Clinical Neurophysiology 130 (2019) 1688–1729

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Standards for quantification of EMG and neurography

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See Editorial, pages 1682–1683

ARTICLE INFO

Article history:
Accepted 9 May 2019
Available online 10 June 2019

Keywords:
EMG
Neurography
Macro EMG
Electrical impedance myography
Mune
MScan
Pediatric neurophysiology
Repetitive nerve stimulation

HIGHLIGHTS

- Standards for EMG and Neurography are suggested.
- Electrophysiological tests in Pediatric practice are summarized.
- Implementation and clinical utility of less common methods for EMG and neurography are presented.

ABSTRACT

This document is an update and extension of ICCN Standards published in 1999. It is the consensus of experts on the current status of EMG and Neurography methods. A panel of authors from different countries with different approach to routines in neurophysiological methods was chosen based on their particular interest and previous publications. Each member of the panel submitted 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.

The document covers EMG topics such as conventional EMG, Macro EMG, applications of surface EMG and electrical impedance myography. Single Fiber EMG is not included, since it is the topic in a separate IFCN document. A neurography section covers topics such as motor and sensory neurography, F wave recordings, H-reflex, short segment recordings, CMAP scan and motor unit number methods. Other sections cover repetitive nerve stimulation and Pediatric electrodiagnostic testing.

Abbreviations: ALS, amyotrophic lateral sclerosis; CI, Clustering Index; cm, centimeter; CMAP, compound muscle action potential; CNE, concentric needle electrode; FFT, fast Fourier transform; GBS, Guillain-Barré syndrome; HUA, humeroulnar aponeurosis; LEM, Lambert-Eaton myasthenia; mA, milliamp; MCD, mean difference of consecutive differences; MG, myasthenia gravis; mm, millimeter; MMN, multifocal motor neuropathy; ms, millisecond; MScan, CMAP amplitude vs stim strength; MU, motor unit; MUP, motor unit potential; MuSK, muscle specific tyrosine kinase; NMT, neuromuscular transmission; RTC, retroepicondylar grove; SFEMG, single fiber EMG; SMA, spinal muscular atrophy; SMUP, single motor unit potential; SPACE, Stimulated potential analysis using concentric needle electrodes; SSS, Short Segment Studies; TA, Turns Amplitude analysis; Tib. Ant., tibialis anterior muscle.

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https://doi.org/10.1016/j.clinph.2019.05.008
1388-2457/

Each method includes a description of methodologies, pitfalls, and the use of reference values. Clinical applications accompany some of these sections.

Contents

1. Introduction

Electrophysiological recordings produce data that lend themselves to quantitative analysis. This may lead to increased accuracy, allows comparison with reference values, and provides objectivity when comparing results in the same patient over time or between different electromyographers. Quantification of many parameters can be performed using automatic methods, but some parameters are still assessed semi-quantitatively using subjective ordinal scales (e.g., +, ++ or slight, moderate and severe).

Automatic analysis is preferred to manual measurements. The advantage of automatic methods is that the analysis is standardized, reproducible, rapid, and may include parameters not measurable manually. With better understanding of the relationship between obtained signals and underlying normal or abnormal signal generators, it has become possible to optimize signal analysis to produce information that is clinically relevant and to suppress redundant or insignificant data. Regardless of the methods of analysis, optimal results require good signal quality based on high standard recording equipment and on the operatoŕs recording skills.

Because of great differences across the world in training, access to equipment, and quantification methods it is difficult to formulate detailed guidelines. Local variations are welcomed as an opportunity for a dynamic continued progress in the area of clinical neurophysiology partly reflected in this document. The authors, who represent laboratories with different and well-established routines, have attempted to reach a consensus regarding principles for quantification of Electromyography (EMG) and Neurography. During this process, each author drafted a technical guideline, based on the best available literature and local practices, on a specific technique of EMG and Neurography. Each guideline was reviewed by all authors and modifications or suggestions were offered and circulated to the group. This process continued until a reasonable consensus was reached.

For practical reasons, we have focused on the most commonly used methods. However, we have also included some less common techniques and techniques currently in development that we felt should be addressed and considered, at least until the next revision of this document. This document is an update and extension from the last Standards for Quantification of EMG and Neurography document published in 1999 (Stålberg et al., 1999).

2. Electromyography

2.1. Conventional needle EMG

In the diagnosis of neuromuscular diseases, muscles are typically examined with concentric or monopolar needle electrodes. Techniques such as high-density surface EMG (see below) are used

for the study of overall activity of the muscle for purposes other than diagnostic evaluation.

The principal changes in nerve and muscle disorders have been established since the 1940′s. Many attempts were made to quantify recorded signals (Buchthal et al., 1954b; Fuglsang-Frederiksen et al., 1976, 1977; Stålberg et al., 1996), but few methods have found general acceptance. Two factors have limited the use of EMG quantification in everyday clinical practice. The first is technical, related to the development of EMG technology. With newer generations of EMG equipment, software has become more robust for fast analysis, so the argument that quantitative procedures are tedious has become irrelevant. For best results the operator also needs to understand the analysis algorithms. The other and even more important factor is the lack of well-defined reference values. Normative data are essential for meaningful quantification. Traditionally, normative reference values have been collected in multicenter studies. Unfortunately, such data are useful only for specified equipment, which is also an important issue in clinical neurophysiology in general. Novel methods that do not require the tedious collection of reference values from healthy individuals have been reported recently (Jabre et al., 2015; Nandedkar et al., 2018b). However, to become more generally useful, these methods must be refined, considering age, height, other demographic variables and combinations thereof (e.g. height and age).

The EMG is performed in three steps: assessment of spontaneous activity with the muscle at rest, MU potential (MUP) analysis at slight voluntary muscle contraction, and interference pattern (IP) analysis at increasing and strong effort. These will be described with a focus on the feasibility and methods for quantification.

2.1.1. Insertional and spontaneous activity

Electrical signals that occur spontaneously or are induced by needle movement in a resting muscle provide important information about the type, severity, and chronicity of underlying disease. Insertional activity is caused by needle tip irritation of the muscle membrane during electrode movement. It lasts usually less than 300 ms, slightly longer than the time for electrode movement (Wiechers, 1979). Sometime a run of positive sharp waves may follow. It is a normal phenomenon that may last longer in denervated muscle, but its diagnostic value has not been demonstrated.

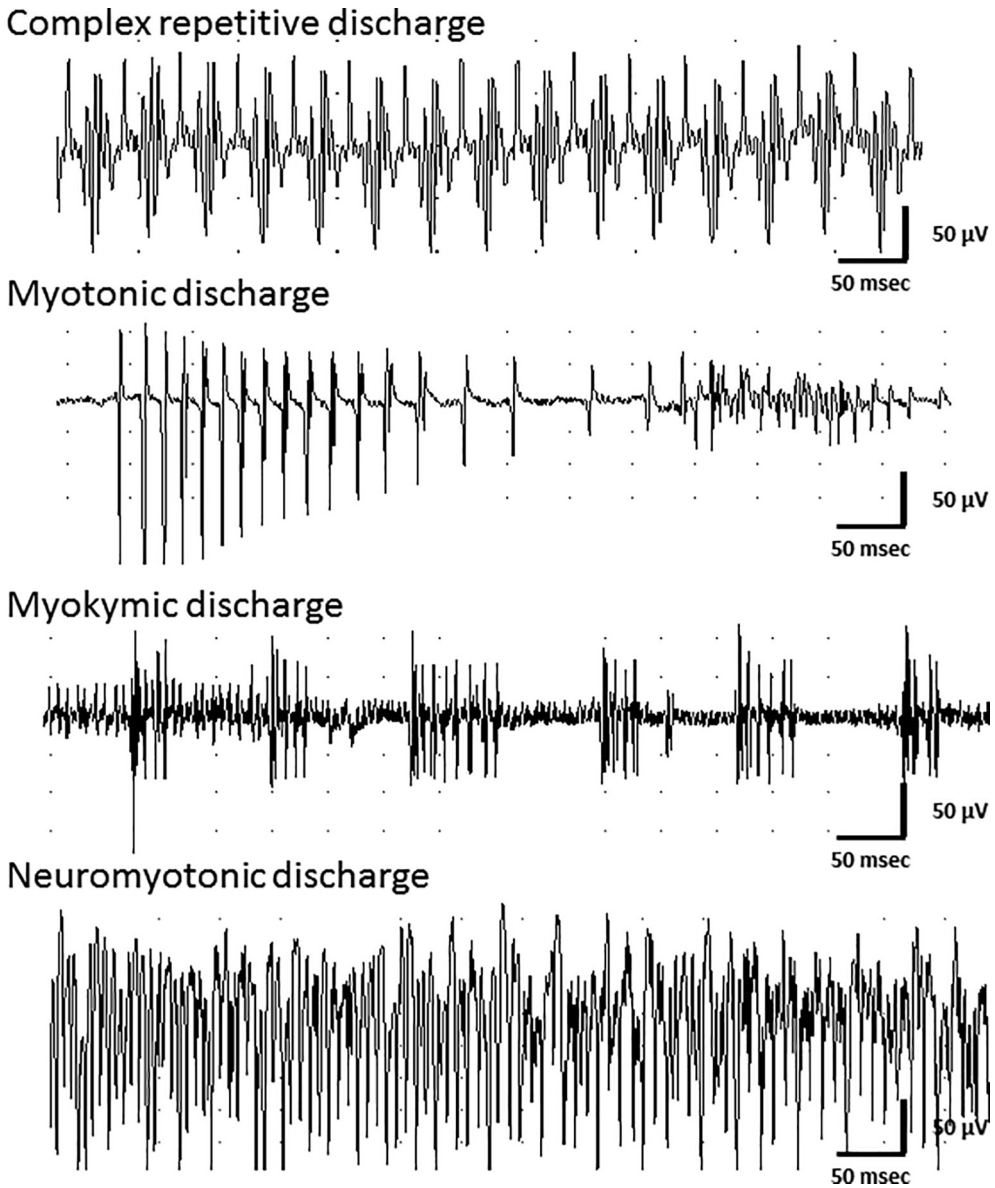


Fig. 1. Different types of spontaneous discharges; the first two examples are generated in the muscle; the others are generated in the nerve.

Table 1
Summary of different types of spontaneous activity.

Type of activity	Description	Pathophysiology	Interpretation
Insertion - normal	Bursts of muscle fiber action potentials occurring with needle movement (<300 ms)	Single muscle fiber action potentials induced by electrode against muscle membrane	Normal, if it ceases shortly after needle movement
Insertion - increased	Bursts of muscle fiber action potentials that continue following cessation of needle movement	Instability of muscle membrane	Denervation or muscle membrane instability Often associated with fibrillation potentials
Insertion - decreased	No or minimal bursts of muscle fiber action potentials during needle movement	Inexcitability or replacement of muscle fibers by fibrosis or fatty tissue	End-stage neurogenic or myopathic disorders Channelopathies (e.g. periodic paralysis) during attack
Fasciculation potentials	Single, spontaneous motor unit potentials. Irregular firing pattern. Morphology varies and reflects the underlying motor unit; often distant fasciculations recorded	Peripheral nerve hyperexcitability (generated anywhere along the lower motor neuron)	Non-specific May be normal (e.g. benign fasciculations) Increased frequency in lower motor neuron disorders
Complex repetitive discharges	Regular firing pattern Abrupt onset, cessation, and/or change in rate Groups of few or many spikes	Groups of muscle fiber action potentials induced by ephaptic conduction between hyperexcitable fibers (Stålberg and Trontelj, 1982)	Non-specific Chronic neurogenic or myopathic disorders (Fellows et al., 2003)
Myotonic discharges	Waxing and waning rate and amplitude Maximum firing rates of 40–100 Hz (Barkhaus and Nandedkar, 2006; Kneiser et al., 2015) Impacted by temperature	Recurrent firing of single muscle fiber action potentials Pathophysiology not completely known; muscle membrane channel dysfunction	Myotonic myopathies Also seen in other myopathies
Myokymic discharges	Bursts of 1 to many MUPs repeating in a regular, semi-rhythmic, or irregular pattern Varying firing rates within and between bursts Interburst spike frequency 2–10 Hz (Gutmann et al., 2001)	Peripheral nerve hyperexcitability at the cell body or axon level	Non-specific. Seen in mononeuropathies, polyradiculopathies, and radiation-induced nerve damage May also be seen with neuromyotonic discharges in "neuromyotonia" (e.g. Isaacs' syndrome)
Neuromyotonic discharges	High frequency discharge (150–300 Hz) of MUs, occurring in bursts or continuous trains (Torbergsen et al., 1996; Daube, 2001; Gutmann et al., 2001; Kneiser et al., 2015)	Peripheral nerve hyperexcitability at the cell body or axon level	Neuromyotonia (e.g. Isaacs' syndrome)
Cramp discharge	High frequency discharge of MUs, occurring in continuous train. Often initiated following voluntary contraction. MUs firing at high frequencies with an irregular firing pattern (Note: electrically silent cramps are seen in "rippling muscle disease"(Torbergsen, 2002) and metabolic myopathies, such as McArdle's disease)	Peripheral nerve hyperexcitability at the axon or spinal level	Non-specific Increased frequency in lower motor neuron disorders

Some spontaneous waveforms are normal (e.g., end-plate noise and spikes) while others indicate a disorder of nerve or muscle, but are not necessarily specific for a single disease or disease process. With a few exceptions, spontaneous activity is not assessed by quantitative methods (Fig. 1).

The presence of any abnormal spontaneous discharge should be reported, given their potential diagnostic significance (Table 1).

2.1.1.1. Fibrillation potentials and positive sharp waves. Fibrillation potentials (fibs) and positive sharp waves (PSWs) are spontaneous waveforms generated by firing of individual muscle fibers due to hyperexcitable muscle membranes (often, but not exclusively in denervation). The fibs consist of biphasic or triphasic spikes of short duration with an initial small positive phase (i.e., downward deflection), followed by a larger negative phase (i.e., upward deflection), which is often followed by a small positive phase. The PSWs are generally biphasic, consisting of an initial large positive phase followed by a smaller and broader negative phase. The mechanism of generation of both forms is similar: Fibs are thought to be muscle fiber action potentials generated at a short distance from the fiber; PSWs, in contrast, are thought to arise from a region immediately adjacent to the needle electrode (Dumitru and King, 1998; Dumitru et al., 1999; Dumitru and Santa Maria, 2007; Willmott et al., 2012; Kneiser et al., 2015). It is suggested that PSWs are generated in a small section of a muscle fiber that is "damaged" by the electrode (Nandedkar et al., 2000). Some literature suggests that PSWs occur earlier after nerve or muscle injury than fibs, but clinical differences between the two have not been clearly defined (Kraft, 1996). Unlike MUPs, morphologic parameters of individual fibs and PSWs (e.g. rise time, amplitude, duration, firing rate) are not quantified or reported.

The fibs and PSWs appear 1–3 weeks after nerve injury, depending on the site of lesion (earlier for a site closer to the muscle). The number of distinct fibs and PSWs recorded at any one time reflects the number of hyperexcitable muscle fibers, which may correlate with the severity of disease. However, they may also reflect the number of muscle fibers irritated by the needle electrode (i.e. the length of needle movement). Cool limb temperature and hypoxemia decrease the density and firing rates of these potentials (Denys, 1991; Izumi et al., 1999).

Several methods are used to grade fibs and PSWs. One common method assesses their density and distribution in different regions of the muscle using an ordinal scale (Table 2). Techniques for grading and reporting these potentials to be considered include:

Needle movement technique: needle movements of 0.5–1 mm are used; the highest level of activity immediately after needle

Table 2
Example of scoring spontaneous activity.

Grade	Degree of fibs/PSWs
0	None
+/–	One - few Fibs/PSWs in only a single area of muscle.
+	One - few Fibs/PSWs in > 1 area
++	Multiple Fibs/PSWs in > 1 area (usually most)
+++	Many Fibs/PSWs in most areas
++++	Profuse Fibs/PSWs (completely filling baseline) in most areas

movement is graded at each site; and the grades of fibs/PSWs from each site are averaged. The reported grade of activity is the average grade from all tested sites (about 10 recordings positions, including 2 skin insertions or 3–4 passes through different muscle areas) in the muscle.

The distribution of fibs/PSWs can also be scored by reporting the number of areas showing this activity over the number of areas examined (e.g. "fibrillation potentials present in 6/10 sites") (Table 2).

2.1.1.2. Fasciculation potentials. Fasciculation potentials (FPs) are spontaneous waveforms produced by single motor units (Kneiser et al., 2015), and may be generated at any point along the motor unit (anterior horn cell or distal portion of the axon) (Denny-Brown and Pennybacker, 1938). While FPs may be recorded in muscles without disease (Fermont et al., 2010), particularly in distal foot muscles of healthy subjects (Falck and Alaranta, 1983) they are commonly associated with focal or generalized neurogenic disorders, such as amyotrophic lateral sclerosis (ALS) (Johansson et al., 2017) spinobulbar muscular atrophy (Kennedy's disease), spinal muscular atrophy, and poliomyelitis. The significance of identifying FPs is most important in ALS as they may be an early indicator of lower motor neuron disease, even before fibrillation potentials or abnormal voluntary motor unit potentials are recorded. As a result, FP potentials are included in the Awaji consensus criteria for ALS diagnosis (de Carvalho et al., 2008; de Carvalho and Swash, 2013b, 2013a). FPs may also be part of a more generalized hyperexcitable peripheral nerve disorder, such as "cramp fasciculation syndrome" or Isaacs' syndrome (de Carvalho and Swash, 2011).

Table 3
Example of scoring amount of fasciculation potentials.

Grade	# per 60 seconds
0	None
+	1–30
++	31–75
+++	76–150
++++	>150

On needle EMG, FP potentials are identified by their irregular firing pattern. The firing frequency varies from a few per second to less than one per minute. To assure a high probability of detecting FP potentials in ALS patients, recordings should last at least 90 seconds without needle movements (Mills, 2011). FPs can be graded by counting the number of FPs per period of time (e.g. per 90 seconds of recording) and the number converted to an ordinal scale (Table 3). The grades of severity may be arbitrarily determined and scales may differ between laboratories.

FP potentials are usually recorded with a needle electrode; however, they may also be recognized in surface electrode recordings and in ultrasonographic recordings (Mateen et al., 2007; Misawa et al., 2011).

2.1.2. MU potential

The MU potential (MUP) is the temporal and spatial summation of individual muscle fiber action potentials belonging to a single MU as recorded by a nearby surface or needle electrode. The needle electrode has a rather restricted uptake radius (Gath and Stålberg, 1978; Nandedkar et al., 1997) and records the bioelectric activity of only a part of the MU. The MUP therefore does not reflect the total size of the MU (Stålberg, 1980; Ertas et al., 1995), but rather the activity of muscle fibers from MUs near the active recording surface of the electrode. The generation of a MUP, its relation to MU micro anatomy and the interpretation of findings in pathology have been discussed extensively in the literature (Nandedkar et al., 1988; Stålberg et al., 1996; Dumitru et al., 1997; Lateva and McGill, 2001; Stålberg, 2004).

EMG recordings are performed with monopolar or concentric electrodes. The monopolar electrodes are non-directional whereas the concentric electrodes have an oval recording surface on the beveled tip, facing 15 degrees from the axis of the cannula. Recordings from both types of electrode have similar MUP duration values regardless of whether or not the muscle fiber passes behind or in front of the tip of the concentric electrode (Dumitru et al., 1997). The amplitude of the sharp components of the MUP is maximum when the action potential is generated by muscle fibers close to the electrode (Dumitru et al., 1997). For the concentric electrode, fibers behind the electrode are "in the shadow" of the electrode, and this will influence the sharp components; this is not the case

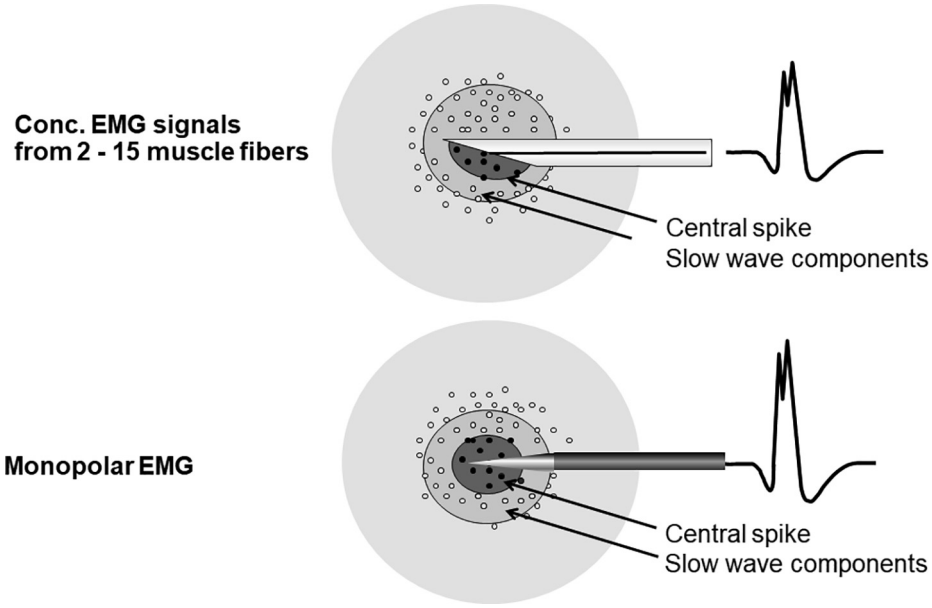


Fig. 2. Schematic presentation of recordings made with monopolar and concentric needle electrodes. Remote activity from fibers behind or in the front of the electrode contribute similar slow wave components (duration) for both electrodes. When the electrical field approaches the concentric electrode, activity from the closest fibers generating sharp components will dominate, making the concentric uptake area hemispherical for this electrode. Copyright Stålberg.

for the monopolar electrode. For the spiky components, the uptake region is hemispheric and for the slow early and late components the regions is circular (Fig. 2).

2.1.2.1. Parameters. MUPs can be quantitatively described by several parameters. Most of the parameters currently considered pertinent for assessment of MU characteristics can be quantified automatically. The parameters, interpretation and mode of quantitation for the most commonly used parameters are listed in Table 4 and are described in many publications (Buchthal et al., 1954a; Stålberg and Erdem, 2002; Nandedkar and Stålberg, 2008).

2.1.2.2. MUP analysis. Three quantitative analysis techniques are generally available for the systematic examination of individual MUPs. All three have a similar sensitivity for detecting neuropathic changes. The first technique is based on manual measurements from a free running sweep display following an algorithm similar to that used by Buchthal (Buchthal et al., 1955). This manual-MUP technique is time-consuming, requiring recordings of 2–3 minutes duration per muscle site. It is also demanding on the operator, as reproducible MUPs must be identified, the one with the smoothest baseline is selected, and duration cursors must be set manually. This technique can only measure low threshold MUs and is inevitably open to operator bias, especially in the determination of MUP duration (Podnar et al., 2002). The second technique uses a trigger and delay function, in which the operator sets a voltage level on a prominent feature of a firing MUP during a constant level of EMG activity (Czekajewski et al., 1969). The MUP is averaged until the baseline becomes smooth, which usually takes about 1 minute per MUP. This technique is also time-consuming, measures only low threshold MUs, one at a time, and is again prone to operator bias (Podnar et al., 2002). Also, averaging techniques will blur any unstable components of the MUP. In the more recent template-matching techniques available on advanced EMG systems (these can all be called multi-MUP analysis, since many MUPs are recorded at each site) (Nandedkar et al., 1995; Stålberg et al.,

Table 4
Parameters used in MUP analysis.

Parameter	Significance	Usually measured as	Analysis
Amplitude	No. of fibers within 0.5 mm	Peak–peak amp (μV)	a/m
Area	No. of fibers within 2 mm	Total area within duration (μVms)	a
Duration	No. of fibers within 2–3 mm	Slope criteria (ms)	a/m
Thickness	No. of closest fibers	Area/amp (ms* μV)/ma	a
Size index	MUP "size"	Normalized thickness	a
No. of phases	Temporal dispersion	Baseline crossings + 1	a/m
No. of turns	Temporal dispersion	No. of direction changes	a/m
No. of satellites	Excessive temporal disp	No. of spikes	m
Jiggle	Neuromuscular transmission	Shape stability	a/visual

MUP, MU potential;"Significance", the main biological counterpart, but usually not the only factor reflected in the parameter;"Usually measured as", the most commonly used algorithm;"Analysis mode", may be measured automatically, by software algorithms (a) or manually (m). Separate references: Thickness and Size index (Sonoo and Stålberg, 1993), Jiggle (Stålberg and Sonoo, 1994). With more than 4 phases the MUP is called polyphasic and with more than 5 turns, the MUP is called complex.

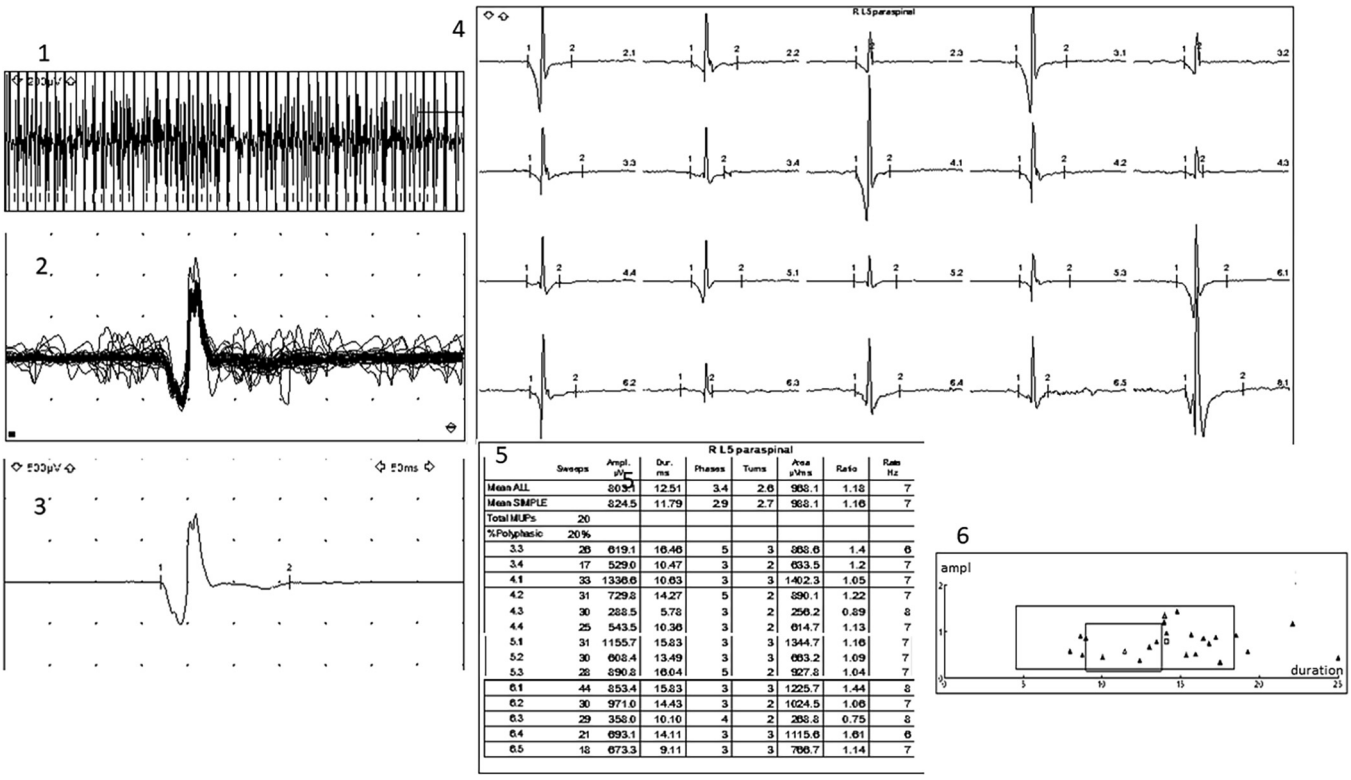


Fig. 3. Principles of Multi-MUP analysis. 1 - free running sweep.2 - automatically extracted MUPS superimposed. 3 - average of these MUPs. 4 - twenty MUPs. 5 - extract from result Table 6 – box of MUP duration vs amplitude. Out and inner boxes represent reference limits for individual MUPs and mean of all MUPs respectively. Copyright Stålberg.

1995), the operator determines when the computer is to analyse the last 5 to 10 seconds of a previously acquired signal. MUPs are first selected and extracted (signal decomposition) based on software definitions, and then analyzed according to various algorithms. MUPs are then sorted into classes, each representing the average of the selected MUPs (Fig. 3). A large number of such algorithms have been developed over the years (McGill and Dorfman, 1989; Nandedkar et al., 1995; Stålberg et al., 1995; Stashuk and Brown, 2002; Nikolic and Krarup, 2011), indicating that none is clearly superior to the others. MUPs with an unsteady baseline (i.e., unclear beginning or end) or MUPs distorted by averaging must be recognized and deleted. Also, minor differences in the MUP shape may misclassify the signals as representing different MUs. Visual inspection during recording and post-processing review is important. Multi-MUP analysis is the fastest and easiest to use of these three quantitative MUP analysis techniques (Podnar et al., 2002) and may take 3–5 minutes for a complete study of 20 accepted MUPs. As with other averaging techniques, any unstable components of the MUP will be blurred.

The values from each measurement technique must be compared with normative data, different for different muscles (Fig. 4) and assessed for robustness and reproducibility in the clinical routine. So far, it has been difficult to develop a test procedure to compare different techniques. Such a comparison should include the entire process: the definition of a MUP to be selected, extraction, averaging protocols, cursor settings, and parameters to be measured.

2.1.3. Analysis of needle EMG at increasing and strong effort

During increasing voluntary activation of the healthy muscle, small MUs are activated first, followed by successively larger units, according to the so-called size principle (Henneman et al., 1965). This cannot be detected with monopolar or concentric needle electrodes because their restricted uptake areas are much less than the total MU territory (Ertas et al., 1995). At slight effort individual MUPs can be extracted and with increasing force an interference pattern (IP) develops. Recordings of MUPs and the IP give information about the orderly physiological recruitment which may be disturbed by peripheral or CNS abnormalities, possible loss of MUs, MUP size, and number of recruited MUs, and possible disturbances in motor control (e.g. tremor).

2.1.3.1. Recruitment analysis. Recruitment analysis is used to indirectly estimate the number of functioning MUs and the integrity of their central control. The *rate* of firing of a MUP reflects the intensity of central excitation of the anterior horn cell and the number of MUs that can be activated. Recruitment analysis determines the relationship between the number of activated MUs and their firing rates, which can be quantified by several methods (Freund et al., 1973; Milner-Brown et al., 1973; Tanji and Kato, 1973; Petajan, 1991; Enoka, 1995; Conwit et al., 1998; De Luca and Hostage, 2010). During low effort, recruitment analysis consists of calculating the *recruitment frequency* and the *recruitment ratio*, both of which are used to determine whether recruitment

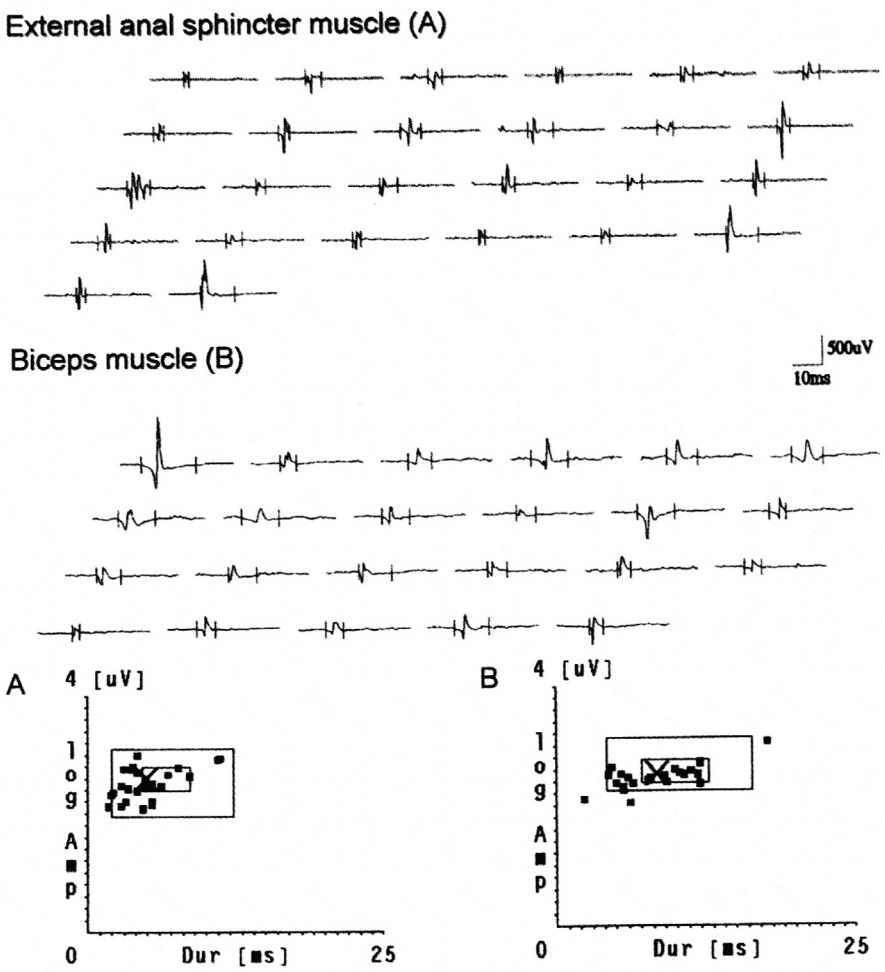


Fig. 4. MUPs from (A) external anal sphincter and (B) biceps muscle of a 26 year old healthy subject. Note similar amplitude but shorter duration (EAS 4.9 ms, biceps 8.4 ms) of MUPS from the EAS. Bottom - graphical plots of duration vs amp (log AMP). 95% confidence limits for individual data (outer rectangle) and mean value (inner rectangle). Note the difference between muscles. (Podnar, 2008 with permission).

is normal or abnormal. Recruitment has been until now interpreted semi-quantitatively since no automated methods have been developed due to lack of an accepted definition of signals to be included in analysis. Automatic recruitment analysis methods are evolving in commercial EMG equipment.

2.1.3.1.1. Recruitment frequency. The *recruitment frequency* or *rate* is the rate of firing of the first activated MUP when the second MUP begins firing (Petajan and Philip, 1969; Petajan, 1991). The steps used to determine the recruitment frequency are: (1) the muscle is contracted at a low level such that only a single MUP with a minimum rise time and maximum amplitude is recorded; (2) the effort is increased slightly until a 2nd MUP with short rise time begins to fire (there is no international consensus on the definition of required rise time); and (3) the maximum firing rate of the first MUP at that time is determined (Petajan, 1991). These steps are repeated in several areas of the muscle to determine the overall recruitment frequency for that muscle. The severity of reduced recruitment can be graded on an ordinal scale based on the recruitment frequency, although the recruitment frequency within each grade is arbitrarily defined (Table 5).

Recruitment frequencies vary in different muscles and for type I and type II MUs. Normal recruitment frequencies are usually between 5–12 Hz for most limb muscles and up to 16 Hz for cranial muscles at low levels of contraction (Petajan and Philip, 1969; Gunreben and Schulte-Mattler, 1992; Sun et al., 2000).

2.1.3.1.2. Recruitment ratio. The *recruitment ratio* is calculated by dividing the number of discharging MUs into the firing rate of the most rapidly discharging (calculated from highest consistent rate) MU in the epoch under study. This is measured during low effort in which 2–4 MUPs are firing at any one time. The steps used to measure the recruitment ratio are: (1) patient contracts the muscle at low force, (2) the needle electrode position in the muscle is adjusted until 2–4 MUPs with short rise time are recorded, (3) the number of distinct MUPs is counted, (4) the firing rate of the fastest firing MUP is calculated, (5) the recruitment ratio is calculated (maximum consistent firing rate/# of firing MUPs). The recruitment ratio from at least 10 areas of the muscle is averaged. For most normal limb muscles, the recruitment ratio is <5 but normal reference values for individual muscles have not been well defined.

When measuring recruitment frequencies and ratios, distant MUPs may be seen in the background; however, only the close MUPs (those with a sharp rate of rise) should be accepted. One possibility for automatic quantification is to use the MUPs that have been extracted and accepted according to algorithms in the multi-MUP program for a given EMG machine. Signals recorded from the cannula of a concentric needle (inverted polarity) or from the reference electrode during monopolar recording should not be included in the recruitment assessment.

During strong contraction, identification of the firing rate of individual MUPs is obscured by overlap of many MUPs. Assessment of recruitment is difficult in patients who are poorly cooperative or are unable to activate more than 1–2 MUPs, and reduced recruitment cannot be accurately assessed if the firing rate of the MUPs is low (e.g. <10 Hz) (termed "poor activation"). In infants and children, the same normal recruitment frequencies and ratios can be used as reference, but controlled low force of contraction may be difficult and recruitment ratios may be more reliable than recruitment frequencies.

Table 5
Example of grading recruitment frequency.

Grade of MU loss	Recruitment frequency
0 (normal)	≤10 Hz
1+ (mildly reduced)	11–15 Hz
2+ (moderately reduced)	16–20 Hz
3+ (severely reduced)	>20 Hz

2.1.3.1.3. Abnormal recruitment. In diseases characterized by a reduction in the number of functional MUs either from loss of MUs or conduction block, the recruitment frequencies and recruitment ratios increase (i.e. *reduced recruitment*, *reduced numbers* or, in severe cases, *discrete recruitment*). This is recognized as a higher than normal firing rate of the initial MUP before the 2nd MUP is recruited and/or an increase in the recruitment ratio (Daube, 2000; Schulte-Mattler et al., 2000). While recruitment frequencies and ratios are best assessed at low effort, moderately or severely reduced recruitment may only be recognized during stronger contraction if higher threshold MUs are lost. Reduced recruitment is characteristic of neurogenic disorders, but may rarely be seen in patients with severe myopathies in muscles that have lost all fibers of a MU.

In myopathies, a *rapid (or early) recruitment* pattern is usually seen. Since loss of fibers within a motor unit generates less force during activation, a higher number of MUPs must be activated *with minimal patient effort* in order to generate a desired force (Kugelberg, 1949; Petajan, 1991). The recruitment frequency and recruitment ratio values are normal but the number of MUPs relative to the level of contraction is increased. Early or rapid recruitment has not been well quantified but 3 or more MUPs present with "barely perceptible muscle contraction" is suggestive of rapid recruitment (Petajan, 1991). Identifying rapid recruitment is mostly subjective and requires assessment of the degree of effort the patient is exerting.

In central nervous system disorders involving the upper motor neuron pathway, such as in patients with spasticity, recruitment of motor neurons may also be affected, especially at minimal contraction and in distal muscles. The onset MUP firing frequency is lower and the second MUP is recruited at lower rates than in normal patients, consistent with loss of ability to modulate the firing frequency (Frascarelli et al., 1998).

2.1.3.2. Strong effort. The characteristics of the signal obtained during strong voluntary activity are dependent on the individual MUP components, their shape and firing pattern. Analysis of these signals can be made in the time domain or in the frequency domain.

2.1.3.2.1. Time domain. These analyses assess features that can be observed on the screen, namely the number of turning points in the signal and the amplitude differences between turns. This was originally defined by Willison (Willison, 1964) and was modified to display a plot of the number of turns vs their amplitude (TA method) (Stålberg et al., 1983) (Fig. 5), and later by including a measure of the fullness of the signal (EQUIP) (Nandedkar et al., 1986a). Typically, 20 recordings are performed during moderate and strong contraction. The boundary of the normal "cloud" in Fig. 5 indicates 95% confidence limits for the reference values. If more than two of the values are outside the boundary on the same side of the cloud, the findings are "abnormal". Other types of display of the Turn and Amplitude values have also been presented (Fuglsang-Frederiksen et al., 1985; Gilai, 1989). Reference values for TA have been developed in 8 muscles for both men and women and for concentric and monopolar electrodes, and for 4 muscles for EQUIP. The time domain method has been accepted as routine in a number of laboratories as a fast, robust method, not heavily dependent on the skills of the operator. The analysis epochs are short (typically 250 msec), and the method can therefore be used in children.

2.1.3.2.2. Frequency domain. Frequency analysis can be performed as frequency band (Johansson et al., 1970) or fast Fourier transform analysis (FFT). The frequency methods have not been implemented for clinical use as much as the time domain methods, but have been used in assessment of muscle fatigue in occupational and sports medicine. Some of these methods are summarized in Table 6.

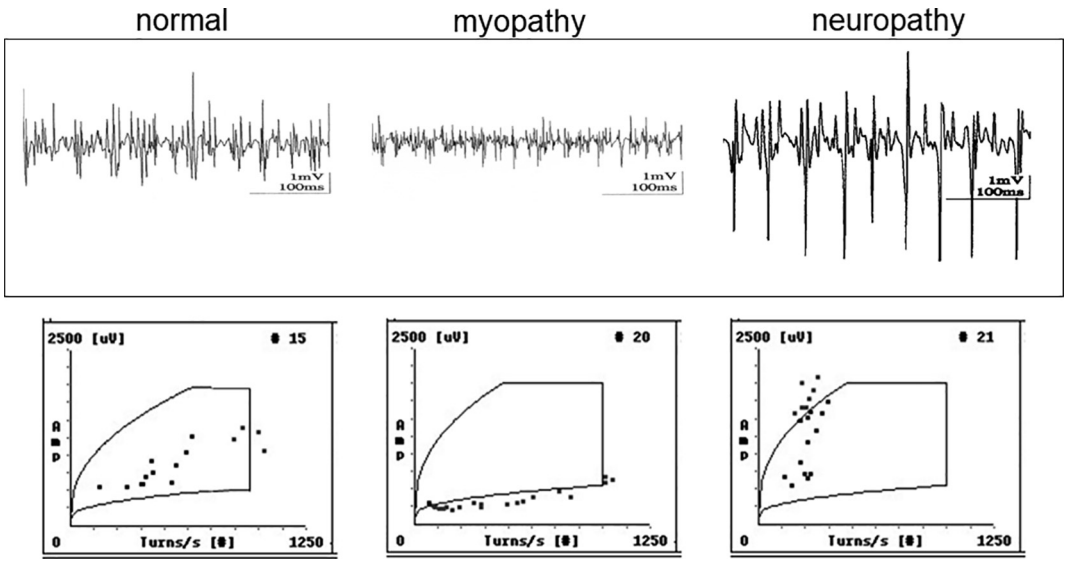


Fig. 5. Examples of three different EMG recordings (Tib.Ant.) showing original signals (above) and TA from each muscle (below). The enclosed area corresponds to 95% confidence age-matched limits. Dots represent the result of TA analysis from 250 msec. epochs. Note the low amplitudes with some high number of turns in myopathy and the opposite in neuropathy. Copyright Stålberg.

Table 6
Methods for quantitation of EMG patterns at increasing and full effort.

Parameter	Significance	Usually measured as	Analysis mode
Increasing activation			
Firing pattern	Central factors/MN size	Discharges/s	a/m
Onset frequency	No. of available MUs/MN size	Discharges/s	a/m
Mean frequency	No. of available MUs/MN size	Discharges/s	a/m
Full activation, time domain			
Turns	Shape of individual MUPs And number of MUs	No. of turning points/s	a
Amplitude	Amplitude of individual MUPs	Ampl/turn or envelope	a
Fullness	No. of MUs	% signal on trace	a
Full activation, frequency domain			
Band power	Shape of MUPs	dB of filtered signals	a
Frequency spectrum	Shape of MUPs	FFT	a
Change in frequency	Fatigue (in the signal)	Continuous FFT	a

MN, motor neurone; FFT, fast Fourier transform; Analysis mode, a = automatic, by software algorithms, m = manual.

2.1.3.3. Comparison between MUP analysis and interference pattern analysis. As may be seen from Table 7, some of the IP results are related to features obtained from MUP analysis. The choice of quantitative methods depends on factors such as availability of the method in the EMG equipment, ease of use, and familiarity with the technique. If both MUP and IP analyses are performed, the electromyographer should use the unique features of each in a rational way and focus the analysis on the parameters that pro-

Table 7
Comparisons of parameters reflected in quantitative EMG analysis.

Parameter	MUP analysis, slight activation, time domain	Turn/amp. analysis, full activation, time domain	FFT, full activation, freq. domain	Recruitment, increasing activity, time domain
MUP amplitude	+	+	—	—
MUP duration	+	—	+	—
MUP rise time	+	—	+	—
MUP complexity	+	+	+	—
MUP stability	+	—	—	—
Extra discharges	+	—	—	—
IP density	—	+	—	—
Early recruitment	—	—	—	+
Loss of axons	—	+	—	+
Central drive	—	+	—	—
Fatigue	—	+	+	—

Table shows analysis using MUP analysis, interference pattern analysis of turns/amplitude (clouds), spectrum analysis (FFT) and analysis of recruitment behavior and firing pattern (recruitment). + , parameter assessed; — , parameter not assessed.

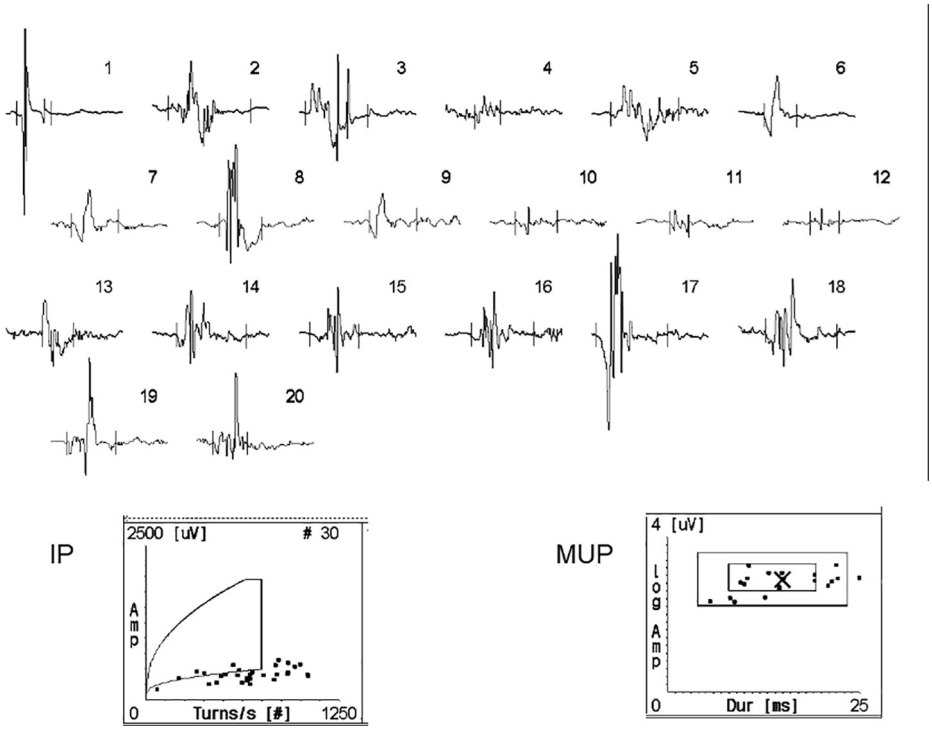


Fig. 6. Recording from tibialis anterior muscle in a patient with myopathy. MUPs are very polyphasic (upper panel). IP analysis shows low amplitudes of individual spikes and increased number of turns, as expected in myopathy. Amplitude and duration parameters appear within normal limits (right lower corner). A combination of methods (visual inspection, MUP analysis, IP analysis) is usually necessary for correct interpretation. Copyright Stålberg.

vide optimal information in a given situation. Different analysis methods may show different results, a situation that usually can be explained (Fig. 6).

2.2. Global recordings

As a complement to the routinely-used, relatively selective EMG recordings with concentric or monopolar electrodes, it is sometimes of value to obtain a non-selective recording from all active muscle fibers in the muscle from individual MUPs. Some techniques are based on triggering with an intramuscular needle electrode and recording with a single surface electrode (Brown, 1972) or multiple surface electrodes, so-called high-density EMG (Stegeman et al., 2000; Zwarts et al., 2000) or needle electrodes, scanning EMG (Stålberg and Dioszeghy, 1991). In another method, called macro EMG, the global recording is made from the cannula of an intramuscular electrode while triggering on signals recorded by a selective electrode surface in the same electrode (Stålberg, 1980).

These methods give information about the electrical size of the MU, comprising number and size of muscle fibers in a MU. It is useful in the assessment of collateral reinnervation and in the indirect estimation of the number of MUs. Quantification aspects of some of these methods are discussed below.

2.2.1. Surface EMG

Surface EMG (SEMG) measures the electrical activity of activated MUs through the skin. Although it doesn't detect spontaneous activity of individual muscle fibers, with SEMG the integrated activity of single MUs and/or the whole muscle can be recorded noninvasively and without pain for long periods. Table 8 presents an overview of its applications in neurology.

The position and size of the recording electrodes that are used depend on the purpose of the measurement (Merletti et al., 2010). It should be realized that the interelectrode distance (IED) acts like a filter; the smaller the distance between the electrodes, the less difference in voltage can be measured, resulting in lower amplitudes and cut-off of lower frequencies. In kinesiological studies, the IED is usually 2 cm. In motor nerve conduction studies, the active electrode is usually over the belly of the muscle (endplate zone) and the reference remotely. The amplifier frequency response should be between 1–2 Hz and 500 Hz. In some cases the high pass filter is increased to prevent movement artifacts.

Table 8
SEMG applications in neurology.

Central nervous system	Peripheral nervous system
Timing	*Evoked activity:*
	CMAP (amplitude/area)
Movement patterns	*Voluntary activity:*
	Amplitude/frequency
	Fatigue
	Endplate localization
Amount of activity/central drive	Muscle fiber conduction velocity
Involuntary movements	MUP variables: amplitude and duration
Central fatigue	
Tremor	

2.2.1.1. Quantification of surface EMG. The main variables quantified are in the frequency and amplitude domain.

2.2.1.1.1. Amplitude domain. With respect to amplitude, the integrated EMG (IEMG) and root mean square (RMS) amplitudes are mostly measured. Sometimes the rectified EMG is measured and the area under the curve is determined. Because amplitude estimation of a random signal shows lower variance, decorrelation or whitening of the EMG signal is sometimes used (mainly in kinesiological studies) to estimate the amplitude more accurately (Clancy et al., 2002).

2.2.1.1.2. Frequency domain. In the frequency domain, estimation of the power spectrum is the most commonly used method, but other measures such as zero crossings are also useful. "Muscle fatigue" is used as a term for changes in frequency content in SEMG recordings (Zwarts and Stegeman, 2003; Marco et al., 2017; Smith et al., 2017). Underlying the decrease of the spectrum during activity is mainly slowing of action potential propagation in the muscle fibers (Stålberg, 1966). A change in firing behavior of the spinal motoneurons during fatigue (e.g., increased synchronization) could also give rise to lower SEMG frequencies (Van Boxtel and Schomaker, 1983; Hermens et al., 1992).

2.2.1.2. Application of surface EMG. SEMG can be used to record the output of the central nervous system with a very high time resolution and on many muscles simultaneously, such as in polymyography. For example, the timing of agonists and antagonists during voluntary movements can be recorded. Also, abnormal movement patterns such as in dystonia, tremor (Thompson et al., 1986) and gait disorders can be quantified and analyzed. The combination of multiple SEMG derivations with readings from an accelerometer gives a good visual representation of difficult movement disorders (Thompson et al., 1986).

With respect to the peripheral nervous system, a single channel mono- or bipolar recording gives limited information about the MUs, and may be used to detect a neurogenic pattern by the so-called Clustering Index (see below). Adding a second channel on a linear array gives additional information about the mean conduction velocity of MUPs (Zwarts and Stegeman, 2003). The mean conduction velocity of MUPs is usually measured by calculating the time difference between two or more bipolar electrodes in a linear array. Due to the small time differences between the two signals a high sample rate is necessary (in combination with linear interpolation) for a reliable estimate. The mean MUP velocity measured with surface electrodes is usually somewhat higher (4–4.5 m/s) compared with needle electrodes (about 3.5 m/s for single muscle fiber conduction velocity (Stålberg, 1966)). The MUP velocity can be reduced in some myopathies, mainly channelopathies (e.g., hypokalemic periodic paralysis).

Lastly, in motor nerve studies, SEMG is a well-established method for recording the CMAP. The amplitude of the CMAP with supramaximal stimulation is related to the number of viable axons and the size of the MUs. Low CMAP amplitudes are typically seen in axonal degeneration, but also in myopathies and synaptic transmission disorders such as Lambert-Eaton myasthenia (LEM).

2.2.2. Multichannel and high-density surface EMG

Although single bipolar SEMG can provide information on the number of active MUs, it provides only limited information about the single MUs. By using multichannel or high-density SEMG this limitation can be overcome. High-density SEMG uses a large number of small electrode contacts to cover a muscle (Blok et al., 2002). The number of electrode contacts used may vary from 16 to 256 or more, with inter-electrode distances as small as 2.5 mm and electrode diameters as small as 1 mm. Linear arrays and 2D-arrays or grids have been developed to be easily applied to the surface of the skin. Covering a single muscle or group of muscles with multiple electrodes results in a more detailed spatial distribution of muscle activity (Holtermann et al., 2008). Moreover, high-density SEMG can be used to determine the neural drive in much more detail than conventional SEMG can (Farina et al., 1985; Merletti et al., 2008). The extra spatial information may for example be used to improve the control of limb prostheses.

High-density SEMG can to some extent be decomposed into the contributions of individual MUPs. Advanced blind source separation, automated and semi-automated algorithms are able to provide information on single MUs (Holobar and Zazula, 2007). For this, the added spatial information is crucial, as the underlying MUs have different spatio-temporal profiles, which can be measured on the skin surface. These so-called fingerprints (Fig. 7) may uniquely identify individual MUs. The decomposition techniques have been validated by simulation studies as well as combined experiments of SEMG and fine wire electrodes (Holobar et al., 2014). It has been shown that decomposition of a sample of MUs is possible during up to 60–100% of maximum voluntary contraction. Detailed information on a single MU level may be obtained and quantified as the MU firing rate, MU recruitment, MU amplitude distribution, MU endplate zone, muscle fiber orientation, MU conduction velocity and MU depth. Of note, only a sample of the MUs active in a muscle may be decomposed. Depending on the type of muscle and contraction level this may vary up to about 20 single MUs. As the signals from muscle fibers close to the surface are larger than those from deeper muscle fibers, the decomposed MUs will be biased toward superficial and larger MUs. However, in a recent study it was shown that with this high-density technique MU activity with muscle fibers up to a depth of 18 mm from the skin surface may be obtained for the masseter muscle (Lapatki et al., 2019). In clinical practice, determination of the MU endplate zone may be beneficial for botulinum injections as effectiveness is improved by precise injections into

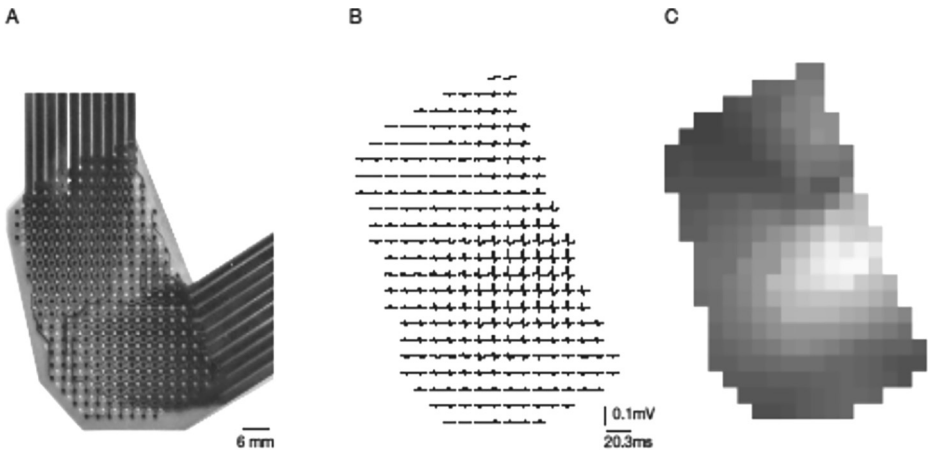


Fig. 7. (A) High-density surface EMG 2D array specifically designed for the masseter muscle. The 2D-array contains 256 contact points with a diameter of 1.2 mm and an inter-electrode distance of 3 mm. (B) Spatio-temporal profile or fingerprint of a single MU after decomposition and spike triggered averaging. (C) 2D spatial amplitude map of the same MU as in B.

the endplate zone (Lapatki et al., 2011). Most applications so far are in the field of movement science although clinical applications, e.g. in children, are within reach. Recent availability of the analysis techniques for other researchers will advance the field and provide additional useful (clinical) applications.

2.2.3. Clustering index

Concentric needle electromyography is the standard diagnostic tool to evaluate neurogenic and myopathic changes in skeletal muscle. However, it is an invasive and may sometimes be experienced as a painful procedure, which may limit its clinical use, such as in follow-up studies or for examinations in children. Therefore, an alternative using SEMG would be very attractive. The "Clustering Index (CI)" method was developed to describe features of MUP as recorded with surface electrodes on one channel (Uesugi et al., 2011). The underlying concept is to quantify the distribution of EMG signals during continuous voluntary activation in relation to total electrical activity. A neurogenic signal is characterized by large and sparse MUPs, and the total area of the signal is clustered into a few large MUPs along the timeline. In a normal or myopathic signal, however, the total area is rather evenly distributed into many small MUPs. In the CI method, the clustering of area values was analyzed for an appropriate window width using differential different time sequences of the signal (Fig. 8). The CI calculated in this way has values from 0 to 1, the higher values representing highly clustered neurogenic signals.

The degree of signal clustering expressed as the CI depends on the contraction level: stronger contractions have lower CI values, regardless of neurogenic, normal, or myopathic signals. Therefore, the results are plotted over a two-dimensional space of the CI vs. total area. The diagnostic yield was the highest when using a time window width of 22.5 ms. The sensitivity was 97% (28/29) for neurogenic recordings and 72% (28/39) for myopathic recordings, with specificity of 97% (64/66).

In summary, the CI method, a one-channel SEMG analysis using ordinary cup electrodes, achieved a reasonably high diagnostic yield in detecting neurogenic or myopathic changes, at least in the tibialis anterior (Tib. Ant.) muscle.

The CI method has also been applied to the abductor digiti minimi (ADM) muscle of patients having spinal and bulbar muscular atrophy (SBMA) (Higashihara et al., 2011). In a comparison between different methods, the sensitivity of CI, CMAP amplitude, and motor unit number estimation (MUNE) using multiple point stimulation method was 100%, 72%, and 93%, respectively (Higashihara et al., 2011).

In a study of Pediatric neuromuscular disorders, the CI method seemed to be able to discriminate between neurogenic and myogenic conditions, although the sensitivity was not so high as in adults (Higashihara et al., 2018). This may be due to thick subcutaneous tissue, which is often seen in children.

In conclusion, the CI method is a promising measure to discriminate between neurogenic and myopathic disorders. It has an advantage over needle EMG in having a wider uptake area and is not much affected by local pathology. CI and MUNE have different roles in clinical practice. MUNE methods are suitable for quantifying the loss of MUs and following the course of neurogenic disorders, such as ALS. The advantage of CI is that it can diagnose both neurogenic and myopathic conditions and time will show its usefulness in the evaluation of suspected neuromuscular disorders.

2.2.4. Macro EMG

The surface recorded spike-triggered MUP is dependent not only on the size of the MU but also on its depth in the muscle and on the conductivity of fat and skin. One way to deal with this would be to have a large recording surface inside the muscle; the cannula of a concentric or SFEMG needle electrode is such a surface. Macro EMG uses a 2 channel recording, with triggering

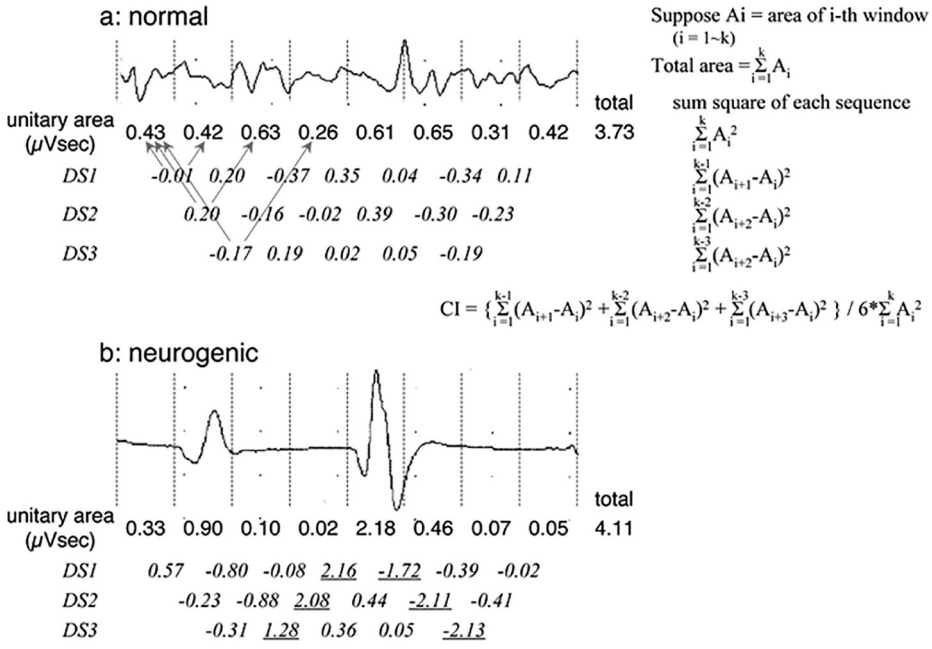


Fig. 8. Schematic explanation of the signal "clustering". Parts of the epochs from a normal (a) and a neurogenic (b) muscle are shown. Windows of 15 msec width were set, and the areas of each unitary window were calculated. In a normal signal, the total area is more or less evenly distributed in each window, whereas the total area is clustered into a few windows containing large MUPs in a neurogenic signal. The differential sequences between two consecutive values (DS1), every other value (DS2), and every third value (DS3) were calculated. The equations used to calculate the Clustering Index (CI) are shown to the right. In a neurogenic signal, terms with large values due to an isolated large MUP would be repeated twice (underlined numbers) for each differential sequence, and six times in all if the three differential sequences (DS1, DS2 and DS3) are considered. This is the reason why the Clustering Index (CI), calculated from the total of the square sum values of the three differential sequences, was divided by six times the square sum of the original sequence (Uesugi et al., 2011), with permission).

SFEMG signals recorded on one channel and the cannula signal vs a remote surface reference electrode on the other (Stålberg, 1980). Fiber density also can be measured from the SFEMG signal. After averaging (usually after 100–500 signals), the cannula-recorded signal from the triggering MU is obtained- as the Macro MUP. Its amplitude (peak-peak) and area under the signal are calculated.

The Macro electrode is Teflon coated except for the distal 15 mm. The side-ported electrode is used for recording the triggering SFEMG signals is 7.5 mm from the tip. These dimensions are based on the average size of a normal MU (5–10 mm) (Stålberg and Dioszeghy, 1991). Filter settings are different for the 2 channels; SFEMG channel 500–10,000 Hz, Cannula channel 5–10,000 Hz.

Alternative method. With restrictions on using reusable material, an alternative has been suggested using the cannula of a concentric needle electrode, Con-Mac (Jabre, 1991). The undefined size of the recording cannula makes the method less standardized than recordings with the Macro EMG electrode.

By applying decomposition, many different triggering signals (SFEMG or concentric EMG) can be used to obtain Multi Macro EMG, to reduce the recording time and to reduce the chance of recording from the same MUs more than once (Stålberg, 2011).

Macro EMG has been used mainly in neurogenic conditions such as ALS and the post-polio syndrome, where it has increased the understanding of reinnervation (Stålberg and Trontelj, 1982). It also gives indirect information about the number of MUs as follows: if 2/3 of all MUs are lost and fully compensated by reinnervation, the Macro signal is increased by a factor of 3. In order to compensate for the general muscle fiber hypertrophy during reinnervation, the relative Macro size thus obtained is divided by 2, so as not to overestimate the loss of axons (Grimby et al., 1998).

In many muscular dystrophies, the Macro EMG value is low, related to fiber diameters and number of fibers in a MU.

2.2.5. Quality control in EMG

Quality control should be applied to the EMG signals themselves and to cursor settings.

Signal quality. This is commonly assessed visually. For MUP analysis it concerns freedom from noise, stability of triggering points and smoothness of the baseline. For Macro EMG, this is quantified by monitoring the difference in area under the signal from two averaging buffers (mean average) to which alternate signals are sent. When the difference has reached a pre-determined minimal value, the signals are considered identical. The two buffers are merged before analysis.

Quality of measurements. This is also commonly assessed visually. For all parameters where reference values are available, deviation from normal is used as an immediate quality check, i.e., is the deviation technical or biological?

Another method that has been used is to plot two parameters against each other, e.g. duration vs amplitude (in MUP analysis there is an expected degree of correlation depending on pathology) or area vs amplitude (Macro EMG). For TA analysis, where turns are plotted against amplitude values, the data points should be distributed in a relatively tight pattern - outliers usually indicate pathology or an error.

2.2.6. Precautions in needle EMG

EMG with needle electrodes is a safe procedure. The only absolute contraindication is infection over the skin in the region of study. Patients with hemophilia and von Willebrand disease should be tested only under the supervision of a hematologist.

Patients on anticoagulation therapy with warfarin can be studied if the INR value is <3.0. If the INR value is >3.0 the EMG can be performed with caution at the discretion of the examiner. For oral anticoagulants such as dabigatran and rivaroxaban, there are no systematic studies, but testing can usually be done with appropriate cautions for bleeding. Testing can be done on all patients on antiplatelet therapy. The anticoagulation and antiplatelet therapies should not be discontinued before the EMG examination (Gertken et al., 2013).

It is good practice to assure safety in patients with anticoagulation therapy by using a small caliber needle electrode, limit the number of skin penetrations, start with superficial muscles and apply external pressure on the examination site before proceeding to the next muscle. Ultrasound may be a useful tool to evaluate hematomas, if they occur.

In most patients with a risk for endocarditis antibiotic prophylaxis is not necessary before needle EMG (Wilson et al., 2007).

3. Electrical impedance myography

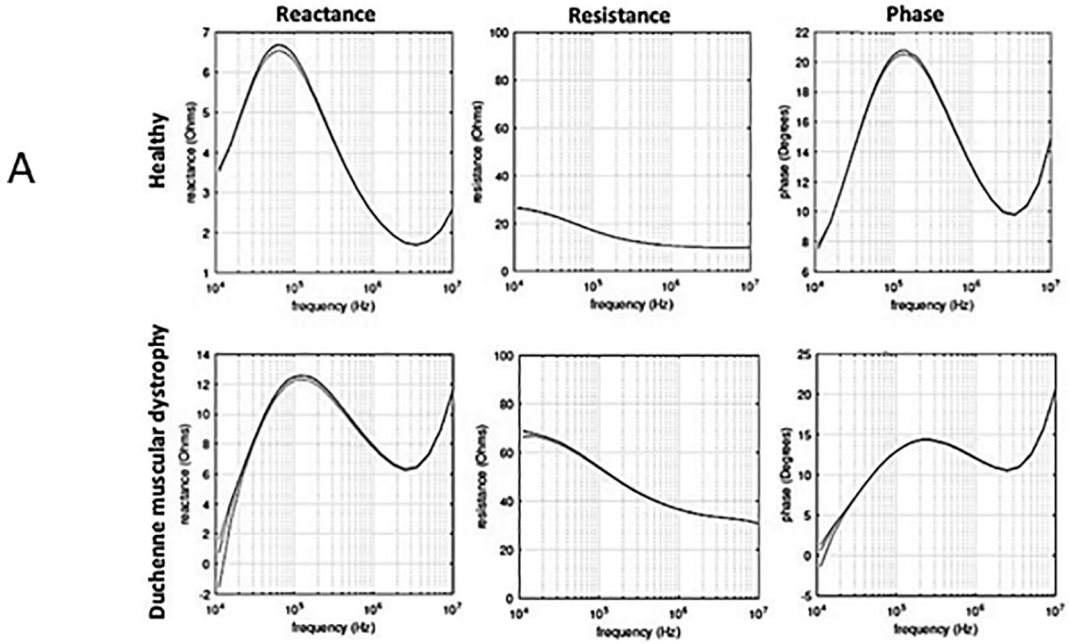
Electrical impedance myography (EIM) provides quantitative data on muscle composition and structure (Sanchez and Rutkove, 2017). In EIM, a high frequency, low-intensity alternating electrical current is passed between one pair of surface electrodes and the resulting voltage is measured by a second pair of electrodes placed over the muscle of interest.

Changes in the voltage amplitude and delay relative to the applied current, collectively termed the impedance, provide information on tissue health. These are captured in three parameters: the resistance, reactance, and phase (Fig. 9 A). Myofiber size, homogeneity, and organization, as well as the presence of edema, connective tissue, and fat all impact these impedance values. EIM has been used to estimate myofiber size without the need for biopsy (Kapur et al., 2018). Electrical current is also applied across a range of frequencies (e.g., between 1 kHz and 2 MHz) and at a minimum of 2 angles (longitudinal and transverse) relative to the major muscle orientation. These two aspects of the impedance provide additional insights into muscle condition since both are altered in disease.

EIM can be performed using an off-the-shelf bioimpedance system. A commercial research medical system is available (Fig. 9B); a system to be submitted for FDA-approval is currently in development. The procedure is automated, takes only several seconds to perform, and can be readily applied to a variety of muscles, including appendicular and truncal muscles as well as the tongue. While standard individual disposable adhesive Ag-AgCl electrodes can be used for measurement, more consistent measurements can be obtained by using a pre-formed electrode array made of stainless steel or other conductive material such that interelectrode spacings are kept constant (Fig. 9B).

Advantages of EIM include its being painless (the current is too weak and high-frequency to induce muscle stimulation), fast, adaptable (virtually any superficial muscle can be studied), easy to use in young children, and requiring minimal technical training. A needle-based version of EIM that records simultaneous EMG is also being developed (Kwon et al., 2018). The EIM technique is highly reproducible, with intra-class correlation coefficients of about 0.90 in both healthy and diseased individuals (Rutkove et al., 2006, 2012; Zaidman et al., 2015; Rutkove et al., 2017). However, it does require consistent electrode positioning, movements of the electrode array causing 2–11% variation/cm displacement (Rutkove et al., 2005).

EIM can serve as a primary diagnostic technique. In radiculopathy, side-to-side comparisons between muscles found a specificity for EIM of 65% and sensitivity of 77% vs EMG in the same individuals, which had 87% sensitivity and 70% specificity (Spieker et al., 2013). In ALS, EIM values have been found to be reduced compared to values in healthy subjects (Shefner et al., 2018). EIM values have also been significantly reduced in other conditions, including



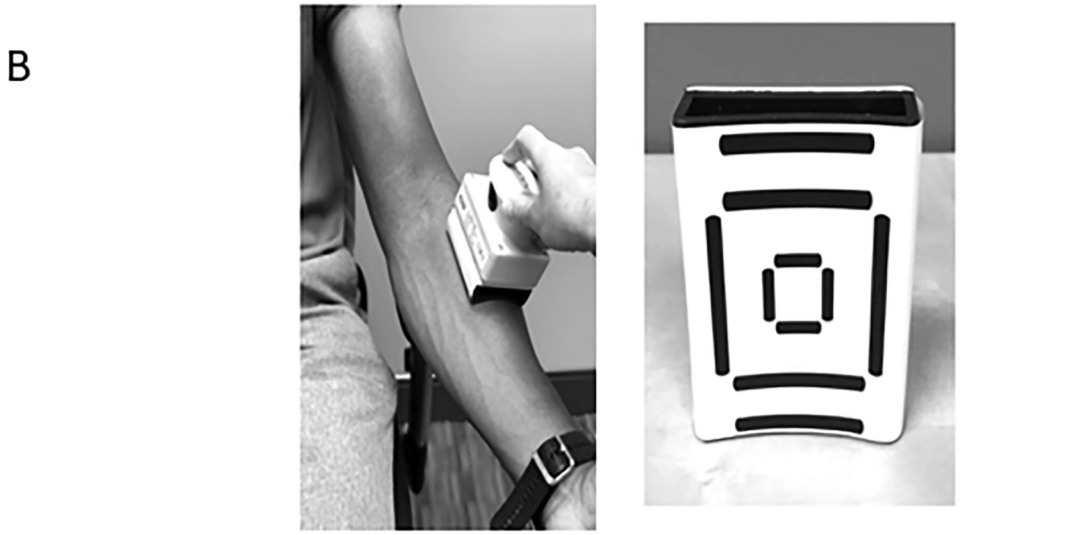


Fig. 9. A. Impedance spectra from a healthy boy (top) and a boy with Duchenne muscular dystrophy (DMD) (bottom). Note marked alteration in the reactance and phase curves in the DMD boy consistent with alterations in the filtering characteristics of tissue due to pathological changes in tissue composition and structure. Also note the major change in the resistance values, with a substantial elevation in DMD, consistent with deposition of fat within the muscle and loss of free water. B. EIM being performed on brachioradialis (left). Detail of electrode array; longer electrodes are current-emitting; the 4 smaller electrodes in the center are voltage-measuring (right).

mononeuropathies and inflammatory myopathy (e.g., one study showed a 31% reduction in abductor pollicis brevis EIM measures in carpal tunnel patients vs a healthy population (Li et al., 2017), but actual sensitivity and specificity values have generally not been reported.

Whereas EIM has the potential to serve as a diagnostic tool, EIM's main value has been in tracking disease status over time such that it can serve as a quantitative biomarker in clinical trials and also potentially for modifying individual patient care. In Duchenne muscular dystrophy (DMD), EIM outperformed the standard functional measure, the 6-minute walk test, for detecting muscle deterioration, offering a 2–3-fold reduction in sample size (Rutkove et al., 2012). Finally, EIM is also sensitive to disuse and myopathic change, alterations that do not have a clear electromyographic signature (Tarulli et al., 2009; Kortman et al., 2013).

Data indicate that EIM can also be used as a drug-effect biomarker. In boys with DMD, EIM could detect the effect of corticosteroids, corresponding to the well-known clinical improvement that occurs with these drugs (Rutkove et al., 2017). Animal studies have also demonstrated high sensitivity to the effect of myostatin pathway inhibition in wild type mice (Nagy et al., 2018) and antisense oligonucleotide therapy in spinal muscular atrophy mice (Arnold et al., 2016).

4. Neurography

Nerve conduction studies (neurography), comprising motor conduction studies (MCS) and sensory conduction studies (SCS) are essential in the diagnosis of focal and diffuse neuropathies. Neurography reflects the functional state of the myelinated motor

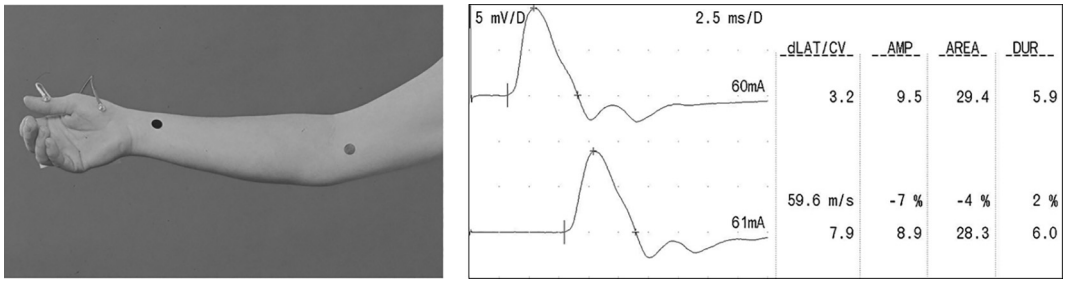


Fig. 10. Median nerve motor study with APB recording and stimulation at wrist and at elbow. Note that the CMAPs from both sites have the same shape in this healthy subject.

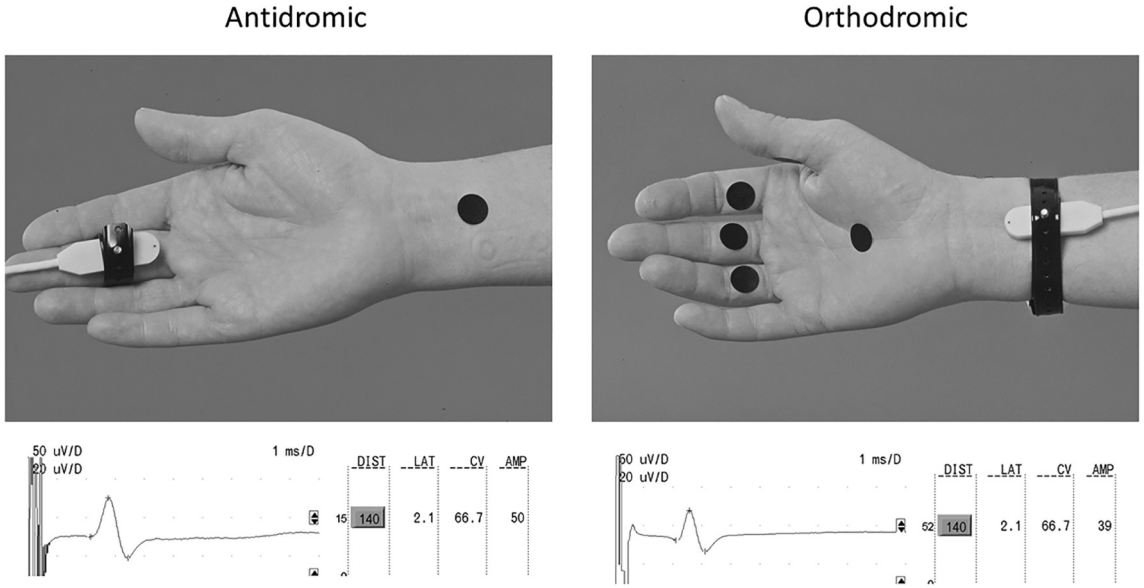


Fig. 11. Sensory antidromic and orthodromic studies. The SNAP has less initial positivity and a higher amplitude with the antidromic method. The peak latency is the same. Black dots indicate the position of the stimulating cathode.

and sensory axons and to some extent neuromuscular transmission and the muscle fibers.

In MCS the nerve is usually stimulated at two or more sites along the nerve and the CMAP is recorded from a distal muscle innervated by the nerve (Fig. 10). In SCS the nerve is stimulated along the nerve and the response is recorded either distal or proximal to the stimulation site directly over the nerve (Fig. 11).

4.1. Stimulation

4.1.1. Stimulating electrodes

In most studies nerves are stimulated with surface electrodes. These are quickly applied, inexpensive and less painful than needle electrodes but still somewhat uncomfortable, particularly when many stimuli are given as in F wave studies. Most stimulating electrodes are bipolar electrodes mounted on a bar or handle with an interelectrode distance of 10 to 40 mm (Fig. 12B and F). The diameter of each electrode is 5–10 mm. In infants smaller stimulating electrodes can be used, but these require a more precise placement close to the nerve. For stimulation of deeply located nerves needle electrodes (Fig. 12A) are used, for instance the inferior alveolar nerve (Jaaskelainen et al., 1995). A monopolar surface stimulator with a surface anode at a distance can also be used. Needle electrodes are also used if two nerves close to each other need to be studied separately, for instance, to stimulate the interdigital nerves in Mortońs metatarsalgia (Falck et al., 1984).

4.1.2. Stimulation parameters

The cathode is placed over the nerve at the site where the nerve is to be stimulated; the anode is proximal to the cathode in MCS and in antidromic SCS, and reversed for orthodromic SCS stimulation. In MCS the length of the distal nerve segment should be standardized by placing the cathode a fixed distance, e.g. 80 mm, proximal to the recording electrode, if possible.

A constant current stimulator that controls the stimulus intensity around the nerve is recommended and is available in most current EMG equipment. Constant voltage stimulators that generate a specified voltage have the drawback that the stimulus current depends on the impedance of the skin.

A rectangular stimulus with duration of 0.1 or 0.2 ms is used for MCS and SCS. The stimulus intensity should be high enough to excite all myelinated axons in the nerve. The stimulus current should be 10–25% greater than that necessary to evoke a maximal response. If such a supramaximal stimulus intensity cannot be obtained with these durations, 0.5 ms or exceptionally even 1 ms (painful) can be used. Stimuli longer than 5 ms are very painful due to generation of multiple discharges and also distort the shape of the response; they should not be used (Winkler and Stålberg, 1985).

Modern EMG equipment usually uses constant current stimulation; the stimulator delivers the desired current. Most equipment deliver currents up to 100 mA. The stimulator adjusts the voltage necessary for the set current depending on the impedance between the electrode and the skin. If the impedance is very high the stim-

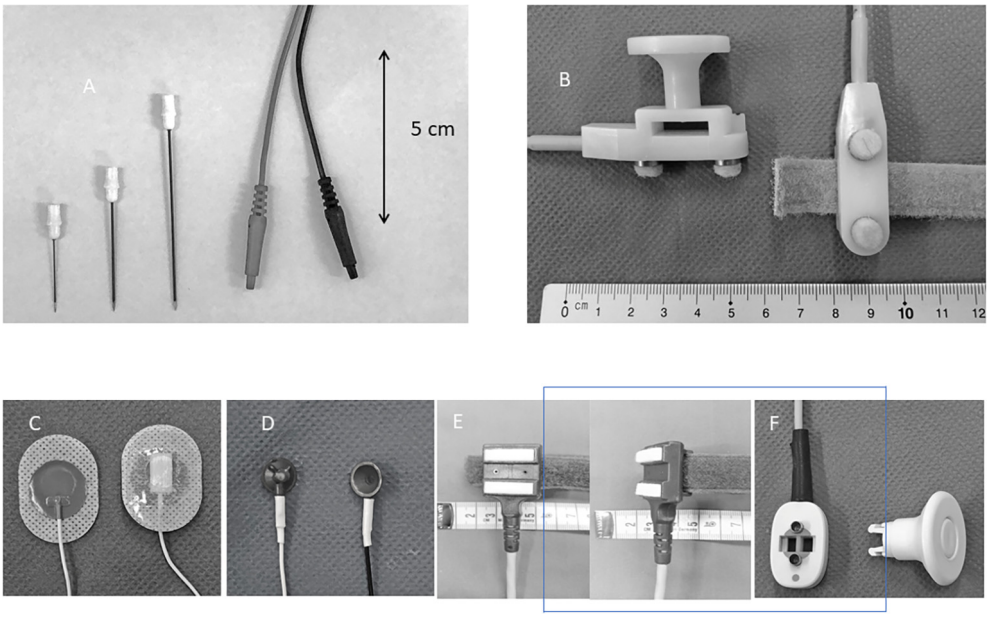


Fig. 12. Different types of electrodes used in MCS and SCS (A) Near nerve monopolar needle electrodes. The shortest, thin electrode (left) is used as the reference for stimulation. The two thicker electrodes are used as the active recording electrode or cathode for stimulation. (B) Electrode to the right is used for SCS and can quickly be fixed with the Velcro strap. The electrode to the left is used for stimulation in both MCS and SCS. (C) Disposable surface electrodes used for MCS. Note that the recording electrode surface is rectangular. These electrodes can be used to test several motor nerves in a given patient. (D) Silver/silver chloride plate electrodes used for MCS. (E) Recording electrode with a large recording felt pad (6 × 20 mm). (F) Small stimulating electrode with felt pads; the distance between the cathode and anode is 10 mm.

ulator may fail to deliver the desired current; it is important that the equipment indicates this to the user. Older and more basic equipment often uses a constant voltage stimulator that deliver intensities up 400 Volts. The drawback of constant voltage stimulation is that the user does not know the current of the stimulator, which is the factor that stimulates the nerve.

High intensity stimuli generate larger stimulus artifacts and are more uncomfortable. Strong stimuli also spread from the cathode and may generate action potentials at a distance from the cathode (cathodal escape), producing erroneously short latency measurements. In demyelinating polyneuropathies, the required intensity is much higher than in axonal neuropathies

The stimulus frequency is adapted to the nerve and method used. For common motor studies the stimulus frequency is often a regular 1 Hz. The alternative is to use manual single stimuli delivered when it is convenient. In sensory nerves the responses are often averaged (from few to several hundred responses), and stimulus frequencies may be higher; 2–7 Hz are usually tolerated by many subjects.

4.1.3. Stimulus artifact reduction

Electrical artifacts are also discussed in a separate IFCN Standards document.

In MCS the stimulus artifact is usually small in proportion to the CMAP and is rarely a problem; SCS is more affected by the stimulus artifact. If the distance between the stimulating and recording electrodes is short, there may be a large artifact that distorts the recorded signal. This stimulus artifact can be reduced by the following maneuvers: (1) Reduce the skin impedance by abrading the skin under the stimulating electrode. (2) Keep the skin between the stimulating and recording electrodes dry. (3) Reposition the patient ground electrode. (4) Rotate the anode while the cathode is maintained over the nerve. (5) Use the biphasic stimulation pulse that is available in many commercial EMG machines (Nilsson et al., 1988). This produces an initial negative rectangular pulse, which is followed immediately by a similar positive rectangular pulse that does not affect the nerve, which is refractory to the rapid change. (6) Software reduction of the stimulus artifact. (7) Special amplifiers with fast recovery and compensators may be used. Some of these latter features have been incorporated into commercial EMG equipment.

4.2. Motor neurography

The CMAP is the temporal and spatial summation of the action potentials generated by all the individual muscle fibers innervated by the stimulated motor axons. The shape and size of the CMAP is determined by the number of MUs and the amplitude, duration and temporal dispersion of their MU potentials (Lee et al., 1975).

4.2.1. Recording electrodes

MCS is usually performed with surface recording electrodes made of silver, silver/silver chloride or stainless steel (Fig. 12D). For clinical studies, 10 mm diameter round cup electrodes or disposable flat stick-on electrodes with variable areas and shapes are used. The intra- and inter-individual variability of the signal amplitudes recorded with these electrodes is high and even small displacements of the electrode affect the shape of the CMAP. The variability of amplitude measurements is decreased with larger electrodes (van Dijk et al., 1995). Stick-on electrodes (Fig. 12C) are commonly used. Round electrodes are direction insensitive, but those with a short and long axis, e.g., Fig. 12C, are sensitive to their direction of orientation.

4.2.2. Electrode placement

The active recording electrode (E1) (Robinson et al., 2016) is placed over the end-plate region of the muscle, usually over the middle of the muscle belly, which produces CMAPs with an initially negative inflection. If distal stimulation produces an initial positive deflection, the recording electrode is not over the endplate and should be repositioned. Sometimes it is not possible to record an initially negative deflection, in which case the latency is measured to the first positive deflection. In recordings with an ill-defined start, any part the CMAP can be used (e.g. the peak) for cal-

culation of conduction velocity, provided that the measurement point is the same for distal and proximal responses.

Optimally, the reference electrode (E2) should be placed over an inactive area outside the muscle, e.g. over the distal interphalangeal joints of the thumb for median nerve MCS. A reference electrode placed over the tendon of the tested muscle is not inactive and will significantly influence the CMAP waveform (Barkhaus et al., 2006b) (Fig. 13). Therefore, fixed plastic bar electrodes with electrode distances of 20–40 mm are not recommended.

An electrode-skin impedance <20 kOhm is desirable. The higher the input impedance of the equipment the less sensitive the measurements are to the electrode-skin impedances. Most modern EMG instruments have an input impedance of 1000 MOhm or more.

4.2.3. Limb position

The position of the joints influences the length measurement of nerve segments that run across joints. The most important segment in this respect is the ulnar nerve across the elbow. Some investigators suggest that the measurement should be performed with the elbow extended, others with 90° flexion (Buschbacher, 1998; Omejec and Podnar, 2016) and others recommend 135° flexion (Kincaid et al., 1986). At 90° elbow flexion the ulnar nerve is tense, but rarely dislocates across the medial epicondyle, which complicates conduction velocity measurements. There are no studies indicating that any position is superior to the other. However, it is important to standardize the position and use the identical position that was used for the reference values.

The muscle(s) that are used for recording should be relaxed and in a neutral position. This is particularly important in the hand when recording responses from the thenar and hypothenar eminences. Passive or active shortening of the muscles increase the amplitude and decrease the duration of CMAP. Stretching of the muscles have the opposite effect.

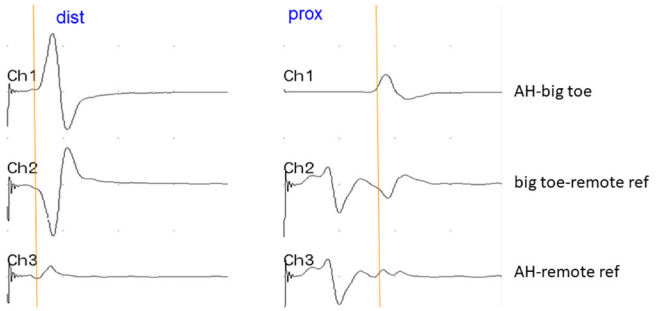


Fig. 13. Effect of reference electrode (E2) position in tibial nerve study – Recording electrode (E1) is placed over abductor hallucis muscle. Recordings obtained with distal stimulation, left, and proximal stimulation, right. Ch1 - standard electrode positions. Ch2 - big toe to a remote reference (other leg). Ch3 - AH to remote reference. In Ch2, note the high amplitude waveform with inverted polarity, generated by intrinsic foot muscles. Vertical lines are inserted to facilitate time comparison of the curves. Copyright Stålberg.

4.2.4. Amplifier and display settings

Amplifier filters influence the signal shape, but are used to reduce artifacts. The high-pass filter is typically set at 2–20 Hz. Compared with the CMAP recorded with a filter of 2 Hz, the CMAP recorded with a filter of 20 Hz has a 15% smaller amplitude, 25% smaller area and 8% shorter duration, but the same distal latency (Pease and Pitzer, 1990). Although a 2 Hz filter is theoretically better for accurate recording of the CMAP shape, 20 Hz is often used to reduce baseline instability. The low pass filter is set to either 10 or 20 kHz.

4.2.5. Parameters

Distal latency (DLAT). DLAT is the time from the stimulus to the onset of the CMAP (Fig. 14). The latency is measured from the stimulus to the first deflection of the signal from the baseline. If the recording electrode is properly placed, the initial deflection is negative at the distal stimulation site. If the electrode cannot be placed over the end-plate region, the distal latency is measured to the first deflection from the baseline

Manual measurement of latency is dependent on the gain used (Maynard and Stolov, 1972). At a high gain the initial signal devia-

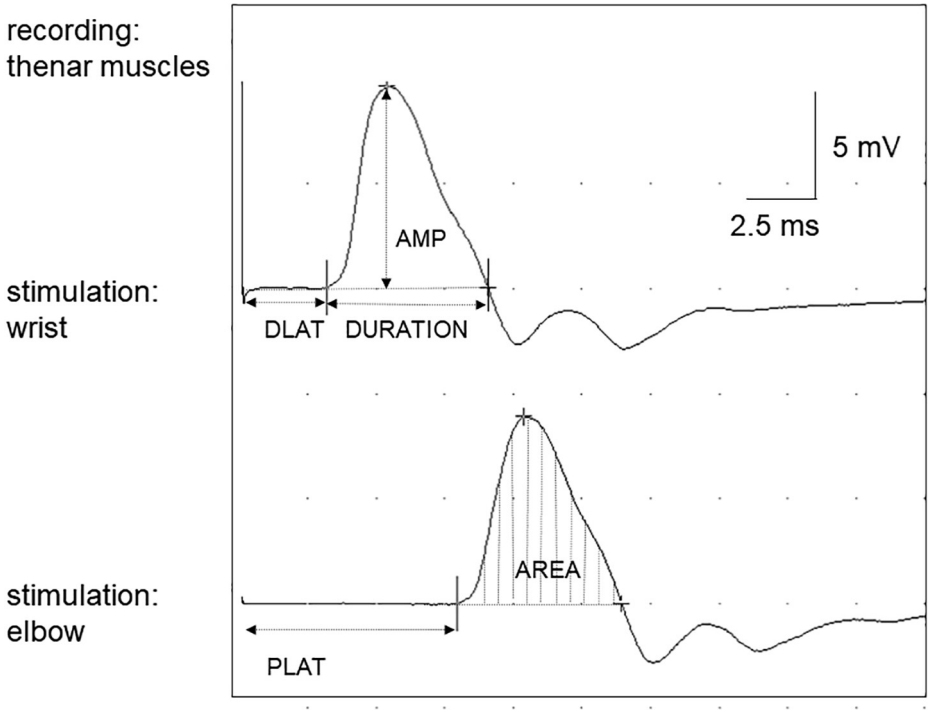


Fig. 14. Parameters used in MCS. Median motor nerve Copyright Stålberg.

tion from the baseline is seen earlier than at a low gain. A standard gain of either 200 μV/div or 2 mV should be used for manual latency measurements. Most commercial instruments have automatic cursor settings for latency using an algorithm based on the slope of the signal which is independent of the display gain.

The conduction time (CT) is the time it takes for the fastest α-motor nerve fibers to conduct over a nerve segment, i.e., the difference between proximal and distal latencies.

The length of a nerve segment is measured from the center of the cathode at one stimulation site along the nerve to the center of the cathode at the next stimulation site.

The motor conduction velocity (MCV) is the conduction velocity of the fastest α-motor fibers, expressed as m/s. MCV = segment length/conduction time. MCV reflects the diameter of the largest axons and the integrity of the myelin.

Methods for measurement of the slower conducting fibers (Hopf, 1962) and the cumulative distribution of motor conduction velocities (Ingram et al., 1987b, a) have been described; these are rarely used clinically and will not be discussed. A method to estimate MCV of individual axons using SFEMG recordings also has been described, but also is not used clinically (Padua et al., 2007).

Amplitude (AMPL). The AMPL of the CMAP may be measured from baseline to the highest negative peak, even if there is a positive going onset for the CMAP. Measurement between minimum and maximum peaks may be used, but the drawback is the variable influence from the E2 electrode, often giving additional positive signals. There are no studies indicating that one or the other of these two definitions is superior.

AMPL reflects the number and diameter of the muscle fibers activated by the stimulus. In an acute neuropathy AMPL reflects the number of MUs in the activated muscle(s). However, in slowly progressing neuropathies the AMPL may remain normal if collateral reinnervation compensates for loss of MUs, then later falls when the MUs have reached their maximum size and cannot compensate for continued loss of MUs.

Negative Duration (DUR) of the CMAP is defined from onset to the *first* negative-to-positive baseline crossing after first negative peak.

Total Duration is measured from onset of signal to its return to baseline after last positive peak.

Duration reflects the dispersion of conduction velocities in the motor nerves and the dispersion of muscle fiber conduction times from endplates to the electrode.

Area (Negative or Total AREA) is the integrated area between the signal and the baseline over the negative or total duration. The physiological interpretation of changes in the area is similar to AMPL.

Decay (% AMPL change). Because of the dispersion of axon conduction velocities (35–65 m/s) there is an increasing temporal dispersion among MUs with increasing segment length (=conduction distance), and AMPL at proximal stimulation is less than at distal stimulation. This change (decay) is expressed as a proportional value. The amplitude decay (AMPDEC) is calculated as AMPDEC = 100*(AMPprox - AMPdist)/AMPdist.

AREADEC is calculated similarly (=100*(AREAprox-AREAdist)/AREAdist) and is the preferred parameter before AMPL (Rhee et al., 1990).

Dispersion (DISP) is the proportional change of the DUR over the nerve segment. DISP is calculated like the decay. Increased dispersion of the conduction velocities will give also an increased DISP.

$$\text{DISPERSION} = 100^{*}(\text{DURprox} - \text{DURdist})/\text{DURdist}$$

AMPDEC or AREADEC reflects conduction block as well as dispersion of conduction velocities. An abnormal decay without increased DISP suggests conduction block, while abnormal AMPDEC or AREADEC with increased DISP suggests increased dispersion of conduction velocities.

4.3. F responses, A waves and H-reflex

4.3.1. Recording and measurement

A supramaximal stimulus applied at any point along the course of a nerve elicits F waves following the M response. Although it has been suggested that proximal orientation of the stimulating cathode be used to avoid the possibility of anodal activation, (Winkler and Stålberg, 1988; Yasunami et al., 2005) some prefer to use standard anode proximal stimulation to elicit M responses as well as F waves. Cathodal monopolar stimulation with the anode placed slightly off the nerve eliminates any ambiguity. The same recording electrode position is used as for motor conduction studies.

To optimally display F waves, use an amplifier setting of 200 or 500 μV/cm and a display sweep of 5 or 10 ms/cm, depending on the nerve length and stimulus point. Most current instruments display the M response and F wave simultaneously, but with different gains. Vertically shifting successive sweeps (rastered display) help to identify the number of F waves seen out of 10–20 volleys and to select minimal and maximal latency responses (Fig. 15).

The level of motoneuron excitability and the number of functional axons determine the F-wave persistence, i.e., the percentage of stimuli that elicit a detectable F wave. F-wave latencies obtained after several stimulations, normally differ by 2–4 ms. The latency is a function of the speed of the impulse along the axon but also of the length of the fine nerve terminals, which may vary a few millimeters, substantially altering the conduction time. Thus, we should not strictly equate the shortest and longest latencies to the fastest and slowest conducting fibers. The difference between shortest and longest F-latencies (measured as minimal F-latency at each trace) is called chronodispersion (Panayiotopoulos, 1979).

F waves characteristically vary in waveform and latency from one stimulus to the next, indicating that they represent different MUs. The frequent appearance of F waves with the same waveform and latency, or repeater F waves, probably indicate a reduced number of excitable motoneurons in patients with some dysfunction of motor fibers (Chroni et al., 2012). In patients with spasticity, stimulation of peripheral nerves may elicit an H reflex, which is not normally seen in most nerves after supramaximal stimulation.

4.3.2. F-wave conduction velocity of proximal nerve segments

The conduction time from the stimulus point to and from the spinal cord can be calculated as F-M, where F and M are the minimal latency of the F wave and the latency of the M response (CMAP), respectively. Assuming a delay of 1.0 ms for the turnaround time at the motor neuron and dividing by two, (F – M–1)/2, yields the conduction time from the stimulus site to the spinal cord. The estimated nerve length divided by the conduction time to and from the spinal cord equals the F wave conduction velocity (FWCV) in the proximal segment as follows:

$$\text{FWCV} = (2\text{D})/(\text{F} - \text{M} - 1)$$

where D represents the estimated distance from the stimulus site to the spinal cord, and (F – M – 1)/2 is the nerve conduction time needed to cover this length. The calculated FWCV over the proximal segment is greater than the motor nerve conduction velocity (MNCV) along the distal segment, which reflects the proximally faster and distally slower nerve conduction characteristics (Kimura et al., 1974, Kimura et al., 1975, Kimura and Butzer, 1975).

4.3.3. Latency –height nomograms and other measures

Of all the measures of NCS in control subjects, the minimal F-wave latency shows the best reproducibility (Andersen et al.,

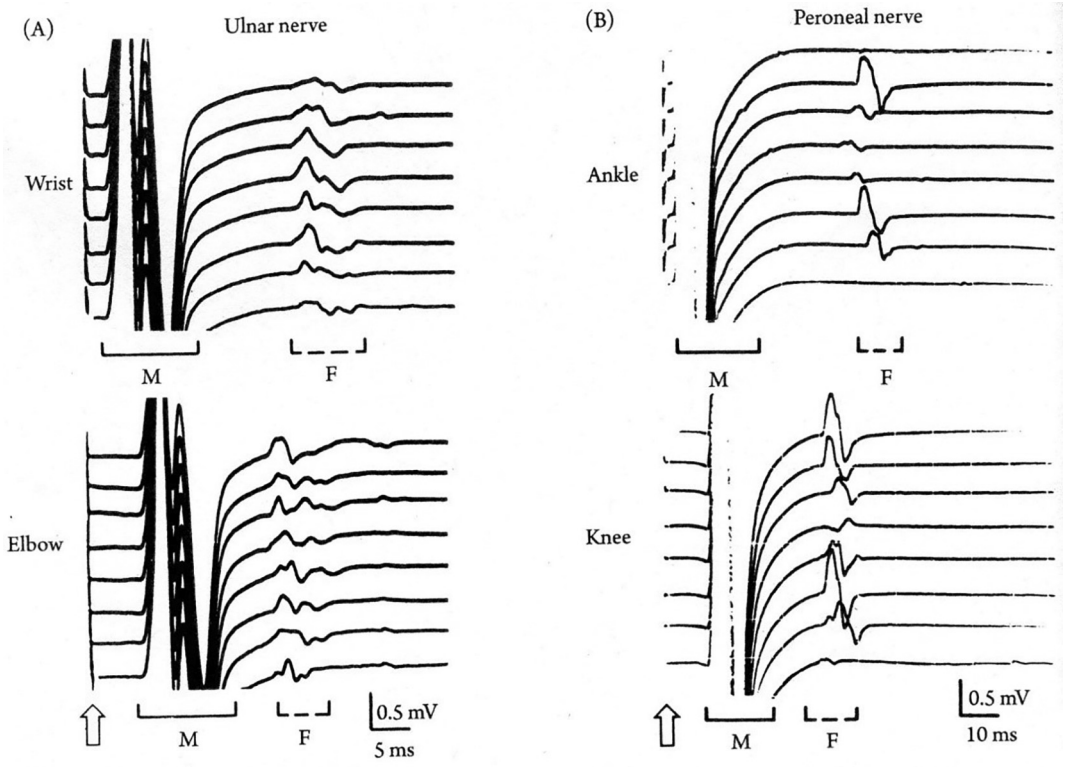


Fig. 15. A. Normal M responses and F waves recorded from the hypothenar muscles after eight consecutive stimulations of the ulnar nerve at the wrist and at the elbow. B. Normal M responses and F waves recorded from the extensor digitorum brevis after eight consecutive stimulations of the fibular nerve at the ankle and at the knee (Kimura, 2013).

1997; Pinheiro et al., 2008; Kimura, 2013). In detecting a lesion affecting only one nerve, latency difference between the two sides and between two nerves in the same limb are the best measures. Latency-height nomograms (Fig. 16) show linear relationships for upper and lower limb nerves (Puksa et al., 2011; Pan et al., 2014).

Published data indicate that at least 10 stimuli are needed to determine the mean latency and 15 stimuli for the minimal and maximal latencies (Nobrega et al., 2004). Based on similar studies many laboratories use 20 stimuli as routine. Clinically relevant and commonly used measures are minimal F latency and persistence. In addition, some measure mean and maximal latencies, FWCV, chronodispersion and mean duration.

F-wave persistence varies widely depending on the degree of central drive not only at the time of (Hara et al., 2010) but also immediately prior to the study (Taniguchi et al., 2008). F-wave persistence reflects the motoneuron excitability and the number of motor units in muscles from which the signals are obtained. For example, the fibular nerve supplying only the extensor digitorum brevis muscle below the ankle gives rise to a low F-wave persistence value as compared to the tibial nerve, which innervates the remainder of the intrinsic foot muscles. Similarly, the median nerve, controlling a smaller number of muscles, has less persistence compared to the ulnar nerve with more intrinsic hand muscles. Deviation from normal in persistence may be due to changed excitability, or loss of axons.

Studies of the F wave help characterize polyneuropathies (Kimura et al., 1974; Lachman et al., 1980; Fisher, 2002; Fisher and Rose, 2005; Puksa et al., 2011; Pan et al., 2013; Jerath et al., 2016) and neuropathic disorders associated with proximal conduction abnormalities. In localized nerve lesions such as radiculopathies, conduction in the remaining normal roots tend to obscure any conduction delay (Tuck et al., 1982; Kimura, 2013).

4.3.4. A waves

The A wave, unlike F waves, has a constant latency and waveform (Figs. 17 and 18). The designation, A wave, has replaced its initial descriptive term, the intermediate latency response, (Fullerton and Gilliatt, 1965) as it does not always appear between the M response and the F wave. The traditional name, axon reflex, also has fallen out of use as it implies a reflexive origin. Possible pathophysiologic mechanisms for A waves include collateral sprouting, ephaptic transmission, and ectopic discharges generated in a hyperexcitable portion of the motor axon (Magistris and Roth, 1992; Bischoff et al., 1996).

Detailed analysis of recorded responses usually uncovers the origin of A waves (Magistris and Roth, 1992). Superimposition of traces usually help to detect A waves (Fig. 18).

4.3.4.1. Clinical significance of A waves. A waves are found in many acute and chronic neuropathies, varying in pathophysiology from nerve regeneration to demyelination (Fullerton and Gilliatt, 1965; Nobrega et al., 2001). In control subjects A waves are commonly seen in leg nerves – they are seen in 25% of tibial nerves and 14% of peroneal nerves - but rarely in the median and ulnar nerves (2%) (Puksa et al., 2003). Multiple A wave are often seen particular in the acute stages of Guillain-Barré syndrome (Kornhuber et al., 1999).

4.3.5. Other repetitive waves that follow the CMAP

Repetitive CMAPs (R-CMAPs) after single nerve stimulation are seen when excess ACh accumulates at the endplate, as in congenital endplate acetylcholinesterase deficiency or acetylcholinesterase inhibition with drugs such as edrophonium or pyridostigmine, (Le Quesne and Maxwell, 1981) or organophosphates (Gutmann and Besser, 1990). They are also seen in the slow channel congenital myasthenic syndrome (Bedlack et al., 2000), in

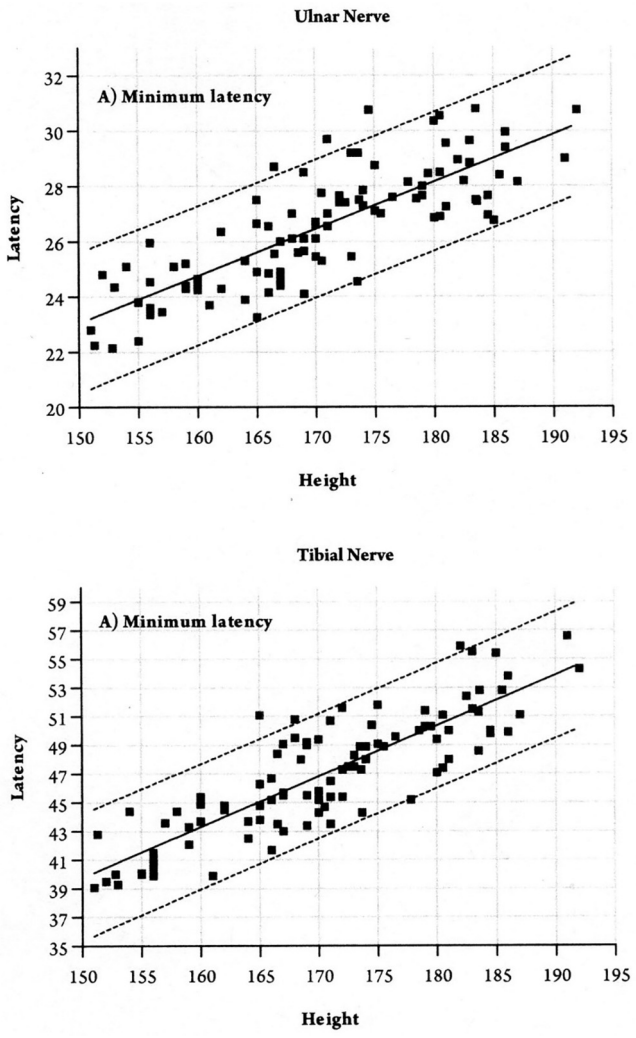


Fig. 16. Height-Minimum F-wave latency nomograms for ulnar (top) and tibial nerves showing upper limit of normal, calculated as mean + 2.5D. Both nerves show a linear relationship between these two measures (Nobrega et al., 2004).

which endplate potentials are prolonged. During RNS, R-CMAPs become progressively smaller, even at rates as low as 0.5/Sec, and may become unapparent after the first 3 or 4 stimuli. Activation also abolishes the R-CMAPs and they may not be appreciated unless single stimuli are delivered at 5 to 10 second intervals in well-rested muscle. R-CMAPs with a broad waveform can be difficult to see when the CMAP waveform itself is broad (Bedlack et al., 2000).

4.4. H-reflex

The H-reflex is a monosynaptic reflex recorded from muscles elicited by electrical stimulation of the muscle spindle afferents described by Hoffman (Hoffmann, 1918). This electrically elicited reflex is similar to the mechanically induced stretch reflex. The main difference is that the H-reflex bypasses the muscle spindle. Another difference is that latency measurements can be made with the H-reflex.

The reflex can easily be recorded from the gastrocnemius or soleus muscles. Recording adjacent to the tibia provides a more consistent, easily measurable response with a well-defined initial negativity. This reflex arc is mediated mainly via the S1 root (Fig. 19).

The H-reflex can also be recorded also from the wrist flexors (Deschuytere et al., 1976). Other muscles such as the thenar muscles, quadriceps femoris and Tib.Ant., show an H-reflex after slight facilitation by voluntary contraction or cortical stimulation (Cowan et al., 1986).

Recordings are made with surface electrodes over the muscle belly. The low frequency filter is set to 10 Hz and the high pass filter to 3 or 10 kHz. The recording window should be at least 50 ms and the gain 0.1–0.5 mV/div. The nerve innervating the muscle is stimulated with surface electrodes at a low frequency. The H-reflex habituates and decreases in amplitude with higher stimulation rates than 0.5 Hz (Olsen and Diamantopoulos, 1967). The H-wave is more easily obtained if the stimulus duration is long, 0.5 or 1.0 ms (Panizza et al., 1989). With this stimulus duration the muscle spindle afferents are preferentially activated rather than the motor axons to the muscle.

The H-reflex is obtained by stepwise increase of the stimulus strength and is maximal at a stimulus strength below threshold for a maximal M-wave. At higher stimulus strength the H-reflex is obliterated by antidromic impulses in the motor axons.

The H-latency is defined as either the H-latency or the H-latency minus the M-latency with stimulation at popliteal fossa. Voluntary activation of the investigated muscle or Jendrassic maneuver will enhance the H-reflex amplitude and shorten the latency. Because these procedures are difficult to standardize, they are not recommended. Complete muscle relaxation is easiest to standardize and should be used. The H-latency to the calf muscles is about 30 ms and is dependent on the age and height of the subject. The H-reflex is absent in many healthy subjects over the age of 69 years.

The H-reflex from the calf muscles is used clinically mainly in the diagnosis of S1 radiculopathies and polyneuropathies. In S1 radiculopathies the H-reflex to the calf muscles is either prolonged or more commonly absent (Braddom and Johnson, 1974; Albers, 1993). The H-reflex from the calf muscles is also abnormal in various polyneuropathies (Albers, 1993). It should be noted that absence of an H-reflex bilaterally has no clinical value, since this may also occur in healthy subjects.

The H-reflex is also used for research purposes to measure the segmental motor excitability and it its control (Schieppati, 1986).

4.5. Sensory neurography

4.5.1. Generation of sensory nerve action potentials

The sensory nerve action potential (SNAP) is the sum of the action potentials generated by individual myelinated axons with a diameter > 9 μm and conduction velocities between 40 and 65 m/s (Behse et al., 1977). The contribution of each axon depends on the amplitude of its action potential, which is proportional to the diameter of the axon and inversely proportional to the distance squared of the axon from the electrode. The action potential of a single axon is triphasic, with positive initial and terminal parts and a large negative peak in between. Because of differences in conduction velocities there is an overlap of negative and positive components of different action potentials that results in cancellation of some components (phase cancellation) (Dorfman, 1984). Orthodromic SNAPs are usually preceded by a volume-conducted positive wave generated by the volley of action potentials approaching the recording electrode. In antidromic recordings from fingers the SNAP is biphasic without a preceding positive peak, due to the volume conduction characteristics of the fingers. The first positive deflection indicates the arrival time of the fastest conducting action potentials. The negative peak is the sum of action potentials generated by thick myelinated axons. In spite of so-called phase cancellation making interpretation somewhat difficult, for practical clinical purposes amplitude and area can be

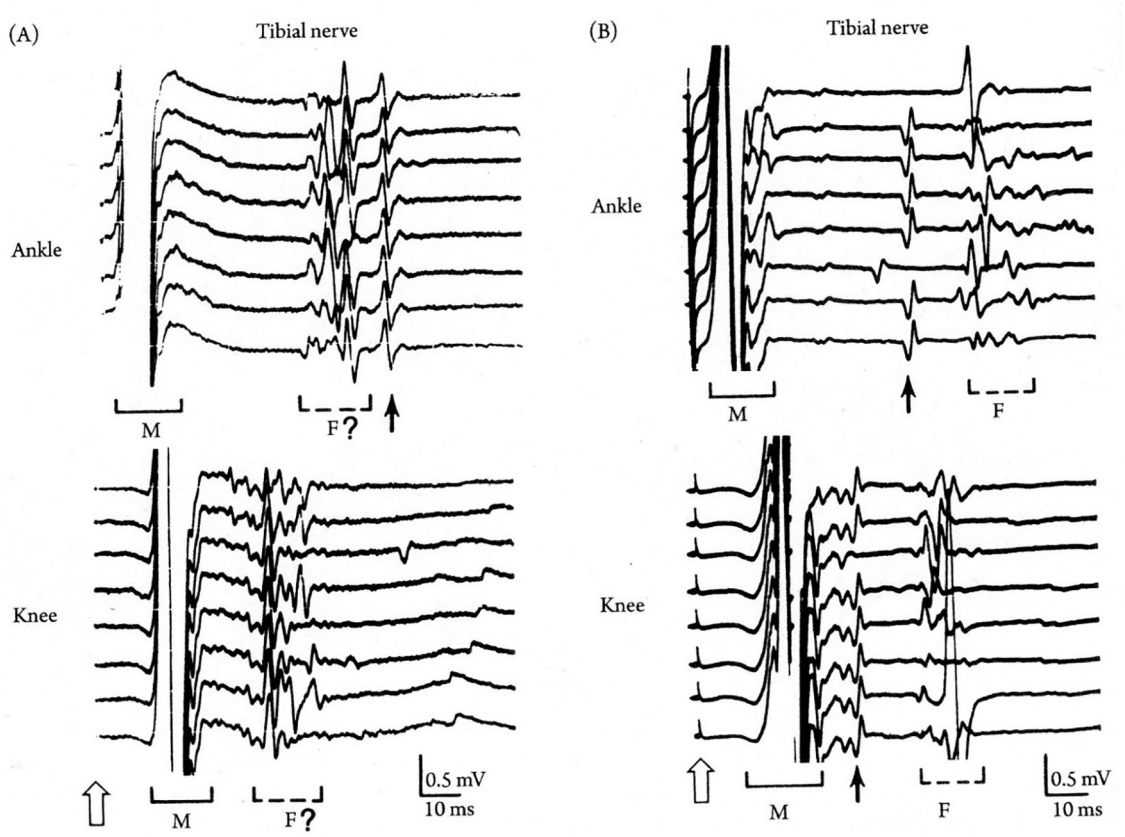


Fig. 17. Figure demonstrating how to localize the site for A wave generation. A. A waves after stimulation of the left tibial nerve at the ankle and knee in a man with low back pain (recording AH muscle). Proximal stimulation eliminated the A waves produced with distal stimulation (black arrow), indicating that they were generated distal to the knee. F wave generated by the proximal stimulation probably contains an A wave making the onset latency difficult to determine (indicated with "?"). B. A waves in a man with recurrent backaches following laminectomy. Stimulation of the tibial nerve at the ankle and knee (recording AH muscle) elicited A waves (black arrows). As with the F wave, the latency of the A wave decreased with proximal site of stimulation, indicating that the A wave was generated proximal to the knee. (Open arrows indicate time of stimulation) (Kimura, 2013). Note that A waves after tibial nerve stimulation is not unusual in healthy subjects.

regarded as parameters that reflect the number of functioning axons of the nerve.

When a sensory nerve is stimulated electrically, action potentials spread not only proximally in the orthodromic direction towards the spinal cord, but also distally in the antidromic direction towards the receptors. In the orthodromic technique the recording electrode is over the nerve and the stimulating electrode is distal. In the antidromic recording the nerve is stimulated proximal to the recording electrode.

Antidromic stimulation is simple because the stimulation sites are the same when motor and sensory nerve conduction studies are performed on the nerve. Another advantage is that amplitudes of antidromic SNAPs are much higher (superficial location of the nerves in the fingers) than orthodromic SNAPs, while the CV is the same for both methods (Buchthal and Rosenfalck, 1966; Murai and Sanderson, 1975). Motor axons are excited by antidromic stimulation of mixed motor-sensory nerves, and volume conducted CMAPs thus elicited may distort the SNAP to some extent.

With antidromic stimulation it is possible to study consecutive segments of a nerve and compare the SNAP amplitude at the different stimulation sites (fractionated test). For example, the ulnar nerve can be stimulated at the wrist, below the elbow and above the elbow, thus three consecutive segments are tested. While it is possible to measure the CV with the orthodromic technique, the amplitudes cannot be used to evaluate conduction blocks.

4.5.2. Recording electrodes

It would be optimal to use a truly monopolar recording electrode over the nerve with the reference electrode at a distance. Unfortunately, such an electrode placement is not practical because the baseline is unstable and artifacts generated by the stimulus and muscles distort the recording. Therefore, bipolar recording electrodes must be used, both of which are active and contribute to the potential. SNAPs recorded with surface electrodes are affected by several factors: distance from the nerve, distribution of conduction velocities, CV and inter-electrode distance (Winkler et al., 1991a) (Fig. 20).

SNAPs can be recorded with several different types of electrodes: (1) bipolar fixed electrodes, (2) plate electrodes, (3) ring electrodes and (4) near-nerve monopolar needle electrodes (Fig. 12A).

Fixed electrodes. Because both electrodes contribute to the SNAP the inter-electrode distance is important for the shape of the recorded potential (Murai and Sanderson, 1975; Winkler et al., 1991b). Increasing the distance between the recording electrodes increases the SNAP amplitude and duration until a maximum amplitude of the SNAP is reached with about 40 mm inter-electrode distance (Note: this is valid for normal conduction velocity. For slow velocity, the maximum amplitude is reached with shorter interelectrode distances). The area and duration increase further with greater inter-electrode distances (Wee and Ashley, 1990). Although there is no optimal inter-electrode distance, for clinical recordings an electrode with a fixed distance of

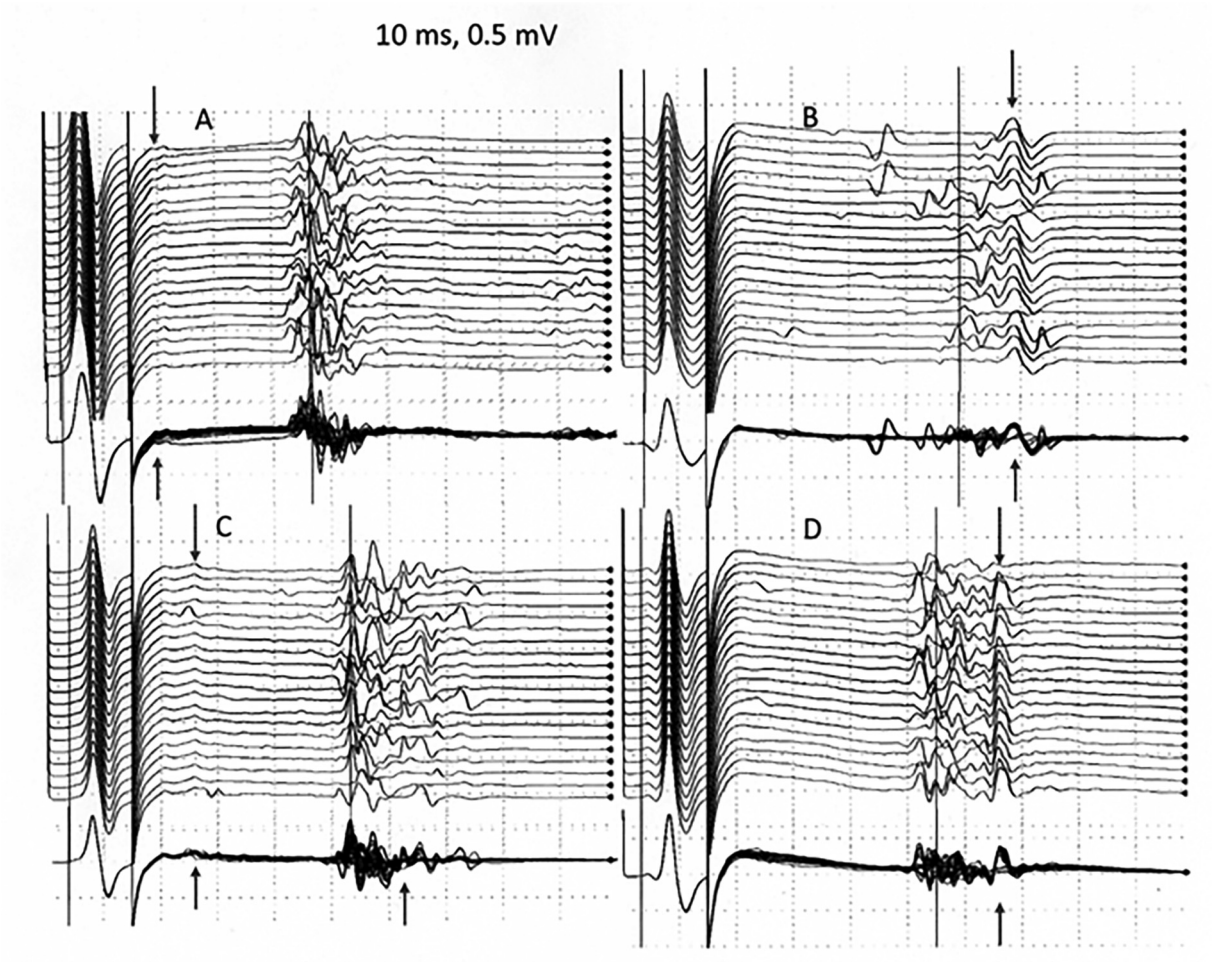


Fig. 18. Four examples of F wave studies, also showing A waves (arrows). Each panel shows 20 traces, displayed consecutively in the upper part, and superimposed in the lower part. Note the advantage of superimposition; it is easier to see A waves and to assure constant CMAP amplitude throughout the study.

20–25 mm is recommended. Commercial electrodes with 23 mm inter-electrode distance are available.

Ring electrodes consist of metal wires that may be soaked in saline or electrode paste; disposable strip electrodes can also be used. These are often used for antidromic recording of SNAPs from fingers because they can quickly be wrapped around the digits. When they are used it is essential to standardize the distance between electrodes. It has been shown that SNAP amplitudes recorded with ring electrodes have a negative linear correlation with the circumference of the fingers (Bolton and Carter, 1980). Since inter-electrode distance is often not standardized, these electrodes are not recommended for sensory nerve conduction studies when the amplitude is measured.

Plate electrodes may be used at sites where it is difficult to attach bar electrodes, for example, to study the lateral cutaneous nerve of the thigh with surface electrodes.

Near-nerve electrodes. Sensory nerve conduction studies (NCS) may also be performed with a near-nerve needle technique (NNT) using monopolar needle electrodes (Buchthal and Rosenfalck, 1971) both for recording and stimulation. Drawbacks of NNT are that insertion of the electrodes is painful and the electrodes are much more expensive than surface electrodes. A third disadvantage of needle electrodes is that studies cannot be done by technicians. In most routine studies surface electrodes are superior to near-nerve needle electrodes. However, needle electrodes have some advantages (Trojaborg and Lange, 1992): with needle electrodes the recording and stimulation points are accurately defined and conduction velocity dispersion and polyphasic late components i.e. from slower conducting axons, can be detected. In severe neuropathies when no SNAP can be recorded with surface electrodes, NNT may show a response (Trojaborg and Lange, 1992). Near nerve recordings and stimulation must be used when the amplitude of the response is too small to be detected with surface electrodes (e.g., recording responses from the plantar digital nerves of the toes in Morton's metatarsalgia), or when the nerve is not close to the skin (the inferior alveolar nerve (Jaaskelainen et al., 1995). NNT may provide recordings of late components by averaging > 500 stimuli.

4.5.3. Electrode impedance, amplifiers, filters and display

The impedance between the electrode and the skin should be less than 10 kOhms. The high pass filter should be set at 20 Hz. In abnormal nerves the SNAP can contain high frequency components; therefore, the low pass filter should be set at a frequency of 3 kHz.

Averaging improves the signal-to-noise ratio and is particularly useful when the SNAP amplitude is small. The first attempt to visualize small nerve signals through the skin was by superimposing sweeps (Dawson and Scott, 1949).

4.5.4. Definition of the SNAP parameters (Fig. 21)

Latency is the time from the stimulus to the first positive peak of the SNAP. Some laboratories measure the latency to the first negative peak. If there is no clear positive peak in an antidromic

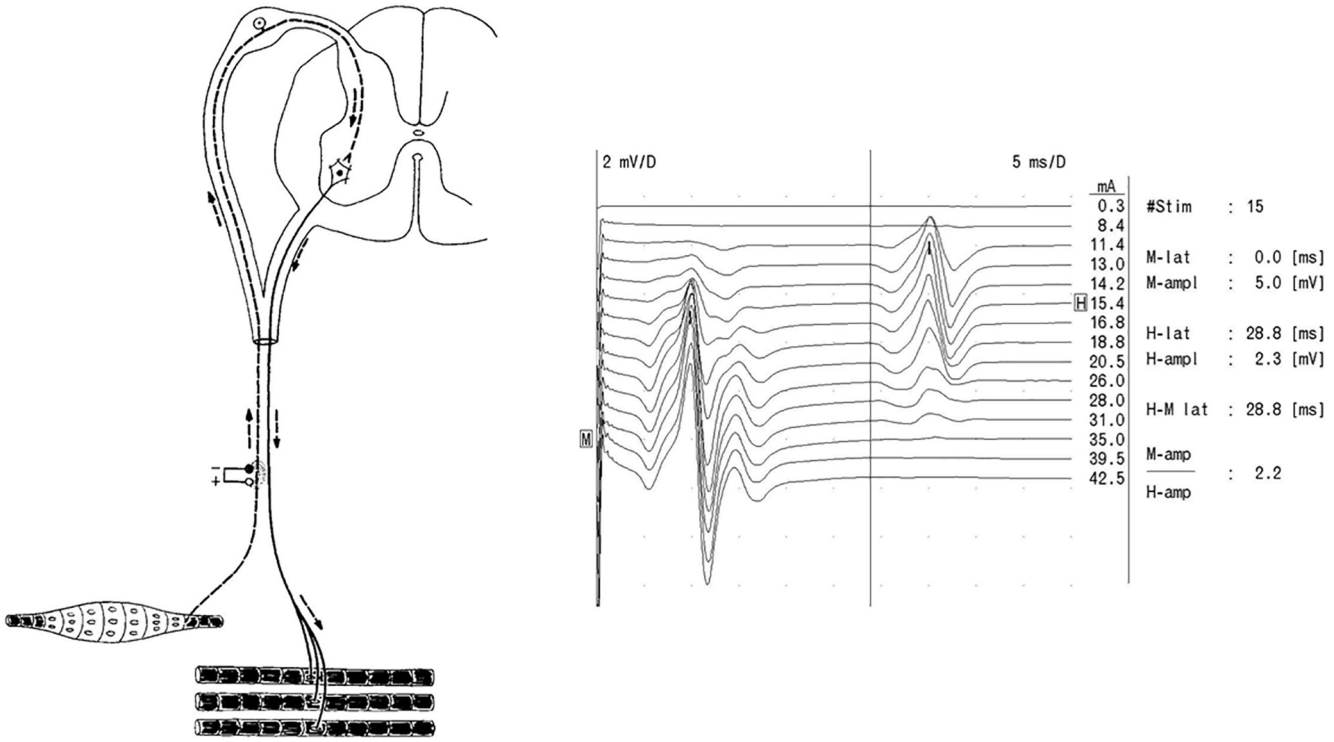


Fig. 19. The H-reflex. Left panel shows the underlying physiology. Stimulation of the afferents that elicit a monosynaptic reflex as a motor response in innervated muscle. Right panel example of H-reflex from Gastrocnemius muscles. The stimulation strength is successively increased and the H-reflex increases up to a maximum, after which the motor responses become blocked.

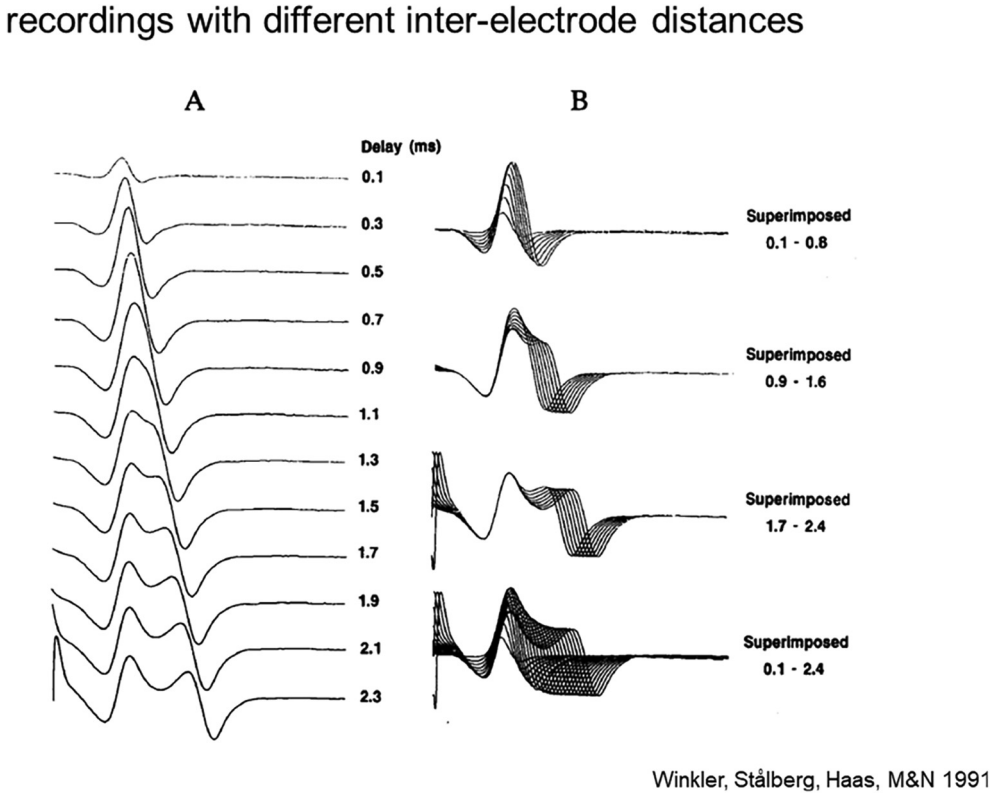


Fig. 20. The shape of the SNAP depends on the distance between the recording electrodes. When the distance is short, the SNAP amplitude is low. With successively increasing inter-electrode distance the amplitude changes from very low to a maximum and to a complex signal of lower amplitude and longer duration (Winkler et al., 1991a).

recording, the latency is measured to the initial take-off from the baseline.

Conduction time (CT) is the difference between the proximal and distal latencies if the nerve is stimulated at two different sites. For calculation of the CT the latency should be measured to the first positive peak.

The length of a nerve segment is measured from the center of the stimulating cathode along the nerve to the center of the closest recording electrode or to the cathode at the next stimulation site.

Conduction velocity of the fastest axons is calculated as follows: CV (m/s) = segment length (mm)/CT (ms). The sensory CV reflects the conduction in the largest myelinated axons.

Amplitude (AMPL) of the SNAP can be measured in several ways: (1) from the baseline to the negative peak, (2) from the first positive peak to the negative peak, (3) from the minimum to the maximum voltage or (4) from the negative peak to the intersection of the line drawn from the first to the last positive peak. There is no international recommendation for the exact measurement of the SNAP amplitude - they are all equally useful.

Duration (DUR) of the SNAP is defined as the time from the first to the last positive peak. If no initial positive peak is present, duration is measured from the take-off from the baseline to the last positive peak.

The duration reflects the dispersion of conduction velocities.

Area (AREA) is the integrated area between the signal and the baseline over the DUR.

Decay. As in MCS the conduction velocity dispersion causes a change of the DUR recorded at two different sites. This AMPL or AREA change is expressed as proportional values.

Dispersion is the proportional change of the duration, DISPERSION, and is calculated in a similar way as amplitude decay.

4.6. Short segment studies

Short segment motor studies (SSS), with 10–20 mm segments, can be used for the exact localization of ulnar nerve lesions at the elbow (Fig. 21.) and fibular nerve lesions at the knee (Miller, 1979; Brown and Yates, 1982; Campbell et al., 1992; Omejec and Podnar, 2015). Similar sensory SSS studies of the median nerve at the wrist (Kimura, 1979) and mixed nerve SSS of the ulnar nerve at the elbow (Omejec et al., 2016) have been used. In ulnar nerve studies mixed nerve SSS were less useful than motor SSS (Omejec and Podnar, 2016).

Although theoretically 10 mm segments are preferable to 20 mm segments, it has been shown that motor SSS localize conduction slowing proximal to the actual site of nerve entrapment using 20 mm, in a study also including ultra sound examination (Podnar et al., 2017). Precise localization is particularly important in ulnar neuropathies at the elbow, in differentiating nerve entrapment under the humeroulnar aponeurosis (HUA) distal to the medial epicondyle from external nerve compression in the retroepicondylar groove (RTC) at or proximal to the medial epicondyle (Omejec and Podnar, 2016). This is important for treatment decisions, as it is suggested that the former be treated with surgical division of the HUA, and the latter with a conservative approach (Omejec and Podnar, 2018).

These methods are sometimes called "inching" because initially the stimulator was moved in about one-inch steps to detect the site of abrupt change in amplitude or configuration of CMAP, but no attempt was made to exactly measure the segment lengths (Miller, 1979). When 10 mm segments are used the term SSS is preferable. A useful maneuver to see a parallel shift indicating a delay is to show responses to stimulation at consecutive positions in a rastered display and draw a line joining the take-off points or peaks (Fig. 22). It is even easier to see the latency shift with the traces superimposed. The position of the elbow must be standardized within the laboratory and reference values should be obtained in the same way. With questionable conduction parameters, the temperature should be checked.

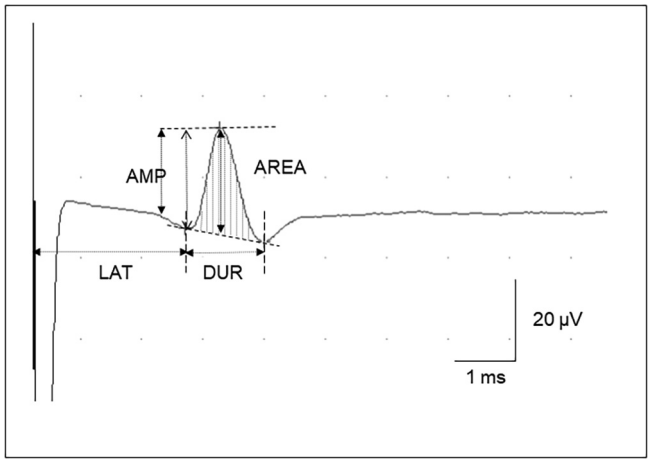


Fig. 21. SNAP parameters. See text for details.

4.7. Factors that influence neurography parameters

Most laboratories use reference values that have been described in the literature, sometimes with slight modifications. Few laboratories have their own reference values for all methods they use.

To optimize the sensitivity of measurements all variables that may influence the results must be considered. The variables that affect nerve conduction measurements are: placement of the electrodes (recording method), biological factors (subject age, height and gender) and physical (position of limb, length of muscle, temperature and nerve segment length).

4.7.1. Age

In preterm neonates the conduction velocities are very slow: at 25 weeks of gestational age the motor CV of the ulnar nerve is 12 m/s and of the tibial nerve 6 m/s (Schulte et al., 1968) In full term neonates the motor and sensory CV is around half the adult values, 25–30 m/s in the upper extremity (Gamstorp et al., 1966; Blom and Finnstrom, 1971). The CV increases to almost adult values by the age of three to five years. Adult values are reached in the teens (Gamstorp, 1963; Gamstorp and Shelburne, 1965; Rosenfalck and Rosenfalck, 1975). Above age 20 there is a slight progressive reduction in the nerve conduction velocity with increasing age, 0.5–1.8 m/s per 10 years of age (Buchthal and Rosenfalck, 1966; Lucci, 1969; Falck et al., 1991). The decrease of conduction velocity is more pronounced in the legs than in the arms (Falck et al., 1991).

The CMAP AMPL from extensor digitorum brevis muscle of the foot decreases significantly with age in adults (Falck et al., 1991) which may be due to age related loss of axons, external pressure from shoes, trauma and so on. The CMAP AMPL of the thenar muscles on median nerve stimulation does not change significantly with age.

No change in DLAT has been found with age (Gutjahr, 1984; Falck et al., 1991).

In adults there is a well-documented progressive decrease of the *sensory* AMPL with increasing age (Buchthal and Rosenfalck, 1966; Kemble, 1967; Gutjahr, 1984). The decrease of amplitudes is due to loss of sensory axons and also due to increased dispersion of sensory nerve conduction velocities (Nielsen, 1973).

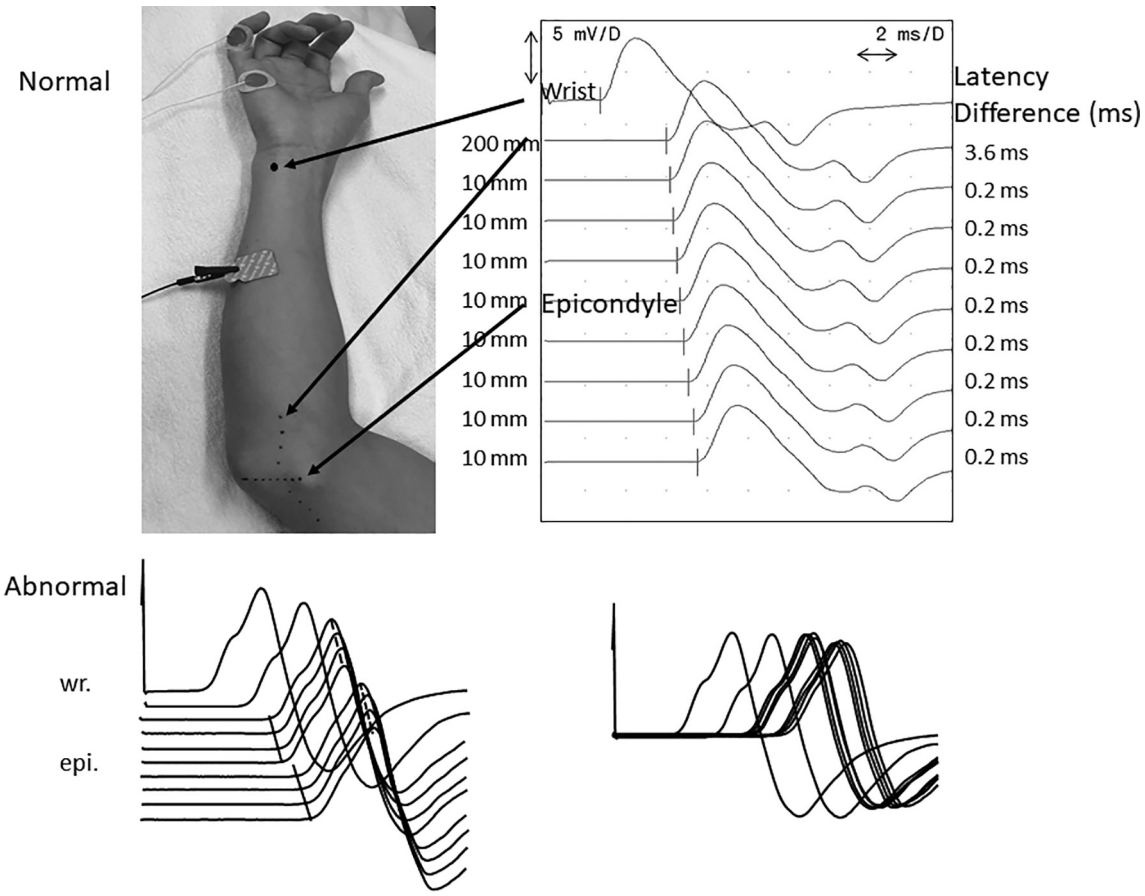


Fig. 22. Short segment motor study of the ulnar nerve. Stimulation at 10 mm intervals around the elbow. The reference line at the elbow is from the midpoint of the medial epicondyle to the distal edge of the olecranon. Normal: The conduction time across each 10 mm segment is 0.2 ms. Abnormal: raster and superimposition modes. There is a delay and amplitude loss at the level of sulcus ulnaris. Note the usefulness of superimposition mode. To see parallel time shift indicating a delay a line is drawn joining the take-off points (or peaks). It is even easier to see the latency shift and amplitude decay with the traces superimposed.

The duration of the SNAP increases and the amplitude decreases with age (Nielsen, 1973).

4.7.2. Height

Taller subjects have slower conduction velocities than short subjects (Lang and Die, 1971; Falck et al., 1991). In several motor nerves height accounts for more variability than age (Falck et al., 1991). The conduction velocity decreases 2–3 m/s per 10 centimeters increase in height in the lower extremities (Falck et al., 1991).

SNAP AMPL is also inversely proportional to height (Stetson et al., 1992).

4.7.3. Sex

No influence of sex on CV has been found in most studies (Stetson et al., 1992). SNAP amplitudes in men are smaller than in women in antidromic sensory nerve studies because of the greater circumference of fingers in men (Bolton and Carter, 1980). Women also have higher amplitudes than men in orthodromic sensory studies (Stetson et al., 1992).

4.8. Body mass index (BMI)

SNAP amplitudes are 20–40% lower in subjects with high body mass than in thin subjects (Buschbacher, 1998) The CMAP amplitude and other nerve conduction parameters are not significantly affected by body mass index.

4.9. Skin thickness

Skin thickness is inversely correlated with the sensory AMPL (Hasanzadeh et al., 2008), persons with thicker skins having smaller sensory AMPs.

4.10. Temperature

Temperature has a dual effect on nerve conduction measurements (Lang and Puusa, 1981). There is a *local effect* on the CMAP and SNAP and a *segmental effect* on the CV. The CMAP AMPL increases 1.7% per °C with decreasing local temperatures down to 18 °C (Ricker et al., 1977). A decrease in temperature of a *nerve segment* decreases the conduction velocity from 1.2 to 2.4 m/s/°C (Dioszeghy and Stålberg, 1992). The local and segmental temperature effects on the amplitude interact and cancel each other. Therefore, there is no significant relationship between temperature and AMPL in reference material. These data refer to healthy subjects. The effects of temperature on normal and abnormal nerves may differ (Dioszeghy and Stålberg, 1992) but the differences are small and insignificant from a clinical point of view.

There are several ways to standardize temperature measurements for nerve conduction studies. One way is to heat the limbs to 35 °C. Warming a limb to a surface temperature of 35 °C with an infrared heater takes 15–20 min (Bjorkqvist et al., 1977) or longer (Geerlings and Mechelse, 1985), which is too long for clinical studies. The limbs can be warmed quickly (1–5 min) by immersion in warm water or by heating pads, but these methods will not give exact temperatures. Unlike the distal sensory nerves, which

are superficial, most of the motor nerve segments are embedded in muscles and only extreme variations in skin temperatures will significantly affect the near nerve temperature of motor nerves. Another way is to include a temperature factor in the reference material. The temperature factor may not be the same for normal and abnormal nerves, and therefore this method is not recommended. A third more practical way is to warm limbs if temperature on the dorsal aspects is below 28 °C for hands and 27 °C for feet, respectively (30 °C for hands and 28 °C for feet are used in some labs). Note that clinical routine and collection of references values must use the same conditions.

4.11. Length of the segment

Conduction velocity is faster in the proximal segments of a nerve (Trojaborg, 1964). Because of this, long nerve segments tend to have higher conduction velocities than short segments (Gutjahr, 1984). AMPDEC and DISP increases with increasing nerve segment length (Kimura et al., 1986). The range of nerve segment length is small in the forearm; therefore the effect of the segment length is insignificant there. However, in the lower extremities the nerve segment length varies between subjects, which influences AMPDEC and DISP measurements.

If the aim is to reduce measurement errors, it is desirable to study segments that are as long as possible. However, slight focal conduction delays due to local nerve lesions may not be detected if the measurements are made over long segments. As an example; the sensitivity of MCV study of the 2 cm segment is about 10% higher compared to regular 10 cm segment (93% vs 82%) in ulnar neuropathy at the elbow (Omejec and Podnar, 2016).

Area and amplitude of antidromic SNAPs decrease and duration increases with increasing distance between the recording and stimulating electrode. This is due to increased dispersion of action potentials with increasing segment lengths. The sensory AMPL decreases exponentially with increasing distance (Horowitz and Krarup, 1992), thus the sensory nerve segment length must be standardized when SNAP amplitude and area are measured. A fixed distance between the recording and stimulating electrode of 120 or 140 mm is recommended for sensory nerve segments.

4.12. Measurement errors in neurography

There are two main sources of measurement error in the calculation of the MCV, distance and latency. The distance can be measured with an accuracy of 2–8 mm, making the error in the distance measurement 3–5% (Buchthal and Rosenfalck, 1966; Gutjahr, 1984). The measuring tape should be made of material that does not easily stretch; if measuring tapes of cloth are used they should be replaced before they stretch. For proximal segments a caliper should be more accurate than tape measures, but is rarely used.

Latency can usually be measured with an accuracy of 0.1–0.2 ms at each point. The error due to latency measurement is around 2–3% (Buchthal and Rosenfalck, 1966; Gutjahr, 1984; Taylor, 1993).

The quality of neurography measurements can be ascertained in different ways (Table 9).

4.13. Types of pathology of nerve segments and nerves

NCS is used to diagnose focal and diffuse neuropathies and to characterize the type, severity and distribution of abnormalities. The main types of pathophysiology are axonal degeneration, demyelination, and conduction block. Abnormalities comprise a combination of different kinds of underlying pathophysiology. The characterization facilitates identifying the etiology.

4.13.1. Axonal degeneration

Following an axonal lesion, the axon distal to the lesion degenerates, Wallerian degeneration (Fig. 23). Distal stimulation of injured axons continues to generate a CMAP for four to six days following an axonal lesion. At this early stage it is not possible to distinguish between a conduction block and an axonal lesion. During Wallerian degeneration neuromuscular transmission fails before the conduction fails in the in the nerve fiber (Gilliatt, 1981). Following the acute stage, the axons degenerate and there will be a reduction of the AMPL (Fig. 23); if there is total axonal loss no CMAP can be elicited at all. In the early stages of a neuropathy AMPL and AREA reflect the number of surviving axons (Cornblath et al., 1988; Miller et al., 1988). With successful collateral or axonal reinnervation the AMPL will eventually increase. Therefore, in chronic axonal neuropathy the AMPL and AREA do not directly reflect the number of surviving axons, but the functioning muscle mass.

Many axonal neuropathies tend to affect primarily the largest myelinated fibers, often with mild secondary myelin abnormalities. Due to these two factors a slight reduction of the conduction velocity is often seen in axonal polyneuropathies, down to 40 m/s in the median nerve (McLeod and Prineas, 1973; Logigian et al., 1994). In severe axonal polyneuropathies the conduction velocity may be reduced to 70–75% of the lower limit of normal if the CMAP amplitudes are markedly reduced (Raynor et al., 1995).

In axonal polyneuropathy there is an additional distal-proximal gradient of conduction velocity slowing which is clearly demonstrated when velocities are measured across the same nerve segment recording from both distal and proximal muscles. On the other hand, no significant gradient in conduction velocity occurs in patients with demyelinating neuropathies, in patients with motor neuron disease, or in controls (Raynor et al., 1995).

4.13.2. Demyelination

In primarily demyelinating polyneuropathies the conduction velocities may be severely reduced, usually by more than 30% of

Table 9
Quality control of neurography.

1. Recording
   - o Check the temperature of the segment studied
   - o Assure that stimulus intensity is adequate
   - o Note and correct stimulus artifacts and background noise.
   - o The automatic cursor settings may be erroneous; these should always be verified by visual inspection and corrected manually if necessary, using standard gain and sweep speed.
2. Signals
   - o Deviation from reference limits should always initially be suspected to be caused by technical factors.
   - o Comparisons between CMAP shapes after distal and proximal stimulation, or asymmetry in shape between sides should alert for technical control.
   - o For F responses, the baseline between CMAP and F should be free from noise from other activity.
   - o In F wave recordings CMAP waveforms should be constant – this is best confirmed by visual inspection of superimposed signals.
   - o For cursor setting for individual F-waves, extreme values should be checked and corrected if necessary.

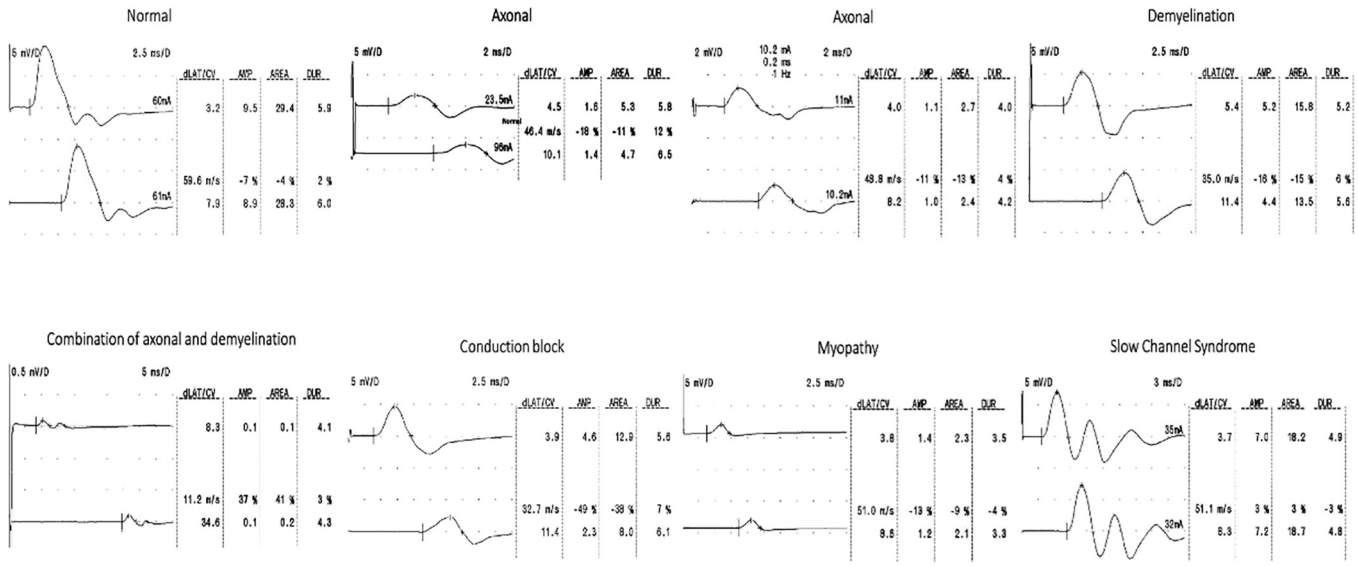


Fig. 23. Examples of motor studies in different types of pathology, indicated above each panel.

the reference values or less than 40 m/s in the upper limb nerves (McLeod and Prineas, 1973; Logigian et al., 1994) (Fig. 22). Also, DLAT will typically be abnormal early in demyelinating polyneuropathy. A median nerve DLAT longer than 7 ms is usually associated with a demyelinating polyneuropathy unless the patient has carpal tunnel syndrome.

4.13.3. Motor conduction block and increased temporal dispersion

Conduction block (CB) is "failure of an *action potential* to propagate past a particular point in the nervous system whereas conduction is possible below the point of the block" (Kneiser et al., 2015). CB is an important parameter often seen in demyelinating polyneuropathies, particularly in immune mediated demyelinating polyneuropathies, while in genetically determined demyelinating polyneuropathies CB is usually not found. CB is demonstrated electrophysiologically by an abnormal AREADECAY or AMPLDECAY.

The quantitative criteria for definition of CB are ambiguous and must always be interpreted in relation to mean conduction velocity and dispersion of velocities because of the physiology of the CMAP generation. The CMAP is the sum of the MUPs generated by the individual MUs in the muscle. Due to the cancellation of the positive and negative phases of the individual MUPs, especially with abnormal slowing of the conduction velocities and increased temporal dispersion among individual MUPs, there is decay in the amplitude and area without conduction block. The influence of mean motor conduction velocity on amplitude decay was investigated in a simulation study (Stålberg and Karlsson, 2001): the normal nerve the conduction velocity was set to 61 m/s. This resulted in amplitude change and duration change of −6% and 19% respectively. With slowing, the corresponding values were 30 m/s, −22% and 40%, not fulfilling criteria for CB. A velocity of 10 m/s produced an amplitude change of −50% and increase in duration of 70%. Thus, in spite of distinct drop in proximal amplitude, changes such as these should still not lead to misinterpretation suggesting CB. Other simulations have suggested that abnormal temporal dispersion alone may cause DECAY values up to 50% (Rhee et al., 1990).

There have been several attempts to define criteria for CB (Olney et al., 1987; Cornblath et al., 1991; Uncini et al., 1993). However, the electrophysiological criteria for conduction block remain ambiguous because the effects of conduction block and increased dispersion of conduction velocities cannot easily be differentiated from each other. To discriminate between "pure" demyelination and demyelination with a conduction block, the following criteria have been suggested (American Association of Electrodiagnostic Medicine, 1999; Olney, 1999): conduction block is present if there is >20% AMPDEC or AREDEC and <15% change in DISPERSION, or if there is >50% AMPDEC or AREDEC, independent of DISPERSION. It has been demonstrated that both criteria are equally sensitive in detecting a conduction block (Uncini et al., 1993). Definition of CB is different for different nerves, overlooked in the rough rules given above. The first criterion (>20% dispersion related) gave several false positive results, while the latter criterion (>50% independent on dispersion) was more specific. In patients with CIDP CB was best detected by AREADEC and abnormal temporal dispersion was detected by negative peak duration method. Similarly, in compressive neuropathies it has been shown that AREADEC allows for more accurate identification and detection of CB than AMPDEC (Olney and Miller, 1984). AREADEC is to be preferred over AMPLDEC in the detection of CB in both polyneuropathies and focal neuropathies.

In multifocal motor neuropathy with conduction block, an immune mediated motor neuropathy, it has been shown that the conduction block can be activity dependent. After a 60 second maximal voluntary contraction there may be prominent fatigue and a significant increase in a focal conduction block, which is due to hyperpolarization of the axonal membrane (Kaji et al., 2000).

4.13.4. Reversible conduction failure

Reversible conduction failure (RCF) is common and important in immune mediated acute motor and sensory axonal neuropathies (AMSAN) and acute immune mediated axonal motor neuropathies (Kuwabara et al., 1998; Uncini et al., 2017). This is due to nodal or paranodal dysfunction caused by an attack by antiganglioside antibodies on at the node of Ranvier; it mimics demyelination but without temporal dispersion. This phenomenon looks similar to a CB but there is no dispersion of conduction velocities and no significant slowing of the conduction velocity. RFC may either be transitory with a quick recovery or progress to axonal degeneration with a delayed recovery (Uncini et al., 2013). Reliable determination of RFC can often be obtained only by serial studies (Uncini et al., 2013).

4.13.5. Criteria for classification of polyneuropathies

The ESTEEM criteria for classification as a polyneuropathy (PNP) require at least two nerves with demyelination or axonal loss

(Tankisi et al., 2005), in agreement with other suggested criteria (see above). In addition, these authors have added limits for probable and definite demyelination separately, as below.

1. Classification of a PNP as primarily demyelinating requires involvement in at least two extremities and one of the following:

(a) Definite demyelination in two nerve segments from different nerves
(b) One definite demyelinated segment and at least two probable demyelinated segments from different nerves
(c) Probable demyelination in at least four segments from different nerves.

Focal neuropathies should be excluded.

2. Classification of a PNP as primarily axonal requires axonal loss in two sensory or motor nerve segments from different nerves. Axonal loss in motor nerve segments with low CMAP amplitude should, however, only be interpreted if myopathy and neuromuscular transmission disorders have been excluded. Involvement in at least two extremities is also required.

3. Classification of a PNP as mixed requires the co-existence of some nerves showing features of demyelination and others being consistent with axonal loss, i.e. criteria for both demyelination and axonal degeneration should be fulfilled.

4 Patients not fulfilling any of the above classifications are defined as "unclassified PNP".

4.13.6. Myopathies

The amplitude of surface recorded potentials generated by individual muscle fibers depend on the muscle fiber diameter (Håkansson, 1956). Atrophic muscle fibers generate small potentials. The abnormalities in various myopathies vary considerably. In many myopathies there is degeneration of muscle fibers that results in a reduced number of muscle fibers per MU and atrophy of the individual muscle fibers. As a result, the AMPL and AREA of the M-wave may be reduced.

5. Repetitive nerve stimulation

5.1. Introduction

Repetitive nerve stimulation (RNS) is the most commonly used electrodiagnostic test of neuromuscular transmission (NMT). Although the RNS technique is straight-forward, it is not without technical pitfalls - the most common errors result from movement of the recording electrode, variations in the stimulus intensity and inadequate warming.

5.2. Background

Compound muscle action potentials (CMAPs) produced by supramaximal stimulation of a motor nerve are the temporal and spatial summation of the action potentials of all muscle fibers activated by the nerve; the size of the negative phase of the CMAP reflects the number of activated muscle fibers. RNS at low rates reduces the store of readily releasable acetylcholine (ACh) in motor nerve terminals and the number of quanta of ACh released decreases during a train of RNS (*rundown*). CMAPs decrease during RNS in diseased muscle if progressively fewer muscle fibers respond to nerve stimulation, producing a *decrementing* pattern (Fig. 24). There is no change in CMAP size during RNS in normal muscle due to the *safety factor* at the motor endplate. Changes in the CMAP size during an RNS train reflect the balance between the rundown, which predominates at low stimulation rates, 1–5 Hz (LF-RNS) and facilitation of transmitter release, which manifests at higher rates and partially counteracts the rundown after several stimuli.

To demonstrate facilitation, the muscle can be activated either by stimulation at 20 to 50 Hz (HF-RNS), or by strong contraction of the muscle (Hatanaka and Oh, 2008), which is less painful. With voluntary activation, facilitation is assessed immediately after the end of activation, with the muscle at rest. With HF-RNS activation, facilitation is assessed either at the end of the HF train, while the muscle is contracting/shortening, or immediately after the end of the HF train, with the muscle at rest. With the former, the CMAP amplitude is subject to *pseudofacilitation*, in which the amplitude increases without commensurate increase in area (McComas et al., 1994), plus increased CMAP amplitude produced by muscle shortening itself. Therefore, with HF-RNS activation, assessment of amplitude facilitation is best made immediately after the end of the HF train.

Activation is immediately followed by a brief period of *post-activation facilitation (PAF)*, during which CMAP amplitude increases and any decrement may be decreased. This is followed by a period of *post-activation exhaustion (PAE)*, during which nerve impulses release less ACh and a decrementing response may be unmasked or enhanced. PAF is maximum immediately after activation; PAE is maximum 2–5 minutes later.

5.3. Technique

5.3.1. Electrode placement

Surface recording electrodes are placed so that the CMAP has an initial sharp negative (upward-going) deflection, indicating that the active electrode is over the motor point of the muscle (Fig. 23). The reference electrode should be placed at a distance where electrical activity from the muscle is minimal. Subcutaneous un-insulated needle electrodes are sometimes useful for recording, particularly for long-term studies: their overall recording area is similar to surface electrodes.

5.3.2. Muscle temperature

The decrementing response in myasthenia gravis (MG) is less when the muscle is cold, and in some cases no decrement is seen unless it is warmed. In Lambert-Eaton myasthenia (LEM) the resting CMAP is much smaller when the muscle is warm. Hand and foot muscles should be warmed to a surface temperature of at least 34 °C before RNS testing; proximal and facial muscles do not need warming. In MG, the decrement is greater at a surface temperature

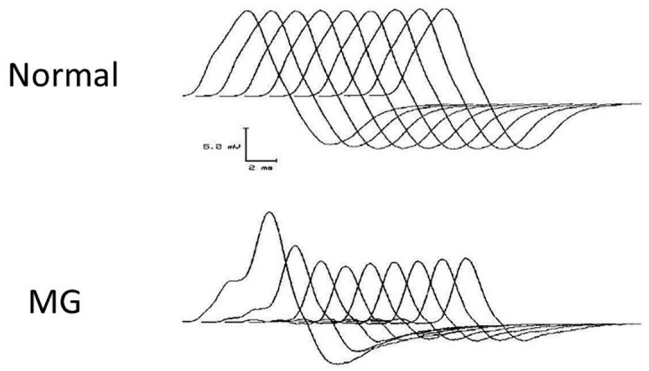


Fig. 24. CMAPS elicited by 3 Hz RNS in a hand muscle. Normal (top) and MG (bottom). In MG, the decrement begins with the 2nd response, is maximum in the 4th or 5th response, and resolves thereafter, producing a characteristic "U-shaped" train envelope. Copyright D.B. Sanders.

of 42 °C than at 32 °C (Rutkove et al., 1998), but heating above 32 °C has no effect on RNS in normal muscle (Rutkove et al., 1997).

5.3.3. Stimulation

The nerve should be stimulated with the least painful intensity that assures that all motor fibers to the tested muscle are depolarized by all the stimuli. This *supramaximal* stimulus is determined by increasing the intensity of a short duration pulse until the CMAP is of maximal size, then increasing the intensity further by approximately 25%. With some muscles, e.g. biceps or rectus femoris, the nerve is best stimulated with a monopolar needle, and some nerves, e.g. the masseteric, can only be stimulated with a needle electrode. The appropriate joints should be immobilized to minimize artifacts due to muscle movement.

The test may be started with a train of 10 LF-RNS stimuli with the muscle at rest (look at amplitude, decrement) and another train of 10 after 10 seconds of voluntary activation (look for increase in amplitude and decrease in decrement) (Fig. 25). More runs do not add any information.

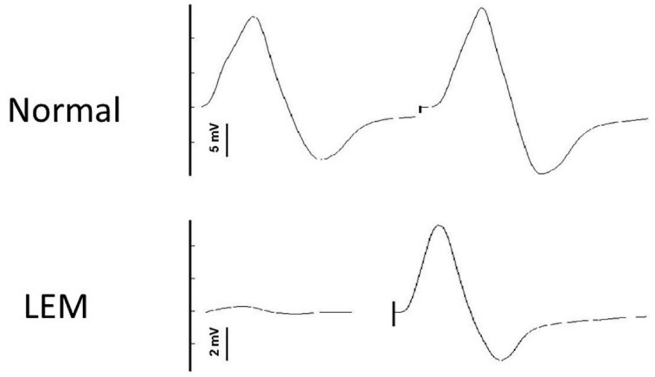


Fig. 25. Schematic presentation of RNS protocol in MG and LEM. Some degree of facilitation is seen in both conditions, being more marked and wearing off more rapidly in LEM. The progressive decrement during the train immediately following activation results from the combination of the facilitation and "rundown".

5.3.4. Activation

In suspected MG without decrement at rest, activation is used to unmask a decrement. Strong muscle contraction for 30 to 120 secs may bring out a decrement that is seen only during the PAE phase (Ambler, 1990; Oh et al., 2014). To demonstrate the maximum PAE, repeat LF-RNS trains at intervals for up to 3 minutes after activation (Fig. 25). The additional yield of this procedure varies by muscle, being approximately 5–8% in the hand, facial, and trapezius muscles and 10 to 20% in the deltoid (Ambler, 1990; Rubin and Hentschel, 2007).

In LEM or other pre-synaptic conditions, activation is performed to demonstrate facilitation. Strong voluntary contraction for 10 seconds usually produces the maximum facilitation (Hatanaka and Oh, 2008), and stimuli should be delivered within 5 seconds after the end of activation to demonstrate the maximum increase in CMAP size; longer activation may lead directly to exhaustion, which can mask the facilitation. The simplest way to demonstrate facilitation in LEM is to compare a single CMAP elicited immediately after activation with a resting pre-activation CMAP (Fig. 26).

5.3.5. Measurement/criteria for abnormality

CMAPs should be observed at a fast oscilloscope sweep speed (50 to 100 ms sweep duration) and superimposed during RNS to detect baseline shift, change in CMAP duration, or other technical artifacts. Changes in CMAP size are quantified by calculating the percentage change in negative peak amplitude (or area) between the first and later responses. In MG, the maximum decrement occurs between the 1st and the 4th or 5th stimuli. Although there is no physiological decrement in normal muscle due to the large safety factor of neuromuscular transmission, up to 10% artifactual technical decrement is accepted as normal in many laboratories; some consider decrements greater than 7% or even 5% to be abnormal if it can be demonstrated that artifacts are not responsible for the change (Abraham et al., 2017).

During LF-RNS, decrements in the area and amplitude of the negative CMAP peak give similar values (Aiello et al., 1986; Cengiz and Kuruoglu, 2006), while amplitude and area increments

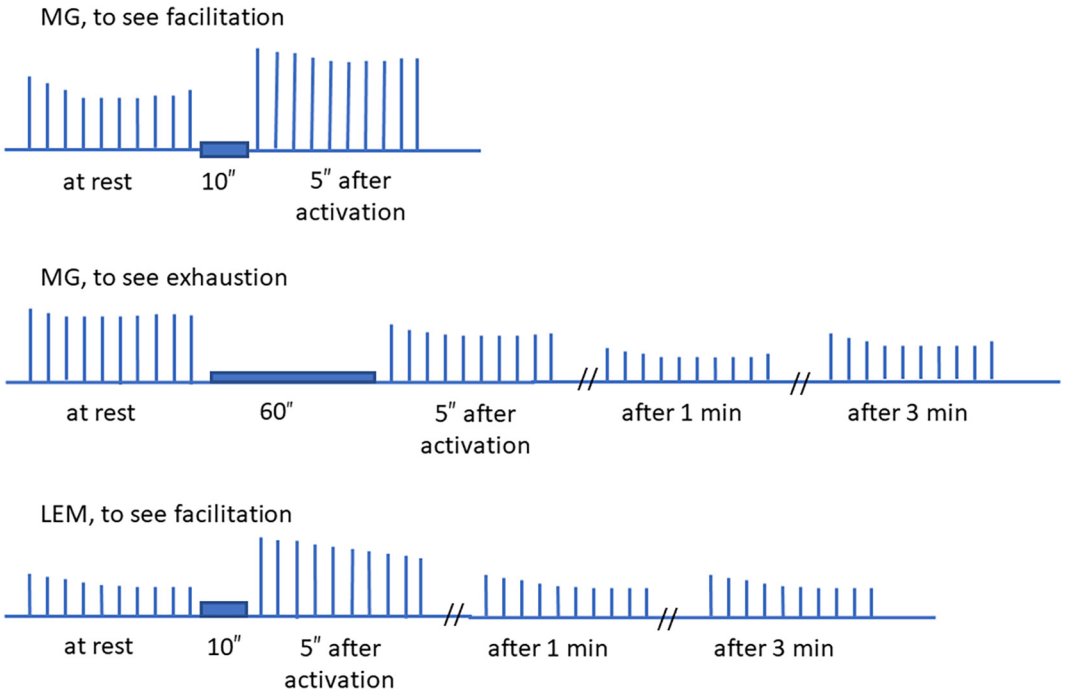


Fig. 26. Single CMAPs elicited before (left) and after (right) strong voluntary contraction in a normal hand muscle (ADM) and in a patient with LEM. Copyright D.B. Sanders.

at HF-RNS do not correlate closely (Cengiz and Kuruoglu, 2006) (Some of this may be due to pseudofacilitation).

While the amplitude of the 5th CMAP of a LF-RNS train varies less than ~2% in normal commonly-tested limb muscles (Baumann et al., 2010), the area of the 5th response in these muscles may fall as much as 6%. To avoid an artifactual decrement, it is preferred to measure the CMAP amplitude in RNS studies.

In presynaptic NMJ disorders (e.g., LEM) the increase in CMAP size during HF-RNS or immediately following activation is measured as percent *increment*. An increment >100% is generally accepted as the criterion for a presynaptic abnormality; however, using a cutoff of >60% increment may increase the sensitivity in LEM without affecting specificity (Oh et al., 2005; Hatanaka and Oh, 2008).

5.3.6. Muscle selection

Many muscles can be tested by RNS, but the results differ in different diseases and among patients. In MG, a decrement is seen more often in proximal (deltoid, trapezius, anconeus muscles) or facial muscles, although testing these muscles is technically more demanding. In LEM, the characteristic abnormalities are found most often in the hand muscles (Tim et al., 2000). In some patients, several muscles must be examined to demonstrate abnormal NMT. Muscle selection is based on the patient's clinical symptoms and distribution of clinical weakness.

The best RNS recordings are made in superficial muscles with a compact endplate zone that produces CMAPs with a predominantly monophasic waveform (Table 10). This makes it easier to measure changes in CMAP size. Movement artifact is less with small muscles and when the stimulus activates only the tested muscle.

5.3.7. Pitfalls and artifacts

Inadequate stimulus intensity or movement of the stimulating electrode or the muscle may produce irregular patterns of change in CMAP size that do not correspond to patterns expected in disease. The following observations help avoid these artifactual changes.

The muscle should be at rest during stimulation, as contraction during RNS can produce changes that mimic a decrement or facilitation. Turn the loudspeaker ON to detect voluntary contraction of the tested muscle.

Reproducibility. The decrement at rest should be the same when the test is repeated.

Envelope shape. Changes during RNS should conform to a pattern seen in disease, without sudden or irregular variations between consecutive responses.

Activation cycle. Changes after activation should conform to an acceptable pattern (Fig. 25).

5.3.8. Clinical findings

5.3.8.1. Myasthenia gravis. The decrementing response in MG is best demonstrated at stimulation rates of 3–5 Hz (Desmedt, 1973). The characteristic pattern in MG is a progressive decrement that is greatest in the 4th or 5th response, with some return toward the initial size during the subsequent responses (Fig. 25), producing a so-called "U-shaped" or "saddle-shape" pattern of the train envelope. Trains of 5 stimuli are sufficient to demonstrate the maximum decrement in MG, but trains of 9 or 10 are required to demonstrate this characteristic train envelope. This pattern is not unique to MG, and may also be seen in other conditions in which neuromuscular transmission is impaired, such as LEM (Lambert et al., 1961), motor neuron disease (Denys and Norris, 1979; Maselli et al., 1993) and, rarely, in peripheral nerve disease (Baginsky, 1968) or radiculopathy (Gilchrist and Sanders, 1989).

The initial CMAP size contains information about the number of functioning end plates at rest. Initially low amplitude indicates blocked or degenerated end plates. The CMAP size immediately

Table 10
List of muscles that may be tested with RNS.

Muscle recorded	Stimulation site	Recording electrode, active	Recording electrode, reference	Immobilization method
ADM or FDI	Ulnar n. at wrist	Over muscle belly	Distally on digit	Tape or hold fingers together
Thenar	Median n at wrist	Over muscle belly	Distally on digit	Tape or hold fingers together
Trapezius	Spinal accessory n. behind sternocleidomastoid below the ear	Over trapezius, at angle between neck and shoulder	Over acromion	Hold bottom of chair, if seated Hold strap around foot, if lying Passive elevation of shoulder in supine position (Ogawa et al., 2013)
Biceps	Musculocutaneous n. in axilla (posterior border of short head of biceps within 1inch of axillary fold)	Over belly of biceps	Over elbow	Hold or strap arm to table
Deltoid	Axillary n. at supraclavicular fossa	Over belly of deltoid	Over acromion or distally over forearm	Hold or strap upper arm to chest
Anconeus (Kennett and Fawcett, 1993)	Radial n. at lateral intermuscular septum (1–2 cm proximal to lateral epicondyle)	Over muscle belly, ~3 fingerbreadths distal to olecranon	Distally on dorsum of forearm	None
Extensor indicis	Radial n. at elbow (between biceps tendon and brachioradialis)	Over muscle belly	Distally on dorsum of forearm	Hold digits against table
Nasalis or orbicularis oculi	Facial n. below the ear or near zygomatic branch close to ear (may use monopolar needle)	Over muscle belly	Tip of the nose, or on contralateral homologous area	None
Masseter (Pavesi et al., 2001)	Trigeminal n with monopolar needle in mandibular notch (between mandibular incisurae and zygomatic arch, approximately 2 cm anterior to ear	Over belly of masseter	Under jaw	None
Extensor digitorum brevis	Fibular n. at fibular head	Over muscle belly	Lateral to base of little toe	
Tibialis anterior	Fibular n. at fibular head	Over muscle belly	Patella. Sometimes Tib.Ant. tendon is used for E2, but this is more prone to record volume conducted activity	Stabilization board with foot fixed in dorsiflexion
Quadriceps (rectus femoris)	Femoral n. in groin, lateral to femoral artery (may require monopolar needle)	Over anterior thigh, to record simplest monophasic CMAP	Over patella	

after activation gives information about the physiological status of the end plates: in MG, a size increase indicates that some end plates are able to respond to increased ACh, predicting an immediate response to cholinesterase inhibitors. If the post activation amplitude becomes "normal", many end plates are viable, a good sign. If little or no increase is seen after brief activation for 10 secs, then most of the end plates are probably degenerated, and little effect or no immediate effect of cholinesterase inhibitors is expected.

The sensitivity of RNS reported in MG varies in the literature and is dependent on the clinical phenotype, distribution of muscle weakness, number of muscle tested, and criteria used for abnormal decrement. RNS is reportedly more sensitivity in generalized MG (53–89% sensitive) than in ocular MG (20%–67%) (Zinman et al., 2006;Bou Ali et al., 2017). RNS is more likely to be abnormal in a proximal or facial muscle (Zambelis et al., 2011; Bou Ali et al., 2017), and in clinically weak muscles. This is especially true for MG patients with MuSK antibodies, in whom RNS is usually abnormal in facial muscles and frequently normal in limb muscles (Oh et al., 2006; Padua et al., 2006). For maximal diagnostic yield, test several muscles, including those that are weak.

5.3.8.2. Lambert-Eaton myasthenia. The characteristic RNS findings in LEM are low CMAP amplitude at rest, a decrement to LF-RNS, and marked facilitation after activation (Lambert et al., 1961). A decrement in at least one hand muscle is the most frequent RNS abnormality in LEM and may be seen even when CMAPs are not low and diagnostic facilitation cannot be demonstrated (Tim et al., 2000). Although a U-shaped pattern may be seen in LEM, the more typical pattern is a progressive decrement that is maximum in the 9th or 10th responses (Fig. 25) (Baslo et al., 2006; Sanders et al., 2014).

5.3.9. Conclusions, RNS

RNS requires meticulous attention to detail during the testing procedure and critical analysis of the results. Abnormalities typical of MG and LEM are well-recognized, but similar abnormalities can be seen in primary nerve and muscle diseases, which must be excluded to accurately interpret abnormal RNS results.

6. MUNE

Motor Unit Number Estimation (MUNE) techniques are electrophysiological approaches to estimate the number of MUs in a muscle or to get an index that is related to the number of MUs. In 1971 the first MUNE method (incremental stimulation MUNE) was introduced by McComas (McComas et al., 1971). Over the following decades, different MUNE variations were developed: a multi-point stimulation technique, a spike-triggered averaging technique, high-density MUNE, MUNIX and others. However, different techniques produced different results in muscles studied in healthy volunteers. Examples are listed in Table 11.

MUNE techniques have been mainly, but not exclusively, applied in motor neuron diseases such as ALS, spinal muscular atrophy (SMA) or post-polio syndrome, but also in demyelinating neuropathies. Users should be aware of the underlying principles and limitations of each technique.

Most MUNE methods are based on the assumption that the CMAP from supramaximal electrical nerve stimulation reflects the total number of single motor unit potentials (SMUP) in the

Table 11
Comparison of different MUNE techniques.

Comparison of MUNE techniques					
MUNE method	Muscle	Age	n	Mean, SD	Author
F-response	APB	19–87	54	253 ± 107	Wang 1995
	APB	19–87	59	278 ± 113	Wang 1995
	APB	21–81	37	288 ± 95	Doherty 1993
	APB	n.a	n.a.	315 ± 48	Daube 1988
Autom. MUNE	APB	21–56	33	228 ± 93	Galea 1991
Microsimulation	Ad. mult. poi. stim.	26–49	10	122 ± 38	Stein 1990
Spike-trigg. averag.	Mult. point stim.	26–49	10	135 ± 27	Stein 1990
Manual incr. stim.	Stat. Estimate	26–49	10	170 ± 62	Stein 1990
Stat. MUNE	APB	n.a	72	101 ± 20	Shefner 2004
MUNIX	APB	23–45	8	190 ± 42	Neuwirth 2010
MUNIX	APB	49 ± 18	66	146 ± 54	Neuwirth 2011
Stat. MUNE	ADM	42 ±13	16	143 ± 26	Shefner 1999
MUNIX	ADM	20–78	33	148 ± 37	Nandedkar 2004
MUNIX	ADM	23–45	7	176 ± 37	Neuwirth 2010
MUNIX	ADM	29–67	34	158 ± 40	Nandedkar 2010
MUNIX	ADM	20–90	62	142 ± 42	Ahn 2010
MUNIX	ADM	49 ± 18	66	163 ± 47	Neuwirth 2011
decomp. quant. EMG	BB	27 ± 4	10	271 ± 125	Boe 2007
MUNIX	BB	49 ± 18	38	172 ± 49	Neuwirth 2011
decomp. quant. EMG	TA	20–30	10	122 ± 46	Boe 2009
MUNIX	TA	49 ± 18	38	144 ± 36	Neuwirth 2011
MScan	APB	55 ± 16	30	93 ± 36	Garg 2017
MScan	APB	65 (44–76)*	20	75 (71–166)*	Jacobsen et al. 2017

MUNE method	Rapidity	Subject Tolerance	Multicenter studies	Standardized technique
F-response	–	+/–	–	–
Ad. mult. poi. stim.	+/–	+	+	+/–
Mult. point stim.	–	+	+	+/–
Stat. Estimate	+	+/–	+	+
Spike-trigg. averag.	–	–	–	–
MUNIX	++	++	++	++
MScan	++	+	–	+

Absolute mean values and standard deviations of different MUNE techniques in different muscles. Summary of attributes of different selected MUNE techniques, modified from de Carvalho et al. (2018).

* *Median* (min–max)

tested muscle. The average amplitude of SMUPS is determined with a procedure specific for each method. For example, the MUNE value is calculated by dividing the CMAP amplitude (or area) by the average SMUP amplitude (or area).

It has to be emphasized that all MUNE methods make assumptions that do not necessarily reflect the real biology. The CMAP is a complex summation of all individual SMUPs (with phase cancellation) and is often not generated by single muscles alone. This is also reflected by the change of the CMAP shape and amplitude seen when the active recording electrode is moved. Further, the reference electrode also contributes to the CMAP (Nandedkar and Barkhaus, 2007). Thus, the position of the recording electrode and the reference electrode must be optimized to obtain the maximal amplitude for reliable results. This is especially true for the MUNIX method and has not been discussed for other MUNE methods. However, all stimulation methods use a certain minimum amplitude for acceptable SMUPs. The CMAP size should be maximized for all MUNE methods; if this is low due to suboptimal electrode positions, the count will be different from that with optimal electrode position.

A further assumption for MUNE is that the average size of the surface recorded SMUPs is representative for the whole muscle and muscle fiber types, which does not reflect the physiological conditions in a muscle. There is also a bias in selection of individual MUPs different for the methods, from low threshold (spike triggered method) to a range of thresholds (stimulation methods). Another problem is the restricted pick-up area of the recording electrode, which is a depth between 15 and 20 mm (Barkhaus and Nandedkar, 1994). Consequently, only the superficial part of larger muscles such as the biceps brachii is measured and used to represent the condition in the entire muscle. The recorded SMUP amplitude is also dependent on the thickness of the overlying skin and subcutaneous tissue. Consequently, none of the MUNE methods provide a true "count" of the motor unit number.

MUNE values in healthy controls exhibit a wide range of values, are age-dependent and have high inter-individual variability. Consequently, low cut-off values for pathological conditions with motor unit loss make these methods unsuitable or less diagnostically sensitive than conventional needle EMG.

The outstanding advantage of MUNE, based on its reproducibility, is the potential to quantify changes over time in diseases such as ALS, when patients are followed longitudinally and serve as their own controls. One could suppose that the simple CMAP size might give the same information. However, in slowly progressive neuronal diseases the CMAP size is dependent on reinnervation with distal sprouting of the remaining nerve fibers, which initially increases the SMUP amplitude and preserves the CMAP amplitude and muscle power, although the number of motor units is reduced. In ALS, 60–70% of motor units must be lost before muscle weakness and wasting appear (Wohlfart, 1957). Presymptomatic motor unit loss has also been shown by two MUNE techniques in ALS (Aggarwal and Nicholson, 2002; Neuwirth et al., 2017; Escorcio-Bezerra et al., 2018).

All MUNE methods have advantages and disadvantages. Some, such as spike-triggered averaging or MUNIX need active subject cooperation, which makes these methods unsuitable for use in animals, young children such as those with spinal muscular atrophy, or when substantial CNS dysfunction affects motor pathways. Other methods are limited to a few distal muscles, such as the incremental, multiple point and F-wave methods.

6.1. MUNIX

Current evidence and literature favor the Motor Unit Number Index (MUNIX) as a MUNE method (Nandedkar et al., 2004). It should be noted that MUNIX, like other MUNE methods, provides an index, which is not an estimate of the number of motor units. Although the underlying mathematical principle is not intuitive and cooperation of the subject is needed, its rapidity and minimal discomfort allow evaluation of multiple muscles in an acceptable amount of time compared to the other methods. This aspect is important, as it allows an evaluation of different regions of the body and could detect regional spread of disease on follow up examination. Growing literature reveals higher change rates in ALS for MUNIX compared to other markers of disease progression, such as functional rating scales (ALSFRS-R), manual muscle testing, or CMAPs.

MUNIX can be measured in almost all muscles where a CMAP can be elicited by supramaximal electrical nerve stimulation, and has even been evaluated in facial muscles, proximal shoulder muscles, and the upper trapezius. However, it is mainly used in the leg and arm muscles.

MUNIX is a two-step process: after measuring the highest CMAP, which is determined by repositioning the recording electrode several times over the muscle belly, 10 or more surface EMG signal epochs are recorded during isometric muscle contractions ranging from minimal to maximal. In the newer software, the MUNIX calculation is done during the recording process: the software uses the calculated area and power of the recorded surface EMG signals along with the area and power of the CMAP to create a regression curve, which provides the index value. Dividing the CMAP amplitude by the MUNIX value adds further valuable information, the Motor Unit Size Index (MUSIX), which is assigned to the average SMUP size, and which is increased in chronic neurogenic conditions.

The procedure generally takes less than 5 minutes per muscle. An example of the method is shown in Fig. 27.

In conclusion, all MUNE methods have different advantages and disadvantages. At this time the MUNIX method has the best evidence for reliability, user-friendliness and detection of change of the lower motor neuron pool. Guidelines for MUNIX have been published recently and a detailed instruction manual is available online (https://www.encals.eu/outcome-measures/) (Neuwirth et al., 2017; Nandedkar et al., 2018a).

6.2. MScan

MScanFit MUNE (MScan) is a recently developed method to demonstrate the loss of functioning MUs in a muscle (Bostock, 2016). MScan resembles Bayesian statistical MUNE (Ridall et al., 2006; Henderson et al., 2007) in fitting a model, made up of MUs with different amplitudes, thresholds, and threshold variabilities, to a detailed stimulus-response curve or CMAP scan (Blok et al., 2007). Previous evaluations of CMAP Scans focused on the number of steps, step sizes, and stimulus intensities (Blok et al., 2007). The 'D50' measurement has been used to study disease progression (Sleutjes et al., 2014). The MScan off-line analysis first generates a preliminary model from the slope and variance of the points in the scan. Then this model is refined by adjusting all the parameters to improve the fit between the original scan and scans generated by the model. Most MUNE methods are based on estimating the size of an average surface-recorded MUP and dividing that value into the maximal CMAP. This makes the estimates strongly influenced by any bias in MU selection and highly dependent on the maximal CMAP amplitude. MScan avoids bias and subjectivity in the selection of MUs by taking account of all the MUs contributing to the CMAP. Like other statistical methods, however, MScan is affected by noisy recordings.

In a recent study, MScan has shown excellent intra- and inter-rater reproducibility, both in healthy controls and in ALS patients (Jacobsen et al., 2017). It has been shown to be a sensitive objective

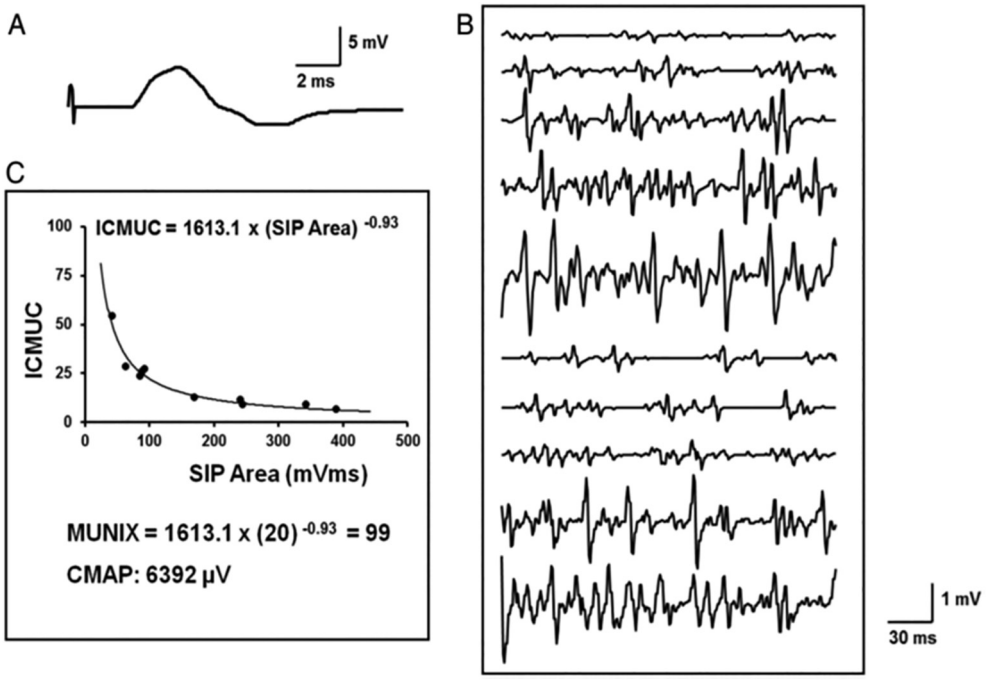


Fig. 27. MUNIX calculation in a healthy abductor pollicis brevis muscle. The CMAP is recorded with the maximal achievable amplitude (A). The surface electromyography interference pattern (SIP) is recorded while the subject gives constant isometric force at different levels from minimal to maximal (B). Area and power of each SIP are measured and used with the CMAP area and power to compute the so-called "ideal case motor unit count (ICMUC)." A regression curve of ICMUC versus SIP area is plotted (C) and the MUNIX value is derived (99 in this example) (Neuwirth et al., 2015).

tool for quantifying MU loss (Jacobsen et al., 2017, 2018a), and disease progression in ALS (Jacobsen et al., 2019). MScans from a healthy control and an ALS patient are illustrated in Fig. 28. MScan has also been used to quantify MU loss in multifocal motor neuropathy (Garg et al., 2017) and neurofibromatosis (Farschtschi et al., 2017).

Important features for a new method are its speed and availability on widely-used equipment. MScan is a quick method to perform the recordings (~6 min) and analysis (<5 min) (Jacobsen et al., 2017) and does not require patient cooperation. However, MScan recordings require up to 500 stimuli, which may be unpleasant. A current limitation of MScan is the requirement of specialized software and associated nerve excitability equipment for optimum recording and analysis. However, a freeware program is applicable to CMAP scans generated with other equipment (Li et al., 2018). Another limitation is that, like most other MUNE methods, it is not suitable for proximal muscles. So far, MScan has mostly been applied to the abductor pollicis brevis (Farschtschi et al., 2017; Garg et al., 2017; Jacobsen et al., 2017), abductor hallucis (Li et al., 2018) and Tib.Ant. muscles (Jacobsen et al., 2018b).

MUNE methods have long been of interest to quantify MU loss, but none has been implemented widely in clinics, probably due to limitations such as subjectivity, dependence on absolute CMAP amplitude, or the long time required for the examinations or analysis. MScan seems to avoid these limitations and may have the potential to be implemented in clinics for follow-up of neuromuscular disorders such as ALS.

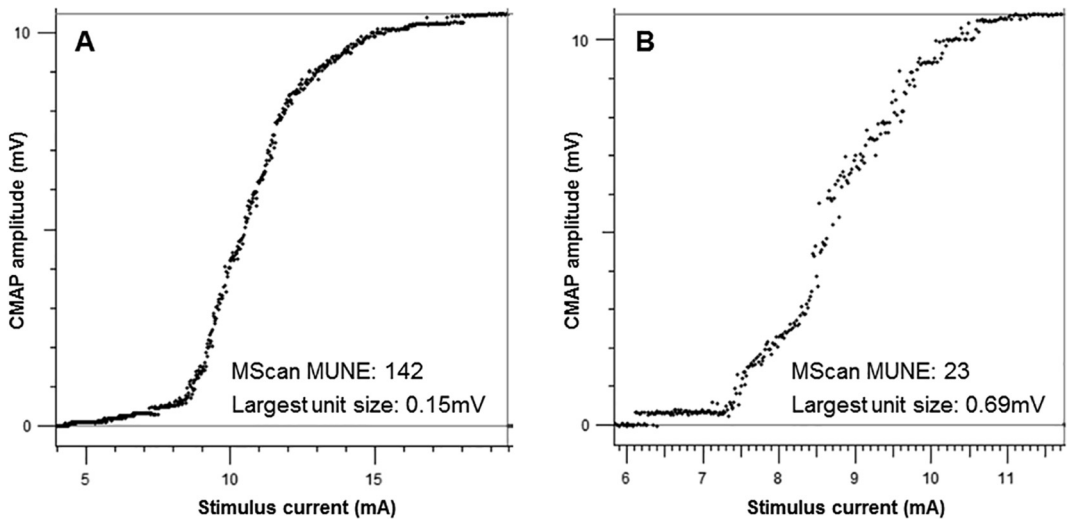


Fig. 28. Examples of MScan MUNE recordings in abductor pollicis brevis muscle from (A) a healthy subject, 803 stimuli, and (B) a patient with ALS, 351 stimuli.

7. Pediatric aspects of electrodiagnostic methods

7.1. Introduction

It is important to recognize the differences between EMG performed in children and in adults as these are crucial to its successful practice. These differences and the methods needed to accommodate them produce a distinct investigative process. This warrants Pediatric EMG being considered a specialty on its own within the broad definition of EMG (Pitt, 2017a).

The child reaches adulthood between 16 and 18 years of age. If of normal behavior a child of seven years or above can be regarded as a young adult. They will also share adult pathologies.

This section dedicated to standards in EMG and nerve conduction therefore applies to the younger subjects in the Pediatric population.

7.2. Equipment

Most commercial EMG machines are suitable for studies in children. The standard programs are applicable but must include MUP analysis, interference pattern analysis algorithms (Nandedkar et al., 1986b, 1986a), and stimulated SFEMG programs. It also must be possible to record most, if not all, of the EMG procedure, allowing retrospective analysis after the child has left the laboratory.

7.3. Electrodes

Stick-on recording electrodes are essential. Those that allow the adhesive surface to be cut down without affecting the recording surface are useful for neonates. For sensory recording, separation between recording electrodes of 3 cm is ideal. Clearly this is not attainable in infants, in whom the distance may be shorter. For motor nerve studies the active electrode is placed over the muscle endpoint and the reference at a standardized remote point, depending on the nerve being tested. Stimulating electrodes should include those with an inter-electrode difference of 1.5 cm as well as the standard 3 cm (Fig. 29). For EMG the so-called facial concentric needle electrode (French gauge 30 or 32) is used exclusively. This is also used to measure jitter, as single-use SFEMG needles are not currently available. Very rarely, and usually only in obese teenagers, is it necessary to use longer needles, which are not available in that diameter.

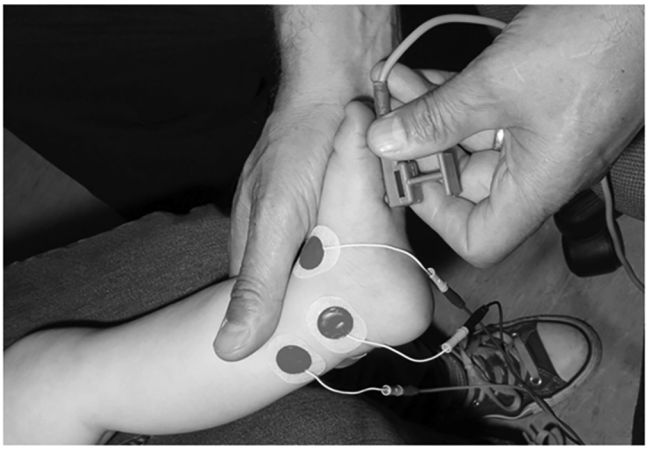


Fig. 29. Recording the left medial plantar response. Recording electrodes are single use adhesive electrodes connected to a shielded cable. Stimulation is shown using a stimulator with a 1.5 cm inter-electrode separation.

7.4. Pediatric neuromuscular disease

The pathologies encountered define methodology. The important elements in each of the important groups of conditions are discussed below.

7.4.1. Neuropathies

Mononeuropathies, with the exception of carpal tunnel syndrome in the mucopolysaccharidoses, are rarely seen in children (Haddad et al., 1997; Davis and Vedanarayanan, 2014). Autoimmune neuropathies, acute and chronic inflammatory demyelinating polyneuropathy are seen, but less commonly than in adults (Jones, 2000; Jo et al., 2010; Rossi et al., 2013; Silwal et al., 2018). Hereditary neuropathies often present late. EMG is used in obstetric brachial plexus palsy, particularly in infants who have poor recovery (Pitt and Vredeveld, 2004, 2005).

7.4.2. Myopathies

In a weak child the primary diagnostic objective is to determine if the child has a myopathy. Here the challenge is to distinguish the EMG changes from those in healthy controls. The small dimensions of muscle fibers in children and particularly in babies, make distinction between these conditions difficult. Motor unit analysis programs and interference pattern analysis are essential for success.

7.4.3. Anterior horn cell disease

Early diagnosis of motor neuronopathies has recently become vital as therapies are now available for spinal muscular atrophy (SMA) (Chiriboga et al., 2016; Finkel et al., 2016; Aartsma-Rus, 2017)and Brown-Vialetto-Van Laere syndrome (Spagnoli and De Sousa, 2012; Ciccolella et al., 2013; Spagnoli et al., 2014), and are more effective the earlier they are given.

7.4.4. Neuromuscular transmission disorders

Many children over age 8–10 can be examined using the same RNS technique as adults, with appropriate adjustments for limb size. To reduce stimulus artifacts, use stimulating electrodes with a shorter inter-electrode distance (1.5–2 cm) in infants and small children. Conscious sedation or general anesthesia is required for uncooperative children. Immobilize the tested limb in unconscious children and assure that temperature is constant in tested limb muscles throughout the procedure.

There are no well-established normative data for RNS in neonates, in whom the neuromuscular junction is immature (Churchill-Davidson and Wise, 1963; Koenigsberger et al., 1973). Decrementing responses up to 25% may be seen in healthy neonates at stimulating frequencies over 20 Hz, and increments of 10% or more are common at 5 Hz to 10 Hz (Koenigsberger et al., 1973).

The protean manifestations of myasthenic conditions, particularly the congenital forms, mean that they may enter the differential diagnosis of many clinical presentations (Kinali et al., 2008; Finlayson et al., 2013; Rodriguez Cruz et al., 2014; Engel, 2018). A screening test that can be used in all ages is essential. The stimulated potential analysis using concentric needle electrodes (SPACE) technique fulfils all of the necessary criteria for such a test (Pitt, 2017a, 2017b). It is applicable without general anesthesia, can be performed in any child, takes a short time to perform and has high sensitivity and negative predictive values. Its specificity is elevated to acceptable levels if the examination includes a general EMG and nerve conduction analysis. The technique is further discussed in a separate IFCN guidelines document on SFEMG.

7.4.5. General considerations in testing children

The luxury of examining multiple nerves and multiple muscles is not available in children. Every action undertaken is driven by

the need to achieve the diagnosis in the fewest number of steps. In many instances examination of one sensory nerve and one motor nerve in the leg followed by EMG of Tib.Ant. is sufficient, certainly to rule out significant pathology. Tib. Ant.is easy to activate unlike many other muscles. The EMG, while restricted to one muscle, must be thorough. It is not uncommon for sampling to exceed three minutes and sometimes longer. Sufficient must be done to allow MUP analysis. Any abnormalities encountered in this preliminary investigation will be supplemented by other tests. The entire examination may be over in less than 15 minutes. The discomfort, when using this strategy, is little more than that of venipuncture and sedation is rarely needed (Alshaikh et al., 2016). Repeat investigation is an option when the patient has a positive experience.

When performing the EMG it is important to respond to genuine discomfort rather than responding to some children's nonspecific reaction to any intervention. This may be enhanced by hunger, lack of sleep, or concomitant conditions such as teething. They also may be sub-consciously picking up anxieties in the parents, particularly the mother. Time spent engaging with the parents before starting is crucial. In this discussion it is important to emphasize the reason for the examination and its importance in guiding further investigation. The parents are told that it is uncomfortable on a par with venipuncture. The short duration of the examination is emphasized.

Parents are forewarned that no results will be given at the end of the examination. This is for two reasons. The first is that only the referring clinician can incorporate the EMG findings into their investigative strategy. The EMG practitioner is not party to that and may give confusing information to the parents. Secondly, even for experienced practitioners the interpretation of the results can be very difficult. It requires detailed examination with quantification techniques, which may not be conclusive because of difficulties in getting clean data in children. Conclusions may require fine judgment, which should not be rushed.

It is important to store EMG data for later examination by MUP and interference pattern programs. It is more important to collect adequate data from one muscle than to get inadequate data from many muscles.

Finally, it is crucial to distract the child, and often the parents. Find what the children love and talk about that. If younger, electronic devices and games achieve the same objective.

7.4.5.1. Sedation. The rules of each institution or the EMG examination requested may force the practitioner to consider some form of sedation. The reason this is considered is because of the pain of EMG. Sedation, by whatever means, makes the child sleepy. Any anesthetic effect is only achieved when a deep level of sedation is reached. This is dangerous outside of an operating theatre or recovery room. A far more logical response to this demand is to give the children effective analgesia. Some institutions prescribe oral morphine, which is given to the child by the parents just before the examination.

The use of local anesthetic cream over the site of the needle insertion is a matter of personal choice. Placebo with reinforcement has been shown to be as effective (Raveh et al., 1995). If the needle is disguised from view many children never feel the insertion. Most of the pain experienced is from deep within the muscle.

General anesthesia, restricting the examination to nerve conduction and SPACE only, should only be resorted to when the latter is not possible because of the risks of injury - it has no other place.

7.4.6. Concluding remarks, Pediatric EDX

Pediatric EMG conducted in a humane, rapid and focused manner is an essential tool in the examination of neuromuscular disease. Often, important information can be obtained within minutes, avoiding the need for other more invasive investigations such as muscle biopsy and MRI, which often need general anesthesia. Clinicians should feel free to use it at the slightest suspicion of an underlying neuromuscular condition. Used sparingly, there is an impression that while diagnoses are reached eventually, these may be delayed by many years.

8. Reference values

8.1. Reference values in EMG

In clinical work the measured values are compared with reference values to determine whether the measurement is normal or not. Reference values have been collected from healthy subjects and published for the parameters of all neurophysiological techniques used in the clinical routine. Statistical methods using data obtained during the clinical routine that include patients to extract reference values have been published recently. The E-norm (Jabre et al., 2015) and e-Ref (Nandedkar et al., 2018b) are possible ways not only to develop local reference values, but also to compare local values with published reference values. The methods are based on the assumption that a significant number of the subjects studied in the clinic are normal. The advantage of these is that reference values can quickly be extracted without the extra effort of studying healthy subjects separately.

Multifactorial analysis is not easily obtained with these techniques.

8.1.1. Reference limits

The simple approach is to use reference limits that are either the 95 %–97.5% confidence limits or the mean ± 2 standard deviations. However, many of the parameters measured are influenced by multiple independent variables (age, gender, height and temperature), which cannot easily be taken into consideration with simple reference limits.

8.1.2. Regression models

Reference values of parameters that are influenced by multiple independent variables (age, gender, height and temperature) can be dealt with by using multiple linear regression models. These models assume that the relationship between the independent value and the dependent value is linear. Linear regression equations have the general format:

y = constant + x1*age + x2*height + x3*temperature.

This equation can be used to calculate the *expected value* for a given subject. The *measured results* are compared with the expected values and the difference between them is used to calculate the Z-score.

Z = (measured value-expected value)/SD,

The Z score is the number of SDs between the measured value and the expected values (Falck et al., 1991). For instance, a measured value with Z score of – 2 has a probability of 0.023 of falling within the reference population. Most doctors are accustomed to using SD in statistics and the interpretation of Z scores is very similar to that.

The Z score may be used to follow a parameter over time. It is not useful to compare parameters or to compare results obtained with different reference materials since it is dependent on the distribution of the reference values.

8.2. Reference values for MUP analysis and SFEMG

SFEMG jitter and fiber density reference values and reference values for MUP analysis have been collected and published by large

international study groups (Gilchrist et al., 1992; Bischoff et al., 1994; Stålberg et al., 2016). Many of the published reference values can be used by other laboratories if the measurements are made exactly the same way as in the reference material. It is important to ascertain that the analysis algorithms for measurements are also identical, which is the case for SFEMG. For other methods, there are no international standards for analysis algorithms used in commercial EMG equipment. For some tests, such as neurography, differences in algorithms do not significantly affect the measurements. On the other hand, in MUP analysis, the results of duration measurements depend greatly on the analysis algorithm (Stålberg et al., 1996), thus reference values obtained with one brand of equipment cannot be used in another. When using reference values from other laboratories, it is recommended that the reference values be tested on a small sample of healthy subjects before using them in the clinic.

Reference data for mean values and outliers (Stålberg et al., 1994) for several muscles (Bischoff et al., 1994; Tomasella et al., 2002; Podnar, 2008) have been published for each of the EMG techniques presented. Each muscle has its own MUP characteristics (Fig. 4). In some laboratories, automatic quantification is performed in almost all muscles under examination, in all patients. In other laboratories, quantification is performed in case of doubtful findings. Most laboratories still rely only on subjective assessment of the EMG.

8.3. Reference values in neurography

Many reviews and manuals for nerve conduction studies with description of methods for individual nerves and reference values have been published (Stålberg and Falck, 1993; Buschbacher, 1998; Oh, 2002; Kumbhare et al., 2015).

8.4. Reference values in Pediatric EDX

The problem of obtaining normative data is particularly relevant to children where ethical boards will not allow studies to be done in healthy control children. Pediatric neurography reference values have also been published (Gamstorp, 1963; Puksa et al., 2011; Pitt, 2012).

The recent development of the E-norms methodology allowing one to extract normative values from clinical data has been valuable in obtaining normative values in children (Jabre et al., 2015; Pitt and Jabre, 2017; Nandedkar et al., 2018b). Without standardization in the collection of these data, the ability to transport normative ranges across laboratories, or even within laboratories, remains elusive (Pitt and Jabre, 2018). A weak point is that multifactorial analysis is not easily achieved, which is particularly important in Pediatric EDX (e.g. age, height).

Declaration of Competing Interest

Dr. Rutkove has equity in, and serves a consultant and scientific advisor to, Myolex, Inc. a company that designs impedance devices; he is also a member of the company's Board of Directors; he also holds several patents in the field of electrical impedance.

All authors have approved the final article.

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