Electrophysiological Assessment of the Neuromuscular Junction

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Outline

1) Normal function and dysfunction of the neuromuscular junction

2) Repetitive nerve stimulation:
   - Technical aspects
   - Post-synaptic assessment (MG)
   - Pre-synaptic assessment (LEMS)

3) Single Fiber Electromyography (intro)
   - Basics
   - Concentric SFEMG (CN-SFEMG)

4) To review additional emerging electrophysiological and imaging test for assessment of MG/NMJ disorders:
   - Ocular evoked myogenic potentials (VEMP)
   - Stimulated Potential Analysis with Concentric needle Electrodes (SPACE)
Assessment of NMJ Transmission

• Clinical assessment, response to treatment and electrodiagnostic methods preceded use of antibodies for diagnosis in MG by nearly a century

• In spite of new antibody discoveries (LRP4, MuSK) and availability of high-affinity cell based assays:
  ○ a proportion of seronegative patients remains
  ○ relevance of antibodies to clinical syndromes and presentations is important for the clinician to establish

• Electrophysiology remains a critical part of identifying congenital myasthenic syndromes
Assessment of the NMJ: Past, present and future

- 1895: RNS by Jolly
- 1960: Continued refinement of techniques
- Use as biomarkers?
- 2019: SFEMG by Stalberg and Eskedt
- VEMP
- SPACE
- Oculography?
Healthy Neuromuscular Transmission : Channels

- Presynaptic nerve terminal responsible for releasing acetylcholine in vesicles

- Requires calcium to facilitate release:
  - Calcium stores
  - Entry of calcium through \( \text{VGCC} \)

- Fast-inactivating \( \text{K}^+ \) channel serves to restore charge balance after Ca influx

- Post-synaptic muscle membrane depends on ligand (acetylcholine) gated sodium channel to generate END PLATE POTENTIAL → engage in contraction of myofibers

Healthy Neuromuscular Transmission: Acetylcholine

- Acetylcholine packaged and released in vesicles (quanta)
  - Immediate release store in nerve terminal (~1000 quanta)
  - Mobilization store (10,000 quanta in the nerve cytosol → transport to terminal)
  - Main reserves (>10,000 quanta in nerve cytosol being recycled & re-synthesized)

- Generation of end-plate potential
  - Depends on ACH release X AChR’s available
  - RESERVE aka safety margin (3-fold) exists to ensure adequate NMJ transmission
  - LOSS of reserve through less ACh or AChR leads to fatigability

**Presynaptic NMJ Pathophysiology (LEMS)**

- Release of acetylcholine from pre-synaptic vesicles depends on presence of calcium and voltage gated calcium channels for entry

- Antibody to VGCC in LEMS prevents Ca influx and prevents Ach efflux → weakness

- Prevention of K+ efflux by targeting VGKC with 3,4 DAP can help maximize Ach release (partial)

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FATIGABILITY

Limb (axial)

Neck

Bulbar (chewing, swallowing, speech)

Resp / sleep

Ocular (diplopia / ptosis)

Decremental response
Technical Aspects of RNS

- Machine set-up
- Basic principles
- Number of stimuli needed
- Artifactual decrement
- Stimulus frequency
- Effects of temperature
- Post-tetanic facilitation
- Post-exercise exhaustion
- Selection of muscles for study
- Electrode placement and stability
- Pre-synaptic testing
- When should one perform RNS
RNS : Machine Set-up

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (mV/div)</td>
<td>2-5</td>
</tr>
<tr>
<td>Sweep time (ms/div)</td>
<td>2</td>
</tr>
<tr>
<td>Low filter (Hz)</td>
<td>2-5</td>
</tr>
<tr>
<td>High Filter (KHz)</td>
<td>2-3</td>
</tr>
<tr>
<td>Stimulus duration (msec)</td>
<td>0.1</td>
</tr>
<tr>
<td>Stimulus rate - low</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Stimulus rate - high</td>
<td>30-50</td>
</tr>
</tbody>
</table>

- Machine set-up similar to routine nerve conduction studies for filters
  - Prolonged sweep and recording in order to capture all the stimuli
- Sensitivity depends on the amplitude of the muscle tested
- Stimuli generally last 0.1 ms
- Stimulus rate depends on what aspect of the NMJ is being evaluated
Principles

• In healthy NMJ transmission, responses should be stable with repeated stimuli due to safety margin.

• Decreased reserve (MG) leads to reduction in amplitude/area of responses.

• Greater than 10% decrement in amplitude or area considered abnormal.
Where does the maximal decrement occur? How many stimuli are needed?

Largest decrement occurs between 1st and 2nd stimulus, less decrement in subsequent stimuli.

Partial repair of amplitude/area occurs after 4th stimulus (U-shaped decrement).

Most common number of stimuli = 5.

Repeating RNS testing in a distal limb when using a cuff can increase yield (not used routinely).
Artifactual Decrement

• When largest decrement occurs out of order, or variable decrement occurs, concern is for technical reasons for result

• Since recurrent stimuli cause movement which can affect subsequent stimuli, movement artifact is most common cause of false decrement:
  • On recording electrode
  • On stimulator
Effects of Stimulation Rates and Temperature
and post-activation exhaustion

- Release of acetylcholine from the mobilization stores in nerve cytosol can partially repair decrement in NMJ

- Activation of muscle under study (10 seconds) can stimulate release of mobilization store of Ach and either repair decrement in MG or increase amplitude in normal or pathological conditions

- 1 minute of exercise is ideal to elicit post-exercise exhaustion
  - Can be identified immediately or up to 3 minutes before repair
  - Testing at baseline, immediate and 1-5 minutes after maximal exercise can be used as testing paradigm
Increment on Repetitive Nerve Stimulation

Botulism

Lambert Eaton Myasthenic Syndrome
NCS

- Given that 30-50 Hz to mobilize Ach stores is painful, can be replaced by maximal activation of muscle in study

- Recurrent stimuli not needed, single stimulation before and after maximal activation (10 seconds) needed

- Greater than 100% increment in amplitude significant for pre-synaptic dysfunction
When should one consider RNS testing?

• Most commonly for assessment of post-synaptic dysfunction myasthenia gravis, with fatigable:
  o ptosis
  o diplopia
  o limb weakness
  o restrictive pattern respiratory dysfunction NYD
  o bulbar dysfunction

• Assessment of pre-synaptic dysfunction (50 Hz / increment on NCS)
  o Botulism
  o LEMS
  o Along these lines, combination of low amplitude NCS and weakness \(\rightarrow\) RNS + increment on RNS testing
Selection of Muscles for RNS

- **Most effective**: TESTING OF CLINICALLY WEAK MUSCLE MOST EFFECTIVE
- **Facial**: nasalis, accessory → trapezius, ulnar → hypothenar
- **Other**: median → APB, axillary → deltoid, MSK → biceps, femoral → quads, peroneal → EDB/TA, phrenic → diaphragm

- **Ocular**: Frontalis / Nasalis
- **Bulbar**: Trapezius
- **Neck**: Limb (ulnar, peroneal / weak muscle)
- **Limbs**
Think of increased jitter as “increased standard deviation away from the mean”

\[
MCD = \frac{(IPI_1 - IPI_2) + (IPI_2 - IPI_3) + \cdots + (IPI_n - IPI_1)}{n - 1}
\]

\[
s = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}
\]
Single Fiber EMG - Electrodes

- Single fiber EMG needle consists of a 0.5-0.6 mm stainless steel cannula with a 25 micrometer fine platinum wire inside its hollow shaft.

- In a side port 3 mm behind its tip, the cut end of the platinum wire is exposed.

- Ensures a very fine pick-up range for analysis of electrical activity between muscle fiber pairs.
SFEMG: Technical Aspects

• Muscle fibers separated by 300 µm, diameter ranging from 20-60 µm in diameter, respectively

• Selectivity of the recording is strengthened by adjusting the filter settings. (Low frequencies filter 500 HZ – high frequencies filter 10 KHZ - abolishes low frequency components from distant muscle fibers).

• Action potentials should be greater than **200 microvolts in amplitude** and **rise time should be less than 300 microseconds**. Normal values apply if the inter-spike interval is up to **four milliseconds**.

• 20 potential pairs are collected from the same muscle by two to four insertions. Abnormalities usually will be seen by pair 16 if they are to occur

• The subject is asked to maintain a steady contraction if volitional SFEMG is undertaken, until 100 consecutive discharges are recorded from each pair.
Single Fiber EMG: Measurements

- Analysis of interpotential difference variability can give an indication of the stability of the neuromuscular junction.
- The NMJ will intrinsically have inefficiency in transmission, leading to “normal” interpotential instability, or “jitter”.
- In pathological states, the jitter is increased due to an inefficiency and impairment in the conduction of a single electrical impulse between 2 adjacent muscle pairs.
- When jitter is increased to the point that it is no longer recordable electrically.

Selvan et al. Ann Ind Acad Neurol 2011;14:64-7.
Single Fiber EMG: Video

2019

SFEMG : What Constitutes an Abnormal Test?

- There is intrinsic variability (jitter) in neuromuscular junction stability, and a greater mean of jitter different muscle normal values considered abnormal (20 pairs collected)
  - Elevated mean jitter
  - >10% abnormal pairs (/20)
  - Any “blocking” pair (when impulses fail to produce an action potential)

- High sensitivity for detection of neuromuscular junction defect if weak muscles tested

- SFEMG may not normalize completely in the setting of MG remission.

- Ask about Botox use as this can affect neuromuscular junction

- In MG, anticholinesterase inhibitors may influence the results of the test, as such it is recommended to discontinue anticholinesterase inhibitors the day of testing with SFEMG (<12 hrs)
Single Fiber EMG Sensitivity vs Other ED Tests

### Table 1

Reference values for Jitter measurement in healthy subjects during voluntary muscle activity. MCD / 95% confidence limits for MCD values of nerve conduction velocities.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>10yr</th>
<th>20yr</th>
<th>30yr</th>
<th>40yr</th>
<th>50yr</th>
<th>60yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontalis</td>
<td>33.6/49.7</td>
<td>33.9/50.1</td>
<td>34.4/51.3</td>
<td>35.5/53.5</td>
<td>37.3/57.5</td>
<td>40.0/63.9</td>
</tr>
<tr>
<td>Obicularis oculi</td>
<td>39.8/54.6</td>
<td>39.8/54.7</td>
<td>40.0/54.7</td>
<td>40.4/54.8</td>
<td>40.9/55.0</td>
<td>41.8/55.1</td>
</tr>
<tr>
<td>Obicularis oris</td>
<td>34.7/52.5</td>
<td>34.7/52.7</td>
<td>34.9/53.2</td>
<td>35.3/54.1</td>
<td>36.0/55.7</td>
<td>37.0/58.1</td>
</tr>
<tr>
<td>Tongue</td>
<td>32.8/48.6</td>
<td>33.0/49.0</td>
<td>33.6/50.2</td>
<td>34.8/52.5</td>
<td>36.8/56.3</td>
<td>39.8/62.0</td>
</tr>
<tr>
<td>Stern Cleido mas</td>
<td>29.1/45.4</td>
<td>29.3/45.8</td>
<td>29.8/46.8</td>
<td>30.8/48.8</td>
<td>32.5/52.4</td>
<td>34.9/58.1</td>
</tr>
<tr>
<td>Deltoid</td>
<td>32.9/44.4</td>
<td>32.9/44.5</td>
<td>32.9/44.5</td>
<td>32.9/44.6</td>
<td>33.0/44.8</td>
<td>33.0/45.1</td>
</tr>
<tr>
<td>Biceps</td>
<td>29.5/45.2</td>
<td>29.6/45.2</td>
<td>29.6/45.4</td>
<td>29.8/45.7</td>
<td>30.1/46.2</td>
<td>30.5/46.8</td>
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<tr>
<td>Ext dig comm</td>
<td>34.9/50.0</td>
<td>34.9/50.1</td>
<td>35.1/50.5</td>
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<td>36.6/54.4</td>
</tr>
<tr>
<td>Abd digit V</td>
<td>44.1/63.5</td>
<td>44.7/64.0</td>
<td>45.2/65.5</td>
<td>46.4/68.6</td>
<td>48.2/73.9</td>
<td>51.0/82.7</td>
</tr>
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Electrophysiology in Diagnosis of MG

RNS and SFEMG in Mild, Moderate and Severe Ocular MG

<table>
<thead>
<tr>
<th>Degree of Test Abnormality</th>
<th>SFEMG Results</th>
<th>RNS Results (Percent Decrement)</th>
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<tbody>
<tr>
<td>Mild</td>
<td>Normal mean jitter and &gt;10% abnormal pairs</td>
<td>11-15</td>
</tr>
<tr>
<td>Moderate</td>
<td>Mean jitter elevated ≥2 × ULN and &gt;10% abnormal pairs</td>
<td>16-25</td>
</tr>
<tr>
<td>Severe</td>
<td>Mean jitter elevated ≥2 × ULN and &gt;60% abnormal pairs and/or study incomplete as all pairs abnormal</td>
<td>≥26%</td>
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• Testing 2 facial muscles with RNS increases yield in patients with high suspicion of OMG and although 10% usually used, 7% can be used if all technical parameters adequate

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<tr>
<td>Mild</td>
<td>93% sensitivity</td>
<td>11–15</td>
</tr>
<tr>
<td>Moderate</td>
<td>97% sensitivity</td>
<td>16–25</td>
</tr>
<tr>
<td>Severe</td>
<td>99% sensitivity</td>
<td>≥ 26%</td>
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• Testing 2 facial muscles with RNS increases yield in patients with high suspicion of OMG and although 10% usually used, 7% can be used if all technical parameters adequate


Repetitive facial nerve stimulation in myasthenia gravis 1 min after muscle activation is inferior to testing a second muscle at rest

Alon Abraham a, Majed Alabdali b, Abdulla Alsulaiman b, Ari Breiner a, Carolina Barnett a, Hans D. Katzberg a, Vera Bril a*

SFEMG : Limited Specificity

• Any disruption of the nerve, muscle OR neuromuscular junction may cause abnormal jitter

• As such, conditions where an inefficient conduction of electrical impulses occurs between muscle fibers (ie motor neuron disease / myositis) may create significantly abnormal jitter

• Most prominent jitter abnormalities seen in neuromuscular junction processes as opposed to most neuropathies or myopathies
How does SFEMG perform as a biomarker?

Minimum Clinical Important Difference cut-off for a minimal clinically significant SFEMG jitter values:

14.2 μs or 16% change from mean


Concentric Needle SFEMG (CN-SFEMG)

• Due to the cost of needles, they are often reused, and as such other alternatives are sought out to use cheaper disposable needles.

• Use of concentric needles EMG cannot reach the level of comparing individual muscle fiber pairs, as such, ESTIMATES must be used by changing filters to calculate “jiggle”.

• Positive and negative predictive values of CN-SFEMG are 0.93 and 0.76:
  • CN-SFEMG showing abnormal jiggle is extremely useful for confirming the diagnosis of MG
  • CN-SFEMG showing normal jiggle has limited utility in excluding the diagnosis.
Reference values for jitter recorded by concentric needle electrodes in healthy controls: A multicenter study

- Recordings from 59 to 92 subjects were obtained for each muscle and activation type.

- Operators worldwide contributed recordings from orbicularis oculi (OO), frontalis (FR), and extensor digitorum (ED) muscles in healthy controls.

- Criteria for acceptable signal quality were agreed upon in advance. Fifteen or 20 recordings of acceptable quality from each muscle were required for voluntary and electrical stimulation recordings, respectively.

- Outlier limits for mean consecutive difference and individual jitter data for voluntary activation were: OO, 31 and 45 µs; FR, 28 and 38 µs; ED, 30 and 43 µs; and for electrical stimulation they were: OO, 27 and 36 µs; FR, 21 and 28 µs; ED, 24 and 35 µs

Stimulation potential < 1 mA; orbicularis oculi used most commonly

After an initial stimulation with single shocks, trains of stimuli are given at 10 Hz, and the potentials recorded (20 stimuli suffice).

When is stimulated single fiber EMG useful:
- Unconscious patients
- Uncooperative patients
- Patients with tremor
- Children/babies

A new test for ocular myasthenia gravis?
OMG!

Ocular vestibular evoked myogenic potentials as a test for myasthenia gravis

Ocular Vestibular Evoked Myogenic Potentials (VEMP) in MG

Bilateral oVEMP decrement shows 100% specificity for MG vs HC with reduced sensitivity of 63%. Sensitivity similar OMG AND GMG.


20 Hz RNS yielded the best differentiation between patients with MG and controls.
Summary

• RNS is an effective, reliable way of detecting pre and post-synaptic neuromuscular junction defect

• Sensitivity of RNS may be low, particularly in ocular MG and subject to many potential technical pitfalls, including:
  - Stabilization of muscle in question
  - Adequate stimulus frequency
  - Temperature control
  - Adequate selection of muscle for testing
Summary

• SFEMG may have better sensitivity than RNS for detection of NMJ defect, however, limited specificity and subject to technical difficulties including:
  - Painful in some cases
  - Operator dependent
  - Expensive needles
  - Using concentric needles can be used to evaluate NMJ function

• The presence of an oVEMP decrement is a sensitive and specific marker for MG.
  • Direct and noninvasive examination of extraocular muscle activity
  • Similarly good diagnostic accuracy in ocular and generalized MG.
  • oVEMP represents a promising diagnostic tool for MG.
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