21

Neurophysiology of Neuromuscular Transmission and Its Disorders

James M. Gilchrist

Summary

Despite several antibody tests being available for the assessment of disorders of neuromuscular transmission, electrophysiological testing of the neuromuscular junction remains a very important part of clinical practice. The neuromuscular junction is a complex structure and an understanding of its anatomy and physiology can assist in better understanding the value of electrodiagnostic testing. The most common disorders include myasthenia gravis, Lambert–Eaton myasthenic syndrome, and botulism, and are usually readily identified using several electrophysiological techniques including slow (2–3 Hz) and fast (20- to 50-Hz stimulation). Single-fiber needle EMG remains an additional powerful and sensitive test for patients with disorders that are more mild, in whom repetitive stimulation testing is negative or indeterminate.

Key Words: Botulism; Lambert–Eaton myasthenic syndrome; myasthenia gravis; neuromuscular junction; repetitive nerve stimulation.

1. NEUROPHYSIOLOGY OF NEUROMUSCULAR TRANSMISSION

1.1. Anatomy of the Terminal End-Plate Region

The physiology of the neuromuscular junction (NMJ) derives from the anatomy of the terminal axon and motor end plate, also referred to as the presynaptic and postsynaptic regions (Fig. 1). Motor nerve fibers end in an arborization of fine intramuscular twigs ending at the terminal bouton. The motor twigs are myelinated until the very terminus, with a Schwann cell covering all but the synaptic interface. The terminal axon is separated from the motor end-plate region of the muscle fiber by an extracellular space approx 70-nm wide, called the primary synaptic cleft. On the far side of this cleft, in the motor end-plate region, are a series of invaginations of the muscle membrane, which are called the secondary synaptic clefts. These junctional folds are unique to NMJs.

The terminal axon membrane contains voltage-gated calcium channels (VGCC) of the P/Q type, which are arranged in active zones in a semigeometric array. Within the terminal axon, synaptic vesicles containing acetylcholine collect at the active zones (Fig. 1). Acetylcholine is synthesized from choline and acetate in the cytoplasm by the enzyme choline acetyltransferase. Acetylcholine enters the synaptic vesicles using the vesicular acetylcholine transporter, which also exports the acetylcholine during exocytosis. Choline is actively transported by an energy-dependent reuptake mechanism.

From: *The Clinical Neurophysiology Primer*
Edited by: A. S. Blum and S. B. Rutkove © Humana Press Inc., Totowa, NJ

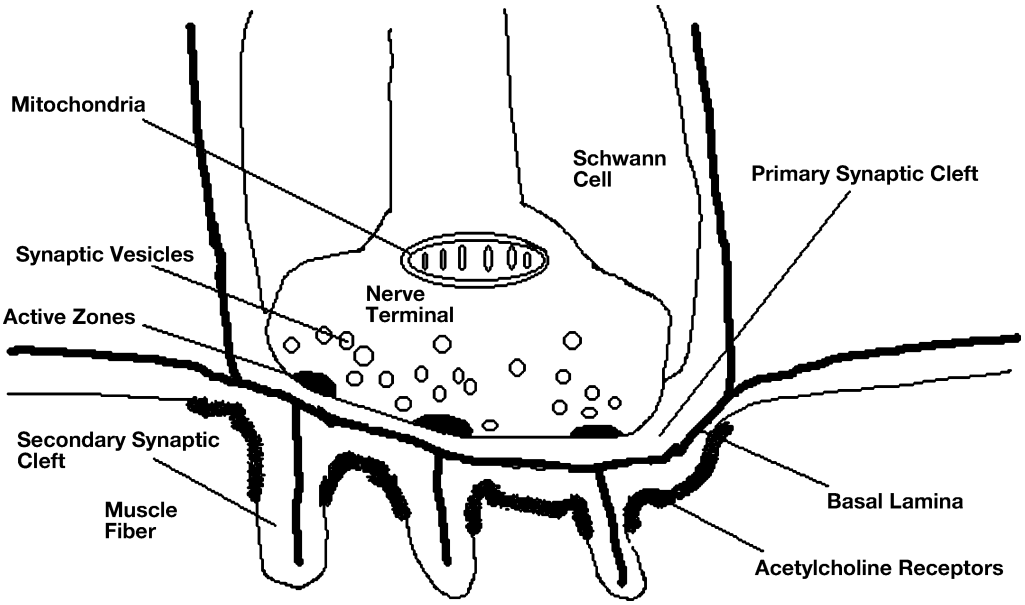

Fig. 1. A cartoon of a neuromuscular junction (NMJ). The synaptic vesicles contain acetylcholine. The active zones contain voltage-gated calcium channels, and part of the apparatus necessary for exocytosis. The basal lamina in the primary synaptic cleft contains acetylcholinesterase. Voltage-gated sodium channels necessary for propagation of the action potential generated at the neuromuscular junction are in the muscle fiber membrane, including the depths of the secondary synaptic clefts.

The primary synaptic cleft is divided by a basal lamina, a loosely organized, porous boundary containing acetylcholinesterase, which catabolizes acetylcholine as it diffuses across the primary synaptic cleft. Approximately 50% of the acetylcholine released from the presynaptic membrane is catabolized before it reaches the postsynaptic membrane.

The most important components of the postsynaptic membrane are the acetylcholine receptors. These receptors are of the nicotinic type, and contain a ligand-activated cation channel. At the base of the secondary clefts are voltage-gated sodium channels, essential for transmitting any action potential along the muscle membrane. Acetylcholine receptors are manufactured by membrane-bound ribosomes in the cytoplasm and then inserted in the postsynaptic membrane. There are approx 10,000 receptors per square micrometer at the terminal and upper areas of the secondary clefts. Each adult acetylcholine receptor (Fig. 2) is a tetramer containing 2 α-subunits, and one each of the β-, δ-, and ε-subunits. Fetal acetylcholine receptors substitute a γ-subunit for the ε-subunit. Of note, ocular muscles, which differ from other skeletal muscles in a number of ways, have an enriched population of fetal-type receptors. The half-life for adult acetylcholine receptors is 8 to 11 d. Ligand sites for acetylcholine are located on each of the α-subunits, and both most be engaged to activate the receptor channel. The main immunogenic region (MIR) is also located on each α-subunit, but separate from the ligand-binding site. After acetylcholine dissociates from the receptor, it is catabolized by acetylcholinesterase. Application of agents that inhibit the activity of acetylcholinesterase will prolong the activity of acetylcholine at the postsynaptic receptors.

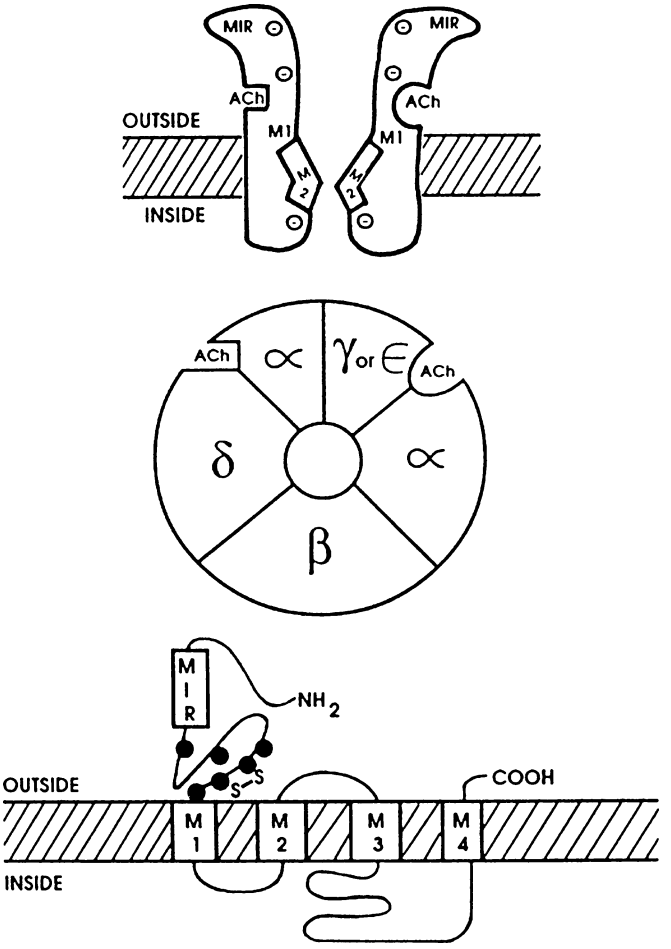

Fig. 2. A cartoon of the acetylcholine receptor, showing a transverse section, a top view, and a deconstructed view of the α-subunit. MIR, the main immunogenic region of the receptor; ACh, the ligand-binding site for acetylcholine. From Engel AG, 1999 with permission.

1.2. Presynaptic Physiology of Neuromuscular Transmission

The motor nerve action potential generated in the cell body is transmitted to the terminal axon membrane. As it traverses the presynaptic membrane, it activates the VGCCs, which open, allowing the movement of calcium into the terminal axon. The influx of calcium activates calmodulin-dependent protein kinase II, which binds with synapsin I, resulting in the docking of the synaptic vesicle with the terminal membrane. Release of the acetylcholine by exocytosis involves a complicated interaction of many proteins, including synaptobrevin, syntaxin, and SNAP-25. The synaptic membrane remains part of the axonal membrane and is recycled.

Acetylcholine-containing vesicles are organized into at least two pools. The immediately available pool is the smallest and consists of those vesicles actually lined up at the active zones for release. During a series of axonal depolarizations, such as when attempting to contract a muscle, the immediately available store will become depleted, and fewer synaptic vesicles will be released. The second pool is considerably larger than the immediately available store

and is called the mobilization or reserve pool. During the course of a few seconds, it will replenish the immediately available pool.

The influx of calcium via the VGCCs is by passive diffusion. In contrast, calcium egress is by active transport and takes longer. During a sustained series of action potentials, the calcium concentration in the terminal axon will continue to increase and facilitate release of synaptic vesicles, partially countering the effects of depletion of the immediately available pool.

1.3. Postsynaptic Electrophysiology of Neuromuscular Transmission

The acetylcholine molecules passively diffuse across the primary synaptic cleft. Those that escape catabolism bind with acetylcholine receptors on the postsynaptic membrane. The activated receptors undergo a conformational change, allowing sodium to enter the cell and potassium to leave. This causes a small depolarization of the immediately adjacent muscle membrane. Release of single synaptic vesicles from the presynaptic membrane causes a reproducible level of depolarization, approx 1 mV, called a miniature end-plate potential (MEPP). This is the basis for the quantal theory of neuromuscular transmission (NMT). Because many synaptic vesicles are released with each depolarization of the terminal axon membrane, many MEPPs are produced. The MEPPs summate temporally and spatially to form an end-plate potential (EPP). If this EPP is sufficient to depolarize the membrane to threshold, an action potential is generated, which is then propagated along the muscle membrane by voltage-gated sodium channels in the sarcolemmal membrane, eventually resulting in muscle fiber contraction, the defining purpose of NMT.

An important concept is that of the safety factor. The EPP normally produced after each action potential activation of the presynaptic membrane is approximately four times that necessary to reach threshold. It is calculated by the formula $m = n \times p$, where m is the quantal of the EPP, and n is the percentage of vesicles released from the immediately available pool, which is determined by p, the probability of release. This excessive EPP derives from the abundance of acetylcholine released presynaptically and of acetylcholine receptors postsynaptically. Thus, even with the normally encountered depletion of the immediately available pool of acetylcholine-containing synaptic vesicles during a rapid train of motor nerve action potentials, the safety factor always provides successful NMT in the healthy NMJ. When healthy muscle fatigues, it is not because of failure of NMT but, rather, because of muscle metabolic issues, such as lactic acid buildup and failure of energy pathways. However, in the abnormal NMJ, if either the amount of acetylcholine released or the number of acetylcholine receptors declines, the safety factor will begin to fall as the size of the EPP falls. If the EPP safety factor falls below 1, that is, that needed to just reach threshold for action potential generation, then NMT will fail. This is the basis for fatigable weakness in disorders of postsynaptic NMT, such as myasthenia gravis (MG). As the number of receptors falls, the EPP also decreases. In many NMJs, the safety factor will fall below 1, and that muscle fiber will be, in essence, denervated. In many other fibers, the safety factor will hover just at or above 1, and initially, there will be successful NMT. However, during a train of motor nerve action potentials, the normal depletion of the immediately available store of acetylcholine-containing synaptic vesicles will result in a decrease in acetylcholine reaching the decreased number of receptors on the postsynaptic membrane. This will cause the safety factor to fall below 1, and NMT will fail, the muscle fiber will not contract, and the muscle will weaken.

The safety factor is also important in understanding incremental strength during sustained effort in disorders of presynaptic NMJ. In these disorders, there are abundant postsynaptic

acetylcholine receptors, but release of the acetylcholine-containing synaptic vesicles is impaired. In a weak muscle, the fibers do not contract because the lack of acetylcholine release causes the safety factor to fall below 1, with resultant failure of NMT. In the case of Lambert–Eaton syndrome (LEMS), this is caused by loss of VGCCs and decreased influx of calcium to start the process of synaptic vesicle release. In botulism, it is impairment of the release of vesicles themselves. In both cases, with rapid repetitive activation of the presynaptic membrane, such as during strong effort, calcium will build up in the terminal axon, potentially increasing to levels that may approximate that observed during normal presynaptic function. At this point, acetylcholine release will improve toward normal levels, as will the EPP. As the safety factor increases and surpasses 1, NMT will successfully resume, though it will quickly fail as soon as the rapid train of motor action potentials ceases. This effect is much more evident in LEMS than botulism, but, if observed, is diagnostic of a presynaptic disorder.

2. NEUROPHYSIOLOGICAL TECHNIQUES TO STUDY NMT

2.1. EMG

The first described clinical neurophysiological abnormality in MG was by Harvey and Masland in 1941, when they reported variability in motor unit potential (MUP) amplitudes. The defect in MG is widespread but not universal; individual motor end plates are affected to varying degrees, even within the same motor unit. Some NMJs may be nonfunctional, some may have marginal safety factors, and others may be healthy. Because of this variable involvement, during muscle contraction, NMT may fail at a variable number of NMJs with each MUP firing, resulting in variation in amplitude and area of the MUP. This is not specific to MG, because it can be observed in any disorder of NMT, including presynaptic and postsynaptic diseases. It is a common finding in ongoing reinnervation after nerve injury, but can also be observed in acute denervation, old polio and postpolio syndrome, amyotrophic lateral sclerosis, and myopathic injury and recovery.

2.2. Repetitive Stimulation

Repetitive stimulation of nerve (RNS) while recording from muscle has been a valuable tool in the assessment of NMT since 1941, and remains the technique in widest use. It is easy to learn, easy to perform, not invasive, and requires no special equipment or training. On the negative side, it is often painful and poorly tolerated, is prone to artifact if not performed properly, and has a limited sensitivity, especially in localized diseases affecting NMT, such as ocular MG. The usual technique is to give a train of supramaximal electrical stimulations to a motor or mixed nerve while recording from an appropriate muscle. The train is usually four to nine stimuli long. For most indications, a rate of 2 to 3 Hz is most appropriate. This should be performed at rest, with the amplitude of the first compound motor action potential (CMAP) compared with the fourth or fifth. Significant decrement is usually defined as exceeding 10% (Fig. 3). After this, the patient should maximally contract the muscle for 1 min, if possible, which is followed by trains of stimuli immediately and at 30-s intervals out to at least 3 min. Decrement may repair immediately after exercise but should reach a maximum amount between 2 and 3 min after exercise, a phenomenon known as postactivation exhaustion. The improvement in decrement is called facilitation and is caused by increased calcium concentration in the terminal axon leading to enhanced release of acetylcholine. Small increments in CMAP amplitude can also be observed in healthy people.

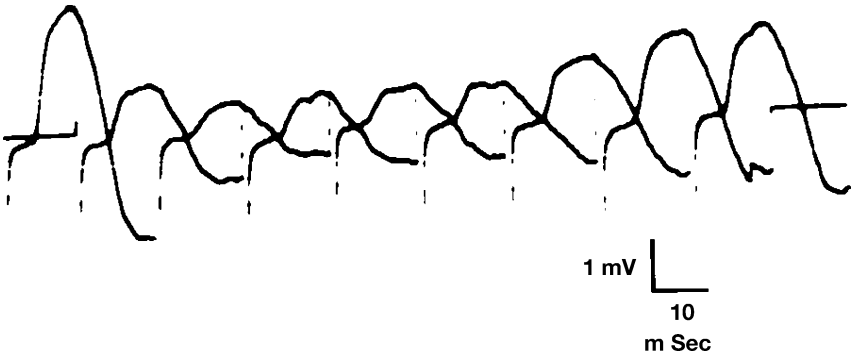

Fig. 3. Repetitive stimulation in a patient with myasthenia gravis demonstrating typical U-shaped decrement: maximal by the third or fourth stimulation in the train of nine, with return toward normal by the ninth stimulation. Decrement, calculated by comparing the fourth evoked response amplitude to that of the first, exceeds 65%.

There are several caveats worth noting regarding RNS:

1. Two- to 5-Hz stimulation is preferred if looking for decrement. One-hertz stimulation is usually too slow to produce decrement, and rates faster than 5 Hz may produce facilitation of the response, masking any decrement.
2. Maximal voluntary contraction for 10 to 60 s (depending on the strength of the patient) followed by a supramaximal nerve stimulus is the preferred method for looking for increment. In patients who cannot cooperate, or are too weak to voluntarily contract the muscle, rapid stimulation up to 50 Hz can be performed but this is exquisitely painful and should not be performed for more than 10 s unless the patient is deeply comatose.
3. Proximal muscles are more likely to show decrement in MG than are distal muscles, but proximal muscles are more prone to technical artifact and are more painful.
4. Decrement disappears as muscle temperature drops, therefore, a cool limb can result in failure to elicit decrement even if there is a defect in NMT. This is a greater problem with distal muscles, which should be warmed to at least 32°C. On the other hand, warming a limb above standard temperature may enhance a mild decrement.
5. In healthy individuals, CMAPs can increase up to approx 40% in amplitude simply because of the phenomenon of pseudofacilitation. Hence, increments of greater than 40% should be considered abnormal, although most patients with LEMS have considerably greater increments, in the range of 100 to 400% (*see* below).
6. Stimulation site and intensity must remain constant throughout the test because decreases in intensity may mimic decrement.
7. A healthy NMJ should have no decrement, but, because of the technical limitations of RNS, a decrement of up to 10% is within normal limits.

There have been several studies of the sensitivity of RNS in MG and LEMS, including comparisons to other techniques. In summary, RNS in MG is more likely to be abnormal in generalized disease than in ocular MG, is marginally more sensitive than measurement of acetylcholine receptor antibodies, is less sensitive than single-fiber EMG (SFEMG) at all levels of disease, and the diagnostic yield increases as more muscles are studied. The yield for a distal muscle RNS in generalized disease is 40% and approaches 70% for a proximal muscle.

NMT is an energy-dependent activity and ischemia will affect it adversely. This is the basis for an uncommonly used procedure, called double-step repetitive stimulation. This method

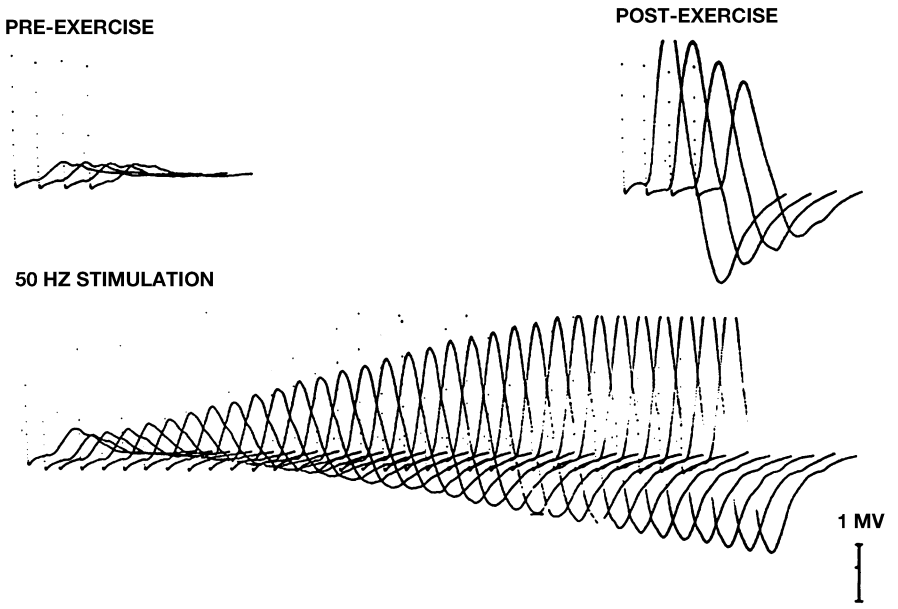

Fig. 4. Repetitive stimulation in a patient with Lambert–Eaton myasthenia syndrome. Pre-exercise shows a very small CMAP with decrement at low rates of stimulation. When repeated after exercise, the CMAP amplitude has increased by several hundred fold but decrement persists. The bottom trace is at 50 Hz stimulation and shows initial decrement followed by dramatic increment in CMAP amplitude. From Maselli R, 1998.

requires near-nerve needle stimulation of the ulnar nerve at the wrist at 3 Hz for 4 min while recording from the abductor digiti quinti muscle, measuring decrement, then repeating the 4 min of 3-Hz stimulation with a sphygmomanometer inflated above systolic blood pressure, measuring decrement after the cuff is deflated. This technique has been shown to increase sensitivity in MG comparable to proximal muscle RNS, but still lags considerably behind SFEMG.

Eaton and Lambert first described RNS in LEMS in 1956. At a low rate of stimulation, these patients will have decrement indistinguishable from MG. At high rates of stimulation, or after maximal voluntary activation of the muscle, there will be an increment in the response reaching and exceeding 100% in most patients (Fig. 4). This facilitation of the CMAP is not unique to LEMS, and up to 90% CMAP facilitation has been reported in MG. However, increase in the CMAP by 100% should be considered diagnostic of a presynaptic defect. A recent large study of LEMS found 98% of patients had decrement with 3-Hz stimulation, 88% of patients had CMAP potentiation greater than 100% in at least one muscle, but only 39% had potentiation greater than 100% in all three muscles studied.

2.3. Single-Fiber EMG

SFEMG is the selective recording of a limited number of single muscle fiber action potentials from one motor unit in vivo. This requires a needle electrode with different specifications from a concentric or monopolar needle electrode, and SFEMG needle electrodes have a dramatically smaller recording area than either. A SFEMG recording surface is 25 μm in diameter, with an effective recording area of 300 μm^3, as compared with a concentric needle

electrode, which records from approx 1 cm^3. A smaller electrode emphasizes the amplitude difference between close and distant fiber potentials. A smaller recording surface will also restrict the number of recordable muscle fiber potentials. In addition, muscle fiber potentials adjacent to the recording electrode will have high amplitudes and short duration, and relatively more high-frequency components compared with more distant potentials. By using a high-pass filter of 500 Hz, much of the amplitude of distant muscle fiber potentials will be attenuated while preserving that of the nearby potentials. This allows single muscle fiber potentials to be selectively studied while the rest of the MUP is effectively dampened to nil.

By counting the number of single muscle fiber potentials observed with each MUP firing, the number of muscle fibers from that MUP within the small recording territory of the SFEMG needle electrode can be determined. For the most part, this should be one or two. Sampling 20 different sites in a muscle allows calculation an average number of single muscle fiber potentials per recording site. This is called the fiber density. In conditions with loss of random distribution of MUP muscle fibers, such as reinnervation, fiber density will increase. Specific disorders that can increase fiber density include anterior horn cell diseases, such as spinal muscular atrophy, polio, postpolio muscular atrophy, and amyotrophic lateral sclerosis; and any peripheral or cranial neuropathy with axonal loss, specifically, those caused by diabetes, alcohol, uremia, toxins, amyloidosis, Guillain–Barré syndrome, chronic demyelinating inflammatory polyneuropathy, and multiorgan failure. The only study to compare etiologies found that alcoholic polyneuropathy produced higher fiber densities than did uremic or diabetic neuropathy, despite (or because of) better nerve conduction velocities. A variety of muscle disorders will also have increased fiber densities, especially as the disease progresses and chronic disability ensues, including muscular dystrophies, inflammatory myopathies, mitochondrial myopathies, and congenital myopathies.

In those instances in which two or more fiber potentials from a single MUP are recorded, an interpotential interval (IPI) can be calculated. By recording multiple consecutive firings of the muscle fiber potentials, the difference between consecutive IPIs can be calculated. The variation amongst these consecutive IPIs is called jitter. Jitter is most accurately determined by calculating a mean consecutive difference using the formula:

$$\text{Mean consecutive difference} = [(IPI_1 - IPI_2) + \ldots + (IPI_{n-1} - IPI_n)]/(n-1)$$

Jitter is thought to derive from variation in the time in takes the NMJ EPP to reach threshold for action potential generation at the postsynaptic membrane. In disorders with disturbed NMT, there will be an increased variation in the time taken to attain an EPP capable of reaching threshold. This will lead to increased jitter (Fig. 5). Therefore, abnormal jitter is an indicator of abnormal NMT.

In those instances in which an EPP fails to reach threshold for action potential generation, one of the muscle fiber potentials in the pair will be absent. This is called impulse blockade (Fig. 5). It is another indicator of abnormal NMT, usually indicating a more severe disturbance than increased jitter alone. Impulse blocking, often referred to simply as blocking, is usually intermittent, with the affected fiber potential appearing and disappearing in an unpredictable pattern. Blocking is uncommonly observed with jitter less than 100 μs.

Increase jitter, and even blocking, are not specific to MG or LEMS, and can be observed in any disorder of NMT. It is an early finding in any neuropathy with axonal loss, including

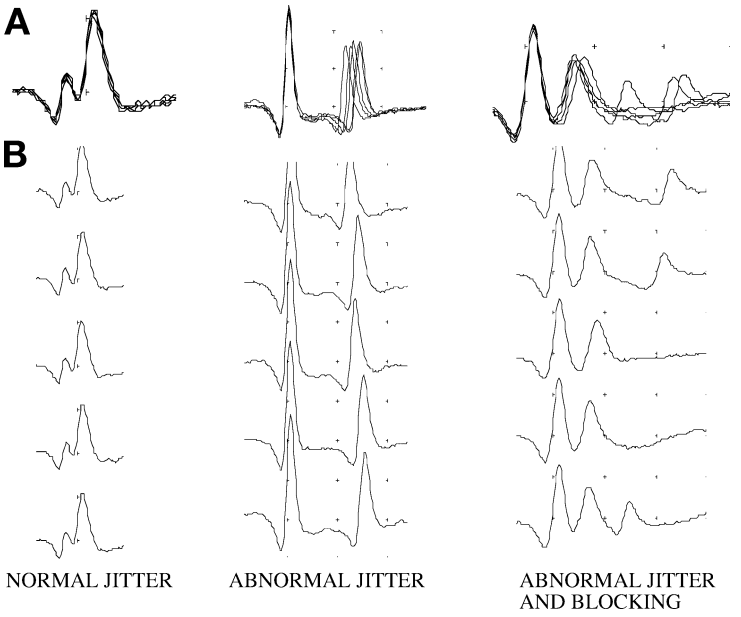

Fig. 5. Single-fiber EMG examination for jitter in normal and abnormal motor end plates. (**A**) Superimposed and (**B**) serial traces indicating (from left to right) normal jitter, increased jitter without impulse blocking, and increased jitter as well as blocking.

acute transection. Wallerian degeneration after nerve fiber transection transpires over 11 to 14 d, and sensory nerve action potentials become unobtainable below the transection by 11 d. However, CMAPs are lost within 7 d of nerve transection, because NMT fails before the nerve fiber becomes inexcitable. Increased jitter will be observed in anterior horn cell disorders, acute and chronic peripheral neuropathies, and myopathies. It is a reflection of acute denervation and subsequent failure of NMT, as the nerve terminus degenerates, and reinnervation, as the nerve terminus regenerates and the NMJ matures. In muscle disease, jitter indicates degeneration of motor end plates caused by myofiber degeneration as well as myofiber regeneration with immature motor end plates. However, it is in the primary disorders of NMT that SFEMG jitter studies are most useful.

SFEMG has been used in the diagnosis of MG since at least 1971. Since then, there have been numerous studies of the sensitivity of SFEMG in diagnosing MG, and comparisons to other diagnostic techniques. The largest series of SFEMG studies in MG reported the results of 788 patients. Results of SFEMG of the extensor digitorum communis muscle was abnormal in 85% of all patients with MG at the time of initial examination. If the extensor digitorum communis muscle was healthy, and a second muscle was studied, 85% of those patients had abnormal jitter studies. Thus, if two muscles were studied when the first was normal, results of SFEMG for jitter analysis were abnormal in 98% of all patients with MG. This far exceeds the sensitivity of all other diagnostic tests for MG, as further illustrated by several comparative studies, which, in one study, found SFEMG to be the most sensitive, at 92% (testing a single muscle); with RNS at 77% (testing multiple muscles) and acetylcholine receptor antibody testing at 73%. SFEMG was sensitive regardless of whether disease was generalized or ocular; the yield was higher than 90%, even in ocular disease, if more than one muscle was studied.

The enhanced sensitivity of SFEMG makes physiological sense: RNS results will not be abnormal until at least 10% of muscle fiber end plates undergo impulse blockade and, therefore, fail to generate or propagate a muscle fiber action potential. Muscle fibers with slowed and unstable NMT, but not so affected that they are blocked, will count as normal. SFEMG not only can determine the fibers with impulse blockade, but, by assessing jitter, will allow those fibers with disturbed but still functional NMT to be measured.

SFEMG studies of jitter and impulse block also correlate well with the clinical severity of the disease. Mean jitter, percentage of fiber pairs with increased jitter, and percentage of fiber pairs with blocking all increase with worsening disease. Mean jitter worsens by at least 10% in two-thirds of patients when their disease worsens, and mean jitter improves by at least 10% in 80% of patients who clinically improve. Despite the sensitivity of SFEMG to changes in NMT, SFEMG does not predict progression of ocular MG to generalized disease.

Results of SFEMG jitter studies are also abnormal in LEMS, often more so than would be expected from the clinical picture. Large case series with SFEMG studies are not available, but virtually all patients reported have had markedly abnormal jitter and large percentages of blocking fibers. Because of the presynaptic nature of LEMS, a relationship between jitter and firing rate would be predicted, but, in fact, this is variable. It is safe to say that a dramatic improvement in jitter and blocking with increasing firing rate is suggestive of a presynaptic defect in NMT.

Botulism arises from defective presynaptic release of acetylcholine caused by the toxin of *Clostridium botulinum.* Results of SFEMG are abnormal in 95% of patients with botulism, and in 100% of botulism patients with clinical weakness. Results of SFEMG studies improve as patients improve. Initially, fiber density is normal but, because botulism causes an irreversible block of acetylcholine release, patients improve by reinnervation and fiber density increases.

3. DISEASES AFFECTING NMT

3.1. Presynaptic Disorders

3.1.1. Lambert–Eaton Myasthenic Syndrome

LEMS was first described in 1951 and received its eponym from the investigators who first described its clinical neurophysiological characteristics. LEMS is caused by a polyclonal antibody attack directed against the P/Q VGCCs located on the presynaptic membrane of acetylcholine terminal nerve axons. VGCCs contain $\alpha 1$-, β- and $\alpha 2/\delta$-subunits, with the $\alpha 1$-subunit containing the calcium conductance channel, as well as being the ligand-binding site. The autoimmune attack results in loss of calcium channels and disorganization of the active zones, leading to inhibition of release of acetylcholine-containing synaptic vesicles. This inability to fully release acetylcholine creates MEPPs that are normal in amplitude but decreased in number, resulting in a decreased EPP. If the EPP is decreased below the level necessary to reach threshold for action potential generation, then NMT is unsuccessful and the muscle remains noncontractile.

The major clinical feature of LEMS is weakness, often generalized and symmetric, and usually affecting proximal muscles more than distal. Affected patients will be areflexic or markedly hyporeflexic, with normal sensation. Signs and symptoms of autonomic dysfunction are also present, such as dry mouth, impotence, constipation, bladder retention, and abnormal papillary reactions, indicating that the autoantibody attack is not limited to VGCCs at NMJs.

Unlike MG, oculomotor function is uncommon and the onset is usually insidious, although acute presentations, including respiratory crisis, are reported. The most striking clinical feature is the improvement in muscle contraction and strength with continued effort. Muscles that were too weak to resist gravity will become nearly normal with sustained effort and reflexes that at first are absent, will steadily improve to normal with repetitive striking of the tendon. All such improvements prove transient and disappear rapidly with cessation of effort. This facilitation with sustained effort is pathognomonic of a presynaptic disorder of NMT.

Unlike MG, the typical patient with LEMS is male and older, often in the fifth or sixth decade of life. This is likely because 50% of LEMS patients have a paraneoplastic syndrome, often associated with a small cell carcinoma of the lung. The 50% of patients without an associated carcinoma are younger, but again with a strong male preponderance. The clinical characteristics do not differ between the cancer and noncancer groups. Survival is determined by the underlying cancer in those patients with one, but is not otherwise shortened.

The diagnosis is never made without a high index of suspicion. Antibodies against the P-type VGCC are found in up to 85% of patients, but the diagnosis is most frequently made in the EMG laboratory. Any patient with small CMAP amplitudes and normal conduction velocities but normal sensory nerve action potential studies should be considered a possible case of LEMS. To screen for LEMS, the muscle being recorded should then be exercised for 10 to 30 s, depending on how weak it is, and an immediate supramaximal stimulus administered. If there is no increment in the CMAP amplitude, a presynaptic defect is not present. If there is more than a 40% increment, a presynaptic defect becomes a strong possibility, and, if the increment exceeds 100%, a presynaptic defect is definite. Any such finding should be confirmed in other muscles by a similar method. Repetitive stimulation (Fig. 4) will show decrement at low rates of stimulation before exercise, with immediate increase in CMAP amplitude and repair of decrement after exercise, and return to decrement after 2 to 3 min. At high rates of stimulation, which are not recommended in the conscious patient because of great discomfort, there will be a dramatic facilitation of the CMAP amplitude. SFEMG will show increased jitter, impulse blockade, and, in some fibers, a decrease in jitter when there is an increase in firing rate.

In patients with cancer, treatment is obviously directed at the cancer, which will often provide improvement and even remission. Symptomatic treatments may also provide clinical benefit. Guanidine enhances release of synaptic vesicles and was shown to be effective, but had severe side effects and is no longer available. Pyridostigmine enhances the amplitude of MEPPs, much as it does in MG, and can provide some benefit. The most effective symptomatic treatment comes from the aminopyridines, which inhibit voltage-gated potassium conductance, lengthening the action potential and prolonging calcium conductance into the terminal axon; 4-aminopyridine is effective but crosses the blood–brain barrier and causes seizures, tremors, and anxiety; 3,4-diaminopyridine has been found to be much safer because it is less capable of crossing the blood–brain barrier and more potent in enhancing release of acetylcholine. The industrial solvent, 3,4-diaminopyridine, is available by the barrel for that purpose, but not approved by the Food and Drug Administration except for individual compassionate use.

3.1.2. Botulism

Botulism is caused by the neurotoxin secreted by the anaerobic bacteria Clostridium botulinum and takes three forms, food-borne, wound, and infantile. Food-borne is the most common worldwide, but in the United States, infantile botulism is most common. The toxin consists of

a heavy and a light chain, and enters the terminal motor axon via receptor-mediated endocytosis. Inside the axon, the light chain interferes with proteins involved in fusion of the synaptic vesicle with the terminal membrane, including synaptobrevin (part of the synaptic vesicle membrane), SNAP-25, and syntaxin (both part of the presynaptic membrane). The effect on the nerve terminal is irreversible and, if severe enough, results in denervation of the myofiber. Recovery in those cases is prolonged, because it requires nerve regeneration and reinnervation.

Infantile botulism occurs between 2 wk and 6 mo of age, and presents as hypotonia, constipation, poor feeding, and dyspnea during the course of hours or days. Food-borne botulism affects older children and adults and begins abruptly, with diplopia, ptosis, dysphagia, limb weakness, and respiratory compromise. External ophthalmoplegia, papillary paralysis, areflexia or hyporeflexia, and limb weakness are found on examination. Respiratory failure can lead to death, but supportive care and use of botulism antitoxin provide for a good prognosis.

The diagnosis should be suspected clinically and can be made by documenting the presence of botulinum toxin. The diagnosis is usually confirmed using electrodiagnosis. CMAPs are always small at rest. Facilitation greater than 100% is observed in 90% of patients but requires a more sustained effort or a longer period of high-rate stimulation. Repetitive stimulation will show a decrement if the CMAP is not too low for decrement to be accurately measured. Routine EMG will often reveal fibrillations and positive waves, with a myopathic recruitment pattern consisting of small, brief, polyphasic MUPs that are early recruited to a full interference pattern. SFEMG will show increased jitter and blocking, which may inversely correlate with MUP firing rate. Fiber density will increase as reinnervation proceeds.

3.2. Postsynaptic Disorders of NMT

3.2.1. Myasthenia Gravis

MG is an autoimmune disorder in which polyclonal antibodies are directed against the nicotinic acetylcholine receptor of skeletal muscle. This results in degradation of the NMJ, with simplification of the secondary synaptic clefts, loss of acetylcholine receptors, and failure of NMT. The loss of receptors results in a MEPP that has decreased amplitude, leading to a decreased EPP. The clinical hallmark of the disease is fatigable weakness, usually after repetitive action, causing intermittent symptoms, such as ptosis, diplopia, dysphagia, dysarthria, and facial and limb muscle weakness. Respiratory compromise can occur in severe cases. The disease has a bimodal peak incidence, affecting older men and young women of childbearing age.

Although the clinical history and examination are often typical and highly suggestive, confirmation of the diagnosis rests on pharmacological, immunological, and electrodiagnostic grounds. Edrophonium (Tensilon) administered intravenously will quickly but briefly reverse the signs of MG and serves as a good bedside test. Assay for the presence of serum acetylcholine receptor antibodies is very specific for MG, and is abnormal in 70 to 90% of cases. Sensitivity is lower in patients with only ocular signs.

Electrodiagnostic methods most useful in the diagnosis of MG are RNS and SFEMG. The results of RNS are most likely to be abnormal in patients with generalized disease, and when testing proximal muscles, but even then sensitivity is only in the 70% range if multiple muscles are tested. Decrement is most likely with low rates of stimulation, 3 to 5 Hz, after a period of maximal muscle contraction. In patients with decrement at rest, exercise will increase the amount of decrement. At high rates of stimulation, facilitation of the CMAP may mask decrement. The decrement tends to be maximal by the fourth stimulus in a train and often will repair by the ninth. This U-shaped decrement is common in MG but not specific.

SFEMG remains the most sensitive test for dysfunction of NMT and the most sensitive diagnostic test for MG, reaching a 98% yield if two muscles are tested when the first is normal. This is true for ocular as well as generalized disease. SFEMG is also useful in the management of MG because it is a faithful and sensitive indicator of the status of NMT, unlike receptor antibody assay and RNS.

Treatment may address symptoms only or may be curative. Anticholinesterases can be used to briefly abate or improve symptoms attributable to MG, but will not affect the underlying immunological dysfunction. These drugs work by inhibiting the breakdown of acetylcholine, the neurotransmitter released by terminal motor nerve fibers. Edrophonium, neostigmine, and pyridostigmine (Mestinon) are all anticholinesterases, the latter most commonly used because of its longer duration of action (2–4 h) and lesser muscarinic side effects. Mestinon is commonly used by itself in mild cases and in conjunction with immune suppression in more severe cases. Side effects include diaphoresis, hypersalivation, diarrhea, nausea, abdominal cramping, bradycardia, and fasciculations. Intravenous dosages of pyridostigmine and neostigmine are 1/30 of the oral dose for both drugs.

Suppression of the immune system attack on the acetylcholine receptor is indicated when the disease is generalized, involves vital functions, such as ventilation or swallowing, or is not amenable to symptomatic treatment alone. Various treatments can be used, including corticosteroids, immune suppressants, such as azathioprine, mycophenylate, mofetil and cyclosporine, plasmapheresis, intravenous human immunoglobulin, and thymectomy. Corticosteroids can cause worsening of symptoms at initiation of therapy and patients must be carefully watched early on, preferably as inpatients. This worsening can be limited by starting patients on very low doses, with a slow titration upward in dose, although this delays clinical benefit. Plasmapheresis is indicated in the severely compromised patient, in the patient refractory to other treatment modalities, and in the patient in whom an immediate response is required.

Thymomas are present in 10 to 15% of patients with MG, and MG occurs in 30% of patients with thymomas. Thymic hyperplasia is present in another 70% of patients with MG. The thymus gland is the likely site for initial sensitization to the acetylcholine receptor. Removal of thymic tissue increases remission rate from 15 to 30%, and results in significant clinical improvement in two-thirds of patients, although the improvement may take up to 5 yr.

3.2.2. Congenital Myasthenic Syndromes

Congenital myasthenic syndromes are genetic disorders affecting components of the NMJ important in the normal function of NMT. The first clinical description was in 1937, but the realization that they were not related to MG occurred only after the autoimmune nature of MG was defined in the 1970s. Many such syndromes have now been defined either by their physiology, their clinical manifestations, or their ultrastructural characteristics, affecting presynaptic, synaptic, and postsynaptic function. Postsynaptic syndromes account for 76% of cases, with end-plate acetylcholinesterase deficiency accounting for 13% of cases and presynaptic syndromes, 8%. There is neither the space nor the inclination to detail each of the syndromes, but a notation of the actual defect usually suffices to illustrate why there is a problem (Table 1).

Onset of symptoms may be in the neonatal period, childhood, or adulthood. The hallmark of all is fatigable weakness, that is, increasing weakness with continued exertion. This can involve ocular, bulbar, limb, and respiratory muscles. Antibodies to acetylcholine receptors are always absent, by definition. The Tensilon test is variably useful depending on the defect, and obviously not useful in end-plate acetylcholinesterase deficiency. A decremental

Table 1
Congenital Myasthenic Syndromes With Location and Description

Presynaptic	Paucity of synaptic vesicles and reduced quantal release
	Defect in ACh resynthesis or packaging
	CMS resembling Lambert–Eaton syndrome
Synaptic	End-plate acetylcholinesterase deficiency
Postsynaptic	Increased response to ACh: slow-channel syndromes
	Decreased response to ACh: low-affinity fast-channel syndromes
	Decreased response to ACh: fast-channel syndrome secondary gating abnormality
	Decreased response to ACh: mode-switching kinetics
	Receptor deficiency secondary recessive mutations in receptor subunits
Unknown	Familial limb–girdle myasthenia
	Benign CMS with facial malformations

ACh, acetylcholine; CMS, congenital myasthenic syndromes.

response to RNS at low rates of stimulation is also important in diagnosis, as are abnormal jitter and blocking during SFEMG. At times, patients with impaired resynthesis of acetylcholine or vesicular packaging will have normal electrodiagnostic studies when asymptomatic. Patients with end-plate acetylcholinesterase deficiency or slow-channel syndrome will have repetitive CMAPs to single stimuli.

SUGGESTED READING

AAEM Professional Practice Committee, Chiou-Tan FY, Gilchrist JM, Tim RW. Practice parameter for repetitive nerve stimulation and single fiber electromyographic evaluation of adult patients with suspected myasthenia gravis or Lambert–Eaton myasthenic syndrome: summary statement. Muscle Nerve, 2001.

Engel AG, ed. Myasthenia Gravis and Myasthenic Disorders. Oxford University Press, New York, NY, 1999.

Fon EA, Edwards RH. Molecular mechanisms of neurotransmitter release. Muscle Nerve 2001;24:581–601.

Gilchrist JM. Myasthenia gravis. In: Current Diagnosis in Neurology (Feldmann E, ed.). Mosby, St. Louis, MO, 1994, pp. 350–352.

Lindstrom JM. Acetylcholine receptors and myasthenia. Muscle Nerve 2000;23:453–477.

Maselli RA. Electrodiagnosis of disorders of neuromuscular transmission. Ann NY Acad Sci 1998; 841:696–711.

Maselli RA, Bakshi N. Botulism. AAEM case report #16. Muscle Nerve 2000;23:1137–1144.

Oh SJ, Doo EM, Kuruoglu R, Bradley RJ, Dwyer D. Diagnostic sensitivity of the laboratory tests in myasthenia gravis. Muscle Nerve 1992;15:720–724.

Sanders DB, Stalberg EV. AAEM minimonograph #25: Single fiber electromyography. Muscle Nerve 1996;19:1069–1083.

Stalberg E, Trontelj JV. Single Fiber Electromyography. Studies in Healthy and Disease Muscle. 2nd Ed. Raven Press, New York, NY, 1994.

REVIEW QUESTIONS

1. Normal presynaptic release of acetylcholine is dependent on:
   A. A VGCC of the P/Q type.
   B. Synaptobrevin.
   C. SNAP-25.

D. Vesicular acetylcholine transporter.
E. All of the above.

2. The immediately available pool of acetylcholine vesicles is:
   A. Rapidly depleted by a train of presynaptic action potentials.
   B. Mobilized to replenish the active zones.
   C. Expanded in proportion to the postsynaptic depolarization.
   D. Held constant by the vesicular acetylcholine transporter.
   E. Irrelevant to the normal function of NMT.
3. NMT safety factor:
   A. Is equal to the number of receptor activations necessary to reach threshold for action potential activation.
   B. Is not a factor in normal NMT.
   C. Refers to the EPP in excess of that necessary to reach threshold for action potential activation.
   D. Fails frequently during exercise in healthy people.
   E. Is determined solely by the number of available acetylcholine receptors.
4. Lambert–Eaton syndrome:
   A. Is a postsynaptic disorder of VGCCs.
   B. Is caused by a defect in acetylcholine vesicle release.
   C. Is not readily diagnosed by repetitive nerve stimulation.
   D. Improves with continued exercise.
   E. Is less likely than botulism to show changes with continued exercise.
5. EMG:
   A. Is irrelevant to the examination of disorders of NMT.
   B. Will show MUP variation only with strong contractions.
   C. Will show abnormalities of motor unit variation that are specific to MG.
   D. Will show denervation potentials in most cases of LEMS.
   E. Can reveal MUP amplitude variation suggestive of defective NMT.
6. Repetitive nerve stimulation:
   A. Is the most sensitive test for disorders of NMT.
   B. Is best performed at high rates of stimulation.
   C. Should be performed both before and after a period of maximum voluntary muscle contraction.
   D. Most commonly shows facilitation 2 to 3 min after exercise.
   E. Does not require impulse blockade to be abnormal.
7. SFEMG:
   A. Is usually performed with a low filter of 20 Hz, using a concentric needle electrode.
   B. Performed by axonal stimulation allows the examiner to determine fiber density.
   C. Is a sensitive measure of NMT safety factor.
   D. Can differentiate presynaptic from postsynaptic disorders.
   E. Is abnormal only in primary disorders of NMT.
8. The clinical characteristics of LEMS:
   A. Are indistinguishable at the bedside from MG.
   B. Often involve oculomotor function.
   C. Always indicate an underlying lung cancer of the small cell type.
   D. Include areflexia and autonomic dysfunction.
   E. Are obvious and rarely missed.
9. Botulism:
   A. Is caused by a toxin directed against acetylcholinesterase in the basal laminar matrix of the primary synaptic cleft.
   B. Is a presynaptic disorder of the VGCCs.
   C. Does not show abnormalities on repetitive nerve stimulation.
   D. Will reveal facilitation after exercise.
   E. Does not involve oculomotor function.

10. MG:
    A. Is characterized by fatigable weakness.
    B. Shows maximal decrement by the fourth stimulus in a train.
    C. Does not require SFEMG for diagnosis.
    D. Is characterized by a MEPP of decreased amplitude.
    E. All of the above.

REVIEW ANSWERS

1. The correct answer is E. VGCCs, synaptobrevin, SNAP-25, and vesicular acetylcholine transporter all participate in the release of acetylcholine from the presynaptic terminal.
2. The correct answer is A. The immediate pool is rapidly depleted by a train of presynaptic action potentials and is not expanded in proportion to the postsynaptic depolarization. The mobilization or reserve pool is mobilized to replenish the immediate pool. The immediate pool is not held constant by the acetylcholine transporter and is, in fact, very relevant to normal NMT.
3. The correct answer is C. The NMT safety factor refers to the EPP in excess of that necessary to reach threshold. It is not the number of receptor activations needed to reach threshold and it is a major factor in normal NMT. However, it does not fail during exercise in healthy people (there continues to be some safety factor normally present even after intense exercise and rapid rates of stimulation). It is determined, in part, by the number of acetylcholine receptors as well as the amount acetylcholine in the NMJ.
4. The correct answer is D. LEMS improves with repeated exercise. It is a presynaptic disorder of VGCC, it is not caused by a defect in acetylcholine release, it is readily diagnosed by repetitive nerve stimulation (with a decrement at slow rates of stimulation and an increment with high rates), and is *more* likely than botulism to show improvements with continued exercise.
5. The correct answer is E. EMG will show unstable MUPs with varying amplitude in patients with defective NMT of any cause and are not necessarily dependent on the strength of contraction (hence, EMG is not irrelevant to examination of disorders of NMT, but there are no abnormalities specific to any one disorder). Denervation potentials would be distinctly uncommon in LEMS (although they are relatively common in botulism).
6. The correct answer is C. To elicit abnormalities on repetitive stimulation, it should be performed both before and after a period of maximum voluntary muscle contraction. It is not the most sensitive test (SFEMG is). It should be performed at slow rates for postsynaptic disorders and rapid rates for presynaptic disorders. Facilitation should occur immediately after exercise (after exercise exhaustion usually is maximal at 2–3 min after exercise). It does require impulse blockade to be abnormal.
7. The correct answer is C. SFEMG provides an excellent measure of the safety factor of NMT. It is usually performed with a single-fiber electrode (not a concentric needle electrode) with a low-frequency filter set to approx 2 kHz. Axonal stimulation is not necessary to determine fiber density. It cannot differentiate presynaptic from postsynaptic disorders. It is a nonspecific finding and can be abnormally elevated in almost any form of neuromuscular disease.
8. The correct answer is D. Areflexia and autonomic dysfunction are usually associated with LEMS. LEMS and MG usually are quite distinct, although, in some rare cases, they can look similar. Oculomotor function is rarely if ever involved in LEMS. It is not always associated with small cell carcinoma of the lung (it can occur as an independent autoimmune disorder). LEMS can be difficult to diagnosis and the disorder can be overlooked.
9. The correct answer is D. Botulism usually demonstrates facilitation after exercise, although in severe cases facilitation may not occur. It is not caused by antibodies directed against acetylcholinesterase or the VGCCs, but rather against the proteins involved in fusion of the synaptic vesicle with the terminal membrane. Abnormalities on repetitive stimulation are to be expected and involvement of oculomotor function is quite common.
10. The correct answer is E. All of the choices are typically observed in MG.