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CUTANEOUS REFLEX PATHWAYS AND THEIR SEGMENTAL CONVERGENCE IN THE INTACT AND CHRONICALLY LESIONED SPINAL CORD

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by

Lisa A. LaBella

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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for both your labors and friendship
with me over the last several years.

- Lisa

ABSTRACT

The present study characterized a cutaneous reflex pathway in the anesthetized, intact, adult cat spinal cord, and investigated its integration with other segmental reflex paths. This analysis was repeated in the adult chronic spinal preparation (6-7 weeks post-transection at L_1-L_2), as an approach to understanding the neuronal basis of paraplegia-induced cutaneous hyperreflexia.

(1) The pattern of short-latency excitatory and inhibitory synaptic input from sural afferents to 115 triceps surae (TS) motoneurons in 10 intact animals was determined with intracellular recording methods. The results revealed a differential sural input to the 3 ankle extensor motor nuclei, with excitation predominating in the medial gastrocnemius (MG) portion.

(2) The technique of spatial facilitation was used to determine whether interneurons in the excitatory sural pathway to MG are accessed by other segmental afferents (70 MG, 12 animals). The results suggest a relatively restricted convergence by nerves with receptive fields near the MG muscle, and with the caudal cutaneous femoral nerve in particular.

(3) Sural synaptic input to 122 TS motoneurons was re-examined in 9 chronic spinal animals to determine the integrity of its distribution after chronic spinal transection. The results show a qualitative integrity of synaptic organization, with evidence for a quantitative enhancement in interneuronal transmission.

(4) Spatial facilitation of excitatory cutaneous pathways was re-examined in 8 chronic spinal animals, in order to explore pathway interaction after a prolonged absence of the descending systems which normally coordinate their activity. Again, the results suggest an integrity of synaptic organization coupled to enhanced synaptic transmission; expressed here as increased convergence of segmental pathways onto common interneurons.

These results provide a solid basis for testing existing theories of neuronal plasticity in poly-synaptic segmental reflex pathways, and suggest that cutaneous hyperreflexia is operating through a spinal network whose organization is highly conserved after chronic spinal lesions.

GENERAL INTRODUCTION

In contrast to our knowledge of the segmental reflex effects produced by muscle receptors, there is a very poor description of those evoked from receptors of cutaneous origin. This is largely because of the difficulty in studying reflex pathways which are at a minimum disynaptic, and usually multi-synaptic (see Baldissera et al. 1981; Fleshman et al. 1988; Lundberg 1975), and because cutaneous afferents are not easily separated into modality-specific categories with different strengths of electrical stimulation (Hunt and McIntyre 1960). Consequently, there is little data available on the specific interneuronal pathways mediating cutaneous reflexes, and so on the integration of cutaneous reflex pathways among themselves, as well as with other segmental, propriospinal, and supraspinal descending systems. This information is necessary to appreciate the role of cutaneous reflex pathways during behavior, and it is also necessary if we are to identify mechanisms which underly altered cutaneous reflex function after central nervous system lesions. Experiments in the first two chapters of this thesis employ electrical stimulation of hindlimb cutaneous afferents for intracellular (motoneuron) recording of cutaneous reflexes in the anesthetized spinal cord-intact adult cat preparation. Electrical stimulation is used in order to achieve a preliminary characterization of a cutaneous reflex system in the spinal cord, and its convergence with other segmental reflex pathways. Similar experiments described in the second two chapters are performed in chronic spinal animals, in order to investigate the neural basis of cutaneous hyperreflexia which presents caudal to chronic spinal lesions in both animal and man. The following *Introduction* is intended to provide some background to our present understanding of the central organization of cutaneous reflex pathways, in particular those arising from the sural nerve and projecting to triceps surae motoneurons.

Peripheral afferents projecting to the central nervous system comprise the first component of segmental reflex pathways to motoneurons, and in the case of polysynaptic pathways, spinal interneurons comprise the second. However, reflexive behavior is limited in the intact adult animal (or man), and Sherrington's early suggestion that a "*simple reflex is probably a purely abstract conception .... a convenient, if not probable fiction*" (Sherrington 1906) reflects his astute hypothesis that segmental reflex pathways function as integrated components of a complex spinal motor system. Indeed it appears that segmental reflex pathways act as substrates for higher levels of motor control, and that purposeful and coordinated movements are achieved through descending control of spinal interneurons in the reflex paths. The question arises as to how multi-sensory afferent input is utilized in the selective control of particular reflex pathways. One possible organization is to have sensory nerves, arising from different receptors on the same or different regions of the body, project to separate or "private" spinal pathways. These pathways could then be activated (or inhibited) independently. However, there is a great deal of evidence which favours the existence of "common" spinal pathways from diverse sources of peripheral afferents. By studying the acute spinal preparation, Sherrington (1910) noted that the dominant action of many peripheral afferents (including cutaneous) was that which promoted a "flexion reflex" (ipsilateral excitation of flexors and inhibition of extensors; contralateral excitation of extensors and inhibition of flexors). Eccles and Lundberg (1959) further described the participating afferents as coming from cutaneous, joint, and high threshold muscle receptors, and collectively referred to these afferents as the "FRA" (flexor reflex afferents; see also Holmqvist and Lundberg 1961; Lundberg 1972; Lundberg 1979). It was logically assumed that the FRA

could exert their common action through shared interneurons, and so the concept emerged of polysynaptic segmental pathways shared by several classes and sources of afferent fibers. However, while the flexion reflex is powerful in the acutely transected spinal preparation, it is not the only action of FRA afferent stimulation, and so reflex pathways arising from these same afferents which promoted the alternate pattern of activity (for e.g. ipsilateral excitation of extensors) were called "alternate FRA" pathways (see Lundberg 1972; 1979; Lundberg et al. 1987). Perhaps the most important general feature of FRA pathways (which distinguishes them from many other known reflex pathways) is that a *convergent* afferent input to interneurons from a variety of muscle, joint and skin receptors from a wide areal source, leads to a wide *divergence* of effects on muscles throughout the limb. However, this converging upon, and diverging from spinal interneurons does not negate the possibility for more "private" spinal pathways, nor does it disallow the opportunity for more discrete reflex actions by afferents within the FRA; it only complicates simplifying generalizations of how spinal integration is achieved. It is worth noting that the term "FRA" has revealed itself to be somewhat of a misnomer; investigators have frequently assumed that these afferents mediate a flexion reflex. The point is that the afferents are able to support behaviorally appropriate patterns of muscle activation by producing similar effects in motoneurons; and the neural circuitry for this is minimized by afferent convergence onto common interneurons.

Thus, while the FRA concept has provided a much needed theoretical framework with which to consider the problem of cutaneous (and other) reflex organization, it does not provide a complete description of segmental pathways arising from peripheral afferents which participate in the FRA. For example, Sherrington observed that pressure on the dog toe pads could elicit an extensor thrust, while

nociceptive stimulation of the pads resulted in limb flexion (Sherrington 1903). More recent studies have demonstrated specific reflex actions of peripheral afferents in restricted groups of motoneurons (e.gs. Eccles and Lundberg 1959; Engberg 1964; Hagbarth 1952; Holmqvist and Lundberg 1961). While it is conceptually attractive to suppose that such special reflex effects comprise private pathways which are entirely segregated from the FRA (or other segmental) spinal systems, there is little evidence that such an organization exists. Determining the true extent of privacy in a polysynaptic pathway would require testing for all possible convergent inputs at each interneuronal relay. It is also possible that special reflex effects in particular motor pools are achieved by having portions of interneuronal pools with widespread convergent input (like those in FRA pathways) project to relatively few motoneurons (unlike those in the FRA); i.e. there may be subpopulations of FRA interneurons which mediate more specific reflex actions.

Early on there was reason to suppose that reflex effects in motoneurons evoked by low (electrical) threshold cutaneous afferents had a role distinct from the FRA-like effects evoked by high threshold cutaneous afferents, when Lundberg and Voorhoeve (1962) showed that cortical stimulation could selectively facilitate excitation of extensor motoneurons produced by low threshold cutaneous afferents (see also Hongo et al 1966; Lundberg 1975); although the division of cutaneous effects into those evoked from low and high threshold afferents has been somewhat arbitary at best. An interesting pathway to consider in the context of "special cutaneous reflexes", is the short-latency (first 10-30 milliseconds) pathway from low threshold afferents in the caudal cutaneous sural nerve (CCS) to triceps surae motoneurons. This cutaneous pathway can produce excitation and/or inhibition in these ankle extensor motoneurons, with inhibitory

postsynaptic potentials (IPSPs) always occurring subsequent to the excitatory postsynaptic potentials (EPSPs) when mixed effects appear. However, characteristics of this pathway have been reported which may suggest it mediates relatively specialized reflex actions. Within the medial gastrocnemius (MG) motor nucleus, motoneurons innervating fast-twitch muscle fibers were found to receive a predominance of excitation from CCS, whereas those innervating slow-twitch fibers were predominantly inhibited (Burke et al. 1970; Burke et al. 1973b; Kanda et al. 1977). Soleus (SOL) motoneurons, which innervate slow-twitch fibers exclusively, also were predominantly inhibited (e.g. Burke et al. 1970). It has been tacitly assumed that the pattern of CCS PSPs in lateral gastrocnemius (LG) motoneurons is similar to that of MG since it is also a functional synergist and like MG, consists of fast and slow motor units (Burke et al. 1973a). Thus, the CCS pathway to triceps surae has been of considerable interest to investigators, since the predominance of excitation in "fast" extensor motoneurons revealed a new level of complexity in the organization of cutaneous reflex pathways to motoneurons and potentially challenges the concept that motor unit recruitment always begins with the slowest, and ends with the fastest motor units (see Henneman and Mendell 1981). Of interest here is that the CCS pathway to ankle extensor motoneurons may mediate highly specialized reflex effects, since it has not been demonstrated that a fractionation of reflex effects on the basis of motor unit type is a general feature of either cutaneous or FRA synaptic organization.

Because reflex pathways do not function independently, knowledge of central convergence upon a given pathway is a step towards elucidating its integrated role during movement. For example, a tendency for either pyramidal tract (Endo et al. 1975) or red nucleus (Hongo et al. 1969) stimulation to produce EPSPs in fast, and IPSPs in slow ankle extensor motoneurons, led to the suggestion that descending

systems may access interneurons in the CCS pathway to override normal recruitment patterns, and selectively activate higher force-producing/faster contracting extensor motor units (cf. discussion Pinter et al. 1982); perhaps during galloping or jumping, or at the onset of these movements, or perhaps during rapid alternating movements such as paw shake. It has been suggested that the preferential inhibition of slow units may be a mechanism to reduce residual tension and so hasten the development of flexor antagonist tension in rapid alternating movements. Pyramidal tract (Lundberg and Voorhoeve 1962; Pinter et al. 1982) and red nucleus (Pinter et al. 1982) convergence upon the CCS pathway to MG motoneurons has been demonstrated, but preferential facilitation of EPSP (compared to IPSP) components was only demonstrated for pyramidal conditioning at extremely low thresholds of CCS stimulation. This may indicate that low and high threshold CCS pathways involve separate sets of interneurons, and that only interneurons in the former pathway are utilized by higher centers for selective activation. It is of interest in this connection that there is previous evidence that low threshold cutaneous afferents have reflex effects in extensor motoneurons distinct from their role in the FRA (Hagbarth 1952; Holmqvist and Lundberg 1961; Engberg 1964).

Allowing that other descending pathways may also have excitatory convergence on the CCS pathway (the vestibulospinal pathway from Dieter's nucleus for example; see ten Bruggencate and Lundberg 1974), it is worth exploring the extent of its segmental convergence with other peripheral afferents. Since reflex effects of other cutaneous inputs to triceps surae motoneurons do not seem to be as tightly coupled to motor unit type (Burke et al. 1970), it is possible that special CCS reflex effects in these motoneurons may be mediated by a relatively restricted set of segmental interneurons. Support for this idea stems from the early work of Sherrington, and later Hagbarth,

which suggested that reflexes evoked by cutaneous stimulation are influenced by the particular area of skin stimulated (Graham Brown and Sherrington 1912; Hagbarth 1952). In particular, Hagbarth showed that in acutely transected/decerebrate animals, stimulation of the skin overlying an extensor tended to facilitate stretch reflexes of that muscle, while stimulation of other skin areas tended to depress them. The latter effect was more in keeping with the flexion reflex-like effects commonly seen in this preparation. Indeed the CCS nerve was one of two major cutaneous nerves he ascribed to this "myotopic" effect of stimulating the skin over the ankle extensors. Flexor muscles on the other hand, contracted upon stimulation of skin spanning the entire limb; inhibited only by skin over their antagonist extensors. Hagbarth's tentative conclusion for the function of this organization was that it might allow for local withdrawal responses of extensors to nociceptive stimulation which did not necessarily require removal of the entire limb. A flexion reflex to remove the entire limb, however, could still be elicited from widespread sensory loci. It is possible then, that the CCS excitatory pathway to triceps surae has a "private" component which is rooted in this myotopic organization described by Hagbarth. If so, cutaneous afferents with excitatory convergence on the CCS pathway may be limited to those with near or overlapping receptive fields, such as the second nerve described by Hagbarth, the caudal femoral cutaneous nerve (CCF). It should be noted that Hagbarth suggested quite a different role for CCS excitation of extensors from that suggested by more recent investigators (described above).

In summary, then, a relatively high degree of privacy may be indicated for the CCS excitatory pathway to triceps surae motoneurons. While privacy in the inhibitory pathway cannot be excluded, CCS inhibition in these cells is more characteristic of pathways from the FRA, and thus may be mediated entirely via "FRA interneurons". There

are further indications that only the lowest threshold CCS afferents may be segregated from the FRA. It is also possible that afferent pathways arising from the ankle extensor muscles themselves may converge on interneurons in the "private CCS" pathway, as part of the same myotopic organization suggested by Hagbarth for cutaneous nerves.

In *Part I* of this thesis, intracellularly recorded short-latency PSPs evoked by low threshold CCS stimulation will be examined in antidromically-identified MG, LG and SOL motoneurons of anesthetized spinal cord-intact animals. This characterization of a cutaneous refex system among functionally related motor nuclei in the unlesioned preparation will be used for comparison in the chronically transected spinal preparation. Short-latency PSPs in these motoneurons evoked by low threshold lateral cutaneous sural (LCS) stimulation will also be examined, since we have preliminary evidence that these effects are primarily inhibitory, and may thus provide a contrast to CCS effects for examination in the lesioned preparation. In addition, motoneuron membrane properties will be measured to predict motor unit type (Zengel et al. 1985), in order to test for the presence of a type-distribution of sural reflex effects. This latter focus will comprise a minor component of this investigation.

A survey of convergence upon the excitatory CCS pathway to MG motoneurons will be examined in *Part II* of the thesis, and as far as we know, will represent the first study to systematically describe segmental convergence among multiple sources of cutaneous afferents. Because of the evidence this pathway might be at least partially segregated from the FRA, analysis of its convergence is an appropriate starting point for understanding the spatial relationships of different cutaneous reflex pathways (since analysis of a pathway predicted to have widespread convergence from many types of peripheral afferents

would not promote our understanding beyond what Lundberg's FRA hypothesis has already given us). As well, the broad assumption in the literature that this pathway conveys special cutaneous reflexes in extensor motoneurons makes an analysis of its convergence by other cutaneous afferents long overdue. Lundberg's technique of spatial facilitation (1975) will be utilized here as an indirect approach to estimate convergence from various hindlimb afferents, and though we will concentrate on facilitating postsynaptic effects in MG motoneurons, we will include measurements in LG and SOL as well. It is hypothesized (on the basis of the early studies of Hagbarth described above) that CCF will have a strong excitatory convergence upon the CCS excitatory pathway to MG. Once patterns of convergence have been identified, future experiments (not part of the present thesis) may concentrate on identifying the responsible interneurons.

Experiments in *Part III* will essentially be a repeat of those conducted in *Part I* but in animals which are 6-7 weeks post-transection of the spinal cord at the L_{1-2} segmental level. This study is motivated by our ongoing interest in "hyperactive reflexes" which present caudal to chronic spinal lesions, the mechanism of which has eluded investigators at least since the turn of the century. The characterization of postsynaptic effects in *Part I* will allow a systematic comparison of postsynaptic effects in triceps surae motoneurons of chronic spinal animals, and the highly specialized nature of CCS synaptic organization, in comparison to LCS reflex effects, may reveal differential changes among these two systems in response to a lesion of descending systems. Because extensor reflexes are generally slower to return than flexor reflexes after the "shock" of spinal transection (e.g. Denny-Brown 1966), this system should provide an adequate model for examining cutaneous reflex pathways which may have undergone plastic change.

Part IV of this thesis capitalizes on the results of all experiments described above, and reflects a new approach to understanding the pathophysiology of cutaneous reflexes present after spinal lesions. The analysis of segmental convergence upon the excitatory CCS pathway to MG will be repeated in chronic spinal animals, to assess the effect of chronic cord transection upon the interactive aspect of segmental reflex pathways. Again, the technique of spatial facilitation will be used to estimate the degree of convergence from multiple sources of (mostly) cutaneous peripheral afferents, and perhaps the "hyperactivity" in cutaneous reflex pathways caudal to spinal lesions is a function of altered and/or enhanced integration with other spinal pathways to motoneurons. However, only through exploration of convergence patterns in the unlesioned and chronically lesioned spinal cord can one begin to understand the mechanisms which eventually result in cutaneous hyperreflexia: the end-result of isolating the interactive cutaneous reflex network.

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PART I) A Differential synaptic input to the motor nuclei of triceps surae from the caudal and lateral cutaneous sural nerves

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SUMMARY AND CONCLUSIONS

1. Postsynaptic potentials (PSPs) were recorded in 115 triceps surae motoneurons of 10 chloralose-anesthetized adult cats (spinal cord intact), upon electrical stimulation of the caudal and lateral cutaneous sural nerve branches (CCS and LCS, respectively).

2. With twice threshold (2T) stimulation of CCS, excitatory PSPs (EPSPs) were the predominant effect in 95% of all medial gastrocnemius (MG) motoneurons tested (min. central latency 1.5 ms; mean 2.4 ms). In only a few MG cells was the EPSP followed by an inhibitory postsynaptic potential (IPSP) and in only one cell was an IPSP the sole effect. Increasing the stimulus intensity to 5T tended to enhance both the later EPSP and IPSP components, with less change in the amplitude or latency of the earliest EPSPs.

3. In lateral gastrocnemius (LG) and soleus (SOL) motoneurons, 2T CCS stimulation led to either inhibition or no potential change in the majority of cells tested: EPSPs were the predominant effect in only 15% and 30% of LG and SOL cells, respectively (min. central latency 2.5 ms; mean 3.0 ms) and rarely occurred without subsequent inhibition. Again, increasing the stimulus intensity to 5T had more of an effect on later rather than earlier PSP components.

4. A predominance of depolarization in MG motoneurons but not in SOL motoneurons is in agreement with previous findings that CCS excitation is more powerful in "fast type" triceps surae motoneurons. However, the strong predominance of hyperpolarizing effects of CCS stimulation in the present LG population is evidence that such an organization does not transcend triceps surae motor nuclei as a whole.

5. Postsynaptic effects of LCS stimulation at 2T were frequently weak or absent but increasing the stimulus intensity to 5T produced predominant inhibition in 71% of all triceps surae motoneurons studied (n = 107). Of the few cells which did receive excitation from this nerve, most were MG, a few were SOL, and none were LG. These EPSPs occurred more frequently at 5T than at lower stimulation strengths.

6. The results indicate that excitation produced by electrical stimulation of the ipsilateral CCS nerve occurs preferentially in the MG portion of triceps surae and with the shortest central latencies. Effects of LCS stimulation are largely inhibitory throughout the motor nuclei comprising triceps surae but even here, the presence of excitation occurs more frequently in MG. A comparison of these results with those in other reports is discussed.

INTRODUCTION

There is substantial evidence that both the pattern of excitatory and inhibitory synaptic inputs, as well as the size of postsynaptic potentials (PSPs) in motoneurons, contribute to variations in recruitment order within single motoneuron pools. For example, it is well known that larger group Ia monosynaptic excitatory postsynaptic potentials (EPSPs) in "slow" compared with "fast" motoneurons (8,19,23), result in the earlier recruitment of slow motor units during stretch activation (3). However, in the case of many polysynaptic pathways to motoneurons, a reversal of this recruitment order would be predicted by the organization of interneuronal projections to the motor nucleus. Thus, preferential excitation of fast motoneurons (and preferential inhibition of slows) has been found upon stimulation of both descending and cutaneous (4,7,11,16,24,25,32-34) pathways to lumbar motoneurons. It has been proposed that convergence on common interneurons by descending and cutaneous pathways so organized (25, see also Ref.32), would allow for selective facilitation of the excitatory drive to fast motor units by higher centers, under conditions requiring modulation of normal force outputs.

The present investigation in animals with intact spinal cords was designed for a future comparative study of low threshold cutaneous reflexes from the caudal and lateral divisions of the sural nerve (CCS and LCS, respectively) in chronic spinal animals. The sural pathway to triceps surae motoneurons was chosen in particular because the tendency of sural afferents to inhibit slow and excite fast motoneurons of triceps surae (4,7,32) allows comparison of a relatively specific reflex action in the two preparations. To our knowledge, similar studies (2,29) have not reported reflex effects in motoneurons upon separate CCS and LCS stimulation, nor have they entailed separate

identification of lateral gastrocnemius (LG) and soleus (SOL) motoneurons. It might be expected that the LG nucleus with its mixed population of fast and slow motoneurons, would receive a differential synaptic input from sural like that for medial gastrocnemius (MG) motoneurons. However, as the present report with chloralose-anesthetized spinal cord intact animals will show, CCS inhibition tends to dominate in LG motoneurons (as it does in SOL motoneurons), regardless of motor-unit type. LCS effects are largely inhibitory throughout triceps surae, with some excitation which occurs mainly in MG.

Although there are known qualitative and quantitative differences in heteronymous monosynaptic Ia input to MG, SOL and LG motor nuclei (12), the present study indicates a difference in multisynaptic cutaneous afferent input as well. This aspect of sural synaptic organization, dependent perhaps on the mediolateral placement in the limb of the particular triceps muscles, must then also be considered for a complete understanding of cutaneous reflexes in these functionally related motoneuron groups. Some of these results have been presented in abstract form (26).

METHODS

Experiments were performed on 10 adult cats of either sex, ranging from 2.3 to 3.0 kg in weight. All experiments were conducted with the animals under chloralose anesthesia and with no lesions of the central neuraxis. An L4-6 laminectomy exposed the spinal cord for intracellular recording and peripheral nerves were dissected in the left (ipsilateral) hindlimb.

Dissection

Animals were anesthetized with halothane delivered in a mixture of oxygen and nitrous oxide. A tracheotomy was performed and one femoral artery cannulated to monitor blood pressure. Intravenous cannulae were placed in forelimb veins for drug and fluid administration as well as for slow infusion of a glucose/bicarbonate buffer. Atropine (0.12 mg) was given subcutaneously and dexamethasone (4 mg) intravenously. Several nerves of the left hindlimb were cut and dissected free of surrounding tissue for subsequent stimulation on bipolar silver electrodes. MG, LG-SOL and SOL nerves were dissected for antidromic identification of motoneurons, and it was ensured that the SOL branch of the LG-SOL nerve was sufficiently dissected away to allow independent activation of SOL efferents.

The CCS nerve was cut at the point where it enters the fat pad of the popliteal fossa and sometimes consisted of more than one branch at this level. The LCS nerve was cut free at its point of entry into the biceps femoris muscle, or in rare cases where this perforation did not occur, it was cut along its distal course over the surface of this muscle (28). Additional nerves were cut and mounted to aid in locating motoneuron nuclei.

Experimental procedure

After completion of the L4-6 laminectomy, halothane was discontinued and replaced with intravenous chloralose (40-50 mg/kg initial dose, increased to a total dose of 60-80 mg/kg during the experiment). Arterial blood pressure and end tidal expired carbon dioxide were continuously monitored. After mounting the animal in a Göteborg type spinal frame (Transvertex Co., Ltd, Stockholm) the animal was paralyzed with gallamine triethiodide and artificially respired, and a bilateral pneumothorax performed. Mineral oil pools were made to prevent the hindlimb nerves and the exposed spinal cord from drying out. Esophageal and back pool temperatures were monitored and regulated by heating lamps.

Intracellular recording

Intracellular recordings from lumbar motoneurons were obtained using glass microelectrodes filled with 2M potassium citrate and having resistances of 2-5 megohms. The microelectrode recordings, as well as the cord dorsum and current monitor, were amplified and displayed on two oscilloscopes at different time bases and were photographed simultaneously. Cord dorsum records were obtained using a large diameter silver electrode placed on the dorsal surface of the mid to rostral L7 spinal cord and used to measure the latency of postsynaptic potentials recorded in motoneurons. Motoneurons were impaled in L7 or S1 and identified by antidromic activation from peripheral nerves. Constant current stimuli (0.2 ms duration) were applied to the peripheral nerves using electrically isolated stimulators (14). Potentiometers across the stimulator output on each nerve were adjusted so that one setting on the stimulator dial would deliver the same threshold of stimulation to all nerves. The individual thresholds for nerve stimulation were determined by the stimulus required to just produce a deflection in the cord dorsum electrode recording.

Data analysis

The intracellular effects in motoneurons were photographed and latency measurements taken from enlargements of the film. Averaged records were sometimes obtained and could be photographed or plotted. Care was taken not to classify reversed inhibitory postsynaptic potentials (IPSPs) as EPSPs by either injecting a constant depolarizing current into the motoneuron through the microelectrode, or by ascertaining that stimulation of some of the nerves produced substantial PSPs in the hyperpolarizing direction. Effects of extracellular fields were assessed by superimposing computer averages of the potential recorded just outside the motoneuron with intracellular records. PSP latencies were measured at the point where the intracellular and extracellular traces deviated.

In order to quantify the distribution of PSPs among the three triceps surae motor nuclei, enlarged photographic records of CCS PSPs were digitized using a digitizing tablet. Records consisted of 4 to 6 single sweeps on one frame of film, or averages of 8 or 16 sweeps. The areas of PSP records were calculated for the first 15 ms beginning from the onset of the PSP and are expressed in terms of mV*ms. Since most PSPs are likely a combination of IPSPs and EPSPs, "depolarizing" areas above baseline and "hyperpolarizing" areas below baseline reflect only the net effect of interacting conductances. It is also acknowledged that small changes in membrane potential will have a greater influence on IPSPs than EPSPs, and thus the size of hyperpolarizations reported here may not be an accurate measure of true IPSP size. Notwithstanding these limitations, this technique of quantifying PSPs is useful in comparing effects between cells. In a total of 11 cells (2 MG, 7 LG and 2 SOL) injected depolarizing current was needed to reveal reversed IPSPs, and thus hyperpolarizing areas were measured in these cells with the minimum injected current so required.

RESULTS

CCS effects in triceps surae motor nuclei

Fig. 1 illustrates intracellular records from MG, SOL and LG motoneurons following twice and five times threshold stimulation of the CCS nerve. The records in the left column are typical of the effects recorded in MG motoneurons and show predominant depolarization at both intensities of nerve stimulation. Note that increasing the stimulus from 2 to 5T led to an increase in the amplitude of the later, rather than the earliest EPSP components. The inhibition of an LG motoneuron in the middle set of records is also typical of CCS effects upon LG: little potential change at twice threshold, and moderate inhibition at higher stimulation strengths. While CCS stimulation often produced EPSPs, IPSPs, or no potential change in SOL motoneurons, the records at the right of Fig. 1 illustrate the commonly found mixed EPSP/IPSP in these cells, with a characteristic increase in the later inhibitory component as the stimulus was increased from 2 to 5T.

The left-hand column of Fig. 2 shows superimposed tracings of CCS PSPs in 89 motoneurons and contains one tracing per cell at each stimulus intensity indicated. Note the overwhelming presence of excitation in MG motoneurons (2A) at both 2 and 5T. In contrast, EPSPs occurring in LG motoneurons (2C) are usually followed by inhibition and the PSPs are relatively small compared with those in MG. PSPs in SOL cells were generally substantial and were either depolarizing or hyperpolarizing or both (2B). It is noteworthy that the weak CCS PSPs at 2T in LG were obtained from nine animals, whereas the few examples of large PSPs at this stimulation intensity were obtained in one animal which had relatively large cutaneous synaptic effects in all three motoneuron groups. Action- potential height was used as an index of membrane potential (21) and is reported as the average of the values

before and after collecting CCS PSP records. Table 1 lists the motoneuron action-potential amplitudes corresponding to PSP recordings in Fig. 2. The similarity of mean values in each motoneuron group suggests that differences in CCS effects between MG, LG and SOL were not likely due to differences in resting membrane potentials.

Variations in stimulation strength did not tend to significantly alter the general conformation of CCS PSPs though excitatory effects usually had a lower threshold (<1.5T) than inhibitory ones. Raising the stimulation intensity to 5T (see Fig. 2) tended to enhance later excitatory and inhibitory components, whereas the early-latency EPSPs rarely grew at these higher strengths. These results concur with those of Pinter and co-workers (32) who also found that early-latency CCS EPSPs in MG are maximal at the lower stimulation strengths and increases in stimulation intensity tend only to enhance the later components of the EPSPs. However, our observation that small PSPs in many LG motoneurons were not substantially enhanced by increasing the stimulus intensity to 5 or even 10T, suggests a weak synaptic input from CCS afferents to many LG cells, rather than a balance of depolarization and hyperpolarization resulting in little net potential change (36).

Predominant effects of CCS stimulation at 2T were estimated by measuring the area of CCS PSPs in each motoneuron and determining the relative amounts of de- and hyperpolarization present (Table 2A). Predominant excitation (>50% PSP area above baseline) was found in 95% of the MG motoneuron sample compared to only 15% of LG motoneurons and 30% of SOL. Conversely, only 5% of the records from MG had predominant inhibition (>50% PSP area below baseline), whereas this number increased to 66% for LG and 48% for SOL. In addition, whereas approximately one fifth of LG and SOL cells received no measurable

effect upon 2T CCS stimulation, no MG cells in the quantitative analysis were without effect (see below). The areas of extracellular fields were not subtracted from the total area because their small size (mean area= 1.0 mV*ms, all depolarizing; n= 17) contributes little error to the reported effects.

Since low resting membrane potentials will disproportionately enhance inhibitory conductances, cells which showed evidence of substantial membrane potential depolarization were excluded from PSP area measurements. Eight cells (of the total 115) which had spike potentials which fell to or below 50 mV during recording of PSPs were rejected for area measurements, and the similarity of median and mean action-potential amplitudes (Table 2A) illustrates that few cells had spike potentials which fell below 60-65 mV. As well, the similarity of these values among the three motoneuron groups suggests that the comparison of synaptic effects between motoneuron species is not biased by large differences in the membrane potential. However, a subpopulation of the entire sample was chosen whose action potential heights were between 80 and 90 mV to test this possibility (see Table 2B). This excludes any cells in which injected current was needed to see reversed IPSPs. Even in this restricted sample there is a clear difference between MG and the other triceps surae motoneurons in the amount of CCS excitation produced; most strikingly between MG and LG (100 versus 13%).

To illustrate both the distribution and magnitude of predominant synaptic currents, predominant polarization (de- or hyper-) has been plotted against its percentage of the total PSP area for each cell in Fig. 3. Cells in which CCS stimulation produced areas >50% in the depolarizing direction are plotted above the abscissa, while cells >50% hyperpolarized appear below. The graph shows an overall predominance

of excitation in MG motoneurons and that depolarization was normally the only effect produced at 2T CCS stimulation in these cells (as depicted by the number of points appearing towards 100%). It is also clear from Fig. 3 that while CCS inhibition was the major effect in the majority of LG and SOL cells, the hyperpolarization was often mixed with depolarization (as depicted by the number of points scattered between 50 and 100%).

The data shows, in agreement with others (eg. 2,4,7,9,29,32,33,35,36,38) that low threshold CCS stimulation activates both excitatory and inhibitory pathways to ipsilateral triceps surae motoneurons. The inhibitory pathway, however, represents a minor input to MG motoneurons, a relatively major input to SOL motoneurons, and is the predominant source of CCS input to LG. The converse appears to be the case for low threshold excitatory pathways. While the unknown admixture of opposing synaptic currents prohibits an accurate assessment of excitatory versus inhibitory CCS input, the data suggests significant differences in CCS reflexes in the three motoneuron populations of triceps surae. This is particularly evident when comparing MG and LG.

Comments on CCS PSPs and motor unit type

Burke and co-workers (4,7) have reported that synaptic input to triceps surae from low threshold fibers in CCS appears to be organized according to motor unit type. In particular, they found a predominance of CCS inhibition in motoneurons innervating slow-twitch muscle fibers, and a trend towards CCS excitation in those innervating fast (7). In this regard, the effects reported here in SOL motoneurons which are exclusively slow in the cat (5,15) are of interest since 30% of the SOL sample received predominant excitation from 2T stimulation of CCS (refer to Table 2A). With regard to MG motoneurons, only 2 of 39 MG

cells received predominant inhibition from CCS stimulation, although more than a quarter of the MG motoneuron pool consists of cells which innervate slow-twitch muscle (6,15). In contrast, 27 of 41 LG motoneurons received predominant inhibition, despite that the percentage of slow LG motor units is in all probability the same as that for MG (6). The general patterns of PSPs were not significantly altered by increasing the stimulus strength to 5T as depicted in Fig.2, and the examples in Figs 1 and 6.

Criteria most recently established in Munson's laboratory (39) were used to assign motoneurons to categories of either "fast" or "slow" motor units. Table 3 illustrates several examples of membrane electrical properties, the resulting classification according to "type", and the predominant PSP in each cell upon both 2 and 5T stimulation of CCS. These examples illustrate that both fast and slow MG motoneurons are primarily excited by low threshold CCS stimulation, whereas many presumed fast-type LG motoneurons receive no excitation at all. The PSPs in the MG and LG motoneurons with asterisks in Table 3 are illustrated in Fig 1.

Latencies of CCS postsynaptic potentials

Latencies of the earliest 2T CCS PSPs recorded in all triceps surae motoneurons (n=115) are represented in the histogram of Fig. 4. Overall mean latencies for EPSPs and IPSPs in triceps surae were 2.6 and 4.3 ms, respectively. Within triceps surae, however, there is a clear tendency for EPSPs to occur at shorter latencies in MG motoneurons than in LG or SOL. The earliest such latency recorded in MG was 1.5 ms (mean latency 2.4 ms) while in both LG and SOL this minimum central latency was 2.5 ms (mean 3.0 ms for both). For the purpose of comparison then, the percentage of all cells with EPSPs occurring at a latency of <3.0 ms at 2T stimulation is 81% for MG and

14% for each of SOL and LG motoneurons, suggesting the presence of a relatively restricted short-latency excitatory pathway from CCS afferents to MG motoneurons. Latency distributions for the three motoneuron groups were not significantly different at 5T stimulation, and a minimum latency of 1.5 ms for CCS excitation in MG is consistent with other reports (e.g., Refs. 31 and 32).

IPSPs recorded in motoneurons as the earliest effect of 2T CCS stimulation had typical latencies of 3.0-5.0 ms, with the occasional IPSP occurring earlier or later than this (lower histogram, Fig. 4). In no instance was such an early-latency IPSP followed by subsequent depolarization and thus from the histogram one can see that pure hyperpolarization was recorded in only 1 of 43 MG cells as compared to 20 of 43 LG cells and 11 of 29 SOL cells.

As mentioned earlier, low threshold CCS PSPs of substantial magnitude were rarely observed in LG motoneurons except in one animal. In this particular animal the effects in all triceps surae motoneurons were relatively enhanced so that, for example, the early EPSP component in MG motoneurons was on average 1 to 2 mV larger than in most other preparations and uncommonly apparent in 3 LG cells at a maximum of 2.5 mV (refer to Fig. 2). Thus, it should be noted that all of the excitation occurring at latencies <3.0 ms in LG (upper histogram, Fig.4) was in this one preparation. In this same animal both excitatory and inhibitory effects were somewhat larger than usual in SOL, but IPSP amplitudes were normally quite large in SOL cells of all preparations. We have no obvious explanation for this variation in the amplitude of effects among preparations other than to propose different sensitivities in responses to anesthetic agents or surgical procedures.

Effects of LCS stimulation

In at least one other study which reported on the effects of sural stimulation in triceps surae motoneurons (4), the CCS and LCS branches were mounted and stimulated together (E. Jankowska, personal communication). However, since the LCS branch innervates a more proximal cutaneous receptive field than CCS (skin lateral to biceps femoris; our own observations), we have examined effects from the LCS branch itself on triceps surae cells.

Figure 2, D-F, illustrates superimposed traces of LCS PSPs in 80 motoneurons (from the same population in which CCS effects were recorded) and comprise one tracing per cell at both 2 and 5T stimulation. Table 1 indicates action potential amplitudes corresponding to PSP recordings traced in each panel, and as in the case for CCS PSPs, the similarity of mean action-potential amplitudes suggests that variations in LCS effects between motoneuron groups are not due to a skewed distribution of resting membrane potentials. While there is an overwhelming predominance of LCS inhibition in SOL and LG motoneurons at both stimulation intensities (2E and 2F), effects are more varied in MG cells (2D) and include a high incidence of "no effect" at 2T. This weak or absent synaptic input to MG from low threshold LCS afferents contrasts sharply with that from the CCS nerve (2A), though raising the strength of LCS stimulation to 5T increased the incidence of both excitation and inhibition. Effects at 2T stimulation in SOL and LG (2E and 2F) were usually small and when present, in the hyperpolarizing direction. Stimulation at 5T tended to increase the size of the inhibition in LG and most of the SOL motoneurons. Intracellular records of LCS PSPs in individual SOL and MG motoneurons are provided in Fig. 6.

Latencies of LCS postsynaptic potentials

Earliest latencies of LCS PSPs are reported for 107 motoneurons

and represented in the histograms of Fig.5. The upper panel in the figure illustrates the latencies of LCS PSPs produced at 2T stimulation, whereas the lower panel illustrates the same at a strength of 5T. Cells with effects at both stimulation intensities are thus represented in both panels. The mean latency of 2T LCS IPSPs is similar to that for CCS IPSPs (4.6 and 4.3 ms, respectively), and the longer mean latency of 5T LCS IPSPs (5.2 ms) is due to the addition of later IPSPs at the higher stimulation strength. Note the infrequent occurrence of excitation at both 2 and 5T stimulation and the wide range of latencies of these EPSPs (1.3 to 5.3 ms). This suggests that LCS synaptic input to triceps surae is unlikely to be organized according to motor unit type, and may resemble a pattern more typical of the flexion reflex, i.e., a generalized inhibition of ipsilateral extensor motoneurons which is more powerful at higher stimulation strengths (13).

A comparison of Fig. 5 and 2D shows that there are some MG cells with apparent effects at 5T that are not listed in the histogram of Fig. 5. This is because latencies were only measured when there was both a clear onset of the PSP and when the PSP was larger than 0.3 - 0.4 mV. Cells with effects failing to meet these criteria are listed as no effect in Fig. 5. and their inclusion would not alter the conclusion that in MG, CCS effects differ substantially from those of LCS.

Comments on LCS effects in triceps surae motoneurons

Despite the clear predominance of LCS inhibition in all triceps surae motoneurons with effects, MG motoneurons stand apart from LG and SOL once again. In only 3 of 107 cells was the first effect upon 2T stimulation excitatory, and all 3 of these cells were MG. In only 9 cells of 107 was the first effect upon 5T stimulation excitatory, and again, 6 of these 9 cells were MG. Unlike the situation with CCS

stimulation where only 1 of 43 MG cells was without effect at 2T, over half the population of MG cells tested showed no effect upon stimulation of the LCS nerve even at 5T (see inset Fig. 5). Thus MG motoneurons in the present preparation have a strong excitatory input from CCS afferents which contrasts with a weak, often inhibitory input from LCS. LG motoneurons on the other hand, were exclusively hyperpolarized upon LCS stimulation, as was the vast majority of SOL cells.

In terms of predominant effects, only 2 of the 8 cells with LCS excitation had EPSPs which were followed by hyperpolarizing potentials and in both cases the IPSP exceeded the EPSP in amplitude. The intracellular records from the SOL and MG motoneurons with this type of "mixed" effect are illustrated in the left columns of Fig.6, A and C, respectively. While LCS EPSPs in the other 6 cells were small (0.5-2.0 mV), even at 5T they remained the only effect in those motoneurons.

Of particular interest is the fact that low strength stimulation of the LCS nerve produced widespread inhibitory effects in the same sample of motoneurons receiving widespread excitation from CCS. This is particularly true in the case of MG where individual motoneurons frequently receive excitation from CCS and inhibition from LCS stimulation. In SOL motoneurons, which also received a strong excitatory input from CCS afferents, LCS EPSPs were present only when CCS EPSPs also occurred although the reverse was not true. An MG motoneuron with predominant excitation from CCS and mixed effects from LCS is shown in Fig. 6C. Figure 6A depicts a similar result of contrasting inputs for a SOL motoneuron, while the panel in 6B shows a more typical SOL motoneuron with inhibition from both divisions of the sural nerve. As mentioned earlier, while there were several

instances of depolarization evoked by CCS stimulation in LG (Fig. 2C) depolarizing effects of LCS stimulation in LG cells were not encountered. While these findings suggest the possibility of common interneurons in some pathways from LCS and CCS afferents to ipsilateral extensor motoneurons, they also emphasize the special character of the CCS excitatory pathway to MG motoneurons within this population.

DISCUSSION

The present results show that in the chloralose-anesthetized, spinal cord intact cat, electrical stimulation of the CCS nerve has different synaptic actions in MG, SOL and LG motoneurons. CCS stimulation consistently depolarized MG motoneurons while producing a variety of effects in SOL and LG. Many LG motoneurons had very weak effects, particularly at the lower stimulation strength (2T). Stimulation of the LCS nerve on the other hand, consistently hyperpolarized LG and SOL motoneurons, while producing a variety of mixed, often weak, effects in MG.

While the present results are in general agreement with others (2,4,7,9,29,32,33,35,36,38) that CCS stimulation has differential effects among triceps surae motoneurons, they do not strongly support a differential organization of excitation and inhibition in fast and slow motoneurons within nuclei. Although CCS IPSPs were often seen in slow motoneurons (eg., SOL) this correspondence was not absolute (see Table 3). It remains possible that a differential distribution was overlooked due to a sampling bias in the present material, as well as the tentative identification of motor units according to motoneuron membrane electrical properties. However, this would unlikely prove to be the case for LG given the infrequent incidence of EPSPs among 43 LG cells at 2T stimulation, and the relatively low amplitude of EPSPs at 5T which were usually followed by hyperpolarization. Furthermore, the potency of the excitatory pathway from CCS afferents to MG may be inferred from the fact that of the entire sample, only 1 MG cell was without effect and only 1 received "pure" inhibition. Overall, our results concur that excitation and inhibition are more powerful in the "fast" MG, and "slow" SOL motor pools, respectively.

In comparing the results presented here and elsewhere, it is important to keep in mind that polysynaptic effects in motoneurons are highly dependent on experimental conditions (e.g., Ref. 13), and preparations used in the studies to which we refer have employed different combinations of anesthesia, different parameters of afferent stimulation, as well as variations in the integrity of the spinal cord, brainstem and cerebrum. For example, three distinct types of synaptic effects with 5T sural stimulation in MG motoneurons in chloralose-anesthetized acute spinal cats have been reported (33). The majority of CCS effects in MG in our experiments fall largely into one (excitatory) category using a chloralose-anesthetized spinal cord intact preparation.

One factor which initially complicated a comparison of our results with those in other studies, was the question of precisely which peripheral afferents comprise the sural nerve. The nerve we refer to here as LCS (see Methods) was mounted and stimulated together with CCS in the experiments which first identified a type-distribution of sural input to triceps surae (4). It is now clear that inclusion of LCS would specifically enhance hyperpolarizing potentials (see Fig. 2) and many cells with mixed effects in this early study may actually have had a predominance of CCS excitation. However, Burke and co-workers later reported a strong type-distribution of PSPs among MG motoneurons with 5T stimulation of only the CCS nerve branch (7). Since they found similar results at lower stimulation strengths approaching 2T (R. Burke, personal communication) stimulation intensity is unlikely to account for observed differences in these and the present results.

Anesthetics may have differing influences upon the distribution of cutaneous PSPs, and while others (32,35) have noted that the type or presence of anesthesia had little effect on the distribution of CCS

PSPs, we have been unable to reach the same conclusion. In (unpublished) experiments in our laboratory where pentobarbital anesthesia was employed, low threshold CCS inhibition was present in many MG motoneurons, either as pure IPSPs or subsequent to earlier excitation, and only IPSPs were recorded in LG and SOL cells. One interpretation is that the actions of barbiturate agents involve a more potent depressant effect upon excitatory, rather than inhibitory interneurons in spinal pathways from peripheral afferents. Alternatively, barbiturate anesthesia may result in the release of a potent inhibitory pathway from CCS afferents to triceps surae motoneurons, which is somehow suppressed in the chloralose preparation. This release might involve a decrease in a tonic inhibitory effect of the dorsal reticulospinal system (DRS) which normally suppresses flexion reflex-like inhibition in these cells (18). Indeed, stimulation of the spinal cord region which contains the DRS was found to largely eliminate any CCS IPSPs in MG motoneurons (7). However, the presence of inhibitory synaptic input from LCS afferents in our chloralose study may contradict this explanation if the DRS has a widespread inhibitory influence upon cutaneous pathways to triceps surae motoneurons in general.

It is well established that cutaneous stimulation can produce excitation of extensor motoneurons in addition to the more general inhibition produced during the flexion reflex (eg., 10,17,22,38). Thus CCS (as well as LCS) excitation of ipsilateral extensor motoneurons seen in the present and other investigations, may be part of this more general observation. However, the consistent presence of EPSPs in MG motoneurons, variable effects in SOL, and variable, often weak effects in LG motoneurons with low threshold CCS stimulation (Fig. 2) suggests the presence of a more specialized excitatory pathway to the MG portion of these ankle extensor motor pools. The observation of a <2.5 ms

excitatory pathway to MG but not to SOL or LG (Fig. 4), supports the notion that at least some low threshold CCS pathways are segregated between these functional synergists. This then raises the question of whether or not CCS afferents projecting to triceps surae motoneurons impinge on separate sets of interneurons projecting to each of MG, LG and SOL. Such interneuron subpopulations might subserve specialization in cutaneous reflexes and/or the initiation of movement by centers higher in the neuraxis, since it is clear that descending motor systems can converge on interneurons in segmental (including cutaneous) pathways (1,20,27). However, the extent to which the differential distribution of CCS synaptic input to triceps surae reflects possible specialization in segmental reflexes, or acts as a substrate for integration with motor control systems in general, awaits further study. It is worth noting in this regard, that the three motor nuclei comprising triceps surae may show differential activity in behaving cat and man. In man for example, a selective inhibition of MG during certain rates of cyclic ankle plantar and dorsiflexion has recently been reported (30) while in the cat, SOL motoneurons are among the first recruited in most movements, but during rapid paw shakes only the gastrocnemius and not soleus motoneurons are active (37).

The presence of a small population of MG motoneurons (presumably slow) which do not receive input from the last-order interneuron in the low-threshold excitatory pathway from CCS has been postulated (see Fig. 6 in Ref. 32). However, with the present results, we are compelled to suggest that synaptic input to the MG nucleus from these afferents is not fractionated among MG motoneurons of different type per se. Because the presence of a differential CCS PSP distribution may indeed depend on the particular patterns of descending, segmental and CCS convergence which are active in a given preparation, it is possible that our particular preparation masks a strong type-distribution of CCS effects

while revealing a pattern which is more divided among the motor nuclei of triceps surae as a whole.

Fig. 7 summarizes the 2T CCS effects recorded in triceps surae motoneurons in the present investigation. The relative strength of synaptic input from the CCS nerve to the motoneuron pools of triceps surae is indicated by the number of "axons" from the last-order interneurons to the motoneurons (large circles). Excitation of motor nuclei by CCS decreases in the order MG, SOL, LG, whereas inhibition is most powerful in SOL and least powerful in MG motoneurons (see overall amplitude differences in Fig. 2). While we have chosen to illustrate a common set of interneurons mediating their effects via variable numbers of axon collaterals to the different motor nuclei, the present results can provide no evidence for or against this alternative. Such conclusions will have to await studies on the interposed interneurons themselves. The discussions in Ref. 20 and 32 should be consulted for evidence on the numbers of interneurons in these pathways. Figure 7 does not depict a "type" distribution of CCS PSPs within individual motoneuron pools. Should a latent type-distribution of CCS effects be revealed with modifications of the present preparation, the circuitry would be rendered much more complex. To give an example, there may be separate populations of inhibitory interneurons which have actions on SOL and MG motoneurons, especially if some descending relays are organized to allow selective activation of fast motor units. One might then postulate that it is only the actions of inhibitory interneurons which project to MG that were turned off in the present study. Further complexity may arise from the finding of shorter CCS EPSP latencies in MG and may indicate a segregation of excitatory pathways to the different motoneuron groups of triceps surae as well.

In summary, the results indicate that excitation produced by electrical stimulation of the ipsilateral CCS nerve occurs preferentially in the MG portion of triceps surae, and with the shortest central latencies. The LCS nerve branch was shown to have a very different pattern of actions throughout triceps surae motoneurons, although here too, excitation was found to preferentially occur in MG. Experiments are in progress to determine the extent to which there is convergence from other segmental afferents to interneurons in these pathways and whether the pattern of synaptic effects from these nerves is altered by chronic spinal transection.

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TABLE AND FIGURE LEGENDS

FIG 1. Typical effects of single-shock stimulation of the CCS nerve at two and five times threshold (T) recorded in ipsilateral triceps surae motoneurons. The *lower traces* in each panel are the cord dorsum recordings obtained during 5T stimulation, and the *upper two traces* the intracellular records. Each trace consists of four to five superimposed records. The species of the motoneuron is indicated above each panel. MG, medial gastrocnemius; LG, lateral gastrocnemius; SOL, soleus. Calibration pulse 2 mV, 2 ms.

FIG 2. The variety of postsynaptic potentials (PSPs) recorded in triceps surae motoneurons upon 2 and 5T stimulation of both CCS and LCS nerves. Enlarged photographic records of sural PSPs in individual motoneurons were traced and superimposed; one trace per motoneuron at each stimulation intensity in each panel (A-F). See Table 1 for numbers of motoneurons in each panel. The small vertical *arrow* indicates the time of arrival of the afferent volleys at the cord dorsum.

FIG 3. Predominant effects of 2T CCS nerve stimulation as assessed by measuring the areas of the first 15 ms of the PSP. Data from motoneurons where the depolarizing component of the PSP was >50% of the total PSP area are plotted in the *upper graph*; those with hyperpolarizing components >50% of the total area are plotted in the *lower*. The area of the predominant effect (*ordinate*) is plotted against its percentage of the total PSP area in that cell (*abscissa*). No PSPs had equal depolarizing and hyperpolarizing areas. Each letter refers to a measurement in one motoneuron (n = 107; M, MG;L, LG;S, SOL). For example, the M with an asterik (*) refers to a medial gastrocnemius motoneuron where the

depolarizing component area (28 mV ms) was ~ 93% of the total PSP area. Mean area measurement values for each motoneuron species are given in Table 2A.

FIG 4. Histogram of the earliest latency PSPs produced by single-shock stimulation of the CCS nerve at twice threshold in triceps surae motoneurons (n=115; abbreviations of motoneuron species as in Fig. 3). Only the earliest latency PSP is reported and thus only one effect per cell indicated. Latencies of EPSPs are given in the *upper* histogram and IPSP latencies in the *lower*. Effects were examined in 29 SOL, 43 LG and 43 MG motoneurons; cells with no PSPs, or PSPs below 0.3-0.4 mV, are listed in the *inset* as NE (no effect).

FIG 5. Latencies of LCS PSPs produced by 2T (*upper* histograms) and 5T (*lower* histograms) single-shock stimulation. Abbreviations as in Fig. 3. Cells in which PSPs were produced at 5T stimulation but not 2T stimulation appear in the *lower* histogram only. Cells in which PSPs were recorded following both 2 and 5T stimulation are plotted in both sets of histograms. There were 28 SOL, 43 LG and 36 MG cells examined. No effect (NE) in the *inset* refers to 5T stimulation.

FIG 6. Intracellular records from SOL (A and B) and MG (C) motoneurons upon stimulation of the CCS and LCS nerves at 2 and 5T. Depolarizing potentials in the uppermost traces in C were in part reversed IPSPs as revealed by the injection of 20 nA of constant depolarizing current through the microelectrode (*middle* and *lower* traces in C). The action potentials produced by 5T stimulation have been truncated. Calibration pulse 2 mV, 2 ms.

FIG 7. Schematic diagram of proposed connections between low-threshold CCS afferents and triceps surae motoneurons. *Large circles* represent motoneuron pools and *small circles* depict interneurons.

TABLE 1. Values are expressed as mean/median with the range in parentheses. Action-potential amplitudes of triceps surae motoneurons averaged from those recorded just before and after collecting CCS- and LCS-evoked PSPs. The table corresponds to PSPs depicted in Fig. 2 only; number of motoneurons correspond to panels A-F; A, n=31[29]; B,n=24; C,n=34; D,n=25; E,n=23; and F,n=32. One action potential/PSP tracing/motoneuron; square brackets indicate a slightly reduced n for CCS PSPs in MG at 5T. CCS, caudal cutaneous sural; LCS, lateral cutaneous sural; PSP, postsynaptic potential; MG, medial gastrocnemius; SOL, soleus; LG, lateral gastrocnemius.

TABLE 2. Areas were measured from the first 15 ms of the PSP produced by 2T stimulation of the CCS nerve and recorded in triceps surae motoneurons. Cells without CCS PSPs are not included in the mean, median, or range of values and are listed as "No effect". Table 2A presents values from the entire sample, whereas Table 2B is a subset of PSPs recorded in cells with action-potential heights between 80 and 90 mV. Depol, depolarization; Hyperpol, hyperpolarization; PSP, postsynaptic potential; CCS, caudal cutaneous sural; MG, medial gastrocnemius; SOL, soleus; LG, lateral gastrocnemius.

TABLE 3. The predominant type of PSP resulting from both 2 and 5T CCS nerve stimulation, i.e., the effect that comprised >50% of the PSP area, is presented in the rightmost column alongside measurements of some electrical properties of these cells. AHP,

afterhyperpolarization; R_{in}, input membrane resistance as measured by a 50-ms hyperpolarizing pulse (1-3 nA); I_{rh}, rheobase current as defined by the magnitude of a 50-ms depolarizing current pulse threshold for action-potential production; F, a presumed "fast" motoneuron; S, presumed slow; F/S, intermediate between fast and slow; NE, no effect. *Cells illustrated in Fig.1.

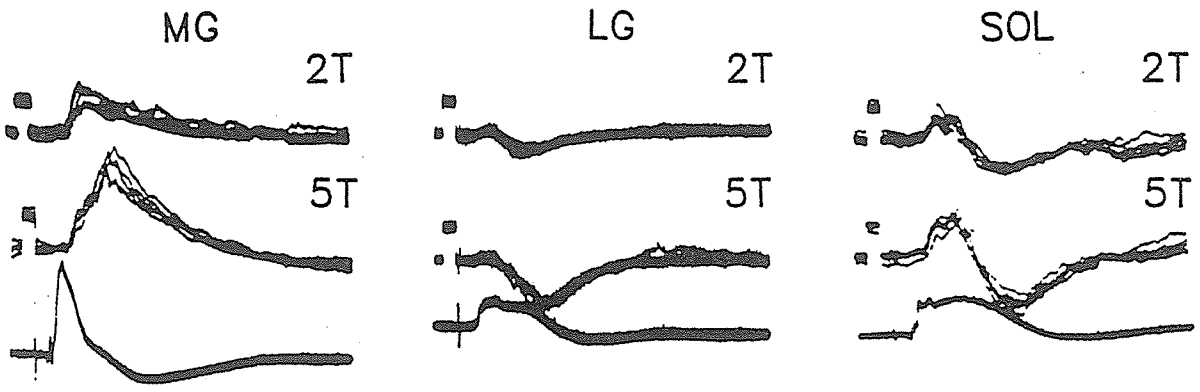

FIGURE 1

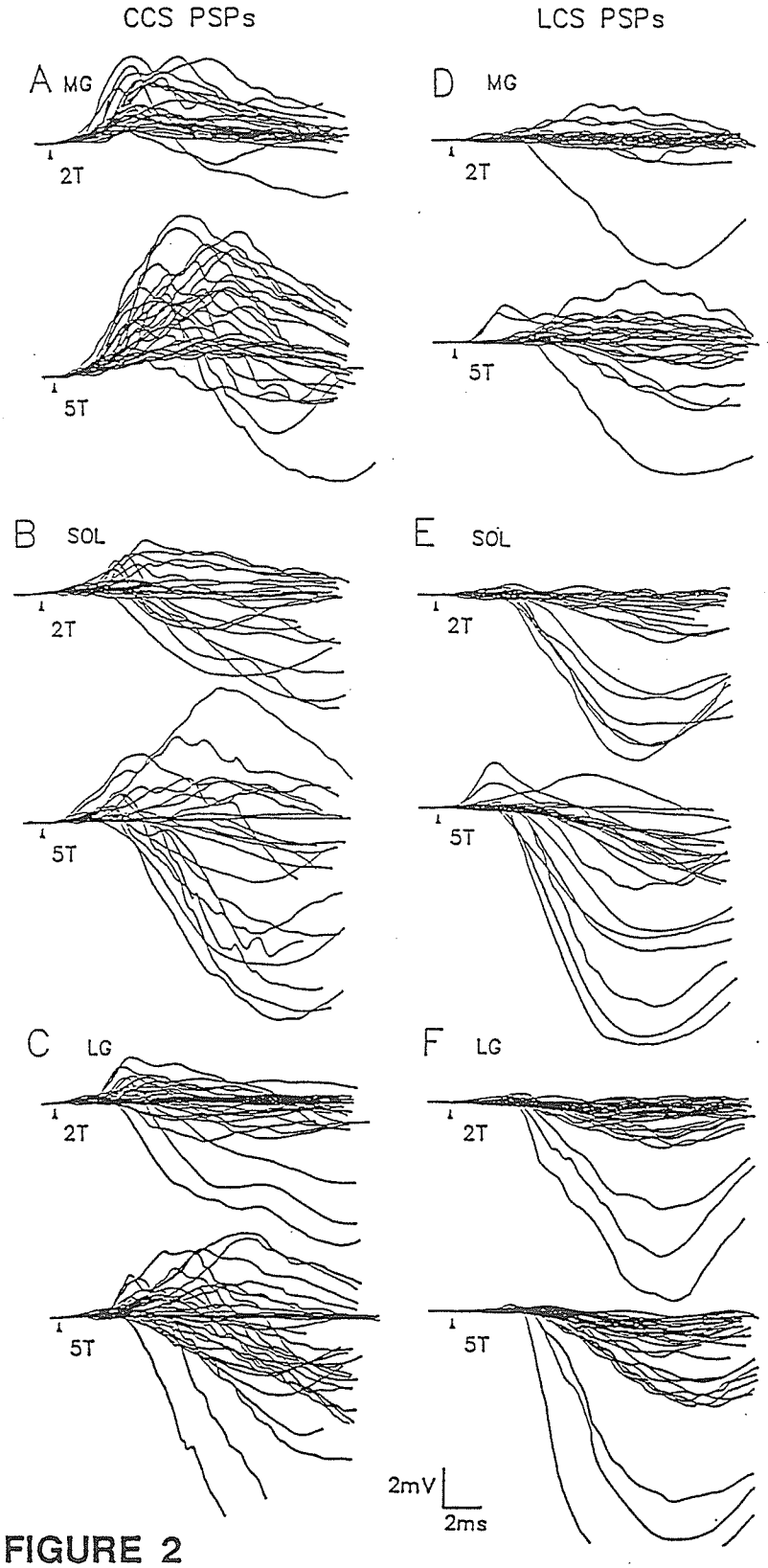
CCS PSPs
LCS PSPs
A MG
2T
5T
D MG
2T
5T
B SOL
2T
5T
E SOL
2T
5T
C LG
2T
5T
F LG
2T
5T
2mV
2ms

FIGURE 2

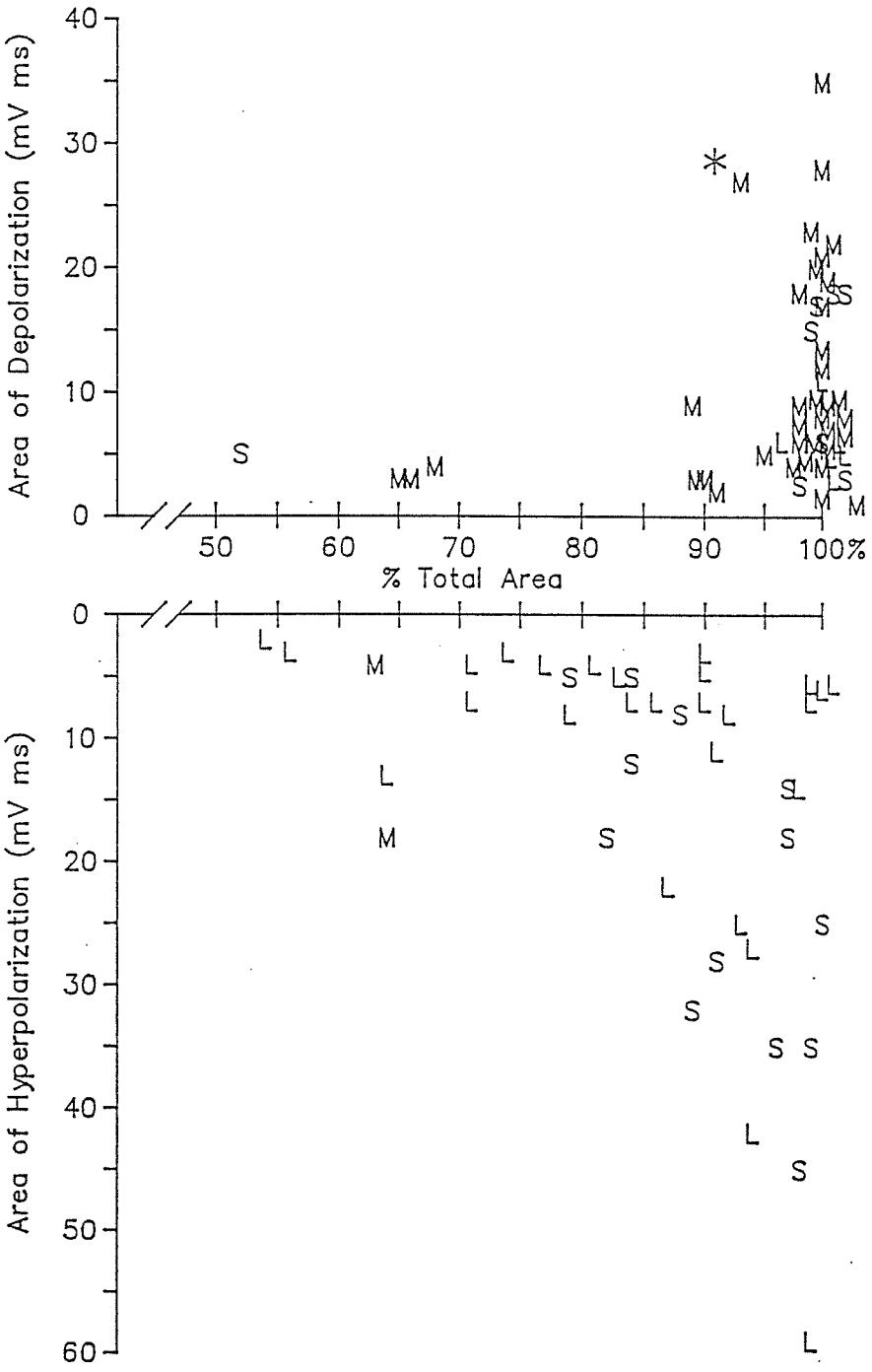
Area of Depolarization (mV ms)
40
30
20
10
0
50
60
70
80
90
100%
% Total Area
0
10
20
30
40
50
60
Area of Hyperpolarization (mV ms)

FIGURE 3

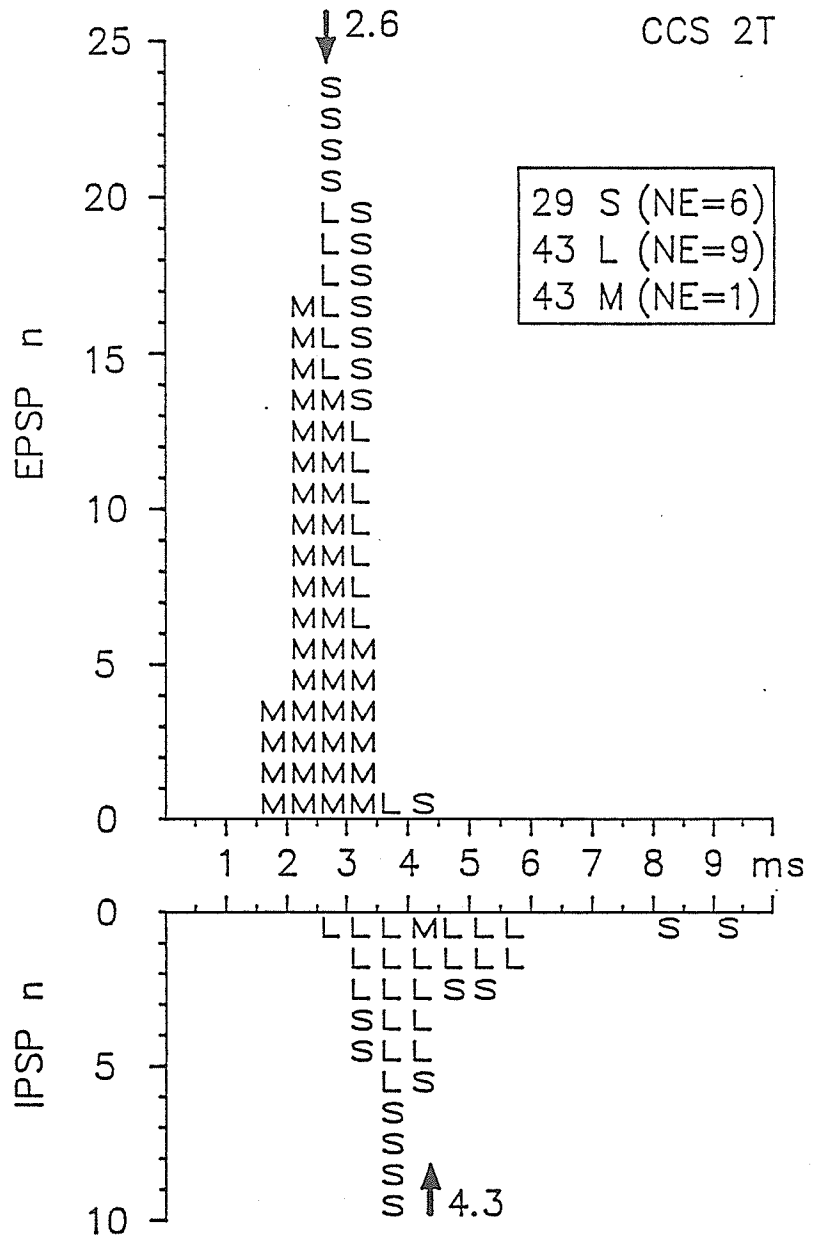
2.6
CCS 2T
29 S (NE=6)
43 L (NE=9)
43 M (NE=1)
EPSP n
IPSP n
25
20
15
10
5
0
0
5
10
1 2 3 4 5 6 7 8 9 ms
4.3

FIGURE 4

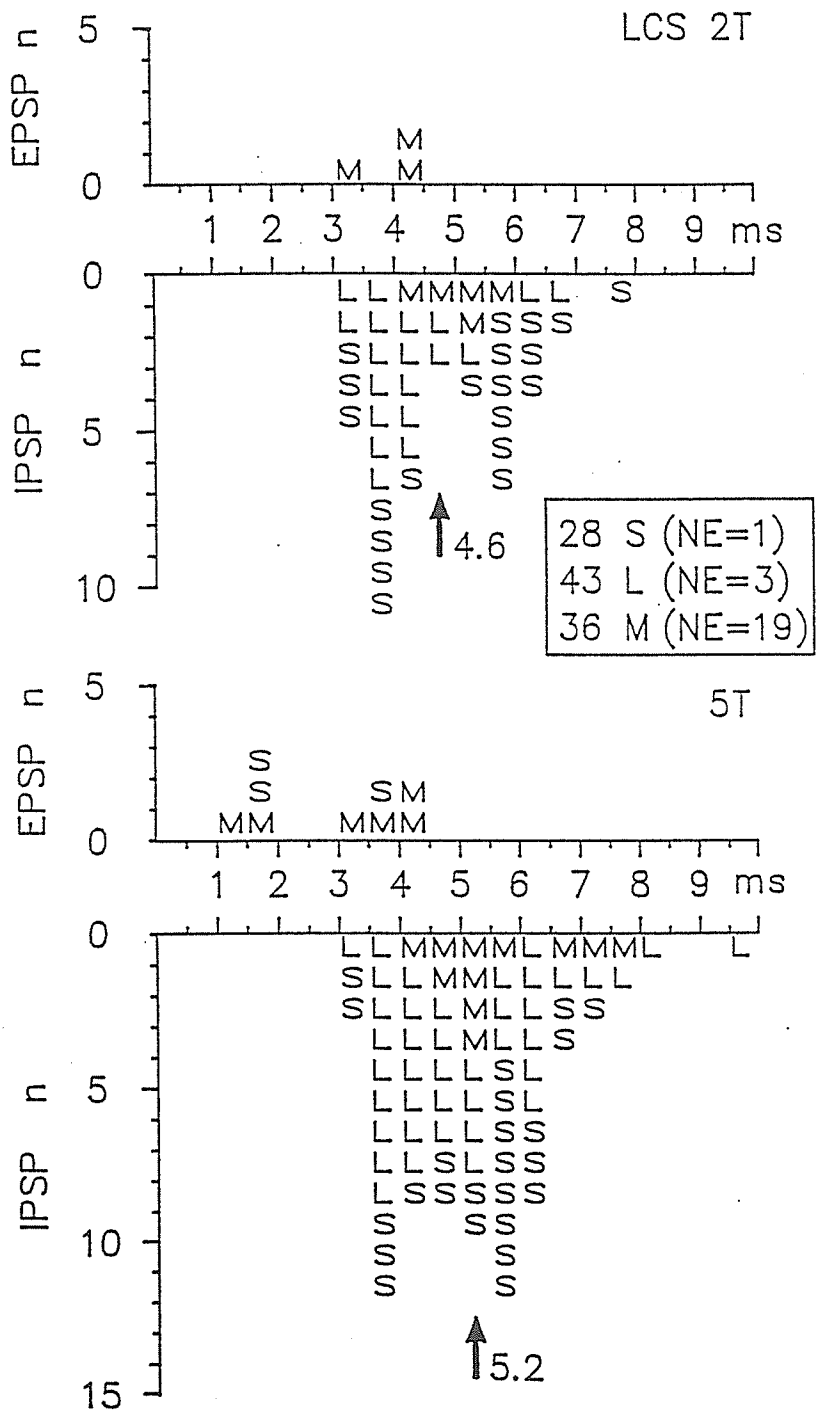

FIGURE 5

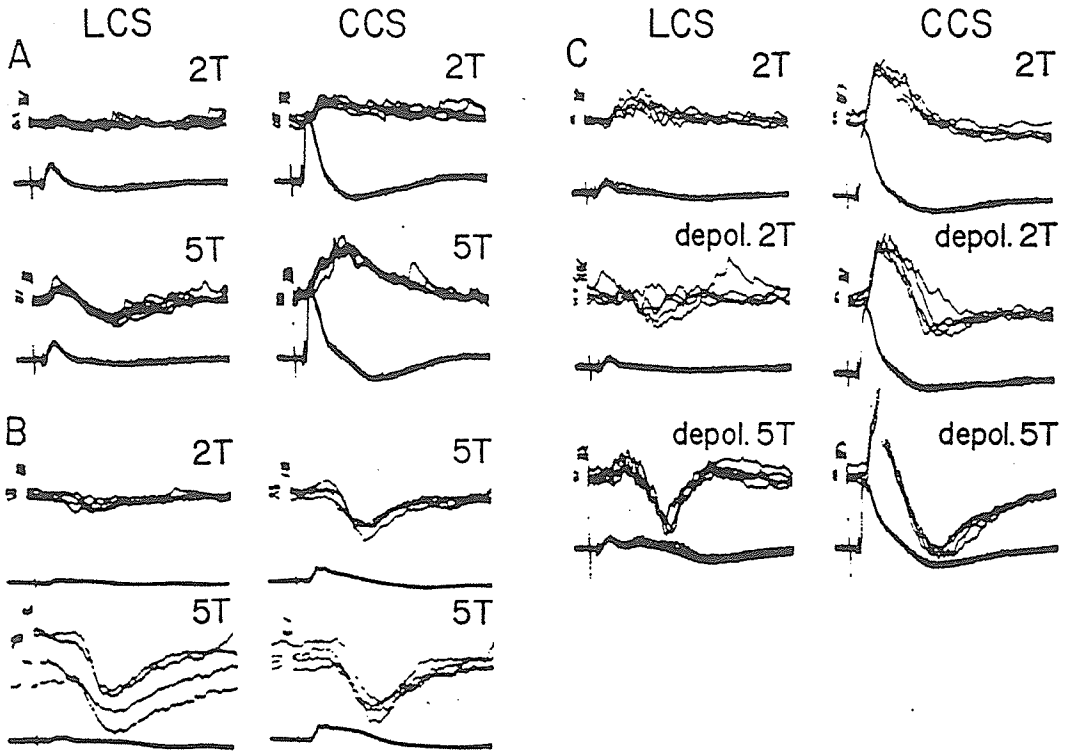
LCS
CCS
LCS
CCS
A
2T
2T
C
2T
2T
5T
5T
depol. 2T
depol. 2T
B
2T
5T
depol. 5T
depol. 5T
5T
5T

FIGURE 6

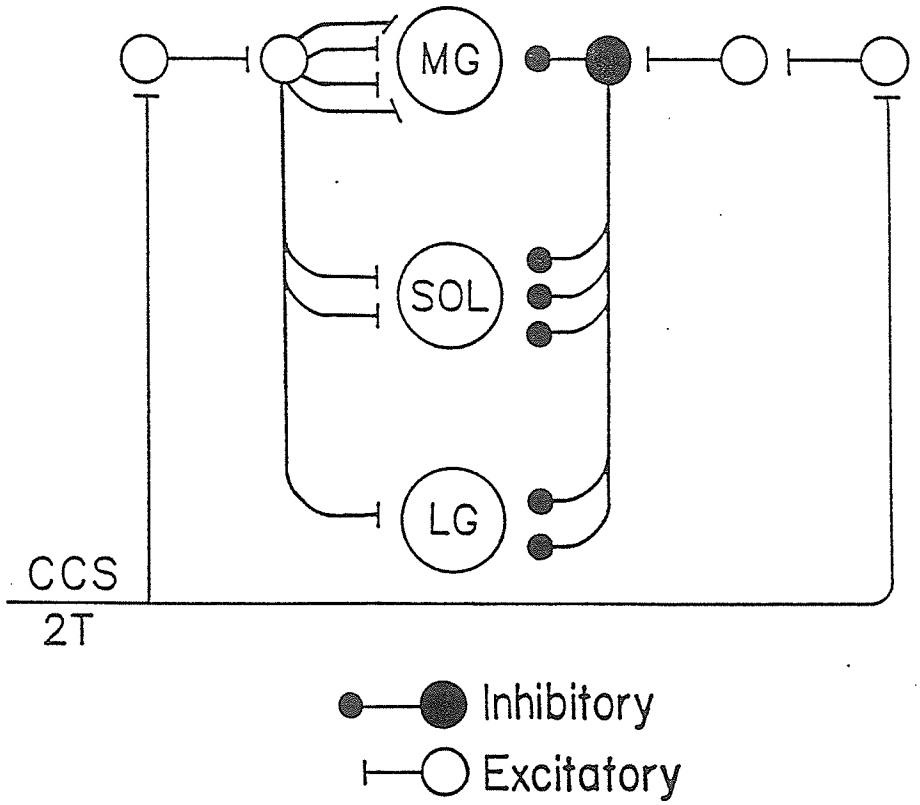

FIGURE 7

TABLE 1. *Action-potential amplitudes of cells with records in Fig. 2*

	CCS PSPs, mV	LCS PSPs, mV
MG	77/80 (61-95)	76/78 (60-88)
SOL	76/77 (60-92)	73/73 (60-92)
LG	77/78 (60-95)	75/79 (60-90)

Table 2A Areas of PSPs elicted by 2T stimulation of the CCS nerve

	MG	LG	SOL
n with effect	39/39	33/41	21/27
AREA (mV*ms):			
MEAN	11.4	11.8	18.5
MEDIAN	7.8	6.4	16.5
RANGE	2.4-34.8	3.0-59.3	3.3-45.5
MEAN DEPOL.	10.6	2.3	5.0
MEAN HYPERPOL.	0.8	9.5	13.5
MEAN %DEPOL.AREA	93%	19%	27%
MEAN %HYPERPOL.AREA	7%	81%	73%
CELLS WITH:			
DEPOL.AREA >50%	37= 95%	6= 15%	8= 30%
HYPERPOL.AREA >50%	2= 5%	27= 66%	13= 48%
NO EFFECT	0= 0%	8= 19%	6= 22%
ACTION POTENTIAL AMP.:			
MEAN/MEDIAN	73/74mV	74/75mV	76/77mV
RANGE	50-95mV	50-96mV	52-103mV

Table 2B 2T CCS PSP areas in cells with action potentials 80 - 90 mV

	MG	LG	SOL
n	13	9	8
AREA (mV*ms):			
MEAN TOTAL	14.0	18.1	15.7
MEAN DEPOL.	14.0	2.3	6.4
MEAN HYPERPOL.	0.0	15.8	9.3
MEAN %DEPOL.AREA	100%	13%	41%

Table 3. Cell membrane properties and predominant effects of CCS stimulation

CELL	SPIKE (mV)	AHP DURATION (ms)	R_{in} (MΩ)	I_{Rh} (nA)	CV (m/s)	"TYPE"	PREDOMINANT PSP
MG-1	75	50	0.6	24.5	100	F	EPSP
MG-2	80	65	2.0	19.0	95	F/S	EPSP
MG-3	90	110	2.0	7.0	---	S	EPSP
* MG-4	89	110	1.5	5.5	77	S	EPSP
MG-5	94	85	2.5	6.0	---	S	EPSP
LG-1	90	65	1.0	7.0	112	F/S	IPSP
LG-2	80	57	0.7	16.0	106	F	IPSP
* LG-3	78	60	0.5	22.0	109	F	IPSP
LG-4	74	45	0.7	13.0	84	F	IPSP
LG-5	88	95	1.0	5.0	85	F/S	EPSP
SOL-1	77	120	1.4	2.1	88	S	IPSP
SOL-2	85	120	2.0	8.2	74	S	EPSP
SOL-3	77	130	2.4	3.0	75	S	EPSP
SOL-4	81	160	1.7	2.5	59	S	NE

PART II) Evidence for restricted central convergence of cutaneous afferents upon an excitatory reflex pathway to medial gastrocnemius motoneurons

SUMMARY and CONCLUSIONS

1. We previously reported that excitatory postsynaptic potentials (EPSPs) produced by low threshold electrical stimulation of the caudal cutaneous sural nerve (CCS) occur preferentially and with the shortest central latencies in the medial gastrocnemius (MG) portion of the triceps surae motor nuclei (LaBella et al. 1989). The present study employs the spatial facilitation technique (Lundberg 1975) to assess interneuronal convergence upon the short-latency excitatory pathway from CCS to MG by several other ipsilateral hindlimb afferents (the lateral cutaneous sural [LCS], caudal cutaneous femoral [CCF], saphenous [SAPH], superficial peroneal [SP], posterior tibial [TIB] and posterior articular [JOINT] nerves).

2. Spatial facilitation of CCS EPSPs in MG motoneurons was demonstrated with conditioning stimulation of the LCS, CCF, SAPH, SP and TIB nerves, but was most readily and consistently observed with CCF conditioning. Facilitation of CCS and CCF EPSPs could also be obtained in individual MG motoneurons with a wide range of condition-test delays.

3. CCF EPSPs in MG motoneurons produced by twice threshold (2T) afferent stimulation had a mean latency of 4.8 ms and often appeared as slowly rising, asynchronous potentials. On the other hand, 2T CCS EPSPs had a mean latency of 2.8 ms and appeared as sharper rising, less variable depolarizations. The optimum delay for facilitation of CCS EPSPs was found to be 5.2 ms on average, with CCS stimulation delayed from that of CCF. The longer latency of CCF EPSPs and the finding that the minimum condition-test delay was on the order of 3.9 ms suggests that convergence is not

likely to occur on the first-order interneurons in the excitatory CCF pathway to MG motoneurons.

4. The evidence for significant convergence between excitatory pathways to MG from CCF and CCS afferents is primarily discussed with regard to the original observations of Hagbarth, who found a relationship between the location of a cutaneous receptive field and excitatory cutaneous reflex effects in functional groups of motoneurons (Hagbarth 1952).

INTRODUCTION

Early studies on spinal reflexes highlighted the similarity of reflexes evoked by stimulation of a wide variety of cutaneous and high threshold muscle afferents, and these observations eventually led to Sherrington's concept of the "flexion reflex": ipsilateral excitation of flexors and inhibition of extensors; contralateral excitation of extensors and inhibition of flexors (Sherrington 1910). Central to this organization of reflex pathways is the idea that multiple classes of afferent fibers converge on common spinal interneurons to produce excitation and inhibition in widespread but functionally related groups of motoneurons. It was also recognized that the intraspinal circuitry involved in the flexion reflex was probably a substrate for more complex behaviors (e.g. Sherrington 1910), and more recently Lundberg has forwarded the "Flexor Reflex Afferent" (FRA) hypothesis (Lundberg 1972; 1979) to give functional meaning to central convergence from many sources and classes of afferent fibers (see also Lundberg et al. 1987c).

Intracellular studies have shown that cutaneous afferents, particularly those with higher electrical threshold, contribute to the FRA spinal network (Eccles and Lundberg 1959; Holmqvist and Lundberg 1961), but additional principles of cutaneous reflex organization are illustrated by the elegant work of Hagbarth conducted in the 1950's (Hagbarth 1952). Hagbarth found that stimulation of skin areas overlying an extensor muscle tended to facilitate monosynaptic reflexes of that muscle, whereas stimulation of other skin areas tended to depress them. A flexor muscle on the other hand, tended to be excited by stimulation of skin spanning the entire limb, inhibited only by skin over its extensor antagonist. An important ramification of Hagbarth's observations, is the demonstration of an organized basis for excitatory

cutaneous reflexes in ipsilateral extensor motoneurons; and from the pioneering reflex studies of Sherrington (1903) to later investigations by Engberg (1964), further evidence of excitatory cutaneous reflexes in ipsilateral extensor motoneurons has been found. In the latter studies a small skin area on the plantar surface of the paw led to reflex contraction of only some intrinsic foot muscles. The term "private" reflex pathway was introduced to describe this type of circumscribed reflex action of cutaneous afferents (see Lundberg 1975).

The present investigation was motivated by the observation that stimulation of the caudal cutaneous sural nerve (CCS) produces a differential distribution of excitatory postsynaptic potentials (EPSPs) within the three extensor motor nuclei comprising triceps surae (LaBella et al. 1989). In anesthetized spinal cord-intact cats, medial gastrocnemius (MG) cells were consistently depolarized by low threshold CCS stimulation, whereas soleus (SOL) and lateral gastrocnemius (LG) motoneurons received a variety of, and in the case of LG, often weak, effects. CCS EPSP latencies were also shortest in MG, and only in a minority of MG cells was the EPSP followed by hyperpolarization. The question now addressed is whether these observations indicate a relatively segregated segmental pathway to MG motoneurons, or one in which there is extensive convergence from other cutaneous afferents via common interneurons. Intracellular recording from MG motoneurons in combination with the technique of spatial facilitation (Lundberg 1975) is used here as an indirect test of interneuronal convergence by other (mostly cutaneous) reflex pathways. As the results will show, CCS EPSPs are more readily facilitated by activation of afferent fibers in the caudal cutaneous femoral nerve (CCF) than by those in other hindlimb cutaneous nerves tested. Together with observations of a predominantly excitatory input from CCF to MG cells, our findings suggest a relatively private excitatory pathway which may be primarily

shared by CCF and CCS afferents. Furthermore, since these cutaneous nerves have overlapping receptive fields in the region of the MG extensor muscle, this pattern of central convergence may be rooted in the myotopic organization of cutaneous afferents described by Hagbarth (1952). Some of these results have been presented in abstract form (LaBella and McCrea 1988).

METHODS

Experiments were performed on 12 chloralose-anesthetized adult cats of either sex, ranging from 1.8 to 3.4 kg in weight, and with no lesions of the spinal cord. Peripheral nerves were dissected in the left (ipsilateral) hindlimb and mounted on bipolar electrodes for electrical stimulation. Intracellular recordings from antidromically identified triceps surae motoneurons were made with 2 M potassium citrate-filled electrodes with resistances of 2-5 MΩs. Threshold (T) for nerve stimulation, as well as the delay between condition and test stimuli, were determined from the earliest deflections on the cord dorsum records. Further details of the dissection and recording techniques can be found in a previous publication (LaBella et al. 1989).

Cutaneous nerves used for conditioning postsynaptic potentials (PSPs) produced by caudal cutaneous sural nerve stimulation (CCS, see LaBella et al. 1989) include: the lateral cutaneous branch of the sural nerve (LCS); the distal branch of the caudal cutaneous femoral nerve (CCF); the saphenous nerve (SAPH) taken approximately one centimeter above the level of the knee; and the superficial peroneal nerve (SP). The mixed musculo-cutaneous nerve, posterior tibial (TIB), was dissected distal to the branches to triceps surae, plantaris, flexor digitorum and hallucis longus muscles. Other nerves mounted for stimulation were: the posterior articular nerve (JOINT); all branches of the quadriceps nerve (Q); posterior biceps and semitendinosus nerves combined (PbSt); anterior biceps and semimembranosus nerves combined (SmAb); medial gastrocnemius (MG), lateral gastrocnemius (LG) and soleus (SOL). Ventral roots were left intact for antidromic identification of motoneurons.

Intracellular and cord dorsum recordings were photographed from the oscilloscope and digitized at 20 KHz in the computer (8 or 16 sweeps per average). The spatial facilitation technique (Lundberg 1975) was used to compare the effect on individual motoneurons of combined stimulation of two nerves at a fixed delay with the algebraic sum of their effects upon separate stimulation. In order to reduce the influence of changing motoneuron impalement throughout data collection, stimuli were delivered in the order of condition, test, then condition-test, and collected in an alternating method of storage. Averaged intracellular records could be added or subtracted to estimate the presence and degree of facilitation. EPSPs were considered to be spatially facilitated when the effect was repeatable on separate trials; substantially greater than any negative deflections occurring in the calculated "difference" trace; and substantially greater than extracellular fields examined after removal of the electrode to an extracellular location. Except in the case of TIB-evoked responses, extracellular fields resulting from low threshold nerve stimulation were usually quite small and often had decayed to baseline by the time PSPs were examined for facilitation (see Fig.4).

RESULTS

Sample and recording procedure

Except for the records in Fig. 7, all data were obtained from 70 antidromically identified MG motoneurons. Because the primary goal of the present study was to assess interneuronal convergence upon the short-latency excitatory pathway from CCS to MG, data collection in all MG cells began by first examining whether twice and five times threshold stimulation of peripheral nerves (2 and 5T, respectively) produced excitation. Those nerves that produced low threshold EPSPs as their earliest-latency effect (at 2 and/or 5T) were then used as the conditioning stimulus in an attempt to spatially facilitate CCS EPSPs.

The threshold for EPSP production was determined and subsequent tests for EPSP facilitation began with each nerve in the condition-test pair stimulated at about its synaptic threshold. The delay between stimuli was varied until facilitation could be seen in the raw records on the oscilloscope. Averages were then taken and subsequent trials attempted in which the delay and stimulation strength were varied to optimize or confirm the presence (or absence) of facilitation. In some cases EPSPs which occurred subsequent to inhibitory postsynaptic potentials (IPSPs) were also tested for spatial facilitation of CCS EPSPs. In addition, a few cases where an increase in synaptic activity which was not clearly discernible as containing IPSP or EPSP components, were also tested for facilitation. In all examples the test nerve refers to CCS.

PSPs in medial gastrocnemius motoneurons

Figure 1 depicts the variety of PSPs produced by peripheral nerve stimulation at 2T with superimposed traces of PSPs from individual MG motoneurons (one trace per motoneuron in each panel). With a stimulus

intensity of only 2T many motoneurons were without effect from peripheral stimulation, as evidenced by the flat lines in all panels. Weak or absent PSPs were most common upon JOINT and LCS stimulation. As reported earlier (LaBella et al. 1989), 2T stimulation of CCS produced EPSPs in the majority of MG motoneurons. Other nerves frequently producing EPSPs in MG were CCF, SP, SAPH and TIB. Note that CCS and SP stimulation resulted in the most frequent occurrence of fast-rising, early-latency excitation, whereas stimulation of CCF and SAPH produced more slowly rising short-latency EPSPs. TIB stimulation produced more variable types of PSPs, while PSPs produced by JOINT and LCS stimulation were primarily hyperpolarizing. The incidence of EPSPs in MG motoneurons produced by stimulation up to 5T is given in the middle panel of Table 1.

Although the time course of records in Fig. 1 is on the order of the first 15 milliseconds, records of longer duration (first 50 ms; not illustrated) revealed that CCF EPSPs in particular, are rarely followed by hyperpolarizing potentials. The number of traces without CCF EPSPs in Fig. 1 is due to the fact that 2T stimulation was frequently below the threshold for excitation. Table 1 (middle panel) documents the 72% incidence of CCF EPSPs as the first effect of 5T stimulation in MG; the only nerves with a higher incidence of EPSPs were SP (91%) and CCS (97%).

Facilitation of CCS EPSPs by CCF stimulation

Effects of stimulating the CCF and CCS nerves in an MG motoneuron are shown in Fig.2. The threshold for CCF excitation in this cell was approximately 2T as evident from records in 2A, and the latency of the EPSP was 7.5 ms. Stimulation of CCS at 1.6T (2B, left) produced a small EPSP with a latency of 4.0 ms. All frames in 2A and B consist of 5 superimposed sweeps. Records in 2C are single consecutive sweeps

showing the sequence of stimulating CCF at 2T (left), CCS at 1.75T (middle), and then both nerves together with the CCS nerve volley arriving at the cord dorsum 7.6 ms after the CCF volley (right). Note the presence of a 2 mV EPSP upon combined stimulation of both nerves. Although the delay in this case was 7.6 ms, the delay producing the largest facilitation in this cell was 6.2 ms. The cord dorsum records of CCS stimulation in middle and right panels of 2C illustrate the commonly seen effect of decreases in the CCS N1 wave when preceded by stimulation of another cutaneous nerve.

Figure 3 illustrates averaged traces from the same MG cell depicted in Fig. 2 with CCS stimulated at a slightly lower threshold. Averaging more clearly reveals the small EPSP evoked by 1.5T CCS stimulation (3B) and the early negative deflection, in part a field potential, produced by 2T CCF stimulation (3A; extracellular records not shown). The average of combined CCF and CCS stimulation is shown in 3C, with the delay of the CCS afferent volley from the CCF volley being 7.6 ms (cord dorsum in 3D). The arithmetic sum of traces 3A and 3B is shown in 3E. Superimposing this arithmetic sum on the combined stimulation trace (3G) reveals EPSP facilitation on the order of 0.75 mV. In 3F, the summed trace has been subtracted from the combined stimulation trace.

Of 30 MG motoneurons tested, it was possible to demonstrate spatial facilitation of CCS and CCF EPSPs in 28. In a total of 71 separate trials where facilitation was obtained the effective threshold of CCF stimulation ranged from 1.2 to 7.0T (mean 2.8T) with the very occasional use of double shocks (usually at 300 Hz). Effective CCS stimulation ranged from 1.1 to 3.7T (mean 1.7T) and the CCS volley was usually delayed from the arrival of the CCF volley at the cord dorsum. Thus, it was frequently observed that a reverse condition-test sequence

(i.e. where CCS stimulation preceded that of CCF) was ineffective for spatial facilitation. The upper panel of Table 2 illustrates the most effective thresholds and delays used with this pair of nerves by grouping parameters according to the amount of EPSP facilitation observed (leftmost column; n= number of observations in the 28 cells). One can see that the average thresholds of CCF and CCS stimulation remained essentially constant, but that as the amount of facilitation observed increased, there was a trend towards longer delays from an average of 3.9 to 5.2 ms (see Discussion).

The lower panel of Table 2 indicates the mean latencies of CCS and CCF EPSPs produced by 2 and 5T stimulation in the same 28 MG motoneurons. The slightly longer average latency of CCS EPSPs at 5T can be attributed to a few cells where longer-latency EPSPs were present at 5T, while no effect was produced at 2T. Since these average latencies indicate that the minimum CCS pathlength is shorter than the minimum CCF pathlength by 2 milliseconds, a fewer number of interneurons likely contributes to the need to delay CCS stimulation from that of CCF to produce faciliation. Together with the observation that the delay producing the greatest facilitation in any particular MG motoneuron was usually similar to the latency of the CCF EPSP, this suggests that the maximum facilitation involves the later rather than the earliest components of CCF excitation.

Facilitation of CCS EPSPs by other hindlimb afferents

Figure 1 shows the patterns of PSPs in MG motoneurons produced by the other hindlimb nerves tested for spatial facilitation of CCS EPSPs. As a means of comparing facilitation by the different nerves, records in Fig. 4 illustrate the best examples of CCS EPSP facilitation found overall for each conditioning nerve indicated (JOINT was the only nerve tested where facilitation was never obtained). The records

include four MG motoneurons in four separate animals. Each panel in Fig. 4 depicts the averaged record of combined stimulation superimposed over the arithmetic sum of the averages of separate condition and test stimulation. The motoneuron of 4C shows that even the best example of facilitation with LCS stimulation was very weak; approximately 0.4 mV. SP conditioning of CCS EPSPs in the motoneuron of 4D was substantial, producing > 0.5 mV facilitation of the later EPSP components. Extracellular records in 4F were taken just outside the motoneuron in 4E where facilitation of the CCS EPSP was obtained with TIB conditioning stimulation. Stimulation parameters in 4F are identical to those indicated in 4E and show that the early field potential is not facilitated upon combined stimulation of TIB and CCS (i.e. combined and arithmetic records in 4F virtually identical). Examples of >3.0 mV and >1.1 mV facilitation with CCF and SAPH conditioning in 4A and 4B respectively, are from the same MG motoneuron. Both of these represent extreme examples of CCS EPSP facilitation and are from an animal in which facilitation became increasingly easy to demonstrate towards the end of the experiment. In the case of SAPH, it was normally quite difficult to obtain facilitatory effects, and when achieved, were usually of much smaller amplitude. It is noteworthy that in MG cells of this animal with exceptionally large CCF and SAPH facilitation, only EPSPs were recorded from stimulation of all peripheral nerves (although only weak EPSPs from JOINT stimulation), and that similiar facilitation could not be obtained with SP or LCS conditioning. JOINT facilitation was not tested in this animal.

In a few cells it was only possible to demonstrate facilitation of CCS EPSPs with CCF stimulation. However, time constraints usually meant that only a few nerves could be thoroughly tested in a particular motoneuron, and/or only a few nerves produced discernible EPSPs upon separate stimulation. While negative results in these kinds of

experiments must be interpreted with great caution, it became clear throughout the experimental series that spatial facilitation of CCS and CCF EPSPs could be predicted for virtually all MG motoneurons, even when facilitation could not be demonstrated, or was difficult to demonstrate, with conditioning stimulation of the other nerves. Thus, whereas the records in Fig. 4 depict the best examples of CCS EPSP facilitation obtained, Table 2 illustrates the overall findings in this study. First of all, note that of all the conditioning nerves, CCF and SP stimulation resulted in the greatest percentage of EPSPs in MG as the earliest-latency effect at 2 and/or 2T (72% and 91% of MG motoneurons respectively; comparable to 97% for CCS). However, facilitation of CCS EPSPs is much more common with CCF conditioning; obtained in 93% of MG motoneurons versus 28% for SP. Although TIB EPSPs occurred in only 50% of the motoneuron sample, we were relatively successful in facilitation trials with TIB conditioning (43% of motoneurons). This would suggest that the probability of convergence on the CCS excitatory pathway is not a strict function of which nerves, like CCS, have strong early-latency excitatory projections to the MG motor nucleus. Occasionally, TIB and LCS stimulation resulted in an increase in the "synaptic noise" of an MG motoneuron without producing discernable EPSPs or IPSPs (incidence not listed in the table). It was in a few such cases that LCS conditioning resulted in facilitation, suggesting perhaps that the "noise" represented a weak excitatory input which was balanced by a parallel inhibitory drive (see Schomburg and Steffens 1986).

Although Table 2 shows that comparable amounts of facilitation could be obtained with conditioning stimulation of most of the afferent nerves tested, the relative ease or difficulty in obtaining facilitation varied considerably. For example, facilitation with TIB, SAPH, SP or LCS conditioning usually required several trials with a

wide variety of stimulation parameters. In the case of TIB, SP and JOINT conditioning, hyperpolarizing potentials frequently followed initial EPSPs even at very low thresholds, rendering it difficult to observe or interpret effects of combined stimulation with CCS. Thus, the consistent and relatively potent facilitation of CCS EPSPs by CCF stimulation suggests that while multiple afferent systems may converge on interneurons mediating short-latency excitation from CCS to MG, CCF projections are most tightly coupled to interneurons in this pathway.

In the present series of experiments only sporadic attempts were made to examine convergence of various muscle afferents onto the CCS excitatory pathway to MG. Because all nerves mounted in each experiment were systematically stimulated at strengths of 2 and 5T, each motoneuron was examined for excitation evoked by group I and group II muscle afferents. However, unlike CCS and CCF EPSPs, group II EPSPs are often absent in gastrocnemius motoneurons and their presence in extensor motoneurons may be highly preparation-dependent (see Lundberg et al. 1987a). Nonetheless, in two MG cells with evidence of group II excitation, CCS EPSPs were facilitated with 5T stimulation of the LG-SOL nerve in one, and more weakly with 5T stimulation of the PbSt and SmAb nerves combined in the other. We were unable to facilitate CCS EPSPs with Q stimulation in two MG cells with Q group II EPSPs. Substantial group I EPSPs were not evoked by any of the muscle afferents tested in these experiments, and thus were not tested for facilitation of CCS EPSPs.

Evidence for a "non-FRA" pathway

Figure 5 illustrates PSPs in a single MG motoneuron produced by stimulation of several ipsilateral hindlimb nerves. Note the short-latency (3.0 ms) excitation from 2T CCS stimulation (upper left panel) typical of that found in MG motoneurons in the anesthetized, cord-

intact preparation (LaBella et al. 1989). In this particular cell, the synaptic threshold for the CCS EPSP was approximately 1.7T (not illustrated). CCF stimulation produced relatively longer latency, smaller amplitude depolarizations, whereas LCS, JOINT, and Q stimulation produced hyperpolarizing postsynaptic effects. Stimulation of SP and TIB at 2T, and SAPH at 5T, did produce small EPSPs as the earliest-latency effect, but these were quickly followed by clear hyperpolarizing potentials. Inhibition produced by 5T but not 2T Q stimulation suggests that group II muscle afferents also inhibit this cell (Lundberg et al. 1987a). The qualitative variety of PSPs produced by different nerves in this cell is typical of that encountered in other MG motoneurons, but PSP amplitudes did vary widely from cell to cell and there tended to be more or less excitation (or inhibition) in some experiments compared to others. However, these observations make it all the more noteworthy that CCS and CCF were the only nerves to consistently depolarize MG motoneurons throughout the 12 experiments.

Thus, while concordant inhibitory effects from a variety of cutaneous, joint and group II muscle afferent inputs might be expected from the operation of flexor reflex afferent (FRA) pathways to extensor motoneurons, EPSPs from low threshold stimulation of CCS and CCF may signify operation of more private cutaneous pathways. Records with 5T stimulation in Fig. 6 further exemplify this point. Note the sharp depolarizations in the MG motoneuron of 6A produced by CCS and CCF stimulation, the similarity of their waveform, and the contrast between these PSPs and those produced by several other nerves. A similar situation prevails in the motoneuron of 6B. While these examples depict quite similar CCF and CCS EPSPs, CCS EPSPs were often earlier and "sharper" as evident with 2T stimulation in Fig.1. Nonetheless, in the majority of MG cells, CCF and CCS produced the same type of membrane polarization when other cutaneous nerves did not. This was particularly

evident at higher thresholds such as 5T when hyperpolarizing potentials produced by these nerves are more pronounced (see LaBella et al. 1989 and Pinter et al. 1982), and because CCF PSPs are frequently absent at 2T. For example, if CCS produced a mixed EPSP/IPSP in a motoneuron with 5T stimulation, usually CCF did as well. These observations strongly support the notion that there is some degree of interneuronal segregation between excitatory reflex pathways to MG shared by CCS and CCF, and those shared by other peripheral afferents.

Lateral gastrocnemius and soleus motoneurons

Compared to MG motoneurons, short-latency CCS excitation in LG and SOL motoneurons is more variable; often weak, absent, or dominated by subsequent inhibition (LaBella et al. 1989). Thus few attempts were made to facilitate CCS EPSPs in triceps surae motoneurons other than MG. Fig. 7 includes records from one SOL and one LG motoneuron in the present series of experiments, illustrating facilitation of CCS and LCS IPSPs in the SOL cell, and CCS and JOINT IPSPs in the LG motoneuron. While examples of IPSP facilitation were thus frequently observed in the small sample of LG and SOL cells tested, EPSP facilitation usually required stimulation of nerves with more consistent excitatory effects in these motoneurons than CCS. In the case of LG, this was often achieved by stimulating the SP and SAPH nerves together (not illustrated).

Short versus longer-latency EPSPs

In some MG motoneurons, and usually towards the end of an experiment, longer-latency EPSPs (approximately 20-30 ms) followed the more common early EPSPs and/or IPSPs upon stimulation of peripheral nerves. Although such EPSPs were only sporadically tested for evidence of convergence, we suspect they are mediated by interneurons common to a variety of peripheral afferents. Figure 8 illustrates records from

two MG motoneurons where such late excitation occurred. In 8A, facilitation of both early and late CCS EPSPs was obtained with stimulation of the CCF nerve. In another MG cell in 8B, facilitation of only the late CCS EPSP was produced by LCS conditioning stimulation. Because these late excitatory effects, when present, occurred in a variety of extensor and flexor motoneurons (i.e. MG, LG, SOL, PbSt and SmAb), we suspect the interneurons involved are not only shared by a variety of peripheral afferents but also diverge to multiple motoneuron pools, thus resembling the FRA spinal network. Lundberg noted that changes in the general state of the spinal cord could greatly influence the patterns of PSPs in extensor and flexor motoneurons produced by afferents participating in the FRA (see Eccles and Lundberg 1959). Thus, the "longer-latency excitation" which appeared in our preparation towards the close of an experiment, may reflect the release of an FRA excitatory network by factors involved in the general regulation of interneuron activity.

DISCUSSION

In summary, the present investigation shows that low threshold, short-latency CCS EPSPs in MG motoneurons can be facilitated by conditioning stimulation of a variety of hindlimb afferents. However, this facilitation was most readily and consistently observed with stimulation of the CCF nerve compared to several other hindlimb nerves tested. Thus, while stimulation of LCS, SAPH, SP and TIB sometimes produced substantial facilitation of CCS EPSPs, such observations were usually difficult to demonstrate and required many variations in the stimulation parameters to be obtained. By contrast, facilitation with CCF conditioning was usually achieved in the very first trial attempted, suggesting a relatively restricted central convergence onto interneurons in the low threshold excitatory path from CCS afferents to MG. The few observations of facilitation by group II muscle afferents are of interest, but as mentioned group II EPSPS are often absent in MG, and their presence may be highly preparation-dependent. This would suggest that conditions would have to be optimized for analyzing group II convergence upon the CCS excitatory path to MG which is itself prevalent in a variety of preparations. As for CCS pathways to motoneurons other than MG, CCS IPSPs were more readily facilitated than EPSPs in the few LG and SOL motoneurons examined. However, facilitation of CCF and CCS EPSPs in the one PbSt/SmAb motoneuron tested raises the possibility of common interneurons in other CCF/CCS excitatory pathways as well.

As discussed by Lundberg and co-workers (Lundberg et al. 1977), the minimum condition-test interval producing facilitation can be used as an indication of the pathlength to the interneurons with convergence from both sets of afferents. However, minimum condition-test intervals were rarely examined in the present study since the primary goal was

to make a qualitative assessment of whether or not low threshold short-latency excitatory reflexes in MG produced by CCS and other hindlimb afferents are mediated by common interneurons. However, upon finding strong evidence for convergence by CCF afferents, the question arises as to the site of convergence in the shared CCF/CCS excitatory path. This is a difficult question in light of the pathlengths of CCS and CCF excitatory projections to MG. Although occasionally shorter (LaBella et al. 1989; Omeniuk et al. 1986), latencies of the earliest CCS EPSPs in MG are commonly 2.2 ms, suggesting a minimum trisynaptic pathway (Fleshman et al. 1988; Lundberg 1975; but see Cavallari et al. 1987). While the slow rising phase of low threshold CCF EPSPs interferes with estimates of minimum latency, CCF effects in MG are of considerably longer latency, and probably involve several interneurons. Because the present study concentrated on facilitation of the EPSPs, the average minimum delay of 3.9 ms needed to see clear facilitation of CCF and CCS EPSPs is most likely an overestimation of the minimum delay possible. Furthermore, delays usually exceeded 3.9 ms (see Table 2) suggesting the presence of several interneurons in the pathway from CCF afferents prior to convergence, and this is consistent with convergence on early, perhaps even first-order, interneurons in the CCS pathway to MG. However, the variety of delays which could be used in any given MG motoneuron suggests there may be multiple sites of convergence, or prolonged intervals allowing facilitation, in the shared CCF/CCS pathway to MG motor nuclei.

According to the general concepts embodied in the FRA framework, a spinal network exists where there is a wide convergence of cutaneous, muscle and joint afferent systems onto common interneurons with a wide divergence of reflex effects in many motoneuron species (Kniffki et al. 1981; Lundberg 1979; Lundberg et al. 1987a,b,c). Furthermore, inhibitory and excitatory effects in extensor (or flexor) motoneurons

from FRA afferents are seen to switch collectively with changes in the preparation, suggesting that FRA interneurons are under common descending control (Eccles & Lundberg 1959; see also Lundberg 1979, 1982). Thus, there are several characteristics of the low threshold excitatory pathway from CCS afferents to MG motoneurons which suggest at least some degree of segregation from the FRA. These include differential effects on MG, LG and SOL motoneurons (LaBella et al. 1989) and the consistent presence of short-latency CCS excitation in MG motoneurons regardless of the preparation used. Thus in intact-anesthetized, acute, or chronic spinal animals, short-latency CCS EPSPs tend to be present in MG cells, even when inhibitory effects are seem to dominate upon stimulation of other peripheral (including cutaneous) nerves (unpublished observations). The predominance of CCS EPSPs in ankle extensor motoneurons innervating fast-twitch muscle fibers (Burke et al. 1970; Burke et al. 1973; Pinter et al. 1982) provides further evidence that the low threshold CCS excitatory pathway represents a "special path" from cutaneous afferents which is not part of the FRA (Hongo et al. 1966; Lundberg et al. 1987c; see also Fleshman et al. 1988).

An early study by Hagbarth offers an additional framework with which to consider excitatory reflexes in extensor motoneurons produced by ipsilateral skin afferents. Hagbarth (1952) demonstrated in decerebrate-acute spinal preparations that stimulation of the skin overlying an extensor tended to facilitate reflex responses of that muscle, while stimulation of all other skin areas on the limb tended to depress them. Effective stimulus modalites included adequate as well as electrical stimulation, suggesting that this reflex pattern was based primarily on receptor locality and not modality. In a collective examination of effects in the ankle extensors, the excitatory region was generally localized near the skin over the heel extending up over

the calf. Hagbarth found that in general, the TIB and CCF nerves innervated this excitatory skin area, but a great part of this region, and especially the region of maximum sensitivity over the heel, was innervated by CCS (alias sural). In animals displaying a relatively large excitatory field over the ankle extensors SAPH was also often involved, but never to the same extent as CCS. Of further interest to the present investigation, is his additional observation that CCF stimulation consistently facilitated spontaneous activity in the MG nerve. Conversely, the fibular femoral nerve was found to strictly depress this activity. Though we have not tested for intracellular effects of stimulating the fibular femoral nerve, it is noteworthy that this nerve, which traverses the ventral thigh, has an overlapping receptive field with that of LCS; a nerve which produces primarily IPSPs in ankle extensor motoneurons (LaBella et al. 1989).

Thus the present finding of extensive spatial overlap in the short-latency pathways from CCF and CCS afferents to MG may be viewed as an intracellular demonstration of Hagbarth's earlier observations of the special excitatory influence of these nerves upon the ankle extensors. Also in accord with his observations are the present findings that SAPH occasionally facilitates CCS EPSPs in MG, whereas SP is often found to inhibit or "occlude" them. Although the present results reveal a strong inhibitory component in the postsynaptic effects of TIB stimulation in MG, there is periodic TIB excitation which can also facilitate CCS EPSPs. This finding is also in accordance with the 1952 observation that pinching the middle plantar region depressed S_1 ventral root activity. It is worth noting that CCS effects in the ankle flexor, tibialis anterior (a muscle which Hagbarth found to be reciprocally affected by cutaneous stimulation when compared to the ankle extensors), are predominantly hyperpolarizing (Dum and Kennedy 1980). Our own recordings from tibialis anterior motoneurons

are consistent with these findings, and we have also noted predominantly hyperpolarizing potentials with CCF stimulation as well (unpublished observations).

Spatial facilitation of reflexes evoked by low threshold stimulation of different cutaneous nerves has received relatively little attention. In contrast, there is substantial evidence for shared pathways between cutaneous and muscle, descending, and propriospinal systems (e.g. Behrends et al. 1983a; Fleshman et al. 1988; Hongo et al. 1972; Jankowska et al. 1973; Kniffki et al. 1981; Lundberg et al. 1977; Pinter et al. 1982; Shomburg et al. 1986), as well as between cutaneous afferents mediating different sensory modalities (Behrends et al. 1983b; Schomburg & Steffens 1986). If the present evidence that CCS and CCF excitatory reflexes in MG are mediated by common interneurons is in fact rooted in the myotopic organization described by Hagbarth, we would still caution along with Hagbarth that the final reflex result for a given muscle cannot be predicted solely on the basis of cutaneous innervation zones. There is a balance of excitatory and inhibitory influences on motoneurons from any skin area, and the final effect is determined not only by the relative strength of these connections but also by the balance of inputs from entirely separate systems interacting in the spinal cord. Furthermore, intracellular studies have now revealed that particular cutaneous reflexes are not necessarily distributed evenly to all ankle extensor motor nuclei, and whereas CCS produces preferential early excitation of MG motoneurons (LaBella et al. 1989), we have preliminary evidence that SP and SAPH preferentially excite LG cells (unpublished observations). The purpose of a differential synaptic input from cutaneous afferents to these functional synergists is unclear, although for the ankle extensors as a group, Hagbarth conceived of local extensor contraction in response to a noxious input on the calf or heel; a situation which would not

benefit from ankle flexion (see also discussion, LaBella et al. 1989). Nonetheless, the evidence for a differential CCS excitatory pathway to triceps surae motor nuclei, and for its relatively restricted convergence with other peripheral afferents, points to cutaneous reflex activity which is distinct from that evoked by the FRA. However, it will only be when convergence patterns are more fully described that the interactive role of cutaneous (and other) reflex systems can be interpreted and understood in the complex context of behavior.

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TABLE AND FIGURE LEGENDS

FIG 1. Postsynaptic potentials (PSPs) recorded in MG motoneurons upon single shock stimulation of seven different peripheral nerves at twice threshold. Enlarged photographic records of PSPs in individual motoneurons were traced and superimposed; one trace per motoneuron in each panel. Number of motoneurons in each panel: CCS 65; CCF 60; TIB 46; SP 61; SAPH 41; LCS 65; JOINT 52. The small vertical *arrow* indicates the time of arrival of the afferent volleys at the cord dorsum electrode. Calibration bar 2 mV, 2 ms.

FIG 2. Example of spatial facilitation between CCF and CCS excitatory postsynaptic potentials (EPSPs) in an MG motoneuron. *Upper traces* in each panel are the intracellular records; *lower traces*, the cord dorsum recordings. Records in A and B show effects of CCF and CCS stimulation respectively, and consist of 5 superimposed sweeps. Single sweep records in C show that combined stimulation of the CCF and CCS nerves produces an EPSP which is larger than the sum of the separate effects. Stimulation strength is expressed as times threshold (T) in each panel. Calibration pulses 2 mV, 2 ms.

FIG 3. Averaged intracellular records from the same MG motoneuron depicted in Fig.2. Trace C shows the effect of combined CCF and CCS stimulation at the thresholds indicated in A and B, respectively. Cord dorsum record of combined stimulation in D, with the delay of the arrival of the CCS afferent volley at the cord dorsum from that of CCF (milliseconds) indicated at the far right of the trace. The arithmetic sum of A and B is depicted in E, and in F the difference of this summed trace from that of

combined stimulation illustrates spatial facilitation of the EPSP. Facilitation can be viewed differently in G, where combined and summed records are placed in a superimposed position. Calibration pulses 2 mV, 2 ms (4 mV, 2 ms for summed trace in E).

FIG 4. Averaged intracellular records depicting the best examples of spatial facilitation for each of the six conditioning nerves, with CCS as the test nerve. In panels A-E, the combined stimulation trace of the condition-test pair is superimposed over the trace depicting the arithmetic addition of the effects of separate stimulation. In each panel the trace of maximum depolarization represents the combined effect. Records in A and B from the same MG motoneuron; those in C, D and E from three other MG motoneurons. Panel F shows the extracellular record of combined TIB and CCS stimulation at the parameters indicated in E, superimposed over the arithmetic addition of separate extracellular TIB and CCS effects. Calibration pulses 2 mV, 2 ms.

FIG 5. Intracellular records from an MG motoneuron upon stimulation of various peripheral nerves at 2T (also at 5T for SAPH and Q stimulation). *Lower traces* in each panel are the cord dorsum recordings. Two different time bases of the same records are presented for CCS, CCF, LCS, JOINT, SP and TIB stimulation. Calibration pulses 2 mV, 2 ms.

FIG 6. Intracellular records (*upper traces*) from an MG motoneuron in A, and from another MG motoneuron in B , upon stimulation of various nerves at 5T. *Lower traces* in all panels are the cord dorsum recordings. Calibration pulses 2 mV, 2 ms.

FIG 7. Averaged intracellular records depicting spatial facilitation

of CCS IPSPs in a SOL motoneuron (A) and an LG motoneuron (B). Format essentially as in Fig. 2. Calibration pulses 2 mV, 2 ms (4 mV, 2 ms for summed trace).

FIG 8. Averaged intracellular records depicting spatial facilitation of late CCS EPSPs from an MG motoneuron in A, and from another MG motoneuron in B. Format essentially as in Fig. 2. Calibration pulses 2 mV, 2 ms (4 mV, 2 ms for summed trace).

TABLE 1. Summary of results for tests of spatial facilitation. Peripheral nerves listed at far left. N= number of MG motoneurons in which postsynaptic potentials were examined. %EP= % of N motoneurons in which the earliest latency effect was an EPSP at 2 and/or 5T stimulation. %IP= % in which only IPSPs were recorded at 2 and 5T stimulation. %NE= % in which there was no measureable effect at 2 or 5T stimulation. n= number of motoneurons in which spatial facilitation of the early-latency CCS EPSP was attempted; %n= % where facilitation was obtained. mV= average of maximum recorded EPSP facilitation in millivolts, in %n motoneurons. (Note for PSP data, total PSP types for TIB and LCS <100% because of a few cells where an increase in synaptic activity was uninterpretable as either EPSPs or IPSPs).

TABLE 2. *Upper panel* summarizes results and parameters of stimulation for 71 successful trials of spatial facilitation between CCF and CCS EPSPs in 28 MG motoneurons. mV= millivolts facilitation in n trials. T= average threshold of stimulation in n trials. DELAY= average delay of the arrival of the CCS afferent volley at the cord dorsum from that of CCF, in milliseconds. *Lower panel* shows average central latencies of EPSPs which occurred as the first effect of CCF or CCS stimulation at twice (2T) and five times

threshold (5T); from total population of 70 medial gastrocnemius motoneurons. CCF 2T (nEPSP= 24); CCF 5T (nEPSP= 43); CCS 2T (nEPSP= 57); CCS 5T (nEPSP=68).

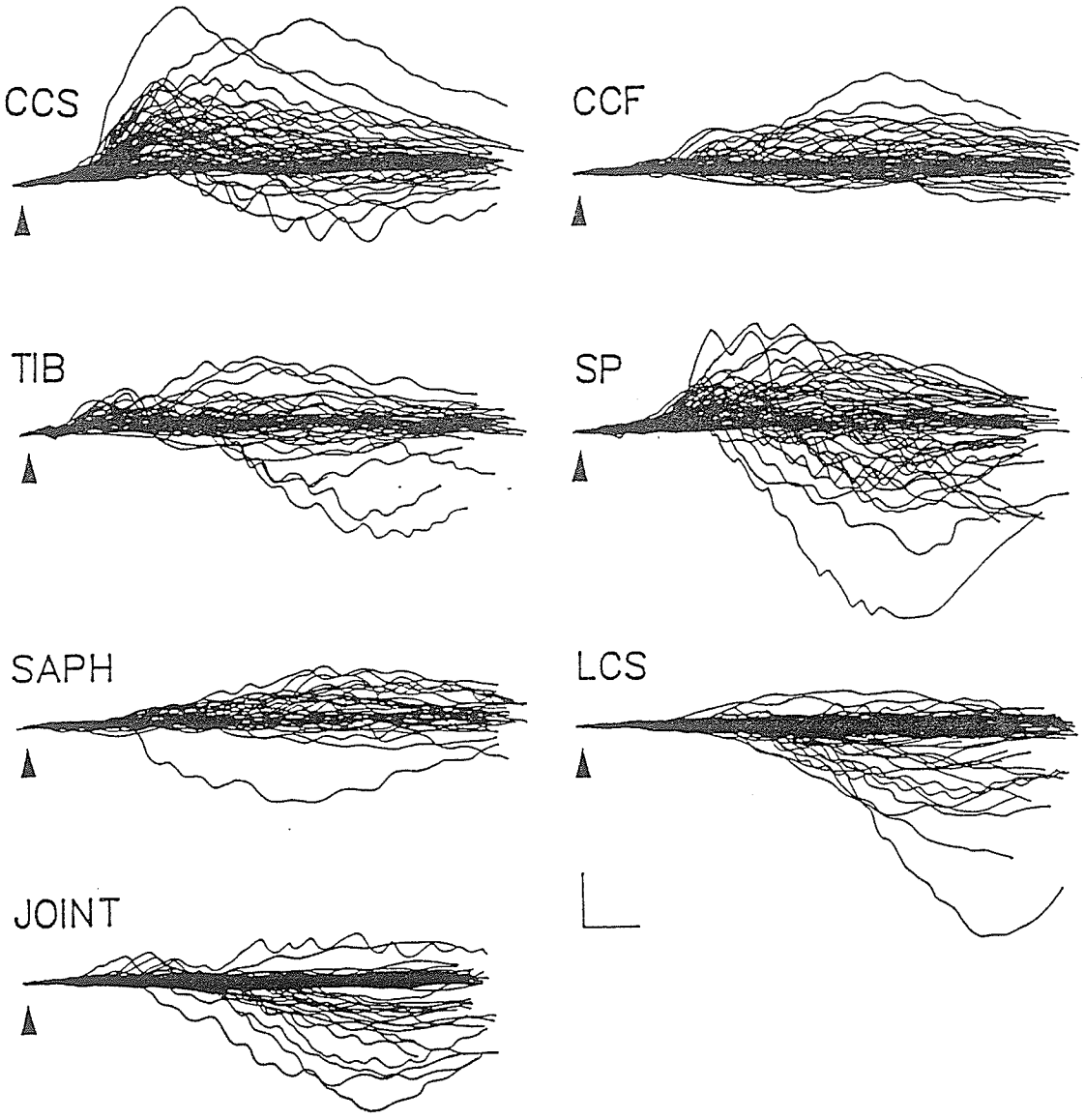

FIGURE 1

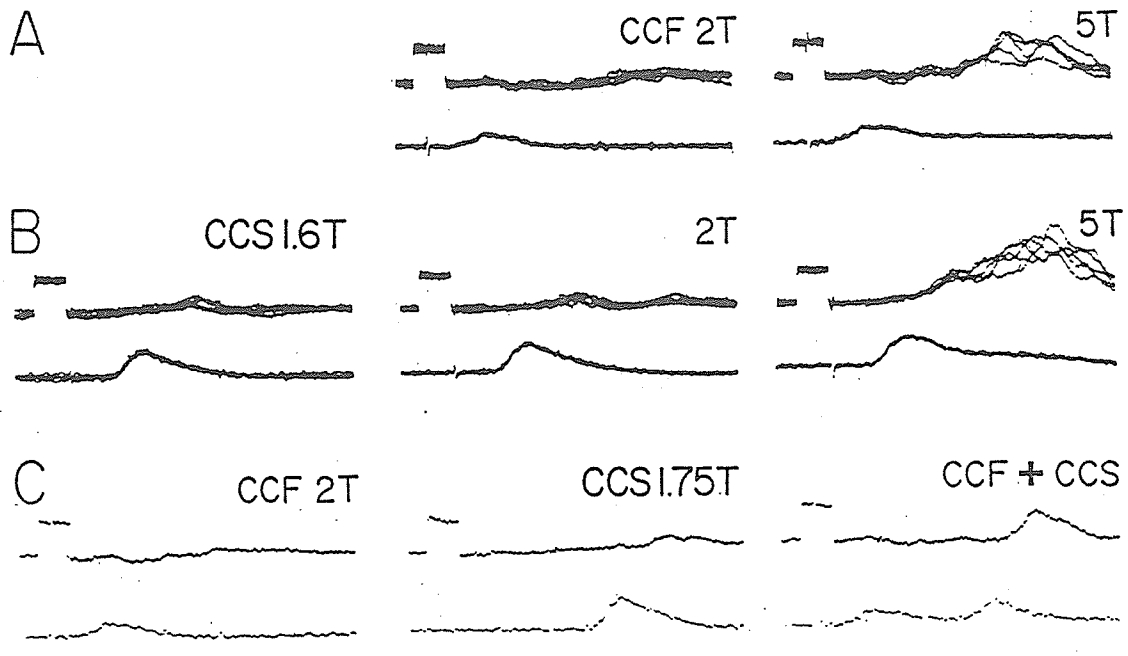

FIGURE 2

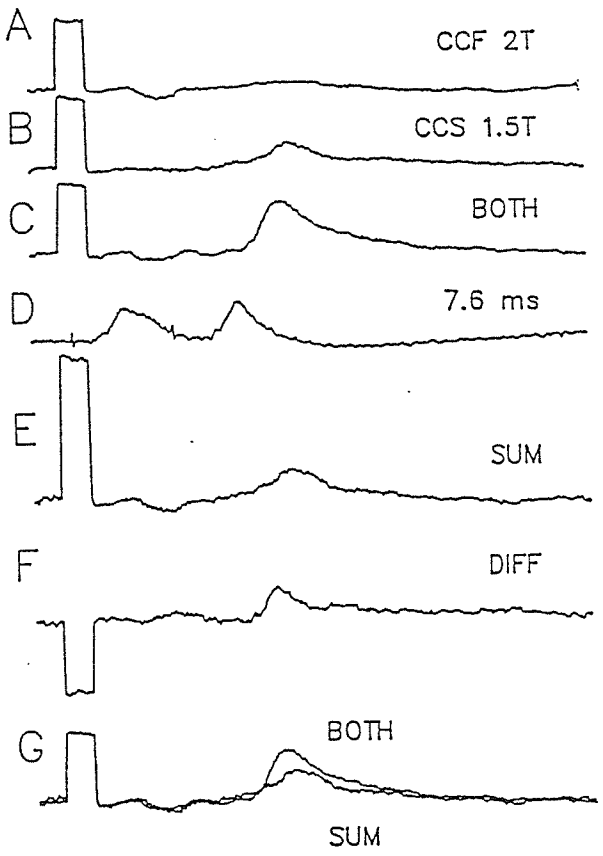

FIGURE 3

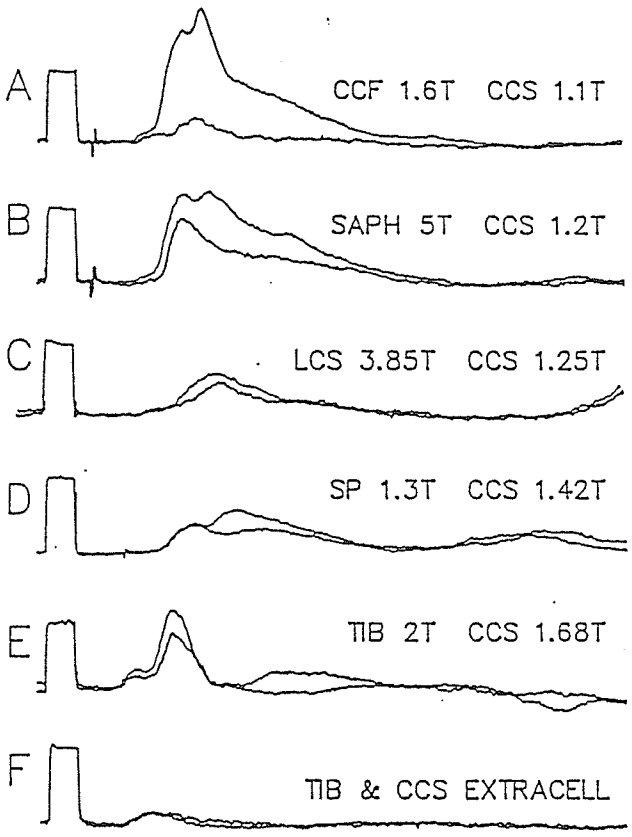
A
CCF 1.6T CCS 1.1T
B
SAPH 5T CCS 1.2T
C
LCS 3.85T CCS 1.25T
D
SP 1.3T CCS 1.42T
E
TIB 2T CCS 1.68T
F
TIB & CCS EXTRACELL

FIGURE 4

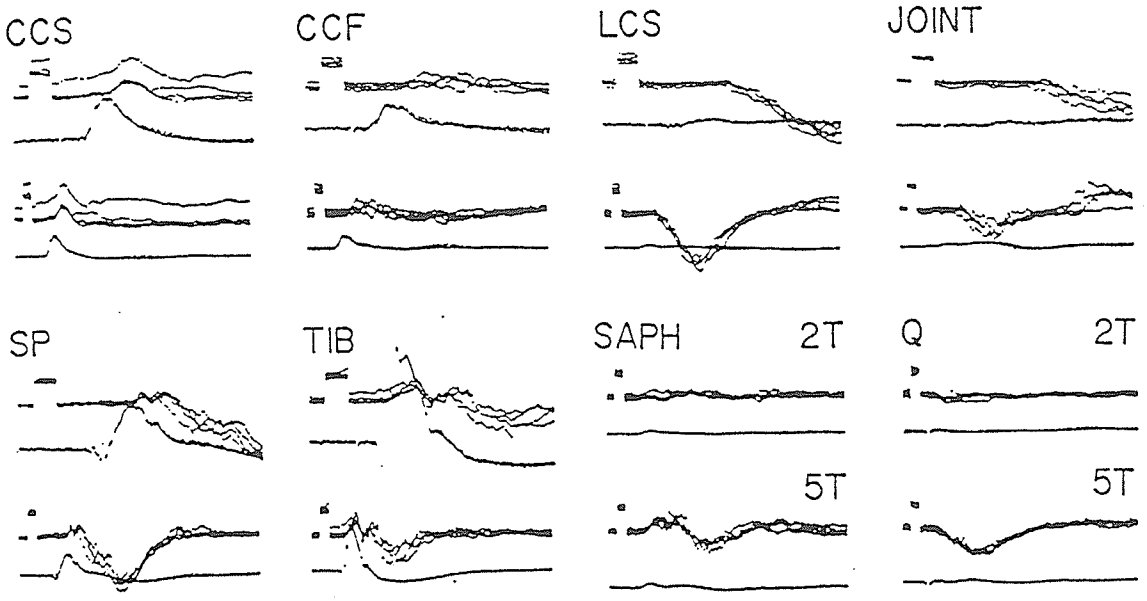

FIGURE 5

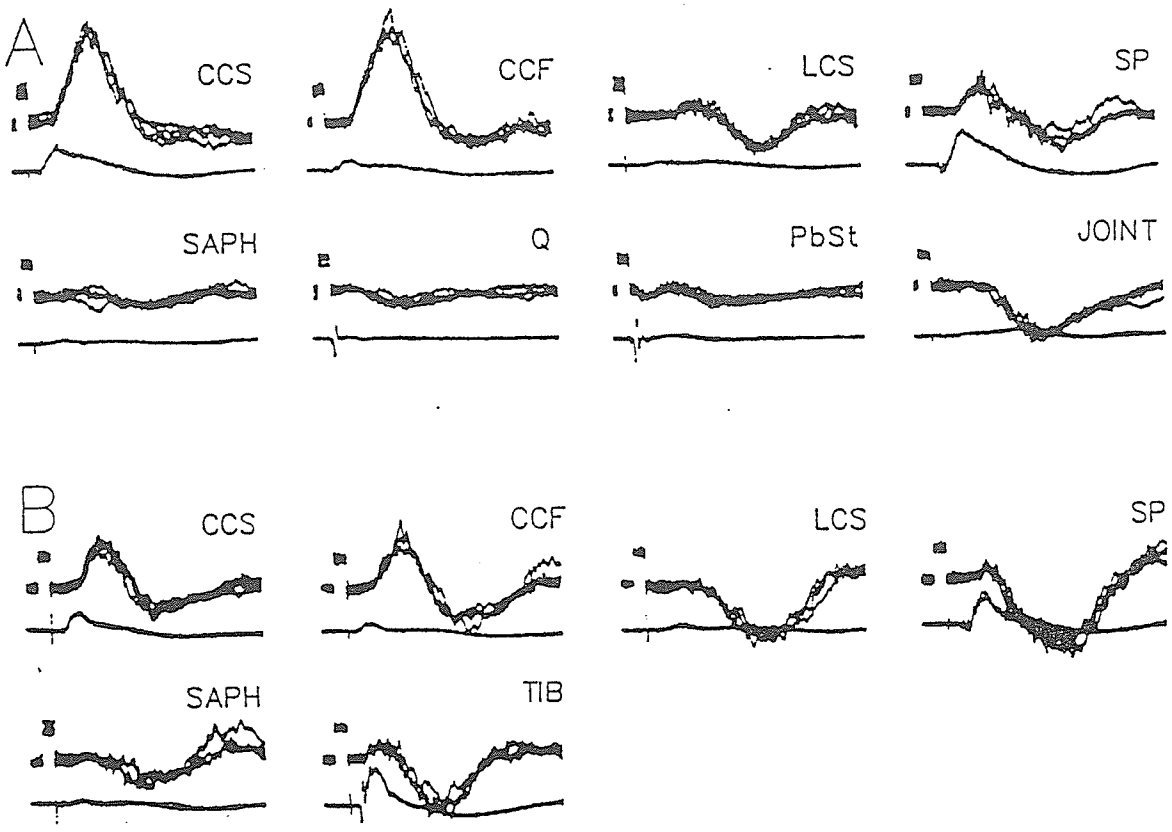

FIGURE 6

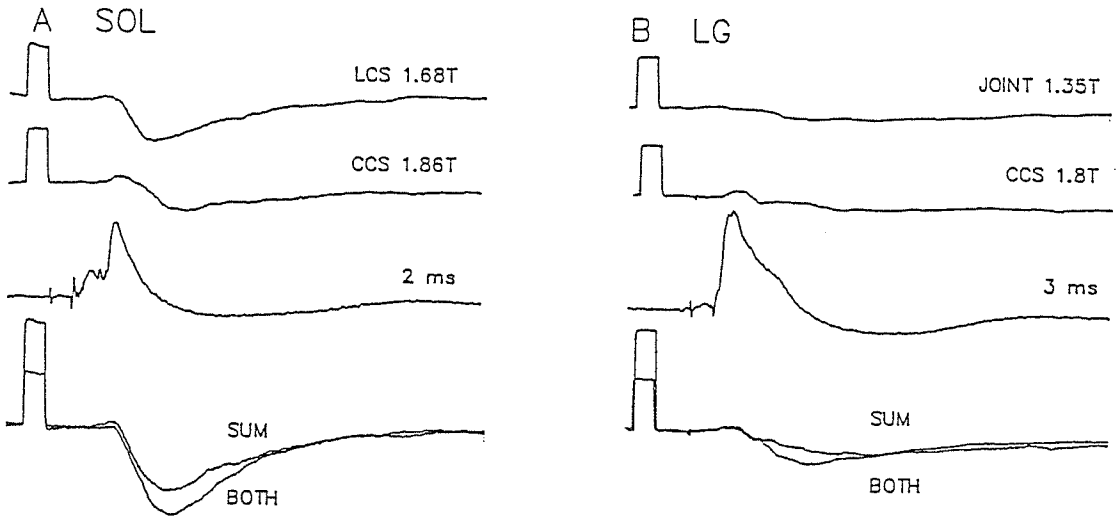
A SOL
LCS 1.68T
CCS 1.86T
2 ms
SUM
BOTH
B LG
JOINT 1.35T
CCS 1.8T
3 ms
SUM
BOTH

FIGURE 7

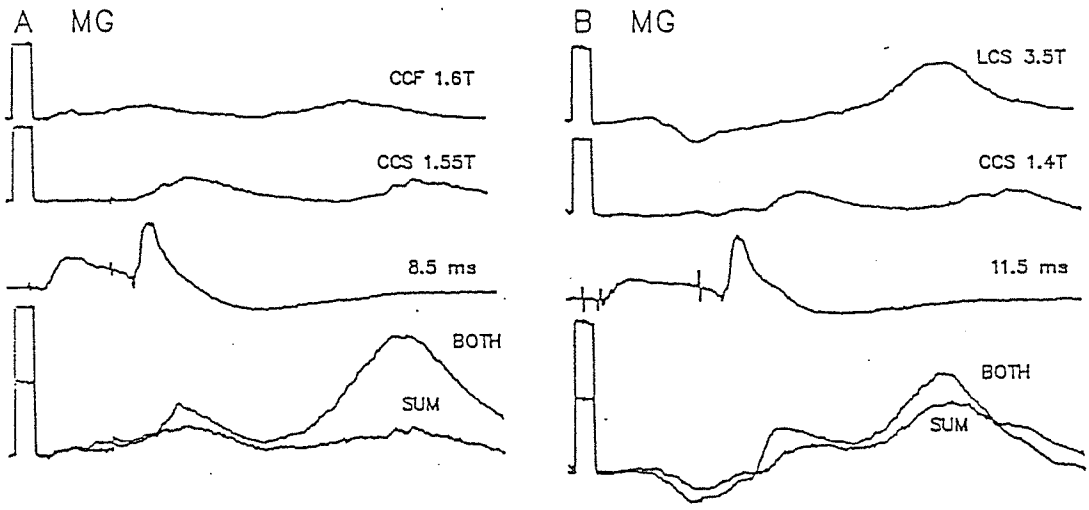
A MG
CCF 1.6T
CCS 1.55T
8.5 ms
BOTH
SUM
B MG
LCS 3.5T
CCS 1.4T
11.5 ms
BOTH
SUM

FIGURE 8

TABLE 1. *Summary of PSPs and spatial facilitation for all condition-test peripheral nerve combinations*

		PSPs			*FACILITATION*		
	N	%EP	%IP	%NE	n	%n	mV
CCS	70	97	--	3	--	--	----
CCF	60	72	7	21	30	93	0.73
LCS	70	13	52	31	9	56	0.38
SAPH	44	59	27	14	9	44	0.69
TIB	46	50	33	9	7	43	0.53
SP	66	91	7	2	25	28	0.42
JOINT	54	19	39	42	3	0	----

TABLE 2. *Stimulation parameters for facilitation of CCF and CCS PSPs*

mV	n	CCF T	CCS T	DELAY
<0.40	19	2.8	1.7	3.9 ms
0.40<0.55	19	3.0	1.6	4.5 ms
0.55<0.70	16	2.8	1.8	4.6 ms
≥0.70	17	2.4	1.6	5.2 ms

AVERAGE LATENCIES OF EPSPs: CCF 2T: 4.8 ms
5T: 4.8 ms

CCS 2T: 2.8 ms
5T: 2.9 ms

PART III) Low threshold cutaneous reflex pathways to the motor nuclei of triceps surae, in the unlesioned and chronic spinal cat

SUMMARY AND CONCLUSIONS

1. Postsynaptic potentials (PSPs) were recorded in 122 triceps surae motoneurons of 9 chronic spinal cats (6-7 weeks post-transection), upon electrical stimulation of the caudal and lateral branches of the ipsilateral sural nerve (CCS and LCS respectively). Results are compared to those from unlesioned animals.

2. With both twice and five times threshold (2 and 5T) CCS stimulation, excitatory PSPs (EPSPs) occurred more frequently as the earliest recorded effect in medial gastrocnemius (MG), lateral gastrocnemius (LG) and soleus (SOL) motoneurons of chronic spinal animals. Average latencies of EPSPs, as well as latencies of inhibitory PSPs (IPSPs) recorded as the earliest effect, were not significantly different in the two preparations. In all three motor nuclei of triceps surae, both EPSPs and IPSPs increased in amplitude and rate of rise and decay. Excitation predominated in the MG portion of triceps surae, as in unlesioned animals (LaBella et al. 1989).

3. Postsynaptic effects of LCS stimulation at both 2 and 5T were primarily inhibitory in LG and SOL, and more variable in MG motoneurons, of either preparation. However, in chronic spinal animals there were more cells with measureable effects of LCS stimulation, IPSPs tended to rise and decay faster, and IPSP latencies were significantly shorter with 5T stimulation (4.5 vs. 5.2 ms). Early-latency EPSPs (<6.5ms) occurred more frequently as the first effect of LCS stimulation in all three motoneuron groups.

4. EPSPs in MG motoneurons produced by double shock CCS stimulation were examined to determine whether or not the generally larger EPSPs in chronic spinal animals involved a concomitant decrease in subliminal fringe interneurons along the reflex path. The results indicate that at various frequencies of double shock stimulation the total amount of depolarization is greatest in motoneurons of chronic spinal animals.

5. In summary, chronic spinal preparations are characterized by an increased incidence, amplitude, and rate of rise and decay of the earliest sural nerve effects in triceps surae motoneurons, with earliest PSP latencies remaining for the most part unaltered. It is suggested that these altered characteristics reflect enhanced recruitment at interneuronal relays which reduces the temporal dispersion of postsynaptic effects in motoneurons.

INTRODUCTION

Chronic transection of the adult mammalian spinal cord is followed by a variety of reflex changes caudal to the lesion, and of these, the increased response to muscle stretch is the most thoroughly documented. By contrast, altered reflexes of cutaneous origin have undergone relatively little investigation, although clinical observations in both man (Kuhn 1950; Riddoch 1917; Walshe 1914) and animal (Afelt 1970; Bailey et al. 1980; Creed et al. 1932; Denny-Brown 1966; Kozak and Westerman 1966; Naftchi et al. 1980; Nesmeyanova 1959; Sherrington 1906) clearly indicate exaggerated responses to cutaneous stimulation after chronic spinal lesions as well. Although widely accepted that the changes in cutaneous reflexes involve centrally located mechanisms, such mechanisms are not clearly defined. The incomplete description of cutaneous reflex pathways in the *intact* spinal cord, and the variety of preparations examined which involve sub-total spinal lesions and/or immature animals, have complicated interpretations relevant to reflex function in the transected adult spinal cord. The reader is referred to several excellent reviews on the subject of mechanisms underlying structural and/or functional plasticity after central lesions (Goldberger and Murray 1985; Marshall 1985; Mendell 1984; Nicholls 1982; Tsukahara 1981; Wall 1987).

The sural reflex pathways to triceps surae motor nuclei are used here as a model system to assess cutaneous reflex changes after chronic spinal transection for several reasons. Initially we were motivated by reports using unlesioned animals that electrical stimulation of the caudal cutaneous branch of sural (CCS) tends to produce more excitation in "fast" triceps surae motoneurons than in "slows" (Burke et al. 1973; see also Burke et al. 1970). This afforded a discrete pattern of cutaneous input discernable at the intracellular level which could be

compared in the chronic spinal preparation. This pattern has subsequently been examined in acute and chronic spinal animals and was essentially found to persist (Baker and Chandler 1987b). However, we remain interested in this pathway upon finding that CCS excitation is exceedingly more prevalent in MG motoneurons than in SOL *or* LG cells, and thus is distributed on a larger scale, in apparent disregard of motor unit type (LaBella et al. 1989). The lateral cutaneous branch of sural (LCS) was found in this same study to produce widespread inhibitory effects in these motoneurons, albeit with some excitation which occurred mainly in MG. Hence, we have an unusually detailed picture of what postsynaptic effects to expect in the unlesioned animal, and from which we might discern similarities and/or departures in the chronic spinal preparation. Since CCS excitation may reflect a "specialized" cutaneous pathway to extensor motoneurons (see Discussion LaBella et al. 1989) while LCS inhibition is more characteristic of a general "flexor reflex afferent" pattern (Eccles and Lundberg 1959a; see also Lundberg 1979) these pathways further provide two contrasting types of synaptic input which may respond differently to chronic spinalization.

Because of the inherent difficulty in interpreting long-latency effects in motoneurons, the majority of this study limits itself to short-latency effects produced by single shock stimulation of the CCS and LCS nerves. However, because of the consistency with which low threshold CCS stimulation produces early-latency excitatory effects in MG cells (which appear to be largely uncomplicated by hyperpolarizing components), we have also used double shock stimulation to examine temporal facilitation of CCS EPSPs in a sample of MG motoneurons. This portion of the study reflects our interest in the interneurons of this excitatory reflex pathway (Part II) and prefaces a comparison of convergence patterns with other cutaneous reflex pathways to MG in the

unlesioned and chronic spinal preparations (Part IV). Details of the sural/triceps surae study in unlesioned animals may be referred to in the report cited previously (LaBella et al. 1989). Portions of the chronic spinal data in the present report have appeared in abstract form (LaBella et al. 1986).

METHODS

Spinalization procedure

The spinal cords of 9 adult female cats (mean weight 2.74 kg) were completely transected at the L_{1-2} level using sterile techniques and sodium pentobarbital anaesthesia (30 mg/kg intraperitoneally, supplemented with Halothane and nitrous-oxide/oxygen). Cords were bluntly dissected with fine forceps and visual inspection under the dissecting microscope ensured that the rostral and caudal cord segments were completely separated. Morphine was given in the immediate postoperative period, and penicillin G was administered for the first five days following transection. No infections were encountered. Nursing care involved twice daily manual expression of the bladder for the 6-7 week period prior to the acute experiment.

Dissection

Animals were anesthetized with halothane delivered in a mixture of oxygen and nitrous oxide. A tracheotomy was performed and one femoral artery cannulated to monitor blood pressure. Intravenous cannulae were placed in forelimb veins for drug and fluid administration as well as for slow infusion of a glucose/bicarbonate buffer. Atropine (0.12 mg) was given subcutaneously and dexamethasone (4 mg) intravenously. An L_{4-6} laminectomy exposed the spinal cord for intracellular recording and several nerves of the left (ipsilateral) hindlimb were cut and dissected free of surrounding tissue for subsequent stimulation on bipolar stimulating electrodes. MG, LG-SOL, and SOL nerves were dissected for antidromic identification of motoneurons, and it was ensured that the SOL branch of the LG-SOL nerve was sufficiently dissected away to allow independent activation of SOL efferents. Peripheral nerves stimulated for the production of PSPs in motoneurons include CCS and LCS; additional nerves were cut and mounted to aid in

locating motoneuron nuclei.

Experimental procedure

After completion of the L_{4-6} laminectomy, halothane was discontinued and replaced with intravenous chloralose (40-50 mg/kg initial dose, increased to a total dose of 60-90 mg/kg during the experiment). Arterial blood pressure and end tidal expired carbon dioxide were continuously monitored. After mounting the animals in a Göteborg type spinal frame (Transvertex Co., Ltd., Stockholm) the animal was paralyzed with gallamine triethiodide and artificially respired, and a bilateral pneumothorax performed. Mineral oil pools were made to prevent the hindlimb nerves and the exposed spinal cord from drying out. Esophageal and back pool temperatures were monitored and regulated by heating lamps.

Intracellular recording

Intracellular recordings from lumbar motoneurons were obtained using 2M potassium citrate microelectrodes with resistances of 2-5 MΩ. The current monitor, as well as microelectrode and cord dorsum recordings, were amplified and displayed at different time bases and were photographed simultaneously. The cord dorsum electrode was placed on the dorsal surface of the mid to rostral L_7 spinal cord and was used to measure the latencies of PSPs in motoneurons. Individual thresholds for peripheral nerve stimulation were defined as the stimulus required to produce the first detectable deflection in the cord dorsum electrode recording.

Data analysis

PSP composites Single sweep records of CCS or LCS PSPs in individual motoneurons were traced and superimposed to provide a composite picture of PSPs in the MG, LG and SOL populations (Figs. 1

and 3). For this purpose records were limited to cells which maintained action potentials between 60 and 95mV during PSP recording, and except for 3 of the 101 cells used for this particular analysis, no injected current was needed to identify reversed PSP components. Composites (as well as latency histograms, described below) from the present chronic spinal experiments are compared to the results from 10 unlesioned animals in a previous report (LaBella et al. 1989).

Latency histograms Intracellular effects in motoneurons were photographed and PSP latencies measured from enlargements of the film records. In addition, PSPs were averaged on-line and stored for later analysis. Care was taken to prevent classification of reversed IPSPs as EPSPs by either depolarizing the motoneuron with injected current or by ascertaining that stimulation of some peripheral nerves resulted in substantial PSPs in the hyperpolarizing direction. PSP latencies were measured from the arrival of the first afferent volley at the cord dorsum. Effects of extracellular fields were assessed by superimposing computer averages of the potential recorded just outside the motoneuron with intracellular records.

Double shock PSPs The CCS nerve was stimulated at 1.4 and 2T at frequencies of 100, 200, and 300 HZ between stimuli, and the effects recorded in a sample of MG motoneurons from each type of preparation. Averaged records of 16 sweeps at each frequency were then stored for later compilation into raster plots. The unlesioned sample is drawn from a pool of 12 animals from a previous study which employed identical experimental conditions (Part II); these additional 12 animals were only used for this portion of the present study. The chronic spinal sample is drawn from a pool of 8 animals used in a subsequent study (Part IV); surgical preparation of these additional 8 chronic spinal animals (which were also only used for this portion

of the present study) are as described above, as are the details of the acute experimental procedure.

RESULTS

Sural PSPs in triceps surae motoneurons

Postsynaptic potentials were recorded in antidromically identified triceps surae motoneurons following single shock stimuli to either the CCS and LCS nerve at 2 and 5T. The sample in unlesioned preparations consists of 43 MG, 43 LG, and 29 SOL motoneurons for a total of 115; the corresponding numbers in chronic spinal cats are 47, 48, and 27 for a total of 122.

CCS PSPs: amplitude and shape

In order to present a general picture of CCS PSPs in the two preparations, film records from individual cells were enlarged and traced, and superimposed with records from other cells of the same motoneuron population. In Fig. 1 these composites are provided for 31 MG, 24 SOL and 34 LG motoneurons in unlesioned animals (1A), and for 36 MG, 25 SOL and 40 LG in chronic spinal animals (1B). One record is provided for each motoneuron at 2 and 5T CCS stimulation; 2T composites in 1A are from a previous report (LaBella et al. 1989). One difficulty with this form of presentation was to select a range of membrane potentials which would provide meaningful comparison of PSPs between cell groups and between preparations. IPSPs are particularly sensitive to the resting membrane potential and may become reversed in motoneurons at high levels of membrane hyperpolarization. However, Gustafsson and Pinter (1984) have demonstrated that action potential amplitude is a fairly reliable index of motoneuron membrane potential. Fig. 1 includes cells which maintained action potentials between 60 and 95 mV throughout recording of CCS PSPs, and injection of depolarizing current through the microelectrode was used in 3 cells only. This procedure did not appear to create any particular bias between unlesioned and chronic spinal preparations and thus the data in Fig.

1 should provide a reasonable comparison of CCS PSPs in different motoneurons and preparations at comparable levels of membrane polarization. Means, medians and ranges of action potential amplitudes for all motoneuron populations are provided in the left panel of Table 1.

The composites of Fig. 1 suggest several differences in CCS PSPs of chronic spinal (1D-1F) compared to unlesioned (1A-1C) data: fewer motoneurons (particularly LG motoneurons) with no effect of CCS stimulation; increased amplitude of PSPs (especially EPSPs); and increased rates of rise and decay for both inhibitory and excitatory PSP components. The faster rates of PSP rise and decay appear to shorten the time course of CCS PSPs in chronic spinal animals; not only are the early EPSPs "cut off" sooner by ensuing IPSPs (compare 1B and 1E), but the hyperpolarization also returns to baseline faster. However, despite differences in amplitude and waveform, the general pattern of synaptic input from CCS is similar in the two preparations. Thus, as reported for unlesioned animals (LaBella et al. 1989), CCS excitation predominates in the MG portion of triceps surae motor nuclei in chronic spinal animals.

CCS PSPs: latencies

The histograms of Fig. 2 show the latencies of CCS PSPs in triceps surae motoneurons. Because only the earliest recorded effects are included, only one effect per cell is indicated at each stimulation intensity (2 and 5T). In motoneurons of unlesioned animals there was a clear tendency for the latencies of 2T CCS EPSPs recorded in MG motoneurons to be shorter than those recorded in LG or SOL (upper panel 2A; data from LaBella et al. 1989). In chronic spinal preparations (upper panel 2B) this difference was greatly reduced by an increased incidence of short-latency EPSPs in LG and SOL cells (and concomitant

decrease in the number of LG and SOL cells with no measureable effect), as well as a trend for slightly longer EPSP latencies in MG. For example, 14% of SOL, 14% of LG, and 81% of MG motoneurons had 2T EPSP latencies <3.0 ms in unlesioned data; corresponding values in the chronic spinal sample were 26%, 33%, and 66%. The average latency of 2.9 ms in all motoneurons of the chronic spinal sample compared to 2.6 ms for unlesioned is significant with $p < .001$ (Student's t-test). When the stimulus intensity was raised to 5T (lower panels Fig. 2) the incidence of EPSP latencies <3.0 ms increased in all three motoneuron groups and in both unlesioned and chronic spinal preparations; values are 21% of SOL, 16% of LG and 85% of MG cells in unlesioned data; corresponding values in chronic spinal data are 54%, 58%, and 93%. Average latencies of 5T EPSPs in all motoneurons were not significantly different in the two preparations (2.8 ms in unlesioned; 2.6 ms in chronic spinal).

[Note: Because average CCS EPSP latencies with 5T stimulation are not significantly different, we cannot conclude the small (0.3 ms) difference with 2T stimulation implies a change in transmission time through the minimum pathlength. This difference with 2T stimulation is likely due to measurement errors introduced by the difficulty in measuring small amplitude, slowly rising EPSPs in unlesioned animals, where even with the aid of extracellular field potentials, latency decisions must sometimes be made on the basis of depolarization amplitude (approximately > 0.3-0.4mV).]

A minimum latency of 1.5 ms for excitation in both preparations is consistent with other reports (e.g. Pinter et al. 1982; R. Burke personal communication; Omeniuk et al. 1986). However, as Fig. 2 indicates, EPSP latencies were usually considerably longer than this and values of 1.5-2 ms were measured more often in motoneurons of

unlesioned than chronic spinal preparations. This appears to be largely due to the reduction in the number of EPSPs recorded before 2 ms in MG cells. However, the average latencies of EPSPs in Fig. 2B for MG motoneurons only, are 2.7 ms for 2T and 2.5 ms for 5T (not indicated in the figure). These averages are identical to those obtained from 70 MG motoneurons in eight chronic spinal cats used in a separate study in this laboratory. Because 23 of these 70 cells had CCS EPSPs < 2.5ms, we do not believe there is evidence in Fig.2B for a decrease in the activity of the shortest-latency pathways from CCS afferents to MG motor nuclei.

Average latencies of IPSPs were not significantly different between the two preparations although IPSPs occurring as the first effect of CCS stimulation did appear less frequently in chronic spinal animals (lower panels in 2T and 5T histograms of Fig.2). This latter observation is probably directly due to the fact EPSPs frequently replaced IPSPs as the first measureable effect in LG and SOL motoneurons. On the other hand, an IPSP was rarely the first effect recorded in an MG motoneuron in either preparation; in fact occurring only once at 2T stimulation in unlesioned animals. Also common to the two preparations was the observation that early-latency IPSPs were never followed by subsequent depolarization (in the first 20-30 ms). Thus, while both latencies and patterns of the earliest CCS PSPs in triceps surae motoneurons are largely unchanged by the transection procedure, PSPs are characterized by an overall increase in incidence which includes earlier excitation and less inhibition of LG and SOL motoneurons.

LCS PSPs: amplitude and shape

The composites of LCS PSPs illustrated in Fig. 3 were made according to the same conditions described for composites of CCS PSPs.

Data from unlesioned animals are depicted in panels A, B and C; data from chronic spinal animals in D, E and F. Action potential amplitudes for records in each of these panels are provided at the right of Table 1.

In triceps surae motoneurons of chronic spinal animals, LCS EPSPs and IPSPs appeared more frequently, and IPSPs had faster rates of rise and decay. There was also a greater incidence of LCS effects, and an average increase in the amplitude of PSPs, particularly IPSPs. For example, many of the largest amplitude LCS IPSPs in unlesioned animals were obtained in only 1 of the 10 animals investigated (see LaBella et al. 1989), while more frequently, there was little or no effect of LCS stimulation seen (number of flat lines in Fig. 3 A, B, C). This contrasts sharply with results from 9 chronic spinal preparations where there were relatively few motoneurons without LCS PSPs, and the IPSPs were of more consistent amplitude (3 D, E, F). The generally faster rise and decay rates of LCS IPSPs is similar to that seen with effects of CCS stimulation (Fig. 1), where the time course of PSPs is both sharper and shorter in chronic spinal animals. LCS EPSPs, while less consistent in waveform, amplitude and incidence than CCS EPSPs, occurred more frequently in chronic spinal preparations. Inhibition, however, remained the dominant overall effect of LCS stimulation in triceps surae motor nuclei.

LCS PSPs: latencies

Latencies of the earliest PSPs in triceps surae motoneurons are reported for LCS stimulation at 2 and 5T in Fig. 4. Figure 4A is reproduced directly from the previous report (LaBella et al. 1989), while 4B consists of data from 113 motoneurons in chronic spinal animals. The upper panels of 4A and 4B depict the latencies of LCS PSPs evoked by 2T stimulation; lower panels those by 5T. Cells with effects

at both 2 and 5T stimulation intensities are thus represented in both upper and lower panels, with "no effect" referring to results at 5T (NE, see boxed inset).

Comparison of LCS histograms in 4A and 4B indicate that chronic spinal section led to an increased incidence of early-latency EPSPs and IPSPs in all three motoneuron groups. The increase in excitation is particularly evident with 5T stimulation (lower histograms) where the incidence of EPSPs observed as the first effect in motoneurons increased from 8% (4A) to 33% (4B). Note that over half of these EPSPs were measured in MG motoneurons. IPSPs, the most prominent early effects of LCS stimulation in both preparations, show a decrease in average latency which is significant for 5T stimulation (4.5 ms compared to 5.2 ms in unlesioned data; $p < .001$), and there is less scatter of IPSP latencies about the mean. Overall, the increased incidence of PSPs is most pronounced in the MG population (compare number of "no effects" in boxed insets; NE); particularly evident with the lower (2T) stimulation strength.

Double shock CCS PSPs

Averaged PSPs in MG motoneurons produced by double shock stimulation of CCS are shown in the raster plots of Figs. 5 and 6. This analysis attempts to address the question of whether or not the larger/sharper PSPs in chronic spinal animals produced by single-shock stimulation (Figs. 1 and 3) reduced the number of subliminal fringe interneurons (Denny-Brown and Sherrington 1928) available for a second stimulus. Early-latency CCS EPSPs in MG were specifically selected for this study because they constitute a relatively consistent postsynaptic effect from cell to cell, and at low stimulation intensities are not usually followed by hyperpolarizing potentials (which will obscure temporal facilitation of depolarizing components).

Figure 5 depicts results with 1.4T stimulation in six raster plots (1.4T was found to be the average threshold for production of CCS EPSPs in MG motoneurons of unlesioned preparations). Records in A, B, and C are from 30 MG motoneurons in chronic spinal animals (one record per motoneuron); records in D, E, and F are from 24 MG cells of unlesioned animals. Records consist of 16-sweep averages with double shocks at 100 Hz (A,D), 200 Hz (B,E) and 300 Hz (C,F) and were ranked in each raster approximately according to amplitude. Note that EPSPs produced by the first shock are generally smaller in D, E and F than in A, B and C. Thus, similar to results for 2 and 5T, stimulation of CCS at 1.4T produces greater excitatory effects in MG motoneurons of chronic spinal animals (compare Fig. 2A and 2D). However, the effect of a second shock in Fig. 5 also tends to be bigger in chronic spinal animals, suggesting that the larger EPSPs produced by a single shock do not compromise the subliminal fringe population for a second stimulus. In theory this could be achieved by a reduced refractoriness of interneuron firing in the chronic spinal preparation and/or a change in the membrane time constant of spinal interneurons (see Discussion). Because 300 Hz stimulation is relatively ineffective in facilitating EPSPs in chronic spinal animals (compare 5C to 5A and B), this may describe the limits of such reduced refractoriness.

Figure 6 depicts averaged PSPs in MG cells produced by double-shock CCS stimulation at 2T; format is identical to that of Fig. 5. Essentially, the differences between unlesioned (D, E and F) and chronic spinal data (A, B and C) persist, with overall depolarization produced by double stimuli being larger in motoneurons of the latter. However, it is noted that actual "facilitation" of EPSPs produced by the first shock is not visibly greater in the chronic spinal data (also true in Fig. 5). This is not surprising given that relatively weak PSPs

in motoneurons of unlesioned animals might be expected to involve a relatively greater subliminal fringe. Nonetheless, it is clear that both the first and second shocks to the CCS nerve are capable of producing larger amplitude effects in MG motoneurons of chronic spinal animals. It is worth noting that the largest PSPs in unlesioned animals (D, E and F in both Fig. 5 and 6) are generally well-facilitated by the second shock, thus appearing much like EPSPs in chronic spinal animals. These few instances of substantially large EPSPs are from only 1 of the 12 unlesioned animals used in this portion of the present study; an animal which had low blood pressure and spinal edema early on in the experiment. This suggests the possibility that altered transmission through interneuronal relays in the chronic spinal animal may not involve a structural change in the number or arrangement of synaptic contacts along the reflex pathway.

DISCUSSION

In summary, sural PSPs recorded in triceps surae motoneurons of the chloralose-anesthetized preparation are altered by chronic spinal transection. In chronic spinal animals they are characterized by a faster rate of rise and decay which decreases their duration, and by an overall increase in amplitude and incidence. This is also the case for PSPs produced by both single and double shock CCS stimulation at low strengths of afferent stimulation (1.4T). Latencies of sural PSPs are for the most part unaltered. Because the general pattern of synaptic input from both CCS and LCS is very similar in unlesioned and chronic spinal preparations, a greater incidence of PSPs and changes in PSP waveform are consistent not with the appearance of novel pathways from sural afferents, but with changes in transmission through pre-existing interneuronal paths. The idea that transmission is altered through mechanisms presynaptic to motoneurons is supported by the observation that passive electrical properties of motoneurons (input resistance and electrotonic length) are largely unchanged six weeks after chronic spinal transection (S. Hochman and D. McCrea, personal communication; see also Baker and Chandler 1987a, b).

The general increase in amplitude and incidence of sural PSPs must be considered carefully, since amplitude, as well as the excitatory/inhibitory balance of polysynaptic PSPs produced by a variety of peripheral afferents, including cutaneous afferents, is highly preparation-dependent (e.g. Eccles and Lundberg 1959a). Thus, while cutaneous-evoked PSPs in ipsilateral extensor motoneurons are often predominantly inhibitory in acute spinal animals (Eccles and Lundberg 1959a; our own observations), it is interesting that both CCS (sural) IPSPs and EPSPs in ipsilateral triceps surae motoneurons have been reported to be larger in chronic spinal animals than in acute

spinal preparations (Baker and Chandler 1987b). However, as mentioned earlier, many of the largest sural PSPs (excitatory and inhibitory) in the present data from unlesioned animals were obtained in only 1 of the 10 animals investigated (for data in Figs. 1-4; see also LaBella et al. 1989). In addition, this was the only animal with CCS EPSPs in LG motoneurons with latencies <3.0 ms. This is an important observation because it suggests that the "wiring" is normally present for relatively large (and early) EPSPs and IPSPs in the anesthetized cord-intact animal, but that these are more frequently observed in the anesthetized chronic spinal preparation. It should be noted that an increased incidence of CCS (sural) EPSPs in MG motoneurons after chronic spinalization motoneurons has been previously documented (Mayer et al. 1984), but PSP amplitudes were not reported by these authors.

It is interesting that LCS IPSPs evoked with 5T stimulation had significantly shorter central latencies in chronic spinal animals (lower panels Fig. 4). Because of the large number of LCS IPSPs recorded in all three motor nuclei of both preparations, and because of the magnitude of the decrease in average latency (0.7 ms), we believe this suggests enhanced transmission through a subset of parallel inhibitory pathways from LCS afferents, rather than the appearance of LCS inhibitory pathways containing fewer interneurons (also note clustering of IPSP latencies around the mean; 5T panel, Fig. 4B). While low numbers of CCS IPSPs in both unlesioned and chronic animals, and low numbers of LCS EPSPs in unlesioned animals, do not allow meaningful comparison of central latencies between preparations, the question arises as to why latencies of LCS IPSPs and not CCS EPSPs are shortened. It is possible that this reflects a greater degree of descending convergence upon the longer inhibitory reflex pathway in the intact animal, which consequently magnifies the effect of spinal lesions upon synaptic transmission. Evidence for potent supraspinal

control of inhibitory pathways from cutaneous afferents to extensor motoneurons is abundant; both indirect evidence from observations of motoneuron PSPs in animals with various segmental and suprasegmental lesions (e.g. Eccles and Lundberg 1959a,b; Holmqvist and Lundberg 1961; Schomburg and Steffens 1986; see also Engberg 1964), as well as direct evidence on the effects of stimulating the dorsal reticulospinal system (Engberg et al. 1968; see also Burke et al. 1973), rubrospinal (e.g Hongo et al. 1969; Pinter et al. 1982), and corticospinal tracts (e.g. Lundberg and Voorhoeve 1962; Pinter et al. 1982). Furthermore, removal of descending systems which exert tonic levels of inhibitory control in the anesthetized unlesioned animal, may also serve to increase transmission through segmental reflex pathways in the anesthetized chronic spinal animal. Lundberg has reported on one such tonic control system operating in the dorsolateral funiculus (see Lundberg 1982). Thus, while descending convergence with the LCS inhibitory pathway to ankle extensor motoneurons has not specifically been reported upon, the long latency of this pathway (see Fig. 4) would allow ample opportunity for interneuronal convergence with a variety of descending systems.

Although there is an increased incidence of sural excitatory effects in triceps surae motoneurons, in particular with LCS stimulation, it is more likely that this reflects enhanced transmission through normally weak excitatory pathways, rather than a reorganization of last-order excitatory projections to these cells. In support of this, is the general observation that patterns of sural synaptic input are similar in the unlesioned and chronic spinal preparations. Thus, in both preparations CCS stimulation produces a predominance of EPSPs in MG motoneurons, and more variable, often inhibitory, effects in LG and SOL cells. Similarly, in both preparations LCS stimulation produces predominantly IPSPs in LG and SOL cells, and more variable, frequently excitatory, effects in MG cells. Thus, while various forms of neuronal

plasticity may be proposed to explain the primarily quantitative changes in PSPs, the integrity of synaptic patterns as well as observations of large post-synaptic effects in two unlesioned animals of a total twenty-two (data from one animal included in Figs. 1-4, and from the other in Figs. 5 and 6) suggest caution in making such proposals. However, even when exceptionally large PSPs are present in unlesioned animals, they do not have the fast rise and decay characteristics of PSPs in chronic spinal animals (see Fig. 1). This then, is evidence that some degree of PSP enhancement in the latter preparations is derived from a mechanism which requires some time to develop after transection. For example, if EPSPs in interneurons also have faster rising phases in chronic spinal animals, interneuronal thresholds may be reached sooner, and could result in faster, more synchronous PSPs in motoneurons. This could be the result of either a change in the membrane time constant of interneurons, or a change in the time course of synaptic transmission at interneuronal relays. On the other hand, threshold itself could be decreased in the interneurons by faster rising EPSPs, if recent evidence for the effect of EPSP slope on motoneuron thresholds (Gustafsson and McCrea 1984) also applies to interneurons. Alternatively, a reduced refractoriness of interneuronal firing could be expected to result in a faster barrage of excitatory inputs to interneurons or motoneurons; and/or more synchronous excitatory inputs to these cells. A reduced refractoriness is certainly consistent with the present finding that a second shock to the CCS nerve still produces larger EPSPs in MG motoneurons of chronic spinal animals. The point is that the loss of descending convergence and/or modulation of interneuronal pathways may result in altered interneuronal activity which does not necessarily involve structural change in the number, kind, or location of axonal terminals in the reflex path; and the cumulative effect of such altered activity through polysynaptic chains may be a significant increase in the amplitude, as

well as rise and decay characteristics, of PSPs in motoneurons.

Afelt (1970) reported that weak tactile stimulation of the skin over the extensor muscles in chronic spinal adult cats usually evoked no response when a single stimulus was applied, and a slow extension of the knee, ankle, or all joints together when repetitive stimulation was applied. Weak pinch of the skin over MG evoked ankle extension during knee flexion and during whole limb extension; and rarely, a flexion of all joints in the limb was observed. Tapping of MG through the skin evoked extension of the ankle followed by slow extension of the entire limb. These observations are essentially in accord with Hagbarth's scheme for excitatory reflexes in MG from the skin afferents overlying it which are largely innervated by the CCS nerve (Hagbarth 1952). In our own chronic spinal cats, we observed that light touch to various regions of the hindlimb skin (including the region over the heel and calf innervated by CCS) could produce rapid and repetitive reflex movements of the entire limb. Slow and powerful reflex movements were most often produced by stimuli to skin of the distal extremities. Our general observations for stimulation of the medial calf skin are in accordance with that described by Afelt.

That EPSPs produced by 1.4T CCS stimulation were enhanced in chronic spinal animals of the present study is of particular interest in the context of behavior. In a few MG motoneurons of both unlesioned and chronic spinal preparations, trials of temporal facilitation had to be abandoned when inhibitory components in the PSP produced by the first shock to CCS, obscured EPSPs evoked by the second shock. However, a more frequent observation was that at both 1.4 and 2T intensities, double shocks to CCS resulted in motoneuron firing; much more so in chronic spinal animals than in unlesioned, and at 1.4T, only in chronic spinal preparations (examples not included in Figs. 5 and 6). Thus,

multi-shock electrical activation of low threshold afferents in CCS results in motoneuron recruitment more often in chronically transected animals. This in turn may have functional implications for the asynchronous volleys produced by natural cutaneous stimulation.

Similar to Denny-Brown's arguments concerning the recovery of segmental reflexes from spinal shock (Denny-Brown 1966), it is important to make a distinction between functional and structural reflex pathology when describing altered cutaneous reflexes in the chronic spinal animal. Because the chronic spinal model for studying segmental reflexes has neither the complexity of spinal integration with higher centers, as in the intact animal, nor the effects of post-lesion trauma, as in the acute spinal preparation, we cannot use preparation differences in reflex effects to assume the presence of an underlying structural pathology for cutaneous hyperreflexia. A recent review by Wall addresses the issue that evidence of morphological change is generally missing to "explain slow changes following damage to the adult central nervous system" (Wall 1987). Various forms of neuronal plasticity after a variety of types of chronic lesions have been identified (for e.g. axonal sprouting, unmasking of latent synapses, neurochemical changes etc...; see Goldberger and Murray 1985; Marshall 1985; Nicholls 1982; Tsukahara 1981; Wall 1987), but evidence that such plasticity is responsible for altered reflex function in the fully transected spinal cord is still missing. Thus, with respect to the present results, we propose that faster/larger sural PSPs in motoneurons of chronic spinal animals can be explained by altered activity in interneuronal relays; which does not necessarily imply a structural change in the number or arrangement of synaptic contacts along the reflex path. Acknowledging that this study has been concerned with subthreshold events in motoneurons, we suggest that such altered activity in excitatory sural pathways may facilitate motoneuron

recruitment in the chronic spinal animal; especially when added to the activity of other segmental excitatory inputs. The observation that sural IPSPs are generally larger and decay faster, could mean that recruitment is also turned off faster by more powerful inhibitory inputs from cutaneous reflex pathways in these animals.

The accompanying report (Part IV) uses the indirect technique of spatial facilitation (Lundberg 1975) to compare convergence on the CCS excitatory pathway to MG motoneurons by other cutaneous afferents in unlesioned and chronic spinal animals (unlesioned data from Part II). As the results in Part IV will show, spatial facilitation is more easily demonstrated after chronic spinal transection for a variety of convergent afferent systems. In a similar vein to the discussion above, we believe this can be explained by enhanced transmission through pre-existing convergent relays, and that qualitative structural reorganization of synapses is not necessarily implied.

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TABLE AND FIGURE LEGENDS

FIG 1. Postsynaptic potentials (PSPs) recorded in triceps surae motoneurons upon single shock stimulation of the CCS nerve at 2 and 5T. Enlarged photographic records of CCS PSPs in individual motoneurons were traced and superimposed. In each panel (A-F) there is one trace per motoneuron at each stimulus intensity. Traces in A, B and C from unlesioned animals; in D, E and F from chronic spinal animals. Number of PSPs (i.e. number of motoneurons) and action potential amplitudes in Table 1. Small vertical arrow indicates time of arrival of the CCS afferent volley at the cord dorsum electrode. MG, medial gastrocnemius; SOL, soleus; LG, lateral gastrocnemius.

FIG 2. Latencies of the earliest PSPs produced by 2T (upper histograms) and 5T (lower histograms) single shock stimulation of the CCS nerve in unlesioned (A) and chronic spinal (B) animals. S, soleus; L, lateral gastrocnemius; M, medial gastrocnemius. Number of motoneurons in A: S, n=29; L, n=43; M, n=43; in B: S, n=27; L, n=48; M, n=47. A few cells were not tested with 5T stimulation, otherwise the total n in each preparation corresponds to the boxed inset under 2T. NE, no effect at each stimulation strength. Arrows point to mean latencies of EPSPs or IPSPs .

FIG 3. PSPs recorded in triceps surae motoneurons upon 2 and 5T LCS stimulation. Details as for Fig. 1. Number of PSPs (i.e. number of motoneurons) and action potential amplitudes in Table 1.

FIG 4. Latencies of the earliest PSPs produced by 2T (upper histograms) and 5T (lower histograms) single shock stimulation of the LCS

nerve in unlesioned (A) and chronic spinal (B) animals. S, soleus; L, lateral gastrocnemius; M, medial gastrocnemius. Number of motoneurons in A: S, n=28; L, n=43; M, n=36; in B: S, n=26; L, n=47; M, n=40. Cells in which PSPs were produced at 5T but not 2T appear in lower histogram only. Cells in which PSPs were recorded following both 2 and 5T stimulation are plotted in both sets of histograms. NE, no effect at 5T stimulation. Arrows point to mean latencies of EPSPs or IPSPs.

FIG 5. Averaged PSPs (16 sweeps) in medial gastrocnemius motoneurons produced by 1.4T stimulation of the CCS nerve with double shocks at 100 Hz (A and D); 200 Hz (B and E); 300 Hz (C and F). Records in A, B and C from chronic spinal (CS) animals; in D, E and F from unlesioned (UL) animals. Ticks on left side of each record mark the arrival of the first CCS afferent volley at the cord dorsum electrode. Number of records (i.e. number of motoneurons) in A, B and C, 30; number in D, E and F, 24.

FIG 6. Averaged PSPs in medial gastrocnemius motoneurons produced by 2T stimulation of the CCS nerve with double shocks. Details as in Fig. 5. Number of records (i.e. number of motoneurons) in A and C, 31; in B, 30; in D, E and F, 28.

TABLE 1. Action potential amplitudes in triceps surae motoneurons averaged from those recorded just before and after collecting CCS and LCS PSPs. Amplitudes are expressed as mean/median with the range directly below. Values in A-F of left and right panels correspond to motoneuron populations in A-F of Figs. 1 and 3, respectively. Number of motoneurons in which action potentials were measured: CCS (Fig.1) A, n=31[29]; B, n=24; C, n=34; D, n=36[30]; E, n=25[24]; F, n=40. LCS (Fig.3) A, n=25; B, n=23;

C, n=32; D, n=30; E, n=23; F, n=37; square brackets indicate a slightly reduced n for CCS PSPs at 5T. UL, unlesioned; CS, chronic spinal.

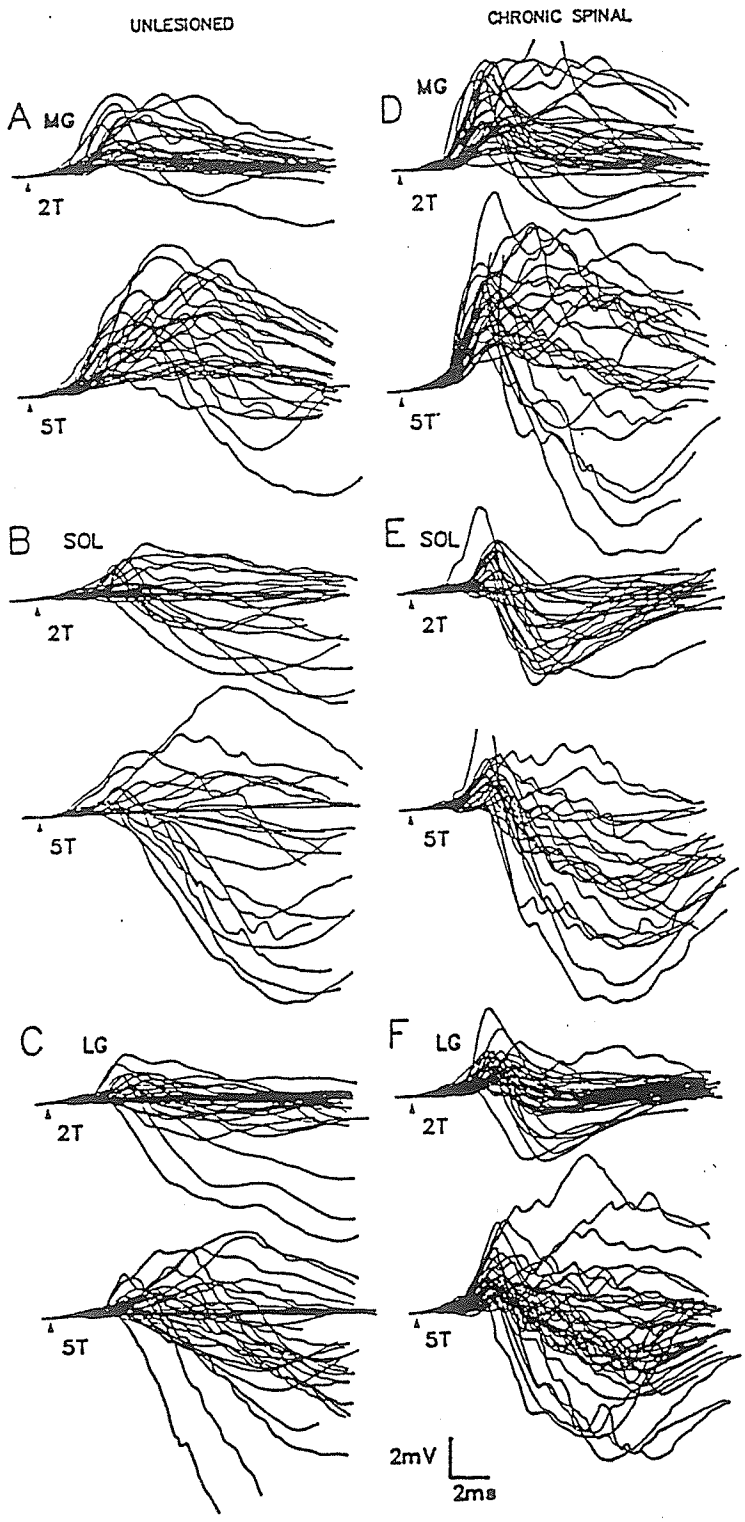

CCS PSPs
UNLESIONED
CHRONIC SPINAL
A MG
2T
5T
D MG
2T
5T
B SOL
2T
5T
E SOL
2T
5T
C LG
2T
5T
F LG
2T
5T
2mV
2ms

FIGURE 1

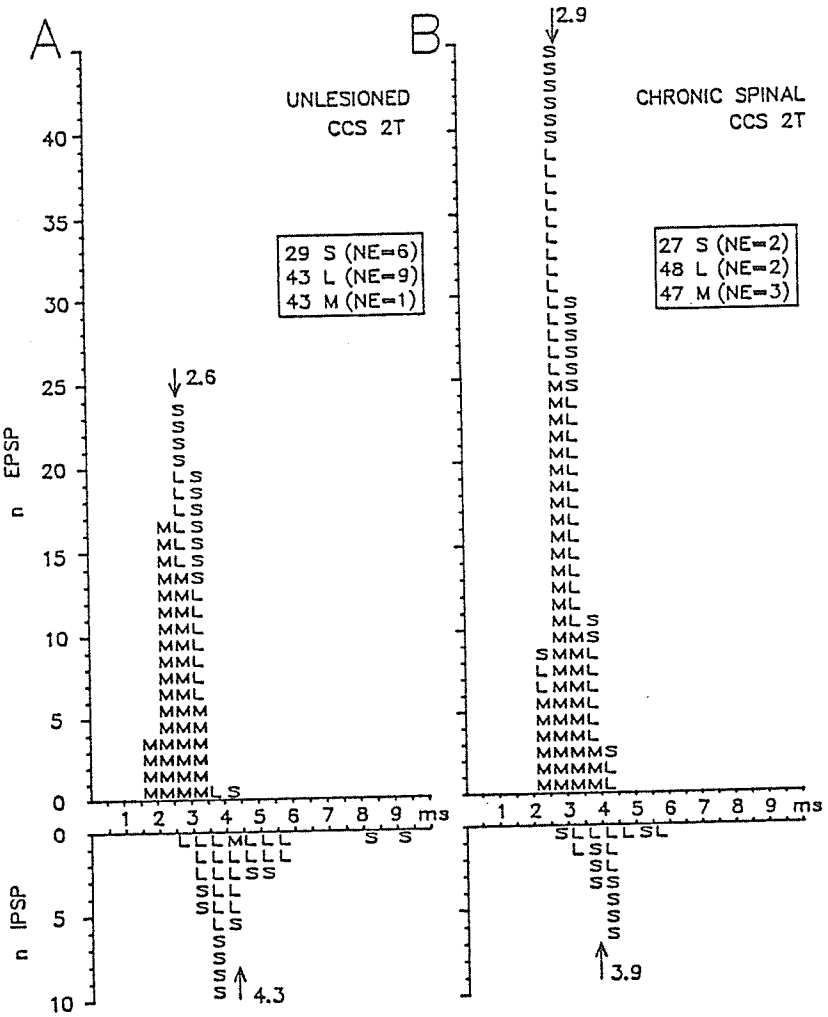
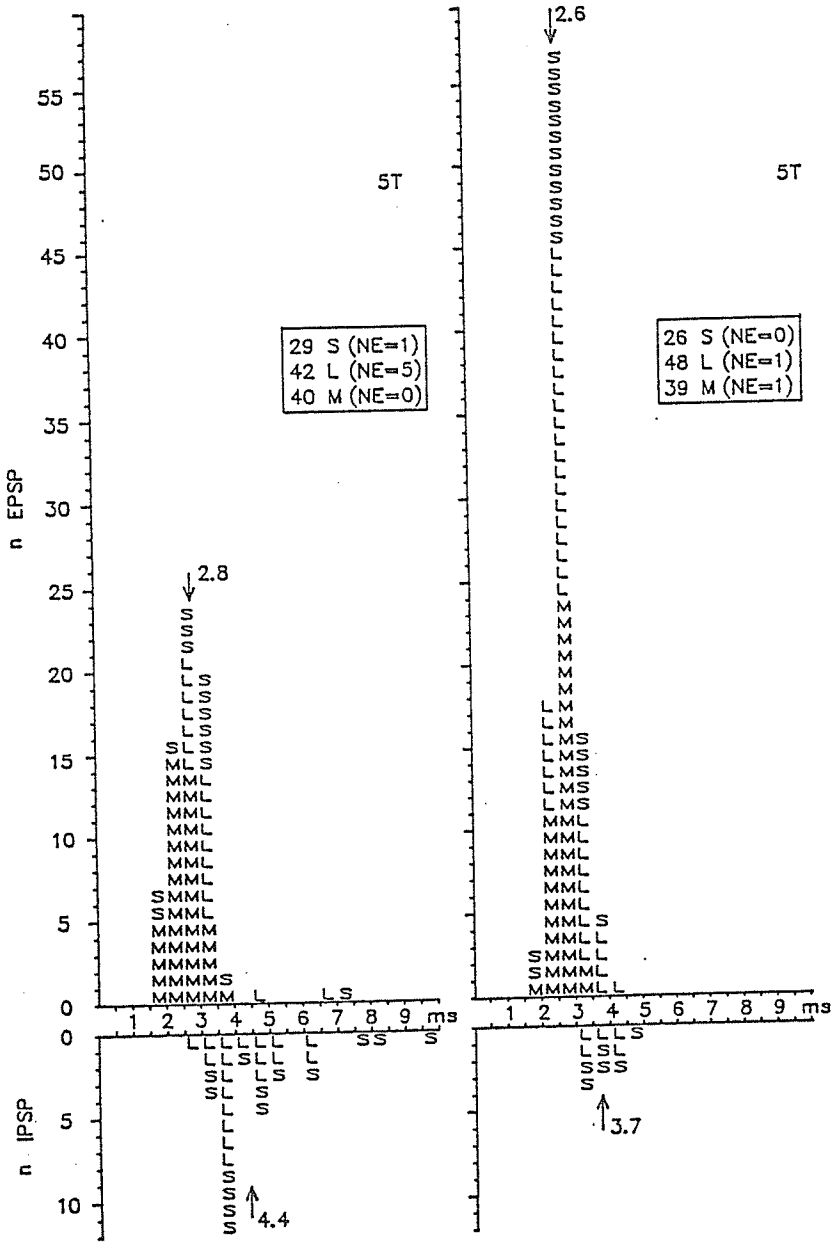

A
B
UNLESIONED
CCS 2T
29 S (NE=6)
43 L (NE=9)
43 M (NE=1)
CHRONIC SPINAL
CCS 2T
27 S (NE=2)
48 L (NE=2)
47 M (NE=3)
n EPSP
n IPSP
2.6
4.3
2.9
3.9
ms

FIGURE 2


5T
29 S (NE=1)
42 L (NE=5)
40 M (NE=0)
2.8
4.4
n EPSP
n IPSP
ms
5T
26 S (NE=0)
48 L (NE=1)
39 M (NE=1)
2.6
3.7
ms

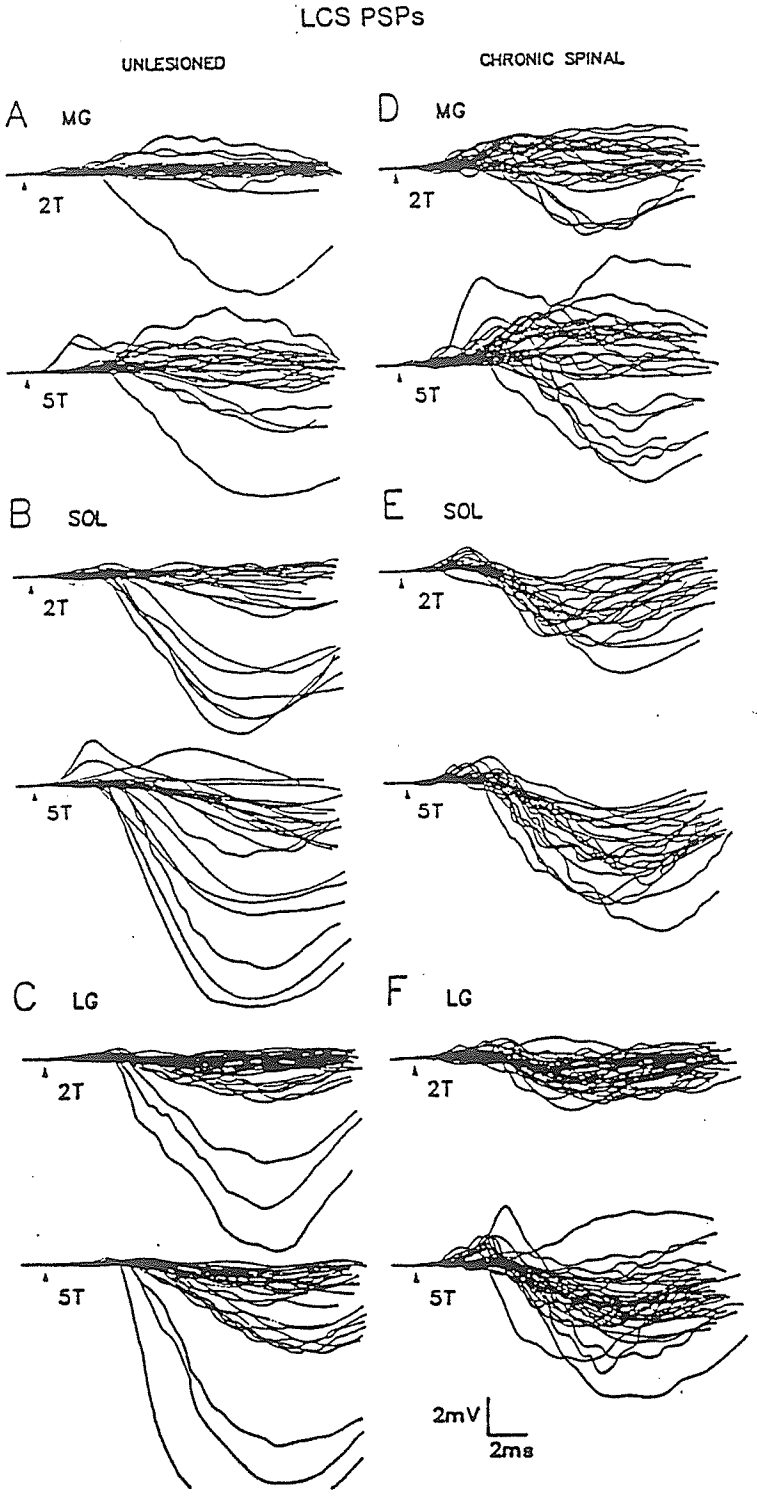

LCS PSPs
UNLESIONED
CHRONIC SPINAL
A MG
2T
5T
D MG
2T
5T
B SOL
2T
5T
E SOL
2T
5T
C LG
2T
5T
F LG
2T
5T
2mV
2ms

FIGURE 3

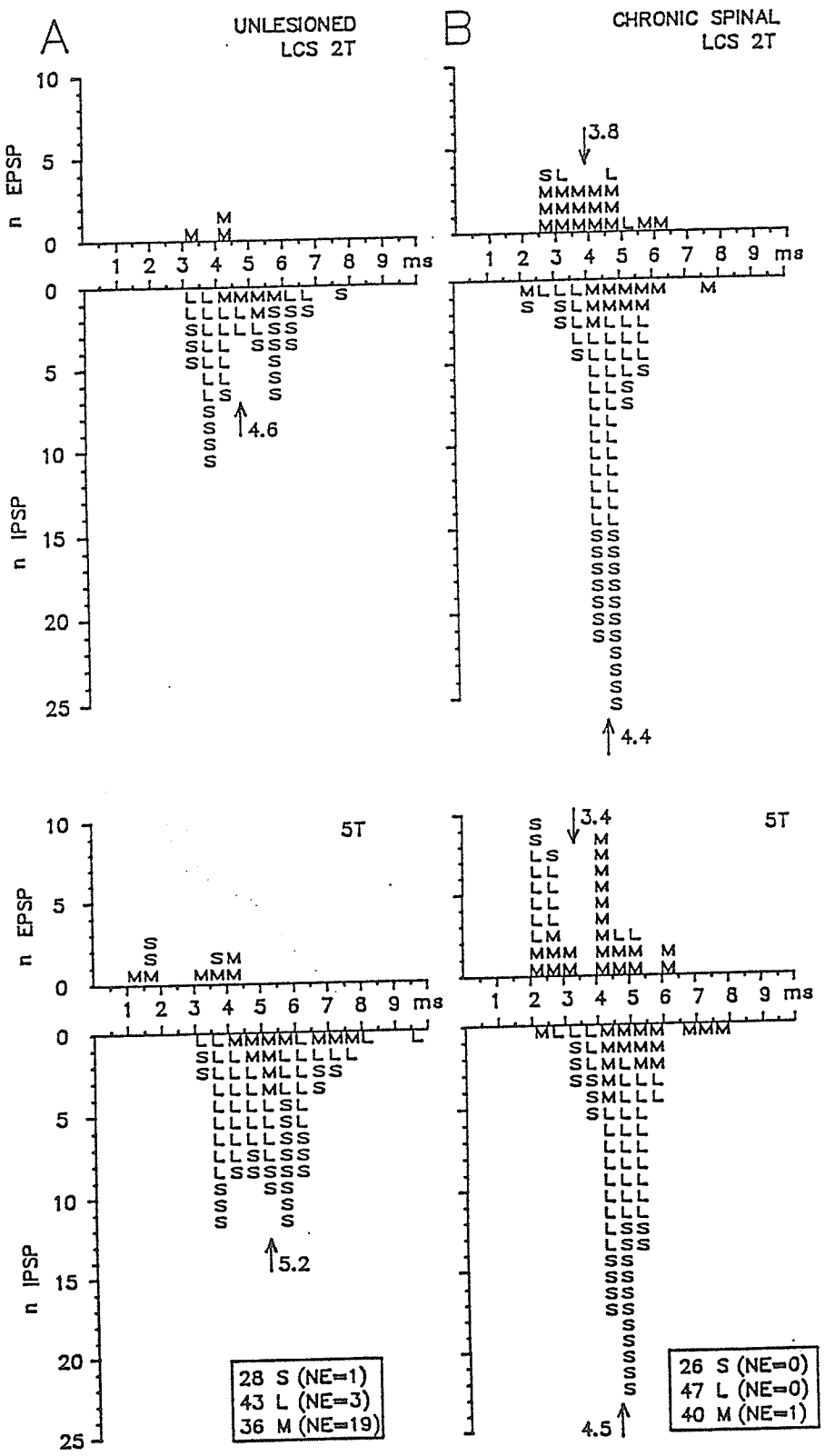

A
UNLESIONED
LCS 2T
B
CHRONIC SPINAL
LCS 2T
n EPSP
n IPSP
ms
4.6
3.8
4.4
5T
5T
5.2
3.4
4.5
28 S (NE=1)
43 L (NE=3)
36 M (NE=19)
26 S (NE=0)
47 L (NE=0)
40 M (NE=1)

FIGURE 4

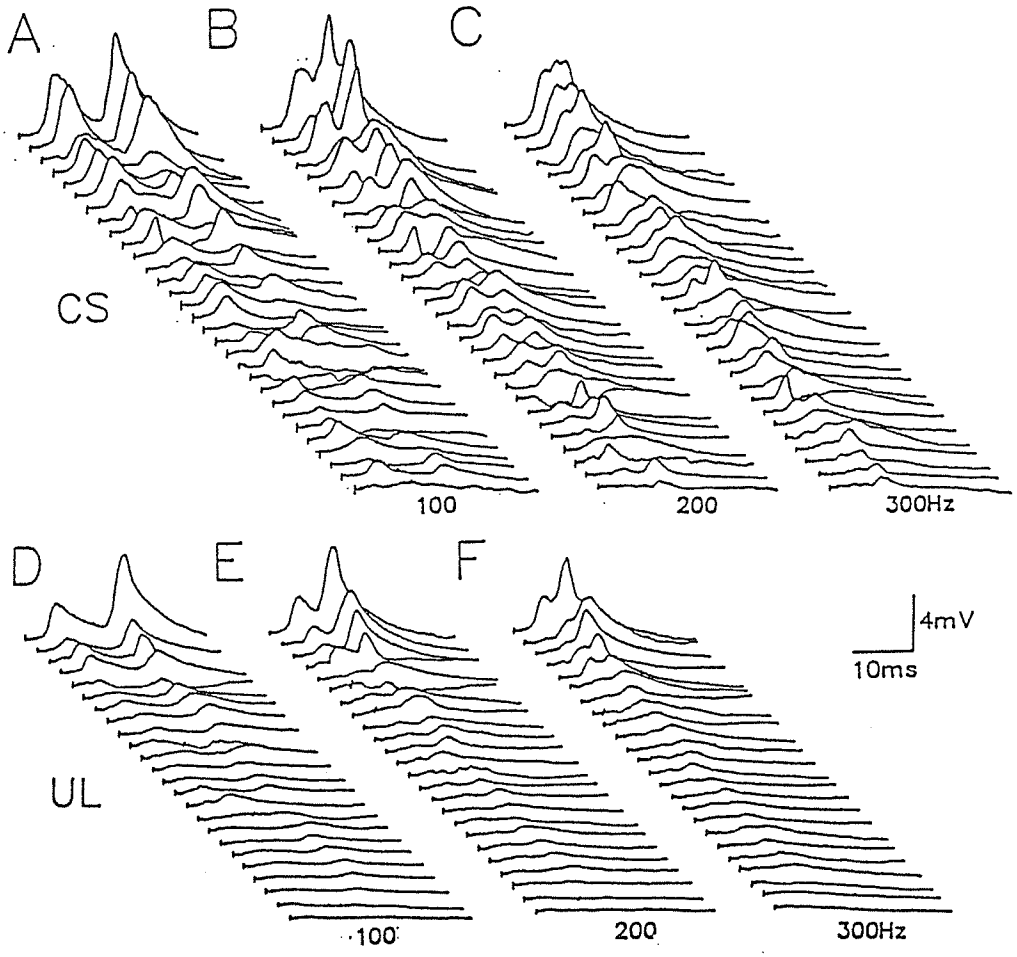


FIGURE 5

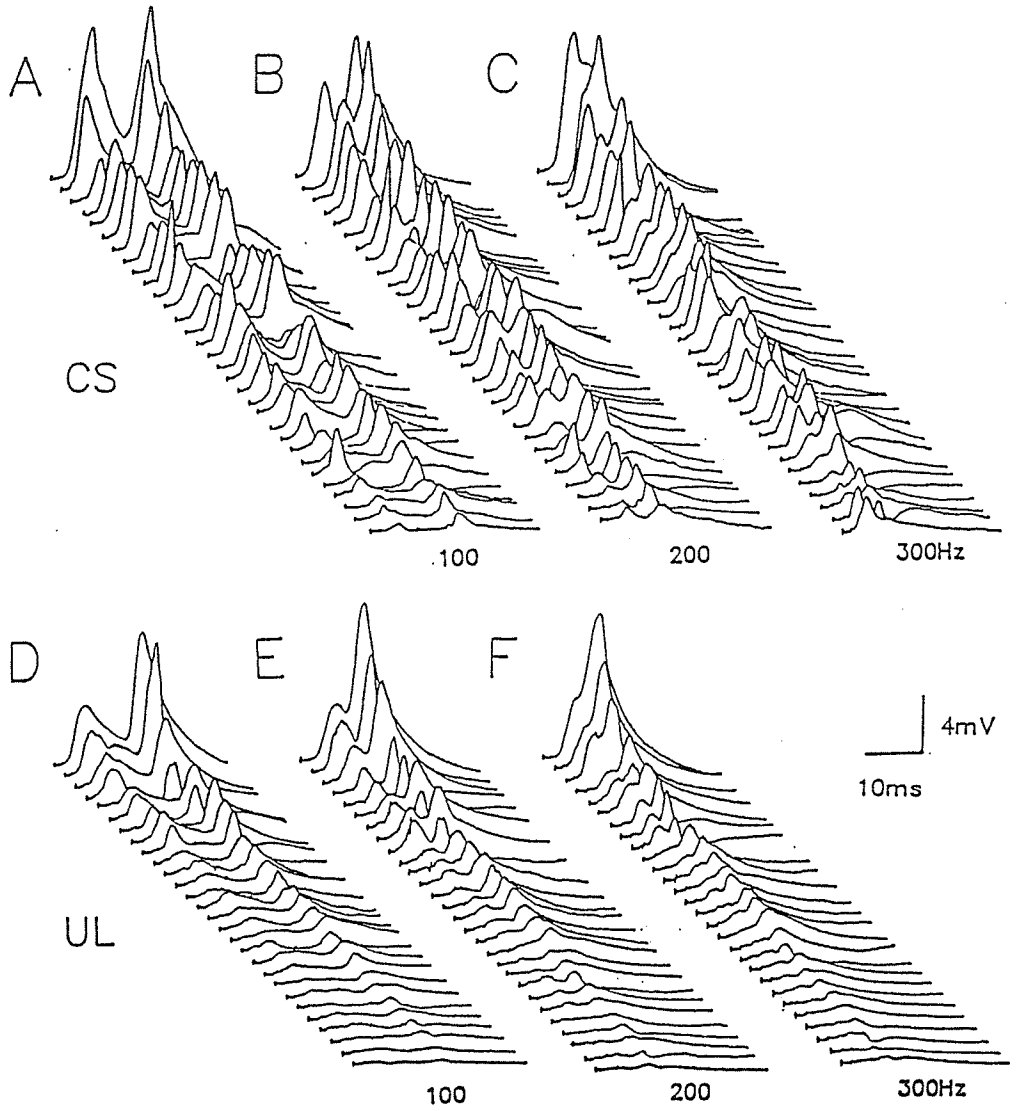


FIGURE 6

TABLE 1. *Action-potential amplitudes of cells with records in Figs. 1 and 3*

	CCS PSPs, mV		LCS PSPs, mV	
	UL	CS	UL	CS
	A	D	A	D
MG	77/80	77/80	76/78	78/80
	61-95	60-95	60-88	60-93
	B	E	B	E
SOL	76/77	77/78	73/73	77/80
	60-92	60-95	60-92	60-95
	C	F	C	F
LG	77/78	76/76	75/79	76/75
	60-95	60-93	60-90	60-93

PART IV) Segmental convergence upon a low threshold, excitatory cutaneous reflex pathway to medial gastrocnemius motoneurons, in the chronic spinal cat

SUMMARY AND CONCLUSIONS

1. The technique of spatial facilitation (Lundberg 1975) was used as an indirect test of interneuronal convergence between several hindlimb reflex pathways producing short-latency excitatory postsynaptic potentials (EPSPs) in medial gastrocnemius (MG) motoneurons. Results are from 64 MG cells in 8 chronic spinal adult cats (6-7 weeks post-transection), and are compared to previous results (Part II) obtained in animals with intact spinal cords.

2. Low threshold test stimuli (electrical) were applied to the caudal cutaneous sural nerve (CCS); conditioning stimuli to the lateral cutaneous sural (LCS), caudal cutaneous femoral (CCF), superficial peroneal (SP), saphenous (SAPH), posterior tibial (TIB), and posterior articular (JOINT) nerves.

3. As reported for unlesioned animals (Part II), facilitation of CCS EPSPs in MG was more readily obtained with conditioning stimulation of the CCF nerve than with the other peripheral nerves examined. However, the average condition-test delay needed to optimize this facilitation decreased in the chronic spinal preparation by approximately 4.8 ms. This observation is most likely related to the observed decrease of 1.8 ms in the average central latency of CCF EPSPs in MG cells (3.0 ms in chronic spinal animals compared to 4.8 ms in unlesioned).

4. In general, spatial facilitation of CCS EPSPs was more readily obtained in chronic spinal animals than in unlesioned animals, with most of the peripheral nerves used for conditioning stimulation. While amplitudes of facilitation were only slightly

larger among this increased incidence of facilitation, delays between condition and test stimuli were generally shorter; most dramatically in the case of CCF conditioning. Results are discussed in the context of enhanced transmission through interneuronal relays caudal to chronic spinal lesions.

INTRODUCTION

We previously reported that the pattern of postsynaptic potentials (PSPs) in triceps surae motoneurons produced by low threshold sural nerve stimulation was essentially unchanged by chronic spinalization (Part III; see also Baker and Chandler 1987), but that sural PSPs occurred more frequently, tended towards greater amplitudes, and had faster rates of rise and decay. This suggests that in animals which are 6-7 weeks post-transection, there are quantitative changes in synaptic transmission through qualitatively unchanged cutaneous reflex paths. Because passive electrical properties of triceps surae motoneurons are also largely unchanged in 6-7 week chronic spinal animals, the mechanisms responsible for altered sural PSP characteristics probably occur at sites presynaptic to the motoneurons (see discussion Part III), and likely involve enhanced transmission through interneuron relays.

This implication of enhanced transmission through spinal interneurons prompted the present study of convergence between segmental reflex pathways to motoneurons of chronic spinal animals. On the one hand, larger/faster sural PSPs suggest that normally weak convergence onto cutaneous reflex pathways by other cutaneous (or non-cutaneous) afferent systems may now be strengthened through a general enhancement of interneuronal transmission in segments caudal to the lesion. On the other hand, the relative integrity of the pattern of sural PSPs among ankle extensor motoneurons speaks to an enormous capacity of the spinal cord to conserve its synaptic organization after chronic central lesions, and the degree of convergence between pathways may be largely unchanged.

The present investigation in animals 6-7 weeks post-transection,

explores the pattern of convergence from various hindlimb afferents (mostly cutaneous) upon the short-latency excitatory pathway from the caudal cutaneous branch of the sural nerve (CCS) to MG motoneurons. We are interested in this particular excitatory pathway because in both unlesioned (LaBella et al. 1989) and chronic spinal animals (Part III), low threshold, short-latency CCS excitation is greatest in the MG portion of the triceps surae motor nuclei. A similar survey using unlesioned animals suggested a relatively restricted convergence by caudal cutaneous femoral afferents (CCF) compared to a variety of other afferent nerves examined (Part II), and the results are now compared between the two preparations.

METHODS

The spinal cords of 8 adult female cats (mean weight 2.73 kg) were completely transected at the L_{1-2} level 6-7 weeks prior to the acute experiment. Details of the spinalization procedure, as well as details of drug administration and preparation of peripheral nerves for the acute experiment as in the preceding report (Part III). Results are compared to those from the 12 unlesioned animals used in Part II.

Dissection and recording procedures

The MG nerve was dissected and stimulated for antidromic identification of motoneurons along with other hindlimb muscle nerves used to aid in locating MG motor nuclei. Peripheral nerves dissected and stimulated for examining PSPs in motoneurons include the caudal and lateral branches of the cutaneous sural nerve (CCS and LCS respectively); the caudal cutaneous femoral nerve (CCF); the saphenous nerve (SAPH) taken approximately one centimeter above the level of the knee; the posterior tibial nerve (TIB), distal to tibial branches to triceps surae, plantaris, flexor digitorum and hallucis longus muscles; the superficial peroneal nerve (SP); and the posterior articular nerve (JOINT). All preparation for the acute experiment was conducted using halothane delivered in a mixture of oxygen and nitrous oxide. Chloralose anesthesia (40-50 mg/kg initial dose, increased to a total dose of 60-90 mg/kg) was then used during data collection. Intracellular recordings were obtained using 2M potassium citrate microelectrodes; all intracellular and cord dorsum recordings were photographed from the oscilloscope and digitized (20 KHz) in the computer. Thresholds for peripheral nerve stimulation were determined by the stimulus required to just produce a deflection in the cord dorsum electrode recording.

Data analysis: histograms and composites

Intracellular effects in motoneurons were photographed and used to measure PSP latencies. Latencies were measured from the arrival of the first afferent volley at the cord dorsum, and care was taken to prevent classification of reversed inhibitory PSPs (IPSPs) as EPSPs by either depolarizing the motoneuron with injected current or by ascertaining that stimulation of some peripheral nerves resulted in substantial PSPs in the hyperpolarizing direction. Effects of extracellular fields were assessed by superimposing computer averages of the potential recorded just outside the motoneuron with intracellular records.

Single sweep records of postsynaptic effects in individual MG cells were traced from film enlargements and superimposed to provide a composite picture of each peripheral nerve's effects in the entire MG motoneuron sample. These composites are presented in Fig. 1 for twice and five times threshold stimulation of peripheral nerves (2 and 5T), and are compared to similar composites made from 12 unlesioned animals from another study (presented in Part II for 2T stimulation only). Care was taken to ensure that composites from the chronic spinal and unlesioned MG populations, as well as composites for the different peripheral nerves within these populations, were not biased by differences in motoneuron membrane resting potential.

Spatial facilitation technique

The spatial facilitation technique (Lundberg 1975) was used to compare the effect on individual MG motoneurons of combined stimulation of two nerves at a fixed delay with the algebraic sum of their effects upon separate stimulation. In all examples of the present study, CCS EPSPs in MG motoneurons comprise the test response, which is conditioned by CCF, LCS, SAPH, SP, TIB or JOINT stimulation; and the

condition-test *delay* refers to the delay of the arrival of the CCS afferent volley at the cord dorsum electrode from that of the conditioning nerve volley. Stimuli were delivered in the order of condition, test, then condition-test, and collected in an alternating method of storage. Averaged intracellular records from condition and test responses were subsequently added together (usually 8 or 16 sweeps per average), and this "summed" record was subtracted from the combined condition-test response to estimate the presence and degree of facilitation (see Fig. 3). Except in the case of TIB stimulation, extracellular field potentials were usually insignificant at the low stimulus intensities used here for facilitation trials. EPSPs were considered to be spatially facilitated when the effect was repeatable on separate trials; substantially greater than any negative deflections occuring in the calculated "difference" trace; and substantially greater than extracellular fields examined after removal of the microelectrode to an extracellular location.

RESULTS

Intracellular records were obtained from 70 MG motoneurons of unlesioned animals, and from 64 MG motoneurons of chronic spinal animals. PSPs evoked with 2 and 5T stimulation of peripheral (mainly cutaneous) hindlimb nerves, were examined and compared between the two preparations. In addition, the spatial facilitation technique was used to examine the segmental convergence of these nerves onto the low threshold excitatory CCS pathway to MG motoneurons in chronic spinal animals, and the results are compared to those obtained in unlesioned animals (Part II).

PSPs in medial gastrocnemius motoneurons

Figure 1 depicts composite records of PSPs from unlesioned (1A) and chronic spinal animals (1B). The figure is intended to catalogue the types of PSPs produced in MG by the different afferent nerves used in trials of spatial facilitation in the two preparations. In each panel of Fig. 1, film records from individual motoneurons were enlarged, traced, and superimposed with traces from other motoneurons. For example, the 65 traces in the CCS panel of 1A are from 65 different MG motoneurons of unlesioned animals, recorded at both 2 and 5T CCS stimulation. Similarly, in the CCS panel of 1B there are 62 traces at each stimulus intensity from 62 MG motoneurons of chronic spinal animals. The smallest sample of motoneurons tested is for SAPH PSPs in 1A (n= 41). Records in 1A and 1B are from motoneurons with similar average action potential amplitudes which were recorded just before and after collecting the PSPs (mean/median mV: 1A(n= 70) 71/70; 1B(n= 64) 72/72). This includes 5 cells in 1A and 4 cells in 1B where depolarizing current was injected to overcome reversed IPSPs. In these 9 cells, PSPs produced by all nerves tested were recorded using the same amount of current, in order to standardize the comparison of

cutaneous PSPs within individual MG motoneurons.

From the composites in 1A, it is clear that both CCS and CCF stimulation consistently produce EPSPs in MG motoneurons at both 2 and 5T stimulation. The other nerves tested also produce EPSPs in MG, but these are comparatively smaller and/or later (e.g. SAPH, TIB, LCS and JOINT), more mixed with hyperpolarizing components (e.g. SP and TIB), or more variable in waveform from cell to cell (e.g. SP and TIB). From the composites of 1B, it is clear that virtually all types of PSPs take on an altered waveform after chronic spinal section, as reported for sural effects in all three motoneuron groups of triceps surae (Part III). Excitatory components of PSPs are faster rising, of increased amplitude, and decay more rapidly, and inhibitory components are similarly larger and faster to decay. In some cases in 1B (e.g. SAPH and LCS) there is also more variety in the effect seen from cell to cell.

Table 1 summarizes the patterns of PSPs illustrated in Fig. 1 (unlesioned data in normal print; chronic spinal data indicated by bold italic print). Peripheral nerves are listed in the leftmost panel (N refers to the number of MG motoneurons tested), and the middle panel lists the frequency of PSP patterns in N motoneurons (recorded within the first 15-20 ms at 2 and 5T stimulation). Note that "%EP" denotes percentage of MG cells in which EPSPs were recorded as the earliest effect of stimulation at 2 and/or 5T; "%IP" denotes percentage where only IPSPs were recorded at 2 and 5T stimulation; and "NE" denotes percentage in which there was no effect of stimulation at 2 or 5T. In a few cases 2 and 5T stimulation of SP, LCS, TIB and JOINT resulted in an increase in synaptic activity which was not intrepretable as containing EPSP or IPSP components and thus the patterns in the table do not add up to 100%.

Note that CCS produced EPSPs as the earliest recorded effect in 97% of the MG motoneurons examined in unlesioned animals, and in 100% of the MG examined in chronic spinal animals. Thus it was possible to try and facilitate CCS EPSPs in almost all MG cells of either preparation. CCF EPSPs were present in the majority of MG cells in both preparations, with a less frequent incidence of "no effects" and IPSPs in chronic spinal animals. Similarly, there were relatively fewer "no effects" and IPSPs in chronic spinal animals with SAPH stimulation, and a much greater incidence of EPSPs recorded as the earliest-latency effect. The distribution of SP PSPs was comparable in the two preparations, as was the distribution of TIB PSPs. However, with both LCS and JOINT stimulation there was a substantial increase in the number of EPSPs recorded as the earliest effect in chronic spinal preparations. In both cases this corresponds to a great reduction in the number of cells with no effect of stimulation (to zero in fact), and with LCS there is a much lower incidence of IPSPs recorded as the only effect of stimulation. Thus, particularly in the case of SAPH, LCS and JOINT, there were more opportunities in chronic spinal animals to test for CCS EPSP facilitation; since the presence of visible EPSPs on the oscilloscope was our first criteria for choosing nerves for conditioning stimulation in each motoneuron.

Because of previous evidence that CCF afferents have a relatively high degree of convergence on the CCS excitatory pathway to MG (Part II), the observation that CCF EPSPs appeared to be of shorter latency in chronic spinal animals was of interest to us (see CCF panel, Fig. 1). The histograms of Fig. 2 show the central latencies of EPSPs (and IPSPs) produced by 2 and 5T CCF stimulation, and depict a shift in average EPSP latency from 4.8 ms in unlesioned (2A) to 3.0 ms in chronic spinal preparations (2B). Note that numerous EPSPs with

latencies < 2.5 ms are present in 2B, and that there are no EPSPs with such short latencies in 2A. Whether or not this implies a reduced number of interneurons in the shortest excitatory pathway from CCF afferents, or an increased efficacy of synaptic transmission, cannot be decided by these types of experiments. However, the overall increase in the incidence of CCF PSPs in chronic spinal animals (compare number of "no effects", NE, in insets of 2A and 2B) is consistent with the latter possibility. It is noteworthy that average latencies are so much shorter for CCF but not CCS EPSPs in MG motoneurons of chronic spinal animals (see below).

Facilitation of CCS EPSPs with CCF stimulation

Figure 3 illustrates the technique of spatial facilition as used in the present investigation. In general, facilitation trials began by stimulating the test (CCS) and conditioning nerve at their approximate thresholds for EPSP production. Stimulus strengths and delays were then varied until combined condition-test stimulation was seen to facilitate the EPSPs, and these were further varied in additional trials in an attempt to optimize the amount of facilitation. In the averaged records of Fig. 3, stimulation of either CCF (3A) or CCS (3B) produced only small EPSPs in this MG motoneuron of a chronic spinal animal. Combined CCF and CCS stimulation is illustrated in 3C, with CCS delayed from CCF by 1.1 ms (cord dorsum records in 3D). The arithmetic sum of traces in 3A and 3B is shown in 3E. The smaller size of this summed trace, evident when superimposed over the combined trace (3G), shows EPSP facilitation on the order of 1.7 to 1.8 mV. The facilitation is similarly shown in 3F where the summed trace has been subtracted from the combined stimulation trace.

Figure 4 shows the condition-test intervals producing spatial facilitation of CCS EPSPs in MG motoneurons, with CCF conditioning.

The abscissa on the graph denotes the delay in arrival at the cord dorsum of the CCS afferent volley from the CCF volley; the ordinate, the amplitude of the observed facilitation (i.e. the difference of the arithmetic sum of condition and test averaged responses from averages of combined stimulation). Open circles depict 71 observations of facilitation in 28 cells of unlesioned animals, from a total of 83 trials in 30 cells. Filled circles depict 85 observations in 34 cells of chronic spinal animals from a total of 92 trials in the same number of cells (see inset Fig. 4). Note that unsuccessful trials are not indicated on the graph. Because there were only two MG cells (in unlesioned animals) where facilitation between CCS and CCF could not be demonstrated, unsuccessful trials in both preparations can be largely attributed to suboptimal condition-test delays or stimulation strengths. EPSP occlusion upon combined stimulation was almost never observed, most likely because the low stimulation strengths used resulted in little overlap in discharge zones of the interneuronal relays.

The most striking difference between unlesioned and chronic spinal preparations in Fig. 4 is the general shortening of the condition-test delay required for facilitation in chronic spinal animals. Whereas the arrival of the CCS afferent volley at the cord dorsum was usually later than that of CCF in unlesioned animals, this order was frequently reversed in chronic spinal animals (see number of filled circles located < 0ms in Fig. 4). In the upper panel of Table 2 stimulation parameters are grouped according to amplitude of facilitation, and several differences between the two preparations are evident. Not only are there more observations of facilitation $\geq$ 0.70 millivolts in chronic spinal animals, but these observations involve lower stimulus strengths, in particular for CCF stimulation, and a decrease in average delay of 4.8 ms (from 5.2 ms to 0.4 ms). The lower

stimulus intensities are consistent with the more general observation that PSPs in motoneurons are frequently produced with weaker electrical stimuli in chronic spinal animals (Part III). However, the substantial decrease in delay is rather interesting. In the lower panel of Table 2, the average minimum latencies for CCF and CCS EPSPs in MG motoneurons at 2 and 5T are indicated. While there is almost no decrease in average CCS EPSP latency in chronic spinal animals (see also Part III), the relatively long average latency for CCF excitation in unlesioned animals (4.8 ms) is reduced by 1.8 ms, to 3.0 ms (see Fig. 2). An average optimum delay of 0.4 ms in chronic spinal animals then, is approximately the difference in minimum latencies (albeit at higher thresholds) between CCF and CCS EPSPs, suggesting that convergence is happening as early as the first-order interneuron relay. However, in unlesioned animals, the average optimum delay for facilitation is 5.2 ms, which is still 3.2 ms longer than the latency differences. Because we did not attempt to rigorously assess minimum condition-test delays in the two preparations, the neuronal length of convergent CCS and CCF pathways in either preparation remains uncertain (see Discussion).

Facilitation of CCS EPSPs with SAPH, SP, and LCS stimulation

Figures 5 and 6 similarly illustrate all recorded examples of CCS EPSP facilitation with SAPH, SP, and LCS conditioning. In Fig. 5A, open circles depict 9 observations of facilitation by SAPH conditioning in 4 cells of unlesioned animals, from a total of 35 trials in 9 cells. Filled circles depict 20 observations in 11 cells of chronic spinal animals, from a total of 40 trials in 17 cells (see inset 5A). In 5A there appears to be a similar trend in the case of SAPH conditioning, as for CCF conditioning: amplitudes of facilitation are often greater in the chronic spinal preparation, and condition-test delays are generally shorter (although the relatively few observations in

unlesioned do not allow much of a quantitative comparison). Here as well, the average threshold of the conditioning stimulus (SAPH) to optimize facilitation (i.e. the best example in each cell) is substantially lower in chronic spinal animals (3.44T in the 4 MG of unlesioned; 1.71T in the 11 MG of chronic spinal), and is probably due to the presence of larger, low threshold SAPH EPSPs in chronic spinal preparations (Fig. 1B). The average threshold of the test (CCS) stimulus was essentially the same in the two preparations (1.36T in the 4 MG of unlesioned; 1.41T in the 11 MG of chronic spinal). However, while short-latency SAPH EPSPs occurred more frequently in the chronic spinal sample of MG cells (Table 1), facilitation of CCS EPSPs was observed in only half the trials (20/40). Only rarely did IPSPs which followed SAPH EPSPs appear to be a factor in unsuccessful trials; the flat lines usually obtained when summed averages were subtracted from the averages of combined stimulation implied little interaction between the CCS and SAPH excitatory pathways to MG with the delays and stimulus intensities employed.

Results with SP conditioning are presented in Fig. 5B. Here, open circles depict 8 observations in 7 MG motoneurons of unlesioned animals from a total of 65 trials in 25 cells; filled circles depict 25 observations in 14 cells of chronic spinal animals, from a total of 41 trials in 17 cells (see inset 5B). Thus SP facilitation of CCS EPSPs was significantly easier to obtain in chronic spinal animals, and again, the amplitude of facilitation was somewhat larger. Condition-test delays were generally shorter, and frequently of reversed sequence, and effective strengths of conditioning stimuli were comparable in both preparations (1.66T in the 7 MG of unlesioned; 1.43T in the 14 MG of chronic spinal). Again the average threshold of CCS stimulation was essentially the same in the two preparations (1.7T in the 7 MG of unlesioned; 1.62T in the 14 MG of chronic spinal). One

notable feature of SP, compared to CCF or SAPH, conditioning, is the narrower range of successful condition-test delays (e.g. -2.0 to 1.3 ms for chronic spinal). This was probably largely a result of the IPSPs which usually closely followed SP EPSPs (see Fig. 1), restricting the range in which facilitation of excitatory components could be assessed. Consequently, negative facilitation results which were sometimes observed with SP conditioning, could have been due to either EPSP occlusion or IPSP facilitation. Thus it is interesting that facilitation was still easier to obtain in chronic spinal animals even though IPSPs which followed SP EPSPs were often much larger than in unlesioned preparations (see Fig. 1).

Figure 6 illustrates results with LCS conditioning of CCS EPSPs. As with SP conditioning, facilitation with LCS stimulation was significantly easier to obtain in chronic spinal animals. Thus, 7/31 successful trials in unlesioned (open circles), compares to 45/65 successful trials in chronic spinal animals (filled circles). Although nearly 3 times as many MG motoneurons were tested in chronic spinal animals (26 cells compared to 9), this was because there were far more MG motoneurons with evidence of early-latency LCS excitation (see Fig. 1 and Table 1). As well, the observations of facilitation in unlesioned animals involved a much greater average LCS stimulus strength (4.12T in the 5 MG of unlesioned compared to 1.73T in the 23 MG of chronic spinal). This is in accordance with the observation of very few LCS EPSPs in MG motoneurons of unlesioned animals, even at thresholds as high as 5T (see number of flat lines in LCS panel of Fig. 1A). The average threshold of CCS stimulation for facilitation was only moderately reduced (1.84T in the 5 MG of unlesioned; 1.37T in the 23 MG of chronic spinal). Note the wide range of condition-test delays in Fig. 6 used to obtain facilitation in either preparation. This is consistent with the irregular amplitudes and latencies of LCS EPSPs in

MG motoneurons of unlesioned or chronic spinal animals.

Facilitation of CCS EPSPs with TIB and JOINT stimulation

Conditioning CCS EPSPs with TIB (Fig. 7A) or JOINT (Fig. 7B) stimulation was not very successful in either preparation. In 7A, open circles depict 8 observations of facilitation in 3 MG motoneurons of unlesioned animals, from a total of 29 trials in 7 cells; filled circles depict 5 observations in 2 cells of chronic spinal animals from a total of 21 trials in 9 cells (see inset 7A). Thus, while TIB conditioning was only successful in about 25% of the trials in either preparation, there are still features in common with conditioning by the other peripheral nerves: larger amplitudes of facilitation in the chronic spinal preparation, which were obtained at shorter condition-test delays. Average thresholds were comparable in the two preparations for both TIB (2.22T in the 3 MG of unlesioned; 2.15T in the 2 MG of chronic spinal) and CCS (1.34T in the 3 MG of unlesioned; 1.45T in the 2 MG of chronic spinal) stimulation. Similar to the case for SP conditioning, large IPSPs which closely followed TIB EPSPs may have masked facilitation of CCS EPSPs; and that TIB conditioning was especially ineffective may relate to the very few observations of TIB EPSPs in MG motoneurons which were not rapidly cut off by ensuing IPSPs (compare TIB and SP panels in Fig. 1). There was no facilitation of CCS EPSPs observed in unlesioned animals with JOINT conditioning (Fig. 7B; 14 trials in 3 MG cells), and only 4 observations (filled circles) in 30 trials with chronic spinal animals (in 3 of 10 cells tested; see inset 7B). However, even JOINT conditioning exemplifies the overall trend for more demonstrable facilitation in the chronic spinal preparation where larger EPSPs produced by separate stimulation of peripheral nerves tend to occur (see Fig. 1). The average stimulation thresholds in the 3 MG of chronic spinal animals where facilitation was obtained were 2.8T for JOINT and 1.98T for CCS.

Summary of facilitation results

The rightmost panel of Table 1 summarizes the results of facilitation trials with each peripheral nerve used for conditioning stimulation: n, number of MG motoneurons tested; %n, number of MG in which facilitation was obtained; and the largest amount of facilitation measured in each cell is averaged at the far right in millivolts. The table shows that CCF conditioning of CCS EPSPs was successful in all MG motoneurons examined in chronic spinal animals, as was nearly the case in unlesioned animals (93%). The percentage of MG in which SAPH conditioning was successful was greater in chronic spinal (65%) compared to unlesioned (44%); as was the case for SP (82% in chronic spinal; 28% in unlesioned) and LCS (88% in chronic spinal; 56% in unlesioned). While this percentage also increased in the case of JOINT conditioning, and decreased in the case of TIB conditioning, the numbers are too small to provide much of a comparison between preparations. However, despite the evidence for varying degrees of convergence on the CCS excitatory pathway to MG by the different afferent nerves examined, it is interesting that in virtually all cases the average amount of CCS EPSP facilitation obtained (optimum values in each cell) was greater in chronic spinal preparations.

Figure 8 depicts examples of CCS EPSP facilitation in an MG motoneuron of a chronic spinal animal, with CCF, LCS, SP, SAPH, and TIB conditioning. The upper three rows are the intracellular records, and the lower two rows are the computer averages. This was one of the few cells in which CCS EPSP facilitation was obtained with most of the nerves used for conditioning stimulation.

DISCUSSION

In summary, the spatial facilitation technique suggests that patterns of convergence upon the CCS excitatory pathway to MG are similar in chronic spinal and unlesioned preparations. Of the six afferent nerves examined, CCF appears to converge most heavily in either preparation, while TIB and JOINT appear to converge the least. However, examination of any one of these six convergent systems suggests a quantitative increase in the degree to which interneurons common to the CCS excitatory pathway are accessed. This is suggested by the larger measurements of EPSP facilitation with generally lower strengths of conditioning stimulation in chronic spinal animals, but even more so by the relative number of successful trials in the two preparations.

Criteria for determining facilitation trials as "unsuccessful" should be justified. First, a trial was regarded as unsuccessful only if both CCS and the nerve used for conditioning stimulation produced measureable EPSPs in the motoneuron, and secondly, if the duration of motoneuron impalement allowed us to try several variations in stimulation parameters with a wide range of condition-test delays. Consequently, there were many more trials attempted than are actually indicated in Figs. 4-7 and in more MG cells than are listed in Table 1. In addition, late EPSPs (>20-30 ms) were sometimes facilitated but not early ones (<10-20 ms), and sometimes IPSPs produced by stimulation of the conditioning nerve facilitated hyperpolarizing components in mixed CCS PSPs. For consistency then, results in the present report only include those which involved EPSPs as the earliest-latency effect of stimulating either CCS or the nerve used for conditioning. It is noteworthy in this regard that facilitation with CCF conditioning became a subjective standard for other condition-test pairs, since CCF

and CCS EPSPs were typically facilitated with the very first stimulation parameters tried in motoneurons of either preparation.

With regard to the shorter (often reversed) condition-test delays in chronic spinal animals, we cannot conclude changes in pathlengths or points of convergence until minimum delays have been assessed in the two preparations and the responsible interneurons identified. However, because delays were determined by the visibility of facilitation on the oscilloscope, there does appear to be at least a quantitative difference in the most prominent points of convergence in the two preparations. As suggested earlier for CCF EPSPs in MG motoneurons, it is possible that there are short-latency CCF/SAPH excitatory pathways in unlesioned animals which become more active in chronic spinal animals, and thus facilitation of CCS EPSPs is enhanced through "normally weak" convergent relays. Although we only report the evidence for shorter-latency CCF EPSPs in chronic spinal animals (Fig. 2), it may be that chronic spinal transection has a greater cumulative effect on longer segmental reflex pathways, such as the CCF excitatory pathway to MG (average minimum latency 4.8 ms in unlesioned; Fig. 2), or the LCS inhibitory pathway to triceps surae (average minimum latency 4.6 ms in unlesioned; see Part III, Fig. 4). Note also that SAPH EPSPs are relatively long-latency and slow rising in unlesioned animals (Fig. 1A) and that these too are larger at shorter latencies in chronic spinal preparations (Fig. 1B). Thus, if transmission is enhanced through some short-latency excitatory pathways, convergence of these pathways onto interneurons in the CCS excitatory pathway to MG may also be enhanced. This alternative is certainly suggested by the one anomalous unlesioned animal in which large facilitation of CCS EPSPs at relatively short condition-test delays was possible for CCS and SAPH conditioning, suggesting that points of convergence may be similar, but differentially active in unlesioned and chronic spinal preparations.

It is noteworthy that in this particular animal, which displayed signs of circulatory disturbances in the spinal cord early on in the experiment, only EPSPs were recorded in MG motoneurons from stimulation of the seven peripheral nerves examined (although IPSPs were produced by the same nerves in LG and SOL cells).

In the present results, evidence for interneuronal convergence upon the CCS excitatory pathway to MG appears to be strongest in cases where the peripheral nerve used for conditioning stimulation produces similar patterns of PSPs (see also Part II). Thus CCF and CCS produce primarily EPSPs in MG of either preparation and these are easily facilitated (refer to Fig. 1). TIB and JOINT PSPs are largely dominated by hyperpolarizing components in both preparations, and the EPSPs are much more difficult to facilitate with CCS EPSPs. SP and SAPH produce EPSPs fairly consistently in the two preparations, and these are facilitated with CCS EPSPs more easily than TIB or JOINT EPSPs. LCS stimulation produces little deflection of resting potential or else IPSPs in unlesioned animals, but in chronic spinals where LCS EPSPs can be quite large, these too are more easily facilitated. That common reflex effects of different afferent nerves in motoneurons may be mediated by common interneurons is not surprising, and in fact was suggested by Lundberg in forwarding his original "Flexor Reflex Afferent" hypothesis (cf. Lundberg et al. 1987). As discussed in an earlier report (Part II), the organization of shared excitatory pathways from cutaneous afferents to extensor motoneurons may further be rooted in the myotopic organization suggested by Hagbarth (1952). Thus, the strong evidence for convergence between CCS and CCF excitatory pathways to MG (in both preparations), may be related to the close proximity of their receptive fields to the MG muscle. Because we have not systematically examined convergence from muscle afferents producing excitation in MG, we do not know if this organization might

possibly extend to include group I, II or III muscle afferents arising from the same local region as CCS and CCF (i.e. afferents in the MG muscle or muscles near it), or if the weak evidence for convergence from JOINT (and TIB) afferents is because the strongest convergence is from pure cutaneous afferents. Nonetheless, of the six afferent systems tested systematically in the present study, the relatively "pure" cutaneous nerves show the greatest extent of convergence, and of these, it is those which most regularly produce short-latency EPSPs in either preparation.

While amplitudes of facilitation were arguably only slightly increased in chronic spinal animals, it is our opinion that it is the relative ease of obtaining facilitation of CCS EPSPs which is the most striking difference in this preparation. Although the relative patterns of convergence among the condition-test pairs are similar in unlesioned and chronic spinal animals, it is difficult to describe the increased incidence of spatial facilitation of CCS EPSPs (for most of the afferents tested), as reflecting either a quantitative or qualitative change in the interactive aspect of segmental reflex pathways. Indeed with the experimental methods presently employed, the evidence for relatively restricted convergence by CCF afferents onto the CCS excitatory pathway to MG (Part II) is greatly reduced in the chronic spinal preparation. However, there are some important considerations in the interpretation of these, and any, results concerned with incidence or patterns of reflex effects in different preparations. In the decerebrate animal, polysynaptic excitation is often the predominant effect in ipsilateral extensor motoneurons upon stimulation of a wide variety of peripheral afferents (see Lundberg 1982), and Hagbarth noted (1952) that in the occasional decerebrate preparation excitatory reflexes in ankle (and other) extensor muscles could be obtained from skin stimulation all over the limb, even though the

excitatory area was usually very small in the decerebrate/acute spinal animal and restricted to the vicinity of the skin overlying the particular extensor muscles (see Discussion Part II; also see Holmqvist and Lundberg 1961). Indeed in the acute spinal preparation, inhibition of extensor motoneurons is the predominant, although not exclusive, effect of stimulating a variety of peripheral afferents (Eccles and Lundberg 1959; Holmqvist and Lundberg 1961). Other investigators have reported additional evidence that in high acute spinal animals, excitatory and inhibitory reflex pathways may be simultaneously open to extensor motoneurons (Schomburg and Steffens, 1986). Although there is evidence that some cutaneous reflex pathways (including that from CCS to triceps surae) may be under a differential descending control from that of other cutaneous or non-cutaneous segmental reflex pathways (see Lundberg 1979; Lundberg 1982), these findings strongly suggest that the neuronal circuitry for excitation (and inhibition) from widespread sensory loci to extensor (or flexor) motoneurons is normally present, but that the particular patterns of reflex effects expressed is highly preparation-dependent. Thus, this could conceivably be the case for patterns of convergence between reflex pathways as well.

Acknowledging that we are using the qualitative technique of spatial facilitation to make a quantitative comparison between preparations, our conclusions remain general, and we believe the comparison does signify a change in the interactive aspect of cutaneous reflexes after chronic spinal transection. Patterns of PSPs in MG motoneurons (Fig. 1), similar to patterns of sural PSPS in all triceps surae motoneurons (Part III, Figs. 1 and 3), display a high degree of integrity after chronic spinal transection when compared to unlesioned animals. However, the PSPs are generally "faster and larger", which implies enhanced transmission through interneuronal relays of both inhibitory and excitatory segmental reflex pathways to motoneurons. The

present results suggest that this enhanced transmission may serve to diminish spatial selectivity among convergent reflex pathways, as assessed in the chloralose-anesthetized preparation. Analysis of the time course of these changes, as well as assessment of minimum condition-test delays and the identification of shared interneurons in the CCS excitatory pathway to MG in the two preparations, should help clarify if this reflects a "re-wiring" of synaptic contacts between segmental reflex pathways below the spinal lesion, or altered expression of pre-existing ones.

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TABLE AND FIGURE LEGENDS

FIG 1. Postsynaptic potentials (PSPs) recorded in MG motoneurons of unlesioned (A) and chronic spinal animals (B) upon single shock stimulation of seven different peripheral nerves at twice and five times threshold (2 and 5T). Enlarged photographic records of PSPs in individual motoneurons were traced and superimposed; one trace per motoneuron at each stimulus intensity in each panel. The small vertical *arrow* indicates the time of arrival of the afferent volleys at the cord dorsum electrode. Calibration bar 4 mV, 4 ms.

FIG 2. Central latencies of PSPs in medial gastrocnemius (M) motoneurons produced by 2T (upper histograms) and 5T (lower histograms) single shock stimulation of the CCF nerve in unlesioned (A) and chronic spinal (B) animals. Cells in which PSPs were produced at 5T but not 2T stimulation appear in the lower histograms only. Cells in which PSPs were recorded following both 2 and 5T stimulation are plotted in both sets of histograms. There were 59 MG cells examined in A; 64 in B (4 cells in B were not tested with 5T stimulation). NE =no effect.

FIG 3. Averaged intracellular records from an MG motoneuron of a chronic spinal animal. Trace C shows the effect of combined CCF and CCS stimulation at the thresholds indicated in A and B, respectively. Cord dorsum record of combined stimulation in D, with the delay of the arrival of the CCS afferent volley at the cord dorsum electrode from that of CCF (milliseconds) indicated at the far right of the trace. The arithmetic sum of A and B is depicted in E, and in F the difference of this summed trace from that of combined stimulation illustrates spatial facilitation of

the EPSP. Facilitation can be viewed differently in G, where combined and summed records are placed in a superimposed position. Calibration pulses 2 mV, 2 ms (4 mV, 2 ms for summed trace in E).

FIG 4. Graph depicting condition-test delays (abscissa) and amplitudes of facilitation (ordinate) of CCS EPSPs with CCF conditioning stimulation. Open circles =observations in MG motoneurons of unlesioned animals; filled circles =observations in MG motoneurons of chronic spinal animals. Delays refer to the time of arrival of the CCS afferent volley at the cord dorsum electrode from the arrival time of the CCF volley. Boxed inset =number of observations of facilitation/number of trials (number of cells with facilitation/number of cells tested).

FIG 5. Graph depicting condition-test delays (abscissa) and amplitudes of facilitation (ordinate) of CCS EPSPs with SAPH (A) and SP (B) conditioning stimulation. Details as in Fig. 4.

FIG 6. Graph depicting condition-test delays (abscissa) and amplitudes of facilitation (ordinate) of CCS EPSPs with LCS conditioning stimulation. Details as in Fig. 4.

FIG 7. Graph depicting condition-test delays (abscissa) and amplitudes of facilitation (ordinate) of CCS EPSPs with TIB (A) and JOINT (B) conditioning stimulation. Details as in Fig. 4.

FIG 8. Spatial facilitation of CCS EPSPs with separate conditioning stimulation of five peripheral nerves in the same MG motoneuron of a chronic spinal animal. Upper three rows illustrate intracellular records; COND. STIM =conditioning stimulation (cord

dorsum records directly beneath in each panel). Bottom row depicts superimposed averages of combined stimulation records (largest in each panel) and summed averages of separate condition and test records. The difference of summed and combined averages is also indicated (DIFF). Calibration pulses in intracellular records, and calibration bar for averaged records, 2 mV, 2 ms.

TABLE 1. Summary of PSP patterns and spatial facilitation results in all MG motoneurons. Peripheral nerves listed at far left. N =number of MG motoneurons in which PSPs were examined. %EP = % of N motoneurons in which the earliest latency effect was an EPSP at 2 and/or 5T stimulation. %IP = % in which only IPSPs were recorded at 2 and 5T stimulation. %NE = % in which there was no measurable effect at 2 or 5T stimulation. n =number of motoneurons in which spatial facilitation of the early-latency CCS EPSP was attempted. %n = % where facilitation was obtained. mV =average of maximum recorded EPSP facilitation in millivolts, in %n motoneurons. First line in every pair of lines refers to results in unlesioned animals; second line in bold italic print refers to chronic spinal results. (Note for PSP data, total PSP types <100% in a few cases because of a few cells where an increase in synaptic activity was uninterpretable as either EPSPs or IPSPs).

TABLE 2. Upper panel: average stimulation parameters used in n observations of CCS EPSP facilitation with CCF conditioning stimulation. Observations from 30 MG motoneurons of unlesioned animals and from 34 MG motoneurons of chronic spinal animals. Parameters are grouped according to amplitude of facilitation in millivolts, at far left. T =average stimulation threshold; Delay =average delay of the arrival of the CCS volley at the cord

dorsum from the arrival of the CCF volley. Lower panel: average central latencies of CCF and CCS EPSPs from the entire sample of MG motoneurons in each preparation (unlesioned CCF 2T, n=24; CCF 5T, n=42; CCS 2T, n=57; CCS 5T, n=67; chronic spinal CCF 2T, n=55; CCF 5T, n=53; CCS 2T, n=61; CCS 5T, n=59).

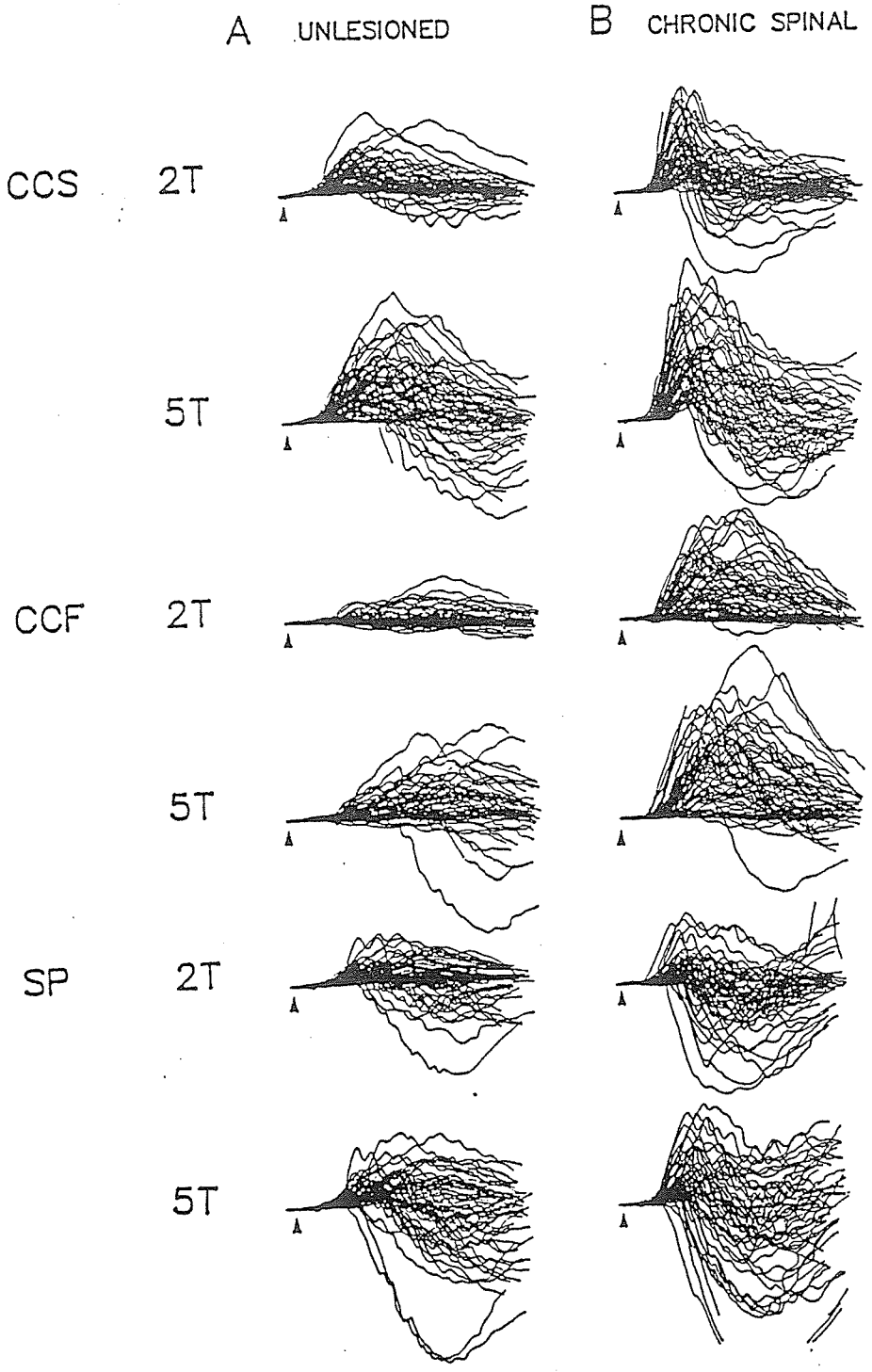
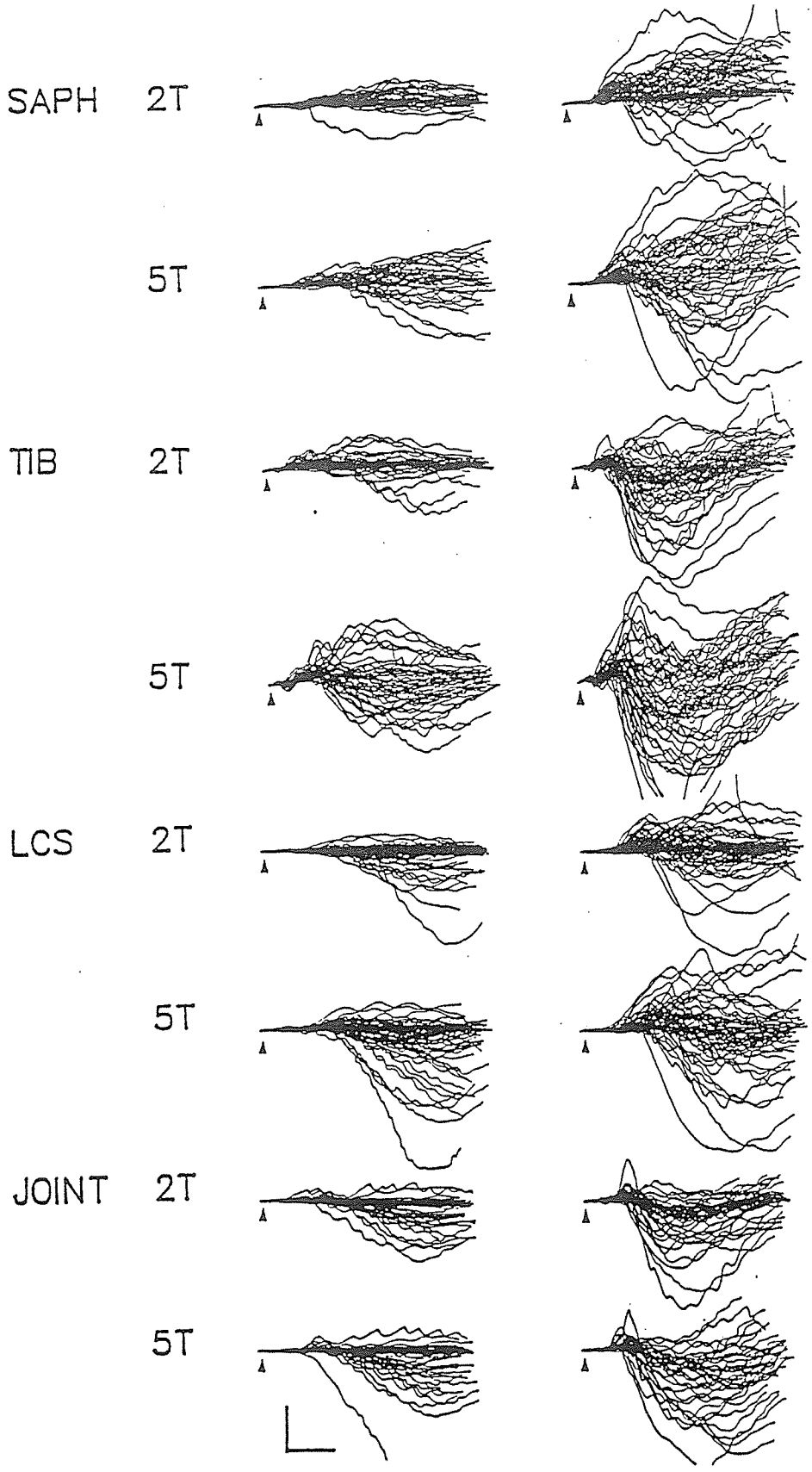


FIGURE 1


SAPH
2T
5T
TIB
2T
5T
LCS
2T
5T
JOINT
2T
5T

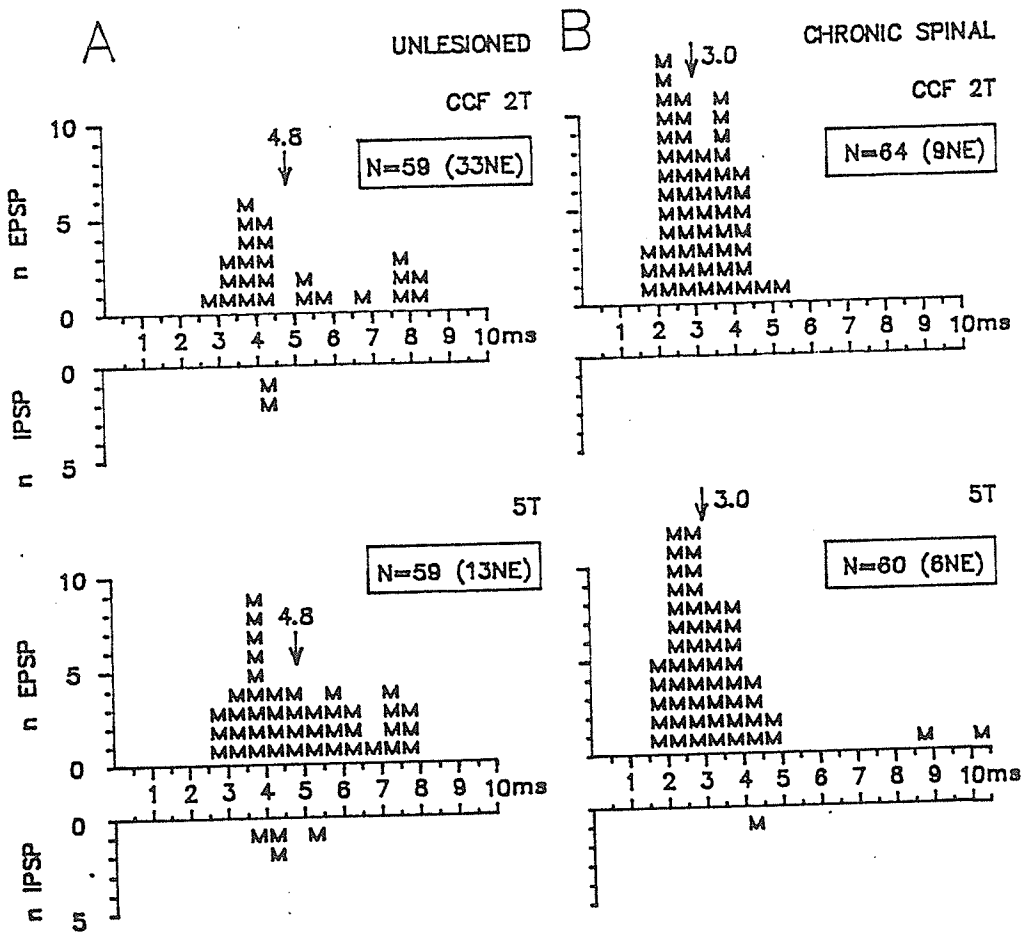

A
UNLESIONED
CCF 2T
4.8
N=59 (33NE)
n EPSP
n IPSP
5T
N=59 (13NE)
B
CHRONIC SPINAL
CCF 2T
3.0
N=64 (9NE)
5T
N=60 (6NE)
1 2 3 4 5 6 7 8 9 10ms

FIGURE 2

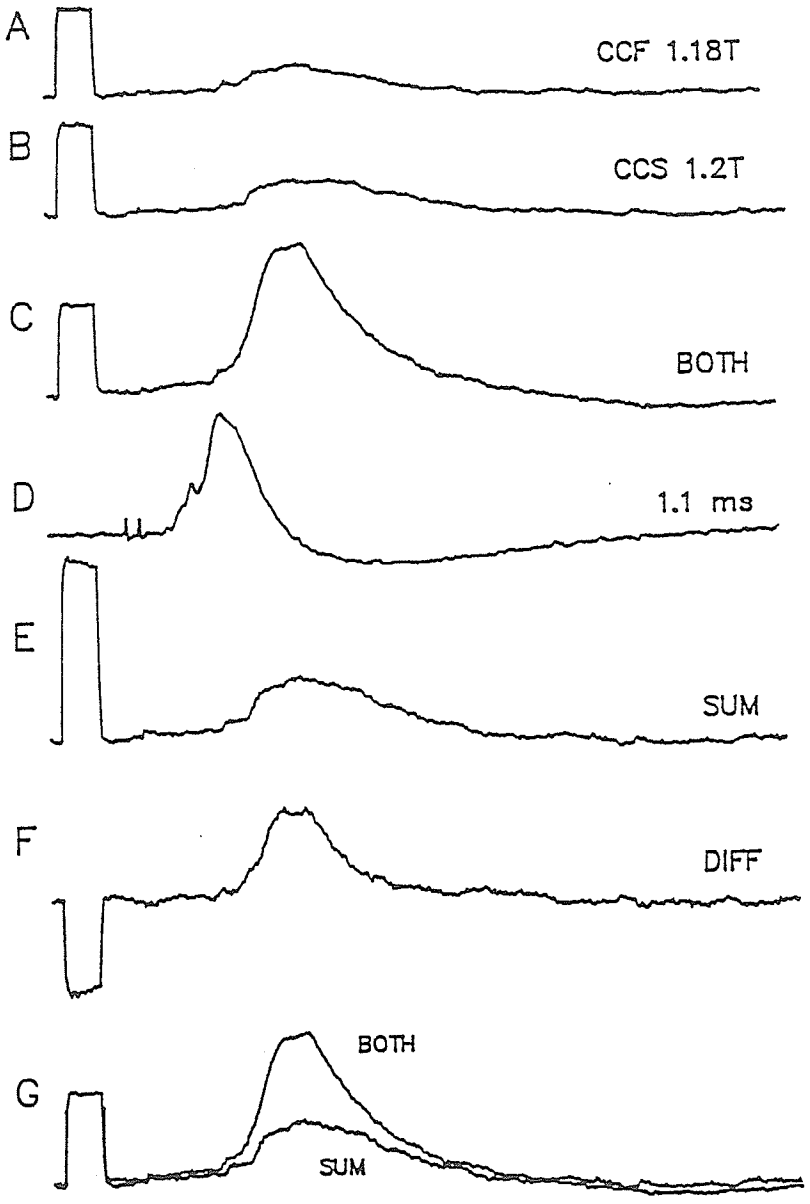


FIGURE 3

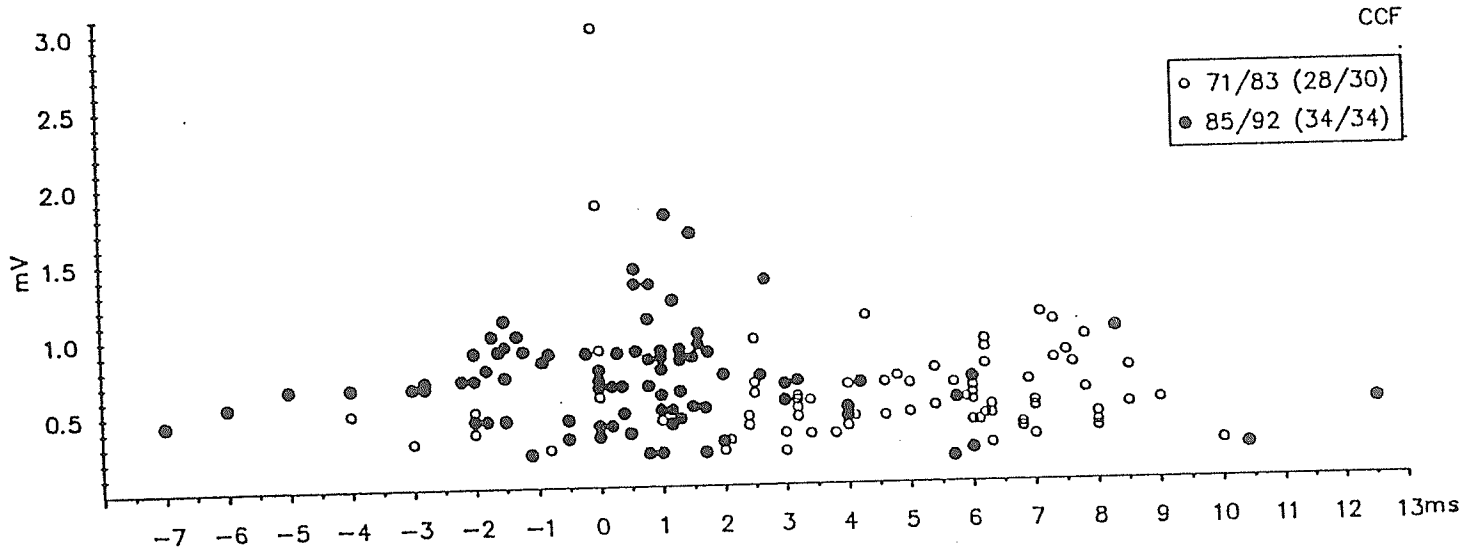
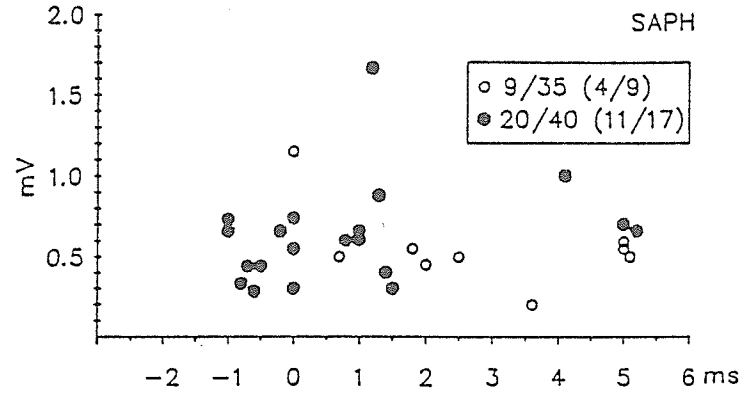

CCF
71/83 (28/30)
85/92 (34/34)
3.0
2.5
2.0
1.5
1.0
0.5
mV
−7 −6 −5 −4 −3 −2 −1 0 1 2 3 4 5 6 7 8 9 10 11 12 13ms

FIGURE 4



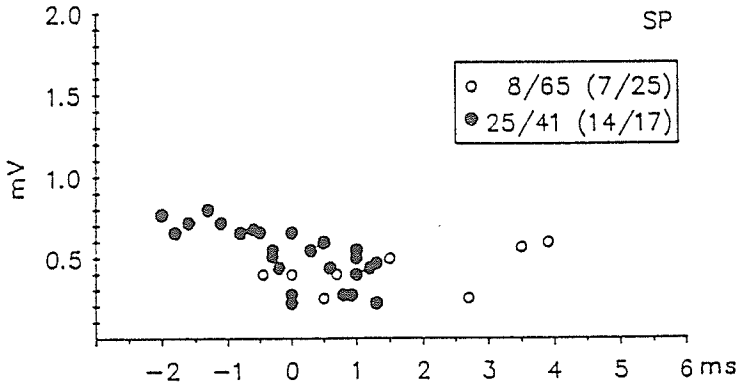


FIGURE 5

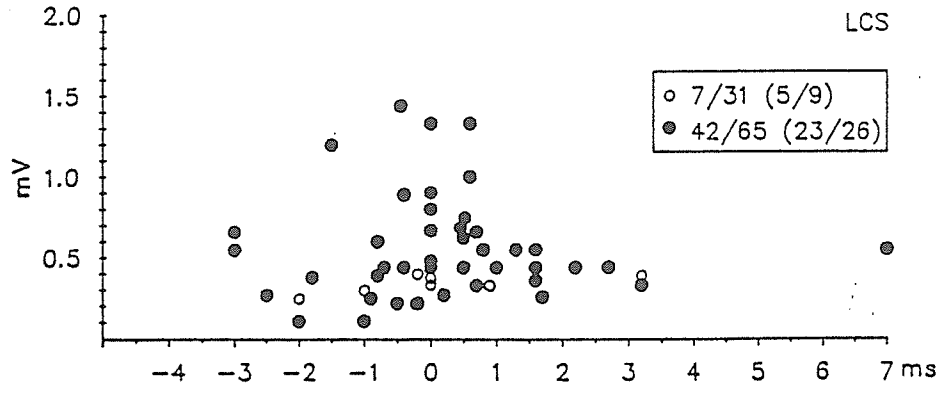


FIGURE 6

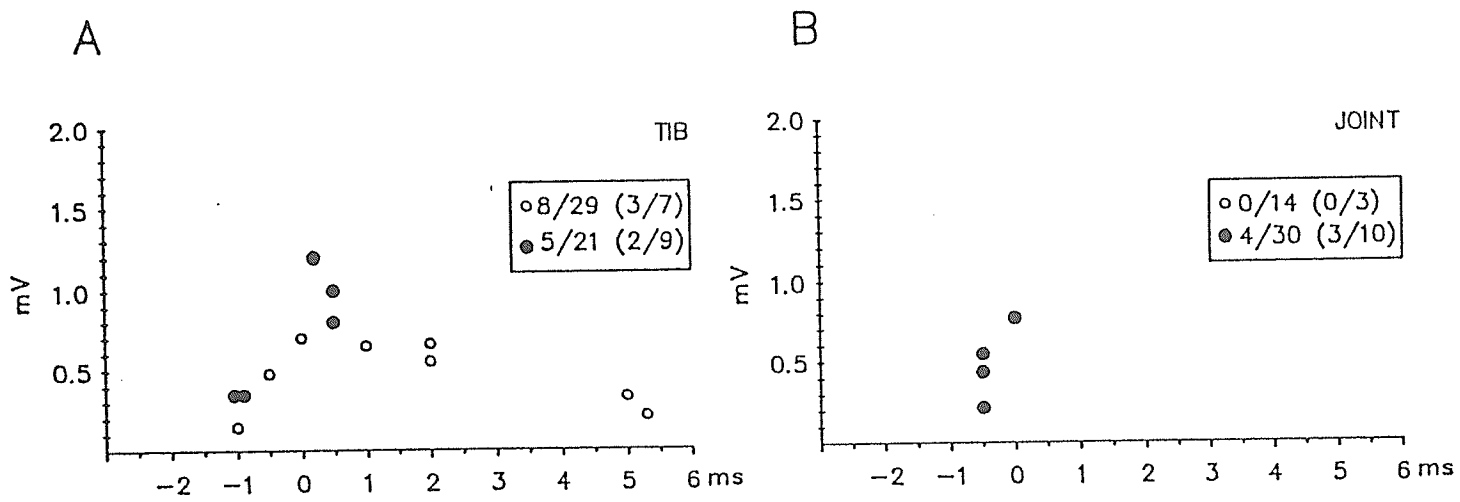

A
TIB
2.0
1.5
1.0
0.5
mV
8/29 (3/7)
5/21 (2/9)
−2 −1 0 1 2 3 4 5 6 ms
B
JOINT
2.0
1.5
1.0
0.5
mV
0/14 (0/3)
4/30 (3/10)
−2 −1 0 1 2 3 4 5 6 ms

FIGURE 7

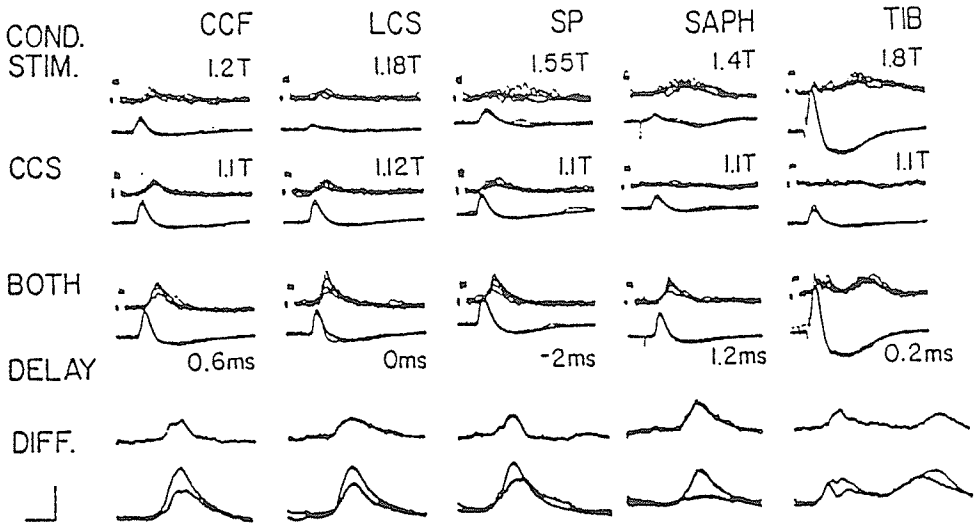

COND.
STIM.
CCF
LCS
SP
SAPH
TIB
1.2T
1.18T
1.55T
1.4T
1.8T
CCS
1.1T
1.12T
1.1T
1.1T
1.1T
BOTH
DELAY
0.6ms
0ms
-2ms
1.2ms
0.2ms
DIFF.

FIGURE 8

TABLE 1. *Summary of PSPs and spatial facilitation for all condition-test peripheral nerve combinations*

		PSPs			*FACILITATION*		
	N	%EP	%IP	%NE	n	%n	mV
CCS	70	97	--	3	--	--	---
CCS	*62*	*100*	–	–	–	–	—
CCF	60	72	7	21	30	93	0.73
CCF	*64*	*89*	*2*	*9*	*34*	*100*	*0.83*
SAPH	44	59	27	14	9	44	0.69
SAPH	*60*	*88*	*9*	*3*	*17*	*65*	*0.72*
SP	66	91	7	2	25	28	0.42
SP	*55*	*89*	*9*	–	*17*	*82*	*0.58*
LCS	70	13	52	31	9	56	0.38
LCS	*64*	*72*	*23*	–	*26*	*88*	*0.70*
TIB	46	50	33	9	7	43	0.53
TIB	*62*	*53*	*42*	–	*9*	*22*	*0.78*
JOINT	54	19	39	42	3	0	---
JOINT	*46*	*50*	*46*	–	*10*	*30*	*0.59*

TABLE 2. *Stimulation parameters for facilitation of CCF and CCS PSPs*

	UNLESIONED				*CHRONIC SPINAL*			
mV	n	CCF T	CCS T	DELAY	n	CCF T	CCS T	DELAY
< 0.40	19	2.8	1.7	3.9 ms	11	1.9	1.4	2.3 ms
0.40 < 0.55	19	3.0	1.6	4.5 ms	18	2.4	1.4	0.8 ms
0.55 < 0.70	16	2.8	1.8	4.6 ms	18	1.6	1.5	0.4 ms
≥ 0.70	17	2.4	1.6	5.2 ms	38	1.5	1.4	0.4 ms
AVG. EPSP LATENCIES:			CCF	2T: 4.8 ms		CCF		2T: 3.0 ms
				5T: 4.8 ms				5T: 3.0 ms
			CCS	2T: 2.8 ms		CCS		2T: 2.7 ms
				5T: 2.9 ms				5T: 2.5 ms

GENERAL SUMMARY AND CONCLUSIONS

In *Part I*, sural nerve inputs to the triceps surae motor nuclei were characterized in animals with intact spinal cords, to provide a data base for examining a cutaneous reflex system in the chronic spinal animal. However, the results revealed an unexpected differential synaptic input to MG, LG and SOL motor nuclei; "unexpected" because of the implicit assumption in the literature that cutaneous reflexes in motoneurons innervating close functional synergists would be essentially uniform. On the basis of existing literature, on the other hand, we had expected patterns of excitatory and inhibitory cutaneous reflex effects which were primarily determined by the specific motor unit type of each motoneuron. While this type-dependent organization was roughly present in our preparation for MG and SOL motoneurons, it was not at all present in our sample of LG motoneurons. This suggests that while a segmental organization on the basis of motor unit type may exist for some cutaneous reflex systems, this organization cannot be predicted to transcend even very closely related groups of motoneurons. The most practical message to be derived from these results, however, is the importance of making a complete identification of individual motoneuron species in experiments concerned with patterns of cutaneous reflex activity; and this is not at all a trivial point. Many studies which have examined "LG-SOL" or "gastrocnemius-SOL" motoneurons may have obscured physiological differences (as well as similarities) in reflex paths from other cutaneous afferents, and perhaps from non-cutaneous peripheral afferents as well.

This study fortifies previous evidence that the CCS excitatory path to MG represents a "specialized" cutaneous pathway, although the results here support that the pathway is specialized for MG, rather than for fast MG motor units. How this pathway might be integrated

during movement remains to be elucidated, but we do not feel that evidence in the cat for supraspinal facilitation of this pathway in order to effect selective recruitment of fast motor units is very strong. Evidence that this is true in humans and non-human primates is much stronger, however, and this may be why some previous authors have focussed heavily on this idea. At the moment we can only suggest that the relative medio-lateral placement of the MG and LG muscles may bear a relationship to asymmetries in the particular reflex effects evoked by different hindlimb cutaneous nerves. This may subserve movements which require asymmetries in MG and LG muscle contraction, perhaps during co-contraction with SOL. Alternatively, there may be a differential segmental input to MG, LG and SOL motoneurons from a variety of cutaneous nerves which is rooted in development, but in the normal behaving adult animal, interneurons in these pathways are utilized by descending systems to activate these groups of motoneurons in concert.

The technique of spatial facilitation was used in *Part II* as an indirect test of convergence by several other (mostly) cutaneous reflex pathways onto common spinal interneurons. Again, the object was to obtain a data base in animals with intact spinal cords for subsequent comparison with data from chronic spinal animals. Using the low threshold excitatory pathway from CCS to MG motoneurons as the test pathway, the results suggest a preferential convergence by CCF afferents compared to the other peripheral afferents tested. This relatively restricted pattern of convergence may be rooted in the organization of excitatory skin reflexes described by Hagbarth (1952), but his studies using monosynaptic test reflexes of combined ventral roots have not, to our knowledge, been previously analyzed or demonstrated at the single motoneuron level with intracellular recording techniques. In reflection of his own findings, Hagbarth

argued that " *the organization of spinal skin reflexes .... demonstrates the presence of a set of rules other than the classical one of ipsilateral flexion and thereby assembles under a single heading a number of disconnected observations on ipsilateral extension. The experiments have also drawn attention to the extreme complexity of afferent skin nerves regarded from the point of view of their reflex effects. They have emphasized a connexion between source and centre at the expense of fibre size and centre*" (Hagbarth 1952). Now, almost forty years later, it appears that spatial facilitation of short-latency excitatory reflex pathways to individual extensor motoneurons may be predicted on the basis of this same organization. An important implication here is that investigators may be able to predict spatial overlap in cutaneous reflex pathways to other extensor (or flexor) motoneurons, if what we have described reflects a more global organization of cutaneous reflex paths through the spinal cord. In addition, identification of the interneurons involved in these excitatory pathways, and full analysis of their convergence with non-cutaneous primary afferents, will help clarify if there is a central circuitry at work here which is separate, or a part of the FRA spinal network described by Lundberg.

Part III was concerned with the neural basis for the well-known, but poorly understood, cutaneous hyperreflexia which occurs caudal to chronic spinal cord lesions. The study in *Part I* was repeated in chronic spinal animals, and the results showed that while sural PSPs in triceps surae motoneurons were substantially enhanced in frequency, amplitude, and rate of rise and decay, their *qualitative* distribution remained essentially unchanged. This suggests that the segmental organization of reflex pathways is highly conserved during development, but after transection there are quantitative changes in segmental synaptic transmission which serve to synchronize afferent inputs to

motoneurons. A behavioral consequence of this could be an amplification of the reflex gain: towards enhanced recruitment of motoneurons in the case of excitatory cutaneous reflex pathways, and towards faster de-recruitment in the case of inhibitory ones. Ways in which altered interneuron activity could account for enhanced transmission were offered previously in this thesis, but there is one more "less exotic" possibility we would like to consider. Specifically, to what extent does the removal of descending control systems which exert tonic inhibitory control over reflex pathways serve to "synchronize" signals coming from the periphery? This may be a simple idea, but the afferent input to interneuronal relays (or primary afferent terminals) must now be greatly "de-modified" by the loss of descending fiber systems with last-order inhibitory connections to the same sites. Afferent input is no longer adapted to purpose through selective descending inhibition of reflex pathways, but is transmitted to motoneurons through a spinal network which is conceivably much less complicated, and more self-organized, and hence, the remaining postsynaptic effects in motoneurons may be more synchronous. Unfortunately, post-transection trauma probably precludes the use of acutely spinalized animals to test this idea (for e.g. by examining shape characteristics of cutaneous PSPs), and in these animals degradation of severed descending axons could not be expected to be complete. Another consideration, is that if motoneurons are now receiving a "more similar" barrage of synaptic input each time a natural peripheral stimulus is applied, to what extent may transmission subsequently be enhanced at the postsynaptic membrane through natural forms of synapse modifiability which occur with repeated use? It is compelling to think about explanations for altered cutaneous reflexes after chronic spinalization that do not involve major structural changes (such as abberant connections of axonal sprouts), which have been largely shown to occur with other types of lesions, and not necessarily in segmental reflex pathways

(e.g. McCouch et al., J. Neurophysiol. 1958; Brenowitz and Pubols, Brain Res. 1981). The results of this study provide a data base of cutaneous reflex characteristics after chronic transection, and may afford a more rational approach to equating functional changes with known forms of neuronal plasticity.

Because individual reflex pathways are not functionally independent, *Part IV* of this thesis compared patterns of peripheral convergence upon the excitatory CCS pathway to MG motoneurons in chronic spinal animals with the results obtained in unlesioned animals (*Part II*). In summary, patterns of convergence were arguably similar in the two preparations, but spatial facilitation of CCS EPSPs in MG was generally much more demonstrable in the transected spinal cord. Similar to the results in *Part III*, if interneurons in cutaneous reflex pathways are now more responsive to a given afferent stimulus, then normally weak points of convergence between pathways may be stronger. However, it is difficult to make a case for altered reflex function on the basis of enhanced convergence, when we do not know the full repetoire of reflex patterns under conditions of descending control. However, there is one particular aspect of the present results which could prove to be interesting in this regard. Although the data was not presented, SAPH EPSP latencies in MG motoneurons were frequently as short as 2 ms in chronic spinal animals (the earliest being 1.8 ms), although such early latencies were rarely measured in unlesioned animals, and were generally much longer. Because SAPH afferents enter the spinal cord primarily via the L_5 dorsal roots (our own observations), this may be preliminary evidence of a disynaptic excitatory pathway from SAPH afferents to MG motoneurons. However, is this pathway present in (chloralose-anaesthetized) unlesioned animals but normally less active? Condition-test delays to facilitate CCS EPSPs in the two preparations incite speculation. SAPH-CCS delays to demonstrate EPSP faciliation

were frequently very short in chronic spinal animals (see *Part IV*, Fig. 5), which was intuitively appreciated by the comparable latencies of CCS and SAPH EPSPs (around 2 ms). However, in the one unlesioned animal where very large facilitation (>1.1 mV) was obtained with SAPH conditioning, the delay was 0 ms but the latencies of the CCS and SAPH EPSPs were 2.0 ms and 7.8 ms, respectively (see *Part II*, Fig. 4; latencies not illustrated). The latency of the combined SAPH-CCS EPSP was also slightly shorter. It is possible then, that short-latency (perhaps disynaptic) segmental pathways from SAPH afferents to MG motoneurons are normally present in the intact spinal cord, but require facilitation by convergent activity in other segmental, propriospinal, or descending pathways to be recorded. Future experiments to identify interneurons in common to CCS and CCF excitatory pathways to MG, as well to CCS and SAPH excitatory pathways to MG, will tell us more about the qualitative integrity of convergent reflex systems after chronic spinalization. However, I think the results in *Part III* of this thesis speak to a high degree of integrity in patterns of cutaneous reflex activity, and cutaneous hyperreflexia probably does not reflect "disordered" patterns of segmental convergence, but rather enhanced transmission through still highly ordered ones.

The organization and integration of segmental reflexes has been a prominent focus of motor control research, but we are still without a unifying framework with which to study the contribution of cutaneous reflex pathways to movement. Indeed, the most useful framework in which we presently study spinal reflexes as a whole (Lundberg's Flexor Reflex Afferent hypothesis), has not provided us with knowledge of interneuronal convergence between cutaneous pathways themselves, and our study should make a significant contribution in this respect. Furthermore, since interneuronal convergence in chronic spinal animals has not, to our knowledge, been previously investigated for either

cutaneous or muscle reflex pathways, our efforts signify a new approach towards understanding the basis of altered cutaneous reflex function.