

# The Control of Neuromuscular Transmission in Health and Disease

MARK M. RICH

*Department of Neuroscience, Cell Biology and Physiology, Wright State University, Dayton, OH*

Multiple techniques are available to study failure of neuromuscular transmission. Electrophysiological techniques used in patients are well suited to detect failure of neuromuscular transmission; however, these methods offer little insight into the mechanisms underlying failure of transmission. More detailed techniques that are better suited for studying the underlying mechanisms can be performed in animal models of neuromuscular disease. However, it is often difficult to compare studies using different techniques to measure neuromuscular transmission. In this review, I discuss different techniques that are available to study failure of neuromuscular transmission. The strengths and weaknesses of various techniques are compared using several diseases as examples. The review concludes with a discussion of mechanisms that may contribute to failure of neuromuscular transmission during repetitive stimulation. *NEUROSCIENTIST* 12(2):134–142, 2006. DOI: 10.1177/1073858405281898

**KEY WORDS** *Neuromuscular junction, Myasthenia gravis, Motor neuron disease, Lambert-Eaton myasthenic syndrome, Synaptic transmission, Neuronal plasticity*

Neuromuscular transmission is the process that translates a motor neuron action potential to action potentials in all the muscle fibers contacted by that motor neuron. The steps in neuromuscular transmission are as follows: 1) an action potential invades the presynaptic motor nerve terminal. 2) Depolarization of the nerve terminal opens calcium channels and causes influx of calcium into the nerve terminal. 3) Influx of calcium triggers fusion of synaptic vesicles to the membrane of the motor nerve terminal and release of acetylcholine. 4) Acetylcholine diffuses across the synaptic cleft, binds acetylcholine receptors, opens them, and causes depolarization of the postsynaptic muscle fiber. 5) Depolarization of the muscle fiber triggers the opening of voltage-gated sodium channels, which trigger an action potential in the muscle fiber, and completes transmission of the action potential from the motor neuron to muscle fibers.

Failure of neuromuscular transmission occurs when depolarization of muscle fibers triggered by a nerve action potential is insufficient to trigger muscle fiber action potentials. Transmission failure during repetitive stimulation is a hallmark of diseases of neuromuscular transmission such as myasthenia gravis (MG) and Lambert-Eaton myasthenic syndrome (LEMS). In this review, I discuss differences in the information acquired using the current techniques available to study neuromuscular transmission. I also discuss some theories

about mechanisms underlying failure of neuromuscular transmission during repetitive stimulation.

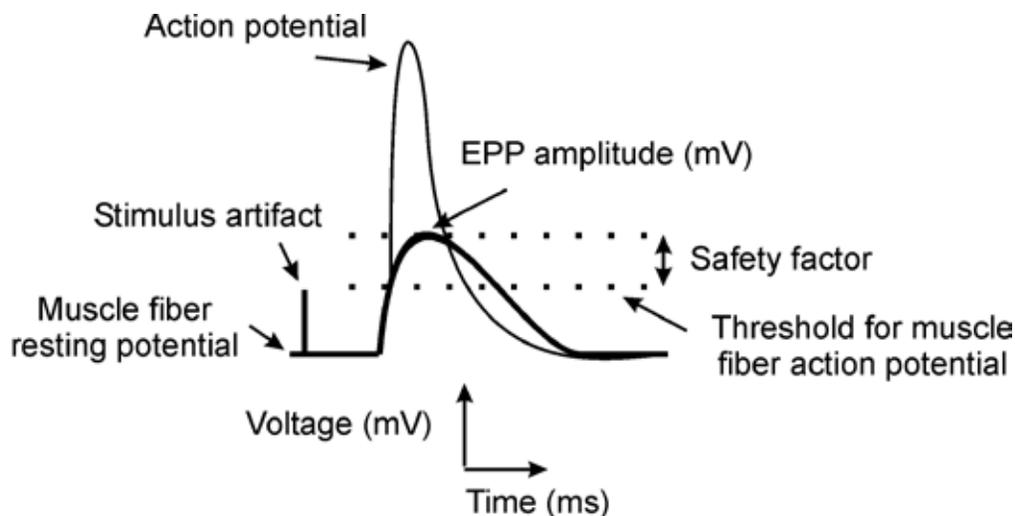
## Techniques Used to Measure Failure of Neuromuscular Transmission in Patients

In patients, the electromyography (EMG) technique most commonly used to detect failure of neuromuscular transmission is repetitive stimulation of the motor nerve. In this technique, the amplitude of the sum of all the muscle fiber action potentials following nerve stimulation is measured. This is known as the compound muscle action potential (CMAP) and is an indicator of the number of muscle fibers firing action potentials. CMAPs range in amplitude in normal muscles from approximately 2 to 8 mV (Preston and Shapiro 1998; Kimura 2001). A decrease in the CMAP amplitude during repetitive stimulation is the gold standard for measuring failure of neuromuscular transmission, in that it indicates that some muscle fibers are no longer firing action potentials in response to nerve stimulation. The percentage decrement in the CMAP corresponds directly to the percentage of neuromuscular junctions (NMJs) at which there is failure of neuromuscular transmission.

Relatively severe disruption of neuromuscular transmission must occur before there is a decrement of the CMAP during repetitive stimulation. The reason for this is that the safety factor for synaptic transmission is high at the NMJ. One definition of safety factor is the difference between the potential at which a muscle fiber action potential is triggered and the potential reached due to opening of acetylcholine receptors (the endplate potential, EPP) in the absence of a muscle fiber action potential (Fig. 1). Another definition of safety factor is

This work was supported by NS40405, MMR. I thank Dr. Kathrin English for her comments on the manuscript.

**Address correspondence to:** Mark Rich, Wright State University, 014 M and M, 3640 Colonel Glenn Hwy, Dayton OH 45435 (e-mail: mark.rich@wright.edu).



**Fig. 1.** Safety factor at the neuromuscular junction. Shown is a cartoon of two superimposed traces from a neuromuscular junction. In one trace, the muscle sodium channels are blocked and there is no action potential (thick line). In the second trace, the sodium channels are active and an action potential is triggered by the endplate potential (EPP) (thin line). The lower horizontal dotted line represents the potential at which an action potential is initiated by the EPP. The higher dotted line represents the potential that is achieved by the EPP in the absence of muscle fiber sodium current. The difference in potential between the two lines is the safety factor. The stimulus artifact is a signal that is caused by nerve stimulation with an electrode that in turn triggers the EPP.

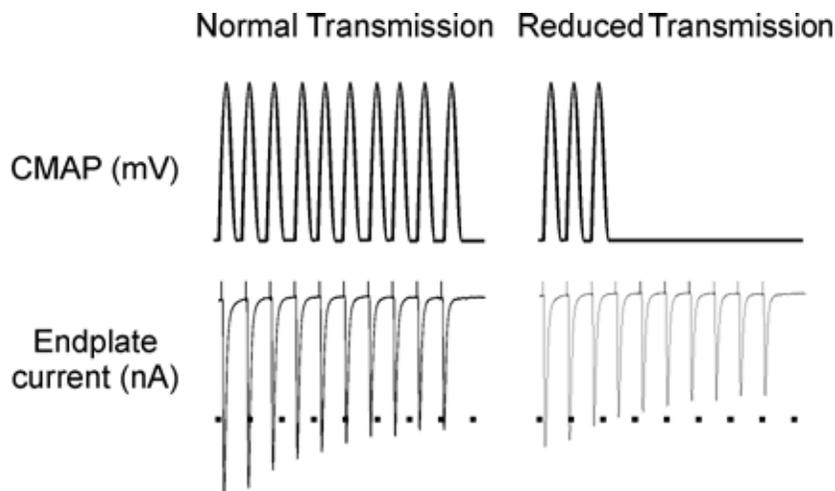
the number of vesicles of acetylcholine released compared to the number required to fire an action potential. For a more detailed discussion of the factors that contribute to safety factor, please see Wood and Slater (2001). In cases where neuromuscular transmission is only mildly affected, there is no decrement of the CMAP during repetitive stimulation because the synaptic current remains sufficient to trigger muscle fiber action potentials in all the fibers. Thus, decrement of the CMAP during repetitive stimulation is an insensitive test for disruption of neuromuscular transmission. The safety factor varies a great deal between species and muscles (Wood and Slater 2001). In man, the safety factor may be as low as a two (Elmqvist and others 1964). Having a relatively large safety factor at the NMJ may serve to allow for significant reduction of synaptic current during high rates of stimulation without failure of neuromuscular transmission (see Fig. 2, normal transmission).

In practice, it is difficult to use CMAP amplitude to study failure of neuromuscular transmission at rates of motor neuron firing that occur during voluntary movement (5 to 40 Hz) (Person and Kudina 1972). Stimulation of nerves at high rates is extremely painful and causes movement of the electrode, which introduces errors in measurement of the CMAP. One way that high-frequency stimulation can be avoided is to measure the CMAP amplitude before and immediately after a brief period of vigorous exercise in patients (Preston and Shapiro 1998; Kimura 2001). In this way, the patient provides the repetitive stimulation without the pain of electrical stimulation. However, this method only allows comparison of one CMAP before and one after exercise. Another difficulty with this method is that there is a delay between the end of the period of exercise and measurement of the second CMAP amplitude. During this brief

(several-second) period of time, NMJs can recover from the synaptic activity. The delay in time allows a process known as posttetanic potentiation to occur. Posttetanic potentiation increases neurotransmitter release and thus obscures depression of synaptic transmission (Zucker and Regehr 2002).

Given that high rates of stimulation are painful and that measurement of the CMAP before and after exercise is complicated by posttetanic potentiation, the most commonly used clinical test of neuromuscular transmission is measurement of the CMAP during low rates of repetitive stimulation (2–3 Hz). An important consideration, however, is that low rates of repetitive firing of motor neurons does not occur *in vivo*, and it is thus difficult to use reduction in CMAP amplitude at 2 Hz to predict the degree of weakness that will be experienced by the patient.

Another concern with use of the CMAP to detect defects of neuromuscular transmission is that once the reduction in neuromuscular function is sufficiently severe, reduction of the CMAP can exaggerate the deficit. For example, in the NMJ with reduced transmission in Figure 2, depression of neuromuscular transmission would be 100% as measured by the CMAP. However, depression of the underlying endplate current is only 30%. The only difference between the NMJ with reduced transmission (with complete loss of the CMAP during repetitive stimulation) and the NMJ with normal transmission (with no reduction in CMAP) shown in Figure 2 is that in the normal NMJ, the initial synaptic current is 30% larger. Thus, because the safety factor plays such an important role in depression of the CMAP during repetitive stimulation, the initial amplitude of the endplate current can greatly affect the percentage decrement seen in the CMAP during repetitive stimulation.



**Fig. 2.** Decrements in compound muscle action potential (CMAP) versus endplate potential (EPP). The top row shows a cartoon of a theoretical decrement of CMAPs in response to repetitive stimulation at 50 Hz of a neuromuscular junction (NMJ) where synaptic transmission is normal and an NMJ where synaptic transmission is reduced. In the lower row are traces of an endplate current (EPC) of a normal mouse NMJ in response to 50 Hz stimulation. The trace shown for the normal is repeated for the reduced transmission panel, except the amplitude of the trace was reduced by 30%. The horizontal dotted line in the lower row represents amplitude of current necessary to trigger an action potential. In the normal NMJ, the EPC remains greater than the current necessary to trigger an action potential. Thus, there is no decrement in the CMAP despite a significant decrement in the EPC. In the NMJ with reduced neurotransmission, the 30% reduction in the EPC causes the endplate current to drop below threshold after the third stimulus. Inasmuch as muscle fiber action potentials are all or nothing, they decrease to 0 on the fourth stimulus. In this theoretical example, there is a much greater decrement in the CMAP than in the EPC that underlies neuromuscular transmission. It should be noted that in this figure EPC is plotted, rather than EPP as in Figure 1. The EPC goes down (to indicate inward current, which depolarizes fibers), whereas the EPP goes up (indicating depolarization of the fiber). In the CMAP trace, it is assumed for simplicity's sake that all neuromuscular junctions are responding in exactly the same way to repetitive stimulation.

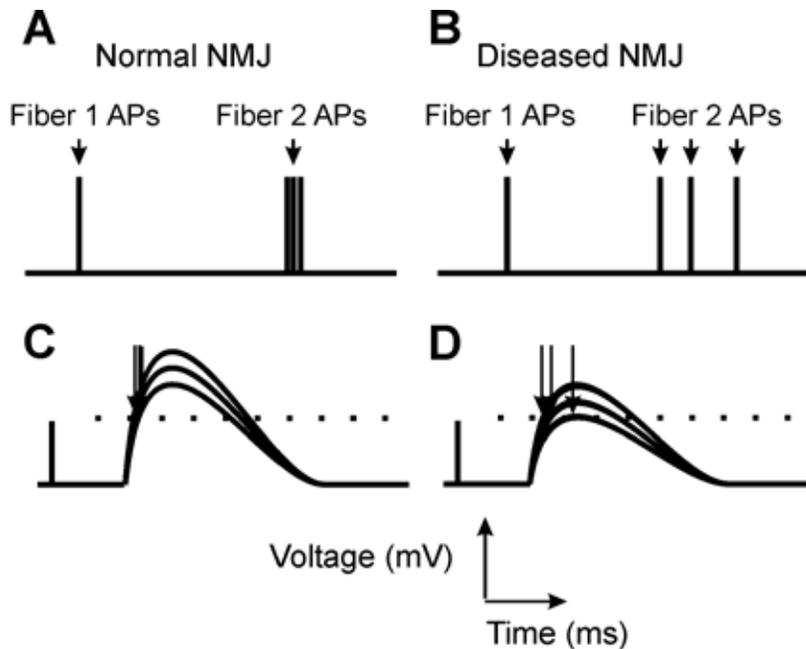
A more sensitive clinical measure of deficits of neuromuscular transmission is mean conduction delay (jitter) on single-fiber EMG (Preston and Shapiro 1998; Kimura 2001). In this test, two muscle fibers from an individual motor unit (a motor neuron and all the muscle fibers it innervates) are studied in isolation by using an EMG electrode that measures muscle fiber action potentials from only a small region of muscle. When the motor unit fires, both muscle fibers fire action potentials that occur with a set delay between them (due to differences in the length of the axons). The motor unit is activated repetitively either voluntarily by the patient or by stimulation with an electrode. In each trial, the difference in the timing of the two action potentials is measured. Variation in the timing between trials is then measured. If neuromuscular transmission is normal, the delay between the firing of the two muscle fibers will always be similar (Fig. 3A, normal NMJ). In diseases that decrease the safety factor for neuromuscular transmission, however, differences in timing between trials are increased (Fig. 3B). Jitter is more sensitive than reduction of the CMAP because it does not require failure of neuromuscular transmission for the diagnosis of neuromuscular dysfunction.

The increased variability in timing of muscle fiber action potentials shown in Figure 3B is caused by reduction of the safety factor for neuromuscular transmission. To understand why reduction of the safety factor leads to more variable delays between the firing of two muscle fibers from the same motor unit, it is necessary to understand the shape of the endplate potential that leads to firing of the muscle fiber action potential. The EPP is

caused by opening of acetylcholine receptors (AChRs) and has a rapidly rising phase followed by a plateau and then a slower decay phase (Fig. 3C). Endplate potentials at both normal and diseased NMJs are variable in amplitude owing to random variations in the number of synaptic vesicles that are released following a presynaptic action potential. As can be seen in Figure 3C, when the safety factor for neuromuscular transmission is high, variation in the amplitude of the endplate current has little effect on the timing of firing of the muscle fiber action potential because the action potential is triggered by the rapidly rising phase of the EPP. However, when the safety factor is low, muscle fiber action potentials are triggered near the plateau of the EPP (Fig. 3D) so that variations in the amplitude of the EPP lead to variable delays in the firing of the muscle fiber action potential (Elmqvist and others 1964).

### Techniques Used to Measure Failure of Neuromuscular Transmission in Animal Models

In animal models (and, rarely, in muscle biopsies from patients), microelectrodes are used to impale individual muscle fibers. The main difficulty in this type of study is that contraction of muscle fibers causes the microelectrodes to come out when the muscle fiber fires an action potential. This makes it necessary to block muscle fiber contraction. Muscle fiber contraction can be blocked by partially blocking AChRs with a toxin such as curare (Boyd and Martin 1956). Use of curare has the disadvantage that the response of the muscle fiber to release



**Fig. 3.** The mechanism underlying jitter. Shown in the top row are cartoons of three superimposed traces during a single-fiber EMG recording from a normal (A) and a diseased (B) muscle where there is neuromuscular dysfunction. In each case, there are two muscle fiber action potentials (APs) (represented as vertical lines). The first APs are used to trigger the sweep and are thus perfectly aligned in each trace and are shown as a single line. The second muscle fiber APs have a variable delay from the first and there are thus three vertical lines. When neuromuscular function is normal, there is little variability in the delay of the second muscle fiber AP, whereas when neuromuscular junctions (NMJs) are diseased, there is much greater variability. In the lower row are cartoons of three superimposed endplate potentials (EPPs) in a single control (C) and single diseased NMJ (D). In each sweep, the EPP is different in amplitude due to the random variability in the number of vesicles released. The horizontal dotted line represents the threshold for AP initiation, which is unchanged in the diseased NMJ. The vertical arrows in C and D represent the point in time when the EPP reaches threshold and triggers an AP. In the normal NMJ, the variability of the EPP causes little variability in the timing of AP initiation, whereas in the diseased NMJ, the variability in the amplitude of the EPP translates into significant variability in the timing of the AP. It is this variability that underlies the jitter seen on single-fiber EMG.

of an individual vesicle (quantal size) cannot be accurately measured. Another toxin that is sometimes used is  $\mu$ -conotoxin GIIIB, which specifically blocks muscle sodium channels and thus muscle contraction (Robitaille and Charlton 1992). This toxin works well but has the disadvantage that it is very expensive. A third method to eliminate muscle contraction is to lower extracellular calcium or raise extracellular magnesium to reduce quantal content below the value necessary to trigger muscle action potentials (Boyd and Martin 1956). This method is not useful for studying failure of synaptic transmission at the NMJ in normal extracellular calcium. The concentration of extracellular calcium profoundly affects behavior of the NMJ to repetitive stimulation such that results obtained in low extracellular calcium cannot be extrapolated to understand weakness in patients. Finally, muscle contraction can be removed by cutting the fibers (Barstad and Lilleheil 1968). This depolarizes muscle fibers and causes inactivation of sodium channels, which blocks action potential initiation. This technique has the disadvantage that depolarization of muscle reduces the driving force for current flow through AChRs to the point that measurement of quantal size is difficult.

With any of the techniques of removing muscle contraction, two methods of recording can be used. The first method uses a single electrode that is inserted into the muscle fiber and the potential of the fiber is measured following nerve stimulation to record the EPP in mV. The EPP is distinct from the CMAP, in that the EPP is

from an individual fiber and can only be measured after blocking muscle action potentials. The second method involves inserting two electrodes into the muscle fiber to voltage clamp the muscle fiber to record the endplate current (EPC) in nA. Voltage clamp has the advantage that changes in passive muscle fiber properties such as input resistance and capacitance do not affect the EPC, whereas they alter the EPP. Changes in muscle passive properties occur when the level of muscle activity is altered (Lomo and Rosenthal 1972). Because muscle activity is likely altered in most diseases of neuromuscular transmission, changes in passive properties complicate measures of the EPP. By combining voltage clamp of muscle fibers with cutting of muscle fibers, it is possible to increase the driving force for current flow through AChRs to the point where quantal size can be recorded and quantal content can be calculated in normal extracellular calcium (Glavinovic 1979b).

Using voltage clamp and the cut fiber preparation, it is possible to record depression of the EPC in normal extracellular calcium during repetitive stimulation of individual NMJs (Fig. 2). In voltage clamp recordings, muscle fiber action potentials are not present, and other muscle fiber properties such as specific membrane resistance have no effect. This allows the experimenter to study performance of the NMJ without complications introduced by changes in muscle excitability. One difficulty introduced in voltage clamp studies is that because muscle fiber action potentials are prevented, the studies

do not allow the experimenter to directly study failure of neuromuscular transmission. To best understand diseases of neuromuscular transmission, nerve conduction/EMG techniques should be combined with microelectrode studies. Comparisons of nerve conduction/EMG techniques to microelectrode studies are presented below for several diseases in which there is dysfunction of the NMJ.

### **Mechanisms Underlying Neuromuscular Dysfunction**

Anything that reduces the safety factor for neuromuscular transmission can weaken neuromuscular transmission. Possibilities include 1) reduction in release of acetylcholine, 2) reduction in sensitivity of the postsynaptic membrane to acetylcholine, or 3) an elevation in the threshold for muscle fiber action potential initiation.

#### *Reduced Release of Acetylcholine—LEMS*

In Lambert Eaton myasthenic syndrome (LEMS), the defect in neuromuscular transmission is due to antibodies to presynaptic Ca channels (Sanders 2003). The antibodies cause a reduction in presynaptic calcium entry, which in turn reduces quantal content. In LEMS, there is often a dramatic increase in CMAP amplitude during high rates of repetitive stimulation. This is known as facilitation and is a hallmark of LEMS and botulism. The facilitation of the CMAP in LEMS can be several hundred percent (Preston and Shapiro 1998; Kimura 2001).

To understand facilitation of neuromuscular transmission in LEMS, it is necessary to understand the behavior of normal NMJs to repetitive stimulation in solution containing reduced extracellular calcium. It has been shown that reducing extracellular calcium at the NMJ causes a change from the normal depression of the EPP that occurs during high rates of repetitive stimulation to facilitation (Lundberg and Quilisch 1953). If the defect in LEMS is due to reduction of calcium entry, one might expect facilitation to occur in solution containing normal extracellular calcium. This is what has been found (Elmqvist and Lambert 1968). However, it has been shown in chromaffin cells exposed to LEMS sera that facilitation in LEMS may be increased beyond what would be expected for the reduction in calcium entry (Engisch and others 1999). Thus, the facilitation in LEMS may be due to several processes. In LEMS, there is no evidence of postsynaptic compensation for the presynaptic defect in neurotransmitter release, so electrophysiological findings are easier to interpret.

#### *Reduced Release of Acetylcholine—Motor Neuron Disease*

The traditional idea is that weakness in motor neuron disease is primarily due to death of motor neurons. However, increased jitter has been reported in patients with amyotrophic lateral sclerosis (ALS) (Stalberg and others 1975). This finding raises the possibility that neuromuscular dysfunction contributes to weakness in ALS.

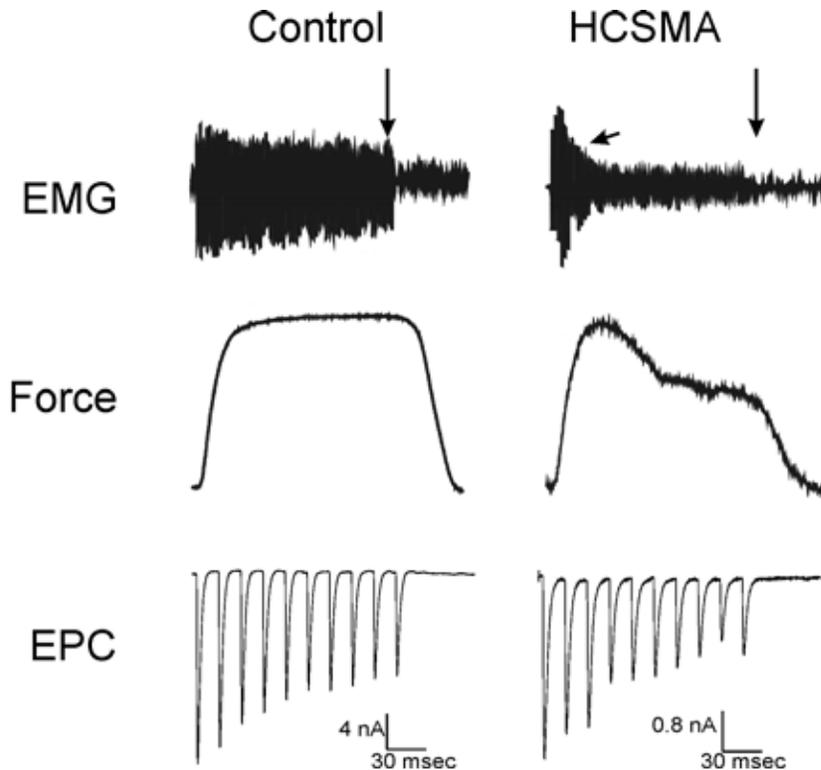
It has been widely assumed that evidence of neuromuscular dysfunction (jitter) in ALS is due to the presence of newly reinnervated NMJs (Kimura 2001). The idea has been that the newly reinnervated NMJs are immature and have failure of neuromuscular transmission with repetitive stimulation. The alternative, however, is that the jitter and CMAP decrements are early stages of ALS in NMJs that have yet not undergone degeneration.

Understanding the cause of weakness in ALS has therapeutic implications. If weakness in ALS is due to motor neuron cell death, then the focus should be on prevention of cell death. If, however, failure of neuromuscular transmission contributes to weakness, then prevention of motor neuron cell death might not be sufficient to maintain strength. One will also need a treatment that keeps motor neurons healthy enough to maintain neuromuscular transmission. One can imagine a situation where one entirely prevents motor neuron cell death but weakness progresses because the loss of neuromuscular function continues. This is the case in an animal model of motor disease known as HCSMA.

Hereditary canine spinal muscular atrophy (HCSMA) is an autosomal-dominant disease of motor neurons occurring in dogs (Pinter and others 2001). The gene defect in the disease has not yet been identified, but dogs that appear to be homozygous for the defect have a rapidly progressive form of motor neuron disease. The first suggestion that weakness in this disease was due to failure of neuromuscular transmission was that there was decrement of individual motor unit amplitudes (equivalent to the CMAP of a single motor unit) during high-frequency repetitive stimulation of the motor nerve (Pinter and others 1997). Such a decrement would not occur if motor neuron loss or neuropathy were the cause of weakness. High-frequency stimulation was possible in this disease model because the dogs were anesthetized for the EMG study. The decrement was often dramatic, indicating that weakness was due to failure of neuromuscular transmission (Fig. 4).

Voltage clamp studies were performed to better understand the mechanism underlying the failure of neuromuscular transmission. Quantal size was normal, suggesting that postsynaptic sensitivity to acetylcholine was unaltered. When quantal content (the number of vesicles released by a nerve action potential) was measured, there was a significant reduction, which indicates the defect in neuromuscular transmission was due to release of fewer vesicles (Rich and others 2002). At first glance, HCSMA thus appears similar to LEMS. However, the response of the repetitive stimulation in the two disorders is very different. In LEMS, there is an increment in the CMAP with high-frequency stimulation, whereas in HCSMA, there is a dramatic decrement.

Given the dramatic decrement of the EMG (often greater than 80%) in HCSMA, one might expect that there is an increase in the depression of the EPP (or EPC in voltage clamp). However, the percentage decrement of the EPC during repetitive stimulation is unaltered in HCSMA (Rich and others 2002) (Fig. 4). The explanation for this difference between the behavior of the



**Fig. 4.** Failure of motor units to maintain force in hereditary canine spinal muscular atrophy (HCSMA). In the top row are shown the EMG traces from a single control and a single HCSMA motor unit during 100 Hz repetitive stimulation. In the control, the amplitude of the EMG signal is maintained until the stimulation is turned off (arrow). In the HCSMA motor unit, however, there is reduction in the amplitude of the EMG signal (short arrow) prior to cessation of stimulation (long arrow). This reduction (depression) is due to failure of neuromuscular transmission at individual neuromuscular junctions (NMJs). The second row shows the force generated by the motor units shown in the top row. The control motor unit maintains force until the stimulation is stopped (with a brief delay for relaxation). The HCSMA motor unit, however, has reduction of force due to failure of neuromuscular transmission. In the bottom row are shown voltage clamp recordings from individual muscle fibers from a control and an HCSMA muscle. The muscle fibers in the bottom row are not from the motor units shown above but are meant to be representative of the underlying process. The amount of depression of the endplate current (EPC) in the HCSMA endplate is similar to the control in the example shown. Note, however, that the scale bars are different for the control and HCSMA EPCs. The control EPC is actually five times the amplitude of the EPC from the HCSMA muscle; quantal content is 26 in the control endplate and is 6 in the HCSMA endplate. From Balice-Gordon and others (2000) and Rich and others (2002).

CMAP and the EPC in HCSMA highlights the difference in the two measures. In HCSMA, quantal content is decreased in diseased NMJs. Thus, the normal decrement of the EPC during repetitive stimulation causes a majority of EPPs to fall below threshold for action potential initiation and causes decrement of the CMAP (see Fig. 2). Thus, failure of neuromuscular transmission appears to account for much, if not all, of the weakness in HCSMA.

Evidence of neuromuscular dysfunction prior to cell death has also been obtained in the superoxide dismutase (SOD) mouse model of ALS. This mouse was generated by overexpressing a mutation of the SOD gene cloned from patients with familial ALS (Gurney and others 1994). There is evidence that degeneration of motor axons precedes motor neuron cell death by a prolonged period in the SOD mouse (Kennel and others 1996; Frey and others 2000; Fischer and others 2004). Although physiological studies have not been done to determine whether there is widespread failure of neuromuscular transmission in the SOD mouse, it seems likely that neuromuscular pathology is an important contributor to weakness in this model as well. The finding of neuromuscular degeneration/dysfunction prior to cell death in at least two animal models of motor neuron disease

raises the possibility that failure of neuromuscular transmission is a significant contributor to weakness in patients with ALS.

#### *Reduced Sensitivity to Acetylcholine—Myasthenia Gravis*

In MG, the defect in neuromuscular transmission is usually due to antibodies directed at the postsynaptic AChR (Vincent and others 2003). The presence of antibodies causes both removal of AChRs (Kao and Drachman 1977; Drachman 1994) and destruction of regions of endplates (Rich and others 1994). The loss of endplate regions and AChRs reduces the postsynaptic response to release of ACh. Decreased ACh sensitivity is manifested as a reduced postsynaptic response to an individual vesicle of neurotransmitter (Cull-Candy and others 1978). Reduction in safety factor due to reduced postsynaptic sensitivity to ACh causes neuromuscular dysfunction such that during repetitive stimulation, the normal decrement of the EPP causes failure of neuromuscular transmission at some junctions. This failure underlies the decrement of the CMAP during repetitive stimulation.

Despite the primary nature of the postsynaptic problem in MG, there is evidence suggesting presynaptic involve-

ment as well. One consistent finding in patients and animal models of MG is an increase in quantal content (Cull-Candy and others 1978; Plomp and others 1992; Plomp and others 1995). One explanation that has been advanced is that there is a presynaptic receptor for ACh that, when blocked, causes an up-regulation of release of ACh (Glavinovic 1979a; Takamori and others 1984; Santafe and others 2003). Another explanation is that the up-regulation of ACh release is due to compensation by the NMJ. It has been found that following a chronic block of activity of both nerve and muscle, there is an increase in quantal content (Snider and Harris 1979; Tsujimoto and others 1990; Wang and others 2004) that could serve to offset the reduction in sensitivity to acetylcholine. The presence of both pre- and postsynaptic changes in synaptic function in MG suggests caution must be used when interpreting alterations in function of the NMJ in other diseases. Whereas some changes may be due to the primary disease process, other changes may be due to compensatory mechanisms.

#### *Elevation in Muscle Fiber Action Potential Threshold—Myasthenia Gravis, Critical Illness Myopathy*

Elevation of the threshold for muscle fiber action potential initiation is a potential cause of failure of neuromuscular transmission that is often not considered. It has been shown that the autoimmune response directed against the endplate AChR in MG may cause loss of sodium channels from the postsynaptic region of the endplate (Ruff and Lennon 1998). The loss of sodium channels from the endplate region significantly increased the amount of current injection needed to initiate an action potential (Ruff and Lennon 1998). Thus, an increase in the depolarization needed to initiate a muscle fiber action potential contributes to the reduction of the safety factor in MG.

It has been found that muscle fiber excitability is reduced in critically ill patients (Rich and others 1996; Rich and others 1997). It seems likely that during recovery from muscle fiber inexcitability, there would be a period where the safety factor for neuromuscular transmission is reduced owing to an elevated threshold for action potential initiation. Decrements of the CMAP during repetitive stimulation have been found in critically ill patients, but these have always been ascribed to persistent neuromuscular blockade (Gooch 1995). It may be that reduced muscle excitability plays a role as well.

#### **Factors That Might Contribute to Failure of Neuromuscular Transmission during Repetitive Stimulation**

##### *Depletion of Synaptic Vesicles*

One of the most prominent ideas is that depression of neuromuscular transmission during repetitive stimulation is due to depletion of synaptic vesicles (Zucker and Regehr 2002). The depletion model in its simplest

form proposes that each synapse has a limited number of vesicles that are capable of being released. When an action potential invades the nerve terminal, a fraction of the vesicles are released, leaving behind fewer vesicles to be released when the next action potential arrives. For example, if an NMJ has 1000 vesicles, each with a 10% probability of release, quantal content in response to a nerve action potential will be 100. If a second action potential invades the nerve terminal soon after the first, there will only be 900 vesicles available, and only 90 vesicles will be released leaving 810 vesicles. A third action potential would release 81 vesicles, and so on. A prediction of this model is that gradual depletion of vesicles leads to a monoexponential decay of the synaptic current. However, synaptic depression usually plateaus after a number of stimuli. This is explained by refilling of the vesicle pool with time, such that during a prolonged period of stimulation, an equilibrium between depletion and refilling is reached (Zucker and Regehr 2002).

In addition to the monoexponential decay, the depletion model makes two predictions:

1. The faster a synapse is stimulated, the greater the depression because there is less time to refill the vesicle pool. Although this is generally the case in normal external calcium, it is not true in LEMS. In LEMS, there is depression of the CMAP during slow rates of stimulation (Preston and Shapiro 1998; Kimura 2001). If the depression in LEMS is due to depletion, faster rates of stimulation should cause greater depression, but instead there is facilitation of the CMAP amplitude. The finding that faster rates of stimulation do not always lead to greater depression has led to the idea of a reserve pool of vesicles that are recruited rapidly during fast rates of repetitive stimulation (Zucker and Regehr 2002). The idea is that during rapid rates of stimulation, the rise in intracellular calcium is greater, and this triggers the recruitment of vesicles to replenish the readily releasable pool.
2. If fewer vesicles are released, depression should be reduced. This prediction has been confirmed at the NMJ when release of vesicles is decreased by lowering extracellular calcium (Lundberg and Quilisch 1953). However, in HCSMA, reduction of the number of vesicles released has no effect on the amount of depression of the synaptic current (Rich and others 2002). In LEMS, fewer vesicles are released than in control, but there is depression of the CMAP at slow rates of repetitive stimulation (Preston and Shapiro 1998; Kimura 2001). Both of these situations suggest that reduction in the number of vesicles released may not always reduce depression of neuromuscular transmission as would be predicted by the depletion model. Thus, although depletion may play an important role in depression of neuromuscular transmission with repeti-

tive stimulation, there are likely other processes that contribute as well.

### *Desensitization of Acetylcholine Receptors*

Desensitization of postsynaptic receptors is another potential cause of depression. At some synapses, this appears to be a prominent mechanism underlying depression (Otis and others 1996; Zucker and Regehr 2002). At the NMJ, there is pronounced desensitization of AChRs during prolonged application of ACh (Pennefather and Quastel 1982). Thus, desensitization is a theoretically plausible cause of depression at the NMJ. However, it has been shown at the NMJ that during trains of action potentials at rates of stimulation of 33 Hz or less, desensitization is not a significant contributor to depression (Magleby and Pallotta 1981).

### *Homosynaptic Modulation*

A third potential mechanism underlying depression is that the contents of synaptic vesicles contain molecules that feed back on the presynaptic terminal to reduce release. This mechanism is termed homosynaptic modulation. Synaptic vesicles at the NMJ contain ATP, which is hydrolyzed to adenosine. It has been suggested that during repetitive stimulation, buildup of adenosine contributes to depression at the NMJ (Hamilton and Smith 1991; Redman and Silinsky 1993). It appears that homosynaptic modulation cannot account for depression during brief trains of stimuli (Malinowski and others 1997).

### *Reduction in Calcium Entry*

A fourth mechanism that may contribute to depression of neuromuscular transmission with repetitive stimulation is reduction in the presynaptic calcium currents that trigger fusion of synaptic vesicles. If inactivation of calcium channels occurred during trains of action potentials, it could account for much of the depression seen with repetitive stimulation. Unfortunately, it is difficult to record presynaptic calcium currents at the NMJ, so it has been difficult to determine whether reductions in calcium currents during repetitive stimulation contribute to failure of neuromuscular transmission. In cells where calcium channels can be studied in detail, it has been found that the channels inactivate during prolonged depolarization of the cell (Hille 2001). However, it has traditionally been believed that during trains of action potentials, changes in calcium entry due to inactivation of presynaptic calcium channels play only a small role in depression (Zucker and Regehr 2002).

The calyx of Held is a synapse in the brainstem that has large presynaptic terminals where voltage clamp and measurement of presynaptic calcium currents is possible. It has recently been found in this preparation that an activity-dependent reduction in calcium entry plays an important role in depression during repetitive stimulation (Xu and Wu 2005). The effect of repetitive stimulation was most pronounced on the P/Q calcium channel type,

which is the calcium channel type that is most important in triggering vesicle fusion at the NMJ (Katz and others 1996; Giovannini and others 2002). Thus, it seems reasonable to propose that reduction in calcium entry during trains of stimuli is an important contributor to depression at the NMJ. Interestingly, the types of calcium channels present at the NMJ may change in LEMS (Giovannini and others 2002). If calcium currents through the up-regulated calcium channel types in LEMS depress less during repetitive stimulation, this may also contribute to the facilitation seen in LEMS.

## **Conclusion**

Failure of neuromuscular transmission plays a role in a number of disease states. In addition to diseases that are classically thought of as diseases of the NMJ such as MG or LEMS, it now appears that defects at the NMJ contribute to weakness in motor neuron disease. Failure of transmission during repetitive stimulation may be due to a variety of causes. A better understanding of the causes of depression at the NMJ during repetitive stimulation is necessary to design better treatments for diseases affecting neuromuscular transmission.

## **References**

- Balice-Gordon RJ, Smith DB, Goldman J, Cork LC, Shirley A, Cope TC, Pinter MJ. 2000. Functional motor unit failure precedes neuromuscular degeneration in canine motor neuron disease. *Ann Neurol* 47:596–605.
- Barstad JA, Lilleheil G. 1968. Transversally cut diaphragm preparation from rat. An adjuvant tool in the study of the physiology and pharmacology of the myoneural junction. *Arch Int Pharmacodyn Ther* 175:373–90.
- Boyd IA, Martin AR. 1956. The end-plate potential in mammalian muscle. *J Physiol* 132:74–91.
- Cull-Candy SG, Miledi R, Trautmann A. 1978. Acetylcholine-induced channels and transmitter release at human endplates. *Nature* 271:74–5.
- Drachman DB. 1994. Myasthenia gravis. *N Engl J Med* 330:1797–810.
- Elmqvist D, Hofmann WW, Kugelberg J, Quastel DM. 1964. An electrophysiological investigation of neuromuscular transmission in myasthenia gravis. *J Physiol* 174:417–34.
- Elmqvist D, Lambert EH. 1968. Detailed analysis of neuromuscular transmission in a patient with the myasthenic syndrome sometimes associated with bronchogenic carcinoma. *Mayo Clin Proc* 43:689–713.
- Engisch KL, Rich MM, Cook N, Nowycky MC. 1999. Lambert-Eaton antibodies inhibit Ca<sup>2+</sup> currents but paradoxically increase exocytosis during stimulus trains in bovine adrenal chromaffin cells. *J Neurosci* 19:3384–95.
- Fischer LR, Culver DG, Tennant P, Davis AA, Wang M, Castellano-Sanchez A, and others. 2004. Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man. *Exp Neurol* 185:232–40.
- Frey D, Schneider C, Xu L, Borg J, Spooren W, Caroni P. 2000. Early and selective loss of neuromuscular synapse subtypes with low sprouting competence in motoneuron diseases. *J Neurosci* 20:2534–42.
- Giovannini F, Sher E, Webster R, Boot J, Lang B. 2002. Calcium channel subtypes contributing to acetylcholine release from normal, 4-aminopyridine-treated and myasthenic syndrome auto-antibodies-affected neuromuscular junctions. *Br J Pharmacol* 136:1135–45.
- Glavinovic MI. 1979a. Presynaptic action of curare. *J Physiol* 290:499–506.

- Glaivovic MI. 1979b. Voltage clamping of unparalysed cut rat diaphragm for study of transmitter release. *J Physiol* 290:467–80.
- Gooch JL. 1995. AAEM case report #29: prolonged paralysis after neuromuscular blockade. *Muscle Nerve* 18:937–42.
- Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, and others. 1994. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science* 264:1772–5.
- Hamilton BR, Smith DO. 1991. Autoreceptor-mediated purinergic and cholinergic inhibition of motor nerve terminal calcium currents in the rat. *J Physiol* 432:327–41.
- Hille B. 2001. Ion channels of excitable membranes. 3rd ed. Sunderland, MA: Sinauer Associates.
- Kao I, Drachman DB. 1977. Myasthenic immunoglobulin accelerates acetylcholine receptor degradation. *Science* 196:527–9.
- Katz E, Ferro PA, Weisz G, Uchitel OD. 1996. Calcium channels involved in synaptic transmission at the mature and regenerating mouse neuromuscular junction. *J Physiol* 497(Pt 3):687–97.
- Kennel PF, Finiels F, Revah F, Mallet J. 1996. Neuromuscular function impairment is not caused by motor neurone loss in FALS mice: an electromyographic study. *Neuroreport* 7:1427–31.
- Kimura J. 2001. Electrodiagnosis in diseases of nerve and muscle: principles and practice. 3rd ed. New York: Oxford University Press.
- Lomo T, Rosenthal J. 1972. Control of ACh sensitivity by muscle activity in the rat. *J Physiol* 221:493–513.
- Lundberg A, Quilisch H. 1953. On the effect of calcium on presynaptic potentiation and depression at the neuro-muscular junction. *Acta Physiol Scand Suppl* 111:121–9.
- Magleby KL, Pallotta BS. 1981. A study of desensitization of acetylcholine receptors using nerve-released transmitter in the frog. *J Physiol* 316:225–50.
- Malinowski MN, Cannady SB, Schmit KV, Barr PM, Schrock JW, Wilson DF. 1997. Adenosine depresses transmitter release but is not the basis for 'tetanic fade' at the neuromuscular junction of the rat. *Neurosci Lett* 230:81–4.
- Otis T, Zhang S, Trussell LO. 1996. Direct measurement of AMPA receptor desensitization induced by glutamatergic synaptic transmission. *J Neurosci* 16:7496–504.
- Pennefather P, Quastel DM. 1982. Fast desensitization of the nicotinic receptor at the mouse neuromuscular junction. *Br J Pharmacol* 77:395–404.
- Person RS, Kudina LP. 1972. Discharge frequency and discharge pattern of human motor units during voluntary contraction of muscle. *Electroencephalogr Clin Neurophysiol* 32:471–83.
- Pinter MJ, Cope TC, Cork LC, Green SL, Rich MM. 2001. Canine motor neuron disease: a view from the motor unit. In: Cope TC, editor. *Motor neurobiology of the spinal cord*. New York: CRC Press. p 231–50.
- Pinter MJ, Waldeck RF, Cope TC, Cork LC. 1997. Effects of 4-aminopyridine on muscle and motor unit force in canine motor neuron disease. *J Neurosci* 17:4500–7.
- Plomp JJ, Van Kempen GT, De Baets MB, Graus YM, Kuks JB, Molenaar PC. 1995. Acetylcholine release in myasthenia gravis: regulation at single end-plate level. *Ann Neurol* 37:627–36.
- Plomp JJ, van Kempen GT, Molenaar PC. 1992. Adaptation of quantal content to decreased postsynaptic sensitivity at single endplates in alpha-bungarotoxin-treated rats. *J Physiol* 458:487–99.
- Preston DC, Shapiro BE. 1998. *Electromyography and neuromuscular disorders: clinical-electrophysiologic correlations*. Boston: Butterworth-Heinemann.
- Redman RS, Silinsky EM. 1993. A selective adenosine antagonist (8-cyclopentyl-1,3-dipropylxanthine) eliminates both neuromuscular depression and the action of exogenous adenosine by an effect on A1 receptors. *Mol Pharmacol* 44:835–40.
- Rich MM, Bird SJ, Raps EC, McCluskey LF, Teener JW. 1997. Direct muscle stimulation in acute quadriplegic myopathy. *Muscle Nerve* 20:665–73.
- Rich MM, Colman H, Lichtman JW. 1994. In vivo imaging shows loss of synaptic sites from neuromuscular junctions in a model of myasthenia gravis. *Neurology* 44:2138–45.
- Rich MM, Teener JW, Raps EC, Schotland DL, Bird SJ. 1996. Muscle is electrically inexcitable in acute quadriplegic myopathy [see Comments]. *Neurology* 46:731–6.
- Rich MM, Wang X, Cope TC, Pinter MJ. 2002. Reduced neuromuscular quantal content with normal synaptic release time course and depression in canine motor neuron disease. *J Neurophysiol* 88:3305–14.
- Robitaille R, Charlton MP. 1992. Presynaptic calcium signals and transmitter release are modulated by calcium-activated potassium channels. *J Neurosci* 12:297–305.
- Ruff RL, Lennon VA. 1998. End-plate voltage-gated sodium channels are lost in clinical and experimental myasthenia gravis. *Ann Neurol* 43:370–9.
- Sanders DB. 2003. Lambert-Eaton myasthenic syndrome: diagnosis and treatment. *Ann N Y Acad Sci* 998:500–8.
- Santafe MM, Salon I, Garcia N, Lanuza MA, Uchitel OD, Tomas J. 2003. Modulation of ACh release by presynaptic muscarinic autoreceptors in the neuromuscular junction of the newborn and adult rat. *Eur J Neurosci* 17:119–27.
- Snider WD, Harris GL. 1979. A physiological correlate of disuse-induced sprouting at the neuromuscular junction. *Nature* 281:69–71.
- Stalberg E, Schwartz MS, Trontelj JV. 1975. Single fibre electromyography in various processes affecting the anterior horn cell. *J Neurol Sci* 24:403–15.
- Takamori M, Sakato S, Okumura S. 1984. Presynaptic function modified by acetylcholine-receptor interaction in experimental autoimmune myasthenia gravis. *J Neurol Sci* 66:245–53.
- Tsujimoto T, Umemiya M, Kuno M. 1990. Terminal sprouting is not responsible for enhanced transmitter release at disused neuromuscular junctions of the rat. *J Neurosci* 10:2059–65.
- Vincent A, McConville J, Farrugia ME, Bowen J, Plested P, Tang T, and others. 2003. Antibodies in myasthenia gravis and related disorders. *Ann N Y Acad Sci* 998:324–35.
- Wang X, Engisch KL, Li Y, Pinter MJ, Cope TC, Rich MM. 2004. Decreased synaptic activity shifts the calcium dependence of release at the mammalian neuromuscular junction in vivo. *J Neurosci* 24:10687–92.
- Wood SJ, Slater CR. 2001. Safety factor at the neuromuscular junction. *Prog Neurobiol* 64:393–429.
- Xu J, Wu LG. 2005. The decrease in the presynaptic calcium current is a major cause of short-term depression at a calyx-type synapse. *Neuron* 46:633–45.
- Zucker RS, Regehr WG. 2002. Short-term synaptic plasticity. *Annu Rev Physiol* 64:355–405.