (or dorso-ventral) position of a motoneuron depended on the rostro-caudal location of its muscle field. These experiments, however, are definitely worth

repeating, for example, using a highly-concentrated solution of WGA-conjugated HRP for tiny intramuscular injections. Such a technique would help to minimize diffusion since the sites of CTM injections of WGA-HRP have been shown to remain very localized (Staines, Kimura, Fibiger and McGeer, 1980). In this manner it would be possible to determine whether motoneurons innervating small areas of the caudal region of the CTM differed in their spinal cord location from those motoneurons innervating small portions of the rostral CTM. From the electrophysiological and behavioral evidence presented in this thesis and in Nixon, Theriault, Jackson and Diamond (1984) it is clear that the CTM motoneuron pool is functionally compartmentalized: afferent input is able to select out small subpopulations of motoneurons which drive small reflex compartments of the muscle. The histochemical and electrophysiological analyses of the major motor nerves described in this study reveal a characteristic pattern of innervation of the muscle according to a distinctive subcolumnar arrangement of the CTM motor pool. At this level then, the intrinsic anatomy of the motor nucleus reflects a functional compartmentalization of the muscle. On the basis of the twig and injection evidence presented it is not possible to conclude that a further level of spatial organization exists in the CTM pool. However, the reflex behavior of the muscle does indicate that afferent input is able to select out a small subpopulation of the CTM motoneuron pool. The actual physical basis of this specificity

remains to be elucidated.

5. IMPLICATIONS FOR THE DEVELOPMENT OF MOTOR CIRCUITRY

The establishment of patterning in the vertebrate nervous system may be considered as occurring in three sequential stages, which overlap considerably in time from one region to another and within any one system (Diamond, 1982). The first stage involves the laying down of the primary cytoarchitecture of the nervous system, in terms of temporal and spatial gradients in the patterns of neurogenesis (Nornes and Das, 1974). The second stage comprises the post-mitotic differentiation of neurons and the projection of their axons to appropriate target regions (Landmesser, 1978a; Hollyday, 1983). The latest-appearing level of development concerns the distribution of nerve endings at their target tissues (see Diamond, 1982; Harris, 1981). With respect to the development of the motor system, it is clear that the columnar and somatotopic arrangement of motoneurons is a fundamental feature of the organization of the spinal cord (Brodal, 1981) which is laid down during early neurogenesis. Studies of the projection patterns of post-mitotic motoneurons reveal no evidence of random outgrowth, but rather clearly demonstrate an early, specific and directed outgrowth of motor axons to their targets (Speidel, 1933; Hibbard, 1965; Landmesser and Morris, 1975; Landmesser, 1978a; Smith and Hollyday,

1983). Now, while there is general agreement that some kind of specificity is involved in the formation of orderly central and peripheral connexions, the nature of the specificity and its mode of operation are widely disputed. A similar situation exists for the understanding of the fine modelling of the nervous system, that which occurs at the level of the target tissue. In the motor system very little is known about how individual motor units are established.

Perhaps the most crucial question facing neurobiologists in this decade, concerns the understanding of mechanisms are that are involved in the development and maintenance of the predictable organization of the nervous system. One indirect aim of this thesis was to gain a better understanding of this second stage of development in the nervous system - in particular, how do the specific projection patterns of motor nuclei became established? A large number of investigations has been reviewed in the Background which indicate that many of the specific patterns of motor connectivity in the adult appear to derive largely from the primary cytoarchitecture of the spinal cord. The craniocaudal differentiation of motoneurons is clearly related to a proximo-distal sequence of limb innervation (Romanes, 1941; Hamburger, 1948), and furthermore, the classical studies of Romanes (1964) have shown that motoneurons in the adult spinal cord are arranged in a columnar fashion, related to the functional characteristics of the muscles themselves (i.e. flexor or extensor; see reviews by Burke, 1981;

Henneman and Mendell, 1981; Schwindt, 1981). An extensive literature exists on the early projection patterns of chick hindlimb motoneurons strongly indicating that the adult pattern is present very early on in embryogenesis (see reviews by Landmesser, 1982; Hollyday, 1982). What of the organization of single motoneuron pools and their projection to individual muscles? Comparatively little is known about the early or the adult projection patterns of single motoneuron pools. The work reported in this thesis sheds light on the adult pattern of connectivity between one motoneuron pool and its skeletal muscle, and may thus provide a basis for speculation about the ontogeny of the reflex circuitry. The medial-to-lateral organization of subcolumns in the CTM motoneuron pool reflects a corresponding dorsal-to-ventral axis of motor innervation in the muscle. Whether this relationship also derives from early embryogenesis is largely a matter of speculation, but the possibility does seem reasonable.

6. QUESTIONS OUTSTANDING

In the final analysis, there are a number of unsettled questions arising from the present work. The most important issue at this time concerns the size and distribution of individual motor units in the CTM. Preliminary electrophysiological and anatomical evidence has been presented which argues for a small size and localized distribution of single motor units. The simplest explanation of the local sign character of the reflex response seen

in this muscle depends upon the demonstration of circumscribed, fast-twitch (or at least focally-innervated) muscle units. Conclusive evidence for this hypothesis could be obtained by microelectrode penetration of a single motoneuron or its axon; by using electrophysiological techniques to map the peripheral locations of the muscle unit, and tetanic stimulation to deplete the muscle fibres of their glycogen content information about the dimensions of a single motor unit, as well as of a single muscle fibre, could be obtained. The distribution of motor units in the CTM also bears upon the size of a behaviorally-defined reflex compartment. Although the preliminary evidence is consistent with the contention that a reflex compartment contains one or more muscle units, the glycogen depletion studies would provide a clearer understanding of this relationship.

Further work needs to be done to show whether or not the spinal cord position of motoneurons innervating rostral versus caudal portions of the muscle is characteristically different. Small localized injections of WGA-HRP would provide further information about a possible organization of motoneurons with respect to the location of their terminals in the muscle. In these experiments it would be of the utmost importance to carefully mark the ventral root entry zones, as the major population of CTM motoneurons is located within two spinal cord segments.

Another aspect of the present thesis that could be improved upon are the ventral root studies. A better technique and a larger

number of animals may reveal some pattern of ventral root innervation in the CTM. Since the motor nerve labeling experiments always labeled motoneurons in all segments (i.e. each longitudinal strip of muscle is supplied by all four ventral roots), the only remaining alternative plan of organization would exist along the rostro-caudal axis. It remains, of course, for the experimental results to confirm or deny this conjecture.

There are a variety of developmental experiments which suggest themselves, a few of which are worth mentioning. Concerning the longitudinal dendritic bundles present in the adult, the question arises as to their functional basis, i.e. does the most medial bundle receive input preferentially from the DCN's (and can the bilaterality of the reflex be localized to this bundle)? If so, what is the developmental timetable for the establishment of this preferential synaptic input - could it be related, for example, to the time period of elimination of polyneuronal innervation in the muscle? This latter question touches on the issue of how muscle fields (and individual motor units) may become established. Another issue concerns the development of local sign in this reflex - what is the developmental onset? Can it be experimentally modified by surgical manipulation (i.e. ablation, redirection) of the sources of afferent input? Yet another area of neurobiology which may be investigated with this preparation relates to the motor innervation of the CTM; for example, what are the neonatal and adult patterns of denervation-induced

sprouting?

The investigations described in this thesis provide evidence for a remarkable intrinsic spatial organization of a motoneuron pool that seems to be directly related to the functional characteristics of the muscle it supplies. While further conclusive studies are required, the present evidence is consistent with the hypothesis that the spatial coordinates of the muscle are in part mapped onto the CTM motor nucleus, at least at one level. These findings raise the possibility that other motoneuron pools may show a similar relationship to their skeletal muscles.

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APPENDICES

APPENDIX I VENTRAL ROOT STIMULATION STUDIES

Animal #	Ventral Root Stimulated	Depleted Muscle Fibres		
		Dorsal CIM	Lateral CIM	Ventral CIM
227	C6	few	few	many
232	C6	"	"	few
231	C7	"	"	"
230	C8	"	"	"
229	C8	"	"	"
228	T1	"	"	"

(n=6)

APPENDIX II. CHARACTERISTICS OF DORSAL MOTOR NERVE (d_3) FIELDS

Animal #	Left d_3 Area (cm^2)	Right d_3 Area (cm^2)	Width of Field at 6 cm from Occiput (cm)		Distance (cm) Separating Muscle Fields at:	
			Left	Right	6 cm	12 cm
265	11.3	9.3	1.3	1.0	-	-
266	9.3	9.8	0.8	1.2	-	-
MH2	11.7	11.0	1.2	1.2	2.5	0.5
282	10.7	11.3	1.3	1.4	-	-
283	13.3	8.7	1.2	0.8	-	-
289	9.4	10.7	1.3	1.4	-	-
294	10.2	11.6	1.1	1.5	-	-
MH1	14.2	12.0	1.2	1.2	3.4	0.8
288	12.2	10.8	1.3	1.3	-	-
MH8	15.0	10.5	1.6	1.0	3.5	1.3
MH4	12.2	15.1	1.4	1.5	2.5	1.1
MH5	10.3	10.6	1.3	1.5	3.0	1.4
MH6	10.8	11.3	1.1	1.3	3.5	1.2
MH7	13.5	13.4	1.4	1.7	4.0	1.8

(n=14)

Area of d_3 muscle field: left 11.7 $\pm$ 0.5 cm^2 (SEM)
Width of d_3 muscle field at 6 cm from the occipital protuberance: left 1.3 $\pm$ 0.5 cm (SEM)
right 1.3 $\pm$ 0.6 cm (SEM)
Distance separating the two d_3 fields at 6 cm from the occipital protuberance: 3.2 $\pm$ 0.2 cm (SEM)
at 12 cm from the occipital protuberance: 1.1 $\pm$ 0.1 cm (SEM)

APPENDIX III CHARACTERISTICS OF LATERAL MOTOR NERVE (l_2) FIELD

Animal #	Left l_2 Area (cm^2)	Width of Muscle Field at 6 cm from Occiput
196	19.8	1.9 cm
487	17.6	1.8
488	15.8	3.1
499	24.8	3.3
500	22.6	3.3
501	24.3	2.2
504	16.3	2.2

(n=7)

Area of the left l_2 muscle field: 20.2 ± 1.4 cm^2 (SEM)

Width of the field at 6 cm from the occipital protuberance: 2.5 ± 0.3 cm (SEM)

APPENDIX IV NUMBER OF CELLS IN THE CIM MOTONEURON POOL

Animal #	Total # Cells	Left (L). Right (R)
274	1045	L
279	1046	L
285	1446	L
287	1193	L
360	1070	L
447	1094	L
448	1389	L
449	1183	L
450	1290	L
451	1234	L
452	1009	L
466	1221	L
468	>725*	L
469	1144	R
474	>853*	L
475	>902*	R
495	1112	L
498	1274	R

* Missing sections of spinal cord

(n=15)

Total # of CIM motoneurons: 1183 ± 33 (SEM)
Length of the CIM pool: caudal C6 to rostral T1 (5200 µm)
Dorso-ventral depth of the CIM pool: 440 µm

APPENDIX V MULTIPLE HRP INJECTIONS INTO THE MUSCLE

Animal #	Location of Injections (cm from Occiput)	µl of HRP	# of Labeled Cells	Location; Organization
L8	3-4 cm	50	114	C6-C8; columnar, medial, mostly dorsal
L1	5-6	50	139	C6-C8; columnar, medial, ventral
372	6-7	4	251	C6-T1; columnar, lateral-medial, ventral-dorsal
373	6-7	5	82	C6-T1; columnar, medial, ventral to dorsal
L4	9-10	50	42	C6-C8; columnar, medial, ventral
L9	9-10	50	86	C6-C8; " , lateral-medial, ventral-dorsal
275	9-10	20	214	C7-C8; columnar, lateral-medial, ventral-dorsal
276	9-10	20	75	C6-C8; " " "
277	9-10	20	161	C6-C8; diffuse, " "
388	10	8	70	C7-T1; " " "
390	10	7	39	C7-T1; columnar, " "
385	13	10	39	C7-C8; mostly columnar, lateral-medial, ventral-dorsal
386	13	10	59	C7-T1; mostly columnar, lateral-medial, ventral-dorsal
387	13	10	65	C7-T1; mostly columnar, lateral-medial, ventral-dorsal
L7	12-13	50	95	C6-C8; mostly columnar, lateral-medial, ventral-dorsal
L10	12-13	50	57	C6-C8; mostly columnar, lateral-medial, mostly ventral

(n=16)

APPENDIX VI SINGLE HRP INJECTIONS INTO THE MUSCLE

Animal #	x,y Location of Injection (cm)	µl of HRP	# of Labeled Cells	Location; Organization
510	7; 1.0	2	48	C6-T1; columnar, medial, dorsal
511	"	"	42	C7-C8; " " "
512	"	"	46	C7-C8; " " "
516	10; 1.0	2	35	C7-C8; columnar, medial, ventral
517	"	"	42	C7-C8; " " "
518	"	"	17	C8 ; " " "
415	13; 1.0	2	55	C7-C8; columnar, lateral, ventral
416	"	"	>33	C7-C8; columnar, lateral to medial, ventral
522	"	"	43	C7-C8; " " "
412	7; 2.0	2	>33	C8-T1; columnar, medial, dorsal
527	"	"		
384	9; 2.5	"	23	C7-T1; columnar, lateral to medial, dorsal
395	10; 2.0	"	10	C8 ; columnar, medial, dorsal
396	"	"	16	C7-C8; " " "
391	13; 2.0	2	17	C7-C8; columnar, lateral, ventral
392	"	"	16	C8 ; columnar, lateral, dorsal to ventral
414	7; 4.0	2	36	C8-T1; columnar, lateral, dorsal
526	"	"	51	C7-T1; " " "
397	10; 4.0	2	11	C8-T1; columnar, lateral, mostly dorsal
398	"	"	4	C8 ; grouped, lateral to medial, dorsal
393	13; 4.0	2	9	C8 ; columnar, mostly lateral, ventral
394	"	"	10	C8 ; columnar, mostly lateral, mostly dorsal
417	"	"	11	C7-C8; columnar, lateral, dorsal

continued on next page

APPENDIX VI (continued)

Animal #	x,y Location of Injection (cm)	µl of HRP	# of Labeled Cells	Location; Organization
513	7; 5.0	2	20	C7 ; columnar, lateral, dorsal
514	"	"	54	C7-T1; columnar, lateral, dorsal
515	"	"	48	C7-T1; columnar, lateral, mostly dorsal
519	10; 5.0	2	38	C7-C8; columnar, lateral, dorsal
520	"	"	21	C8-T1; " " "
521	"	"	32	C8-T1; " " "
523	13, 5.0	2	19	C7-C8; columnar, lateral, mostly dorsal
524	"	"	16	C7-T1; " " "
525	"	"	>9	C7-C8; " " "

(n=16)

APPENDIX VII HRP DIFFUSION STUDIES

Animal #	x,y Location of Injection (cm)	Distance from Border of Field	# of Labeled Cells
528	4; 2.0	1.0 cm from l_1	12
529	4; 1.0	1.0 cm from l_1	none
530	4; 0.5	1.5 cm from d_3	none
531	6; 5.0	2.0 cm from d_3	none

(n=4)

APPENDIX VIII HRP LABELING OF THE DORSAL MOTOR NERVES

Animal #	Nerve	EMG Map Done	# of Labeled Cells	Location; Organization
478	d_1, d_2, d_3	no	805	C7-T1; columnar, lateral to medial, dorsal to ventral
479	"	"	369	C6-T1; " " "
306	d_1, d_2	"	305	C6-C8; columnar, medial, ventral
307	"	"	470	C6-C8; " " "
308	"	"	>289	C6-C8; " " "
309	"	"	637	C8-T1; diffuse, medial, dorsal to ventral
322	"	"	398	C8-T1; $\pm$ columnar, medial to lateral, dorsal to ventral
323	"	"	292	C6-T1; $\pm$ columnar, lateral, mostly ventral
427	d_1	"	23	C7-C8; columnar, medial to lateral, mostly dorsal
428	"1	"	165	C7-T1; columnar, medial, dorsal to ventral
429	d_2	"	$\geq$ 16	C7-C8; columnar, medial, mostly dorsal
430	"2	"	$\geq$123	C7-T1; no organization
364	d_3	"	>128	C8-T1; diffuse, medial, dorsal to ventral
365	"3	"	187	C8-T1; " " "
366	"	"	181	C8-T1; " " "
367	"	"	251	C7-T1; " " "
369	"	"	181	C8 ; $\pm$ columnar, medial to lateral, dorsal to ventral
444	"	"	111	C8-T1; " " "
480	"	yes	55	C8-T1; columnar, medial to lateral, dorsal
481	"	"	123	C7-T1; columnar, medial to lateral, dorsal
483	"	"	49	C7-T1; " " "
502	"	"	21	C7-T1; columnar, medial, dorsal

(n=22)

APPENDIX IX HRP LABELING OF THE LATERAL MOTOR NERVES

Animal #	Nerve	EMG Map Done	# of Labeled Cells	Location; Organization
339	l_1, l_2	no	418	C7-T1; columnar, lateral, ventral
460	"	"	296	C7-T1; " " "
472	"	"	468	C7-T1; " " "
473	"	"	251	C7-T1; " " "
476	"	"	217	C7-T1; " " "
471	"	"	788	C7-T1; " " "
338	l_1	"	>134+	C8 ; diffuse, medial, dorsal to ventral
420	"	"	298	C6-T1; columnar, lateral, dorsal to ventral
431	"	"	> 49+	C8-T1; columnar, lateral, ventral
432	"	"	> 12*	C8-T1; columnar, lateral, ventral
419	l_2	"	125	C7-T1; columnar, lateral, ventral
433	"	"	> 20*	C7-T1; columnar, lateral, ventral
439	"	"	239	C6-T1; columnar, lateral, dorsal to ventral
477	"	"	> 66+	C6-T1; columnar, lateral, dorsal to ventral
487	"	yes	> 45*	C6-T1; columnar, lateral, mostly dorsal
488	"	"	> 38+	C7-T1; columnar, lateral, ventral
499	"	"	195	C7-T1; " " "
500	"	"	92	C7-T1; " " "
501	"	"	165	C7-T1; columnar, lateral, mostly dorsal

(n=19)

* Missing sections of the spinal cord

+ Cells faintly stained with HRP

APPENDIX X HRP LABELING OF THE VENTRAL MOTOR NERVES

Animal #	Nerve	EMG Map Done	# Labeled Cells	Location; Organization
375	V_1,V_2	no	123	C6-C8; columnar, medial to lateral, dorsal
376	"	"	185	C6-T1; columnar, medial, dorsal
421	"	"	280	C7-T1; columnar, lateral, dorsal
435	"	"	> 50*	C7-C8; columnar, lateral, ventral
436	"	"	>157*	C7-T1; " " "
486	V_1	yes	182	C7-T1; columnar, lateral, ventral
489	V_1	yes	171	C7-T1; columnar, lateral, dorsal

(n=7)

* Missing sections of the spinal cord.

APPENDIX XI MOTOR NERVE TWIGS

Animal #	x,y Location of Twig* (cm)	Length & Width of Twig Field (cm)	Area of Twig Field (cm^2)	# Labeled Cells	Location; Organization
409	6; 1.5	7.5 x 1.4	8.0	27	C8; columnar, medial, ventral
410	6; 2.0	9.0 x 1.5	9.9	31	C8; " " "
383	9; 2.5	-	-	15	C8-T1; columnar, lateral to medial, dorsal
490	9; 3.0	7.4 x 1.6	9.1	28	C7-T1; columnar, lateral to medial, ventral
403	10; 2.0	4.5 x 1.6	5.7	17	C7-C8; columnar, lateral to medial, ventral
404	10; 1.0	6.5 x 1.5	7.1	8	C7-C8; columnar, medial, ventral
405	10; 1.0	5.0 x 1.3	4.7	32	C7-C8; columnar, medial, ventral
491	11; 1.5	5.0 x 1.5	5.3	13	C7-C8; columnar, lateral, ventral
409	7; 4.5	7.1 x 1.9	9.1	-	-
410	8; 3.0	7.0 x 1.5	8.7	-	-
504	10; 4.0	4.5 x 1.4	4.7	-	-

(n=11)

* See text, Figure 35

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