Enhanced Synaptic Effectiveness Following Prolonged Changes in Synaptic Use

One of the many unresolved problems in synaptic physiology is the relationship of prolonged synaptic usage to synaptic effectiveness. Although it has often been postulated that prolonged increases in synaptic usage lead to increased synaptic effectiveness[1-3], a careful review of the literature on peripheral junctions lead SHARPLESS[4] to the opposite conclusion, namely that prolonged disuse leads to a compensatory increase in synaptic effectiveness.

There have been a number of attempts to test these ideas using the monosynaptic reflex pathway[5-7]. 2 groups using chronic tenotomy (i.e. transection of tendons) to decrease activation of muscle spindles independently observed an increase in the amplitude of both the monosynaptic reflex[8-10] and the monosynaptic group I ascending dorsal spinocerebellar tract response[10]. These effects were *highly specific*, being confined to the actions of group IA afferents supplying the tenotomized muscles.

However, tenotomy alone does not guarantee that the annulospinal endings are silenced in the resting muscle[11]; therefore the extent of afferent fibre disuse is unknown. Indeed, nerves from tenotomized muscles showed an increased overall resting integrated afferent fibre activity compared to control nerves[10,12], although this was not confirmed in studies of dorsal root activity[13,14]. Finally, because gamma motoneurone activity could theoretically still profoundly influence the tonic activity of group IA fibres in these experiments, it is uncertain whether the enhanced monosynaptic responses observed following tenotomy could result from chronic *disuse* or from *excessive use* of muscle stretch afferents.

To obtain a more rigorous test of these conflicting hypotheses, we attempted to provide an unequivocal and profound disuse of group I muscle afferents through opening the gamma loop by transecting the appropriate ventral roots (de-efferentation) as well as cutting the Achilles tendon. This should greatly decrease afferent discharges from muscle stretch receptors by completely eliminating gamma control[11,15]. Since ventral root transection leads to retrograde chromatolysis of motoneurons, and to changes in their excitability[16,17], we studied the ascending monosynaptically relayed discharges in the dorsal spinocerebellar tract following activation of group I fibres.

In adult cats the Achilles tendon was cut on one side and intradural section of ventral roots L6, L7 and S1 made ipsilateral to the tenotomy under sterile conditions. A sham operation was performed on the opposite side. 4–10 weeks later, acute experiments were carried out in these animals under pentobarbital anaesthesia. The ascending monosynaptic mass discharge relayed by cells of Clarke's column was recorded monophasically with bipolar electrodes. Single stimuli that were supramaximal for group I fibres were applied to soleus and both gastrocnemius nerves. Data were used only from experiments in which there were symmetrical afferent volley spikes recorded from a monopolar electrode on the L5 cord dorsum.

We found that following chronic tenotomy and de-efferentation, the amplitude of the monosynaptic ascending response was considerably and consistently increased (approximately 50%) on the operated side as compared to the control side (Figure 1). This difference persisted unchanged throughout acute experiments lasting up to 10 h. Only the earliest (group I) component showed significant and consistent asymmetry. The monosynaptic ascending responses to sural nerve volleys were symmetrical, indicating that experimental conditions did not artifactually favour one side. In non-operated control preparations the monosynaptic ascending group I responses were symmetrical. The monosynaptic response asymmetry was preserved throughout the course of post-tetanic potentiation following application of a 10 sec train of stimuli at 500 per second to soleus and both gastrocnemius nerves (Figure 2). This demonstrates that the asymmetry will remain even during a markedly different functional state. Statistical analysis, using both parametric and non-parametric tests, of response amplitudes

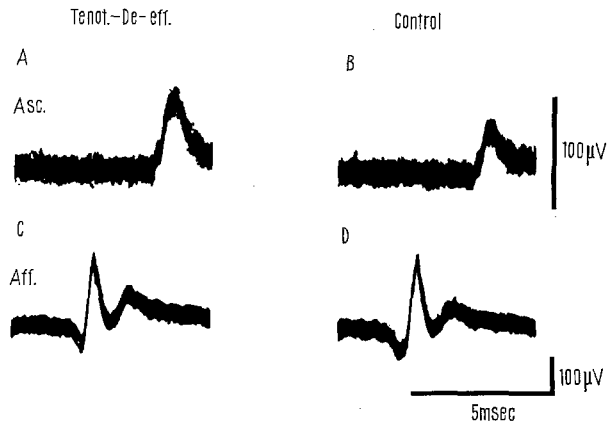

Fig. 1. Monosynaptic ascending responses recorded from acutely transected thoracic spinal cord (A and B) and L5 cord dorsum potentials (C and D) to single shock stimulation of soleus and gastrocnemius nerves 47 days after unilateral Achilles tenotomy and transection of ventral roots L6, L7 and S1. Traces A and C, operated side; traces B and D, non-operated side. Each record consists of 20 superimposed individual responses. The amplitude of the monosynaptic ascending response of the operated side is approximately 1.5 times larger than that of the non-operated side.

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in each of 7 critical experiments using tenotomy and de-efferentation, and in each of 6 control experiments revealed that all animals in the experimental group showed significant group I response asymmetry ($P < 0.01$), whereas none in the control group did. Comparison of mean response amplitude on the two sides for all the operated and all the non-operated groups by the t-test showed the response amplitudes to be significantly different ($P < 0.01$) in the experimental group, but not in the control group.

Our data demonstrate that combined tenotomy and ventral root transection (de-efferentation) lead to enhancement of the monosynaptic ascending group I response. Our result with the combined procedure is identical to that found after tenotomy alone[10]. The additional de-efferentation in our series conclusively rules out an effect mediated through the gamma loop, and thus diminishes the likelihood that the increased-use hypothesis is a satisfactory explanation for the results in the original tenotomy experiment[10,18]. These findings therefore suggest that chronic *disuse* by means of tenotomy alone or combined tenotomy and de-efferentation leads to increased synaptic effectiveness.

To test this hypothesis further it is necessary to rule out the possibility that a trophically induced increased activity in some group of muscle afferents might be responsible for the increased effectiveness of group I actions in our experiments. Therefore, we measured the resting discharge frequencies and functional properties of over 100 group I and II afferents from muscles previously tenotomized and de-efferented; we found no evidence of any significant increase in the resting discharge frequency of such units. Indeed, histograms of spontaneous resting discharge frequency of these units were nearly identical to those derived from normal and tenotomized muscles[12], except that there were more 'silent' units in our experimental group. Moreover, in experiments in which the muscles were not exposed, passive rotation of the ankle joint through ranges of motion judged to be similar to those occurring during unrestrained, normal postural movements produced no, or very little, increase of resting discharge in afferents from operated muscles. In contrast, very slight ankle rotation in normal control preparations was associated with the usual very large increases in discharge frequency of afferents from comparable normal muscles. We therefore assume that, in tenotomized, de-efferented muscles, postural ankle rotation would fail to increase discharge frequency in muscle stretch afferents to a level comparable to that which would occur on the normal control side during natural rotational movements. Detailed studies of ankle rotation in animals with tenotomy alone have also shown that ankle rotation produces less discharge increase in afferents supplying tenotomized muscles than in afferents supplying control muscles[13,14]. Therefore, it seems reasonable to postulate that the enhanced monosynaptic group I responses seen in experiments in tenotomy alone[8-10], and in our experiments are associated with chronic synaptic disuse.

As a final control procedure we have performed experiments on 5 cats subjected to chronic separation of the tip of the os calcis as an alternative means for unloading the triceps surae without actually cutting the tendon. Since our results again duplicated those reported for tendon transection[10], they rule out any trophic effect specific to the tendon transection itself.

Our observations on the resting discharge frequency of fibres are confined to group I and II afferents. However, it seems doubtful that mechanisms related to increased spontaneous activity in higher threshold, non-stretch afferents could account for either our results or the highly restricted distribution of increased synaptic effectiveness seen in tenotomy experiments[8-10]. Thus, at least at these spinal synapses and with these procedures, our data favour the hypothesis that prolonged *disuse* is associated with increase in the effectiveness of the disused afferent fibres in discharging their postsynaptic cells. From these studies alone we cannot specify a pre- or postsynaptic locus for the critical change; neither can we delineate the mechanisms involved.

If it can be shown that synaptic disuse at other junctions also leads to increased synaptic effectiveness, then this reciprocal relationship could prove to be an important homeostatic principle governing long-enduring changes in synaptic transmission[19].

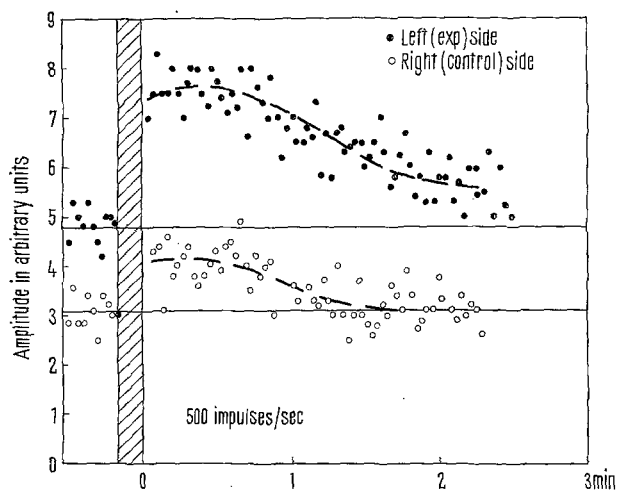

Fig. 2. Post-tetanic potentiation of monosynaptic ascending responses to soleus and gastrocnemius nerve stimulation on operated side (filled circles) and non-operated side (open circles). Baseline amplitudes are plotted to left of hatched column which represents 10 sec of stimulation at 500 impulses per second. The figure shows the potentiated response of the non-operated side. The time course of the potentiated responses is comparable on both sides.

Résumé. Pour mieux étudier le rapport entre la diminution de l'utilisation chronique d'une synapse et son efficacité, l'amplitude de la réponse monosynaptique ascendante des fibres afférentes de Groupe I a été mesurée plusieurs semaines après ténotomie et déefférentation. La conclusion de cette étude est qu'une plus grande efficacité monosynaptique est associée à la diminution de l'utilisation des fibres afférentes du Groupe I.

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