Jitter in the muscle fibre

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Abstract
Direct stimulation of muscle fibres with a regular 10 Hz rate, three computer generated random rhythms and a sequence of voluntary discharges was used to quantify the interdischarge interval (IDI) dependent jitter due to velocity recovery function (VRF). This jitter was found to depend on conduction time and strength of VRF, but not on propagation velocity. The results suggest that in the usual jitter study in voluntarily activated muscle fibre pairs, with moderately irregular discharge rate and interpotential intervals below 3 ms the IDI dependent jitter contributes on average less than 10 μs, but can be so large as to produce false abnormal values at more irregular rates, longer interpotential intervals and pronounced differences in VRF. It can be effectively removed by mathematical algorithms or, better still, by using electrical stimulation.

The jitter between the action potentials of two muscle fibres of a voluntarily activated motor unit, as recorded in the standard way by single fibre electromyography (SFEMG), represents the variability of difference in time taken in the two impulse pathways from the branching point of the two axonal twigs to the electrode recording the action potentials from the two muscle fibres. Thus, it reflects variability of time needed for impulse conduction along the respective axonal twigs, for transmission across the motor end-plates and for propagation of muscle fibre action potentials from the two motor end-plates to the electrode. It has been shown that, except in some specific circumstances, the variability of time taken for neuromuscular transmission at the motor end-plate accounts for most or nearly all of the jitter. This has made the measurement of jitter a valuable method of quantitative estimation of reliability of neuromuscular transmission in a variety of clinical and experimental settings, particularly when it became apparent that even normal jitter values reflect the safety factor of the motor-end plates. Indeed, at regular discharge rates such as with electrical stimulation, it has been shown that variability of conduction time along the muscle fibres, at least when expressed as mean consecutive difference (MCD) of latencies, contributes very little, less than 5 μs. Moreover, it has been estimated that the jitter in the axonal twigs is of a similar, if not smaller, magnitude, unless there is pathological conduction, as in partly demyelinated or quite recently regenerated axons, or newly grown axonal sprouts. However, propagation velocity in the muscle fibres has been found to change with the length of preceding interdischarge intervals due to the so-called propagation velocity recovery function (VRF). Its impact upon the measured jitter depends on the degree of irregularity of interdischarge intervals (IDI) and the difference in conduction times along the two muscle fibres (mean interpotential interval, MIPI). In practice, the "myogenic" part of the jitter is minimised by asking the patient to keep the discharge rate as constant as possible by means of auditory and visual feedback, and by excluding potential pairs with MIPI exceeding 4 ms. This limit, based on calculations and actual measurements was set as a practical guide and was supported by experiments with electrical stimulation. The effect of the myogenic jitter was also reduced mathematically by calculating mean consecutive differences on data sorted in increasing order of interval to preceding discharge (mean sorted-data difference, MSD). This study was undertaken with the aim of further quantifying the myogenic contribution to the jitter as measured in the standard way in voluntarily activated muscle fibre pairs.

Method
Direct electrical stimulation of muscle fibres was used to measure precisely the variability of conduction times along the muscle fibres, the "myogenic jitter", uncontaminated by the motor-end plate jitter.

A pair of fine monopolar needle electrodes, made of 0.2 mm tungsten wire insulated to about 1 mm from the tip, or similar, slightly thicker steel needle electrodes (Medelec MF37) were inserted perpendicularly into the extensor digitorum communis or extensor carpi radialis longus, about mid way from elbow to wrist. The distance between the anode and the cathode was 7–10 mm, and their insertion sites were at right angle to the course of the muscle fibres. A position of the cathode was then sought from which stimuli (rectangular pulses of 50 μs at 3 or 5 Hz) already at amplitudes as low as 0.5–9 V produced small twitches, visible as a rule only as fine jerking of the stimulating cathode. The anode occasionally had a lower threshold, in which case stimulus polarity was changed and that electrode was used as a cathode. Stimulation at such low threshold sites was not only con-
considered helpful in obtaining responses of just a small bundle of muscle fibres and thus allowing for selective recording from single muscle fibres but also essential in assuring a stable starting point for the muscle fibre action potential. This was proved by the fact that its latency remained unchanged when stimulus amplitude was varied over a range of values, up to double or triple the threshold. The responses which showed progressive uniform or stepwise latency shortening on increasing stimulus strength were discarded. For the actual measurement, the stimulus amplitude was adjusted well above the threshold, and its adequacy was tested before each recording sequence by determining the threshold value and raising the amplitude again well above it.

Recording was made with a single fibre EMG electrode (Medelec SF25), inserted 5-15 mm proximally to the stimulating cathode in the presumed direction of the muscle fibres. The high pass filter was set to 2000 Hz, to make the baseline more stable and to minimise the possible interference of the action potentials from distant muscle fibres. Both stimulation and recording were done with a Medelec Mystro electromyograph.

The following stimulation rhythms were used: (1) regular at 10 Hz, (2) three computer-generated random sequences with a mean value of 10 Hz and ranges of 2 Hz, 3 Hz and 5 Hz, and (3) a sequence of voluntary discharges at a mean of 10.5 Hz, range 5 to 17 Hz. The latter was taken from SFEMG recording of a voluntarily firing motor unit in the EDC muscle of one of the subjects, when she produced a moderately irregular discharge rate around an average of 10 Hz, using a rate meter for visual feedback. The computer-generated random sequences had a rectangular distribution, while the “voluntary” sequence contained typical short trends (fig 1). The computer-generated random sequences as well as the voluntary rhythm were recorded on tape which was used to externally trigger stimulation and recording on the EMG machine. Each of the five sequences contained 501 consecutive triggering pulses and they were identical for all subjects and all the tested muscle fibres.

The SFEMG signals were fed to a Tektronix 565 oscilloscope whose second sweep was used to select the time window with the studied muscle fibre's action potential, and to set the voltage level at which latency readings were taken, monitored by Z-modulation of the sweep. The mean latency, mean interdischarge interval, mean consecutive difference (MCD) and mean sorted-data difference (MSD) were computed by a microprocessor-based jitter-meter for 10 series of 50 responses for each of the stimulation sequence. The resolution of time measurement was 0.1 µs, however, the measurement system in this configuration had its own technical jitter of 2.5-3.0 µs, mainly due to the slight variability of time taken for external triggering of the digital electromyograph. Each of the random sequences was immediately preceded by a repeated regular sequence, to confirm the constancy of recording conditions and the adequacy of stimulus amplitude and to assure equal preceding activity for all testing sequences. In each sequence, computation was started after about one second of stimulation to exclude from the result the brief initial shortening of latency of the responses to the first few stimuli. Means of 10 series were computed, each value thus representing 501 consecutive responses for each sequence. The advantage of breaking the computation in 10 series of 50 responses was that it allowed a better control of the quality of data. Some of the sequences were repeated, with closely similar results.

Seven healthy young females volunteered as subjects. Two to five muscle fibres were studied in each. An effort was made to collect muscle fibres responding at latencies between 0.5 and 5.0 ms, by trying different distances between the stimulating cathode and the recording SFEMG needle, as well as sampling muscle fibres with different latencies from fixed individual stimulation and recording sites. Approximate propagation velocity was calculated for each muscle fibre from the conduction distance and the latency.

Results

Thirty three different muscle fibres were studied, with all experimental sequences completed in 26. The results are shown in table 1 and fig 2.

The jitter on regular stimulation at 10 Hz was within the expected limits, that is, less than 5 µs, mean for the 33 fibres being 3.4 µs. This includes the technical jitter of the system which was 2.5-3.0 µs, so the real latency variation was below 1 µs (when checked with an analogue EMG machine it was below 0.7 µs for all muscle fibres). At the beginning of a series of stimuli there was usually some shortening of the latency for the first few responses (which
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Latencies, propagation velocities and jitter in the different stimulation sequences for the 33 muscle fibres studied

<table>
<thead>
<tr>
<th>Latency (ms)</th>
<th>Propagation velocity (m/s)</th>
<th>10 Hz reg. MCD</th>
<th>10 Hz vol. MCD</th>
<th>10 Hz</th>
<th>10 Hz</th>
<th>10 Hz</th>
<th>10 Hz</th>
<th>10 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>0-5</td>
<td>2-5</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Maximum</td>
<td>4-4</td>
<td>10-0</td>
<td>5</td>
<td>5</td>
<td>25-21</td>
<td>35-28</td>
<td>73-66</td>
<td>41-46</td>
</tr>
<tr>
<td>Mean</td>
<td>2-3</td>
<td>4-9</td>
<td>3-4</td>
<td>3-4</td>
<td>9-0</td>
<td>10-4</td>
<td>13-7</td>
<td>13-3</td>
</tr>
<tr>
<td>SD</td>
<td>1-0</td>
<td>1-7</td>
<td>0-5</td>
<td>0-6</td>
<td>5-4</td>
<td>5-0</td>
<td>8-7</td>
<td>6-4</td>
</tr>
<tr>
<td>Number of fibres</td>
<td>33</td>
<td>32</td>
<td>33</td>
<td>33</td>
<td>31</td>
<td>28</td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2 MCD in 33 muscle fibres, directly stimulated with different stimulation sequences. The bars indicate range, circles median values and crosses median + 1 SD.

was excluded from computation) and this was followed by a period of rather stable latency. The irregular rhythms resulted in an increase in the jitter, which was greatest for the random 10 ± 5 Hz sequence. The increase in the jitter was much less marked with the “voluntary” rhythm, which contained trends, that is, brief sequences of increasing, or less often, decreasing rates (fig 1). There was a highly significant correlation between the increase in jitter and the latency of the response of the stimulated muscle fibres (fig 3). An example of a recording is shown in fig 4.

Fibres with different propagation velocities, as computed from latency to the positive peak of the action potential and distance between the stimulation and recording electrodes, ranging from 2-5 to 10 m/s were tested and found to behave in a similar way: there was no correlation between the propagation velocity and the size of the myogenic jitter. Thus, at a given latency, both fastest and slowest fibres showed a similar increase in the jitter.

For the computer-generated random sequences, the mean MSD values for all muscle fibres were rather close to those of MCD. On the other hand, in the “voluntary” sequence the MSD value was significantly higher than MCD.

In the course of the protocol, when responses to at least 4000 stimuli were measured and an additional 2000–3000 stimuli were presented at 10 Hz during the initial adjustment of the recording position and between the individual sequences, the latency of 21 out of the 29 muscle fibres gradually increased. The mean increase was 16%, in 6 muscle fibres it exceeded 25%, and the extreme value was 41% in one muscle fibre, which corresponded to a decrease from an initial 3-6 m/s down to 2-1 m/s. The increase in latency was smooth and initially slow, but became progressively faster towards the end of the protocol. In a few fibres it was rather fast from the beginning and became extreme enough to prevent the completion of the protocol. The latency prolongation was associated with progressive reduction of action potential amplitude and an increase in its duration. The increase in latency was not associated with any increase in the MCD on regular stimulation. These changes were not

Figure 3 MCDs of individual muscle fibres at different stimulation rhythms plotted against their latencies. Correlation coefficient (r) and level of significance (p) are included.
reversible by increases in stimulus amplitude. In three fibres the latency change was less than 1%, and five behaved in a reverse fashion, their latency becoming progressively shorter by up to 13%, mean 12%.

**Discussion**

When jitter measurement is done in the standard way, that is, between two muscle fibres of a voluntarily activated motor unit, there may be a myogenic contribution due to VRF in the two muscle fibres. VRF will affect the result if (1) the discharge rate is non-uniform, (2) its variability has a different impact on the two muscle fibres. The latter will happen either if velocity recovery function for the two fibres or their conduction times are different, or both. The conduction times may be different due to different lengths of the muscle fibre segments from the motor end-plates to the recording electrode and/or due to different propagation velocities in the two muscle fibres. Discharge rate during voluntary activity, even when an effort is made to maintain it steady, varies usually by about 10–20% in either direction around the mean, which at 10 Hz amounts to 1–2 Hz, that is, between about 80–90 ms and 110–125 ms in terms of IDIs, enough for the successive action potentials to find the muscle fibre at different levels of supernormality. VRFs of different muscle fibres are known to differ within the same muscle, and even within the same motor unit. In voluntarily activated muscle, the jitter is studied between muscle fibres with different conduction times producing sufficient delay of the second potential to allow undisturbed measurement. The typical delays (MIPIs) vary from 0.5 to 2 ms, with occasional larger values, especially in certain kinds of pathology. Thus a mean MIP1 value for extensor digitorum communis muscle was 0.65 ms in normal subjects, 0.66 ms in myasthenic patients, 1.58 ms in ALS patients and 3.68 ms in patients with limb-girdle dystrophy (Stålberg, unpublished material).

The aim of this study was to estimate the size of the myogenic jitter under circumstances of jitter study in voluntarily activated muscle, by simulating the IDI irregularity during direct muscle fibre stimulation.

When a muscle fibre is activated directly, bypassing the motor end-plate, as with electrical stimulation, there is very little variation of the latency (jitter), provided that stimulus strength is well above the threshold and stimulation rate is regular. After a brief initial shortening of the latency of the first few responses in a series following a pause, which is due to rapidly accumulating supernormality of propagation velocity, there was a stable period with very small jitter and little change in the latency, lasting several minutes at 10 Hz in a large majority of muscle fibres. In a proportion of muscle fibres, the stable period was much shorter and was followed by gradual increase in latency, associated with a progressive decrease of amplitude and increase in duration of the action potential. These changes were smooth, but their rate often progressively increased. They reflect the well known progressive decrease of propagation velocity in the muscle fibres during continuing activity and are a result of progressively accumulating supernormality. These changes were observed in 21 out of the 29 muscle fibres studied at 10 Hz, but their extent and rate of development were quite variable. They were at least partially reversible by introducing a rest period of a few seconds or minutes, but not by an increase in stimulus strength, therefore they could not have been due to the excitability recovery function (ERF) of the stimulated point on the muscle fibre. The jitter measured as MCD of the latencies was not influenced by the gradual prolongation of the latency. Three of the studied muscle fibres showed no appreciable change in propagation velocity, while a further five exhibited a significant progressive increase. Fibres with decreasing and increasing velocities could coexist in the same fascicle and in the same recording sequence. The nature of the increasing velocity was not studied.

The MCD values on regular stimulation at 10 Hz were between 3 and 5 µs, however, after subtraction of the technical jitter in the measuring system most of the values fell below 1 µs and all below 2 µs. The low jitter was the obligatory criterion to distinguish between direct stimulation of the muscle fibres and stimulation of the intramuscular motor axons. As observed in this as well as some previous studies, the jitter of action potentials with longer latencies (5 ms and above), either due to longer segments of muscle fibres involved or due to slower velocity in thin muscle fibres, is not greater than that in fibres with short latencies, and remains small in dystrophic as well as denervated muscle fibres.

The irregular rhythms produced a significant increase in the jitter, different for different muscle fibres, but correlated to the degree of IDI variation, except for the "volun-
myogenic jitter activity were and latter, not but, short should remove tary" rhythm in which the increase was relatively less marked, being similar to the 10 (3) Hz sequence. There was a highly significant positive correlation between the jitter in the irregular sequences and the latency, that is, conduction time in the muscle fibres. On the other hand, the velocity itself did not seem to be a factor of any significance.

The magnitude of the myogenic contribution to the jitter due to irregular IDIs, as suggested by these results, may seem to be somewhat larger than expected in voluntarily activated muscle fibre pairs with IPI under 4 ms. However, the IDI irregularity in the computer-generated sequences is non-physiological, containing sharp transitions between extremes, while the irregularity in the voluntary sequence is exaggerated, perhaps similar to that in a difficult patient. The voluntary rhythm in this study produced a considerably smaller myogenic jitter than the computer-generated random sequence with similar range of IDI variation. The physiological IDI variation contained some smooth transitions from long to short intervals (trends), the effect of which was compensated for by MCD. At latencies below 2 ms, the mean myogenic jitter was 8.5 µs, after subtraction of the technical jitter about 7 µs. The corresponding value for latencies between 2 and 3 ms would be about 8 µs. It could be concluded that a similar myogenic jitter may be expected in voluntarily activated action potential pairs with IPI of 1–2 ms and 2–3 ms, respectively. However, with smaller variability in discharge rate than in the tested sequence, which is the rule in most clinical studies, the myogenic jitter would be even smaller. For statistical reasons, its effect is further reduced when combined with the random end-plate jitter. It is thus reasonable to assume that in the usual circumstances of a clinical jitter study the average net effect of the myogenic jitter should not exceed about 5 µs, which is a negligible artifact in clinical work. It should, however, be remembered that even in these circumstances the myogenic jitter in an occasional potential pair may be considerably larger and may actually reach the range of a normal motor end-plate jitter. Exaggerated differences in the VRF, long MIPIs and erratic discharge rates in pathology, especially in muscle disorders, may be expected to produce a very significant myogenic jitter, pushing a normal or even low jitter value beyond the upper normal limit.

MSD in this study failed to extract much of the myogenic jitter due to random IDI variation. The MSD values were surprisingly close to the MCD values in the random sequences and were higher in the voluntary sequence. Irregularity of discharge rate during voluntary activity contains both random changes and short trends; MCD effectively compensates for the latter, but is not immune to the effect of random IDI changes. MSD, on the other hand, should remove these IDI changes but, being computed from reordered data, it loses immunity to trends. Thus it is not unexpected that MSD values were higher in the voluntary sequence. However, even in the random sequences MSD did not perform well; this may be due to the fact that not only the immediately preceding IDI but also the history of one or more previous IDIs determine the velocity for the succeeding action potential. In the random sequence of a rectangular distribution including rapid alternation of extremes the contribution of the previous history may be exaggerated, as can be seen from fig 4. The very slow trends due to progressive changes in propagation velocity constitute another reason for the relative inefficiency of MSD to compensate for the myogenic jitter. These changes were evident in most of the muscle fibres studied.

Davies et al recently proposed a computer algorithm for reducing the myogenic jitter, or, as they called it, the velocity recovery function artifact. The algorithm is designed to be used on voluntarily activated muscle fibre pairs and, when tested on muscle fibres stimulated either directly or through their axons at regular and pseudo-random rhythms, it was shown to effectively remove the pseudo-random component of jitter due to VRF in the muscle fibres. The study of over 100 pairs in normal extensor digitorum communis muscle, however, showed negligible disparity between the jitter as measured in the original way and the corrected jitter.

In practice, the elimination of the IDI dependent component of jitter becomes important in the following two situations: (1) when it is large enough to push the measured value outside the normal range; and (2) when it obscures the low jitter between branches of split muscle fibres. The latter may present a particularly difficult task for computer methods, for example, in cases of long IPIs between the two components of a split fibre, which are not unusual (such as in muscular dystrophies) and at high and irregular firing rates including trends and pauses. To unmask the low jitter of a split fibre, the error of correction must be very small, not exceeding 2–3 µs. Indeed the incidence of split muscle fibres in muscular dystrophies, detected by using the algorithm already mentioned, seemed to be significantly lower than in another study using electrical stimulation.

It should also be noted that not all IDI dependent component of the jitter is necessarily due to VRF in the muscle fibre. A part may occur in the axonal branches, particularly when abnormal and at the motor end-plate, due to rate dependent changes in quantal acetylcholine release. Another source of a potentially large myogenic jitter, which is often not realised, is the disruption of the discharge rhythm due to intermittent blocking, either at the motor end-plate or in the axon, and due to extra-discharges (Trontelj et al, in preparation).

It seems therefore that when exact information is needed about the motor end-plate jitter, uncontaminated as far as possible by the myogenic jitter, as in some experimental situations, electrical stimulation of the motor axons is preferable to voluntary activation. Even this
method, however, is not quite immune to the myogenic jitter: there is IDI related jitter due to intermittent blocking, extra-discharges and recurrent (F) responses of the muscle fibre studied, all of which disturb the regularity of the rhythm. The F-responses are, however, infrequent, often following less than 5% of direct responses, and this source of artifact is much less significant than some other possible pitfalls with this method (Stålberg and Trontelj, in preparation). For clinical purposes, however, jitter measurement in the standard way is accurate enough, provided that the caveats mentioned are respected. The exactness of measurement can be improved by using a computer algorithm to extract the IDI dependent jitter\textsuperscript{3} or, even better, by resorting to electrical stimulation of the motor axons.\textsuperscript{4,5}

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