Repetitive Stimulation

Repetitive stimulation is used in the presence of unexplained weakness which may be on the basis of a defect of neuromuscular transmission, a sarcolemmal membrane abnormality or abnormality of nerve terminals. When disorders such as myasthenia gravis, the myasthenic syndrome, periodic paralysis, myotonias, botulinum intoxication or diseases affecting the nerve terminals are present, abnormalities may be seen on repetitive stimulation. The extent, duration and specific pattern of testing depend entirely on the clinical evaluation. The electromyographer must first define the clinical problem in each patient in order to determine which of the following tests are needed. No specific routine is appropriate for each patient. Therefore, the following is meant only as a general outline which must be modified for individual patients.

I. ELECTRODE LOCATION

Repetitive stimulation may be performed on any nerve/muscle combination on which stimulation and recording can be made. In general the electrode placement is the same as that used for standard nerve conduction studies. However, particular care must be taken to immobilize the sites of stimulation and recording as much as possible. The placement of recording and stimulating electrodes will be described for some of the more commonly used sites.

A. Ulnar/Hypothenar

Because of the ease of stimulation and restraint of movement, this is technically the most reliable muscle to test with repetitive stimulation, and should generally be used initially. The recording electrodes are placed in the routine positions over the hypothenar muscle for ulnar nerve conduction studies. The stimulating electrode is placed over the ulnar nerve at the wrist. Digits 2 through 5 are bound together closely, but not tightly, with a Velcro® strap which prevents abduction. The hand is laid flat on a restraining board with a sponge rubber/board clamp over the knuckles holding the hand firmly on the board. Care must be taken not to dislodge the recording electrodes during placement of the hand on the board. When the hand is in place, attempts by the patient to abduct the fingers should result in no significant movement of the electrodes, the fingers or the hypothenar muscles. The patient is asked to exercise by spreading his fingers apart as vigorously as possible without moving his hand, wrist or arm.

B. Facial/Nasalis

In patients with predominantly bulbar or facial symptoms in whom abnormalities cannot be found peripherally, it is sometimes necessary to stimulate the facial nerve. Because of difficulty with immobilization and relaxation, this is technically unreliable muscle to test and a decrement must be unequivocal before it can be called abnormal. Recording electrodes are placed over the nasalis muscles as for routine facial nerve stimulation with the stimulating electrodes at the mastoid bones. No attempt at immobilization is made. The patient is asked to contract the muscles by squeezing the eyes shut tightly and wrinkling the nose.
II. TEST EXECUTION

A. Technical Check

After stimulating and recording electrodes have been placed over the appropriate nerves and muscles, the most important step is to be certain that stimulation is supramaximal. It is quite common for apparent abnormalities to occur because of improper stimulation with a resultant fall or change in amplitude of the evoked response. Therefore, it is necessary to be certain that no further change in stimulus occurs with increase in voltage and duration. This should be checked regularly during the course of repetitive stimulation on each nerve tested, particularly those in which movement is commonly a problem.

B. Baseline Testing

Once a supramaximal stimulus has been obtained, repetitive stimulation is applied at slow rates, usually two per second at rest. These stimuli are applied in groups of three with at least ten seconds between groups. The evoked responses are watched carefully as they occur to determine what the heights and shapes are of each sequential evoked response. When recording the responses on paper, they should be recorded as responses separated in time using the continuous/space option on the Teca machines.

The response is abnormal only if there is a consistent fall in amplitude between the first, second and third shocks in each of a series of repetitive shocks. Variation in the pattern or an increase in the amplitude is an indication of a technical error. Three consistent responses should be recorded for the baseline.

C. Exercise

After giving preliminary repetitive shocks at a slow rate at rest, a period of exercise should be used. The duration of the exercise will be a function of the clinical problem and the evoked response. If the responses are of low amplitude and a myasthenic syndrome is suspected, the exercise should initially be brief (approximately 10 to 15 seconds). If there is a prominent decrement, the exercise should also be brief, even if the amplitude is normal. However, in patients suspected of having myasthenia gravis in whom the amplitude is quite large and there is no apparent decrement, the exercise may begin with a one minute exercise. Each period of exercise should be a maximal effort on the part of the patient. Because of difficulty in maintaining a full minute of strong exercise, the one minute exercises are best divided into three equal periods of approximately 20 seconds each with 3 to 4 seconds rest between them. If the initial exercise was a brief exercise, and no facilitation occurred or if no repair of a marked defect occurred, then the exercise can be continued out to 30 seconds or a minute.

D. Post-Exercise

Immediately after exercise repetitive stimulation at 2 per second is once again applied just as before exercise. However, if there is any suspicion of a technical problem, it should first be determined if the shock is still supramaximal. Repetitive stimulation is then given at intervals with no further exercise. These intervals are similar, whether the exercise has been a brief, 10 second exercise or a more prolonged one minute exercise.
Three shocks at 2 per second should be given immediately after exercise, and at 15 seconds, 30 seconds, one minute, two minutes and three minutes after exercise. If a prominent defect persists at three minutes, the responses should be followed to four or five minutes. At this time, an additional brief (10 to 15 second) exercise may again be performed to look for repair of the defect.

E. Rapid Stimulation

More rapid rates of stimulation may be applied in some instances. In patients with botulinum intoxication, facilitation may be seen only at rapid rates of stimulation. In patients with the myasthenic syndrome rapid rates of stimulation may make the facilitation characteristic of this disorder much more obvious. Abnormalities of the nerve terminal membrane may be enhanced by more rapid rates of stimulation. However, in patients with myasthenia gravis, rapid rates of stimulation may or may not demonstrate a decrement in response, while slow rates of stimulation will more commonly do so. Rapid stimulation may be at rates of 5, 10, 20 or 40 per second. It is important to remember that as the rate of stimulation increases, the pain and discomfort the patient is subjected to become much greater. All these tests should, therefore, only be done with adequate forewarning to the patient and only when clinically indicated.

F. Evaluation

The goal of the brief and prolonged exercise is to show the repair of any defect that is already present or the facilitation of a low amplitude response immediately after exercise. In both cases, at two to three minutes after exercise an enhancement of the decrement or the occurrence of a decrement that was not seen before exercise is sought. However, in disorders of the sarcolemmal membrane or in disorders of the nerve terminal neither of these patterns will occur, but other patterns such as a fall in the response immediately after exercise may be seen.

III. Interpretation

While most laboratories require a 10% decrement to call a response abnormal, in fact if there are no technical errors, any decrement is abnormal. Because of the wide range of disorders that may show abnormalities on repetitive stimulation, it is not possible to fully discuss these responses here. In general, the following disorders show patterns of the type described. However, more extensive description and interpretation than that noted here are necessary for each problem.

A. Myasthenia Gravis

The typical finding is a consistent decrement in amplitude at slow rates of stimulation at rest, which is repaired immediately after exercise, and accentuated two to four minutes after exercise. There may or may not be a decrement at rapid rates of stimulation.

B. Myasthenic Syndrome

Usually has a low amplitude evoked response with only a small or minimal decrement at slow rates of stimulation. Prominent facilitation immediately after exercise (greater than 200 %) with an accentuation of the defect, and a fall below the resting
amplitude by 2 to 4 minutes after exercise. There is also marked facilitation at high rates of stimulation.

C. Botulinum Intoxication:
   Very low amplitude evoked responses with minimal decrement at slow rates of stimulation. Minimal increase in amplitude after exercise, and variable increase in amplitude with high rates of stimulation. Little repair after exercise, and variable enhancement of the decrement 2 to 4 minutes after exercise.

D. Periodic Paralysis (Myotonias):
   Variable small decrement at slow rates of stimulation. Enhancement of the decrement immediately after exercise with a reduction in the amplitude of the evoked response. Gradual return to the baseline state at one to three minutes after exercise.

E. Neuropathies Involving Nerve Terminals:
   Variable decrements at slow rates of stimulation that are of increasing amount with increasing rates of stimulation. No enhancement with exercise.

F. Amyotrophic Lateral Sclerosis (and other neurogenic atrophies):
   May show abnormalities much like those described above for myasthenia gravis.
WORKSHOP ON REPETITIVE STIMULATION

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I. INTRODUCTION

The purpose of the Workshop is to demonstrate specific electrodiagnostic techniques and to allow direct application and practice by course participants using conventional equipment. The technique of repetitive motor nerve stimulation has application to a wide variety of clinical disorders that affect the neuromuscular junction. The most common use involves evaluation of patients with suspected myasthenia gravis (MG) or Lambert-Eaton myasthenic syndrome (LEMS), although the studies routinely are used in the electrodiagnostic evaluation of patients who are weak or demonstrate abnormal fatiguability. Abnormal results are not diagnostic of specific clinical disorders, and abnormalities may be detected in patients with motor neuron, peripheral nerve, or muscle disease, in addition to patients with other primary neuromuscular junction disorders such as botulism, arthropod envenomation, congenital myasthenic syndromes, and impaired neuromuscular transmission caused by certain commonly used medications.

The techniques of repetitive stimulation used in the evaluation of neuromuscular transmission are similar to those used in conventional nerve conduction velocity studies, differing only in the application of paired stimuli or stimuli trains, the use of conditioning exercise, and the use of careful immobilization to reduce movement artifact. The techniques will be described in detail and the reasons for selecting one test over another will be examined, after a simple model of neuromuscular transmission is presented. The latter will be used to develop a rationale for selection of stimulation rates in repetitive stimulation studies.

Because the Workshop will emphasize demonstration and technique, there will not be time to discuss the physiology of neuromuscular transmission in detail during the Workshop. Section V of the handout (Neuromuscular Junction Overview) therefore should be reviewed in detail before attending the workshop.

II. REPETITIVE STIMULATION TECHNIQUE

A. General principles.

1. Selecting a muscle depends upon several factors.
   a. Distribution of involvement; want to test the most involved site if possible.
   b. Ease of performance of test.
      (1) If muscle difficult to immobilize, movement artifact may cause problems.
      (2) Small muscles usually easier to immobilize.
      (3) Stimulation of deep nerves or those carrying many sensory fibers may be painful.
2. Identify abnormality in at least two muscles if possible.

3. Begin with distal muscles, followed by proximal and facial muscles.

4. Once an abnormality is identified, does it make sense?
   a. Does the decrement proceed in an orderly fashion with evidence of subsequent partial repair ("early dip phenomenon" representing the ACh depletion effect, followed by ACh mobilization-related facilitation)? (Figure 1)

Figure 1. Compound Muscle Action Potentials (CMAPs) evoked by repetitive supramaximal stimulation of the ulnar nerve at low rates (3 Hz), recording from hypothenar muscle in a patient with myasthenia gravis. In the Figure, each triggered sweep is displaced to the right with each new stimulus so that sequential CMAPs can be identified. The initial large decrement (stimuli 1 to 3) represents the depletion effect of a previous stimulus. The stabilization of the evoked response amplitude (stimuli 4 to 8) represents mobilization-related facilitation.
b. Can you demonstrate immediate postactivation facilitation (partial repair of decrement) and late (2 to 4 minutes after exercise) postactivation exhaustion? (Figure 2)

At Rest
Median

After 60 Seconds of Exercise
2 Seconds Later
2 Minutes Later
4 Minutes Later

Ulnar

Ulnar

Figure 2. Compound Muscle Action Potential (CMAPs) evoked by repetitive supramaximal stimulation of the median and ulnar nerves at low rates (3 Hz), recording from thenar and hypothenar muscles respectively in a patient with myasthenia gravis. Superimposed responses (4 stimuli) are shown at rest, and after 60 seconds of volitional exercise (recording 2 seconds, 2 minutes, and 4 minutes after exercise). Postactivation facilitation results in partial repair of the decrement immediately after exercise, followed by postactivation exhaustion and an increased decrement 2 minutes later.

c. If postactivation exhaustion is present, does additional brief exercise again produce postexercise facilitation?

5. Repetitive stimulation studies usually are used for diagnostic purposes, although determination of the average decrement for multiple muscles can be used to follow disease progression or monitor therapy.
B. Technique of Repetitive Nerve Stimulation.

1. Discontinue anticholinesterase medications for at least 12 hours, if clinically safe.
2. Check muscle temperature and warm limb if necessary to maintain limb temperature of 32 to 36 degrees C.
   a. Slight cooling enhances neuromuscular transmission (?decreased activity of AChE).
   b. Moderate cooling may impair neuromuscular transmission.
   c. Warming over 36 degrees C may impair neuromuscular transmission.
3. Tape surface electrodes over clean skin; active electrode over end-plate and reference electrode over distal tendon.
4. Carefully restrain to prevent movement of electrodes.
   a. Poor restraint may result in CMAP amplitude variability that is related to change in electrode position.
   b. If testing hypothenar muscles, tape fingers together and use a handboard to further reduce movement.
   c. Some muscles (e.g. nasalis) cannot be restrained.
5. Proceed with routine conduction study to determine whether the nerve and muscle are "normal".
6. Maintain position of stimulating electrodes.
   a. Movement may result in submaximal stimulation and decreased CMAP amplitude.
   b. When possible (most distal recordings), tape simulating bar electrodes over nerve.
7. Use supramaximal stimulation intensity (about 25% above maximum stimulation).
8. Begin with single stimulus; observe CMAP amplitude.
   a. Defective neuromuscular transmission may result in reduced amplitude.
   b. The reduction usually is minimal in MG, but substantial in disorders of impaired ACh release (LEMS or botulism).
9. Perform screening examination to evaluate ACh release.
   a. Have patient contract muscle for 5 to 10 seconds and then relax; use EMG amplifier and loudspeaker to monitor level of contraction.
   b. Immediately after relaxing, repeat single stimulus.
      (1) Facilitation > 100% (ratio post- to pre-exercise x 100) definitely abnormal.
      (2) Slight "pseudo-facilitation" may result from increased synchronization of ACh release.
          (a) CMAP amplitude increases but area unchanged.
          (b) Normal physiologic phenomenon.
      (3) Producing facilitation with brief voluntary exercise instead of 50 Hz stimulation reduces discomfort and increases the likelihood of performing a complete evaluation.
10. Record superimposed CMAPs using 3 Hz stimulation rate; observe for decrement and late partial repair.
a. Need to record only 4 or 5 responses to evaluate decrement (do not need prolonged, uncomfortable stimuli trains).
b. Best observed using storage oscilloscope while simultaneously recording on photographic film, fiberoptic recorder, or digital storage equipment.
c. Observe CMAP amplitude as a function of stimuli number; a decrement following the second and fourth stimuli but not the third would not make physiologic sense.

11. If reproducible decrement present, calculate the % decrement as:
   \( \frac{\text{Amplitude initial} - \text{Amplitude smallest}}{\text{Amplitude initial}} \)
   a. If unequivocal (>10%) decrement, demonstrate repair immediately after 5 seconds of maximal volitional contraction.
   b. If no repair, suspect technical difficulty.

12. If equivocal or no decrement, exercise muscle for one minute.
   a. Perform repetitive stimulation trial (e.g. 4 stimuli @ 3 Hz) immediately after stopping exercise, and again at 0.5, 1, 2, 3, 4, and 5 minutes.
   b. Expect maximal decrement 2 to 4 minutes after exercise (postactivation exhaustion)
   c. If decrement found, perform an additional brief exercise, demonstrating immediate partial repair.

13. Normal muscle shows no decrement and any reproducible decrement without technical difficulty is abnormal.
   a. Some laboratories use 5%, others use 10% criteria as abnormal.
   b. Either represents a conservative approach and smaller decrements may be significant if all criteria above are fulfilled consistently (initial decrement with stabilization or repair, postactivation facilitation, postactivation exhaustion).

C. Specific Nerve/Muscle Evaluations.

1. Ulnar nerve, recording hypothenar muscles, stimulating at wrist.
   a. Most accessible, easily immobilized, well tolerated.
   b. Often uninvolved.
      (1) Immobilize by taping fingers together, use of hand-board with foam padding optional.
      (2) Secure stimulating electrode with wrist strap after optimizing placement; use of needle stimulating electrodes optional.

2. Median nerve, recording thenar muscles, stimulating at wrist.
   a. Very accessible, well tolerated.
   b. Difficult to immobilize, often uninvolved.
      (1) Use of hand-board that immobilizes thumb in palmar plane may reduce artifact.
      (2) Taping thumb to hand of limited value.

3. Musculocutaneous nerve, recording biceps brachii muscle, stimulating in axilla.
   a. Frequently involved (clinically and electrically).
   b. Poor access, difficult to immobilized, unstable stimulus, painful.
(1) Secure arm in extended position with arm-board, restraining above and below elbow to reduce movement.
(2) Main problem involves unstable stimulation because of stimulating electrode movement in axilla.

4. Upper trunk of brachial plexus, recording deltoid muscle, stimulating at Erb point.
   a. Same as musculocutaneous (even more commonly involved?)
   b. Immobilize arm over abdomen; have patient grasp wrist with opposite hand.

5. Spinal accessory nerve, recording trapezius muscle, stimulating at the posterior border of the sternocleidomastoid muscle.
   a. Excellent access, very well tolerated, often involved.
   b. Difficult to immobilize.
      (1) Best performed in sitting position.
      (2) Rest arm in lap; have patient grasp wrist with opposite hand.

6. Facial nerve, recording nasalis or orbicularis oculi muscles, stimulating anterior to the stylomastoid foramen.
   a. Often involved.
   b. Difficult access, unable to immobilize, unstable stimulus, painful.
      (1) If baseline shift is excessive, use baseline "clamp" on oscilloscope if available.
      (2) Movement artifact may be less with nasalis than orbicularis oculi muscle.

7. Peroneal nerve, recording anterior tibialis or EDB muscle, stimulating at knee or ankle.
   a. Good access, easy to immobilize, well tolerated.
   b. Often uninvolved.

8. Femoral nerve, recording vastus medialis muscle, stimulating over nerve at level of inguinal ligament.
   a. Often involved.
   b. Poor access, unable to immobilize, painful.

D. Technical and physiologic factors resulting in incorrect interpretations:

1. False-positive results:
   a. Poor temperature control.
   b. Unstable or submaximal stimulation.
   c. Movement artifact; poor immobilization.
   d. Inappropriate simulation rate (too fast).
   e. Test positive, but interpreted to represent a specific disease (e.g. MG), when impaired neuromuscular transmission actually related to another disorder.

2. False-negative results:
   a. Poor temperature control.
   b. Muscles tested uninvolved.
   c. Anticholinesterase medications.
E. Decrement enhancing techniques.

1. Exercise (described above).
   a. Used routinely to increase the magnitude of an equivocal decrement and to demonstrate a decrement when none was present in rested muscle.
   b. Safe and easy; may be performed in minutes.
   c. Limitations are technical; related to electrode movement during exercise.

2. Limb ischemia following inflation of pressure cuff above systolic pressure.
   a. 3 Hz stimulation during 4 minutes of ischemia; then standard repetitive stimulation test.
   b. Resultant depletion of ACh store and decreased re-synthesis.
   c. Reduced safety factor.
   d. Painful and poorly tolerated by most patients.
   e. Results unreliable; must carefully establish laboratory criteria for positive test.

3. Repetitive stimulation studies after increasing muscle temperature.
   a. Difficult to control.
   b. Unreliable and unpredictable.

4. Repetitive stimulation after systemic injection of curare.
   a. Decreased safety factor due to competitive, non-depolarizing blocking agent.
   b. Rarely used now that SFEMG available.
   c. Potentially dangerous because of sensitivity of susceptible patients to neuromuscular transmission blocking agents of any type.
      (1) Resultant neurologic impairment may be prolonged.
      (2) Respiratory impairment may develop in patients with minimal or no evidence of respiratory or bulbar involvement.
   d. 1/10th the normal curarizing dose is used by continuous injection during continual repetitive stimulation monitoring.

5. Regional (forearm) curare administration using tourniquet.
   a. Felt by most investigators to be simple and safe.
   b. Nevertheless, has not had widespread application.

III. REVIEW OF REPETITIVE STIMULATION ABNORMALITY IN NEUROMUSCULAR TRANSMISSION DISORDERS

A. Myasthenia Gravis. (Figures 1 and 2)

1. Decrement to 3 Hz stimuli trains.

2. Partial repair (early dip phenomenon) after 3rd or 4th response.

3. Repair immediately after 5 to 10 seconds of voluntary exercise.
4. Reproducibility of decrement after 15 seconds rest.

5. Postactivation exhaustion 2 to 4 minutes after 1 minute of voluntary exercise.

B. Lambert-Eaton Myasthenic Syndrome. (Figures 3-5)

1. Low amplitude CMAP to single stimulation.

2. Decrement to 3 Hz stimuli trains.

3. Abnormal facilitation to 50 Hz stimuli train or significant postactivation facilitation immediately after 10 seconds of voluntary exercise.

4. Abnormalities relatively uniform in all muscles of given patient with LEMS.

Figure 3. Compound Muscle Action Potentials (CMAPs) evoked by repetitive supramaximal stimulation of the ulnar nerve a low rates (3 Hz), recording from hypothenar muscle in a patient with myasthenic syndrome. Response is of low amplitude and a small decrement is present.
Figure 4. Compound Muscle Action Potentials (CMAPs) evoked by repetitive supramaximal stimulation of the ulnar nerve at low rates (3 Hz), recording from hypothenar muscle in the patient with myasthenic syndrome from Figure 3. Lower tracings represent the low amplitude CMAPs with decrement in rested muscle. Upper tracings demonstrate marked facilitation immediately after 10 seconds of maximal voluntary contraction.
Figure 5. Compound Muscle Action Potentials (CMAPs) evoked by repetitive supramaximal stimulation of the ulnar nerve at high rates (50 Hz), recording from hypothenar muscle in the patient with myasthenic syndrome, demonstrating marked facilitation similar to that shown in Figure 4.

C. Botulism.

1. Low amplitude CMAP to single stimulation.
2. Occasional presence of a small decrement to 3 Hz stimuli train.
3. Variable presence of abnormal facilitation following exercise or rapid repetitive stimulation (less than typically recorded in LEMS).

D. Amyotrophic Lateral Sclerosis.

1. Severely involved muscles in some patients demonstrate a decremental response at low rates of stimulation.
2. Postactivation facilitation and exhaustion identical to MG.
3. Presence of decrement may reflect recent re-innervation and signify a poor prognosis. → rapid turnover
E. Myotonic Dystrophy.

1. Progressive decremental response to repetitive stimulation.
2. Abnormality due to membrane inactivation, not defective neuromuscular transmission.
3. Further reduction of CMAP amplitude immediately after voluntary contraction (no postactivation facilitation).

IV. DOUBLE OR PAIRED STIMULI TECHNIQUE

A. Similar to repetitive stimulation techniques; comparison is made of CMAP amplitudes evoked by paired stimuli at different interstimulus intervals.

1. More detailed evaluation of neuromuscular transmission over a greater range of stimulation frequencies.
2. Less painful than stimulation trains used in repetitive stimulation studies.

B. Can generate "stimuli interval-amplitude response" curves by comparing the second CMAP to the initial (control) response over a wide range of stimuli intervals.

1. Stimuli intervals between 1 ms and 2 seconds typically studied.
   a. < 1 ms interval (>1,000 Hz), no response; nerve refractory period.
   b. 2 to 20 ms interval (50 to 500 Hz), second response normally smaller; muscle refractory period.
   c. 20 to 100 ms interval (10 to 50 Hz), second response sometimes slightly higher; synchronization of MFAPs (CMAP area unchanged).
   d. 100- to 2,000 ms interval (0.5 to 10 Hz), no change normally.

2. Important intervals in LEMS and MG:
   a. 5 to 10 ms interval (100 to 200 Hz), maximal facilitation in LEMS.
   b. 200 to 500 ms interval (2 to 5 Hz), maximal depression in MG.

V. NEUROMUSCULAR JUNCTION OVERVIEW

A. Morphology.

1. Nerve terminal (presynaptic portion).
   a. Contains mitochondria, cisternal structures, vesicles, and free acetylcholine (ACh).
      (1) Vesicles contain ACh, adenosine triphosphate (ATP), and proteins.
      (2) Vesicles are associated with quantal ACh release; they are distributed in clusters or "active zones".
   b. In resting muscle, random vesicles fuse with the presynaptic membrane and ACh is released into the synaptic cleft (exocytosis).
   c. During nerve stimulation, a greater number of synaptic vesicles fuse and release ACh.
2. Synaptic cleft.
   a. Approximately 50 nanometer width.
   b. Contains acetylcholinesterase (AChE) that degrades ACh to acetate and choline.

3. Postsynaptic end-plate
   a. Convoluted portion of muscle that has specialized membrane properties.
   b. Contains acetylcholine receptors (AChRs).
      (1) About 1.5 x 10^10 receptors per end-plate.
      (2) AChRs span the postsynaptic membrane and extend into cleft.

B. Physiology.

1. ACh is synthesized from choline and acetyl-coenzyme A by choline acetyl transferase (CAT).

2. ACh is released in discrete packets (quanta).
   a. Some quantal release occurs spontaneously.
   b. Release of a large number of quanta follows invasion of the nerve terminal by a nerve action potential (NAP).
   c. Some free ACh (not contained in vesicles) may also be released.

3. ACh diffuses across synaptic cleft (50 microseconds) and binds with AChRs.
   a. Each ACh molecule may "activate" none, one, or many AChRs.
      1. The ACh channel on the AChR opens after 2 ACh molecules bind with another receptor.
      2. The channel then closes and ACh can theoretically re-bind with another receptor.
   b. ACh/AChR interaction results in membrane depolarization.
      (1) A single quantal release results in a miniature end-plate potential (MEPP).
      (2) The summation of multiple MEPPs following the near synchronous release of many quanta by a NAP results in an end-plate potential (EPP).
   c. The EPP amplitude depends upon:
      (1) Number of ACh molecules/quanta (usually 5,000 to 50,000).
      (2) Availability of ACh quanta for release and number released.
      (3) Inactivation of ACh by AChE.
      (4) Number of functional AChRs.
   d. If the EPP exceeds a certain level (threshold), a muscle fiber action potential (MFAP) is generated.
   e. The normal EPP amplitude ordinarily is 3 fold greater than the 10 to 20 mv depolarization required to activate a MFAP (large "safety factor").

C. Physiologic Model of Neuromuscular Transmission.

1. Assumes three ACh storage sites within the presynaptic terminal.
   a. A primary (immediately available) store contains 1,000 quanta; releases ACh following NAP (stimulus-evoked release).
   b. A secondary (readily available) store contains 10,000 quanta that can be "mobilized" to replenish primary store; probably represents re-synthesized quanta moving toward the membrane for release.
c. A tertiary (main) store contains 100,000 quanta; these quanta are relatively unavailable for release.

d. Released ACh is metabolized into choline and acetate by AChE; choline is taken back into the nerve terminal for resynthesis.

2. Following nerve depolarization, a fixed percentage of the primary store ACh is released.
   a. The actual percentage released in dependent upon influx of calcium.
      (1) 4 calcium ions required to couple excitation with release of a single ACh quanta.
      (2) Calcium diffuses out of the presynaptic region in 100 to 200 microseconds.
      (3) In rested muscle, approximately 20% of the primary store is released per NAP.
   b. Another NAP arriving before calcium diffuses out will result in release of a greater percentage of the available ACh (calcium-dependent facilitation).
   c. After several NAPs, ACh release may be increased for several minutes (post-tetanic potentiation).

3. Mobilization of ACh.
   a. Takes 1 to 2 seconds.
   b. After an initial discharge and prior to mobilization, there are fewer ACh quanta available in the primary store for release (depletion effect of a previous impulse).
   c. During prolonged activity, mobilization reaches steady state (ACh release = ACh mobilization).

4. Immediately after prolonged activity:
   a. Quantal release is facilitated because of residual bound calcium (calcium-dependent facilitation [brief] and post-tetanic potentiation [long] and ongoing mobilization).
   b. Mobilization probably continues for seconds.
   c. Spontaneous MEPP frequency is markedly increased for 2 to 3 minutes (e.g. from 4/second to over 40/second).
   d. 2 to 4 minutes later, ACh availability falls and the EPP amplitude decreases.
      (1) Probably related to decreased ACh stores due to random release and decreased mobilization.
      (2) This normal phenomenon is called postactivation exhaustion.

D. Use of the Model to Explain the Optimal Repetitive Stimulation Rate.

1. Want maximal "challenge" to the neuromuscular junction (enhance the ACh depletion effect of a previous impulse while minimizing the facilitory effect of calcium-dependent ACh release).
   a. Simulation rates > 2 Hz allow several stimuli to arrive before mobilization becomes effective.
   b. Simulation rates < 5 Hz allow subsequent stimuli to arrive after calcium effect has diminished.
2. Example of challenge resulting from 3 Hz stimulation:
   a. First stimulus releases 20% of 1,000 primary store ACh quant
   b. Second stimulus releases 20% of remaining 800 ACh quanta (prior to mobilization).
   c. Third stimulus releases 20% of remaining 640 ACh quanta (still prior to mobilization).
   d. About this time, ACh mobilization begins to replenish primary store.
   e. Therefore, fourth stimulus releases 20% of somewhat greater number of ACh quanta, e.g. 640 to 1,000.
   f. The EPP amplitude transiently diminishes between stimuli 1 and 3, and then returns toward baseline.
      (1) In a normal NMJ, this decrease is insignificant.
      (2) In an abnormal NMJ, the EPP may drop below threshold and result in conduction block.
   h. Summary of effect of 3 Hz stimulation upon the EPP:

<table>
<thead>
<tr>
<th>Stimuli #</th>
<th>Available ACh</th>
<th>ACh Release</th>
<th>EPP Amp (Approx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,000</td>
<td>200</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>800</td>
<td>160</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>640</td>
<td>128</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
<td>&gt;640 (mobilization)</td>
<td>&gt;128</td>
<td>&gt;38</td>
</tr>
</tbody>
</table>

3. The compound muscle action potential (CMAP) is a summation of the MFAPs.
   a. The CMAP negative phase area is the best measure of the number of muscle fibers activated.
   b. CMAP amplitude is a reasonable approximation if CMAP configuration is stable.

4. Stimulation rates of 10 to 50 Hz enhance the calcium-dependent ACh release (postactivation facilitation).
   a. In disorders of ACh release, abnormal facilitation observed.
   b. The same effect can be demonstrated by comparing the CMAP amplitude before and immediately after a brief (5 to 10 second) trial of volitional activation.
REFERENCES


Clinical disorders of neuromuscular transmission and their diagnosis are difficult to understand without some knowledge of fundamental aspects of the morphology and physiology of the normal neuromuscular junction at the subcellular level. This basic information provides a framework for describing not only subcellular abnormalities of neuromuscular transmission but also the rationale of single-fiber electromyography (SFEMG) at the cellular level. SFEMG in turn helps explain the more conventional clinical testing of neuromuscular function in whole muscles by repetitive nerve stimulation (RNS).

This review will therefore begin by summarizing the extensive information available about ultrastructure and subcellular physiology first at normal and then abnormal neuromuscular junctions. SFEMG will be described briefly in terms of this basic information and used to interpret the normal and abnormal responses to RNS. After this background has been presented, the clinical technique of RNS will be discussed to illustrate the practical aspects of neuromuscular pathophysiology.

NORMAL NEUROMUSCULAR TRANSMISSION

Compared with the integration of impulses which takes place at other synapses, the neuromuscular junction is a relatively simple relay between one of the nerve terminals of a motoneuron and the skeletal muscle fiber which the nerve terminal innervates. Figure 1 diagrams the morphological aspects (on the left) and the biochemical aspects (on the right) of this connection between the presynaptic nerve terminal and the specialized postsynaptic muscle membrane, the endplate, underlying the nerve terminal. Chemical transmission across the synaptic cleft overcomes the large electrical mismatch between the very fine nerve fiber and the large muscle fiber by introducing an amplification of an order larger than 100-fold.11

Presynaptic Features. The chemical transmitter at the neuromuscular junction is acetylcholine (Ach), which is synthesized in the nerve terminal from acetyl coenzyme-A and choline by the enzyme choline acetyltransferase (Fig. 1, right). Acetylcholine behaves as if it were partitioned in the presynaptic terminal into at least three main compartments. The largest compartment is “reserve” Ach which is not directly available for release. A
NEUROMUSCULAR JUNCTION

MORPHOLOGY

presynaptic nerve terminal ending

microtubules and microfilaments

mitochondrion

primary synaptic cleft

postsynaptic membrane folds

receptors

muscle fibers

BIOCHEMISTRY

ChAT = choline acetyltransferase

CoA = coenzyme A

ChAT

cytosolic CoA

AchE

Acetylcholine

Choline

AchR = acetylcholine receptor

AchE = acetylcholinesterase

FIGURE 1. Diagram of the neuromuscular junction between a nerve terminal ending and a postsynaptic muscle endplate. Morphological aspects are presented to the left of the vertical dashed line and biochemical aspects to the right. See text for explanation.

smaller compartment, called the "mobilization store," in turn supplies the smallest portion of Ach immediately available for release. This readily releasable portion behaves as if it were released in units of several thousand molecules of Ach at a time. Most scientists believe that this packaging takes place in the synaptic vesicles which can be seen by electron microscopy adjacent to the nerve terminal membrane (Fig. 1, left). Vesicles attach to specific sites on the presynaptic membrane called active zones and release their contents into the primary synaptic cleft. In the resting state individual multimolecular packets or "quanta" of Ach are released intermittently, and the frequency of this release is dependent upon extracellular calcium concentration and temperature.\(^3\)

**Postsynaptic Features.** The released Ach diffuses across the primary synaptic cleft and can bind to transmembrane glycoproteins called acetylcholine receptors (AchRs)\(^2^5\) concentrated at the crests of postsynaptic folds opposite the presynaptic active zones (Fig. 1; see also Fig. 4A). When two molecules of Ach bind to specific extracellular sites on an AchR molecule, a funnel-shaped ion channel in the center of the receptor opens for a few milliseconds (Fig. 2). The resulting fluxes of sodium down its electrochemical gradient, occurring through a few thousand AchR ion channels in response to a "quantum" of Ach, briefly depolarizes the muscle membrane only at the junctional region (Fig. 3, top left and right). Each postsynaptic nonpropagating depolarization is called a miniature endplate potential (MEPP). Measured intracellularly by microelectrodes, MEPP amplitude provides a useful estimate of the response of the postsynaptic membrane to a single quantum of Ach. The action of Ach on the postsynaptic membrane is normally terminated within a few milliseconds of its release from the nerve terminal by the enzyme acetylcholinesterase, which breaks Ach down into acetic acid and choline.

**Response to Nerve Stimulation.** A nerve impulse propagating down the motor axon invades and
FIGURE 2. Diagram of three acetylcholine receptors in the postsynaptic membrane demonstrating one possible arrangement of the alpha, beta, gamma, and delta subunits organized like barrel staves around a cation channel through the center of the molecule. The acetylcholine (Ach) binding sites and the main immunogenic region (MIR) are located on the protruding extracellular surface of alpha subunits. Sugars on the extracellular surface of the subunits are indicated by branched zigzag lines. The cigar-shaped structures on the cytoplasmic surface represent cytoskeletal components. (Reprinted from Hermann by permission of the Western Journal of Medicine.)

depolarizes the nerve terminal, which causes a voltage-sensitive influx of calcium ions across the presynaptic nerve terminal membrane. In contrast to the intermittent random release of quanta of Ach at rest, up to 100 quanta of Ach are released into the primary synaptic cleft in response to a nerve impulse. This Ach diffuses across the synaptic cleft and binds to about 100,000 AchRs, which causes a larger nonpropagating depolarization of the postsynaptic membrane called the endplate potential (EPP in Fig. 3, bottom left). The amplitude of the endplate potential tends to be a multiple of the amplitude of a MEPP under similar conditions, which is one of the key pieces of evidence for the quantal theory of neuromuscular transmission. If the endplate potential is large enough to reach a critical level of depolarization called the “threshold” for activa-

FIGURE 3. On the left of the figure are postsynaptic intracellular recordings made at a muscle fiber endplate compared with similar recordings on the right made 2 mm away from the endplate in the same muscle fiber. Upper portions were recorded at low sweep speed and high amplification; they show that spontaneous miniature endplate potentials are recorded only at the endplate. Lower records show the electrical response to a nerve impulse at high sweep speed and lower gain; the stop-like endplate potential (EPP) leading to a propagating muscle fiber action potential is seen only near the endplate. (Reproduced from Fatt P, Katz B: Spontaneous subthreshold activity at motor nerve endings. J Physiol (Lond) 1952;117:109–126, with permission.)
tion, an all-or-none action potential (Fig. 3, bottom left) will be propagated in both directions along the nonjunctional muscle membrane (Fig. 3, bottom right). When this action potential invades the transverse tubule system of the muscle, another voltage-sensitive calcium influx triggers mechanical contraction of the muscle fiber.

**Safety Factor.** Normally an excess of Ach is released, and several times as many AchRs are activated, as would be necessary for an endplate potential to reach the threshold for action potential propagation. This is called the “safety factor” of neuromuscular transmission. If the safety factor is greatly decreased, as in the abnormal conditions described below, this may lead to blocking of neuromuscular transmission.

**ABNORMAL NEUROMUSCULAR TRANSMISSION**

Studies of human disorders of neuromuscular transmission at the subcellular level, based on information gained from normal ultrastructure and microelectrode physiology, have contributed greatly to the understanding of myasthenia gravis, Lambert–Eaton myasthenic syndrome, a growing number of congenital myasthenic syndromes, and botulism. This knowledge has made clinical diagnostic testing for many of these conditions more rational.

**Myasthenia Gravis.** Myasthenia gravis (MG) is characterized clinically by asymmetric weakness and fatigability of extraocular, bulbar, masticatory, or limb muscles with normal reflexes and sensation and a 15% association with thymoma. MG is an autoimmune disease of the postsynaptic membrane. Antibodies to human acetylcholine receptor are detected in the blood of 80% of patients with generalized MG, and immune complexes (IgG and complement) have been localized to the postsynaptic membrane, which shows degraded remnants of junctional folds in the synaptic cleft on electron microscopy of muscles from patients with MG (Fig. 4B). The number of acetylcholine receptors on the postsynaptic membrane in MG is decreased. This correlates closely with the amplitude of MG MEPPs, which are on the average one-fifth smaller than normal MEPPs. The frequency of spontaneous MEPP release (a presynaptic function) is normal in MG, as are presynaptic Ach content and synaptic vesicles. Although the number of quanta released following a nerve impulse is normal, the endplate potential response to nerve stimulation is decreased in MG in proportion to the small size of the MEPP. Therefore some endplate potentials are too small to initiate a muscle action potential, and weakness or fatigue results.

Anticholinesterases, by inhibiting the breakdown of Ach, increase the amplitude and duration of endplate potentials and increase the chances of small endplate potentials reaching the threshold for propagation of muscle action potentials.

**Lambert–Eaton Myasthenic Syndrome.** Lambert–Eaton myasthenic syndrome (LEMS) is characterized clinically by weakness and fatigability of proximal limb muscles with relative sparing of extraocular and bulbar muscles and by hyporeflexia, dry mouth, and a 70% association with oat cell carcinoma of the lung. Freeze-fracture electron microscopy of the presynaptic nerve terminal membranes of intercostal muscles from patients with LEMS (Fig. 5) demonstrate a disruption of the “active zones,” the rows of large intramembranous particles arranged in parallel double rows on the presynaptic nerve terminal membrane where vesicles attach. There is also a decreased number of particles which make up the active zones in LEMS presynaptic membrane (Fig. 5). The same findings were present in mice which were injected with LEMS IgG. These treated mice also showed the electrophysiological characteristics seen in vitro at the neuromuscular junction of patients with LEMS, namely normal presynaptic Ach stores, normal postsynaptic response to individual Ach quanta (normal MEPPs and normal MEPP frequency), but reduced release of Ach quanta from the nerve terminal by a nerve impulse, and therefore reduced endplate potentials. The reduction in the size of the endplate potentials was proportional to the decreased number of active zone particles, which may represent the calcium channels for voltage-sensitive calcium-dependent Ach release. This and other evidence indicates that LEMS is probably an autoimmune disease of the presynaptic nerve terminal.

**Congenital Myasthenia.** Congenital myasthenia comprises a heterogeneous group of disorders in which the symptoms of muscle weakness and fatigue have been present from birth, often with a positive family history of similar disorder but without autoimmune MG in the mothers, and without circulating AchR antibody in the patients. Immunootherapy (prednisone, thymectomy) is usually ineffective, and the response to anticholinesterase medication is variable. Those
disorders which have been investigated thoroughly (with ultrastructural studies and in vitro electrophysiology) are of interest for the unique information which they provide about the subcellular dysfunction at different sites of the human neuromuscular junction. Thus, the few cases of "familial infantile myasthenia" (severe respiratory and feeding problems) which have been so
studied\textsuperscript{16,34} demonstrated deficient presynaptic Ach storage, mobilization, and/or resynthesis. Other families showed long-duration MEPPs and prolonged endplate potentials which produced repetitive compound muscle action potentials thought to be on the basis of prolonged opening of \(\text{AchR} \) ion channels.\textsuperscript{17} Greatly prolonged MEPPs and endplate potentials, as well as repetitive action potentials, were also found in a boy who had congenital absence of endplate acetylcholinesterase.\textsuperscript{15} thereby allowing repetitive binding of Ach to \(\text{AchR} \). Other cases of congenital myasthenia\textsuperscript{51,51} have had reduced numbers of \(\text{AchRs} \) or \(\text{AchRs} \) with altered affinity for cholinergic ligands.

**Botulism.** Botulism is a disease of neuromuscular transmission caused by the potent toxin of the bacterium *Clostridium botulinum*, either ingested preformed in contaminated food or produced anaerobically in vivo by bacteria in wounds or in the gastrointestinal tract. Affected infants fail to suck and become constipated, weak, and hypotonic. Adults show a descending paralysis first affecting the eye muscles and then other muscles of the head, neck, trunk, and limbs. The type of toxin determines the extent of accompanying autonomic cholinergic involvement. When measured in vitro, the size of MEPPs at neuromuscular junctions of patients poisoned with botulinum toxin (type A) was almost normal,\textsuperscript{29} but MEPP frequency was greatly reduced, and the number of Ach quanta released by a nerve impulse was reduced profoundly. With high doses of toxin to animals experimentally, MEPP amplitude was also greatly reduced.\textsuperscript{7} The onset of paralysis is faster in actively firing nerves and at higher temperatures. Ach synthesis and postsynaptic Ach sensitivity are normal, but the toxin irreversibly blocks Ach release.\textsuperscript{45} Apparently calcium influx into the nerve terminal is not inhibited, but fusion of synaptic vesicles with the presynaptic nerve membrane is impaired in some unknown way which appears to be different from the mechanism occurring in LEMS.\textsuperscript{10} Ach release may be blocked so completely that signs of denervation may appear on needle electromyography. Recovery requires sprouting of nerve terminals and the formation of new neuromuscular junctions.

**SINGLE-FIBER ELECTROMYOGRAPHY**

Single-fiber electromyography (SFEMG) is a method of monitoring the action potentials of single muscle fibers extracellularly. As such it may be thought of as a conceptual transition from the subcellular focus of intracellular microelectrodes to the conventional recording from motor units by needle electromyography (EMG). Ordinary EMG concentric needles or Teflon-coated monopolar needles may be used to record from nearby single muscle fibers by filtering out the lower frequencies (below 500–1000 Hz) coming from distant muscle fibers.\textsuperscript{47} Selectivity may also be increased by using a smaller recording surface (25 micrometers in diameter) to obtain a steeper relationship between the amplitude of the potential and the distance of the electrode from the potential generator.\textsuperscript{47} SFEMG requires a time base with a resolution of a few microseconds, a stable trigger, a delay line, and a method of recording and counting the potentials obtained.

**Fiber Density.** Single-fiber action potentials are obtained by slight voluntary activation of the muscle in which the recording needle has been placed or by intramuscular electrical stimulation of motor axons to the muscle fibers being examined.\textsuperscript{50} Single-fiber action potentials should have a duration of about 1 millisecond, a peak-to-peak rise time of 100–200 microseconds, and an amplitude of between 1 and 5 mV. Electrical activity from only one muscle fiber is recorded in about two-thirds of random insertions in a normal muscle, a reflection of the electromyographic fiber density of the muscle. Measured according to certain strict criteria, fiber density can be a reproducible measure of the average number of single fiber action potentials in that muscle within the recording radius of the electrode.\textsuperscript{42} It may be greatly increased in reinnervation or dystrophies, but in neuromuscular disorders it is usually normal or only slightly increased.\textsuperscript{24}

**Jitter.** In normal muscle, pairs of potentials will be recorded approximately 30% of the time, indicating that at those sites the needle is recording from two muscle fibers sharing the same motor axon and motor unit (Fig. 6, top). The time between the two potentials, the interpotential interval, varies from discharge to discharge, and this variability is called the electromyographic jitter (Fig. 6A). Most of the jitter is produced by fluctuations in the time which endplate potentials take to reach the threshold for action potential propagation (Fig. 7A). Jitter is usually expressed as the mean consecutive difference (MCD) to compensate for any slow change in the mean interpotential interval with time.\textsuperscript{42,47} Normal jitter is between 10 and 60 microseconds. It will be
FIGURE 6. In the diagram at the top of the figure, electrode E can record electrical activity from two muscle fibers belonging to the same motor unit. Such recordings are demonstrated in the rows below the diagram. In the upper row 10–15 oscilloscope sweeps have been superimposed, and in the lower row the sweeps move downward with time. The oscilloscope sweep is triggered by the first of the two muscle action potentials, and variability in the interval between potentials is seen as a variable position of the second potential. (A) Normal jitter in a normal muscle. (B) Increased jitter without impulse blocking in a myasthenic muscle. (C) Increased jitter and occasional blockings (arrows) in MG muscle. Bar = 50 μsec. (Reprinted from Stålberg47 with permission of Elsevier Scientific Publishing Company.)

increased in any conditions in which the safety factor of neuromuscular transmission is lessened and the size and rise time of the endplate potential is decreased (Fig. 7B). At low rates of firing these include all known disorders of neuromuscular transmission. Therefore, an increased amount of jitter on SFEMG is a nonspecific measure of neuromuscular dysfunction.

Blocking. In neuromuscular disorders in which the jitter exceeds 80–100 microseconds, one of the single-fiber potentials of a pair may intermittently fail to appear along with the recorded unchanged discharge of the other potential (Fig. 6C, arrows). This is called blocking. Blocking occurs, presumably, when the endplate potential for the disappearing potential fails to reach the threshold for propagation of the action potential (Fig. 7B). Blocking is the SFEMG manifestation of clinical fatigue and weakness. It is also the basis for the decremental response which can be seen on repetitive nerve stimulation as described in the next section. It follows that increased jitter without blocking (Fig. 6B) can occur in muscles which are not clinically weak or abnormal by other clinical electrophysiological techniques. Increased jitter thus represents the most sensitive clinical indicator of impaired neuromuscular transmission, even though it remains very nonspecific.

Amounts of jitter can vary widely even among neuromuscular junctions of the same motor unit. Standardization of jitter measurements has been most extensive in the extensor digitorum muscle of the forearm. Different results are obtained by different laboratories and techniques, but in general the study is considered to reflect abnormal function of neuromuscular transmission if more than 1 of 20 (95% confidence level) potential pairs
FIGURE 7. Drawing to explain neuromuscular jitter and blocking based on superimposed intracellular recordings near the endplates of human intercostal muscle fibers during indirect stimulation at 5 Hz. A fluctuating threshold for the amount of endplate potential (EPP) depolarization which will produce a propagated muscle fiber action potential (AP) is supposed to account for normal variation in the latency of the action potential (A). In conditions in which the endplate potential amplitudes are decreased (B), this variation is greater, resulting in increased jitter, and some endplate potentials are not large enough to produce an action potential, resulting in intermittent blocking. (Reprinted from Stålberg47 with permission of Annals of the New York Academy of Sciences.)

in this muscle have a calculated MCD of greater than 55 microseconds or if the mean MCD of the 20 pairs is greater than 35 microseconds. Almost any other muscle can be used for jitter measurements as well if normal values for various age groups are available. Normal individuals over the age of 50 tend to have increased jitter and blocking.

REPETITIVE NERVE STIMULATION

The firing rate of muscle action potentials is an important variable in assessing abnormal neuromuscular function. SFEMG of MG muscles usually shows increasing jitter with increasing firing rate, whereas muscles from patients with LEMS or botulism demonstrate decreasing jitter with increasing firing rate. These contrasting responses to repetitive stimulation reflect a differential effect of firing rate upon two main physiologic factors affecting transmitter release at the normal nerve terminal. These two factors are the amount of Ach transmitter available for release and the amount of available calcium affecting the probability of transmitter release.

Transmitter Depletion. Quantal Ach release (and the number of synaptic vesicles) is gradually depleted during repetitive depolarization of the normal nerve terminal. This is most prominent during the first few stimuli at slow rates of repetition (1–4 Hz, or 1000–250 millisecond intervals between stimuli), after which Ach mobilization has responded to the stimulation by increases which match Ach discharge. Because of the decreased safety factor for transmission in MG, however, a decrease in the size of the endplate potential with repeated stimulation may lead to subsequent blocking of action potential production when the endplate potential falls below threshold. This normal process of quantal Ach depletion during slow repetitive stimulation may not be as prominent in conditions such as LEMS or botulism in which transmitter release is defective.

Calcium Facilitation. The other important change occurring at the nerve terminal during repetitive stimulation is an increase of calcium influx during Ach depolarization caused by a nerve impulse. This increased calcium facilitates transmitter release for about 100–200 milliseconds following each impulse, after which time the calcium has been sequestered by mitochondria. Repetitive stimuli occurring less than 200 milliseconds apart (i.e., 5–50 Hz) may therefore produce a cumulative facilitation of transmitter release. (Facilitation decreases above 50 Hz because impulses begin to overlap each other; above 200 Hz the refractory periods of nerve and muscle inhibit repetitive discharges). Facilitation by increased calcium is not so important in normal neuromuscular transmission, which is already operating optimally, as it is in conditions in which transmitter release is defective. In LEMS and botulism, for instance, facilitation by calcium influx into nerve terminals during 40–50 Hz stimulation may improve transmitter release that neuromuscular
**Diagnosis of Myasthenia Gravis by Repetitive Nerve Stimulation.** Blocking which occurs at the neuromuscular junctions of many muscle fibers during repetitive voluntary contractions can be detected in principle by routine needle EMG, which should show a progressively decreasing amplitude of an isolated motor unit potential. However, in practice changes in motor unit potential amplitude are more likely to be caused by needle movement than by dropout of individual muscle fiber potentials, so motor unit potential amplitude is not a useful test for defects of neuromuscular transmission. Conventional needle EMG is used to exclude the presence of concomitant nerve or muscle disease.

A more reliable way to detect blocking of the neuromuscular junctions of many muscle fibers is to record from *surface electrodes* on the skin over the endplate zone of a whole muscle during repetitive stimulation of the appropriate nerve to that muscle. Normally each successive muscle response should be the same. In MG at rest, the size of the initial evoked compound muscle action potential so recorded is usually normal or near normal. During the first two to five stimuli of a low-frequency repetitive train, however, a typical decrement occurs over some weak muscles (Fig. 8, upper left). This initial decrement is the reflection of progressive blocking at the neuromuscular junctions of hundreds of muscle fibers as transmitter depletion (as described in a previous section) causes increasing numbers of endplate potentials to become subthreshold. The initial fall in the size of the compound muscle action potential then levels off or even slowly increases during subsequent stimuli (Fig. 8, upper left). Presumably Ach mobilization has now caught up with Ach quantal depletion.

Voluntary exercise of the measured muscle for several seconds produces a rapid neuromuscular stimulation at rates between 20 and 40 Hz. This is much less uncomfortable than tonic 50 Hz external stimulation to the nerve and is the preferred method of rapid stimulation. Immediately after such exercise, the size and area of the evoked myasthenic response may be larger and the decrement less (Figure 8, upper right). This is called *postactivation potentiation* and probably reflects the calcium facilitation described above with rapid stimulation. By assessing the area under evoked responses before and after exercise, postactivation potentiation can be differentiated from “pseudo-facilitation,” in which the amplitude of the response is greater after exercise because of increased synchronization of its components but the area of the response remains the same.

A few minutes after exercise, the size of the evoked response in a myasthenic muscle may be smaller and the decrement greater than at rest (Fig. 8, lower left). This is called *postactivation exhaustion*, the basis for which is unknown, although it has been attributed to receptor desensitization. Postactivation exhaustion may be the only abnormality to occur during repetitive testing of mildly involved myasthenic muscles, and so after 60 seconds of exercise a train of low-frequency repetitive stimulation every minute for up to 5 minutes may be useful to bring out a marginal decrement during postactivation exhaustion.

**Comparison of Repetitive Nerve Stimulation with Other Laboratory Tests for Myasthenia Gravis.** The clinical hallmark of MG is fluctuating specific skeletal muscle weakness made worse by repeated use and relieved at least partially by rest. The diagnosis is easy to make clinically in obvious cases, but in patients in whom weakness is mild or restricted to only a few muscles such as the ocular muscles, the presence of myasthenia may be questioned.

Unfortunately, RNS is not a very sensitive confirmatory test for MG (Table 1). Thirty-two percent of patients with definite myasthenia and 69% of patients with myasthenia were normal on RNS of a distal hand muscle. Use of a proximal muscle for RNS was more sensitive, but still 11% of patients with definite MG and 32% of patients with mild myasthenia were normal. Ocular myasthenia was even more difficult to pick up by RNS (Table 1). Various techniques to decrease the number of these RNS false negatives are described, but because they are based on increased blocking rather than increased jitter, none has proved to be more sensitive than SFEMG.

Even SFEMG, however, is normal in a few patients with mild myasthenia (8–12%) and quite a few patients with ocular myasthenia (23–41%), the categories in which SFEMG is most likely to be employed (Table 1). Serum antibodies to human AchR are also absent in about one-quarter of patients in these categories. Since individual MG patients may be confirmed by one of these tests and not by another, a battery of tests may be required in questionable cases. The elevated antibody titer has an advantage over the electrical...
tests in that it confirms a diagnosis of autoimmune MG, whereas SFEMG is a sensitive but nonspecific test for general neuromuscular dysfunction.

**Diagnosis of Lambert–Eaton Myasthenic Syndrome by Repetitive Nerve Stimulation.** In comparison to MG, in which a decremental response to RNS occurs in some weak muscles of some patients, a characteristic response to RNS is practically essential for the diagnosis of LEMS and is said to occur in all measurable extremity muscles. The compound muscle action potential amplitude in LEMS

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<th>Table 1. Results of laboratory testing for myasthenia gravis.</th>
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<td>Procedure</td>
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Source: Data are from Stålberg£6 and Kelly.£5 Table reprinted from Hermann£5 with permission of the Western Journal of Medicine.
at rest is often 10% of normal, apparently because many muscle fibers are blocked as a result of decreased quantal release of transmitter on nerve stimulation (see LEMS above). At slow (2–3 Hz) rates of stimulation there may be a decremental response as additional transmitter depletion occurs (Fig. 9). After a brief period of voluntary exercise (10–30 seconds), the compound muscle action potential in LEMS increases to almost normal size (usually over double what it was at rest), presumably as a result of calcium facilitation of available transmitter release at rapid rates of stimulation. This postactivation potentiation is one of the electrical hallmarks of LEMS, the other being the low amplitude initial response. The development of potentiation at rapid rates of stimulation can also be displayed as an incremental response to rapid electrical stimulation in patients who are not able to activate the measured muscle voluntarily. Postactivation exhaustion may occur in LEMS, but it is less important for potential diagnosis than it is in MG.

Amounts of postactivation potentiation greater than 200% can also be seen in other presynaptic conditions such as botulism, hypermagnesemia, and hypocalcemia. It has been reported to occur in some muscles of cases of otherwise typical MG; this does not represent a basic change in the character of the disease, but only a change in the number of quanta released by subsequent nerve impulses in some neuromuscular junctions of rare patients with MG.

**Diagnosis of Botulism by Repetitive Nerve Stimulation.** Compared with LEMS, the electrical abnormalities to RNS in botulism are not found in all muscles; they depend upon the severity of the intoxication, and they evolve with time after absorption of the toxin. Very mild cases may be normal on RNS, and a neuromuscular defect is detected only by increased jitter on SFEMG, which decreases at higher rates of stimulation. The amplitude of the initial compound motor action potential may be small or even absent in some affected muscles (particularly muscles of the head and normal in other muscles for example, leg muscles), depending upon the area of the body tested. Decremental responses at slow rates of stimulation usually do not occur (Fig. 10, top). Postactivation potentiation and incremental responses to fast rates of stimulation are signs that transmitter release can be increased by repetitive stimulation (Fig. 10) and therefore represent moderately involved cases with damaged but still-functioning nerve terminals. In severe cases, transmitter release may be so severely blocked that no increment or potentiation occurs at rapid rates of stimulation, even though the initial response is small.

The magnitude of the postactivation potentiation is usually less than that observed in LEMS, but it can last several minutes rather than the few seconds seen in LEMS. RNS of several muscles is a useful diagnostic test for botulism, since it can indicate the presence of neuromuscular transmission defects many days or even weeks before the toxin or organism is identified.

Needle electromyography is also a useful diagnostic indicator of botulism, particularly infantile botulism. Short-duration low-amplitude polyphasic motor unit potentials, accompanied by abnormal spontaneous activity, may be prominent in severe cases even within a few days of clinical intoxication, presumably because the botulinum toxin functionally denervates muscle fibers at the presynaptic terminals.

**Technique of Repetitive Nerve Stimulation.** It should be readily apparent from the foregoing that much judgment is required at each step of performing repetitive nerve stimulation for clini-
FIGURE 10. Repetitive stimulation of the median nerve at four rates of stimulation (listed on the left) in a patient with infantile botulism, with subcutaneous recordings over the abductor pollicis brevis muscle. An 8% decrement was obtained at 5 Hz, a 25% increment at 10 Hz, a 38% increment at 20 Hz, and a 94% increment at 50 Hz. (Reprinted from Cornblath with permission.)

Three things can be done before testing begins to increase the chances of detecting a defect of neuromuscular transmission:

1. **Withdraw anticholinesterase medications** at least 8 hours and preferably 24 hours before the test if this is possible without compromising the patient’s ability to breathe and swallow. If these functions are borderline on medication, the doctor ordering the test should decide the amount and duration of medication withdrawal.

2. **Make sure that the muscles being tested are warm** by placing the patient’s limbs under blankets or by using a heating pad or infrared lamp.

Cooling improves neuromuscular transmission in all the conditions described above, probably by decreasing acetylcholinesterase activity and increasing MEPP and endplate potential amplitude and duration. Limb temperature should be monitored closely during the test.

3. **Select appropriate nerve-muscle combinations for testing.** Any accessible combination may be used, but weak muscles are preferred. The small muscles of the hand are usually tested first because they are easy to immobilize. However, the small muscles of the hand are often not abnormal in MG during RNS, whereas proximal MG muscles are more likely to show decremental responses (Table 1), perhaps because they are warmer. Therefore, even though immobilization of proximal muscles is difficult, a proximal muscle should always be tested when MG is a possibility.

Stimulation of the upper trapezius muscle via the accessory nerve in the neck is better tolerated than stimulation of other proximal muscles at Erb’s point, in the axilla or in the leg. Facial muscles are often involved in MG and can show a decremental response when limb muscles are normal on RNS. The chances of detecting an abnormal response in MG increase with testing of two or three nerve-muscle combinations.

In addition, steps should be taken during testing to obtain satisfactory results:

1. **Neither the stimulus site nor the tested muscle should move,** even during rapid stimulation. Special clamps, armboards, and straps should be used to immobilize the recorded muscle whenever possible.

2. **Supramaximal stimulation is absolutely necessary** at a level of stimulation intensity 25% greater than that at which no further increase is obtained in the amplitude of the evoked compound muscle action potential. This should be checked frequently during the course of testing. Submaximal or threshold stimulation may cause the amplitude of the evoked response to vary in a misleading manner.

3. **Display the whole waveform of each muscle response at maximum amplitude.** The supramaximally stimulated evoked electrical muscle response should be displayed at maximum amplitude compatible with complete visualization of the whole response during repetitive stimulation.

4. **Trains of at least 4 supramaximal stimuli at a repetitive rate of 2 or 3 Hz should be given** to the nerve and the electrical muscle responses recorded once the amplitude and waveform of sin-
gle compound muscle action potentials are clearly discernible.

5. The course of response amplitude during a train should show a smooth progression and be reproducible on repeated testing, with at least 15 seconds of rest between each train of stimulation. Even so, the examiner should be constantly on the alert for possible movement artifacts at both the stimulating and recording sites.

6. The effect of fast rates of stimulation should be determined after the RNS response to slow rates of stimulation has been obtained at rest. This can take the form of maximal voluntary isometric contraction (20–40 Hz) of the measured muscle if the subject is cooperative or else 50 Hz electrical stimulation if the subject cannot cooperate (as with infants).

7. The duration of the fast stimulation should be only 10 seconds if postactivation potentiation is to be detected by 2 Hz stimulation immediately afterwards, but it can be up to 60 seconds long if an incremental response or subsequent postactivation exhaustion is the purpose of this part of the test. The longer periods of activation (namely, 60 seconds) should be used for stronger muscles, shorter duration of stimulation being adequate for demonstrated postactivation exhaustion in weaker muscles.

8. Postactivation exhaustion may take several minutes to manifest itself after exercise, and so without further exercise trains of 2 Hz stimulation should be repeated every minute for at least 5 minutes after the period of exercise before concluding that postactivation exhaustion is absent.

9. Record the whole waveform of each response as seen on the oscilloscope screen rather than recording each response as a straight line without a timebase. A real decremental or incremental response should involve a change in the area of the negative phase of the evoked compound muscle action potential rather than just a change in the maximal amplitude of each response. Commonly there is an increase in amplitude and decrease in duration following exercise because of increased synchronization of muscle fiber action potentials, but the area of the responses (measured by computer) in such normal cases remains constant. In actual practice, the maximal amplitudes of the negative phase of the first and fourth responses are measured manually from baseline to negative peak, and the percent change of the fourth response compared with the first represents the decrement or increment. Everyone accepts a decrement of greater than 10% as abnormal and most would accept 5% change, although any technically perfect reproducible decrement or increment deserves clinical correlation.

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