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EFFERENTS AND AFFERENTS IN AN INTACT MUSCLE NERVE: BACKGROUND ACTIVITY AND EFFECTS OF SURAL NERVE STIMULATION IN THE CAT

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SUMMARY

1. The background activity was observed in γ and α efferent fibres and in group I and II fibres innervating the muscle gastrocnemius lateralis or medialis. The reflex effects of ipsilateral and contralateral sural nerve stimulations on the muscle efferents were analysed together with their consequences upon the afferents of the same muscle. The observations were made in the decerebrated cat without opening the neural loops between the muscle and the spinal cord.

2. The multi-unit discharges of each category of fibres were obtained, on line, by an original electronic device (Joffroy, 1975, 1980) that sorted the action potentials from the whole electrical activity of a small branch of gastrocnemius lateralis or medialis nerve according to the direction and velocity of propagation of the potentials.

3. The small nerve may be regarded as a representative sample of different functional groups of fibres conducting faster than 12 m . sec^{-1} and supplying gastrocnemius muscles.

4. Some γ efferents were always tonically firing except when a transient flaccid state developed. Usually the α efferents were silent, probably because the muscle was fixed close to the minimal physiological length.

5. Separate and selective stimulations of Aβ, Aδ and C fibres of ipsilateral and contralateral sural nerve showed that each group could induce the excitation of γ neurones. The reciprocal inhibition period of α efferents during a flexor reflex was only once accompanied by a small decrease in γ-firing.

6. The reflex increase of over-all frequency of γ efferents resulted from an increased firing rate of tonic γ neurones and from the recruitment of γ neurones previously silent. When the γ efferents in the small nerve naturally occurred in two subgroups, the slower-conducting subgroup (mainly composed of tonic γ axons) was activated before the faster-conducting subgroup (mostly composed by γ axons with no background discharge). Some rare exceptions were found, however.

7. The selective activation of γ efferents could be obtained with short-and low-frequency stimulation. When, with stronger stimulations, γ–α co-activation was observed, the onset of the γ-firing increase preceded by 100–600 msec that of the α discharge in the small nerve. Likewise, the onset of the γ-efferent response preceded the increase of over-all electromyographic activity of the whole triceps muscle but

only by 10–100 msec. The discrepancy could be due to the soleus α motoneurones being activated earlier than the α-motoneurones of gastrocnemius muscle, according to the size principle. In only one experiment, the α-firing onset preceded the γ-firing increase.

8. Stimulations of ipsilateral or contralateral nerve, whatever the α or γ reflex patterns, always led to increased firing rates of group I and II fibres of the small nerve. The origins of the discharge of group I and II muscle afferents and the excitation mechanisms of the receptors involved are considered. Some aspects of the mechanism of the reflex control of movement are discussed in the light of these results.

INTRODUCTION

The reflex responses of γ motoneurones to a variety of afferent stimuli are not so well documented as those of α motoneurones. This is mainly due to (i) the lack of a simple method to examine in isolation the total γ activity in a muscle nerve or its consequences on sensory endings in muscles spindles and (ii) the scarcity of intracellular recording from γ motoneurones (Eccles, Eccles, Iggo & Lundberg, 1960; Grillner, Hongo & Lund, 1969; Appelberg, Johansson & Kalistratov, 1977).

Most direct investigations of reflex effects on γ motoneurones have been carried out by recording single unit activity in a ventral root or muscle nerve filament as originally used by Hunt (1951). The most sophisticated analysis of the results has used peristimulus time histograms and their cumulative sum derivative to assist the detection of minimal responses and to determine precisely their time course and extent (Ellaway, 1977). In addition, indirect information has been obtained from single unit afferent activity recorded from transected dorsal root filaments in the animal (Granit & Kaada, 1952) or '*en passant*' from a muscle nerve through a tungsten micro-electrode percutaneously implanted in the man (Hagbarth & Vallbo, 1969).

Both these direct and indirect methods have some limitations and disadvantages. The preparation of nerve filaments requires transection of at least part of the nerve or spinal root and entails at least partial interruption of structures forming reflex arcs. Furthermore, the susceptibility of recording techniques to mechanical disturbance does not allow observation over a long time unless movements are greatly reduced. In experimental animals this is usually achieved by curarization with or without extensive denervation. The processes induce a decrease in fusimotor activity because spinal afferent inputs are reduced (Hunt, 1951).

Three criticisms may be made against the methods used in observing single unit γ activity: (i) some uncertainty persists about the fusimotor function of the observed activity if the sole criterion for identification is the action-potential amplitude; (ii) samples of γ axons tend to be biased in favour of those having tonic discharges; (iii) reflex responses of γ motoneurones to similar stimuli are heterogeneous perhaps because they have not been recorded at the same time and often not in the same experiment.

Two objections may be made to the methods in which γ activity is estimated from muscle-spindle afferent response: (i) the amplitude and the temporal relationships of the reflex reponses of γ motoneurones cannot be precisely ascertained; (ii) the

fusimotor origin of the observed afferent response may be attested only when α motoneurones are not activated so that the length and the tension of the muscle are kept constant (Granit & Kaada, 1952).

We have developed a new method to investigate directly the activity of γ motoneurones with the aim of avoiding the disadvantages described above. The method allows the study of simultaneous activity in small populations of γ and α efferent axons and in groups I and II afferent axons in a small, intact, muscle-nerve. Thus, it is now possible to investigate, in closed loop, the activities of the various components of the servoregulatory loop controlling muscle contraction (Matthews, 1972).

In the present work, the background activity in the efferent and afferent fibres of a fine branch of the gastrocnemius lateralis and, rarely, gastrocnemius medialis muscle-nerves, in the decerebrate cat, was observed. Reflex responses, especially of γ efferents, following electrical stimulation of cutaneous afferent fibres in the ipsilateral or contralateral sural nerves, are described together with their consequent effects on muscle-afferent activity. Preliminary results have been described in short reports (Banks, Bessou, Joffroy & Pagès, 1979; Bessou, Joffroy & Pagès, 1981).

METHODS

Anaesthesia. The experiments were performed on twenty-five adult cats under preliminary anaesthesia by halothane (Fluothane, I.C.I. Ltd.) to allow surgical procedures on the left or both hind limbs, the decerebration by intercollicular section of the brain stem after the common carotid arteries have been tied and the ablation of the cranial part of the C.N.S. The electrocardiogram was continuously monitored during anaesthesia to avoid halothane overdose signalled by bradycardia and arrhythmia.

Surgical procedures. The left or right sural nerve was dissected over a 10 cm length. The left hind limb was firmly fixed in semi-extended position by clamps at the knee and ankle. The adipose tissue of the popliteal fossa was removed to expose the left gastrocnemius lateralis nerve. On the lateral side of this nerve, just before it enters the muscle body, a thin branch innervating a few motor units located in the upper and medial part of gastrocnemius lateralis was found eight out of ten times. The small nerve was freed from adjacent tissues over 8–10 mm (under microscopic control). When it was absent a gastrocnemius medialis small nerve was prepared in a similar manner. No denervation was performed except for the transection of the ipsilateral or contralateral sural nerve. Exposed tissues were protected from drying by mineral oil maintained at 37 °C. Rectal temperature was monitored and automatically maintained at 38 °C.

Experimental protocol. Sural nerve stimulation with progressive increase of stimulus strength allowed successive recruitment of Aβ, Aδ and C fibre groups. Also, in half of the experiments, the different groups were excited separately, using the anodal block method of Kuffler & Vaughan Williams (1953) as modified by Accornero, Bini, Lenzi & Manfredi (1977). Afferent sural volleys were monitored by recording the electroneurogram. In this paper, the afferent cutaneous fibres of sural nerve are classified according to conduction velocity as Aβ (60–30 m . sec^{-1}), Aδ (30–5 m . sec^{-1}) and C (2–0·4 m . sec^{-1}) groups (Erlanger & Gasser, 1937; Burgess & Perl, 1973). The muscle afferent fibres (from gastrocnemius muscles) are classified following Lloyd (1943) into group I (120–72 m . sec^{-1}), group II (72–30 m . sec^{-1}), group III (30–4 m . sec^{-1}) and group IV (2–0·4 m . sec^{-1}) (see also Burgess & Perl, 1973).

The electromyogram of triceps surae muscle was recorded by two needles inserted between soleus and gastrocnemius muscles. In this way, it was possible to detect the activity of the totality of triceps but not to detect which head of triceps was activated first.

Two monopolar recordings of the whole electrical activity of the intact branch of the gastrocnemius lateralis or gastrocnemius medialis nerves were made 4 mm apart. Each electrode was made of enamelled copper wire (diameter 60 μm) bared for 1 mm at its tip. The electrodes were glued on the short sides of a rectangular frame, 4 mm long, 3 mm wide, 1 mm thick, made of four cotton

threads embedded in polystyrene. The frame was slipped between the small nerve and the underlying tissues. The nerve was grounded midway between the two electrodes by a very small cotton wick soaked in Ringer solution. Because of its lightness and the flexibility of its wires, the frame could follow all displacements elicited by reflex contraction of adjacent muscle, and the electrodes remained in contact with the nerve. The recording from the electrode nearer to the central nervous system was called proximal, and that from the electrode nearer to the muscle was called distal. During experiments the recordings were processed on line with the aim of separating the potentials of each group of fibres composing the small nerve and so to observe the effects of the stimulation of ipsilateral or contralateral sural nerve on the activity of each fibre group. The recordings were also stored on magnetic tape using an FM recorder (DC, 20 kHz) and were subsequently displayed and processed.

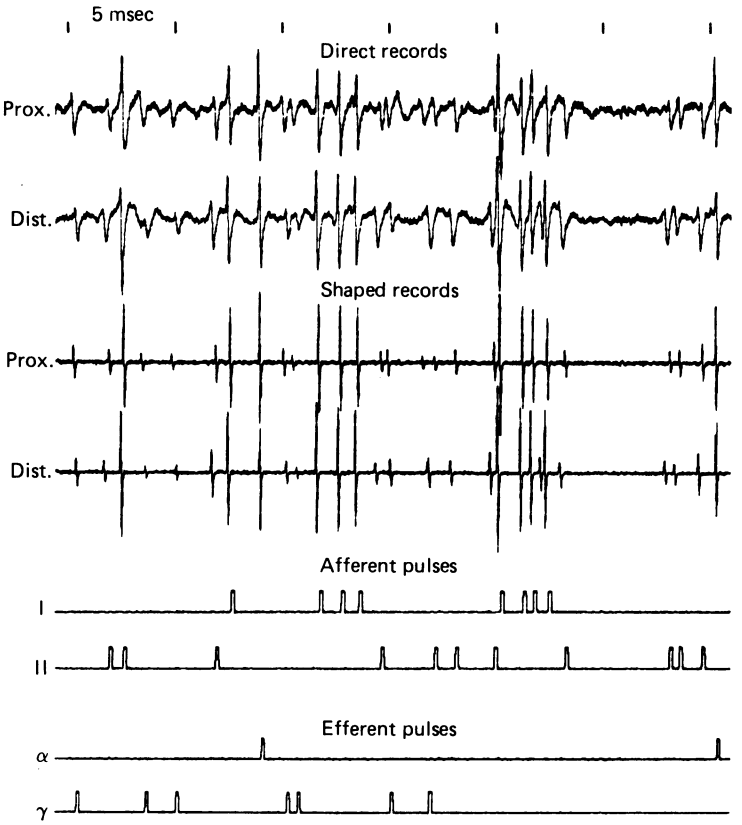

Fig. 1. Sorting, from two recordings of the over-all discharge of a small muscle nerve, of action potentials conducted by group I and II afferent fibres and by α and γ efferent axons. Direct records: two monopolar records, made 4 mm apart, of the over-all discharge of a small nerve branch supplying the muscle gastrocnemius lateralis. Prox: proximal record from the electrode nearer to the C.N.S.; Dist.: distal record from the electrode nearer to muscle. Shaped records: proximal and distal records after shaping as described in the text. Note the recording order of potentials; distal then proximal for afferent potentials; proximal then distal for efferent potentials. Afferent pulses: 150 μsec pulses elicited by the action potentials conducted by group I and group II afferent fibres. Efferent pulses: 150 μsec pulses elicited by the action potentials conducted by α and γ efferent axons.

Processing of the records. The purpose of the processing was to isolate the action potentials of the different groups (α, γ, I and II) of efferent and afferent fibres that composed the gastrocnemius small nerve. The sorting of potentials was performed by an electronic device designed and operated by one of us (M.J.). The principles of the method, and the potential discriminator have already been described (Joffroy, 1975, 1980) so that only a brief description will be given here.

The triphasic potentials of the proximal and distal records (Fig. 1, two upper traces) were shaped by transforming the negative-positive reversal phase into a diphasic impulse that crossed zero potential at exactly the same time. Shaping of the potentials reduced the background noise, removed the second positive-negative reversal of polarity of the triphasic potentials, and so lessened the superimposition of action potentials (Fig. 1, two middle traces). An angular window admitted only shaped potentials with reversal phases characterized by a falling slope, the value of which was at least equal to that of the slowest potentials. The angular window eliminated unwanted e.m.g. potentials. The point of reversal of each diphasic potential thus selected triggered both a 1 μsec pulse, used in the following steps of potential sorting, and a 150 μsec pulse for visual observation. Then, afferent and efferent action potentials were sorted according to their direction of propagation and the time taken to travel between the recording electrodes. Time windows, adjustable for their onset delay and their duration, allowed the selection of action potentials propagated from one recording electrode to the other in the pre-set time. The time windows were triggered by the 1 μsec pulses generated by the shaped potentials. Those triggered by the distal recording admitted the distally–proximally propagated potentials i.e. afferent potentials. Whereas those triggered by the proximal recording admitted the proximally–distally propagated potentials i.e. efferent potentials.

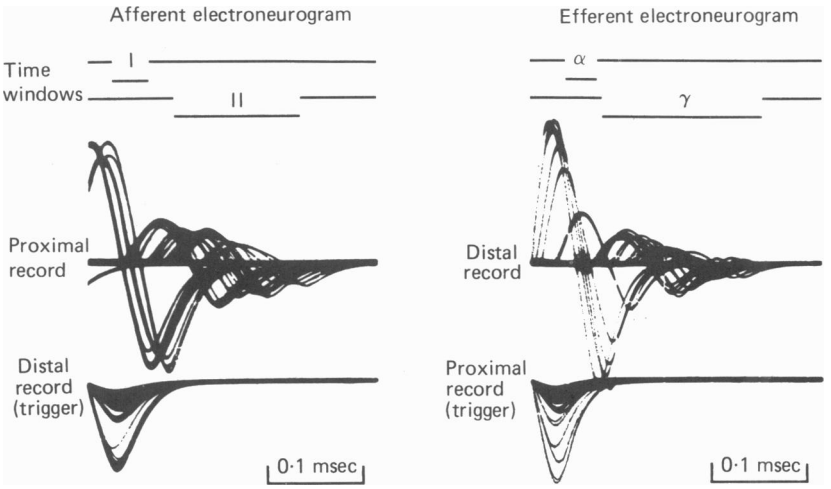

Fig. 2. Visualisation, on two oscilloscope screens, of some steps of the sorting of group I and II afferent potentials (left panel) and of α and γ efferent potentials (right panel) constituting the over-all discharge of a small nerve branch supplying the muscle gastrocnemius lateralis (description in the text).

Some steps of the processing are illustrated in Fig. 2. The sorting of afferent potentials is shown on the left. The 1 μsec pulse elicited by each selected potential of the distal record triggered the cathode-ray sweep (lower trace). Each afferent potential appears on the proximal record after a latency depending on the conduction velocity of the axon. Thus, an electroneurogram of the active afferent fibres in the small nerve was displayed. In this case the delay times and durations of two time windows were adjusted to separate the potentials with conduction times of 30–62 μsec and 83–224 μsec, i.e. potentials propagated with velocities between 130 and 64 m . sec^{-1} (group I fibres) and between 48 and 17 m . sec^{-1} (group II fibres). On the right of Fig. 2 is shown the sorting of efferent potentials. Here the zero line crossing of each shaped potential of the proximal recording produced a 1 μsec pulse which triggered the cathode-ray sweep so that an efferent electroneurogram was produced in the distal recording. Again, by adjusting the delay times and durations of two other time windows, it was possible to separate the efferent potentials with conduction times of 35–70 μsec and 75–240 μsec, i.e. the potentials respectively propagated at 114–57 m . sec^{-1} (α axons) and at 53–16 m . sec^{-1} (γ axons).

The 150 μsec pulses triggered by the selected potentials were sometimes displayed on an electrostatic paper recorder (Gould ES 1000) (Fig. 1, four bottom traces). Usually, however, they were used as the inputs of frequency meters (time constant 0·44 sec), each of which provided an

output voltage continuously proportional to the over-all firing frequency in one group of active fibres in the small nerve.

Efficiency limit of the sorting process. The smallest potentials that could be sorted had to be 1·5 times the background noise. That condition limited the maximum size of the nerve used in the recording to 0·2 mm in diameter. Such nerves allowed easy separation of potentials propagated at velocities as low as 12 m . sec^{-1}. High-speed records, with the aim of adjusting the sorting device, showed some mistakes. They were always produced by superposition of potentials propagated in different axons and passing the recording electrodes almost simultaneously. Since the mean duration of action potentials was about 0·5 msec, theoretically the highest possible frequency for sorting without error was 2000 impulses . sec^{-1}. It has been observed empirically that the errors of counting were those of omission and that the error augmented as the frequency of over-all activity of the small nerve increased. For instance, when the frequency was 800 impulses . sec^{-1} the error of potential counting was 5–6 % for groups I and II afferent fibres and 8–10 % for α and γ axons. When the frequency reached 1,300 impulses . sec^{-1} the error was 8–10 % for afferent fibres, 15 % for α and 30 % for γ fibres. Peak amplitudes of the frequencies in the experiments reported here were usually 600–900 impulse . sec^{-1}. Estimation of the value of γ firing frequency was most disturbed by counting errors because when a γ potential was superimposed on a large afferent or efferent potential its peak-to-peak amplitude could be reduced sufficiently that the signal-noise ratio was less than the minimal value required to detect the potential. That happened when the negative-positive reversal phase of a γ potential was superimposed with opposite (positive-negative) reversal phase of a large potential.

RESULTS

The small nerve as a representative sample of the whole gastrocnemius innervation

The gastrocnemius lateralis and gastrocnemius medialis small nerves had diameters approximately $\frac{1}{10}$ that of the whole nerve. It may be asked whether all functional groups of nerve fibres were present in these samples. The electroneurograms of the active fibres (see Fig. 2) showed that at least four fibre groups were represented: α and γ efferent axons, and groups I and II afferent fibres. However, three exceptional cases occurred: active group I fibres were absent in one nerve and group II fibres in two nerves. Fibres conducting at less than 12 m . sec^{-1} could not be detected (see Methods).

A comparison of the distribution of conduction velocities in the small nerve with the distribution of axonal diameters in gastrocnemius lateralis or medialis nerves (Eccles & Sherrington, 1930) ought to indicate whether the sample is representative of the whole nerve. Unfortunately, when the electroneurograms of spontaneously firing afferent or efferent fibres were observed at high sweep speed (20 μsec/division) it appeared that the conduction time of potentials of a given fibre was not absolutely constant but fluctuated around a mean value because of the background noise in the recordings. Moreover, potentials propagated along nerve fibres, differing only slightly from one another in diameter, appeared with approximately similar shape and after almost identical delays owing to the short conduction distance (4 mm). Thus, the fluctuation of positions of the potentials on the cathode-ray screen led to spatial superimposition and to visual fusion of the potentials. Consequently, it was not possible to separate potentials propagated along afferent or efferent fibres, the diameters of which were very similar. It follows that to count precisely the active fibres in the small nerve and to construct the histogram of conduction velocities was impossible.

However, observation of electroneurograms of twenty-five small nerves gave some information about the distribution of conduction velocities of active fibres. Afferent

fibres had conduction velocities of 130–15 m . sec^{-1}. The distribution was bimodal: 130–72 m . sec^{-1} for the faster group (group I fibres) and 70–30 m . sec^{-1} for the slower group (group II fibres). Generally, separation of group I from group II was easy because there were few or no fibres with velocities between 70 and 60 m . sec^{-1}. The diameters of these rare fibres, established by dividing their conduction velocities by Hursh's conversion factor of 6 (Hursh, 1939), were 12–10 μm. The gap in the distribution of afferent velocities therefore corresponds to the gap found by Eccles & Sherrington (1930) in the distribution of the diameters of afferent fibres of gastrocnemius medialis nerve.

Efferent axons conducted at velocities between 110 m . sec^{-1} and 12 m . sec^{-1}. Using Hursh's factor again, diameter ranging from 20 to 2 μm were estimated. Usually, the distribution of conduction velocities was bimodal; the faster group conducting at 110–50 m . sec^{-1} (α fibres) and the slower group at 50–12 m . sec^{-1} (γ fibres). Efferent fibres conducting in the range 70–45 m . sec^{-1} (calculated diameter 11–7·5 μm) were uncommon. This gap in the distribution of conduction velocities was in agreement with the small number of efferent fibres of diameter 11–8 μm found in gastrocnemius medialis nerve (Eccles & Sherrington, 1930). In four cases, the efferent electroneurogram showed three groups of potentials (for instance, Fig. 2). Conduction velocities of the middle group ranged from 60 to 48 m . sec^{-1}. These potentials were sorted arbitrarily into either the α group or the γ group, according to whether their conduction velocities were faster or slower than 50 m . sec^{-1}. The group might be formed by skeleto-fusimotor (β) fibres but this could not be decided on the present evidence. In thirteen small nerves the γ axons could be divided into two subgroups, as indicated by a gap in the range of conduction velocities. Since the position of the gap varied from one nerve to another, the γ fibres of the two subgroups were simply called the slower and the faster γ fibres.

The above observations allow us to consider the small nerves as representative samples of the different functional groups of nerve fibres conducting faster than 12 m . sec^{-1} and composing the gastrocnemius lateralis or medialis nerves. However, owing to the small size of each sample, all conduction velocities were not invariably encountered. Those which were most usually present in any sample paralleled the maxima in the distribution of the conduction velocities of the whole nerve. Thus, the gaps observed in the electroneurograms in the distribution of conduction times of afferent or efferent potentials occurred precisely at the boundaries between the group I and the group II fibres and between the α skeleto-motor axons and the γ fusimotor axons.

The background discharge in the small nerve

The over-all firing frequencies of the four functional groups of active fibres were measured 2–3 hr after the decerebration and before any stimulation. Triceps muscle was at its minimal physiological length (tibio-tarsal angle, 120 °) hence with only feeble or no tonic activity of gastrocnemius motor units. From these measurements the sum of the total firing frequencies of all the active fibres of each nerve could be established (range, 50–480 impulses . sec^{-1}). The dispersion of values arose from variations in both the nerve branch diameter and the level of decerebrate rigidity in each preparation. As expected, the number of active fibres increased with the size of the branch, and the number as well as the firing rate of fibres increased with the level of rigidity.

Group I afferent fibres (about one to eight; approximative estimation based upon the latency times and the shapes of action potentials on the electroneurograms) showed tonic activity in most nerves (eighteen out of twenty). The over-all firing frequency of this group ranged from 20 to 250 impulses . sec^{-1}. Of the two nerves that lacked resting activity in group I fibres, one apparently did not possess group I fibres, since neither muscle stretch nor contraction produced potentials of the appropriate velocity; the other possessed fibres that were inactive owing to the short length and weak tone of the muscle.

Generally, the total activity of group II fibres was higher than that of group I (range, 40–400 impulses . sec^{-1}). This resulted from the number of active group II fibres (about two to fifteen) being greater than that of active group I fibre (about one to eight) and not from a higher firing rate of individual group II than group I fibres.

Alpha axons showed no tonic activity in most of the small nerves. Some activity (10–50 impulses . sec^{-1}) in a few axons (about one to four) was observed in two nerves at the same time as the periods of high γ-firing rate (see below).

Gamma tone was present in all the small nerves. This finding was in agreement with the observations of Hunt (1951), Kobayashi, Oshima & Tasaki (1952), Voorhoeve & Van Kanten (1962). The number of tonic γ fibres ranged approximatively from one to fifteen. The over-all firing frequency of the population was 5–250 impulses . sec^{-1}.

Without any stimulation or length change of triceps muscle, the over-all firing frequency in each nerve branch underwent slow and sometimes rythmical changes which varied with time and in different animals. Variations in amplitude of as much as 20–40 % of the mean resting discharge were the most usual. Transition between two levels of activity could take from a few seconds up to 10 min. The changes of the over-all firing rate mostly accompanied spontaneous variations of muscle tone. After decerebration, and during the 6–8 hr recording, the animals passed several (one to five) times from a marked rigidity to a profound flaccidity, the duration of which considerably varied (10–90 min).

Fig. 3 illustrates such a change of rigidity. In this experiment the tension in triceps was maintained at 0·5 kg. After a long period of high rigidity, during which the muscle response to the constant weight was a tonic myotatic contraction, the muscle showed a series of phasic contractions elicited by bursts of α activity as monitored in the nerve and in the triceps e.m.g. (only the four last bursts are shown in the Figure). Then, the α activity in nerve and triceps e.m.g. activity suddenly disappeared and the animal went into a profoundly flaccid state for 7 min 20 sec. Following the sudden suppression of α activity, the γ activity underwent a period of slow decrease. Whereas the over-all firing of approximately twenty γ axons was 210 impulses . sec^{-1} at the beginning of this period, 2 min later the frequency was 10 impulses . sec^{-1}, resulting from the activity of only one γ axon. During the period of phasic contractions, the over-all firing frequency of group I and II fibres increased each time the muscle was relaxed and consequently stretched by the 0·5 kg weight. When the rigidity disappeared, although the triceps length increased, the mean firing frequency of group I and II decreased (from 300 to 75 impulses . sec^{-1} and from 400 to 270 impulses . sec^{-1}, respectively) because of the fall of γ tone. Six minutes thirty seconds after the

beginning of the flaccidity the γ-firing frequency increased, eliciting increased afferent fibre discharges. Only 1 min later the α axons were firing again and the triceps e.m.g. activity reappeared, both features accompanying the first appearance of a further period of rigidity. Hunt (1951) has observed, in decerebrate preparations showing periodic fluctuations in extensor tone, that the activity increase in small efferent fibres accompanied the discharge in large efferent fibres.

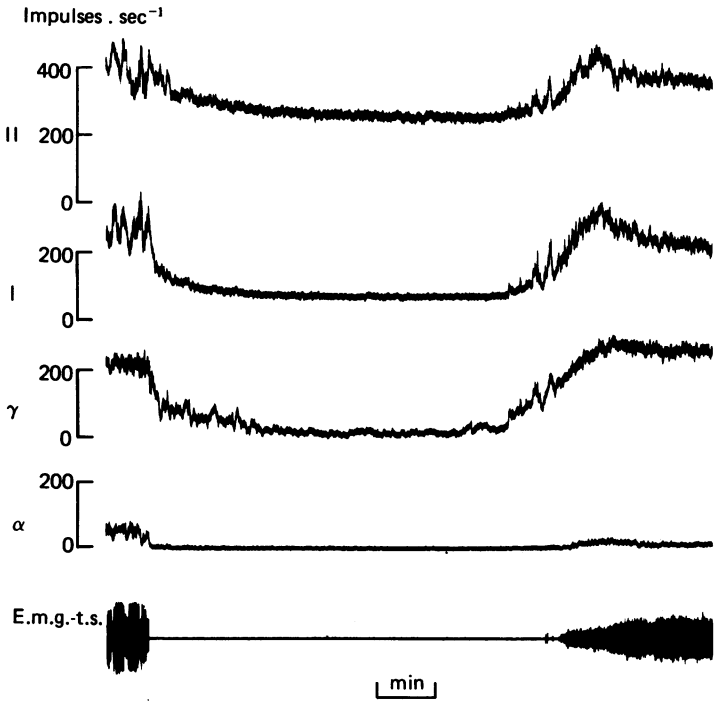

Fig. 3. Changes of discharges of the population of group I and group II afferent fibres and of α and γ efferents axons found in a small nerve branch innervating the muscle gastrocnemius lateralis during spontaneous variations of rigidity, in a decerebrate cat. *First and second traces*, mean firing frequencies respectively of group II and I afferent fibre populations; *third and fourth traces*, mean firing frequencies respectively of γ and α efferent axon populations; *fifth trace*, e.m.g.-t.s. = direct electromyogram of muscle triceps surae.

Several experiments gave evidence that γ-firing changes were accountable not only by variation of the firing frequencies of tonic γ neurones but also by the number of active γ neurones. Thus, an increase of γ tone in part results from the recruitment of previously silent γ neurones. Frequently the conduction velocities of recruited axons were faster than those of tonic γ axons.

Reflex effects on γ motoneurones of gastrocnemius muscle induced by stimulating the ipsilateral or the contralateral sural nerve

The stimulation of the ipsilateral (seven experiments) or contralateral (eight experiments) sural nerve induced γ-reflex response in all but one experiment in which an ipsilateral sural nerve was stimulated. The reflex change evoked by contralateral as well as ipsilateral stimulation almost always consisted of an increase of the over-all firing frequency of γ axons in the nerve. However, in one experiment, a few ipsilateral

stimulations elicited a small (10–20%) decrease followed by an increase of γ discharge.

The dispersion of time intervals separating successive action potentials in the γ multi-unit discharge makes it difficult to determine precisely a threshold stimulation for inducing a reflex response. Therefore, sural stimulation was considered to be at threshold when, in a reproducible manner, it elicited an increase of about 15 impulses . sec^{-1} of the γ-firing frequency.

At stimulus strengths that were only activating Aβ fibres, γ reflex excitation were observed in twelve of fifteen experiments. Repetitive stimulation (two to twenty stimuli at 10–50 Hz; fifty stimuli at 100 Hz in one experiment) was necessary to obtain the responses, except in one experiment in which a single stimulus was effective. Stretching the triceps muscle increased the response to Aβ volleys.

With a stimulus strength sufficient to activate both Aβ and Aδ sural fibres, it was possible to elicit a γ activation with repetitive stimulation of shorter duration and lower frequencies than those used when only Aβ fibres were excited. This occurred in seven out of eleven experiments where the comparison was possible. In four of these eleven experiments a single stimulus was effective, but otherwise three to four stimuli at 12–20 Hz were required.

When the C fibres were recruited in addition to the Aβ and Aδ fibres, γ reflex activation was elicited by a single stimulus in one third of cases and by two to four stimuli at 5–15 Hz in the remainder.

Fig. 4. shows the γ-reflex responses evoked by suprathreshold repetitive stimulation of the ipsilateral sural nerve. The response latency varied widely (range 50–560 msec), depending on the stimulation rate (temporal facilitation) and on the temporal and individual variations of the rigidity. When Aβ fibres were excited (Fig. 4*A*) a 35% increase of over-all firing frequency of the γ population lasted about 1·6 sec. The stimulation elicited neither α activity in the gastrocnemius lateralis small nerve nor change in the triceps e.m.g. However, three stimuli at 40 Hz produced α and γ activations (Fig. 4*D*). When both Aβ and Aδ fibres were excited (Fig. 4*B*), the amplitude of the γ response reached about 66% of resting discharge. The return to resting firing level was very slow, so that 8 sec after the end of the stimulation the firing frequency of the γ population was still 20% higher than that before the stimulation. Alpha-motoneurones were also activated as shown by the activity in the small nerve and by the triceps e.m.g. Alpha and gamma responses reached their greatest amplitudes and longest durations when Aβ, Aδ and C fibres were stimulated together (Fig. 4C). Thus, it seemed probable that all or almost all the fibres constituting the sural nerve were excitatory actions on the γ motoneurones of gastrocnemius lateralis muscle. However, the earlier effects of the fast conducting Aβ fibres could have been conditioning the reflex effects of the slower Aδ and C fibres. It was also possible that the excitatory action of some fibres could have concealed an inhibitory action of slower conducting fibres. To investigate these possibilities we used an anodal block to stimulate Aδ and C fibres separately. Both groups (Aδ, Fig. 4*E* and C, Fig. 4*F*) still induced an excitation of the γ motoneurones of gastrocnemius lateralis muscle. The amplitudes of the responses were very close to those induced when respectively stimulating Aβ and Aδ fibres (Fig. 4*B*) and Aβ, Aδ and C fibres (Fig. 4*C*). Similar results have been observed with the stimulation of four contralateral

and six ipsilateral sural nerves. These selective stimulation experiments provide evidence that each group of fibres of the sural nerve induced the excitation of gastrocnemius γ motoneurones and that reflex actions of Aδ fibres and of C fibres were independant of the activation of faster fibres. Our results agree with those of Catley & Pascoe (1978) who recorded single-unit γ activity in the rabbit.

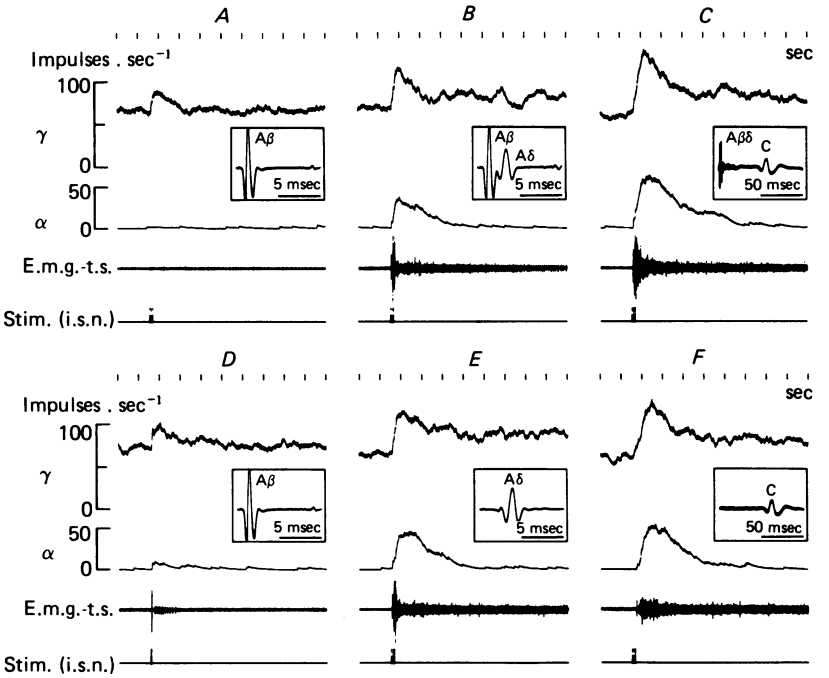

Fig. 4. Reflex changes of discharges of the population of γ and α axons in a small nerve branch innervating the muscle gastrocnemius lateralis elicited by stimulating different groups of cutaneous afferent fibres of the ipsilateral sural nerve. In each panel *A*, *B*, *C*, *D*, *E*, and *F*: *first trace*, time marker, 1 sec calibration; *second and third traces*, mean firing frequency respectively of γ and α axon populations; *fourth trace*, e.m.g.-t.s. = direct electromyogram of muscle triceps surae; *fifth trace*, Stim (i.s.n.) = stimulation of the ipsilateral sural nerve; *inset*, electroneurogram of the ipsilateral sural nerve to monitor the afferent volleys. *A*, *B*, and *C*, repetitive stimulation of the i.s.n. (three stimuli, 12 Hz) with increase of stimulus strength to recruit successively smaller fibre groups: Aβ (*A*), Aβ+Aδ (*B*), Aβ+δ+C (*C*). *D*, *E*, and *F*, repetitive stimulation of the i.s.n. (three stimuli, 40 Hz in *D*; 12 Hz in *E* and *F*) with a modified anodal block method to excite separately and selectively the fibre group Aβ (*D*), Aδ (*E*) and C (*F*).

However, both with ipsilateral and contralateral stimulation, the amplitudes of the responses of γ and α motoneurones of gastrocnemius muscle to stimulation of all sural fibres (Fig. 4*C*) were less than the corresponding sums of amplitudes of the responses to selective stimulation of the three groups of fibres in the sural nerve (Fig. 4*D*, *E* and *F*). This indicated that occlusion probably occurs in reflex responses of both γ and α motoneurones.

Patterns of activation of γ motoneurones

Comparison of the efferent electroneurograms before and during sural nerve stimulation showed that the reflex increase in γ activity resulted from the increased

firing of tonic γ neurones and from the recruitment of previously silent ones. A few tonic units were insensitive to the afferent stimulation. No γ motoneurone was found to decrease its firing rate when the sural nerve was stimulated, despite careful observation of the discharges of as many single axons as possible. However, a few such units may have been missed due to the difficulty of separating action potentials with very similar conduction velocities (see Methods), and this must have occurred in one experiment where there was some over-all inhibition (*vide infra*).

Recruitment of silent γ motoneurones in particular was studied in seven experiments using contralateral (four experiments) or ipsilateral (three experiments) sural nerve stimulation. Recruited γ axons did not belong to any special category that could be characterized by clearly defined conduction velocities, nor did there seem to be any correlation between the conduction velocity of recruited γ axons and the afferent group(s) stimulated in sural nerve, or the length of triceps muscle. In three experiments in which the conduction velocities of tonic γ axons were rather slow (30–15 m . sec^{-1}) the recruitment of faster γ axons (40–30 m . sec^{-1}) was easily shown. The discharge of the fast γ group generally started 30–360 msec after the beginning of the response of the slow group, which itself was due both to increase of firing rate of tonic γ neurones and to recruitment of previously silent ones, though the relative importance of these two processes could not be determined because of similar conduction velocities.

Fig. 5 shows the different latencies of the reflex responses of two γ axon populations with different conduction velocity ranges. On stimulation of the contralateral sural nerve the reflex response of a tonically active population of γ axons conducting at 21–17 m . sec^{-1} starts about 60 msec after the first stimulus whereas that of a second, originally silent, population of γ axons conducting at 48–36 m . sec^{-1} begins some 220 msec later (Fig. 5*A*). When there was no tonic γ activity before sural stimulation, the slower group of γ axons (17 m . sec^{-1}) was recruited before the faster one (28 m . sec^{-1}) by about 70 msec (Fig. 5*B*).

In four experiments, the population of tonically firing γ axons could be divided into two subgroups. Sural nerve stimulation elicited responses in both subgroups, but to what extent the responses were due to recruitment of silent axons or to increase of firing rate of tonic axons could not be ascertained. However, it was easy to compare the response latencies in the two subgroups: two thirds of sural nerve stimulations induced a response of the slower subgroup 20–200 msec before that of the faster subgroup. (In the same experiments, the latency times of the responses of the two subgroups and the delay between the onset of the two responses underwent changes of more than 50%, probably on account of excitability variations of the decerebrate preparation). In one third of sural nerve stimulations, the responses of the two subgroups began, at the level of the recording electrodes, at approximately the same time; therefore, as with the above examples, the slower subgroup was activated before the faster subgroup. This chronological order was reversed in only one experiment.

Comparison of reflex responses of γ and α motoneurones

(*a*) *Stimulation of the contralateral sural nerve*

(i) *Selective activation of gastrocnemius γ motoneurones.* In six experiments, small reflex responses of the γ population of the small nerves could be evoked without any activity change in either the α population or the triceps e.m.g. The responses were usually obtained when repetitively stimulating Aβ sural fibres with less than ten stimuli at frequencies below 50 Hz. When Aδ and C fibres were successively recruited (one experiment) or when Aδ and C fibres were separately excited (two

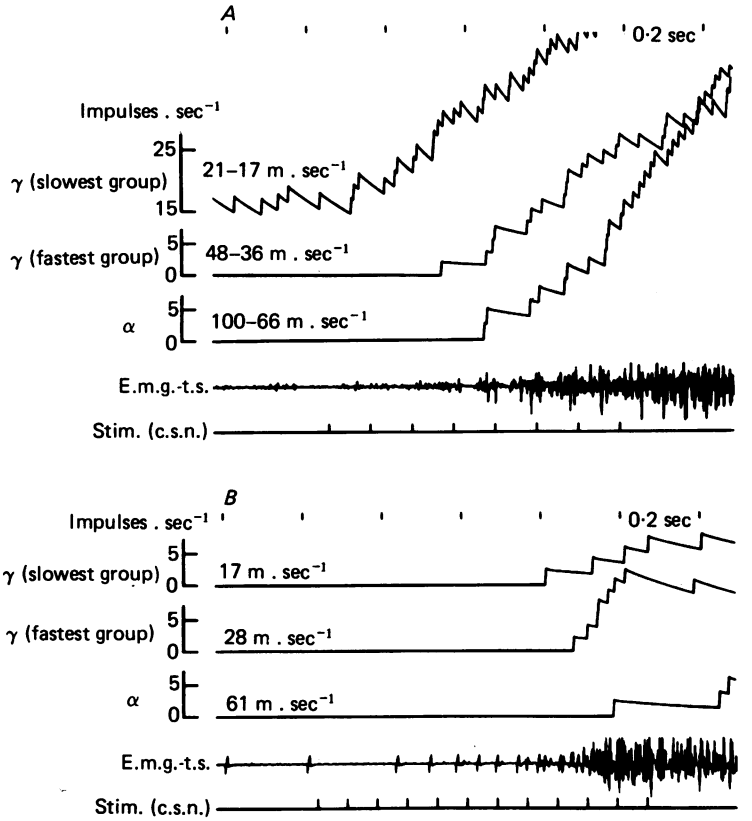

Fig. 5. Chronological order of the reflex excitation of α- and γ-motoneurones. *A* and *B* illustrate two experiments in which efferent potentials of a small nerve branch supplying the muscle gastrocnemius lateralis naturally occurred in three groups: one of α velocity range (*A*, 100–66 m . sec^{-1}; *B*, 61 m . sec^{-1}), and two of γ velocity range, named the faster group (*A*, 48–36 m . sec^{-1}; *B*, 28 m . sec^{-1}) and the slower group (*A*, 21–17 m . sec^{-1}; *B*, 17 m . sec^{-1}). In each panel *A* and *B*: *the first, second and third traces*, mean firing frequency respectively of the slower γ group, the faster γ group and the α group; *fourth trace*, e.m.g.-t.s. = direct electromyogram of the muscle triceps surae; *fifth trace*, Stim. (c.s.n.) = repetitive stimulation (*A*, eight stimuli, 10·6 Hz; *B*, twelve stimuli, 13 Hz) of Aδ (*A*) and C (*B*) fibres of contralateral sural nerve.

experiments) selective γ responses were obtained with two to six stimuli at 1–12 Hz. Figure 6 illustrates one of these latter experiments. The contralateral sural nerve was repetitively stimulated with 12 Hz trains consisting of increasing numbers of stimuli. Five Aδ volleys (Fig. 6*A*) did not elicit any α- or γ- reflex response, whereas six Aδ volleys (Fig. 6*B*) provoked selective γ activation shown by the lack of α recruitment or of e.m.g. change. Finally, seven Aδ volleys (Fig. 6*C*) induced a reflex response of γ motoneurones followed by one of α motorneurones (see below). C volleys were more effective than Aδ volleys to induce reflex responses, since five C volleys (Fig. 6*D*) were enough to activate γ motoneurones selectively and six C volleys (Fig. 6*E*) to elicit a reflex response of γ motoneurones then one of α motoneurones. These results show that by using temporal facilitation the reflex activation of γ motoneurones may be separated from that of α motoneurones, and that the threshold

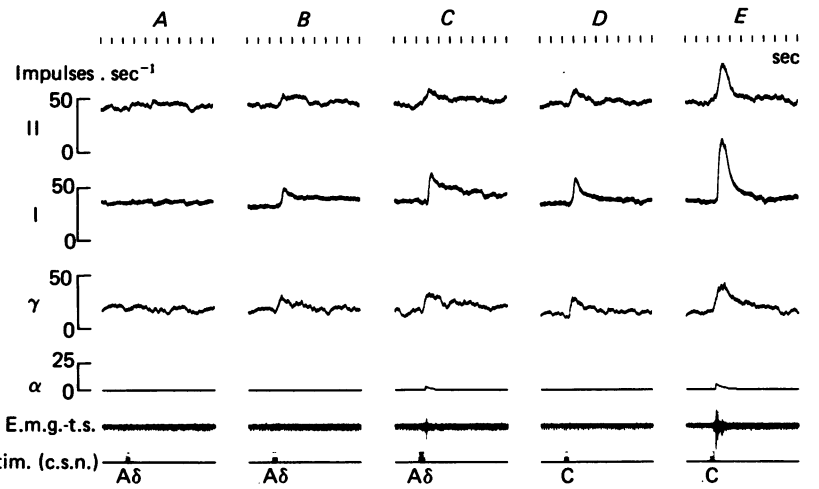

Fig. 6. Changes of discharges of the population of group I and II afferent fibres and of α and γ efferent axons in a small nerve branch innervating the muscle gastrocnemius lateralis elicited by the separate and selective stimulation of Aδ fibres and of C fibres of the contralateral sural nerve. In each panel *A*, *B*, *C*, *D*, and *E*: *first trace*, time marker, 1 sec calibration; *second and third traces*, mean firing frequency respectively of group II and I afferent fibre populations; *fourth and fifth traces*, mean firing frequency respectively of γ and α axon population; *sixth trace*, e.m.g.-t.s. = direct electromyogram of muscle triceps surae; *seventh trace*, Stim. (c.s.n.) = stimulation of contralateral sural nerve. *A*, *B*, and *C*, repetitive stimulation of Aδ fibres with five (*A*), six (*B*) and seven (*C*) stimuli at 12 Hz. *D* and *E*, repetitive stimulation of C fibres with five (*D*) and six (*E*) stimuli at 12 Hz.

for reflex activation by contralateral sural nerve stimulation was lower for γ motoneurones than for α motoneurones. Selective activation of γ motoneurones was usually obtained with triceps muscle close to minimal physiological length and consequently exhibiting feeble e.m.g. activity. Selective activation of gastrocnemius γ-motoneurones could be obtained in all but two experiments.

(ii) *Temporal relationships of reflex responses of α and γ motoneurones.* Adequate repetitive stimulation of each fibre group of the sural nerve elicited a crossed extensor reflex as shown by the α discharge in the small nerves (Fig. 5*A* and *B*; Fig. 6*C* and *E*; Fig. 7). Muscle stretch facilitated the crossed extensor reflex by increasing the afferent discharges from muscle spindles supplying gastrocnemius α motoneurones. With only one exception, the α-reflex response resulted from recruiting α motoneurones since these neurones were silent before sural stimulation. In these conditions, α and γ populations of the small nerves were excited.

Fig. 7*B* shows the chronological order of onset of the excitations of α and γ motoneurones, elicited by repetitive stimulation (six stimuli, 12 Hz) of the Aδ group of fibres of contralateral sural nerve. The firing increase of γ axons of the gastrocnemius lateralis small nerve started about 200 msec before the onset of α activity in the nerve. A similar relationship was observed in four out of five preparations used to investigate this problem. The average delay between the onset of γ and α responses, at recording level, was 0·4 sec (range 0·2–1 sec). This corresponds to a slightly longer delay time at the central level because of the differences of conduction velocity of α and γ axons. The α reflex frequently did not last as long as that of the γ reflex, indeed the α

response started after and faded before the γ response (Fig. 6*C* and *E*). In one experiment, γ neurones of the gastrocnemius lateralis muscle were found to respond less to sural stimulation than the α neurones of the same muscle and consequently pure α responses could be easily induced.

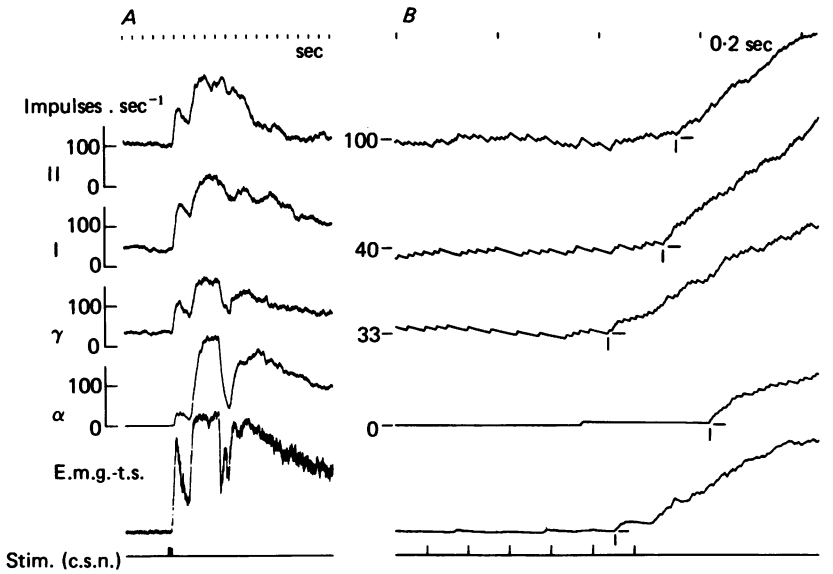

Fig. 7. Changes of discharges of the populations of group I and II afferent fibres and of α and γ efferent axons in a small nerve branch innervating the muscle gastrocnemius lateralis elicited by the repetitive stimulation of Aδ fibres of the contralateral sural nerve. In each panel *A* and *B*: *first trace*, time marker; *second and third traces*, mean firing frequency respectively of group II and I afferent fibre populations; *fourth and fifth traces*, mean firing frequency of γ and α efferent axon populations; *sixth trace*, e.m.g.-t.s. = electromyogram of muscle triceps surae (rectified and smoothed with a low-pass filter, time constant: 1 sec); *seventh trace*, Stim. (c.s.n.) = stimulation of contralateral sural nerve (six stimuli, 12 Hz). In panel *B*, a short part (about 0·8 sec during and after sural stimulation) of curves of panel *A* is shown with expanded time (× 49) and frequency (× 4) scales; zero lines of frequency curves are not represented; at the left of each curve the number and the marks indicate the average level of frequency during the 10 sec before stimulation.

(iii) *Temporal relationships of the reflex activation of gastrocnemius α and γ motoneurones and of soleus α-motoneurones.* Simultaneous recording of the firing in the small nerve and of the triceps e.m.g. allowed us to analyse the pattern of the skeleto-motor reflex in a pale and in a red muscle, induced by sural nerve stimulation. The α axons of the small nerve are supplying a pale muscle (gastrocnemius lateralis or medialis) formed of motor units mainly of A type that are characterized by numerous muscle fibres, rapidly contracting, activated at low frequencies and easy to fatigue (Wuerker, McPhedran & Henneman, 1965; Henneman & Olson, 1965). The triceps e.m.g. is a sample of electrical activity in pale (gastrocnemius) muscles but also in red (soleus) muscle formed of motor units mainly of B type which are characterized by a few muscle fibres, slowly contracting, activated at high frequencies and resistant to fatigue (McPhedran, Wuerker & Henneman, 1965; Henneman & Olson, 1965). In addition, because of the size-cell principle (Henneman, Somjen & Carpenter, 1965*a*, *b*), the recruitment of α-motoneurones of soleus muscle is easier than that of α-motoneurones of gastrocnemius muscles. The participation of B-type motor units in the triceps e.m.g. probably explained the reflex increase of the electromyographic activity that preceded the onset of the α-reflex discharge in the small nerve. That occurred in spite of conduction and synaptic delays added when recording the e.m.g.

We could compare, therefore, the beginning of the reflex response of the γ axons in the small nerve with the onset of activity of both fast and slow motor units. Figure 7*B* illustrates the most frequent chronological order; the triceps e.m.g. response (lower trace) started hardly (about 15 msec) after the γ response, but well before (185 msec) the α response in the gastrocnemius lateralis small nerve. In five experiments, the triceps e.m.g. activity (and therefore the soleus muscle activity) began 10–25 msec after the first appearance of γ activity and 100–250 msec before the onset of α activity in the small nerve.

When the γ axons of the small nerve could be divided into two subgroups, the beginning of the activity of the faster subgroup was later than that of the slower subgroup (see above) and concomitant with that of the triceps e.m.g. response (Fig. 5*A* and *B*).

Recruitment of the thinnest fibres of sural nerve together with triceps muscle stretch reduced the delay between the first appearance of the responses of the triceps e.m.g. and of the γ activity recorded from the nerve, so that both responses might appear simultaneously. Exceptionally, and in a way neither reproducible nor correlative with any physiological events, the triceps e.m.g. response could start before the γ response from the nerve.

The total duration of the triceps e.m.g. response was longer than the α response but shorter than the γ response in the small nerve.

(*b*) *Stimulation of the ipsilateral sural nerve*

Seven experiments were performed to study the reflex response to stimulation of the ipsilateral sural nerve.

(i) *Selective activation of gastrocnemius γ motoneurones.* In five experiments, selective activation of γ neurones of the small nerve could be obtained. The characteristics of these responses and the conditions needed to elicit them were similar to those described in relation to contralateral stimulation. For example, Fig. 4*A* illustrates a selective γ activation by sural Aβ volleys. A similar result was found by Hunt (1951) who reported that a stimulus eliciting an extensor thrust response could be graded so as to produce an increase in the discharge of small axons only.

(ii) *Temporal relationships of γ and α responses.* Stimulation of cutaneous afferents (particularly those of sural nerve) evokes ipsilaterally the activation of flexor α-motoneurones while extensor motoneurones are first inhibited and then undergo a rebound excitation.

Figure 8 shows the responses induced in populations of α and γ motoneurones of gastrocnemius lateralis muscle by stimulating the C fibres of sural nerve. This experiment was the only one in which α axons of the small nerve were tonically firing though the muscle was at minimal physiological length. Inhibition of the α motoneurones started about 165 msec after the onset of the stimulation and lasted 580 msec. A very long (6 sec) period of α-motoneuronal excitation followed the inhibition. Since it began more than 200 msec after the end of the repetitive stimulation, it was representative of the excitation rebound following reflex inhibition. In this experiment about one third of the sural nerve stimulations elicited small (10–20 %) decreases of the firing rate of γ motoneurones. This was the only example found of γ inhibition, which started at the same time and usually lasted the same duration as that of the α motoneurones. The period of γ inhibition was followed by a small but long increase of the γ firing frequency (20–30 % above the resting rate for 7–10 sec). The remaining sural nerve stimulation evoked only γ motoneurone excitations that were of small amplitude and with latencies similar to those of activation rebounds (Fig. 8). The triceps e.m.g. in Fig. 8 shows that the sural stimulation elicited an inhibition of a motor unit that previously fired a large amplitude potential at regular rate. This was followed by marked triceps e.m.g. activity beginning 330 msec after the onset of stimulation and lasting 25 sec.

In most of the experiments (five out of six) the gastrocnemius α motoneurones were silent or exhibited very low frequency of firing (less than 1 Hz), therefore any possible reflex inhibition of these motoneurones could not be distinguished. An example is shown in Fig. 9 where repetitive stimulation (three stimuli, 12·7 Hz) of sural C fibres elicited a late activation (300 msec latency) of some α motoneurones. Taking into account that the firing began 145 msec after the end of the stimulation, the α activation was probably an activity rebound following an undetected reflex inhibition. The stimulation similary induced γ activation with 105 msec latency, thus preceding the activation of the α motoneurones by about 195 msec. Triceps e.m.g. activity appeared at the same time as the α discharge in the small nerve (apart from a minor burst 100 msec earlier).

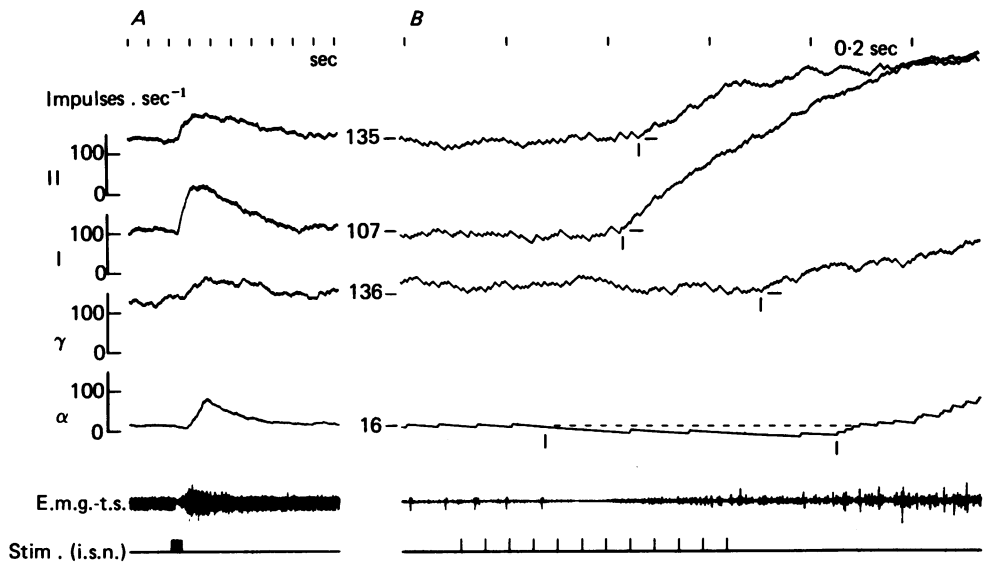

Fig. 8. Changes of discharges of the populations of group I and II afferent fibres and of γ and α efferent axons in a small nerve branch innervating the muscle gastrocnemius lateralis elicited by repetitive stimulation (twelve stimuli, 12 Hz) of C fibres of ipsilateral sural nerve (i.s.n.). Same arrangement as Fig. 7.; e.m.g.-t.s. = direct electromyogram of muscle triceps surae.

Consequences of sural nerve stimulation on discharges of group I and II muscle afferents

The stimulation of ipsilateral and contralateral sural nerves, whatever the α or γ reflex patterns induced, led to the increased discharge of group I and II fibres in the small nerves. The firing frequency increase of group I fibres was usually two or three times greater than that of group II fibres (Figs. 8 and 9). Both groups usually began to respond simultaneously, but sometimes, as indicated in Figs. 7, 8 and 9, the group I response preceded by 20–200 msec that of the group II fibres.

Group II afferents of gastrocnemius nerves are almost exclusively derived from secondary endings of muscle spindles, whereas group I afferent fibres originate both from primary endings of muscle spindles and from Golgi tendon organs. Since the angles of the ankle and knee were kept unchanged during each experiment, the

responses of group I and II afferents of triceps muscle did not result from changes in muscle length. The activation of muscle spindle afferents might be due to: (i) intrafusal muscle contraction elicited by reflex excitation of γ motoneurones; (ii) asynchronous activation of motor units driven by reflex response of α motoneurones; such contractions in the vicinity of muscle spindle capsules might cause the excitation of primary and secondary endings by localized changes of pressure or length; (iii) conjugate contractions of intrafusal and skeletal muscle fibres innervated by β skeleto-fusimotor axons; this activation mechanism cannot be discriminated in the present experiments since efferent fibres were identified only by conduction velocity. Tendon organ activation was essentially due to the tension developed by active motor units.

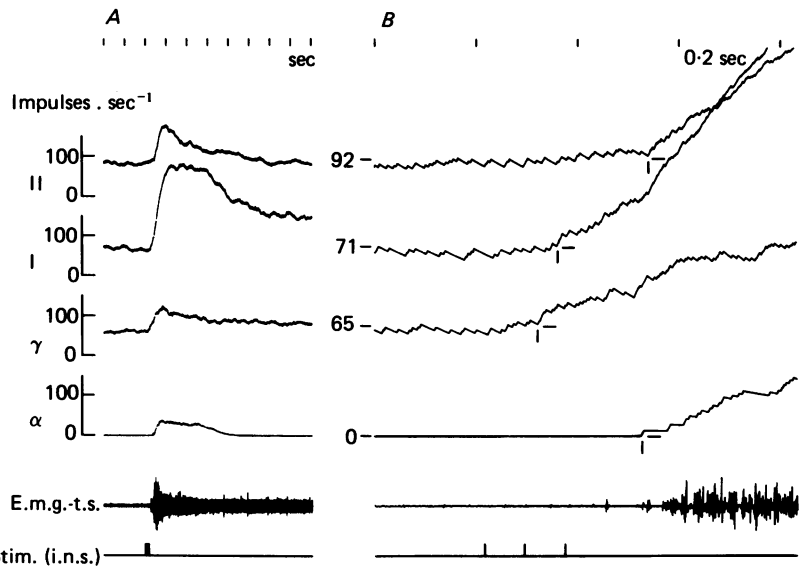

Fig. 9. Changes of discharges of the populations of group I and II afferent fibres and of α and γ efferent axons in small nerve branch supplying the muscle gastrocnemius lateralis elicited by the repetitive stimulation (three stimuli; 12·7 Hz) of C fibres of the ipsilateral sural nerve (i.s.n.). Same arrangement as Fig. 7.; e.m.g.-t.s. = direct electromyogram of muscle triceps surae.

When sural nerve stimulation evoked selective γ activations, the increases of group I and II discharges resulted from the excitation of sensory endings of muscle spindles. When stimulation elicited both α and γ reflexes, some clues as to both the origins of the discharge of muscle afferents and the excitation mechanisms of receptors may be gained from a comparison of the onsets of the activations of α and γ axons and of group I and II muscle afferents. For instance in Fig. 9, ipsilateral sural nerve stimulation elicited an increase of group I fibre discharge with 140 msec latency and rising with a slope of 195 impulses . sec^{-1} . sec^{-1} for 175 msec. This was accompanied by a γ-reflex firing increase that began before the group I fibre response, but there was no discharge of α motoneurones of gastrocnemius lateralis muscle. About 320 msec after the start of the stimulation, the slope of group I fibre discharge was abruptly accentuated (410 impulse . sec^{-1} . sec^{-1}) and the firing rate of the group II afferent fibres began to rise. A few milliseconds before these afferent changes a reflex

excitation of α motoneurones of gastrocnemius lateralis muscle had begun, almost simultaneously with strong electromyographic activity in triceps muscle. From this time mechanical changes in triceps muscle, resulting from the reflex contraction, were at least partly responsible for the group I and II afferent discharges. It might be supposed that the change of the slope of group I discharge arose exclusively from the activation of Golgi tendon organs due to development of tension; however, the simultaneous activation of group II fibres argues against that interpretation as it shows that muscle-spindle activity increased. The activation of sensory endings of muscle spindles could be explained by possible β innervation of intrafusal muscle fibres and/or, since the contraction was isometric, by deformations of muscle spindles brought about by internal length changes of different muscle parts as well as by changes of pressure on muscle spindle capsules when motor units were asynchronously contracting in triceps (Binder, Kroin, Moore, Stauffer & Stuart, 1976).

In Fig. 7 the responses of group I and II fibres followed (by 110 and 140 msec respectively) the increase of activity of gastrocnemius lateralis γ motoneurones and of the triceps e.m.g. In this case, even though the α axons of the gastrocnemius lateralis small nerve were activated only after the onset of the afferent responses, it was not possible to attribute the afferent responses to the action of γ motoneurones exclusively, because of the early electromyographic activity. In addition, the respective contributions of muscle spindles and tendon organs to the response of group I fibres could not be estimated.

Fig. 8 clearly shows that group I and II afferents may be activated without simultaneous increase of the discharge of γ motoneurones. The onset of the group I fibre activation could not be ascribed to excitations of Golgi tendon organs because, at this time, there was a decrease in active tension of gastrocnemius lateralis muscle due to the reflex inhibition of the α motoneurones. On the contrary, the onset of the group I fibre activation most likely resulted from excitation of spindle primary endings as the muscle relaxation was probably accompanied by local length changes. However, it should be noted that the firing increase of group I fibres coincided with the reflex increase of the triceps e.m.g. activity. So, deformations induced by asynchronous contractions of motor units of soleus and gastrocnemius muscles could be transmitted to the part of gastrocnemius lateralis muscle innervated by the small nerve, thereby stimulating the spindle primary endings. Later, when the α discharge was stronger while the γ discharge was present but weak, tendon organ activation probably accounted for a significant part of the group I discharge.

DISCUSSION

Knowledge of the reflex responses of γ motoneurones has been acquired by the use of various methods (see review by Murthy, 1978), the disadvantages of which have been argued in the Introduction to this paper. In this study, a method of sorting of potentials which participate in the over-all discharge of a small muscle-nerve has been used. The method enables us to observe separately the potential traffic in four groups of fibres found in a thin branch of the gastrocnemius lateralis nerve that has often been used for physiological studies of fusimotor neurones (Hunt, 1951; Voorhoeve & Van Kanten, 1962). However some restrictions have to be mentioned. Functional

identification of nerve fibres participating in the recorded discharge is necessarily limited since the experimental protocol does not allow the use of any functional test. Thus, it is impossible to distinguish: (i) among group I potentials, those of muscle-spindle primary afferents from those of Golgi's tendon organs; (ii) among efferent potentials, those conducted by β axons innervating both skeletal muscle fibres and intrafusal muscle fibres; (iii) among γ potentials those conducted by static γ axons and those by dynamic γ axons.

Reflex responses of gastrocnemius γ motoneurones to stimulation of contralateral or ipsilateral sural nerves have been characterized by increase of the overall firing rate. However, in one experiment few stimulations of ipsilateral sural nerve elicited a weak decrease before the usual increase of γ firing. The predominantly excitatory effects of stimulation of sural nerve are in keeping with the observations of Kobayashi, Oshima & Tasaki (1952) in anaesthetized cats and Hunt & Paintal (1958) in spinal cats. The chronological order of activation of α and γ axon populations in the small nerve was mostly consistent with the size principle (Henneman *et al.* 1965*a*, *b*). Indeed, (i) when decerebrate cats spontaneously went from a flaccid to a rigid state, γ axons were recruited before α axons (Fig. 3); (ii) by lightly stimulating sural nerve, fusimotor activity often increased alone without any α-motoneuronal activation (Figs. 4*A* and 6*B* and *D*); (iii) with stronger sural nerve stimulation, activation of γ axons preceded that of α axons (Figs. 7 and 9); (iv) when the γ population could be separated into two subgroups, activation started in the slower subgroup earlier than the faster one (Fig. 5).

During a flexor reflex elicited by stimulating the ipsilateral sural nerve the reciprocal inhibition period of gastrocnemius α motoneurones was not usually accompanied by any decrease of firing of the γ population in the small nerve. That does not necessarily signify a complete lack of inhibitory influences of ipsilateral sural afferents on the γ motoneurone pool of gastrocnemius. Since the method used in these experiments provides a measure of the over-all activity of a γ population, it is possible that an increase in the activity of some units is simultaneously accompanied by a decrease in the activity of other units. According to the relative strengths of the two antagonistic effects, either no change (Fig. 8) or exceptionally a slight decrease (not illustrated) or an increase (Fig. 9) of the over-all firing frequency of the γ population would be seen.

Henneman *et al.* (1965*b*) have shown that excitability and inhibitability of α motorneurones are strictly size dependent. The excitability of α motorneurones is an inverse function of the cell size and their ability to be inhibited is a direct function. This rule, established by comparing the order in which numerous pairs of α units in a ventral root filament were recruited or silenced during various types of reflex excitation and inhibition, seems valid when comparing two populations of motoneurones (α and γ) whose range of axon conduction velocity and consequently of cell size is wide. Indeed, when stimulating the ipsilateral sural nerve, the α population was inhibited while the γ population was more often activated.

Recording from a sufficiently fine intact nerve provides the advantage pointed out by Steg (1964) to observe simultaneously activities in efferents to, and afferents from, muscle, and thus to examine some aspects of the mechanisms underlying reflex movement. Merton (1951, 1953) suggested that α motorneurones could be activated via the excitation of muscle spindles by γ motorneurones, and Granit (1955) used the

expression 'γ-route' for such a motor-control pathway. For a muscle contraction to be initiated by the γ-route the activations in the different parts of the command loop must appear in the following chronological order: γ motoneurones–muscle spindle primary afferents–α motoneurones–skeletal muscle fibres. When recording from the small nerves, the first appearance of changes of activity in the different axonal populations induced by stimulations of sural nerve were very often in agreement with this chronological order. This finding was in favour of the activation of gastrocnemius α motoneurones by the reflex γ-route. A similar observation has yet to be made for the activation of soleus muscle. Indeed, some uncertainty exists concerning the initiation of soleus muscle contraction, since, when comparing the onsets of afferent and efferent responses in the small nerve with that of the electromyographic increase of triceps muscle, the chronological order is: gastrocnemius γ motoneurones–triceps electromyogram–group I and II muscle afferents–gastrocnemius α motoneurones. The difference in the latencies of the onset of activity of gastrocnemius and of triceps muscles is probably explained both by differences of physiological properties of motor units in pale (gastrocnemius) and in red (soleus) muscles forming triceps (Wuerker *et al.* 1965; McPhedran *et al.* 1965) and by the orderly recruitment of α motoneurones according to the cell size (Henneman *et al.* 1965*a*). In that case, if group I and II muscle afferents as well as γ axons of the small nerve are considered as a representative sample of the soleus nerve it would be concluded: first, that the activation of soleus α motoneurones cannot be via the γ-route since it precedes the muscle spindle activation; secondly, that α and γ motoneurones are reflexly co-activated. However, since the firing increase in the γ motoneurone population of the small nerve starts before the triceps e.m.g. response, the response may be called a γ pre-activation, thus describing a temporal difference in the activation of the α and γ motoneurone populations without implying activation of α motoneurones via the γ-loop. Such a γ pre-activation could prepare muscle spindle sensitivity for mechanical changes induced by the subsequent α motoneurone activation. During this later period of co-activation, continued increase of γ activity could counteract the depressive action on muscle-spindle afferents of the muscle shortening elicited by reflex responses of α motoneurones, following the original idea of Kuffler & Hunt (1952).

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