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ACTION OF VIBRATION ON THE RESPONSE OF CAT MUSCLE SPINDLE Ia AFFERENTS TO LOW FREQUENCY SINUSOIDAL STRETCHING

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SUMMARY

1. A study has been made of the effect of continuous vibration, at 150 Hz, upon the response of muscle spindle afferents to low frequency sinusoidal stretching (1 and 8 Hz). Using the soleus muscle of the anaesthetized cat, with severed ventral roots, recordings were made of single Ia afferents and of the massed Ia afferent discharges in the main bulk of the cut L7 dorsal root.

2. When the amplitude of vibration was large (50 μm, short pulses) and that of the sinusoidal stretching was not too great (50–100 μm, peak-to-peak) the discharge of the afferents was largely locked 1:1 to the vibration and the response to the sinusoidal stretching was abolished.

3. When the amplitude of vibration was reduced to below that eliciting continuous afferent driving, then the response to sinusoidal stretching of any amplitude was often markedly increased. This arose through the vibration having a much more powerful excitatory action during the rising phase of the sinusoidal stretch than it did during the falling phase.

4. Averaged over a full cycle, the phase of the response to the sinusoidal stretching tended to be delayed during the vibration in comparison with the normal. This was largely dependent upon the afferents continuing to respond maximally to the vibration around the peak of the sinusoidal stretch, at which stage their unvibrated response is declining, rather than to a phase lag of the whole pattern of response.

5. The results are discussed in relation to the effects of vibration on tremor and the human stretch reflex, and on the determination of the frequency–response of spindle afferents.

INTRODUCTION

Recent experiments in man have shown that vibration applied to the tendon of biceps or of triceps may increase the amount of spontaneous elbow tremor when the arm is loaded compliantly, and with isometric recording alter the reflex response to an imposed sinusoidal displacement of the arm (Cussons, Matthews & Muir, 1980; Matthews, Muir & Watson, 1979). Both effects were felt likely to be attributable to an augmentation, during the vibration, of the response of the human muscle spindle afferents to the sinusoidal movement, irrespective of whether the latter was generated

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inside or outside the body. The present experiments on the cat were performed to observe the actual behaviour of spindle afferents to small amplitude sinusoidal stretching applied in the presence of continuous vibration. As anticipated, they have shown that when the relative amplitudes of the vibration and of the stretching are varied the response to a given sinusoidal stretch may either be increased above the normal (by virtue of the stretching affecting the degree to which the afferent is excited by the vibration), or alternatively reduced or abolished (by virtue of the occurrence of secure 1:1 driving of some or all of the Ia afferents by the vibration frequency-clamp). In addition, as is relevant for reflex control, it was found that during vibration the falling phase of the envelope of the afferent response to sinusoidal stretching may be delayed in comparison with the normal.

METHODS

The experiments were performed on cats deeply anaesthetized with sodium pentobarbitone. Five were used. The methods followed those used earlier (Hulliger, Matthews & Noth 1977*a*). The right hind limb was widely denervated except for the soleus muscle and the rest of the triceps surae was removed. The soleus tendon was connected to an electromagnetic stretcher, controlled by feed-back from a length transducer, which was used to apply both the sinusoidal stretching and the vibration.

Observations were made with the muscle within a few mm of its maximum *in situ* length, as judged from marker threads attached before cutting the tendon; between observations the muscle was relaxed by 5 mm. The sinusoidal stretch was left on continuously, but the vibration was only turned on 1–2 sec before stretching the muscle at 10 mm/sec up to the experimental length. Next, after an equilibration period of 2–3 sec, the responses were studied for 10–12 sec, after which the vibration was turned off and the muscle was released. The cycle was repeated every 30–45 sec, the precise value varying between experiments. The response of the endings showed only very slight adaptation during each individual 10 sec period of data collection and no major shift in behaviour was seen on repeating observations with one set of parameters, after using other parameters. Normally, the amplitude of the sinusoid was set to a particular value and a range of vibration amplitudes tested in increasing and then decreasing order. The vibration was usually given at 150 Hz and as before consisted of a stream of discrete pulses, each lasting about 3·5 msec (Clark, Matthews & Muir, 1981); their sharply rising wave form allows them to produce 1:1 driving with a smaller peak-to-peak amplitude than does vibration of sinusoidal wave form (McGrath & Matthews, 1973). The amplitude employed ranged from 2·5 to 50 μm. Sinusoidal stretching at 8 Hz was studied in most detail because of its applicability to tremor and peak-to-peak amplitudes of movement of 20, 50, 100, 200 and 500 μm were usually tested with a range of amplitudes of vibration. 1 Hz sinusoidal stretching was also used routinely (50, 100, 200 and 500 μm peak-to-peak).

A lower lumbar laminectomy was performed and the soleus de-efferented by severing the L6, L7 and S1 ventral roots ipsilaterally; the corresponding dorsal roots were also cut. Functionally single spindle afferents were obtained by splitting dorsal root filaments. They were shown to be primary spindle afferents by measurements of their conduction velocity (over 70 m/sec, on stimulating the nerve close to the muscle) and on their general pattern of behaviour. The responses of two, three or four afferents were recorded simultaneously during the experiment, using separate electrodes, and stored on separate channels of a 7-channel tape recorder for subsequent analysis. Fifteen afferents were studied in detail.

In four of the five experiments an assessment of the behaviour of a much larger number of afferents was obtained by recording from the whole of the remaining part of the L7 dorsal root that had not been consumed in the search for unitary discharges. This was placed on a pair of silver wires, separated by about 10 mm, and the free end of the root crushed. The massed monophasic action potentials were then amplified (band-pass 25 Hz to 3 kHz), full-wave rectified, and smoothed (low-pass filtered, cut-off 17 Hz) to give a moving average of the summed electrical activity in the root which was recorded along with the unitary discharges. This measure of the afferent firing was further related to the sinusoidal stretching by averaging the response to a number of cycles of stretch with a small hard-wired averager (NL 750; Digimiter Ltd). The flexibility of operation of the averager was greatly improved for this purpose by supplying it with externally generated clock pulses, the frequency of which could be adjusted so as to make each sweeep of the averager correspond to just fractionally less than a full cycle of stretch. This both allowed about 98 % of the data to be included in the average and also permitted the cycle average to be played back repetitively with the correct time relations for it to appear as if the average consisted of several cycles in succession, thus greatly aiding visual inspection (see Figs. 3 and 6).

Cycle histograms, relating the times of occurrence of the unitary spikes to the sinusoidal stretching were constructed by essentially the same means. These histograms were relatively 'noisy' because of the limited number of cycles employed (10–12 for 1 Hz, 80 for 8 Hz) and the large number of bins in the averager sweep cycle (256). For the 8 Hz stretching this 'noise' was reduced by using each real spike to trigger four separate pulses which were then counted into four successive bins, starting with that in which the spike would have been counted, thus quadrupling the number of counts in the histogram. This has much the same effect as reducing the number of bins to sixty-four and increasing their duration from approximately 0·5 to 2 msec, which would equally have the effect of delaying the mid-point of the response by 1½ original bin widths. It was not, however, feasible to do this for the 1 Hz stretching. Further smoothing of both the 1 Hz and the 8 Hz histograms was produced by the electrical damping of the pen recorder used in plotting; this was set so that, at the plot speeds used, wide variations between the values of successive bins could not be followed fully. This did not, however, prevent the display of real variations in the course of the cycle, as can be judged in the illustrations (Figs. 2 and 6) from the speed with which the recorder followed the jump to zero counts at the end of the cycle.

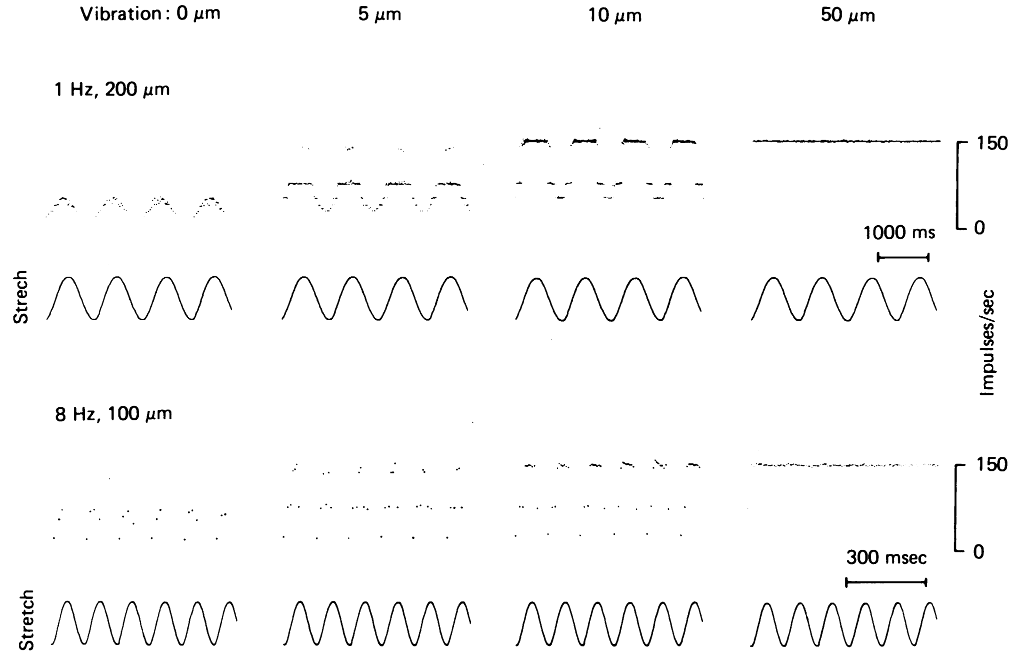

Fig. 1. Instantaneous frequency display of the response of a Ia afferent to sinusoidal stretch in the presence of various amounts of vibration. The figures at the top give the peak-to-peak amplitude of the vibration pulses, derlivered at 150 Hz. 1 Hz stretching above, 8 Hz stretching below, each with a diagrammatic stretch signal (note different time scales; all amplitudes peak-to-peak. Dot size deliberately increased in some places).

RESULTS

The discharges of fifteen single soleus Ia afferents, from five cats, were recorded while stretching the muscle sinusoidally at 1 or at 8 Hz, at the same time as vibrating it at 150 Hz. The displays of instantaneous frequency in Fig. 1 illustrate the contrasting effects that vibration, depending upon its amplitude, may have on the response to the low frequency sinusoidal stretching. When the amplitude of vibration is large (50 μm, far right) the afferent is driven in 1:1 synchrony with the vibration so that its frequency of discharge is clamped at that of the vibration; the tightness of the synchronization was readily confirmed by observing the spike on an oscilloscope, the sweep of which is triggered by the vibration (not illustrated). Thus these large vibration pulses abolish the normal prominent cyclic response of the afferent to the

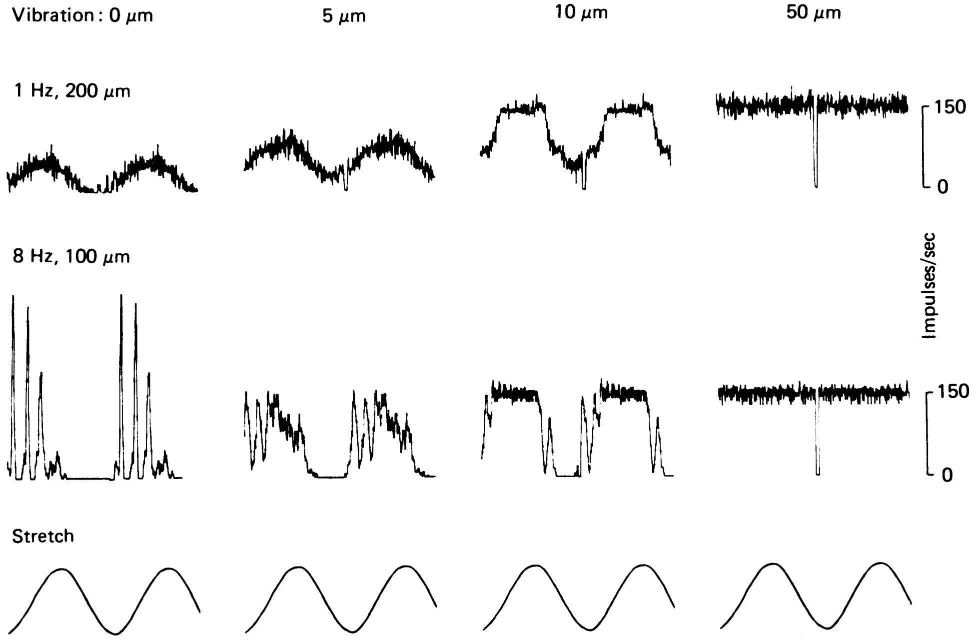

Fig. 2. Cycle histograms relating the Ia afferent discharge to a sinusoidal stretch that was applied during vibration at 150 Hz and at the peak-to-peak amplitudes shown. Top, 1 Hz stretching; middle, 8 Hz stretching; bottom, diagrammatic stretch marker. The 1 Hz histograms are based on 12 cycles of stretching, and the 8 Hz histograms on 80 cycles. Each histogram was computed for a single cycle of stretch, but for ease of visual inspection the response is shown as if it were obtained for nearly two successive cycles of stretching. This was done by repeating the play-back of the histograms from the memory store, and so displaying the same data twice; in between, the value for zero counts is displayed for the short part of the cycle which was omitted from the histogram. Bin width 3·7 msec for 1 Hz histograms, and 0·46 msec for 8 Hz histograms. However, the 'noisiness' of the 8 Hz histograms has been reduced by using each real spike to trigger four separate pulses which were then located in four successive bins (see Methods); this is approximately equivalent to increasing the bin width fourfold. The damping of the pen recorder also helped smooth the records but this had no discernible effect on the main features of response. Same afferent as Fig. 1.

stretching (Fig. 1, 0 μm, far left). In contrast, when the amplitude of vibration is small (5, 10 μm, middle) the afferent responds more vigorously than normal to the low frequency stretching. This happens because the afferent is readily excited by the vibration during the rising phase of the stretch and so here fires at the vibration frequency or its sub-harmonics, and at a higher frequency than in the absence of vibration. But the ending is less readily excited by the vibration during the releasing phase of the stretch. Thus the vibration increased the modulation of Ia firing by the sinusoidal stretching and hence the size of the afferent stretching signal, at any rate from the point of view of a detector that was unconcerned with the occurrence of the vibration, and operated by taking a moving average of the frequency of afferent discharge. Such contrasting modes of response to the sinusoidal stretching have been seen for every afferent studied for appropriate combinations of stretching and of vibration on varying the amplitudes of each. The precise parameters of stimulation required to elicit each mode of behaviour varied slightly for different endings, including for those which were studied simultaneously.

Cycle histograms

The behaviour of the afferents under a range of conditions was more fully explored by constructing cycle histograms displaying the time of occurrence of spikes in relation to the phase of the low frequency sinusoidal stretching. By averaging a number of cycles this allows a measure of the afferent response at times when it is alternating its discharge between the vibration frequency and its sub-harmonics; a display of instantaneous frequency provides rather little help in this situation. Fig. 2 shows cycle histograms, with the ordinate calibrated in impulses/sec, for the unit of Fig. 1. Frequency clamping is again seen when the amplitude of vibration is large (50 μm) though in comparison with the records of instantaneous frequency the noise level is increased. This arises from the inevitable statistical variations introduced by the binning, and the chance relation between the bin width and the interspike interval; some of this noise has been arbitrarily reduced by smoothing (see legend and Methods). For smaller amplitudes of vibration a marked cyclic modulation of firing can be seen to be produced by the sinusoidal stretching. For the 1 Hz stretching the response during vibration is clearly seen to be increased above its value in the absence of vibration, particularly with 10 μm vibration. However, for the 8 Hz stretching such comparison is complicated by the fact that in the absence of vibration the afferent fired at very constant times with regard to the sinusoidal cycle, so that some bins during the rising phase of stretch have a very high probability of containing a spike, momentarily equivalent to a very high frequency of firing, whereas other bins are empty. Nonetheless, on two criteria the modulation can still be seen to have been increased by the vibration. First, the peak firing during the stretch on its own can be determined from the interspike intervals corresponding to the separation of the humps in the histogram; these show the maximum discharge frequency in the absence of vibration to be only 55/sec, in comparison with a maximum of 150/sec during vibration of 5 and 10 μm, in agreement with Fig. 1. Secondly, the peaks of the response without vibration can be smoothed by eye or otherwise to give an average value over the rising phase of the stretch which is below the value found during vibration. However, neither of these provides a satisfactory continuous measure of the response of the unvibrated ending suitable for comparison with a response of the same order of size obtained during vibration. Even so, the construction of cycle histograms served to show that vibration may increase the response to sinusoidal stretching under conditions for which this might not have been readily demonstrable with displays of instantaneous frequency. However, both methods readily showed 'frequency clamping' and that the precise amplitude of vibration required to achieve it varied from unit to unit.

Massed response

Rather smoother cycle histograms could be obtained by including all the spikes discharged by the three separate Ia afferents that were normally studied simultaneously, via separate recording channels. Such composite histograms improved the situation because the different units normally differed slightly in their responsiveness and so did not discharge their spikes at precisely the same time; these fully confirmed that vibration could increase the Ia sensitivity to sinusoidal stretching. This led us on to obtain a yet more widely based average Ia response by monophasically recording the gross electroneurogram from the remaining part of the L7 dorsal root that had not been split in the search for single units. Comparison of the unitary all-or-none spikes in the base-line 'noise' with the monophasic compound action potential produced by a maximal pulse of vibration suggested that usually about thirty Ia afferents contributed to the composite response. A running measure of the mean level of neural discharge was obtained by rectifying and then smoothing the amplified electrical signals from the root (i.e. a leaky integrator type of rate-meter). During sinusoidal stretching this gave a cyclic response which may be presumed to depend largely upon the activity of the soleus Ia fibres (see later). The cyclic responses were readily observable in the raw 'integrated' record, but their precise shape was more reliably displayed by averaging a number of cycles as shown in Fig. 3. In the absence of vibration the original dorsal root record could be considered as a continuous analogue signal and to be directly represented by the average traces. But during vibration the original signal consisted of a series of monophasic waves, each elicited by a separate pulse of vibration and similar to those illustrated earlier (Matthews, 1975). The smoothing (low-pass filter, cut-off 17 Hz) and averaging transforms this stream of pulses into a continuously varying signal which reflects the variation in size of the monophasic response at different phases of the cycle.

Fig. 3 shows again how, with increasing amplitude, vibration first leads to an augmentation and then to a progressive suppression of the afferent response to the sinusoidal stretch, but this is now in a form permitting ready comparison of the response to a range of parameters of stimulation. It should be emphasized, however, that the records do not necessarily provide an exact linear transformation of the average level of Ia discharge; but they are thought to preserve its main features. The massed records have the particular advantage of allowing a rapid pictorial presentation of matters which can only be grasped with much greater effort from the single unit recordings. The residual modulation with 50 μm vibration in Fig. 3 corresponds to the failure of secure driving of only one to two units out of the twenty-five estimated to be active for this massed response; in line with this all three of the single units that were recorded concurrently were securely driven, as already shown for one of them in Figs. 1 and 2.

Calibration of massed responses and questions of validity of linear interpolation

On increasing the amplitudes of stretching while also applying vibration of appreciable amplitude both the peak and the trough of the modulated massed response tended to constant values. The peak was presumed to correspond to firing at 150 Hz of all the Ia afferents in the portion of root studied, corresponding to secure 1:1 driving by the vibration. The trough was assumed to correspond to a momentary silence on the part of all the Ia afferents. Both these features of response were regularly seen in the unitary recordings. These two levels were taken as the two fixed points for calibration of the massed responses, and a linear interpolation was then performed between 0 and 150 Hz. But given the complexity of the situation no claim is made that this procedure will necessarily have yielded an accurate linear scale of the Ia average firing frequency. However, it is felt to have provided at least an approximate scale and one adequate to allow ready assessment of the main features of response, all of which could also be observed in the single unit recordings.

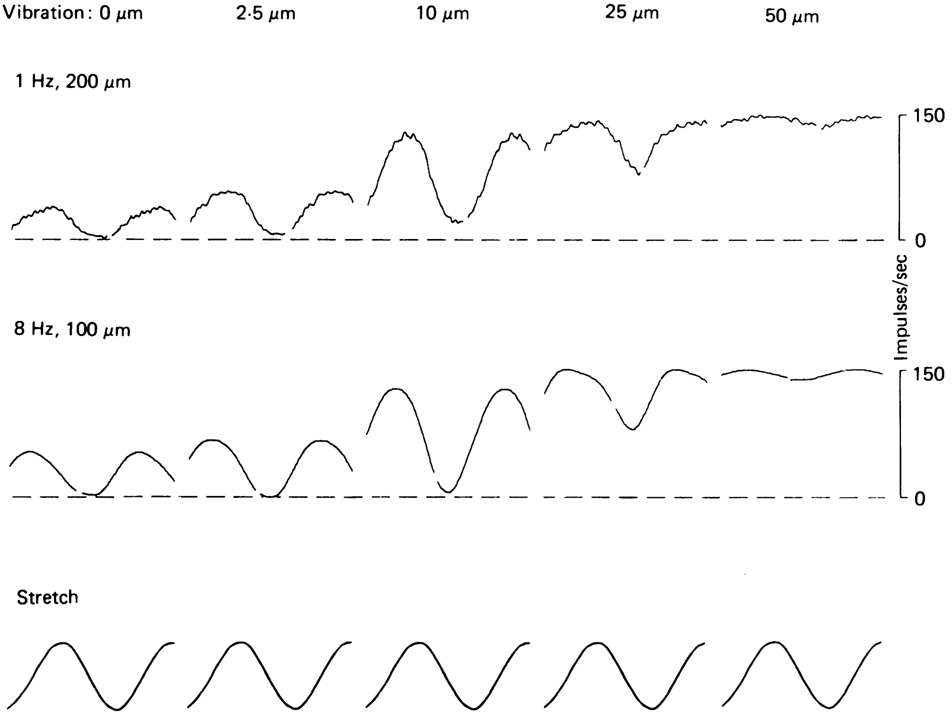

Fig. 3. The averaged massed response to sinusoidal stretching recorded, in the presence of varying amounts of vibration, from the major part of the L7 dorsal root. Top, responses to 1 Hz stretching; middle, responses to 8 Hz stretching; bottom, diagrammatic stretch. The largely monophasic action potentials were rectified, low-pass filtered and then averaged with respect to the stretch cycle; for 1 Hz, 12 cycles were averaged, and for 8 Hz, 80 cycles. For ease of visual inspection the response to about one and a half cycles of stretch is shown; this was obtained by repeating the play-back of a single averaged cycle. The very short part of the cycle omitted from the averaging is left blank. The stretch signal has been positioned so as to compensate, at the stretch frequency, for the phase lag introduced by the analysis. Same experiment as Figs. 1 and 2. The calibration bars give the average level of unitary Ia firing (see text).

The first requirement for linear scaling with regard to Ia firing is that no other afferents should be contributing appreciably to the sinusoidally modulated signals. The Ib afferents from the tendon organs seem unlikely to have been contributing appreciably, since the muscle was not contracting (Brown, Engberg & Matthews, 1967). The contribution from the group II afferents from the spindle secondary endings also seems likely to have been negligible, both because they are unlikely to have been modulating their firing appreciably with the stretching and because of the smallness of their unitary spikes as recorded extracellularly so that any modulation on their part would not appreciably augment the modulation in the electroneurogram due to cyclic Ia activity. Next, for the electrical activity of different Ia axons to sum linearly during asychronous activity their spikes need to be monophasic, since with diphasic or triphasic spikes the statistical degree of interference between the positive and negative phases of different unitary spikes will increase with their frequency of firing. In the present experiments the spikes were reasonably monophasic with little or no undershoot. This was assessed from examination of the compound action potential set up on stimulating the nerve to soleus or in response to pulses of vibration (cf. Matthews, 1975, Fig. 2). Vibration introduces the additional factor that the spikes are then synchronized with the vibration and so with each other, even when it is not strong enough to produce 1:1 driving. The gross electroneurogram then consists of a series of waves at the vibration frequency, with the amplitude of the wave increasing with the amplitude of the vibration as more and more Ia afferents are brought to secure driving. The degree of interference between the positive and any negative components of different spikes will then depend crucially upon their precise time of arrival at the dorsal root in response to the same pulse of vibration. Among other things this will vary slightly with the conduction velocity of the afferents in question, and it seems unlikely that the arithmetic of the addition of the activity will be exactly the same as in the absence of vibration. Thus quantitative comparisons between the response obtained in the absence of vibration and those obtained in its presence are inevitably suspect. However, less doubt attaches to comparison amongst the responses obtained with different amplitudes of vibration, since even very small amounts of vibration normally suffice to produce phase locking of the unitary responses onto the vibratory stimulus, and so put the different spikes into an approximately constant temporal relation for different amplitudes of vibration. Thus the scale between 0 and 150 Hz should hold reasonably reliably during vibration. The progressive changes in the size and phase of the response

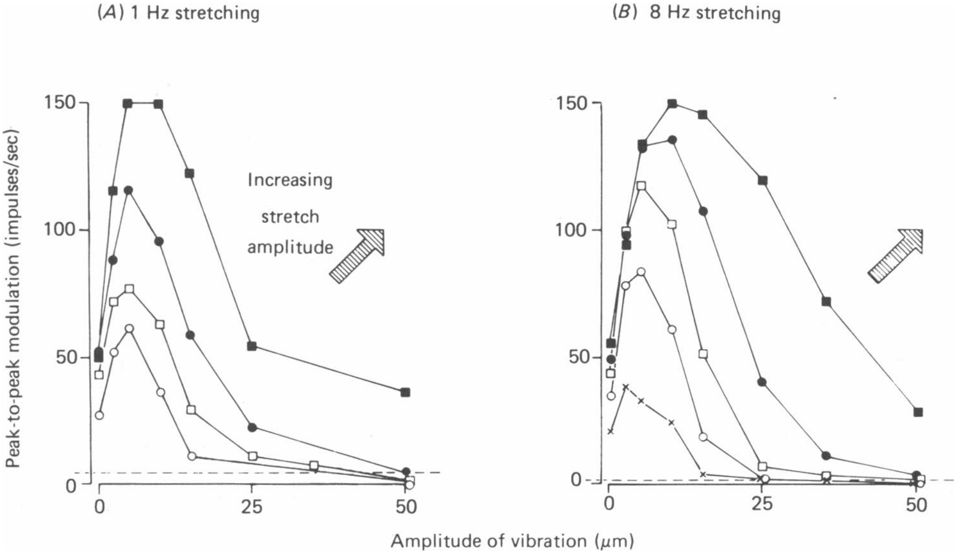

Fig. 4. The effect of increasing amplitude of vibration on the massed response to sinusoidal stretching, at 1 Hz and at 8 Hz, for various sizes of stretch. The ordinate shows the peak-to-peak modulation measured directly from records such as those as in Fig. 3; it is calibrated in impulses/sec firing for an average Ia afferent. The peak-to-peak extent of the sinusoidal stretching is as follows: ×, 20 μm; ○, 50 μm; □, 100 μm; ●, 200 μm; ■, 500 μm. The dashed line shows the noise level of the histograms, different for the two frequencies, below which the modulation could not be differentiated from zero. Vibration, 150 Hz. Different experiments from Fig. 3.

on increasing the amplitude of vibration from zero upwards all hold equally well if the reference for comparison is the response obtained with 2·5 or 5 μm vibration rather than zero. Moreover, the use of a.c. recording does not seem to have influenced the results. No appreciable change in size or phase of the response to 1 or 8 Hz stretching was produced when the time constants of the a.c. amplifiers were changed (between 10 and 1 Hz low-cut for the main amplifier and 1 and 25 Hz for the input circuit to the rectifier). There was, however, considerable phase lag introduced into the responses by the smoothing which was performed after the rectifier to reduce the pulsatile nature of the electroneurogram before averaging; but this was, of course, without effect on the linearity of the response. It may be concluded that there is little likelihood that the gross changes seen in the electroneurogram on altering the amplitude of vibration, as in Figs. 3 – 6, could be attributed to changes in the mode of summation of the different unitary spikes, especially as they are entirely supported by the unitary recordings. But the quantitative calibration of the massed records in terms of average Ia firing may conceal a degree of non-linearity, the extent of which has not been assessed.

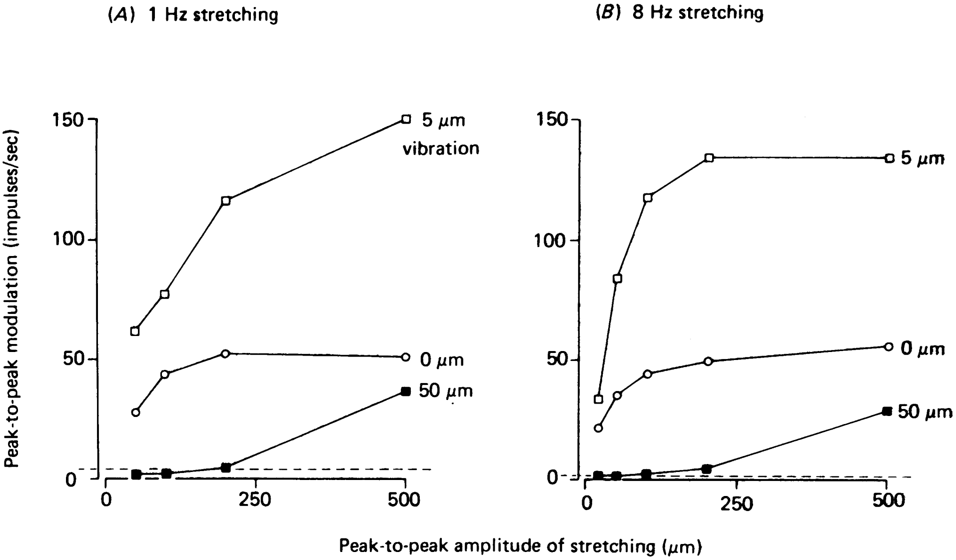

Fig. 5. The relationship between the modulation of the massed response and the amplitude of stretching for three different levels of vibration. Same experiment as Fig. 4.

Fig. 4A shows the peak-to-peak modulation of the massed response plotted against the amplitude of vibration for 1 Hz stretches of a peak-to-peak amplitude varying from 50 to 500 μm; the preparation is a different one from that used for Figs. 1 – 3 and one for which driving was slightly more readily produced. For each size of stretch the afferent modulation first increased and then decreased with increasing amplitude of vibration until, for all except the largest stretch, it became indistinguishable from the background 'noise' level of the histogram (dashed lines). This was entirely in accordance with the three simultaneously recorded unitary Ia responses. It is notable that vibration of 2·5 and 5 μm amplitude augmented the average response to stretching, with the latter being slightly the more effective; this again was borne out by the unitary recordings, though then impossible to quantify. Fig. 4B shows similar data for 8 Hz stretching of amplitudes of 20–500 μm, using the same preparation.

Fig. 5 repeats some of the data of Fig. 4, but now with the afferent modulation plotted against the size of the stretch for three amplitudes of vibration. When the vibration was small (5 μm) the massed response was invariably larger than in the absence of vibration. But when the vibration was large (50 μm) the response to a small stretch was suppressed; and even for the 500 μm stretch the response was below its normal level. Put another way, in the presence of a small amount of vibration the 'gain'or sensitivity of the afferents to sinusoidal stretching was increased, whereas in the presence of a large amount of vibration the sensitivity to stretching is reduced, even down to zero when the stretch is small.

Consistency between preparations. The findings shown in Figs. 3 – 5 were typical for all four preparations in which the massed neural activity was studied, and were always in accordance with the two or three simultaneous unitary recordings. The precise form of the curves such as those in Figs. 4 and 5, however, varied from preparation to preparation. In part this probably reflected the slightly different lengths, all close to physiological maximum, at which the various muscles were studied since this effects both the response to vibration and that to sinusoidal stretching (Brown *et al.* 1967; Goodwin, Hulliger & Matthews, 1975). This meant that there was little point in attempting to present an overall average of the response for the various parameters of stimulation. However, 50 μm vibration always abolished modulation for 1 Hz stretches of up to and including 100 μm amplitude, but never did so for 500 μm stretches. For 8 Hz stretching such clamping was only invariably produced for amplitudes of up to and including 50 μm. All fifteen units studied were likewise securely driven for this range of stretches for which modulation of the massed discharge was abolished.

2·5 and 5 μm vibration always augmented the massed response to all amplitudes of stretching, at both frequencies, and the response with 5 μm vibration was almost always larger than that with 2·5 μm. Likewise, for almost all of the units an increase of response with vibrations amplitudes of 2·5 and 5 μm was readily apparent for 1 Hz stretching of all amplitudes. But for 8 Hz stretching, the effect was not always immediately discernible by simple observations of cycle histograms when the amplitude of stretching was small, and the matter was not tested further.

The transition from augmentation to reduction and then suppression of the massed sinusoidal response consistently required progressively larger amplitudes of vibration with increasing stretch amplitude, as Fig. 4. For stretch amplitudes up to and including 200 μm the peak of the curve always lay below 25 μm vibration amplitude. In one cat, probably with the soleus relatively less stretched than in the others, the curves for 500 μm stretching showed an extended plateau with increase of vibration amplitude, rather than a peak. This was associated with two of the three units studied concurrently continuing to alternate their firing between secure driving and complete silence.

Phase of response

As the amplitude of vibration was increased so the afferent modulation progressively ceased to be at all closely sinusoidal in its wave form. In particular, a hump appeared on what had been the declining phase of the massed response and its wave form became assymetrical with the slowing of I a firing occupying a progressively smaller part of the cycle, as can be seen in the superimposed records of Fig. 6*A*. The rising phase of the response, however, showed much less change, with a very small 'phase advance' with increasing amplitudes of vibration. The overall effect of vibration was assessed by fiting a pure sine wave, at the frequency of the stretching, to the massed response and measuring its phase angle with respect to the stimulus. For example, in Fig. 6*A* the response was advanced by 106° on the stretch in the absence of vibration, but this was reduced to 78° with the 15 μm vibration. Fig. 6*B* shows the progressive reduction of the normal phase advance with increasing amount of vibration for the series of records of Fig. 6*A* (□, 8 Hz, 100 μm peak-to-peak stretching) and for three other amplitudes of stretching at the same frequency. Such a reduction of the overall phase advance of the fundamental of the response was found in each of the four experiments, for both 1 Hz and 8 Hz stretching. Fig. 6*C*, showing the cycle histograms for the response of single afferent, illuminates the 'cause' of this phase lagging action of vibration by showing that a given unit may continue to be 'driven' by the vibration up to and beyond the point of maximum stretch, whereas without vibration the firing of an afferent normally decreases from its maximum value well before the maximum stretch is reached (cf. Hulliger *et al.* 1977*a*). On the other hand, at the beginning of the stretching phase of the cycle the ending only begins to respond to the vibration very shortly before it would at any rate be responding to the stretch on its own. It would appear that the effect of stretch in increasing the sensitivity of the spindle to vibration does not precisely parallel the effect of stretch in exciting the spindle *per se*, at any rate for vibration and stretch applied

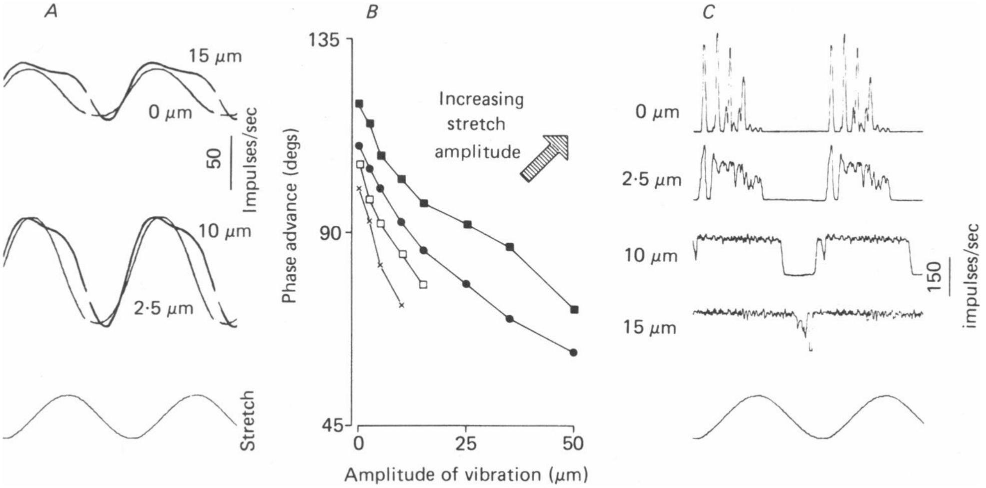

Fig. 6. The progressive changes in the time course of the afferent response to sinusoidal stretching, in this case at 8 Hz, on increasing the amplitude of vibration. A, superimposed averaged massed responses to stretching of 100 μm peak-to-peak extent with 150 Hz vibration of 0 and 15 μm (top) and of 2·5 and 10 μm (middle); bottom, diagrammatic stretch signal. The records have been superimposed so as to have approximately the same mean level, though this actually increased with the amplitude of vibration as in Fig. 3. *B*, the relation between the 'phase advance' of the massed response, with respect to stretch cycle, and the amplitude of vibration for four different amplitudes of stretching (×, 20 μm; □, 100 μm; ●, 200 μm; ■, 500 μm; all peak-to-peak). The phase was measured by fitting a pure sinusoid to the response and ignoring the harmonics. *C*, cycle histograms for a single unit that was studied concurrently with the massed responses of *A*. Bin width 0·46 msec, but as in Fig. 2 this has effectively been increased fourfold. Same experiment as Figs. 4 and 5. As in Fig. 3 the stretch cycle has been positioned so as to compensate for any lags introduced into the fundamental by the analysis.

to the whole muscle. Put another way, the response during vibration is more dependent upon the length of the muscle and less dependent upon the velocity of stretching than is the unmodified response of the ending itself.

Frequency of vibration

The effect of varying the frequency of vibration from 50 to 250 Hz was studied in two experiments, both employing vibration consisting of a series of discrete pulses of the usual wave form (total duration 3·5 msec). The same general effects were seen as already described for 150 Hz vibration. However, the higher the frequency of vibration the more marked was the augmentation of the response to sinusoidal stretch. The usual single unit recordings showed, as to be expected, that the afferents were still driven by the vibration on the rising of the stretch, so that the increase above the normal level was greater the greater the frequency of vibration. However, increasing the frequency of vibration tended to increase the threshold for 1:1 driving so that for a given amplitude a high-frequency vibration might produce 1:2 driving under conditions for which a low-frequency vibration produced 1:1 driving, so that the potentiating effects of vibration did not increase in direct proportion with the frequency.

Wave form of vibration

In four preparations the effect of sinusoidal vibration was compared with the effect of the discrete pulses that were normally employed. Frequencies of 100 and/or 150 Hz were tested on selected amplitudes of stretch at 1 and at 8 Hz. The same kind of responses were observed with sinusoidal vibration as just described for pulsed vibration, with a steady progression from augmentation of the sinusoidal response with low amplitudes of vibration to reduction or suppression of modulation with large amplitudes of vibration. However, as noted before (McGrath & Matthews, 1973), a sinusoidal vibratory wave form has to be about twice the peak-to-peak extent of a pulsed wave form to produce the same effect. Another point of difference was that the sinusoidal vibration, especially at 100 Hz, was less efficacious at producing complete suppression of modulation in the massed response and 'frequency clamping' in the unitary responses. This was because the sinusoidal vibration sometimes produced 'double driving' with two spikes per cycle of vibration when its amplitude was large so that large stretch might lead to alternation between 2:1 and 1:1 driving on its rising and falling phases respectively, and so overcome the normal tendency to frequency clamping at the frequency of the vibration itself.

With the pulsed vibratory wave form even large pulses did not directly excite two spikes in the present experiments. However, in one preparation when the 8 Hz stretching was at its largest the units fired at above the vibration frequency of 150 Hz in response to the stretch on its own when, as might be expected, frequency clamping failed and there were two spikes per pulse for a part of the stretching cycle. McGrath & Matthews (1973) attributed the efficacy of the pulsed waveform in avoiding direct double driving to the short duration of its rising phase (about 1·5 msec).

DISCUSSION

As was expected, vibration of a suitably large amplitude has been found to provide a 'frequency clamp' so that the Ia afferents continue to fire at the frequency of vibration in spite of a certain amount of external disturbance, the occurrence of which they thus completely fail to signal. This is in line with studies on the tremor of the tonic vibration reflex of the decerebrate cat, recorded isometrically, where the spindle primary afferents are unaffected by the continuous quivering of the muscle due to unfused motor unit activity (Clark *et al.* 1981). Confirmation has also been obtained for the more debatable suggestion that with suitably reduced amplitudes of vibration the response to sinusoidal stretching may be increased above its normal value (Cussons *et al* 1980); equally, this may be presumed to occur for other rhythmically varying wave forms. Whether vibration produces augmentation or reduction of the spindle response to another mechanical input depends upon the interaction of a number of factors. One-to-one driving with frequency clamping is, of course, best seen when the amplitude of vibration is large and the amplitude of sinusoidal stretching is small. Also, driving would seem to persist slightly more readily in the face of low rather than high-frequency disturbances of a given amplitude. This is shown in the comparison between 1 and 8 Hz stretching and follows equally from *a priori* considerations. Because of its visco-elasticity the faster the release of a muscle the slacker it is likely to fall, with reduction of its stiffness, and so the less readily might it be expected to transmit the vibration to the spindle. Likewise, the visco-elasticity of the spindle itself makes it less likely to be excited by vibration of a given amplitude when the release is fast rather than slow.

When 1 : 1 driving breaks down, the modulation evoked by the sinusoidal stretching may be either larger or smaller than the normal, depending upon the precise parameters of stimulation (see Fig. 4). What happens in any particular case will depend especially upon the relative efficacy of the vibration during the stretching and releasing phases of the sinusoidal cycle. But a precise prediction cannot be made as to what will happen under any given set of conditions, as defined in mechanical terms. In the present experiments, however, augmentation of the sinusoidal response has been regularly found for a wide range of combinations of stretch and of vibration amplitude for both 1 and 8 Hz stretching, and did not require any careful adjustment of the conditions to be obtained. For the de-efferented spindle, therefore, such augmentation by vibration of the response to other stimuli may be considered to be a commonplace mode of behaviour.

The question next inevitably arises as to how the endings would behave if they were also being influenced by tonic fusimotor action. To test the matter experimentally would require a considerable body of work, but certain suggestions may be offered. Frequency clamping seems likely to occur yet more readily, since stimulation both of static and dynamic axons increases the sensitivity of Ia afferents to vibration (Crowe & Matthews, 1964; Brown *et al.* 1967). However, there can be little doubt that augmentation of the response to sinusoidal stretching would still readily be obtained during steady activity of dynamic fusimotor axons. The Ia afferent then still remains silent on the releasing phase of a sinusoidal stretch of any appreciable magnitude (Hulliger *et al.*, 1977*a*) and so it is then also likely to be rather unresponsive to vibration. At higher frequencies of stretching, however, the response of the activated ending to stretch alone might be so great as to leave little scope for increase with vibration. During steady static axon activity the Ia afferent fires throughout the cycle for even quite large amplitudes of sinusoidal stretching, and so it must be expected to be continuously susceptible to vibration. But some variation in vibration responsiveness would still presumably occur between stretch and release, and since during steady static activity the modulation produced by sinusoidal stretching on its own is quite modest there is ample scope for augmentation by vibration. During combined activation of static and dynamic axons the situation is, however, less favourable for augmentation of the sinusoidal response. The ending might then be expected to be appreciably sensitive to vibration on the falling phase of the stretch (because of the static fusimotor action) while already firing at an appreciable rate on the rising phase of the stretch (because of the dynamic fusimotor action), and at a frequency that might be hard to better with vibration (cf. Hulliger *et al.* 1977*b*). But, all in all, it may be suggested that the action of vibration in augmenting the response of the spindle to other mechanical inputs will occur for a range of fusimotor activity, and that the possibility of such behaviour has to be allowed for in all experiments when vibration is present, whether it is introduced deliberately or occurs adventitiously.

Implications for frequency–response studies

A particular situation where vibration could potentially distort the findings is in the determination of the frequency–response of the spindle to mechancial inputs, since some degree of background noise is always inevitably present. Goodwin *et al.* (1975) in studying the linear range of the spindle reduced the higher frequency noise in their stretcher to 0·2–0·5 μm peak-to-peak, which is below the levels currently studied, and performed control experiments showing that at these levels the spindle behaved 'linearly' so that the deliberate input and the unwanted noise acted independently and the wanted spindle signal could be extracted without distortion. An essential difference from the present experiments was that the noise in the earlier experiments was sufficiently small not to produce any frank over-all excitation of the ending, with an increase of its mean firing rate, and simply modulated the probability of spikes being discharged at certain times. When Goodwin, Hulliger & Matthews (1976) subsequently introduced noise that fell just, but only just, outside the linear range they observed changes in the response to high frequency inputs but not to low frequency signals; these latter would presumably also have been altered with further increase in the noise input, as in the present experiments. In other experiments in which the spindle has been tested seriatim with pure sinusoids over a range of frequencies the noise level also seems unlikely to have been such as to interfere with the results (cf. Chen & Poppele, 1978; Matthews & Stein, 1969) since noise of the requisite amplitude should have been detected and eliminated.

But effects such as those currently described would seem to be inescapable when the frequency response is determined in the non-linear range of spindle response by using white noise, which is an attractive engineering technique that has recently been exploited by Poppele (1981). In effect, a number of frequencies are then applied simultaneously, rather than sequentially, and in computing the response of the spindle to each particular frequency there is no simple answer to the question as to whether the calculated response represents the 'true' response to that frequency of stretching *per se*, or whether it partly represents a modification by a lower frequency signal of the response to the concomitant higher frequency inputs. An effect of this kind might well be responsible for the somewhat puzzling finding that on using noise analysis the form of the frequency–response varies with the band-width of the noise input (Poppele, 1981). Moreover, the results seem to be in the direction to be expected for such an action, with the low-frequency response being relatively enhanced on increasing the proportion of higher frequency signal in the input (although the signal only contained frequencies up to about 20 Hz rather than the present 150 Hz, the same principles would seem to apply).

A wider point is that it may be suggested that what matters from the functional point of view is not the absolute frequency response obtained in isolation, but that appertaining to the normal physiological types of input, whatever these may happen to be. But since for different movements these may well vary in their spectral composition, no unique frequency–response can be expected for the non-linear range, quite apart from the effects of amplitude of input and variations of fusimotor activity. A further dimension to the problem is that the 'noise' introduced into the spindle by intrafusal twitching, especially of the nuclear chain fibres, might perhaps have some of the same effects as an externally introduced noise and vibration; it has long been known that this internally generated vibration can 'drive' the Ia afferent. For the signalling of external disturbances falling in the linear range of the primary ending, however, Goodwin *et al.* (1975) argued that this internally generated noise was not a crucial factor in determining the frequency response, since they obtained curves of the same shape irrespective of whether or not the static fusimotor axon studied produced significant driving effects on the primary ending. But the question would seem to remain for the behaviour of the spindle in its non-linear range.

Relation to human studies

In view of the numerous factors involved there can be no direct quantitiative transfer of the present measurements to the situation at the human elbow, which was the starting point for our interest Cussons *et al.* 1980; Matthews *et al.* 1979). But rough calculation suggests that vibration might be expected to augment the spindle response to movement under the conditions then studied. Although the amplitude of vibration typically employed in man might initially appear rather large, it is applied transversely to the tendon; only a small proportion of the movement generated by the vibrator can be transformed into the longitudinal stretch of the muscle as, used in the present experiments, which can be presumed to be what excites the spindles. On simple geometrical considerations and making favourable assumptions, the 0·5 mm or so peak-to-peak movement applied earlier to the flexor or extensor tendons at the elbow would produce no more than 15 μm longitudinal stretch of the muscle (this assumes that the vibration transversely displaces a rigid tendon 1 cm from its insertion and that the more compliant muscle, 15 cm long, absorbs the whole of the stretch; the wave form of vibration might also be distorted, probably so as to have a sharper rising phase). On less favourable assumptions the vibratory muscle stretch would be below 5 μm. Given the greater lengths of the human biceps and triceps than the cat soleus there seems little possibility of widespread 1:1 driving of the human spindles when the muscle is passive, although it might well occur locally. When the vibrated muscle is contracting under voluntary drive the concomitant fusimotor activity will increase the spindle sensitivity to some degree, although this will be partially offset by the mechanical effects of the contraction (Brown *et al.* 1967) and universal driving still seems improbable. On the other hand, there is no reason to think that the vibration was too small to be effective in the human experiments, since the augmenting effects were currently seen for vibration of down to 2·5 μm amplitude (the lowest tested). Moreover, recording of human spindle discharges for muscles acting at the ankle shows that tendon vibration can produce a variety of patterns of sub-harmonic driving, as well as some driving, and that the efficiency of the vibration can be markedly altered by imposed movement (Burke, Hagbarth, Löfstedt & Wallin, 1976).

The approximately sinusoidal length changes of the muscles in the human experiments were small and comparable to the smaller stretches used in the present experiments. Matthews *et al.* (1979) studied the response to sinusoidal displacement at the wrist of 1 mm peak-to-peak which, with a lever ratio of 5:1 (assessed by measurements of the skeleton), corresponds to a 200 μm stretch of biceps. In comparative terms these values may be scaled down by a further factor of 3 to allow for the greater length of the human muscle, thus making the stretch equivalent to about 70 μm amplitude for the cat. In the experiments on tremor (Cussons *et al* 1980) the average peak-to-peak movement was about twice as great (2 mm at the wrist). It may be noted that human spindles tend to fire at lower absolute frequencies than do cat spindles for comparable inputs (Vallbo, Hagbarth, Torebjörk & Wallin, 1979) so that on their own such small stretches would not be expected to cause them to fire at 100 Hz which was the frequency of vibration used in man, thus leaving scope for an augmentation of firing with vibration. In the human experiments the effects of vibration were found both when it was applied to the contracting biceps, acting as agonist, and when it was applied to the triceps acting as antagonist and showing no significant contraction. Especially in the latter case, where fusimotor activity may be presumed to be in abeyance (Vallbo *et al.* 1979), it would appear that the parameters both of the movement and of the vibration were of the right order of magnitude for the vibration to have augmented the spindle response to the movement. The present experiments thus support the previous suggestion that such augmentation provides an acceptable explanation for the observed effects of vibration on tremor and on the stretch reflex. In addition, the effect of vibration in delaying the 'phase' of the spindle response with regard to the movement might also be important.

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