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MAINTAINED CHANGES IN MOTONEURONAL EXCITABILITY BY SHORT-LASTING SYNAPTIC INPUTS IN THE DECEREBRATE CAT

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SUMMARY

1. During investigation of the tonic stretch reflex in the unanaesthetized decerebrate cat we observed that a short train of impulses in Ia afferents from the soleus muscle (or its synergists) may cause a prolonged activity in the soleus muscle as judged by EMG and tension recordings. This excitability increase, which outlasted the stimulus train, could stay virtually constant during long periods (even minutes), but could be terminated at any time by a train of impulses in, for example, the peroneal nerve.

2. Gradation of the strength of stimulation and the duration of the train of impulses show that the amount of maintained excitability increase depends – within some limits – on the total amount of Ia impulses.

3. In paralysed preparations a short train of impulses in Ia afferents from any part of the triceps surae, caused a maintained increase of the efferent activity in the nerves to triceps surae and a maintained increase of the triceps surae monosynaptic test reflex. These experiments demonstrate the existence of a central mechanism (in the spinal cord and/or the brain stem), which is responsible for the maintained excitability increase seen in motoneurones to the homonymous and synergic muscles.

4. In acute spinal preparations it was not possible to demonstrate any long-lasting excitability increase by a train of Ia impulses. Following intravenous administration of the serotonin precursor 5-hydroxytryptophan, mimicking the tonic activity of these pathways in the decerebrate state, it was again possible to elicit the long-lasting excitability increase by a train of impulses in Ia afferents. A subsequent I.V. injection of methysergide (a serotonin receptor blocker) abolished the long-lasting excitability increase. This set of experiments demonstrates that the basic mechanism responsible for the maintained excitability increase is located at segmental level, and involves serotonergic systems.

5. It was demonstrated that activation of several ipsilateral and crossed reflex

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pathways by trains of impulses in cutaneous or high-threshold muscle afferents could trigger a maintained excitability increase of those motoneurone pools which were activated by the stimulation. Trains of stimuli to facilitatory regions in the brain stem could also cause a long-lasting excitability increase of motoneurones. Furthermore, activation of all reflex pathways which mediate postsynaptic inhibition to a motor nucleus (including recurrent inhibition via Renshaw cells) could terminate the prolonged excitability increase of that particular motor nucleus.

6. The soleus EMG and tension were recorded during ramp-and-hold stretches. It was found that higher velocities of stretch in the ramp caused stronger tonic stretch reflexes during the subsequent holding phase. It is assumed that several motor units which are recruited by the intense Ia activity during the dynamic stretch remain active (self-sustained activity) during the holding phase. Recording of single motor units during the stretch reflex presented in the accompanying paper (Hounsgaard, Hultborn, Jespersen & Kiehn, 1988) support this conclusion.

INTRODUCTION

The high degree of tone which promptly develops in extensor muscles following a mechanical transection of the brain stem at the level of the corpora quadrigemina was described by Sherrington in 1898. He soon established the importance of the afferent input for the maintenance of tone; transection of the appropriate dorsal roots caused a local reduction of rigidity (Sherrington, 1898), while the tone remained after extensive denervation only sparing the innervation of the muscle under investigation (Sherrington, 1909). With improved myographs Liddell & Sherrington (1924, 1925) were able to prove that receptors in the muscle accounted for the muscle contraction in response to extension; the alternative terms 'stretch reflex' and 'myotatic reflex' were introduced. It is now firmly established that the tonic stretch reflex is dependent both on an excitatory input to motoneurones from muscle spindles and a particular set of descending activities from the brain stem facilitating both α-motoneurones and static γ-motoneurones as well as inhibiting transmission in pathways mediating autogenetic inhibition from the stretched muscle (a vivid and critical account of the development of this research is given by Peter Matthews in his monograph, 1972).

The present series of experiments started with the observation that a very short-lasting activation of triceps surae Ia afferents in the decerebrate cat could trigger a long-lasting increase of the triceps surae contraction. The long-lasting excitability increase initiated by a short-lasting vibration was so conspicuous (especially in anaemic decerebrates where decerebration occurs at more caudal levels; Pollock & Davis, 1923, 1930) that it seemed possible to analyse the underlying mechanisms, and to determine its role in the classical tonic stretch reflex.

Initially the maintained excitability increase resulting from stimulation of muscle spindle Ia afferents was interpreted as a result of long-lasting polysynaptic excitation (Hultborn, Wigström & Wängberg, 1975; Hultborn & Wigström, 1980). However, our recent analysis of this phenomenon at cellular level (Hounsgaard, Hultborn, Jespersen & Kiehn, 1984, 1988) has shown that intrinsic membrane properties of the motoneurones themselves can be responsible for self-sustained firing

following a short-lasting synaptic excitation. This motoneuronal property is, however, contingent upon their innervation from the serotonergic raphe–spinal projection, which is active in the decerebrate preparation. Our understanding of the underlying mechanisms has thus changed fundamentally. In this paper we therefore restrict ourselves to a descriptive account of maintained excitation by Ia input in the decerebrate preparation. The mechanisms and the general significance of the serotonergic raphe–spinal control of the intrinsic properties of the α-motoneurones will be discussed in the accompanying paper (Hounsgaard *et al.* 1988).

The present report is based on sixty-nine cat experiments over a period of 10 years. Some of the results have been published in a short communication (Hultborn *et al.* 1975) and two reviews (Hultborn & Wigström, 1980; Hounsgaard, Hultborn & Kiehn, 1986).

METHODS

General preparation. The experiments were performed on sixty-nine unanaesthetized decerebrate cats (2·5–4·0 kg); ten cats were decerebrated by classic intercollicular section with removal of the anterior part of the brain, while the remaining fifty-nine were anaemically decerebrated. The cats were initially anaesthetized with ether and N_2O, which was maintained throughout the surgical procedure. Intercollicular decerebration was performed at the end of the operation, while the anaemic decerebration was accomplished as soon as surgical anaesthesia was reached. Briefly, the anaemic decerebration is achieved by ligating the basilar artery in conjunction with both common carotid arteries. The ligature on the basilar artery was placed rostral to the branchpoint of the inferior cerebellar arteries (Pollock & Davis, 1923). Following the completion of surgery anaesthesia was discontinued and a strong extensor rigidity developed. Anaemic decerebration was characterized by the appearance of rigidity in conjunction with the lack of spontaneous movement and large non-reactive pupils. In the majority of experiments these criteria for accepting the animal as decerebrate were met. However in very few animals some spontaneous movements were apparent. In these cases gaseous anaesthesia was immediately reinstated and an intercollicular decerebration was performed. In several animals with anaemic decerebration Evans Blue was injected intravenously at the end of the experiment. The post-mortem dissection of the brain showed that there was no staining of the brain rostral to the basilar artery ligature indicating an irreversible destruction of that part of the brain. When the cats had remained non-reactive for $\frac{1}{2}$ to 1 h some of them were paralysed (Pavulon, Organon, 0·15–0·2 mg/kg supplemented every 40 min or Flaxedil, Gea, 5–10 mg/kg supplemented every 20 min) and artificially ventilated. The end-tidal CO_2 concentration was kept within 3·5–4·5 vol% and blood pressure drops below 80 mmHg were counteracted by infusion of Dextran or noradrenaline. The rectal temperature was kept within 36–38 °C.

Nerve and muscle preparation. Both hindlimbs were extensively denervated and selected nerves were dissected and mounted on silver electrodes for stimulation and recording. The nerves to the following muscles were usually dissected: soleus, or soleus and lateral gastrocnemius together, medial gastrocnemius, plantaris, flexor digitorum longus, the tibial nerve to intrinsic foot muscles, the common peroneal nerve or its branches to tibial anterior and extensor digitorum longus, and the branches to peroneus brevis and tertius, semimembranosus (often together with the nerve branch to the anterior part of biceps), semitendinosus (often together with the branch to the posterior part of biceps) and quadriceps. The sural nerve and the superficial peroneal nerve (without the muscle branches) were used to stimulate cutaneous afferents.

In several experiments the innervation of the soleus muscle (or the soleus and lateral gastrocnemius muscles) was left intact to allow selective activation of muscle afferents by vibration and ramp-and-hold stretches in addition to allowing EMG and tension records to be made. The muscles were dissected free from surrounding muscles and connective tissue without compromising their blood supply. The tendon was attached to a force transducer mounted on a muscle puller. The tibia was fixed rigidly by steel pins.

A laminectomy exposing lumbar spinal segments L4–S1 was performed. In some experiments the

ventral roots were sectioned and the proximal part of the L7 and S1 ventral roots mounted for recording of monosynaptic reflexes. In one experiment dorsal roots were sectioned in order to obtain a selective activation of Renshaw cells by antidromic motor volleys following stimulation of peripheral motor nerves. In some animals a complete spinal section was performed at Th12 level during the course of an experiment. The skin flaps around exposed areas of the cord and the hindlimb were sewn to form pools which were filled with warm paraffin oil.

In some experiments the mid-line structures of the brain stem were stimulated. In these cases the cerebellum was exposed and a vermal cerebelloctomy performed to visualize the floor of the fourth ventricle.

Stimulation of peripheral nerves and brain stem. Peripheral nerves were stimulated with square pulses of 0·1 ms duration. The strength of nerve stimulation is expressed in multiples of threshold for the lowest threshold fibres ($\times T$). Orthodromic and antidromic nerve volleys were recorded from the surface of the spinal cord. The procedures described by Bradley & Eccles (1953) and Eccles, Eccles & Lundberg (1957*a*) for differentiating between afferent fibre groups was followed.

Thin tungsten electrodes, with diameter approximately 10 μm and a resistance of 60–150 kΩ, were used for stimulation of brain stem structures. For stimulation, trains (50–200 s^{-1}) of 0·2 ms negative square pulses were delivered and the current intensity varied from 5 to 100 μA.

The brain stem electrodes were positioned according to Horsley–Clarke co-ordinates as well as the gross landmarks of the exposed fourth ventricle. Small electrolytic lesions were made at the stimulus sites at the end of the experiments and the electrode positions were checked on histological sections stained with Cresy Violet. Schematic reconstructions were compared with appropriate sections from the atlases of Berman (1968) and Snider & Niemer (1961).

Recording. EMG signals were recorded with thin copper wires inserted in the muscle. In paralysed preparations the motoneuronal excitability was monitored either from the tonic activity in the peripheral motor nerves (electroneurograms, ENG), or by the size of monosynaptic test reflexes recorded from the ventral roots. The EMG and ENG signals were sometimes rectified and smoothed (RC integration) to obtain a semiquantitative measure of the activity. The size of the monosynaptic reflexes (the area) was measured by a computer system. Original data were stored on a 7-channel Racal tape-recorder on magnetic tape (bandwidth DC to 2·5 kHz) for off-line analysis.

Drugs. Intravenous injection of 5-HTP (5-hydroxytryptophan) and L-DOPA (L-β-3,4-dihydroxyphenylalanine), precursors of serotonin (5-HT) and noradrenaline, respectively, enhances the fluorescence in serotonergic and noradrenergic terminals (Fuxe, 1965*a*, *b*) in the spinal cord and it has therefore been assumed that injection of the precursors would force synthesis and release of monoamines from presynaptic terminals of the respective reticulospinal fibres. This could possibly reproduce (in the acute spinal cat) the physiological effects that would follow selective activation of the two descending monoaminergic systems (Andén, Jukes & Lundberg, 1964*a*; see also Baldissera, Hultborn & Illert, 1981, pp. 562–563 for a summary of evidence). With this purpose 5-HTP (50–100 mg/kg; Sigma) was given to some acute spinal cats in the present series of experiments.

The α-adrenergic blocker phenoxybenzamine (25–40 mg/kg, $n = 2$; a gift from A/S Alfred Benzon) and the serotonergic blocker methysergide (1·5–4 mg/kg, $n = 4$; a gift from Sandoz) were given intravenously. The drop in blood pressure following the α-adrenergic blockers was counteracted by I.V. infusion of angiotensin II (10 ng kg^{-1} min^{-1}). Strychnine (I.V. 0·05–0·1 mg/kg) was administered in three experiments.

The particular experimental condition for each type of experiment is described in the text and diagrammatically illustrated in some of the figures.

RESULTS

(1) *Long-lasting excitability increase of triceps surae motoneurones evoked by impulses in Ia afferents and recorded by electromyogram (EMG), electroneurogram (ENG) and monosynatic test reflexes*

Figure 1*A–D* illustrates the basic finding that a short train of impulses in Ia afferents in the decerebrate cat may trigger a sustained increase in the EMG activity. The example in Fig. 1*A–C* is from an anaemically decerebrated cat in which the soleus muscle was kept at a length close to the threshold for the stretch reflex to

ensure an efficient background excitation of the motoneurones. When the vibration (100 μm at 200 Hz for 200 ms, indicated by the arrow) was applied there was not only a strong EMG response *during* the vibration, but also a powerful sustained EMG activity *after* the cessation of vibration. Usually the maintained response after a vibration was much smaller in relation to the response during vibration than in the illustrated example. In addition, there was often a short silent period (around 50 ms) in the EMG between the cessation of vibration and the maintained response. The time course of the long-lasting activity seemed to depend on the general 'excitability' of the preparation and could only partly be controlled by changing the initial length of the soleus muscle. It was possible to encounter a stable maintained EMG activity in most anaemically decerebrate preparations (indeed in all of twenty-three cats in which a tonic stretch reflex could be recorded). In the intercollicularly decerebrate cat the long-lasting response usually decayed within a few seconds unless an additional cerebellar ablation had been performed (cf. Matthews, 1966). Therefore we have mainly used the anaemically decerebrated preparation (Pollock & Davis, 1923, 1930), which generally shows a high excitability with powerful stretch reflexes.

It was thus a striking feature that the long-lasting EMG activity could be maintained regularly at a virtually constant level – often for a minute or more (Figs 1 and 3; see also Fig. 2 in Hultborn & Wigström, 1980). In such cases it was possible to reset the activity level back to normal (i.e. before vibration) by a train of impulses to cutaneous afferents and/or group II (and III) muscle afferents. A full account of the sources for this resetting will be given in section (3). In Fig. 1*B* and *C* it is seen that a short train of stimuli to the superficial peroneal nerve (for about 500 ms, indicated by the arrow) abolished the long-lasting EMG activity thus resetting the excitability to the pre-vibratory level. In the following sections we will often use the terms 'on' and 'off' stimulus when we refer to trains of stimuli that initiate and terminate the long-lasting activity (cf. Fig. 1).

Small amplitude vibration is a selective means of activating muscle spindle Ia afferents (Brown, Engberg & Matthews, 1967; Matthews, 1972). We therefore measured the maintained EMG activity 20 s after the end of a vibratory stimulation (200 Hz for 300 ms) as a function of vibration amplitude (Fig. 1*D*). The sustained EMG activity as well as tension increased steadily with vibration amplitudes from 15 to 70 μm; larger vibration amplitudes did not further increase the amplitude of the maintained response in this experiment. In several preparations effective vibration could be as small as 10 μm.

Figure 1*E–G* illustrates the regular finding that short trains (100 ms at 300 Hz) of electrically evoked group I volleys in the nerve from the synergist medial gastrocnemius could evoke a similar course of events as vibration of the soleus muscle, i.e. a large response *during* and a smaller maintained activity *after* the stimulus train. The effective stimulus for the long-lasting response could sometimes be as low as $1{\cdot}1$–$1{\cdot}2 \times T$ for the lowest threshold fibres (Fig. 1*E*) and was maximal at about $1{\cdot}5$–$1{\cdot}7 \times T$ (Fig. 1*G*), i.e. at strengths that were often submaximal for group I fibres when delivered in a train at 300 Hz. Similar results were obtained with stimulation of the plantaris nerve (three cats) and occasionally from the quadriceps nerve. Stimulation of group I afferents from flexor digitorum longus, pretibial flexors, knee flexors and hip extensors were ineffective (see further in section (3)).

Figure 2 illustrates that the amplitude of the maintained response is very much

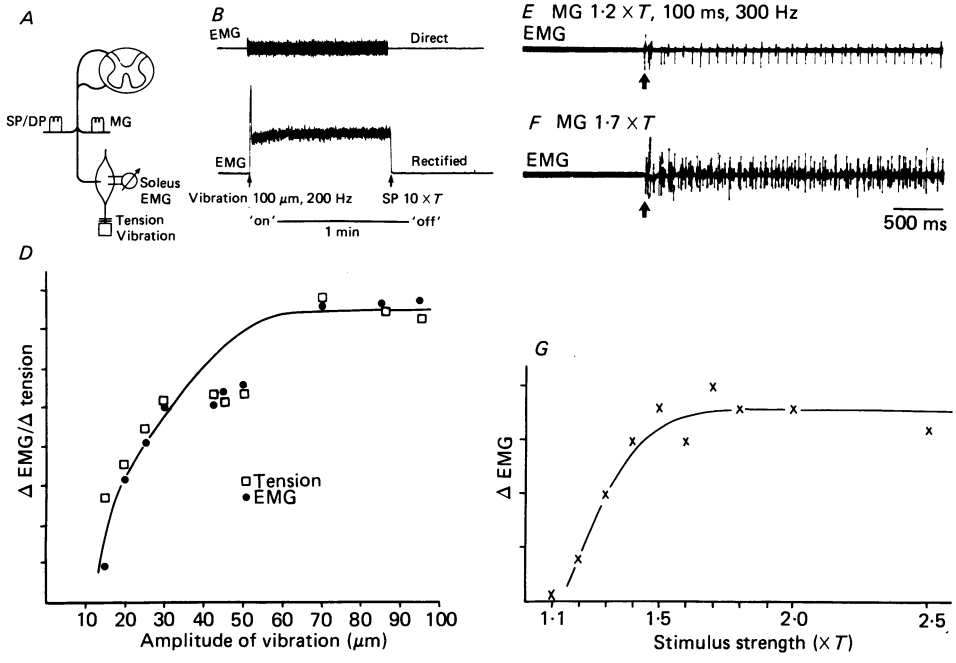

Fig. 1. Long-lasting excitability increase following vibration of the soleus muscle (*A–D*). *A*, experimental arrangement: unanaesthetized anaemically decerebrate (*B* and *C*) or intercollicularly decerebrate and decerebellated (*D–G*) cats in which the left soleus muscle was freed and fixed to a puller and a strain gauge. The EMG was recorded in the soleus muscle. Stimulation of either the superficial (SP) and deep peroneal nerves (DP) or the tibialis anterior nerve were used to reset increased excitability back to pre-vibratory level. *B*, direct EMG showing maintained increase in excitability following vibration of the tendon (100 μm, 200 Hz). *C*, same signal as in *B*, rectified and RC integrated. *D*, increment of EMG activity (rectified; arbitrary units) and tension (arbitrary units) plotted as a function of vibration amplitude (μm). Muscle vibration (200 Hz for 300 ms) caused maintained activity increase which was reset by stimulation of the tibialis anterior nerve ($5 \times T$, 200 Hz) before the next vibration. Scales for the EMG activity and tension were adjusted so that the curves are superimposed with the largest vibration amplitude (> 70 μm). Relation between EMG increment and strength of 'on' stimulus (*E–G*). The medial gastrocnemius nerve was used as 'on' stimulus; these trains are indicated by the arrows in *E* and *F* (direct EMG activity). The 'on' stimulus train had a duration of 100 ms and a frequency of 300 Hz; the strength was $1{\cdot}2 \times T$ in *E* and $1{\cdot}7 \times T$ in *F*. *G*, the increment in EMG activity (ordinate, the rectified EMG response) plotted against the stimulus strength to the medial gastrocnemius (MG) nerve. The EMG activity was reset between the trials by stimulation of deep peroneal nerve (DP $2 \times T$, 300 Hz).

dependent on the duration of the 'on' stimulus train. The graph in Fig. 2*A* shows how the maintained EMG activity increases almost linearly with duration of the stimulus train to the quadriceps nerve in the interval between 20 and 120 ms (abscissa); a train of 500 ms caused a further, but less than linear, increase. The graph in Fig. 2*B* shows a similar relation between the maintained response (estimated from active tension) and the duration of the stimulus train to the medial gastrocnemius nerve. In this case the response is growing linearly when the train duration increases from 25 to 100 ms; additional increase of the duration seems

inefficient. The duration of the stimulus train at which the response was saturated varied considerably from one experiment to the other; in preparations with high excitability a maximal response could be reached with a stimulus train of about 100 ms, while trains of several seconds would be needed when the general excitability was low.

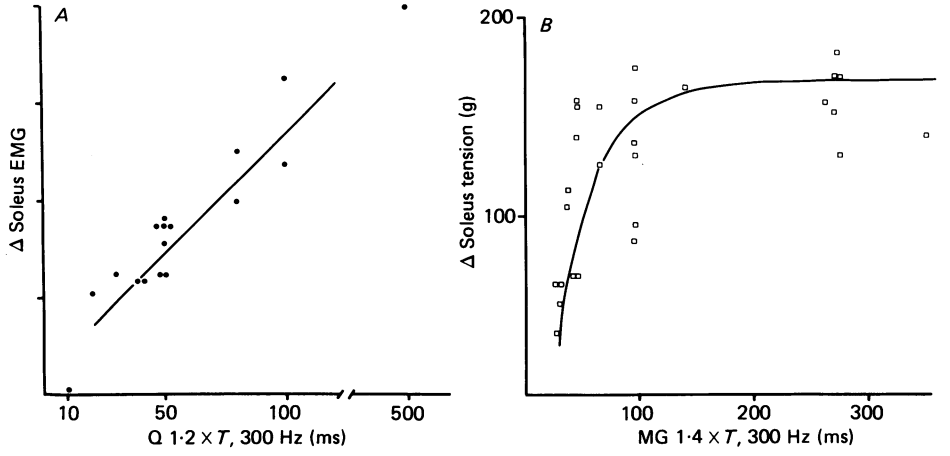

Fig. 2. Relation between EMG and tension increments in the soleus muscle and duration of the 'on' stimulus train. Experimental arrangement as in Fig. 1. *A*, increment in EMG activity measured 6 s after the end of 'on' stimulus (quadriceps nerve, Q $1{\cdot}2 \times T$, 300 Hz) as a function of different durations of the train. *B*, increment in tension measured 15 s after the 'on' stimulus (medial gastrocnemius nerve, MG $1{\cdot}4 \times T$, 300 Hz) plotted as function of different durations.

Figure 3*A–C* illustrates that the response may be growing (summating) even though the stimulus train is fractionated, i.e. several short stimulus trains given at rather long intervals. In the illustrated example vibratory bursts given at 10 s intervals (arrows below Fig. 3*C*) resulted in a stepwise increase of the ensuing maintained response. Thus the mechanism summating the afferent input operates with a long time constant, which allows additive responses even when the repeated 'on' stimuli are separated by 10 s, or more (Figs 3, 4 and 5).

By analysing the activity of several individual motor units on records with a fast time base it is evident that stepwise increase of the summated rectified EMG response depends on recruitment of new motor units. The firing frequency of already recruited units did not increase further with subsequent 'on' stimuli. An example of this behaviour is illustrated in Fig. 5; the large unit in *C* is recruited by the first train of vibration and its firing frequency did not increase by the second train (*D*) although several additional motor units were recruited.

The 'off' stimulus, used to reset the excitability to the level of activity before the 'on' stimulus, was rather long-lasting (1–5 s in the examples illustrated in Figs 1 and 3); in every case it resulted in a complete EMG silence. When the stimulus strength of the 'off' stimulus is decreased and/or the train duration shortened it is possible to demonstrate that the maintained motor response can be decreased stepwise. In the

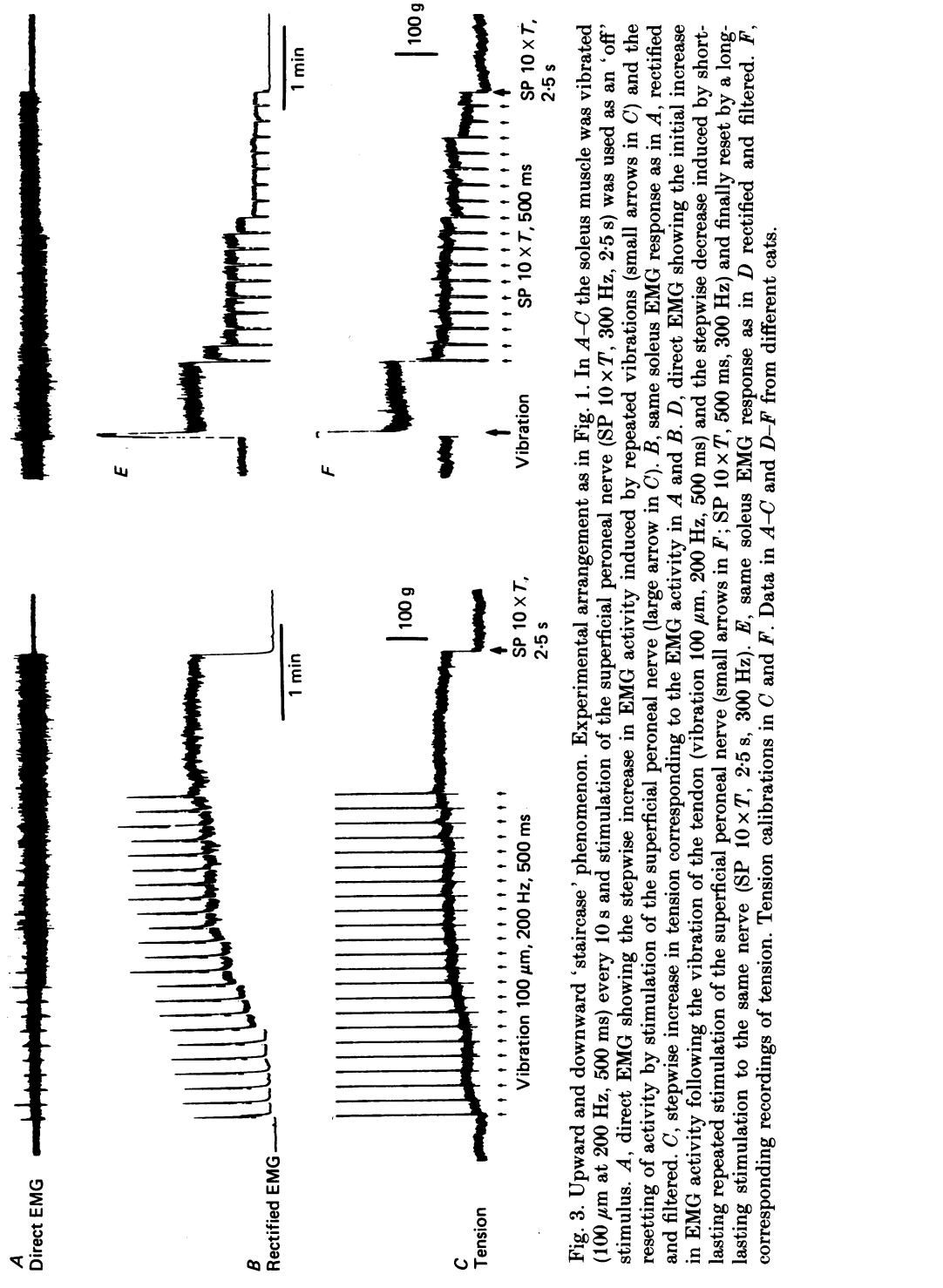

Fig. 3. Upward and downward 'staircase' phenomenon. Experimental arrangement as in Fig. 1. In *A–C* the soleus muscle was vibrated (100 μm at 200 Hz, 500 ms) every 10 s and stimulation of the superficial peroneal nerve (SP $10 \times T$, 300 Hz, 2·5 s) was used as an 'off' stimulus. *A*, direct EMG showing the stepwise increase in EMG activity induced by repeated vibrations (small arrows in *C*) and the resetting of activity by stimulation of the superficial peroneal nerve (large arrow in *C*). *B*, same soleus EMG response as in *A*, rectified and filtered. *C*, stepwise increase in tension corresponding to the EMG activity in *A* and *B*. *D*, direct EMG showing the initial increase in EMG activity following the vibration of the tendon (vibration 100 μm, 200 Hz, 500 ms) and the stepwise decrease induced by short-lasting repeated stimulation of the superficial peroneal nerve (small arrows in *F*; SP $10 \times T$, 500 ms, 300 Hz) and finally reset by a long-lasting stimulation to the same nerve (SP $10 \times T$, 2·5 s, 300 Hz). *E*, same soleus EMG response as in *D* rectified and filtered. *F*, corresponding recordings of tension. Tension calibrations in *C* and *F*. Data in *A–C* and *D–F* from different cats.

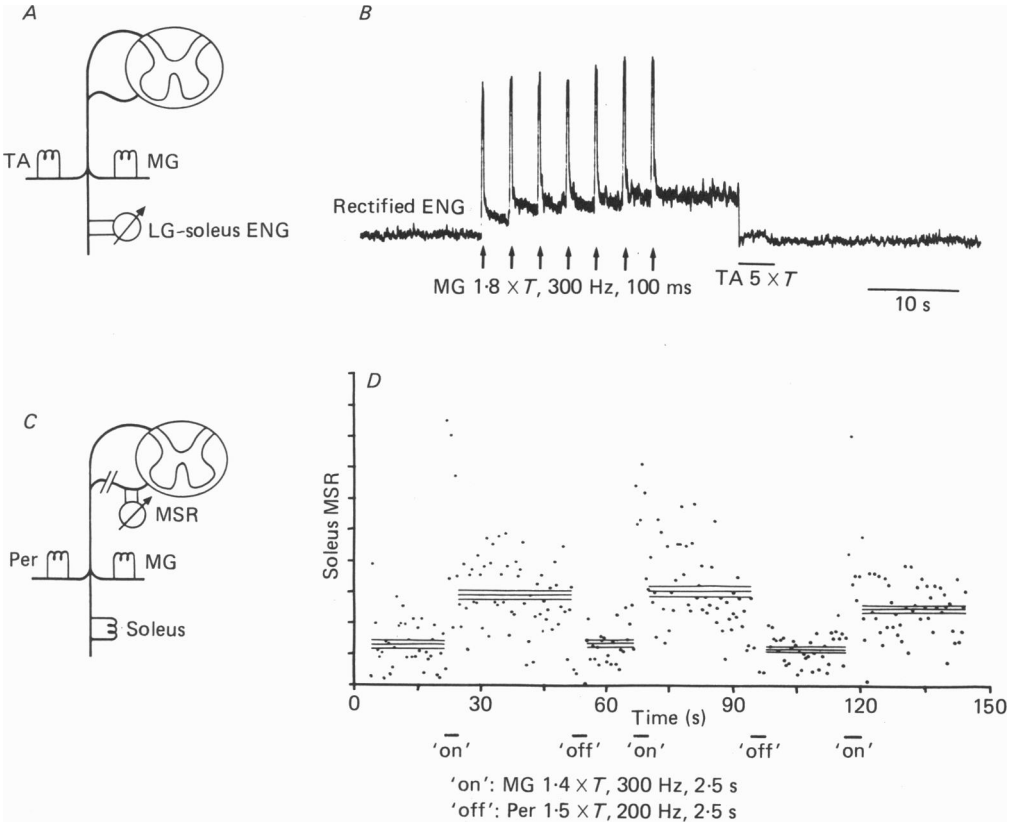

Fig. 4. Upward 'staircase' phenomenon monitored from the sectioned muscle nerve (*A* and *B*). *A*, experimental arrangement (anaemically decerebrate cat). The medial gastrocnemius (MG) and the tibialis anterior (TA) nerves were mounted for stimulation and the lateral gastrocnemius–soleus nerve (LG–soleus) for recording (ENG). *B*, rectified and filtered lateral gastrocnemius–soleus ENG showing the 'staircase' phenomenon. The excitability increase was induced by stimulation of the medial gastrocnemius nerve every 3 s (arrows, MG $1{\cdot}8 \times T$, 300 Hz, 100 ms) and terminated by stimulation of the tibialis anterior nerve (TA $5 \times T$ as indicated by a bar below the ENG). Long-lasting excitability shift measured by monosynaptic test reflexes (*C* and *D*). *C*, experimental arrangement (unanaesthetized intercollicularly decerebrate and decerebellated cat). The soleus, medial gastrocnemius (MG) and the peroneal (Per) nerves were mounted for stimulation. The monosynaptic test reflex (MSR) from soleus was recorded from the central part of the cut S1–L7 ventral roots. *D*, the integrated amplitude of the soleus test reflex response (arbitrary units) plotted against time during several 'on' (MG $1{\cdot}4 \times T$, 300 Hz, 2·5 s) and 'off' stimulations (Per $1{\cdot}5 \times T$, 200 Hz, 2·5 s). Horizontal lines indicate mean ± S.E.M. Values during 'on' and 'off' stimulus trains to medial gastrocnemius nerve or peroneal nerve were not included in the calculation.

experiment of Fig. 3*D–F* the maintained soleus activity was initiated by a vibratory burst. The 'off' stimulus ($10 \times T$, 300 Hz to the superficial peroneal nerve) was kept as short as 500 ms and repeated every 10 s (arrows below Fig. 3*F*). Although the EMG activity was completely silenced *during* the train it is seen that the remaining 'maintained activity' shows a stepwise decrease (although some of the 'off' trains are

seemingly ineffective). This stepwise decrease is best seen in the rectified EMG (*E*) or in the tension recording (*F*); a detailed comparison of these records shows that some of the active motor units certainly contribute rather differently to the recorded EMG and force.

The maintenance of the prolonged excitation was also established in experiments with sectioned peripheral nerves/ventral roots and/or curarization. With sectioned peripheral nerves (and intact ventral roots) the efferent activity can be monitored directly by the ENG from the central end of the motor nerve (Fig. 4*A*). Figure 4*B* illustrates the rectified ENG from the nerves to soleus and lateral gastrocnemius, while the prolonged activity is triggered by short trains of stimuli to the nerve from the synergist medial gastrocnemius. It is also seen that repeated trains causes a stepwise increase of the maintained response. In paralysed preparations with intact innervation of lateral gastrocnemius–soleus muscles it was also found that small amplitude vibration could initiate a maintained activity in the nerve to medial gastrocnemius. The ENG recording has proved to be an easy and reliable way of monitoring the maintained motor activity following an 'on' stimulis in paralysed preparations.

The long-lasting response could be elicited in virtually every experiment just as described for the EMG recordings. Since these 'on' stimuli were effective without producing a contraction and a subsequent sensory feed-back (mainly through Ib afferents) it must be concluded that the 'on' stimulus itself, i.e. the activity in muscle spindle Ia afferents, is initiating the long-lasting excitability increase.

With severed ventral roots the excitability of the motor nuclei was monitored by recording the size of monosynaptic test reflexes (Fig. 4*C*). Each dot in Fig. 4*D* shows the size of the monosynaptic reflexes in the S1–L7 ventral roots upon stimulation of the soleus nerve (stimulation 12 Hz). The 'on' stimulus trains and 'off' stimulus trains are marked below the diagram. Even though the variability in reflex amplitude is rather large, the maintained effects by the 'on' and 'off' trains are obvious. The horizontal lines show the mean (±S.E.M.) of the reflexes for the periods indicated by the length of these lines.

The long-lasting excitability changes have been successfully monitored by monosynaptic test reflexes in several experiments (about four experiments on decerebrates without spinal lesion and six experiments on acute spinal cats plus 5-HTP (see section (2)) although it was often difficult to obtain unambiguous results, even when ENG recordings had shown large and maintained activity following the 'on' stimulus just before ventral root section and recording of monosynaptic test reflexes.

The experiments using ENG recording and monosynaptic test reflexes in paralysed preparations are important, because they demonstrate unequivocally the *existence of central mechanisms* for the maintenance of excitability levels triggered by short-lasting afferent input. This conclusion does not refute the possibility that a peripheral loop formed by the efferent and afferent innervation of the muscle(s) may contribute to an excitability increase in the intact preparation. However, such a hypothetical contribution (cf. Hultborn & Wigström, 1980) is not critical for the phenomenon under study.

(2) *Long-lasting excitability increase of triceps surae motoneurones in the acute spinal cat following* I.V. *administration of 5-hydroxytryptophan*

The experiments described in section (1) demonstrated the existence of a central mechanism that maintains a lasting excitability increase of triceps surae motoneurones when activated by a train of impulses in Ia afferents. Since all the experiments discussed above were performed on decerebrate cats it was an open question in which part of the remaining CNS this mechanism was located. An acute spinal transection at low thoracic level immediately abolished the long-lasting response by an 'on' stimulus and therefore it may be concluded that the brain stem is involved either by mediating the effect or by upholding a supraspinal facilitation of primarily intrinsic spinal mechanisms.

To test if an 'unspecific' high excitability of the spinal cord was sufficient to reveal maintained excitability changes strychnine (0·05–0·10 mg/kg I.V.) was administered in three experiments. Although a spontaneous motor discharge indeed appeared after strychnine (recorded from peripheral nerves or ventral roots) the maintained excitability shifts following trains of 'on' and 'off' stimuli were never seen.

Another possibility would be that intrinsic spinal mechanisms are dependent on tonic activity in specific descending systems, as for example the serotonergic raphe–spinal projection, which is tonically active in the decerebrate preparation (see further in Discussion). Intravenous injection of the serotonin precursor 5-hydroxytryptophan (5-HTP, 50–100 mg/kg) to acute spinal cats was used to mimic such a tonic activity (see Methods).

Following administration of 5-HTP to acute spinal cats the maintained excitability shifts by 'on' and 'off' stimuli indeed appeared in fifteen out of twenty preparations. Figure 5 illustrates the stepwise increase of maintained soleus EMG activity following repeated periods of vibration (arrows below *B*) in an acute spinal cat after intravenous administration of 50 mg/kg 5-HTP. With equal ease it was possible to monitor the maintained excitability shifts by the ENG from the sectioned motor nerve in paralysed preparations (cf. Fig. 6). Confirmative results were also obtained when monosynaptic test reflexes were used to monitor maintained excitability shifts following repeated 'on' and 'off' stimulus trains in a spinal preparation following 5-HTP administration.

Intravenous injection of the noradrenaline precursor L-DOPA and the serotonin precursor 5-HTP in spinal cats produces strikingly similar effects with regard to the reflex transmission to α-motoneurones (Andén *et al.* 1964*a*; Andén, Jukes, Lundberg & Vyklický, 1964*b*, 1966) and the tonic stretch reflex (Grillner, 1969; Ahlman, Grillner & Udo, 1971). Nevertheless the effects of L-DOPA and 5-HTP seem to be exerted through different systems since the effects are counteracted by specific blockers, (Andén *et al.* 1964*a*, 1966; Lundberg, 1965). We have confirmed in two control experiments on acute spinal cats that pre-treatment with phenoxybenzamine (which blocks the noradrenergic effect, Andén *et al.* 1966; see also Conway, Hultborn, Kiehn & Mintz, 1988) did not prevent the effect of 5-HTP. Figure 6*A* illustrates repeated excitability shifts to 'on' and 'off' stimuli in one of these experiments. Furthermore, in Fig. 6*B* it is shown that the maintained activity following an 'on' stimulus train rapidly disappears (without an 'off' train) even within 15 s after an

intravenous injection of methysergide (a serotonergic receptor blocker; 3 mg/kg). Repeated 'on' stimulus trains shortly afterwards only caused a phasic activation of the motoneurones, without any maintained activity. It is noteworthy that the phasic response during the 'on' stimulus is as large as before injection of methysergide and the suppression of the maintained activity can thus not be ascribed to an unspecific decrease in general excitability.

In two decerebrate preparations without spinal lesion, the long-lasting maintained activity in response to 'on' and 'off' trains where similarly blocked by methysergide.

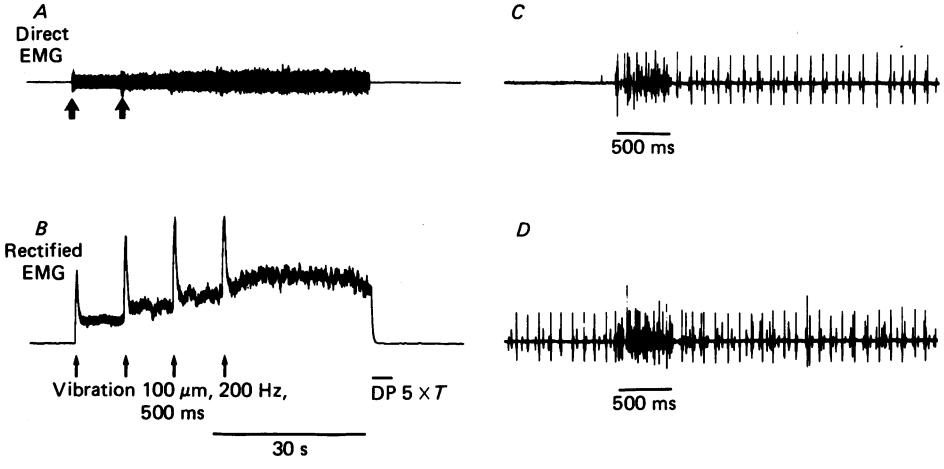

Fig. 5. Long-lasting excitability increase and 'staircase' phenomenon in 5-HTP-treated acute spinal cat. Experimental arrangement: unanaesthetized anaemically decerebrate cat, with an acute spinal transection (Th12) and 5-HTP (50 mg/kg I.V.). The soleus muscle was vibrated every 10 s (100 μm at 200 Hz for 500 ms, arrows in *B*). Stimulation of the deep peroneal nerve (DP $5 \times T$, 300 Hz) was used as 'off' stimulus. *A*, direct soleus EMG showing stepwise increase in EMG activity by repeated vibration. The activity was reset by stimulation of the deep peroneal nerve (DP). *B*, same signal as in *A*, rectified and filtered. *C* and *D* show at different time scales the EMG activity following the first and second vibration (shown by large arrows in *A*). The time calibration in *B* applies for *A* and *B*.

(3) *Afferent and central sources of effective 'on' and 'off' stimuli*

As mentioned in section (1) it has been possible to elicit the maintained excitability increase in soleus motoneurones by short vibratory periods of the soleus muscle itself (or the whole triceps surae) or by trains of group I volleys by electrical stimulation of nerves from the synergist muscles medial gastrocnemius, plantaris and, occasionally, quadriceps; group I volleys in other hindlimb muscle nerves were ineffective (flexor digitorum longus, pretibial flexors, knee flexors and hip extensors). It is striking that this pattern is identical with the origin of *mono*synaptic Ia excitation to soleus motoneurones (Eccles, Eccles & Lundberg, 1957*b*). The medial gastrocnemius (ENG activity) was influenced in the same way, and from the same sources, as the soleus activity (or soleus and lateral gastrocnemius together). Thus, the most efficient 'on' stimulus for medial gastrocnemius was a train of group I volleys in the soleus–lateral gastrocnemius nerve. However, we have never recorded

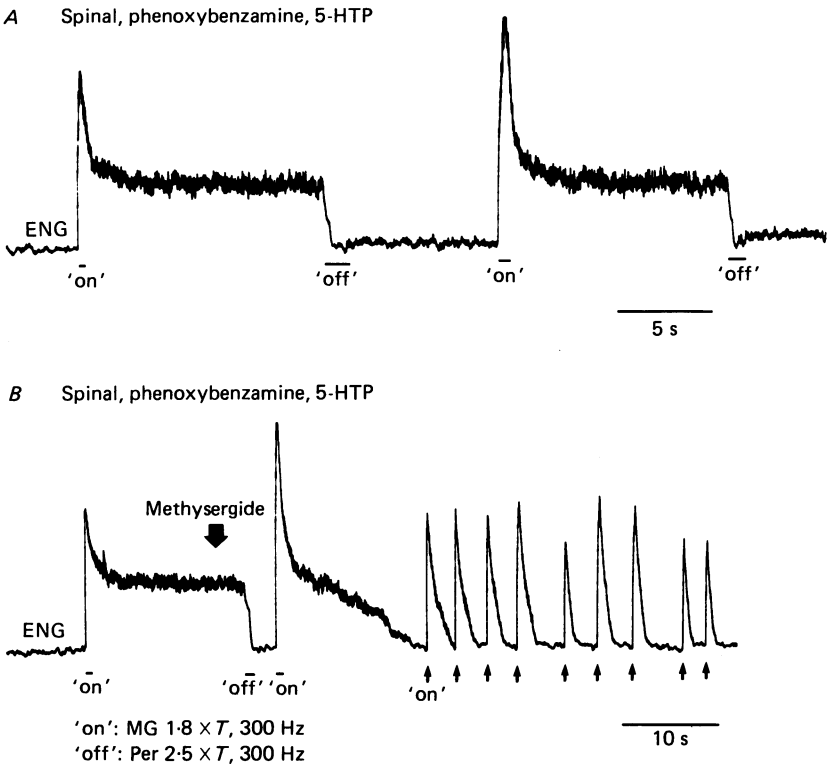

Fig. 6. Long-lasting excitability increase in acute spinal cat following phenoxybenzamine and 5-HTP and blocked by methysergide. Experimental arrangement: unanaesthetized anaemically decerebrate preparation with an acute spinal transection. The nerves to medial gastrocnemius (MG) and to peroneal (Per) nerves were used as 'on' and 'off' stimuli, while the ENG activity was recorded in the nerve to lateral gastrocnemius–soleus. *A*, long-lasting excitability increase recorded as lateral gastrocnemius–soleus ENG activity, elicited by an 'on' stimulus (MG $1{\cdot}8 \times T$, 300 Hz) and terminated by an 'off' stimulus (Per $2{\cdot}5 \times T$, 300 Hz). Phenoxybenzamine (25 mg/kg I.V.) was administered before 5-HTP (70 mg/kg I.V.). The blood pressure was 'clamped' to 100 mmHg with continuous I.V. administration of angiotensin II. *B*, decrease and disappearance of the long-lasting excitability increase following methysergide (3 mg/kg was given intravenously at the bold downward arrow). Same 'on' and 'off' stimuli as in *A*. Note different time calibration in *A* and *B*. The timing of 'on' and 'off' stimuli are indicated by bars below the ENG recordings.

any maintained medial gastrocnemius ENG activity in response to a quadriceps group I train.

In the decerebrate state the triceps surae can also be activated in the crossed extensor reflex (Sherrington, 1910). When this reflex was elicited by stimulation of high-threshold afferents (cutaneous or muscle) in the contralateral hindlimb it was possible to demonstrate a maintained activity outlasting the stimulus. However, in the unanaesthetized preparation stimulation of high-threshold afferents often gives rise to violent 'rebound' effects at the end of the stimulus train and therefore the crossed extensor reflex was not studied in detail.

The excitability increase of triceps surae motoneurones could be 'reset' by various afferent 'off' stimuli. The most efficient and reliable resetting was obtained with stimulation of the common peroneal nerve or its branches. Trains of stimuli to the deep peroneal nerve (only muscle branches) were sometimes efficient at a strength of $1{\cdot}4$–$1{\cdot}6 \times T$ thus implying that group I fibres could be effective; usually stimulus strengths of 2–$10 \times T$ were necessary. Also stimulation of cutaneous fibres in the superficial peroneal nerve could be effective although the risk of a violent 'rebound excitation' seemed to be very large if the 'off' train was terminated abruptly. In order to avoid the rebound excitation the stimulus strength was often gradually decreased at the end of the 'off' train. Stimulation of the nerves to peroneus brevis and tertius (5–$10 \times T$) usually produced the best resetting with the smallest tendency for a rebound excitation. Group II stimulation of other hindlimb nerves (which did not trigger the maintained excitation) was occasionally effective, but the frequency of rebound excitation was large.

Descending 'on' and 'off' stimuli. Mori, Kawahara, Sakamoto, Aoki & Tomiyama (1982) have described brain stem areas subserving the setting of extensor muscle tone related to the standing posture in acute decerebrate cats. Depending on the site of stimulation, it was possible to increase ('ventral tegmentum'; mid-line, P3 to P7, H-7·5 to H-9·5) or decrease ('dorsal tegmentum'; mid-line, P3 to P7, H-4·5 to H-6) the postural tone. The changes persisted for long periods after cessation of the stimulus train, i.e. the behaviour resembled very much that of the long-lasting excitability changes following segmental 'on' and 'off' stimuli.

Figure 7*A–E* illustrates an experiment where we have confirmed the findings of Mori *et al.* (1982) in our experimental situation, and furthermore demonstrated the interaction between 'on' and 'off' stimuli to segmental afferents and the brain stem centres.

The rectified ENG from the nerve to lateral gastrocnemius and soleus in Fig. 7*B* illustrates the usual effects of a group I 'on' stimulus train and a peroneal 'off' train. In *C* virtually identical responses are evoked from the brain stem. The 'on' stimulus was a brief train of pulses to the 'ventral tegmentum' (P7, H-8, mid-line). The 'off' stimulus was given at the same level, but more dorsal (P7, H-5, mid-line). The excitability increase following an 'on' stimulus to the brain stem can be cancelled by a peripheral 'off' stimulus (Fig. 7*D*) and *vice versa*, i.e. the excitability increase by a train of Ia volleys can be turned off from the brain stem (Fig. 7*E*). The fact that the central and peripheral 'on' and 'off' stimuli are interchangeable strongly suggest that they operate on a common neuronal mechanism at spinal level.

In five experiments the effective brain stem sites for 'on' and 'off' effects were searched for by systematic tracking. The effective 'on' and 'off' centres in the brain stem were in the regions described by Mori *et al.* (1982). A maintained increase of triceps surae excitability was elicited from a mid-line strip 5–6 mm below the surface of the fourth ventricle from P3 to P9. In the ventral part of this field the threshold could be as low as 2 μA for a significant 'on' response. In the more rostral planes (P5–P8) effective sites (threshold $< 20\,\mu$A) could extend 2–3 mm lateral from the mid-line (only the ipsilateral side). The effective sites for 'off' stimuli were limited to a narrow mid-line strip 1·5–2·0 mm below the surface of the fourth ventricle. It extended from at least P3 to P9; more rostral and caudal levels were not

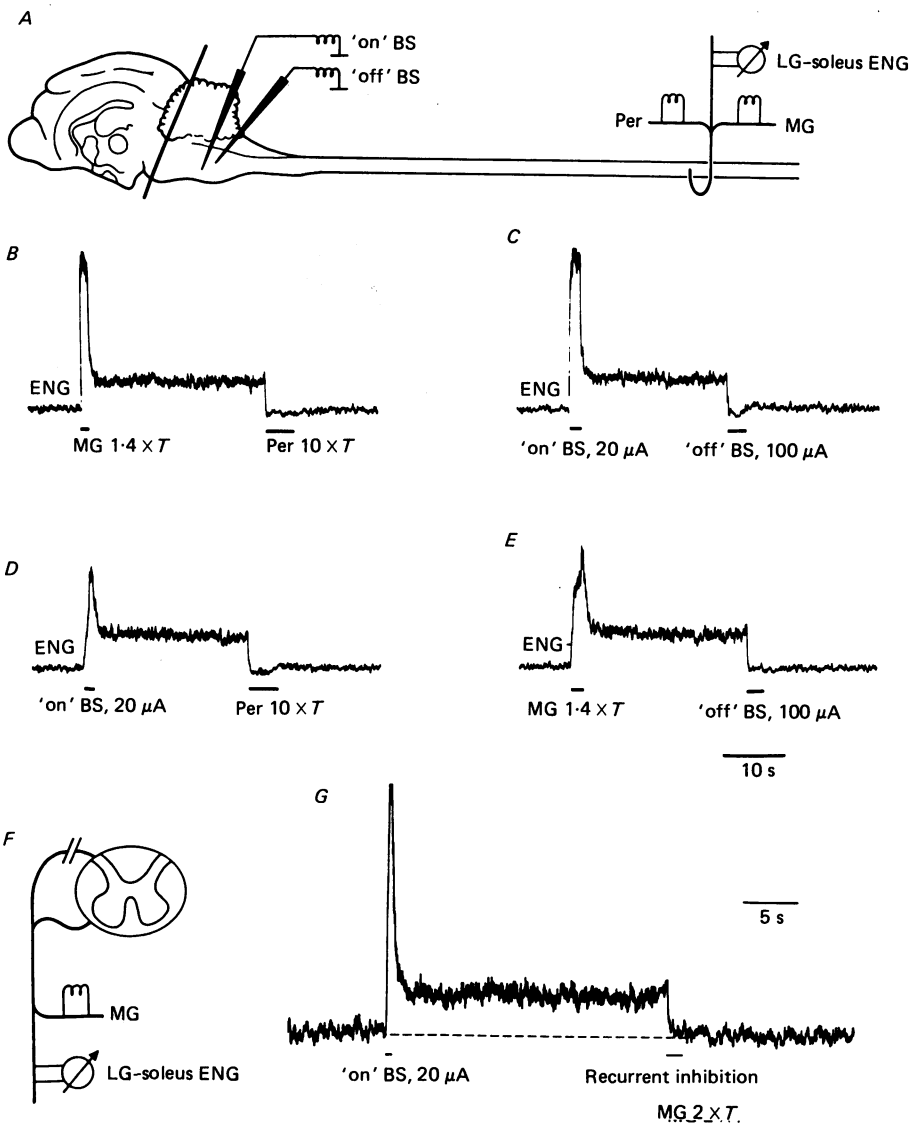

Fig. 7. Long-lasting excitability changes elicited by stimulation of the brain stem (BS) and peripheral nerves. *A*, experimental arrangement (unanaesthetized anaemically decerebrate cat). The 'on' stimulus electrode was placed in the 'ventral tegmentum' (P7, H-8, mid-line) and the 'off' stimulus electrode in the 'dorsal tegmentum' (P7, H-5, mid-line). The peroneal (Per) and medial gastrocnemius (MG) nerves were mounted for stimulation and the lateral gastrocnemius–soleus nerve for recording. *B–E*, rectified electroneurograms (ENGs) show maintained changes in excitability induced and terminated by stimulation of the medial gastrocnemius nerve (MG $1{\cdot}4 \times T$, 300 Hz) and peroneal nerve (Per $10 \times T$, 200 Hz) (*B*); the 'ventral tegmentum' (on BS 20 μA, 200 Hz) and 'dorsal tegmentum' ('off' BS 100 μA, 200 Hz) (*C*); the 'ventral tegmentum' and the peroneal nerve (*D*); the medial gastrocnemius nerve and the 'dorsal tegmentum' (*E*). Long-lasting excitability increase elicited by brain stem stimulation and terminated by recurrent inhibition. (*F–G*). *F*, experimental arrangement (unanaesthetized anaemically decerebrate cat). The brain stem was stimulated in the 'on' centre. The medial gastrocnemius nerve (MG) was mounted for stimulation and the lateral gastrocnemius–soleus nerve for recording. Dorsal roots to L5–S1 were cut. *G*, increased ENG activity (rectified) following stimulation of the 'on' centre in the brain stem ('on' BS, 20 μA, 200 Hz, 100 ms) and terminated by stimulation of the medial gastrocnemius nerve (recurrent inhibition MG $2 \times T$, 300 Hz).

investigated. The possible anatomical structures responsible for the 'on' and 'off' responses are briefly considered in the Discussion.

As mentioned above the finding that segmental and descending 'on' and 'off' stimuli actually are interchangeable suggest that they operate on a common neuronal mechanism at spinal level. In this context it would be of particular interest to test whether recurrent inhibition of motoneurones (via motor axon collaterals and Renshaw cells) could serve as an 'off' stimulus. Renshaw cells project to motoneurones (both α and γ), interneurones mediating reciprocal Ia inhibition, other Renshaw cells and some spinocerebellar tract neurones (see Baldissera *et al.* 1981). Since excitatory interneurones in reflex pathways to motoneurones are *not* subject to recurrent inhibition (Hultborn, Jankowska & Lindström, 1971) an efficient excitability resetting seen with recurrent inhibition would strongly suggest that the mechanism of the maintained excitability shifts resides with the motoneurones themselves. In order to obtain a selective recurrent inhibition following stimulation of a peripheral motor nerve it was necessary to prevent afferent volleys from reaching the spinal cord by sectioning the dorsal roots (Fig. 7*F*); therefore the 'on' stimuli were given to the 'ventral tegmentum'. As illustrated by Fig. 7*G* recurrent inhibition was effectively resetting the excitability level, thus implying that the maintained excitability shifts rely on mechanisms in the motoneurones themselves. This view was indeed fully corroborated by the following investigation on the intrinsic motoneuronal properties (Hounsgaard *et al.* 1988).

Maintained excitability shifts in other motoneurone pools. Most of our results on the maintained excitability shifts have focused on the triceps surae motoneurones. However, some observations (four cats) on quadriceps motoneurones (ENG and monosynaptic test reflexes) have shown that group I trains to some quadriceps branches cause a powerful maintained response in the remaining nerve branches. The ENG was also recorded from several flexor nerves (pretibial flexors, knee flexors). Trains of group I volleys to close synergists could evoke a (monosynaptic) activation during the 'on' train, but (almost) never any maintained response. Only in one exceptional case a maintained EMG response in tibialis anterior was triggered by vibration of its tendon. The difference between flexor and extensor nerves will be considered further in the Discussion.

The ENG was monitored from several extensor and flexor nerves during stimulation of the 'on' and 'off' areas in the brain stem. It was found that the stimulation of the 'ventral tegmentum' caused a maintained excitability increase of all extensor nerves (hip, semimembranosus and anterior biceps; knee, quadriceps; ankle, all triceps surae and plantaris; toes, flexor digitorum longus and nerve branches in the tibial nerve supplying intrinsic foot muscles). These stimuli did not activate flexor nerves.

(4) *The soleus stretch reflex in response to ramp-and-hold stretches of different velocity*

It is well known that the muscle spindle Ia afferents are particularly sensitive to the velocity of stretch (see in Matthews 1972, 1981). From the results presented in section (1) it could therefore be expected that the 'tonic' phase of the stretch reflex during a ramp-and-hold stretch would not only depend on the final length, but also on the velocity of stretch in the initial ramp. In five out of six experiments it was

indeed possible to demonstrate that a higher velocity of stretch (with intense Ia activity, not recorded) also caused a stronger maintained reflex during the holding phase. In the last experiment the variability in excitability was too large to allow any conclusion. In the three positive experiments it was necessary to 'titrate' the optimal stretch amplitude to demonstrate the effect (e.g. the tonic stretch reflex might attain the same level independently of the initial stretch velocity when the final stretch amplitude was 'too' large).

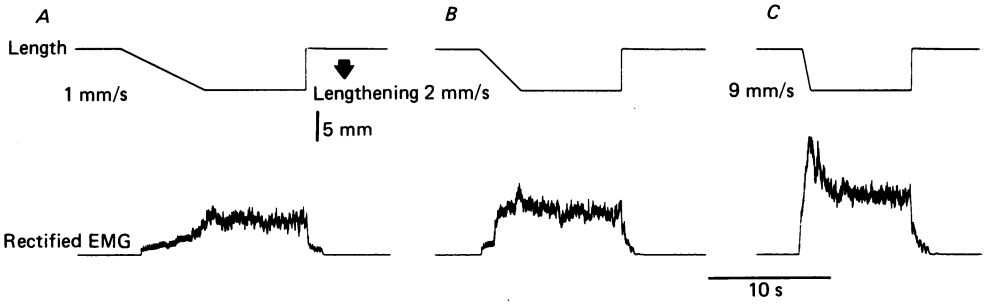

Fig. 8. Stretch reflexes of the soleus muscle in response to ramp-and-hold stretches with different velocities of the ramp phase. Experimental arrangement: unanaesthetized anaemically decerebrate cat. *A–C*, upper traces show the length of the muscle and the velocity of the ramp phase. Downward deflection indicates lengthening of the muscle (calibration in *A*). Lower traces show rectified soleus EMG.

Figure 8 illustrates one of the experiments in which the stretch velocity during the ramp ranged from 1 to 9 mm/s. It is seen that the tonic component of the EMG (rectified) during the holding phase increases with higher velocity during the ramp. Velocities higher than 9 mm/s were not more effective in this experiment, but in the two other experiments the optimal velocity was around 30–50 mm/s.

DISCUSSION

Our understanding of the mechanism underlying the maintained excitability shifts has changed fundamentally during the series of experiments starting in 1975. We initially thought that the maintained excitability increase was caused by long-lasting polysynaptic excitation (Hultborn *et al.* 1975), while later investigations (Hounsgaard *et al.* 1984, 1986, 1988) have clearly demonstrated that the mechanism for the excitability shifts resides with the motoneurones themselves. In order to keep this Discussion within the conceptual frame of our present understanding it is necessary to start with a brief comment on the basic mechanisms underlying the maintained excitability changes.

Hounsgaard *et al.* (1984, 1986, 1988) have shown that in decerebrate preparations – or acute spinal preparations after administration of 5-HTP – a short-lasting synaptic excitation may trigger a depolarizing plateau potential in the motoneurone itself. This plateau potential is caused by a voltage-sensitive non-inactivating Ca^{2+} conductance (Hounsgaard & Kiehn, 1985). If the background synaptic excitation (or the continuous depolarizing current through the microelectrode) is high enough,

the plateau potential can be maintained for very long periods and support a self-sustained firing of the motoneurone, which is triggered by the initial synaptic excitation. A synaptic inhibition may terminate the plateau potential. With weaker background depolarization the plateau potential will fall off spontaneously; its duration may be more or less related to the background excitability.

The finding that intrinsic motoneuronal properties are important in maintaining a self-sustained activity following a short depolarization does not *per se* exclude the possibility that in addition a maintained synaptic excitation contributes to the final excitability increase seen with ENG or EMG recordings. However, several observations summarized in the Discussion of the following paper (Hounsgaard *et al.* 1988) strongly argue against an important contribution from a maintained synaptic excitation in the present preparation.

As described in section (3), a maintained excitability increase of various species of extensor motoneurones can be evoked by volleys in Ia afferents from those muscles which contribute to monosynaptic Ia excitation of the tested motoneuronal pool. In addition the crossed extensor reflex, and stimulation of a mid-line strip in the 'ventral tegmentum' of the brain stem could trigger a maintained excitability increase. The common feature of these sources seems to be that they all produce a 'pure' excitation during the stimulation without any rebound inhibition (that would immediately terminate the plateau potential). Similarly, all sources of 'pure' synaptic inhibition (i.e. without rebound excitation which is often seen, for example, *after* a train of cutaneous volleys) are efficient in terminating the excitability increase and thus resetting the original excitability level. The sources for such inhibition include reciprocal Ia inhibition, recurrent inhibition, group II inhibition (especially from peroneus brevis and tertius and the mid-line strip in the 'dorsal tegmentum' of the brain stem).

The 'on' and 'off' responses from the brain stem were obtained from the regions described by Mori *et al.* (1982) as increasing and decreasing postural tone. The later work from Mori's group suggest that the inhibitory response from the mid-line strip in the dorsal tegmental field (P3 to P7, H-4·5 to 6·0 mid-line) is at least partly due to stimulation of passing fibres from nucleus reticularis pontis oralis projecting to reticulospinal neurones in nucleus reticularis gigantocellularis (see Mori, 1987, and personal communication). These cells cause inhibition of flexor as well as extensor motoneurones via one interposed inhibitory interneurone at segmental level (Ohta, Takakusaki, Matsuyama & Mori, 1986, and personal communication). The structural basis for the 'on' response from the 'ventral tegmental' field is more uncertain. Anatomically the rostral part of the effective region overlaps with a part of nucleus raphe magnus and the caudal part overlaps with both nucleus raphe pallidus and obscurus. However there are many passing fibres in this region originating in hypothalamus, pons and medulla (cf. review by Mori, 1987) and conclusions on the origin of the 'on' response thus have to await analysis. We have no evidence suggesting that electrical stimulation of the descending serotonergic fibres are of importance; however it seems necessary to have tonic activity of the serotonergic projection of the motoneurones (following section, Hounsgaard & Kiehn, 1985; Hounsgaard *et al.* 1988).

The serotonergic raphe–spinal projection (or at least parts of it) seems to be

tonically active in the unanaesthetized preparation. This conclusion rests on the observations that (1) the serotonin blockers (but not noradrenergic blockers) partly abolish the tonic decerebrate inhibition of reflex transmission (Engberg, Lundberg & Ryall, 1968) and the tonic stretch reflex (although less effectively, Ellaway & Trott, 1975), and (2) that administration of the monoamine oxidase inhibitor, nialamide, enhances the tonic decerebrate inhibition of reflex transmission (Engberg *et al.* 1968). When 5-HTP is given intravenously to acute spinal preparations – thus mimicking tonic serotonergic raphe–spinal activity (see Methods) – several aspects of the decerebrate control of spinal mechanisms are indeed restored. This applies both to the inhibition of transmission in several reflex pathways in the decerebrate state (Andén *et al.* 1964*a*, *b*) and the reappearance of the tonic stretch reflex (Ahlman *et al.* 1971; Ellaway & Trott, 1975). In section (2) we also described that the maintained excitability shifts seen in the decerebrate preparation, were abolished following an acute spinal transection and restored after 5-HTP administration. Tonic activity of the serotonergic raphe–spinal projection thus seems to be an essential prerequisite for the maintained excitability shifts in the unanaesthetized decerebrate preparation. The pivotal importance of serotonin is further established by the analysis of individual motoneurones in the cat (*in vivo*, Hounsgaard *et al.* 1984, 1988) and in the turtle (*in vitro*, Hounsgaard & Kiehn, 1985).

Long-lasting motor discharge (5 s or more) following brief afferent stimulation has been described in reduced preparations, such as decerebrate or spinal animals (see Sherrington 1906; Creed, Denny-Brown, Eccles, Liddell & Sherrington, 1932). More recently, and more closely related to the present study, Matthews (1966) demonstrated that the EMG activity evoked by muscle vibration in the intercollicular decerebrate cat may outlast the stimulus (vibration). Usually the EMG activity ceased immediately (i.e. 10–20 ms) on stopping the vibration, but in some cases it reappeared after about 50 ms and lasted for a few seconds. This late activity was more pronounced in animals with a high excitability as reflected by some EMG activity present before vibration. With a cerebellar ablation added to the intercollicular decerebration (which further increase excitability) Matthews described that the contraction could outlast the vibration for 10 s or more. These observations were much extended by Carp & Rymer (1986) who showed that the prolonged EMG activity was also dependent on serotonin; serotonin agonists enhanced and serotonin antagonists decreased the long-lasting activity after vibration. There is no doubt that the observations by Matthews (1966) and Carp & Rymer (1986) are directly related to the results presented in the present report.

In 1943, Lloyd conclusively demonstrated that the tendon jerk was caused by a monosynaptic transmission from the fastest conducting muscle afferents to homonymous motoneurones. Following Lloyd's demonstration of this response and his idea of it as representing 'the reflex response to stretch' it was often assumed that the monosynaptic connection from the muscle spindle Ia afferents was the exclusive afferent source accounting for the excitability increase during the tonic stretch reflex. In addition to the monosynaptic excitation (also established from muscle spindle group II afferents, Kirkwood & Sears, 1975; Stauffer, Watt, Taylor, Reinking & Stuart, 1976; Lüscher, Ruenzel, Fetz & Henneman, 1979; Munson, Sypert, Zengel, Lofton & Fleshman, 1982) it has been proposed, now and then, that

the muscle spindle afferents contribute excitation by more complex (polysynaptic) pathways.

One line of evidence was provided by Granit, Phillips, Skoglund & Steg (1957). They described that repeated brief pulls of triceps surae muscles (compare vibration in this study) potentiated the tonic stretch reflex during a subsequent ramp-and-hold stretch. Furthermore the potentiation by brief pulls was shared for several different reflexes to triceps surae (the tonic stretch reflex, the crossed extensor reflex and the pinna reflex). Granit *et al.* interpreted their finding as being due to a (post-tetanic) potentiation at a common pre-motoneuronal level, but most probably these results can be ascribed to a final convergence onto the α-motoneurones from these sources, contributing sufficient excitation to reach the threshold to generate long-lasting plateau potentials. Another line of evidence originates from the observation that the motoneuronal discharge in triceps surae is only partly time-locked to the afferent volleys following either vibration or electrical nerve stimulation (Kanda, 1972; Homma, Mizote & Watanabe, 1975). It was thus assumed that the motor unit spikes that were not time-locked (or occurred at latencies too long to be explicable by monosynaptic transmission) were elicited through polysynaptic pathways and this was further strengthened by their susceptibility to anaesthesia as compared to the short-latency time-locked discharges. Again it seems possible that the spikes that were not time-locked actually reflect the spike discharge on the top of a plateau potential in the motoneurone, which is triggered by the monosynaptic Ia excitation (cf. further in Hounsgaard *et al.* 1988). The slow gradual onset (15–60 s) until a steady contraction is reached when vibration is applied to muscles in human subjects (the tonic vibration reflex, TVR; see Hagbarth, 1973; Lance, Burke & Andrews, 1973, for reviews) was interpreted as being due to a slow recruitment of polysynaptic pathways, but it may also be explained by slow increase of the number of motoneurones in which plateau potentials are triggered (cf. the 'staircase' phenomenon by recruitment of new motor units in Figs 3 and 5 and the Discussion of Hounsgaard *et al.* 1988). Thus, most of the evidence for contribution by polysynaptic pathways from muscle spindles to excitation in the tonic stretch reflex is indeed indirect and the observations may well be explained by the plateau potentials triggered by short-lasting synaptic excitation.

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