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Guidelines for single fiber EMG

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HIGHLIGHTS

- This paper is an IFCN-endorsed Guideline written by international experts.
- It reviews the current status of single fiber EMG (SFEMG) and the measurement of jitter with concentric needle electrodes.
- It presents pitfalls of measuring jitter with single fiber and concentric needle electrodes.

ABSTRACT

This document is the consensus of international experts on the current status of Single Fiber EMG (SFEMG) and the measurement of neuromuscular jitter with concentric needle electrodes (CNE – CN-jitter). The panel of authors was chosen based on their particular interests and previous publications within a specific area of SFEMG or CN-jitter. Each member of the panel was asked to submit a section on their particular area of interest and these submissions were circulated among the panel members for edits and comments. This process continued until a consensus was reached. Donald Sanders and Erik Stålberg then edited the final document.

Abbreviations: μm, micrometer; μSec, microsecond; μV, microvolt; AAEM, American Association of Electrodiagnostic Medicine; ALS, amyotrophic lateral sclerosis; AP, action potential; ASFAP, apparent single fiber action potential; Cm, centimeter; CMAP, compound muscle action potential; CNE, concentric needle electrode; CR, cervical radiculopathy; ED, extensor digitorum; FD, fiber density; GBS, Guillain-Barré syndrome; IDI, interdischarge interval; IPI, interpotential interval; LEM, Lambert-Eaton myasthenia; mA, milliamp; MCD, mean difference of consecutive differences; MG, myasthenia gravis; MISI, mean interspike interval; mm, millimeter; MMN, multifocal motor neuropathy; Ms, millisecond; MSD, mean difference of sorted differences; MU, motor unit; MUP, motor unit potential; MuSK, muscle specific tyrosine kinase; Na+, sodium; NMT, neuromuscular transmission; OO, orbicularis oculi; PV, propagation velocity; R2, late component of the blink reflex; SFAP, single fiber action potential; SFE, single fiber EMG electrode; SFEMG, single fiber EMG; SPACE, Stimulated potential analysis using concentric needle electrodes; TA, tibialis anterior; VRF, velocity recovery function.

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Contents

1. Introduction and background

When beginning their study to quantify muscle fatigue, Ekstedt and Stålberg developed a multielectrode to record action potentials from single muscle fibers (SFAPs), inspired by an electrode used by Buchthal et al. (1957). The criteria for a SFAP were a fast-rising positive-negative spike of constant shape at consecutive discharges (Ekstedt, 1964). Variations in the timing of SFAPs produced the "jitter phenomenon" (Ekstedt and Stålberg, 1965), which was attributed to variations in the time at which muscle action potentials are initiated at the motor end-plate. This multielectrode was also used in the analysis of propagation velocity of individual muscle fibers (Stålberg, 1966). Development of the SFEMG technique required novel technical features, some of which, such as signal trigger and signal delay, have become standard in current EMG equipment.

During its development, SFEMG was found to be remarkably sensitive in detecting disturbed neuromuscular transmission (NMT) and the method was introduced into clinical use to identify myasthenic disorders (Ekstedt et al., 1969). SFEMG also demonstrated the activity-dependent decrease in muscle fiber propagation velocity (Stålberg, 1966) that explains changes in the frequency spectrum of EMG signals that is used in the assessment of muscle fatigue (Lindström et al., 1970), and short-term variability in the propagation velocity along muscle fibers during activation, the so called "velocity recovery function (VRF)," which will be discussed later. The SFEMG technique also led to the development of new concepts about motor unit organization, particularly by means of the Fiber Density (FD) parameter (Stålberg et al., 1976a).

The SFAP has also been used as a marker of active MUs, such as in spike-triggered averaging of surface signals, Macro EMG (Stålberg, 1980a), Scanning EMG (Stålberg and Eriksson, 1987), reflex studies at the single cell level (Trontelj, 1968), studies of central pathways acting on single cell final common pathways (Zidar et al., 1987), and in assessing conduction velocity in single axons (Padua et al., 2011). These subtopics will be briefly discussed later (See Other Uses for SFEMG, below).

SFEMG recordings may be performed either during slight voluntary contraction or during near-nerve intramuscular or surface stimulation of the motor axon. Both methods can be used to assess NMT, but they differ in a number of aspects, which will be discussed later.

Most current EMG machines have software that supports the application of SFEMG. Although they differ in some technical features, all record and analyze jitter. The nomenclature and quantification principles (Ekstedt et al., 1974) have not changed since the technique was first presented, thus, it is now relatively noncontroversial to formulate standards for the quantification of SFEMG parameters.

The use of CNEs as a replacement for SFEMG electrodes (SFE) to measure jitter, which is a consequence of restrictions on the use of reusable material in many countries (Stålberg and Sanders, 2009), makes it necessary to consider new standards for signals measured with these electrodes.

Different filters are used when measuring jitter with CNEs, new definitions of accepted signals have been suggested (Stålberg and Sanders, 2009), and new reference values have been developed (Stålberg et al., 2016). This paper will focus on these aspects of jitter recordings. Although the title is "SFEMG," this paper will also include jitter analysis with CNEs, since this will be the routine for the foreseeable future.

1.1. The motor unit

The muscle fibers of a motor unit (MU) are randomly distributed within a restricted area of the muscle that is mainly circular in cross section in the middle of the muscle, becoming oval or irregular in the periphery of the muscle. The distribution within the muscle of fibers belonging to one MU has been demonstrated in animals (Edström and Kugelberg, 1968) and man (Brandstater and Lambert, 1969; Garnett et al., 1979) by repeatedly stimulating one motor axon to deplete its muscle fibers of glycogen, and by scanning EMG in healthy subjects and in pathology (Stålberg, 1980b). The abnormal distribution of muscle fibers reflects muscle or nerve disease and is a useful quantitative parameter in assessing pathology, as further described elsewhere (Stålberg et al., 2010).

2. Fiber density

The SFE has a small uptake area, within which action potentials (APs) from muscle fibers at some distance are recorded with lower amplitude than adjacent fibers. A practical definition of action potentials produced by single muscle fibers has been determined, namely spikes with amplitude >200 μV and rise time <300 μs, and a constant shape at consecutive discharges without notches or inflections. Signals meeting these criteria are usually generated by muscle fibers within 250–300 μm from the recording electrode (Gath and Stålberg, 1978). The FD technique and values discussed below apply only to recordings made with SF electrodes; fiber density cannot be measured with CN electrodes.

2.1. Recording procedure

The subject activates the tested muscle with slight voluntary contraction and the amplitude of the AP from one activated muscle fiber is made maximal by adjusting the electrode position. Note: this position is different from that used in jitter studies, where an electrode position is sought where more than one AP is recorded. One then counts the number of time-locked APs, including the triggering potential; thus, the lowest possible count at each site is 1. This is repeated at 20 different sites throughout the muscle and the mean of the 20 counts is the FD (Fig. 1).

2.2. Requirements for measuring the FD:

- Set the display trigger level as low as 200 μV to capture SFAPs.
- Use a signal delay of at least 5 ms while triggering the display on a stable spike component that meets the following SFAP criteria; this is not necessarily the first SFAP in the recorded signal.
- Use a sweep speed that is slow enough to allow visualization of at least 5 ms after the triggering potential. Adjust the sweep speed accordingly for signals of greater duration in order to see all time-locked components.
- Aim to record clean SFAPs.
- Use at least 2–4 separate skin penetrations. When moving to the next recording site, advance the electrode far enough to lose all previously recorded potentials.
- It is essential that the amplitude of one of the SFAPs be made maximal, i.e., one fiber must be approached as closely as possible by the recording electrode.

2.3. Definitions of acceptable signals for FD measurement

The following defining criteria of SFAPs for FD measurement have been derived, in part, from simulation studies:

- The amplitude should exceed 200 μV.
- The rise time should be less than 300 μs.
- The shape should be constant in consecutive discharges.
- If the spike contains a distinct "notch," even of low amplitude, this is considered to represent a separate SFAP with amplitude exceeding 200 μV, and counted as such. (Fig. 2)

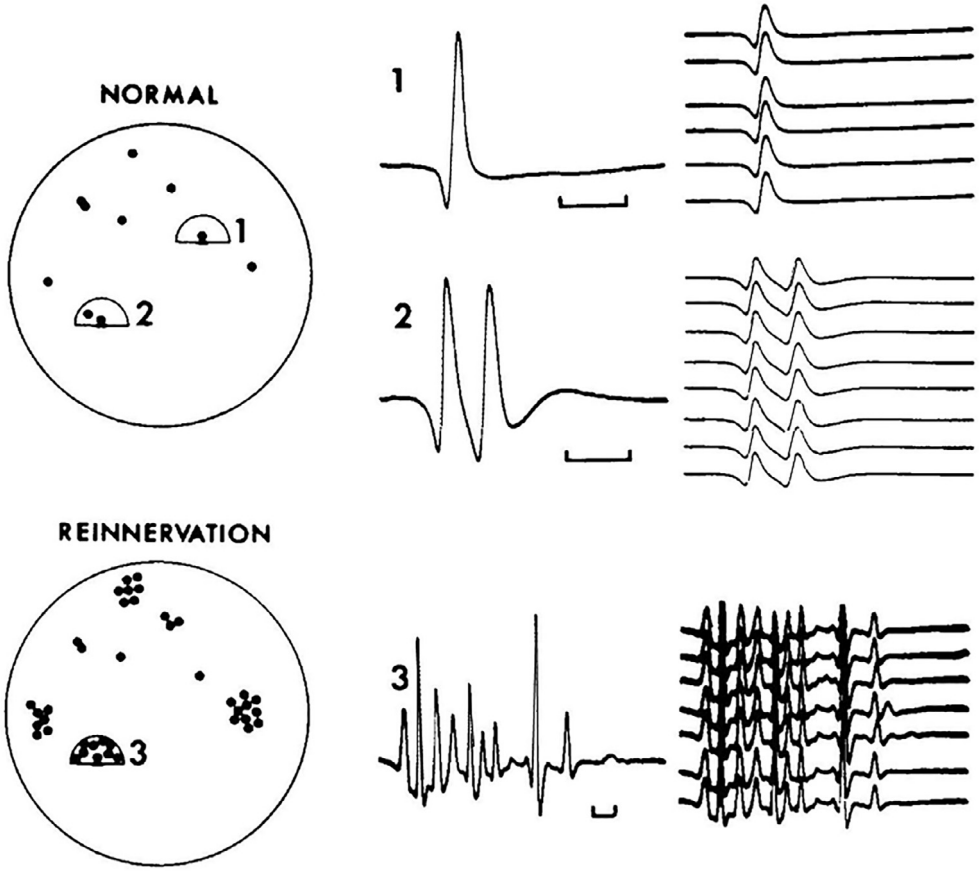


Fig. 1. Fiber density measurements in normal and reinnervated muscle. The diagram illustrates the number of muscle fibers of one motor unit (filled circles). Semicircles represent the uptake area of the recording electrode. In Normal, 1 and 2, only action potentials from one or two fibers are recorded. In Reinnervation and 3, action potentials from many fibers are recorded because of grouping of muscle fibers within the MU due to collateral sprouting. Reproduced from Stålberg et al, Single Fiber EMG, 2010, with permission from Edshagen Publishing House.

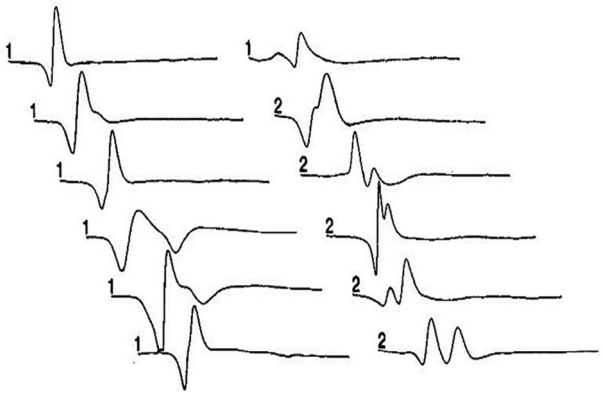


Fig. 2. SFEMG recordings that are classified as 1 or 2 fibers in FD measurements. Reproduced from Stålberg et al, Single Fiber EMG, 2010, with permission from Edshagen Publishing House.

- If a superimposing signal causes only slight distortion or instability in an otherwise smooth spike, it is considered too small to count as a separate SFAP (Fig. 2).
- Uncertainties may arise in any FD study; despite these, normal values obtained from different laboratories in a multi-center study are very similar (Gilchrist, 1992).

The FD is not a measure of the total number of fibers in an MU, but of the average number of fibers within the electrode pick-up area wherever there is at least one fiber belonging to that MU. In the normal extensor digitorum (ED), only a single AP from individual MUs is recorded in 65–70% of sites and two APs are recorded in about 30% of sites (Stålberg and Thiele, 1975b); this corresponds to an average of 1.5 fibers per uptake area of the electrode. Three or 4 fibers are seldom recorded in any site in normal muscles.

The FD increases with age – this is most pronounced in the tibialis anterior and ED, presumably the result of reinnervation associated with normal age-related neuronal drop-out (Fig. 3) (See Reference Values and Limits, below). There is no correlation between FD and the size of MUs as assessed by the amplitude of Macro EMG MUPs. This is presumed to indicate that larger MUs have larger territories rather than a higher concentration of fibers within a given territory (Stålberg, 1986). The reason for the slightly higher FD in young children is not clear.

2.4. Explanations for increased FD in nerve and muscle diseases

After reinnervation, the FD is increased due to collateral sprouting, and SFAPs from 3 to 10 fibers in the same MU may be recorded at one electrode position (Stålberg et al., 1975a). There is a correlation between histochemical fiber-type grouping and increased FD, however the FD value is a more sensitive index of reinnervation,

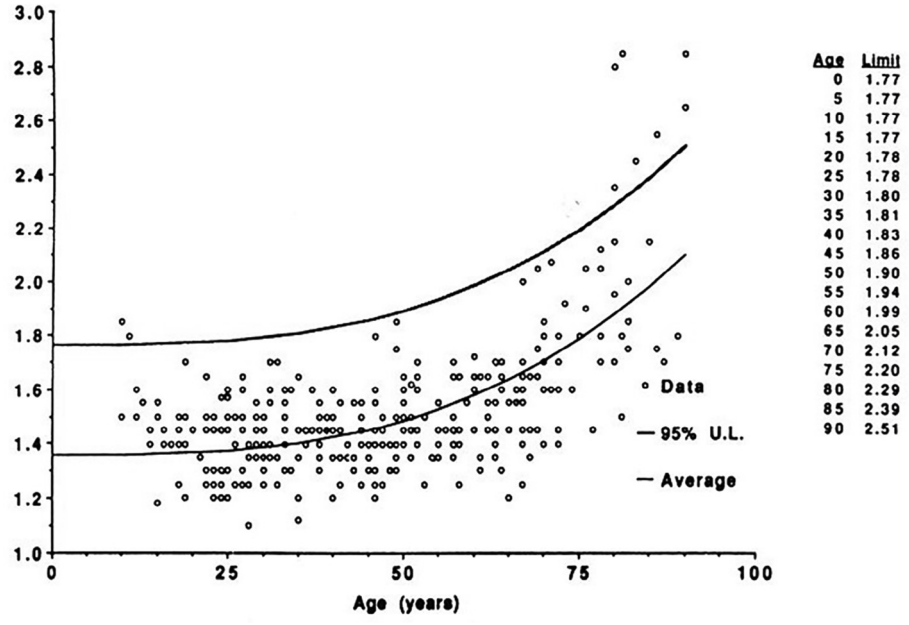


Fig. 3. Fiber density in the extensor digitorum at different ages. Mean and +95% confidence limits are indicated. Reproduced from Gilchrist et al, Single fiber EMG reference values: A collaborative effort. Muscle Nerve. 1992;15:151–61, with permission from Wiley Periodicals, Inc.

showing slight rearrangement within the MU even before fiber-type grouping is evident on muscle biopsy.

The FD is slightly or moderately increased in muscular dystrophies and other myopathies (Bertorini et al., 1994). Muscle fiber atrophy, as in disuse, does not increase the FD, since their small size makes the atrophic fibers weaker generators. The resultant smaller uptake area at the electrode counteracts the increase in concentration of fibers.

Possible causes for increased FD in myopathy (Fig. 4):

- Splitting of fibers, seen particularly in muscular dystrophies.
- Reinnervation after secondary denervation from local fiber necrosis.
- Denervation in late stages with fibrosis.
- Possible ephaptic activation of neighboring fibers, seen as increased FD but not necessarily as fiber type grouping in biopsies.

3. Voluntary jitter studies

Jitter recordings are made while the subject slightly activates the tested muscle. Time-locked signals from at least 2 muscle fibers are recorded. Time intervals between APs (interpotential intervals – IPIs) are measured either between points on two signals determined by the voltage level of each ("voltage level method") or between mathematically-defined peaks of the signals ("peak detection method") (Fig. 5). To minimize the effect of slow trends

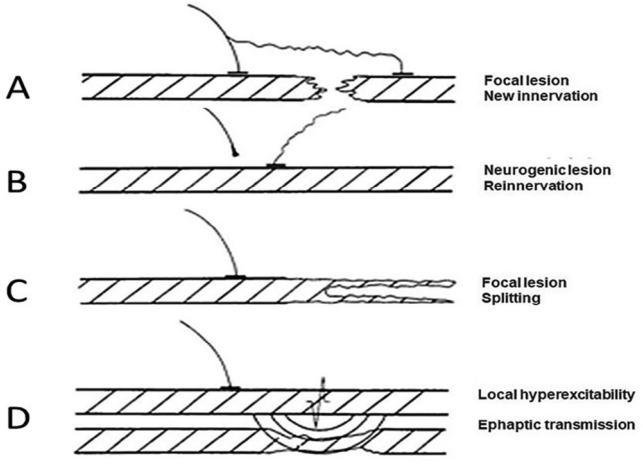


Fig. 4. Possible explanations for increased FD in myopathies. A – focal lesions with segmental necrosis produce "myogenic denervation" of the distal part of the muscle fiber, which is subsequently reinnervated by a sprout from the same or another motor axon. B – regeneration by satellite cells produce new muscle fibers which are innervated by sprouts of existing axons. C – branching or splitting of (hypertrophic) muscle fibers, each branch giving rise to an AP. D – muscle fibers are activated ephaptically by APs of adjacent muscle fibers. Reproduced from Stålberg et al, Single Fiber EMG, 2010, with permission from Edshagen Publishing House.

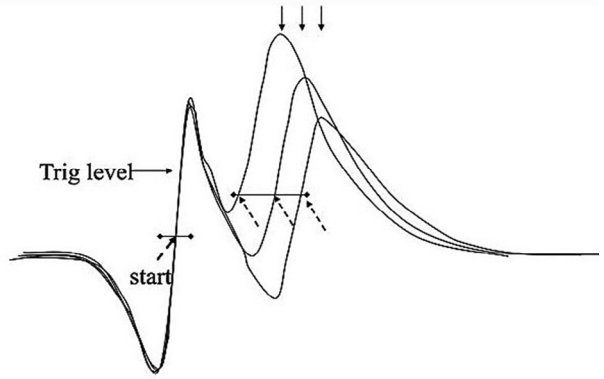


Fig. 5. "Riding potentials" showing the different points used for IPI calculations by the amplitude level and peak-detection techniques of time interval measurements. For both methods, the signal is extracted and displayed using an amplitude level trigger ("Trig level"). Dashed arrows indicate IPI start and stop points for the amplitude level method. These measurements are affected by the voltage level of the falling phase of the first spike and should not be used for jitter calculations. In the peak-detection method, IPI stop points (vertical arrows) are determined from peaks defined mathematically from the rising and falling phases of the spike and are relatively unaffected by the shape of the first spike. Reproduced from Stålberg et al, Pitfalls and errors in measuring jitter. Clin Neurophysiol;128:2233–41, 2017, with permission from Elsevier Ireland Ltd.

on the IPIs, IPI variability is calculated as the mean value of differences between consecutive IPIs (MCD). The IPI may also be affected by changes in the firing rate, due to the velocity recovery function; to minimize this effect, the jitter calculation is also performed after the IPI values are sorted based on the interdischarge interval (IDI value) (the mean sorted difference – MSD). In practice, we calculate both the MCD and MSD and report the lower value as the *jitter* value of that particular recording.

Optimally, jitter should be measured from 20 potential pairs during voluntary activation and 30 endplates for stimulation jitter (Stålberg & Trontelj, 1994). However, collaborative studies to define reference values for CN jitter have accepted 15–20 pairs from each muscle for voluntary activation and >20 spikes for stimulation studies (Kouyoumdjian and Stålberg, 2008; Stålberg et al., 2013; Stålberg et al., 2016; Abraham et al., 2017). To assure adequate sampling of the muscle, recordings should be made from different portions of the muscle using 3–4 skin insertions. MCD is usually computed from a series of 50 responses from each endplate. A full study in a muscle should include MCD values for 30–40 different endplates (20 signal pairs in a voluntarily activated muscle represents 40 endplates). A study of voluntary jitter showed that calculations from 60 to 100 consecutive traces give nearly the same jitter values, but fewer than 60 traces gave lower and variable values (Baslo et al., 2003). A stimulation jitter study reported that calculations from 80, 50 or even 20 consecutive traces showed no significant difference in mean MCD values (Patel et al., 2016). We prefer to measure jitter from at least 50 discharges and to record up to 100 discharges, which will permit deletion of some during editing, if necessary.

If the recording contains more than 2 spikes, jitter values are calculated between one of them, the triggering spike, and each of the others. Thus, for example, a signal with 4 spikes gives 3 jitter values for each triggering spike. (See Influence from the Triggering Spike, below.)

For voluntary signals with 3 or 4 spikes, we accept jitter values from all IPIs if all are normal or if only one is abnormal. If jitter is abnormal in more than one IPI in such recordings, we accept only the abnormal IPI with the lowest jitter value in order to avoid an excessive contribution from reinnervated MUs. (See Selecting abnormal intervals in multi-spike potentials, in Pitfalls in Measuring Jitter with voluntary activation, below.) This is uncommonly an issue in CNE recordings since the individual spikes in multi-spike signals rarely fulfill the ASFAP acceptance criteria. In stimulation jitter studies, jitter values in all acceptable spikes are included, provided they are stimulated supraliminally.

4. Stimulation jitter studies

For stimulation jitter studies, motor axons can be activated by electrical stimulation with a needle electrode inserted near the motor point within the muscle or with a surface or needle electrode near the nerve trunk. Selectivity is enhanced by close approximation of the stimulating needle cathode to the nerve trunk (Trontelj et al., 1988; Stålberg et al., 1992). For facial muscles, surface stimulation can also be used to find a low-threshold stimulation site in branches of the facial nerve for subsequent needle stimulation. Stimulation is delivered as 0.05 ms or less duration 2–3 Hz pulses, and the stimulus intensity is progressively increased in 0.1 mA steps until small muscle twitches are seen, usually at <10 mA stimulus intensity. The recording electrode is inserted into the twitching part of the muscle and positioned to record acceptable spikes.

For intramuscular nerve stimulation, a monopolar needle electrode is inserted close to a motor nerve twig near the endplate zone as the active stimulating electrode, and a subcutaneous or intramuscular monopolar needle reference electrode is placed a few cm lateral to the active stimulating electrode. The ED and tibialis anterior muscles are commonly tested in this way. The recording electrode is inserted within 2–3 cm of the stimulating electrode, usually distally in the muscle. Muscle fibers may be inadvertently stimulated directly with intramuscular stimulation, producing "jitter" less than 5 μs, which must not be included in the jitter calculations (Trontelj et al., 1986; Stålberg et al., 1992).

Extramuscular nerve trunk stimulation avoids direct muscle fiber activation but has the disadvantage of stimulating many motor axons, resulting in multi-spike recordings. In facial muscle studies this can be partially overcome by stimulating a facial nerve branch anterior to the ear or the more distal branches – the temporalis branch to the frontalis muscle or zygomaticus branch to the orbicularis oculi (OO) muscle – which contain few axons.

The recruitment order of MUs in stimulation studies is quite different from that of voluntary activation, thus different populations of muscle fibers are recorded with the two techniques: small MUs are initially activated with slight voluntary contraction (according to the size principle (Olson et al., 1968)), while electrical stimulation variably activates axons depending on their electrical excitability and distance from the stimulating cathode (Trontelj and Stålberg, 1983).

For jitter measurements in MG, use a 10 Hz stimulation rate, which approximates the normal physiologic firing rate. To get reliable results, it is essential to make the stimulus well suprathreshold for all accepted spikes (see Pitfalls in Measuring Jitter, below).

Jitter for stimulation studies is calculated as the variability in latency between the stimulus and accepted signals. In equipment with peak detection capability all peaks are analyzed simultaneously; in equipment without this capability, individual spikes are analyzed from stored signals.

Advantages of stimulation jitter studies include avoiding the IDI-dependent jitter that is produced by the VRF (Trontelj et al., 1986). However, the VRF effect does introduce spurious jitter when stimulation begins or the stimulation rate changes, or when there is any intermittent impulse blocking (Stålberg et al., 2017) (see Pitfalls in Measuring Jitter, below).

Jitter measured during axonal stimulation is less than that measured during voluntary activation because the contribution from only one endplate is being assessed. The theoretical relationship between these two values is expressed by the formula (Trontelj et al., 1986):

$$Mean\ MCD\ stim = Mean\ MCD\ vol/\sqrt{2},$$ or 71% of voluntary activation.

Stimulation jitter studies can be particularly useful in patients who have difficulty maintaining constant voluntary activation of the muscle; in movement disorders; in children too young to cooperate; or when it is desirable to control the firing rate, as when assessing the effect of the firing rate on jitter to distinguish between pre- and postsynaptic abnormalities (Trontelj and Stålberg, 1983; Sanders and Stålberg, 1996) (see Jitter in Lambert-Eaton myasthenia, below).

Disadvantages of stimulation studies are possible misinterpretation of the jitter produced by subliminal stimulation, and underestimation of jitter secondary to direct muscle fiber stimulation (Trontelj et al., 1986) (see Pitfalls in Measuring Jitter, below).

4.1. Specific recommendations for electrical activation

- Be sure that you are delivering suprathreshold stimulation for each measured spike.
- Do not analyze signals during the initial second after beginning activation or after any change in stimulation rate.

- For intramuscular stimulation, be aware of possible axon reflexes (see Pitfalls in Measuring Jitter, below).

5. Measuring jitter with concentric needle electrodes

The use of concentric needle electrodes (CNE) to assess MUP stability was reported by Payan, in 1978, who offered it as a bridge between two complementary disciplines (Payan, 1978). Although it is preferable to use SFE for measuring jitter, CNEs are now being increasingly used instead. The main reasons are concern about transmissible diseases such as bovine spongiform encephalopathy and the cost of SFEs.

The SFE recording surface exits through a port on the side of the cannula, a few millimeters back from the tip, and is circular with a 25 μm diameter = 0.0005 mm^2 area. Diameters of the smallest commercially available CNEs differ among manufacturers, and the recording surface area of the smallest ones with a 300 μm shaft diameter varies from 0.019 mm^2 to 0.03 mm^2, which is smaller than that of the 460 μm shaft diameter CNE used in routine EMG practice, but still much larger than that of the SFE. The CNE with the smallest recording area is recommended for jitter studies.

For SFE recordings, the filter settings are: 500 Hz high-pass and 10 kHz low-pass. Because the larger pick-up area of the CNE can record signals from more than one muscle fiber belonging to the same MU, the spikes recorded by this electrode frequently contain APs from more than one muscle fiber. To better define spike distortions produced by contributions from more than one AP, a high-pass filter of 1 or 2 kHz has been used for CNE jitter recordings, while using the same 10 kHz low-pass filter. We have used a high-pass filter setting of 1 kHz to maintain a balance between removal of slow-wave components, which makes recordings more selective, while preserving the main features of the signal. High-pass filter settings greater than 1 kHz will reduce the signal amplitude to a far greater extent and may introduce extra phases (so-called "ringing") that may be misinterpreted as APs. Although a 2 kHz high-pass filter was used in initial reports (Ertas et al., 2000; Patel et al., 2016), for both theoretical and practical reasons we recommend a 1 kHz high-pass filter for CNE jitter recording (Stålberg and Sanders, 2009).

Because spikes from CNE recordings may be produced by more than one muscle fiber, those acceptable for jitter recordings are referred to as "apparent single-fiber action potentials (ASFAPs)" (Ertas et al., 2000). CNE signals are smaller than those recorded with SFEs because of the effect of the higher high-pass filter setting (1 kHz vs 500 Hz), among other things. Different reported CNE jitter studies used amplitude criteria of 50 μV, 100 μV or 200 μV, but it is better not to use strict amplitude criteria, instead, acceptable ASFAPs should be clear solitary spikes with a fast-rising slope to a well-defined negative peak with a constant shape in consecutive discharges (Fig. 6). If an amplitude level technique is used to measure jitter, the time between the triggering signal and individual ASFAPs should be at least 150 μs to avoid having one signal riding on the other (Fig. 5) (see "Measurements from riding signals," in Pitfalls in Measuring Jitter, below.).

As with the SFE, the CNE is inserted at an angle into the muscle (like "holding a pencil") and positioned as close as possible to individual muscle fibers. Although one to three insertions may be enough to collect 20 ASFAP pairs during voluntary activation, more than one insertion point should be used because more APs are within the recording territory of the larger CNE and their frequent interference with each other makes it harder to record clear ASFAPs.

As with SFE recordings, aim to analyze at least 50 traces with acceptable ASFAPs for CNE jitter analysis. The total number of ASFAP pairs recorded in any muscle should ideally be 20, preferably 15 or more, but not less than 10.

Because the shaft diameter of the CNE is smaller and the tip is sharper than the SFE, the CNE is easier to insert into the muscle, less painful and better tolerated. However, it is not as easy to "aim" the CNE at the closest muscle fiber, thus it requires more time to position, and the CNE does not maintain a constant position in the muscle as well as does the SFE.

The reported sensitivity of CNE and SFE jitter studies in myasthenia gravis (MG) with either voluntary or stimulation activation are virtually identical (Tables 1 and 2), as were the results of SFE and CNE stimulation jitter studies performed in the same muscle in normal subjects and patients with MG (Ertas et al., 2000).

Several reports have concluded that monopolar needles electrodes are suitable for determining volitional jitter, using either a 1 kHz or 3 kHz high-pass filter (Buchman and Garratt, 1992; Tutkavul et al., 2006; Tutkavul and Baslo, 2010; Oliveira Santos et al., 2018). However, the amplitude of signals recorded with a 3 kHz high-pass filter become quite small while the amplifier noise is unchanged, leading to a lower signal:noise ratio. Also, reference

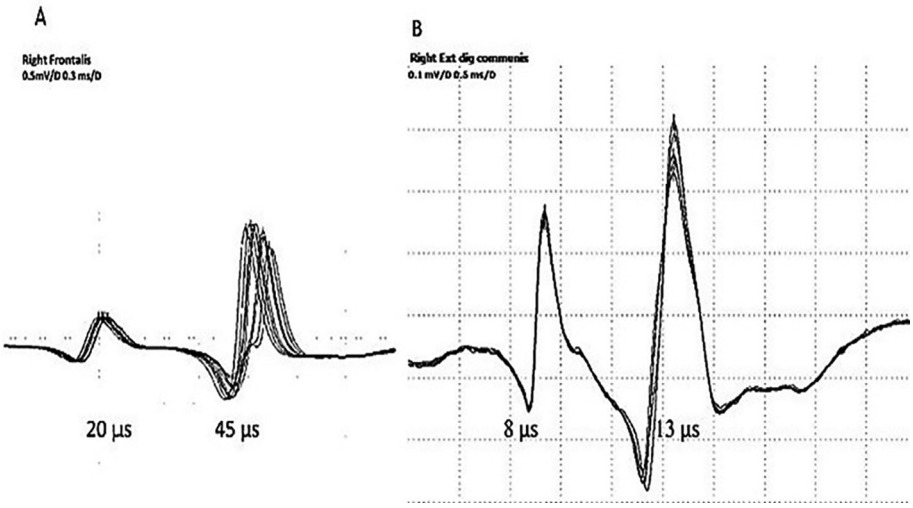


Fig. 6. Spike summation, concentric electrode recording during electrical stimulation. A – the first spike with parallel rising phases is acceptable as an apparent single fiber action potential, but not the second. B – the first spike is acceptable, but the second spike is produced by summation of more than one action potential, which produces a slight variation in signal shape in consecutive responses that is easily seen when the signals are superimposed. Thus, this spike is unacceptable. Reproduced from Stålberg et al, Pitfalls and errors in measuring jitter. Clin Neurophysiol;128:2233–41, 2017, with permission from Elsevier Ireland Ltd.

Table 1
Reported Sensitivity of SFEMG in Myasthenia Gravis.

Reference	Population Studied	Diagnostic Criteria	Muscle	Activation	MG Class	Sensitivity	Specificity
Nogues et al. (1991)	MG N = 70	Clinical, response to treatment	ED	Stim	Ocular Mild GMG Moderate GMG Severe GMG	72% 95% 93% 100%	ND
Oh et al. (1992)	MG N = 120	AChR antibody, or clinical plus RNS and/or response to ch'ase inhibitors	ED	Vol	OMG, Mild GMG Severe GMG	89% 100%	ND
Milone et al. (1993)	OMG (treated) N = 14	AChR antibody, or clinical plus RNS and/or Tensilon test	OO	Vol	OMG	93%	ND
Sanders and Stålberg (1996)*	MG (Untreated except those in remission) N = 788	AChR antibody, or clinical plus response to ch'ase inhibitors and/or RNS.	ED	Vol	Remission (Treated) N = 21 OMG N = 107 GMG N = 660	52% 61% 88%	ND
	MG (Untreated) N = 362		ED + Front	Vol	ED Front Both Neither	76% 93% 72% 4%	ND
Valls-Canals et al. (2003)	OMG N = 20	AChR antibody, or clinical plus RNS and response to ch'ase inhibitors	Front OO	Stim	OMG	90% 100%	ND
Cui et al. (2004a)	OMG N = 90	AChR antibody, or clinical plus RNS	ED Front	n/a	OMG	61% 83%	ND
Katzberg and Bril (2005)	OMG (treated) N = 121	Clinical, response to treatment	Front (OO in 2)	Vol	Mild OMG Moderate OMG Severe OMG All	93% 96% 100% 95%	ND
Hiroko et al. (2007)	OMG N = 16	n/a	Front OO	Vol	OMG	Front 62% OO 50% Either 81%	ND
Padua et al. (2014)	Suspected MG N = 100	AChR or MuSK antibody, or clinical plus RNS and/or response to ch'ase inhibitors	OO	Vol	OMG GMG All	97% 100% 98%	70%
Mercelis and Merckaert (2011)	MG N = 456	AChR antibody or clinical plus response to treatment	OO	Stim	MG	80%	97%
Morren et al. (2016)	Suspected MG N = 348	AChR antibody, or clinical plus Tensilon test or RNS.	ED, Front or OO	Vol	OMG GMG All	73% 85% 78%	91%
Lo et al. (2017)	MG N = 99	AChR antibody, or clinical plus response to ch'ase inhibitors and/or treatment. AChR antibody plus clinical	OO	Stim	OMG + GMG	97% 94%	21% 17%

AChR – acetylcholine receptor; ch'ase – cholinesterase; Clinical – fluctuating weakness by history and examination; front – frontalis muscle; GMG – generalized myasthenia gravis; n/a – not available; ND – not done; OMG – ocular myasthenia gravis; OO – orbicularis oculi; stim – stimulation; vol – voluntary.
* J.F. Howard and J.M. Massey contributed data in this report.

jitter values may be significantly lower with this filter setting (Tutkavul et al., 2006). The reported experience with jitter measured with monopolar electrodes is quite limited.

6. Jitter in myasthenia gravis

6.1. Sensitivity and specificity of jitter measurements in MG

A number of papers have reported the sensitivity of jitter studies in MG, and a small number have also addressed their specificity (Tables 1 and 2). Measuring the sensitivity of a diagnostic test requires that it be compared with a "gold standard" diagnostic criterion that is independent of the test in question. Tables 1 and 2 summarize the results of reports in which jitter measurements were not used to confirm the diagnosis.

Another factor to be considered is the specific muscles and number of muscles in which jitter was tested. Most studies report the results of jitter testing in the same one or two muscles in all patients, even though no one muscle or combination of muscles is more likely to be abnormal in all MG patients.

It also must be recognized that NMT is impaired in neuropathic and some myopathic conditions: Thus, the specificity of increased jitter for MG will depend on the proportion of patients with neuropathy or myopathy in the population being studied. To determine the specificity of jitter testing for diagnosing MG, the tested muscles should be free of nerve or muscle disease.

In an early report, SFE jitter was increased in at least one muscle in up to 99% of MG patients if 3 muscles, selected by the distribution of weakness, and without nerve or muscle disease, were studied (Sanders and Howard, 1986). When jitter was normal in the ED, testing the frontalis detected abnormalities in 85% of MG patients, and when both of these muscles were normal, testing of a third muscle (OO) showed increased jitter in almost all patients (Sanders and Stålberg, 1996).

6.2. Muscle selection for jitter testing in MG

No one muscle is more abnormal or more likely to be abnormal in every MG patient. In patients with mild disease or weakness in

Table 2
Reported sensitivity of CNE jitter studies in myasthenia gravis.

Reference	Population Studied	Diagnostic Criteria	Muscle	Activation	MG Class	Sensitivity	Specificity
Sarrigiannis et al. (2006)	56 MG vs 20 normal	AChR antibody	ED (N = 53) OO (N = 30)	Vol	All	96%	100%
Benatar et al. 2006	21 MG vs 30 non-MG	AChR antibody or response to treatment	Front	Vol	OMG GMG All	62% 75% 67%	96%
Witoonpanich et al., 2011	MG N = 112	Clinical, plus response to ch'ase inhibitors and treatment	ED or OO	Vol	OMG (N = 42) GMG (N = 70)	93% 99%	ND
Kouyoumdjian et al. (2011)	MG N = 20	AChR antibody, or RNS plus response to treatment	ED or Front	Stim	OMG GMG All	75% 94% ED – 85% Front – 85% Either – 90%	ND
Kouyoumdjian and Stålberg (2013)	MG N = 42	AChR antibody, or clinical plus response to ch'ase inhibitors or RNS	ED or Front	Stim	OMG GMG	ED – 67% Front – 83% Either – 90% ED or Front 89%	ND
Machado et al. (2017)	33 MG vs 20 normal	AChR antibody, or clinical plus response to ch'ase inhibitors or RNS	OO	Vol	OMG GMG	92.3% 100%	96%
Sirin et al. (2018)	MG (untreated) N = 30	AChR or MuSK antibody, or clinical plus response to ch'ase inhibitors	ED Front	Stim	OMG GMG	ED or Front –93% ED or Front – 100%	ND

AChR – acetylcholine receptor; ch'ase – cholinesterase; Clinical – fluctuating weakness by history and examination; ED – extensor digitorum muscle; front – frontalis muscle; GMG – generalized myasthenia gravis; MuSK – muscle specific tyrosine kinase; ND – not done; OMG – ocular myasthenia gravis; OO – orbicularis oculi; stim – stimulation; vol – voluntary.

only a few muscles, it is particularly important to test a symptomatic muscle.

Jitter is increased in the facial muscles more often than in the limb muscles in most MG patients, but this is not always true (Sanders et al., 1995). Some studies report that the OO shows abnormalities more often than the frontalis (Trontelj et al., 1988; Oey et al., 1993; Valls-Canals et al., 2003), but this has not been confirmed in all studies (Hiroko et al., 2007).

SFE jitter was increased in the ED in 94% of patients with generalized MG (GMG) but without weakness in the tested muscle, and in 100% of those with weakness in the ED (Sanders and Howard, 1981). If jitter is normal in a weak muscle, one can be confident that the weakness is not due to MG (Stålberg et al., 1976b; Sanders et al., 1979). If jitter is normal in all the strategically-selected muscles, then it is highly unlikely that the patient has MG (Sanders et al., 1995).

Jitter is increased in limb, as well as periocular muscles in at least 60% of patients with OMG (Tables 1 and 2), indicating that subclinical abnormal NMT is widespread in these patients.

Because it is difficult to get good signals for CNE jitter studies in the ED, we recommend that special care be taken to assure good signal quality in this muscle, and that it not be the only muscle used for diagnostic CNE studies for MG. Fortunately, jitter is usually more pronounced in facial muscles than in the ED in MG (Sanders, 1996).

Based on the above observations, it is recommended that the OO or frontalis be examined first if weakness is mild or limited to ocular muscles. Most examiners find the frontalis to be easier to examine than the OO with voluntary activation, but there is little difference with stimulation activation. In any case, any difference in sensitivity between these muscles is unlikely to affect user preference, and if the first of these muscles tested is normal, testing should then include the other or a weak muscle.

In a patient with generalized or predominantly oropharyngeal weakness, nearly any muscle that is convenient to study may be examined first. In about 5% of MG patients weakness is limited to a few muscles; this is particularly true in some patients with anti-MuSK antibodies (Sanders et al., 2003; Stickler et al., 2005; Sanders and Juel, 2008). In these patients the physiologic abnormality is not as diffusely distributed as in non-MuSK MG and electrodiagnostic abnormalities may not be found in the muscles that are usually examined for MG. For example, jitter may be normal in the ED but abnormal in another limb muscle that is weak (Stickler et al., 2005; Sanders and Juel, 2008).

If jitter is increased, it is essential that neuropathic or myopathic abnormality be excluded in the tested muscle by conventional electrodiagnostic testing. FD and quantitative MUP measurements are useful in this regard.

Jitter is usually abnormal in affected muscles even when the patient is taking cholinesterase inhibitors. However, in the rare patients with only ocular or mild limb weakness, jitter may be increased in the tested muscle only after these medications have been discontinued (Massey et al., 1989). For maximum sensitivity, we recommend that cholinesterase inhibitors be discontinued for 12 hours before jitter testing if the clinical condition allows.

7. Reference values and limits

A quantitative diagnostic test with high sensitivity and specificity is likely to become a gold standard exam for an applicable disease. This is particularly true for electrodiagnostic tests, including jitter measurements. For the diagnostic test to be reliable, reference values based on acquisition of data from normal or control subjects must be determined. This seems a disarmingly simple proposition, as minor variations in normal may exist across different populations, and between equipment, techniques and operators. The definition of normal implies that no disease is present. Such hyper-healthy individuals are unlikely to be a suitable proxy for most of the population at risk for a particular disease. Thus, it is preferable to use control subjects, who do not have a disease likely to affect the result of the test in question, and to construe the results as reference values rather than normal limits.

Efforts to define reference values and limits began early in the use of SFEMG, and Stålberg and Trontelj included reference limits for SFE voluntary jitter (6 muscles) and FD (8 muscles) in the first

edition of their monograph, *Single Fibre Electromyography*, in 1979 (Stålberg and Trontelj, 1979).

These reference values were the standard for the next decade for anyone who had not acquired their own. In 1986, the AAEM Special Interest Group for SFEMG began an effort to improve reference values for jitter and FD by developing a multi-institutional collaboration to pool control data (Gilchrist, 1992). Nine laboratories were recruited based on their prior work on SFEMG. Prospective data acquisition was performed using standardized SFEMG methodology, with muscles and age groups assigned to individual investigators so that at least two investigators acquired jitter and FD values for each muscle and age group, including age greater than 50. At least 20 pairs were collected from each muscle for voluntary jitter and 20 sites were assessed for FD. A variety of machines and jitter analysis methods were included. FD values were collected for 11 muscles and in general, FD was found to increase with age (Fig. 3). Jitter values were also collected from 11 muscles during voluntary activation and the mean MCD was found to increase after age 60 in all muscles. As expected, there was variation in results among laboratories and investigators, which was more prominent for FD than for jitter. Data from this study were subsequently reformatted in tabular form (Bromberg and Scott, 1994). These reference data became the accepted standard for SFEMG voluntary jitter and FD, and were included in subsequent editions of *Single Fiber Electromyography* (Stålberg and Trontelj, 1994; Stålberg et al., 2010). Later published reference values for the frontalis muscles in 32 control subjects at least 70 years of age were quite similar to those from the collaborative effort, though FD results were a bit higher; all values increased further above age 80 (Balci et al., 2005).

Reference values for SFE stimulation jitter are more limited, coming for the most part from single laboratories and covering only a few muscles. As previously mentioned, stimulation jitter values should theoretically be approximately 71% of voluntary jitter values. However, stimulation jitter values in the ED from 15 healthy volunteers between the ages of 17 and 39 were 77% of previously reported voluntary jitter values from a similar population (Trontelj et al., 1986). Later, reference SFE values for the OO (Trontelj et al., 1988; Valls-Canals et al., 2003) and frontalis (Valls-Canals et al., 2000) were reported. When there are no stimulation SFE reference values for a muscle, it was recommended that the collaborative voluntary reference values be multiplied by 0.8 (Stålberg and Trontelj, 1994).

Two multicenter studies have reported reference CNE jitter values with somewhat different results (Kokubun et al., 2012; Stålberg et al., 2016). Results of these two studies are difficult to compare because of differences in filter settings, specific CNE used, definitions of signal acceptance, and analysis method. According to the more recent of these studies, the CNE jitter values for a given muscle were somewhat lower than those for SFEs (Stålberg et al., 2016).

We recommend that each laboratory obtain results from a small group of healthy subjects and compare these to published reference values using the same procedures that were used to acquire those values, including specific type of electrode, filter settings, and criteria of acceptable APs (Stålberg and Sanders, 2009).

Reference values for some populations are difficult to obtain, particularly in pediatric and very elderly age groups (see Measuring Jitter in Children, below). Recent studies have used mathematical techniques to derive normative limits from jitter values obtained from patients being evaluated for suspected NMT disorders. The E-Norms method used stimulation CNE jitter values from the OO to derive normative limits that compared favorably to those of a healthy adult cohort (Jabre et al., 2015). A similar mathematical technique, the E-Refs method (Nandedkar et al., 2018), used a large number of non-selected normal and abnormal clinical SFE jitter values to derive reference limits for voluntary SFE that were virtually identical to the previously published collaborative reference values (Gilchrist, 1992). These mathematical techniques show great promise not only for developing reference jitter values for specific populations, but also for individual laboratories. When multiple factors affect a parameter, e.g., age and height, reference values for specific subpopulations can be developed by these techniques if adequate numbers of clinical values are available.

8. Measuring jitter in children

Pediatric EMG is an uneasy compromise between the demands of scientific rigor and the practicalities of performing EMG tests in uncooperative subjects. Satisfactory co-operation for volitional SFEMG can often be achieved in children as young as 6–7 years. Stimulation activation, which bypasses the need for cooperation, allows measuring jitter in younger children, but usually requires general anesthesia or conscious sedation (Stålberg et al., 2010). The location of the recording surface behind the needle tip in the SFE may present problems in babies, where very thin muscle may mean that the recording port is not within the muscle even if the needle is inserted as far as possible. Small CNEs with the recording surface in the tip do not have this problem.

Reference values for jitter or FD in infancy and early childhood have not been obtained for SFEs or CNEs. However, if weakness is due to abnormal NMT, jitter measured with either electrode should be clearly abnormal on visual inspection.

8.1. Stimulated potential analysis using concentric needle electrodes (SPACE)

This acronym is used to describe a technique used in children that assesses the variability in signal waveforms recorded with small CNEs during axonal stimulation (Pitt, 2017; Pitt and Jabre, 2018). Although it has been described as stimulation SFEMG, single fiber potentials are not identified by this technique. Instead, what is measured is the variability of timing among the spike components of MUPs, which are produced by variable and undeterminable numbers of ASFAPs. The variability of these spikes is measured using a peak detection algorithm – it may not be suitable to use a voltage-level algorithm.

The muscle of choice is OO. Until recently it was felt that abnormal jitter is detectable in this muscle in nearly all myasthenic conditions encountered in children, even when there is a limb-girdle distribution of weakness. However, more recently described congenital myasthenic syndromes such as GMPPB may have abnormalities only in a peripheral muscle such as ED (Belaya et al., 2015).

The skin is anesthetized with a local anesthetic preparation. A single-use 300 μm diameter "facial" CNE is used to record the potentials. For studying the OO, a 300 μm diameter 15 mm long monopolar needle is used to stimulate the zygomatic branch of the facial nerve, referred to a surface electrode placed near the tragus (Fig. 7). A surface ground electrode is placed on the forehead. The stimulating needle is positioned to produce a visible twitch of the OO with stimulation intensity less than 1 mA. The recording electrode is then inserted into the twitching area of the muscle and moved to allow different populations of potentials to be collected. 20 repetitions of each signal are recorded and at least 10 individual signals should be obtained. As the stimulus intensity is increased the initial signals are sub-threshold and should not be used. Duplicate signals must be identified and removed. Placement of the cursor by the peak detection algorithm is accepted unless it is clearly alternating between two peaks of a potential (Fig. 8).

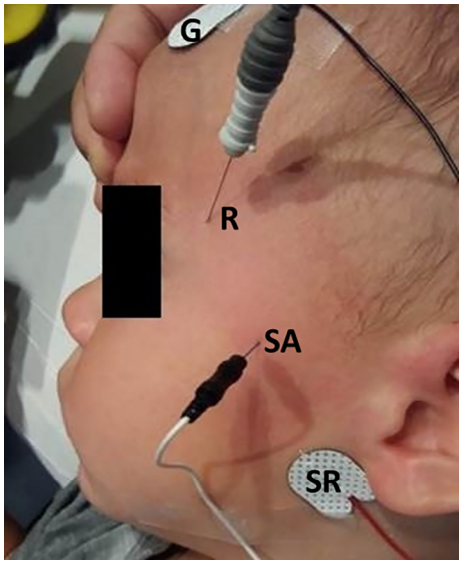


Fig. 7. SPACE examination setup for the left orbicularis oculi in a two-year-old. A ground electrode (G) is placed on the forehead. Stimulation is delivered via a monopolar needle anode (SA) inserted near the zygomatic branch of the facial nerve with reference to a surface electrode below the tragus (SR), and a recording CNE (R) is inserted into the twitching portion of the muscle. From Pitt et al., Use of stimulated electromyography in the analysis of the neuromuscular junction in children. Muscle Nerve. 2017;56:841–7, with permission from Wiley Periodicals, Inc.

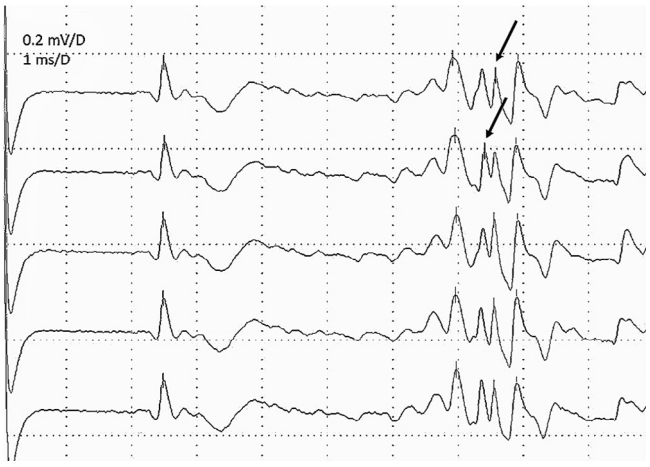


Fig. 8. Peak detection of concentric needle electrode recordings from orbicularis oculi during stimulation of the facial nerve. Vertical marks indicate peaks that have been identified by the software. Arrows indicate two adjacent peaks being alternately identified – these should be analyzed separately or only one should be analyzed. Modified from Pitt, 2018. Paediatric Electromyography, 2018, with permission from Oxford University Press.

When studying the ED, the recording electrode is placed near the motor point and the stimulating electrodes proximally.

A major advantage of the SPACE technique is that it is possible to examine nearly all children of all ages using only local anesthesia. General anesthesia may be needed if it is not possible to perform the study under local anesthesia.

The E-Norm method was used to determine the normative range of signal variability for this technique in children (Jabre et al., 2015; Pitt and Jabre, 2017). The upper limit of the mean variability in OO is 45 μs from birth to one year, 33 μs from 1 to 2 years and 26 μs above that age. The mean signal variability is the only parameter measured in these studies.

Studies have shown this jitter technique to have a sensitivity of 84%, positive predictive value 36%, negative predictive value 96% and specificity of 71% (Pitt and Jabre, 2017).

SPACE is usually performed as the initial part of the EMG examination, but is incorporated later if the initial EMG suggests a myasthenic syndrome.

The SPACE technique fulfils most of the requirements as a screening test for jitter abnormalities in children. Its relatively low specificity can be improved if a jitter measurement more than 115% of the reference limit is considered to be evidence of myasthenia (Pitt, 2017). Measurements less than that but still above the upper reference limit are more likely to be due to myopathy or other pathology.

9. Jitter in other conditions

9.1. Neuropathy

SFEMG findings are not diagnostic of specific neuropathic conditions, but add useful information about the pathophysiology.

Reinnervation of denervated muscle fibers takes place by collateral sprouting of intramuscular nerve fibers and regeneration from the end of transected nerve fibers. Collateral sprouting produces remodeling of the MU, increased MU territory and increased numbers of muscle fibers per MU. On muscle biopsy, this is seen as type grouping and increased terminal innervation ratio; with SFEMG, this is seen as increased FD. FD may be increased 3–4 weeks after nerve injury, before changes of reinnervation are seen on muscle biopsy or conventional EMG studies (Hakelius and Stålberg, 1974). Thus, increased FD may be the earliest and most subtle evidence of reinnervation.

Jitter is increased during ongoing reinnervation, implying that NMT is uncertain or impaired in immature neuromuscular junctions. To a lesser extent disturbed conduction along the nerve twigs may also increase jitter in neuropathy. As reinnervation becomes established and FD increases, the jitter becomes less. Jitter may also be increased during acute denervation, e.g. within days after nerve injury.

SFEMG can be useful in demonstrating or excluding abnormalities in patients with mild or questionable neuronal disease. The distribution of abnormality can be demonstrated by testing several muscles even when these are mild or subclinical. For example, in peripheral neuropathies, the more distal muscles have the most marked abnormalities. The combination of jitter and FD can also be used to infer the stage and completeness of reinnervation – increased FD with low jitter indicates that reinnervation is complete. Most neuropathies are progressive, however, and increased jitter and blocking due to simultaneously ongoing degeneration and reinnervation may be seen at all stages of these conditions.

Jitter and blocking are greatest when the disease is progressing most rapidly. As reinnervation becomes established and FD increases, the jitter becomes slightly decreased or almost normal.

9.1.1. Motor neuron diseases

Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive disease, advancing from one region to another. When muscle strength is decreased in one region, it may still be normal in other regions, and different patterns of SFEMG changes can be seen in different muscles at the same stage of the disease. Increased jitter, blocking, and FD vary in ALS, depending on the strength of the tested muscle, the rate of progression, and the stage of disease (Swash, 1980; Cui et al., 2004b). In early stages, jitter and FD can be normal in muscles with normal strength, but in others FD may be increased in the absence of increased MUP size. Increased jitter may be the earliest electromyographic abnormality in some

muscles, suggesting that NMT is abnormal during neuronal degeneration. This is also seen in conventional EMG as MU instability ("jiggle") (Stålberg and Sonoo, 1994), which is included in the Awaji criteria for ALS (de Carvalho et al., 2008). ("Jiggle" is the term used to describe variability of MUP shape, as distinct from "jitter," which is the variability in time relationships between MUP components.)

Markedly increased FD has also been suggested to be one of the earliest findings of reinnervation in ALS. When muscle strength is even slightly decreased, FD is always increased while jitter may be normal or only slightly increased, indicating that loss of MUs is at least partially compensated by reinnervation. When strength is further decreased, increased jitter, blocking, and FD are seen. In some cases, when the FD is very high, the recorded SFEMG signal contains many APs and although jitter is easily heard and seen on the screen, it cannot be precisely calculated (Fig. 9). Reinnervation is near maximum at this stage of the disease but is still not sufficient to compensate for the loss of MUs. Higher FD with mildly increased jitter and little blocking is usually seen in cases with slow progression, indicating better collateral sprouting and NMT, so that the loss of neuromuscular junctions has been compensated for, resulting in relatively less severe weakness. Increased FD with significantly increased jitter and blocking are the result of reinnervation and immature nerve terminals, and suggests the inability to compensate for impaired NMT and weakness from serious denervation; this always indicates rapid progression and a worse prognosis (Cui et al., 2004b).

Jitter recording with CNEs in ALS showed similar waveforms as SFE recordings (Liu et al., 2016). Recordings with both electrodes show increased jitter in ALS patients compared with healthy controls.

FD is also markedly increased in other chronic, slowly progressive motor neuron diseases, such as spinal muscular atrophy, Kennedy disease and post-polio syndrome. In most of these conditions, the jitter is normal or slightly increased, with little blocking. However, when the muscle is severely weak, increased jitter and blocking can also be seen in these conditions (Shields, 1984). In syringomyelia, there is increased FD and occasional increased jitter, with marked variability among different muscles (Schwartz et al., 1980).

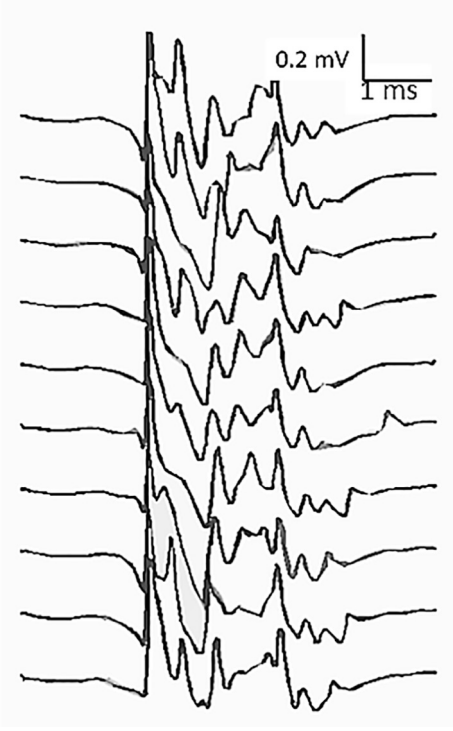


Fig. 9. SFEMG recorded in a weak extensor digitorum muscle in ALS, showing markedly increased jitter and blocking. There are too many spikes to identify individual SFAPs and jitter cannot be precisely analyzed.

9.1.2. Cervical radiculopathy

SFEMG in cervical radiculopathy (CR) shows increased FD and normal to slightly increased jitter and rare blocking in the involved muscles, consistent with reinnervation after denervation with many mature end-plates. The SFEMG findings can help differentiate CR from ALS, and is particularly useful when both are present, to avoid unnecessary surgery (Liu et al., 2013). Markedly increased jitter and blocking with increased FD support the diagnosis of ALS. In ALS patients who also have CR, SFEMG changes are the same as in ALS, with increased jitter, marked blocking, and increased FD in the involved muscles, suggesting that the SFEMG changes are due mainly to progressive denervation.

9.1.3. Multifocal motor neuropathy (MMN)

MMN with persistent conduction blocks is a chronic neuropathy in which an immunological process inhibits remyelination and reorganization of Na^+ channels, which damages the axonal membrane. Jitter and FD are abnormal in MMN in both clinically affected and unaffected ED muscles, indicating ongoing denervation and reinnervation (Lagueny et al., 1998). Jitter is increased during relapses and decreases as strength improves. Jitter in MMN may be partly due to blockade of Na^+ currents in the distal part of the motor nerve and consequent impaired action potential electrogenesis.

9.1.4. Diabetic neuropathy

Diabetic neuropathy is mainly autonomic and sensory, thus SFEMG is usually normal in its early stages. Jitter and FD are increased in the tibialis anterior muscle of diabetic patients with clinical peripheral neuropathy, but may be normal in the ED muscles in the same patients, consistent with length dependent axonal loss (Bril et al., 1996). SFEMG is more sensitive than conventional nerve conduction studies in detecting reinnervation changes in clinically overt diabetic neuropathy but may not show abnormal changes in all diabetic patients. This may be due to the variable presence or absence of neuropathy, as well as to the severity of neuropathy in the tested muscles, an important consideration in a length-dependent process.

9.1.5. Guillain-Barré syndrome (GBS)

Jitter is usually normal or near normal acutely in patients with GBS (Kuwabara et al., 2011), however stimulation SFE studies have shown blocking with relatively normal jitter in such patients, presumably due to axolemmal dysfunction (Spaans et al., 2003). In most cases of GBS there is some axonal loss secondary to demyelination or conduction block, in which case FD will increase as reinnervation takes place from collateral sprouting. If conduction block or demyelination is the only abnormality, recovery is not accompanied by increased FD.

9.1.6. Other neuropathies

In other neuropathies, such as those due to uremia or alcoholism, FD and jitter changes are variable depending on the motor nerves involved and whether there is axonal loss and reinnervation. Reports of SFEMG in neuropathy are usually single case reports or small series. SFEMG has limited usefulness in the diagnosis and differential diagnosis of peripheral neuropathy.

9.2. SFEMG in myopathy

The diagnosis of myopathy is based on clinical symptoms and signs, laboratory findings, conventional EMG, muscle biopsy, and molecular genetics. SFEMG may give additional information about the reorganization of the MU and the pathophysiology of muscle fibers, complementing information from conventional EMG, thus increasing our understanding of the disease process.

9.2.1. Fiber density in myopathy

Fiber density is increased in some muscle diseases, but in muscles of similar weakness the FD tends to be much lower in myopathies than in neuropathies. The total number of muscle fibers per MU is not generally as increased in myopathic as in neuropathic conditions.

Fiber density is increased in muscular dystrophies (Stålberg, 1977) and myositis (Henriksson and Stålberg, 1978; Hatanaka and Oh, 2007); FD values are related to muscle fiber diameter and histochemical fiber type grouping (Bertorini et al., 1994). The increased FD in myopathies suggests that there is focal grouping in some areas and probably loss of fibers in other parts of the MU territory. Grouping of muscle fibers can be due to new innervation of muscle sequestered by focal lesions and/or regeneration of satellite cells (Fig. 4). Splitting of muscle fibers is commonly seen on muscle biopsy in muscular dystrophies and is correlated with recordings with low jitter. The split fibers may contribute to the increased FD (Hilton-Brown et al., 1985).

9.2.2. Jitter in myopathy

Jitter is usually normal or only slightly increased, mainly in limb muscles, in most myopathies. However, jitter is increased in most patients with mitochondrial disease that predominantly affects the extraocular muscles (progressive external ophthalmoplegia – PEO) (Krendel et al., 1987; Ukachoke et al., 2015), which also has clinical findings similar to MG. Most patients with PEO have EMG findings of myopathy in proximal limb muscles (Krendel et al., 1987; Torbergsen et al., 1991), whereas mitochondrial disease with more widespread weakness is associated with increased FD and other EMG findings of neuropathy (Torbergsen et al., 1991; Girlanda et al., 1999). Although jitter would be expected to be more abnormal in MG than in CPEO, the overlap between the SFEMG findings in these two conditions makes it impossible to distinguish individual cases by this technique alone (Krendel et al., 1987). The following characteristics of PEO help distinguish it from ocular MG: 1) slowly progressive, symmetrical extraocular muscle weakness; 2) small, complex MUPs in limb muscles; and 3) ragged red or ragged blue fibers, or COX-negative fibers in limb muscle biopsy (Sundaram et al., 2011). The definitive diagnosis of mitochondrial disease requires mitochondrial DNA analysis.

Jitter is often slightly increased in muscular dystrophies (Stålberg, 1977; Stålberg et al., 2010) and myositis (Henriksson and Stålberg, 1978; Hatanaka and Oh, 2007). Some reports of congenital myopathies showed normal or only slightly increased jitter. In muscular dystrophies, VRF is prominent in a higher proportion of fibers (Stålberg, 1977) and dystrophic muscle fibers have a pronounced supernormal part of the VRF (Mihelin et al., 1991). This abnormal VRF produces IDI-dependent jitter (myogenic jitter). Another source of increased jitter and blocking in myopathies is abnormal endplate function, however, the degree of NMT failure is not as pronounced as in primary NMJ disorders. Abnormal jitter could also be due to uncertain transmission in regenerating fibers derived from satellite cells or threshold ephaptic transmission. Concomitant blocking due to axonal block and blocking due to extra-discharges are also reported in muscular dystrophies, suggesting some neurogenic involvement. Low jitter, i.e. MCD < 5 μs, is seen more often than normal in muscle dystrophies, probably due to muscle fiber splitting (Hilton-Brown et al., 1985); frequent abnormally low jitter strongly suggests myopathy.

Neuromuscular junction disorders such as limb-girdle myasthenia may mimic myopathies on routine EMG (Rodolico et al., 2002); SFEMG can be useful in differentiating these disorders (Mongiovi et al., 2014).

9.2.3. Mean interspike intervals (MISI)

The MISI is the mean value of the total duration of each SFEMG recording divided by the number of intervals in the recording, i.e., the number of SFAPs in the recording minus 1. The duration of multi-spike potentials results from the different conduction times along the peripheral nerve branches, the anatomic locations of the endplates, and different propagation times along the muscle fibers. High MISI values are frequently seen in myopathies due to degeneration of muscle fibers and increased muscle fiber diameter (Cruz-Martinez and Lopez-Terradas, 1992; Stålberg et al., 2010). Increased MISI values with normal or slightly abnormal jitter is highly characteristic of myopathy (Stålberg et al., 2010).

9.2.4. Clinical usefulness of SFEMG in myopathy

SFEMG demonstrates abnormalities in muscle disease that may not be apparent by other electromyographic techniques. Although individual SFEMG findings are not specific for a given myopathy, the relative degree of abnormality of individual parameters, the distribution of abnormalities among different muscles, and the combination of these frequently assist in making the correct diagnosis when assessed along with the clinical picture and the results of other diagnostic procedures.

9.3. Botulism and botulinum toxin-treated patients

Botulism results from intoxication with one of eight toxins produced by *Clostridium botulinum* that interfere with NMT by inhibiting ACh release from presynaptic nerve terminals. Botulism occurs in five forms: food-borne, infant, wound, hidden, and iatrogenic.

Electrodiagnostic abnormalities of botulism include small CMAPs and post-activation facilitation during tetanic stimulation that persists for at least 2 minutes after activation, with no post-activation exhaustion (Gutmann et al., 1992; Gutierrez et al., 1994). These findings progress over time and may not be present early in the condition. Jitter is maximally increased within the first 2 weeks after onset of weakness and at slower discharge rates (Schiller and Stålberg, 1978; Girlanda et al., 1983; Cruz-Martinez et al., 1985; Vita et al., 1987). Jitter usually improves 3–8 weeks after onset of weakness and is nearly normal after 3–4 months (Ibid) but FD may increase over the subsequent 2–4 months, as jitter and strength are improving (Cruz-Martinez et al., 1985).

Fibrillations and positive sharp waves are seen in most cases of infant botulism (Cornblath et al., 1983), suggesting that profound neuromuscular blockade produces functional denervation. Markedly increased jitter and blocking have been found in all reported cases of food-borne, infant and wound botulism who had jitter testing, including patients in whom RNS did not show diagnostic findings (Schiller and Stålberg, 1978; Chaudhry and Crawford, 1999; Padua et al., 1999; Verma and Lin, 2016). Jitter and blocking typically decrease at higher firing rates in most cases (Fig. 10), but not in all (Mandler and Maselli, 1996; Chaudhry and Crawford, 1999).

Botulinum toxin is used in the treatment of strabismus, blepharospasm, hemifacial spasm, torticollis, writer's cramp, Meige syndrome and other forms of focal dystonia, spasticity, headache and hyperhidrosis. One of the most common uses is to remove facial wrinkles.

Injected botulinum toxin may produce focal or regional weakness (Borodic et al., 1990), including diplopia, dysphagia, urinary incontinence, and brachial plexopathy, and may unmask or exacer-

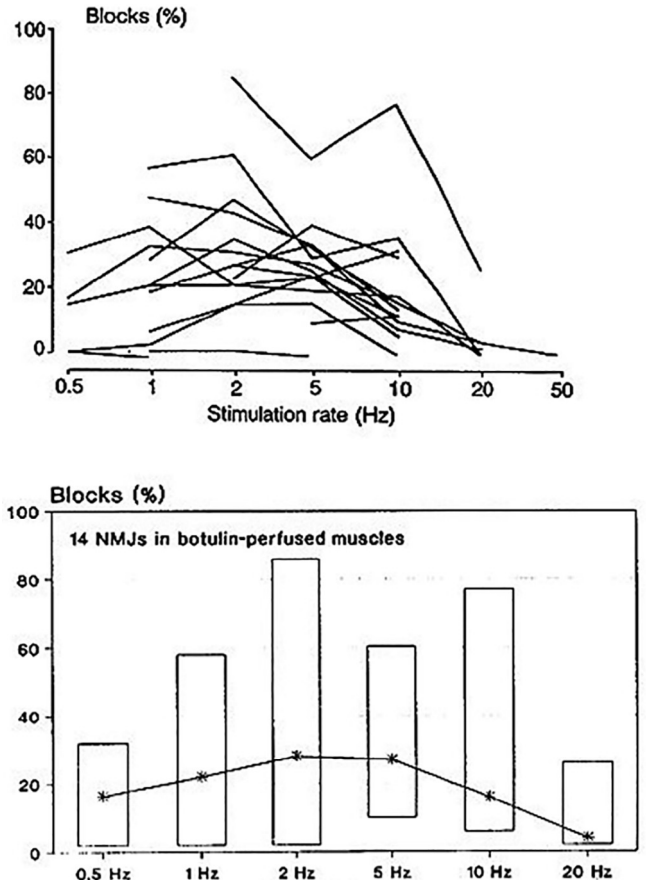


Fig. 10. Frequency of blocking studied at 16 endplates of muscles injected with botulinum toxin. Top: values for individual endplates connected with lines. Bottom: median values and ranges (Krzan and Trontelj – personal communication).

bate other neuromuscular diseases, such as MG (Borodic, 1998; Tarsy et al., 2000), LEM (Erbguth et al., 1993) and motor neuron disease (Mezaki et al., 1996). Increased jitter is also seen in muscles remote from the site of injection (Sanders et al., 1986; Lange et al., 1987; Olney et al., 1988; Girlanda et al., 1992; Singer and Weiner, 1993), and persists for at least 6 months (Sanders et al., 1986; Lange et al., 1987; Girlanda et al., 1992).

9.4. Jitter in Lambert-Eaton Myasthenia (LEM)

Jitter is markedly increased in all tested muscles in patients with LEM, with frequent blocking, typically out of proportion to the severity of weakness in the tested muscle. In many endplates, the jitter and blocking decrease as the firing rate increases (Trontelj and Stålberg, 1991; Sanders, 1992); however, this is not seen in all endplates nor in all patients with LEM (Sanders, 1992). In MG, most motor endplates have the largest jitter (and blocking) at 5–10 Hz (Trontelj and Stålberg, 1991). In contrast, in LEM jitter in nearly all endplates is most abnormal at low stimulation rates (0.5–1 Hz) and decreases significantly at 10–20 Hz (Trontelj and Stålberg, 1991). Moreover, jitter and blocking may also improve at higher firing rates in some endplates in MG; thus improvement of jitter and blocking at higher rates, unless dramatic, does not necessarily indicate a presynaptic abnormality.

10. Serial jitter studies in MG and LEM

A number of studies have reported the use of serial jitter studies to demonstrate, confirm or predict changes in clinical weakness in MG and LEM.

10.1. Serial jitter studies in MG

In MG the degree of abnormal jitter in a given muscle correlates with the disease severity – improvement of symptoms is usually accompanied by improvement in jitter (Howard and Sanders 1981; Massey, Sanders and Howard 1989). Although jitter falls as the disease improves, it is usually increased even if strength becomes normal. Thereafter, jitter typically continues to fall toward normal as long as therapy is adequate; deviation from this expected pattern suggests that treatment may not be adequate.

Serial jitter studies may be of value in predicting or confirming change in disease severity under certain circumstances. For example, when jitter in a muscle has been relatively constant for several months, a subsequent increase usually accompanies or heralds clinical deterioration.

The effect of cholinesterase inhibitors on jitter must be taken into account when comparing the results of serial jitter studies. Ideally all jitter studies should be performed after the same interval following a dose of these medications.

Serial jitter measurements may be of particular value when the severity of disease is difficult to assess objectively or when change in symptoms cannot be otherwise assessed (Massey and Sanders, 1993). Thus, it may be useful to obtain baseline jitter values for comparison with subsequent studies, even when not required diagnostically.

Several parameters are measured for a jitter study: the mean MCD of all the recordings, the percent of recordings with increased jitter, and the percent of recordings with blocking.

All of these parameters improved in patients who had significant improvement in strength after treatment with prednisone or plasma exchange (Howard and Sanders, 1981) and changes in jitter were less marked in patients who had only a slight response to treatment. There was a strong correlation between overall clinical change and a change of at least 10% in mean jitter in any muscle (Sanders and Howard, 1986).

In a retrospective study of patients treated with cyclosporine, the MCD fell more then 10% from the pretreatment value in all patients (Ciafaloni et al., 2000). Jitter has been measured in several therapeutic trials in MG. In a pilot trial of mycophenolate mofetil (MMF), the mean MCD was significantly lower in patients receiving MMF vs those receiving placebo (Meriggioli and Rowin, 2003). In another trial of MMF, there was a significant correlation between change in all jitter parameters and change in clinical outcome measures (Massey et al., 2008).

Serial jitter studies in a patient with refractory MG who received eculizumab in a prospective trial demonstrated normalization of previously markedly abnormal jitter that paralleled marked improvement in clinical outcome measures (Juel et al., 2017).

In a recent retrospective study of patients with MG, change in all jitter parameters in the ED or frontalis muscle predicted clinical change (Sanders and Massey, 2017). Absolute or percentage change in mean MCD appeared to be the best jitter parameter to follow.

In MG patients who improve after immunotherapy, jitter typically continues to fall toward normal as long as adequate immunotherapy is continued; deviation from this pattern suggests that treatment may not be adequate. Exceptionally, all jitter parameters improve during clinical remission (Emeryk et al., 1985; Sanders and Howard, 1986; Kostera-Pruszczyk et al., 2002) but most patients in remission still have some endplates with increased jitter and a few with blocking (Table 1).

In summary, jitter is a sensitive measure of disease severity in MG and has a potential role as a biomarker in treatment trials and in the clinic.

10.2. Serial jitter studies in LEM

Several small or single-case studies in patients with LEM have reported correlation between clinical status and the mean MCD and/or number of blocking fibers (Phillips, 1982; Oh, 1989; Bird, 1992).

In a study of 82 jitter tests in the ED in 30 patients with LEM, jitter was abnormal in all cases at the first evaluation and became increasingly abnormal with worsening disease severity (Oh and Ohira, 2012); the mean MCD correlated well with clinical severity.

In summary, jitter correlates with clinical changes in LEM. However, change in resting CMAP amplitude or amount of CMAP facilitation are also correlated to change in LEM, and are more easily measured.

11. Other uses for SFEMG

Since SFEMG identifies the electrical activity from one muscle fiber and thus the activation of individual motor units, SFAPs can be used to "mark" individual motor units in a number of scenarios. For example, APs from individual MUs recorded with various electrodes during voluntary activation can be identified by triggering on an SFAP recorded with the same electrode (e.g., Macro EMG (Stålberg, 1980a)) or a separate SFE (e.g., Scanning EMG (Stålberg and Dioszeghy, 1991)). The voluntarily SFAP has also been used for triggering in studies of multiple end-plates within a single MU (Stålberg et al., 1976a).

11.1. Single axon conduction studies

This technique (SF-CV) was developed to improve the sensitivity of nerve conduction studies by recording SFAPs in a distal muscle while stimulating the nerve at a proximal and a distal site (Figs. 11 and 12) (Padua et al., 2007). SF-CV testing was abnormal in 64% of nerves in patients with peripheral neuropathy that were normal by conventional NCV testing. This procedure may be useful in detecting early, mild, or partial myelin damage when conventional nerve conduction tests are normal.

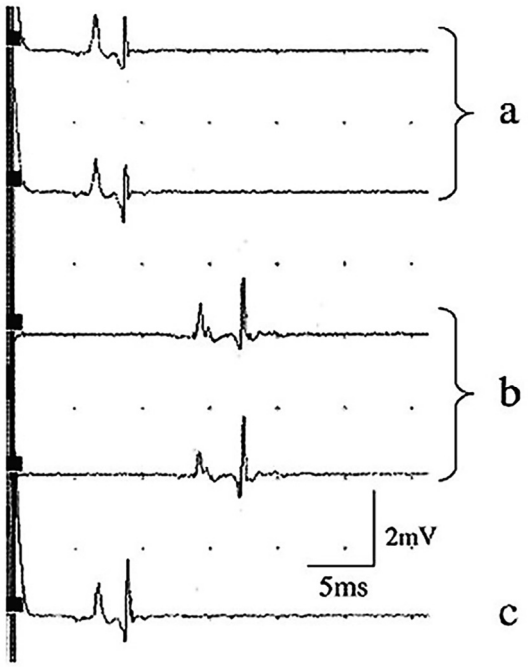


Fig. 12. Example of SF-CV evaluation from one muscle site: (a) responses from nerve stimulation at distal site, (b) responses from nerve at proximal site, (c) response from nerve stimulation at distal site to check that the electrode did not move. From Padua et al., A novel approach to the measurement of motor conduction velocity using a single fibre EMG electrode. Clin Neurophysiol. 2007;118:1985–90 with permission from Elsevier Ireland Ltd.

11.2. Reflex studies

The physiology of individual anterior horn cells can be studied by selective SFEMG recordings of F responses (Fig. 13) (Trontelj, 1973a) and H reflexes (Trontelj, 1973b). Polyneuronal reflexes such as the blink reflex, sacral reflexes and flexion reflex can be studied by measuring jitter in the SFAP responses after reflex activation

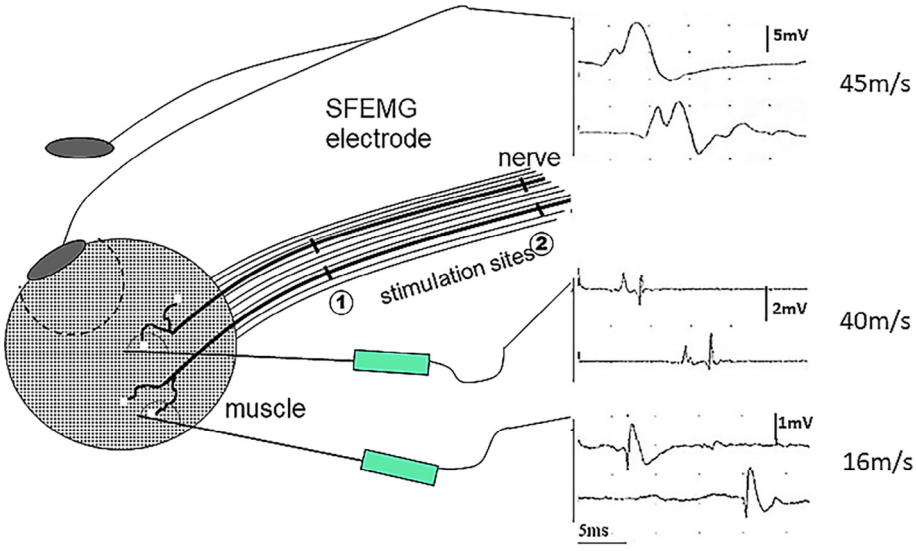


Fig. 11. Diagram of recordings and responses to nerve stimulation using conventional surface electrodes and an SFEMG electrode (in two recording sites for the latter electrode). From Padua et al., A novel approach to the measurement of motor conduction velocity using a single fibre EMG electrode. Clin Neurophysiol. 2007;118:1985–90 with permission from Elsevier Ireland Ltd.

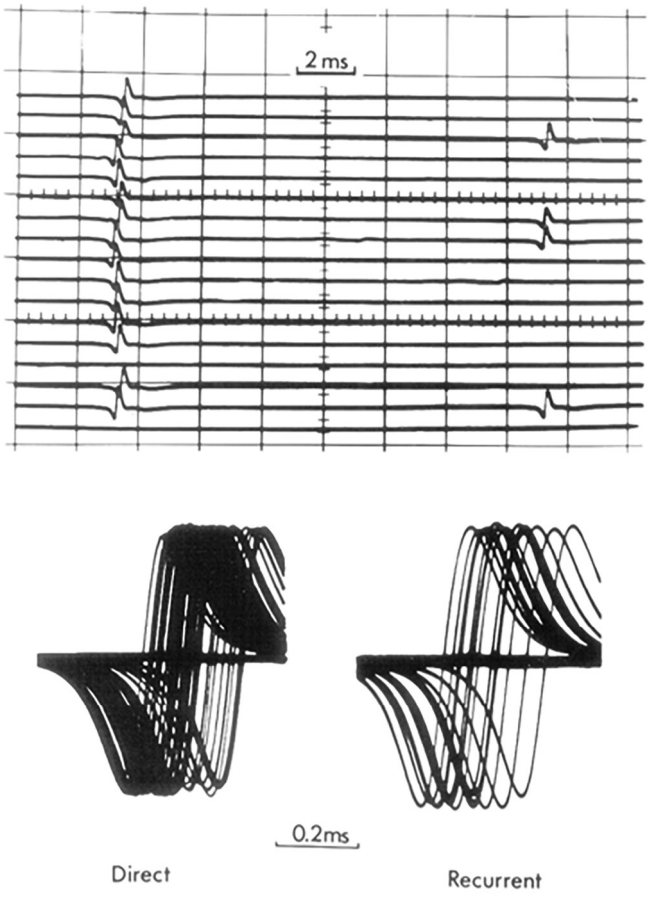


Fig. 13. Direct and F responses of a muscle fiber in abductor pollicis brevis. Top – if at threshold stimulus the direct response is missing, the late recurrent response is also missing. Bottom – the direct and late recurrent responses of the same muscle fiber superimposed at a higher sweep speed. The jitter is similar in both responses since both are from the same end-plate. Reproduced from Stålberg et al, Single Fiber EMG, 2010, with permission from Edshagen Publishing House.

(Trontelj and Trontelj, 1978; Vodušek et al., 1982; Janko and Trontelj, 1983).

11.3. Transcortical stimulation responses

The SFE jitter in responses to transcortical electrical stimulation has been studied to assess the physiology of the entire motor pathway (Rossini et al., 1988). When SFE recordings were compared with CNE recordings a constant shape of the more complex CNE signal actually was a better indication that the recording was from just one MU since SFEMG signals from two adjacent fibers belonging to different MUs could theoretically be very similar. At this time, measuring jitter after transcortical stimulation has no clinical applications.

12. Pitfalls in measuring jitter

This section identifies pitfalls that can be seen in jitter measurements made with both voluntary activation and electrical stimulation, and with both SFEs and CNEs.

Many pitfalls cannot be avoided during recording but can be corrected during post-processing. Artefacts that are common for SFE and CNE recordings will be discussed together, although they are more frequent in CNE recordings.

12.1. Pitfalls in measuring jitter – general

12.1.1. Summated spikes

Signal spikes may be comprised of more than one SFAP – such summated spikes are common in CNE recordings and rare in SFE recordings. Measurements from summated spikes produce erroneous jitter values and should be avoided.

To avoid measuring summated signals in CNE jitter studies, spikes to be measured should have a fast-rising phase without notches or shoulders, and consecutive discharges should have parallel rising phases (Fig. 6) (Stålberg and Sanders, 2009; Stålberg et al., 2018). These features are best observed in a display in which 5–10 consecutive signals are superimposed.

Summated spikes are more difficult to recognize during stimulation activation when two or more MUs are activated simultaneously, which does not occur with voluntary activation.

Recommendation: Make sure that the signals measured with a CNE fulfill the criteria for acceptable ASFAPs.

12.1.2. Repeated measurements from the same signal

Signals recorded within the territory of a MU change configuration with only slight movement of the recording electrode, and thus may be considered to originate from different muscle fibers. Jitter measured from the same muscle fibers will have the same MCD and, with voluntary activation, similar firing rates. It important to recognize these as being duplicate measurements and not include them in the results.

Recommendation: Move the recording electrode sufficiently (perpendicularly to the muscle fiber direction, if possible) between jitter measurements to assure that repeated measurements are not being made from the same muscle fibers.

12.1.3. Other conditions with abnormal neuromuscular transmission

Jitter is increased in some myopathies and in muscles with active denervation or ongoing reinnervation (Stålberg et al., 1975a; Stålberg et al., 2018), making it difficult to diagnose or exclude coincidental MG. (See Jitter in Other Conditions, above.) These conditions should be excluded by neurography and routine EMG examination whenever jitter is increased.

Jitter is also increased after previous treatment with botulinum toxin, which may produce long term local and remote effects (Sanders et al., 1986; Lange et al., 1987). (See SFEMG in Botulism and Botulinum-Treated Patients, above.) The duration of increased jitter in the injected or remote muscles after botulinum toxin injection is not known, but could be as much as 12 or more months.

Recommendation: If jitter is increased, neuropathy and myopathy should always be excluded by routine EMG examination of the tested muscle(s), particularly if the SFEMG signals are complex or if increased jitter is unexpected. Always ask about previous botulinum toxin injections; avoid muscles that have been injected or adjacent muscles and interpret increased jitter in remote muscles with caution.

12.1.4. Triangular after-potentials

One frequently sees SFAPs with a following triangular potential having large jitter and frequent blocking (Fig. 14); this after-potential is actually a component of the immediately preceding spike and should not be used for jitter analysis. The mechanism for its generation is unknown and it is seen in both normal and diseased muscle.

Recommendation: Do not measure jitter in triangular after-potentials.

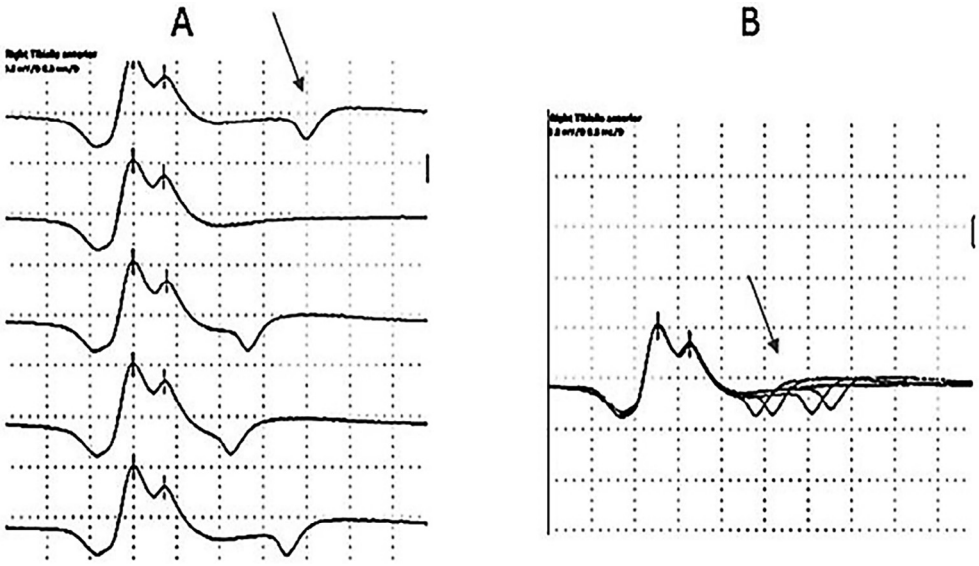


Fig. 14. Positive triangular after-potentials (arrows) belonging to the immediately preceding spike. These should not be used for jitter analysis. Reproduced from Stålberg et al, Pitfalls and errors in measuring jitter. Clin Neurophysiol;128:2233–41, 2017, with permission from Elsevier Ireland Ltd.

12.1.5. Measurements from riding signals

Amplitude level and peak-detection techniques for measuring spike intervals give the same jitter results for well-separated spikes. However, if the signals are so close together that the second spike begins during the falling phase of the first, a "riding" signal, the amplitude level trigger will occur at different points on the signal relative to its negative peak (Fig. 5), and the resulting measurement error may be as much as 100 μs, depending on the shape of the signals. With the peak-detection technique, the position of the measurement trigger point is much less influenced by the DC level of the signal, and the error in jitter measurements from riding signals is minimal. If the peak-detection technique is available, it should be used to measure riding signals. If not, only signals separated by more than 150 μs should be used for jitter measurements. Riding signals occur more frequently in CNE recordings.

Recommendation: Use well-separated signals (>150 μs) or the peak-detection technique for jitter measurements.

12.1.6. Recordings with low jitter

Sometimes the jitter is less than 5 μs. In normal muscle it is most likely that spikes with such low jitter are generated by branches of longitudinally split muscle fibers (Stålberg et al., 2010). Low jitter can also be seen when muscle fibers are directly stimulated during intramuscular axonal stimulation. These low values should not be included in the jitter results.

Recommendation: Do not include jitter values less than 5 μs.

12.1.7. Interpretation of results

The results of jitter testing should be interpreted in light of the clinical findings. Slightly abnormal or borderline results, especially, should be interpreted with great caution, and would be expected only in patients with mild disease. Patients with clinically significant MG should have definitely abnormal jitter, especially if clinically involved muscles are tested.

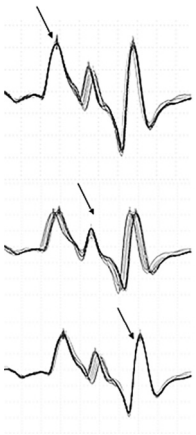
If jitter is normal in all muscles tested as recommended above, it is highly unlikely, but not impossible, that the patient has MG, especially if jitter was not tested in the most involved muscle. However, if all jitter tests are normal in a patient with MG, the disease is so mild that most patients would not require treatment at this point. The best approach in such situations usually is to repeat the studies after several months, or earlier if the symptoms progress and the diagnosis is still in question.

Recommendation: Be sure that the results of the testing make sense in light of the clinical findings.

12.2. Pitfalls in measuring jitter with voluntary activation

12.2.1. Measuring jitter in multi-spike potentials

12.2.1.1. Influence from the triggering spike in multi-spike potentials. With voluntary activation, jitter is measured between a triggering spike and another acceptable spike. The jitter in the triggering spike contributes to the paired jitter between it and all the other spikes. When there are only 2 spikes, triggering on either spike gives the same result. However, in multi-spike signals high jitter in the triggering spike produces erroneous results and the spike that produces the lowest summed jitter between spikes should theoretically be chosen as the triggering signal (Fig. 15). Most EMG equipment allows post-processing to select the trigger-



Jitter between spikes		Summed jitter
1-2 71.2μS	1-3 32.8μS	104μS
2-1 71.2μS	2-3 83.0μS	154.2μS
3-1 32.8μS	3-2 83.0μS	115.8μS

Fig. 15. Effect of the triggering potential (arrows) on the calculated jitter of a multi-spike potential. In this example, the sum of the jitter between different pairs of spikes is least when spike 1 is used as the trigger Reproduced from Stålberg et al, Pitfalls and errors in measuring jitter. Clin Neurophysiol;128:2233–41, 2017, with permission from Elsevier Ireland Ltd.

ing signal. Triggering on each of the spikes in a multi-spike signal in turn can thus be tested to see which produces the lowest summed jitter – some equipment can make this calculation automatically. (Use of this procedure would not affect published reference values since most normal recordings have only two spikes in which the choice of triggering signal does not affect the calculated jitter.)

Recommendation: In signals with more than 2 spikes, avoid triggering on the most unstable spike, i.e., test different spikes for triggering if the jitter is large.

12.2.1.2. Selecting abnormal intervals in multi-spike potentials. Multi-spike potentials with increased jitter in several spikes are usually produced by reinnervation. If all the abnormal intervals in such signals were included in jitter analysis, 1 or 2 reinnervated MUs could unduly affect the overall jitter measurement in that muscle, leading to a misleading diagnosis of MG. To avoid this, in multispike potentials with no or only one spike with increased jitter, we accept jitter values from all intervals, but in potentials in which jitter is increased in 2 or more spikes, we accept all the normal jitter values but only the lowest abnormal jitter value.

Recommendation: Accept all jitter values in multi-spike potentials in which jitter is increased in 1 or no spikes. In potentials with increased jitter in 2 or more spikes, accept all the normal jitter values but only the lowest abnormal value.

12.2.2. Effect of the velocity recovery function

The propagation velocity (PV) of an impulse along a muscle fiber is affected by the interval from the previous discharge; the PV increases over the interval range of 10–1000 ms, the so-called *velocity recovery function* (VRF) (Stålberg, 1966). (See VRF Effect, below.) This will artefactually increase the calculated jitter if the firing rate is very irregular. Most equipment with jitter software can calculate both the MCD and MSD; the lesser of these values is used as the jitter for each pair.

Intermittent impulse blocking produces an irregular firing rate, which then exaggerates the jitter via the VRF. To reduce the effect of these exaggerated jitter values, we recommend that jitter values exceeding 150 μs be set to 150 μs when calculating the mean jitter for the tested muscle. Statistical analysis has shown no correlation between the IPI and jitter until the IPI exceeds 4 ms, which therefore has been chosen as the maximum acceptable IPI for signals during voluntary activation. For longer IPIs the recording should be skipped or the MSD should be used. This is not an issue for stimulation jitter studies where the activation rate is constant.

Recommendation: For voluntary jitter measurements, only use IPIs < 4 ms. Use the lesser of the MCD and MSD values for each recording. When calculating the mean jitter in a muscle, set individual jitter values > 150 μs to 150 μs.

12.2.3. Dual IPI latency, the "flip-flop" phenomenon

IPIs occasionally have a bimodal latency in normal and in pathological muscle (Thiele and Stålberg, 1974). In healthy subjects, the latency shift it is less than a few hundred μs (Fig. 16); in complex recordings due to reinnervation, it may be many ms long. The underlying physiology of this phenomenon has not been established. It may sometimes be unrecognized and misinterpreted as increased jitter, especially when the difference between the two latency positions is small. If jitter can be measured at both of the latency positions, only one should be used. Otherwise, the recording should not be used for jitter measurements.

Recommendation: In signals with flip-flop, do not measure jitter or measure jitter only for one of the latency positions.

12.2.4. Quality control for blocking

Before accepting a recording, visually assess its quality. Check for possible disturbances from non-time-locked signals. If the jitter value is more than 100 μs and no blocking is seen, check the traces visually for disturbances. Conversely, blocking should not occur unless the jitter is increased; if spurious signals trigger the sweep, the other expected spikes are not present and this is misinterpreted by the software as blocking. Blocking must be verified by the operator.

Recommendation: Visually inspect all traces before accepting the software analysis of blocking.

12.3. Pitfalls in measuring jitter with electrical stimulation

12.3.1. Insufficient stimulation

When measuring jitter with axonal stimulation, very weak stimulus intensity is used so as to elicit spikes only from a few motor axons. If the axon is subliminally stimulated, impulse initiation is uncertain, producing both increased jitter and intermit-

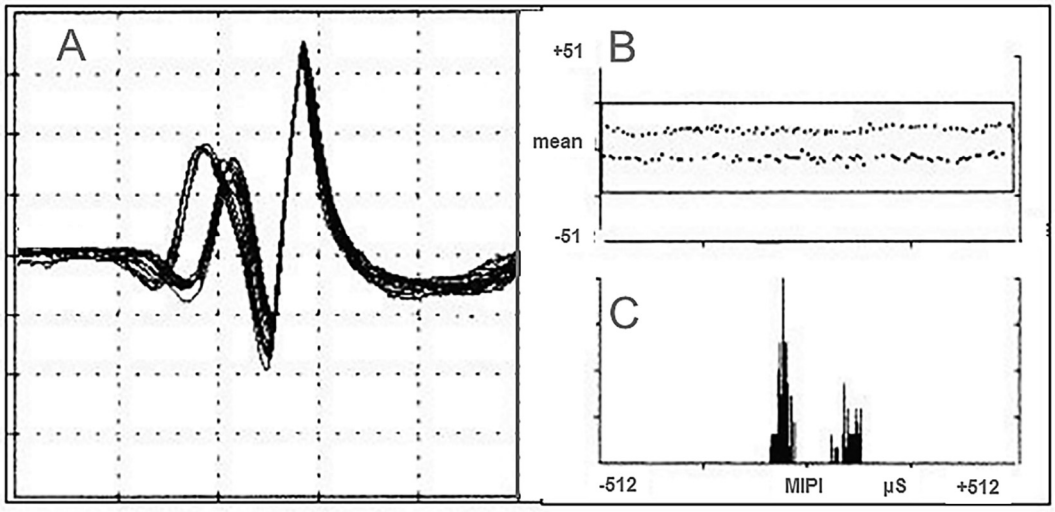


Fig. 16. Bimodal jitter ("flip-flop"). SFAPs from a normal patient. Ten traces superimposed, triggered on the 2nd spike (A). There is a difference of 140 μs between the 2 positions of the 1st spike. The bimodal distribution is seen in the plot of sequential interpotential intervals (B) and non-sequential histograms (C) of the interpotential intervals. Copyright DBSanders, 2019.

tently unsuccessful activation, which may be misinterpreted as blocking (Fig. 17). By increasing the stimulation intensity slightly (less than 10% or 0.3–2 mA for needle stimulation and somewhat more for surface stimulation, depending on the stimulation electrode position, skin conditions, subcutaneous fat, etc.), the stimulation becomes supraliminal and the artefactual jitter approaches zero. When the stimulation intensity is thus increased, however, the newly activated axons may be subliminally stimulated – the operator must again assure that stimulus intensity is adequate for each of the accepted spikes. This is more difficult in recordings with increased jitter and/or blocking due to neuromuscular disease; in such cases, supraliminal stimulation is verified by seeing a stable stimulus-response latency that does not shorten further when the stimulus is increased.

Recommendation: Verify that stimulation is supraliminal for each measured spike.

12.3.2. VRF effect

Although a constant firing rate eliminates the VRF effect, there are a few situations in which this effect can be seen during electrical stimulation, potentially leading to erroneous results. One such situation is when stimulation begins or the stimulation rate changes. If the rate is changed, e.g., from 10 to 20 Hz, the latency shortens after the rate change (Fig. 18). If jitter is measured immediately following the rate change, it will be artefactually increased. To avoid this, spikes elicited during the initial second after beginning stimulation or after a rate change should not be included in the jitter calculation.

Another situation is when individual discharges are missing, either due to insufficient stimulus intensity or when there is impulse blocking due to disturbed NMT. In these situations, the IDIs will be doubled or even tripled, and the first spike after such a long interval will have a longer latency due to the VRF effect, add-

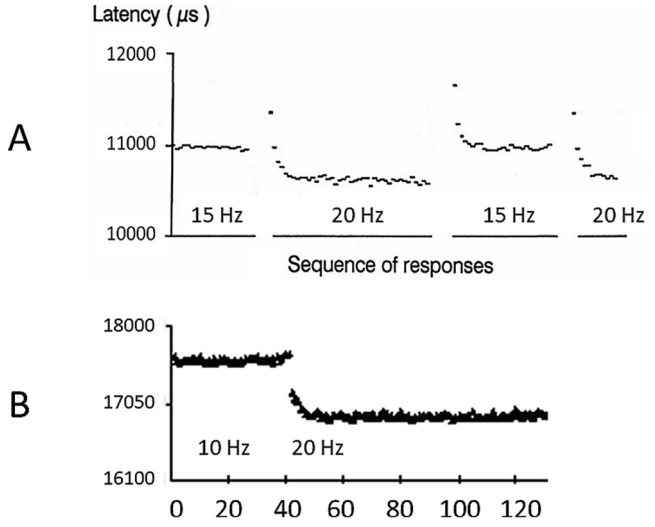


Fig. 18. Velocity recovery function effect during axonal stimulation. A – SFE responses to trains of stimulation of the same endplates at 2 stimulation rates. The intervals progressively shorten during the initial second of each train and most intervals shorten at the higher rate, demonstrating the effect of the velocity recovery function. B – Intervals measured before and after the stimulus rate is increased from 10/s to 20/s. Intervals measured during constant stimulation at each show little variation but progressively shorten immediately after the rate changes. Jitter should not be measured during the transition period.

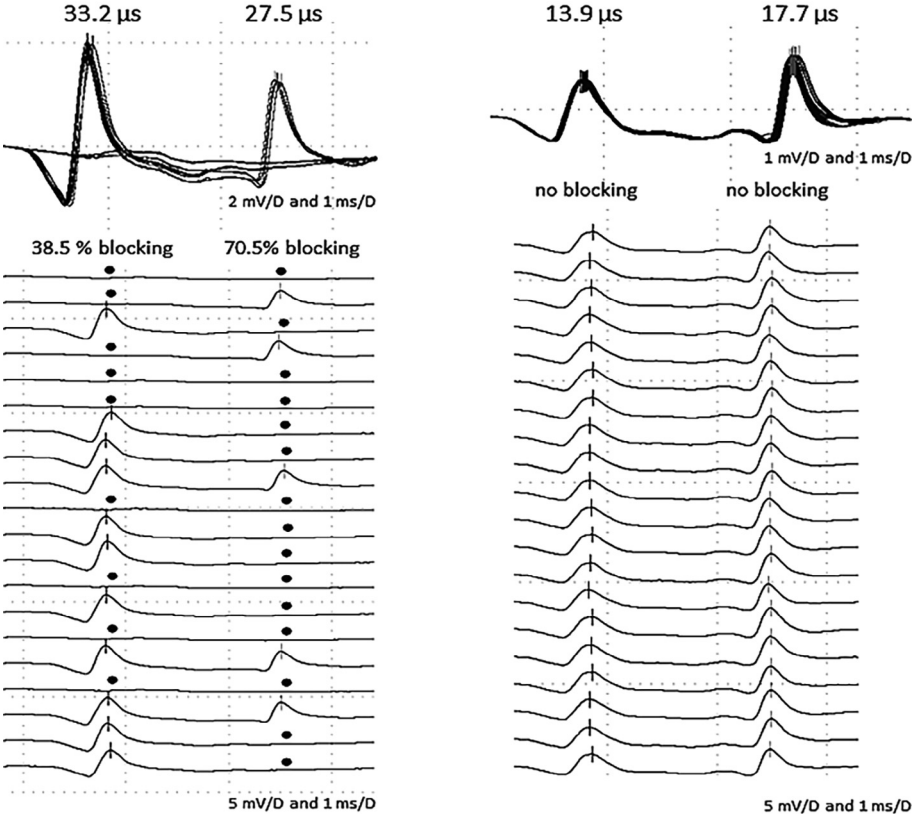


Fig. 17. Effect of liminal stimulation. Concentric needle recordings in the extensor digitorum muscle, 10 Hz intramuscular needle stimulation. Left – liminal stimulation, with erroneously increased jitter and the two spikes blocking independently (dots). Right – suprathreshold stimulation, correct jitter and no blocking.

ing artifactual jitter to the already increased jitter value typical of blocking fibers.

A third situation is the occurrence of F responses between regular stimulus pulses (Blijham et al., 2006; Stålberg et al., 2010). In this case, the interval from the previous muscle fiber discharge is shortened and the stimulus-response latency becomes shorter because of the VRF effect. This normally occurs in fewer than 5% of traces and is relatively easy to recognize in a continuous stimulus-response display or in the sequential IPI histogram (dot plot) (Fig. 19). It should be emphasized that F wave interference can only be visualized and verified if the sweep lasts at least 20 ms, but its effect is seen even with sweeps of shorter duration. It should be noted that facial F waves may be contaminated by the blink reflex, particularly the R2 component (Trontelj and Trontelj, 1978). However, the blink reflex interference does not occur with 10 Hz stimulation, which is usually used for stimulation jitter studies.

A fourth situation is when the muscle is not relaxed during electrical stimulation. In this case a muscle fiber under study may be voluntarily activated immediately before the electrical stimulus arrives. The critical time for this to occur ranges from the F latency (during which time no voluntary impulses can pass) to the refractory period for the muscle fiber. Thus, it is important that the muscle be at complete rest during electrical stimulation. Note that this discussion concerns only the extra activation of the muscle fiber under study.

Recommendations: Do not measure spikes during the initial second after beginning stimulation or a rate change. Be aware that blocking (for any cause) adds to the real jitter. Check for the presence of F waves or disturbing voluntary activity.

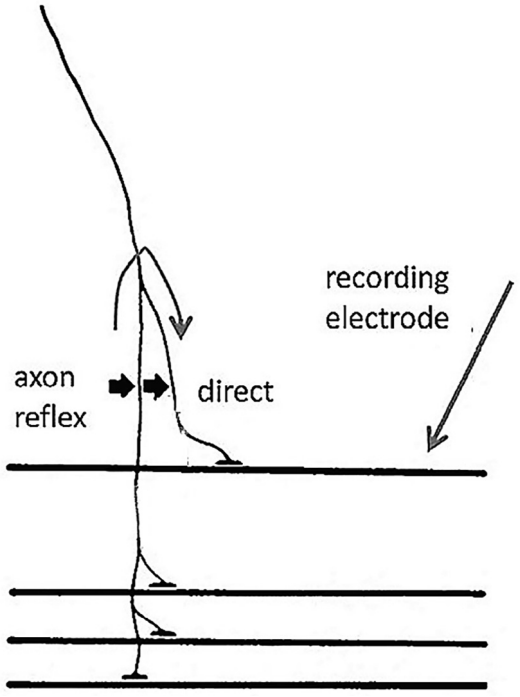


Fig. 20. Diagram of the so-called axon reflex. Weak stimulation produces retrograde activation of the recorded fiber (long arrow). Stronger stimulation gives direct activation and a shorter stimulus-response latency. Reproduced from Stålberg et al, Pitfalls and errors in measuring jitter. Clin Neurophysiol;128:2233–41, 2017, with permission from Elsevier Ireland Ltd.

12.3.3. "Axon reflex"

During electrical stimulation of an intramuscular axon, the evoked spike may sometimes occur at one of two latency positions. This is seen both in normal and pathological conditions. The operator can control the latency position to some extent by slightly changing the simulation intensity. This is the so-called axon reflex* (Fig. 20) (Stålberg and Trontelj, 1970), in which the muscle fibers are stimulated by nerve impulses traveling alternatively orthodromically to the muscle fiber or antidromically to the nearest nerve branching point and then orthodromically to the muscle fiber. If the latency difference is on the order of many hundred ms it is easily detected (Fig. 21), but if it is very short, the latency jump may not be appreciated and will be mistaken for increased jitter. Therefore, if a jitter value is more than 100 μs and there is no blocking, the trace should be carefully inspected visually. Superimposition of 5–20 consecutive traces is very useful in identifying latency jumps, and is routinely used by some operators as a quality

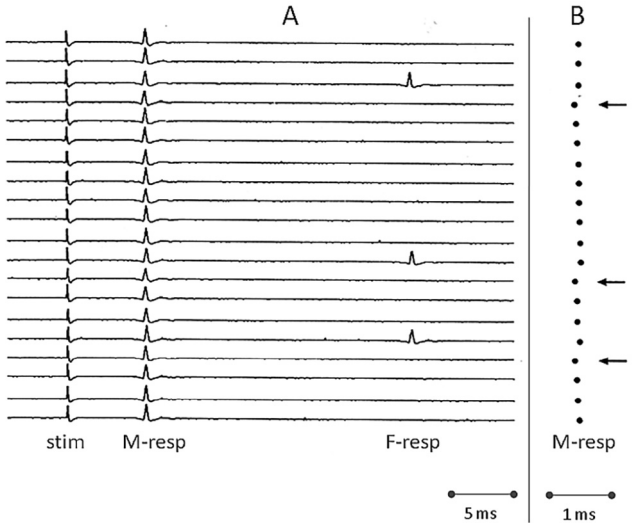


Fig. 19. The effect of F responses on the M-response latency, stimulation SFE recording. A – rastered display, B – dots representing latency to M response. The latency to the M-response following an F response (arrows) is shortened due to the velocity recovery function effect. Reproduced from Stålberg et al, Single Fiber EMG, 2010, with permission from Edshagen Publishing House.

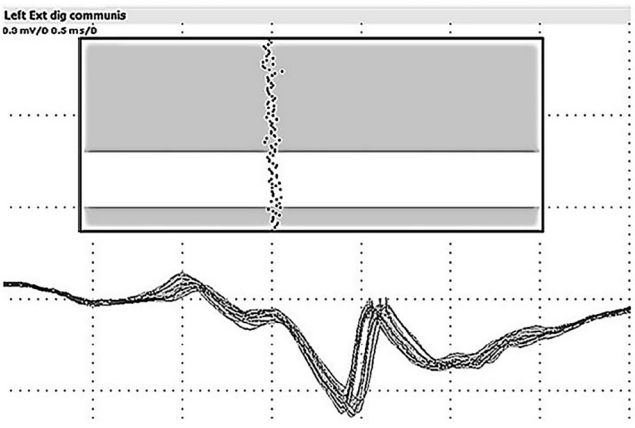


Fig. 21. "Axon reflex." 15 traces superimposed. The jitter is not normally distributed and the superimposed traces (below) show a bimodal distribution. Such recordings should not be accepted for analysis. Reproduced from Stålberg et al, Pitfalls and errors in measuring jitter. Clin Neurophysiol;128:2233–41, 2017, with permission from Elsevier Ireland Ltd.

control. (Superimposition of all traces will make detection more difficult, since slight trends in IPI duration will blur the picture.)

*This not a true reflex, since the impulses travel in the peripheral branches of the motor axon.

Recommendation: Do not include recordings with findings indicating an axon reflex.

13. What does the future hold for SFEMG?

The performance of jitter measurements with SFEs or CNEs is technically demanding, requiring considerable time and experience to develop the skills necessary to obtain quality recordings within a reasonable period of time. Signal analysis techniques should be developed to automatically evaluate the quality of recorded signals to detect artefacts and composite signals, and thus reduce the operator time necessary to assure the quality of CNE signals for jitter analysis.

"Disposable," reasonably-priced SFEMG electrodes should be developed to permit recordings consistent with the original jitter analysis technique.

Conflict of interest

None of the authors have potential conflicts of interest to be disclosed.

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