

## RESPONSE OF SOLEUS Ia AFFERENTS TO VIBRATION IN THE PRESENCE OF THE TONIC VIBRATION REFLEX IN THE DECEREBRATE CAT

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### SUMMARY

1. Micro-electrode recordings were made from single Ia afferents in the intact nerve to the soleus muscle in the decerebrate cat while the muscle was developing a tonic vibration reflex. This was done in order to test how effectively the afferents were excited by the vibration, and to see if any insecurity in driving might be related to tremor.

2. When the amplitude of vibration was 50  $\mu\text{m}$ , and the tonic vibration reflex was reasonably well developed ( $> 1$  N of active tension) all but one of forty-four Ia afferents were driven 1:1 by the vibration. Most were still driven by 30  $\mu\text{m}$  vibration. The vibration, consisting of a train of discrete pulses at 150 Hz, was applied longitudinally in combination with a stretch of 1 mm to make the muscle taut.

3. If the reflex was poorly developed (active tension  $< 1$  N) the driving was on average less secure. However, fourteen of eighteen afferents then studied were still driven 1:1 by 50  $\mu\text{m}$  vibration. The lower level of excitation by vibration was thought to be due to a deficiency of spontaneous fusimotor activity, because stroking the cat's tail or other similar gentle manipulation led each of the three misbehaving afferents so tested to be driven securely by 50  $\mu\text{m}$  vibration; at the same time the reflex tension increased.

4. Additional, indirect evidence favouring widespread security of Ia driving by 50  $\mu\text{m}$  vibration in the presence of the reflex was obtained by modulating the amplitude of the 150 Hz vibration with a 7–10 Hz square wave and detecting any tension fluctuations at that frequency by spectral analysis. Small degrees of modulation (e.g.  $< 10\%$ ) produced little if any effect, although larger depths of modulation had a powerful action.

5. When the amplitude of vibration was reduced to permit insecure driving but still to elicit a reflex response, the fluctuations in Ia firing pattern were unlike those previously seen in the de-efferented muscle. Spectral analysis showed that these firing fluctuations bore a general similarity to the tremor in the same preparation, but measurement of coherence demonstrated that the tremor and Ia firing were not well related. This was probably because individual Ia afferents were primarily influenced

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by local factors, and provides further evidence against the tremor of this preparation being attributable to the action of the stretch reflex.

#### INTRODUCTION

In a de-efferented passive muscle the spindle primary endings are highly sensitive to vibration. For example, Brown, Engberg & Matthews (1967) found that when the soleus muscle of the cat was well stretched virtually all its primary endings were driven in 1:1 synchrony with sinusoidal vibration of 200–400 Hz at a peak-to-peak amplitude of 50  $\mu\text{m}$ ; usually 25  $\mu\text{m}$  sufficed. When the vibration is delivered as a series of discrete, rapidly rising pulses the requisite amplitude is yet lower (McGrath & Matthews, 1973). Moreover, vibration has much less effect on the other two main mechanoreceptors in muscle, namely the spindle secondary ending and the Golgi tendon organ. In consequence, vibration has come into widespread use as a simple way of exciting spindle primary endings so as to study their central effects in both man and the experimental animal. However, the behaviour of the primary ending is still not certain in the physiologically important situation where the reflex loop is intact and the muscle is contracting, and this situation is also much less well documented. Fusimotor action increases the sensitivity of the primary ending to vibration but extrafusal contraction decreases it, even when the spindle is being kept taut by fusimotor stimulation (Brown *et al.* 1967). In addition, even with appreciable amplitudes of vibration 'misbehaviour' of primary endings, i.e. failure to follow vibration 1:1, has been reported in both man and cat (Burke, Hagbarth, Löfstedt & Wallin, 1976; Jack & Roberts, 1978).

Knowledge of whether or not significant misbehaviour occurs in any particular situation is of importance for the quantitative interpretation of experiments with vibration. For the decerebrate cat, it is of topical interest in relation to a possible contribution of the spindle secondary ending to the stretch reflex (cf. Matthews, 1969; Jack & Roberts, 1978) and to the origin of the tremor seen in the tonic vibration reflex (Cussons, Matthews & Muir, 1979; Clark, Matthews & Muir, 1981). A study has therefore been made of the response to vibration of a number of Ia fibres from the soleus muscle of the decerebrate cat while the reflex pathways have been left in continuity so that the vibratorily induced Ia firing could still elicit a tonic vibration reflex. This was done by recording single unit responses with a capillary micro-electrode inserted into the nerve to soleus 2–3 cm above the muscle. Each afferent was tested for its response to 50  $\mu\text{m}$  pulsatile vibration at 150 Hz, as used previously for experiments on tremor (Cussons *et al.* 1979). When the central state of the preparation was such that there was a significant tonic vibration reflex then virtually all the primary afferents were found to be securely driven in synchrony with the vibration. However, misbehaviour was observed for a few afferents when fortuitous changes in the central state of a preparation led to a virtual disappearance of the tonic vibration reflex. This failure to follow was attributed to a decrease in spontaneous fusimotor activity. Some afferents were also studied with low amplitudes of vibration to examine the pattern of misbehaviour which then occurred. In addition to direct recording, the extent of driving in the entire population of Ia afferents was roughly assessed by using spectral analysis of the tension recording to detect the effects of low-frequency modulation of the amplitude of vibration.

## METHODS

The unitary recordings to be described were obtained from six cats; another seven cats were either employed in preliminary experiments or failed to yield useful results, usually because of vagaries of the decerebrate preparation rather than because of any failure of the recording technique *per se*. Analysis of the reflex response to modulation of the amplitude of vibration was performed on seven cats, four of which were subsequently used successfully for micro-electrode recording. An earlier paper (Cussons *et al.* 1979) describes the general features of the preparation and apparatus. In brief, under halothane anaesthesia the right hind leg was denervated except for the soleus muscle and the cat was then decerebrated by intercollicular section whereupon anaesthesia was discontinued. The soleus tendon was attached to an isometric myograph mounted upon a vibrator pulling along the longitudinal axis of the muscle. This could be used both to vibrate and to stretch the muscle. The maximum length at which the muscle was studied was always several mm short of maximum physiological extension, as judged by marker threads attached to the tendon and to the adjacent tissues. As before, the vibration consisted of a train of discrete pulses each lasting about 3.5 msec, rather than a sinusoidal waveform. The various recordings were observed at the time on oscilloscopes and stored on a 7-channel tape recorder for subsequent analyses.

*Micro-electrode recording and identification of Ia fibres.* Because of the limitations arising from the use of micro-electrode recording the present methods of isolating and identifying the Ia afferents differed from those normally employed when the afferents are isolated from dorsal root filaments (cf. Brown *et al.* 1967). The present methods are thus given in some detail to show that they should have provided both reliable sampling of the Ia population and accurate identification of the units studied. The electrical recording technique broadly followed that used before for cutaneous and joint afferents (Burgess & Perl, 1967; Burgess & Clark, 1969). The nerve to soleus was laid upon a rigidly held plate 2–3 cm above its point of entry to the muscle and a small slit was made in the upper surface of its sheath with a fragment of razor blade. Warm agar solution was then poured over the nerve for support. The remaining part of the muscle was covered with liquid paraffin or Ringer solution. Due to the smallness of the residual pool its temperature was not well controlled and on occasion fell below the desired 35–37°C. Conventional glass micro-capillary electrodes of about 10–15 M $\Omega$  resistance were inserted into the nerve using a hand-driven hydraulically operated microdrive.

Unitary responses were sought with the muscle set to within a few mm of its maximum *in situ* length and with an additional excitatory stimulus of either 10  $\mu$ m pulses of vibration at 150 Hz, or larger pulses (50  $\mu$ m) at 8–10 Hz; it did not appear, however, that the larger pulse permitted the detection of any more units. For a Ia fibre to have remained undisclosed it would have had to remain silent in spite of the combination of moderate steady stretch and background fusimotor activity, and to lack any excitatory response to vibration; the 10  $\mu$ m pulses did not elicit an appreciable reflex response and so would not have been expected to have led to unloading of the spindle. Ib afferents from tendon organs and spindle group II afferents would be expected to be sampled much less efficiently; the former because of their failure to fire under the circumstances studied, and the latter through being vibration insensitive and because of their smaller size. Few Ib or II afferents were encountered although several identified fusimotor fibres were seen indicating that the electrodes were functioning well.

When a suspected Ia unitary discharge was obtained it was usually confirmed to be an afferent fibre by showing that it could be excited by local probing of the muscle with a glass rod. In addition, for most single units (54/62 Ia fibres) the direction of spike transmission was confirmed by using spike triggered averaging to observe the later arrival of the spike in the sciatic nerve, and the spike conduction velocity was estimated. The averager (NL 750, Digitimer Ltd) was triggered by the spike from the micro-electrode in the soleus nerve. The recording electrodes for the averaging consisted of a pair of silver wires placed under the intact sciatic nerve at the level of its junction with the severed hamstring nerve. All the presumed Ia afferents had latencies corresponding to a velocity of above 60 m/sec. The method, however, was not felt to be reliable enough, as we employed it, to provide a secure basis for separating all group I and II afferents. This was because of the relative shortness of the conduction distance (6–8 cm), the suspicion that the temperature of the nerve within the agar might sometimes have been unduly low, and the uncertainty and variability of the precise time of triggering of the averager from the spike recorded from the micro-electrode; this last difficulty was sometimes further compounded by the presence of a vibration artifact on the micro-electrode record.

No attempt was made to demonstrate that the presumed Ia afferents showed a silent period during an evoked muscle twitch, because of the fear of dislodging the micro-electrode. However, many of the units were shown to be spindle afferents rather than tendon organ afferents by observing an increase in their discharge on gentle manipulation of the pinna or other part of the cat. This occurred without the development of appreciable tension by the soleus, and may be attributed to the reflex activation of fusimotor fibres. *To summarize*, the criteria used for Ia identification were local excitation by muscle probing, estimation of conduction velocity, some degree of excitation by vibration (though not, of course, necessarily to the extent of 1:1 driving), and fusimotor induced excitation. In the majority of cases all four tests were successfully applied. Thus we have considerable confidence that those units we classified as Ia afferents from spindle primary endings were indeed such, and we are quite reasonably confident that we were not neglecting any sub-group of such fibres either by failing to recognize them for what they were or by virtue of our sampling procedure failing to detect them in the first place.

Once obtained, Ia afferents could be 'held' reasonably stably in the face of vibration of up to 50  $\mu\text{m}$  extent and of the resulting tonic vibration reflex. However, the electrode stability tended to be much less with stretch of the muscle and its concomitant displacement of the nerve. But as before (Cussons *et al.* 1979) we regarded it as important to stretch the muscle after commencing the vibration so as to make the spindles taut and counteract any unloading effect of the tonic vibration reflex on the afferents which might lead them to 'misbehave' in their response to the vibration. However, the stretch was restricted to 1 mm and was usually applied rather slowly (1 mm/sec). A favourable unit could be held for half an hour or more in the face of repeated stretches and periods of vibration. The loss of a unit did not, however, appear to mean that it had been damaged since it could often be obtained once more by slight adjustment of the electrode; this was also the case when the electrode was driven past a unit and then withdrawn again. Thus it is believed that the majority of the nerve fibres remained in functional continuity in spite of continued probing, and it may be noted that appreciable tonic vibration reflexes could still be obtained after over 6 hr of recording. However, since the afferents were believed to remain in good condition after we had finished studying them it becomes impossible to guarantee that we never studied the same afferent a second time once the positional reference within the nerve was lost on changing the micro-electrode. But on statistical grounds it seems unlikely that this would have happened often since in any one experiment we never studied more than fourteen of the fifty or so soleus Ia axons.

*Computing methods.* Spectral analysis of 10 sec segments of tension recording was performed with a PDP 12 computer in the same way as before (Cussons *et al.* 1979); the analysis was sometimes performed 'on-line' during the experiment and sometimes 'off-line' from high gain analogue tape recordings. A moving average of the firing rate of the Ia afferents that were responding insecurely to low-amplitude vibration was sometimes generated by filtering out the higher frequencies in the spike train, as illustrated in Fig. 4. This was done by converting each spike into a standard pulse and then low-pass filtering the resulting train of constant sized pulses (cut-off 23 Hz, as for spectral analysis). This 'demodulation' preserved fluctuations in firing at tremor frequencies, but eliminated the very rapid changes seen when an ending was alternating between 1:1 and 1:2 firing in relation to the vibration pulses (cf; Cussons *et al.* 1979; Matthews & Muir, 1980). For spectral analysis of these 'tremor band' fluctuations in firing the demodulated Ia record was analysed by the computer in the same way as the tension record, but without further filtering.

*Modulation of vibration.* In some experiments the amplitude of the vibratory pulses was square-wave modulated at a frequency of 6–10 Hz by using a relay driven by an oscillator to introduce an attenuating resistor into the pulse-amplitude control circuit. When the relay happened to switch in the middle of a pulse a single slightly abnormal pulse might occur but this is not thought to have contributed any significant features to the experimental records. A further potential source of artifact arose from the fact that during vibration the average length of the muscle was very slightly greater than in its absence, by an amount which increased with pulse amplitude, since the pulses were unidirectional. This created a small tension signal at the modulating frequency which, for a passive muscle, could just be detected using spectral analysis when the result was displayed at a much higher than normal gain. But since the size of the artifact will have varied with the stiffness of the muscle its precise size during a reflex contraction was not known. However, it is considered likely to have been too small to have contributed appreciably to clear peaks at the modulation frequency, such as those illustrated in Figs. 2 and 3. This was supported by the finding that during a series of relatively consistent reflex contractions the precise size of the modulation peak varied from trial to trial, each based on 10 sec recording, whereas the artefactual peak for the passive muscle should be constant.

## RESULTS

*Driving with 50  $\mu$ m vibration*

For comparison with previous experiments on tremor (Cussons *et al.* 1979) every Ia afferent isolated was tested for its response to vibration at 150 Hz and 50  $\mu$ m amplitude, with the muscle being stretched by 1 mm shortly after the beginning of the vibration in order to keep the spindles taut. As before, the vibration consisted of a train of pulses each of approximately 3.5 msec total duration rather than a sinusoidal movement; pulsed vibration and sinusoidal vibration produce indistinguishable tonic vibration reflexes, though the amplitude of movement required to produce a maximal reflex is appreciably lower for the pulsed vibration (Cussons *et al.* 1979; McGrath & Matthews, 1973). Such vibration proved to be remarkably

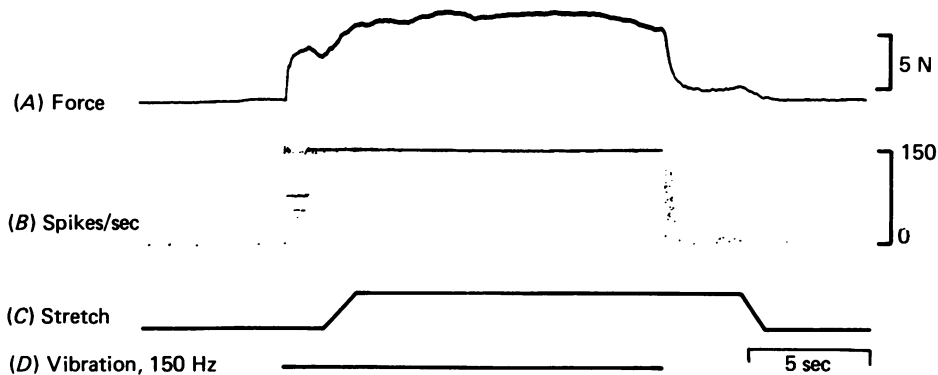


Fig. 1. Secure 1:1 driving shown during the plateau of a tonic vibration reflex by a presumed Ia afferent fibre. The vibration consisted of a stream of discrete pulses of 50  $\mu$ m amplitude delivered at 150 Hz. *A*, isometrically recorded tension developed by the soleus. *B*, instantaneous frequency of firing of the Ia afferent, with each dot representing a spike and its height above zero giving the reciprocal of the interval since the immediately preceding spike; the banded appearance at the beginning arises from the afferent initially responding to every second, third or fourth pulse, rather than showing secure 1:1 driving. *C*, stretch applied to make the muscle taut (1 mm at 1 mm/sec). *D*, period of vibration (*C* and *D* diagrammatic).

efficacious in eliciting 1:1 driving from virtually all the endings studied, irrespective of the occurrence of the tonic vibration reflex. Fig. 1 shows a typical example. At its onset the vibration did not immediately produce maintained 1:1 driving, but after some variable insecurity soon came to do so. This was then maintained during the rising phase of the stretch and subsequent changes in muscle tension and the afferent continued to discharge at the frequency of the vibration until the vibration was turned off some 10 sec later. Inspection of an oscilloscopic trace triggered by the vibration pulses confirmed that the discharge was temporally locked one-to-one with the vibration. The tonic vibration reflex was developing an active tension of over 5 N. Not all afferents settled to secure driving at the initial length before the application of stretch, but these then usually did so within 1–2 sec after completion of the stretch and resulting changes in reflex tension. Presumably the intrafusal contraction elicited by spontaneous fusimotor activity takes a little time to compensate for the unloading of the spindle produced by the extrafusal contraction, and possibly the spindle is also adjusting itself to reflexly elicited changes in fusimotor activity.

Table 1 brings together the findings with 50  $\mu\text{m}$  amplitude of vibration on the sixty-two presumed primary afferents studied. It was compiled on the basis of the following criteria. The driving was considered as 'secure' if it remained so throughout the period of stretch after neglecting the first 1–3 sec while the tension was equilibrating; it was commonly secure throughout this early period also. In three cases where the unit was lost in the course of the first period of stretch its behaviour was counted as secure on the basis of a minimum of 3 sec of uninterrupted 1:1 driving (i.e. > 450 spikes); a few other units were lost yet earlier and have not been included in the series. No other presumed Ia afferents have been rejected, though seven presumed secondary afferents have not been included (see later). Security was judged at the time of the experiment both by listening to the afferent discharge relayed by

TABLE 1. Numbers of Ia afferents observed with secure 1:1 driving in response to pulsed vibration at 150 Hz of 50  $\mu\text{m}$  amplitude combined with 1 mm stretch applied after the beginning of the vibration. The spindle endings have been subdivided according to the size of the tonic vibration reflex recorded at the same time. Three of the four 'misbehaving' endings in experiment 6 showed secure driving during gentle reflex fusimotor excitation combined with vibration at which time the active tension of the muscle also rose to above 1 N; the fourth afferent of this experiment was not so tested, nor was the misbehaving afferent of experiment 4.

Expt.	No. of Ia afferents	TVR > 1 N		TVR < 1 N	
		Secure	Insecure	Secure	Insecure
1	13	13	—	—	—
2	4	4	—	—	—
3	10	4	—	6	—
4	12	11	1	—	—
5	9	6	—	3	—
6	14	5	—	5	4
Totals	62	43	1	14	4

a loudspeaker and by observing an oscilloscopic display of the spikes with the sweep triggered by the vibration pulses; during secure driving spikes occurred at fixed places on the trace. In any case of doubt the matter was checked retrospectively from tape recordings of the spike train and the vibration pulses. The discharge was accepted as 'secure' if, after the reflex tension had equilibrated, not more than three or four spikes were dropped from the 8–10 sec period of vibration (> 1200 spikes); a few records with violent swings of tension associated with walking type movements were discarded. The afferents have been subdivided according to the size of the tonic vibration reflex at the time of the observations. This was done chiefly because it was felt that reflexes developing less than 1 N were unduly small to be relevant to the earlier experiments (Cussons *et al.* 1979), though they continued to show appreciable tremor. Recordings made when the reflex was below 0.1–0.2 N have not been included. In experiment 4 the muscle was regularly developing an active tension of 5–8 N, and in experiment 1 of 2–3 N; otherwise when above 1 N the tension was in the range 1–2 N, at least for the first part of the reflex (cf. Fig. 1, Cussons *et al.* 1979). In assessing the absolute size of these reflexes in relation to other published series it should be noted that the muscle was usually studied several mm below its maximal physiological length and that the tautening stretch was only 1 mm; we had the impression that the reflex would have been appreciably larger if we had applied a

larger tautening stretch terminating near physiological full extension, but this would have caused problems with the micro-electrode recording.

It may be seen in Table 1 that of the forty-four afferents that were studied in the presence of a vibration reflex of above 1 N, forty-three (98 %) were driven securely by 50  $\mu\text{m}$  vibration. Moreover, for the majority of them there was an appreciable margin of safety for driving since on testing the response to 30  $\mu\text{m}$  vibration thirty-two of the thirty-nine afferents for which this was done (80 %) remained securely driven; the size of the reflex was not usually appreciably altered with such reduction of vibration amplitude. Three of the forty-three noted as secure with 50  $\mu\text{m}$  vibration had, however, a rather fine margin of security and though completely secure on most trials, showed brief periods of insecurity on some others. Even if these be subtracted the proportion of behaving endings remains at 91 % of those observed during an appreciable tonic vibration reflex. However, of the eighteen afferents observed during a weak vibration reflex only fourteen or 78 % were securely driven by 50  $\mu\text{m}$  vibration in the first instance. Nor was the margin of security statistically as great as when the reflex was large; of the twelve tested with 30  $\mu\text{m}$  vibration, six (50 %) then became insecure in comparison with a figure of 20 % for larger reflexes.

We suspected that the poorer security of driving with the smaller reflexes might be related to a paucity of fusimotor activity leaving the spindles free to be unloaded relatively easily by the concomitant extrafusal contraction, even when it was not a particularly strong one. Accordingly in experiment 6 of Table 1 the reflex centres were gently facilitated by stroking the cat's ears, the contralateral hind limb, or tail in an entirely non-noxious manner; such excitation was started before and continued throughout the standard period of vibration. In all three of the cases of initial misbehaviour of experiment 6 that were tested in this way the vibration reflex increased appreciably (to 1.5–3 N) and the afferent became securely driven by the 50  $\mu\text{m}$  vibration; the fourth unit was not so tested. Without such reflex facilitation these afferents showed occasional periods of insecure driving (cf. Fig. 4). Such procedures normally increase the Ia firing rate in the absence of vibration and can be presumed to excite reflexly the soleus fusimotor neurones, probably along with other spinal facilitatory actions. The effect of such reflex facilitation was also tested on the five afferents of experiment 6 which were securely driven in the presence of a poor vibration reflex. The reflex contraction then increased to 2–3 N and the afferents remained securely driven. The same result was obtained in experiment 5 for the two units so tested of the three that were observed initially in the presence of a poor reflex. When such facilitation was tested on the response of afferents that were initially secure in the presence of a reasonable reflex, the reflex usually increased and the units continued to respond securely to the vibration.

The one afferent in the whole series which misbehaved in the presence of an appreciable reflex (in experiment 4) was not tested with reflex facilitation. It differed from the other misbehaving afferents in falling completely silent during the period of the tonic vibration reflex after showing an initial period of 1:1 driving at the very beginning of the reflex; in this it seems to have resembled that illustrated by Jack & Roberts (their Fig. 2, 1978). It was, however, moderately powerfully excited by vibration of 10 or 15  $\mu\text{m}$  amplitude throughout the whole period of vibration, though not to the extent of 1:1 driving. All this suggests that its fusimotor supply was insufficiently activated to overcome the unloading effect of a powerful reflex (7.5 N).

The reverse effect of 'too much' excitation by the vibration and the appearance of two spikes

per pulse was only seen for one ending and only during the falling phase of swings of contractile tension of 5 N and above, occurring during stroking of the cat's tail to increase fusimotor excitation. This absence of such 'double driving' for other units in the present experiments contrasts with the experience of McGrath & Matthews (1973) and is presumably attributable to the small tautening stretch used here and to the presence of a reflex contraction. On using pulsed vibration McGrath & Matthews found double driving to be restricted to the rising phase of a large tautening stretch; they used decerebrate preparations in which the tonic vibration reflex had been inactivated by dorsal root section.

In summary, when the tonic vibration reflex is spontaneously well developed in the present preparation a trivial number of Ia afferents fail to respond securely to vibration of 50  $\mu\text{m}$  amplitude, though a few may be just on the verge of showing insecurity. However, when the reflex is poorly developed but present a small proportion show misbehaviour, but this can be eliminated by reflexly elicited augmentation of fusimotor activity; at the same time the tonic vibration reflex increases to a reasonable size.

*Secondary endings.* Seven presumed spindle secondary afferents were observed briefly, but sufficiently to establish that they were not primary afferents that were misbehaving in response to the vibration. Five were unequivocally shown to be secondary afferents by virtue of their conduction velocity which was appreciably less than that of all the primary afferents studied in the same experiment and beyond the level of uncertainty (see Methods). The velocity of the other two just overlapped with the range of velocity of the primary endings observed in the same experiments and therefore was not a reliable indicator. None of these seven was excited to fire appreciably faster by vibration of 50  $\mu\text{m}$  amplitude. In fact, five of them slowed their firing, one to complete silence, but without any of the initial excitation which was characteristic of the misbehaving primary afferent of experiment 4. No attempt was made to decide whether the slowing was attributable to a slight unloading of the spindle in question by the mechanical effects of the tonic vibration reflex or to an inhibitory reflex action onto soleus fusimotor neurones from the vibratorily induced afferent discharge. Four of these afferents were tested to see if their spikes were temporally locked onto the vibration pulses; two showed phase-locking and two did not. Phase-locking without appreciable change of frequency has been observed previously for secondary endings recorded from dorsal root filaments with more accurate measurement of conduction velocity (McGrath & Matthews, 1973; Dale, P. & Matthews, P. B. C., unpublished). Thus, as in previous studies, the secondary endings were little influenced by vibration.

*The effect on the tremor spectrum of modulating the amplitude of vibration.* When a primary afferent is being securely driven by vibration a small change in the amplitude of vibration produces no effect on the frequency of firing of the afferent, since it remains locked at the vibration frequency. However, if the amplitude is reduced so that an ending starts to 'misbehave', or is on the verge of doing so, then any further reduction in the amplitude of vibration will reduce the mean frequency of firing of the afferent and hence also the reflex action of its discharge; and if the vibration amplitude is modulated cyclically there should, therefore, be a cyclic reflex response at the same frequency. Thus examination of the tonic vibration reflex during low-frequency modulation of the amplitude of vibration provides a test for the security of driving for the whole population of Ia afferents, though not one which is readily quantifiable in terms of the extent of Ia misbehaviour. Fig. 2 shows the effect on the tension spectrum, recorded isometrically, of progressively increasing the depth of modulation, at 7 Hz, of the vibration with the maximum amplitude kept at 50  $\mu\text{m}$ . Fig. 2A shows a typical tremor spectrum for a constant level of vibration (cf. Cussons *et al.* 1979) and with a reflex of about 2 N. Fig. 2B shows the spectrum obtained on alternating the amplitude of vibration between 50  $\mu\text{m}$  and 45  $\mu\text{m}$  (i.e.



square-wave modulation). The differences between *A* and *B* fall entirely within the range of normal variability; there is, however, the suspicion of a small hump at the modulation frequency in *B* which, apart from random 'noise', might possibly be attributable to a very small mechanical artefact (see Methods) or to a miniscule reflex action. On increasing the modulation to 10  $\mu\text{m}$  (Fig. 2*C*) a small but definite hump appeared in the spectrum which became progressively larger with further increase in depth of modulation (*D*, *E*, *F*). With the greater depth of modulation there were also recognisable swings of tension in the myographic output which were about 0.1 N peak-to-peak for *F*, or some 5% of the mean tension; the smallness of the fluctuations in relation to the mean tension seems attributable at least in part to the low fusion

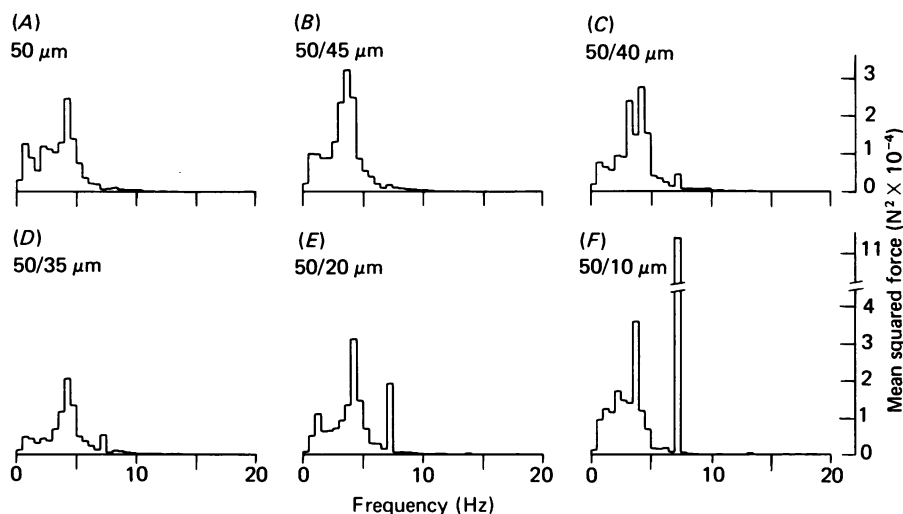


Fig. 2. Effect on the tremor spectrum of modulating the amplitude of vibration to a progressively greater extent. *A*, control spectrum during the tonic vibration reflex elicited by a constant 50  $\mu\text{m}$  150 Hz vibration and developing about 2.5 N tension. *B*, comparable spectrum obtained during vibration at 150 Hz with the pulse amplitude alternating between 50 and 45  $\mu\text{m}$  (i.e. square wave modulation) at a frequency of 7 Hz. *C*, *D*, *E*, *F* similar spectra observed with vibration of a maximum amplitude of 50  $\mu\text{m}$  but modulated at 7 Hz down to amplitudes of 40, 35, 20 and 10  $\mu\text{m}$  respectively. The reflex developed a tension of 1.5–2.0 N throughout. Same expt. as Fig. 1.

frequency of soleus, but any Ia excitation of soleus motoneurons via polysynaptic pathways could contribute to the smoothing of the reflex response. Related experiments demonstrated that changes like these in the distribution of tremor frequencies on modulating the vibration are accompanied by changes in motor unit firing (Clark *et al.* 1981). These results are in accordance with the direct evidence that in the presence of an appreciable reflex nearly all the Ia afferents are securely driven by 50  $\mu\text{m}$  vibration, but that misbehaviour progressively increases as vibration amplitude falls below 40  $\mu\text{m}$ .

Fig. 3, derived from another preparation, extends the argument by contrasting the effects of a 5  $\mu\text{m}$  modulation of vibration amplitude when the maximum amplitude was 50  $\mu\text{m}$  (*C*) and when it was 25  $\mu\text{m}$  (*D*); in both cases there was an appreciable tonic vibration reflex. On the basis of the single fibre recordings the large amplitude

vibration would be expected to produce secure driving, irrespective of the modulation, and in accordance with this there is no significant hump on the tremor spectrum. But the small amplitude vibration should produce misbehaviour of an appreciable number of afferents the extent of which should vary with the modulation and, accordingly, produce a well developed spectral peak at the modulation frequency as is indeed seen in Fig. 3.

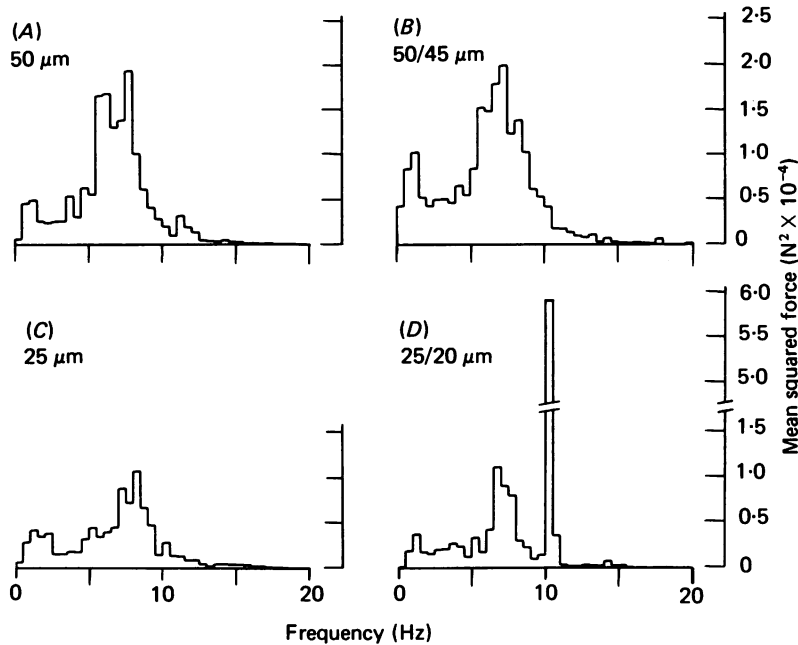


Fig. 3. Comparison of the effect of 10 Hz modulation of the amplitude of vibration by 5  $\mu\text{m}$  when the maximum amplitude was 50  $\mu\text{m}$  (B) with that found when the maximum amplitude was 25  $\mu\text{m}$  (D). A, C, control spectra in the absence of modulation for the reflexes elicited respectively by steady 50 and 25  $\mu\text{m}$  amplitude vibration at 150 Hz. Both in the presence and absence of modulation the smaller amplitude of vibration elicited a smaller reflex (about 3.5 N as compared with about 4.5 N). Different expt. from that of Fig. 2.

It may be noted, in confirmation of previous work (Cussons *et al.* 1979), that for steady unmodulated vibration the tremor during 25  $\mu\text{m}$  vibration shows no augmentation over the level for 50  $\mu\text{m}$  vibration (in this case it is markedly less), though the modulation experiment suggests that there was far more potential for variable misbehaviour with the smaller amplitude of vibration. This supports the previous view that the normal tremor cannot be attributed to phasic Ia reflex action, for this would be expected to be much the greater when the spindle endings were misbehaving and thus could be readily influenced by muscle shortening than when they were being securely driven 1:1 by the vibration (see later).

Altogether, seven experiments were performed in which the vibration amplitude was alternated between 50 and 45  $\mu\text{m}$  at a frequency of 7–10 Hz (chosen to be just above the main peak of spontaneous tremor so as to make the artificial peak more readily distinguishable). All developed a reflex contraction of 1 N or more in response to steady 50  $\mu\text{m}$  vibration. In four of the experiments any possible modulation peak

was at the level of the background noise; as in the experiments of Figs. 2 and 3 the relevant bin was sometimes just perceptibly higher for the spectrum obtained during modulation than in the control spectrum obtained with unmodulated vibration. In the remaining three experiments there was a definite but very small peak at the modulation frequency; relative to the spontaneous tremor, which varies from preparation to preparation, its size was about half that of the peak in Fig. 2C. For all of these small effects the extent of any contribution from mechanical artefact was unknown (see Methods) and may have been significant. However, in one of the experiments in which there was not initially a clear modulation peak, a small peak was found when the muscle was slackened by 2 mm thus encouraging Ia misbehaviour; the peak was then slightly larger than that of Fig. 2C. This must have betokened some misbehaviour for the 45  $\mu\text{m}$  vibration, though not necessarily for the 50  $\mu\text{m}$  vibration.

In attempting to assess the extent of Ia misbehaviour underlying such small peaks it may be noted that the 7 Hz peak of Fig. 2C has an r.m.s. value of 6.3 mN whereas the average value for the firing of a single motor unit is of the order of 3.5 mN (Cussons *et al.* 1979). Thus very roughly the peak corresponds to the on-off modulation of the firing of two motor units out of the some tens that may be expected to have been firing. In contrast, in the same experiment 100% modulation (50  $\mu\text{m}$  to zero) produced a peak with an r.m.s. value of 52 mN, equivalent to the synchronised firing of fifteen units. Assuming that this is attributable to 100% modulation of every one of the fifty soleus Ia afferents and that simple proportionality applies then the peak of Fig. 2C would correspond to the modulation of seven soleus afferents. In view of the many uncertainties involved this value cannot be taken as definitive, but it indicates that a very considerable amount of misbehaviour would be required to account for the normal spontaneous tremor (typically around 20 mN n.m.s.) if it were to depend upon the stretch reflex.

It may be concluded that the experiments with amplitude modulation support the more direct evidence of single fibre recording that the great majority of Ia fibres are responding securely during vibration of 45–50  $\mu\text{m}$  amplitude. The contribution provided by the indirect method is that it is much the more rapid, and so can readily provide a large sample in the presence of a respectably sized tonic vibration reflex; the seven experiments may be deemed to have sampled the behaviour of some 350 afferents. The method, however, would not have shown up any afferents which fell completely silent during the vibration, but any such would not have been contributing to the production of the spontaneous tremor. The irrelevance of any lesser degree of misbehaviour for the genesis of the spontaneous tremor is shown by the finding, already detailed in the specific (Fig. 3), that in all five experiments in which the matter was tested a 5  $\mu\text{m}$  modulation of 20 or 25  $\mu\text{m}$  vibration always produced a definite tremor peak which was very appreciably larger than any produced by the same modulation of 50  $\mu\text{m}$  vibration, whereas the spontaneous tremor was never the larger.

#### *Pattern of misbehaviour with low amplitude of vibration*

In a de-efferented muscle, reduction of the amplitude of vibration so that driving ceases to be secure leads to a pattern of firing in which a given muscle spindle ending fires on every second or third cycle of vibration, say, or may alternate between these. There is no sign of any low frequency irregularity of behaviour (< 10 Hz) either in the passive state when the ending is responding to stretch and vibration alone, or during the super-added activation of the spindle by stimulation of a single fusimotor axon at a rate of 50–100 Hz. This may be seen, for example, in Fig. 5 of Brown *et*

*al.* (1967), based on records of instantaneous frequency on a slow time scale, and has been fully confirmed by subsequent re-examination of the original records from those experiments with this point in mind (these included a raster display of the temporal sequence of spikes on a fast time scale). In contrast, in the present experiments with the reflex arc intact the level of Ia afferent firing changed quite markedly from moment-to-moment when the amplitude of vibration was set below that producing secure driving. This is illustrated in Fig. 4 where *A* shows the timing of the spike train and *B* gives a display of the moving average of firing frequency. The latter was produced by filtering out frequencies above 23 Hz, as is commonly done by feeding

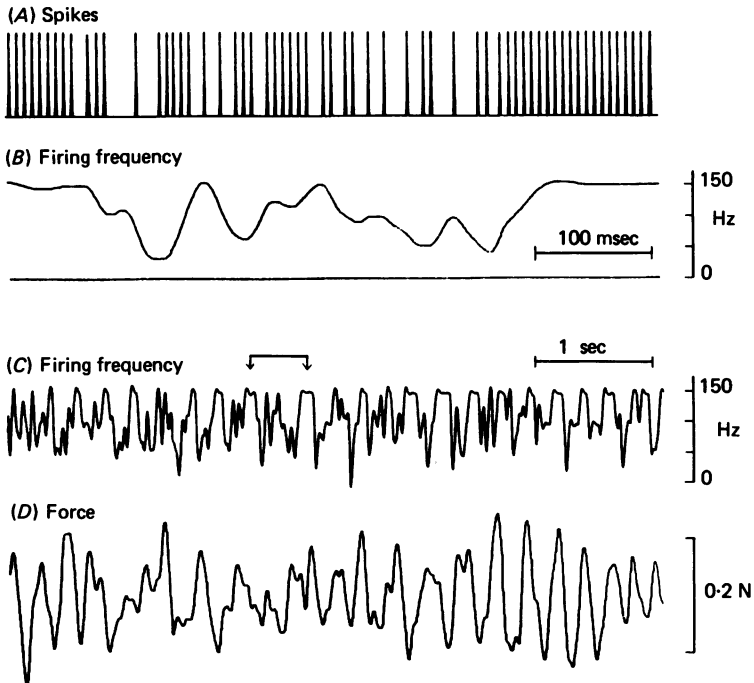


Fig. 4. Fluctuations in Ia afferent firing when the amplitude of vibration was reduced to below the level required to elicit continuous 1:1 driving, but while it remained large enough to elicit an appreciable tonic vibration reflex. *A*, the timing of the original spikes shown by a train of standard pulses, each triggered by a spike; the vibration frequency was 150 Hz. *B*, moving average of the frequency of Ia firing produced by filtering out frequencies above 23 Hz from trace *A*. *C*, longer period of 'demodulated' Ia firing, including the record of *B* as indicated by the arrowed bar (the small deflections above the 150 Hz level were due to the filtering). *D*, high gain record of the tremor variations in the isometric tension developed by soleus at the same time as record *C* (the record has been band-pass filtered, 1.5–23 Hz). The amplitude of vibration was 15  $\mu\text{m}$ ; 30  $\mu\text{m}$  vibration produced secure driving of the afferent. During the period shown the tonic vibration reflex was developing 1.7 N tension; 50  $\mu\text{m}$  vibration at 150 Hz elicited a reflex of about 2.5 N.

a spike train into a 'leaky integrator'; we refer to this process as 'demodulation' (see Methods, cf. Matthews & Muir, 1980). In Fig. 4 periods of secure driving alternate with those in which the frequency of firing was much lower; inspection of an oscilloscope display locked to the vibratory pulse train showed that the spikes were at all times phase locked to the vibration and so may be presumed to have been excited by it throughout.

Such irregularity of afferent behaviour would be expected if the spindles were being influenced by irregularities in the contraction of the muscle, whether local or general. Fig. 4C and D shows the relationship between the firing of the Ia afferent and the isometric force fluctuations recorded over a longer period. Some segments of traces C and D look as if they might be related, but there is no consistent one-to-one relation throughout. On inspection of still longer periods of record, the two types of trace again sometimes gave the impression of being correlated, but at other times they did not; moreover, the phase relation between the two types of record was not consistent, either on examining the same ending at different times or for different endings.

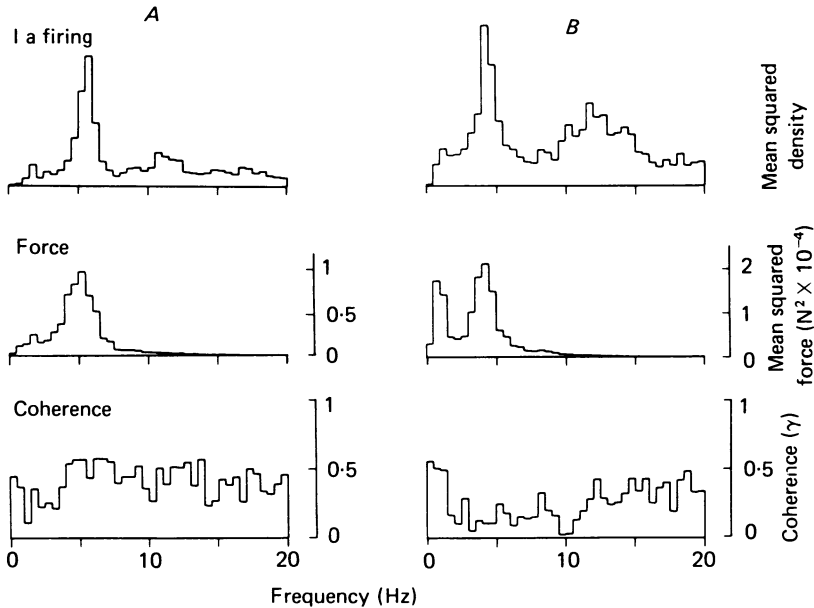


Fig. 5. Spectra comparing the irregularities of afferent firing with the tension tremor when the amplitude or vibration was reduced so as to make the afferent firing insecure with respect to the vibration. *A, B*, results for two different afferents both from the same preparation. Vibration, 15  $\mu$ m at 150 Hz for both. Top, spectra of the demodulated spike train. Middle, concurrently obtained spectra of the tremor tension. Bottom, spectra showing at each frequency the coherence between the tension and demodulated Ia firing recordings. The spectra in *A* were based on 80 sec recording (i.e. on eight separate 10 sec segments of data) and in *B* on 70 sec. The tonic vibration reflex was developing about 0.4 N active tension during *A* and about 1.5 N during *B* (same afferent as in Fig. 4).

The matter was more fully examined by studying the irregularities in the two types of trace by spectral analysis and estimating the degree of relationship between them by determination of their coherence spectrum. This is illustrated in Fig. 5 for two separate afferents which were studied at different times in the same experiment. The spectra of demodulated afferent firing confirm that there is considerable low frequency variation in the rate of moment-to-moment firing, and show that it contains frequency components similar to those of the concurrently recorded tremor tension. However, there is no apparent relationship between the irregularities underlying these two spectra since the coherence between them remains at a low level throughout, particularly for the ending of Fig. 5*B*. The maximum value that the coherence ( $\gamma$ ) can take is 1.0, which indicates a linear, noise-free relation between two

variables, and none of the present values can be taken to indicate a significant relationship between afferent firing and the ongoing small irregularities in isometric tension recorded at the tendon. A further seven afferents were studied in the same experiment with the same results. In this fortunate preparation the registration of the unitary spikes by the micro-electrode proved to be very stable in the face of stretch and vibration. Moreover, the tonic vibration reflex was large, so that for most of the time it was above 1 N even when the amplitude of vibration was reduced so as to make the recorded afferents misbehave; and the level of tremor was entirely typical (cf. Cussons *et al.* 1979). Another four afferents were studied in this manner in three other preparations and found to behave similarly. For two of the afferents the reflex was in excess of 1 N, but for the other two the active tension was only about 0.5 N, though still showing tremor as in Fig. 5A. It may be concluded that irrespective of their precise origin these irregularities of Ia firing cannot be ascribed a major role in the genesis of tremor since the timing of the two events is not well correlated.

#### DISCUSSION

*Relation to tremor.* The present recordings from Ia afferents confirm the earlier indirect arguments of Cussons *et al.* (1979) that a variable misbehaviour of Ia afferents to vibration is unlikely to be responsible, via the stretch reflex, for the tremor of the tonic vibration reflex. Indeed, under conditions directly comparable to theirs (50  $\mu\text{m}$  pulses at 150 Hz with the muscle stretched) there was a trivial amount of failure of 1:1 driving when the tonic vibration reflex was of reasonable size (1–8 N). We believe our sampling of the Ia afferents was adequate (see Methods and Table 1). In addition, the observation that modulation of the amplitude of vibration between 45 and 50  $\mu\text{m}$  at a frequency in the tremor range produces little or no corresponding peak in the tremor spectrum provides further indirect evidence that the spontaneous tremor of the reflex is not due to Ia misbehaviour, as well as being in line with the direct evidence that there is very little misbehaviour for this amplitude of movement. When Ia misbehaviour was created by using a maintained small-amplitude vibration there was no increase in tremor although there were tremor frequency fluctuations in the firing of individual afferents. But these latter were not well correlated with the tremor recorded at the tendon, as would be required for them to be the main cause of the tremor. Thus there seems little possibility that the tremor of the tonic vibration reflex can be attributed to stretch reflex activity. This view is in line with the study of the firing rates of individual motor units and the correlations between them (Clark *et al.* 1981). Rather, all the evidence points towards the tremor being due at least in part to the chance beating of different motor units firing at frequencies below their tetanic fusion frequency; indeed the tonic vibration reflex would now seem to be the best documented example of such a mechanism in operation.

It remains, however, slightly puzzling that there was not an obvious reflex tremor during low amplitudes of vibration since the Ia afferents were modulating their firing at tremor type frequencies. It seems likely that the various Ia afferents were varying their patterns of firing independently of each other, since otherwise a clear correlation would have been expected between their discharges and the tremor. Probing the surface of the soleus with a glass rod showed that the spindles studied lay in many

different parts of the muscle, so that their only common input would be from the length changes of the tendon in response to the variations in isometric tension. Rather any individual spindle would appear to be primarily influenced by local mechanical factors such as the contraction of motor units that lie in its immediate vicinity. The response of a given spindle afferent to the contraction of a single motor unit is known to vary greatly for different motor units and independently of the strength of the unit (Binder, Kroin, Moore, Stauffer & Stuart, 1976) thus strongly suggesting that a given motor unit influences different Ia afferents quite differently, as also to be expected *a priori*. In addition, some of the irregularity of firing might reflect low-frequency variations in the strength of contraction of the intrafusal muscle fibres within a given spindle; this might arise from a particularly potent fusimotor axon happening to fire at a low frequency, or perhaps from low frequency beats between the discharges of several fusimotor axons firing at higher and slightly different frequencies. But in spite of the presumed sensitivity of the spindles to local factors they are still all coupled together functionally by virtue both of lying 'in parallel' with muscle fibres that are connected to a common tendon, and also by feeding their afferents into the same motoneurone pool. Stretch reflex action might therefore be expected to bring their discharges into synchrony. But since this does not appear to happen the properties of the whole physiological system must somehow be such that this expected tendency to synchronisation does not lead to a reflex effect that is large enough to manifest itself in the face of the other factors responsible for tremor.

*Wider aspects.* The recording of such uniformly good behaviour from our Ia afferents may also help to dispel the fear that Ia misbehaviour might be common and of gross degree during the tonic vibration reflex; the present results with pulses seem likely to be as applicable to sinusoidal stretching of suitably enhanced amplitude. This doubt was raised in acute form by Jack & Roberts' (1978) brief report of rather frequent misbehaviour from among a small sample of Ia afferents in their decerebrate preparations in which part of the ventral root supply to the soleus had usually been severed. As they themselves recognised this was probably attributable to a deficiency of fusimotor bias to the spindles, which cannot then readily take up the slack on being unloaded by extrafusal contraction. But misbehaviour clearly can occur when a muscle is not well stretched and when there is a paucity of fusimotor activity. It might indeed become a problem in preparations such as the anaemic decerebrate cat or that with cerebellar damage which may show an excess of alpha motor activity (encouraging spindle unloading and the study of the muscle at the bottom of its physiological range of length) but rather little fusimotor activity (Granit, Holmgren & Merton, 1955). Other authors using vibration (cf. Kanda & Rymer, 1977; Westbury, 1972) have tended to take an optimistic view of the security of Ia driving, on the basis of varying quantities of evidence. In considering the interpretation of earlier work on the tonic vibration reflex it may be noted that an unduly high degree of misbehaviour would be required to explain on its own the findings of Matthews (1969) which were then used to argue for a contribution of the spindle secondary ending to the stretch reflex; for example, if 20% of the Ia afferents were to misbehave and fire 1:2 rather than 1:1 in relation to the vibration, then on making them all secure the overall Ia afferent input would increase by only 10%, whereas considerably larger increases would seem to be required. However, the interpretation

of the experiments by McGrath & Matthews (1973) is considerably more problematical since it was then assumed that driving remained secure after paralysis of fusimotor axons in the nerve by procaine. It may be concluded that with suitable awareness of the potential pitfalls, vibration remains a useful tool for the quantitative study of Ia reflex action in spite of the presence of a reflex contraction of the vibrated muscle.

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