ELECTRODIAGNOSTIC APPROACH TO NEUROMUSCULAR JUNCTION TESTING

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Repetitive nerve stimulation (RNS) testing

RNS depletes immediate acetylcholine (ACh) stores with low stimulation frequencies to unmask the reduced safety factor common to all NMJ disorders. The reduced number of end plate potentials (EPP) that successfully elicit muscle fiber action potentials (MFAPs) is reflected by a corresponding decrement of compound muscle action potential (CMAP) amplitudes. In presynaptic disorders, presynaptic calcium concentration is augmented by high stimulation frequencies or following brief exercise. This results in increased EPP amplitudes that may unblock MFAPs with a corresponding increase in CMAP amplitude or facilitation.

In RNS testing, a motor or mixed nerve is stimulated supramaximally (10-25% above the level needed to activate all muscle fibers) and surface CMAPs are measured with a recording electrode placed over the corresponding muscle belly with a reference over the distal tendon. The negative peak amplitude of each CMAP represents the summation of MFAPs responding to motor nerve stimulation and serves as an index of successful neuromuscular transmission. Reduced limb temperature may augment NMT and render RNS testing less sensitive. Muscles should be warmed to 34-36°C, and this temperature should be maintained throughout RNS testing. Proximal limb, shoulder, and craniobulbar muscles do not require warming.

Stimulation.
Nerve stimulation is typically performed with surface stimulating electrodes. Low-frequency RNS at stimulation rates of 2-3 Hz are best to elicit decremental responses in MG.[1] In low-frequency RNS for MG, no more than 5-10 stimuli are needed to demonstrate significant decrement. High-frequency stimulation (> 5 Hz) should be avoided due to pseudofacilitation where CMAP negative peak amplitude increases and duration decreases with no net change in CMAP negative peak area.

High-frequency (greater than 10 Hz) RNS should be reserved for evaluation of suspected presynaptic NMT disorders in patients who are unable to perform brief isometric exercise as in infants, adults with attenuated consciousness, or with severe paralysis related to possible botulism or LEMS. In such patients, the optimal frequency is 20-50 Hz for two to ten seconds. In other situations, ten seconds of MVC represents an equivalent to high-frequency RNS, and it is far more comfortable for patients.

Immobilization.
Repeated movement in RNS testing may induce recording artifacts and contaminate the findings. Securing the stimulating and recording electrodes and immobilizing the relevant joint is essential. Artifact owing to electrode movement or to limb movement appear as an abrupt change in CMAP waveform during a train of stimuli. Movement of the stimulating electrodes may result in submaximal nerve stimulation and generate pseudo-decrement.[2]

Activation methods.
Activation methods such as exercise may increase the sensitivity of RNS testing. Isometric exercise with maximum voluntary effort against an examiner's resistance may elicit post-activation or post-exercise exhaustion (PAE) with increased CMAP amplitude decrement at two to five minutes following exercise periods of 30-60 seconds. In patients with low amplitude CMAPs or decremental responses at baseline, post-activation or post-exercise facilitation (PAF) may unblock MFAPs following ten seconds of exercise. PAF may be quite marked in presynaptic NMT disorders, and generally exceeds 100% in LEMS.

Data analysis.
Decrement represents the percentage of amplitude reduction between the initial CMAP in a train of nerve stimulation ($CMAP_1$) and the lowest amplitude CMAP generated ($CMAP_n$), and is computed from the formula: % $Decrement_n = [(CMAP_n - CMAP_1)/CMAP_1] \times 100\%$. With appropriate technique and quality control, the fourth or

fifth CMAP amplitude should represent the lowest CMAP amplitude reflecting depletion of immediate ACh stores. Mobilization ACh stores become available after the fourth or fifth stimulation, and CMAP amplitudes increase slightly giving rise to a characteristic saddle- or U-shaped series of CMAP waveforms. Decrement is typically calculated based on the amplitude of the first CMAP and the fourth or fifth CMAP in a 2-3 Hz RNS train. Greater than 10% decrement is often considered abnormal.

Facilitation represents the percentage of amplitude increase and is calculated from the formula: % Facilitation_n = $[(\text{CMAP}_n - \text{CMAP}_1)/\text{CMAP}_1] \times 100\%$, where CMAP_n is the highest CMAP amplitude generated by a train of nerve stimulation. When high-frequency RNS testing must be performed, it is important to assess for pseudofacilitation by comparing the amplitude facilitation with area facilitation. These values should be comparable in the absence of pseudofacilitation. Whenever possible, facilitation is best assessed following ten seconds of maximum voluntary isometric contraction (MVC) of the tested muscle to avoid patient discomfort and pseudofacilitation associated with high-frequency RNS. The percentage of facilitation following MVC is calculated from the same formula: % Facilitation = $[(\text{CMAP}_{\text{Post}} - \text{CMAP}_{\text{Pre}})/ \text{CMAP}_{\text{Pre}}] \times 100\%$, where $\text{CMAP}_{\text{Post}}$ is the first post-exercise CMAP elicited after the 10 second exercise period, and CMAP_{Pre} is the first CMAP in the baseline, pre-exercise RNS train.

Quality control issues.
Quality control to eliminate technical error is essential when performing RNS testing. Each CMAP waveform train should be inspected. The baseline should be stable; a shifted baseline suggests movement artifact involving the recording electrodes and/or leads. Sudden changes in CMAP amplitude and/or morphology within trains suggest understimulation related to movement of the stimulating electrodes. Inadequate muscle warming renders RNS testing insensitive and may eliminate abnormal findings. Muscle shortening due to poor immobilization, patient discomfort, or high frequency RNS testing may elicit pseudofacilitation. In MG, a regular, orderly decline in CMAP amplitude is expected over the first four stimuli with an increase in CMAP amplitude after the fourth or fifth stimulus. Findings should be reproducible after appropriate rest periods.

Assessment of neuromuscular jitter

Single-fiber electromyography (SFEMG) is a sensitive technique for evaluating neuromuscular transmission. SFEMG facilitates recording of individual MFAPs within the same motor unit. The ability to selectively record individual MFAPs is achieved by the use of the SFEMG electrode: a specialized concentric needle electrode with a 25 µm recording surface on a side port about 3 mm from the needle tip. By comparison, the recording surface of a standard concentric needle is about 120 µm in diameter. A low frequency filter setting of 500 Hz rejects low frequency signals that originate in distant muscle fibers and increases the selectivity of the technique[10]

Nearly all of the temporal variability between firing of MFAPs innervated by the same motor neuron is related to neuromuscular transmission. This temporal variability, or neuromuscular jitter, reflects the fluctuations in the time needed for EPPs to reach threshold to generate MFAPs. When processes affecting the NMJ reduce the safety factor, some EPPs take longer to reach threshold, MFAP generation is delayed, and jitter correspondingly increases. When EPPs fail to reach threshold following a nerve impulse, blocking occurs. Clinical weakness in a muscle occurs when a critical number of endplates are blocked. Along with jitter, SFEMG may be used to demonstrate fiber density (FD), the concentration of MFAPs within the small recording field of the single-fiber (SF) electrode. FD may be increased with reinnervation and in some myopathies.

Voluntary-activated SFEMG.
Jitter is best assessed with voluntary muscle activation in cooperative patients. In this technique, the recording needle electrode is inserted in a muscle and positioned to record from a pair of two muscle fibers innervated by the same motor neuron as the patient minimally contracts the muscle. The oscilloscope is triggered by a MFAP, and that single MFAP is displayed in a fixed position on the oscilloscope screen. MFAPs from other muscle fibers innervated by the same motor neuron are relatively time-locked to the triggering potential, but exhibit some temporal variability or jitter with respect to the triggering potential. MFAPs that have a rise time less than 300 µs and amplitude greater than 200 µV are suitable for jitter analysis, and a minimum of 50 discharges of the triggering potential are recorded. An increased degree of jitter is seen when neuromuscular transmission is abnormal, and when the abnormality is severe, impulse blocking may also be seen. Blocking must occur in order for RNS to elicit a decrement in a train of CMAPs. Blocking is only seen with jitter values greater than 100 µs.[3] The normal amount of jitter varies with age and between muscles.[4]

Stimulated SFEMG.
In patients unable to cooperate with voluntary muscle activation procedures such as young children, patients with severe tremor, or patients with disturbed consciousness, stimulated SFEMG may facilitate jitter assessment. In this technique, axonal stimulation of an intramuscular motor nerve branch (e.g. posterior interosseous nerve branch within the extensor digitorum communis muscle) or of a motor nerve proximal to a muscle (e.g. facial nerve) is used to trigger the oscilloscope.[5] Jitter is then measured between the stimulus and a single MFAP. A monopolar stimulating needle is placed just proximal to the endplate zone for intramuscular stimulation and just anterior to the tragus for facial nerve stimulation. A surface or second monopolar electrode serves as the anode. The nerve is stimulated between 2 and 10 Hz to elicit subtle twitches of the muscle, and the SFEMG needle is inserted for recording. Liminal or direct stimulation of muscle fibers and electrode movement related to muscle twitching are technical pitfalls, particularly with the intramuscular stimulating technique.[6] Stimulated SFEMG is therefore only recommended for experienced electromyographers, as it is subject to fewer technical issues compared to the voluntary activation technique.

Jitter calculation. Jitter is calculated as the mean difference between consecutive interpotential intervals (IPI) or MCD:

$$MCD = \frac{\left|IPI_1 - IPI_2\right| + \ldots \left|IPI_{n-1} - IPI_n\right|}{(n-1)}$$

For stimulated SFEMG, the mean difference between the stimulus and the single MFAP responses is calculated.

Influence of firing rate.
With voluntary activation, differences in firing rates of MFAPs may influence the IPI variability due to changes in MFAP propagation. This influence can be minimized by sorting the IPI by the interdischarge interval (IDI), and then calculating the mean sorted-data difference between interpotential intervals (MSD). MCD is normally reported for each pair of potentials, but when slow trends such as variable firing rate influence IPI and the MCD:MSD ratio is greater than 1.25, the MSD should be reported.[6] Fiber pairs with very long IPIs greater than 4 msec should not be analyzed, as MFAP propagation significantly influences IPI in such fiber pairs. In stimulated SFEMG, MFAP propagation issues are avoided by using a constant stimulation rate and by excluding the initial ten stimulated MFAP responses from analysis.[3,5]

Data collection and reporting.
At least twenty different potential pairs are assessed from different areas of each muscle. This requires about three separate needle insertions in many cases. Results are reported as the mean jitter (MCD) for all fiber pairs tested, the percentage of pairs with normal jitter, the percentage of pairs with abnormal jitter, and the percentage of pairs with impulse blocking. The SFEMG study is abnormal when greater than 10% of fiber pairs (three or more pairs) exhibit increased jitter for the muscle studied, or when the mean jitter exceeds the upper limit of normal in patients less than age 60 years. Any impulse blocking is abnormal. Abnormally low jitter (less than 5 μs) may be seen in myopathy with fiber splitting. Such data should be excluded from analysis, as it does not reflect actual neuromuscular transmission. Jitter reference values have been determined for both the voluntary activation technique and for axonal stimulation.[4,7,8]

Jitter measurements with concentric needle electrodes.
The use of concentric needle electrodes to isolate MFAPs from recorded motor unit potentials (MUPs) has become popular in light of sterilization and maintenance issues related to true SFEMG electrodes. These recordings may be performed by increasing the low frequency filter to 1kHz and using the smallest concentric needle available to minimize the size of the recording surface.[9] Because the concentric needle recording surface remains much larger than the SF electrode recording surface, it cannot be determined whether a single spike recorded with this technique represents a single MFAP or a composite of several MFAPs. These composite spikes are therefore referred to as apparent single fiber action potentials (ASFAPs).[10,11] ASFAPs are lower in amplitude than MFAPs due to the effect of the additional low frequency filtering. Jitter analysis with the concentric needle technique can demonstrate abnormal neuromuscular transmission, but mild increases in jitter are best demonstrated with SF electrodes due to the effects of superimposed ASFAPs. Jitter measured with concentric needles is about 5μs lower than with SF electrodes, and reference values derived for concentric needle electrodes should therefore be used.[10]

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