THE RECURRENT INHIBITION OF MONOSYNAPTIC REFLEXES AND ITS ALTERATION AFTER PERIPHERAL NERVE CRUSH IN DECEREBRATE RATS

YUN WANG, BSc

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ABSTRACT

During a critical period of development in the rat, nerve injury results in a permanent enhancement of polysynaptic reflex responses in reinnervated muscles (Navarrete et al., 1990). Such enhanced reflex activity may result from alterations in intrinsic motoneuronal properties (e.g., input resistance and afterhyperpolarization) and from changes in the behaviour of interneuronal pathways. Studies in cat have shown that axotomy leads to an elimination of intramedullar axon collaterals innervating Renshaw cells from motoneurones (Havton & Kellerth, 1984, 1990a). In this study, the hypothesis of whether recurrent inhibition (RI) in the rat is altered following recovery from peripheral nerve crush in both adult and 5-day-old rats was examined. In both these preparations peripheral nerve crush does not result in the loss of motoneurones (Lowrie et al., 1982; 1987). This point was also confirmed in this study by conventional HRP retrograde labelling techniques.

In decerebrate rats, recurrent inhibition between lateral gastrocnemius-soleus (LG-S) and medial gastrocnemius (MG) motor pools was assessed by conditioning monosynaptic reflexes elicited from the cut dorsal roots and recorded either from the LG-S and MG nerves by antidromic volleys delivered to the synergist muscle nerve. The results show that following sciatic nerve crush in both experimental groups there is a significant and sustained depression in the level of recurrent inhibition. The reduction in recurrent inhibition observed is less in adult animals when comparisons are made with the results from rats given the nerve injury early in postnatal life. However, following lateral gastrocnemius-soleus nerve crush instead of sciatic nerve crush, which shortened the dennervation time respectively, the reduction of recurrent inhibition was not observed in adult rat group and was much less in 5-day-old rat group. In addition, the different contributions of the recurrent inhibition between LG-S and MG motor pools are dedifferentiated as a result of nerve injury.

It is proposed that this reduction of recurrent inhibition occurs due to degeneration of motor axon collaterals following the nerve injury. Such degeneration has been observed in the cat following axotomy (Havton & Kellerth, 1984, 1990a). The maintained reduction of recurrent inhibition may therefore be a consequence of a failure of regenerating axon collaterals to re-establish contact with Renshaw cells and/or alterations in the membrane properties of the injured motoneurones. However, this kind of degeneration may not occur if dennervation is short enough.

In conclusion, this observed reduction of recurrent inhibition may contribute to the hyperreflexia reported in young animals following nerve crush (Navarrete et al., 1990) and that the results in this study support the view that peripheral nerve injury induces plastic changes in the behaviour of spinal circuitry.

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ABBREVIATION IN TEXT AND FIGURES :

1.5xT	1.5 times threshold
2.5xT	2.5 times threshold
5xT	5 times threshold
5LC	5-day-old LG-S nerve crush
5LC7	7 weeks after 5-day-old LG-S nerve crush
5SC	5-day-old sciatic nerve crush
5SC7	7 weeks after 5-day-old sciatic nerve crush
5SC30	30 weeks after 5-day-old sciatic nerve crush
5TC	5-day-old tibial nerve crush
5TC14	14 weeks after 5-day-old tibial nerve crush rat
ALC	adult LG-S nerve crush
ALC5	5 weeks after adult LG-S nerve crush
ALC6	6 weeks after adult LG-S nerve crush
ALC12	12 weeks after adult LG-S nerve crush
ASC	adult sciatic nerve crush
ASC5	5 weeks after adult sciatic nerve crush
ASC6	6 weeks after adult sciatic nerve crush
ASC14	14 weeks after adult sciatic nerve crush
ATC	adult tibial nerve crush
ATC6	6 weeks after adult tibial nerve crush
ATC12	12 weeks after adult tibial nerve crush
DR	dorsal root
EDL	extensor digitorum longus
G	gastrocnemius
G-S	gastrocnemius-soleus
HRP	horseradish peroxidase
IPSPs	Inhibitory post synaptic potentials
L	lumbar segment
LG	lateral gastrocnemius

LG-S	lateral gastrocnemius-soleus
MG	medial gastrocnemius
MSR(s)	monosynaptic reflex(s)
n	number of animals
normal	data from normal animals
P	peroneal nerve
RI	recurrent inhibition
RIPSP	recurrent inhibitory post synaptic potential
S	soleus
SDH	succinic dehydrogenase
SEM	standard error of mean
SU	sural nerve
T	threshold
TA	tibialis anterior
Tib	tibial nerve
VEN	ventral root

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CHAPTER 1 INTRODUCTION

1.0. General introduction

A single motoneurone supplies many muscle fibers. The neurone, together with these fibers, forms a functional unit commonly referred to as a motor unit. When the neurone is activated, excitation is transmitted to all the muscle fibers it supplies. The motor unit is then the final functional element that produces movement. A given muscle is composed of many motor units, and graded contraction is achieved by changing the rate of firing of individual motor units and by recruitment of additional units within the same muscle. The variability of firing rate is an important control mechanism of all muscles therefore of coordinated movement. This frequency modulation is, in part, contributed by recurrent inhibition pathways.

Although some abnormal function has been demonstrated in both regenerated muscles and motoneurones following peripheral nerve injury, little has been known about the functional change in the recurrent inhibition pathways after regeneration. The main purpose of the experiments discussed in this thesis is to study the role of the alteration of recurrent inhibition as a result of temporary denervation in adult and neonatal rats. The over view of the literature relating to the recurrent inhibition pathways and to its developmental changes will be considered in the following sections.

1.1. Recurrent inhibition of spinal motoneurones

In the early 1940's, Renshaw (1941) first demonstrated that antidromic impulses in motor axons reduced the monosynaptic reflex discharge, not only of their own motoneurones but also of those in the same or in neighbouring motor nuclei. Inhibition typically occurred if the tested and the conditioning motor nerves were branches to the same muscle or muscle groups. In a later investigation, Renshaw (1946) reported that a number of interneurone-like cells located in spinal ventral horn could be excited antidromically at a latency of less than 1 ms. These interneurones, characterized by their high initial firing frequency were then designated by Eccles and co-workers (1954) as 'Renshaw cells'. "In addition to confirming almost all of Renshaw's findings, a detailed study of the interneuronal discharges has estab-

lished that these interneurones form a specialized group mediating the inhibitory path from motor axons. They may appropriately be given the distinguishing title of 'Renshaw cells'" (Eccles et al., 1954).

1.1.1 Renshaw cells receiving convergence input from motoneurones via axon collaterals

The axon collaterals of mammalian spinal α-motoneurones, which were originally discovered by Golgi in 1880's, were first systematically investigated morphologically by Cajal in the first decade of this century (see Haase et al., 1975). They were found to exist in many parts of the nervous system. Later evidence confirmed Cajal's description of axon collaterals branching from the axon of the motoneurones prior to the axon reaching the ventral root exit zone (Prestige, 1966; Scheibel & Scheibel, 1971). These collateral branches were found to be limited to the ipsilateral ventral horn and the longitudinal range of most of the ramifications of motor axon collaterals in prenatal kittens was limited to about half a segment, while some 10 to 20% of them encompassed up to three segments along the rostrocaudal axis (Scheibel & Scheibel, 1966, 1971). This agreed well with physiological results that recurrent inhibition extended to motoneurones two or three segments above or below the incoming level of the antidromic volley, whereas a contralateral activation of Renshaw cells was never observed (Renshaw, 1946; Eccles et al., 1954; Wilson et al., 1960; Willis & Willis, 1966).

Following introduction of horseradish peroxidase (HRP) as an intracellular marker in the spinal cord (Cullheim & Kellerth, 1976; Snow et al., 1976; Cullheim et al., 1977) Cullheim and Kellerth (1978 a,b,c) investigated the intramedullary axonal system of sciatic α-motoneurones in the cat. They found that most of the motor axons gave off one to five collaterals and the branching patterns of the axon collaterals showed considerable variation and the number of end branches from a single collateral ranged between 1 and 39. They also reported that the number of axon collateral outbuglings, interpreted as synaptic boutons, which originated from a single α-motoneurone showed large variation within each pool that possessed axon collaterals, the total range being from 17 to 158 (Cullheim and Kellerth, 1978b). These axon collateral outbuglings were distributed not only in the Renshaw cell area ventromedial to the main motor nuclei but also in those parts of the motor nuclei which were

located in the vicinity of the parent cell bodies. Synaptic boutons of collaterals in ventral part of Rexed's lamina VII exhibited spherical synaptic vesicles and made synaptic contacts with cell bodies and proximal dendrites of Renshaw cells (Lagerback et al., 1978, 1981).

Both extracellular recording results, in which single antidromic motor axon volleys produced a characteristic burst of firing in Renshaw cells at very high frequency with 0.5-0.7 ms central latency (Renshaw, 1946; Eccles et al., 1954, 1961a), and intracellular recording results, in which a repetitive discharge of a single motoneurone caused firing of the Renshaw cell at a much higher frequency (Ross et al., 1976; van Keulen, 1979b), indicated that the recurrent axon collaterals had strong monosynaptic linkage with Renshaw cells.

It was found that not all motor axons gave off recurrent collaterals (Scheibel & Scheibel, 1966, 1971; Prestige, 1966; Eggar et al., 1980). Morphological studies showed, for instance, that 20-30% of ankle extensor motoneurones (Prestige, 1966) lack recurrent collaterals. In support of these anatomical findings, collateral free motoneurones physiologically did not exhibit recurrent inhibition (Sasaki, 1963). The existence of recurrent collaterals were found to correlate with motoneurone type (Cullheim & Kellerth, 1978b). In particular, collaterals were always given off by motoneurones innervating long muscles at the ankle and knee, but were absent in the case of motoneurones of short plantar muscles of the foot. This finding indicated that recurrent inhibition was primarily concerned with the control of proximal muscles which are involved in limb position rather than of the distal ones which are involved with the movement of the digits (Pompeiano, 1984; Rossi & Mazzocchio, 1991).

Evidence also suggests that each individual Renshaw cell could be excited not only by motor axons in an individual nerve but also by stimulation of motor axons in different peripheral nerves (Renshaw, 1946; Eccles et al., 1954, 1961a,b; Ryall, 1970, 1972, 1979, 1981). Recording from single Renshaw cells suggested that a given Renshaw cell might be activated by collaterals from one or at most a few muscle nuclei in which the input motoneurones need not be strict synergists but rather represent neighbouring nuclear groups (Eccles et al., 1961b). The convergence of recurrent collaterals was found to be organized in specific patterns, in particular, the greatest excitatory convergence was seen with axons supplying functionally synergistic muscles, but not with axons supplying strict antagonists (Ryall,

1981).

In addition, Granit et al. (1957) and Eccles et al. (1961b) also investigated whether all the motoneurones within a same nuclei contributed the same effect to the Renshaw cell their axon collaterals connected. They proposed that activation of large motor axons produced a much larger excitatory effect on Renshaw cell firing than activation of small motor axons. This hypothesis was supported by many investigations (Cullheim & Kellerth, 1978c; Hultborn et al., 1988a). The most direct evidence supporting this idea came from anatomic observations by Cullheim and Kellerth (1978c), that recurrent collaterals, which were given off from type-identified cat triceps surae α-motoneurones and which were classified according to Burke et al. (1971), of FF (fast, fatiguing)-type motoneurones (which were of large size) had presumably more synaptic endings (92.9) on to Renshaw cells than did those of FR (fast fatigue resistant)- (45.3) and S (slow)-type (32.2) motoneurones (which were of smaller size). This result indicated that the excitatory input to an average Renshaw cell (or the Renshaw cell pool) was largest from FF motoneurones and smallest from S motoneurones (see Windhorst, 1990). This hypothesis was also recently verified by electrophysiological data obtained by Hultborn and co-workers (Hultborn et al., 1988a). Their results indicated that the excitatory inputs to Renshaw cells from (cat triceps surae) FF, FR, and S motoneurones on average were scaled 4:2:1 (see figure 1.1).

The hypothesis that large phasic motoneurones were more effective in exciting Renshaw cells than small motoneurones, was also supported by the results of experiments in which the activation of Renshaw cells was recorded during static and dynamic muscle stretches (Pompeiano & Wand, 1976; Wand & Pompeiano, 1979). In their experiments, responses of the same Renshaw cells to static stretch and vibration indicated that the orthodromic excitation of Renshaw cells was much greater (4.3 times) during vibration than during static stretch for a comparable increase in firing of Ia afferents. Static stretch was mainly effective in exciting small tonic α-motoneurones by excitation of the static component of primary endings and secondary endings of muscle spindles. Vibration, principally driven by the dynamic component of the primary muscle spindle ending discharge, was effectively excited the large phasic motoneurones. It appeared that the larger the size of the motoneurones recruited by a vibratory stimulation, the greater was the amount of Renshaw inhibition produced by these motoneurones, while the small-size motoneurones, participating in the motoneuronal response during static stretch, were relatively weak in exciting Renshaw cells (Wand & Pompeiano, 1979). Thus it could be concluded that Renshaw cells were activated more effectively by large phasic α-motoneurones than by small tonic ones (see Pompeiano, 1984, Hultborn et al., 1988a).

In contrast to α-motoneurones, gamma motoneurones only occasionally gave rise to recurrent collaterals (Cullheim & Ulfhake, 1979; Westbury, 1979,1982). In their experiments following intracellular injection of HRP, only one out of 13 gamma motoneurones investigated were found to have axon collaterals. Electrophysiological studies demonstrated that when both alpha and gamma motor axons were excited, there was no greater influence on Renshaw cells than when alpha motor axons alone were activated (Eccles et al., 1954; Granit et al., 1957). In addition, when gamma motor axons alone were activated, there was a limited excitation of a few Renshaw cells only (Kato & Fukushima, 1974). These results suggested that maximal alpha efferent antidromic volleys elicited the maximum degree of recurrent inhibition (Eccles et al., 1954; Granit et al., 1957). It led Pompeiano (1984) to the conclusion that "gamma motoneurones do not significantly contribute recurrent collaterals to Renshaw cells".

In addition to their excitation from motor axon collaterals, Renshaw cells also receive excitatory and inhibitory synaptic inputs from many other sources. Several polysynaptic excitatory and/or inhibitory effects onto Renshaw cells could be driven from segmental afferents and supraspinal systems. From segmental afferents, polysynaptic excitation happened after stimulation of 1) dorsal roots; 2) ipsilateral group II and III muscle afferents; 3) cutaneous afferents; 4) contralateral high-threshold afferents. Polysynaptic inhibition was from both ipsi- and contralateral segmental afferents (see Baldissera et al., 1981).

Evidence has suggested that Renshaw cells could be under the control of several supraspinal systems that could modify transmission in the recurrent pathway. In particular, excitatory and/or inhibitory effects had been evoked by stimulation of the cerebral cortex, the internal capsule, the red nucleus, the vestibular nuclear complex, the cerebellum, the reticular formation, and the thalamus (Pompeiano, 1984). Four major areas of the brain were found to profoundly inhibit Renshaw cell discharge. These included the pericruciate cortex, ventral thalamus, mesencephalic and bulbar reticular formation (MacLean & Leffman, 1967). The coeruleospinal system was also reported to suppress the recurrent inhibitory pathway in cat (Fung et al., 1987). In human, recurrent inhibition was found to be increased in patients with spinal cord injury (Shefner et al., 1992). It was also found that the amount of recurrent inhibition in rat spinal cord could be enhanced by the preceding stimulation of medulla raphe

nuclei (Kaneko et al., 1987) and the synaptic transmission in recurrent pathway was enhanced by activation of bulbospinal noradrenergic pathways.

1.1.2 Renshaw cells activity and their efferent action on α-motoneurones

Physiologically identified (by their discharge pattern) Renshaw cells were reported to be small neurones located in the ventral portions of lamina VII, medial to the motor nuclei (Eccles et al., 1954; Jankowska & Lindstrom 1971). This finding was verified in part by morphological studies which showed that the terminal arborization of motor axon collaterals were located in this part of the ventral horn (Szentagothai, 1967; Cullheim & Kellerth, 1978 a,b,c). Renshaw cells had axon conduction velocities that ranged between 6 and 30 m/sec which projected to the same or adjacent segment of spinal cord in which the cell body was located (Jankowska & Lindstrom, 1971). Both extracellular and intracellular recording from Renshaw cells indicated that their characteristic firing response, to each antidromic stimulation on motor axons, was a burst of spikes at very high frequencies (Renshaw, 1946; Eccles et al., 1954) They responded in a similar manner to intracellular current injection into α-motoneurones (Ross et al., 1975, 1976) or intracellular injection of depolarizing current in Renshaw cells themselves (Walmsley & Tracey, 1981). Intracellular recordings indicated that Renshaw cells probably exhibited postspike afterhyperpolarization, at least under certain conditions such as after the discharge of a small burst (Hultborn & Pierrot-Deseilligny, 1979b; Hultborn et al., 1979b; Walmsley & Tracey, 1981).

Anatomical studies by several groups of investigators by using either lesion or extracellular dye injection at identified sites (Willis & Willis, 1964, 1966; Willis, 1969) or intracellular injection of fluorescent dye (Jankowska & Lindstrom, 1971; van Keulen, 1971) or HRP (van Keulen, 1979a; Lagerback & Kellerth, 1985a, b; Fyffe, 1991 a, b) indicated that Renshaw cells were located in the ventral part of Rexed's lamina VII. Renshaw cells were found to be small neurones which had the diameters of about 20x26 μm (van Keulen, 1979a; Lagerback & Kellerth, 1985a, b; Fyffe 1991, a, b). In addition they were found to have 4-8 dendrites in each cell and the dendrites extended up to 0.7 mm from the cell body into the neighbouring parts of lamina VII and IX as well as into more dorsal parts of lamina VII (van Keulen, 1979a; Lagerback & Kellerth, 1985b), where part of the α-motoneurone axon

collaterals were known to terminate (Szentagothai, 1967; Cullheim & Kellerth 1978a). Within the Renshaw cell region the α-motoneurone axon collaterals were found to make synaptic contact not only with proximal dendrites of Renshaw cells but also with cell bodies of Renshaw cells implying that the α-motoneurone axon collateral boutons might be situated on any part of the dendritic tree of Renshaw cells (Lagerback et al., 1981).

Following intracellular staining with either fluorescent dyes (Jankowska & Lindstrom, 1971) or HRP (van Keulen, 1979a,b; Lagerback & Kellerth, 1985a), Renshaw cell axons could be traced a rostrocaudal distribution length of about several mm. They mainly ran in the ventral funiculus before turning into grey matter again to reach their target neurones. Renshaw cell axons came off both soma and dendrites and provided terminal endings within Lamina IX as well as within the more medial regions of the spinal grey (lamina VII and VIII). A larger number of axon endings from Renshaw cells was found among the motor nuclei (lamina IX) compared to Lamina VII and VIII.

In order to determine the spatial distribution of recurrent inhibitory synapses on spinal motoneurones, intracellular HRP staining technique was employed to label a Renshaw cell and one or more possible target motoneurones after physiological identification. These labelled neurones were reconstructed and measured at the light microscopic level (Fyffe, 1991a). The author reported that, on average in his experiments in cat, each Renshaw cell made three synaptic contacts (range 1-9) on each motoneurone. Thus both anatomic and physiological data implied that individual motoneurones received convergent inputs from numerous Renshaw cells. Conversely, a single Renshaw cell gave rise to sufficient synaptic boutons to contact a large number of motoneurones distributed over a considerable length of spinal cord. All of the identified synapses observed in Fyffe's (1991a) experiments were located on motoneurone dendrites and the synapses were electronically close to the soma. These results provided the direct evidence that Renshaw cell synapses on motoneurones were located on the dendrites and not on the cell body as proposed by Burke et al. (1971). Fyffe suggested that one possible functional proposal of the recurrent inhibitory pathway was to selectively inhibit particular dendritic inputs to the motoneurone.

It was found that the excited Renshaw cells not only inhibited the neurone which gave off the

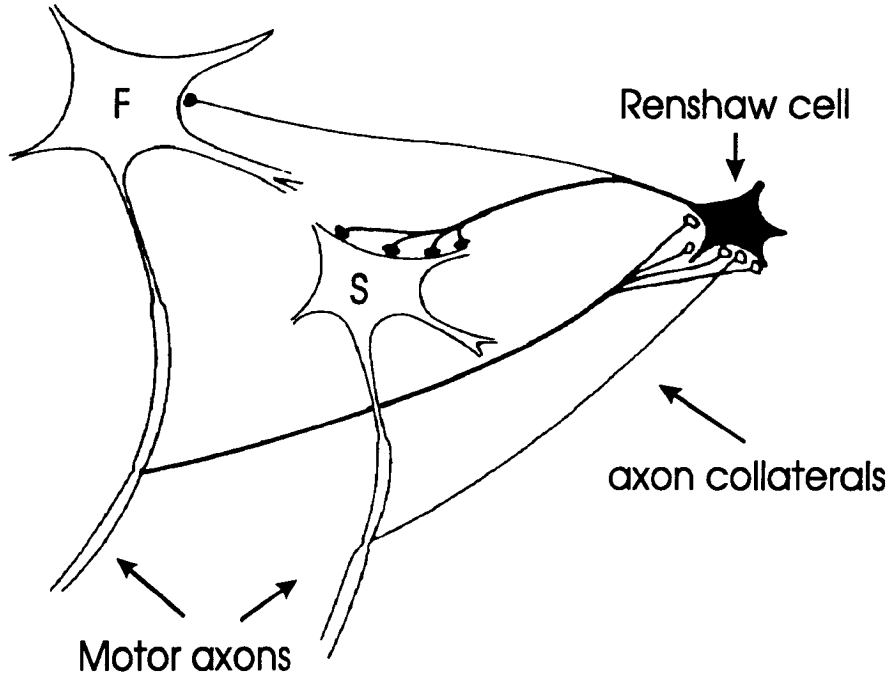
axon but also the neighbouring motoneurones (van Keulen, 1981; Hamm et al.,1987 a,b). However for a given nucleus the most pronounced recurrent inhibition was evoked in the motoneurones activated antidromically and in the motor nuclei of close synergists. This suggested that its contribution depended on functional factors (Eccles et al., 1954, 1961b; Ryall, 1970; Pompeiano, 1984). In whole spinal motoneurone pools, it had been indicated that recurrent inhibitory connections would never happen between nuclei supplying muscles acting as strict antagonists at the same joint even these motor nuclei had a similar segment location. However among those homonymous and synergist motoneurones, not all the neurones were affected by Renshaw inhibition (Eccles et al., 1954; Kuno, 1959; Sasaki, 1963 Rossi & Mazzocchi, 1991). Eccles et al. (1954) could find no recurrent inhibition in some 20% of the α-motoneurones, and Kuno (1959) found about 25% of the soleus motoneurones and 5% of lateral gastrocnemius motoneurones were not inhibited by antidromic stimulation of medial gastrocnemius. In human, it was also found that motoneurones acting on the knee and ankle muscles received recurrent inhibition, but that this was lacking in motor nuclei innervating the more distal muscles, such as the intrinsic foot muscles (Rossi & Mazzocchio, 1991). In support of these physiological findings, morphological studies were shown that not all motor axons gave off recurrent collaterals (Scheibel & Scheibel, 1966, 1971; Prestige, 1966; Eggar et al., 1980). For example, that 20-30% of ankle extensor motoneurones lack recurrent collaterals (Prestige, 1966).

However, among the neurones do receive the recurrent inhibition, some evidence suggests that some motoneurones are more strongly inhibited than others: e.g., earlier investigations demonstrated that the small, tonic α-motoneurones were as a rule strongly influenced by recurrent inhibition, whereas motoneurones which responded physically to muscle stretch seem to be less easily inhibited (Eccles et al., 1961b; Granit et al., 1957). Moreover, recurrent inhibitory postsynaptic potentials were larger in small motoneurones that innervated the 'red' soleus muscle than in large motoneurones that innervated the 'pale' lateral gastrocnemius muscle (Kuno, 1959). Later, Friedman and co-workers (1981) provided evidence that S-type motoneurones of the cat medial gastrocnemius motoneurone pool were more strongly inhibited by Renshaw cells than were FR- and FF-type motoneurones. They reported that recurrent IPSPs were generally larger in S-type than FR-type, and FR-type than in FF-type motoneurones. Similar confirming results were reported by Hultborn et al.

Fig.1.1

Diagram of recurrent inhibitory pathways to small S-type and large F-type (FF- &FR-) G-S motoneurones. Recurrent collaterals of S-type and F-type G-S motoneurones synapse on the same Renshaw cell. In turn, the Renshaw cell axon inhibits both types of motoneurones. Recurrent collaterals and axonal branches of the Renshaw cell may have different effectiveness as indicated by their relative thickness. It is postulated that Renshaw cells which are known to affect mainly small motoneurones, are in their turn more powerfully excited by the recurrent collaterals originating from large motoneurones. Excitatory synapses are drawn as empty structures; inhibitory synapses are filled in. (From Pompeiano et al., 1984)

Fig.1.1



F = F-type motoneurone, S = S-type motoneurone

(1988b) that the recurrent IPSP increased in the order of FF < FR < S which recorded from motoneurones even at resting membrane potential.

It has been concluded in figure 1.1, with the results of motor axon collaterals convergence to Renshaw cells, that FF-type motoneurones (largest cell size) appear to exert the strongest recurrent inhibitory effect on S-type motoneurones (smallest cell size) and receive the weakest effect from the latter, FR-type motoneurones (cell size in middle) taking an intermediate position (Pompeiano, 1984; Windhorst, 1990). This phenomenon has been explained that small motoneurones are less sensitive to inhibitory/excitatory synaptic effects than large ones because the input resistance of motoneurones is inversely related to their size (Kernell, 1966; Burke, 1968 a, b) in which small motoneurones have higher input resistance than large ones (Haase et al., 1975).

In addition to their well-known projection to α-motoneurones, Renshaw cells also have connection with γ-motoneurones (Brown et al., 1968; Ellaway, 1971), Ia-inhibitory interneurones (IaINs) (Hultborn et al., 1971 a,b,c), other Renshaw cells (Ryall, 1970), cells within the ventral spinocerebellar tract (VSCT neurones) (Lindstrom & Schomburg, 1973), and sympathetic (Lebedev et al., 1980) and parasympathetic (de Groat & Ryall, 1968; de Groat, 1976) preganglionic neurons.

1.1.3 Recurrent inhibition in rat

Most of the investigations on recurrent inhibition were carried out on anaesthetized or decerebrated/spinal cat preparations (eg. Renshaw, 1941, 1946; Eccles et al., 1954, 1961a, b; Cullheim & Kellerth, 1981; Havton & Kellerth, 1984, 1990b). Using the cat preparation, recurrent inhibition and its pathway have been studied systematically in fields of physiology, pharmacology, morphology, anatomy, and etc. (for review see Hasse et al., 1975; Baldissera et al., 1981; Pompeiano, 1984; Windhorst, 1990).

However, in the rat preparation, there are only a few papers published reporting investigations on recurrent inhibition of spinal motoneurones (Kaneko et al., 1987; Schneider & Fyffe 1990, 1992; Fyffe, 1991 a,b; Sanna et al., 1993). Kaneko et al. (1987) demonstrated

that recurrent inhibition was enhanced by preceding stimulation of the medullary raphe nucleus. It suggests that in rat preparation there is supraspinal modulation of spinal recurrent inhibitory pathways as exists in cats.

After successful intracellular staining with HRP, Schneider and Fyffe (1992) traced and showed the morphological features of the motoneurones and also the recurrent inhibitory pathway in the lumbar enlargement of neonatal rat spinal cord. The dendritic trees of the motoneurones were extensive, and although most dendritic branches had a smooth appearance, some exhibited spiny appendages and/or varicosities. 36% of stained motor axons which came from both 5-day or $\leq$ 13-day old postnatal rats, were observed to give off recurrent collaterals branches which arborized in the vicinity of the dendritic tree.

In the isolated lumbar spinal cord preparations from neonatal rats, a group of neurones in ventral horn, located medial to the motoneurone pools, had responses which resembled the characteristic discharge of Renshaw cells in cat spinal cord when ventral roots were stimulated with a single pulse (Schneider & Fyffe, 1992). In this preparation, the maximal recurrent IPSPs which were recorded from motoneurones located in the same spinal segment as the stimulated ventral root filaments was normally attained with ventral root stimuli of three to five times threshold. As in the cat (Eccles et al., 1954; Brooks & Wilson, 1959; Kellerth, 1968; Cullheim and Kellerth, 1981), RIPSPs recorded from isolated neonatal rat spinal cord, could be attenuated by giving strychnine or bicuculline and almost eliminated by administrating these two drugs together (Jahr & Yoshioka, 1986; Schneider & Fyffe, 1990; 1992). These results suggested that the recurrent inhibitory pathway, at least in the neonatal rat, had a similar pattern to that described in cat.

The most recent work in literature related to recurrent inhibition in rat was reported by Sanna et al. (1993). They demonstrated that Renshaw cells were inactive following peripheral nerve injury and the recovery of their activity was associated with motoneurone regeneration and reinnervation of the targets (also see section 1.2.4).

1.1.4 Possible function of recurrent inhibition

Ever since Renshaw (1941) discovered the recurrent inhibition of α-motoneurones -- transmitted via axon collaterals and Renshaw cells -- there has been much interest in the possible function of this neuronal pathway. Although the general feature of a negative feedback is obvious, the implications of such a feedback are not.

The hypothesis that recurrent inhibition might act as a variable gain regulator at the motoneuronal level rather than to modify the pattern of motor activity was postulated by Hultborn and co-workers (1979a, 1988a,b, 1989). They suggested that the gain of the input-output curve relating to the excitatory input of the activity of the pool of motoneurones (output) would increase when the Renshaw cells were inhibited, but would decrease when Renshaw cells were facilitated. It was supported by the results of experiments which demonstrated that during the postural adjustments evoked by stimulation of macular labyrinthine reflexes in cat (Pompeiano et al., 1983) as well as during voluntary movements in man (Bussel & Pierrot-Deseilligny, 1977; Hultborn & Pierrot-Deseilligny, 1979a) weak muscle contractions were accompanied by an increased discharge of Renshaw cells. Conversely, strong muscle contractions were associated with a reduced discharge of Renshaw cells which would reduce the gain of the recurrent loop, and thus enhance the motoneuronal discharge. Hultborn et al. (1979a) pointed out that a 'low gain' condition (facilitation of Renshaw cells) would allow supraspinal force-generating circuits to play over a considerable part of their working range and yet cause only small changes in muscular force, whereas a 'high gain' state (inhibition of Renshaw cells) would allow the central command to generate larger forces for a given drive. Such a variable gain control at motoneuronal level might thus optimize the resolution of the motor output during weak as well as strong contractions (Baldissera et al., 1981). Since the activity of α- and γ-motoneurones and Ia inhibitory interneurones might act in parallel and balance each other to control postural muscle contraction, and the same Renshaw cells probably inhibit all these neurones, Renshaw cells could serve as a variable gain regulator at the output level which formed by all those three groups of neurones (Hultborn et al., 1979b).

1.2. Effect of temporary denervation on recurrent inhibitory pathway on adult and neonatal motor units

During development, immature muscles and nerves develop their specific characteristics as a result of maturation. When this maturation process has been disturbed, unrecovered impairment has been observed (Lowrie et al., 1982, 1987, 1990; Albani et al., 1988). Also if the mature motoneurone and its target muscle has been temporary disconnected, there will undergo a process of regeneration which is similar to that of maturation progress. Thus, this section will deal with the normal postnatal development first followed by briefly an over-view of the effect of nerve crush on motor units and also the recurrent inhibitory pathways in either adult or young animals.

1.2.1. Postnatal development of motor units

Mammals are born with immature muscles and nerves. The characteristic properties of adult rat muscles develop during the first few weeks of postnatal life (Navarrete & Vrbova, 1983) as appropriate neural activity becomes imposed upon them from their motoneurones. Muscles develop their specific characteristics as postural or flexor muscles as a result of maturation and interaction with their motoneurones during development. This development really begins postnatally. Muscles eventually fall into two general functional and biochemical categories: slow and fast muscles. The most comprehensive study on the development of slow and fast skeletal muscles in the rat was done by Close (1964). He found that initially all muscles are slow. Then after an initial increase in speed slow soleus muscles develop slow contraction speed. Fast muscle such as the EDL however continue to develop increased speed of contraction and relaxation until adult values are reached.

Functionally, mature skeletal muscles can be divided according to their physiological properties into slow contracting and relaxing and fast contracting and relaxing. Biochemically, they can be divided into those with high levels of oxidative enzymes and those with high levels of glycolytic enzymes. Slow fibers are also called Type I (Dubowitz & Pearse, 1960 a,b) and are characterised by high oxidative and low glycolytic enzyme activity. Type II fibers are generally fast, have high glycolytic and low oxidative enzyme

activity. Slow muscles, such as soleus, which contain predominantly slow-contracting muscle fibers, tend to be tonically active and are used in the maintenance of posture, whereas fast muscles such as extensor digitorum longus (EDL) and tibialis anterior (TA) contain predominantly fast contracting muscle fibers, active physically and produce large forces of short duration. The distinction between fast and slow muscles is a general one, as most muscles contain a percentage of slow and fast contracting muscle fibers (for review, see Burke, 1981).

In the mammalian spinal cord, neonatal motoneurones have been shown to be immature in their intrinsic electrophysiological properties (Kellerth et al., 1971; Fulton & Walton, 1986; Kenell, 1990), synaptic connectivity (Conradi & Ronnevi, 1975, 1977; Kudo & Yamada, 1987) and the polyneural innervation of skeletal muscle fibers (Bagust et al., 1973) compared to those of adult motoneurones. In adults, motoneurones are differentiated with respect to their intrinsic electrophysiological properties and recruitment order (for review see Burke, 1981; Kernell, 1990). Motoneurones to slow muscles have a much longer AHP and are recruited more readily than those supplying fast muscles (Eccles et al., 1958a,b). Studies of unidentified motoneurones in foetal and new born kittens (Naka, 1964; Kellerth et al., 1971) and neonatal rats (Fulton & Walton, 1986) indicate that their intrinsic properties undergo changes with age that lead to their differentiation into functional subclasses. Unlike in adults, neonatal motoneurones have high input resistance and low maximum firing rate. (Fulton & Walton, 1986). They are smaller and also more easily excited by afferent stimulation (Kellerth et al., 1971). Kellerth et al.(1971) showed that all neonatal motoneurones of kittens have very similar electrophysiological properties, and none of the differences between the firing patterns of "slow" and "fast' motoneurones that they mature into, exists. In young kittens, the duration of AHP was short in motoneurones to both future "slow"'and "fast" muscles (Huizar et al., 1975), within the further development, the AHP duration increased in motoneurones to slow muscles while it remained short in motoneurones to fast muscles (Hammarberg & Kellerth, 1975).

The control of motoneurone firing patterns depends not only on their intrinsic electrophysiological properties but also on the source and distribution of the synaptic input that activates them. Morphological and physiological evidence exists to show that

synaptogenesis on motoneurones in the lumbar spinal cord, of descending and segmental connections, is complete only by the first few weeks of postnatal life (e.g. Saito, 1979) with many of the descending pathways to motoneurones being established after birth. For example, the cortico-spinal tract is known to be functional 9-14 days after birth and sensory afferents, if they are present at birth, are not functional until the second postnatal week (Fitzgerald & Gibson, 1984). Although interneuronal synapses onto motoneurones are formed early in embryonic life and primary afferent monosynaptic connections to motoneurones occur just before birth (Kudo & Yamada, 1987), evidence exists that up to 50% of synapses onto motoneurones are eliminated during the first 3 weeks of postnatal life in kitten (Conradi & Ronnevi, 1975). In addition, synaptic inputs to kitten motoneurones have been shown to become reorganised during early postnatal development; as the neurone matures and extends its dendritic tree, synapses are thought to move away from the cell body, possibly onto the dendrites (Ulfhake et al., 1988). This was supported by the evidence that a large reduction of the number of synaptic boutons on the motoneurone cell body surface (Conradi & Ronnevi, 1975) and a total elimination of boutons on the initial axonal segment (Conradi & Ronnevi, 1977). Thus, developmental changes in synaptic inputs to motoneurones have to be considered in order to account for the differentiation in motoneurone firing patterns. It may indeed be the changes in afferent synaptic activity associated with the maturation of descending and interneuronal inputs to motoneurones that may regulate the functional specialization of the motoneurones. These synaptic rearrangements have been proposed to occur within the spinal cord itself (Saito, 1979; Fitzgerald, 1985; Navarrete et al., 1987).

One of the major developmental process in the postnatal maturation of hindlimb motor unit in the mammals is the elimination of polyneuronal innervation of skeletal muscle fibers (Bagust et al., 1973). This takes place mainly during the first 2-3 weeks of life in kitten. However, in both kitten and adult cat the motoneurones make synaptic contacts not only with muscle fibers but also with Renshaw cells in spinal cord (Cullheim & Ulfhake, 1982, 1985). By using the intracellular deposition of horseradish peroxidase (HRP), Cullheim and Ulfhake (1982, 1985) found that during the first few weeks of postnatal life as much as half or even the majority of the contacts, the axon collateral end branches and swellings interpreted as synaptic boutons between axon collaterals and Renshaw cells present at birth have been eliminated. In addition they found that there is a total elimination of short and thin axonal

processes without swelling within the collateral system (Cullheim & Ulfhake, 1985). However, during the substantial postnatal reduction in the number of axon collateral swellings, the projection field of the axon collaterals of the triceps surae motoneurones did not change. They suggested that their presence reflected a remodelling process of the synaptic connections and represented retracting axons.

In adult animals, the motoneurones of fast-twitch motor units seem to produce stronger and receive weaker recurrent inhibition than motoneurones of slow-twitch units (Eccles et al., 1954; Hultborn, et al., 1971c; Kuno, 1959; Ryall et al., 1972). A morphological investigation in the adult cat has shown that motoneurones of slow-twitch motor units had fewer axon collateral swellings than motoneurones innervating fast-twitch muscle units (Cullheim & Kellerth, 1978b). This difference in the effect of recurrent inhibition seen in adult cat may be explained by the report that motoneurones of slow-twitch motor units were subjected to a large elimination of axon collateral swellings with a substantial reduction of their recurrent effects while the motoneurones innervating fast-twitch muscle units displayed less prominent changes (Cullheim & Ulfhake, 1985).

In association with the morphological findings that the recurrent axon collaterals undergo elimination during postnatal development (Cullheim & Ulfhake, 1985), physiological data also demonstrated that the recurrent inhibitory effect in kitten was changed postnatally (Wilson, 1962). Recurrent inhibition in foetal cats was found to be very powerful and long lasting (Naka, 1964). Postnatally, recurrent inhibition of kitten extensor MSRs showed a two phase development (Wilson, 1962; Mellstrom, 1971b). The recurrent inhibition of ankle extensor (Gastrocnemius to plantaris) was obtained in the new born kitten (10% of MSR amplitude reduction) and increased (to about 30%) within the first postnatal week, but disappeared during the first postnatal month. It reappeared during the first half of the second month postnatally and increased to reach adult values (60%) at the end of second month. The observed elimination of collaterals (Culheim & Ulfhake, 1982, 1985) may in part contribute to the recurrent inhibition change during the development.

1.2.2. Effects of adult denervation

Depriving the interaction between motoneurone and muscle fibre by way of axotomy, has a detrimental effect on the normal functioning of the neuromuscular system, as it results in muscle paralysis and an alteration in motoneurone properties. In adult animals, these effects are reversed if nerve-muscle contact is restored by reinnervation (Lowrie et al., 1982). It is also reported that there was no retrograde cell death could be observed if the nerve injury occurred in adult animals and the reinnervation was not prevented (Carlson et al., 1979).

1.2.2.1 Effects of nerve injury on the electrophysiological properties of mature neurones

The characteristic changes provoked in the pericarya of mature neurones by axotomy are collectively referred to as the axon or retrograde reaction of the cell body response (Titmus & Faber, 1990). The effects of nerve injury on the properties of mature motor units are different depending on the type of mechanical response (fast or slow) (Kuno et al., 1974b; Foehring et al., 1986 a,b). In the adult mammal, type different (FF-, FR-, S-) α-motoneurones not only have different sizes but also have different intrinsic electrophysiological properties. For example, S-type motoneurones have a higher input resistance (Burke et al., 1982; Gustafsson & Pinter, 1984a; Ulfhake & Kellerth, 1984; Foehring et al.,1986a,b), longer AHP duration (Eccles et al., 1958a; Foehring et al., 1986b 1987a) and slower axonal conduction velocity (Gutmann & Holubar, 1951; Cragg & Thomas, 1961; Kuno et al., 1974a,b) than that of F-type motoneurones. In general, after axotomy when neurones do not successfully regenerate their axons, there was a reduction in axon conduction velocity, increased input resistance, reduction in AHP parameters increased soma-dendritic excitability and decreased IS excitability (Kuno et al., 1974a,b; Titmus & Faber, 1990). However the difference of the motor unit properties within the type different motor units were found to be "dedifferentiated" as a result of axotomy (Cragg & Thomas, 1961; Kuno et al., 1974a,b; Gustafsson & Pinter, 1984b; Foehring et al., 1986a,b). For example, the input resistance of MG motoneurones (predominant of F-type) was increased significantly but not that of the soleus motoneurones (S-type dominant) (Kuno et al., 1974b). Foehring and co-workers (1986b) also found that input resistance values for FF-, FR, and S-type motoneurones in the MG motoneurone pool became dedifferentiated after axotomy. The

changes in properties of motor units were reported to functionally benefit to the regeneration (Kelly et al., 1986; Gordon et al., 1987; Titmus & Faber, 1990). It was suggested that this dedifferentiation as the result of axotomy may possibly mean (i) a return to a more immature or embryonic state, with the connotation that this state is more conductive to growth, or (ii) simply the loss of properties which distinguish functional classes of neurones, such as fast and slow type motoneurones (Titmus & Faber, 1990).

Although recurrent IPSPs (RIPSPs) recorded from type identified cat MG motoneurones indicated that RIPSPs were largest in S motoneurones, smallest in FF motoneurones, and intermediate in FR motoneurones (Friedman et al., 1981), it still remains unclear whether there is a similar "dedifferentiation" within the recurrent inhibitory pathway following axotomy. Some evidence may favour this hypothesis. The axon collateral end branches from type identified cat MG motoneurones normally have a different distribution in which FF-type was much more than FR-type and FR-type was more than S-type. However, following nerve section, the mean number of axon collaterals were dropped below the normal S-type numbers (Havton & Kellerth, 1984, 1990a). This result may suggest that F-type motoneurones tend to loss much more collaterals than S-type motoneurones.

1.2.2.2 Recovery time course for axotomized motoneurones

All the changes in the intrinsic properties of motoneurones listed above occur because of peripheral nerve transection and prevention of its reinnervation to target tissue. In the adult animal axotomized motoneurones allowed to reinnervate their original targets will eventually recover their normal or nearly normal electrical properties. For example, nine months after self-reinnervation to MG muscle the mean values for the various membrane properties of each functional type of motoneurone (FF, FR and S) are not significantly different from those of their normal counterparts (Kuno et al., 1974b; Foehring et al., 1986a). However, additional time is required for full restoration of normal motoneurone properties, though the functional connection to the muscle appears to be necessary for the recovery of these properties (Foehring et al., 1986b).

Foehring and associates (1986b) found that during early stages of reinnervation (5-6 weeks

postaxotomy) cat MG motoneurones with and without functional reconnection have membrane properties similar to those of the axotomized motoneurones. Several weeks later (9-10 weeks postaxotomy), although the proportions of fast and slow motor units are similar to normal, neither the motoneurone's electrical properties nor muscle's contractile properties have reached normal value, although both are changed in that direction from the early stage. Following 9 months after nerve section, self-reunion results in most motoneurone and muscle unit properties recovering to the control level and the normal relationship between motoneurone and muscle is established (Foehring et al., 1986a,b).

In the rabbit, peroneal nerve crush (Cragg & Thomas, 1961) induced a decrease in conduction velocity by 10% and 20% at 30 and 100 days following injury. It remained impaired for up to 150 days, but normal impulse velocity was regained by 200 days. In frog, Farel (1978) found that the increased latency of spinal reflex activity (accounted for by an observed 60% drop in axonal conduction velocity) as a result of axotomy was reversed to normal at 61-75 days postoperatively.

The degree of restoration of neuronal properties after reinnervation may also be dependent on the period of denervation. Gutmann and Young (1944) found that recovery of muscle function after nerve crush occurred earlier when the nerve lesion was made close to the muscle. Some workers have reported that temporary disruption of nerve-muscle interaction during early postnatal life did not result in the profound impairment of either, if the lesion was made close to the muscle (Brown et al., 1976). A more recent report by Lowrie and co-workers (1990) indicated that when the nerve was crushed close to the muscles, the muscle recovered significantly better than when the site of injury was further away. They concluded that the permanent impairment of fast muscles seen after neonatal nerve injury depended upon the length of time that the muscle were separated from their motoneurones.

1.2.2.3 Morphology of motoneurones subjected to axotomy and subsequent reinnervation

Peripheral axotomy induces a swelling of the cell body as indicated by the increase in its diameter, surface area, and volume of the soma at 3 weeks after the axotomy in cat spinal α-motoneurone (Brannstrom et al., 1992a). The soma size of axotomized neurones, however,

returns to normal after reinnervation (Lieberman, 1974). In addition to the recovery of the reinnervated cell size to normal control values, Bowe et al. (1988, 1992) found that following long term reinnervation from nerve crush that the mean cross-sectional area of rat sciatic motoneurones was remained within the normal distribution, but that in age-matched control motoneurones' size was reduced with increasing age. This failure of the ageing processing may reflect the findings in neonatal nerve crush that nerve injury "arrests" the maturation of the motoneurones (Dekkers & Navarrete, 1993).

Following permanent axotomy of cat spinal motoneurones, a marked reduction in dendritic size was observed by Brannstrom and co-workers (1992a). In comparison with normal MG α-motoneurones, the dendritic membrane area and volume of the axotomized cells has decreased by 36% and 29%, at 12 weeks after the axotomy. This reduction in dendritic size is thought to be due to a loss of preterminal and terminal dendritic segments (Brannstrom et al., 1992a). After muscle reinnervation, the retracted dendritic trees in axotomized neurones re-expanded (Sumner & Watson, 1971). Recently, Brannstrom et al. (1992b) reported that peripheral reinnervation caused the dendritic value and membrane area to return to normal values. However, the values for combined dendritic length and number of dendritic end branch still remained reduced by more than 25% as compared with those uninjured motoneurones. Bowe et al. (1988) also reported "thicker dendritic processes" in rat sciatic motoneurones after peripheral nerve crush. These changes may be associated with altered synaptic inputs to the injured motoneurones.

1.2.2.4 Alterations at the synaptic input level in motoneurones

In general, synaptic depression occurred in the transmission between presynaptic neurones and axotomized neurones. Injuries to a muscle nerve caused a progressive decrement in the amplitude of monosynaptic EPSPs evoked in the homonymous and heteronymous motoneurones by afferent volleys from the muscle nerve (Kuno & Llinas, 1970; Gallego et al., 1979; 1980). Similar alterations were also found in IPSPs (Kuno & Llinas, 1970). The evoked EPSPs had virtually disappeared by the eighth postoperative month after nerve section where reinnervation was prevented (Goldring et al., 1980). Functionally, synaptic depression resulted in a marked depression of firing of axotomized neurones within the first month after

axotomy (Acheson et al., 1942; Gordon et al., 1980). The number of synaptic profiles of axotomized neurones were found to decline sharply in the first few days following axon injury (Mathews & Nelson, 1975), so that loss of synaptic contact was thought to be the major factor responsible for synaptic depression (Gordon, 1983). In adult animals, peripheral nerve lesions (crush or axotomy) resulted in a temporary decrease in the amplitude of the monosynaptic EPSP, indicating a "plasticity" of the different synapses onto motoneurones following disconnection from their target muscle (Eccles et al., 1958b; Kuno & Llinas, 1970; Mendell, 1984). Some of the depression in synaptic transmission is partially compensated for by an apparent hyperexcitability of axotomized motoneurones. Monosynaptic reflexes were either lost or very depressed but polysynaptic reflex discharge were even larger than normal. Loss of monosynaptic reflexes and enhancement of polysynaptic reflexes may indicate selective loss of some terminals as suggested by ultrastructural studies and by electrophysiological studies (Kuno & Llinas 1970; Gordon, 1983).

Although the degree of central synaptic depression initially (weeks 2-3) occurring after crush of a peripheral nerve is indistinguishable from that produced by section of the nerve (Gallego et al., 1979), the initial reaction of central synapses to the crush procedure is followed by a supernormal phase (weeks 8-12) before slowly declining to about 70% of the normal values later (by week 30), whereas the reaction to section is characterized by a monotonic decrement in synaptic efficacy as long as peripheral regeneration is prevented (Gallego et al., 1980). Gallego and co-workers (1980) suggest that the enhanced monosynaptic EPSPs amplitude seen following nerve crush is contributed by those sensory fibers that regenerate into the muscle but do not achieve functional reinnervation. The experiments from Beranek and Hnik (1959) using tenotomy supported this view. They demonstrated that spinal monosynaptic reflexes evoked by afferent volleys from chronically tenotomized muscles were significantly enhanced, due to prolonged disuse of the sensory pathway. However such enhanced reflexes can be seen only for several weeks after tenotomy, not in the longer post-operative periods (Beranek et al., 1961).

In contrast, if the cut peripheral nerve is allowed to regenerate into a muscle, the maximum amplitude of monosynaptic EPSPs recorded from the homonymous motoneurones remains about half the normal size even several hundred days after the reunion of a sectioned muscle

nerve to its muscle (Eccles et al., 1962). The failure of complete recovery of synaptic function has been attributed to degeneration of some dorsal root ganglion cells subsequent to the axonal injuries.

It is unclear whether reactive deafferention (which is the apparent rejection of some synapses by the cell body and dendrites) involves only excitatory inputs in motoneurones. Most studies have reported that it results in the depression of synaptic transmission at proximally located synapses with the EPSPs decreased in amplitude (Titmus & Faber, 1990). The morphological correlate of this is the selective stripping of synaptic boutons from the soma and proximal dendrites with an associated glial proliferation (Kerns & Hinsman, 1973). Kuno and Llinas (1970) reported that along with Ia EPSPs, proximally located IPSPs (generated mainly by Renshaw cells and Ia inhibitory interneurones) were also affected by axotomy. In agreement with these results, Chen (1978) showed that there was a loss of terminals containing flattened as well as spherical vesicles. A recent study (Brown & Fyffe, 1981) has shown that Renshaw cells appear to synapse to proximal dendrites and provide direct evidence to support the early electrophysiological results (Burke et al., 1971), and this may be significant in relation to physiological data from axotomized motoneurones (Titmus & Faber, 1990). It is of interest to note that the overall spatial distribution of Renshaw synapses closely resembles the distribution of excitatory Ia-afferent synapses (Brown & Fyffe, 1981).

1.2.2.5 Muscle fiber change as the result of adult axotomy

Adult skeletal muscles depend on their motor innervation for the maintenance of their function. However, they can also survive temporary denervation provided that reinnervation is allowed to proceed unhindered. For example, after sciatic nerve crush in adult rats, muscles recover virtually completely their weights and tensions upon reinnervation (Beranek et al., 1957; Lowrie et al., 1982). In adults, recovery following nerve crush is *so good* that not only do the muscles regain their original strength (Lowrie et al., 1982) but little fiber grouping can be seen (Karpati & Engel, 1968).

When adult skeletal muscles are reinnervated after nerve section, regenerating axons contact muscle fibers in a non-specific manner. Thus fibers of different biochemical properties may

be reinnervated by branches of the same axon and the distribution of muscle fibers of a given motor unit within the muscle is altered (see Albani et al., 1988). During the restoration of function there is a gradual transformation of those muscle fibers of inappriate biochemical type so that the motor units become histochemically homogeneous (Karpati & Engel, 1968). Albani et al. (1988) reported that after nerve section in the adult, the twitch tension of reinnervated muscle was only 61% of the control, and the muscle weight showed a similar difference which was 80% of control. They also found that after nerve section, the reinnervated muscle fibers were grouped and there was a large number of type I (more oxidative) fibers in these muscles. However, after nerve reinnervation in adult rats, the distribution of motor unit force and their physiological properties were restored to normal (Gordon et al., 1980; Albani et al., 1988).

1.2.3. Regeneration of motor unit in young animals

After nerve crush in adult rats, the distribution of motor unit force is restored to normal but this does not occur following nerve crush in neonatal animals. Thus following nerve injury during the neonatal period the muscles are not only permanently weaker, but the distribution of motor unit size is also abnormal (Albani et al., 1988). However changes of activity patterns and muscle properties are not seen after nerve injury in animals older than 12 days (Navarrete & Vrbova, 1984; Lowrie et al., 1987). The motoneurone loss is also not seen following peripheral nerve crush performed at and after 5-day postnatally (Lowrie et al., 1982, 1987), indicating that long lasting alterations of motor unit activity occur only after lesions in the immediate postnatal period.

1.2.3.1. Alterations in neonatal motoneurones as a result of axotomy

During early postnatal life, if nerve-muscle interaction is disrupted, both the muscle and the motoneurones that innervate them become severely impaired. Thus, for example, in rats, injury to immature motoneurones by sciatic nerve crush immediately after birth results in the degeneration and death of 70% of the motoneurones (Romanes, 1946; Lowrie et al., 1987). The number of motoneurones dying decreases if the injury is inflicted later after birth so that by day 5 in rats no motoneurone loss is seen as a result of sciatic nerve crush (Romanes,

1946; Lowrie et al., 1982, 1987; Krishnan et al., 1985). In addition, if these 5-day crush motoneurones are prevented from reinnervating their targets for more than two weeks, they degenerate and die (Kashihara et al., 1987). However, no motoneurone loss has been shown to occur in new born animals if the injury is made very close to the target muscle and reinnervation is left to proceed unhindered (Brown et al., 1976; Kashihara et al., 1987).

There is very little direct evidence as to the effects of nerve injury on membrane properties of neonatal motoneurone membrane properties. However this could be inferred from studies on adult motoneurones as it has been shown that functional connection with their targets is necessary for adult motoneurones (Czeh et al., 1977), and if it is prevented, they revert to the membrane properties of immature motoneurones (Kuno et al., 1974a,b; Huizar et al., 1975). The evidence on the membrane properties of neonatal motoneurones shows that they are as yet immature and activated by general stimulation. Evidence from the study of motor unit properties of reinnervated fast muscles after a sciatic nerve crush in early postnatal life shows a tonic firing more characteristic of slow muscles which is maintained throughout the life of the animal (Navarrete & Vrbova, 1984). In addition, normally weak cross-excitatory polysynaptic reflexes on flexor motoneurones become permanently enhanced after sciatic nerve crush in new born rats (Navarrete et al., 1990) suggesting an alteration in the intrinsic properties of the motoneurones or in their synaptic connectivity as a result of a temporary disruption of the developmental process. It has also been shown that a permanent effect of early neonatal nerve injury is to alter the dendritic arborization of regenerated motoneurones when these are compared with those of normal age-matched control animals (O'Hanlon & Lowrie, 1993).

The changes in the morphology of motoneurones when reducing its target during early development are paralleled by a permanently altered activity pattern of the motoneurones. These were revealed by recording EMG activity from freely moving animals that had their nerves crushed either at birth, or 5-6 days later by Krishnan and co-workers (1985). Motoneurones to fast muscles fired in short bursts of high frequency whereas motoneurones that were crushed in early life fired for much longer periods of time. In addition the EMG activity in reinnervated muscle was 2-3 times greater than normal (Navarrete & Vrbova, 1984). This firing pattern change may have been related to the change in intrinsic

motoneurone excitability following neonatal nerve injury. Krishnan et al. (1985) suggested that the reinnervated motor unit firing pattern change may be important in that the functional readjustment to a greater than normal activity which provided the stimulus for the maintenance of the axonal expansion and metabolic capacity of the motoneurones.

Lowrie et al. (1987) also reported that, after neonatal sciatic nerve injury in rat, the mean cell body area was smaller than that of the contralateral control motoneurones. It is due to a selective reduction in the size of the larger cells and was significant whatever the degree of motoneurone loss, even in 5-day old rats whose sciatic nerve had been crushed in which no motoneurone loss was seen (Krishnan et al., 1985; Lowrie et al., 1987).

1.2.3.2 Muscle regeneration in neonatal animals

Previous investigators have shown that skeletal muscles recover function less well after neonatal nerve injury than after the same injury in adult animals (Bueker & Meyers, 1951; Zelena & Hnik, 1963; McArdle & Sansone, 1977; Lowrie et al., 1990). The severity of the impairment varies with the age at which injury is inflicted. In the rat sciatic nerve injury at birth results in permanent impairment of the fast (TA and EDL) muscles, but when the nerve was injured a little later i.e. at 5 days the recovery was better, although the muscle still only developed about 50% of normal weight and tension (Lowrie et al., 1982, 1984, 1987). In contrast slow (soleus) muscles reinnervated after a 5 day crush recovered to 80% of their contralateral controls. This was compatible with the finding that, after neonatal nerve crush reinnervated fast muscles development remained significantly slower than normal age-matched controls (Lowrie et al., 1987). Reinnervated fast muscles also showed an increase in oxidative enzyme activity (Lowrie et al., 1982). This contrasted with normal fast muscles which were very low in oxidative enzyme activity. Thus, upon reinnervation, no large pale fibers were seen in fast muscles and the remaining fibers were regrouped into clusters (Lowrie et al., 1982). The suggestion from this study was therefore, that the reinnervated muscle fibers that remained were gradually being converted to slow ones. Evidence from adult mixed muscles indicated that slow motor units had a selective advantage during reinnervation (Lowrie et al., 1982; Foehring et al, 1986a,b).

Consistent with the alteration of their biochemical properties reinnervated fast muscles also showed an increase of up to 2 to 3 times in their electrical activity (EMG) compared to their contralateral controls or to normal animals (Navarrete & Vrbova, 1984). Since a muscle was thought to mirror the activity of its motoneurone, it was thought that after neonatal nerve crush, the motoneurone had become more excitable (Navarrete & Vrbova, 1984; Vrbova et al., 1985).

1.2.3.3. Effect of neonatal nerve injury on spinal reflexes

Following sciatic nerve crush in newborn rats, a high percentage of axotomized motoneurones degenerate and the motor activity permanently impaired due to muscle fiber loss, reduction of motor unit size and a decrease in the number and diameter of motor axons. Sciatic nerve lesions in newborn animals not only affected motoneurones but also interrupted the sensory afferent input to the spinal cord from muscle and skin targets innervated by this nerve. Moreover, it is known that peripheral nerve lesions in neonatal animals lead to the death of a significant number of motor and sensory neurones (Zelena & Hnik, 1963; Bondok & Sansone, 1984; Yip et al., 1984). It is likely that loss of sensory neurones results in the permanent loss of synaptic inputs to the spinal neurones onto which they normally project, and it may be that the vacated synapses become occupied by other inputs (Navarrete et al., 1990). In deed Miyata et al. (1986) found a significant depression of monosynaptic EPSPs in adult rat motoneurones after neonatal sciatic nerve crush. Thus it is possible that nerve injury during early stages of development may result in a permanent alteration of the synaptic input to the motoneurones. Consistent with such an idea was evidence indicating that after neonatal nerve injury intact sensory afferent fibers were able to expand their central projections in the spinal cord (Fitzgerald, 1985). Moreover, synaptogenesis in the spinal cord was still in progress during the first 2 weeks after birth (Stelzner, 1982) and it may be that regenerating motoneurones were more likely to receive new synaptic inputs than those cells that had already terminated their growth. Since the vast majority of synaptic inputs to motoneurones was mediated via interneurones, an indication of abnormal synaptic drive to the injured motoneurones would be proved by changes in polysynaptic reflexes (Navarrete et al., 1990). Navarrete and co-workers (1990) reported that, following neonatal nerve crush, reflexes recorded from reinnervated muscles by stimulation of the branches of the uninjured

sciatic nerve were 3-7 times greater than those recorded from the uninjured muscles or the muscles in normal animals. These results indicated that sciatic nerve crush during a critical developmental period leads to a permanent enhancement of reflex responses from reinnervated fast flexor muscles which was not seen after similar injury in adults.

Recently, Vejsada and co-workers (1991) reported that neonatal sciatic nerve crush markedly altered the activity pattern in fast flexors (TA), whereas the slow (Soleus) and fast extensor (LG) were much less affected. The functional changes in the tibialis muscle may reflect increased motoneuronal excitability, altered neuronal connectivity and/or lack of inhibition in the spinal cord as a result of peripheral nerve injury during a critical developmental period.

1.2.5. Axotomy induced alteration of recurrent inhibitory pathway: its morphology and function

By using the calbindin-expression method, Sanna and co-workers (1993) indirectly demonstrated that Renshaw cells in rat lamina VII undergo complex morphological and physiological changes after sciatic nerve crush. A subpopulation of 28-KDa calbindin-immunoreactive neurones located in the ventral portion of lamina VII, medial to the motoneurone column, has been proposed to be Renshaw cells based on anatomical location (Antal et al., 1990; Arvidsson et al., 1992). Calbindin-expression is thought to appear as a result of motoneuronal activity. One week after nerve crush calbindin immunoreactivity was strongly reduced on the lesioned side while calbindin-containing neurones and fibers were still numerous contralaterally and cranially to the lesion level. With the progression of regeneration, calbindin immunoreactive neurones reappeared, reaching a normal distribution 6-8 weeks after the crush (Sanna et al., 1993).

A series of investigations on recurrent inhibitory reflexes and alterations in recurrent axon collaterals following axotomy in the cat were reported by Havton and Kellerth (1984, 1990a,b). Following axotomy, the retrograde axon reaction causes a gradual elimination of intramedullary axon collaterals from the lesioned motoneurones (Havton & Kellerth, 1984; 1990a). This elimination process, which at 12 weeks postoperatively has caused about 40%

4of the motor axon collaterals to disappear. However, shortly after nerve section, there is not any signs of degeneration of axon collaterals could be observed. It indicates that the elimination of axon collaterals appears to progress gradually (Havton & Kellerth, 1990a).

By using intracellular recordings, Havton and Kellerth (1984, 1990 a,b) found that the recurrent inhibitory postsynaptic potentials (RIPSPs) elicited from the chronically axotomized motoneurones were greatly reduced at 3 and 6 weeks postoperatively which were paralleled the successive elimination of intramedullary axon collaterals in the same cells. Surprisingly, the amount of recurrent inhibition (obtained by means of intracellular recording and monosynaptic reflex testing) produced by axotomized motoneurones at 12 weeks postoperatively seemed to be unaffected by the pronounced reduction in the number of intramedullary recurrent axon collaterals originating from the same cells. They proposed that postoperative enhancement of reflex actions may take place in the recurrent inhibitory pathways persisting in the axotomized motoneurones as well as in those originating from synergistic non-lesioned motoneurones and the site of compensatory enhancement was at the synaptic contacts between the motor axon collaterals and the inhibitory Renshaw interneurones (Havton & Kellerth, 1990b).

1.3.0. Aim of this thesis

Since the monosynaptic reflex is one of the simplest reflexes in the mammalian body, and this reflex could be modulated by various input sources including the spinal recurrent inhibitory input, monosynaptic reflexes have been widely used as a model in investigation effects of recurrent inhibition first by Renshaw (1941) and more recently by Havton and Kellerth (1990b). Monosynaptic reflex discharges elicited by exciting (test pulses; Ia afferents could be reduced by delivering antidromic (conditioning) impulses in motor axons. In this study, monosynaptic reflexes were used as a tool to assess the spinal recurrent inhibition on spinal motor pools and subsequently the change of recurrent inhibitory effect after nerve crush.

The aim of the study for this thesis is to 1) test the recurrent inhibition of spinal monosynaptic reflexes in decerebrate rat in comparing with that in cat and anaesthetized rat; 2) examine the proposal that following peripheral nerve crush, recurrent inhibitory pathways

would be affected as a result of nerve injury; 3) investigate if there is some degree of impairment in RI pathways after nerve crush, the possible recovery time course; 4) find the relationship between impairment of RI pathways and the length of time of neurone-muscle disconnection; and 5) detect the possible "dedifferentiation" in recurrent inhibition pathway following nerve crush.

The results discussed in the chapters 4 and 5 indicate that regenerated motoneurones in general produce reduced recurrent inhibition with the most dramatic reduction being on motoneurones injured in early postnatal life. In chapter 3, recurrent inhibition of MSRs in decerebrated rat has been described in which it has been found that virtually there is no difference between rat and cat in generating of recurrent inhibition in spinal cord.

CHAPTER 2 MATERIALS AND METHODS

2.0. Introduction

There are three main methods described in this chapter. They are divided into three sections which are as follows: (1) animal preparation; (2) electrophysiological data collection and analysis; (3) histology of spinal motoneurones and hindlimb muscles.

2.1. Animal preparation

The results presented in this thesis are based on the experiments performed on a total of 164 male or female adult Wistar rats weighing between 180g and 400g.

2.1.1. Anaesthesia

Two kinds of anaesthetic were used in the experiments and are described below:

1. Cold anaesthesia: 5-day old rats received cold anaesthesia during nerve crush. Baby rats were put in a small dry beaker placed on a block of ice (under 0°C). An adequate depth of surgical anaesthesia was considered to be achieved when their breathing was slow, the colour of skin pale and all reflex activity ceased (Wetsner, 1934).

2. Hypnorm/Diazepam: Adult rats either for initial nerve crush or re-anaesthetized for acute electrophysiological experiments were anaesthetized using a combination of Hypnorm and Diazepam. Anaesthesia was induced by an intramuscular (i.m.) injection of Hyponorm (Janssen Pharmaceutical Ltd, which included Fentanyl Citrate 0.315mg/ml and Fluanisone 10mg/ml) 0.1ml per 100 gram body weight, and an intraperitoneal (i.p.) injection of Diazepam (Roche Products Ltd, which included Diazepam 5mg/ml BP with benzyl alcohol and sodium benzoate) 0.1ml per 100 gram body weight (personal communication with Dr Shahani). The rat was judged to be anaesthetized on disappearance of withdrawal reflexes in the limbs after pinching. Subsequent doses of both drugs were given at 0.1ml each if it was needed. After decerebration the anaesthetic agents were discontinued.

2.1.2. Nerve crush

Two groups of rats, 5-day old and 2-month-old, were used initially for recovery experiments. Peripheral nerve crush was performed on these rats under deep anaesthesia by using Hypnorm/Diazepam (8 weeks old rats) or by using ice (5-day old rats). In each animal group, rats were further divided into three sub-groups in which they received nerve crush on sciatic or tibial or lateral gastrocnemius-soleus nerve.

In each animal, the left hindlimb was exposed in the middle of the thigh and the nerve (sciatic or tibial or LG-S) was then crushed with fine watchmakers forceps for five seconds (Lowrie et al., 1982) either in the popliteal fossa or just before its entry into the muscle. Care was taken to preserve the epineurium, to facilitate regeneration of the nerves along their endoneural sheaths. This was done by visual control under a dissecting microscope. The skin was then sutured. When the animals recovered from the anaesthetic, 5-day-old rats were returned to their mother and adult rats were returned to their own cages. Following nerve crush, rats were allowed to recover for at least 5 weeks and reinnervation of muscles was allowed to proceed unhindered. The animals were then used for either acute electrophysiological or histological experiments. In rats, reinnervation is known to be complete 6 weeks after nerve injury (Lowrie et al., 1982, 1987).

2.1.3. Animal arrangement for the experiments

The animals for acute electrophysiological or histological experiments were divided into 3 groups. Group 1 contained normal adult animals which were used as a control group. Group 2 contained animals with peripheral nerve crushed in adulthood. Group 3 contained animals which received a peripheral nerve crush at 5 days postnatally. Both normal control and experimental rats were deeply anaesthetized prior to, and during any surgical procedure before decerebration. The results obtained from these three groups were presented separately in the following three chapters.

In order to test the possibility that the different denervation time may induce the different impairment in recurrent inhibitory pathway, the animals were further divided into three sub-

groups in either adult or 5-day-old rats according to the nerve crush site: A. sciatic nerve crush (far crush); B. tibial nerve crush (mid crush); and C. lateral gastrocnemius-soleus nerve crush (near crush).

In group 2 in which animals with nerve crush in adulthood were subgrouped as: (1) sub-group A contained animals with the whole sciatic nerve crushed in the popliteal fossa about 20 mm away from the gastrocnemius-soleus muscles. This operation reflected the temporary disconnection of muscles innervated by peroneal, tibial, and sural nerves; (2) sub-group B contained animals with tibial nerve crushed about 10 mm away from the triceps sural muscle; (3) sub-group C contained of animals with lateral gastrocnemius-soleus nerve crush at about 1 mm above the entry of the lateral gastrocnemius-soleus nerve into the muscle.

Group 3 contained animals with peripheral nerve crushed at 5-day old. In group 3, the injury sites were at about 10 mm (sciatic crush), 3 mm (tibial crush), and less than 1 mm (LG-S crush) away from the gastrocnemius-soleus muscles.

It is known from the previous work that the crushed nerve will fully reinnervate its target muscle and regeneration will be complete in about 6 weeks time after nerve crush in rat (Lowrie et al., 1982, 1987). Recurrent inhibition from axotomized motoneurones is reported to be markedly reduced 6 weeks after axotomy (Havton & Kellerth, 1990b). Thus, in each experimental animal group, recurrent inhibition were tested mainly at 6 or 7 postoperative weeks when regeneration had presumely finished. However, in the sciatic or LG-S adult nerve crush groups, recurrent inhibition was also tested at 5 weeks postoperatively in a small group of animals. In this case regeneration was not complete. The recurrent inhibition generated from axotomized motoneurones is reported to recover to its normal size at about 12 weeks after nerve section in cat although the reduced number of axon collaterals remained at about 40% (Havton & Kellerth, 1990b). In order to examine the time course of recovery in recurrent inhibition after a nerve crush, a number of experimental animals in each group were tested for recurrent inhibition after a long recovery period (at least 12 weeks after nerve crush) which made several sub-groups. The details of the animal grouping are as follows:

Group 1. Normal adult rats (Normal)

Group 2. Rats with nerve crush in adulthood (ANC):

A. Rats with sciatic nerve crush in adulthood (ASC):

a). 5 weeks after crush (ASC5)

b). 6 weeks after crush (ASC6)

c). 14 weeks after crush (ASC14)

B. Rats with tibial nerve crush in adulthood (ATC)

a). 6 weeks after crush (ATC6)

b). 12 weeks after crush (ATC12)

C. Rats with LG-S nerve crush in adulthood (ALC):

a). 5 weeks after crush (ALC5)

b). 6 weeks after crush (ALC6)

c). 12 weeks after crush (ALC12)

Group 3. 5-day-old nerve crush rats (5NC)

A. 5-day-old sciatic nerve crush rats (5SC):

a). 7 weeks after crush (5SC7)

b). 30 weeks after crush (5SC30)

B. 5-day-old tibial nerve crush rats (5TC)

a). 7 weeks after crush (5TC7)

b). 14 weeks after crush (5TC14)

C. 5-day-old lateral-gastrocnemius nerve crush rats (5LC):

a). 7 weeks after crush (5LC7)

The animals used for motoneurone labelling and muscle histology were taken from normal,

ASC6 and 5SC7 groups.

The animals used for acute electrophysiological experiments were at the age between 8 and 20 weeks old for normal group, 14 weeks old for ASC6 group, 22 weeks old for ASC14 group, 8 weeks old for 5SC7 group and 31 weeks old for 5SC30 group, etc, respectively. In order to test whether different age will induce different results in generating MSR and RI, the contralateral uninjured side of MG and LG-S motor pools were also examined in both ASC6 and 5SC7 group animals to compare with normal animals.

2.1.4. Acute surgical procedures

The surgery was basically similar for all of the experiments. The anaesthetized rat was placed on a cork and wood experimental table. The body temperature was maintained at $37\pm0.5^{\circ}C$ by a servo-controlled unit monitoring the rectal temperature.

Tracheostomy was performed on all animals used for acute electrophysiology experiments. The trachea was cannulated below the larynx with polythene tracheostomy tubing for artificial ventilation later and in order to facilitate the removal of any mucus build up during the course of an experiment. A tube was attached on the side of the tracheal cannula and connected to a rodent O_2/CO_2 meter for monitoring end tidal CO_2. The animals breathed spontaneously until positioned in the stereotaxic frame. During the experiment, the rat was artificially ventilated with room air and the end tidal CO_2 was maintained at 4% by adjusting the ventilating rate.

The common carotid arteries on both sides were permanently ligated on each rat prior to decerebration. Thus, the main blood supply to the cortex was cut off to reduce blood loss during the process of decerebration. One of the common carotid arteries was cannulated by inserting a cannula towards the heart for monitoring the arteria blood pressure during the experiment.

On the ventral side of the hindlimbs, both sides of the femoral nerves were exposed and sectioned for denervation to minimize movement artifacts later during the electrophysiological

recording. The right femoral vein was usually cannulated for injection of drugs and fluids respectively.

The rat was then turned on to its abdomen on the table. The ***decerebration*** would be carried out next. The technique employed in decerebration was essentially the same as the classical method described by Sherrington (1898).

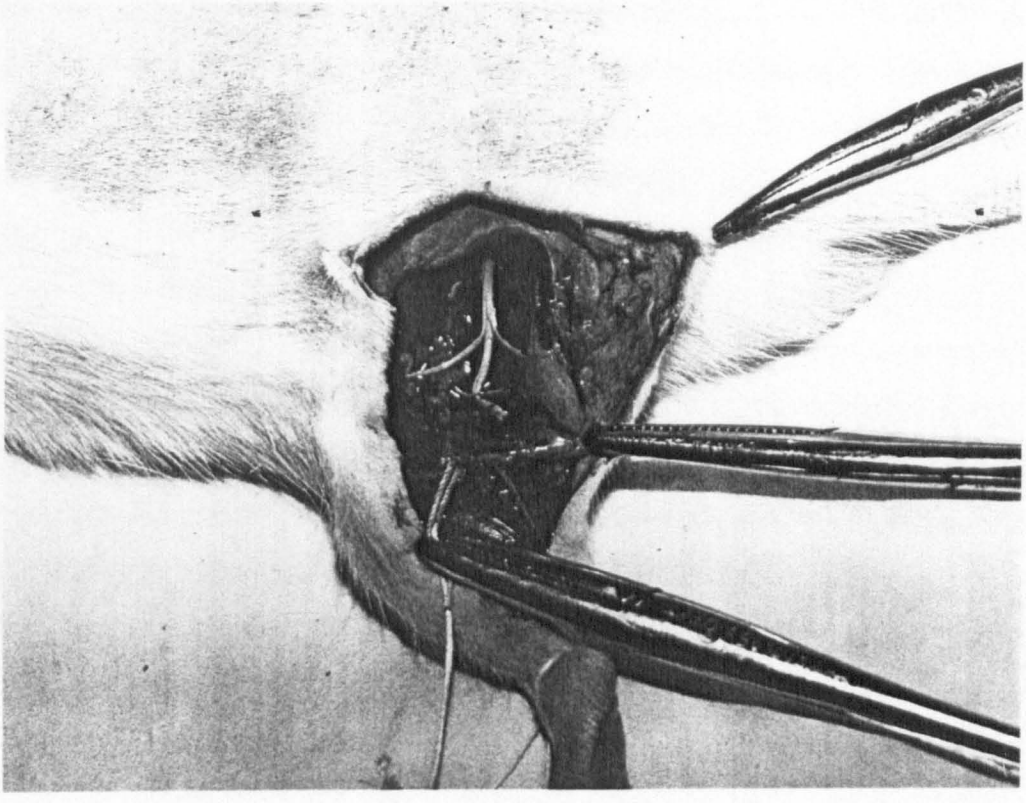
The head was shaved and cleared of all loose hairs. A midline longitudinal incision was made over the top of the head and the skin was retracted to expose the right temporal muscle. The muscle and the periosteum were then scraped from the underlying skull and removed on the left side. The parietal bone was thinned by bevelling it with a dental-drill. The bone was then removed by using a pair of bone-cutters until a hole was made that was big enough for the removal of cerebral tissue. The bone edges were then sealed with bone wax to stop bleeding from the diploic veins and to minimize the risk of air embolus.

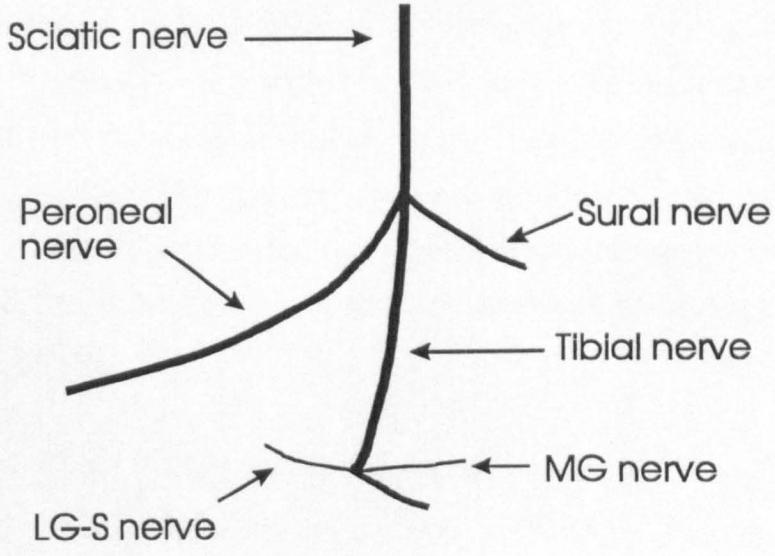
A cruciate incision was made in the dura to expose the brain tissue and then the temporal and occipital cortex either scooped out or reflected until the colliculi were exposed. An anteriorly sloping cut was made either between the inferior and superior colliculus (intercollicular), or immediately anterior to the superior colliculus (precollicular). The section was carried through to the base of the skull and all brain tissue above the section was then removed. A small cotton wool pad was then used to mop up the blood and some pieces of absorbable gelatine sponge were used to control the bleeding. Once the bleeding was controlled, the cavity was loosely packed with saline soaked cotton wool and the surface of the hole in the bone was covered with a small quantity of vaseline to prevent the remaining brain tissue from drying out.

In a few animals respiration ceased following the transection and they did not spontaneously breathe again. The rat was then artificially ventilated by massaging the chest. In these cases spontaneous respiration always started again after a few seconds. In very few cases, the spontaneous breathing did not start again, animals were artificially ventilated using a ventilator.

Fig.2.1

Photograph showing nerve branches of left sciatic nerve of rat. All the nerve branches of sciatic nerve were dissected free from each other and the peripheral ends were sectioned. The draw of these nerve branches has been put at the bottom panel and each nerve end indicates by arrow.




Sciatic nerve
Peroneal nerve
Sural nerve
Tibial nerve
MG nerve
LG-S nerve

No more anaesthetic was given to the animals after decerebration. On average, the surgery for acute electrophysiological experiment lasted about one and a half hours in which decerebration was normally finished in the first half an hour.

In normal rats, the right sciatic nerve was exposed in the thigh and sectioned for denervation. The left hindlimb muscle nerves were dissected out and used for recording/stimulating. The skin of the left hindlimb was shaved and all loose hair were cleared. A midline incision was made in the thigh and the skin was retracted to expose the whole biceps muscle. This muscle was then separated from the underlying nerves, muscles, and connecting tissues and removed. The sciatic nerve was dissected free using the fine forceps and spring scissors and was prepared for later recording or stimulation. The peroneal(P), tibial(Tib), and sural(SU) nerves, which are the three main branches of the sciatic nerve, were dissected free from each other. The lateral gastrocnemius-soleus nerve (LG-S) and medial gastrocnemius nerve (MG) were also dissected free from the remaining tibial nerve (Tib). All the separated nerve branches were then sectioned (Fig.2.1). The proximal end of either LG-S or MG was dissected out as long as possible to put onto the stimulating or recording electrodes. All nerve dissection was done under the dissecting microscope to prevent damage to the nerves. The exposed tissues were then covered with warmed saline soaked cotton wool pads.

In both normal animals and experimental animals, lateral-gastrocnemius nerve and soleus nerve were not dissected out separately and served as a whole nerve trunk. In addition, since lateral-gastrocnemius, medial-gastrocnemius and soleus muscles functionally are synergist to each other, this makes it possible to investigate the recurrent inhibition between the lateral gastrocnemius-soleus and medial gastrocnemius motor pools. Previous studies in cat has shown that recurrent inhibition between LG-S and MG pools exists (Cullheim & Kellerth. 1981; Havton & Kellerth, 1990b).

In experimental animals, sciatic, tibial, MG and LG-S in both operated side and contralateral uninjured side were dissected free as above described, especially LG-S and MG nerves were kept long enough for late use.

Laminectomy were then performed in order to expose the lumber spinal cord. Laminectomy

made it possible to dissect out the dorsal roots for stimulating in order to evoke monosynaptic reflexes and dissect out ventral roots for MSR recording.

The skin was cut longitudinally over the lower lumbar and the upper sacral vertebrae. The tendinous attachments to these vertebrae were cut and then cleared with a dental pick. The articular, spinous process, and arches of the L3-L6 vertebrae were removed with bone forceps and a special laminectomy forceps in order to expose the dura. While removing the bone, the vertebra was gently picked up using tooth forceps. This was done to make a small gap between the spinal cord and vertebrae and to prevent damage to the spinal cord. The edges of the cut bone were then covered with bone wax to prevent bleeding and the entry of small air bubbles into the vessels.

After laminectomy, the rat was transferred to a special rat supporting frame (Narashige ST7). The head was supported by the head holder and two ear bars (Fig.2.2). The body weight was supported by two clamps which were attached to the exposed vertebrae, one on L2 and the other one on S1. The skin and surrounding muscles of the exposed lumbar spinal cord and the hindlimb(s) were sutured to the frame or several special holder bars in order to make paraffin pools (Fig.2.2). The pre-warmed paraffin (37°C) was then filled into pools to cover the exposed tissues. Radiant heating lamps were set above the frame to maintain body and paraffin pool temperature at 37 ± 0.5°C by a servo-controlled unit monitoring the rectal and paraffin pool temperature.

In the paraffin pool, the dura covering lumbar L4 to L6 was gently cut to open with fine forceps and spring scissors. The dorsal roots of spinal segments L4-L6 were dissected and cut to leave the proximal stumps long enough for later stimulation. In several animals in order to study the recurrent inhibition within the whole motor pools in lumbar segment L5, ventral root L5 was dissected out and sectioned. The proximal stump was then further gently split into two parts, one for MSR recording and another one for antidromic stimulating.

2.2. Experiment set up and procedures

2.2.1 Data capture

Four pairs of silver wire bipolar electrodes were normally used for stimulating or recording (see Fig.2.2).

1. *Test stimulating electrode* (E1):
This was set in the spinal paraffin pool above the spinal cord. The free central ends of dorsal roots L4, L5, and L6 were mounted on it to evoke, by electrical stimulation, the monosynaptic reflexes.

2. *MSR recording electrode* (E2):
This was set in the leg paraffin pool (for MG or LG-S nerve) or spinal paraffin pool (for L5 ventral root). One of the dissected nerve proximal stumps (or ventral root) was mounted on it to record the monosynaptic reflexes evoked by dorsal roots stimulation.

3. *Electrode for antidromic stimulation* (E3):
This was set in the leg paraffin pool (for MG and LG-S pair) and in the spinal paraffin pool (for L5). The proximal stumps of sectioned MG or LG-S nerve (or part of L5 ventral root) was mounted on the electrode to generate the antidromic volleys.

4. *Electrode used to record antidromic spikes* (E4):
The whole sciatic nerve was mounted on this electrode in order to record the antidromic spikes in the leg paraffin pool.

Both test and antidromic stimuli were single square wave pulses generated separately by two Digitimer DS2 isolated stimulators triggered from a Digitimer D4030. The Digitimer D4030 was programmed to deliver the trigger pulse at 1 pulse per second. A four channel storage oscilloscope, a CED1401, and a VCR Recorder Adapter were also triggered by this Digitimer D4030 (Fig. 2.3, dotted line). The stimulators which were driven by the Digitimer D4030 reproduced the stimulating pulses which were 0.1ms in width. According to previous

work, the recurrent inhibition of spinal monosynaptic reflexes has a time course of about 50 msec in rat (Kanoke et al., 1987). Thus, the time interval between antidromic (conditioning) and test stimulus trains was set to vary between 0-50msec. The test stimulus was delivered to sectioned dorsal roots and was preceded by a conditioning stimulus delivered on one muscle nerve with the interval varied among 0, 1, 2, 3, 4, 5, 8, 10, 15, 20, 30, 40, and 50 msec.

In this study, recurrent inhibition has been assessed by monitoring the change of amplitude of the maximal monosynaptic reflexes elicited with supramaximal test stimulation conditioned by maximal antidromic stimulation. The strength of the test stimulus was usually set at 5 times threshold (5xT). This was supramaximal to evoke the monosynaptic potential of the entire motoneurone pool with square wave shocks at 0.1 msec width. However, in a group of animals (5SC7 group), the test stimulus strength was varied at supramaximal (5xT) and sub-maximal (2.5xT and 1.5xT) in order to generate monosynaptic reflex (MSR) ~~as~~ of different amplitude. Subsequently, the recurrent inhibition has been tested in this group with constant antidromic conditioning stimulus strength and varied test stimulus strength. The threshold was judged as the point at which increase the stimulus strength resulted in the monosynaptic reflexes being just recorded from the muscle nerves.

The strength of the conditioning stimulus was set to supramaximal (5xT) for the antidromic spikes recorded on a more proximal part of the sciatic nerve, since the frequency and duration of firing recorded from Renshaw cells was known to be positively correlated to the stimulus strength of the antidromic volley (Renshaw, 1946; Eccles et al., 1961b) and the maximum RIPSP amplitude could be normally attained with ventral root stimuli of three to five times threshold in rat (Schneider and Fyffe, 1992). Monosynaptic reflexes were recorded from one of the muscle nerves and the antidromic spikes were evoked by stimulating a synergist to the recording one (LG-S and MG pair) (Fig.2.4). In several other cases, MSRs were recorded from one half of a lumbar ventral root and the conditioning stimulus was delivered to the other half of this ventral root.

Fig.2.2

Photograph showing the experiment setting. Rat was put on the frame. Electrodes were positioned in two paraffin pools for either recording or stimulating.

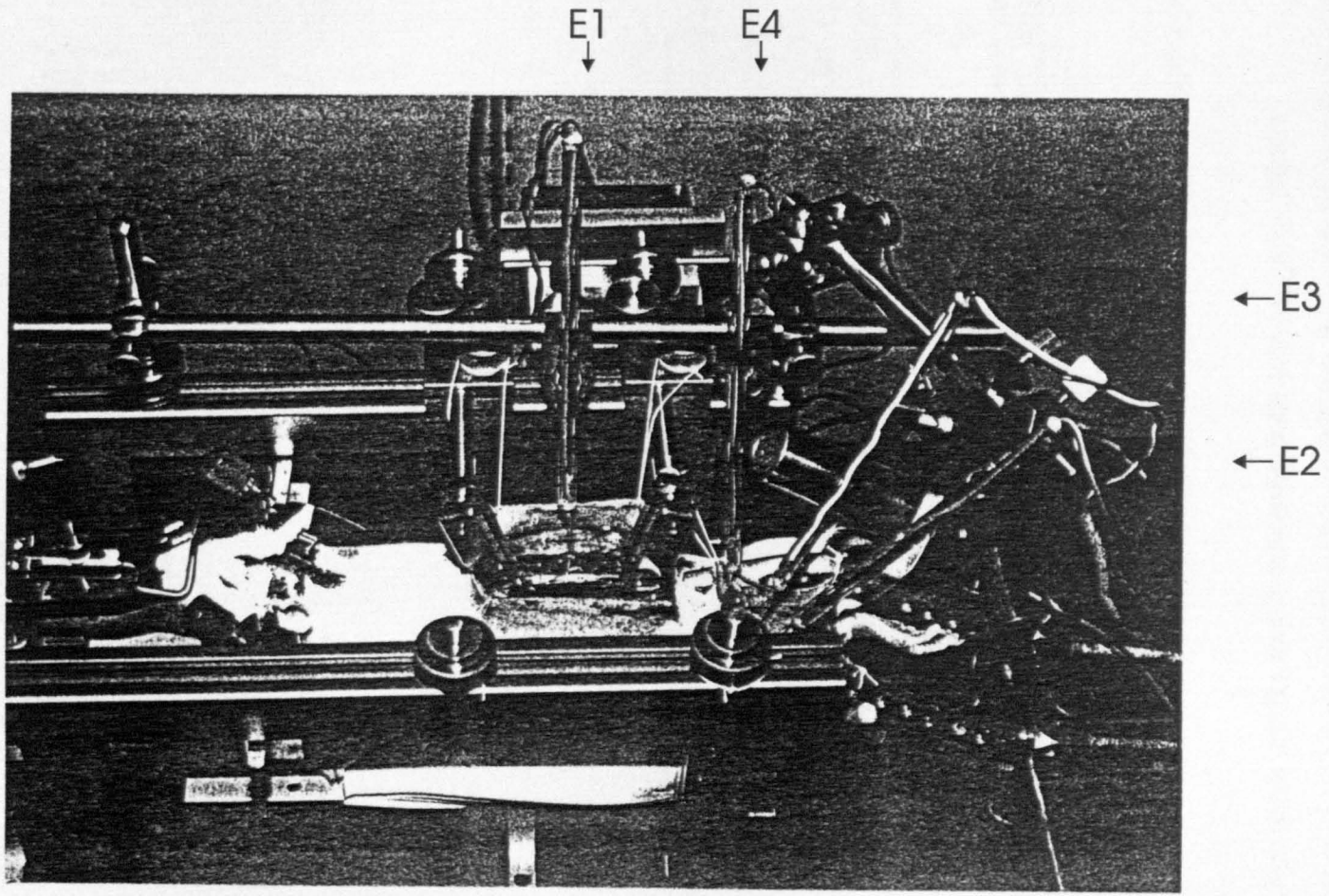
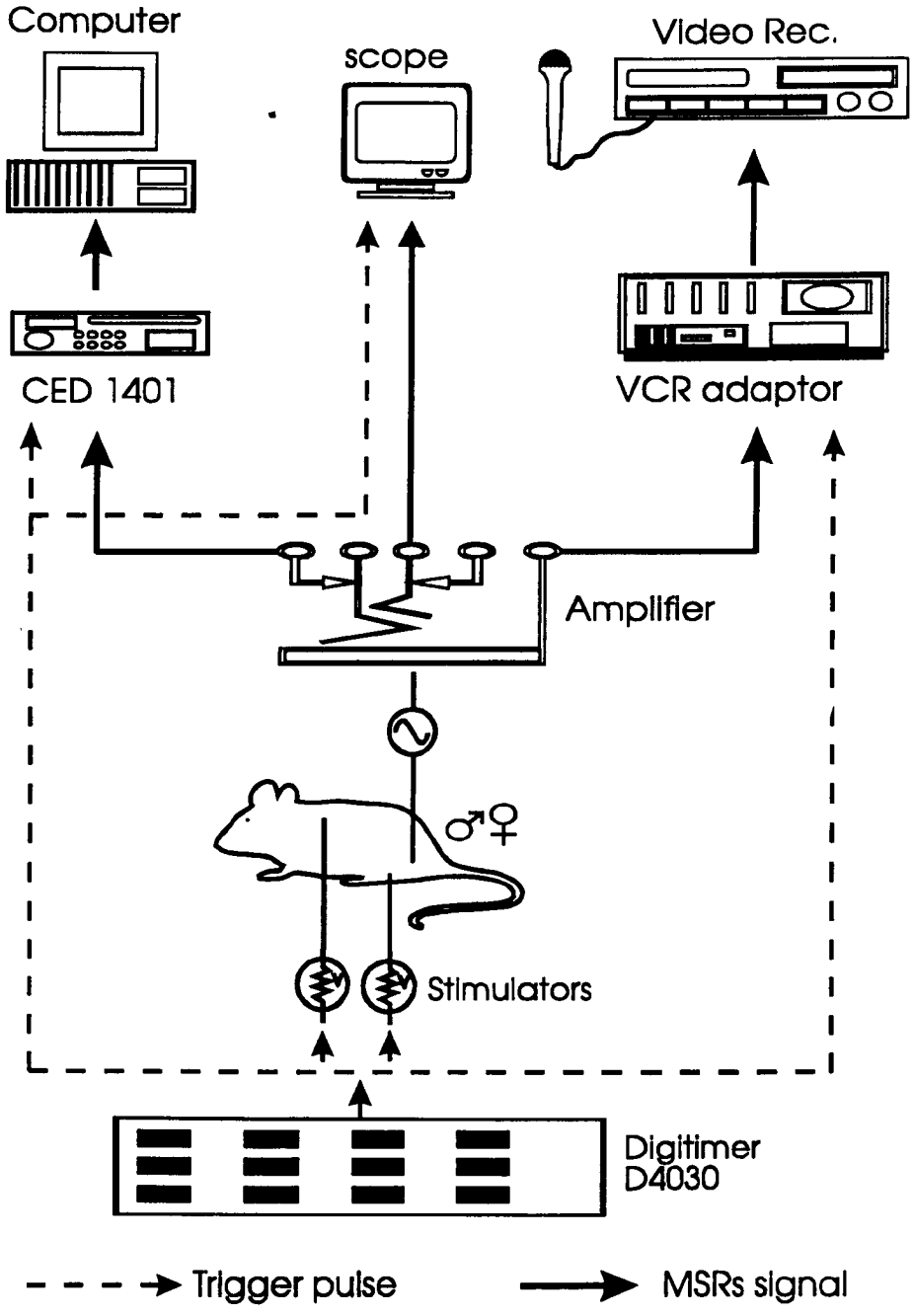

E1
E4
E3
E2

Fig.2.3

Diagram showing the settings for generating and recording MSR signals from rat. Dotted lines indicate the trigger pulses and solid lines indicate MSR signals.


Computer
scope
Video Rec.
CED 1401
VCR adaptor
Amplifier
♂♀
Stimulators
Digitimer
D4030
Trigger pulse
MSRs signal

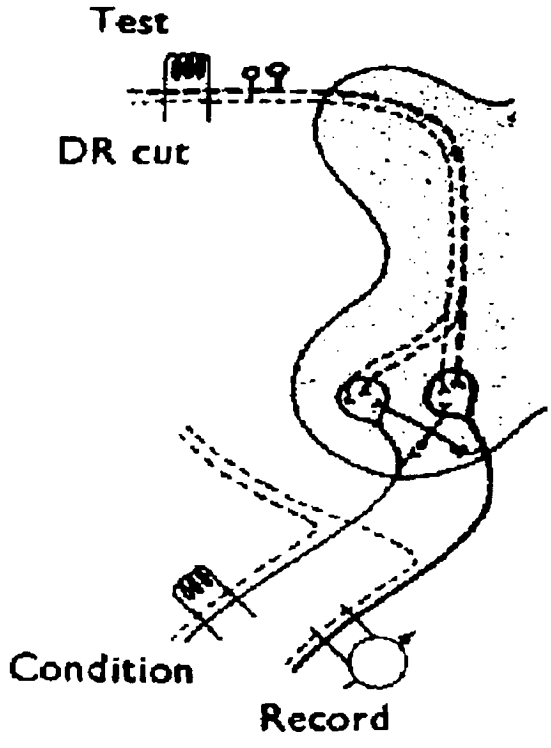
Recorded monosynaptic reflexes and antidromic spikes were passed through a DC/AC preamplifier and monitored on a **storage oscilloscope** *(Tektronix 5113)*. The amplified monosynaptic reflex compound potentials were simultaneously recorded on to a video tape using a **video recorder** transferred by a 8-channel **VCR Recorder Adapter** for later computational analysis. Re-played monosynaptic reflex signals were averaged every 10 consecutive sweeps by using a **signal-average** (SIGAVG CED) program on a **IBM-PC compatible computer** linked with a **CED1401** (Fig. 2.3).

2.2.2. Data analysis

Monosynaptic reflexes were recorded in the LG-S, MG nerves or a ventral root of L5 during single-shock stimulation of the transectioned central ends of L4, L5, and L6 dorsal roots at supramaximal strength. The size of the unconditioned test monosynaptic reflexes often varied somewhat between corresponding nerves of the individual animals. Recurrent inhibition could be elicited from each of the normal or regenerated MG, LG-S, and ventral roots L5 by conditioning antidromic stimulation preceding the test stimulus. By varying the time interval between the conditioning and test stimuli (0, 1, 2, 3, 4, 5, 8, 10, 15, 20, 30, 40, and 50 msec) and simultaneously recording the amount of depression of the MSR amplitude at each interval, the magnitude and time course of the recurrent inhibitory effects produced by each nerve were estimated. The unconditioned test reflexes were obtained immediately before and after the test series. Thus any long term variations in the amplitude of monosynaptic potential were as far as possible excluded. In a few cases, if the amplitude of monosynaptic potentials varied more than 5% between the first and last test potential, the data were discarded. At each time interval 10 consecutive sweeps of MSRs were recorded. The recorded MSR potentials were played back and fed into a PC computer via a CED1401 for data processing. A CED signal average program was used to average the MSRs at each time interval. The amplitude of the averaged unconditioned test MSRs served as the reference value of 100%. The averaged conditioned MSRs amplitude at each time interval was then calculated to express as a percentage of the unconditioned test MSR and plotted against the test-conditioning stimulus interval to create the ***recurrent inhibitory curve***. A recurrent inhibitory curve has been used to present the recurrent inhibition of MSRs in this study. Within the recurrent inhibitory curve, X-axis is the time interval between test stimulus and conditioning

Fig.2.4.

Diagram showing how recurrent inhibition was assessed. Dorsal roots of the spinal cord were cut and the central ends were used for generating monosynaptic reflexes. Monosynaptic reflexes were recorded from one peripheral nerve (eg. LG-S) and the conditioning volley was delivered on to one of the synergist nerves (eg. MG).


Test
DR cut
Condition
Record

stimulus and Y-axis is the percentage change of monosynaptic reflex amplitude. The area between 100% line (unconditioned reflexes) and conditioned reflexes change curve expresses the strength and time of the recurrent inhibitory effect. The maximal recurrent inhibitory effect is the point that amplitude of conditioned reflexes is smallest. When the reduction of the amplitude of MSRs was less than 5%, the monosynaptic reflexes were considered to have recovered from inhibition.

The recurrent inhibition (RI) originating from one motoneurone pool and influencing the MSRs of another motoneurone pool was studied in the following RI/MSR (recurrent inhibition / monosynaptic reflexes) combinations: MG/LG-S, LG-S/MG, and L5/L5. The recurrent inhibitory projections from regenerated MG or LG-S motoneurone pool to regenerated LG-S and MG motoneurone pool were studied to analyses the physiological consequences of the peripheral nerve injury on recurrent inhibition pathways.

All comparisons of the effectiveness of recurrent inhibition in this study were based on the maximal effect of recurrent inhibition on MSRs amplitude. The data on monosynaptic reflexes and on recurrent inhibition from animals with regenerated peripheral nerves was compared to those from normal animals and the statistical difference was tested using student t-test. Recurrent inhibition of different motoneurone pools (LG-S or MG) was also compared in both normal and experimental animals. In addition the time course of effectiveness of nerve crush on recurrent inhibition was also studied.

The Student's t-test for paired and unpaired comparisons (two-tailed) was used to compare the difference of maximal effects of recurrent inhibition in between different group animals. In each pair of data sets, the statistical difference of the mean values of corresponding conditioning and test stimulus intervals were tested. The significant difference between two tested means was considered at $P<0.05$ and presented as **$*=P<0.05$, $**=P<0.01$, and $***=P<0.001$** (Fraunhofer and Murray, 1976).

2.3. HRP retrograde labelling Gastrocnemius-soleus motoneurone pool

The purpose of labelling the gastrocnemius-soleus motoneurone pool was to confirm that the

peripheral nerve crush either in early postnatal life or in adulthood did not affect the number of motoneurones in G-S motor pool.

2.3.1 Injection of Horseradish Peroxidase

Both adult normal control rats and adult experimental rats at least 6 weeks post nerve crush were prepared for localization studies of the gastrocnemius-soleus pool. Motoneurones were identified by retrograde labelling with horseradish peroxidase (HRP). The amount of HRP (20%) solution injected into each of the gastrocnemius-soleus muscles was estimated to be about 1 μl of HRP solution for each 1 gram of estimated muscle weight. The weight of the muscle (LG, MG, and soleus) were estimated according to the animal body weight since the muscle weight has a certain relationship with the body weight (personal communication with Dr. U. Shahani).

The 20% HRP (SIGMA TYPE VI) solution was made up in sterile 0.9% NaCl for injection to the muscle. The animals were anaesthetized with Hyponorm and Diazepam and the HRP injection made using sterile precautions. A longitudinal incision was made on the dorsolateral aspect of one side (or both sides) legs to expose the gastrocnemius-soleus muscles. By using a microsyringe (Hamilton), the HRP was injected directly into the body of both lateral and media gastrocnemius and soleus muscles of left leg. Care was taken to ensure that minimal leakage of HRP occurred by injecting it very slowly while gradually withdrawing the needle. When spillage occurred it was immediately mopped up with cotton wool and the area was rinsed with sterile saline. The incisions were then closed and the animals allowed to recover. They were allowed to survive for 40 to 48 hours.

2.3.2 Perfusion and preparation of the spinal cord

40 to 48 hours after the HRP injection, the animals were reanaesthetized using Hypnorm (i.m.) and Diazepam (i.p.), and then perfused through the heart. Each animal was first perfused briefly with heparinised (500 unit/ml) normal saline to flush the blood out of the vessels and followed by a fixative made up of 2.5% glutaraldehyde in Millonigs phosphate buffer (pH 7.3), for approximately 30 to 40 minutes. About 1 ml of the fixative was used per gram of body weight.

The lumbar region of the spinal cord was carefully dissected out and placed in a small volume of the fixative and postfixed at 4°C for 4 hours. The spinal cord was then transferred to a 30% sucrose + Millonigs phosphate buffer (pH 7.3) solution and stored overnight at 4°C.

A section of the spinal cord was then cut to get a block of the spinal cord of L3 to L6 under a dissecting microscope. The segments were identified by the exit of their ventral roots. It was in L4 and L5 that the gastrocnemius motoneurone pool was known to be located (Swett, et al., 1986). The uninjured control right side of the block was marked for identification by penetrating the dorsal horn with a micropin along the length of the block.

Each individual block of spinal cord was dipped in tissuetek (OCT compound) then mounted on a cryostat chuck. The cord was cut in a cryostat at temperature of between -20 and -25°C. Serial section of 50μm thickness were cut and immediately reacted for HRP histochemistry using a modified Hanker-Yates method (Hanker et al., 1977) (for a detailed protocol see Appendix I).

The sections were then mounted onto poly-L-lysine coated slides and air-dried for 24 hours, after which they were counterstained with gallocyanin between 15 to 25 minutes until the nucleolus was well stained and the Nissl substance lightly stained. Coverslips were placed on the slides using Permount and the slides allowed to dry for 24 hours.

2.3.3 Motoneurone counting

The stained sections were examined under a light microscope for the HRP reaction product in the motoneurones of the spinal cord. The precise location of the motoneurone pool was confirmed (Nicolopoulos-Stournaras & Iles, 1983; Swett et al., 1986). The labelled cells were normally localized in the central area of the grey matter within the ventral horn.

Only those cells that were labelled and in which the nucleolus could be clearly seen were counted. In those labelled cells where the cytoplasm was very densely "packed with granules" and the nucleolus could not be distinguished, sections immediately rostral and caudal were

reviewed in order to make sure that each cell was counted only once. Labelled cells found outside the gastrocnemius pool as defined above were discounted and considered to be due to spread of HRP.

2.4. Muscle histology

At the end of the acute electrophysiological study in several experiments, the soleus and gastrocnemius muscles of both sides (pre-crushed and uninjured) legs of the experimental rats were removed and weighted separately. The gastrocnemius muscles were then immediately stored in liquid nitrogen for later succinic dehydrogenase (SDH) staining. The protocol for preparing gastrocnemius for SDH staining is as following: corresponding muscles from each side of the legs of each animal were mounted side by side against a pin embedded in a cork block, and at the same length. They were then quickly frozen in melting isopentane which had been cooled with liquid nitrogen. They were stored in liquid nitrogen until used for histochemical study. Each pair of muscles were subsequently processed as a single block of tissue. Transverse sections from the middle third of each muscle pair were cut at a thickness of 10μm in a cryostat at temperatures of -20°C.

The sections were mounted onto glass slides air-dried and were stained routinely by haemotoxylin and Van Gieson, and for succinic dehydrogenase (SDH) an enzyme which indicates oxidative activity in muscles by the method of Nachlas et al. (1957) (for detailed protocol refer to Appendix II).

RESULTS

The results to be presented describing recurrent inhibition of monosynaptic reflexes of spinal motoneurones with and without pre-peripheral nerve crush are divided into three different chapters: (I) in normal rats, (II) in rats with nerve crush in adulthood and (III) in rats with nerve crush performed at 5 days postnatally. The effects of peripheral nerve crush on recurrent inhibition, spinal motoneurone pool and relative muscles will be dealt with in (II) and (III).

CHAPTER 3. RESULTS I.
RECURRENT INHIBITION OF SPINAL MSRs IN DECEREBRATE RATS

3.0. Introduction

In this chapter, spinal monosynaptic reflexes (MSRs) and recurrent inhibition of MSRs in adult decerebrate rats are detailed in following sections. Since recurrent inhibition between motoneurone pools was assessed by conditioning MSRs elicited from cut dorsal roots and with antidromic volleys delivered to one of the synergist nerves, spinal monosynaptic reflexes have been first studied. Recurrent inhibition between MG and LG-S motor pools and within motor pools in the spinal segment L5 are described. The motoneurones of gastrocnemius-soleus motor pool labelled by HRP in adult Wistar rats are also presented.

3.1. Spinal monosynaptic reflexes

In the rat it is well documented that the spinal segments L4, L5 and L6 contain all the motoneurones that supply the sciatic nerve (Greene, 1935; Nicolopoulos-Stournaras and Iles, 1983). In this study, spinal monosynaptic reflexes were evoked by stimulating sectioned dorsal roots L4, L5 and L6 and were recorded from proximal peripheral (LG-S and MG) nerve stumps (Fig.2.4) in decerebrate rats. The mean latency and amplitude of MSRs in this preparation are presented in Table 3.1 (also see Fig.4.2 (unfilled)).

Figure 3.1 are the photographs taken from storage oscilloscope showing evoked potentials recorded from LG-S (left) and MG (right) muscle nerve by stimulating sectioned dorsal roots in one rat. The test stimulus was applied at the time 0 and the conditioning stimulus was applied preceding the test stimulus by 15 msec (B) and 4 msec (C). Figure 3.1.A shows the typical unconditioned MSRs recorded from LG-S and MG nerves.

In some studies, the compound potentials evoked by stimulating dorsal roots and recorded in peripheral nerves displayed a twin peak. In these cases, the second peaks of the compound potentials generated by polysynaptic pathways (see Kaneko et al., 1987) are not counted in this study. All the results presented in this thesis were based on monitoring the first peak

(with shortest latency) of the evoked potentials which are considered to be monosynaptic responses.

The MSRs recorded in LG-S and MG muscle nerves evoked by stimulating dorsal roots in decerebrate rats have mean latencies (presented as mean±SEM) of 2.30±0.05 ms (n=7) and 2.30±0.06 ms (n=8) and mean amplitudes of 0.89±0.15 mv (n=11) and 0.87±0.11 mv (n=12) (table 3.1, also see Fig.4.2).

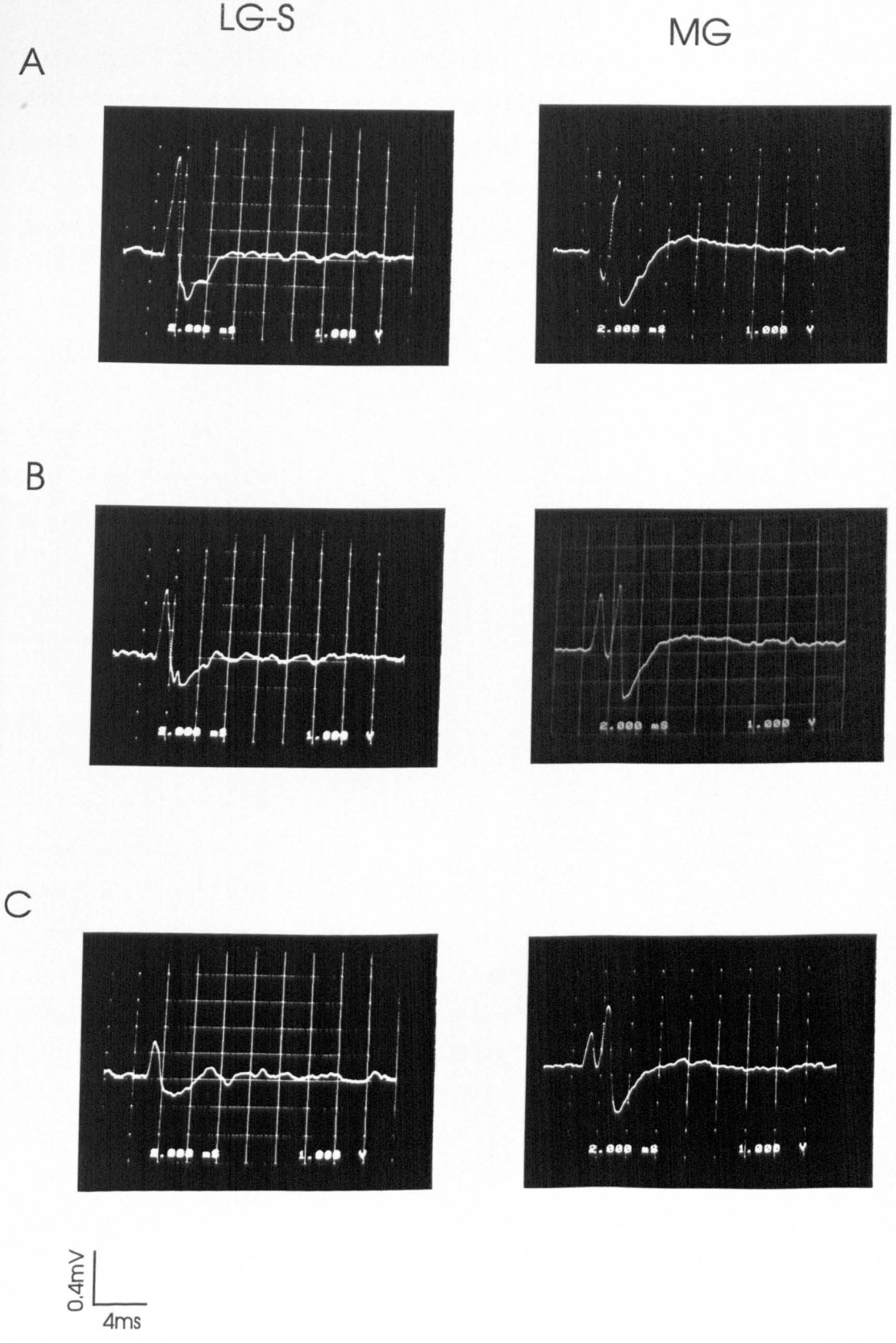
Table 3.1 Amplitude and latency of MSRs in MG and LG-S muscle nerves in normal decerebrate rats

Nerve	Latency of MSR (mean±SEM, ms;(range))	Amplitude of MSR (mean±SEM, mv; (range))
LG-S	2.30±0.05 (2.1-2.5) (n=7)	0.89±0.15 (0.32-1.82) (n=11)
MG	2.30±0.06 (2.0-2.5) (n=8)	0.87±0.11 (0.32-1.62) (n=12)

The phenomenon that a monosynaptic reflex, evoked by stimulating dorsal roots and recorded from a peripheral muscle nerve, is attenuated by a preceding antidromic volley in the synergist or homonymous nerve in cat (Renshaw, 1941; Brooks and Wilson, 1959; Kuno, 1959) and anaesthetized rat (Kaneko et.al. 1987) was also observed in this study in this decerebrate rat preparation (Fig.3.1 B and C). Figure 3.1.A shows monosynaptic reflexes recorded from LG-S and MG muscle nerves without antidromic motor axon stimulation (unconditioned reflexes). The amplitude of the MSRs are reduced following a preceding (15 msec) antidromic stimulation (conditioning stimulation) of synergist muscle nerves (Fig.3.1.B). The reduction of the MSRs amplitude as a result of antidromic volleys invasion could reach a maximal (Fig.3.1.C) at a certain interval (4 msec) between conditioning and test stimuli. The reduction of the MSR amplitude by a conditioning volley on the test motor pool is the result of the recurrent inhibition which acts via recurrent collaterals exciting Renshaw cells in deafferented animal preparations.

Fig.3.1.

Photographs showing the monosynaptic reflexes elicited from dorsal roots L4,L5 and L6 and recorded from either LG-S (left) or MG (right) muscle nerves in adult decerebrate rat. (A) is the unconditioned reflexes; (B) and (C) indicate the reduced size of monosynaptic reflexes following preceding antidromic volleys of the synergist nerves. The test stimulus was at the beginning of each trace and the conditioning stimulus was preceding as the interval between these two stimuli were 15 msec (B) and 4 msec (C).


LG-S
MG
A
B
C
0.4mV
4ms

3.2. Recurrent inhibition of LG-S and MG motoneurones

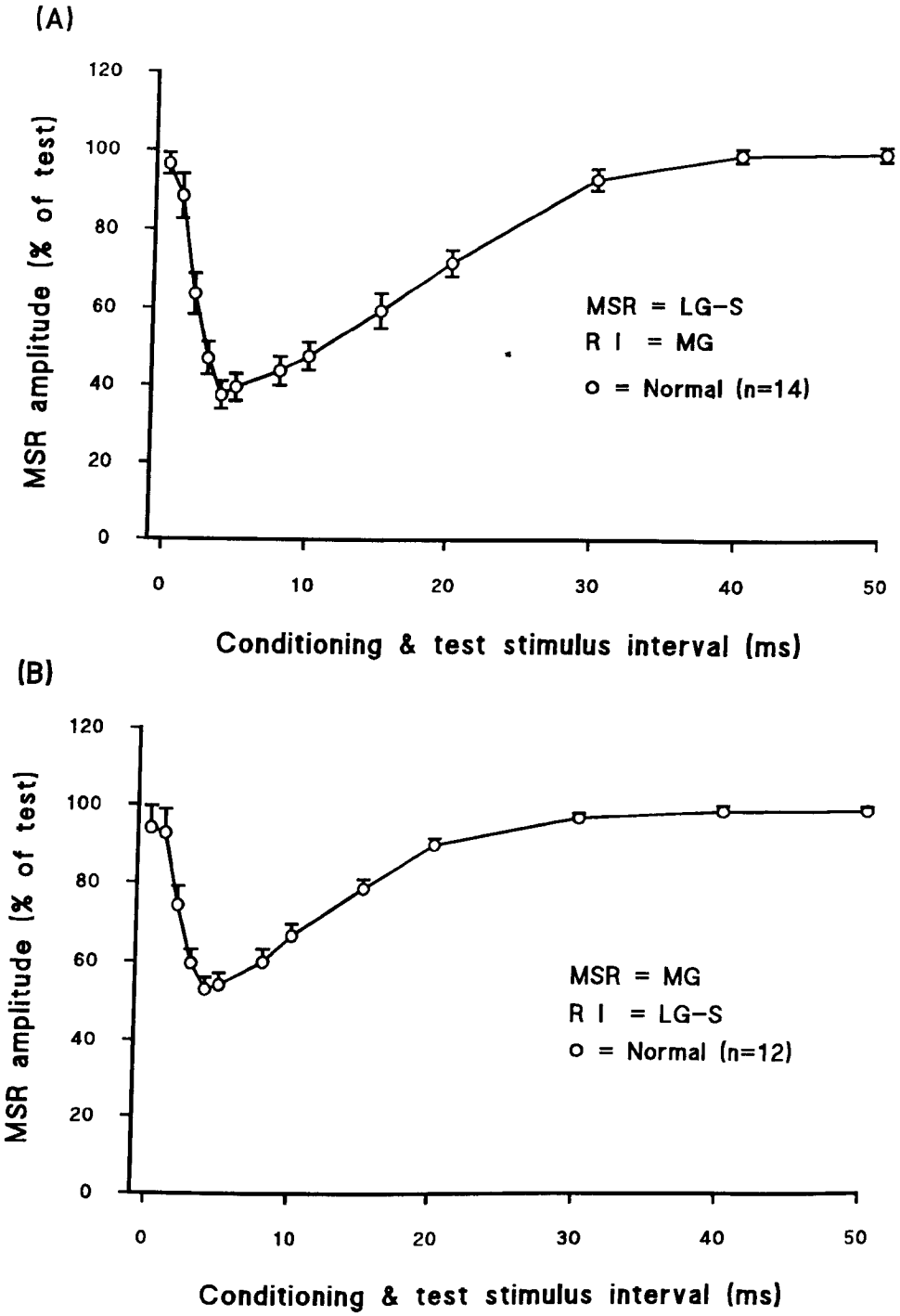
Since the most conspicuous effect of an antidromic volley in some motoneurones on the discharges of others has been found when the conditioning and tested nerves are branches to the same muscle group and the two pools of motoneurones then occupy the same portion of the ventral horn (Renshaw, 1941), lateral gastrocnemius-soleus (LG-S) and medial gastrocnemius (MG) motor pools, two groups of motoneurones innervating two different heads of the gastrocnemius muscles, were chosen to investigate the recurrent inhibition in this study.

In 14 decerebrate adult rats, following preceding antidromic volleys delivered on MG nerve and the MSRs recorded from LG-S nerve, recurrent inhibition from MG motor pool to LG-S motor pool was examined. By altering the intervals between antidromic (conditioning) stimulus on MG nerve and test (unconditioning) stimulus on dorsal roots, the reduction of MSRs amplitude has been observed varied within the intervals between 0 to 50 msec (Fig.3.2.A). The reduction of MSRs amplitude as a result of recurrent inhibition started at about 1 msec of conditioning and test stimuli interval, reached maximum at about 4 msec and lasted until the interval was 40 msec. In this preparation, recurrent inhibition of MSRs from MG motoneurones to LG-S motoneurones maximally reduced the amplitude of MSRs to $37.4 \pm 3.6\%$ (mean$\pm$SEM) of the size of the unconditioned reflexes (Fig.3.2.A and Table 3.2).

Similar results were obtained with MSRs recorded from the MG nerve when the conditioning stimulus was applied to LG-S nerve (Fig.3.2.B). Recurrent inhibition from LG-S motoneurones to MG motoneurones has the same time course as MG to LG-S in which reduction of monosynaptic reflexes was maximum while the test-conditioning stimuli interval is 4 msec. The reduction in MSR amplitude disappeared at a test-conditioning interval of about 40 msec. In this preparation, the maximal reduction of MSRs amplitude as a result of recurrent inhibition was to $52.9 \pm 3.0\%$ ($n=12$) of the size of the unconditioned reflexes. It is of interesting to note that the strength of recurrent inhibition from LG-S motoneurones to MG motoneurones is weaker than that from MG to LG-S.

Fig.3.2.

Recurrent inhibition between MG motoneurones and LG-S motoneurones in normal decerebrate rat. Recurrent inhibitory effect on the % of the maximal monosynaptic potential (ordinate) versus interval in msec between conditioning and test stimuli (abscissa). Inhibitory curves obtained by conditioning a monosynaptic reflex, elicited by dorsal roots stimulation and recorded in either branch of the gastrocnemius-soleus nerve, by antidromic stimulation of the other branch. A. MG conditioning LG-S reflex; B. LG-S conditioning MG reflex.


(A)
MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
MSR = LG–S
R I = MG
o = Normal (n=14)
Conditioning & test stimulus interval (ms)
(B)
MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
MSR = MG
R I = LG–S
o = Normal (n=12)
Conditioning & test stimulus interval (ms)

3.3. Recurrent inhibition of MSRs of L5 segment motoneurone pools

This section deals with the recurrent inhibition elicited from part of sectioned central end of ventral root L5 in depressing monosynaptic reflexes of spinal motoneurones which were evoked by stimulation dorsal root L5 and recorded from the other part of sectioned ventral root L5.

Monosynaptic reflexes recorded from ventral root L5 have a shorter latency and larger amplitude compared to those from peripheral muscle nerves. The mean reflex response latency is 1.00±0.04 ms (mean±SEM, n=5) and the mean amplitude is 1.72±0.90 mv (mean±SEM, n=5). This reflex response latency value is quite close to a synaptic delay. It is not surprising to obtain a large reflex amplitude comparing to the MSRs in peripheral MG or LG-S nerves since monosynaptic spikes from all motoneurones of L5 segment have summated and contributed to this reflex response.

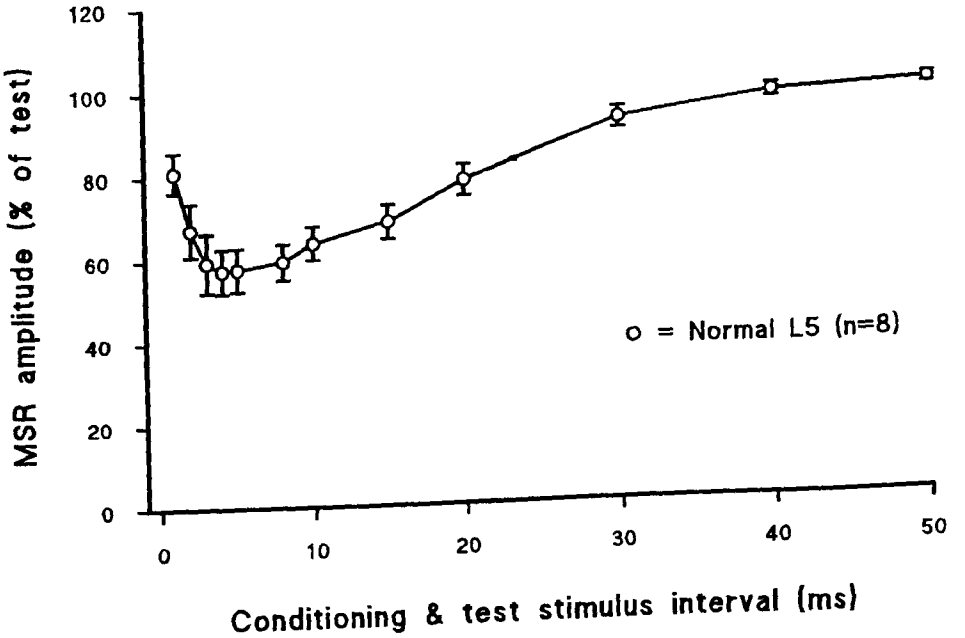
In this preparation, the peak suppression ratio of the MSR is 42.5±5.1% (mean±SEM, n=8) as a result of recurrent inhibition which occurred at 5 ms of conditioning and test stimuli interval (Fig.3.3). The duration of recurrent inhibition is about 40ms.

Table 3.2 Summary of recurrent inhibition in normal decerebrate rat

RI/MSR	Number of rats	T-C interval to Max. RI (ms)	Max. RI (% of MSR reduction) (mean±SEM)	RI duration (ms)
MG/LG-S	14	4	62.7±3.6%	40
LG-S/MG	12	4	47.1±3.0%	40
L5	8	5	42.5±5.1%	40

Fig. 3.3.

Recurrent inhibition of MSRs within spinal segment L5 motor pools. Recurrent inhibitory curve illustrates of recurrent inhibition of monosynaptic reflexes recorded from part of the ventral root L5 by an antidromic volley in the other part of the ventral root L5. X-axis indicates the interval time between test and conditioning stimuli in millisecond and Y-axis indicates the percentage reduction of the amplitude of the MSRs of spinal segment L5 motor pools.


120
100
80
60
40
20
0
MSR amplitude (% of test)
o = Normal L5 (n=8)
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)

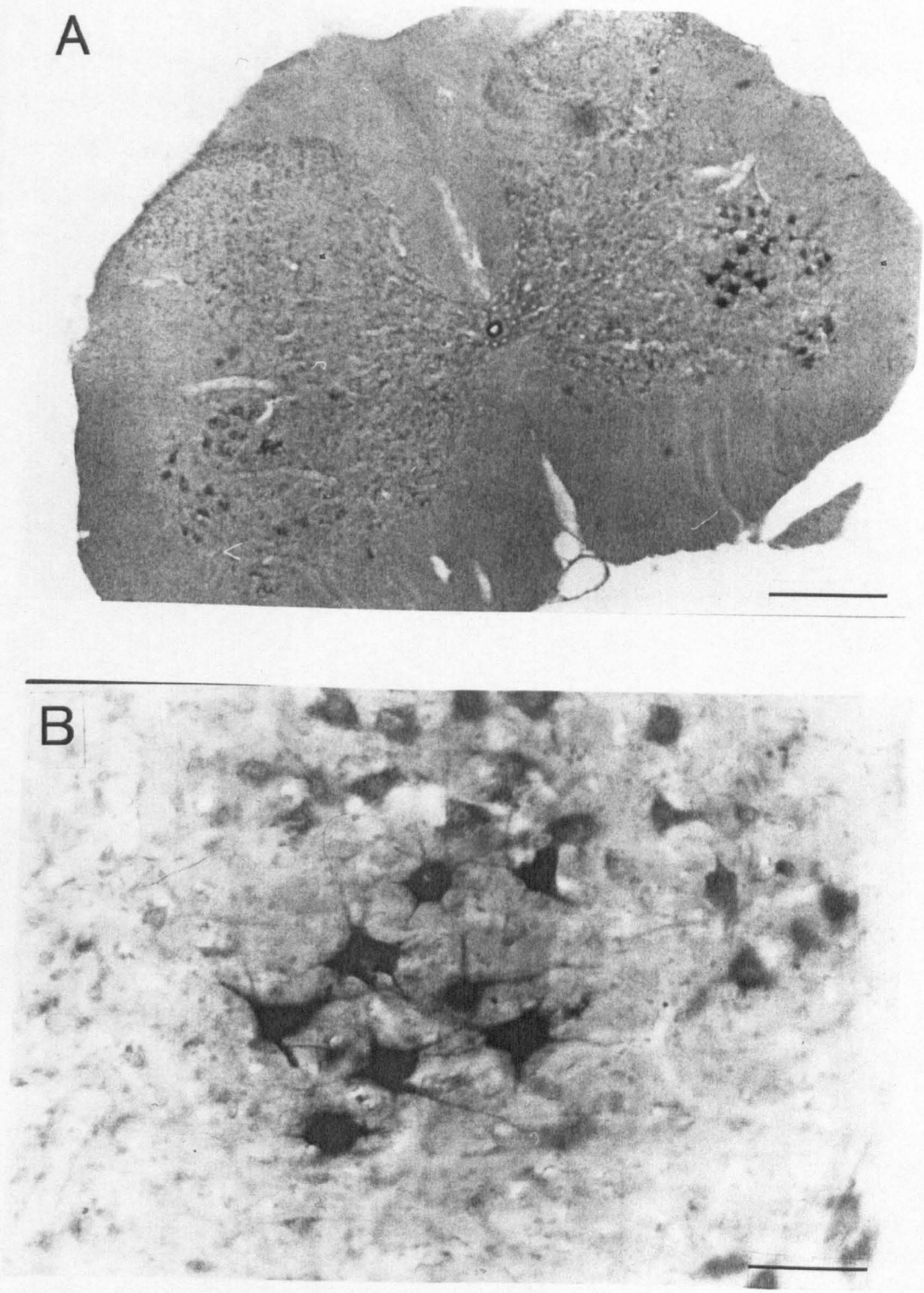
3.4. Motoneurones in rat spinal Gastrocnemius-soleus pool

The motoneurones innervating the Gastrocnemius-Soleus muscle have been labelled retrogradely with the HRP. HRP labelled motoneurones contained in spinal cross sections were found at the dorsal lateral part of the anterior horn in lumbar segments L4 and L5. Rostra-caudally the majority of labelled cells were observed in the L5 segment. Figure 3.4 illustrates the localization of the pool of the motoneurones to the gastrocnemius-soleus muscles in one rat. Although the motoneurones in Figure 3.4.A were visualized at a low magnification (2.5x1.6) individual labelled motoneurones could also be distinctly identified. Figure 3.5.B shows the same labelled cells at a higher magnification (10x2).

The mean number of motoneurones labelled by injection of 20% HRP into gastrocnemius-soleus muscles in normal Wistar rats was found to be 167 ± 27 (mean$\pm$SEM, n=3). This mean number compares well with previous observations in which HRP was used to label motoneurones to the gastrocnemius-soleus muscles (Nicolopoulos-Stournaras & Iles, 1983).

Fig.3.4.

Location of the HRP labelled Gastrocnemius-soleus motoneurones in rat spinal cord. Cross-sections of rat spinal were counterstained with gallocyanin showing the blue staining background with dark brawn HRP labelled G-S motoneurones. (A) showing the whole section with low magnification (2.5x1.6), and (B) showing the individual HRP labelled motoneurones at high magnification (10x2). The scale for the bar in (A) is 500μm and in (B) is 100μm.


A
B

3.5. Summary

In this study, results indicate that spinal monosynaptic reflexes generated by stimulating sectioned dorsal roots and recorded from either ventral root or peripheral nerves are capable of being attenuated by the electrical volleys invasion through the motor axons in decerebrated rat preparations as a result of recurrent inhibition. The amplitude of the recurrent inhibition varied depends the motor pools investigated. In tested motor pools in this study, the maximal recurrent inhibition from MG motor pool is capable to reduce 62.7% of the size of MSRs in LG-S. However the corresponding value from LG-S to MG is 47.1%. In addition, the maximal recurrent inhibition among spinal segment L5 motor pools is 42.5%.

CHAPTER 4. RESULT II.
RECURRENT INHIBITION OF MSRs AFTER A NERVE CRUSH IN ADULTHOOD

4.0. Introduction

The results presented in this chapter are divided into three parts according to the site of the nerve crush performed. They are (1) sciatic nerve (far crush), (2) tibial nerve (mid-crush), and (3) lateral gastrocnemius-soleus nerves (near crush). The time course of recovery of recurrent inhibition following nerve crush were also investigated between 5 weeks and 14 weeks postoperatively. In addition, the change in motoneurones number in the G-S motor pool and gastrocnemius muscle weight and fiber type have also been examined after regeneration.

4.1. Monosynaptic reflexes of regenerated motoneurones

Following the nerve crush, the motor nerves will regenerate and reinnervate their original muscles. Since reinnervation is complete 6 weeks after nerve injury (Lowrie et al., 1982, 1987), monosynaptic reflexes of LG-S and MG motor pools were tested at 6 weeks after sciatic nerve crush. Monosynaptic reflexes evoked by stimulating sectioned dorsal roots L4, L5 and L6 were recorded from the regenerated LG-S and MG muscle nerves (Fig.4.1). Test stimuli on dorsal roots in figure 4.1.A were at time 0. Following a short delay, the evoked monosynaptic reflexes were observed. Monosynaptic reflexes recorded from regenerated LG-S and MG nerves have a mean latency of about 2.80 ± 0.10 ms (mean$\pm$SEM, n=11) and 2.70 ± 0.06 ms (mean$\pm$SEM, n=15) and an amplitude of about 0.50 ± 0.13 mv (mean$\pm$SEM, n=11) and 0.88 ± 0.19 mv (mean$\pm$SEM, n=15) (Fig.4.2 and table 4.1). Compared with results from normal control rats, the MSRs' latencies of regenerated motoneurones in rats after a sciatic nerve crush in adulthood are significantly longer ($P < 0.01$ t-test) than those in normal rats (Fig.4.2.A). However, there is no significant difference in MSR amplitude between in normal rats and in rats after a sciatic nerve crush in adulthood (Fig.4.2.B).

In adult sciatic nerve crush rat preparations, after regeneration from nerve crush, the

amplitude of monosynaptic reflexes in LG-S or MG motoneurones were observed to be reduced following antidromic stimulating the nerve of either MG or LG-S. Figure 4.1.A shows the unconditioned monosynaptic reflexes recorded from regenerated LG-S and MG nerves 6 weeks after nerve crush. In addition, in Figure 4.1.B and 4.1.C monosynaptic reflexes from same preparation as in figure 4.1.A were conditioned by an antidromic volley delivered onto synergist nerve 15 msec and 4 msec prior to the test stimuli. When test-conditioning stimuli interval was at 4 msec, the maximal reduction of MSR amplitude has been observed and shows in figure 4.1.C.

Table 4.1. Mean amplitude and latency of MSRs of LG-S and MG motor pools in adult sciatic nerve crush rats

Nerve	Latency of MSR (mean±SEM, ms;(range))	Amplitude of MSR (mean±SEM, mv; (range))
LG-S	2.80±0.10 (2.4-3.4) (n=11)	0.50±0.13 (0.08-1.23) (n=11)
MG	2.70±0.06 (2.4-3.1) (n=15)	0.88±0.19 (0.13-2.73) (n=15)

Fig.4.1.

Photographs taken from oscilloscope showing the monosynaptic reflexes elicited from dorsal roots L4, L5 and L6 and recorded from LG-S and MG muscle nerves 6 weeks after adult sciatic nerve crush. (A) is the unconditioned monosynaptic reflexes in LG-S (left) and MG (right); (B) and (C) are conditioned monosynaptic reflexes in which the test-conditioning stimuli interval at 15 msec and 4 msec respectively. The test stimulus is at time 0 in each picture and the conditioning stimulus on the synergist nerve is prior to the test stimulus.

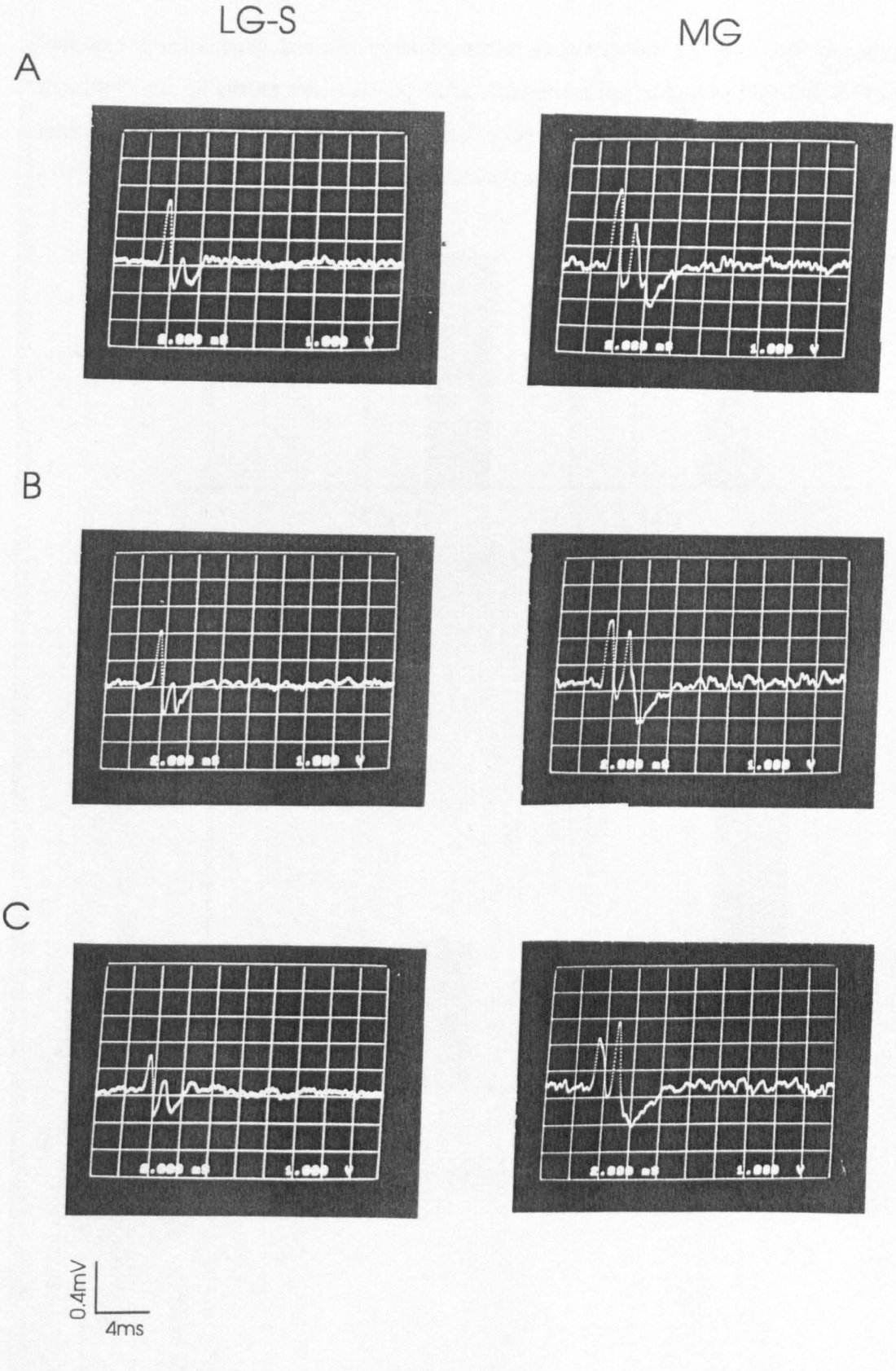
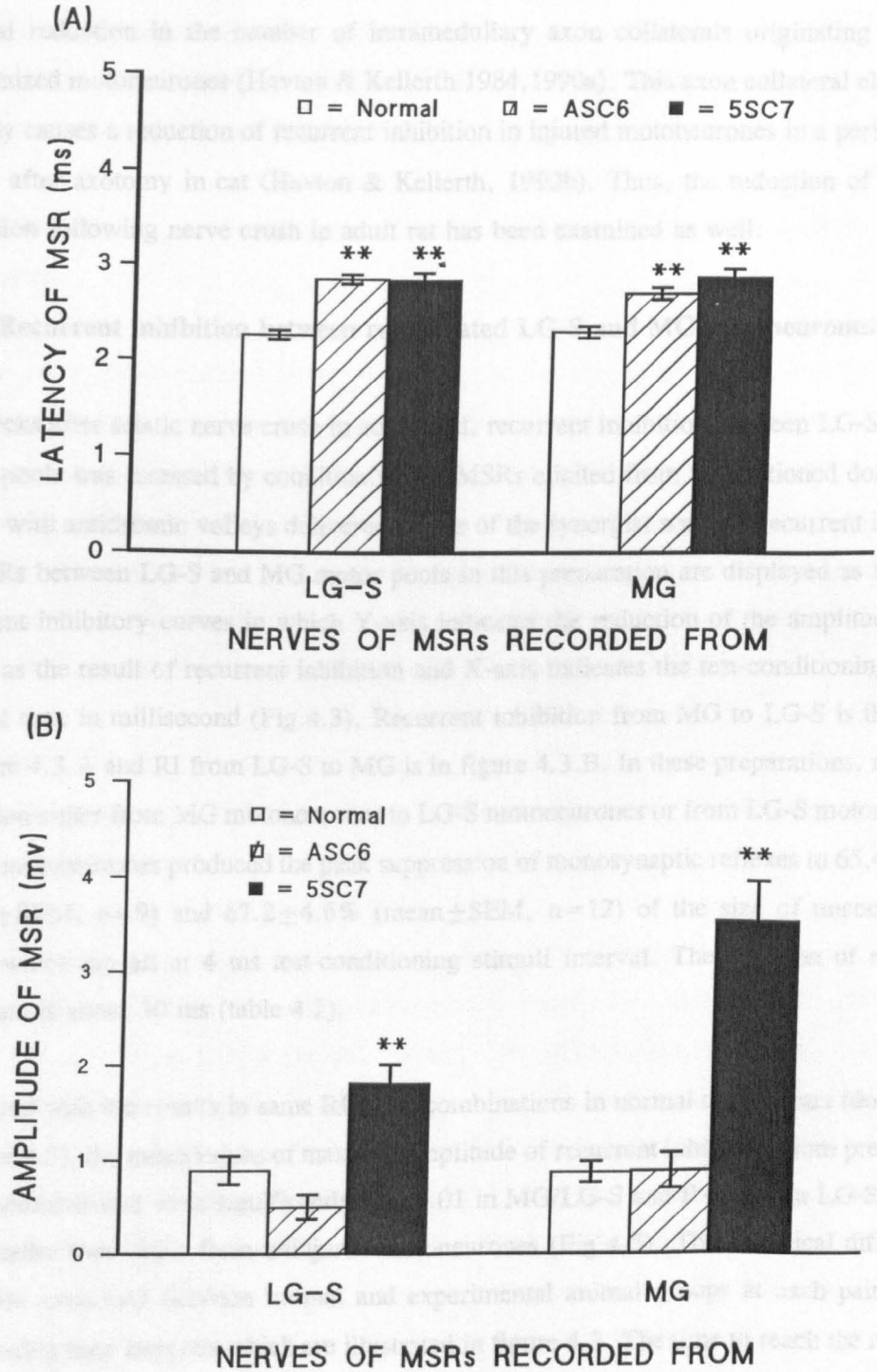

LG-S
MG
A
B
C
0.4mV
4ms

Fig. 4.2.

Bar histogram showing the monosynaptic reflexes latency (A) and amplitude (B) recorded from LG-S and MG nerves in normal (open), adult sciatic nerve crush (/), and 5-day-old sciatic nerve crush (filled) rats. The t-test for paired comparisons were employed to analyses the MSR between normal and experimental animals.(** = $P<0.01$).


(A)
□ = Normal
▨ = ASC6
■ = 5SC7
LATENCY OF MSR (ms)
0
1
2
3
4
5
**
**
**
**
LG-S
MG
NERVES OF MSRs RECORDED FROM
(B)
□ = Normal
▨ = ASC6
■ = 5SC7
AMPLITUDE OF MSR (mv)
0
1
2
3
4
5
**
**
LG-S
MG
NERVES OF MSRs RECORDED FROM

4.2. Recurrent inhibition of the MSRs after sciatic nerve crush

Previous work reported that chronic axotomy of a peripheral motor nerve in cat caused a gradual reduction in the number of intramedullary axon collaterals originating from the axotomized motoneurones (Havton & Kellerth 1984,1990a). This axon collateral elimination directly causes a reduction of recurrent inhibition in injured motoneurones in a period of 3-6 weeks after axotomy in cat (Havton & Kellerth, 1990b). Thus, the reduction of recurrent inhibition following nerve crush in adult rat has been examined as well.

4.2.1 Recurrent inhibition between regenerated LG-S and MG motoneurones

Six weeks after sciatic nerve crush in adulthood, recurrent inhibition between LG-S and MG motor pools was assessed by conditioning the MSRs elicited from the sectioned dorsal roots L4-L6 with antidromic volleys delivered to one of the synergist nerves. Recurrent inhibition of MSRs between LG-S and MG motor pools in this preparation are displayed as in typical recurrent inhibitory curves in which Y-axis indicates the reduction of the amplitude of the MSRs as the result of recurrent inhibition and X-axis indicates the test-conditioning stimuli interval time in millisecond (Fig.4.3). Recurrent inhibition from MG to LG-S is illustrated in figure 4.3.A and RI from LG-S to MG is in figure 4.3.B. In these preparations, recurrent inhibition either from MG motoneurones to LG-S motoneurones or from LG-S motoneurones to MG motoneurones produced the peak suppression of monosynaptic reflexes to $65.4\pm5.4\%$ (mean$\pm$SEM, n=9) and $67.2\pm4.6\%$ (mean$\pm$SEM, n=12) of the size of unconditioned MSRs which are all at 4 ms test-conditioning stimuli interval. The duration of recurrent inhibition is about 30 ms (table 4.2).

Compared with the results in same RI/MSR combinations in normal control rats (dotted line in figure 4.3), the mean values of maximal amplitude of recurrent inhibition from pre-injured adult motoneurones were significantly ($P<0.01$ in MG/LG-S and $P<0.05$ in LG-S/MG, t-test) smaller than those from uninjured motoneurones (Fig.4.5). The statistical differences were also examined between normal and experimental animal groups at each paired test-conditioning time intervals which are illustrated in figure 4.3. The time to reach the maximal recurrent inhibitory effect was as same as that in normal rats at about 4 msec test-

conditioning interval. However, the duration of recurrent inhibition elicited by regenerated motoneurones in adult sciatic nerve crushed animals was less (30 ms vs 40 ms) than that in normal animals. These results indicate that recurrent inhibition within gastrocnemius-soleus motor pools has been reduced by a previous sciatic nerve crush in adulthood although the regeneration has completed (Lowrie et al., 1982, 1987) and no motoneurone loss (see section 4.5) has occurred as the result of adult nerve crush.

4.2.2. Time course of recovery of recurrent inhibition after a sciatic nerve crush in adulthood

Havton and Kellerth (1990b) has reported that, in cat, the initial reduction of the effect of recurrent inhibition generated from sectioned non-reinnervated motoneurones could recover to the normal level 12 weeks after nerve section. The possibility that the observed reduction in recurrent inhibition 6 weeks after nerve crush in rat would recover to the normal level was examined 14 weeks after nerve crush. In addition, the recurrent inhibition among gastrocnemius-soleus motoneurones was also tested at 5 weeks after sciatic nerve crush in which the regeneration may not complete (Lowrie et al., 1982,1987).

Figure 4.4 shows that recurrent inhibition (A) from MG to LG-S and (B) from LG-S to MG tested at 5 weeks (filled triangles), 6 weeks (filled circles), and 14 weeks (open triangles) post-operatively.

In rats 5 weeks after sciatic nerve crush, monosynaptic reflexes have less attenuation in amplitude after an antidromic volley in the synergist nerve than those either in normal or 6 weeks after nerve crush animal groups. The amplitude of conditioned MSRs were maximally reduced to $84.1 \pm 2.7\%$ and $92.7 \pm 1.5\%$ (mean$\pm$SEM%, n=3) of the size of unconditioned reflexes as a result of recurrent inhibition from MG to LG-S and from LG-S to MG. These values were all significantly ($P < 0.001$, t-test) smaller than those in normal control rats (Fig.4.4.A1,B1 and Fig.4.5). They were also differed significantly ($P < 0.05$, MG to LG-S; $P < 0.001$, LG-S to MG) from those 6 weeks after sciatic nerve crush rats group (Fig.4.4. A4,B4). It is of interest to note that, before the injured motoneurones fully regenerated, the recurrent inhibition of MSRs between MG and LG-S motor pool is much smaller than that

in a regenerated one. This may due to the unrecovered Renshaw cells activity following nerve injury (Sanna et al., 1993).

It was surprising that at 14 weeks post-operatively, monosynaptic reflexes from MG to LG-S and from LG-S to MG after antidromic activity had the peak reduction, as a result of recurrent inhibition, only to 67.3±7.4% (mean±SEM, n=5) and 67.5±6.2% (mean±SEM, n=5) of the size of unconditioned test reflexes (Fig.4.4 and Fig.4.5). The recurrent inhibition between MG and LG-S motor pools in this animal group were all significantly ($P<0.01$ and $P<0.05$, t-test) smaller than those from normal rats (Fig.4.5). However the amounts of recurrent inhibition from animal group of 14 weeks after sciatic nerve crush well matched the value in animals 6 weeks postoperatively. That there is no difference between 6 weeks and 14 weeks post-operatively animal groups in the amount of recurrent inhibition may suggest that the impairment of recurrent inhibition as a result of sciatic nerve crush in adulthood is permanent.

In conclusion, after sciatic nerve crush in adulthood, the results indicated that, following initial huge amount loss, recurrent inhibition was re-established between weeks 5 and 6 after nerve crush. However, that recurrent inhibition remained permanently and significantly depressed at least up to 14 weeks postoperatively compared that in normal rats indicated failure of fully recovery.

4.2.3. Recurrent inhibition of MSRs of contralateral uninjured motoneurones with left sciatic nerve crush in adulthood

Since nerve crush performed on left side of sciatic nerve markedly reduced recurrent inhibition ipsilaterally, whether there is any crossed effect of nerve injury onto the contralateral uninjured side recurrent inhibitory pathways has been examined in ASC6 group animals. In addition, both MSR and recurrent inhibition from contralateral side ASC6 group rats could be also compared with those from normal animals.

Monosynaptic reflexes evoked by stimulation of uninjured control (right) side dorsal roots L4, L5, and L6 and recorded from ipsilateral LG-S and MG muscle nerves have a latency

of about 2.20±0.10 ms (mean±SEM, n=8) and 2.24±0.06 ms (mean±SEM, n=9) (Fig.4.6.A) and an amplitude of about 1.25±0.36 mv (mean±SEM, n=8) and 1.30±0.35 mv (mean±SEM, n=9) (Fig.4.6.B). Neither the latency nor the amplitude of the monosynaptic reflexes differ ($0.5>P>0.4$) from those obtained from normal rats.

Recurrent inhibition of MSRs of uninjured side MG motoneurones had the effect to reduce monosynaptic reflexes of LG-S motoneurones to 40.8±8.9% (mean±SEM, n=9) of the size of unconditioned test reflexes (Fig.4.7.A). LG-S motoneurones also could reduce monosynaptic reflexes of MG motoneurones to 51.7±6.8% (mean±SEM, n=11) of the size of unconditioned test MG reflexes (Fig.4.7.B). The maximal recurrent inhibitory effects on MSRs compared well ($P>0.5$, t-test) with those from normal decerebrated rats. These results indicated that after injury in adulthood recurrent inhibitory pathways on the contralateral side are unaffected.

4.2.4. Recurrent inhibition of MSRs of L5 segment motoneurone pools

In rats with sciatic nerve crush at adulthood, recurrent inhibition of MSRs of motoneurones in whole L5 spinal segments has been studied. In this preparation, MSRs were evoked by stimulating dorsal root L5 and recorded from half of the split ventral root L5. Recurrent inhibition was assessed by antidromic stimulating the other half of the ventral root L5.

Recurrent inhibition of MSRs of L5 motoneurones were examined in two groups of rats (1) 6 weeks. (2) 14 weeks after sciatic nerve crush. In the 6 weeks postoperative group, the peak suppression ratio of the MSR was 26.8±4.2% (mean±SEM, n=10) as a result of recurrent inhibition which occurred at 4 ms test-conditioning interval and the duration of recurrent inhibition was about 30 ms (Fig.4.8). With 14 weeks postoperative group, the peak suppression ratio of the MSR was 25.0±3.9% (mean±SEM, n=4) which was at 4 ms test-conditioning stimuli interval and the duration of recurrent inhibition was also about 30 ms (Fig.4.7). The maximal effect of recurrent inhibition was significantly smaller in both 6 weeks ($p<0.05$, t-test) and 14 weeks ($p<0.05$, t-test) postoperative groups than in normal rats. However, within in these two groups, results are correlated well. These results indicated that following sciatic nerve crush, the permanent depression of recurrent inhibition could be

detected in whole motoneurone pools in the spinal segment L5, although some motor pools in this segment were not affected by sciatic nerve crush.

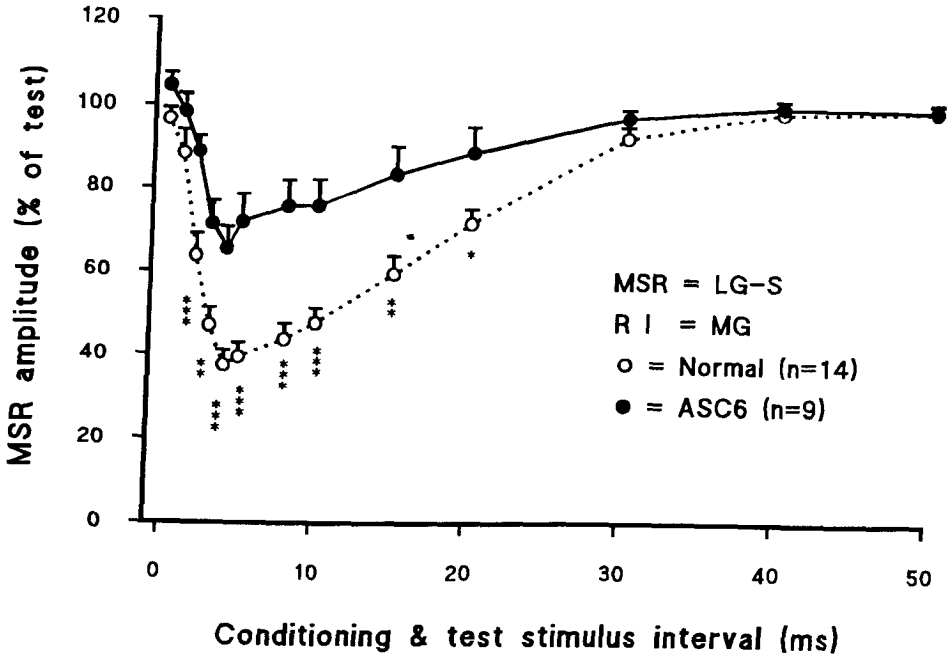
Table 4.2 Summary of recurrent inhibition of MSRs after sciatic nerve crush in adult rat

RI/MSR	number of rats	Post operative time (weeks)	T-C interval to Max. RI (ms)	Max. RI (% of MSR reduction) (mean±SEM)	RI duration (ms)
MG/LG-S	3	5	4	15.9±2.7%	30
	9	6	4	34.6±5.4%	30
	5	14	5	32.7±7.4%	30
control	9	6	5	59.2±8.9%	40
LG-S/MG	3	5	5	7.3±1.5%	30
	12	6	4	32.8±4.6%	30
	5	14	5	32.5±6.2%	20
control	11	6	4	48.3±6.8%	30
L5	10	6	4	26.8±4.2%	30
	4	14	4	25.0±3.9%	30

Fig. 4.3.

Recurrent inhibitory curves show the amount (mean±SEM) of recurrent inhibition (RI) expressed as the reduction of monosynaptic reflexes (MSR) amplitude in the adult sciatic nerve crush rats (filled circles with solid line) reduced as a result of sciatic nerve crush 6 weeks postoperatively. The RI/MSR combinations tested were MG/LG-S (A) and LG-S/MG (B). The student t-test was employed to compare the difference between in experimental and normal (open circles with dotted line) animals. * = $P<0.05$, ** = $P<0.01$, and *** = $P<0.001$.

(A)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = LG–S
R I = MG
o = Normal (n=14)
● = ASC6 (n=9)

(B)

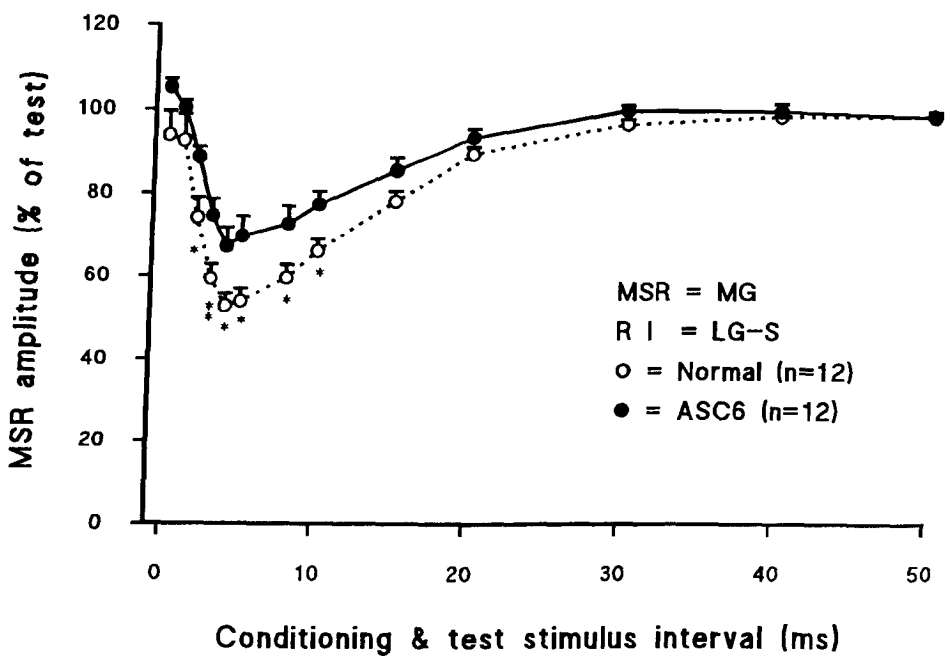
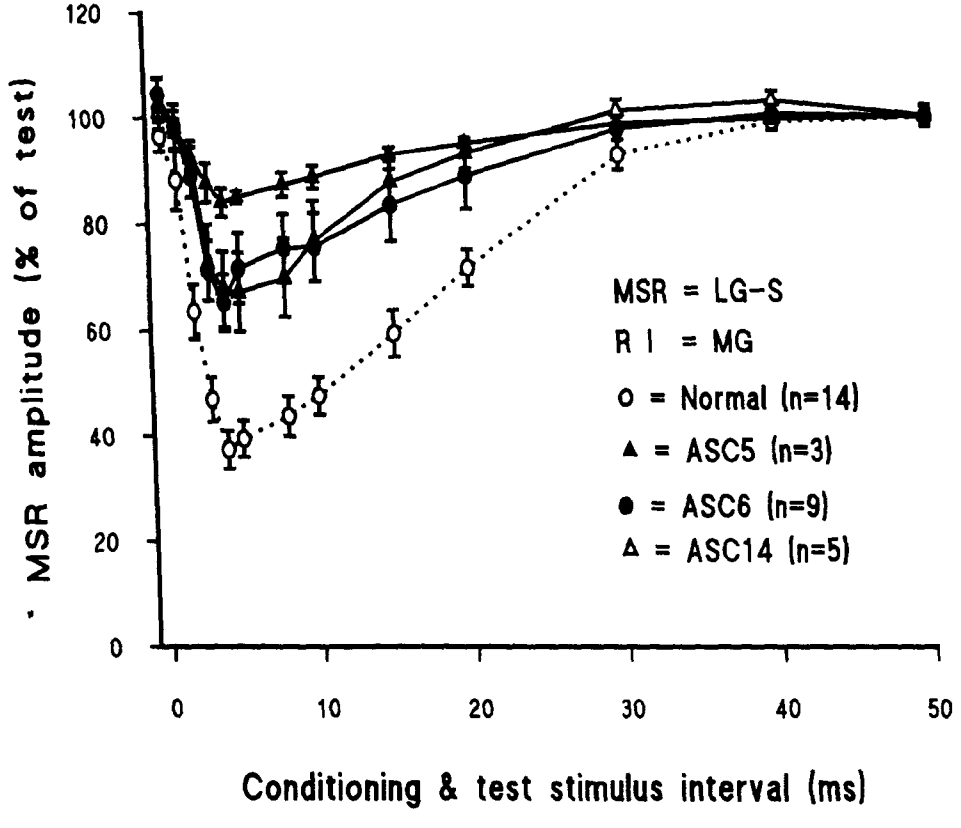
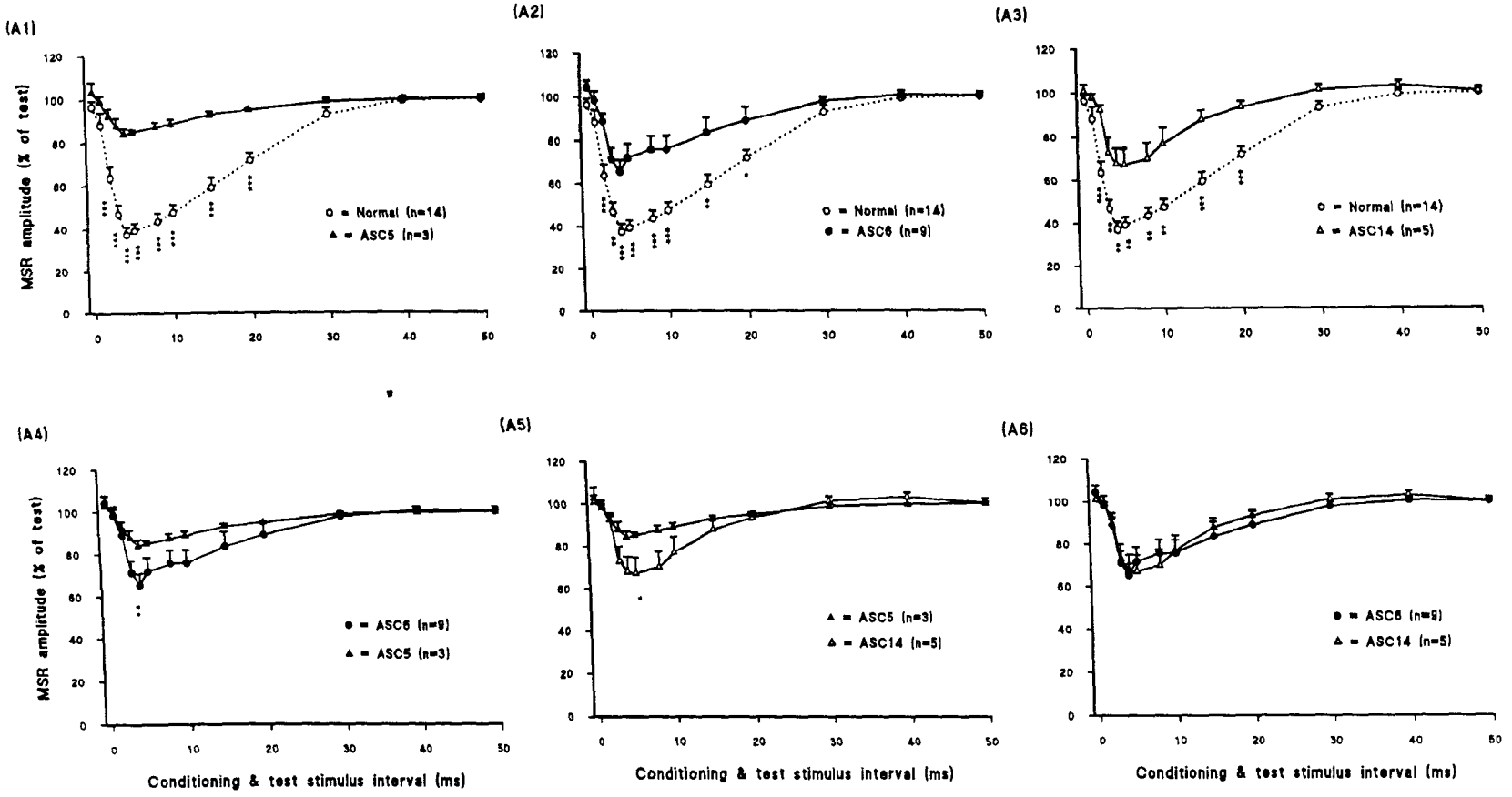

MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = MG
R I = LG–S
o = Normal (n=12)
● = ASC6 (n=12)

Fig. 4.4.

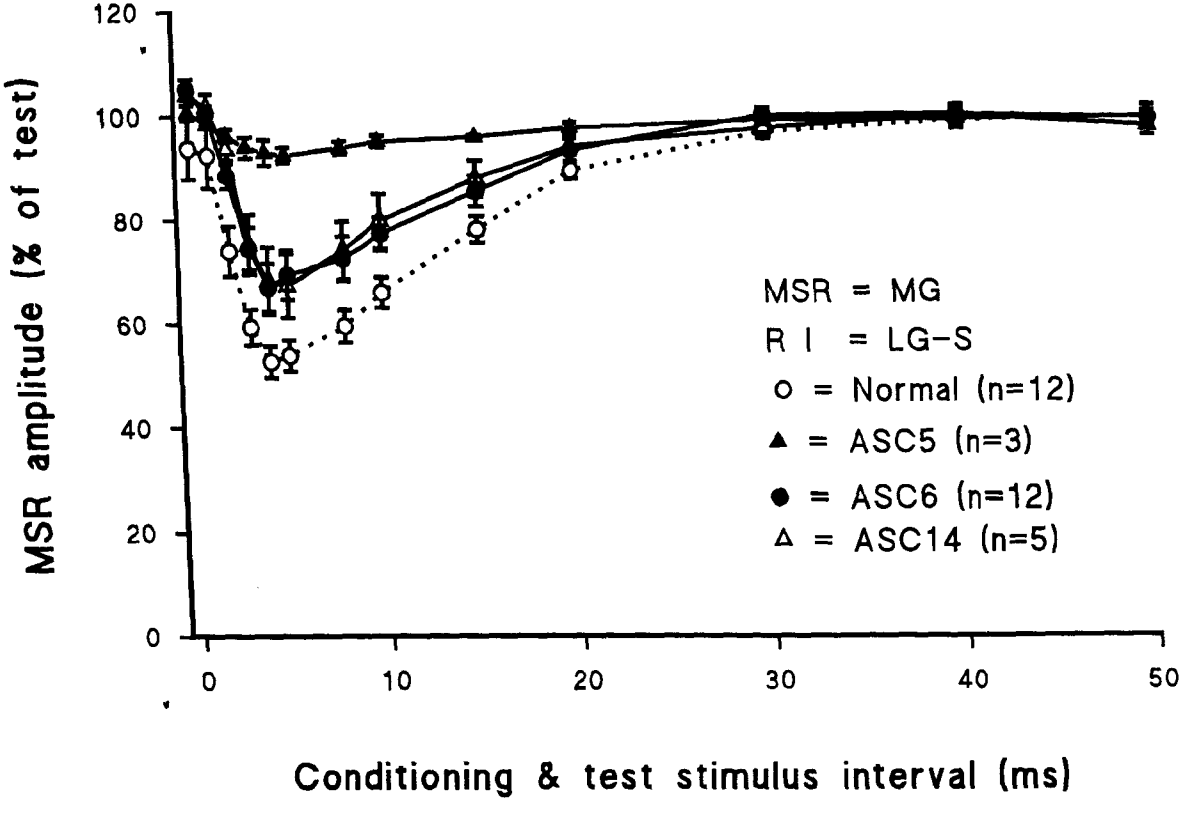
The recovery time course of the recurrent inhibition in adult sciatic nerve crush rats. The RI/MSR combinations tested were MG/LG-S (A) and LG-S/MG (B) and animals with different postoperative time tested was 5 weeks (filled triangles), 6 weeks (filled circles), and 14 weeks (open triangles). The corresponding recurrent inhibitory curves obtained from normal rats is presented as control (open circles and dotted line). In both A and B, first part shows the recovery time course of recurrent inhibition of MSRs following sciatic nerve crush in adult rats and second part shows the statistical analysis (two-tailed student t-test) of the results between experimental animals and normal animals and between each postoperative groups in experimental animals. * = $P<0.05$, ** = $P<0.01$, and *** = $P<0.001$.

(A)


120
100
80
60
40
20
0
MSR amplitude (% of test)
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = LG-S
R I = MG
o = Normal (n=14)
▲ = ASC5 (n=3)
● = ASC6 (n=9)
Δ = ASC14 (n=5)


(A1)
(A2)
(A3)
(A4)
(A5)
(A6)
MSR amplitude (% of test)
o = Normal (n=14)
▲ = ASC5 (n=3)
● = ASC6 (n=9)
△ = ASC14 (n=5)
Conditioning & test stimulus interval (ms)

(B)


120
100
80
60
40
20
0
MSR amplitude (% of test)
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = MG
R I = LG-S
o = Normal (n=12)
▲ = ASC5 (n=3)
● = ASC6 (n=12)
△ = ASC14 (n=5)

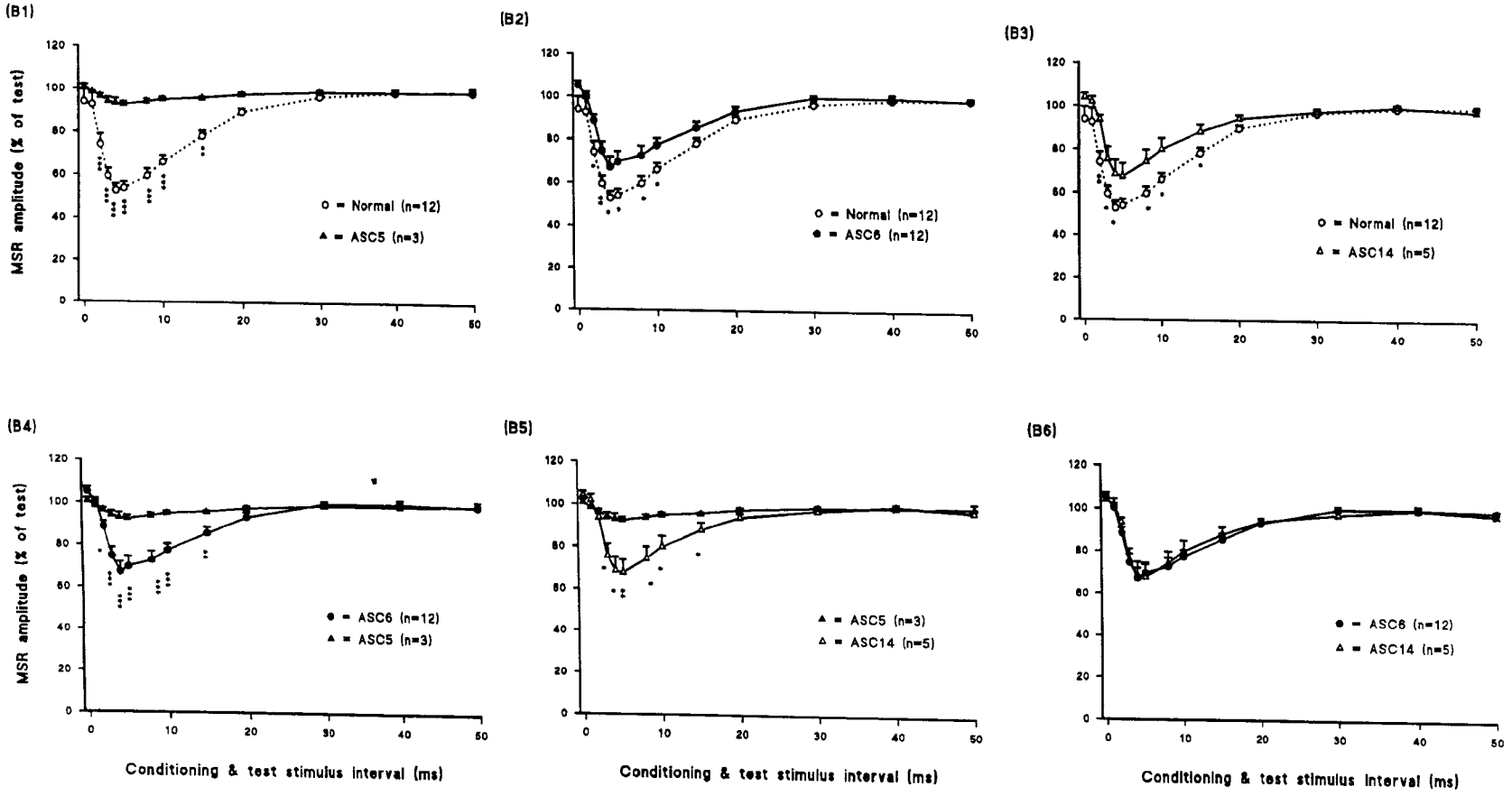

(B1)
(B2)
(B3)
(B4)
(B5)
(B6)
MSR amplitude (% of test)
Conditioning & test stimulus interval (ms)
o = Normal (n=12)
▲ = ASC5 (n=3)
● = ASC6 (n=12)
△ = ASC14 (n=5)

Figure 4.5.

Bar diagram showing the maximal MSR amplitude reduction as a result of recurrent inhibition in either normal or experimental decerebrate rats. Statistical difference was tested (two tailed Student t-test) between normal and experimental animals. X-axis indicates the animal groups according to the postoperative time and Y-axis indicates the maximal amount of recurrent inhibition expressed by % reduction of amplitude of MSRs. *=$P<0.05$; **=$P<0.01$; ***=$P<0.001$.

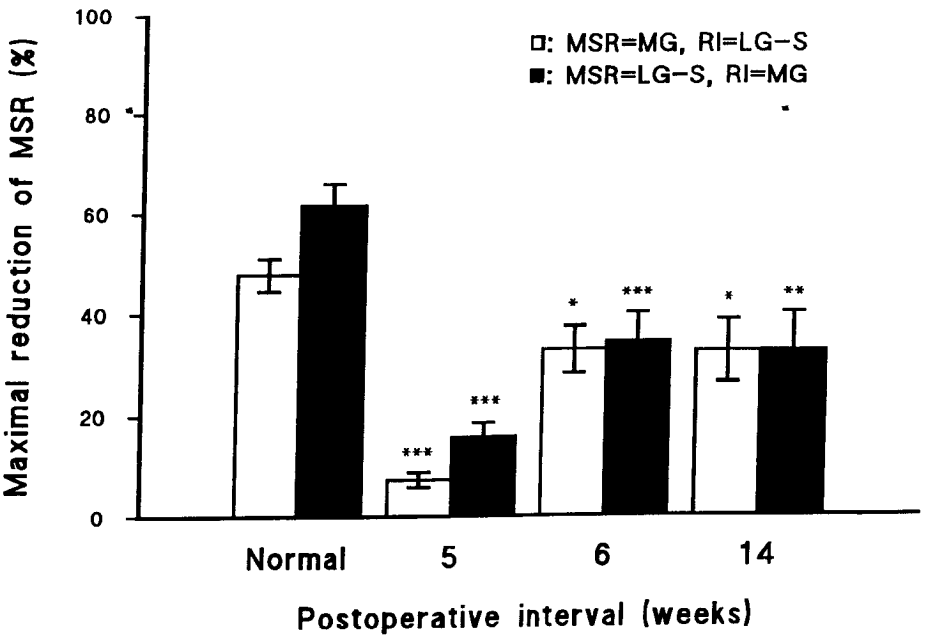
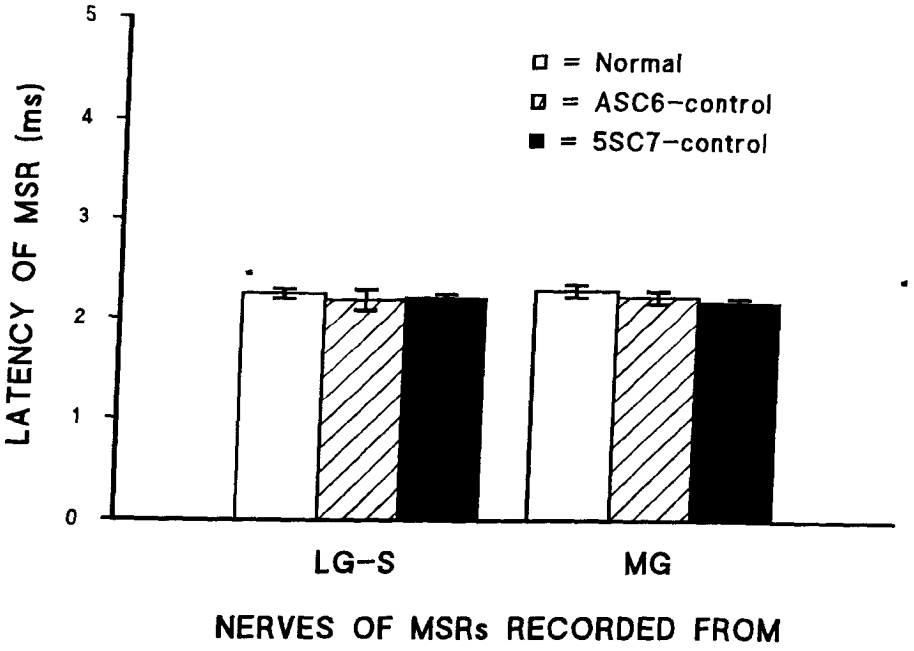

□: MSR=MG, RI=LG-S
■: MSR=LG-S, RI=MG
Maximal reduction of MSR (%)
100
80
60
40
20
0
***
***
*
***
*
**
Normal
5
6
14
Postoperative interval (weeks)

Fig. 4.6.

Bar diagram showing the monosynaptic reflexes latency (A) and amplitude (B) recorded from LG-S and MG nerves in normal (open); contralateral control side of adult sciatic nerve crush (/); and contralateral control side of 5-day-old sciatic nerve crush (filled) rats. The Student t-test for unpaired two comparisons is employed for analysis of the difference between normal and experimental animals. (** = $P < 0.01$).

(A)


5
4
3
2
1
0
LATENCY OF MSR (ms)
□ = Normal
▨ = ASC6-control
■ = 5SC7-control
*
*
LG-S
MG
NERVES OF MSRs RECORDED FROM

(B)

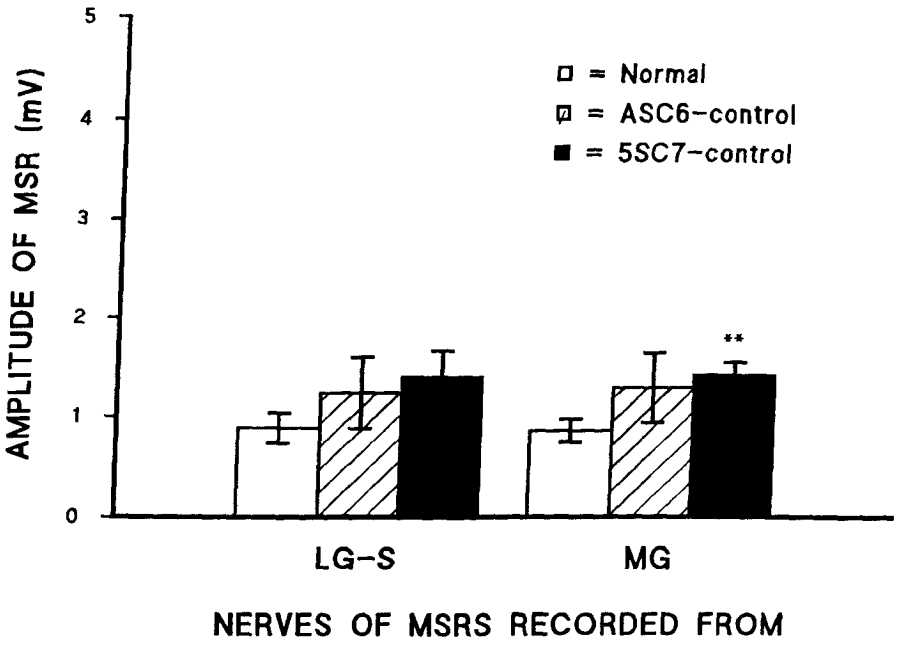
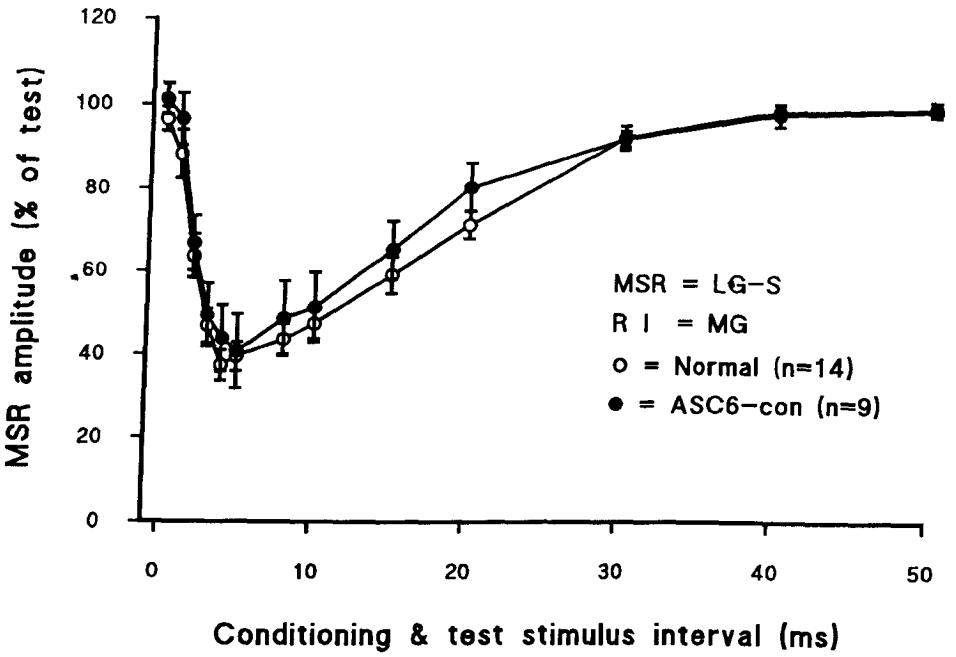

5
4
3
2
1
0
AMPLITUDE OF MSR (mV)
□ = Normal
▨ = ASC6-control
■ = 5SC7-control
**
LG-S
MG
NERVES OF MSRS RECORDED FROM

Fig. 4.7.

Recurrent inhibitory curves show the recurrent inhibition of MSRs in normal and in contralateral uninjured side of adult sciatic nerve crush rats. Recurrent inhibition were tested with the RI/MSR combinations as MG/LG-S (A) and LG-S/MG (B). Statistic test (t-test, two-tailed) is failed to detect the significance ($P > 0.5$) in these two preparations.

(A)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = LG-S
R I = MG
o = Normal (n=14)
● = ASC6-con (n=9)

(B)

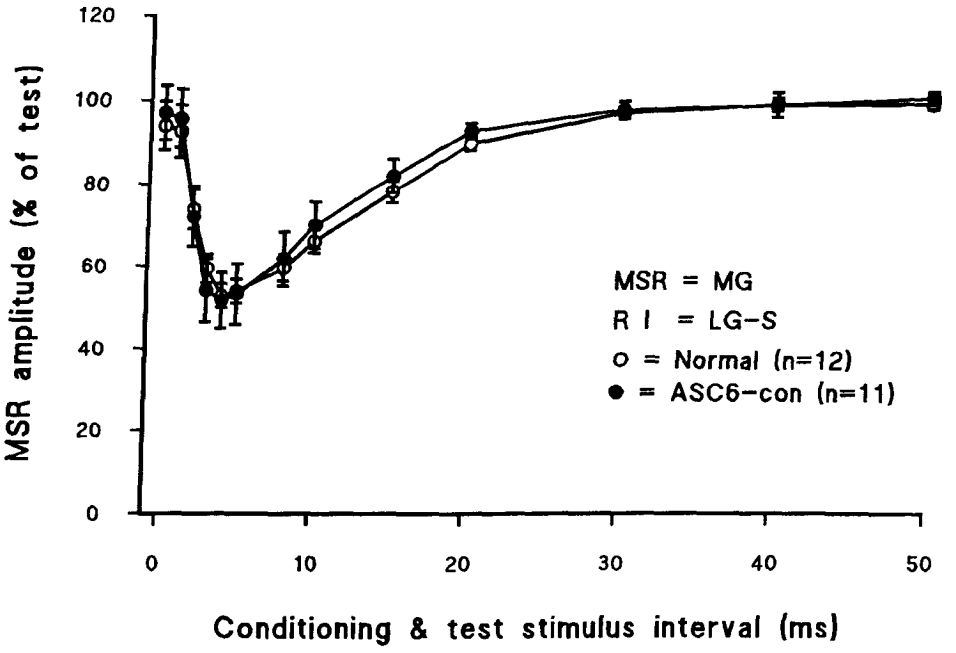
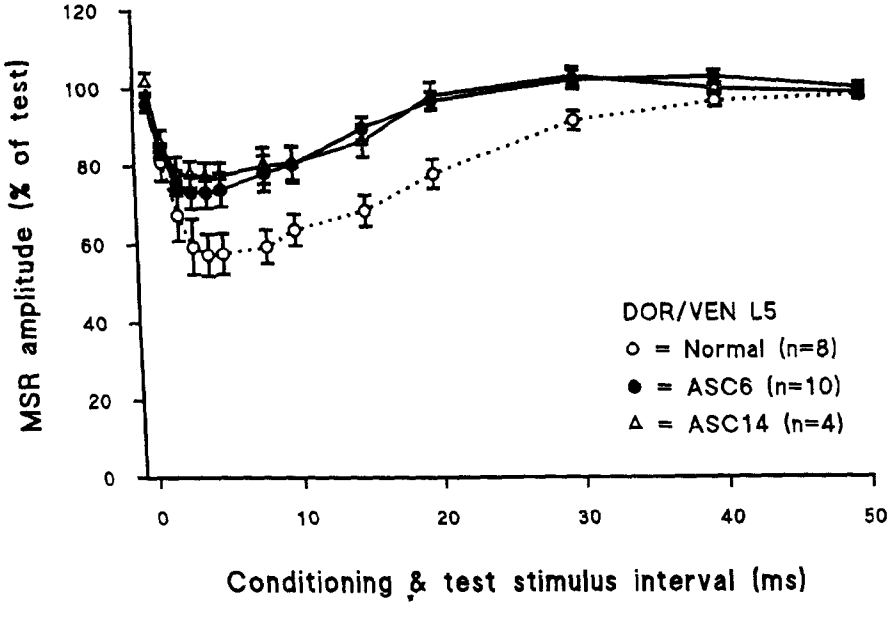
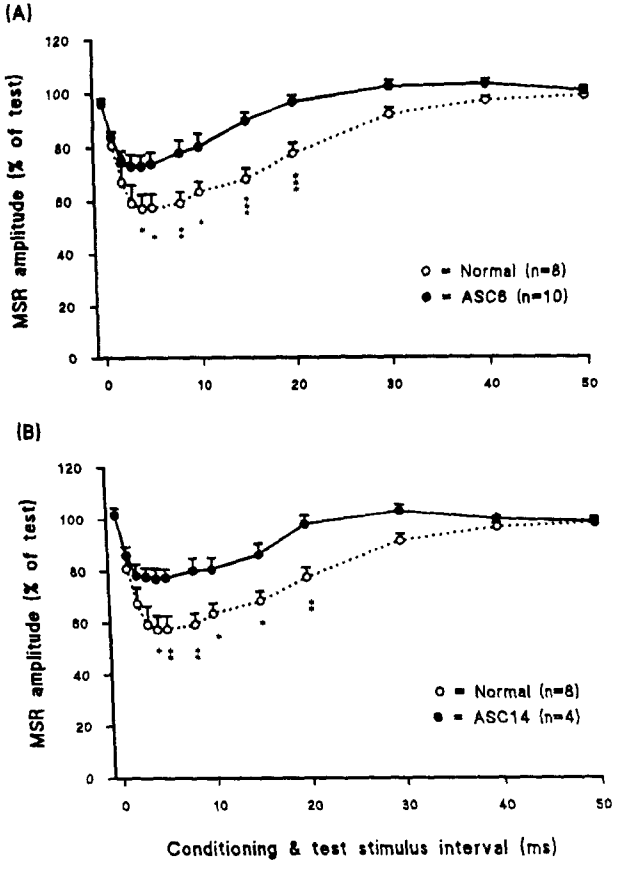

MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = MG
R I = LG-S
o = Normal (n=12)
● = ASC6-con (n=11)

Fig. 4.8.

Recurrent inhibition of MSRs of whole spinal segment L5 motor pool in adult sciatic nerve crush rats. The recovery time course of RI were tested 6 weeks (filled circles) and 14 weeks (open triangles) after nerve crush. The corresponding normal curve (open circles and dotted line) is presented as control. The two small diagrams (A) & (B) showing at right side illustrates the statistic difference between experimental and normal animals (t-test), * = $P<0.05$, ** = $P<0.01$, and *** = $P<0.001$. The amount of recurrent inhibition between 6 and 14 weeks postoperatively animals does not differ significantly ($P>0.5$).


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
DOR/VEN L5
o = Normal (n=8)
● = ASC6 (n=10)
Δ = ASC14 (n=4)


(A)
MSR amplitude (% of test)
o = Normal (n=8)
● = ASC6 (n=10)
(B)
MSR amplitude (% of test)
o = Normal (n=8)
● = ASC14 (n=4)
Conditioning & test stimulus interval (ms)

4.3. Recurrent inhibition of MSRs in rats with tibial nerve crush in adulthood

It was reported that there were different effects of nerve crush on the peripheral nerve if crush was carried out distant or near to the muscle (Brown et al., 1976; Lowrie et al., 1987 1990). This difference has been proposed due to the different length of time during which the muscle and motoneurones are disconnected. The longer the period of separation the poorer is recovery (Lowrie et al., 1990).

In this section, results are presented that recurrent inhibition examined in rats with nerve crush performed on tibial nerve in adult rats in which the crush site was below branching point of the perineal and tibial nerves and above the branching point of gastrocnemius-soleus nerves. The crush site on the tibial nerve was about 10 mm away from the point nerve enters into gastrocnemius-soleus muscles. 6 or 12 weeks after tibial nerve crush, recurrent inhibition of MSRs between regenerated MG and LG-S motoneurones was examined.

In the group of animals 6 weeks after tibial nerve crush, the amplitude of LG-S monosynaptic reflexes was reduced, as a result of recurrent inhibition, to $72.4 \pm 2.4\%$ (mean$\pm$SEM, n=3) of unconditioned test MSR value by MG conditioning; and the corresponding LG-S to MG value was $76.6 \pm 2.7\%$ (mean$\pm$SEM, n=3) (Fig.4.9 and table 4.3).

Similarly, in the animal group 12 weeks after tibial nerve crush, the recurrent inhibition of RI/MSR combination on MG/LG-S and LG-S/MG were tested. In 3 animals recurrent inhibition from MG motoneurones to LG-S motoneurones reduced the amplitude of the MSRs to $66.1 \pm 4.9\%$ (mean$\pm$SEM, n=3) of the size of the unconditioned reflex. The corresponding value for LG-S to MG was $72.6 \pm 7.1\%$ (mean$\pm$SEM, n=3) (Fig.4.9 and table 4.3).

Comparing the results from both 6 weeks and 12 weeks after tibial nerve crush animal groups with the results from normal rats (dotted line in Fig.4.9), the peak reduction of amplitude of MSRs as a result of recurrent inhibition (effect of recurrent inhibition) was significantly ($P < 0.001$, t-test, Fig.4.9.) smaller than that from normal rats at same RI/MSR (MG/LG-S, LG-S/MG) nerve combinations. However, there were no differences in the maximal recurrent

inhibitory effect that could be detected between these two groups (6 and 12 weeks after tibial nerve crush. These results indicated that after tibial nerve crush in adulthood, the amount of recurrent inhibition was reduced as a result of tibial nerve crush and the reduction of recurrent inhibition may be permanent.

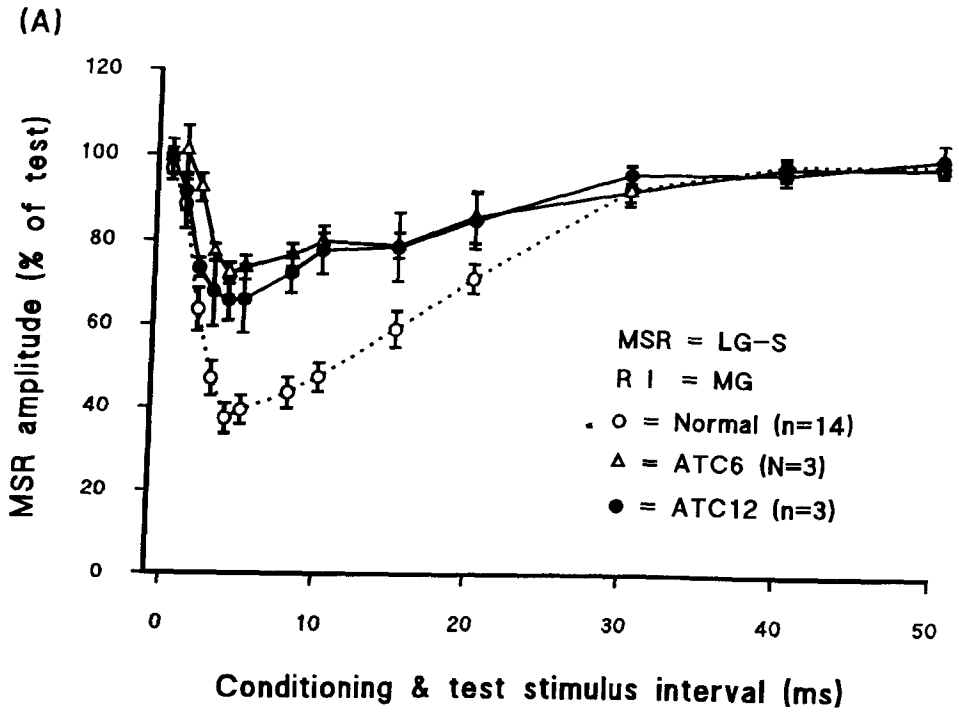
As described above, the effect of recurrent inhibition obtained from rats with sciatic nerve crush was significantly reduced 6 and 14 weeks after nerve crush. Surprisingly, the reduction of recurrent inhibition between LG-S and MG motor pools had a same degree ($P > 0.1$, t-test) following nerve crush performed on either sciatic or tibial nerve. This result may suggest that the reduction of recurrent inhibition in regenerated motoneurones may not be affected by shortening the neurone-muscle disconnection length of time in this particulary period (from sciatic crush to tibial crush) or the length of time of dennervation after tibial nerve crush may be still long enough to produce a maximal reduction on recurrent inhibition.

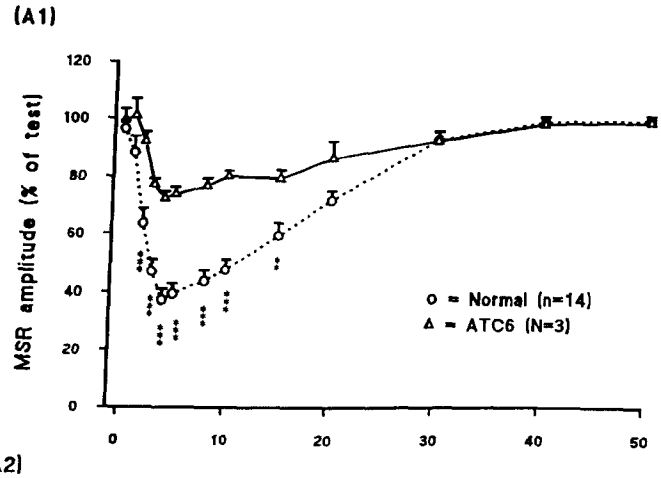
Table 4.3. Summary of recurrent inhibition of regenerated LG-S and MG motoneurone pool in adult tibial nerve crush rats

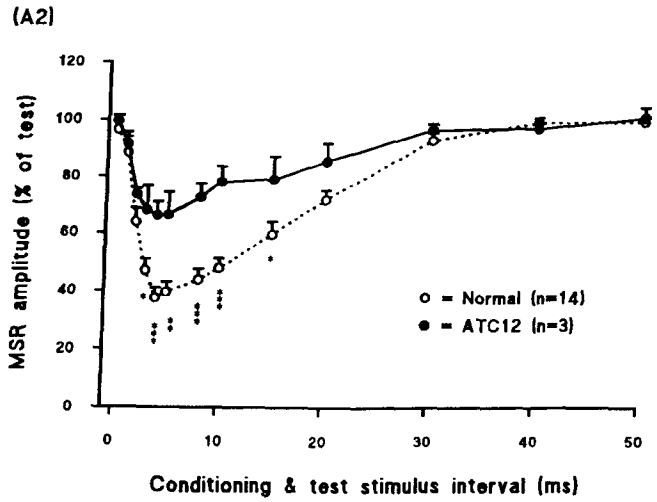
RI/MSR	Number of Rats	Post operative time (weeks)	T-C interval to Max. RI (ms)	Max. RI (% of MSR reduction) (mean±SEM)	RI duration (ms)
MG/LG-S	3	6	4	27.6±2.4%	40
	3	12	4	33.9±4.9%	40
LG-S/MG	3	6	5	23.4±2.7%	40
	3	12	5	27.4±7.1%	30

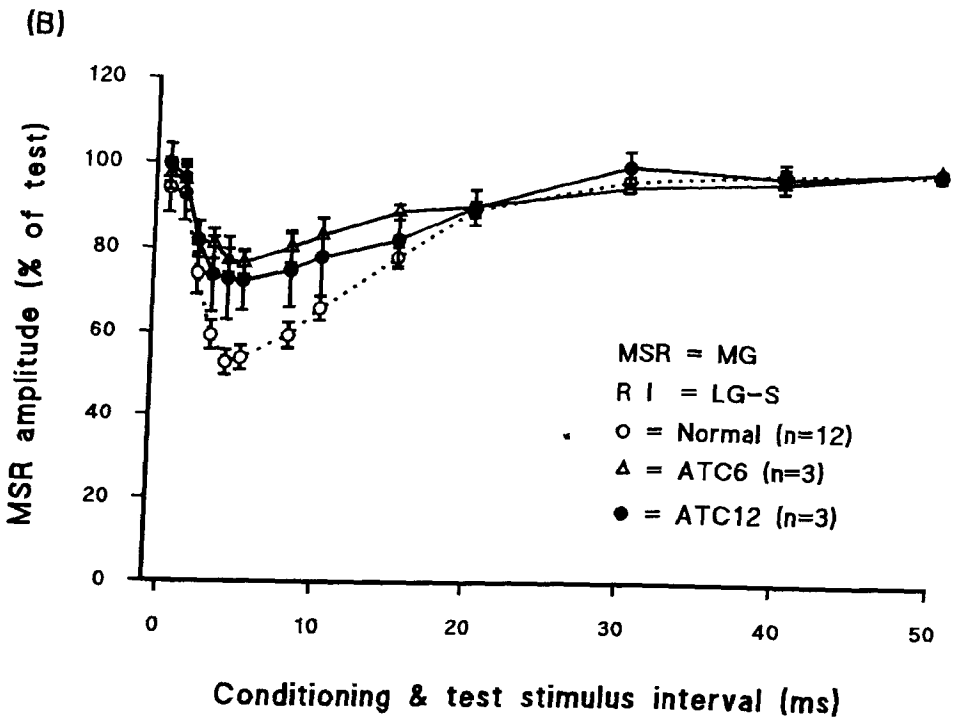
Fig. 4.9.

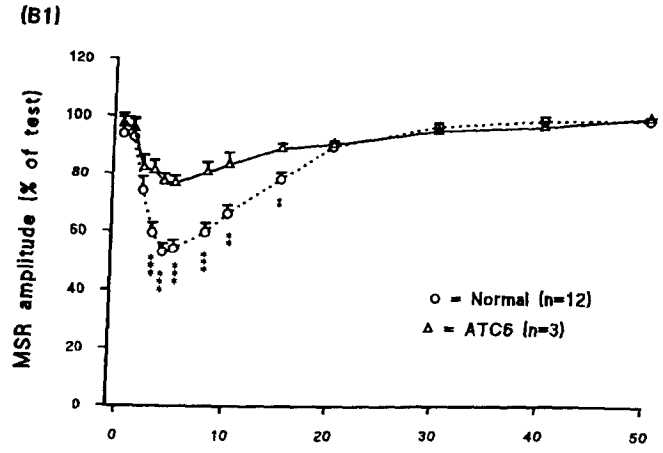
Figures showing the recurrent inhibition of MSRs from MG motoneurones to LG-S motoneurones (A) and from LG-S to MG (B) in adult tibial nerve crush rats. The recovery time course of RI were tested 6 weeks (filled circles) and 12 weeks (open triangles) after nerve crush. The corresponding normal curve (open circles and dotted line) was also presented as control. The two small diagrams showed at bottom illustrates the statistic difference between experimental and normal values (t-test), * = $P<0.05$, ** = $P<0.01$, and *** = $P<0.001$. The amount of recurrent inhibition between 6 and 12 weeks postoperatively animals does not differ significantly ($P>0.5$).

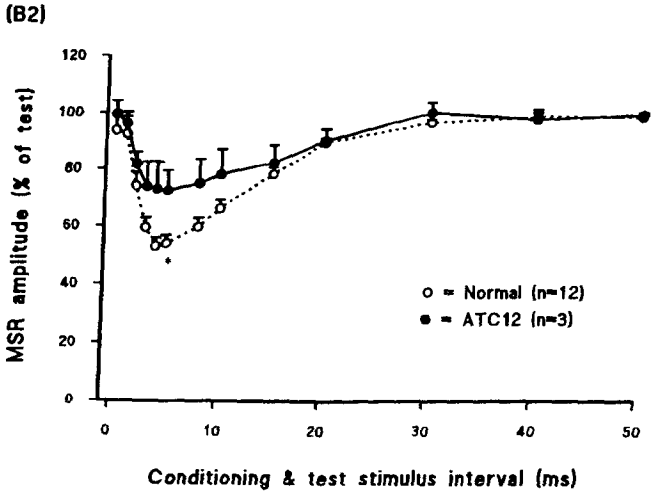

(A)
MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
MSR = LG–S
R I = MG
o = Normal (n=14)
Δ = ATC6 (N=3)
● = ATC12 (n=3)
Conditioning & test stimulus interval (ms)


(A1)
MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
o = Normal (n=14)
Δ = ATC6 (N=3)


(A2)
MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
o = Normal (n=14)
● = ATC12 (n=3)
Conditioning & test stimulus interval (ms)


(B)
MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
MSR = MG
R I = LG-S
o = Normal (n=12)
Δ = ATC6 (n=3)
● = ATC12 (n=3)
Conditioning & test stimulus interval (ms)


(B1)
MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
o = Normal (n=12)
Δ = ATC6 (n=3)


(B2)
MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
o = Normal (n=12)
● = ATC12 (n=3)
Conditioning & test stimulus interval (ms)

4.4 Recurrent inhibition of MSRs between regenerated LG-S and normal MG motoneurones in rats with LG-S nerve crush in adulthood

As presented above, shortening the motoneurone to target muscle disconnecting time by crushing tibial nerve instead of sciatic nerve, the detected recurrent inhibition reduction was not affected. In order to test whether the observed relation between functional impairment and the disconnection period of neurone-muscle (Lowrie et al., 1990) also exists in recurrent inhibitory pathway following nerve crush, the crush site had been moved further down to close to the muscle by crushing the LG-S nerve to make the dennervation time even shorter. The crush site in this preparation was about 1 mm away the LG-S muscle on the LG-S nerve.

Three groups of rats performed with LG-S nerve crush in adulthood according to the postoperative period have been studied and presented below. They are 1) 5 weeks; 2) 6 weeks; and 3) 12 weeks after LG-S nerve crush.

Recurrent inhibition between normal MG and regenerated LG-S motor pools was assessed by conditioning the MSRs elicited from the cut dorsal roots with antidromic volleys delivered to LG-S or MG nerve. In the 5 weeks postoperative group, recurrent inhibition from MG motoneurones to LG-S motoneurones reduced the amplitude of the MSRs to $54.3 \pm 7.2\%$ (mean$\pm$SEM, n=3) of the size of the unconditioned reflexes. The corresponding value for LG-S to MG was $68.6 \pm 8.4\%$ (mean$\pm$SEM, n=3) (Fig.4.10 and table 4.4). Compared with the results in normal rats, the amounts of recurrent inhibition from either MG to LG-S or LG-S to MG have a tendency to be decreased although they are not statistically different. However, the amounts of recurrent inhibition between LG-S and MG are significantly larger in LG-S nerve crush rats than those in sciatic nerve crush rats in which all at 5-weeks postoperatively. The reduced recurrent inhibition seen 5 weeks after LG-S nerve crush indicated that the lower activity of the Renshaw cells seen after sciatic nerve crush (Sanna et.al., 1993) occurred in LG-S nerve crush preparation too, although the depressive effect on activity of Renshaw cells may be much less or recover much faster in LG-S nerve crush preparations than that in sciatic nerve crush rats.

In the animal group of 6 weeks after LG-S nerve crush, recurrent inhibition from MG

motoneurones to LG-S motoneurones reduced the MSRs amplitude to 38.0±1.8% (mean±SEM, n=4) of the unconditioned control size. The corresponding value for recurrent inhibition from LG-S to MG was 50.9±5.1% (mean±SEM, n=3) (Fig.4.10 and table 4.4). In addition, in the animal group of 12 weeks after LG-S nerve crush, these two values were: MG to LG-S 40.0±5.6% (mean±SEM, n=3) and LG-S to MG 51.2±10.1% (mean±SEM, n=3) (Fig.4.10 and table 4.4).

Comparing the results from rats at 6 weeks postoperatively with results from normal animals, recurrent inhibition from MG to LG-S in LG-S nerve crushed animals (62.0%) is as great as normal (62.7%). This was not changed up to 12 weeks postoperatively (60%). It is not surprising that the MG motoneurones were not injured while LG-S nerve were crushed and regenerated and therefore the axon collaterals of MG motoneurones would retain their original distribution.

In addition, recurrent inhibition from LG-S to MG motoneurones 6 weeks after LG-S nerve crush has the value 49.1% which was also as great as the value (47.1%) from the same combination in normal rats. Even 12 weeks after nerve crush, the recurrent is still remain at 48.8% which is as great as either 6 weeks after crush or normal rats. However, they were significantly larger than those obtained from either sciatic or tibial nerve crush rats. This result indicated that, after LG-S nerve crush, the reduction in recurrent inhibition could fully recover in a short postoperative period. Takeing the results from all three groups together (sciatic, tibial and LG-S nerve crush), these results consistent with the view that shorter the period of denervation, better the recovery of the motor unit function (Lowrie et al., 1990).

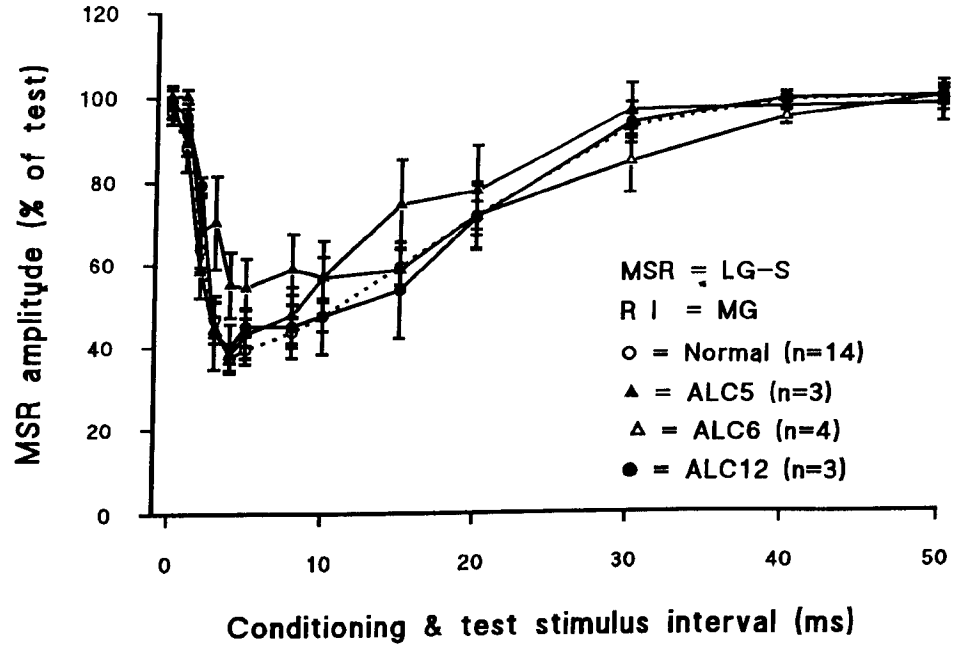
Table 4.4 Summary of recurrent inhibition of MSRs after LG-S nerve crush in adulthood

RI/MSR	Number of rats	Post operative time (weeks)	T-C interval to Max. RI (ms)	Max. RI (% of MSR reduction) (mean±SEM)	RI duration (ms)
MG/LG-S	3	5	5	45.7±7.2%	40
	4	6	4	62.0±1.8%	50
	3	12	4	60.0±5.6%	40
LG-S/MG	3	5	4	31.4±8.4%	20
	3	6	5	49.1±5.1%	40
	3	12	4	48.8±10.1%	30

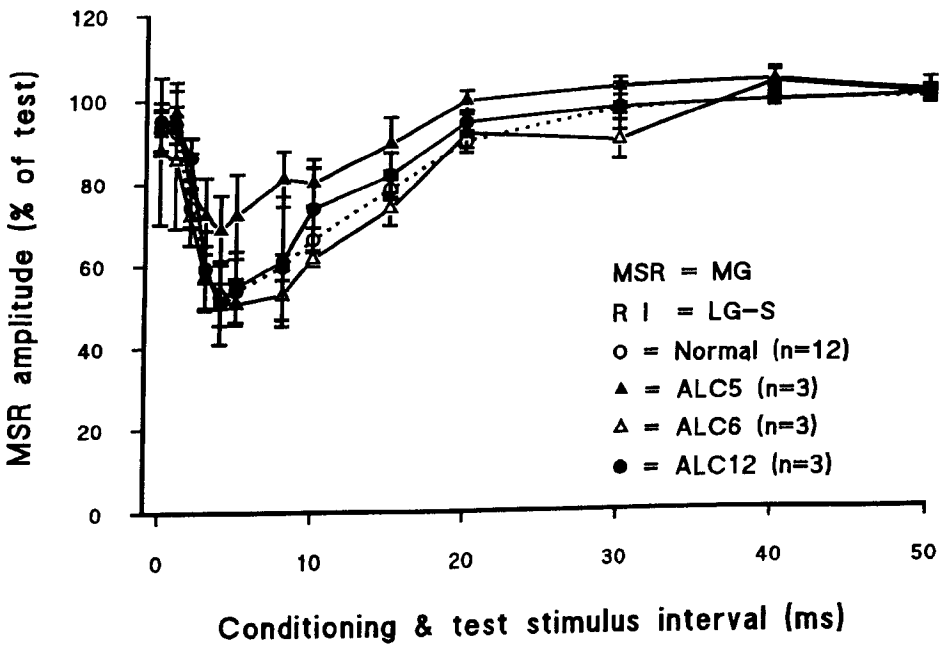
Fig. 4.10.

The time course of recovery of the recurrent inhibition in adult LG-S nerve crush rats. The RI/MSR combinations tested were MG/LG-S (A) and LG-S/MG (B) and the postoperative time tested was 5 weeks (filled triangles), 6 weeks (open triangles), and 12 weeks (filled circles). The corresponding recurrent inhibitory curves obtained from normal rats were also presented as control (open circles and dotted line). There was no statistical difference ($P > 0.05$, t-test) could be detected between the data set in both figures.

(A)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = LG–S
R I = MG
o = Normal (n=14)
▲ = ALC5 (n=3)
△ = ALC6 (n=4)
● = ALC12 (n=3)

(B)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = MG
R I = LG–S
o = Normal (n=12)
▲ = ALC5 (n=3)
△ = ALC6 (n=3)
● = ALC12 (n=3)

4.5 Effect of sciatic nerve crush in adulthood on motoneurone pools, muscle weight and muscle fiber type in rat

4.5.1 Gastrocnemius-soleus motoneurones number and location after adult sciatic nerve crush

The motoneurones innervating the gastrocnemius-soleus muscle were labelled retrogradely with the HRP at least 6 weeks after sciatic nerve crush in adult rats. HRP labelled gastrocnemius-soleus motoneurones were found in the dorsolateral part of the anterior horn in rat spinal lumbar segment L4 and L5 (Fig.4.11.A). Rostra-caudally the majority of the labelled motoneurones were in the segment L5 just as in normal rats. Figure 4.11 illustrated the localization of the pool of the motoneurones to the gastrocnemius-soleus muscles in one adult sciatic nerve crush rat: (A) at a low magnification (2.5x1.6), showing the whole spinal cord section containing HRP labelled motoneurones in both side anterior horn; (B) at a high-magnification (10x2) to give a close view of labelled G-S neurones at injured side.

The mean number of HRP labelled motoneurones counted on injured side spinal ventral horn in adult sciatic nerve crush rats was 181 ± 10 (mean$\pm$SEM, n=4). The mean number of labelled motoneurones in the contralateral uninjured gastrocnemius-soleus pool was 177 ± 10 (mean$\pm$SEM, n=4) (Fig.4.12.B). The number on the regenerated side was not significantly ($P > 0.1$, t-test) different from that obtained either in normal rats or from the contralateral control side. In addition, the mean number from normal animals was not different to that from contralateral control side in adult sciatic nerve crush rats. This result indicated that nerve crush on sciatic nerve in adult rat does not lead to motoneurone death.

4.5.2 Muscle weight and muscle fiber change after adult sciatic nerve crush

At the end of each acute experiment, gastrocnemius and soleus muscles on both sides were dissected out and weighted separately. Gastrocnemius muscles then quickly stored in liquid nitrogen for later SDH staining. Figure 4.12.A shows the percentage change of the weight of the reinnervated gastrocnemius and soleus muscles against the contralateral uninjured muscles in adult sciatic nerve crush rats. At least 6 weeks after sciatic nerve crush, the

weight of reinnervated gastrocnemius muscle was 73.2±3.6% (mean±SEM, n=8) of the weight of contralateral gastrocnemius muscle; and the soleus muscle was 68.7±4.2% (mean±SEM, n=8) of the control (Fig.4.12.A). This result indicated that, although there was no motoneurone loss after nerve crush in adult, the muscle weight was still decreased after reinnervation. Loss of muscle weight after peripheral nerve crush in adult sciatic nerve crush rats observed in this study is consistent with a previous report (Albani et al., 1985).

Figure 4.13 shows cross-sections of both reinnervated and contralateral control gastrocnemius muscles stained with succinic dehydrogenase (SDH). Staining for SDH shows that normal gastrocnemius muscle in the rat contains a mixture of fibers which have either a low (pale staining) or high (dark staining) oxidative capacity (fig.4.13.A) while after sciatic nerve crush in adult virtually all the fibers in the reinnervated muscle stain darkly (fig.4.13.B). This result indicated that following nerve crush in adult, although there is no motoneurone loss, reinnervated muscle fiber type has changed.

Fig.4.11.

Cross sections of spinal cord processed to demonstrate HRP and counter-stained with gallocynin. HRP had been injected into the gastrocnemius and soleus muscles of both sides of animals 6 weeks after left sciatic nerve crushed at adulthood. (A) is the photograph taken under low magnification (2.5x1.6) showing both side of the spinal cord. The HRP labelled cells are located on both sides of dorsolateral part of the anterior horn. The hole pointed by the finger in the dorsal horn indicates the uninjured side contralateral to the nerve crush. (B) is the photograph taken under high magnification (10x2) from the same section as in (A) showing the close view of the labelled G-S motoneurones of injured side. The scale for the bar in (A) is 500μm and in (B) is 100μm

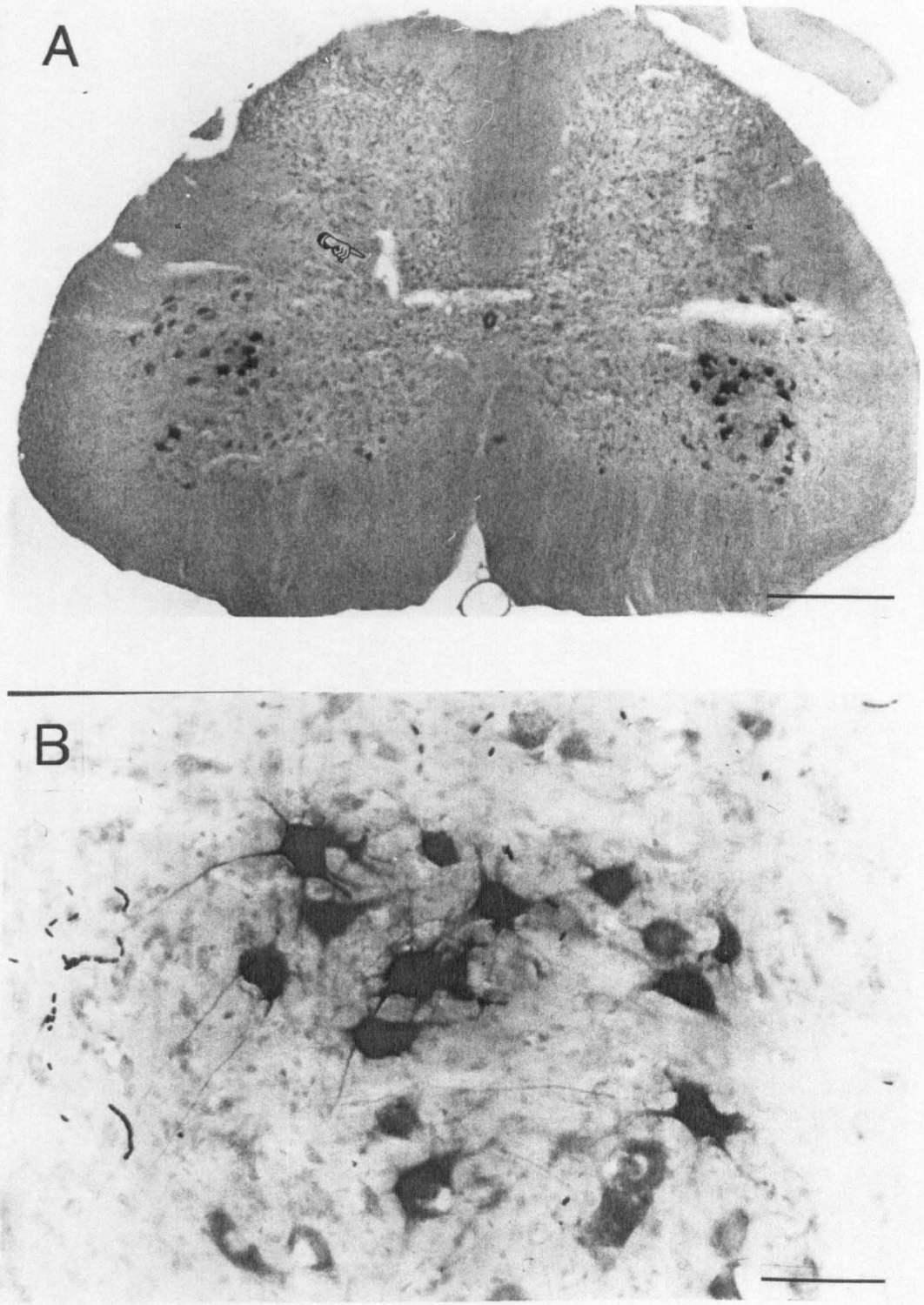
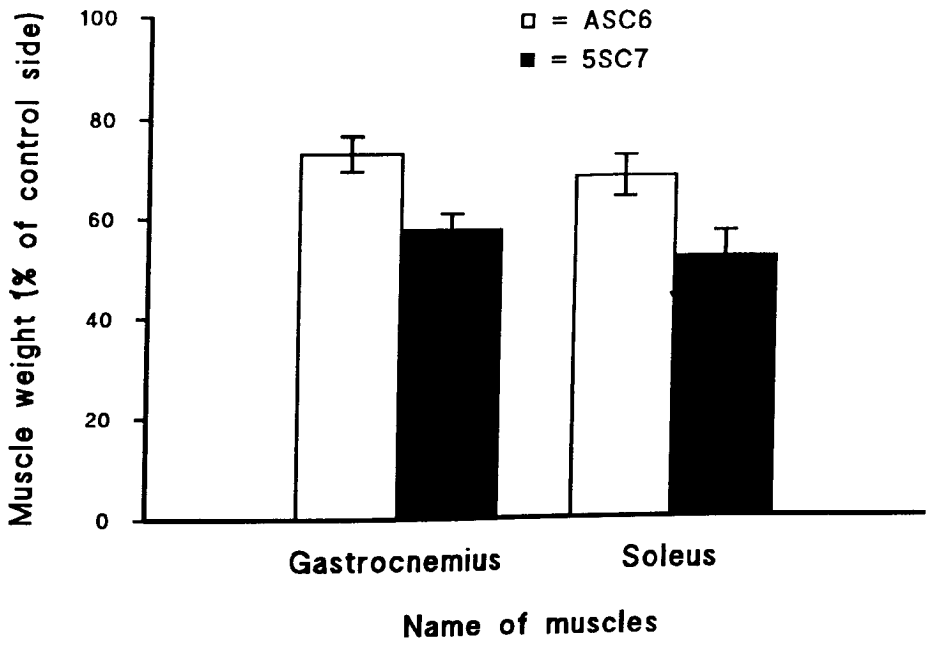

A
B

Fig. 4.12.

Bar diagrams showing the gastrocnemius and soleus muscle weights (A) and motoneurone number of the gastrocnemius pool (B) in both adult and 5-day-old sciatic nerve crush rats. In (A), sciatic nerve crush side G and S muscle weight was expressed as the percentage of contralateral control side corresponding muscle weight. The open bar were used as for adult nerve crush and filled bar for 5-day-old nerve crush rats. In (B), bar diagram showing the gastrocnemius motoneurone number on nerve crush side motor pool (filled bar) and in the contralateral control side (open bar). The neurone number come from normal animals was illustrated at first bar at left.

(A)


□ = ASC6
■ = 5SC7
Muscle weight (% of control side)
100
80
60
40
20
0
Gastrocnemius
Soleus
Name of muscles

(B)

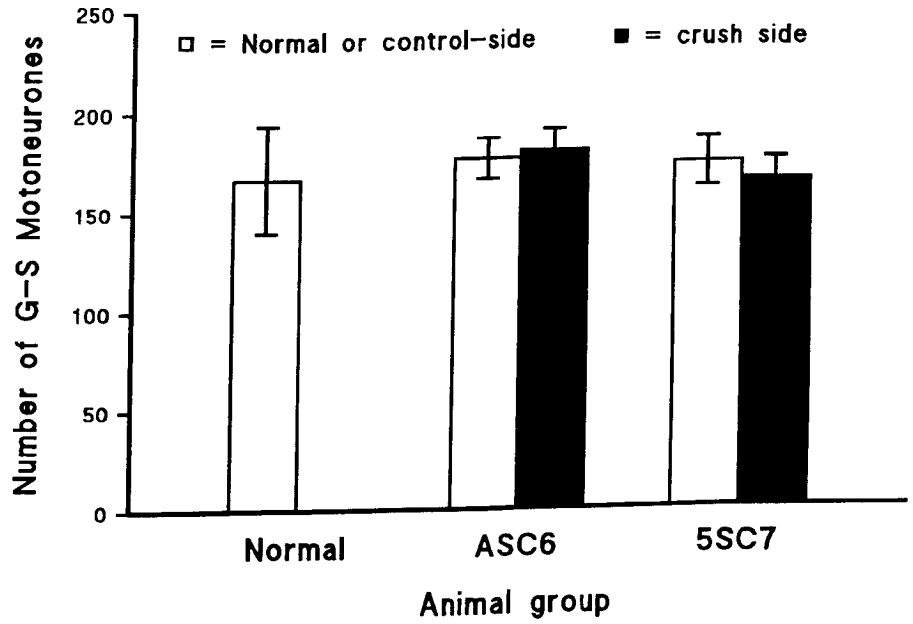
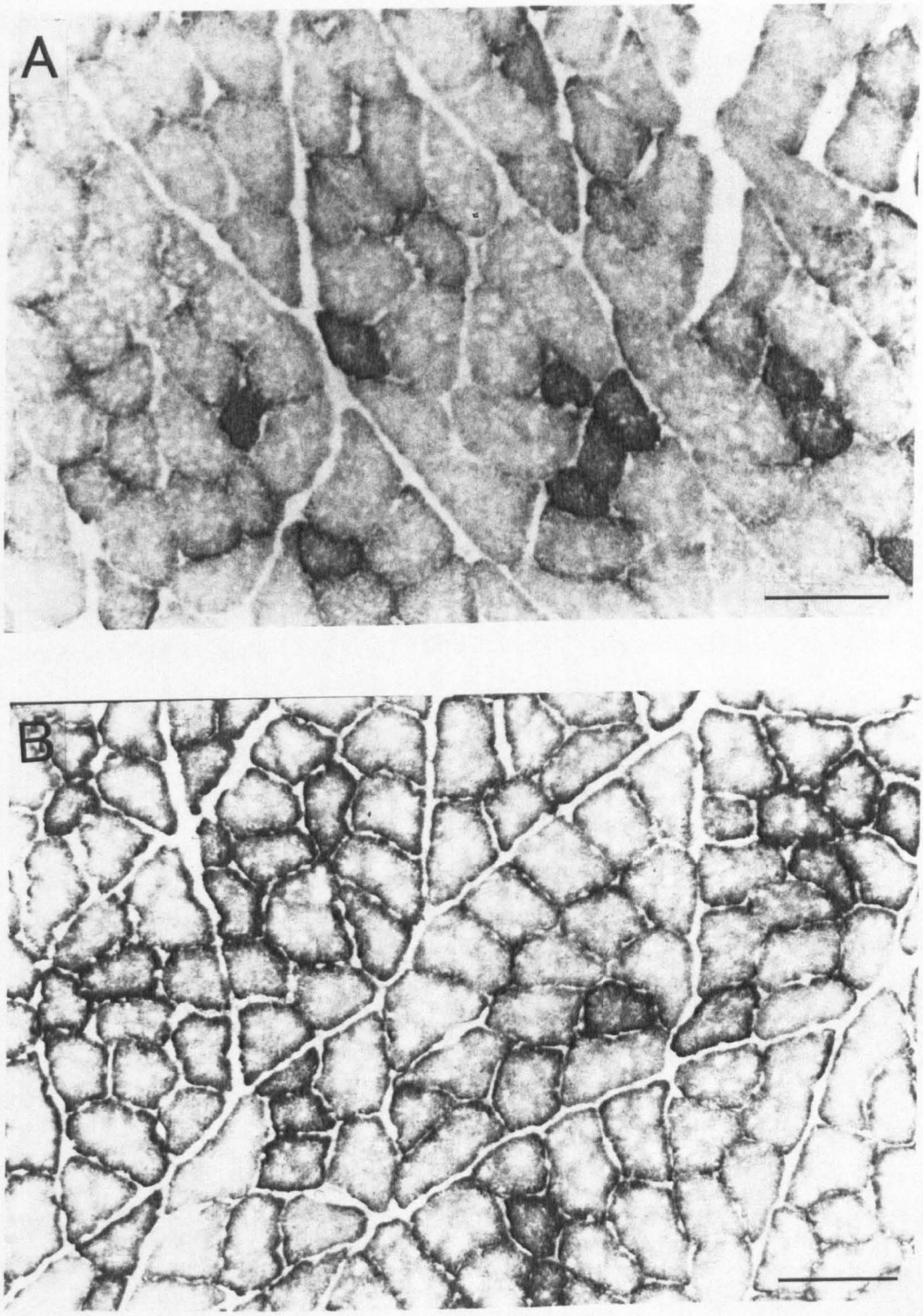

□ = Normal or control-side
■ = crush side
Number of G-S Motoneurones
250
200
150
100
50
0
Normal
ASC6
5SC7
Animal group

Fig.4.13.

Examples of cross-sections from reinnervated (B) and contralateral control (A) gastrocnemius muscles from adult sciatic nerve crush rat stained for succinic dehydrogenase (SDH). The muscles were taken 6 weeks after sciatic nerve crush in adult. SDH staining showing the oxidative activity of muscle fibers in either reinnervated or normal muscles. The scale for the bar in both (A) and (B) is 80μm.


A
B

4.6. Summary

The results presented in this chapter were based on the experiments carried out in rats in which the sciatic nerve or one of its branches had been previously crushed and the reinnervation was not prevented. In this preparation, monosynaptic reflexes generated from regenerated motoneurones are as large as those from normal motoneurones.

Following nerve crush on sciatic nerve in adult rat, there were different responses of injured motoneurones in generating and receiving recurrent inhibition. Before full regeneration, recurrent inhibition was nearly completely lost. When regeneration was complete, recurrent inhibition partially recovered from the initial large reduction but was still markedly depressed and there was no further recovery been observed.

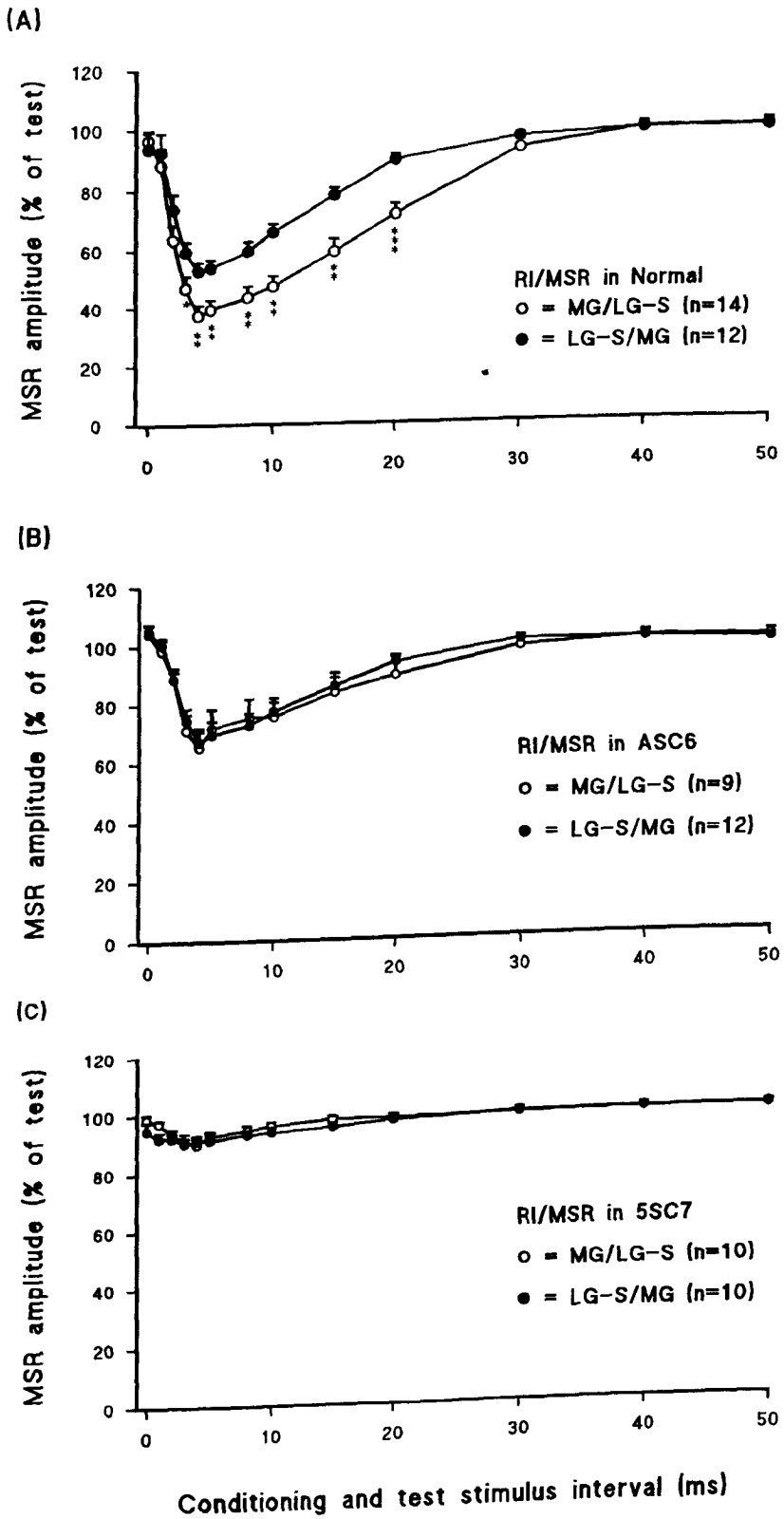
The depressive effect of peripheral nerve crush on recurrent inhibition seen in sciatic and tibial nerve crush preparations could not been detected in LG-S nerve crush animals after regeneration. Since the neurone-muscle separation time in LG-S nerve crush preparation was much shorter than that crush either on sciatic or tibial nerve, this result indicated that LG-S nerve crush left the neurone-muscle disconnected for such a short time that there was no time for irreversible changes to develop and recovery was therefore much better.

It is of interest to find that the original difference in recurrent inhibition between different motor pools have been "dedifferentiated" as a result of adult nerve crush (Fig.4.14). Following sciatic nerve crush in adult, recurrent inhibition from MG to LG-S was as large as that from LG-S to MG (Fig.4.14.B), in which in normal rat, recurrent inhibition from MG to LG-S was significantly larger than that from LG-S to MG (Fig.4.14.A). This dedifferentiation occurred in recurrent inhibitory pathway may be in association with the progress of motor unit properties dedifferentiation following nerve injury (Kuno et al., 1974b; Foehring et al., 1986a,b).

It is concluded that nerve crush in adult rat results in a permanent reduction in recurrent inhibition and this impairment depends upon the length of time that the motoneurones are separated from their targets.

Fig.4.14.

Dedifferentiation of recurrent inhibition following peripheral nerve injury. Recurrent inhibitory curves show that the different effects of recurrent inhibition from MG to LG-S and from LG-S to MG in normal rats (A) have been dedifferentiated after adult (B) and neonatal (C) sciatic nerve crush.


(A)
MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
RI/MSR in Normal
o = MG/LG-S (n=14)
● = LG-S/MG (n=12)
(B)
MSR amplitude (% of test)
RI/MSR in ASC6
o = MG/LG-S (n=9)
● = LG-S/MG (n=12)
(C)
MSR amplitude (% of test)
RI/MSR in 5SC7
o = MG/LG-S (n=10)
● = LG-S/MG (n=10)
Conditioning and test stimulus interval (ms)

CHAPTER 5. RESULT III.
RECURRENT INHIBITION OF MSR OF REGENERATED MOTONEURONES AFTER A NERVE CRUSH IN 5-DAY-OLD POSTNATAL RATS

5.0. Introduction

All the animals used in these experiments had their left hindlimb nerve crushed at 5-day of age. Recurrent inhibition of MSRs of regenerated motoneurones was examined 7 to 30 weeks after sciatic nerve crush. Rats with tibial and lateral gastrocnemius and soleus nerve crushed at 5-day old were also studied. Motoneurones and muscles following nerve injury were tested after regeneration has completed.

5.1. Spinal monosynaptic reflexes

7 weeks after nerve crush performed on sciatic nerve at 5-day of age, monosynaptic reflexes evoked by stimulating dorsal roots L4, L5 and L6 were recorded from the regenerated LG-S and MG muscle nerves. In figure 5.1., photographs taken from storage oscilloscope demonstrated the evoked monosynaptic reflexes from LG-S (left) and MG (right) pools in this preparation. The test stimulus on dorsal roots was at time 0 and the conditioning stimulus on motor axons, if it was applied, was prior to the test stimulus which was not showed on the picture. Figure 5.1.A illustrated the typical unconditioned monosynaptic reflexes recorded from LG-S and MG nerves. Monosynaptic reflexes generated from pre-injured LG-S and MG motoneurones had a latency of about 2.80 ± 0.10 msec (mean$\pm$SEM, n=10) and 2.90 ± 0.08 msec (mean$\pm$SEM, n=10) and an amplitude of about 1.82 ± 0.19 mV (mean$\pm$SEM, n=10) and 3.54 ± 0.42 mV (mean$\pm$SEM, n=10) (table 5.1 and Fig.4.2). Compared with results obtained from normal adult rats, both latency and amplitude of MSRs obtained from rats with regenerated nerves after a sciatic nerve crush at 5-day of age were significantly increased ($P < 0.01$, t-test) (Fig.4.2).

The monosynaptic reflexes, in these experimental animals, evoked by stimulating dorsal roots and recording in one of the regenerated muscle nerves, could be slightly inhibited by a preceding antidromic volleys in one of its synergist muscle nerves which was also

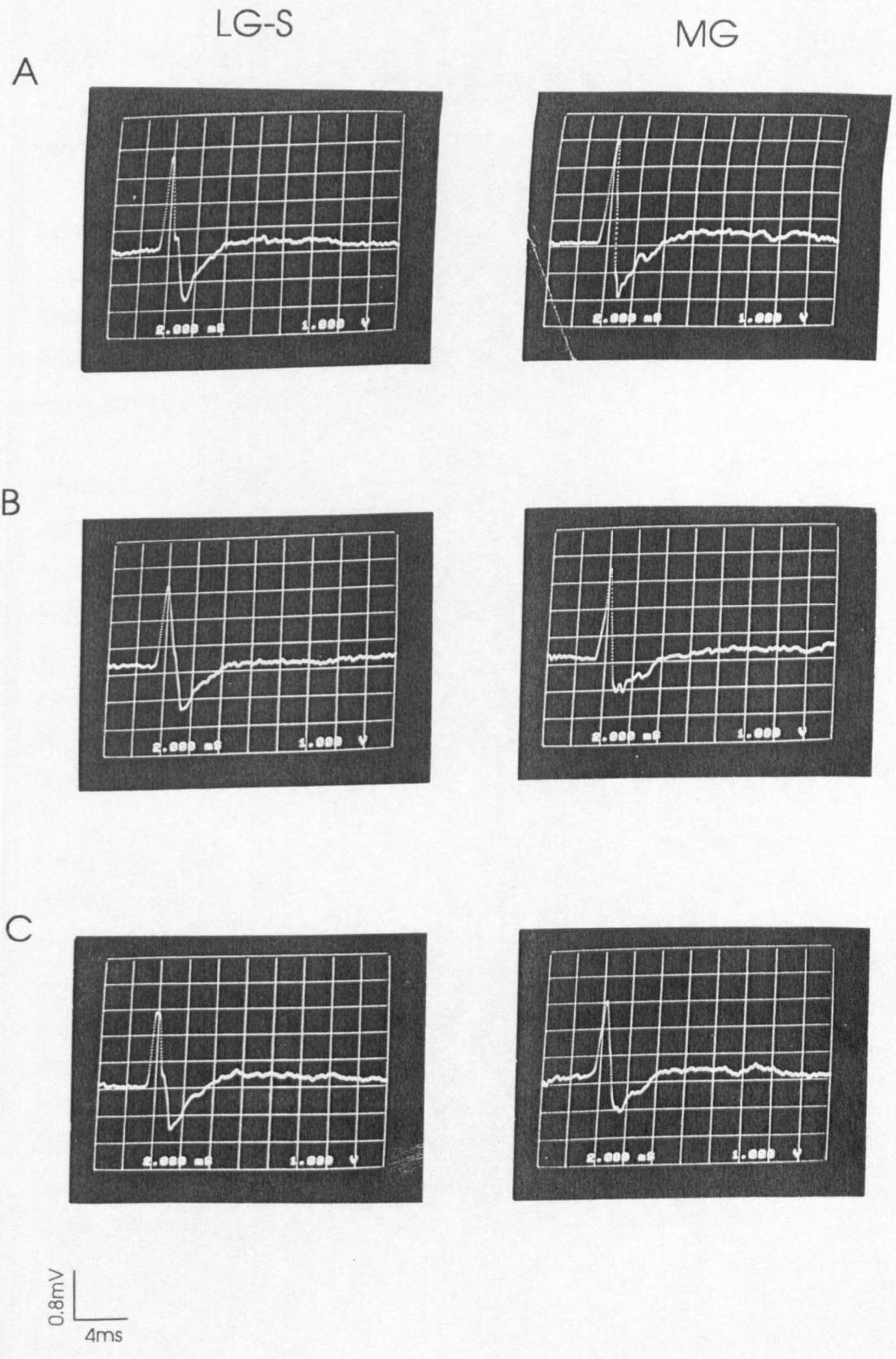
regenerated from previous sciatic nerve crush at 5-day of age. Figure 5.1.B and C show conditioned monosynaptic reflexes on LG-S (left) and MG (right) nerves with reduced amplitude (B) and maximum reduction of monosynaptic reflex amplitude (C) at test-conditioning stimuli interval 15 msec and 4 msec respectively. The reduction of the monosynaptic reflex amplitude following antidromic volley delivered into spinal cord was much smaller in this preparation than those in normal rats. The results indicated that following nerve crush performed on sciatic nerve at 5-day of age, monosynaptic reflexes generated from regenerated motoneurones not only had increased amplitude, but wee difficult to be influenced by recurrent inhibitory inputs.

Table 5.1 Amplitude and latency of MSR in LG-S and MG nerves in rats with sciatic nerve crush at 5-day of age

Nerve	Latency of MSR (mean±SEM, ms; (range))	Amplitude of MSR (mean±SEM, mv; (range))
LG-S	2.8±0.10 (2.4-3.2) (n=10)	1.82±0.19 (0.94-3.19) (n=10)
MG	2.9±0.08 (2.5-3.3) (n=10)	3.54±0.42 (1.41-5.75) (n=10)

Fig.5.1.

Photographs showing the monosynaptic reflexes elicited by stimulating dorsal roots and recorded from LG-S (left) and MG (right) nerves in 5-day old sciatic nerve crush rat. A. shows the unconditioned reflexes, B. shows conditioned reflexes with reduced amplitude, and C. shows the maximal reduction of reflex amplitude as a result of recurrent inhibition. In each figure, the test stimulus was at time 0 and the conditioning stimulus for (B) and (C) was 15 and 4 msec prior to test stimulus. Note the degree of the reduction of MSR amplitude is much small in this preparations.


LG-S
MG
A
B
C
0.8mV
4ms

5.2 Recurrent inhibition of regenerated motoneurones after sciatic nerve crush at 5-day of age

At least 7 weeks postoperatively, the rats in which the sciatic nerve was crushed at 5-day of age were re-used for acute electrophysiological experiments to examine the changes of recurrent inhibition as a result of postnatal peripheral nerve crush.

5.2.1 Recurrent inhibition of MSRs between regenerated LG-S and MG motoneurones

Rats with sciatic nerve crushed at 5-day postnatally were divided into two groups according to the regeneration period. One group contained animals for acute electrophysiological experiments 7 weeks postoperatively and the other group was 30 weeks postoperatively.

7 weeks after sciatic nerve crush performed at 5-day of age, recurrent inhibition between MG and LG-S motoneurones were tested in 10 rats. Recurrent inhibition either from MG to LG-S motoneurones or from LG-S to MG motoneurones caused the peak suppression of the amplitude of conditioned monosynaptic reflexes to $89.9\pm2.0\%$ (mean$\pm$SEM, $n=10$) and $90.5\pm1.8\%$ (mean$\pm$SEM, $n=10$) of the size of unconditioned MSRs (Fig.5.2). The test-conditioning stimuli interval was at 4 ms when recurrent inhibition matched its maximal. The duration of recurrent inhibition in this preparations was only about 15-20 msec (Fig.5.2. and table 5.2).

Compared with the recurrent inhibition obtained from normal decerebrate rats in the same RI/MSR combinations in which recurrent inhibition either from MG to LG-S or from LG-S to MG (dotted line in Fig.5.2), the mean values of maximal reduction of MSRs amplitude as a result of recurrent inhibition between regenerated LG-S and MG motoneurones were all significantly ($P<0.001$, t-test) smaller than those from normal animals. The results of statistical analysis were shown in figure 5.2. The duration of recurrent inhibition in this preparation was also much smaller than that in normal animals. The test-conditioning stimuli interval time to get to maximal MSR depression, however, had the same value as that in normal rats. These results indicated that when the nerve crush was applied to sciatic nerve at its early postnatal life, the recurrent inhibition could be depressed dramatically even when

the regeneration has completed. In this case, the strength of recurrent inhibition was only about 20.2% of the size of normal recurrent inhibition from LG-S to MG and 16.1% of size of normal value from MG to LG-S.

A group of rats with sciatic nerve crush at 5-day of age were allowed to survive for 30 weeks before testing the effect of nerve crush on recurrent inhibition between MG and LG-S motoneurone pools. In this preparation, a successive reduction in the amount of recurrent inhibition has been observed in which recurrent inhibition from MG to LG-S and from LG-S to MG was only capable of reducing the amplitude of MSRs to about 92.0±2.6% (mean±SEM, n=3) and 94.3±2.0% (mean±SEM, n=2) of the size of unconditioned monosynaptic reflexes (Fig.5.3). In other words, the maximal effects of recurrent inhibition were only 12.8% (MG/LG-S) and 12.0% (LG-S/MG) of the corresponding value of the recurrent inhibition in normal decerebrate rat respectively. The amounts of recurrent inhibition from this preparation were all significantly ($P<0.001$, t-test) smaller than those from normal rats (Fig.5.3 and Fig.5.4). However, although there was a tendency for further reduction of recurrent inhibition 30 weeks after nerve crush compared with results from 7 weeks postoperative rats, they were not different statistically ($P>0.5$ in MG/LG-S, $0.2>P>0.1$ in LG-S/MG, t-test). This indicated that the depressive effect of nerve injury on recurrent inhibition could not recover following regeneration. In other words, this impairment seems permanent.

The maximal effect of recurrent inhibition expressed as a percentage change in MSRs amplitude from both normal and experimental (7 and 30 weeks postoperatively) animals were presented in figure 5.4. The results of statistical analysis (between experimental and normal animals) were shown in this figure as well.

5.2.2 Recurrent inhibition of uninjured control side motoneurones in rats with sciatic nerve crush at 5-day of age

In order to examine whether there is cross effect on recurrent inhibitory pathway, recurrent inhibition of MSRs between LG-S and MG motoneurone pools were examined in the uninjured control side in rats with one side sciatic nerve crushed at 5-day of age. In addition,

since animals in 5SC7 group were considerably younger than ASC6 group and normal rats, the MSRs and RI obtained from contralateral uninjured side in 5SC7 group were compared with those from either normal or ASC6 group animals in order to monitor some age related change in generating MSRs and RI.

Monosynaptic reflexes evoked by stimulation of uninjured control (right) side dorsal roots L4, L5, and L6 and recorded from ipsilateral LG-S and MG muscle nerves had a latency of about 2.23±0.03 ms (mean±SEM, n=6) and 2.20±0.03 ms (mean±SEM, n=7) and an amplitude of about 1.42±0.26 mv (mean±SEM, n=6) and 1.44±0.12 mv (mean±SEM, n=7). The results of MSRs latency are in good agreement with those obtained from both normal and ASC6 group rats. However, the amplitude of MSRs is greater than those obtained from normal rats but not those from ASC6 groups.

Table 5.2 Summary of recurrent inhibition of MSRs after sciatic nerve crush at 5-day old

RI/MSR	Number of rat	Post operative time (weeks)	T-C interval to Max. RI (ms)	Max. RI (% of MSR reduction) (mean±SEM)	RI duration (ms)
MG/LG-S	10	7	4	10.1±2.0%	15
	3	30	4	8.0±2.6%	20
control	6	7	5	70.1±8.4%	40
LG-S/MG	10	7	3	9.5±1.8%	20
	2	30	5	5.7±2.0%	10
control	7	7	5	57.3±6.4%	30

Recurrent inhibition of MSRs of uninjured side MG motoneurones reduced ipsilateral LG-S monosynaptic reflexes amplitude to 29.7±8.4% (mean±SEM, n=6) of the size of unconditioned test reflexes. Also, LG-S motoneurones could reduce MG monosynaptic

reflexes amplitude to 42.7±6.4 (mean±SEM, n=7) of the size of unconditioned test MG reflexes (Figure 5.5). Compared with the corresponding value in normal rats, although the mean amount of recurrent inhibition showed a tendency to increase in both MG/LG-S and LG-S/MG pair, they were not statistically different ($0.5 > P > 0.4$ in MG/LG-S, $0.2 > P > 0.1$ in LG-S/MG, t-test) between the normal animals and control side of experimental animals. In contrast, the mean value of recurrent inhibition between MG and LG-S motor pools from injured and control side in experimental rats differed statistically ($P < 0.001$, t-test). These results indicated that nerve injury in one hindlimb, although causing a reduction in recurrent inhibition on this side, does not affect the recurrent inhibitory pathway on the other side. These results also suggested that the massive reduction of RI and increasing of MSR amplitude were not because of the younger animals used in 5SC7 group rather than the effect of nerve crush on sciatic nerve early at 5-day of age.

5.2.3 Effect of motoneurone pool excitability on spinal monosynaptic reflexes and recurrent inhibition

Monosynaptic reflexes elicited in 5-day old sciatic nerve crush rats were found to be much bigger than those in normal rats (see section 5.1). It has been reported that a diminished monosynaptic excitation of the motoneurones might enhance the recurrent inhibition (Mellstrom, 1971a,b). Also, evidence suggested that when the amplitude of the MSRs was at a very high level, the recurrent inhibition would be totally "blocked" (Crone et al., 1990). Thus, one question raised is whether the dramatic reduction of the recurrent inhibition seen in this regenerated preparation was simply because the increased monosynaptic reflex amplitude over ride the inhibitory effect generated by Renshaw inhibitory pathways. In order to get rid of this possibility, recurrent inhibition between MG and LG-S motor pools were studied when monosynaptic reflexes were elicited either supramaximum or submaximum.

It has been well known that not only muscle but also nerve can be maximally excited when the stimulus strength are up to 5 times its threshold. During the experiments in this study, it was also found that the amplitude of monosynaptic reflexes could reach maximum value while the test stimulus strength was up to 5 times the threshold. In this section, experiments in rats with sciatic nerve crush at 5-day of age were tested in three series in

nerve crush at 5-day of age were tested in three series in which the test stimuli applied on dorsal roots were with strength at 5, 2.5, and 1.5 times threshold (T) in order to generate different magnitude of the monosynaptic reflexes respectively.

The mean amplitude of monosynaptic reflexes recorded from LG-S and MG nerves in which elicited by stimulating dorsal roots L4, L5 and L6 at different stimulus strengths which were 5xT (5 times threshold), 2.5xT and 1.5xT are displayed in figure 5.6.A and table 5.3. Briefly, between the threshold and supramaximal, the bigger the stimulus strength, the larger the monosynaptic reflexes amplitude. If taking the monosynaptic reflexes in the 5xT group as supramaximal as one hundred percent, the monosynaptic reflex amplitude recorded in 2.5xT group were 94.1% (MG) and 89.0% (LG-S) and in 1.5xT group were 65.0% (MG) and 56.6% (LG-S) of the corresponding value of 5xT group (Fig.5.6.B). The amplitude of monosynaptic reflexes were not different statistically ($P > 0.4$, t-test) between those evoked with 5xT and 2.5xT groups, although there was a tendency to decrease in magnitude of monosynaptic reflexes when test stimulus strength was 2.5xT. When the test stimulus strength was only 1.5xT, monosynaptic reflexes decreased to significantly ($P < 0.01$, LG-Sol; $P < 0.02$, MG; t-test) smaller than those from 5xT group. Compared with the result that MSR generated with supramaximal test stimulation in normal rats, the amplitude of MSRs in 1.5xT group of LG-S motor pool was not different statistically from that in normal. Although, in MG motor pool, the amplitude of MSRs in 1.5xT group was still bigger than that in normal, the difference was getting less.

In order to examine whether the amount of recurrent inhibition will increase in relation to the monosynaptic reflex amplitude decrease, recurrent inhibition was tested with the test stimulus strength changed as above described (5xT, 2.5xT, and 1.5xT) and with a constant supramaximal (5xT) conditioning input volley delivered into spinal cord.

The results of recurrent inhibition with three different test stimulus strength are displayed in figure 5.7 and table 5.3. The magnitude of recurrent inhibition from MG to LG-S, tested as MSRs reduction, induced by supramaximal antidromic stimulation of MG nerve and with test stimulus at 5xT, 2.5xT, and 1.5xT on dorsal roots were $10.1 \pm 2.0\%$ (mean$\pm$SEM, n=10), $8.0 \pm 1.8\%$ (mean$\pm$SEM, n=10), and $14.5 \pm 3.5\%$ (mean$\pm$SEM, n=10). The recurrent

inhibition from LG-S to MG had the corresponding values as 9.5±1.8% (mean±SEM, n=10), 8.4±1.3% (mean±SEM, n=10), and 16.9±2.7% (mean±SEM, n=10).

Although there is a tendency towards an increased recurrent inhibitory effect in group with 1.5xT test stimulus strength compared to those in 2.5xT and 5xT groups, they were not statistically different ($P>0.05$, t-test) in the amount of recurrent inhibition between MG to LG-S. In contrast, the maximal recurrent inhibitory effect were significantly bigger ($P<0.05$,t-test) in 1.5xT group than those in either 5xT or 2.5xT groups. However, all the figures from these three groups, compared with the recurrent inhibition in either normal or adult sciatic nerve crush rats, were much much smaller ($P<0.001$, t-test).

This result may indicate that the degree of recurrent inhibition to a given motor pool is, at least in part, related to the degree of the excitability of the motoneurones in the pool. The lower motoneuronal excitability, the stronger recurrent inhibition is. However the increasing in recurrent inhibition after MSR amplitude reduction were limited. For example, the maximal recurrent inhibition from MG to LG-S in 1.5xT group was still only 23% of the normal RI value and even the RI from LG-S to MG was only 34% of the normal value. In contrast, the amplitude of MSR of LG-S in 1.5xT group was as big as in normal rats. The amplitude of MSR of MG motor pool also decreased largely in 1.5xT group. Taking the results of MSR amplitude and recurrent inhibition together indicates that the increased amplitude in neonatal regenerated motoneurones could not be counted as a main factor contributing to the dramatic reduction of the recurrent inhibition seen in 5-day old sciatic nerve crush rats.

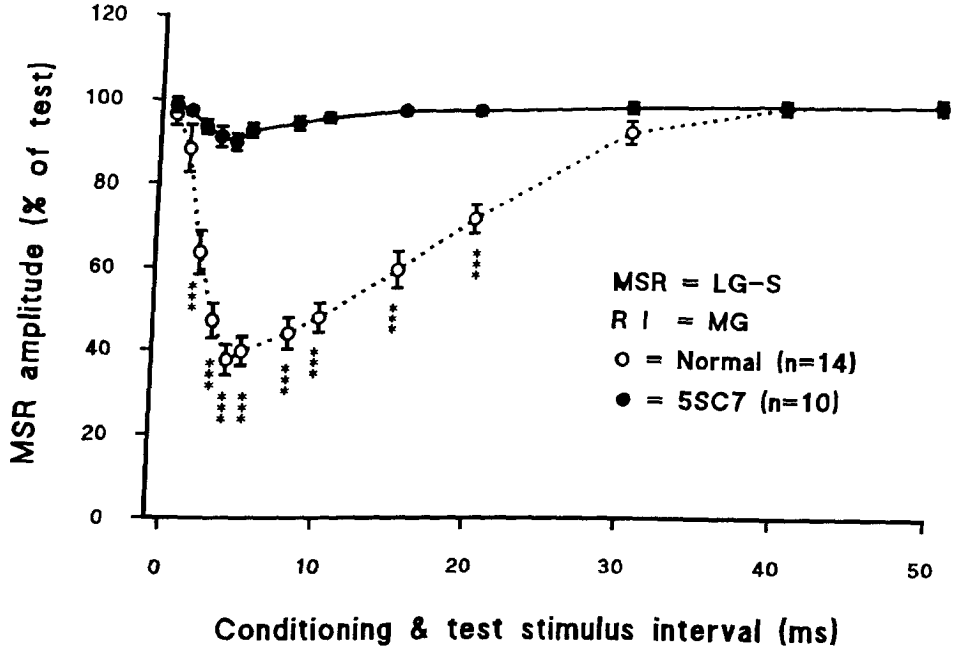
Table 5.3 Changes of MSR and RI in different test stimulus strength

RI/MSR	Test stimuli strength (Xt)	Rats number (n=)	Amplitude of MSR (mean ± SEM) (mV)	% 5xT	Max. RI (% of MSR reduction) (mean±SEM)
MG/LG-S	5xT	10	1.82±0.19	100%	10.1±2.0%
	2.5xT	10	1.62±0.20	89.0%	8.0±1.8%
	1.5xT	10	1.03±0.16	56.6%	14.5±3.5%
LG-S/MG	5xT	10	3.54±0.42	100%	9.5±1.8%
	2.5xT	10	3.33±0.38	94.1%	8.4±1.3%
	1.5xT	10	2.31±0.28	65.0%	16.9±2.7%

Fig. 5.2.

Recurrent inhibitory curves illustrate the amount (mean$\pm$SEM) of recurrent inhibition (RI) of monosynaptic reflexes (MSR) in the 5-day-old sciatic nerve crush rats (filled symbols with solid line) 7 weeks after nerve crush. The RI/MSR combinations tested were MG/LG-S (A) and LG-S/MG (B). The Student t-test was employed to compare the difference with the corresponding inhibitory effects in normal animals (open symbols with dotted line). ** = $P<0.01$ and *** = $P<0.001$.

(A)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = LG-S
R I = MG
o = Normal (n=14)
● = 5SC7 (n=10)

(B)

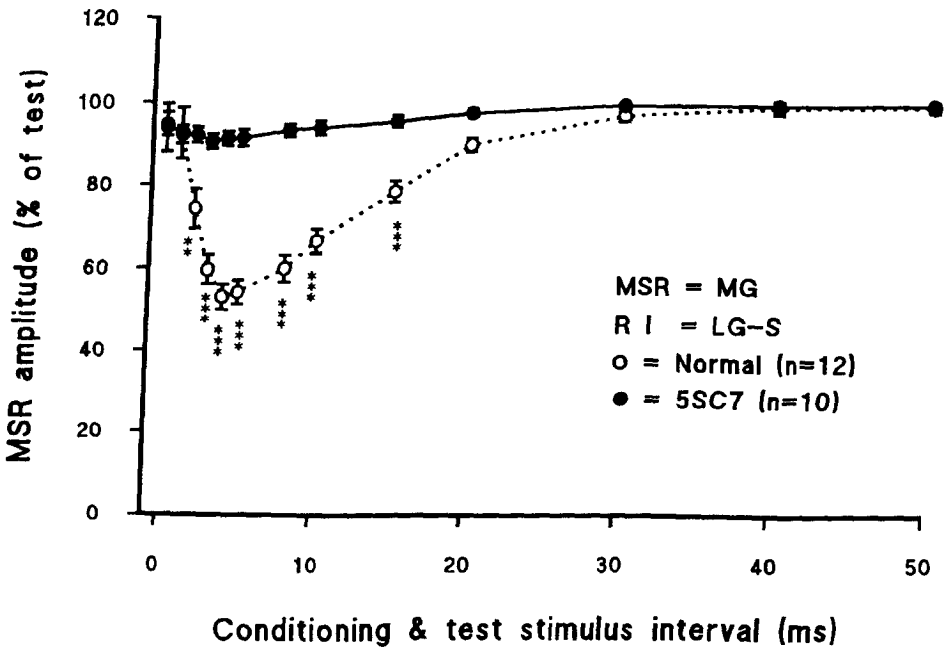
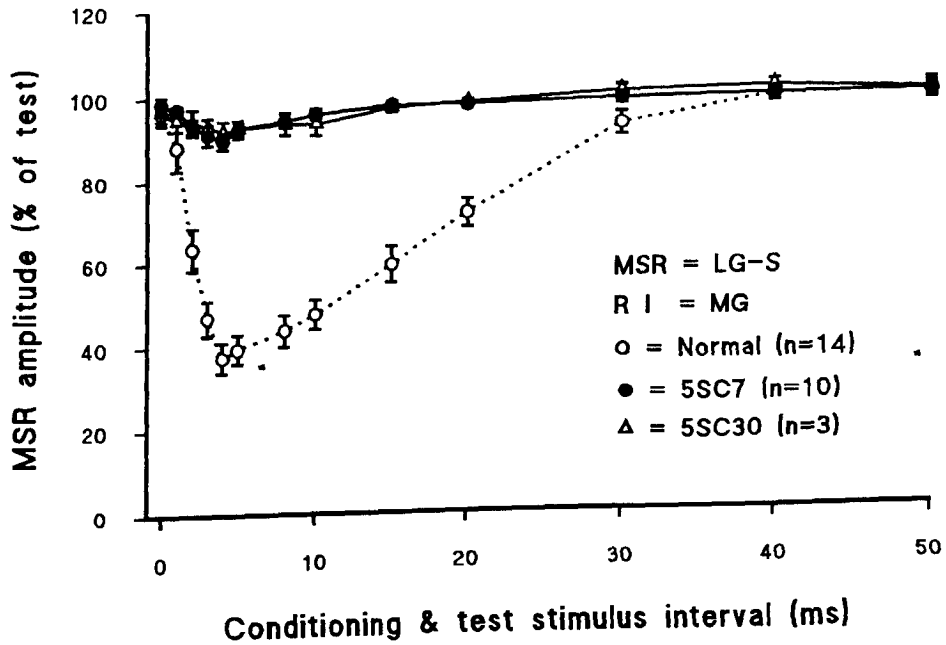

MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = MG
R I = LG-S
o = Normal (n=12)
● = 5SC7 (n=10)

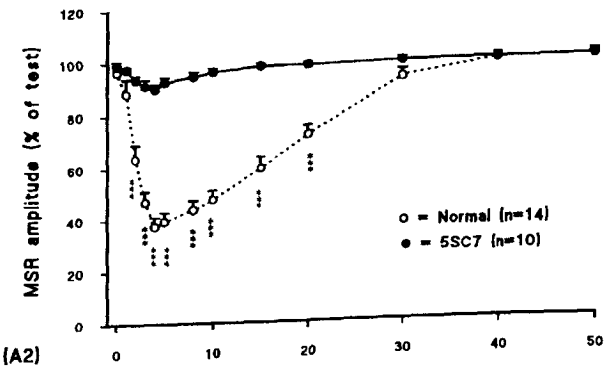
Fig. 5.3.

Recurrent inhibitory curves show the recurrent inhibition of MSRs from MG motoneurones to LG-S motoneurones (A) and from LG-S to MG (B) in 5-day-old sciatic nerve crush rats. The recovery time course of RI were tested 7 weeks (filled circles) and 30 weeks (open triangles) after nerve crush. The corresponding normal curve (open circles and dotted line) is also presented as control. The small diagrams (A1 and A2; B1 and B2) showing at bottom illustrate the statistic difference between experimental and normal values (t-test), ** = $P<0.01$, and *** = $P<0.001$. The amount of recurrent inhibition between 7 and 30 weeks postoperatively animals is not differed at all ($P>0.5$).

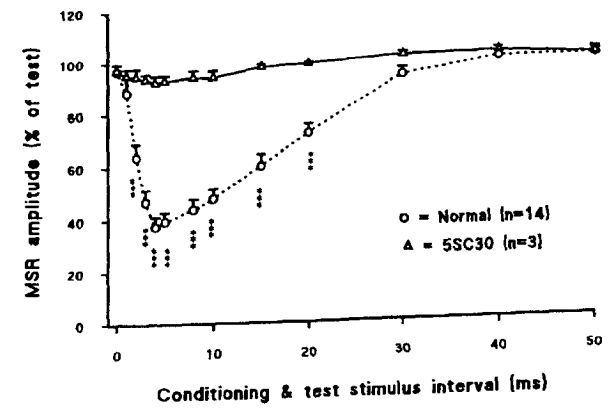
(A)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = LG-S
R I = MG
o = Normal (n=14)
● = 5SC7 (n=10)
Δ = 5SC30 (n=3)

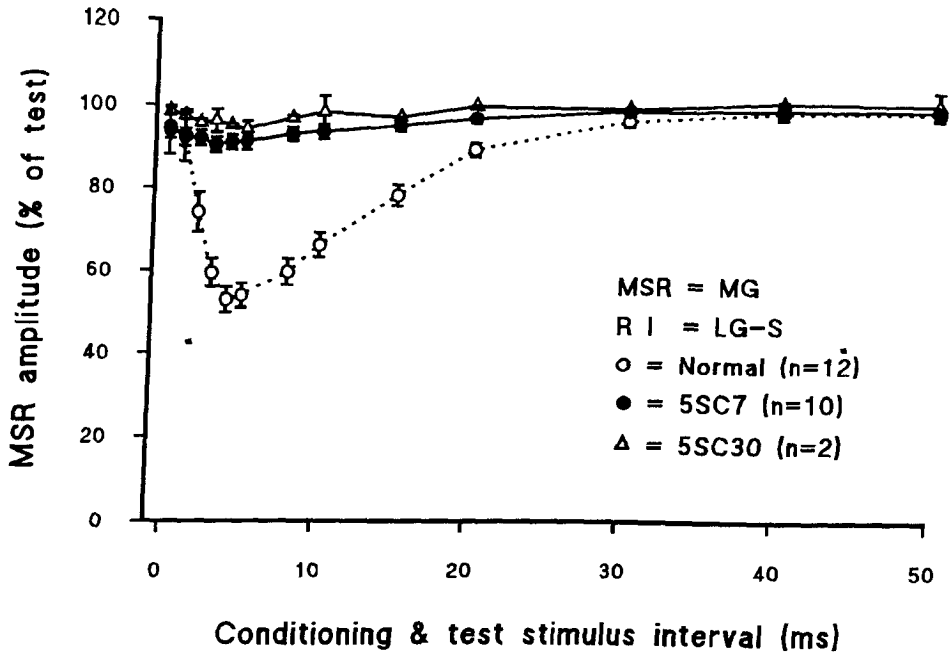
(A1)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
o = Normal (n=14)
● = 5SC7 (n=10)

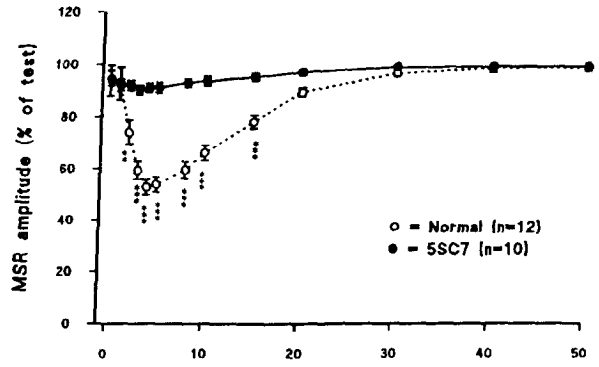
(A2)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
o = Normal (n=14)
Δ = 5SC30 (n=3)
Conditioning & test stimulus interval (ms)

(B)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
MSR = MG
R I = LG-S
o = Normal (n=12)
● = 5SC7 (n=10)
Δ = 5SC30 (n=2)
Conditioning & test stimulus interval (ms)

(B1)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
o = Normal (n=12)
● = 5SC7 (n=10)

(B2)

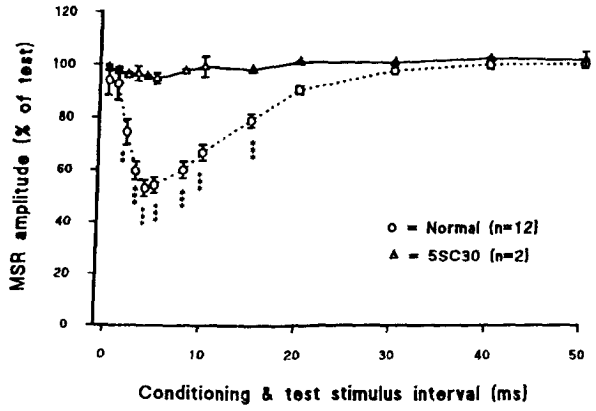

MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
o = Normal (n=12)
Δ = 5SC30 (n=2)
Conditioning & test stimulus interval (ms)

Fig. 5.4.

Bar diagram showing the recovery time course of the maximal mean amount of recurrent inhibition, expressed as MSR amplitude reduction, 7 weeks and 30 weeks after sciatic nerve crush at 5-day of age. The RI/MSR combinations tested were MG/LG-S (filled symbol) and LG-S/MG (open symbol). *** = $P < 0.001$, two tailed Student t-test.

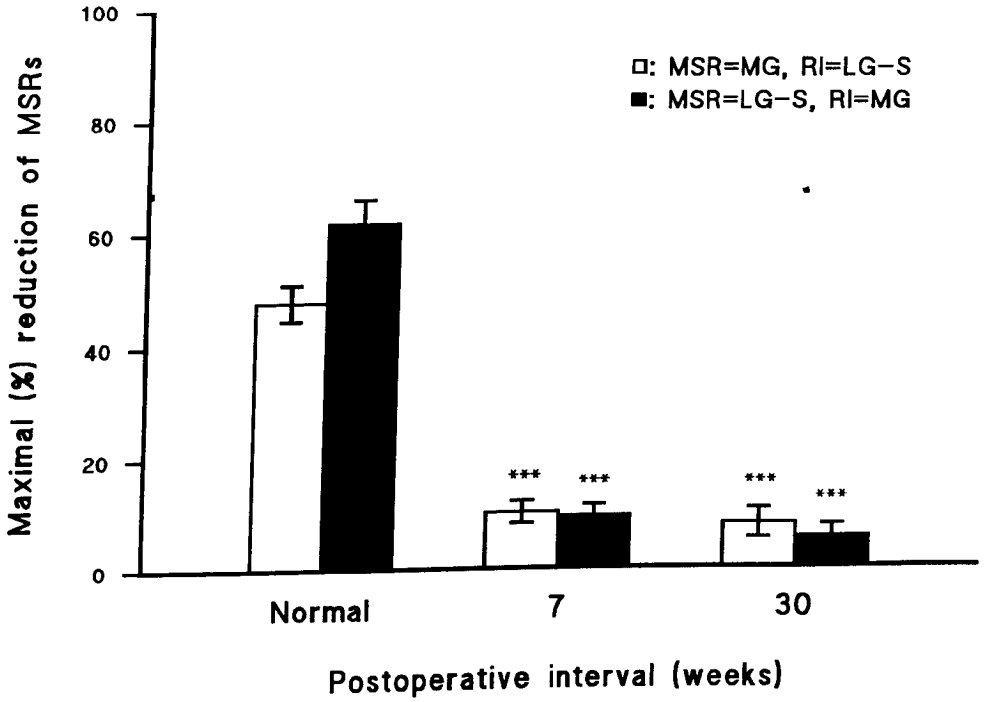
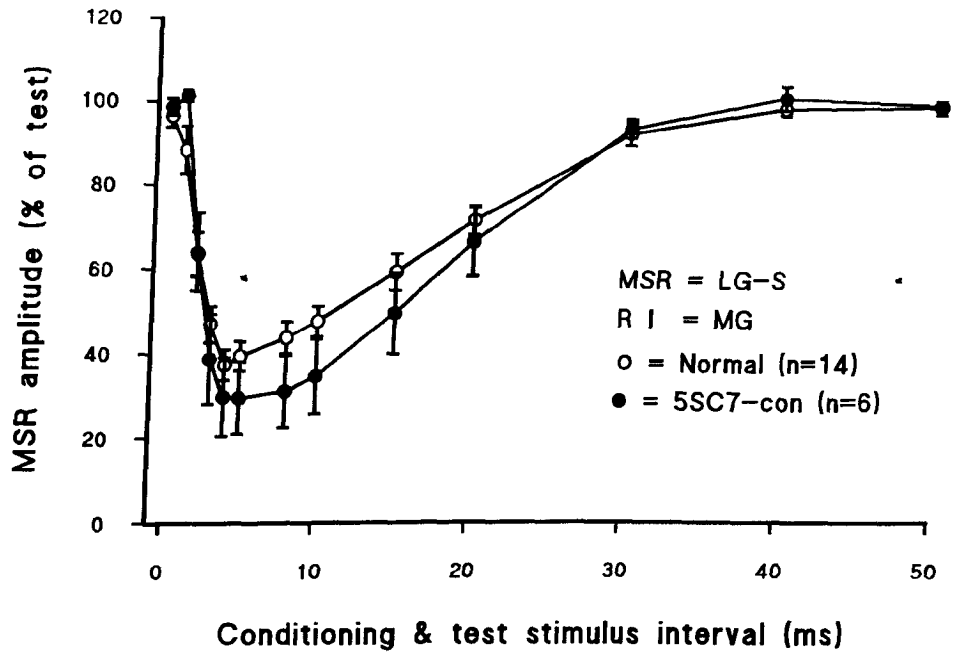

100
80
60
40
20
0
Maximal (%) reduction of MSRs
□: MSR=MG, RI=LG-S
■: MSR=LG-S, RI=MG
***
***
***
***
Normal
7
30
Postoperative interval (weeks)

Fig. 5.5.

Diagrams showing the recurrent inhibition of MSRs in normal and contralateral uninjured side of 5SC7 group rats. Recurrent inhibition were tested with the RI/MSR combinations as MG/LG-S (A) and LG-S/MG (B). There is no statistical difference ($P>0.1$, two-tailed Student t-test) between these two groups of data.

(A)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = LG-S
R I = MG
o = Normal (n=14)
● = 5SC7-con (n=6)

(B)

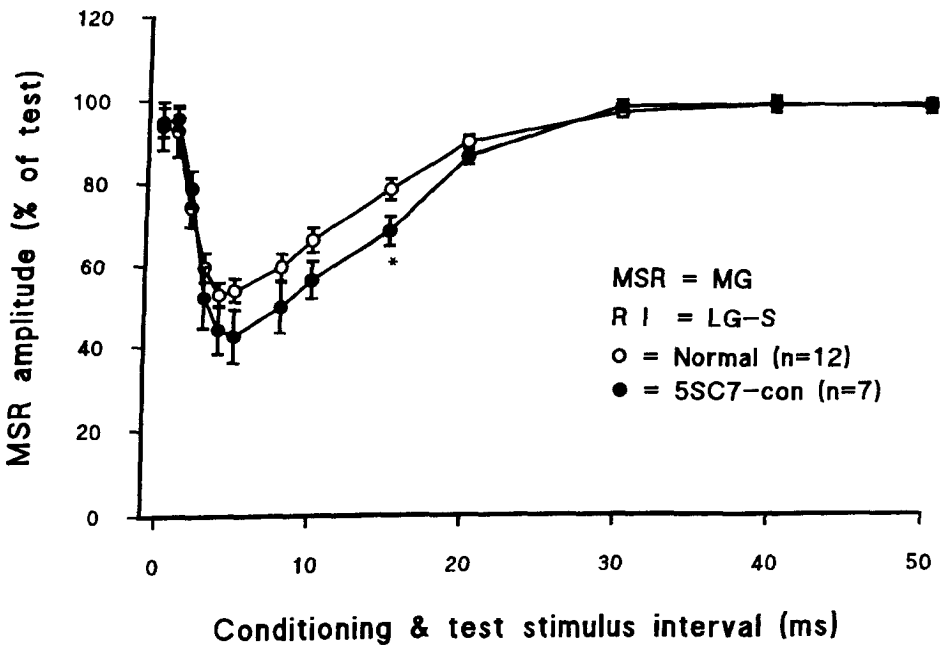
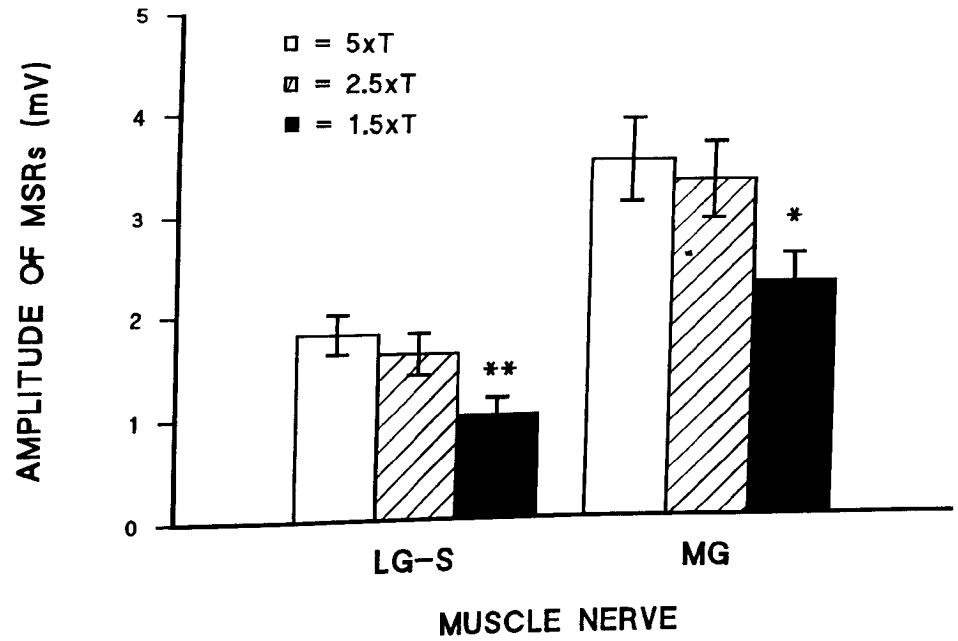

MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = MG
R I = LG-S
o = Normal (n=12)
● = 5SC7-con (n=7)

Fig. 5.6.

Diagrams showing the amplitude of MSRs with different stimulus strength. (A) is the MSR amplitude in mV; (B) is the MSR amplitude in 2.5xT and 1.5xT groups expressed as a percentage of the maximal amplitude value at 5xT. Student t-tests were applied, * = $P<0.05$ and ** = $P<0.01$. Monosynaptic reflex amplitude is significantly reduced when the test stimulus strength is at 1.5 times threshold.

(A)


AMPLITUDE OF MSRs (mV)
5
4
3
2
1
0
□ = 5xT
▨ = 2.5xT
■ = 1.5xT
**
*
LG-S
MG
MUSCLE NERVE

(B)

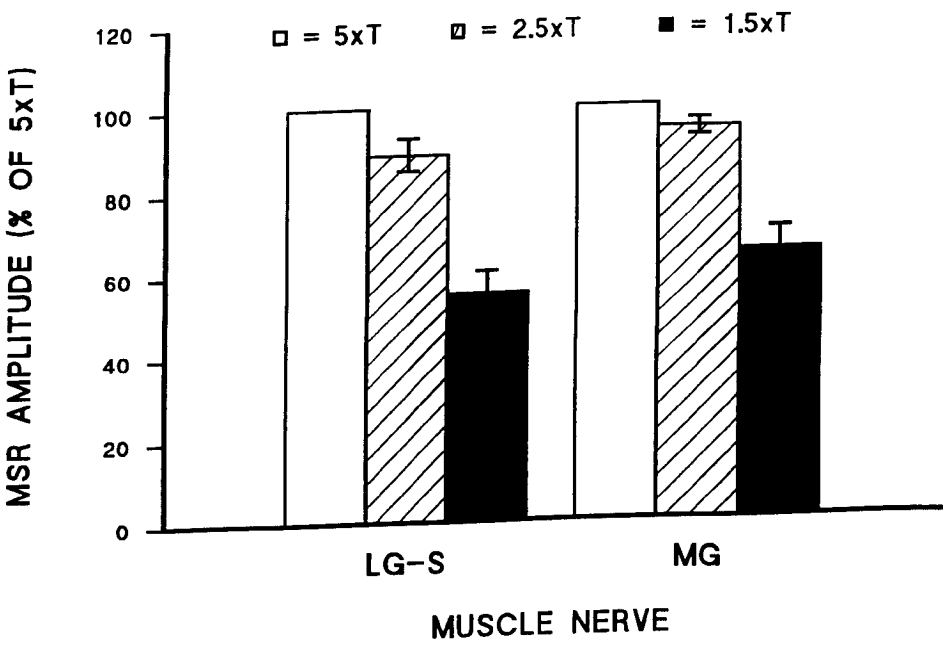
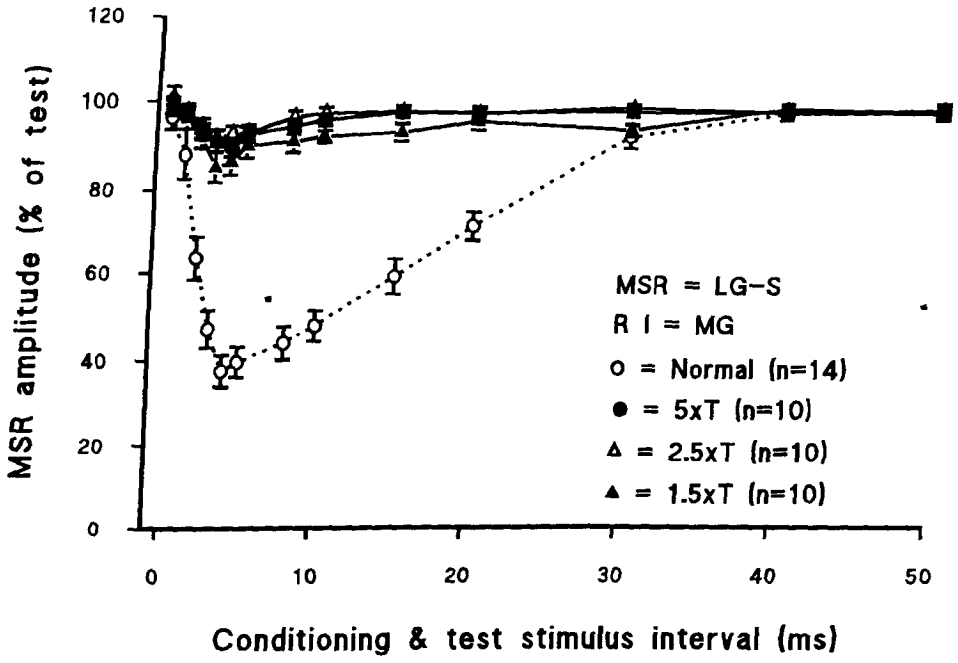

MSR AMPLITUDE (% OF 5xT)
120
100
80
60
40
20
0
□ = 5xT
▨ = 2.5xT
■ = 1.5xT
LG-S
MG
MUSCLE NERVE

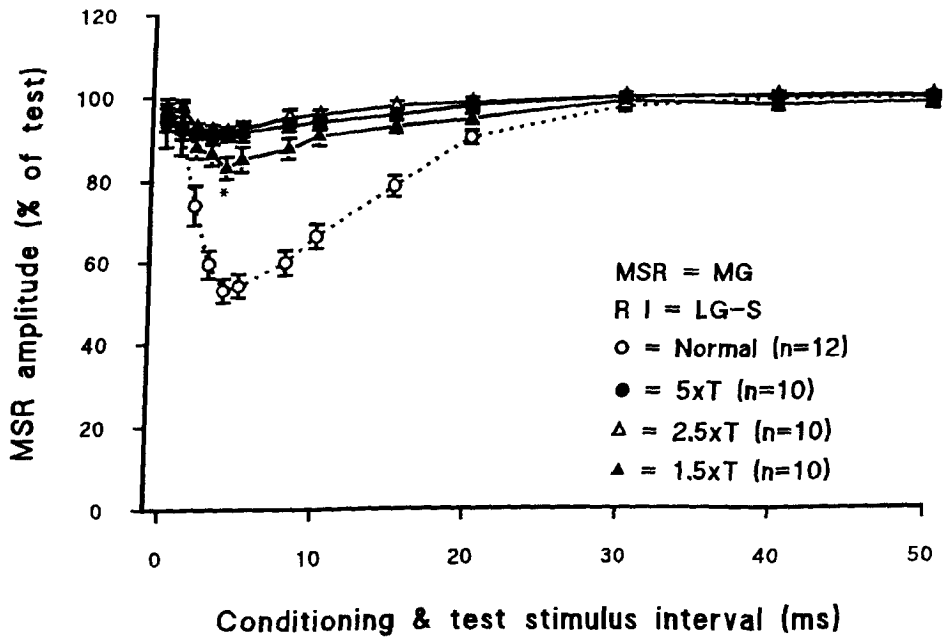
Fig. 5.7.

Recurrent inhibitory curves show recurrent inhibition (RI) of MSRs from MG to LG-S (A) and from LG-S to MG (B), occurring at various levels of test stimulus strength, in 5SC7 group rats. Test stimulus strength to elicit MSRs are 5xT (5 times threshold), 2.5xT and 1.5xT. The conditioning stimulus strength is supramaximal at 5xT. The corresponding recurrent inhibitory curve from normal animals is presented with open circles and dotted line. (* = $P < 0.05$, t-test).

(A)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = LG-S
R I = MG
o = Normal (n=14)
● = 5xT (n=10)
Δ = 2.5xT (n=10)
▲ = 1.5xT (n=10)

(B)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = MG
R I = LG-S
o = Normal (n=12)
● = 5xT (n=10)
Δ = 2.5xT (n=10)
▲ = 1.5xT (n=10)

5.3 Recurrent inhibition between regenerated LG-S and MG motoneurones in 5-day-old tibial nerve crush rat

The depressive effect of nerve crush on recurrent inhibition in adult nerve crush rats was found to depend on the length of time which the neurone-muscle was separated (section 4.4). The longer the period of separation the poorer the recovery. Whether this phenomenon also happens in 5-day-old nerve crush rats has been studied and presented in this and next sections.

In this section, recurrent inhibition was examined in rats with nerve crush performed on tibial nerve at 5-day of age. Nerve crush was carried out on tibial (Tib) nerve where the crush site was below the branching point of perineal and tibial nerves and above the branching point of gastrocnemius-soleus nerves. It was (about 3mm) closer to the muscles than that crushed on sciatic nerve (about 10 mm). It led to the result that the disconnected muscle after tibial nerve crush could be reinnervated in shorter time than that in sciatic nerve crush.

14 weeks after tibial nerve crush, recurrent inhibition of MSRs between regenerated MG and LG-S motoneurones were examined. Recurrent inhibition from MG motoneurones to LG-S motoneurones reduced the amplitude of the MSRs to $62.0 \pm 9.2\%$ (mean$\pm$SEM, n=4) of the size of the unconditioned reflexes. The corresponding value from LG-S to MG was $68.4 \pm 5.3\%$ (mean$\pm$SEM, n=5) (Fig.5.8 and table 5.4).

Comparing these results in neonatal tibial nerve crush rats with the results from normal rats (dotted line in figure 5.8), the peak reduction of amplitude of MSRs as a result of recurrent inhibition (effect of recurrent inhibition) were significantly ($P < 0.05$, t-test) reduced in either LG-S to MG and MG to LG-S combinations.

As described in section 5.2 in this chapter, the effect of recurrent inhibition obtained from rats with sciatic nerve crush at 5-day of age had been significantly depressed to nearly absent. In contrast, although recurrent inhibition was also strongly depressed as a result of tibial nerve crush, the amounts of recurrent inhibition retained a certain degree (60-70% of normal RI size) after regeneration. Compared with sciatic nerve crush rats, recurrent

inhibition in regenerated 5-day-old tibial nerve crush rats were significantly ($p<0.02$ in MG/LG-S and $P<0.01$ in LG-S/MG, t-test) bigger. This result suggested that after motoneurones regenerated from tibial nerve crush, the amount of recurrent inhibition between these neurones was somewhat recovered or less affected as a result of shorter neurone-muscle disconnection.

Table 5.4 Summary of recurrent inhibition of MSRs after tibial nerve crush in rats at 5-day of age

RI/MSR	Number of rat (n)	Post operative time (weeks)	T-C interval to Max. RI (ms)	Max. RI (% of MSR reduction) (mean±SEM)	RI duration (ms)
MG/LG-S	4	14	5	38.0±9.2%	40
LG-S/MG	5	14	4	31.6±5.3%	30

5.4. Recurrent inhibition between regenerated LG-S and normal MG motoneurones following LG-S nerve crush in 5-day-old rats

In this section, recurrent inhibition is examined in rats with nerve crush on LG-S nerve at 5-day postnatally. LG-S nerve crush in this preparation was carried on at the site on LG-S nerve which was less than 1 mm away from the point nerve entering the LG-S muscle. Following LG-S nerve crush, regeneration time would be expected to be less compared with either sciatic or tibial nerve crush preparations. In 2 animals recurrent inhibition from MG motoneurones to LG-S motoneurones 7 weeks after LG-S nerve crush reduced the amplitude of the MSRs to 49.4±11.7% (mean±SEM, n=2) of the size of the unconditioned reflex. The corresponding value for recurrent inhibition from LG-S to MG was 71.7±2.8% (mean±SEM, n=2) (Fig.5.9 and table 5.5)

Comparing the result in 7 weeks postoperatively in this preparation to results from normal

rats, recurrent inhibition from MG to LG-S in LG-S nerve crushed animals (50.6%) was not statistically different ($0.4>P>0.3$, t-test) to the value from normal rats (62.7%). It was not surprising that the MG motoneurones were not affected by the LG-S nerve crush and the normal development progress were not interrupted.

However, the amount of recurrent inhibition from LG-S to MG expressed as MSR amplitude reduction 7 weeks after LG-S nerve crush was only 28.3±2.8% (mean±SEM, n=2) which was significantly ($p<0.001$, t-test) smaller than that (47.1%) in normal rats. This result suggested that nerve injury like LG-S nerve crush was sufficient to induce the impairment in recurrent inhibitory pathways. In addition, compared with the results that recurrent inhibition from LG-S to MG in sciatic and tibial nerve crush rats, recurrent inhibition in LG-S nerve crush rats was significantly ($p<0.001$, t-test) bigger than that (9.5%) in sciatic nerve crush rats and as great as ($P>0.5$) that in tibial nerve crush rats. This indicates that the nerve crush performed on LG-S nerve which is close to its target muscle in 5-day old rats causes less injury and/or more recovery in recurrent inhibition pathway than that in sciatic nerve crush rats.

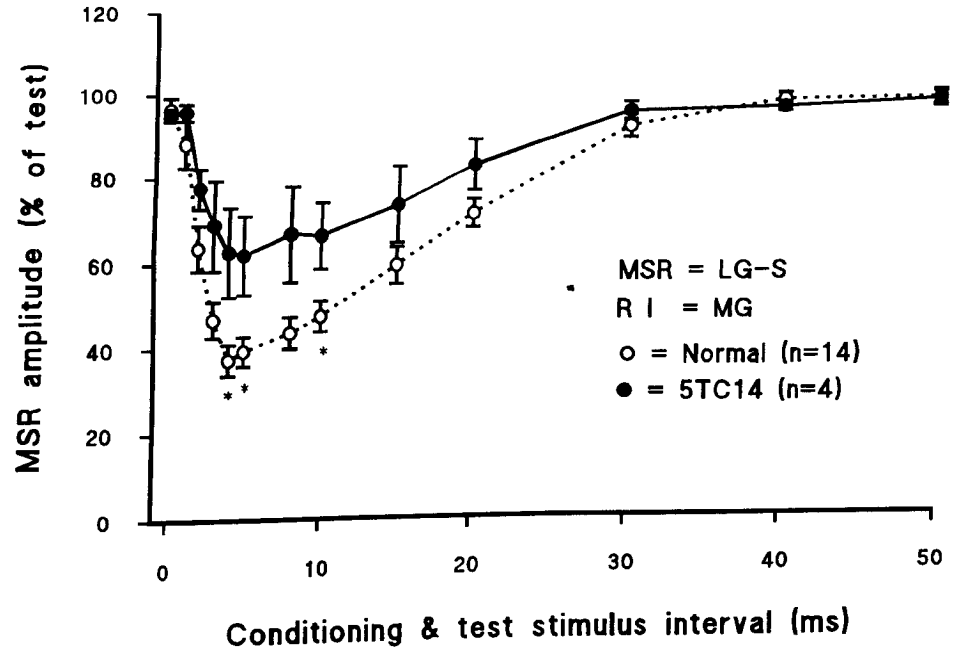
Table 5.5 Summary of recurrent inhibition of MSRs after LG-S nerve crush in rats at 5-day of age

RI/MSR	Number of rat (n)	Post operative time (weeks)	T-C interval to Max. RI (ms)	Max. RI (% of MSR reduction) (mean±SEM)	RI duration (ms)
MG/LG-S	2	7	5	50.6±11.7%	50
LG-S/MG	2	7	5	28.3±2.8%	30

Fig. 5.8.

Diagrams showing the % reduction of monosynaptic reflexes (MSR) as the result of recurrent inhibition (RI) in the 5-day-old tibial nerve crush rats (filled symbols and solid line) 14 weeks after nerve crush. The RI/MSR combinations presented are MG/LG-S (A) and LG-S/MG (B). Two tailed Student t-test was applied to compare the difference with the corresponding inhibitory effects in normal animals (open symbols and dotted line). * = $P<0.05$ and ** = $P<0.01$.

(A)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = LG-S
R I = MG
o = Normal (n=14)
● = 5TC14 (n=4)

(B)

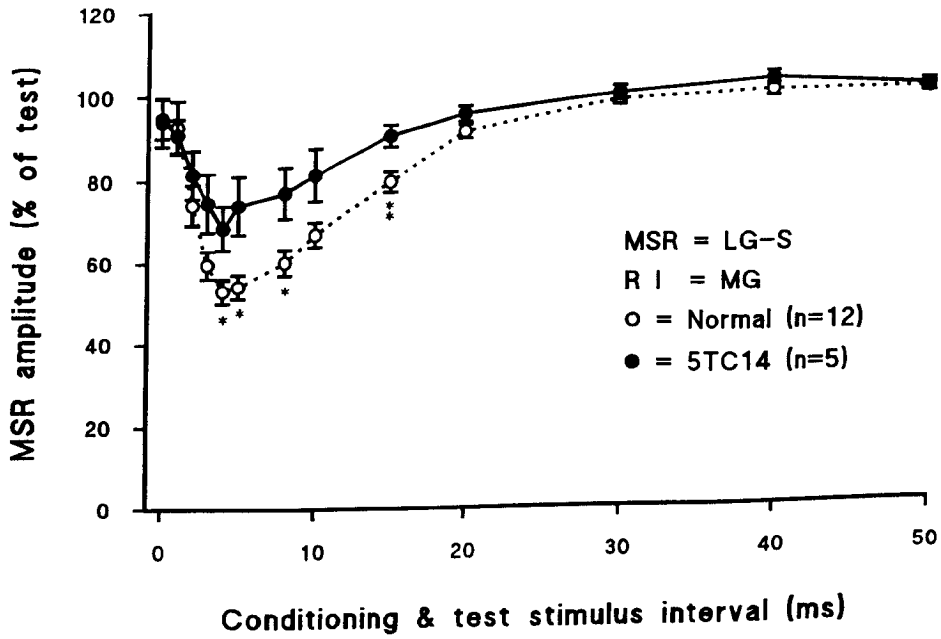
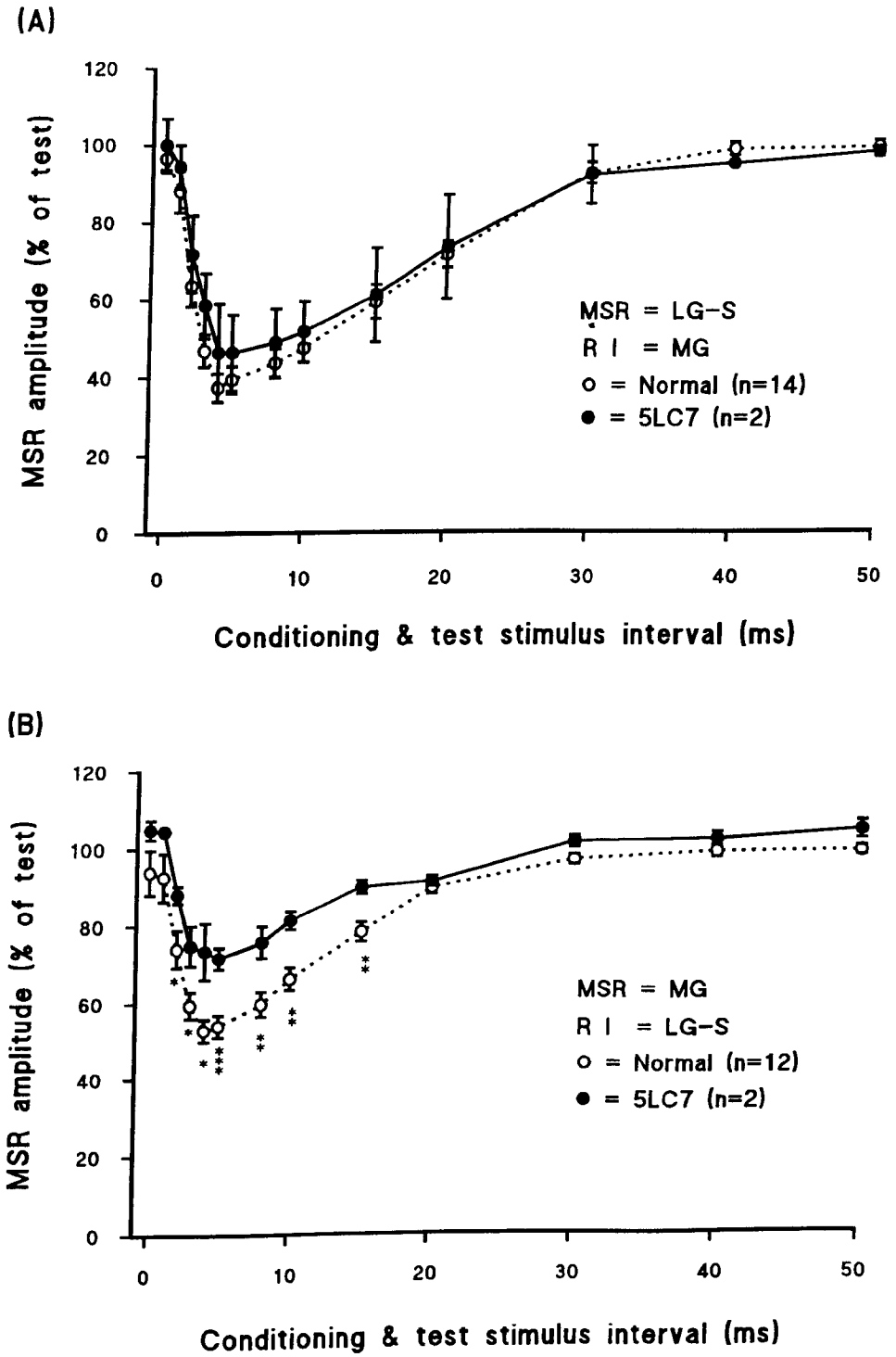

MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = LG-S
R I = MG
o = Normal (n=12)
● = 5TC14 (n=5)

Fig.5.9.

Recurrent inhibition in 5-day old LG-S nerve crush rat. The amount of recurrent inhibition reduced as a result of LG-S nerve crush at 5-day of age 7 weeks postoperatively. The different RI/MSR combinations presented in recurrent inhibitory curves are as MG/LG-S (A) and LG-S/MG (B). Two tailed Student t-test were applied between each corresponding data point in regenerated (filled circles with solid line) and normal (open cycles with dotted line) animals, * = $P<0.05$, ** = $P<0.01$, and *** = $P<0.001$. Y-axis indicates the % monosynaptic amplitude change and X-axis is the interval between test and conditioning stimuli.


(A)
MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = LG-S
R I = MG
o = Normal (n=14)
● = 5LC7 (n=2)
(B)
MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = MG
R I = LG-S
o = Normal (n=12)
● = 5LC7 (n=2)

5.5. Effect of nerve crush at 5-day of age on spinal motoneurone pools, muscle weight and muscle fiber type in rat

5.5.1 Gastrocnemius-soleus motoneurones number and location after 5-day-old sciatic nerve crush

The motoneurones innervating the gastrocnemius-soleus muscle were labelled retrogradely with the HRP at least 7 weeks after sciatic nerve crush in 5-day-old rats. Although on the injured side the spinal cord in cross sections was smaller in size than on uninjured side, the HRP labelled gastrocnemius-soleus motoneurones were found to be located in the similar area in dorsal-lateral of the anterior horn in both side of spinal lumbar segment L4 and L5. Rostral-caudally the majority of the labelled motoneurones were found in the segment L5 just as in normal rats and as in contralateral control side in ventral horn. Figure 5.10 was an example of HRP retrograde labelled gastrocnemius-soleus motoneurones in one 5-day old left sciatic nerve crush rat. The hole in the dorsal horn pointed out by the finger indicates the contralateral uninjured side. Figure 5.10.A was at a low magnification (2.5x1.6) showing the whole section which containing labelled motoneurones and figure 5.10.B was at a high-magnification (10x2) showing a close view of the labelling motoneurones at the crushed side.

The mean number of motoneurones counted as HRP labelled neurones in spinal ventral horn in 5-day-old sciatic nerve crush rats was 166 ± 10 (mean$\pm$SEM, n=4). The mean number of motoneurones in contralateral uninjured gastrocnemius-soleus pool was 174 ± 12 (mean$\pm$SEM. n=4) (Fig.4.12.B). The mean number from regenerated side was not significantly different from that obtained either from the normal rats or from the contralateral control side ($p > 0.1$, t-test). In addition, the labelled motoneurone number from normal animals was also not different from the contralateral control side in 5-day-old sciatic nerve crush rats. This result confirmed the reported that nerve crush performed on neonatal rats age at 5-day old would not lead to motoneurone death (Lowrie et al., 1982, 1987).

5.5.2 Muscle weight and muscle fiber change after sciatic nerve crush in 5-day-old rats

At the end of each acute experiments, the gastrocnemius and soleus muscles on both sides

were dissected out and weighted separately. Gastrocnemius muscles were then quickly stored in liquid nitrogen for later SDH staining. Figure 4.11.A illustrates the percentage change of the weight of the reinnervated gastrocnemius and soleus muscles against the contralateral uninjured muscles in 5-day-old sciatic nerve crush rats. Although there was no motoneurone loss after nerve crush in 5-day-old rats, the muscle weight was still reduced as a result of nerve crush even after reinnervation. At least 7 weeks after sciatic nerve crush, the gastrocnemius muscles had the weight at 58.0±3.1% (mean±SEM, n=8) of the contralateral control value, and the soleus muscles were 52.4±5.0% (mean±SEM, n=8) of the control (Fig.4.12). Loss of muscle weight after peripheral nerve crush in 5-day-old sciatic nerve crush rats observed in this study is in agreement with the other results. Lowrie et.al. (1982, 1987, 1990) reported that the fast muscles such as TA and EDL, have 50% of the normal level and slow muscles as soleus recovery well have about 80% of the contralateral level.

Figure 5.11 shows the cross-sections of both reinnervated and contralateral control gastrocnemius muscles stained with succinic dehydrogenase (SDH). Staining for SDH showed that normal gastrocnemius muscle in the rat contained a mixture of fibers which have either a low (pale staining) or high (dark staining) oxidative capacity (Fig.5.11.A) while after sciatic nerve crush at 5-day of age virtually all the fibers in the reinnervated muscle stained darkly (Fig.5.11.B). The result of muscle fiber type change is in good agreement with previous reports (Lowrie et al., 1982, 1987, 1990).

Fig.5.10.

Cross sections of spinal cord processed to demonstrate HRP and counter-stained with gallocynin. HRP had been injected into both side gastrocnemius and soleus muscles at 8 weeks of age in which left sciatic nerve was crushed at 5-day of age. (A) is the photograph taken under low magnification (2.5x1.6) showing both side of the spinal cord. The HRP labelled cells are located at both side of dorsolateral part of the anterior horn. The hole in the dorsal horn indicates the uninjured side contralateral to the nerve crush. (B) is the photograph taken under high magnification (10x2) from the same section as in (A) showing the close view of the labelled G-S motoneurones of injured side. The scale for the bar in (A) is 500μm and in (B) is 100μm.

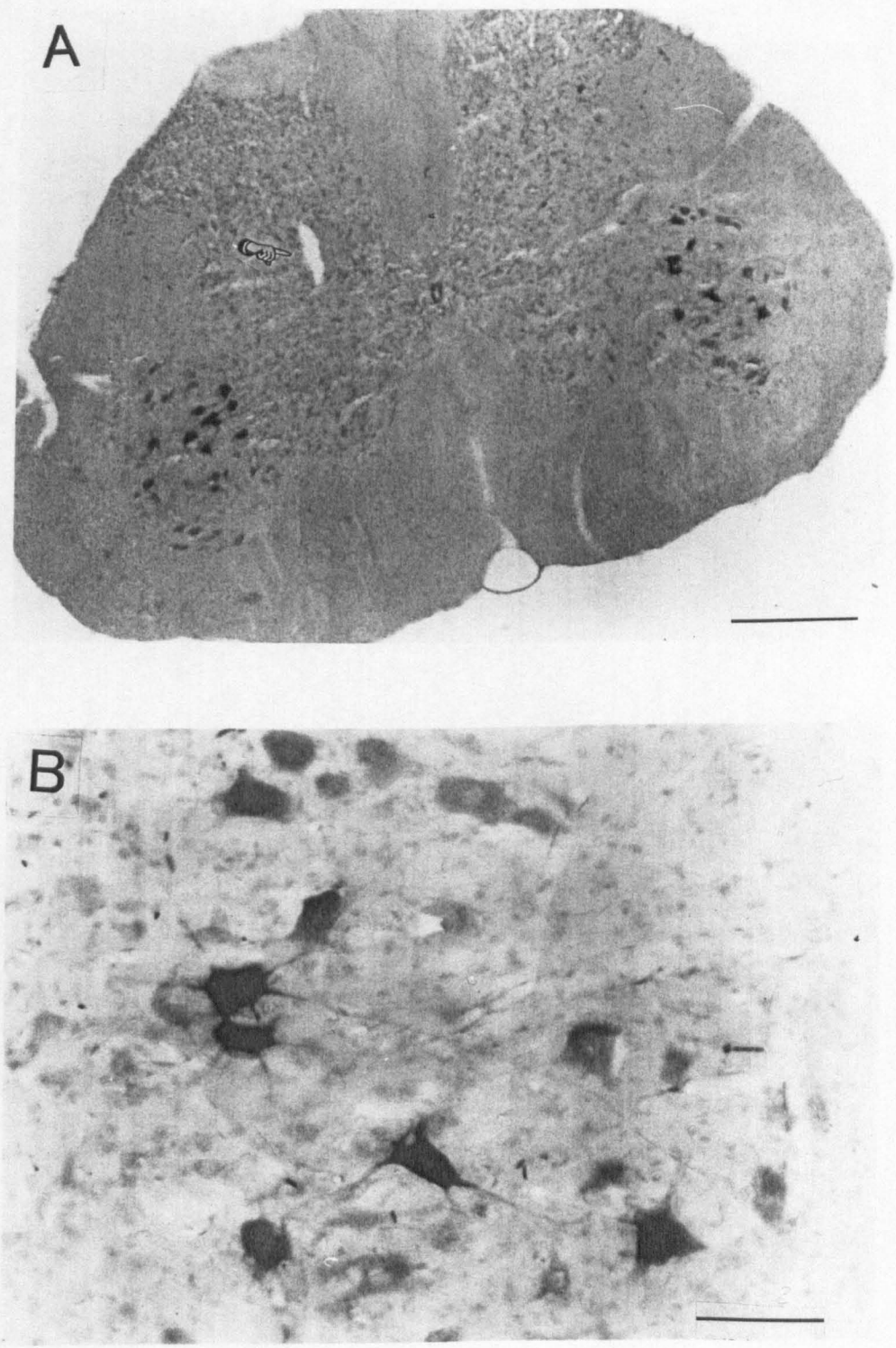
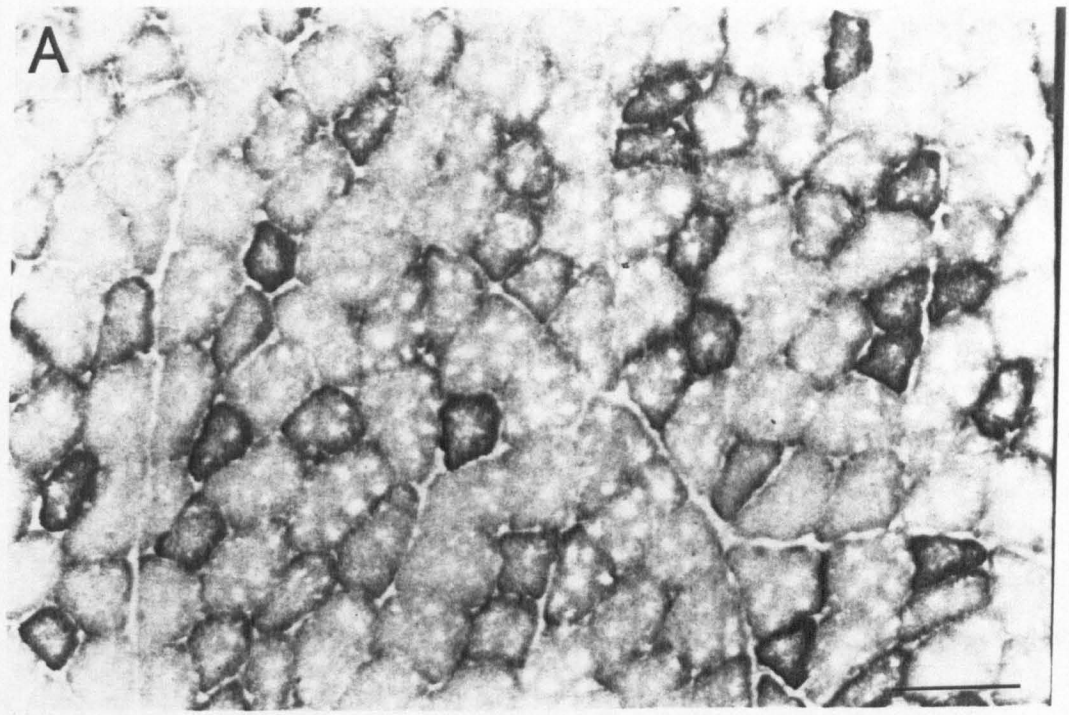
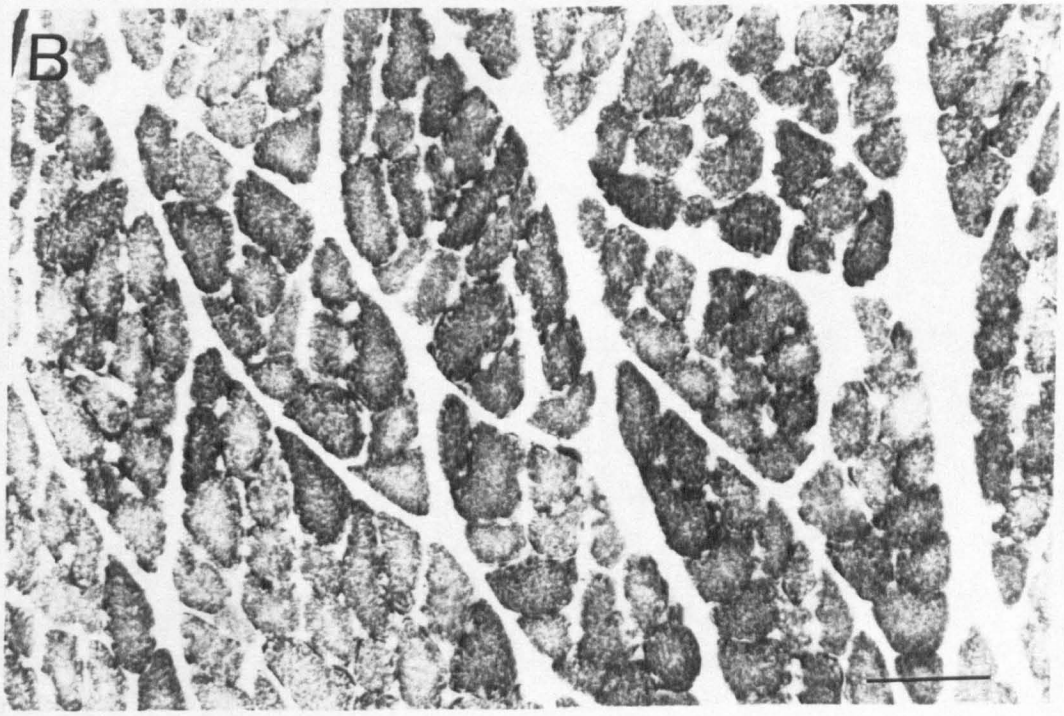

A
B

Fig.5.11.

Examples of cross-sections from reinnervated (B) and contralateral control (A) gastrocnemius muscles from 5-day-old sciatic nerve crush rat stained for succinic dehydrogenase (SDH). The muscles were taken 7 weeks after sciatic nerve crush at 5-days. SDH staining showing the oxidative activity of muscle fibers in either reinnervated or normal muscles. The scale for the bar in both (A) and (B) is 80μm.


A


B

5.6. Summary

The results presented in this chapter are based on the experiments carried on in rats in which the sciatic nerve or one of its branches had been previously crushed at 5-day-old postnatally. In this preparation with sciatic nerve crush, the amounts of recurrent inhibition between regenerated motor pools were very small. They were virtually lost compared with the normal RI size (15-20% of the normal RI size) even after a long recovery period (10-15% of normal at up to 30 weeks after nerve crush).

In 5-day old sciatic nerve crush preparation, the monosynaptic reflexes generated by regenerated motoneurones not only had a longer latency but also had a larger amplitude. However, the increased MSR amplitude was not the main factor in contributing to the recurrent inhibition reduction, since at different MSR amplitude level in the same preparation, the amounts of recurrent inhibition were virtually same.

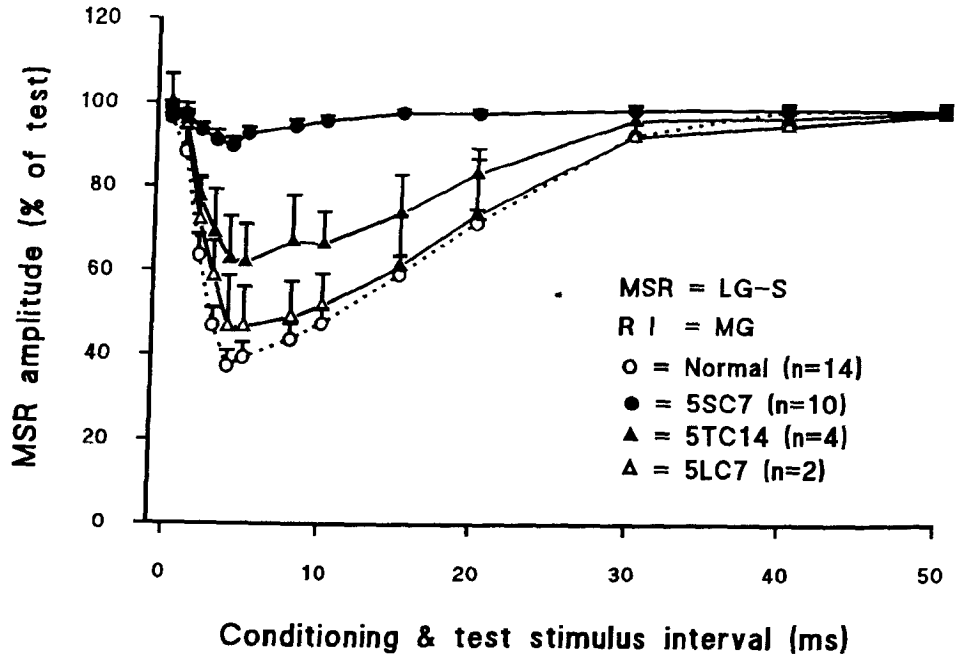
In contrast to the huge reduction in sciatic nerve crush preparation, the amounts of recurrent inhibition following tibial and lateral gastrocnemius-soleus nerve crush at 5-day of age were only reduced to about 60-70% of the normal RI size (Fig.5.12). This may suggest that shortening the disconnection time between motoneurones and their targets would lead to less impairment in the recurrent inhibitory pathway after nerve crush. The lack of any difference of recurrent inhibition between tibial and LG-S nerve crush preparations (Fig.5.12.B) may suggest that the depressive effect of nerve crush on recurrent inhibitory pathways is only in part related to the length of time that neurone and muscle are disconnected.

The effect of dedifferentiation in recurrent inhibition seen following adult nerve crush was observed after nerve crush at 5-day of age as well. The original different distribution of recurrent inhibition between LG-S and MG motor pools (Fig.4.13.A) were dedifferentiated as a result of nerve crush in early postnatal life (Fig.4.13.C). This dedifferentiation occurring in the recurrent inhibitory pathway may be part of the dedifferentiation progress in motor unit during regeneration.

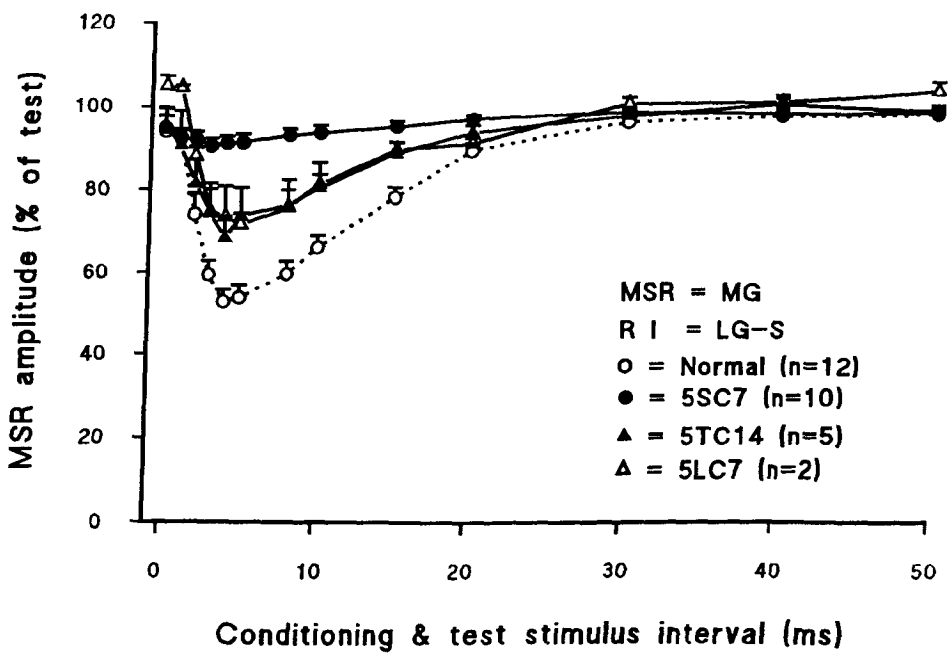
Fig. 5.12.

Recurrent inhibitory curves show recurrent inhibition from 5-day-old nerve crush rats with different nerve injury site: sciatic nerve (filled cycles), tibial nerve (filled triangle), and LG-S nerve (open triangle). Recurrent inhibitory curve obtained from normal animals is presented as a control (open cycle with dotted line). (A) is recurrent inhibition from MG to LG-S and (B) is from LG-S to MG. Y-axis indicates the % monosynaptic amplitude change and X-axis is the interval between test and conditioning stimuli. RI = recurrent inhibition, MSR = monosynaptic reflex, normal = data from normal animals, 5SC7 = 5-day-old sciatic nerve crush rat at 6-weeks postoperatively, 5TC14 = 5-day-old tibial nerve crush rat at 14 weeks postoperatively, and 5LC7 = 5-day-old LG-S nerve crush rat at 7 weeks postoperatively.

(A)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = LG-S
R I = MG
o = Normal (n=14)
● = 5SC7 (n=10)
▲ = 5TC14 (n=4)
Δ = 5LC7 (n=2)

(B)


MSR amplitude (% of test)
120
100
80
60
40
20
0
0
10
20
30
40
50
Conditioning & test stimulus interval (ms)
MSR = MG
R I = LG-S
o = Normal (n=12)
● = 5SC7 (n=10)
▲ = 5TC14 (n=5)
Δ = 5LC7 (n=2)

CHAPTER 6 DISCUSSION

The results presented in this thesis are the first report of investigations of recurrent inhibition of spinal monosynaptic reflexes in decerebrate rats and the subsequent changes of recurrent inhibition following peripheral nerve injury. The results of this study can be summarised as: 1). The recurrent inhibition of MSRs in decerebrate rat preparation is as great as that in decerebrate or spinal cat preparations (Cullheim & Kellerth, 1981; Havton & Kellerth, 1990b) and is greater than that in anaesthetized rat (Kaneko et al., 1987) and cat (Havton & Kellerth, 1990b) preparations; 2) The recurrent inhibition of MSRs in decerebrate rat could be effectively and permanently depressed as the result of peripheral nerve crush and the most dramatic effect is in rats with nerve crush in young life; 3) The impairment within recurrent inhibitory pathways following nerve crush depends the neurone-muscle disconnection time: the shorter the disconnection time, the less the impairment; 4) The different distribution of recurrent inhibition between the motor pools is dedifferentiated after regeneration.

RENSHAW RECURRENT INHIBITION IN DECEREBRATE RAT

Renshaw (1941, 1946) first reported that an antidromic volley delivered to motor axons could excite a group of interneurones (Renshaw cells) and in turn reduce the monosynaptic reflex discharge in cat. Since then recurrent inhibition has been detailed mainly in cat preparations. Only very recently, Kaneko et al. (1987) by using anaesthetized rat preparation studied the descending effect on to spinal recurrent inhibitory pathway from medullary raphe nuclei.

The present study first demonstrated the recurrent inhibition of spinal MSRs in decerebrated rat preparations. The amount of recurrent inhibition generated in the spinal motor pools in this preparation was found as great as that in decerebrate or spinal cat preparations. For example, the results presented in this thesis showed that the recurrent inhibition between spinal LG-S and MG motor pools in decerebrate rat reduced the MSRs amplitude by 62% (MG to LG-S) and 53% (LG-S to MG). They were in the same range as in cats where was about a 50-60% MSRs reduction (Renshaw, 1941; Brooks & Wilson, 1959; Cullheim & Kellerth, 1981; Havton & Kellerth, 1990b). These results may suggest that there is a similar distribution of recurrent inhibition in spinal cord motor pools in these two species (rat and

cat). However, the recurrent inhibition (42%) of MSRs of spinal segment L5 in decerebrate rats was found to be smaller than those in spinal segment L7 (80%) in decerebrate cat (Brooks & Wilson, 1959). This difference may have occurred because the motor pools of rat L5 segment and cat L7 segment may not have been directly comparable in that they contain different motor pools since different motor pools give and receive recurrent inhibition with different effect.

In contrast, recurrent inhibition of MSRs of whole spinal segment motor pools tested by sectioned dorsal and ventral (L5) root pair in decerebrate rats (current study) was found to be much greater than that obtained from anaesthetized rats (Kaneko et al., 1987). In anaesthetized rats the recurrent inhibition reduced the amplitude of monosynaptic reflexes from ventral root L5 elicited by stimulating dorsal root L5 by 18.7±2.2% (n=4) (Kaneko et al., 1987). The corresponding value in decerebrate rats was 42.7±5.3% (n=8). In addition, in the cat, recurrent inhibition from MG motoneurones to LG-S motoneurones from anaesthetized preparations (Havton & Kellerth, 1990b) was less than that obtained from spinal (Cullheim & Kellerth, 1981) or decerebrate cats (Brooks & Wilson, 1959). The different effect of recurrent inhibition in between decerebrate/spinal preparations and anaesthetized preparation in either rat or cat may due to (1) loss of supraspinal descending inhibitory mechanisms to recurrent inhibitory pathway after decerebration or spinal transection, and/or (2) anaesthesia which may inhibit the recurrent inhibitory pathway's activity.

The stronger recurrent inhibition may be as a result of decerebration or spinal transection in which removed some descending input into recurrent inhibitory pathway. Kaneko et al. (1987) reported that [redacted] (unpublished observations) [redacted], the recurrent inhibition observed in C1 transected preparations was greater than that obtained in intact, anaesthetized animals. These authors postulated that the differences observed in the magnitudes of recurrent inhibition could, at least in part, be ascribed to the difference in the preparations (Kaneko et al., 1987). Previous studies also supported the view that there was a transient increase in recurrent inhibition of monosynaptic reflexes in acute and chronic spinal cats due to the increased efficacy of axon collaterals to Renshaw cells and an increased sensitivity of Renshaw cells to neurotransmitters (Goldfarb & Sharpless, 1971; Goldfarb, 1976). In

addition, in the human it was found that recurrent inhibition is increased in patients with spinal cord injury (Shefner et al., 1992).

The results presented in this thesis verified the findings that in different animal preparations recurrent inhibition of MSRs varied in strength. It has been reported that tonically active inhibitory or facilitatory control of recurrent inhibitory pathways might be mediated by central regions via descending pathways (MacLean & Leffman, 1967; Pompeiano, 1984; Kaneko et al., 1987). MacLean and Leffman (1967) described that there were four major areas of the brain (Pericruciate cortex, ventral thalamus, mesencephalic and bulbar reticular formation) that profoundly inhibited Renshaw cells discharge. There was also evidence that transmission in recurrent inhibitory pathways could be enhanced by activation of bulbospinal noradrenergic pathways and medullary raphe-spinal serotonergic pathway (Pompeiano, 1984; Kaneko et al., 1987). These supraspinal effects may serve as a selector and as an amplifier for a particular form of output required of the motoneurone pool. Thus, after removal of the whole or part of the supraspinal influence on to spinal recurrent inhibitory pathway, the recurrent inhibition must then be changed. Over all, this suggests that the supraspinal structures may play an important role in descending inhibition on spinal recurrent inhibition and absence of this inhibition, the spinal recurrent inhibition would be enhanced.

The other possible explanation for this difference in effect in recurrent inhibition in anaesthesitized and decerebrated animals is that anaesthesia may inhibit the Renshaw cell activity directly or indirectly by a central mechanism. The different results that Cullheim and Kellerth (1981) and Havton and Kellerth (1990b) reported may be explained as an effect of of the anaesthesia. Evidence exits that different anaesthesitic agents (sodium pentobarbitone vs chloral hydrate) could alter dorsal raphe serotonergic neuronal activity pattern in rat (unpublished result by Yun Wang) may support this hypothesis, since central serotonergic pathways could enhance spinal recurrent inhibition (Kaneko et al., 1987). MacLean and Leffman (1967) also observed that the effectiveness of the supraspinal modulating Renshaw cell activity varied directly with the depth of the barbiturate anaesthesia. In addition, more directly, intracellular recorded recurrent IPSPs from MG motoneurones by stimulating single motor-axon in chloralose-urethane anaesthetized cats (mean=12.0μV) were much smaller than those (mean=38.5μV) in ischaemic-decapitate cats (Hamm et al., 1987a). Thus, the

results suggest that anaesthesia regulation of spinal recurrent inhibition may occur by modulating the central descending control pathways to indirectly act on to recurrent inhibitory pathways. The fact that anaesthesia modulates the recurrent inhibition locally in spinal cord is not clear yet but may exist.

These two possibilities, absence of descending inhibitory influence and direct or indirect anaesthetic agents affect, may work together.

It is of interest to see that recurrent inhibition from MG motoneurones to LG-S motoneurones (62% reduction in MSR) was significantly greater than that from LG-S to MG (42% reduction in MSR) (Fig.4.14.A). Previous work on cat also reported this difference in the effect of recurrent inhibition between these two motoneurone pools. Brooks and Wilson (1959) demonstrated recurrent inhibition from MG to LG (65% reduction in MSR) was much stronger than that from LG to MG (40% reduction in MSR) in decerebrate cat. Havton and Kellerth (1990b) by using anaesthetized cat also found that recurrent inhibition from MG motoneurones to LG-S motoneurones (50% reduction in MSR) was bigger than that from MG motoneurones to plantaris (Pl) motoneurones (20% reduction in MSR).

The reason for the different distributions of recurrent inhibition among motor pools are still not clear, however, the observed difference in the distribution of recurrent inhibitory input/output relationship within type different motoneurones may contribute to it (see Fig.1.1). The strength of excitation of a Renshaw cell pool produced by a motoneurone correlates with the complexity of the motor axon's recurrent collateral arbour (McCurdy et al., 1992). It is well known that α-motoneurones are of three types depending on their properties and the muscle fibers they innervate --- fast fatiguable (FF), fast fatigue resistant (FR) and slow (S) (Burke et al., 1971; Burke, 1981; Balldissera et al., 1981). FF-type motoneurones are mostly large cells and S-type are small cells. In the case of motoneurones of triceps surae in rat, MG motoneurones are mostly FF-type (3/4 F-type and 1/4 S-type) and soleus containing about 80% S-type (Close, 1964). Thus, axon collaterals for motoneurones in this pool are much more numerous for FF units than for FR and are particularly sparse for S units of the soleus (S < FR < FF in the number of recurrent collateral terminals) (Cullheim & Kellerth 1978c). Large motoneurones have been reported to have a greater

effect on Renshaw cell pools than small motoneurones (Ryall et al., 1972; Pompeiano et al., 1975; Hultborn et al., 1988a). Both Granit et al. (1957) and Eccles et al. (1961b) observed that activation of large motor axons produced a much larger excitatory effect on Renshaw cell firing than activation of small motor axons. In cat triceps surae motor units, Hultborn and co-workers (1988a) found that the excitatory inputs to Renshaw cells from type identified FF, FR, and S type motoneurones were increased in order ($S < FR < FF$) of the motor unit type. During locomotion, it was also found that Renshaw cells were firing at much higher frequency (4.3 times) to vibration (both small tonic and big phasic motoneurones activated) than in static stretch (small tonic motoneurones activated) (Pompeiano & Wand, 1976; Wand & Pompeiano, 1979; Pratt & Jordan, 1980).

In contrast, small motoneurones have been found to receive more recurrent inhibition from Renshaw cells than large motoneurones (see Fig.1.1). Kuno (1959) recorded larger RIPSPs from small motoneurones (soleus) than that from big motoneurones (gastrocnemius). Friedman et al. (1981) confirmed that S-type motoneurones in MG motoneurone pool were more strongly inhibited by Renshaw cells than were FR- and FF-type motoneurones. Hultborn et al. (1988b) indicated that the recurrent inhibition from Renshaw cells to motoneurones increased in the order of $FF < FR < S$.

Intracellular recording of recurrent inhibitory postsynaptic potentials (RIPSPs) is the direct evidence to show that recurrent inhibition from MG (mainly large) motoneurones to LG-S (mainly small) motoneurones are stronger than LG-S to MG. Lindsay and Binder (1991) found that both the effective synaptic current and the RIPSPs recorded in LG-S motoneurones by stimulating MG nerve were larger than those recorded in MG motoneurones and stimulation on LG-S nerve. Similarly, Eccles et al.(1961b) found that in spinal cat RIPSPs elicited by stimulating all other ankle nerves to LG motoneurones was bigger (4.1mV) than that to MG (2.9mV). In an individual nerve pair, Eccles et al. (1961b) found, for example, RIPSP from MG to LG was 0.74mV, to soleus was 2.0mV; RIPSP from LG to MG was 0.68mV; and RIPSP from soleus to MG was 0.23mV. These results clearly show that the difference of recurrent inhibition between MG and LG-S motoneurones is mainly due to the dramatic difference of contribution and effectiveness of RIPSPs between MG motoneurones (big) and soleus motoneurones (small). In addition, Friedman et al. (1981) and Hultborn et

al. (1988b) demonstrated that, in MG motor units the mean amplitude of RIPSPs differed among motor-unit types, increasing in the order of FF<FR<S. They also indicated that input resistant was not the major determinant of the amplitude of RIPSPs in α-motoneurones. In addition to the RIPSPs recording, Kuno (1959) reported that recurrent inhibition of spinal monosynaptic reflexes from MG motoneurones to LG motoneurones was much ~~stronger~~ less than to soleus motoneurones.

It can be concluded that Renshaw cells are activated more effectively by large F-type α-motoneurones (sending more axon collaterals) than small S-type ones and recurrent inhibition is acting more powerfully on small S-type motoneurones than on large F-type ones (see Fig 1.1). Since 75% of MG motor units are F-type and 25% are S-type and 80% of soleus is S-type, assuming that the composition of the LG nerve is similar to that of the MG nerve (Hultborn et al., 1988a), and as the motoneurone number of LG and soleus are closely similar (Nicolopoulos-Stournaras and Iles, 1983), the LG-S motoneurone pool contains over 50% of the S-type motoneurones. Thus it is not surprising that recurrent inhibition from MG motoneurones (F-type in majority) to LG-S (S-type predominantly) motoneurones will be greater than vice versa.

MONOSYNAPTIC REFLEXES OF REGENERATED MOTONEURONES

Monosynaptic reflexes generated from regenerated motoneurones and recorded in a peripheral nerve in adult nerve crush rat had a similar strength as in normal (Fig.4.2). However, the latency was prolonged following regeneration. In contrast, monosynaptic reflexes recorded from regenerated motoneurones in 5-day-old sciatic nerve crush rats are much different from those in normal rats not only by their latency, but also by their amplitude. The latencies of MSRs in regenerated motoneurones (either from adult or 5-day old sciatic nerve crush) were significantly longer than that in normal rats. This may be due to the observed reduction of axon conduction velocity in injured motoneurones (Kuno et al., 1974b; Foehring et al., 1986a,b). However, the largely increased amplitude of the MSRs seen in 5-day-old sciatic nerve crush rats were not seen in adult crush rats (Fig.4.2). This is not simply because the rats used for test the MSRs in ASC6 and 5SC7 groups had a different age. The rats of 5SC7 group were usually 7 weeks younger than those of ASC6 group rats. However, the amplitude

of MSRs recorded from contralateral uninjured side of MG and LG-S nerves was not different between these two groups animals. Although the amplitude of contralateral MSRs in both ASC6 and 5SC7 groups rats was larger comparing to that in normal animals, this is not the case of age difference. Since the age of the rats in ASC6 and 5SC7 groups was at 14 weeks old and 8 weeks old respectively, and was well fitted into the age range of the normal rats (between 8 and 20 weeks old), the difference of MSR amplitude seen between normal and contralateral regenerated motor pools may contributed by the effect of nerve crush carried out on the other side of sciatic nerves. The evidence that following nerve crush in young animals, that the normal weak cross-reflexes recorded from injured muscles were permanently enhanced (Navarrete et al., 1990) may support this view. They observed that at what ever the level of the stimulus intensity, from lower for just suprathreshold to a higher level, the injured motoneurones will have an enhanced response to stimulation of the homonymous nerve on the contralateral side. This may refelect the fact that, following nerve injury in young animals, the injured motoneurones increased their excitability. In addition, the amplitude of MSRs between operated and contralateral side was much different in which that in the operated side was larger than that in uninjured side. Overall, these results ruled out the possibility that the largely increased MSR amplitude seen in 5SC7 group rats is because the younger animals been used. Instead, this MSR amplitude increase is in agreement with the early report that after temporary loss of contact with the target in young animals the surviving motoneurones became more active than normal; however, in animals which had their nerves crushed as adults the motoneurone activity was as normal (Navarrete & Vrbova, 1984). It is possible that nerve injury in neonatal animals disrupted the normal developmental process and prevented the usual elimination of synapse from the surface of the motoneurones. This would result in enhancement of monosynaptic EPSPs (Kuno & Llinas, 1970; Gallego et al., 1980; Navarrete & Vrbova, 1984). The enhanced monosynaptic EPSPs evoked from crushed nerve are reported to be seen only in a period of 8 to 12 weeks after nerve crush (Gallego et al., 1980). A recent study (Hellgren & Kellerth, 1989) showed that partial deafferentation by way of rhizotomy of L7 and S1 dorsal roots resulted in the enhancement of MSRs and monosynaptic EPSPs as well. Since the sciatic nerve crush carried out on 5-day-old rats in this study led to both deafferentation and denervation of the sciatic motor pool, albeit briefly, these changes of motoneurone synaptic input may result in the enhancement of MSRs.

ALTERATION OF RECURRENT INHIBITION FOLLOWING NERVE INJURY

The major contribution of this study was to demonstrate that the recurrent inhibitory pathways following nerve crush were effectively and permanently impaired in rats. The

younger the injury applied, the greater impairment. The results showed that recurrent inhibition was depressed to about 55% to 70% of its normal value in adult sciatic nerve crush rats and was nearly abolished (only 15-20% of normal value) by neonatal sciatic nerve crush. The reduction of recurrent inhibition seen in either adult or 5-day-old sciatic nerve crush rats did not recover even after a long postoperative period (14 weeks in adult and 30 weeks in young).

The initial reduction of recurrent inhibition seen in this study 6 weeks after adult nerve crush is in good agreement with those of a recent study (Havton & Kellerth, 1990b) where recurrent inhibitory postsynaptic potentials (RIPSPs) of LG-S motoneurones in cat elicited by stimulating the chronically sectioned MG nerve 6 weeks postoperatively were smaller than those from normal. The mean values of RIPSPs area and maximal amplitude were reduced to 53% and 60% of the control values 6 weeks postoperative as a result of MG nerve transection (Havton & Kellerth, 1990b).

The possible mechanisms behind the reduction of recurrent inhibition have been proposed by Havton and Kellerth (1984, 1990 a,b). The finding that a gradual elimination of intramedullar axon collaterals from the lesioned motoneurones in cat has been suggested to produce this recurrent inhibitory strength reduction (Havton & Kellerth, 1990b). However in nerve crushed rat preparations, there is no direct evidence in the literature to show that there is a gradual elimination of intramedullar axon collaterals. It seems to be the case since the recurrent inhibitory strength from regenerated motoneurones are greatly reduced postoperatively and this functional change might be expected to parallel alterations in morphological indices such as the elimination of the axon collaterals. Indirect evidence that changes in motoneurone membrane properties (Kuno et al., 1974a,b; Foehring et al., 1986a,b), muscle tension, and muscle fiber type (Gallego et al., 1980; Lowrie et al., 1982, 1987, 1990; Lowrie & Vrbova, 1984; Albani et al., 1988) seen in nerve sectioned animals were also seen in nerve crushed animals indicated that the elimination of axon collaterals may happen in reinnervated motoneurones in adult nerve crush rats as well. In addition, the reduced activity of Renshaw cells following nerve crush would be expected because of the reduction of synaptic input from motoneurones to Renshaw cells (Sanna et al., 1993). The reduced synaptic input from motoneurones to Renshaw cells is due to, at lease in part, loss

of axon collaterals. If it is the case, the reduction of recurrent inhibitory effect 6 weeks and longer after sciatic nerve crush in adult seen in this study could be counted, at least in part, by the elimination of axon collaterals from injured motoneurones as proposed by Havton and Kellerth (1990b).

In addition, the synaptic contacts between Renshaw cell axon terminals and regenerated target neurones may be reduced as the result of temporary denervation, and therefore, also could contribute to observed depression of recurrent inhibition. Electrophysiological (Burke et al., 1971) and morphological (Fyffe, 1991a) evidence exists that Renshaw cell synapses on motoneurones are located on the dendrites and not on the cell body. Brannstrom et al. (1992a,b) reported that the α-motoneurone dendritic length and number of dendritic end branches were permanently reduced as the result of denervation even after reinnervation. Bowe et al. (1988) reported "thicker dendritic processes" in rat sciatic motoneurones after peripheral nerve crush. Most recently, Dekkers and Navarrete (1993) demonstrated that neonatal nerve injury arrested the maturation of the motoneurone somatodendritic receptive surface in neonatal nerve crush rat and then this might be associated with altered synaptic inputs to the injured motoneurones. These changes in dendritic tree of motoneurones may lead to reduced synaptic input from Renshaw cells to regenerated motoneurones. Losing synapses as the result of regeneration may be selective. Peripheral nerve injury induced depressed monosynaptic reflexes and enhanced polysynaptic reflexes, that may indicate a selective loss of synaptic contact between afferent and motoneurones (Kuno & Llinas, 1970; Gordon, 1983). Since it has been reported that the overall spatial distribution of Renshaw cell synapses closely resemble the distribution of excitatory Ia afferent synapses (Brown & Fyffe, 1981; Burke et al., 1977), selective loss of recurrent inhibitory synapses (reduce the amount recurrent inhibition) may benefit Ia EPSPs to compensate for the loss of normal force.

Whether Renshaw cells respond to peripheral nerve injury and how they cope with it during the period in regeneration is not clear. In a very recent study by monitoring the calbindin-expression alteration as the index of Renshaw cells activity change, Sanna and co-workers (1993) found that Renshaw cell activity was strongly reduced one week after sciatic nerve crush and, with the progression of regeneration, Renshaw cells activity regained and reached

a normal distribution 6-8 weeks after crush. This result may explain the observation that recurrent inhibition generated from injured motoneurones 5 weeks after adult sciatic nerve crush was only about 20% of the normal RI level and less than 50% of the value after regeneration, since the Renshaw cell activity still could not be observed in that period after nerve crush (Sanna et al., 1993). Havton and Kellerth (1990a,b) also reported the gradually elimination of axon collaterals and the associated recurrent inhibition reduction occurred at 3 to 6 weeks postoperatively. In the week-3 postoperatively, there was a 25% reduction of axon collaterals and increased to 35% in the week-6 (Havton & Kellerth, 1990a). A similar progress of axon collaterals elimination would be expected before 6 weeks postoperatively in rat sciatic nerve crush preparation as well. Since reinnervation will be completed 6 weeks after nerve injury (Lowrie et al., 1982, 1987; Navarrete et al., 1990), the motoneurones undergo progression of regeneration and therefore the function of injured motoneurones might remain unrecovered from the injury 5 weeks after crush. Taken together, the huge reduction of recurrent inhibition observed in 5 weeks after nerve crush in rat may due to impairment involved the whole recurrent inhibitory pathways. The Renshaw cell inactivity may be the major contributor in addition to the eliminated axon collaterals and the abnormal function of motoneurones in resulting this recurrent inhibition depression. In contrast, the reduced activity of Renshaw cells following nerve crush could not counted to contribute to the reduction of recurrent inhibition after regeneration, since Renshaw cell activity could fully restored 6-8 weeks after sciatic nerve crush (Sanna et al., 1993).

Havton and Kellerth (1990b) have shown that despite an initial loss of recurrent inhibition up to 6 weeks post axotomy which corresponded to a loss of axon collaterals of the injured motoneurones, it was found that the recurrent inhibition from chronically lesioned MG motoneurones to LG-S motoneurones to be similar in magnitude as the control 12 weeks after nerve section and prevented reinnervation in cat. This was the case despite the fact that about 40% of the intramedullar axon collaterals were eliminated (Havton & Kellerth, 1984, 1990a). These authors suggested that the initial reduction in the strength of recurrent inhibition following postoperative elimination of axon collaterals might be compensated for by reactive changes occurring in the inhibitory pathways of the axotomized motoneurones as well as from uninjured intact synergist motoneurones. However, results obtained in this study are not in agreement with their findings (Havton & Kellerth, 1990a,b). The reduction of

recurrent inhibition appears to be permanent both in adult and in 5-day old sciatic nerve crush rats. For example, the recurrent inhibition of MSRs in this study either 14 weeks after adult sciatic nerve crush (see section 4.2) or 30 weeks after 5-day old sciatic nerve crush (see section 5.2) were found to have same value as those in rats 6/7 weeks after nerve crush. They all significantly decreased from the normal recurrent inhibition value.

One simple explanation for the discrepancy between the results at this study with that of the previous study in cat (Havton & Kellerth, 1990b) could be that in this study the whole sciatic nerve was injured and allowed to reinnervation. Despite regeneration of the nerve and reinnervation of the appropriate targets, this would lead to the entire sciatic motor pool being injured. Consequently, all motoneurones in the sciatic nucleus would perhaps lose a proportion of intramedullar axon collaterals, a factor which may have prevented compensatory effects occurring from synergist motoneurones within the sciatic pool. In addition, between LG-S and MG motor pools, motoneurones which either send out recurrent inhibition or receive recurrent inhibition were pre-injured.

In this study recurrent inhibition did not recover 14 weeks after sciatic nerve crush in ASC14 group tested within whole spinal segment L5 motor pools as might have been expected from experiments in cat (Haverton & Kellerth, 1990b). Haverton and Kellerth (1990b) reported a compensatory mechanism from uninjured motoneurones underlying the recovery of recurrent inhibition 12 weeks after axotomy. Since the motor pools in L5 containing both uninjured and pre-injured motoneurones, it was surprise that there was no recovery of recurrent inhibition observed 14 weeks after nerve crush. In fact, since the compensatory effect may occur within the close homonymous and synergist motoneurones (Havton & Kellerth, 1990b), recurrent inhibition from pre-injured motoneurones (including LG-S, MG etc.) are not or very little compensated by those uninjured motoneurones. Thus, it is not surprising that the recovery of recurrent inhibition from whole L5 motoneurones could not be detected in this study even 14 weeks after nerve crush.

It is clear, from the data in this study, that peripheral nerve injury in young animals affects the recurrent inhibitory pathway more than in adult. This result may relate to the findings in muscles and motoneurones (Lowrie et al., 1982, 1987, 1990; Navarrete & Vrbova, 1984;

Albani et al., 1988; Navarrete et al., 1990; Schmalbruch, 1990; Dekkers & Navarrete, 1993). For example, immature motoneurones are more likely to die as a result of axonal damage than mature ones (Lowrie et al., 1982, 1987) and even the surviving motoneurones are unable to recover the original size of their peripheral field (Zelena & Hnik, 1963). Adult mammalian muscles recover virtually completely from nerve injury if the reinnervation is allowed to proceed unhindered (Gutmann & Young, 1944; Beranek et al., 1957; Lowrie et al., 1982; Albani et al., 1988). However, the reinnervated muscles in neonatal nerve injury animals are affected to a much greater extent than after similar injury in adult animals. The effects include gross loss of weight, loss of muscle tension, and muscle fiber grouping (Lowrie et al., 1982, 1987, 1990; Lowrie & Vrbova, 1984; Albani et al., 1988). The reason why the nerve injury applied in adult and young animals induces such a different impairment to motor units has been explained as a consequence of the development of the muscle fibers being arrested while the motoneurones continue to develop after nerve injury (Lowrie et al., 1982). Upon reinnervation the still immature muscle fibers may not be able to match the functional demands imposed upon them by the now mature nervous system. In the recurrent inhibitory pathway in spinal cord, it has been found that after nerve injury the Renshaw cells in adult rat appear to be inactive during reinnervation (Sanna et al., 1993) and motor axon collaterals are eliminated (Havton & Kellerth, 1984, 1990a). There is also evidence shows that postnatal elimination of a large number of terminal arborization and synaptic boutons of recurrent motor axon collaterals occur during the first two weeks of postnatal life (Cullheim & Ulfhake, 1982, 1985). This elimination of terminal axon collaterals appears to be coincident with the elimination of polyneuronal innervation which is known to occur at the neuromuscular junction (Bagust et al., 1973). That may be the factor that during the period of inactivity of Renshaw cells following the neonatal sciatic nerve crush, the elimination of terminal axon collaterals process underwent. Without interaction with the target (Renshaw cells), more axon collaterals of the motoneurones may be eliminated than in the normal developmental process, since the activity of the target plays a very important role in the development of motor units (for review see Lowrie & Vrbova, 1992; Navarrete & Vrbova, 1993). In addition, at the Renshaw level, after Renshaw cells restored the ability to respond synaptic input, which in adult is about 6-8 weeks after nerve injury, the postnatal recurrent axon collaterals elimination process has already finished (Cullheim & Ulfhake, 1982, 1985) and the immature Renshaw cells thus could not undergo the developing process to match their

mature function. If the denervation time is long enough, it may cause the permanent change in Renshaw cell firing properties that never recover to match the mature state. In contrast, in adult animals, both motoneurones and Renshaw interneurones are mature type at the time of nerve injury and after recovery from temporary "arrest", Renshaw cells recover to active as normal (Sanna et al., 1993). Thus, the large reduction of recurrent inhibition seen in this study in 5-day-old nerve crush rats may be accounted for by (1) the elimination of recurrent axon collaterals, (2) loss of synaptic contact with regenerated motoneurones by Renshaw cells as those in adult nerve crush rats, and (3) the abnormal excitability of Renshaw cells themselves.

Since the monosynaptic reflex amplitude following sciatic nerve crush in 5-day old rats has increased significantly compared with that from either normal or adult sciatic nerve crush rats, what this is the relationship between monosynaptic reflex enhancement and recurrent inhibition reduction? Is the massive reduction of amount of recurrent inhibition expressed as MSR amplitude change seen in this study due simply to the increased MSR amplitude in motoneurones regenerated from neonatal nerve crush? Renshaw (1941) demonstrated that, with increasing amplitude of the MSR, there was a profound decrease in the percent inhibition produced by a constant ventral root volley. Mellstrom (1971b) also reported that recurrent inhibition could overcome reduced monosynaptic excitation of the motoneurones which might just about to fire. More recently, Crone and co-workers (1990) demonstrated that RI was more effective at a certain level of MSR amplitude than that at other level. For example, RI from biceps to soleus was maximal when the amplitude of MSRs of soleus at 60% of the maximal and there was no recurrent inhibitory effect when MSR of soleus was maximal. In this study, this possibility has also been investigated by testing the recurrent inhibition at different level of the MSR amplitude in neonatal nerve crush preparations. The MSR compound potentials were generated when the test stimulus applied on dorsal roots were at 5xT, 2.5xT and 1.5xT. The amplitude of the MSRs was reduced as a result decreasing the stimulating strength in which the MSR amplitude was about 60% of the maximal and near the normal MSR amplitude when the stimulus was set at 1.5xT. In 1.5xT situation, the recurrent inhibition of MSRs between LG-S and MG motor pools tended to increase the amount recurrent inhibition (see section 5.2.3), however, the amount of the recurrent inhibition seen in 1.5xT group animals was still far more smaller than the recurrent

inhibition value in either normal or adult nerve crush rats. These results were not in agreement with the report by Crone et al. (1990), since the effect of recurrent inhibition virtually no difference when the MSR amplitude whatever maximal or 60% of the maximal. These difference may be due to the different preparation used (normal cat vs regenerated rat). Thus the observed dramatic reduction in amount of recurrent inhibition in 5-day-old sciatic nerve crush rats in this study seems not due to the increased monosynaptic reflex amplitude. The other evidence also supported this view. Although, the amplitude of MSRs recorded from contralateral side of 5SC7 group animals was larger than that from normal animals, the RI in this preparation was not differ from that in normal rat. This result may indicate that the reduction of recurrent inhibition seen in neonatal nerve crush motoneurones is mainly the result of impairment of recurrent inhibitory pathways following nerve crush.

DEPRESSIVE EFFECT ON RECURRENT INHIBITION AS A RESULT OF NERVE CRUSH DEPENDS UPON THE PERIOD OF DENERVATION

In this study the length of time during which muscles were separated from their neurones was varied using three different nerve crush sites along the nerve innervating the G-S muscles in either adult and 5-day of age rats. In each case, shortening the period of dennervation improved recovery of the recurrent inhibitory effect. Taken together these experiments show that the degree of permanent impairment of recurrent inhibitory pathway following temporary dennervation either during the neonatal period or in adult is related to the length of time during which the motoneurones and muscles are disconnected. The longer the period of separation the more severe the impairment.

These results are supported by the other findings. Lowrie et al. (1990) reported that crushing of the peroneal nerves at 3 mm away from the EDL muscle at 5-day of age was followed by a much better recovery in EDL muscle than that crushing site at 9 mm away the muscle. Brown et al. (1976) demonstrated a similar result in soleus muscle in 2-day-old rat in which soleus muscle was nearly complete recovery after a crush on soleus nerve at its point of entry into the muscle. In this study, nerve crush carried out in either adult or 5-day of age rats, motoneurone death does not occur (Carlson et al., 1979; Lowrie et al., 1982, 1987; also see section 4.5.1 and 5.5.1). In addition, the Renshaw cell activity does recover to normal

following a initial depression, at least in adult, after the sciatic nerve crush (far crush) (Sanna et al., 1993). Thus it could be concluded that nerve crush far from the muscle leads to poor recovery on recurrent inhibition, not because of motoneurone death or Renshaw cell permanent inactivity, but because it disrupts the interaction between the motoneurone and its target for a longer time.

However, the impairment in sciatic and tibial never crush pair in adult and in tibial and LG-S nerve crush pair in 5-day-old rats had the similar degree although the length of time of dennervation were different within each pair. The rate of regeneration of the crushed nerve was found to be at 1.5-1.6 mm a day in rat and no change whether the crush was close to or far from the muscle (personnel communication with Dr. V. Shahani). Thus, the sciatic nerve crush (20mm) and tibial nerve crush (10mm) in adult rat in this study certainly had a different neurone-muscle disconnection time in which one was about 14 days and the other was about 7 days. The result that the reduction of recurrent inhibition in adult tibial nerve crush rats was as large as that in sciatic nerve crush rats may suggest that tibial nerve crush (1 week dennervation) is long enough in producing the maximal impairment in recurrent inhibitory pathway. In contrast, the result that the recovery of recurrent inhibition after 5-day-old tibial nerve crush was as good as that after LG-S nerve crush may also suggest that, in young animals, the length of time of dennervation after LG-S crush is long enough to generate a depressive effect on recurrent inhibition.

DEDIFFERENTIATION IN RECURRENT INHIBITORY PATHWAY FOLLOWING NERVE CRUSH

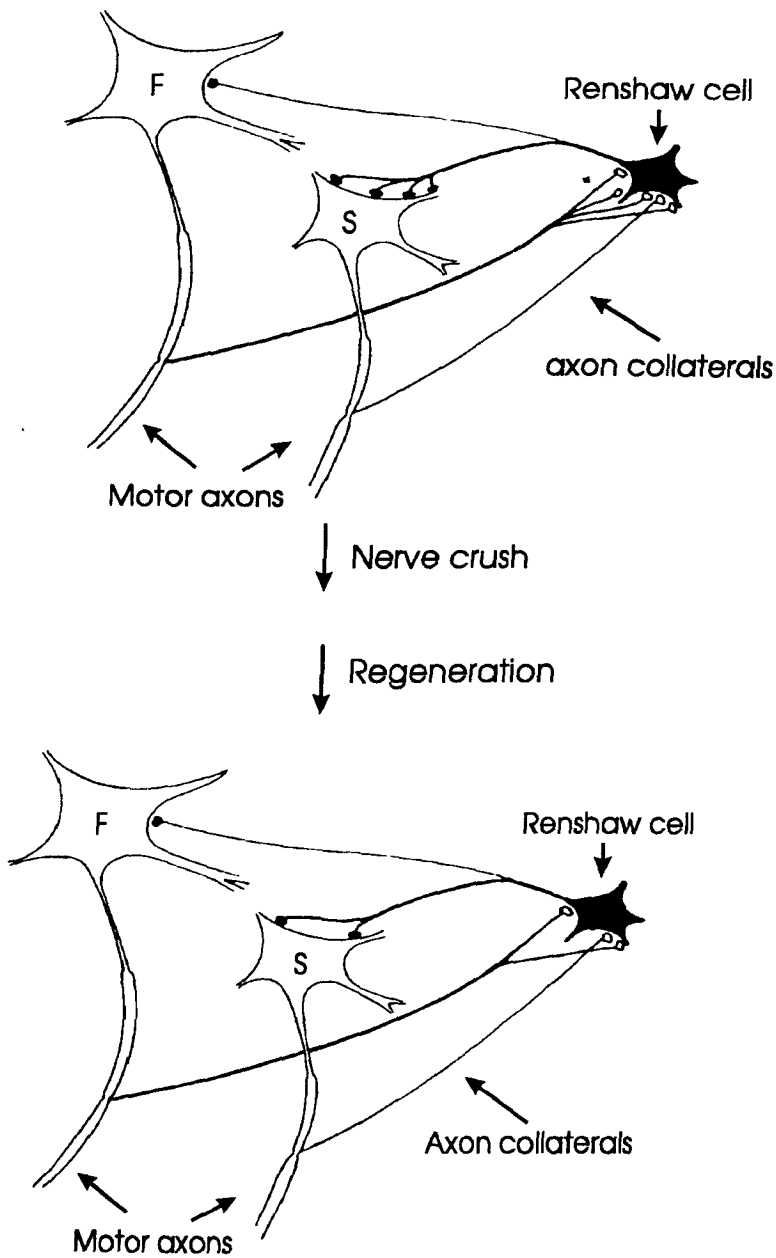
"Dedifferentiation" after peripheral nerve injury have been reported widely not only in motoneurones but also in muscles. The different membrane intrinsic properties of FF-, FR-, and S-type motoneurones (Burke & Rudomin, 1977, Burke, 1981) were reported to be dedifferentiated in axotomized motoneurones (Kuno et al., 1974a,b; Foehring et al., 1986a,b; for review see Titmus & Faber, 1990). For example, the wide range of motoneurone input resistance, conduction velocity, and afterhyperpolarization duration was found to be narrowed as the result of axotomy. One example of muscle "dedifferentiation" is that normal fast TA and EDL muscle staining for succinic dehydrogenase (SDH) shows a mixture of fibers which

have either low (pale staining) or high (dark staining) oxidative capacity, while after sciatic nerve crush at 5-days virtually all the fibers in the reinnervated muscle stain darkly (Lowrie et al., 1982, 1990; Albani et al., 1988). Functionally, following nerve injury, fast muscle exhibits great increase in fatigue resistance (Lowrie et al., 1990). This "dedifferentiation" also happens in the recurrent inhibitory pathway of regenerated animals. In this study, results indicated that the different distribution of recurrent inhibition between different motoneurone pools was virtually lost as a result of nerve crush (Fig.4.14). In normal decerebrated rat, recurrent inhibition from MG motoneurones to LG-S motoneurones is significantly bigger ($P < 0.01$, t-test) than that from LG-S to MG. However, recurrent inhibition between LG-S and MG motor pools tested after either adult sciatic nerve crush or 5-day of age sciatic nerve crush does not differ statistically (Fig.4.14). The different recurrent inhibitory effect between MG and LG-S motor pools is due to the different distribution of F-type or S-type motoneurones containing in these motor pools. In general, the strength of recurrent inhibition to α-motoneurones increases in the order of FF < FR < S (Friedman et al. 1981; Hultborn et al., 1988b). It is because that the number of Renshaw cells projecting to a given motoneurone and the number of synaptic terminations on a given motoneurone by an individual Renshaw cell increase in the order of FF < FR < S (Friedman et al., 1981) and the recurrent axonal collateral synaptic terminals among motor unit types increase in order S < FR < FF (Cullheim & Kellerth, 1978b). Ryall and co-workers (1972) also demonstrated that large motoneurones excited could recruit a large percentage of the Renshaw cell pool while small motoneurones excited only recruited a small fraction of the pool. Thus, it could be proposed that following nerve injury either in adult or in neonatal animals, the number of eliminated axon collaterals may increase in the order of S < FR < FF, and the projection from Renshaw cells to regenerated motoneurones decrease in the order of FF < FR < S (Fig.6.1). These changes could directly result in a loss of the different distribution of recurrent inhibition between different type of motor units. Evidence exists to support this proposal that, from morphological study by Havton and Kellerth (1984, 1990a), the number of end branch of axon collaterals and the number of axon collateral boutons from axotomized motoneurones 12 weeks postoperatively were significantly smaller than those in normal FF- and FR- type neurones in gastrocnemius motor pool but undistinguishable from those of normal gastrocnemius type S neurones. After 5-day-old sciatic nerve crush, the mean cell body area was found smaller than that of the contralateral control motoneurones and this

Fig.6.1

Diagrams of recurrent inhibitory pathways to small S-type and large F-type G-S motoneurones before and after nerve crush and regeneration. Recurrent collaterals of S-type and F-type G-S motoneurones synapse on the same Renshaw cell. In turn, the Renshaw cell axon inhibits both types of motoneurones. Following nerve crush, it is postulated that there are more motoneuronal axon collaterals eliminated in large F-type motoneurones than in small S-type motoneurones and there is less synaptic input loss from renshaw cells to large motoneurones than to small motoneurones. Excitatory synapses are drawn as empty structures; inhibitory synapses are filled in.

Fig.6.1



F = F-type motoneurone, S = S-type motoneurone

change was believed due to a selective reduction in the size of the larger motoneurones (Lowrie et al., 1987). In addition, although the RIPSPs recorded in cat type identified LG-S motoneurones which elicited by axotomized MG motoneurones 12 weeks postoperatively were found no significant different with those of normal type identified LG-S motoneurones, the tendency in reducing the mean RIPSPs amplitude in type S neurones was bigger than in type F neurones (Havton & Kellerth, 1984). Thus, it is not a surprise that the distribution pattern of recurrent inhibition of MG and LG-S motoneurones has been altered as the result of the nerve crush seen in this study. In addition, the possibility that motoneurone membrane properties change following denervation could not be excluded to contribute to this alteration in recurrent inhibition. Since the excitability of motoneurones is known to be inversely related to their size (Henneman et al., 1965; Luscher et al., 1979), the possible changing motoneuronal excitability following nerve injury may lead to an alteration of the motoneurone response to recurrent inhibitory input from Renshaw cells.

REGENERATED MOTONEURONES AND MUSCLES

The mean number of motoneurones labelled by injection of 20% HRP into gastrocnemius-soleus muscles in normal Wistar rats in this study is 167 ± 27 ($n=3$). This mean number compared well with previous observations in which HRP was used to label motoneurones to the gastrocnemius-soleus muscles (Nicolopoulos-Stournaras & Iles 1983).

Following sciatic nerve crush in either adult and 5-day-old rats, gastrocnemius motor pools in both pre-injured side and contralateral uninjured side contain as many HRP labelled motoneurones as those observed in normal rats. This result has confirmed the previous reports that crushing of peripheral nerve in rat older than 5-day postnatally does not lead to motoneurone death (Carlson et al., 1979; Gutmann & Young, 1944; Lowrie et al., 1982, 1984, 1987; Romanes, 1946; Schmalbruch, 1984). In this study, the regenerated muscle weight and its fiber type have also studied as well. After reinnervation from adult sciatic nerve crush reinnervated muscles not only changed its fiber type from type-II (fast) towards type-I (slow) but also lost their weight while no motoneurones lost been observed.

The other findings are consistent with these results. Albani et al. (1988) reported that in

reinnervated fast EDL muscle after adult axotomy, the twitch tension was 61% of control and the maximal tetanic tension was 77%. The corresponding muscle weight after reinnervation was 80% of the control. In contrast, in this study, the reinnervated gastrocnemius muscles after adult nerve crush had a muscle weight of 73% of the control.

Normal gastrocnemius muscles show a typical mosaic pattern while staining for SDH in which a small population of type I fibers are randomly distributed. However, after reinnervation, the mosaic pattern has been changed to group and there ~~is~~ are a large number of type-I fibers ~~are~~ distributed in random groups through the muscle. This phenomenon was also found in rat EDL muscle by Albani et al. (1988) after adult nerve crush and by Lowrie et al. (1982,1990) following 5-day-old nerve crush. Since type-I fibers are thought to be small and oxidative and type-II fibers are large and non-oxidative, muscle type change may contribute to the muscle weight lost. In addition, the consequent relative increase in oxidative capacity may explain the increase in fatigue resistance found in the reinnervated muscles (Lowrie et al., 1982).

FUNCTIONAL ASPECTS

The control of motoneurone firing patterns depends not only their intrinsic biophysical properties, eg. the firing threshold and the afterhyperpolarization (AHP), but also on the source and distribution of the synaptic input that activates them, to which Renshaw cells only contribute a minor fraction. The function of synaptic inhibition is to decrease firing rate or prevent activation altogether. One of the four major elaborate hypotheses on possible functions (Hultborn, 1989) that the Renshaw system would serve as variable gain regulator at the output level were postulated by Hultborn et.al. (1979b). They suggested that the gain of the input-output curve relating the excitatory input to the activity of the pool of motoneurones (output) would increase when the Renshaw cells were inhibited, but would decrease when Renshaw cells were facilitated. A "low gain" condition (facilitation of Renshaw cells) allows supraspinal force generating circuits to play over a considerable part of their working range and yet cause only small changes in muscular force, whereas a "high gain" state (inhibition of Renshaw cells) allows the central command to generate larger forces for a given drive. Thus it appears to be that the decreased recurrent inhibition could facilitate

the α-motoneurones output, in association with the gamma-motoneurone and Ia inhibitory interneurone (Hultborn et.al., 1979b), which allows generate the high force.

After reinnervation, there was an overall increase in the impulse traffic to the reinnervated muscle accompanied by an increase in the fatigue resistance and the activity of oxidative enzymes in the muscle which are usually a consequence of increased muscle activity could be observed. This increased impulse traffic to the reinnervated muscles always thought to be an indication of the alteration in the activity patterns of the pre-injured motoneurones (Lowrie et al., 1982,1987, 1990; Navarrete & Vrbova, 1984; Pette & Vrbova, 1985; Navarrete et al., 1990; Vejsada et al., 1991). These changes may reflect a compensatory mechanism for the lost forces of motor unit output after nerve injury. Reduced recurrent inhibition in regenerated motoneurone pools may benefit and contribute to this functional compensation. As a gain regulator, recurrent inhibitory pathway by reducing its activity presents a "high gain" state in generating large forces for a given drive to compensate the lost forces due to impaired motor unit activity following denervation. Evidence exists that the locomotor EMG pattern in the flexor TA muscle was served impairment following neonatal nerve crush (Vejsada et al., 1991). Vejsada and co-workers (1991) postulated that the extensor-like EMG burst in the TA muscle could be the result of enhanced excitability of ankle flexor motoneurones and/or interneurones. This would imply that the central pattern generator neurones that generate the TA locomotor bursts are being triggered by inputs which normally only drive the extensor. Impaired reciprocal inhibition between central pattern generator neuronal circuits that control antagonistic muscles was suggested to be involved (Vejsada et al., 1991). Since Renshaw cells not only inhibit the synergist α-motoneurones but also inhibit Ia inhibitory interneurones, the reduced Renshaw cell activity may induce the enhancement in reciprocal inhibition pathway and then in turn increase the antagonistic muscle activity. Navarrete et al. (1990) also reported that usually small crossed-reflex responses were permanently enhanced by temporary interruption of the motoneurone's contact with its target muscle during early postnatal development. They proposed that this long lasting alterations in reflex responses in young nerve crush animals may be due to a permanent alteration of the synaptic inputs to the injured motoneurones. In fact, the permanent reduction of recurrent inhibition in regenerated motoneurones may contribute to this enhanced crossed-reflexes in which the normal inhibitory input from Renshaw cells are disappeared in injured

motoneurones. Since Renshaw cell terminals are located closely to the Ia afferent synapses, the absence of inhibition from recurrent inhibitory pathway indeed increases the possibility for motoneurones to response the weak excitatory input from contralateral Ia afferents.

It is of interesting that the permanent impairment of recurrent inhibitory pathway in regenerated motoneurone pools has been also observed in adult nerve crushed rats. It has been reported that the outcome of reinnervation after peripheral nerve crush in adult animals is relatively satisfactory (Beranek et al., 1957; Lowrie et al., 1982). However, although nerve injury in adult animals is not as severe as nerve injury in neonatal animals (Beranek et al., 1957; Lowrie et al., 1982, 1987, 1990), the muscle fiber property change (Lowrie et al.,1982), muscle weight and forces lost (Albani et al., 1988), and some impairment features of motoneurones (Brannstrom et al., 1992a, b) were still observed in adult nerve crush animals, even after regeneration. In this study, we also observed that the reinnervated muscles lost their weight and changed their fiber type not only in 5-day-old crush rats but also in adult sciatic nerve crush rats. Thus, it is not a surprise that in association with the lost forces (lost weight), the motor units, in order to keep their normal function, may have to increase the motoneurone output, in part, by reducing the inhibitory feed back effects from Renshaw cells. This may also happen in adult nerve injury animals, although the changes in young regenerated animals are most dramatic.

FUTURE WORK

The results presented in this thesis indicate that regenerated motoneurones in general showed reduced recurrent inhibition with the most dramatic effect being on motoneurones injured early in postnatal life. We hypothesise that the reduction of recurrent inhibition seen in regenerated motoneurones is due to permanently morphological and physiological change within recurrent inhibitory pathway. Possible elimination of recurrent axon collaterals from regenerated motoneurones and elimination of synaptic connection from Renshaw interneurones to regenerated motoneurones may exist. The excitability of Renshaw cells may also be permanently depressed after nerve crush performed in early postnatal life.

This hypothesis needs to be tested and verified. Physiologically intracellular recording

applied on to Renshaw cells and regenerated motoneurones will monitor their activity with antidromic volley on either normal muscle nerve or pre-injured nerves in either adult or neonatal nerve crushed rat to determine the alteration in membrane excitability (intrinsic properties) and in response to synaptic input (EPSPs, IPSPs) following nerve crush. Morphologically, combined intracellular staining of Renshaw cell and regenerated or normal α-motoneurones after peripheral nerve injury in rat, as reported in normal (Fyffe, et al., 1991a,b) and axotomized (Havton & Kellerth, 1984, 1990a) cat , will demonstrate whether 1) elimination of recurrent axon collaterals does happen in peripheral nerve crush rat; 2) the alteration of distribution of recurrent inhibitory synapses on injured or uninjured spinal motoneurones exists in young and adult nerve crush rat.

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Appendix I.

Modified Hanker-Yates Method for Visualising Motoneurones Filled with Horseradish Peroxidase.

Solutions

1. Millonig's phosphate buffer: $NaH_2PO4.2H_2O$ 19.08g in 903.7ml distilled water;
   1M NaOH 96.3ml (for 200ml 1M NaOH, 8g NaOH in 200ml distilled water).

Adjust to PH 7.3 and make up to 1 litre with distilled water.

2. Cobalt/nickel solution: 1% cobalt chloride 300ml and 1% ammonium nickel sulphate 200ml.
   Make fresh and mix together just before use.

3. Cacodylate buffer: *Solution A* - 0.1M sodium cacodylate (21.4g/500ml distilled water).
   Solution B - 0.2N HCl (17.22ml 10N Hcl/litre distilled water).

Add 500ml of solution A to 440ml of solution B and adjust to PH 5.1-5.2. Make up to 2 litres with distilled water and keep in fridge.

4. Hanker-Yates solution: Hanker-Yates combined reagent (Sigma) 150mg;
   Cacodylate buffer (PH 5.1-5.2) 100ml;
   1ml of 1% solution H_2O_2.

Make up just before use, discard after one hour.

5. Counterstaining solution: 0.3g Gallocyanin; Chromalum (CrK(SO4)2.12H2O) 10g.
   Dissolve chromalum in 100ml distilled water by heating. Add 0.3g gallocyanin, bring to boil and allow to simmer for 20-30 min. Cool, add distilled water to the original volume and filter.

Method.

1. Perfuse animal through heart with 2.5% gluteraldehyde in Millonig's buffer, preceded by a short flush with saline or buffer. Remove spinal cord and post fix blocks for about 4 hours at 4°C, followed by 30% sucrose in Millonig's buffer at 4°C overnight (see also section 5.2 Methods in chapter 5).

2. Cut frozen (-20°C) sections at approximately 50μm. Collect in Milloig's buffer.

3. Brief wash in distilled water. Incubated in cobalt/nickel solution for 15 min.

4. Brief wash in distilled water. Rinse in 2 changes Millonig's of buffer, 10 min each.

5. React in Hanker-Yates solution 10 to 25 minutes, stopping before background darkens.

6. Rinse in 2 changes of Millonig's buffer, 5 min. each.

7. Mount sections on gelatinized slides and dry overnight at 37°C.

8. Next day, brief wash in distilled water. Counterstain in Gallocyanin for 10 to 25 min, depending on age of the stain (the older the stain, the longer the counterstaining time).

9. Brief wash in distilled water. Dehydrate in 70%, 95% and 2 changes of 100% alcohol and 2 changes of Histoclear, 2 min each.

10. Coverslip mounted with Permount.

Appendix II.

Succinic Dehydrogenase Stain for Oxidative Capacity of Muscles.

Stock Solutions.

1. 0.1M Phosphate Buffer pH7.6: a) Dissolve 1.42g Na_2HPO4 in 100ml distilled water.
   b) Dissolve 0.468g NaH2PO4 in 30ml distilled water.
   Add (b) to (a) a little at a time until PH 7.6 is reached.

2. 1M Na succinate: 1.62g in 10ml buffer; keep in fridge.

3. 15mM nitroblue tetrazolium: 246mg in 20ml distilled water. May need to warm up to 45°C to dissolve; keep in fridge.

4. 0.1M KCN: 13mg in 2ml buffer. Make fresh.

5. 10mM phenazine methosulphate: 12.2mg in 4ml distilled water. Must be made up fresh. Very sensitive to light, so protect while making up working solution. If colour changes from yellow to green, discard.

Working Solution.

1. Take 32.8ml of 0.1M phosphate buffer Ph 7.6
2. Add 2ml 1M Na succinate in buffer

3. Add 4ml 15 Mm nitroblue-tetrazolium
4. Add 0.4ml 0.1M KCN in buffer
5. Add 0.8ml 10mM phenazzine methosulphatee
6. Filter and keep in dark bottle at 4°C.

Method.

1. Put few drops of working solution over sections on slide. Incubate at 37°C for 5 min.

2. Wash in 0.9% saline. Dehydrate in 70%, 95% and 2 changes of 100% alcohol and 2 changes of Histoclear, 2 min each.

10. Coverslip mounted with Permount. The background is clear and the SDH stained fibres are blue.

Appendix III.

RELATED PUBLICATIONS

1. Shahani, U., Wang, Y., Rosenberg, J.R. & Conway, B.A. (1992) Reduction in recurrent inhibition of motoneurones following peripheral nerve crush in the rat. *Neurosci. Lett.* **(supplement) 42**, S16.

2. Shahani, U., Conway, B.A., Wang, Y. & Rosenberg, J.R. (1993) Changes in Renshaw cell recurrent inhibition following peripheral nerve injury in rats. *J. Physiol.*, **459**, 500P.